Study of Muscle Physiology

by

Research and Travel

A Sabbatical Project

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Acknowledgements

I am so grateful for the opportunity to participate in a cutting-edge research study in the field of Muscle Physiology as well as gaining valuable insights and updates in my field of expertise through travel. This sabbatical project would not have been possible without the support of some very important people who deserve special mentions:

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Sabbatical Proposal for 2008 – 2009

Carola Wright, Ph. D.

Rationale:

"The Great Teacher Inspires". This quote by William Arthur Ward is engraved on the Educator of Distinction Award my students presented to me in May of this year.
Being an inspiration to my students has always been my goal and has been the center of my teaching philosophy ever since I joined the Mt. SAC faculty in 2001 to teach Anatomy and Physiology.
My ability to inspire students largely stems from over 10 years of research experience in the field of Physiology. My research projects involved studies at the cellular and molecular level in several laboratories at the University of Goettingen, Germany (1988 – 1991) and at the University of California, Irvine (1992 – 2001). The most recent research experience stems from my dissertation project in the Department of Physiology and Biophysics at UCI (1994 – 2000).
This research experience in combination with my love for teaching has influenced my teaching style and has been able to inspire so many of my students at Mt. SAC.
After seven years of teaching the time has come to refresh this research experience. Therefore my proposed sabbatical activities are centered on participating in a current research project and on once again learning some of the most cutting edge technologies in the field of Physiology.

Proposed Sabbatical Activities:

A. Research

For the Fall semester 2008 I will join the Baldwin lab in the Department of Physiology and Biophysics at the University of California, Irvine to conduct research in the field of muscle Physiology for a time that is equivalent to one and one-half teaching semesters. In a recent meeting with Dr. Baldwin at UCI I discussed the possible research projects with him and obtained permission to join his research group at UCI.
If this sabbatical leave is granted, I will participate on a full-time basis in at least one of the ongoing research projects in the Baldwin lab. Each project is introduced below with a short description. Please refer to appendix A for a more technical and detailed description of the projects.

Project 1: Gene regulation of the cardiac myosin heavy chain genes.

The human heart is composed of interconnected muscle cells whose rhythmic contractions enable the heart to pump blood through the whole body.
The exact contractile properties of the heart depend largely on the precise composition of contractile proteins in the heart muscle cells. Specifically, the composition of the

myosin isoforms determines the contractile properties of the working heart. It is known that the composition of myosin isoforms in the heart changes in response to conditions such as diabetes, hypertension (high blood pressure), hyperthyroidism and hypothyroidism.
Since the myosin heavy chain composition of the heart affects its contractile properties it is very important medically and physiologically to understand how high blood pressure and different hormone states can change the composition of myosin heavy chains in the heart.
The Baldwin lab studies the regulation of gene expression of the myosin heavy chain genes in the heart with the goal of gaining a better understanding of the cellular effects of hypertension, diabetes and different thyroid states on the heart.

Project 2: Myosin heavy chain gene regulation in skeletal muscle

Similar to the heart, the contractile properties of skeletal muscle also depend on the exact composition of myosin isoforms in the muscle cells. Skeletal muscle fibers can be slow, intermediate or fast depending on the myosin isoforms that are expressed in the fiber. Factors such as loading conditions, motor neuron innervation and hormone states determine the myosin heavy chain profile that is expressed in a muscle fiber.
Research in the Baldwin lab focuses on molecular mechanisms of gene switching in response to various physiological stimuli.

Project 3: Prevention of unloading-induced muscle atrophy

When human skeletal muscle is not used it quickly loses its strength.
In studies on human subjects exposed to space flight (zero gravity) or ground-based analogs such as bed rest, it has been shown that both muscle mass and strength are significantly reduced within as little as 5-7 days. These changes occur even when various types of exercise are imposed. This is of practical importance because it drastically limits the time flight crews can spend on space flights. It is currently impossible to send astronauts on a mission to Mars (which would require space travel of several months) or even to keep them for extended time periods at the international space station.
NASA is very interested in research that is aimed at preventing this rapid muscle loss and therefore has been supporting research projects in the Baldwin lab on this topic. Current research projects are aimed at understanding the underlying mechanisms for the changes in muscle structure and function that occur during unloading (or zero gravity). The Baldwin lab is also working on exercise training programs that could be carried out on space flights and would prolong the time astronauts could spend in a zero gravity environment.

B. Travel

Towards the middle of the second teaching semester I will travel to Germany and visit the following places that are related to my teaching assignment at Mt. SAC and to my Sabbatical project:

1. Technical University Munich
    a. Department of Sports science / Sports Medicine.
        Of particular interest are the research projects on muscle mechanics and muscle dynamics in combination with resistance training and sport orthopedics.
        The Department offers lectures and seminars.
        I am planning to attend the Bavarian Sports Congress (a 3-day event) in 2009. The exact schedule is not yet available.
        More information can be found at http://www.sport.tu-muenchen.de/

2. German Heart Center (Deutsches Herzzentrum) in Munich (http://www.dhm.mhn.de/ww/de/pub/dhm.htm)
    a. The German Heart Center in Munich specializes in molecular cardiology and in the diagnosis and treatment of cardiovascular disease. The center also contributes to the advancement of diagnosis and treatment through its own patient – related research activities.
        I am planning on visiting the center and participating in at least one workshop and / or Symposium hosted by the center during the time of my visit. The exact Symposium schedule for 2009 is not available yet.

3. Munich Center of Molecular Life Sciences
    a. The Center offers research and facilities in the areas of functional genomics, molecular medicine, protein science, Biotechnology and Cell Biology (http://www.ch.tum.de/lifesciences/). They offer many lectures and seminars in the above areas. I will attend at least two events the center offers during my stay in Munich (exact calendar of events is not available yet).

B. Travel Updated Plan (approved by Salary and Leaves Committee)

During the second teaching semester I will travel to Germany and visit the following places that are related to my teaching assignment at Mt. SAC and to my Sabbatical project:

4. Goethe University Frankfurt and Sportklinik Bad Nauheim
   a. Department of Sports science / Sports Medicine.
   Of particular interest are the research projects on muscle mechanics and muscle dynamics in combination with resistance training and sport orthopedics.
   The facilities offer ongoing lectures and seminars.
   Currently posted offerings that are relevant to my project inclcude
   - Sport therapy in cardiology and
   - Sport and Diabetes mellitus

   More information can be found at http://www.sportmedizin.uni-frankfurt.de/ and http://www.sportklinik-badnauheim.de/

   I am planning on visiting the centers and participating in at least one public lecture, workshop and / or Symposium offered during my time in Frankfurt. Events are offered on an ongoing basis.

5. German Heart Center (Deutsches Herzzentrum) in Frankfurt, also known as CCB (Cardioangiologisches Zentrum Bethanien)
   http://www.herzzentrum-frankfurt.de/

   a. The German Heart Center in Frankfurt specializes in the diagnosis and treatment of cardiovascular disease. The center also contributes to the advancement of diagnosis and treatment through its own patient – related research activities.
   I am planning on visiting the center during my stay in Frankfurt. If a public lecture, workshop and / or Symposium is offered by the center I will participate in at least one such event.

6. The *'Excellence Cluster Cardio-Pulmonary System'*
   of the University Frankfurt and the Max-Planck-Institute (MPI) for *Heart and Lung Research* in Bad Nauheim (near Frankfurt) constitutes a unique translational research center, dedicated to combine cutting edge basic sciences with preclinical and clinical studies in the field of *vascular and parenchymal heart and lung diseases.* Research is organized on different levels of complexity such as molecular signatures and target structures, cellular phenotypes, integrative - including developmental - biology, disease models and preclinical studies as well as clinical trials.
   They offer many lectures and seminars in the above areas. I will attend at least two events as offered during my stay in Frankfurt.
   More information at http://eccps.de/index.htm

If any of the above places are unavailable for visit or a major event I was planning on attending is cancelled I agree to contact the Salary and Leaves committee in advance regarding approval of any changes that may have to be made.

Proposed Timeline

I am planning to spend at least three fourths of my sabbatical leave time on the research projects outlined above. For the remaining time I will travel to Germany and visit the places mentioned in section B above.
Since the nature of research is to explore and study the unknown it is hard to predict the exact nature and time line of the project. A reasonable time line to the best of my knowledge is listed below:

Fall 2008:

Week 1: Begin research activities in the Baldwin lab in the Department of Physiology and Biophysics at UCI. The exact project / projects will depend on the progress the Baldwin lab has made in the different research areas up to that point.
I will begin with a review of the literature related to my research project. Specifically, I will be reviewing relevant journal articles published in the American Journal of Physiology, the Journal of Applied Physiology and the Journal of Biological Chemistry.

Week 2: Review of research tools needed for the particular project. Develop a research strategy to answer questions regarding the project.

Weeks 3 & 4: Preliminary tests on the methods as they apply to my project.
Refine lab protocols. Learn new PCR techniques and test them on control samples.

Weeks 5 & 6: Work on animal models. Learn surgical techniques as necessary for my project. The Baldwin lab uses rats as animal models for studies on the heart and on skeletal muscle.

Weeks 7 & 8: Molecular biology. Work on plasmid constructs. Design PCR primers. Extract RNA / DNA. Manipulate DNA as necessary for the project.

Week 9: Test DNA constructs. Sequence DNA as necessary.

Weeks 10 & 11: In vivo gene transfer experiments. Test DNA constructs in animal models.

Weeks 12 & 13: Isolate heart muscle or skeletal muscle tissue. Analyze muscle tissues for reporter gene activities, protein levels or RNA content as appropriate for the project.

Week 14: Analyze data. Make adjustments for next round of experiments.

Weeks 15 & 16: Refine PCR techniques. Test alternative protocols. Work on plasmid constructs. Set up a new set of test animals. Perform surgeries and/ or experimental procedures on animals.

Spring 2009

Weeks 1 & 2: Continue the research project. Extract muscle tissues and test for reporter gene activities, protein levels or RNA content as appropriate for the project.

Weeks 3 - 6: Collect more data. The goal will be to produce quality data that can ultimately be submitted for publication in a peer reviewed journal article. However, given the nature of research, the outcome of the research project is impossible to predict. And while I am hoping that I can produce publishable data during my sabbatical leave I cannot promise that this will be the case.
Towards the end of my research project I will make final arrangements for my travel to Germany, including housing arrangements and applications for participation in the relevant symposia, conferences etc. as outlined above.

Week 7 & 8: Finish up work in the Baldwin lab. Begin writing up the results.

Week 9: Travel to Germany. Settle into my new living arrangements in Munich. Since I am bilingual English and German there is no need for a language course and I will be able to visit the places and attend the seminars and symposia as outlined in section B above.

Weeks 10 - 13: Visit the Technical University Munich, Department of Sports science / Sports Medicine, the German Heart Center and the Center of Molecular Life Sciences in Munich. Attend Seminars and Symposia as they are offered during that time.

Week 14: Return to California. Begin writing the sabbatical report.

Week 15: Prepare research results for publication, if possible. Continue writing on the sabbatical leave report.

Week 16: Complete sabbatical leave report.

Anticipated Value and Benefit of the Proposed Sabbatical Activities:

A. Benefits to my personal and professional development

Physiology is a dynamic field that has experienced much growth and accumulation of new knowledge over the past decades. It is a foundation science for the field of Medicine and as such a course in Human Physiology is a major prerequisite course for all students entering nursing programs or pursuing a career in any of the health care professions.
At Mt. SAC the majority of my teaching assignments are lectures and labs in Human Physiology.
When I teach Physiology I find it extremely helpful to have a research background in the field. My enthusiasm for teaching Physiology also stems from my research

background. Students catch on easily to this enthusiasm by asking questions and showing a genuine interest in the course material.
My sabbatical project will help me to stay in touch with my field of expertise. This will translate into enthusiastic lectures and labs in which students are presented with current information and up-to-date knowledge in the field of Physiology.
The travel component of my proposed sabbatical activities will add broader knowledge at an international level to my expertise in the field.

B. Benefits to the Biology Department

Given the constant changes and increase in available knowledge in the field of Physiology it is important to keep our lab manuals and lab exercises current. While teaching, it is difficult to stay current in the field. My sabbatical project will be beneficial to the Department because I will be able to share my knowledge and expertise when we update the lab exercises for the Physiology students.
I am also planning to share the results of my research project and my travel experiences with my colleagues in form of a PowerPoint presentation and/or a written report.

C. Benefits to the College

An enthusiastic instructor with updated knowledge in a dynamic field presents an asset to the College.
Through my interactions with faculty from various Departments I will be able to share my research experience with colleagues outside of the Biology Department.
Over the years teaching at Mt. SAC several of my students have become interested in research themselves. My experiences and connections will help them to find research and transfer opportunities at four-year Universities.
In addition, as an instructor with international experience I have a better understanding of the cultural and language barriers that so many of our international or immigrant students face. On numerous occasions I have been able to encourage international students to face their challenges and to try until they succeed.

Abstract

This sabbatical proposal is a combination of project and travel. The project component will encompass research in the field of muscle Physiology at UC Irvine and will take about 75% of the time. The travel component will include travel to Germany for the remaining time. I will visit research facilities and institutions in the Munich area that are relevant to my research project and my teaching assignment.

Abstract

This report is about a research project in the field of skeletal muscle Physiology and travel in Germany relating to the fields of muscle physiology, cardiology and exercise physiology.

Recent advances in the field of epigentics have provided a novel way to study the complex cell physiological processes that lead to plasticity in muscle cells. Differential epigenetic modifications of histones at the myosin heavy chain genes were examined in slow skeletal muscle fibers and in response to muscle unloading. Chromatin Immunoprecipitation assays were used to study complex gene regulation of myosin heavy chain genes in a model of hind limb suspension and thyroid treatment.

Travel in Germany provided updates and new insights into the fields of cardiology, exercise physiology and sports medicine.

Statement of Purpose

Physiology is a dynamic field in which new research data constantly adds novel information to the existing body of knowledge.

My students have always enjoyed and benefited from my frequent references to recent or ongoing research in the field of Physiology. Many have reported that it has stimulated their critical thinking and their general interest in the subject matter. In depth knowledge of the research methods as well as the current publications in the field are essential to being able to apply this information in the classroom setting.

This sabbatical project was an opportunity to participate once again in cutting-edge research and to get an update in my specialty field of muscle physiology through research and travel. By joining a muscle physiology research group at the University of California Irvine, I was able to learn some of the most advanced methods and technologies in gene expression and apply them to a study of skeletal muscle tissue. In addition I had access to very advanced equipment and learned how to use it for my research project. This also included learning to use software programs such as GraphPad Prism, ImageQuant and Photoshop.

The travel time in Germany further broadened my horizon as it exposed me to different approaches to research and study in the fields of cardiology, muscle physiology and exercise science.

This new knowledge and experience will be incorporated into my lectures and enrich the classroom experience of my future students.

Summary of Research Activities

The Baldwin Lab at UC Irvine focuses on research in the field of muscle physiology. There are three major branches dividing up the lab activities and research efforts: 1. cardiac muscle physiology, 2. skeletal muscle physiology and 3. exercise physiology and sports science.

As outlined in my original proposal I was going to participate in a research study in at least one of these three areas. When I joined the Baldwin lab it became quickly clear that I would be able to get the best experiences and learn the most advanced techniques by participating in the ongoing skeletal muscle project.

Skeletal muscle has plasticity and is able to adapt to various physiological and pathophysiological stimuli. For example, it is well known that muscle quickly atrophies when exposed to a zero gravity environment such as a space flight. It is also known that the slow type I myosin heavy chain isoform is almost completely shut down in a model of hind limb suspension and thyroid treatment. Although the effects are often apparent the causes are usually poorly understood. However, it is absolutely necessary to understand the causes if we want to be able to interfere and ultimately prevent the effects of zero gravity or hind limb suspension on a muscle.

The soleus muscle in the leg is a slow muscle that mostly consists of slow type I fibers (endurance fibers). Expression of the slow type I myosin heavy chains (MHCs) is almost completely shut down in the model of hind limb suspension (which simulates space flight) and thyroid hormone treatment. The question is what makes the type I MHC turn off in the T3 + HS model? It is known that the MHC I gene is regulated at various levels including transcription factors, pre mRNA, alternative splicing, antisense RNA and

intergenic transcription (2). Very little is known about whether epigenetic mechanisms also regulate MHC expression in skeletal muscle with altered muscular activity.

It became my project to find out whether epigenetic modifications play a role in the expression of the MHC type I genes in response to altered muscular activity and thyroid treatment.

Relevance of Epigenetics

Until recently, chromatin has rarely been thought of as much more than just genomic DNA and protein packaging. However, an emerging field in biological research over the past decade, termed "epigenetics," has given new-found respect to chromatin structure as being a major player in the control of transcriptional activity and gene expression (2).

The term Epigentics means literally "above the genetics". This indicates that there are factors controlling the activity of genes (on and off switches of genes) that are not part of the DNA sequence itself. Epigenetic modifications, unlike genetic mutations or single nucleotide polymorphisms, do not result in changes in the DNA sequence. Instead, DNA structure is indirectly modified by histone modifications (e.g. acetylation or methylation) to alter the expression of genes. Histone modifications can be both transient, rapidly responding to changes within the cell or surrounding environment, or stably maintained throughout life when obtained in early or fetal life.

Histone acetylation can occur at many different lysine residues (e.g., lysine 4, 9, or 27), creating a negative electrostatic charge that repels the negatively charged DNA backbone, thus producing a loosely packed chromatin that allows easier access for transcription factors and the RNA polymerase complex to increase transcription, also providing a site for recruitment of other proteins to the promoter region of a given gene. Conversely, histone deacteylation results in a compact, tightly bound histone:DNA complex

and general repression of gene transcription (2). The acetylation status of core histones is based on the balance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity in the nucleus. Although it is known that changes in muscle activity can alter the activity of HATs and HDACs there are no reports in the literature demonstrating that histone modifications occur in skeletal muscle at the locus of the MHC genes and that they concur with changes in MHC gene expression.

It became my project to study whether histone modifications at the MHC type 1 gene are responsible for the almost complete shut down of the gene that occurs with hind limb suspension and thyroid treatment of rat soleus muscle. The specific modifications of interest included acetylations and methylations at specific lysine residues on the histone H3 protein on the type I MHC gene. The way to study this is by chromatin immuno-precipitation assay (ChIP assays).

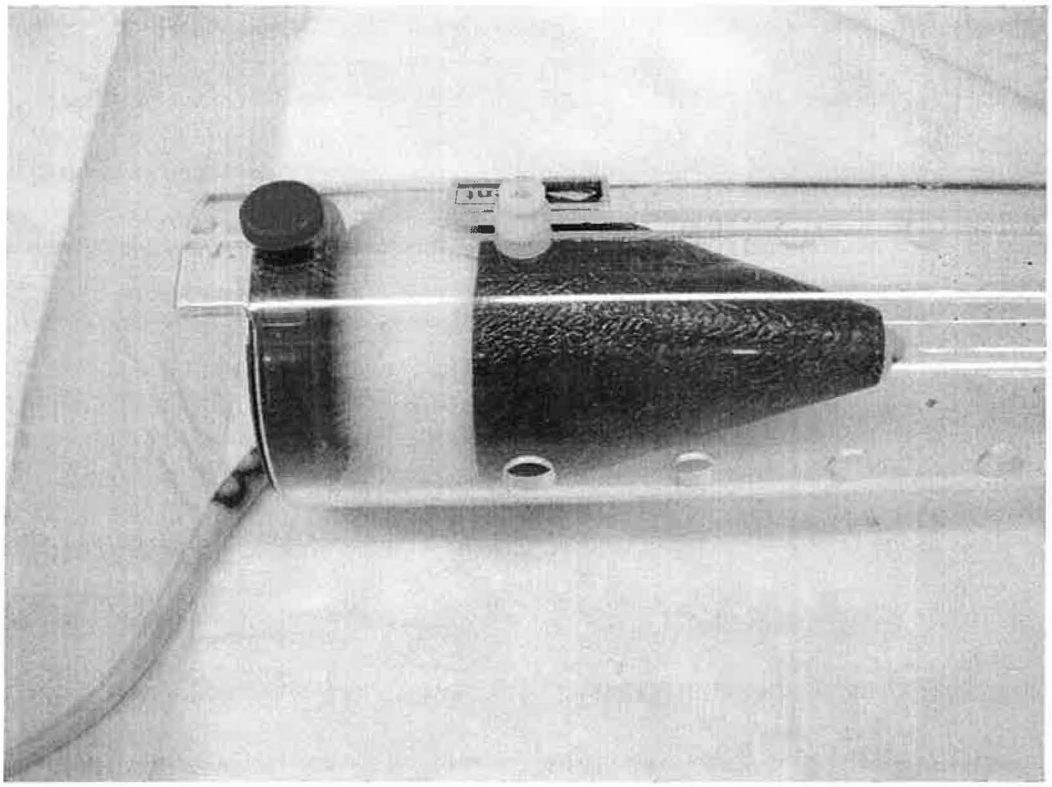
Research methods

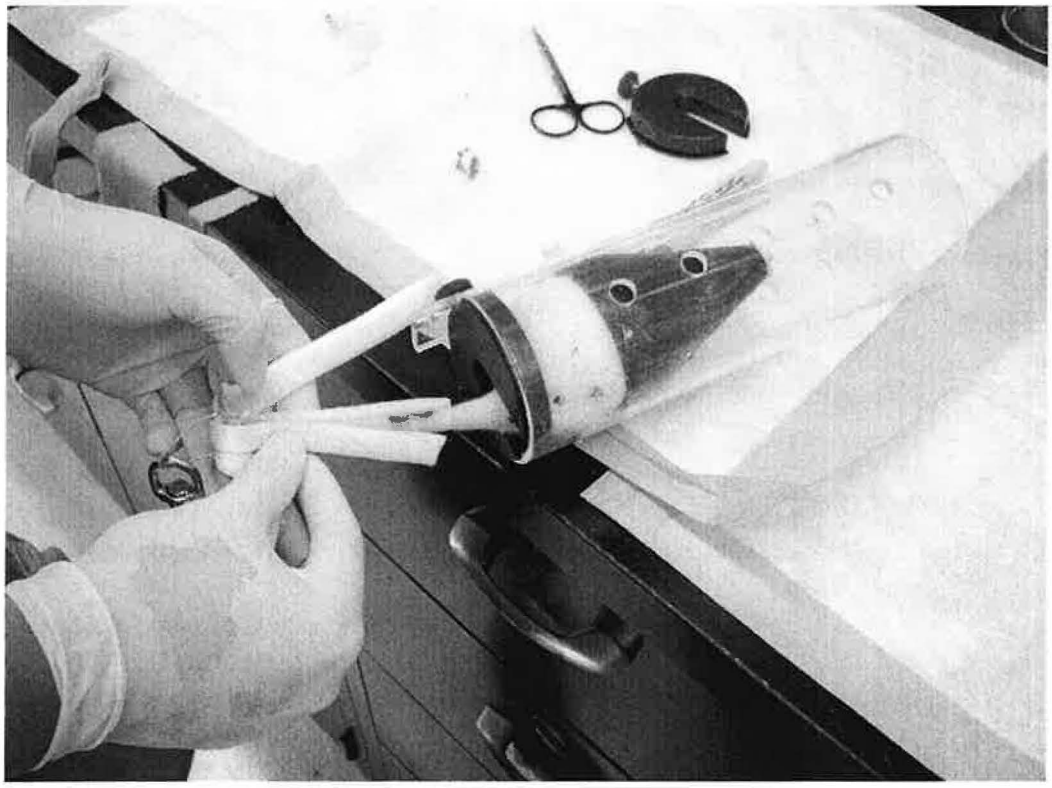
The animal model

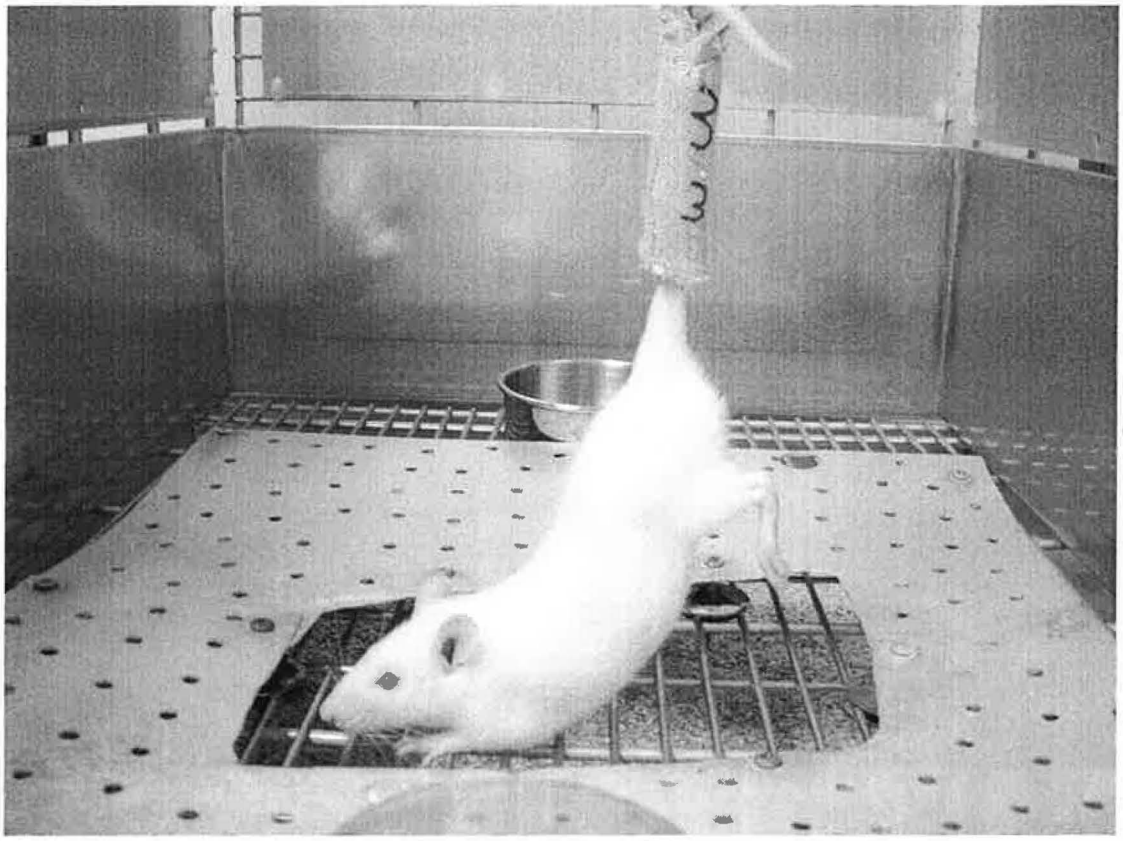
In order to have tissue to work with for my project I had to obtain animals (rats). The animals were divided into two groups of seven rats each. Group A is the untreated normal control group while group B is subjected to hind limb suspension and thyroid hormone treatment.

The animal model of hind limb suspension and thyroid treatment is well established in the Baldwin lab and further described in appendix A and reference 1 of this report.

The sample pictures below illustrate the method.







Rats in the experimental group are not allowed to use their hind limbs during the time of the study which simulates weightlessness or a situation of complete unloading of the muscle such as during a hospital stay for a broken leg bone.

Tissue Collection

After seven days of hind limb suspension and thyroid treatment the animals of both control and experimental groups were sacrificed and tissues collected. All collected muscle tissues were frozen at -80 °C and stored at that temperature until used for the experiments.

Please refer to appendix A for details of the animal and tissue collection procedures and protocols. Below is a table that summarizes the tissues collected on 9-18-08. The study was carried out twice with another 14 rats, each time with similar results and tissue harvest.

Studied carried out to collect tissue for CHIP assay for Carola's epigenetics GC vs. HS-T3 study. Animals were euthanized on 9-18-08

GC= Ground Control BW = Body weight MG = Medial Gastrocnemius

HS= hind limb suspension T3 = thyroid hormone treatment by injection

		Left	Left	Left	Right	Right	Right
GC	BW	Soleus	Plantaris	MG	Soleus	Plantaris	MG
1	168	69	170	368	68	173	375
2	165	65	162	342	74	152	356
3	177	68	197	401	71	203	414
4	168	70	172	376	74	185	377
5	184	72	193	430	77	199	427
6	198	83	204	430	81	214	444
7	189	71	188	426	72	194	434
8	171	65	168	411	66	174	420
Averages	**178**	**70**	**182**	**398**	**73**	**187**	**406**

7day treatment		Left	Left	Left	Right	Right	Right
HS + T3	BW	Soleus	Plantaris	MG	Soleus	Plantaris	MG
1	167	47	150	299	45	155	300
2	133	35	100	229	31	92	210
3	160	42	112	253	35	117	253
4	164	45	157	272	44	143	266
5	150	35	122	264	29	125	253
6	131	30	91	209	30	98	214
7	145	44	121	298	40	124	280
8	142	59	132	268	56	123	359
Averages	**149**	**42**	**123**	**256**	**39**	**122**	**265**

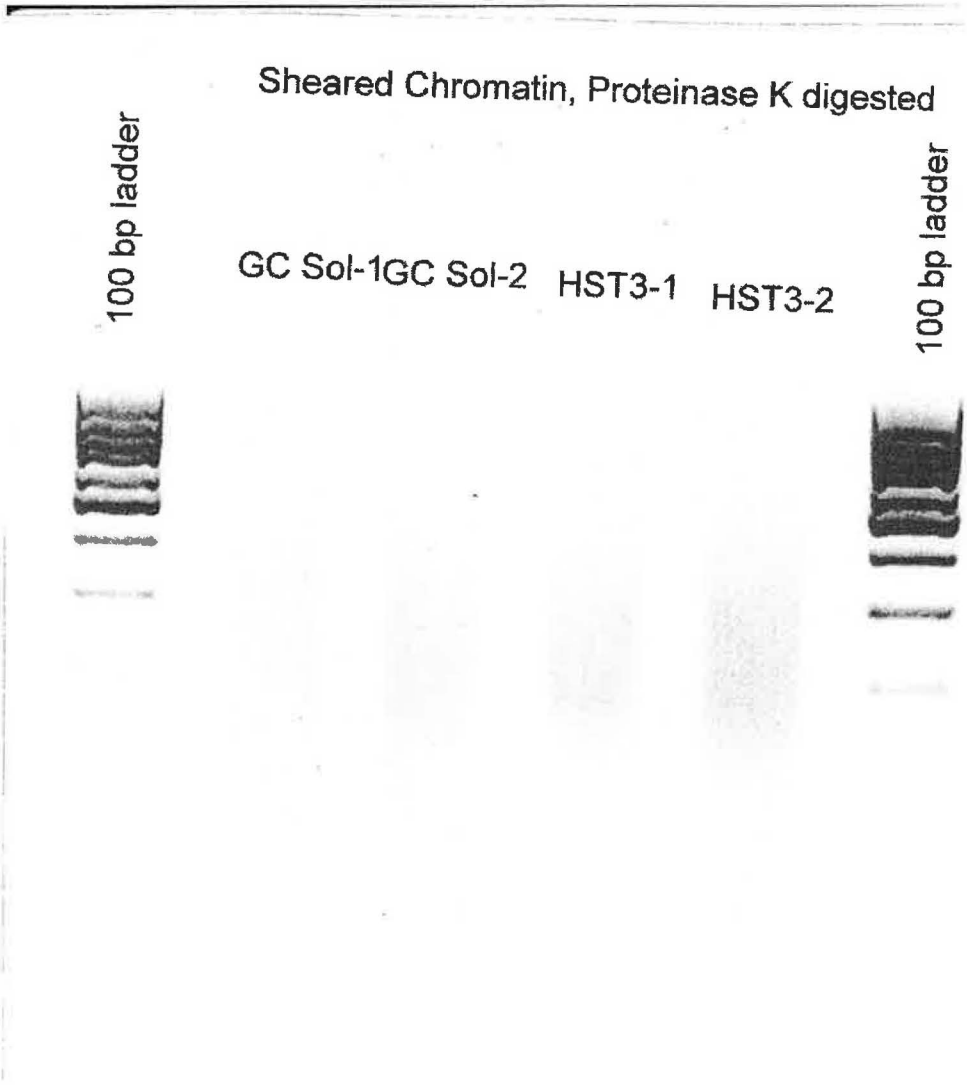
 # 8 rat got out of its cast several times, not a good suspension. Therefore was not used for data collection.

Summary of Tissue preparation

For each set of new experiments I selected 100 mg of frozen GC Soleus muscle and another 100 mg of frozen HS + T3 Soleus muscle (pool several muscles samples to get 100 mg).

Following an elaborate procedure that involves cutting, washing, chemically cross-linking protein and DNA, homogenizing and centrifuging the tissue samples the muscle cell nuclei are being prepared for the chromatin immuno precipitation assay.

Additional steps before the ChIP procedure can be started involve sonication of the protein DNA complexes to break them down into pieces of 200 – 1000 base pairs. To ensure effectiveness of sonication, an aliquot of the supernatant is reverse cross-linked by incubation at 65° C overnight, and RNAse treated. Then the protein is digested and run on a 2% agarose gel to confirm size of DNA fragments to be between 200 and 1000 bp. A representative gel picture is shown below:



Another aliquot of the same sample is then used to measure the DNA concentration of the chromatin-DNA. This is necessary because of the varied muscle mass to DNA ratio in the three types of muscle samples analyzed. HS Soleus tissue is more enriched in DNA than ground control Soleus. Therefore in order to equalize the starting DNA concentrations for the ChIP assay SYBR green I is used to bind DNA. A Stratagene Mx3000p real-time PCR machine is used in the quantitative plate read mode to accurately measure DNA concentration, with thymus calf DNA (Sigma) used as a standard (1).

For more detail on the tissue preparation and analysis please refer to appendix D and reference 1.

Chromatin Immuno-Preciptitation ChIP assay

The ChIP assay is an extremely involved multi-step procedure. Each step has to be optimized and applied to the given tissue and study. The exact protocols including preparation of all solutions, materials and equipment used are detailed in Appendix D and in reference 1.

At the end of each ChIP procedure, the Chromatin-DNA complexes are eluted from agarose beads and cross-links are reversed by incubation at 65°C overnight. Samples are then RNAse A treated, protein is digested (proteinase K), and the DNA is purified by using a spin column. Immunoprecipitated DNA for specific genes is then analyzed and quantified by PCR. PCR primers used with ChIP samples are shown in the table below (1). The number of PCR cycles and amount of ChIP DNA has to be adjusted so that the accumulated product is in the linear range of the exponential curve of the PCR amplifications. PCR products are separated by electrophoresis on agarose gels and stained with GelGreen. The ultraviolet light-induced fluorescence of stained DNA is captured by a digital camera, and band intensities are quantified by densitometry with ImageQuant software on digitized images.

PCR primer sequences, their specific target, and PCR product size, for primers used for ChIP of MHC genes.

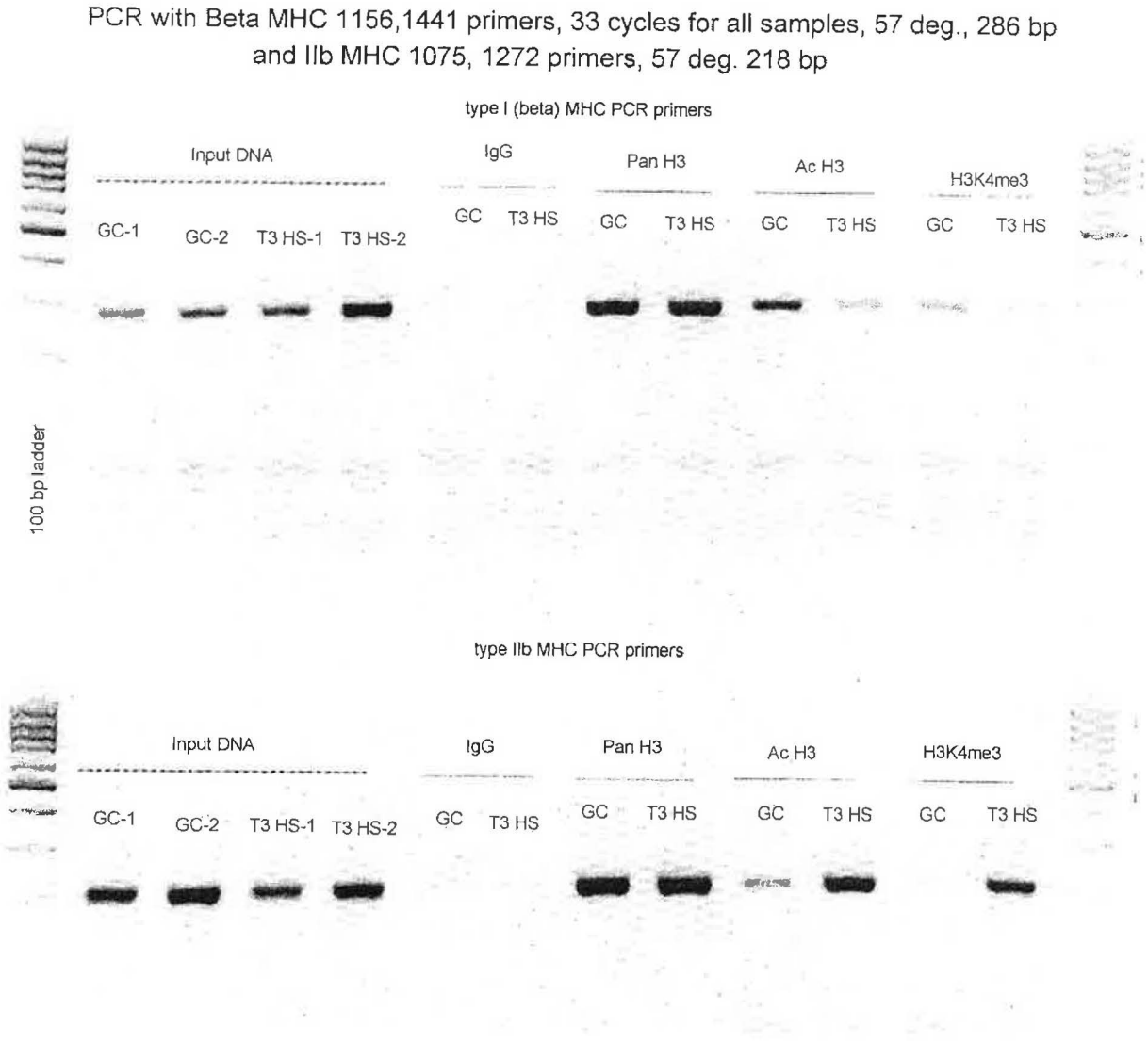
Target	RT-PCR primers: 5'→3'	PCR product size (bp)
Type I	Fwd: GGCCTGGGCCTACCTCTTTATCC Rev: TATTCAATTGGGGCACTCTTCGGGTGTAT	286
IIa	Fwd: ATCATTACCCCAAATATCACCCTATCC Rev: GGCCCCAGATGCACATTACACTA	323
IIx	Fwd: TGCCACAGAAAGAGGGACGC Rev: CTGGCTGTGGTGTGGCTGAAA	290
IIb	Fwd: AGGGAATAAATGTTAACTTGTTGACACTGG Rev: GGGGGCGGGGCTAATGAAGC	218
β-actin	Fwd: CACGCCCTTTCTCAATTGTCTTTCT Rev: GGCCATTTATCACCAGCCTCATTAG	225

With the ChIP assay, primers can be targeted to any genomic region. The histone modifications H3ac and H3K4me3 have been shown previously to peak immediately downstream of the transcription start sites of active genes (see ref 1, sub ref 6,13 and 51). A preliminary analysis of these histone modifications in the MHC genes resulted in the same conclusion. Thus, PCR primers were designed to target the second intron of each MHC gene studied, which occurs before the translation start site (ATG) of each gene. This location occurs at 1.1 to 1.4 kb from the transcription start site of the ~25kb MHC genes.

For each sample four ChIP assays were carried out in parallel reactions with antibodies to H3ac, H3K4me3, core histone H3, and normal rabbit IgG. The latter serves as a negative control for specificity of antibody binding. For analysis, the normal rabbit IgG IP

signal was subtracted from the specific antibody IP signals. In all cases the normal IgG precipitated negligible levels of DNA for the targeted genes analyzed. Then this value was divided by the input DNA PCR signal, to correct for any differences in starting DNA concentrations between samples, and for differences in DNA accessibility at the PCR-targeted genomic sites (1). Please see reference 1 for more detail on this procedure.

A representative agarose gel showing the PCR products as the ultimate result of each ChIP assay procedure is shown below:



Statistical Analyses

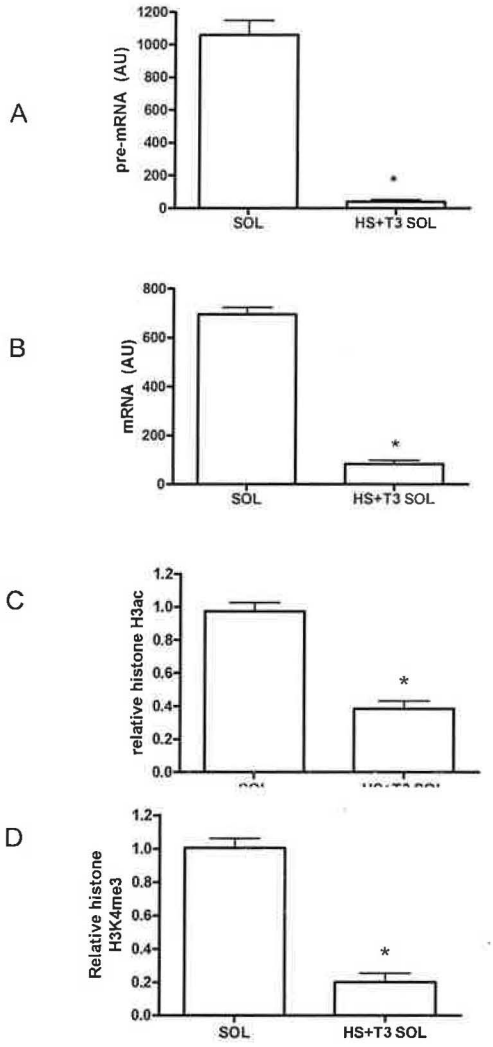
Data are reported as mean ± SEM. Differences between three muscle groups (Plantaris, Soleus, HS Soleus) were analyzed using one-way ANOVA, with Newman-Keuls post hoc test. Differences between two groups were analyzed using an unpaired t-test. Statistical significance was set at $P<0.05$.

Summary of the Results

It is the nature of original research to explore the unknown and to move on to uncharted territory without any guaranties for the outcome. Therefore, the above described methods and protocols all had to be carried out multiple times to optimize each step for the best possible results. Please refer to Appendix D for a complete record of all protocols, changes to protocols and optimizations for each step.

In the Baldwin lab I was part of a research group studying the epigenetics of MHC isoforms. Our results were collectively published in the American Journal of Physiology (July issue). This original research paper of which I am a co-author is attached as appendix B. Since the findings and results of our paper are highly relevant and important to the field, the editors of the American Journal of Physiology decided to write an editorial focus paper about our publication. This editorial focus article highlights the importance of the results of our research study and is attached to my report as appendix C.

My contribution to the American Journal of Physiology paper is the comparison of ground control soleus muscle versus hind limb suspended and thyroid hormone treated (HS + T3) soleus muscle. A summary of my results is shown in the figure below.



Type I MHC transcription and relative modifications to H3ac and H3K4me3 in SOL and HS+T3 SOL. A. pre-mRNA. B. mRNA. C. Histone H3ac. D. Histone H3K4me3. Bar graphs show mean ± S.E. N = 6 / group. Histone H3ac and histone H3K4me3 are corrected for core histone H3 occupancy, as described in the Methods. *, significantly different from SOL, ($p < 0.01$).

Repression of type I MHC with HS + T3. Hind limb suspension combined with thyroid hormone treatment (HS+T3) results in virtually complete repression of type I MHC transcription (Panel A and B in the figure above). This also resulted in a significant reduction of both H3ac (Panel C) and H3K4me3 (Panel D) at the type I MHC.

Histone H3 acetylation. The H3ac data shown is expressed relative to the core histone H3, in order to account for variation in histone H3 occupancy between the different muscle samples with differing rates of gene transcription. Histone occupancy is subject to alteration depending on the transcriptional state of the gene, such that nucleosome occupancy inversely correlates with transcription rate (1). When an inactivity model is imposed on the slow soleus muscle transcription of the slow type I MHC is greatly reduced at the level of the pre-mRNA and RNA (panel A and B above) and a faster MHC transcriptional pattern emerges.

Histone H3 lysine 4 trimethylation. Similar to H3ac, H3K4me3 was highly enriched at the second intron of the IIx and IIb genes in the fast plantaris relative to the slow soleus muscle, which similarly corresponds with the transcriptional activity of these MHC genes in each muscle type (1). H3K4me3 at the type I MHC also corresponds with type I transcription, such that trimethylation is high in the ground control soleus as compared to a significant reduction in the HS + T3 soleus muscle.

Histone modifications at β-actin gene. In order to validate the results of the ChIP assays in determining the levels of enrichment of H3ac and H3K4me3 at the MHC genes, I examined the H3ac- and H3K4me3- ChIP DNA for another target gene, β-actin, which is less prone to dynamic change. The β-actin gene is constitutively active in skeletal muscle, and its expression is unaltered in HS soleus compared to ground control soleus. Thus, β-actin serves as a control gene unaffected by HS (1).

For all the details on these results please refer to my research journal which is attached as Appendix D.

References

1. **Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading. Am J Physiol Cell Physiol (April 15, 2009). doi:10.1152/ ajpcell.00075.2009.**
   Pandorf CE, Haddad F, Wright C, Bodell PW, Baldwin KM.

   A complete copy of this publication is attached to this report as Appendix B.

2. **Novel epigenetic regulation of skeletal muscle myosin heavy chain genes. Focus on "Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading"**
   Kevin A. Zwetsloot, Matthew J. Laye and Frank W. Booth
   Am J Physiol Cell Physiol 297:1-3, 2009. First published Apr 29, 2009; doi:10.1152/ajpcell.00176.2009

   A complete copy of this publication is attached to this report as Appendix C.

3. **Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle.**
   Baldwin KM and Haddad F. *J Appl Physiol* 90: 345-357, 2001.

Travel

During the spring semester 2009 I travelled to Frankfurt, Germany.

This was a change from my original proposal of travelling to Munich, Germany. The change of location was approved by the salary and leaves committee in January 2009.

Frankfurt is the most international city in Germany, the largest financial center in Europe, the city of Goethe and the Frankfurt School. Since the city also plays host to many international science and sports events it was the ideal location for me to further my studies of Physiology at an international level.

In my travel plans I proposed to visit three places related to exercise physiology, sports medicine and physiology of the heart. My experiences at each place / center and what I learned at each of the events I attended are summarized below.

Program information, list of speakers and certificate of attendance letters ("Teilnahmebescheinigung") are attached as applicable to each place I visited.

References with relevant publications are also listed for further information.

1. Exercise Science and Sports Medicine

The Goethe University Frankfurt has a large Department of Sports Science / Sports Medicine. In addition there is a famous sports clinic in Bad Nauheim (near Frankfurt) treating patients with sports related injuries.

Since I had just completed a research project on the epigenetics of skeletal muscle gene regulation I became particularly interested in any sports science project or research that would focus on environmental or even psychological factors affecting muscle (i.e. athletic) performance. These could include particular exercise regimen as well as special foods or diets aimed at enhancing muscle / athletic performance.

In my search for an expert in the field I met Martin Lobstedt who had just attended a symposium of the Bundesinstitut für Sportwissenschaft (BISp) covering that topic. He is a sports scientist and state trainer working for the Landes Sportbund (LSB) in Frankfurt. Since my research was of interest to him and his knowledge and experience was of interest to me we formed a working relationship discussing the epigenetics of muscle physiology and the implications for the training of top athletes in the various sports.

In several seminar style meetings our talks / discussions centered around „Talentdiagnose und Talentprognose im Nachwuchsleistungssport".

In particular I learned in these meetings:

- How talent in sports can be recognized and advanced.
- How the environment contributes to development of athletes.
- How sport science responds to advances in muscle physiology research.
- The relevance of diet in athletic performance

These discussions were valuable because they helped me to gain an understanding of how

- sports scientists in Germany respond to research in the field of muscle physiology
- the knowledge affects their training plans

References:

The National Institute for Sports Science in Germany web site.

http://www.bisp.de/cln_090/sid_2D4CE0D246E0D6E00192487939E5196D/DE/Home/homepage__node.html?__nnn=true

All publications including the entire sports science symposium 2009 are listed here.

http://www.bisp.de/cln_090/nn_1181334/SharedDocs/Downloads/Publikationen/sonstige__Publikationen__Ratgeber/BISp__Symposium__2009,templateId=raw,property=publicationFile.pdf/BISp_Symposium_2009.pdf

2. Cardiac Physiology

The German Heart Center and the Heart Center of the Kerkhoff Klinik Bad Naueim sponsored a Symposium on the heart called "Herzinsuffizienz – Update" which I attended on June 6 2009. This was an all-day symposium which featured over 40 different speakers that are all experts in the field of cardiology.

In particular I learned:

- About non-ischemia heart failure – new therapy approaches
- Heart failure with ischemia: therapy options in patients with acute heart failure and / or cardiogenic shock
- Heart failure due to valve defect – patho-physiology, diagnostics and treatment options
- Terminal heart failure: options for heart transplants and electro stimulation
- Each topic had case studies which were helpful for understanding and applying the information given in the presentation.
- International comparison Differences in the diagnostic philosophies and resulting treatment plans between Europe and the United States.

Overall this symposium focused only on the very latest information and technology available in the field of cardiology. It was an excellent update for anyone teaching anatomy, physiology and patho-physiology of the heart.

Deutsche Gesellschaft für Kardiologie
– Herz- und Kreislaufforschung e. V.
GERMAN CARDIAC SOCIETY

WEITER- UND FORTBILDUNGS
AKADEMIE KARDIOLOGIE

Anerkannt nach § 10 der Fortbildungsordnung durch die Landesärztekammern Nordrhein (seit April 2001), Bayern (seit Juli 2003) und Westfalen-Lippe (seit Juni 2005)

Teilnahmebescheinigung

Herr/Frau Dr. Carola Wright
Titel/Name/Vorname

EFN

geboren am: 23. Dezember 1964 EFN.....
Datum

wohnhaft in: Schüttenhelmweg 51, 60529 Frankfurt /Main
Straße/Hausnr., PLZ, Ort

hat an der Fortbildungsveranstaltung zum Thema:

Herzinsuffizienz - Update 2009

Akademie-Veranstaltungsnummer: FGK - 09 - 06 - 06 - 24133

vom 06. Juni 2009 Beginn: 09:00 Uhr bis 06. Juni 2009 Ende: 17:00 Uhr

in Bad Nauheim teilgenommen.

**Die Veranstaltung wurde von der
Weiter- und Fortbildungs-Akademie *„Kardiologie"* bewertet,
und ist im Rahmen der „Zertifizierung der ärztlichen Fortbildung"
unter der Voraussetzung der vollständigen Veranstaltungsteilnahme
durch die Landesärztekammer Hessen mit
8 Fortbildungspunkt(en) anrechenbar.**

Prof. Dr. Christian Hamm
Kerckhoff-Klinik GmbH
Ärztlicher Direktor

Bad Nauheim, 06.06.2009
Datum

Stempel/Unterschrift
Wissenschaftlicher Leiter

VNR *27606020090447300000*

Kategorie: A	Fortbildungspunkt(en): 8	Zusatzpunkte: 0

Wir möchten ausdrücklich darauf hinweisen, dass bei nicht aufgeklebtem LÄK-EFN-Barcode-auf der Teilnehmerliste die automatische Übermittlung der CME-Punkte durch den Veranstalter an den EIV nicht vorgenommen werden kann.
In diesem Fall müssen Sie diese Teilnahmebescheinigung direkt bei Ihrer Ärztekammer einreichen.

Deutsche Gesellschaft für Kardiologie – Herz- und Kreislaufforschung e. V. • Achenbachstr. 43 • 40237 Düsseldorf
Vorstand: Prof. Dr. Dr. h. c. Gerd Heusch (Präsident) • Prof. Dr. Rainer Dietz (Stellvertreter) • Prof. Dr. Michael Böhm
Geschäftsführer und besonderer Vertreter nach § 30 BGB: Dipl.-Math. Konstantinos Papoutsis
Sitz: Bad Nauheim • Eingetragen beim AG Friedberg unter VR 334 • Steuer-Nr: 105 5888 1351

3. The *'Excellence Cluster Cardio-Pulmonary System'*

This is a cluster of public and private research facilities, departments and institutions of the University Frankfurt, the Max-Planck Institute and affiliated organizations.

I visited several departments of the Univeristy and the Max-Planck institute and I attended an all-day event sponsored by "dbgs Gesundheitsservices" which is a "Tochter der IAS Institut für Arbeits- und Sozialhygiene Stiftung".

Topics covered at the event were

- Stress tests (measured by electro cardiograms) for children, athletes, adults and elderly and the implications for physical fitness and ability to perform specific work
- Pulmonary function tests for children, athletes healthy adults and adults with compromised lung function (e.g. smokers, asthma patients, cystic fibrosis patients etc.)
- Sensory Physiology tests including hearing, seeing for the different age groups and patients with deficiencies in seeing and hearing.
- Drug testing including doping related drug testing.

The above topics and tests were covered in theory and in praxis with test patients. Copies of some of the results are attached in Appendix E.

The "Teilnahmebescheinigung" (= certificate of attendance) is on the next page.

References with further content and publications can be found at:

http://www.dbgs.eu/content/

http://www.mpibp-frankfurt.mpg.de/

http://www.klinik.uni-frankfurt.de/

dbgs:
GesundheitsService

Ein Unternehmen der IAS-Gruppe.

dbgs GesundheitsService GmbH · Mainzer Landstraße 181 · 60327 Frankfurt

Frau
Carola Wright
Schüttenhelmweg 51
60529 Frankfurt / Main

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Dr. med. Uta Ziegler

Telefon (069) 265-25820
Telefax (069) 265-25826

uta.ziegler@ias-gruppe.de

23.4.2009

Teilnahmebescheinigung

Frau Carola Wright hat am 23. April 2009 an einer ganztägigen Veranstaltung der DBGS teilgenommen.

Themen der Veranstaltung waren die Durchführung von Belastungs-EKGs und die Messung von physiologischen Parametern (Lungenfunktionstests, Seh- und Hörfähigkeit etc.) im Rahmen von Sporttauglichkeitsuntersuchungen und Arbeitstauglichkeitsuntersuchungen.

Mit freundlichen Grüßen

U. Zie

Dr. med. Uta Ziegler

dbgs GesundheitsService GmbH
Gesundheitszentrum Mitte
Mainzer Landstraße 181
60327 Frankfurt am Main
Tel.: 069/265-25800
Fax: 069/265-25826

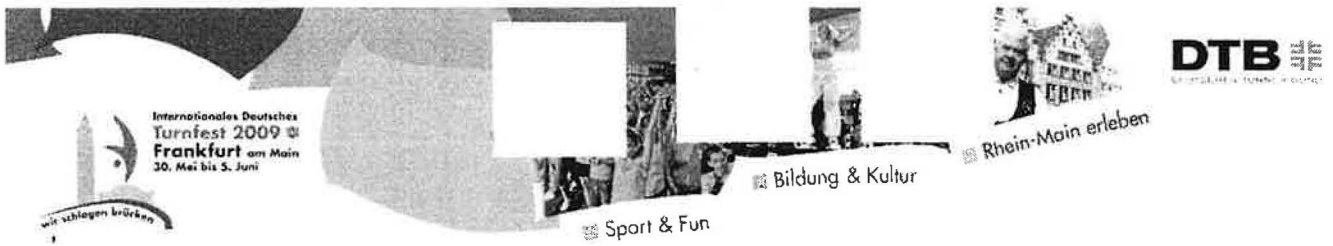
Another event sponsored by the University Frankfurt and the City of Frankfurt was the International deutsche Turnfest 2009. This was a huge event across the entire city lasting for one whole week with emphasis on sports, athletics (especially gymnastics) and health for all ages. The catalog of events spanned over 200 pages with many topics related to exercise physiology and sports science. Some international presentations included:

- Developing the Resource of youth sports: Gender, Opportunity and Public Health
- Health Sport: An effective concept of health promotion
- Growing together through sport: Reciprocal learning through sport with young Muslim women.

I attended the International Turnfest in Frankfurt and visited many of the offered events in various locations throughout the city. Most of the talks and exhibits were located at the Messe Zentrum in Frankfurt (the fair complex).

A copy of one of my Turnfest tickets is below:

http://www.turnfest-congress.de/index.php



Education **Health** **Integration** **Akademie**

- Greetings
- News
- Organisation
- Aim & Theme
- Important Dates
- Call for Abstracts
- Fees
- Registration
- Keynotes
- Program
- Venue
- Arrival
- Accomodation
- Contact
- Imprint
- My Conference

Bundesministerium des Innern

Stadt Frankfurt am Main

Hessen

International Sport Scientific Turnfest-Congress

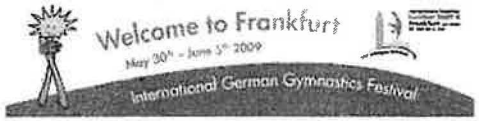
Facing New Challenges: Education, Health Promotion and Integration in Gymnastics and Sports

June 4 - 5, 2009 - Chamber of Industry and Commerce (IHK) Frankfurt

Greetings

It is our pleasure to invite you to the Turnfest-Congress of the German Gymnastics Federation. The congress "Facing new Challenges: Education, Health Promotion, Integration in Gymnastics and Sports" is to be held in Frankfurt, Germany from June 4 -5, 2009. We are looking forward to a congress which aims at international cooperation and scientific multidisciplinarity. It is intended to discuss links between theoretical approaches and practical projects in the three main conference topics. Scholars from a variety of disciplines and interdisciplinary "fields" examining issues relevant to the study of sports are encouraged to present their work.

Dr. Annette Hofmann



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News

April 21, 2009

Turnfest-to-go

(only for Festival-card holder)

On two days, Sunday 31st of May and Wednesday 3rd of June, you can get guided tour through the Turnfest and experience a feeling for the Turnfest-atmosphere.

An English-speaking Volunteer will take care of the participants and accompany through the program. He/ She will take the lead to the venues, convey a feeling for the International German Gymnastics Festival – the Turnfest and be available the whole day as guide, adviser and translator.

Meeting point on both days will be the International Meeting Point at the riverside at 9.30 am. Please register via e-mail to info@turnfest-congress.de. The price is 20.00 Euro. It is possible to register last-minute at the International Meeting Point one day before the program starts at the latest.

Deadline for registration is May, 15th.
Minimum attendance: 3 persons
Price: 20.00 Euro

Download program (pdf)
Download registration form (pdf)

Conclusions

Summary:

Epigenetics is still an emerging field of study within the biological sciences. It is a relatively new and different way of studying gene expression. The classic way of studying gene expression includes reporter gene essays, transcription factor studies, promoter analyses, protein-DNA binding studies etc. Before coming to Mt. SAC to teach I was involved in these classic research approaches to study gene expression. In the past decade much has changed in the field and new methods and approaches to study gene expression have been developed.

This sabbatical project has given me the opportunity of getting once again involved with cutting edge research in the field of cell physiology. During my time in the Baldwin lab at UCI I learned about the field of epigenetics and how ChIP assays can be used to answer epigenetic questions. I was able to get first-hand experiences in applying the method to a muscle physiology research setting. In addition to the research protocols, I learned how to use advanced equipment such as a real time PCR machine and software programs for data analysis such as GraphPad Prism, ImageQuant and Photoshop.

Within the time allotted for the research project I was able to generate original research results that became part of an important peer – reviewed publication in the American Journal of Physiology (see appendix B). The findings of this research study have significant physiological relevance in skeletal muscle biology because they extend our current understanding of the molecular mechanisms that regulate skeletal muscle remodeling and MHC gene expression in muscle tissue with altered muscular activity.

In addition, an editorial focus paper was written by leaders in the field of skeletal muscle biology emphasizing the importance of the work (see appendix C). Their concluding remark about the publication states: "Answering broad questions such as whether epigenetics play a role in the regulation of other genes involved in skeletal muscle remodeling, and further yet, whether epigenetic regulation is involved in muscle phenotypes with pathological consequences, such as the failed regrowth of muscle from atrophy with aging, muscular dystrophies, or cardiac myopathies, is beginning to come within reach thanks to studies such as this one."

Value of this project to the Applicant

I sincerely thank the Sabbatical and Leaves Committee and the Board of Trustees for approving this sabbatical project. My sabbatical leave was a valuable and prolific break from teaching and commuting. I updated my knowledge, participated in University laboratory research and got creative in the research project I participated in. This new knowledge gives me confidence in teaching my Physiology course at Mt. SAC and also made it easy to update the Physiology course contents.

The travel component of my sabbatical further broadened my horizons and gave me up to date insights into the fields of cardiology, exercise science and sports medicine at an international level. The value of international travel lies not only in the information received but also in the interaction with people of a different culture and an inherently different way of doing things. At the places I visited and the events I attended I learned a lot of factual information about the various topics. In addition I learned about different ways of thinking and presenting or studying a particular subject matter.

Value of this project to the Biology Department

The Natural Sciences division at Mt. SAC has state of the art labs and new buildings. As a faculty teaching in these labs and buildings I strive to provide state of the art instruction and expertise for my students. This sabbatical project involving advanced research in the field of muscle physiology provides both my students and my colleagues with a valuable resource in a constantly evolving field. For example, the Biology department is offering a new genetics course for the first time in the fall semester 2009. Several of my colleagues in the department – especially the ones teaching in the area of cell and molecular biology and / or genetics - are interested in hearing about my epi-genetics project and my research results. Therefore, at our fall flex day I gave a presentation about my sabbatical project to my colleagues.

My students have let me know many times how much they appreciate my references to current research and how it motivates and inspires them. Specifically, I have updated my lectures on muscle physiology (Anatomy 36, Human Physiology) to provide my students with the most current knowledge in the field. I addition I have updated the lectures on cell physiology of the anatomy 36 course to include information about epigenetics. In the physiology laboratory I am providing students with information on how research is done in the field of muscle physiology and how new information can be incorporated into student laboratory exercises. Specifically, I show students how they can use and analyze data they have collected during a laboratory in a way that creates a meaningful presentation or could be used in a publication. As an additional exercise in my physiology laboratory course I have included a group research presentation project where students are required to research a topic of their choice (within the scope of Physiology) and present the information they have collected in PowerPoint format to their class mates. The presentation is followed by

questions from the audience and usually quickly reveals the depth of knowledge of the presenters. This gives students a rare opportunity to present information they have collected to others and defend their knowledge in front of a group.

In the near future I am planning to also teach the Biology 8 course (Cell and Molecular Biology). This course requires frequent updates as new knowledge becomes available and enters the text books. Since I am now up to date on some important advances in the field I am very comfortable in including a new and updated section on our current understanding of the mechanisms of epigenetics.

In addition to my updated knowledge in my field of expertise my students also benefit from my international experiences. Many of my students have English as their second language. Due to my own experiences of studying in a foreign country I can relate to their difficulties in language and culture and help them to better understand and overcome the challenges they face.

Value of this project to the College

The mission of Mt. San Antonio College is to welcome all students and to support them in achieving their personal, educational, and career goals in an environment of academic excellence. My sabbatical project has given me the opportunity to get valuable updates for the classes I teach. This supports the mission of Mt. SAC to provide academic excellence.

In addition, one of the core values of Mt. SAC is to "respect and welcome all differences, and to foster equal participation throughout the campus community". The campus community at Mt. SAC is very international with many students from diverse cultural backgrounds. My travel in Germany has given me not only a knowledge update in

my teaching area of physiology and cell biology but also a deeper understanding of different cultures, different ways of thinking and different ways of life. This understanding of different cultures is a value that is appreciated especially by my international students.

In conclusion, having faculty that stay current in their field through research and education is a benefit to the reputation of the department and the college as a whole.

Appendix A

Technical Detail for the Proposed Research Actvities

(This was also Appendix A of the original sabbatical proposal)

Appendix A:

Technical detail for the proposed research activities.

1. Gene regulation of the cardiac myosin heavy chain genes.

Adult mammalian cardiac muscle expresses two genes encoding for myosin heavy chains (MHC)s, which have been designated as α- and β MHC. The αMHC and βMHC genes are members of the MHC multi-gene family in which each of the genes is expressed and highly regulated in a muscle-type specific fashion. While αMHC is only expressed in the heart, βMHC is expressed in the heart and is also the major myosin isoform expressed in slow twitch skeletal muscle.

In the myocardium, the MHC isoform composition affects the physio-dynamics and energetics of the working heart, which is of great physiological significance to cardiac performance. The αMHC isoform is characterized by a higher ATPase activity, and faster shortening speed than the β MHC isoform. Thus hearts rich in the α isoform have a high intrinsic contractility, while those rich in the βMHC have a lower contractility, but a higher economy of tension development. In the adult rodent, the α MHC is the predominant isoform expressed in the ventricles, accounting for ~85-90% of the total MHC protein pool, while the β MHC accounts for the remaining 10-15%. In cardiac cells of different mammalian species, the expression of the two MHCs is developmentally regulated, and can be altered by a variety of patho-physiological conditions including abnormal thyroid status, diabetes, and hemodynamic overload.

Chronic hemodynamic overload as occurs in hypertension, is a complex physiological stimulus that triggers significant changes in myocardial structure and function. The visible changes include cardiac hypertrophy, which is expressed as an increased heart weight to body weight ratio, while changes on the molecular level include altered phenotype expression of specific cardiac genes that include several contractile and regulatory proteins. For example, in the hypertensive rodent heart, βMHC gene expression is significantly increased relative to normal control values. The up-regulation of β MHC gene expression in hypertensive adult mammalian hearts has been well documented; however the molecular mechanisms driving this up-regulation are poorly understood.

During my time as a graduate student in the Baldwin lab I was involved with this research project which ultimately resulted in the publication of 3 peer reviewed journal articles and my dissertation in the year 2000 with the title: In vivo characterization and activity of the cardiac beta myosin heavy chain gene promoter.

Since then, significant progress has been made and the project has advanced in several areas. Recent results show evidence for a novel mechanism of regulation of the α and β MHC genes by naturally occurring antisense transcription. Pre-mRNA analysis of antisense β MHC expression under pressure overload, altered thyroid state and in diabetes show direct correlation (positively or negatively) with α and β MHC pre-mRNA expression. This suggests that antisense RNA is involved in the coordination of cardiac myosin heavy chain gene switching in various physiological and patho-physiological states of the heart.

While I am familiar with in-vivo gene expression studies in rodent heart using reporter gene assays I will need to learn several new methods in order to catch on to antisense RNA studies.
Some of the experimental procedures I would lilke to learn and participate in involve amplification of α and β MHC intergenic DNA, pre-mRNA targeted PCR, 5'RACE PCR, real-time PCR and RNA analyses.

2. Myosin heavy chain gene regulation in skeletal muscle

Skeletal muscle is highly adaptable when subjected to altered loading and hormone states. Its size, metabolic makeup, and contractile properties can all be altered to optimize function. Variability in contractile properties is mainly achieved by diversification in the myosin heavy chain (MHC) protein, where different isoforms are encoded by distinct genes. Of this family of eight MHC genes six are tandemly linked and span about 420 kb of DNA in the rat chromosome # 10. The genomic order and orientation on the chromosomes of the MHC genes are conserved in all mammalian species, leading researchers to suspect that this organization might be an important feature in the strategy for the coordinated regulation of these genes.
MHC gene expression is regulated at the transcriptional/pre-translational level. The isoform profile is dynamically altered to confer optimal function inresponse to varying conditions. In rodent muscle, loading conditions, motor neuron innervation and hormone states determine the MHC profile that is expressed in a muscle fiber. For example, disuse, inactivity, lack of innervation and hyperthyroidism result in a shift of the MHC profile from slow to fast MHC isoforms. In contrast, increased loading state, electrical stimulation and hypothyroidism can cause a shift to expression of slower MHC isoforms in fast muscle fibers. It is poorly understood how this complex and apparently coordinated expression pattern is regulated. Research in the Baldwin lab focuses on molecular mechanisms of gene switching. Current research projects examine the role of antisense RNA in MHC gene regulation. The studies are conducted using animal models (rats).
Methods to be used include preparation of the animal models, isolation of particular muscles of interest. RNA analysis of muscle tissues, design of PCR primers. One-step RT-PCR, quantitative Real-time RT-PCR, genomic DNA PCR, DNA sequence analysis and Statistical analysis of the obtained data.

3. Prevention of unloading-induced muscle atrophy

Human skeletal muscle undergoes adaptive changes in both fiber size and contractile properties in response to conditions of altered loading states. In studies on human subjects exposed to space flight (zero gravity) or ground-based analogs such as bed rest or lower limb unloading, it has been well established that both muscle mass and strength are significantly reduced within as little as 5-7 days. These changes occur even when various types of exercise are imposed. This is of practical importance because it drastically limits the time flight crews can spend on space flights and makes it currently impossible to send astronauts on a mission

to Mars (which would require space travel of several months) or even to keep them for extended time periods at the international space station.
NASA is very interested in research that is aimed at preventing this rapid muscle loss and therefore has been supporting research projects in the Baldwin lab on this topic.
The Baldwin lab has demonstrated that an isometric resistance training program is effective in stimulating muscle hypertrophy in ambulatory rats but is not effective in completely preventing muscle atrophy during unloading. They have also demonstrated that combined isometric, concentric and eccentric resistance exercise prevents unloading-induced muscle atrophy in rats.
Current research projects are aimed at understanding the underlying mechanisms for the changes in muscle structure and function that occur during unloading (or zero gravity). The Baldwin lab is also working on exercise training programs that could be carried out on space flights and would prolong the time astronauts could spend in a zero gravity environment. These studies involve human subjects as well as animal models.
Methods include unilateral lower limb unloading, various resistance exercise protocols, percutaneous muscle biopsies, molecular analyses of muscle tissues, RT-PCR protocols and statistical analyses of the data.

Appendix B

Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading. Am J Physiol Cell Physiol (April 15, 2009). doi:10.1152/ ajpcell.00075.2009.
Pandorf CE, Haddad F, Wright C, Bodell PW, Baldwin KM.

Am J Physiol Cell Physiol 297: C6–C16, 2009.
First published April 15, 2009; doi:10.1152/ajpcell.00075.2009.

Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading

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Pandorf CE, Haddad F, Wright C, Bodell PW, Baldwin KM. Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading. *Am J Physiol Cell Physiol* 297: C6–C16, 2009. First published April 15, 2009; doi:10.1152/ajpcell.00075.2009.— Recent advances in chromatin biology have enhanced our understanding of gene regulation. It is now widely appreciated that gene regulation is dependent upon post-translational modifications to the histones which package genes in the nucleus of cells. Active genes are known to be associated with acetylation of histones (H3ac) and trimethylation of lysine 4 in histone H3 (H3K4me3). Using chromatin immunoprecipitation (ChIP), we examined histone modifications at the myosin heavy chain (MHC) genes expressed in fast vs. slow fiber-type skeletal muscle, and in a model of muscle unloading, which results in a shift to fast MHC gene expression in slow muscles. Both H3ac and H3K4me3 varied directly with the transcriptional activity of the MHC genes in fast fiber-type plantaris and slow fiber-type soleus. During MHC transitions with muscle unloading, histone H3 at the type I MHC becomes de-acetylated in correspondence with down-regulation of that gene, while upregulation of the fast type IIx and IIb MHCs occurs in conjunction with enhanced H3ac in those MHCs. Enrichment of H3K4me3 is also increased at the type IIx and IIb MHCs when these genes are induced with muscle unloading. Down-regulation of IIa MHC, however, was not associated with corresponding loss of H3ac or H3K4me3. These observations demonstrate the feasibility of using the ChIP assay to understand the native chromatin environment in adult skeletal muscle, and also suggest that the transcriptional state of types I, IIx and IIb MHC genes are sensitive to histone modifications both in different muscle fiber-types and in response to altered loading states.

hindlimb suspension; chromatin immunoprecipitation; in vivo; multigene family

MUSCLE CONTRACTION PROVIDES the basis for all movements and postural support in animals and humans. A broad range of contractile intensities can exist in different muscle fibers, primarily attributed to diversity in the motor protein myosin heavy chain (MHC). Four isoforms of MHC (I, IIa, IIx, IIb), each encoded by a distinct gene, can be expressed in adult skeletal muscle. Intrinsic differences in the ATPase (and shortening velocity) properties of the MHC isoforms have led to the classification of slow and fast fiber-types in muscle (5). For example, slow type I MHC predominates in the soleus, whereas fast IIx and IIb are the primary MHCs expressed in the plantaris. The transcriptional products of the MHC genes are also easily altered by perturbations to environmental stimuli, such as altered mechanical loading (5, 16). DNA regulatory sequence, transcription factors, and other mechanisms that are important in the transcriptional regulation of the MHC genes in skeletal muscle have been studied; however, virtually no information is known about the role of chromatin remodeling at these highly regulated genes.

Recent advances in the understanding of the dynamic nature of DNA regulatory regions have highlighted the importance of chromatin structure and associated histone modifications in influencing gene expression. Numerous chromatin-bound protein complexes and their enzymatic activities have been identified, which can covalently modify certain histone sites that, in turn, determine whether a specific gene will be transcribed or not (28). Posttranslation modifications such as acetylation, methylation, and phosphorylation to histone tails can exert control on a given gene's expression. The combination, sequence and extent of various histone modifications, referred to as the histone/epigenetic code hypothesis, is proposed to result in distinct functional consequences impacting various cellular processes (35, 42). Of particular relevance in understanding the regulation of the MHC genes is that these histone modifications are predicted to ultimately result in varying degrees of transcriptional activation or repression.

Histone hyperacetylation is correlated positively with actively transcribed genes (2, 22, 38). Acetylation alters the charge of histone tails, and thus interaction properties of both histone-DNA contacts within a given nucleosome, and also internucleosomal contacts to result in relaxing of a compact chromatin structure (10, 35) This enables access of compacted DNA to different factors that promote transcription of the coded gene. The activation of muscle-specific genes during myofiber differentiation is dependent on the activities of enzymes that regulate histone acetylation, i.e., histone acetyltransferases (HATs) and histone deacetylases (HDACs) (26). Myogenic basic helix-loop-helix (bHLH) MyoD, Myf5, myogenin, and Mrf4 proteins and members of the MEF2 family of MADS-box transcription factors bind to the regulatory regions of muscle-specific genes to activate them (3, 7, 26, 37). These muscle-specific proteins are reported to activate and repress muscle-specific genes by associating with HATs and HDACs, respectively, to control the acetylation state of histones during differentiation (26, 37), and in an activity-dependent manner in adult muscle (27, 44).

Several recent reports have provided evidence that indicate that particular HDAC isoforms may act as nodal points of control for skeletal muscle fiber-type phenotype in vivo by mediating, via muscle-specific transcription factors, the expression of genes involved in the neuromuscular junction, metabolism (glycolytic vs. oxidative), and contraction (slow vs. fast) (20, 33, 43, 44). A critical role for HDACs in the coordinated

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regulation of MHC genes has been demonstrated with various manipulations to HDAC isoforms in both skeletal (20, 33, 44) and cardiac muscle (21, 25). It is not clear where the effects of manipulations to HDACs, which oppose the enzymatic activity of HATs, are mediated. Such effects could be mediated at the level of signaling intermediates, at the level of transcription factors (e.g., MEF2, myogenin) that bind the promoters of MHC genes, at the level of the histones of the MHC genes themselves, or at any combination of the above. Furthermore, the acetylation state of signaling proteins and transcription factors themselves may be altered in addition to the histones at their promoters (41). In the present study we initiate a series of experiments to examine the acetylation state of histone H3 (H3ac) of the MHC genes themselves.

In addition to H3ac, we examined trimethylation of histone H3 at lysine 4 (H3K4me3). This histone modification also is associated with active gene transcription (6, 28, 32, 36). Although the precise role for H3K4me3 marks has not been fully characterized, H3K4me3 has been shown to be coupled to transcription through the recruitment of effectors, which are subunits of chromatin remodeling complexes that are required for transcriptional competence (34). Recruitment of effector proteins via H3K4me3 may also stabilize the association of sequence-specific trans-acting factors/complexes with the chromatin template, and provide the cell with a gene-specific regulated response (34). A high correlation is observed in genome-wide analyses between H3ac and H3K4me3 within the 5′-regions of active genes (13, 38, 48). Recent reports have further demonstrated that H3K4me3 may be a modification that serves as a target for HATs and HDACs (9, 15). Thus, genomic regions trimethylated at H3K4 may be subjected to the opposing actions of acetylation and deacetylation in a dynamic manner, such that turnover of acetyl groups is high, thereby ensuring a rapid transcriptional response to altered conditions.

Since the histone tail modifications H3ac and H3K4me3 have both been shown to occur at active genes, and may be functionally interconnected with each other to recruit and stabilize transcription complexes, we chose to focus on these two modifications in the context of the four MHC genes that have varying levels of transcription among its members, and between muscles of different type as well as different environments. To our knowledge, a survey of histone modifications at MHC genes in skeletal muscle has not been carried out. Therefore, we sought to determine whether H3ac and H3K4me3 are differentially enriched at MHC promoters in vivo in the following conditions: *1*) under steady-state transcriptional states where the MHC genes in different fiber types have a stable yet divergent MHC transcription patterns, and *2*) in response to altered mechanical loading states, where transcription of the MHC genes become dynamically altered.

We report that both H3ac and H3K4me3 enrichment at each of the four MHC genes corresponds with transcript abundance in the fast fiber-type plantaris (Pla) and slow fiber-type soleus (Sol). A slow to fast fiber type shift in the Sol (i.e., with unloading or unloading plus thyroid hormone) similarly induced alterations to H3ac and H3K4me3 at the type I, IIx, and IIb MHC genes, but not the IIa MHC.

METHODS

Animal procedures. Female Sprague-Dawley rats (140–150 g) were used for all experiments. Animals were randomly assigned to either control or hindlimb suspension (HS) groups (n = 7/group). Control animals were housed in groups of four in a temperature- and light-controlled environment (i.e., 12:12 h light-dark cycle). All animals in a given experiment were allowed food and water ad libitum, and all procedures were approved by the Institutional Animal Care and Use Committee. HS was carried out for 7 days, which was shown in prior experiments to be sufficient to induce measurable alterations in the endogenous MHC genes expression (authors' unpublished observations). Animals subjected to thyroid hormone treatment were administered 150 $\mu g \cdot kg^{-1} \cdot day^{-1}$ of triiodothyronine (T_3) by intraperitoneal injection. At the end of the experiment, rats were euthanized and the muscles were rapidly removed, weighed, and frozen at −80°C for later analysis.

Hindlimb suspension protocol. The HS model used employed a tail traction method using a noninvasive tail casting procedure described previously (46). The technique used a swivel harness system incorporated into the casting materials, which was attached to a hook at the top of the cage. The hook was adjusted to allow only the forelimbs of the animal to reach the floor of the cage. Suspended animals were free to move about the cage using their forelimbs to obtain food and water.

RNA analysis. Total RNA was extracted from frozen control plantaris (Pla), control soleus (Sol), and from HS soleus (HS Sol) using the Tri Reagent protocol (Molecular Research Center). Extracted RNA was DNase-treated using one unit of RQ1 RNase-free DNase (Promega) per microgram of total RNA and was incubated at 37°C for 30 min followed by a second RNA extraction using Tri Reagent LS (MRC).

RT-PCR was used to assess pre-mRNA and mRNA of target genes. RT-PCR reactions were performed with the OneStep RT-PCR Kit (Qiagen), where the RT and PCR are performed in a single reaction tube, with some modifications to the manufacturer's protocol, and as described previously (31). This protocol has been optimized to avoid amplification of nonspecific transcripts, which are known to be coamplified with pre-mRNA and mRNA transcripts, and can thus preclude accurate measurement (14, 31). These one-step RT-PCR analyses were performed using 10 ng to 200 ng total RNA and 15 pmol of specific primers in 25-μl total volume and were carried out on a Robocycler (Stratagene). Samples to be compared were run under similar conditions (template amounts, PCR cycle numbers). RT reactions were performed at 50°C for 30 min followed by 15 min of heating at 95°C, followed by PCR cycling for a varied number of cycles (20–32 cycles). The annealing temperature was based on the PCR primers optimal annealing temperature. PCR primers used for RNA analysis are shown in Table 1. The amount of RNA and the number of PCR cycles were adjusted so that the accumulated product was in the linear range of the exponential curve of the PCR amplifications. PCR products were separated by electrophoresis on agarose gels and stained with ethidium bromide. The ultraviolet light-induced fluorescence of stained DNA was captured by a digital camera, and band intensities were quantified by densitometry with ImageQuant software (Molecular Dynamics) on digitized images.

Posttranscriptional control of mRNA steady-state levels can occur at many steps after the synthesis of the initial pre-mRNA and is subject to stability regulation (10). The pre-mRNA transcript abundance serves as a better marker of a gene's level of transcriptional activity than the mRNA because its half-life is much shorter. The nuclear run-on assay is another method to quantitate a gene's transcriptional activity; however, in our hands it is a technically unreliable measurement tool for the MHC genes, due to the inability to detect outcomes with consistent fidelity. Moreover, assessing the transcriptional activity of other genes by measuring pre-mRNA with RT-PCR has been validated as an alternative to the nuclear run-on approach (12).

Table 1. *PCR primer sequences, their specific target, and PCR product size, for primers used for RNA analysis of MHC genes*

Target	RT-PCR Primers: 5′→3′	PCR Product Size, bp
Type I pre-RNA	Forward: CCTGGTCCTATGTGCCGATCTCTAACGA	215
	Reverse: CGGTCCCCAATGGCAGCAATAAC	
Type I mRNA	Forward: GGAGCTCACCTACCAGACAGA	308
	Reverse: CTCAGGGCTTCACAGGCATCC	
IIa pre-RNA	Forward: TGCTTCCCAATGCTGCCATATCTACAT	295
	Reverse: TTCCTACTGCTTCCCTTGGTCTTGTCA	
IIa mRNA	Forward: CCTCTTACTTCCCAGCTGCACCTTCT	239
	Reverse: ACTTTCCCTGCGTCTTTGCTCTGAAT	
IIx pre-RNA	Forward: TGCCACAGAAAGAGGGACGC	290
	Reverse: CTGGCTGTGGTGTGGCTGAAA	
IIx mRNA	Forward: ACGGTCGAAGTTGCATCCCTAAAG	263
	Reverse: CACCTTCGGTCTTGGCTGTCAC	
IIb pre-RNA	Forward: GGCCATGCCAGCTAGCTTTTACG	270
	Reverse: GCGTTTTGATTGGTGGAAGAGTCC	
IIb mRNA	Forward: AGCCTGCCTCCTTCTTCATCTGG	229
	Reverse: CACGGTTGCTTTCACATAGGACTC	
β-Actin	Forward: CACGCCCTTTCTCAATTGTCTTTCT	225
	Reverse: GGCCATTTATCACCAGCCTCATTAG	

MHC, myosin heavy chain.

MHC mRNA isoform distribution. The MHC mRNA isoform distribution was evaluated by RT with oligo(dT)/random primers followed by PCR with primers targeting the embryonic, neonatal, I, IIa, IIx, and IIb MHC mRNAs, as described previously (11, 49). In these PCR reactions, each MHC mRNA signal was corrected to an externally added control DNA fragment that was coamplified with the MHC cDNAs using the same PCR primer pair. This approach provides a means to correct for any differences in the efficiency and/or pipetting of each PCR reaction. A correction factor was used for each control fragment band on the ethidium bromide-stained gel to account for the staining intensity of the variably sized fragments (224 to 324 bp), as reported previously (11).

Tissue preparation. Frozen muscle tissue was minced, and then washed in ice-cold PBS. All solutions were supplemented with protease inhibitors (leupeptin, AEBSF, and aprotinin, each at 1:1,000). Minced tissue was then incubated for 10 min in 1% formaldehyde to cross-link chromatin-DNA. Cross-linking was stopped by addition of glycine to 0.125 M for 5 min. This solution was exchanged with cold PBS, and then repeated a second time to remove all the formaldehyde. Tissue samples were then homogenized in PBS (20 vol of the muscle weight) with a Dounce homogenizer. The homogenate was then pelleted by centrifugation at 1,500 *g* for 10 min. The pelleted muscle tissue was resuspended in cold SDS lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris, pH 8.1), and sonicated (model VCX 130, Sonics Vibracell) to fragment the DNA. Samples were centrifuged at 12,000 *g* for 10 min to remove insoluble material. To ensure effectiveness of sonication, an aliquot of the supernatant was reverse cross-linked by incubation at 65°C overnight, and RNase treated (RNase A). Then the protein was digested (proteinase K) and then run on a 2% agarose gel to confirm size of DNA fragments to be between 200 and 1,000 bp. This aliquot was also used to measure the DNA concentration of the chromatin-DNA. This is necessary because of the varied muscle mass to DNA ratio in the three types of muscle samples analyzed. HS Sol tissue is more enriched in DNA than Sol, and Sol has more DNA than Pla per unit muscle mass. Therefore, to equalize the starting DNA concentrations for the chromatin immunoprecipitation (ChIP) assay, we used SYBR green I to bind DNA. A Stratagene Mx3000p real-time PCR machine was used in the quantitative plate read mode to accurately measure DNA concentration, with thymus calf DNA (Sigma) used as a standard.

Chromatin immunoprecipitation. Approximately 25 μg of DNA of each muscle sample was used to perform chromatin immunoprecipitation. Normal rabbit IgG (12-370) and anti- H3ac (06-599) was obtained from Millipore (Billerica, MA). The H3ac antibody detects diacetylation at lysines 9 and 14. Anti-histone H3 (ab1791) and anti-H3K4me3 (ab8580) was obtained from Abcam (Cambridge, MA). Chromatin was precleared with protein A/G agarose (Pierce, Rockford, IL) by incubation for 30 min at 4°C on a rotating platform, in a volume of 1 ml with ChIP dilution buffer (0.01% SDS, 1.1%, Triton X-100, 1.2 mM EDTA, 16.7 mM Tris·HCl, pH 8.1, and 167 mM NaCl). After agarose removal by centrifugation, 1% of the precleared chromatin was saved and used as input DNA. Antibody was added to chromatin and incubated at 4°C with rotation for ~1 h. Protein A/G agarose was added and incubated at 4°C with rotation for ~2 h. Three separate washes with low-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl, pH 8.1, and 150 mM NaCl) were then performed, followed by separate washes for 15 min with rotation with the following buffers. High salt (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl, pH 8.1, and 500 mM NaCl), LiCl [0.25 M LiCl, 1% Igepal-CA630, 1% deoxycholic acid (sodium salt), 1 mM EDTA, and 10 mM Tris, pH 8.1], and two separate washes with TE buffer (10 mM Tris·HCl, 1 mM EDTA, pH 8.0).

Chromatin-DNA complexes were eluted (0.1 M $NaHCO_3$, 1% SDS) from agarose beads, and cross-links were reversed by incubation at 65°C overnight. Samples were RNase treated (RNase A), protein was digested (proteinase K), and DNA was purified by using a spin column (Qiaquick PCR purification kit). Immunoprecipitated DNA for specific genes was analyzed and quantified by PCR. PCR primers used with ChIP samples are shown in Table 2. The number of PCR cycles and amount of ChIP DNA was adjusted so that the accumulated product was in the linear range of the exponential curve of the PCR amplifications. PCR products were separated by electrophoresis on agarose gels and stained with GelGreen (Biotium). The ultraviolet light-induced fluorescence of stained DNA was captured by a digital camera, and band intensities were quantified by densitometry with ImageQuant software (Molecular Dynamics) on digitized images.

With the ChIP assay, primers can be targeted to any genomic region. The histone modifications H3ac and H3K4me3 have been shown previously to peak immediately downstream of the transcription start sites of active genes (6, 13, 51). A preliminary analysis of these histone modifications in the MHC genes resulted in the same conclusion. Thus, PCR primers were designed to target the second intron of each MHC gene studied, which occurs before the translation

Table 2. *PCR primer sequences, their specific target, and PCR product size, for primers used for ChIP of MHC genes*

Target	RT-PCR Primers: 5′→3′	PCR Product Size, bp
Type I	Forward: GGCCTGGGCCTACCTCTTTATCC	286
	Reverse: TATTCAATTGGGGCACTCTTCGGGTGTAT	
IIa	Forward: ATCATTACCCCAAATATCACCCTATCC	323
	Reverse: GGCCCCAGATGCACATTACACTA	
IIx	Forward: TGCCACAGAAAGAGGGACGC	290
	Reverse: CTGGCTGTGGTGTGGCTGAAA	
IIb	Forward: AGGGAATAAATGTTAACTTGTTGACACTGG	218
	Reverse: GGGGGCGGGGCTAATGAAGC	
β-Actin	Forward: CACGCCCTTTCTCAATTGTCTTTCT	225
	Reverse: GGCCATTTATCACCAGCCTCATTAG	

ChIP, chromatin immunoprecipitation assay.

start site (ATG) of each gene. This location occurs at 1.1 to 1.4 kb from the transcription start site of the ~25 kb MHC genes.

For each sample, four ChIP assays were carried out in parallel reactions with antibodies to H3ac, H3K4me3, core histone H3, and normal rabbit IgG. The latter serves as a negative control for specificity of antibody binding. For analysis, the normal rabbit IgG IP signal was subtracted from the specific antibody IP signals. In all cases, the normal IgG precipitated negligible levels of DNA for the targeted genes analyzed. Then this value was divided by the input DNA PCR signal, to correct for any differences in starting DNA concentrations between samples, and for differences in DNA accessibility at the PCR-targeted genomic sites. Previous studies have shown that variation in histone occupancy at different genomic regions, or in response to varied gene activity, can lead to misinterpretation of histone modifications measured with ChIP (32, 51). This is because nucleosome density inversely correlates with transcription rate (23). Therefore, all data are expressed relative to core histone H3 ChIP results at each PCR-targeted genomic site.

Statistical analyses. Data are reported as means ± SE. Differences between three muscle groups (Pla, Sol, and HS Sol) were analyzed using one-way ANOVA, with Newman-Keuls post hoc test. Differences between two groups were analyzed using an unpaired *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

RNA transcripts of the MHC genes. The profile of pre-mRNA and mRNA transcripts of the Pla, Sol, and HS Sol are shown in Fig. 1. The unspliced pre-mRNA products (Fig. 1, *A–D*) show congruency with the spliced mRNA products (Fig. 1, *E–H*) when comparing expression level between the three different muscle groups examined. Pre-mRNAs are the nascent, unprocessed, transcriptional products, and they serve as an indirect measure of the level of transcriptional activity of the corresponding gene.

The classification of fast and slow fiber types is illustrated by the distinct expression of MHC transcripts in the Pla and Sol (Fig. 1 and Table 3). As a percentage of total MHC, the fast fiber-type Pla expresses a predominance of IIx and IIb MHC mRNA, while the slow fiber-type Sol muscle expresses primarily the slow type I MHC mRNA, and smaller proportion of the fast IIa MHC (Table 3). Differential expression of the type I, IIx, and IIb MHC between the Pla and Sol is also observed in comparing the relative abundance of pre-mRNA and mRNA between these two muscle types (Fig. 1). Expression of IIa MHC pre- and mRNA is similar between the Pla and Sol (Fig. 1, *B* and *F*).

With HS, the Sol is remodeled to a muscle more resembling the fast Pla, where IIx and IIb MHC become strongly expressed (Fig. 1, *C* and *D*, and Table 3). Type I MHC is downregulated in HS Sol as compared with Sol, although its pre-mRNA is still transcribed in the HS Sol, indicating that the type I MHC gene remains transcriptionally active after 7 days of HS (Fig. 1*A*). Transcriptional products of IIa MHC are significantly reduced in HS Sol as compared with Sol (Fig. 1, *B* and *F*).

The proportion of the developmentally regulated embryonic and neonatal MHCs is also shown in Table 3. Although expression of the embryonic MHC is downregulated in HS Sol compared with Sol as a percentage of total MHC (Table 3) and at the absolute level of pre- and mRNA abundance (data not shown), we have previously shown that it is not translated into protein in adult muscle and therefore does not contribute to the functional performance of the muscle (11).

Histone H3 acetylation. The H3ac data shown are expressed relative to the core histone H3, to account for variation in histone H3 occupancy between the different muscle samples with differing rates of gene transcription. Histone occupancy is subject to alteration depending on the transcriptional state of the gene, such that nucleosome occupancy inversely correlates with transcription rate (23). H3ac was assessed at genomic regions of the second intron of each of the MHC genes. Significant fiber-type differences in H3ac were observed at each of the MHC genes with differential transcriptional expression between the slow Sol vs. fast Pla muscles (i.e., I, IIx, and IIb; Fig. 2). The H3ac pattern in the type I, IIx, and IIb MHC genes corresponds to its MHC expression profile (compare Fig. 1 and Fig. 2). Thus, the fast Pla, which maintains an abundance of IIb and IIx MHC, was highly enriched in H3ac at both the IIb and the IIx MHC genes in contrast to the Sol. The slow Sol has relatively little expression of the fast IIx and IIb MHCs, and in correspondence to this expression the fast IIx and IIb MHCs are deacetylated relative to the Pla. A reciprocal pattern is observed in the slow type I MHC, where type I mRNA expression is low in the Pla and highly abundant in the Sol, and H3ac is similarly low in the Pla and relatively enriched in the Sol. H3ac at the IIa MHC did not differ significantly however, between the Sol and Pla, which corresponds to the lack of difference in IIa MHC transcription in the two muscle types. The directional shifts of mRNA and the corresponding H3ac among the MHCs in Pla versus Sol are summarized in Table 4.

When an inactivity model is imposed on the slow Sol muscle, a faster MHC transcriptional pattern emerges (Fig. 1 and Table 3). The H3ac pattern was similarly altered for the type I, IIx, and IIb MHCs. H3ac is more highly enriched in the IIx and IIb MHC genes in HS Sol muscle as compared with Sol (Fig. 2, *C* and *D*, respectively). Conversely, the type I MHC became deacetylated in the HS Sol as compared with the Sol. In both of these cases, MHC transcription positively corresponds with H3ac (compare Fig. 1 and Fig. 2). However, H3ac in the IIa MHC did not correspond with its transcription pattern. Transcription of the IIa MHC is significantly decreased in the HS Sol compared with Sol, while H3ac was unchanged with HS. Thus, the directional shifts in types I, IIx and IIb pre-mRNA and mRNA in HS Sol as compared with Sol correspond to that of H3ac (see Table 4).

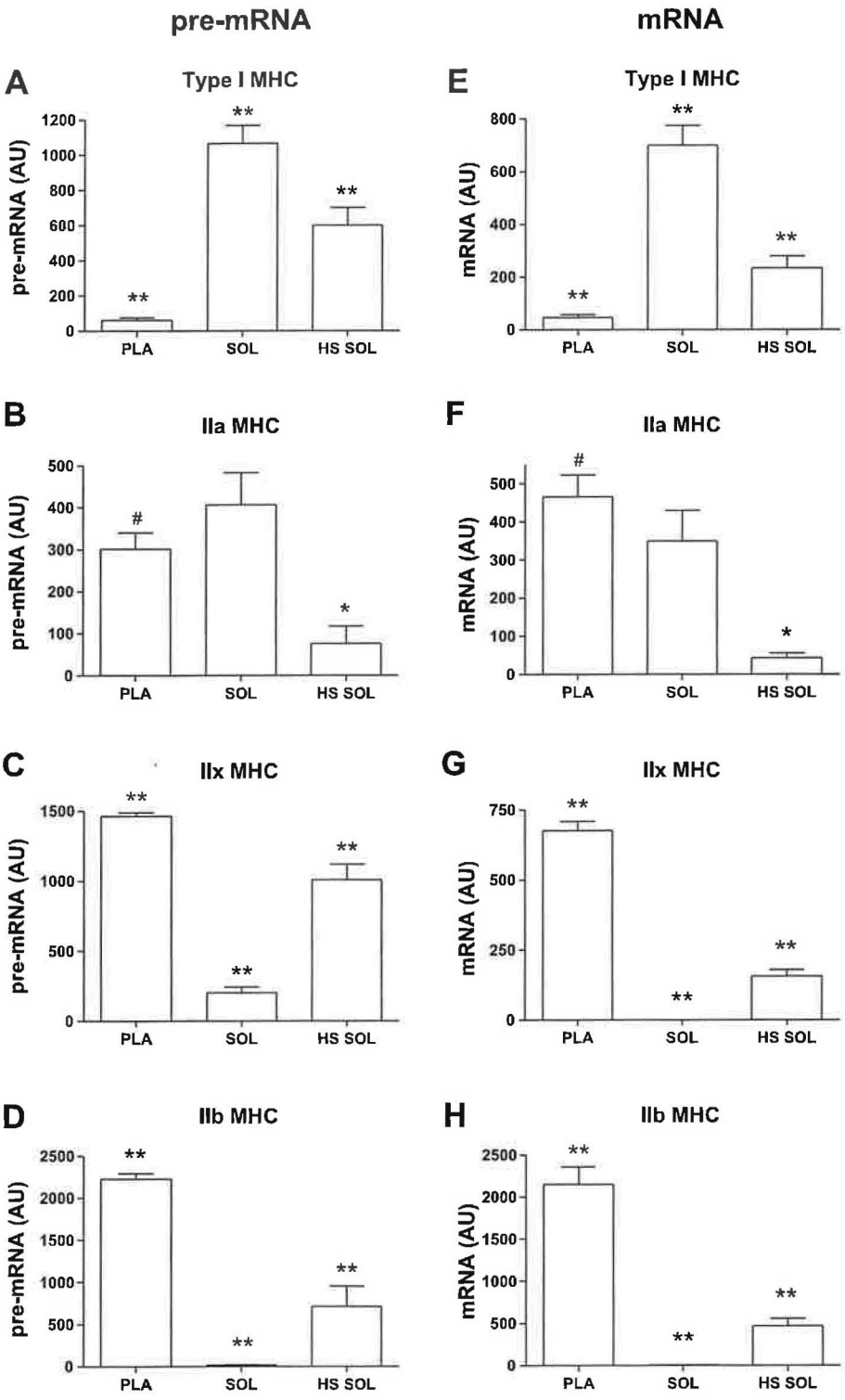

Fig. 1. pre-mRNA and mRNA transcripts of the myosin heavy chain (MHC) genes in plantaris (Pla), soleus (Sol), and hindlimb suspension soleus (HS Sol) muscle groups. Bar graphs show means ± SE of RT-PCR analyses. *A–D*: pre-mRNA. *E–H*: mRNA. AU, arbitrary units. *Significantly different from Sol; **all groups significantly different from each other; #significantly different from HS Sol ($P < 0.05$); $n = 6$/group.

Histone H3 lysine 4 trimethylation. Together with histone H3 acetylation, we assayed the MHC genes for H3K4me3. Similar to H3ac, H3K4me3 was highly enriched at the second intron of the IIx and IIb genes in the Pla relative to the Sol, which similarly corresponds with the transcriptional activity of these MHC genes in each muscle type (compare Fig. 1 and Fig. 3). H3K4me3 at the type I MHC also corresponds with type I transcription, such that trimethylation is increased in the Sol as compared with the Pla, where type I MHC mRNA is expressed at low levels. Upon induction of type IIb and IIx MHC transcription, which occurs upon unloading of Sol, we observed significantly increased enrichment of H3K4me3 at

Table 3. *MHC mRNA isoform distribution expressed as a percentage of total MHC mRNA*

	I	IIa	IIx	IIb	Embryonic	Neonatal
Pla	3%	12%	31%	54%	0%	0%
Sol	88%	4%	0%	0%	8%	0%
HS Sol	65%	0%	15%	18%	2%	0%

Pla, plantaris; Sol, soleus; HS, hindlimb suspension; $n = 6$ per group.

these genes in HS Sol as compared with Sol (Fig. 4). There was no statistically significant difference in H3K4me3 in the IIa MHC between the three muscle types studied or type I MHC between the Sol and HS Sol (Fig. 4). The directional shifts of mRNA and the corresponding H3K4me3 among the MHCs in Pla versus Sol and HS Sol versus Sol are summarized in Table 4.

Histone modifications at β-actin gene. To validate the results of the ChIP assays in determining the levels of enrichment of H3ac and H3K4me3 at the MHC genes, we examined the H3ac- and H3K4me3- ChIP DNA for another target gene, β-actin, which is less prone to dynamic change, particularly in response to unloading of Sol (Fig. 4). The β-actin gene is constitutively active in skeletal muscle, and its expression is unaltered in HS Sol compared with Sol (Fig. 4*A*). Thus, β-actin serves as a control gene unaffected by HS. ChIP analyses showed that enrichment of H3ac and H3K4me3 at the 5′-region of the β-actin gene also does not differ between Sol and HS Sol (Fig. 4, *B* and *C*). The Pla had significantly greater β-actin pre-mRNA expression than either Sol or HS Sol, and this corresponded with the significantly increased enrichment of H3ac although not H3K4me3.

Repression of type I MHC with HS + T_3. Since there was a lack of correspondence between H3K4me3 and type I mRNA between Sol and HS Sol, we then determined whether an alternative model, in which the type I MHC could be more robustly repressed than HS alone, would result in a loss of H3K4me3 enrichment at the type I MHC promoter. In a separate experiment we found that HS combined with thyroid hormone treatment (HS + T_3) results in virtually complete repression of type I MHC transcription (Fig. 5, *A* and *B*). This also resulted in a significant reduction of both H3ac (Fig. 5*C*) and H3K4me3 (Fig. 5*D*) at the type I MHC. Thus, the loss of H3K4me3 at the type I MHC with more robust repression indicates that HS alone likely resulted in insufficient repression of type I MHC to alter H3K4me3.

DISCUSSION

As an initial survey of histone modifications involving the chromatin of the MHC genes, we examined acetylation of histone H3 and trimethylation of H3 lysine 4 in each of the four MHCs expressed in adult rodent skeletal muscle. We compared the extent of these chromatin modifications, previously shown to positively correlate with gene activity, in three types of muscle: fast versus slow fiber-type muscles together with muscle unloading, which is shown to downregulate types I and IIa MHC and upregulate types IIx and IIb MHC. We report that enrichment of H3ac corresponds to mRNA abundance in the type I, IIx, and IIb MHCs in each of the three muscle groups (Pla, Sol, HS Sol). As the soleus myofiber is remodeled in response to unloading, downregulation of the type I MHC corresponds with deacetylation of histones, while upregulation of the IIx and IIb MHC genes occurs in concert with enhanced histone acetylation (see Table 4).

In comparing the fast fiber-type Pla with the slow fiber-type Sol, mRNA/pre-mRNA abundance of each of the four MHCs

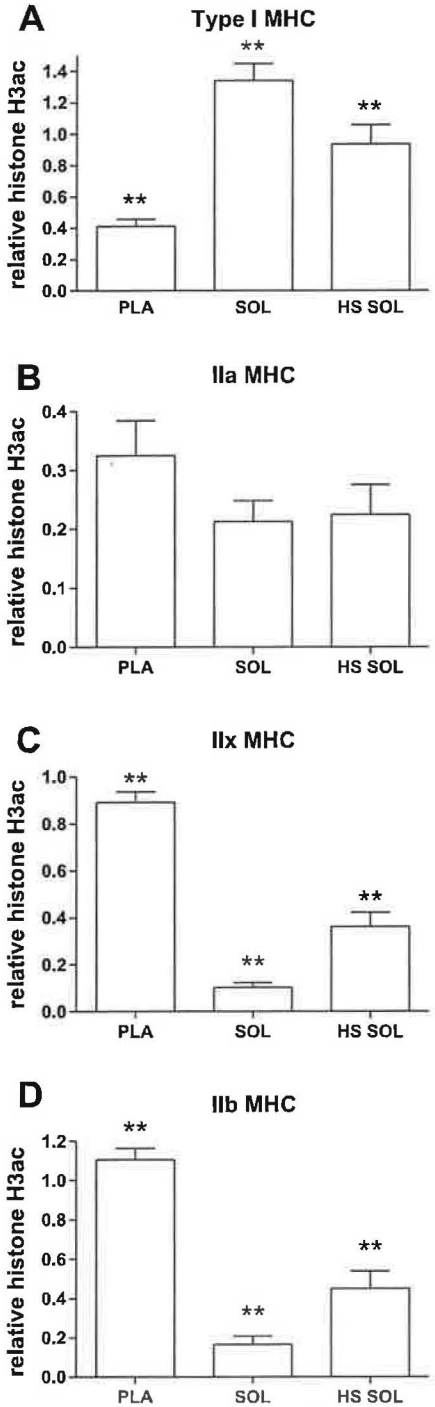

Fig. 2. Relative histone H3 acetylation (H3ac) at MHC genes. *A*: type I MHC. *B*: IIa MHC. *C*: IIx MHC. *D*: IIb MHC. Bar graphs show means ± SE of chromatin immunoprecipitation (ChIP) analyses; $n = 7$/group. Histone H3 acetylation is corrected for core histone H3 occupancy, as described in METHODS. **All groups significantly different from each other ($P < 0.05$).

Table 4. *Summary of directional changes in mRNA abundance and enrichment of H3ac and H3K4me3 in each of the four MHCs in Pla and HS Sol as compared with Sol*

MHC Gene	Variable Assessed	Pla versus Sol	HS Sol versus Sol
I	mRNA	↓↓	↓
	H3ac	↓↓	↓
	H3K4me3	↓↓	⇆
IIa	mRNA	⇆	↓↓
	H3ac	⇆	⇆
	H3K4me3	⇆	⇆
IIx	mRNA	↑↑	↑
	H3ac	↑↑	↑
	H3K4me3	↑↑	↑
IIb	mRNA	↑↑	↑
	H3ac	↑↑	↑
	H3K4me3	↑↑	↑

Directional arrows indicate directional change in indicated variable, relative to control Sol. ↓↓, highly decreased; ↑↑, highly increased; ↓, decreased; ↑, increased; ⇆, no change. H3ac, histone H3 acetylation; H3K4me3, trimethylation of lysine 4 on histone H3.

corresponds with both H3ac and H3K4me3. This comparison demonstrates that the fiber-type differences in MHC transcription are linked to modifications at the level of the chromatin of the genes that primarily define the fast vs. slow fiber phenotype. H3K4me3 is also increased in HS Sol as compared with Sol in both the type IIx and IIb MHCs, appearing in correspondence with the induction of these two genes that occurs in HS Sol. An almost identical pattern of histone acetylation is observed in the Pla to that seen in the HS Sol, when compared with the Sol, although the directional change is more exaggerated in the Pla as compared with HS Sol (summarized in Table 4). The directional difference of mRNA/pre-mRNA is similarly exaggerated in the Pla, indicating that the level of transcriptional activity of the MHCs is reflected in the degree of enrichment of epigenetic markers of gene activity.

The globin genes are another multigene family that undergoes coordinated shifts in gene expression, in liver tissue. In the fetal vs. adult stages of development, expression of the globin genes switches in correlation with the level of histone acetylation at the developmentally regulated globin genes (51). Hyperacetylation of histone H3 was observed in the active β-globin genes in adult tissue, while the inactive embryonic globin genes exist in hypoacetylated regions (18, 51). H3K4me3 also correlated with transcriptional activity across the β-globin locus in cultured cells (19). We report that in skeletal muscle, where MHC gene expression undergoes alterations in expression during unloading, a similar pattern of histone acetylation is observed. The IIx and IIb MHC, which have very little transcriptional activity in the Sol, are relatively hypoacetylated. Upon induction of the IIx and IIb MHC genes in HS Sol, these genes become hyperacetylated. In the tissue comparison between the Pla and Sol, differential patterns of acetylation are also observed. The type I MHC is hyperacetylated in the Sol relative to the Pla, while the opposite is observed in the fast IIx and IIb MHCs, i.e., the IIx and IIb are hyperacetylated in the Pla relative to the Sol.

Given that the finding that relative enrichment of histone H3 acetylation at the type I, IIx, and IIb MHC genes corresponds with each MHC's respective expression level in each muscle type examined, it is surprising that in HS Sol, where IIa MHC

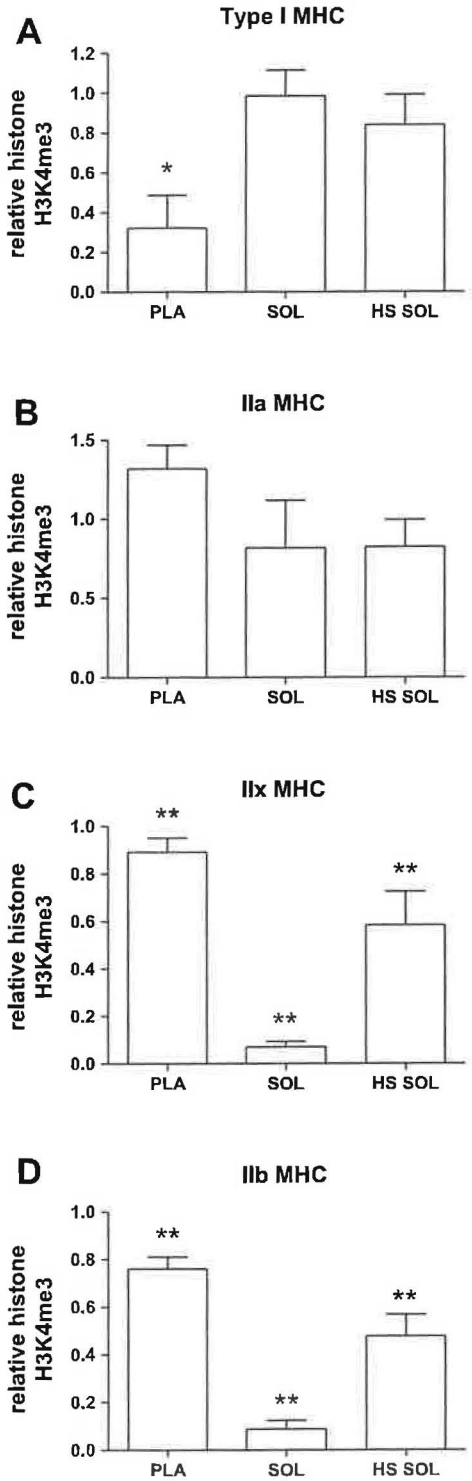

Fig. 3. Relative trimethylation of lysine 4 on histone H3 (H3K4me3) at MHC genes. *A*: type I MHC. *B*: IIa MHC. *C*: IIx MHC. *D*: IIb MHC. Bar graphs show means ± SE of ChIP analyses; n = 6/group. Histone H3K4me3 is corrected for core histone H3 occupancy, as described in METHODS. *Significantly different from Sol ($P < 0.05$). **All groups significantly different from each other ($P < 0.05$).

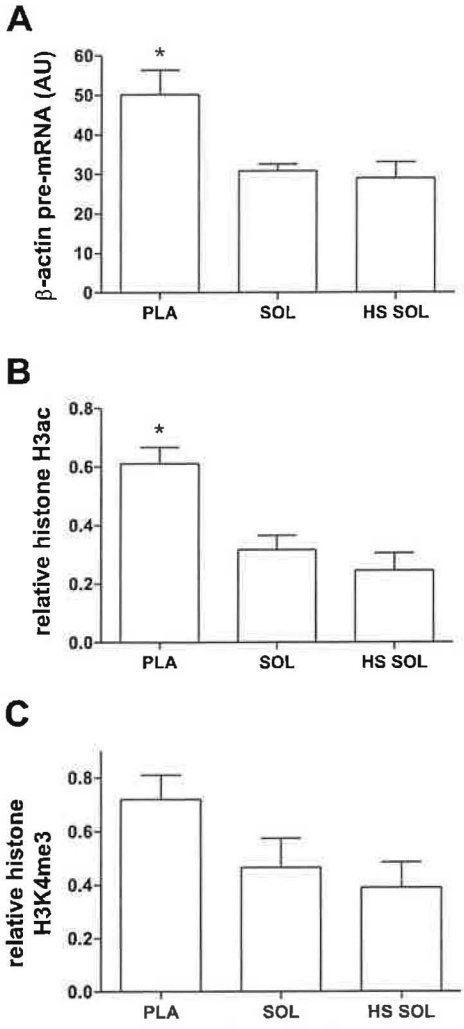

Fig. 4. β-Actin transcription and relative modifications to H3ac and H3K4me3 in Pla, Sol, and HS Sol. *A*: pre-mRNA. *B*: histone H3ac. *C*: histone H3K4me3. Bar graphs show means ± SE; n = 6/group. Histone H3ac and histone H3K4me3 are corrected for core histone H3 occupancy, as described in METHODS. *Significantly different from Sol and HS Sol ($P < 0.01$).

mRNA is significantly decreased compared with Sol, we did not observe a corresponding deacetylation at the IIa MHC. This suggests that transcriptional downregulation of the IIa MHC is not dependent on histone deacetylation. Furthermore, enrichment levels of H3K4me3 were also not changed at the IIa MHC. This seeming contradiction may indicate an alternative regulatory mechanism for the IIa MHC in unloaded slow muscle. Indeed, as previously reported, antisense RNA is transcribed across the IIa MHC and its promoter at significantly increased levels in the inactive Sol (31). This antisense RNA likely mediates transcriptional silencing of the IIa MHC (31). Thus, the effect of the antisense RNA may negate the need for a histone acetylation/deacetylation strategy to regulate the IIa MHC in the unloaded slow myofiber. However, it is still not clear by what mechanism the antisense RNA exerts control. Preliminary ChIP analyses with histone modifications known to repress transcriptional activity, H3K9me2, H3K9me3, and H3K27me3 were not enriched at the IIa MHC gene in HS Sol vs. Sol (data not shown). Perhaps, other histone modifications or methylation of the DNA itself may mediate repression of IIa MHC induced by antisense RNA.

The pattern of H3K4 trimethylation across the three muscle types studied was similar to that of histone H3 acetylation for the IIx and IIb MHCs, and followed the same pattern as mRNA/pre-mRNA expression. In type I MHC there was a

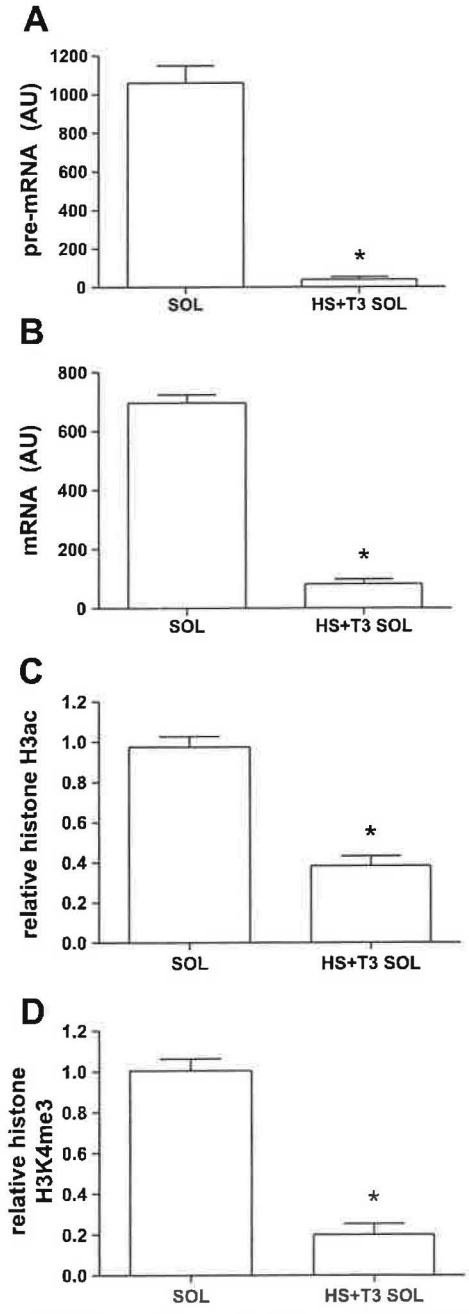

Fig. 5. Type I MHC transcription and relative modifications to H3ac and H3K4me3 in Sol and HS + triiodothyronine (T_3) Sol. *A*: pre-mRNA. *B*: mRNA. *C*: histone H3ac. *D*: histone H3K4me3. Bar graphs show means ± SE; n = 6/group. Histone H3ac and histone H3K4me3 are corrected for core histone H3 occupancy, as described in METHODS. *Significantly different from Sol ($P < 0.01$).

reduction in both mRNA and H3ac in HS Sol as compared with Sol, but H3K4me3 was not correspondingly decreased. While type I MHC pre-mRNA and mRNA were downregulated, the gene is apparently not fully repressed, because transcripts continue to accumulate, indicating that transcription is still active and merely attenuated in HS Sol (see Fig. 1). This suggests that H3K4me3 at the type I MHC is less sensitive to the attenuated transcription than H3ac. To address this, we examined H3K4me3 (and H3ac) under conditions where transcription of the type I MHC is almost completely abolished. This model, where HS is combined with thyroid hormone (T_3) treatment, resulted in a significant reduction in both H3K4me3 and H3ac at the type I MHC gene compared with Sol. This suggests that the lack of differential enrichment of H3K4me3 in Sol and HS Sol is due to the maintenance of transcription initiation of the type I MHC gene in HS Sol, albeit with reduced transcriptional activity (i.e., pre-mRNA). This is consistent with previously defined roles for H3K4me3 in transcription initiation (13, 30).

H3K4me3 along with K36 methylation is implicated in a regulatory phase before transcription elongation to ensure controlled onset of transcription (30). This is supported by a whole genome ChIP-chip approach in human cells, where H3K4me3 is shown to be associated with transcription initiation, but not elongation (13). Furthermore, a H3K4me3-specific effector, the bromodomain PHD finger transcription factor (BPTF), is a subunit of nucleosome remodeling factor (NURF), a chromatin remodeling complex which facilitates transcription (50). It is proposed that H3K4me3 serves as a docking site for NURF, recruited to promoters by sequence-specific transcription factors, which mediates transcription initiation via energy-dependent disruption of nucleosomes (50). Given this role for H3K4me3, it may not be surprising that this histone modification is not diminished in the type I MHC of HS Sol, since transcription initiation is still necessary. Conversely, expression is strongly increased in the IIx and IIb MHCs in HS Sol, compared with Sol, and H3K4me3 is proportionally increased, perhaps resulting in more frequent transcription initiation at each gene and/or indicating that a greater number of myonuclei are actively transcribing IIx and IIb MHC.

H3K4me3 has been shown to be positively correlated with H3 acetylation using ChIP methods (13, 38, 48) and mass spectroscopy (17, 45, 52). H3K4me3 and H3ac can also exist on the same H3 tail (15, 17, 45). Hazzalin and Mahadevan (15), in a series of experiments, show that all detectable H3K4me3 is targeted for rapid turnover of acetylation at H3. Thus, H3K4me3 may serve as a target for continuous acetylation and deacetylation by HATs and HDACs (15). All of the MHC genes in all of the muscle tissues examined had some degree of both H3ac and H3K4me3. In response to 7 days of HS, the slow soleus muscle is phenotypically remodeled, and we observed induction of the IIx and IIb MHC genes. This corresponds positively with both increased histone H3 acetylation and trimethylation at H3K4. Upon repression of the type I MHC with HS, histone H3 is deacetylated in HS Sol. These findings are consistent with the model of rapid turnover of acetyl groups at sites that are also trimethylated at H3K4. Rapid turnover of acetyl groups may have important implications for genes with regard to their response to treatment with HDAC inhibitors.

Transcriptional therapies using HDAC inhibitors to influence skeletal muscle remodeling or to treat muscular dystrophies are being actively explored (4, 20, 29, 33). However, there are functional implications to histones subject to dynamic turnover of acetylation, as opposed to more stably enhanced acetylation, which occurs when HDACs are inhibited (8, 9). Hazzalin and Mahadevan (15) have demonstrated that the opposing actions of HATs and HDACs are necessary for normal expression of some genes. For example, upon treatment of mouse fibroblasts with the HDAC inhibitor trichostatin A, which allows the activity of HATs to be unopposed, the induced expression of c-*jun* was inhibited, though the histones of c-*jun* remained hyperacetylated (15). These results illustrate the complex manner by which inhibitors of HDACs can influence gene expression, by resulting in either activation or repression, depending on the target gene. As research progresses in this area, it will be important to consider dynamic turnover of acetyl groups in MHC chromatin and the potential unintended consequences of unopposed acetylation at MHC loci that may require dynamic turnover of acetylation for proper regulation.

The fact the H3K4me3 and H3ac can occur on the same histone tail, and are associated with active transcription, supports the emerging view of a histone code defined by multivalent histone modifications. Multivalency may impart greater specificity to the coactivators that recognize specific histone modifications via bromodomains, which recognize acetylated lysine, and PHD finger domains, which recognize H3K4me3 (35). Importantly, many chromatin-associated proteins have been identified that contain both bromodomains and PHD fingers (34). For example, one identified effector, BPTF, contains the domains to recognize both histone marks (24). Furthermore, multivalent binding may result in enhanced affinity of such interactions, while remaining more dynamic than a correspondingly tight monovalent interaction (35).

The role of histone methylation on gene transcription has, until recently, centered on that of a highly stable histone modification, with its primary role in the storage of epigenetic information, and propagation of such information across cell divisions as well as in epigenetic inheritance (1, 39, 47). Thus, histone lysine methylation was thought to be primarily a one-way process carried out by histone methyltransferases. However, histone methylation is now known to be enzymatically reversible and dynamically regulated (39). For example, a mechanistic role for dynamic regulation of H3K4me3 has been described for gene induction in yeast (30). Little is known about the regulation of demethylation, as only recently has the first histone demethylase been identified and characterized (40). More recently yet, a new family of lysine demethylases, the Jumonji family, has been identified that can demethylate trimethylated histone tails, including a subfamily of these demethylases that can specifically revert H3K4me3 (1, 39). This has shifted the thinking of histone trimethylation to provide the basis for a dynamic view of H3K4me3 in the regulation of gene expression. Our data on the dynamically regulated MHC genes would support this view. We report that with strong upregulation of the IIx and IIb MHCs in HS Sol as compared with Sol, H3K4me3 was also strongly increased at the 5′-end of these genes. We also observed a loss of H3K4me3 in the type I MHC under conditions (HS + T_3), where the activity of this gene is markedly repressed. Clearly,

regulation of H3K4me3 does occur in correspondence with expression of the MHC genes.

In conclusion, we provide evidence in this report that the chromatin immunoprecipitation assay can be used to accurately examine in vivo native histone modifications at specific genomic sites in skeletal muscle. We show that H3 acetylation and H3K4me3 are dynamically altered in conjunction with slow to fast shifts in MHC gene expression. Furthermore, the maintenance of the slow versus fast MHC phenotype is linked to the MHC chromatin environment by the degree of H3 acetylation and H3K4me3, which corresponds to MHC transcription levels in different fiber types. In addition to the increase in histone acetylation-induced accessibility of DNA by transcription factors, the recruitment of effectors to marks such as H3K4me3 may impart MHC-specific activation in response to specific loading and/or hormonal states. Thus, epigenetic mechanisms are another factor to be considered in the coordinated regulation of the types I, IIa, IIx, and IIb MHC genes. This initial survey of H3 acetylation and H3K4me3 in the MHC gene locus provides a starting point for further research into the factors that influence MHC gene regulation in their natural in vivo setting. Skeletal muscle is particularly sensitive to many stimuli, including loading state, that provide constant feedback on gene expression and cell signaling programs. Therefore, the sum influence of the entire system can only be faithfully represented in intact muscle. For this reason, the ChIP assay should prove to be an extremely useful tool to examine protein-DNA interactions under varying environmental conditions in skeletal myofibers.

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REFERENCES

1. **Agger K, Christensen J, Cloos PA, Helin K.** The emerging functions of histone demethylases. *Curr Opin Genet Dev* 18: 159–168, 2008.
2. **Allfrey VG, Faulkner R, Mirsky AE.** Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci USA* 51: 786–794, 1964.
3. **Arnold HH, Winter B.** Muscle differentiation: more complexity to the network of myogenic regulators. *Curr Opin Genet Dev* 8: 539–544, 1998.
4. **Avila AM, Burnett BG, Taye AA, Gabanella F, Knight MA, Hartenstein P, Cizman Z, Di Prospero NA, Pellizzoni L, Fischbeck KH, Sumner CJ.** Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy. *J Clin Invest* 117: 659–671, 2007.
5. **Baldwin KM, Haddad F.** Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle. *J Appl Physiol* 90: 345–357, 2001.
6. **Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K.** High-resolution profiling of histone methylations in the human genome. *Cell* 129: 823–837, 2007.
7. **Black BL, Olson EN.** Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF2) proteins. *Annu Rev Cell Dev Biol* 14: 167–196, 1998.
8. **Bresnick EH, John S, Berard DS, LeFebvre P, Hager GL.** Glucocorticoid receptor-dependent disruption of a specific nucleosome on the mouse mammary tumor virus promoter is prevented by sodium butyrate. *Proc Natl Acad Sci USA* 87: 3977–3981, 1990.
9. **Clayton AL, Hazzalin CA, Mahadevan LC.** Enhanced histone acetylation and transcription: a dynamic perspective. *Mol Cell* 23: 289–296, 2006.
10. **De la Cruz X, Lois S, Sanchez-Molina S, Martinez-Balbas MA.** Do protein motifs read the histone code? *Bioessays* 27: 164–175, 2005.
11. **Di Maso NA, Haddad F, Zeng M, McCue SA, Baldwin KM.** Role of denervation in modulating IIb MHC gene expression in response to T_3 plus unloading state. *J Appl Physiol* 88: 682–689, 2000.
12. **Elferink CJ, Reiners JJ Jr.** Quantitative RT-PCR on CYP1A1 heterogeneous nuclear RNA: a surrogate for the in vitro transcription run-on assay. *Biotechniques* 20: 470–477, 1996.
13. **Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA.** A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* 130: 77–88, 2007.
14. **Haddad F, Qin AX, Giger JM, Guo H, Baldwin KM.** Potential pitfalls in the accuracy of analysis of natural sense-antisense RNA pairs by reverse transcription-PCR. *BMC Biotechnol* 7: 21, 2007.
15. **Hazzalin CA, Mahadevan LC.** Dynamic acetylation of all lysine 4-methylated histone H3 in the mouse nucleus: analysis at c-fos and c-jun. *PLoS Biol* 3: e393, 2005.
16. **Huey KA, Roy RR, Baldwin KM, Edgerton VR.** Temporal effects of inactivity on myosin heavy chain gene expression in rat slow muscle. *Muscle Nerve* 24: 517–526, 2001.
17. **Jiang L, Smith JN, Anderson SL, Ma P, Mizzen CA, Kelleher NL.** Global assessment of combinatorial post-translational modification of core histones in yeast using contemporary mass spectrometry: LYS4 trimethylation correlates with degree of acetylation on the same H3 tail. *J Biol Chem* 282: 27923–27934, 2007.
18. **Kim A, Dean A.** Developmental stage differences in chromatin subdomains of the beta-globin locus. *Proc Natl Acad Sci USA* 101: 7028–7033, 2004.
19. **Kim A, Kiefer CM, Dean A.** Distinctive signatures of histone methylation in transcribed coding and noncoding human beta-globin sequences. *Mol Cell Biol* 27: 1271–1279, 2007.
20. **Kim MS, Fielitz J, McAnally J, Shelton JM, Lemon DD, McKinsey TA, Richardson JA, Bassel-Duby R, Olson EN.** Protein kinase D1 stimulates MEF2 activity in skeletal muscle and enhances muscle performance. *Mol Cell Biol* 28: 3600–3609, 2008.
21. **Kong Y, Tannous P, Lu G, Berenji K, Rothermel BA, Olson EN, Hill JA.** Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation* 113: 2579–2588, 2006.
22. **Kurdistani SK, Tavazoie S, Grunstein M.** Mapping global histone acetylation patterns to gene expression. *Cell* 117: 721–733, 2004.
23. **Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD.** Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat Genet* 36: 900–905, 2004.
24. **Li H, Ilin S, Wang W, Duncan EM, Wysocka J, Allis CD, Patel DJ.** Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. *Nature* 442: 91–95, 2006.
25. **Majumdar G, Johnson IM, Kale S, Raghow R.** Epigenetic regulation of cardiac muscle-specific genes in H9c2 cells by interleukin-1β and histone deacetylase inhibitor *m*-carboxycinnamic acid *bis*-hydroxamide. *Mol Cell Biochem* 312: 47–60, 2008.
26. **McKinsey TA, Zhang CL, Olson EN.** Control of muscle development by dueling HATs and HDACs. *Curr Opin Genet Dev* 11: 497–504, 2001.
27. **Mejat A, Ramond F, Bassel-Duby R, Khochbin S, Olson EN, Schaeffer L.** Histone deacetylase 9 couples neuronal activity to muscle chromatin acetylation and gene expression. *Nat Neurosci* 8: 313–321, 2005.
28. **Mellor J, Dudek P, Clynes D.** A glimpse into the epigenetic landscape of gene regulation. *Curr Opin Genet Dev* 18: 116–122, 2008.
29. **Minetti GC, Colussi C, Adami R, Serra C, Mozzetta C, Parente V, Fortuni S, Straino S, Sampaolesi M, Di Padova M, Illi B, Gallinari P, Steinkuhler C, Capogrossi MC, Sartorelli V, Bottinelli R, Gaetano C, Puri PL.** Functional and morphological recovery of dystrophic muscles in mice treated with deacetylase inhibitors. *Nat Med* 12: 1147–1150, 2006.
30. **Morillon A, Karabetsou N, Nair A, Mellor J.** Dynamic lysine methylation on histone H3 defines the regulatory phase of gene transcription. *Mol Cell* 18: 723–734, 2005.
31. **Pandorf CE, Haddad F, Roy RR, Qin AX, Edgerton VR, Baldwin KM.** Dynamics of myosin heavy chain gene regulation in slow skeletal muscle: role of natural antisense RNA. *J Biol Chem* 281: 38330–38342, 2006.

32. **Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, Bell GW, Walker K, Rolfe PA, Herbolsheimer E, Zeitlinger J, Lewitter F, Gifford DK, Young RA.** Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* 122: 517–527, 2005.
33. **Potthoff MJ, Wu H, Arnold MA, Shelton JM, Backs J, McAnally J, Richardson JA, Bassel-Duby R, Olson EN.** Histone deacetylase degradation and MEF2 activation promote the formation of slow-twitch myofibers. *J Clin Invest* 117: 2459–2467, 2007.
34. **Ruthenburg AJ, Allis CD, Wysocka J.** Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Mol Cell* 25: 15–30, 2007.
35. **Ruthenburg AJ, Li H, Patel DJ, Allis CD.** Multivalent engagement of chromatin modifications by linked binding modules. *Nat Rev Mol Cell Biol* 8: 983–994, 2007.
36. **Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL, Mellor J, Kouzarides T.** Active genes are tri-methylated at K4 of histone H3. *Nature* 419: 407–411, 2002.
37. **Sartorelli V, Caretti G.** Mechanisms underlying the transcriptional regulation of skeletal myogenesis. *Curr Opin Genet Dev* 15: 528–535, 2005.
38. **Schubeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F, Gottschling DE, O'Neill LP, Turner BM, Delrow J, Bell SP, Groudine M.** The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev* 18: 1263–1271, 2004.
39. **Shi Y, Whetstine JR.** Dynamic regulation of histone lysine methylation by demethylases. *Mol Cell* 25: 1–14, 2007.
40. **Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y.** Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 119: 941–953, 2004.
41. **Spange S, Wagner T, Heinzel T, Krämer OH.** Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *Int J Biochem Cell Biol* 41: 185–198, 2009.
42. **Strahl BD, Allis CD.** The language of covalent histone modifications. *Nature* 403: 41–45, 2000.
43. **Tang H, Goldman D.** Activity-dependent gene regulation in skeletal muscle is mediated by a histone deacetylase (HDAC)-Dach2-myogenin signal transduction cascade. *Proc Natl Acad Sci USA* 103: 16977–16982, 2006.
44. **Tang H, Macpherson P, Marvin M, Meadows E, Klein WH, Yang XJ, Goldman D.** A histone deacetylase 4/myogenin positive feedback loop coordinates denervation-dependent gene induction and suppression. *Mol Biol Cell* 20: 1120–1131, 2009.
45. **Taverna SD, Ueberheide BM, Liu Y, Tackett AJ, Diaz RL, Shabanowitz J, Chait BT, Hunt DF, Allis CD.** Long-distance combinatorial linkage between methylation and acetylation on histone H3 N termini. *Proc Natl Acad Sci USA* 104: 2086–2091, 2007.
46. **Thomason DB, Herrick RE, Surdyka D, Baldwin KM.** Time course of soleus muscle myosin expression during hindlimb suspension and recovery. *J Appl Physiol* 63: 130–137, 1987.
47. **Trojer P, Reinberg D.** Histone lysine demethylases and their impact on epigenetics. *Cell* 125: 213–217, 2006.
48. **Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K.** Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet* 40: 897–903, 2008.
49. **Wright C, Haddad F, Qin AX, Baldwin KM.** Analysis of myosin heavy chain mRNA expression by RT-PCR. *J Appl Physiol* 83: 1389–1396, 1997.
50. **Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, Kauer M, Tackett AJ, Chait BT, Badenhorst P, Wu C, Allis CD.** A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* 442: 86–90, 2006.
51. **Yin W, Barkess G, Fang X, Xiang P, Cao H, Stamatoyannopoulos G, Li Q.** Histone acetylation at the human beta-globin locus changes with developmental age. *Blood* 110: 4101–4107, 2007.
52. **Zhang K, Siino JS, Jones PR, Yau PM, Bradbury EM.** A mass spectrometric "Western blot" to evaluate the correlations between histone methylation and histone acetylation. *Proteomics* 4: 3765–3775, 2004.

Appendix C

Novel epigenetic regulation of skeletal muscle myosin heavy chain genes. Focus on "Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading"

Kevin A. Zwetsloot, Matthew J. Laye and Frank W. Booth

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Novel epigenetic regulation of skeletal muscle myosin heavy chain genes. Focus on "Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading"

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SKELETAL MUSCLE IS A HIGHLY plastic tissue, capable of responding and adapting to a variety of physiological stimuli, e.g., muscle loading and unloading. Multiple functional phenotypes of skeletal muscle exist throughout the body at the same time. For example, muscles required for antigravity support of the skeleton or sustained locomotion are generally slow-contracting, low force-generating, and relatively resistant to fatigue, while other muscles required for quick, powerful movements are generally fast-contracting, high force-generating, and easily fatigued. In fact, skeletal muscle phenotypes have been characterized using combinations of muscle properties, including metabolic, fatigability, color, innervation, or predominant contractile protein expression. Myosin heavy chain (MHC) is the most abundant protein in skeletal muscle, comprising ~25% of the total protein content, and is a major component of the complex responsible for generating contractile force in skeletal muscle. At least nine MHC isoforms, each transcribed from their own gene, exist in mammalian striated muscle; but only slow type I MHC and various isoforms of fast type II (IIa, IIx/d, and IIb MHC isoforms) are present in adult limb skeletal muscle that is not undergoing regeneration (16). It is well established that skeletal muscle phenotype, particularly MHC gene expression, can be altered by different states of muscular activity or inactivity, as well as various hormonal and metabolic factors (1).

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In response to altered muscular activity, skeletal muscle undergoes MHC remodeling to adapt to the new physiological demands on the tissue. The final composition of various MHC protein isoforms after adaptation is dependent on the species, on the environmental stimulus type and intensity (whether it is physiological or supra-physiological), and on contractile activity (whether it is increased or decreased). Resistance training switches rat MHC from type IIb to IIx MHC in red and white portions of rat gastrocnemius muscle (4). Importantly, no increase in type I MHC occurs in response to physiological resistance training. However, supra-physiological models of muscle hypertrophy (functional overload) do increase type I fiber percentage (17) in rats. Similarly, endurance training, a physiological stimulus, for <3 mo exhibits no increase in type I MHC (13; see discussion in Ref. 9), whereas, in contrast, supra-endurance models (chronic stimulation for >12 h/day) have major increases in the percentage of type I MHC (13). Physiological decreases in muscle loading by hindlimb suspension or spaceflight do decrease type I fiber percentage in rodents (3, 5) and humans (8, 18, 19). Taken together, the question then is how does each nucleus decide which MHC gene to express and not to express in a muscle fiber with multiple nuclei during reductions in contractile activity?

Six of the nine MHC genes are arranged in tandem on the same chromosome, and their order and orientation are highly conserved. Multiple control mechanisms have been shown to exist beyond transcription factors. Surprisingly, while pre-mRNA (10), alternative splicing (7), antisense RNA (11), and intergenic transcription (15) are all known to regulate MHC,

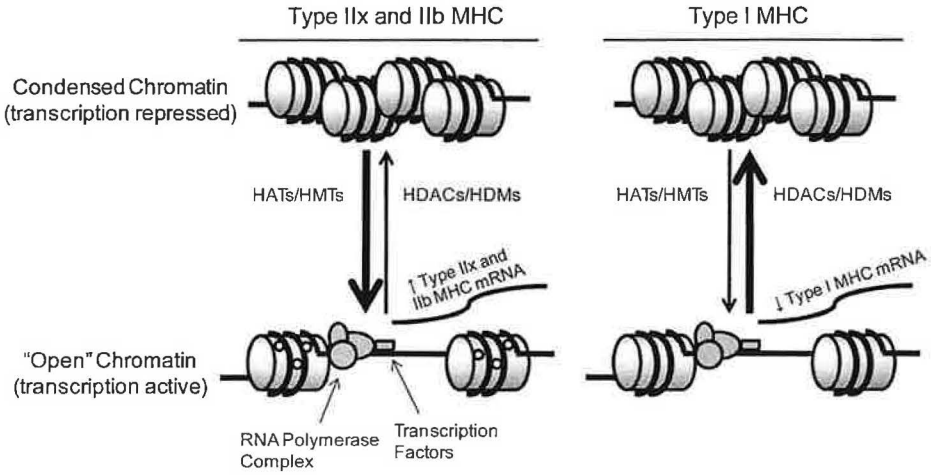

Fig. 1. Schematic representing epigenetic histone modifications occurring in the slow fiber-type soleus muscle in response to reduced muscular activity/unloading (hindlimb suspension). Upon induction of hindlimb suspension, histone acetylation and/or histone methylation (*left*), by histone acetyltransferases (HATs) and histone methyltransferases (HMTs), respectively, are associated with an "open" chromatin structure and increased transcriptional activity of type IIx and IIb myosin heavy chain (MHC) genes. On the other hand, histone deacetylation and/or histone demethylation (*right*), by histone deacetylases (HDACs) and histone demethylases (HDMs), respectively, are associated with a condensed chromatin structure and repressed transcriptional activity of the type I MHC gene in response to hindlimb suspension. ○, Acetyl or methyl groups covalently bound to histones.

little is known about whether epigenetic mechanisms also regulate MHC gene expression in skeletal muscle with altered muscular activity. Pandorf et al. (12) describe a novel mechanism by which epigenetic modifications play a role in the expression of MHC genes in response to altered muscular activity (12).

Until recently, chromatin has rarely been thought of as much more than just genomic DNA and protein packaging. However, an emerging field in biological research over the past decade, termed "epigenetics," has given new-found respect to chromatin structure as being a major player in the control of transcriptional activity and gene expression. Epigenetic modifications, unlike genetic mutations or single nucleotide polymorphisms, do not result in changes in the DNA sequence. Instead, DNA structure is indirectly modified by histone modifications (e.g., acetylation or methylation) or directly modified by methylation of the cytosine base in cytosine-guanine dinucleotides (CpG) of DNA to alter the expression of genes. CpG methylation of DNA is a stable covalent modification more associated with disease states, such as cancer or atherosclerosis (2, 14), while histone modifications are more akin to protein phosphorylation. Histone modifications can be both transient, rapidly responding to changes within the cell or surrounding environment, or stably maintained throughout life when obtained in early or fetal life (6). Histone acetylation can occur at many different lysine residues (e.g., lysine 4, 9, or 27), creating a negative electrostatic charge that repels the negatively charged DNA backbone, thus producing a loosely packed chromatin that allows easier access for transcription factors and the RNA polymerase complex to increase transcription (Fig. 1), also providing a site for recruitment of other proteins to the promoter region of a given gene. Conversely, histone deacteylation results in a compact, tightly bound histone:DNA complex and general repression of gene transcription (6).

The acetylation status of core histones is based on the balance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity. Although it has been known that skeletal muscle contraction during exercise can regulate the activity of HATs and HDACs, this report by Pandorf et al. (12) is the first to show with direct evidence that histone modifications occur in skeletal muscle at the locus of MHC genes, concomitantly with changes in MHC gene expression. Using chromatin immunoprecipitation (ChIP), the authors demonstrate that both histone H3 acetylation and histone H3 methylation are directly related to the steady-state transcriptional activity of the MHC genes in the fast fiber-type plantaris and in the slow fiber-type soleus muscles under normal conditions. Of particular interest is the finding that the acetylation and methylation status at specific sites of histone H3 also directly coincides with the altered transcriptional activity of type I, IIx, and IIb MHC genes in the soleus in response to reduced muscular activity/unloading (hindlimb suspension).

These findings have significant physiological relevance in skeletal muscle biology because they extend our current understanding of the molecular mechanisms that regulate skeletal muscle remodeling and MHC gene expression with altered muscular activity. Also, Pandorf et al. (12) demonstrate the feasibility of using ChIP assays to analyze native chromatin structure in frozen samples as it pertains to transcriptional activity of skeletal muscle genes. Certainly, studies elucidating the mechanisms by which specific HATs and HDACs change their activity in response to a physiological stimulus, such as hindlimb unloading or endurance exercise, are needed. Additionally, answering broad questions such as whether epigenetics play a role in the regulation of other genes involved in skeletal muscle remodeling, and further yet, whether epigenetic regulation is involved in muscle phenotypes with pathological consequences, such as the failed regrowth of muscle from atrophy with aging, muscular dystrophies, or cardiac myopathies, is beginning to come within reach thanks to studies such as this one.

GRANTS

This work was supported by National Institutes of Health Grant AG-18780 (to F. W. Booth), American College of Sports Medicine (to M. J. Laye), and American Heart Association (to M. J. Laye).

REFERENCES

1. **Baldwin KM, Haddad F.** Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle. *J Appl Physiol* 90: 345–357, 2001.
2. **Baylin SB.** DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol* 2, *Suppl* 1: S4–S11, 2005.
3. **Caiozzo VJ, Baker MJ, McCue SA, Baldwin KM.** Single-fiber and whole muscle analyses of MHC isoform plasticity: interaction between T_3 and unloading. *Am J Physiol Cell Physiol* 273: C944–C952, 1997.
4. **Caiozzo VJ, Haddad F, Baker MJ, Baldwin KM.** Influence of mechanical loading on myosin heavy-chain protein and mRNA isoform expression. *J Appl Physiol* 80: 1503–1512, 1996.
5. **Caiozzo VJ, Haddad F, Baker MJ, Herrick RE, Prietto N, Baldwin KM.** Microgravity-induced transformations of myosin isoforms and contractile properties of skeletal muscle. *J Appl Physiol* 81: 123–132, 1996.
6. **Clayton AL, Hazzalin CA, Mahadevan LC.** Enhanced histone acetylation and transcription: a dynamic perspective. *Mol Cell* 23: 289–296, 2006.
7. **Dennehey BK, Leinwand LA, Krauter KS.** Diversity in transcriptional start site selection and alternative splicing affects the 5′-UTR of mouse striated muscle myosin transcripts. *J Muscle Res Cell Motil* 27: 559–575, 2006.
8. **Edgerton VR, Zhou MY, Ohira Y, Klitgaard H, Jiang B, Bell G, Harris B, Saltin B, Gollnick PD, Roy RR.** Human fiber size and enzymatic properties after 5 and 11 days of spaceflight. *J Appl Physiol* 78: 1733–1739, 1995.
9. **Garcia-Roves PM, Huss J, Holloszy JO.** Role of calcineurin in exercise-induced mitochondrial biogenesis. *Am J Physiol Endocrinol Metab* 290: E1172–E1179, 2006.
10. **Haddad F, Roy RR, Zhong H, Edgerton VR, Baldwin KM.** Atrophy responses to muscle inactivity. II. Molecular markers of protein deficits. *J Appl Physiol* 95: 791–802, 2003.
11. **Pandorf CE, Haddad F, Roy RR, Qin AX, Edgerton VR, Baldwin KM.** Dynamics of myosin heavy chain gene regulation in slow skeletal muscle: role of natural antisense RNA. *J Biol Chem* 281: 38330–38342, 2006.
12. **Pandorf CE, Haddad F, Wright C, Bodell PW, Baldwin KM.** Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading. *Am J Physiol Cell Physiol* (April 15, 2009). doi:10.1152/ajpcell.00075.2009.
13. **Pette D.** Historical perspectives: plasticity of mammalian skeletal muscle. *J Appl Physiol* 90: 1119–1124, 2001.
14. **Post WS, Goldschmidt-Clermont PJ, Wilhide CC, Heldman AW, Sussman MS, Ouyang P, Milliken EE, Issa JP.** Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. *Cardiovasc Res* 43: 985–991, 1999.
15. **Rinaldi C, Haddad F, Bodell PW, Qin AX, Jiang W, Baldwin KM.** Intergenic bidirectional promoter and cooperative regulation of the IIx and IIb MHC genes in fast skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 295: R208–R218, 2008.
16. **Schiaffino S, Reggiani C.** Molecular diversity of myofibrillar proteins: gene regulation and functional significance. *Physiol Rev* 76: 371–423, 1996.

17. **Stone J, Brannon T, Haddad F, Qin A, Baldwin KM.** Adaptive responses of hypertrophying skeletal muscle to endurance training. *J Appl Physiol* 81: 665–672, 1996.
18. **Trappe S, Costill D, Gallagher P, Creer A, Peters JR, Evans H, Riley DA, Fitts RH.** Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. *J Appl Physiol* 106: 1159–1168, 2009.
19. **Zhou MY, Klitgaard H, Saltin B, Roy RR, Edgerton VR, Gollnick PD.** Myosin heavy chain isoforms of human muscle after short-term spaceflight. *J Appl Physiol* 78: 1740–1744, 1995.

Appendix D

Research Journal

Activities Journal

Monday, Aug 4 2008

Start in Baldwin lab at UCI.

Intro to Chip assay, read papers on the assay

Tuesday, Aug. 5, 2008

Shadow Clay as he begins a Chromatin Immuno Precipitation assay (Chip assay) on White Vastus Lateralis muscle vs. Soleus muscle (50 mg each muscle tissue).
Start with frozen tissue samples and follow the assay protocol.
Formaldehyde cross-linking for 5 min. vs. 10 min., tissue homogenizer.....Freeze overnight.

Wednesday, Aug. 6, 2008

Sonicate DNA Run sample on 2% agarose gel, quantitate Chromatin with real time PCR.
Use ImageQuant and Adobe Pgotoshop for image processing.
DNA is visualized under UV light with GelGreen as dye.
Image results on next page.

File/Range: G:\Carola\08-06-08 TEST of WVL _Sol shearing at 80% with no formaldehyde and 5 min.gel / 1028-20658 /Magn
User Name: Geldoc user
Image Name: G:\Carola\08-06-08 TEST of WVL _Sol shearing at 80% with no formaldehyde and 5 min.gel
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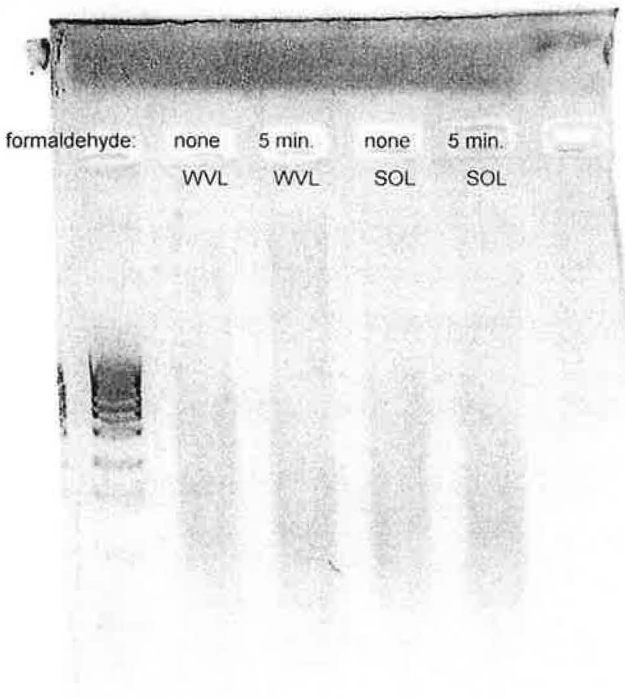
Scan Date/Time: 2008:08:06 14:24:41
Pr ate/Time:

this is from spare tissue



400 ul lysis buffer for shearing. samples were frozen in lysis buffer prior to sonication each done for 10 pulses, x-linked for 5 min, and with no formald.

10 ul taken for reverse x-link (20hrs 65 deg.), rnase treat, protein dig.

loaded 38ul on gel

Thursday, Aug 7, 2008

Finish Chip procedure on samples
Ready for PCR

Monday, Aug 11, 2008
Evaluate results
Run PCR reactions again, side by side with Clay

Tuesday, Aug 12, 2008

Run 2% Agarose gel with PCR products
See printout of results (next page)

Collect samples for new Chip assay procedure: Control soleus vs. HS + T3 Soleus vs. a mix of normal control soleus and white vastus lateralis (WVL).
The idea is that HS and T3 synergistically turn off slow type I MHC and turn on fast type 2 fibers. This should be detectable on the transcriptional level with the Chip assay. The soleus WVL mix is included as an additional control to the ChIP procedure.

PCR Reactions	1x	3x	8x	9x	12x
Water	6.1	18.3	48.8	54.9	73.2
10x PCR Buffer	2.5				
10 mM dNTP	0.5	11.25	30	33.75	45
50 mM MgCl2	0.75				
5+3' primer mix at 5 pmol/ul	3	9	24	27	36
Template (IP DNA/input DNA)	**12**	Add individually			
Biolase DNA Poly	0.15	0.45	1.2	1.35	1.8
SUM		39	104	117	156

PCR Reactions	1x	3x	5x	7x	8x
Water	1.1	3.3	5.5	7.7	8.8
10x PCR Buffer	2.5				
10 mM dNTP	0.5	11.25	18.75	26.25	30
50 mM MgCl2	0.75				
5+3' primer mix at 5 pmol/ul	3	9	15	21	24
Template (IP DNA/input DNA)	**17**	Add individually			
Biolase DNA Poly	0.15	0.45	0.75	1.05	1.2
SUM		24	40	56	64

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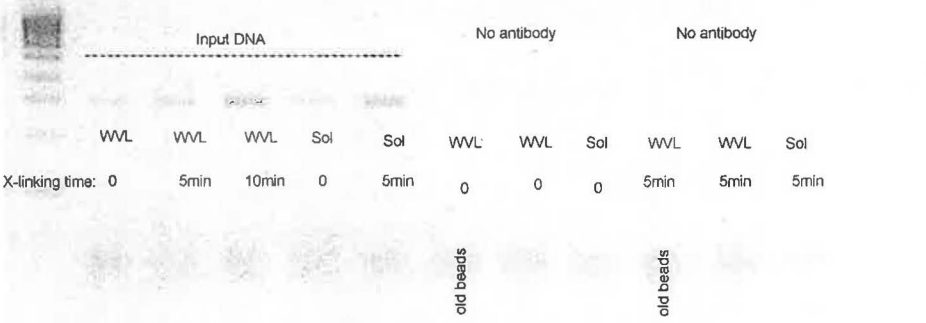
Image Name: G:\Carola\08-11-08 TEST of WVL Sol with diff xlinking times, ChIP Ac H3 _H3K4me3, PCR with beta (1156,1
Image Comment:
Present Date/Time: 2008:08:28 12:49:08
Scan Date/Time: 2008:08:11 19:37:16
Pr ate/Time:

PCR with Beta MHC 1156,1441 primers, 30/33 cycles, 57 deg., 286 bp



ChIP date 8-7-08; with Acetyl H3 (Upstate 06-599, 1.0 ul), H3K4me3 (1.0ul) (1hr incubation)

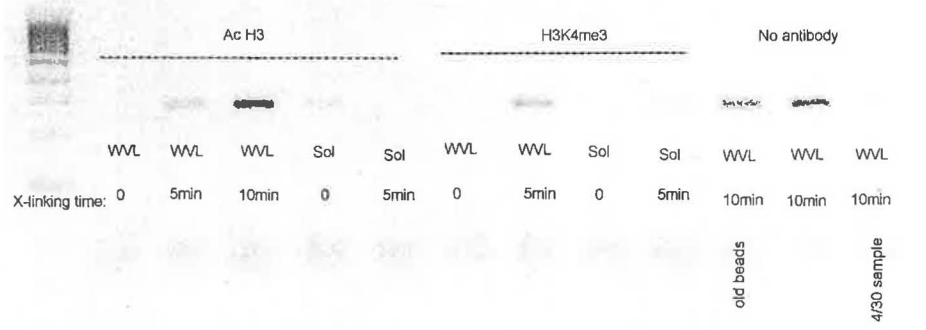
2hr incubation with protein A beads

30 cycles for Ac H3; 33 cycles for H3K4me3

12 ul DNA template for Ac H3, 17 ul for H3K4me3, from 135 ul eluted DNA

started with 25 ug chromatin DNA

old beads = 5-23-08 recieved (protein A agarose). others are new beads, rec. 8-7-08



WVL with 10min xlinking time is the 6/30 sample, done with previous ChIP

File/Range: G:\Carola\08-11-08 TEST of WVL Sol with diff xlinking times, ChIP Ac H3 _H3K4me3, PCR with IIb (1075,1272)
User Name: Geldoc user
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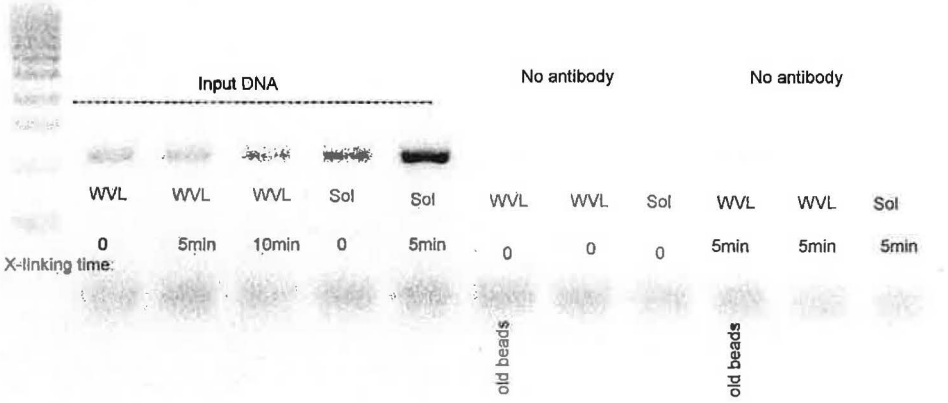
Image Comment:
Present Date/Time: 2008:08:28 12:50:05
Scan Date/Time: 2008:08:11 19:15:45
Pr ate/Time:

PCR with IIB 1075/1272, 30/33 cycles, 57 deg., 218 bp, 1045-1262 from IIb TSS



ChIP date 8-7-08; with Acetyl H3 (Upstate 06-599, 1.0 ul), H3K4me3 (1.0ul) (1hr incubation)

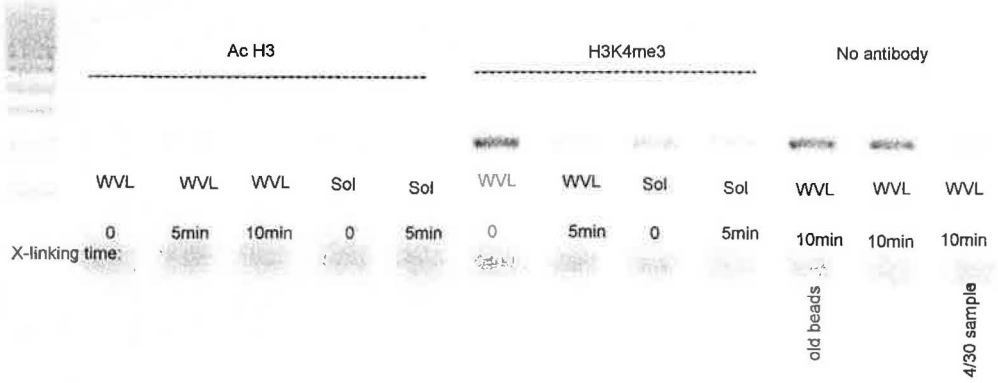
2hr incubation with protein A beads.

30 cycles for Ac H3; 33 cycles for H3K4me3

12 ul DNA template for Ac H3, 17 ul for H3K4me3, from 135 ul eluted DNA

started with 25 ug chromatin DNA

old beads = 5-23-08 recieved (protein A agarose). others are new beads, rec. 8-7-08



WVL with 10min xlinking time is the 6/30 sample, done with previous ChIP

this gel was made 8-6, so 5 days old.

File/Range: G:\Carola\08-12-08 TEST of WVL Sol with diff xlinking times, ChIP Ac H3 _H3K4me3, PCR with beta (1156,144
User Name: Geldoc user
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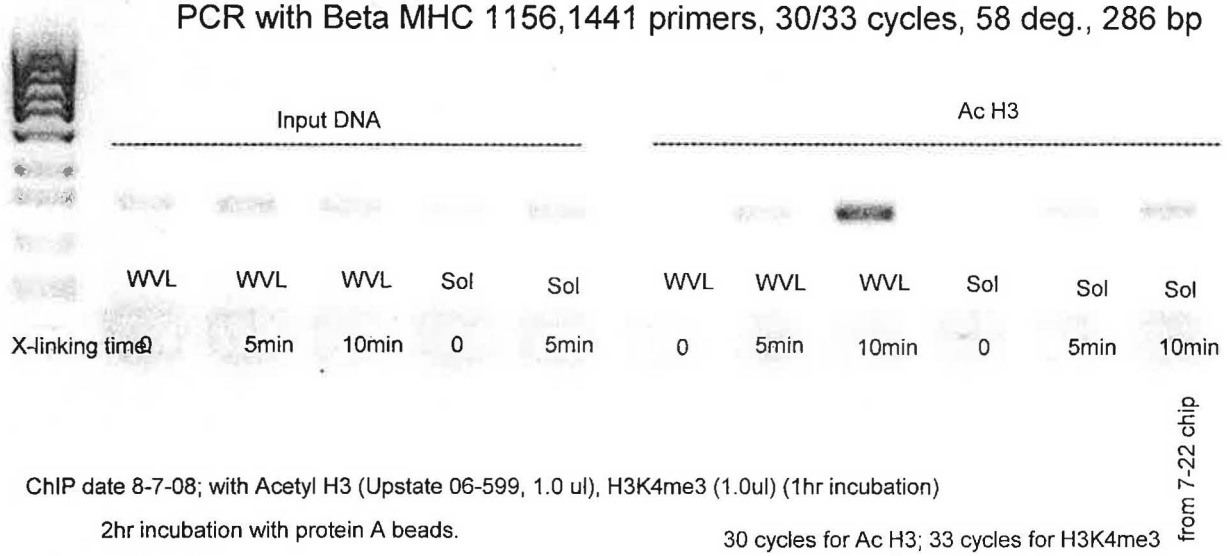
Scan Date/Time: 2008:08:13 11:49:39
Pr ate/Time:

PCR with Beta MHC 1156,1441 primers, 30/33 cycles, 58 deg., 286 bp



ChIP date 8-7-08; with Acetyl H3 (Upstate 06-599, 1.0 ul), H3K4me3 (1.0ul) (1hr incubation)

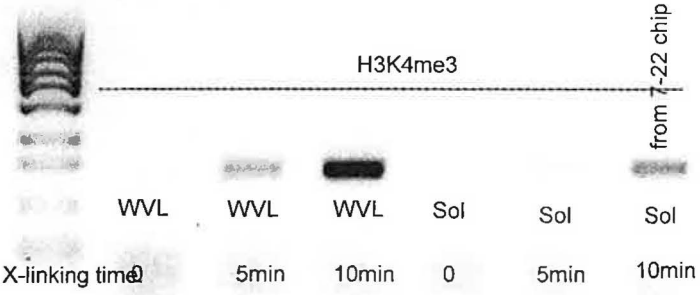
2hr incubation with protein A beads.

30 cycles for Ac H3; 33 cycles for H3K4me3

12 ul DNA template for Ac H3, 17 ul for H3K4me3, from 135 ul eluted DNA

started with 25 ug chromatin DNA

old beads = 5-23-08 recieved (protein A agarose). others are new beads, rec. 8-7-08



Duplicate of 08-12-08 Test of WVL & Sol...

This gel by Carola

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Image Comment:
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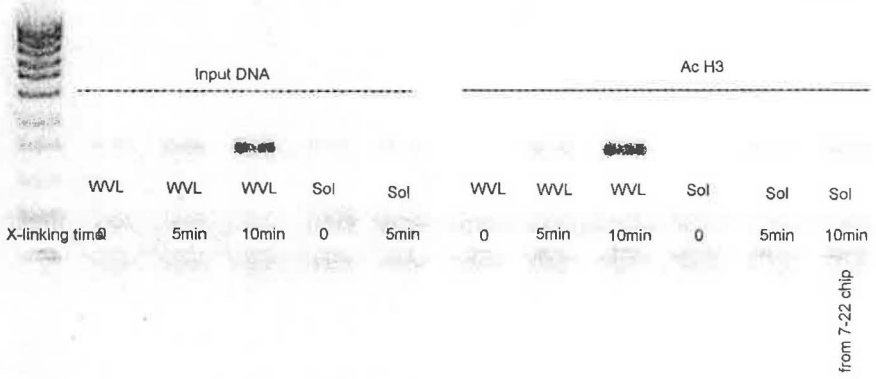
Pr ate/Time:

PCR with Beta MHC 1156,1441 primers, 30/33 cycles, 58 deg., 286 bp



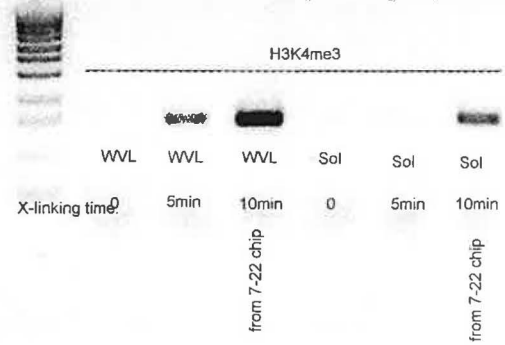
ChIP date 8-7-08; with Acetyl H3 (Upstate 06-599, 1.0 ul), H3K4me3 (1.0ul) (1hr incubation)

2hr incubation with protein A beads.

30 cycles for Ac H3; 33 cycles for H3K4me3

12 ul DNA template for Ac H3, 17 ul for H3K4me3, from 135 ul eluted DNA started with 25 ug chromatin DNA

old beads = 5-23-08 recieved (protein A agarose). others are new beads, rec. 8-7-08



See also duplicate gel by Carola

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Image Name: G:\Carola\08-12-08 TEST of WVL Sol with diff xlinking times, ChIP Ac H3 _H3K4me3, PCR with IIx (pre2).gel
Image Comment:
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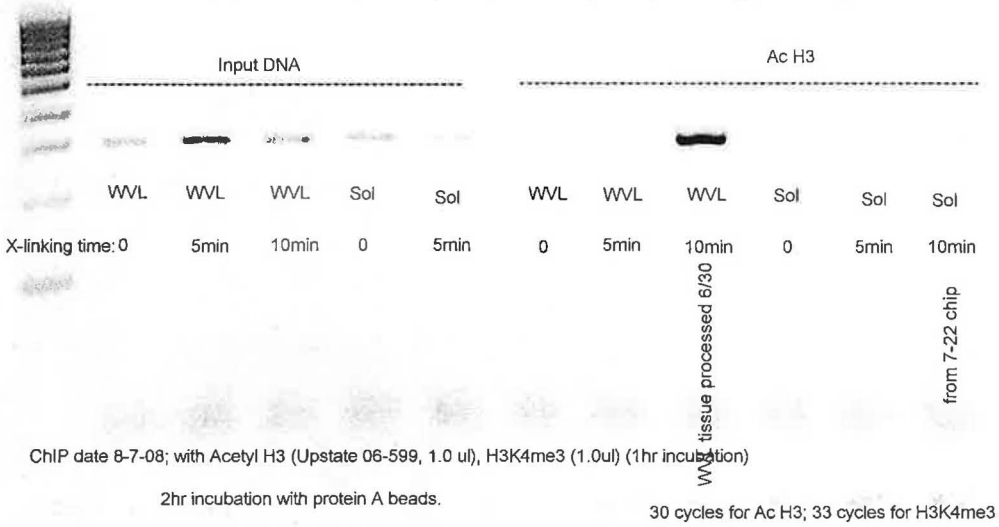
Scan Date/Time: 2008:08:13 17:20:36
Pr ate/Time:

PCR with IIx MHC pre2, 30/33 cycles, 57 deg., 254 bp

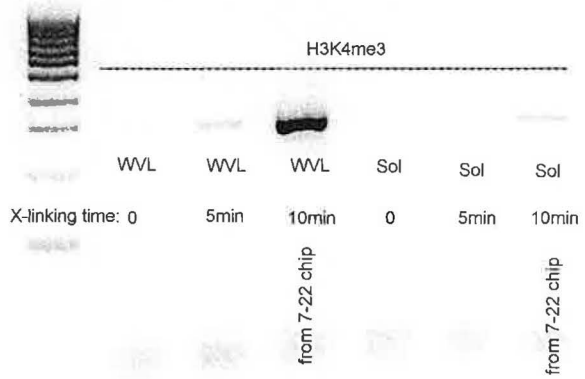


ChIP date 8-7-08; with Acetyl H3 (Upstate 06-599, 1.0 ul), H3K4me3 (1.0ul) (1hr incubation)

2hr incubation with protein A beads.

30 cycles for Ac H3; 33 cycles for H3K4me3

12 ul DNA template for Ac H3, 17 ul for H3K4me3, from 135 ul eluted DNA started with 25 ug chromatin DNA



Premixed 10x buffer, dNTP, MgCl2

PCR Reactions	1x	120x
Water	6.1	
10x PCR Buffer	2.5	300
10 mM dNTP	0.5	60
50 mM MgCl2	0.75	90
5+3' primer mix at 5 pmol/ul	3	
Template (IP DNA/input DNA)	**12**	Add individually
Biolase DNA Poly	0.15	
SUM	25	450

PCR Reactions	1x	3x	8x	9x	13x	14x
Water	6.1	18.3	48.8	54.9	79.3	85.4
10x PCR Buffer	2.5					
10 mM dNTP	0.5	11.25	30	33.75	48.75	52.5
50 mM MgCl2	0.75					
5+3' primer mix at 5 pmol/ul	3	9	24	27	39	42
Template (IP DNA/input DNA)	**12**	Add individually				
Biolase DNA Poly	0.15	0.45	1.2	1.35	1.95	2.1
SUM		39	104	117	169	182

PCR Reactions	1x	3x	5x	8x	13x	14x
Water	1.1	3.3	5.5	8.8	14.3	15.4
10x PCR Buffer	2.5					
10 mM dNTP	0.5	11.25	18.75	30	48.75	52.5
50 mM MgCl2	0.75					
5+3' primer mix at 5 pmol/ul	3	9	15	24	39	42
Template (IP DNA/input DNA)	**17**	Add individually				
Biolase DNA Poly	0.15	0.45	0.75	1.2	1.95	2.1
SUM		24	40	64	104	112

15x	16x	18x	21x	
91.5	97.6	109.8	122	Water
				10x PCR Buffer
56.25	60	67.5	75	10 mM dNTP
				50 mM MgCl2
45	48	54	60	5+3' primer mix at 5 pmol/ul
				Template (IP DNA/input DNA)
2.25	2.4	2.7	3	Biolase DNA Poly
195	208	234	260	

15x	16x	18x	32x
16.5	17.6	19.8	35.2
56.25	60	67.5	120
45	48	54	96
2.25	2.4	2.7	4.8
120	128	144	256

Tissue available for Chip assay

Tissue weight
ChIP tissue in the -80 in Tower 4 in the autoclave room

These were cut to use a piece for RNA.

		left over after using some for RNA	
control soleus		original weight	estimated weight
animal #		mg	mg
1	GC-1	89	29
2	GC-2	86	19
3	GC-3	112	51
4	GC-4	79	18
5	GC-5	94	43
6	GC-6	104	57
7	GC-7	89	20
8	GC-8	74	27
	GC-9	92	92
	GC-10	90	90

7d T3+HS Soleus samples These animals were on the ground for 3 hours before sac
these are also label 0d from T3+HS+CSA reload project
whole group fro carola's use

tissue dated 4/9/2008

	Left	**Right**
HS+T3 0 Day	Sol	Sol
HS-1	45	45
2	44	39
3	48	41
4	40	41
5	49	50

Wednesday Aug 13

Prepare samples for ChIP assay	Weigh muscle tissue	
50 mg GC Sol	50 mg HS + T3 Sol	50 mg GC Sol + WVL mix

Prepare 1% Formaldehyde solution as follows:

to make 10 ml 1% formaldehyde		
37% formaldehyde	0.27	ml
10xPBS	1	ml
water	8.73	ml

Prepare necessary amount of 1xPBS solution and put on ice, to be used for different washes.
Need about 4.5 ml 1x PBS per sample.
When using PBS, always supplement with protease inhibitors
protease inhibitors: use leupetin, AEBSF, and aproteinin each are at 1000x stock solutions in the -80 C freezer.
Keep AEBSF and leupetin on dry ice, thaw and refreeze as necessary.
Keep aprotinin on ice and use as necessary.

Isolating and lysing cells from tissue
Mince tissue (small pieces) using a razor blade
Put minced tissue in 1.5ml tubes in ~1 ml cold PBS supplemented with protease inhibitors
Keep on ice until all pieces are minced
Drain cold PBS, add 1 ml 1% formaldehyde buffer (freshly made)
Incubate at room temperature for 10 minutes with mixing every few minutes
After 10 minutes incubation, add 110 ul 10x glycine (1.25M glycine) prepared in a 15 ml tube.
Incubate 5 minutes at room temperature
Change solution, take out the formaldehyde PBS, replace with 1 ml cold PBS (+protease inhibitors)
Repeat PBS wash once more (use PBS supplemented with protease inhibitor)
Add 19x cold PBS (plus inhibitors) to samples
Transfer to homogenizing pestle tube, and homogenize on ice, transfer to clean 1.5ml tube.

Spin down the homogenate at 1500g for 10 minutes in order to collect the cells as a pellet
Take out supernatant, use a pipet to leave a clean pellet (no liquid should be left)
Suspend the pellet in 400 ul lysis buffer (supplemented with protease inhibitors)
Make sure samples are in 1.5 ml tubes. The sonicator does not work well with the 2ml tubes due to shape of tube bottom.
Incubate 10-30 minutes on ice
Remove 5ul from the lysate to be used as unsheared DNA (high molecular weight)
Freeze lysate at -80 (since no time to continue).

Thursday Aug 14,2008

Shearing the DNA with sonication:
Use the Sonics Vibracell, 130 watts ultrasonic from sonics and materials (VCX 130)
The converter is connected to a 2mm probe
2mm probe is to process samples with volumes of 150ul to 5 ml

Sonication:
Put the samples on ice at all time to keep it cool
Immerse the probe tip in the sample
Sonicate using the following protocol:

amplitude	time	# of pulses	
80% max	15 sec		10

25 sec rest on ice between pulses
(monitor tube temperature, as it can heat up quickly)
Set sonicator to do 2 pulses at a time. 2 pulses = 55 sec, with 25 sec rest set. Then vortex/mix sample, and return to ice, and start next sample. Rotate thru samples like this. Make sure that all of the sample is in the lysis buffer, as it tends to stick to the sides of tube (use pipette tip to clears sides).
Have water in the ice, so that the tube is immersed in ice-cold temp, otherwise air pockets in ice around tube can heat up.
Sonication will solubilize the chromatin and break up the DNA
The goal is to find the condition that shears the DNA to 400-1000 bp size fragments
After sonication, spin the samples in a cold microfuge at 12,000g for 10 minutes to remove insoluble material.

Remove 10ul aliquot of sheared DNA to analyze on agarose gel
Transfer the supernatant (containing soluble chromatin) to a fresh microfuge tube.
Freeze at -80 to be used later for the IP procedure.

Friday Aug. 15, 2008

DNA analyses: necessary to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.

To 10ul total sheared lysate, add 90ul nuclease free water, and 4ul 5M NaCl (94ul premix)
(Better to use **10 ul** sheared lysate, as that will yield enough to see easily on the gel to confirm shearing size. Same volume of H2O and NaCl works fine.)
Incubate at 65oC for 4-5 hours to **overnight** at 65oC to reverse the DNA-protein crosslinking.
Freeze at -80 C.

Monday Aug. 18, 2008

Continue DNA analysis to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.
Add 1 ul RNAse A (10ug/ul) and incubate 30 minutes at 37oC
Add 7ul (digestion buffer, see below) & incubate at 45oC for 1-2hours
digestion buffer=2ul 0.5M EDTA, 4ul 1M Tris-HCL pH 6.5, and 1ul proteinase K(10ug/ul)
load 20 or 40 ul on 2 % agarose gel with a 100 bp ladder
loading different amouts to increase the chance of being in the visibility range of gel-green (preferred to ethidiumbromide) staining.

to 5 ul lysate, add 94 ul premix (NaCl 4, water, 90)
incubate at 65oC overnight

premix	**1x**
5M NaCl	4
H2O	90
	94

digestion buffer

premix:	**1x**
0.5M EDTA	2
1M Tris pH6.5	4
proteinase K	1
	7

all suspension buffer must be supplemented with protease inhibitors

Tuesday Aug 19, 2008

Prepare necessary reagents for ChIP assay scheduled for Wednesday.

This tissue preped on 8-13-08

	Sample	muscle frozen weight
ul lysis buffer	GC Sol	50
80 % amplitude	HS + T3 Sol	50
15 sec pulse	WVL + Sol	32, sol + 25 WVL
10 pulses		

Tried a smaller Plan and WMG this time (50mg) instead of 80+ mg, in case the size of the tissue makes a difference.

Use 25ug of DNA for this ChIP	ng/ul of original chromatin sample	ul of chromatin for 25 ug	total dilution buffer to yield 1ml
GC Sol	**538.1**	**46.5**	954
HS Sol + T3	**891.6**	**28.0**	972
WVL + Sol	**547.0**	**45.7**	954

Preclear chromatin for **45 min** with 50 ul Protein A beads (pre-washed in chip dilution buffer).
Normal Rabbit **IgG** from Upstate (12-370) used **1.0 ul** (1.0 ug).
ChIP with Anti-**Histone H3 K4 me3** antibody (Upstate #07-473), use **1.0ul**.
ChIP with **Anti-Acetyl Histone H3** antibody (Upstate #06-599), used **1.0 ul** (~1 ug).
Incubate 1 hr with antibodies.
added protein A agarose beads (Pierce) 50 ul, incubate **2 hr**. These are new agarose beads, that worked great with trial run

ed in 135 ul water.

SYBR green I, dilute 1:10,000 in filtered 1x TAE (same buffer as gel running buffer)
add 50 ul sybr green mix to well, 5ul of sample/standard
generally dilute sample 1:40

8-13-08 samples, read 8-18-08
using standards from 3-11

	ng/well/5ul				average		
water blanl	0	6478	6184	6107	**6256**	Net	predicted r
S0.5	0.5	7003	6678	6858	**6846**	6846	1.2
S1	1	6868	6670	6993	**6844**	6844	1.4
S2.5	2.5	7420	8262	7690	**7791**	7791	2.5
S5	5	9454	8931	8761	**9049**	9049	4.2
S10	10	12523	14513	12712	**13249**	13249	9.9
S15	15	16979	15788	14821	**15863**	15863	13.4
S20	20	21777	23552	20098	**21809**	21809	21.4

r^2 **0.9833**

10 ul sheared DNA taken, in final volume of 112 ul
samples diluted 1:40 (5:200), measured 5ul in well

				average	Net		**ng/ul of original chro**mahn
GC Sol	10598	10605	9898	**10367**	4111	6.01	**538.1**
HS Sol + T3	12572	13566	13757	**13298**	7042	9.95	**891.6**
WVL + Sol (32mg Sol	9581	11550	10193	**10441**	4185	6.10	**547.0**

Chip assay

- 25 µg Chromatin

test 3 antibodies : ① Rabbit IgG control

② anti Histone H3 K4 me3 antibody

③ Anti-acetyl Histone H3 antibody

File/Range: I:\Clay\ChIP Assay PCR\8-13 tissue\08-18-08 Sheared samples, GC SOL, HS SOL, WMG, PLAN.gel / 2315-2441
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8-18-08

Sheared chromatin, Proteinase K digested

1	2	3	4
100 bp ladder	GC	WVL +Sol	HS+ T3

GC: ground control soleus

HS+ T3: Soleus from hindlimb suspended + T3 treated animals

WVL +Sol: white vastus lateralis + Soleus from ground control animals

← these are Clay's samples on same gel

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P[illegible]ate/Time:

ng
0.5
1
2.5
5
10
15
20
water

GC HS HTg Sol WK

File/Range: G:\Carola\DNA check 8-18-08.gel / 2315-24415 /Magnification - 2.31
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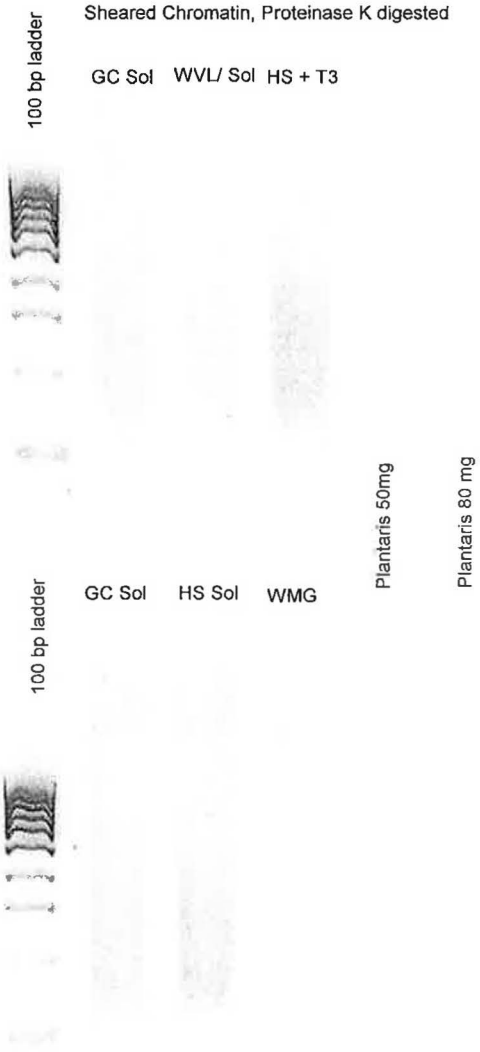


Mx3000P

Fluorescence
Cybr green dye

1:40 diluted DNA sample (ChIp assay sample)
for DNA quant

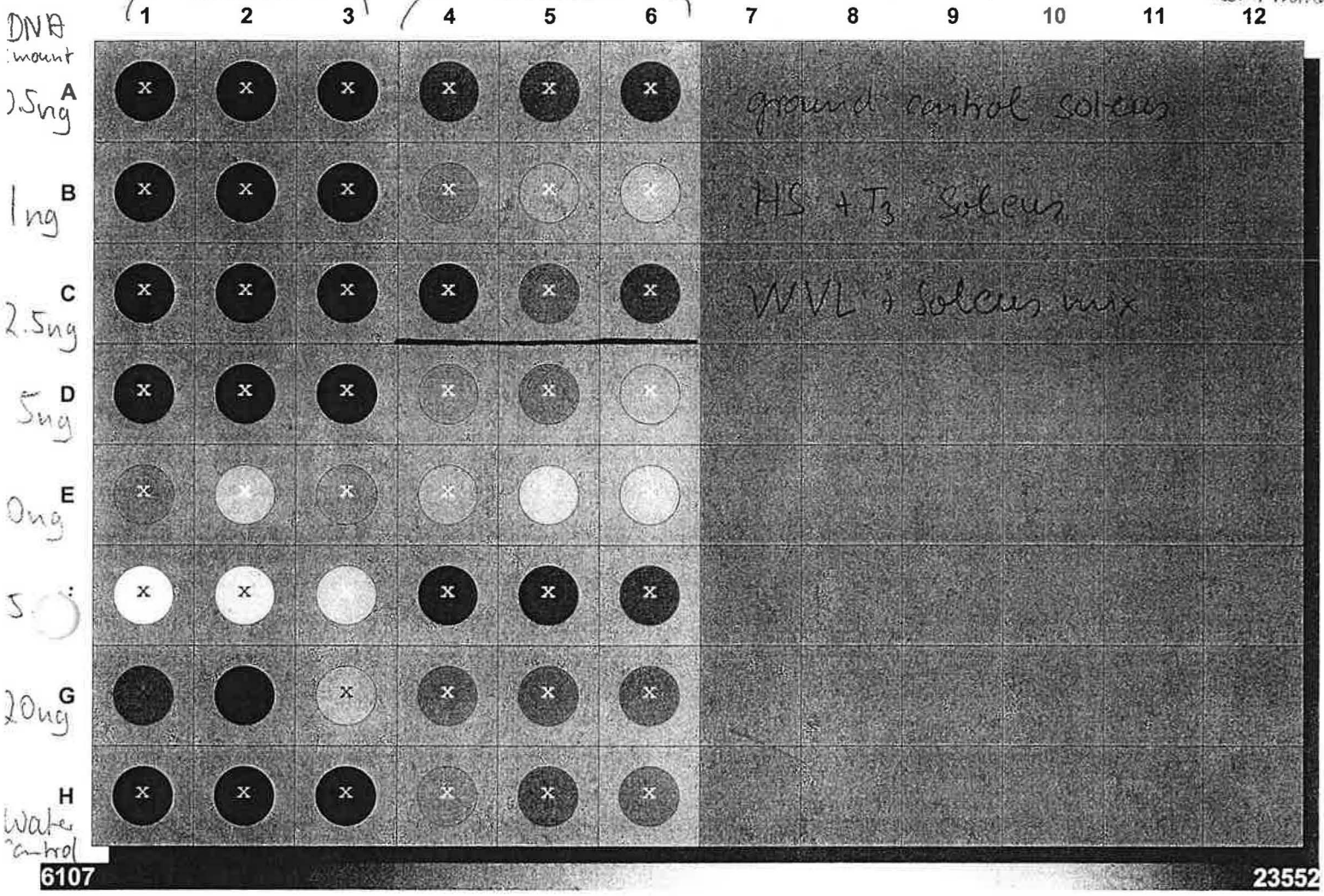
5 µl + 50µl 1x PBS buffer

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Fluorescence intensity values
F:\Carola\ChIP\Quantitative Plate Read, 08-18-2008.mxp
Run date: August 18, 2008

55µl in each well for quant real time PCR determination

Replicates: Treated individually (since no replicates in selection)

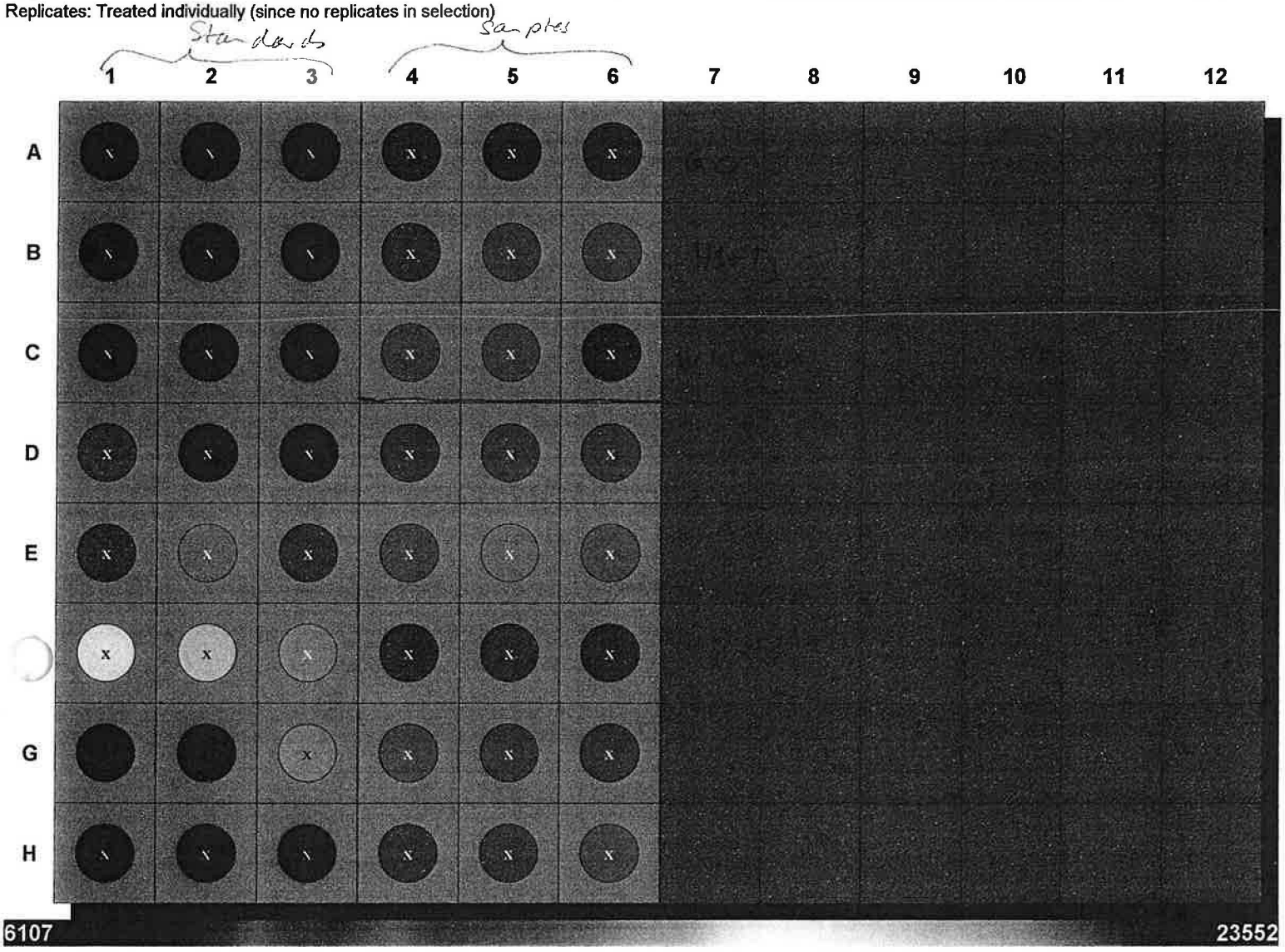
triplicate control | triplicate samples



Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Fluorescence intensity values
H:\Carola\ChIP\Quantitative Plate Read, 08-18-2008.mxp
Run date: August 18, 2008

Replicates: Treated individually (since no replicates in selection)



Wednesday Aug 20, 2008 (long day, cannot stop procedure once started)

Each tube will contain a total of 1mL
Dilute ~10 fold with ChIP dilution buffer
Do each sample/antibody reaction in a separate 1.5 ml tube. For a given sample, all antibody reactions could be precleared simultaneously in a large 15 ml tube, but I found that the beads didn't pellet well in those tubes, and thus was difficult to separate from the chromatin sample.

ul buffer	ul chromatin sheared lysate
1000 - (volume of lysate)	equiv. to ~25 ug DNA

1 ul protease inhibitor/each

always have the samples in pairs: +(+Ab), - (+ normal IgG)

Pre-clear the chromatin:
Add 50ul protein A agarose (Perice) or 30ul ProA/G (Perice)/1ml of the above dilution
Beads should be washed in ChIP dilution buffer prior to use. With pipete tip cut (to accommodate beads), add 50 ul protein A to tube, then add ~600 ul Chip dil buffer. Spin at 1200 g for 40 sec, and remove buffer. Then sample can be added to the beads.

Make sure protein A/G slurry is well mixed gently by inversion
Incubate 30min -1 hour at 4oC with rotation/gentle shaking

Spin at 1200g for 20-50 sec to pellet the protein agarose
Do not use higher speed, it crushes the beads. According to Pierce the beads can be spun at 2500 g for 2-3 min.

Collect the supernatant into 1 ml aliquots store into 1.5 ml microtube
Remove 10 ul of the supernatant and **save at 4oC as input DNA**
For input DNA we need to save 1% of the total DNA used in each IP

Chromatin Immunoprecipitation:

positive control	to each 1 ml cleared chromatin, add 1 ug anti RNA polymerase/tube
negative control "-"	to each 1 ml cleared chromatin, add 1 ug normal IgG/tube
negative control "0"	to each 1 ml cleared chromatin, add No IgG/tube

I am using three antibodies to start:
1) rabbit IgG as control
2) anti-Histone 3 acetylation antibody (H3-Ac)
3) anti Histone 3 Lysine #4 tri-methylation antibody (H3 K4 me3)

May need to vary the amount of Ab/incubation because we're not sure of the number of cells/IP
Incubate 1 hour
Add 50 ul protein A or 40ul protein A/G agarose and incubate 1-2hour at 4oC with rotation.
Two hrs seems to have worked best for me. Upstate recommends 1hr in their protocol.
This serves to collect antibody/antigen/DNA complex.

Pellet protein agarose by spinning at 1200 g for 40sec at 4oC
Discard the supernatant
Wash the protein agarose-antibody chromatin samples by resuspending the beads in 1 ml of the cold buffers in the order listed
First use 1 ml Low salt Immune complex Wash buffer wash 10sec and spin, discard supernatant, then wash as list:
For each wash, incubate for 15 minutes on a rotating platform followed by spin 1000g for 20sec at 4oc
Discard the supernatant after each wash
Wash buffer in the order to be used:

a	Once wash with Low salt Immune complex Wash buffer
b	Once wash with high salt Immune complex Wash buffer
c	Once wash with LiCl complex Wash buffer
d	twice wash with TE buffer

remove supernatant and procede to elution of the protein/DNA from the agaraose beads

Elution of Protein/DNA complexes
Bring 1M NaHCO3 solution to room temperature. Vortex at room temperature to dissolve precipitate
Set water bath to 65oC to use for reverse linking(next section)

Prepare elution buffer for all IP tubes as well as for input DNA tube (which was 1% of DNA in the IP)
Count how many tubes you have, make 0.5 extra

elution buffer	1x	6.5	
20% SDS	10	65	
1M NaHCO3	20	130	+NaCl
sterile di Water	170	1105	52
Sum	200	1300	

For input DNA, add 200ul elution buffer and set aside at room tempertaure until reverse cross-linking
Add 100ul of elution buffer to each IP tube containing the agarose pellet

Mix by flicking the tube gently
Incubate at room temperature for 15 minutes
Pellet the agarose by brief centrifugation 1200 g for 40sec at 4oC)
Collect and save the supernatant into a new microfuge
Repeat the elution with another 100ul elution buffer.
Collect the supernatant and combine with the previous step 100ul, to make a total of 200ul eluate

Reverse crosslinks of Protein/DNA complexes to free the DNA

To all the tubes (input DNA and IP), add 8ul 5M NaCl
Incubate at 65oC overnight (4-5 hours sufficient, but overnight is better)

Thursday Aug 21, 2008

To all tubes, add 1 ul RNAse A and incubate at 37oC for 10-30 minutes.
Add 13ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 1-2 hours.

digestion buffer premix:	**1x**
0.5M EDTA	4
1M Tris pH6.5	8
proteinase K	1
	13

DNA purification using spin columns

Use Qiagen miniprep columns

To each of the above tubes (input DNA and IP DNA) add 1 ml binding reagent"A"
Mix well.
A precipitate might form, this will not interfere with the procedure
Transfer 600ul of sample/Bind reagent A mixture to the column
Spin 30 sec at 12,000 g
Discard flow through
Add the remaining 600 ul to the column
Spin again and discard flow through
Add 750ul wash buffer of Qiagen to the column
Spin at 12,000 g
Discard flow through
Spin again for 12,000 g for 2min.
Discard the collection tube
Put the column in a new 1.5 ml collection tube
Add 135 ul water to elute the DNA off the column.
Spin at 12,000g for 2 minute (be careful to not zip the lids off the tubes.)

Eluate is pure DNA that can be analyzed by PCR
Use 12 µl of this DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples.
General set-up for PCR reactions

PCR Reactions	**1x**
Water	6.1
10x PCR Buffer	2.5
10 mM dNTP	0.5
50 mM MgCl2	0.75
5+3' primer mix at 5 pmol/ul	3
Template (IP DNA/input DNA)	**12**
Biolase DNA Polymeras	0.15
SUM	25

Friday Aug 22, 2008

Set up PCR reactions to test the eluted DNA.
I have 12 different samples total:

3 different muscle types X 3 different antibodies:
Ground control Soleus
T3 and HS treated Soleus muscle
WVL and control Soleus mix

Tested with
Rabbit IgG antibody
Anti acetylation antibody
Anti tri-methylation antibody

Plus 3 input DNA samples (see above).

The 12 samples will be tested by PCR for type I and type IIb MHC.

PCR protocol: see above
Use 12 µl of eluted DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples.

30/33 cycles of

60 sec. 96 oC
45 sec. 57 oC
45 sec. of 72 oC

(Program # 33)
Use 2 different PCR machines:
33 cycles for trimethylation samples and 30 cycles for all other samples

Monday Aug 25, 2008

Run PCR products on 2% Agarose gel

Results on next pages

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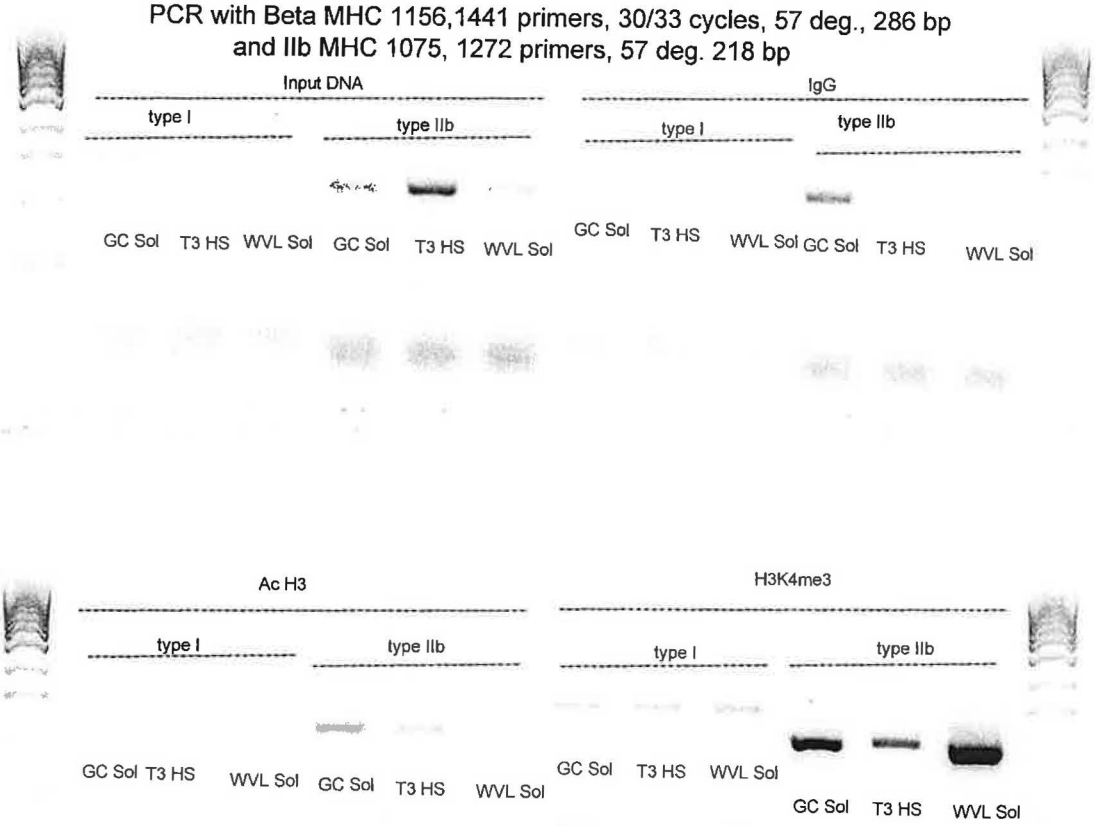

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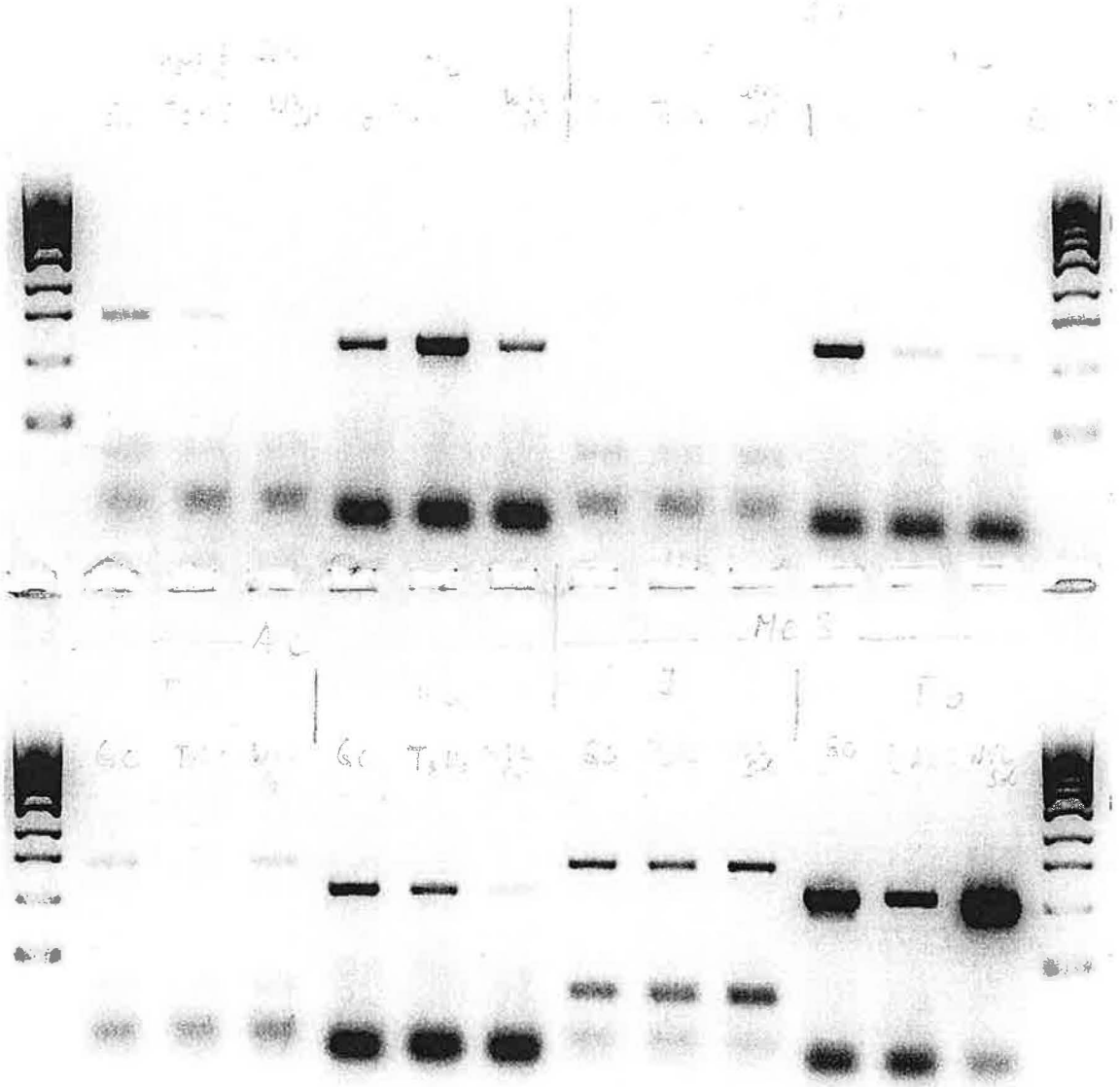

Tuesday Aug 26, 2008

More PCR reactions with ChIP samples.

I have 12 samples: three different tissues (GC Sol, T3/HS Sol and Sol/WVL mix) processed with 3 different antibodies and the 3 input DNA samples.

Set up PCR for all 12 samples with three different PCR primer sets:

1) Beta MHC / Type 1 primer
2) IIb MHC primer
3) Beta actin primer

Total of 36 samples.
Each tube has a total of 25 µl plus mineral oil on top.
PCR mix is

PCR Reactions	**1x**
Water	6.1
10x PCR Buffer	2.5
10 mM dNTP	0.5
50 mM MgCl2	0.75
5+3' primer mix at 5 pmol/ul	3
Template (IP DNA/input DNA)	**12**
Biolase DNA Polymeras	0.15
SUM	25

Use 12 µl of eluted DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples (use 5µ less water per reaction for those samples).

30/33 cycles of

60 sec. 96 oC
45 sec. 57 oC
45 sec. of 72 oC

(Program # 33)
Use 2 different PCR machines:
33 cycles for trimethylation samples and 30 cycles for all other samples.

Wednesday Aug 27, 2008

Run PCR products on 2% Agarose gel

Results on next pages

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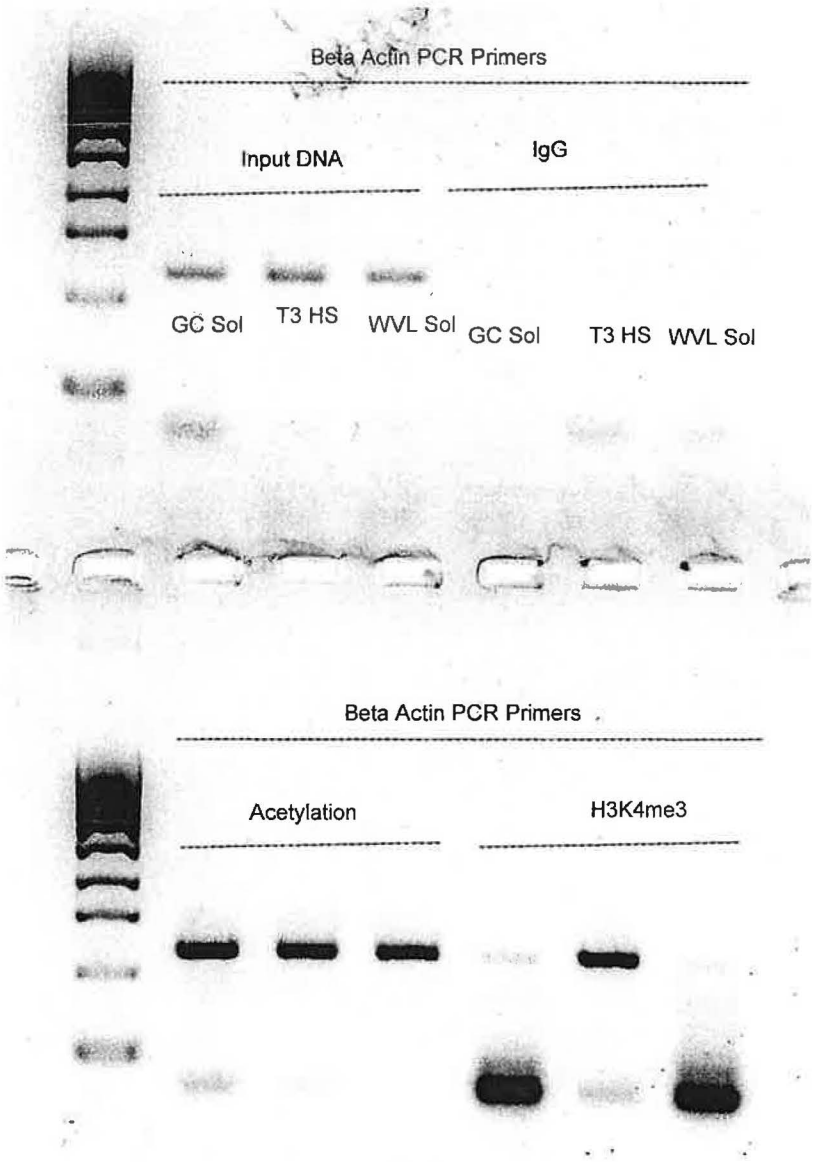
Date/Time:



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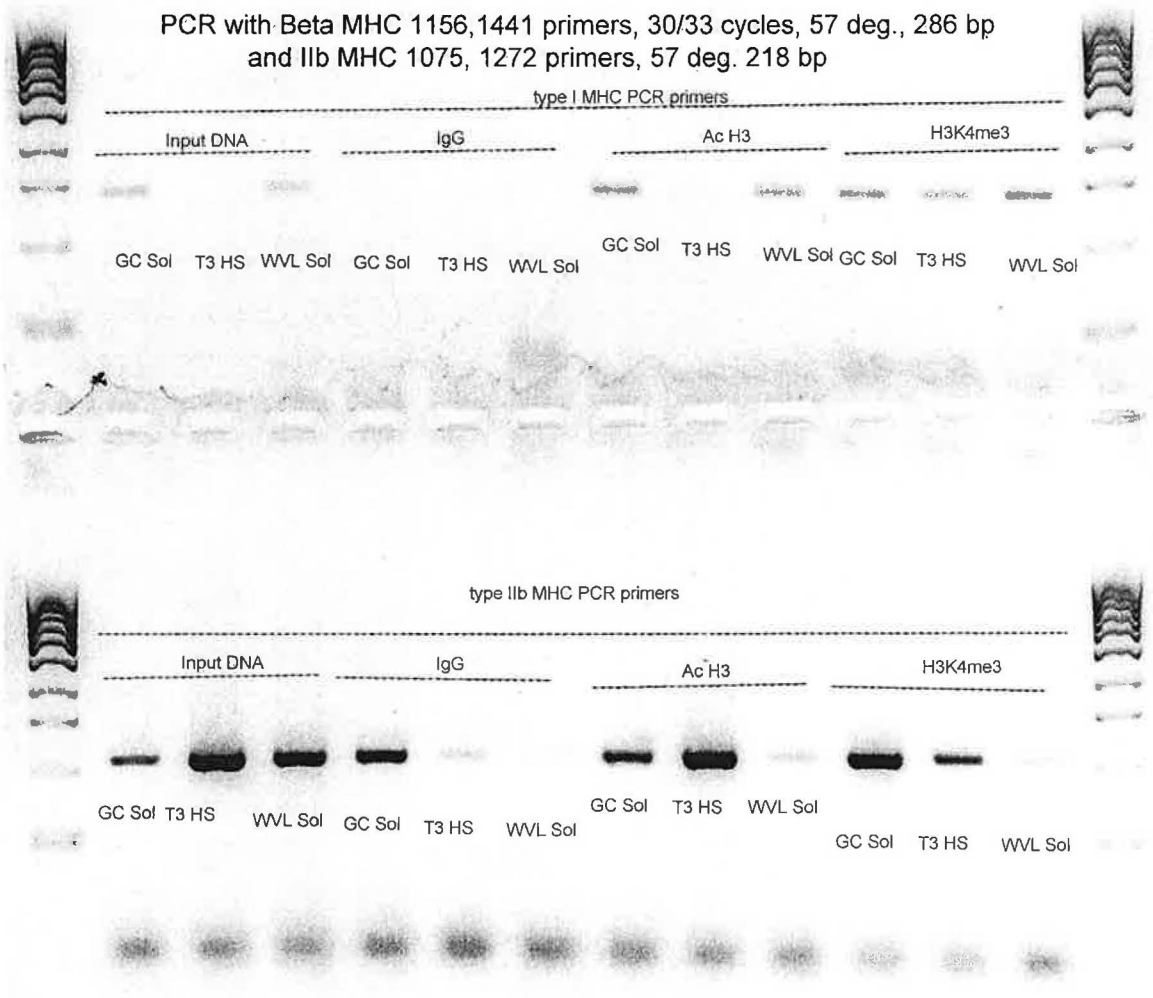

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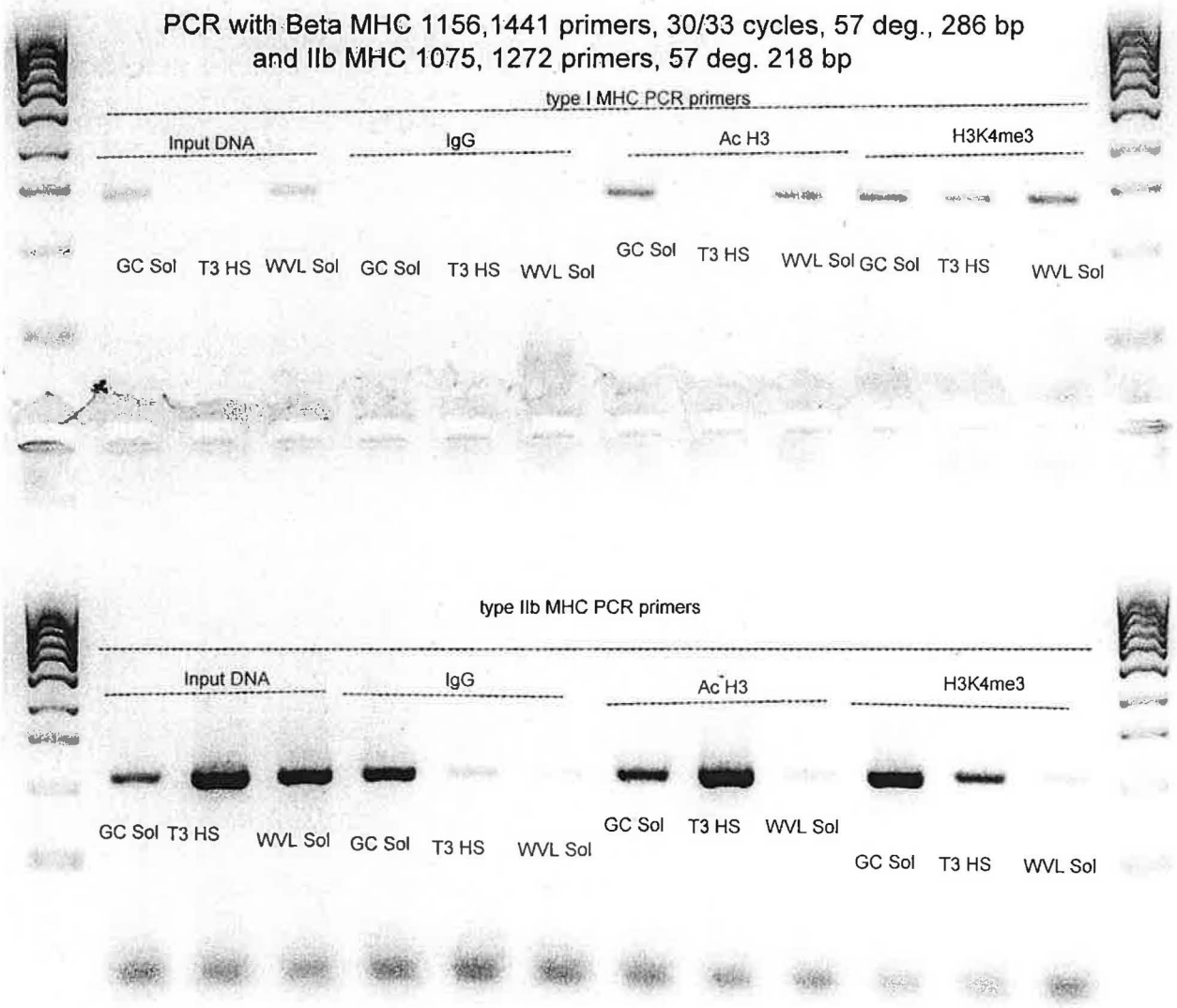

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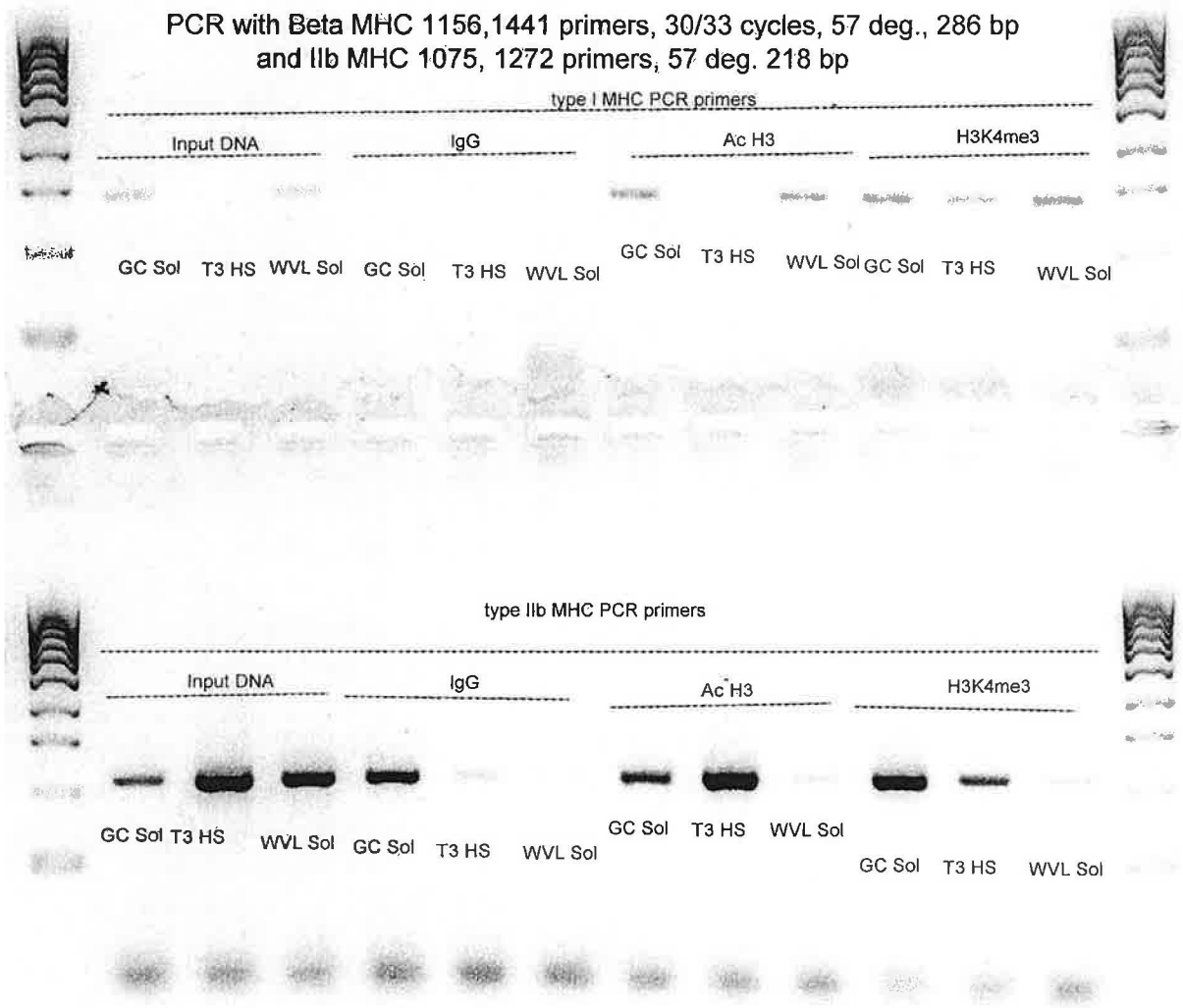

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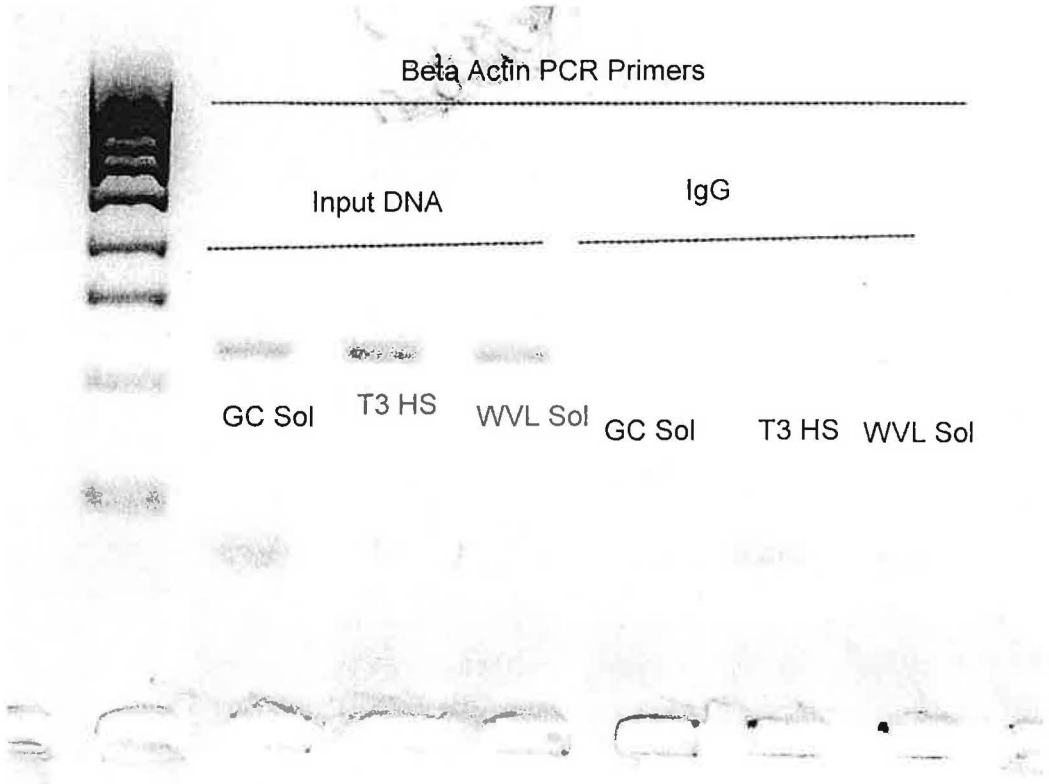
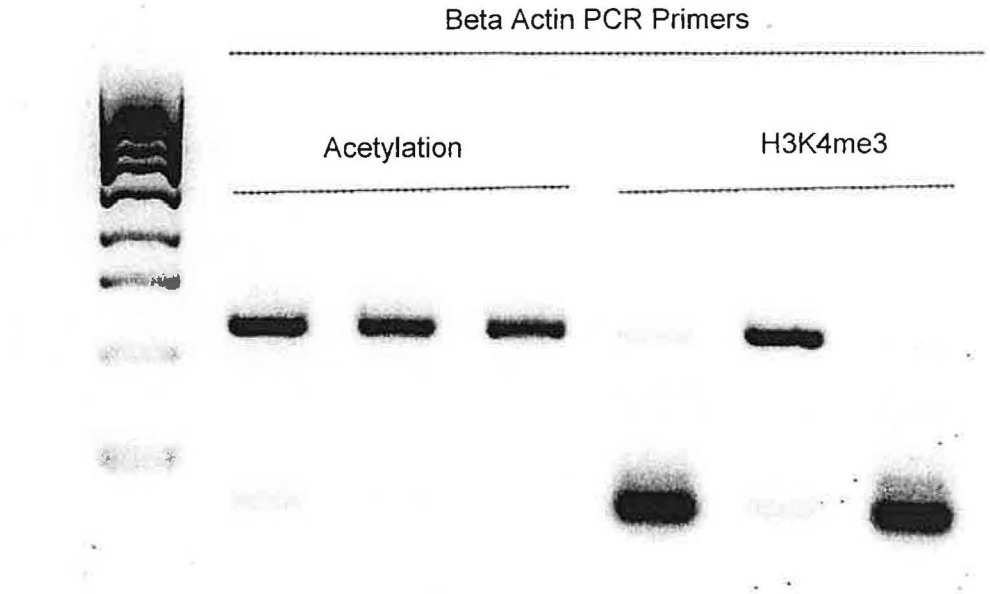
β-Actin primers +15/+241
61°C





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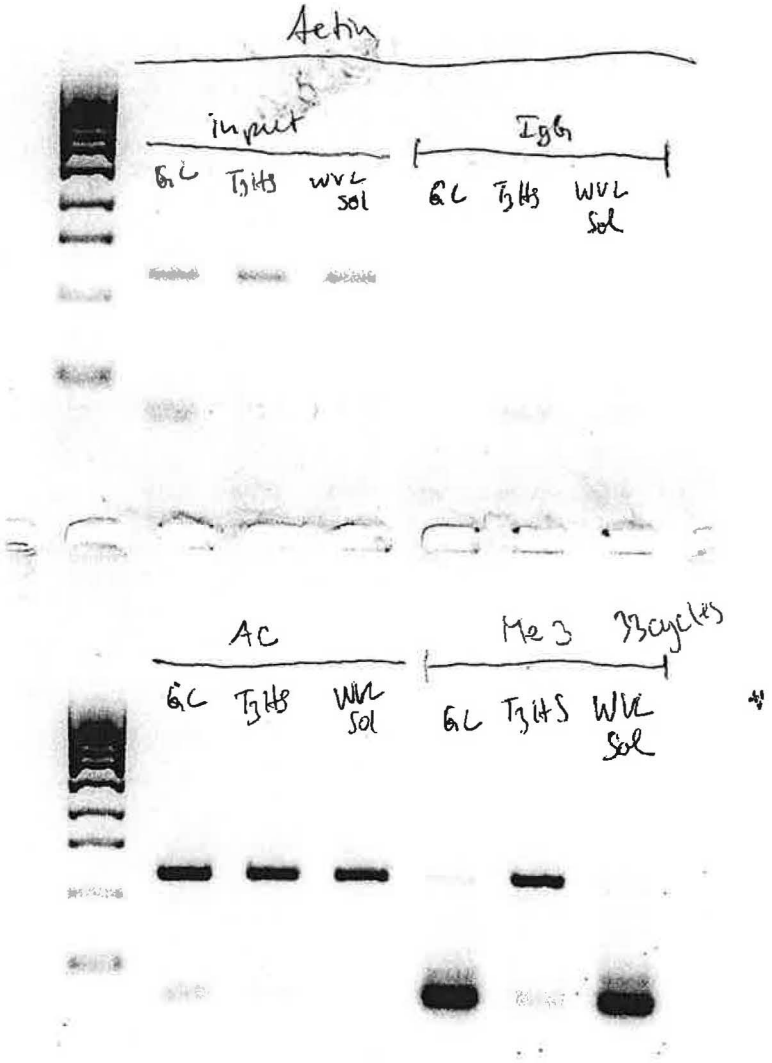

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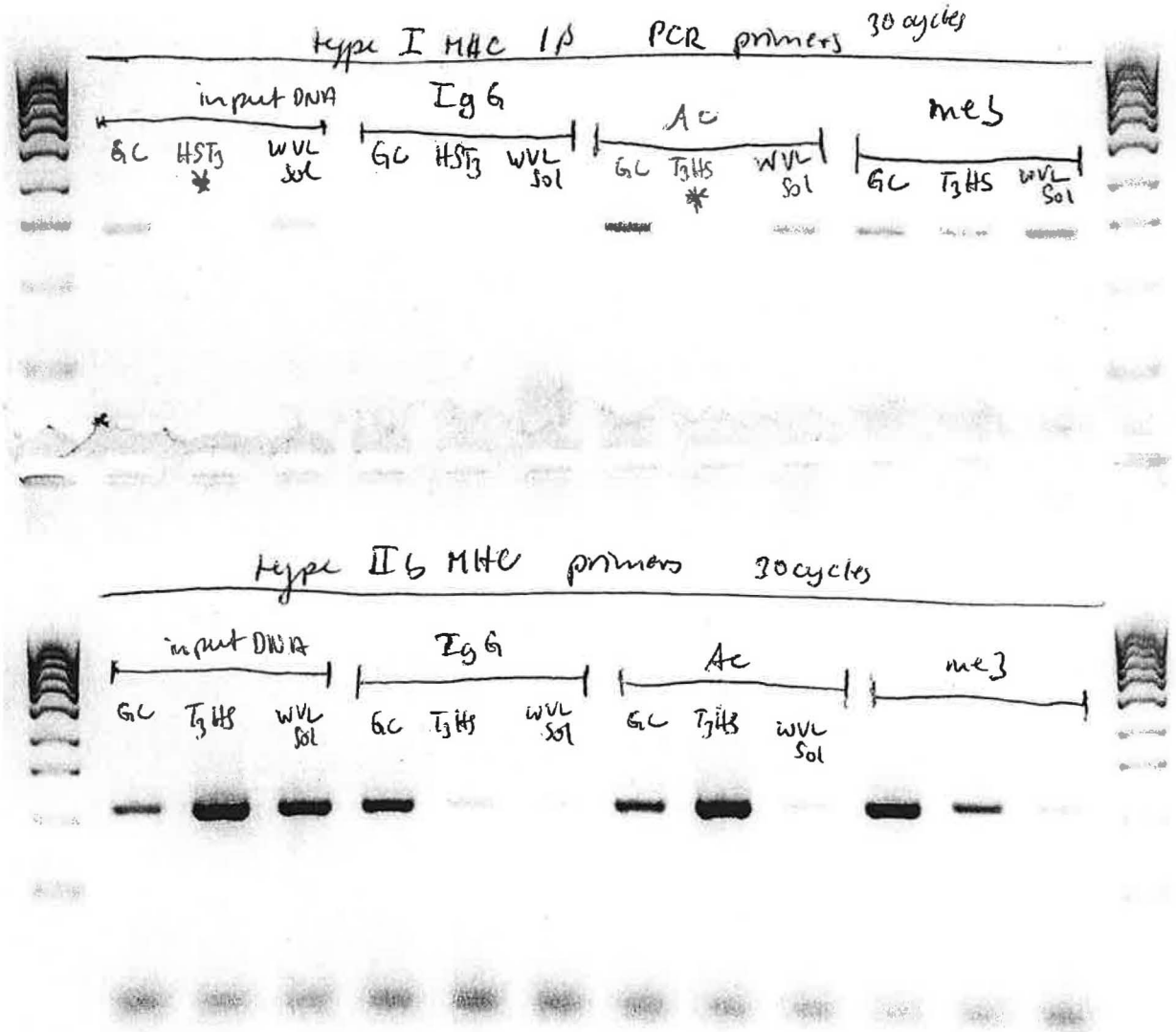
p no oil



me3
33 cycles

Ary IIx

Thursday Aug 28, 2008

More PCR reactions with ChIP samples.
The purpose is to verify the quality of the ChIP samples and the eluted DNA.

Set up PCR for all 12 samples with two more PCR primer sets:

1) IIx MHC primer
2) RPL28 primer

PCR reaction mix is the same as Aug 26, 2008

Use 12 µl of eluted DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples (use 5µ less water per reaction for those samples).

Fore IIx primer set use:

30/33 cycles of

60 sec. 96 oC
45 sec. 57 oC
45 sec. of 72 oC

(Program # 33)

The RPL28 primer requires 55oC annealing temperature

30/33 cycles of

60 sec. 96 oC
45 sec. 55 oC
45 sec. of 72 oC

Friday Aug 29, 2008

Run PCR products on 2% Agarose gel

Results on next pages.

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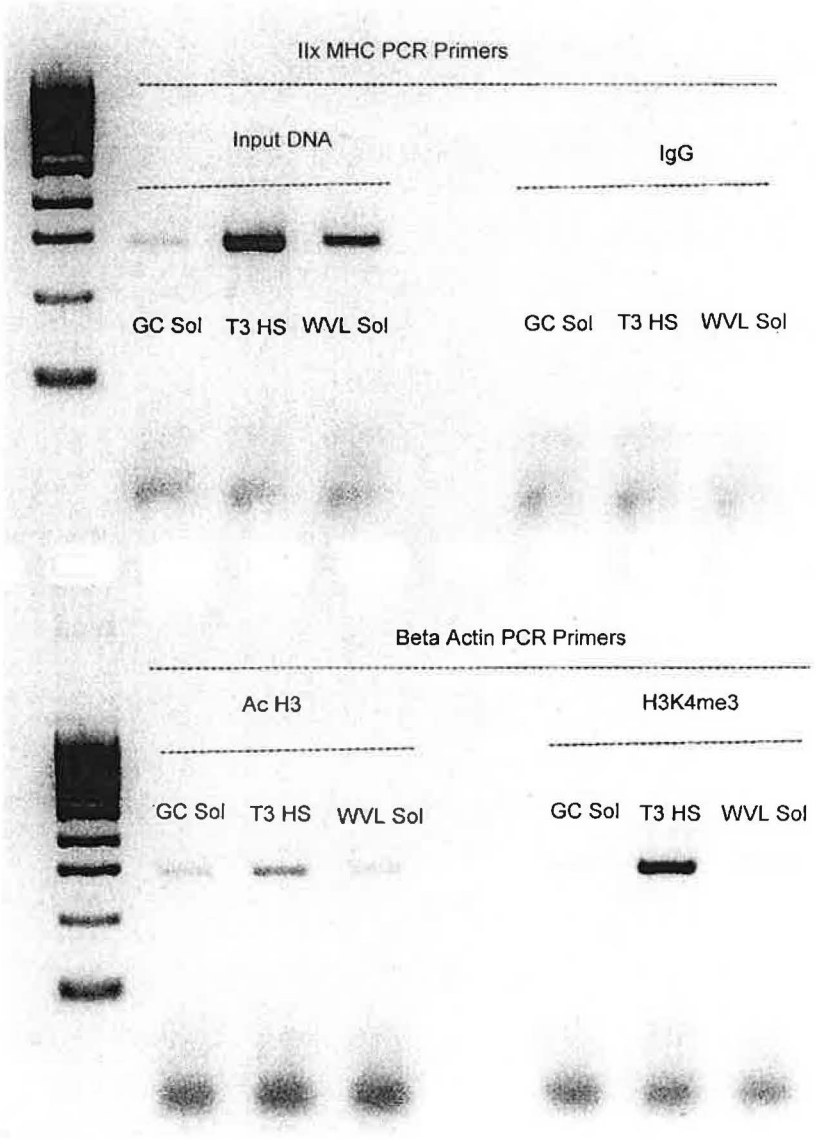

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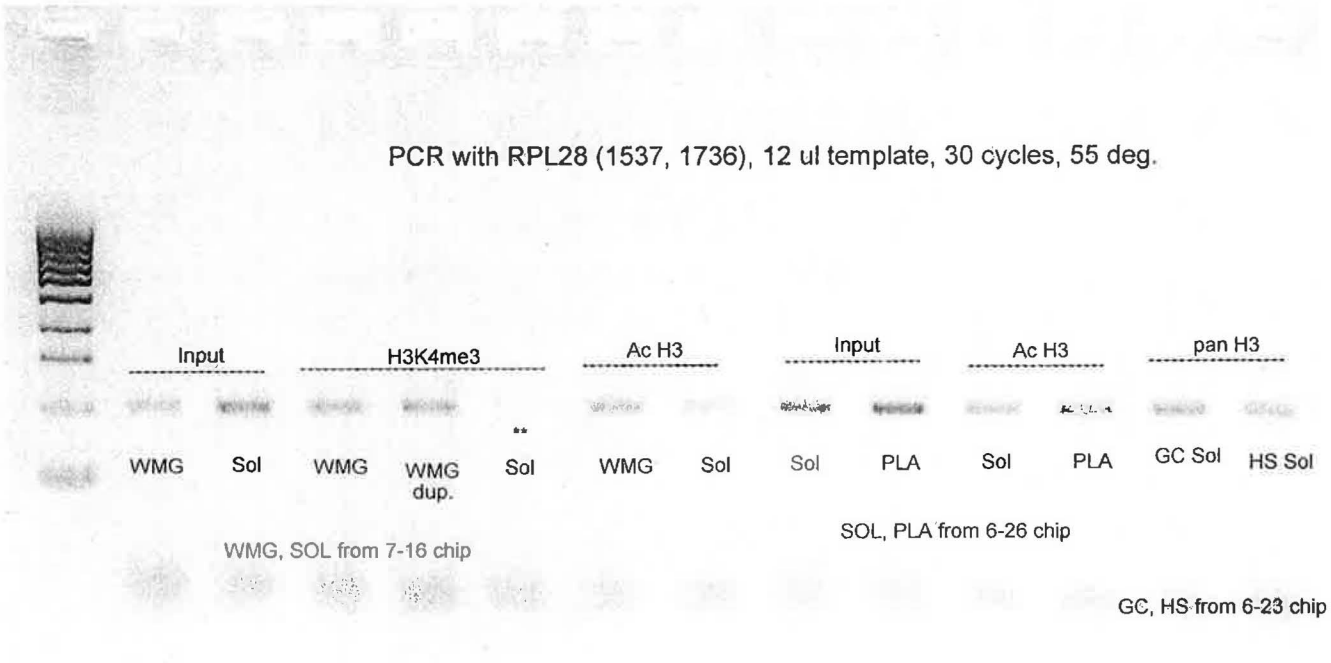

** This probably didn't work due to only 30 cycles, 12 ul (vs. 33, and 17)

Monday Sept. 1, 2008

Labor Day Holiday

Tuesday Sept. 2, 2008

Discuss results of previous week with Clay.
The beta actin results are positive except that the primer tends to form primer dimers during the PCR reaction, especially with the tri-methylation precipitated DNA (33 cycles in the PCR reaction protocol). The results with the IIX primers are also in line with the expected. However, the results with the type I and IIb primers are inconclusive and may be due to tissue/essay processing issues or due to primer issues.

Plan strategy on how to proceed.
Look at the beta actin genomic sequence for design of a new primer pair with less likelihood of forming primer dimers.

It seems to be a good idea to use the same tissue (NC Soleus, T3/HS Soleus and WVL/NC Soleus mix) and repeat the Immuno-precipitation procedure.

Wednesday Sept. 3, 2008

Read and prepare for next round of ChIP essay.

Thursday Sept. 4, 2008

Prepare solutions, antibody, materials etc. for ChIP assay tomorrow.

Friday Sept. 5, 2008

Use homogenized and sonicated tissue from 8-13 for a new ChIP procedure.

Wash 9 x 50µl protein A agarose (Perice) in 9 separate1.5 ml tubes with 500 µl ChIP dilution buffer.
Spin at 1200g for 30 sec to pellet the agarose. Remove and discard supernatant.
Add chromatin and dilution buffer based on measurements made with processed tissue samples on Aug. 18.

This tissue preped on 8-13-08

	Sample	muscle frozen weight	in mg
400 ul lysis buffer		GC Sol	50
80 % amplitude		HS + T3 Sol	50
15 sec pulse		WVL + Sol	32, sol + 25 WVL
10 pulses			

Use 25µg of DNA for this ChIP

	ng/µl of original chromatin sample	for 25 µg	
		µl of chromatin	µl buffer to yield 1ml
GC Sol	538.1	46.5	954
HS Sol + T3	891.6	28.0	972
WVL + Sol	547.0	45.7	954

Prepare three tubes for each muscle. Add the above amounts of chromatin solution and ChIP buffer to the washed agarose beads. Preclear chromatin for 45 min at 4 oC on rotator.
Spin at 1200g for 30 sec to pellet the agarose.
Collect the 1 ml supernatants into fresh 1.5 ml microtubes.
Remove 10 µl of the supernatant and **save at 4oC as input DNA** (one input DNA sample per muscle). For input DNA we need to save 1% of the total DNA used in each IP.

Add the following antibodies to the pre-cleared chromatin samples

Normal Rabbit IgG from Upstate (12-370) used 1.0 µl (1.0 µg).

Anti-Histone H3 K4 me3 antibody (Upstate #07-473), use 1.0 µl.

Anti-Acetyl Histone H3 antibody (Upstate #06-599), used 1.0 ul (~1 ug).

Incubate 1 hr with antibodies. Tubes are in refrigerator on rotator.

Wash another 9 x 50µl agarose beads with ChIP dilution buffer. Spin and discard supernatant. After the 1 hour incubation add the chromatin antibody mixtures to the washed beads and incubate for another 2 hours in the refrigerator on rotator.

Pellet protein agarose by spinning at 1200 g for 40sec at 4oC.Discard the supernatant.
Wash the protein agarose-antibody chromatin samples by resuspending the beads in 1 ml of the cold buffers in the order listed
First use 1 ml Low salt Immune complex Wash buffer wash 10sec and spin, discard supernatant, then wash as list:
For each wash, incubate for 15 minutes on a rotating platform followed by spin 1000g for 20sec at 4oc
Discard the supernatant after each wash
Wash buffer in the order to be used:

a	Once wash with Low salt Immune complex Wash buffer
b	Once wash with high salt Immune complex Wash buffer
c	Once wash with LiCl complex Wash buffer
d	twice wash with TE buffer

remove supernatant and procede to elution of the protein/DNA from the agaraose beads

Elution of Protein/DNA complexes

Bring 1M NaHCO3 solution to room temperature. Vortex at room temperature to dissolve precipitate

Set water bath to 65oC to use for reverse linking(next section)

Prepare elution buffer for all IP tubes as well as for input DNA tube (which was 1% of DNA in the IP)

Count how many tubes you have, make 0.5 extra

elution buffer	1x	6.5	
20% SDS	10	65	
1M NaHCO3	20	130	+NaCl
sterile di Water	170	1105	52
Sum	200	1300	

For input DNA, add 200ul elution buffer and set aside at room tempertaure until reverse cross-linking

Add 100ul of elution buffer to each IP tube containing the agarose pellet

Mix by flicking the tube gently
Incubate at room temperature for 15 minutes
Pellet the agarose by brief centrifugation 1200 g for 40sec at 4oC)
Collect and save the supernatant into a new microfuge
Repeat the elution with another 100ul elution buffer.
Collect the supernatant and combine with the previous step 100ul, to make a total of 200ul eluate

Reverse crosslinks of Protein/DNA complexes to free the DNA

To all the tubes (input DNA and IP), add 8ul 5M NaCl
Incubate at 65oC overnight.

Saturday Sept. 6, 2008

Take samples out of waterbath and freeze at -80 oC.

Monday Sept. 8, 2008

RNA and Protein digestion:
To all tubes, add 1 ul RNAse A and incubate at 37oC for 30 minutes.
Add 13ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 2 hours.

digestion buffer premix:	**1x**
0.5M EDTA	4
1M Tris pH6.5	8
proteinase K	1
	13

DNA purification using spin columns

Use Qiagen miniprep columns

To each of the above tubes (input DNA and IP DNA) add 1 ml binding reagent"A"
Mix well.
A precipitate might form, this will not interfere with the procedure
Transfer 600ul of sample/Bind reagent A mixture to the column
Spin 30 sec at 12,000 g
Discard flow through
Add the remaining 600 ul to the column
Spin again and discard flow through
Add 750ul wash buffer of Qiagen to the column
Spin at 12,000 g
Discard flow through
Spin again for 12,000 g for 2min.
Discard the collection tube
Put the column in a new 1.5 ml collection tube
Add 135 ul warm water to elute the DNA off the column.
Spin at 12,000g for 2 minute (be careful to not zip the lids off the tubes.)

Eluate is pure DNA that can be analyzed by PCR
Use 12 µl of this DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples.

Freeze samples at -20 oC.

Tuesday Sept. 9, 2008

Set up PCR reactions with the new samples.
Use the beta MHC +1156 / +1441 primer set at 57 oC annealing and
The IIb 1075 F / 1272 R primer set also at 57oC annealing.

Total of 24 samples.
Each tube has a total of 25 µl plus mineral oil on top.

PCR mix is

PCR Reactions	1x
Water	6.1
10x PCR Buffer	2.5
10 mM dNTP	0.5
50 mM MgCl2	0.75
5+3' primer mix at 5 pmol/ul	3
Template (IP DNA/input DNA)	**12**
Biolase DNA Polymeras	0.15
SUM	25

Use 12 µl of eluted DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples (use 5µl less water per reaction for those samples).

30/33 cycles of

60 sec. 96 oC
45 sec. 57 oC
45 sec. of 72 oC

(Program # 33)
Use 2 different PCR machines:
33 cycles for trimethylation samples and 30 cycles for all other samples.

Wednesday Sept. 10, 2008

Run PCR products on 2% agarose gel.
Results on next page.

Set up more PCR reactions:

Use the beta Actin +1517 F / 1741 R primer set at 56 oC annealing and
The IIx 5'end I 2 pre F + R primer set also at 56oC annealing.

Total of 24 samples.
Each tube has a total of 25 µl plus mineral oil on top.
Same PCR mix as yesterday (see above).

Use 12 µl of eluted DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples (use 5µl less water per reaction for those samples).

30/33 cycles of
60 sec. 96 oC, 45 sec. 56 oC, 45 sec. of 72 oC (Program # 33)
Use 2 different PCR machines: 33 cycles for trimethylation samples and 30 cycles for all other samples.

Thursday Sept. 11, 2008

Run Wednesday's PCR products on 2% agarose gel.
Results and gel analysis on next page.

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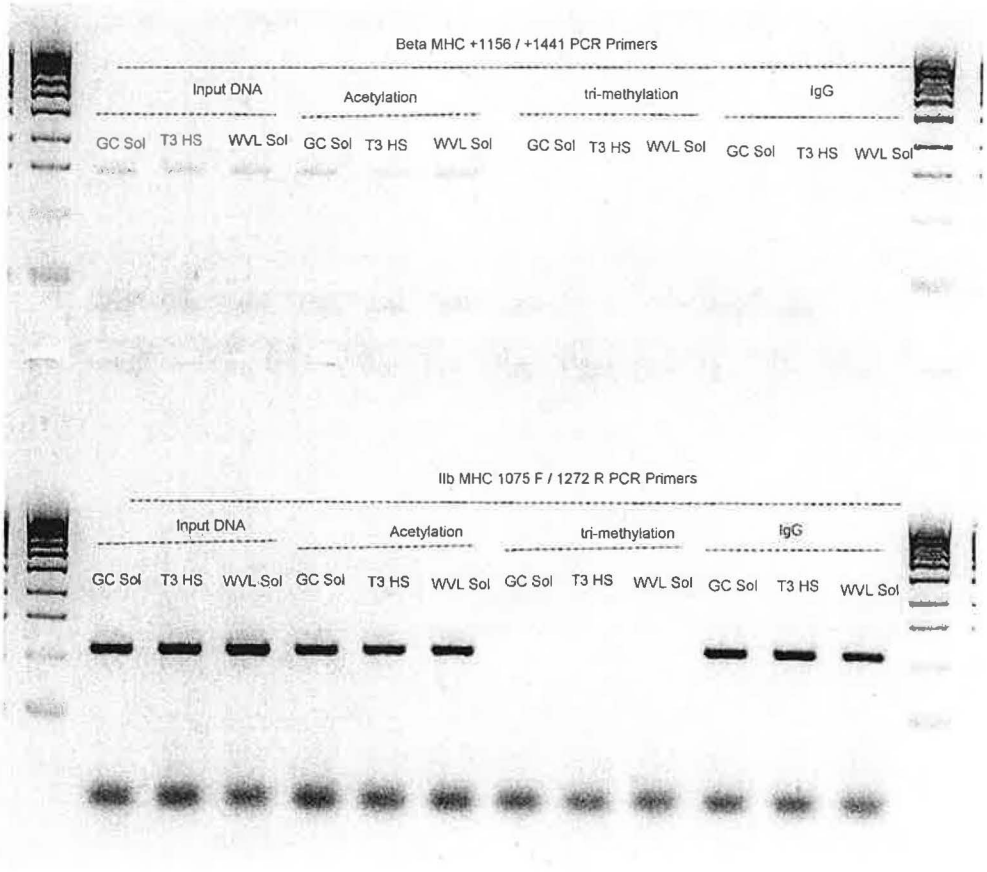

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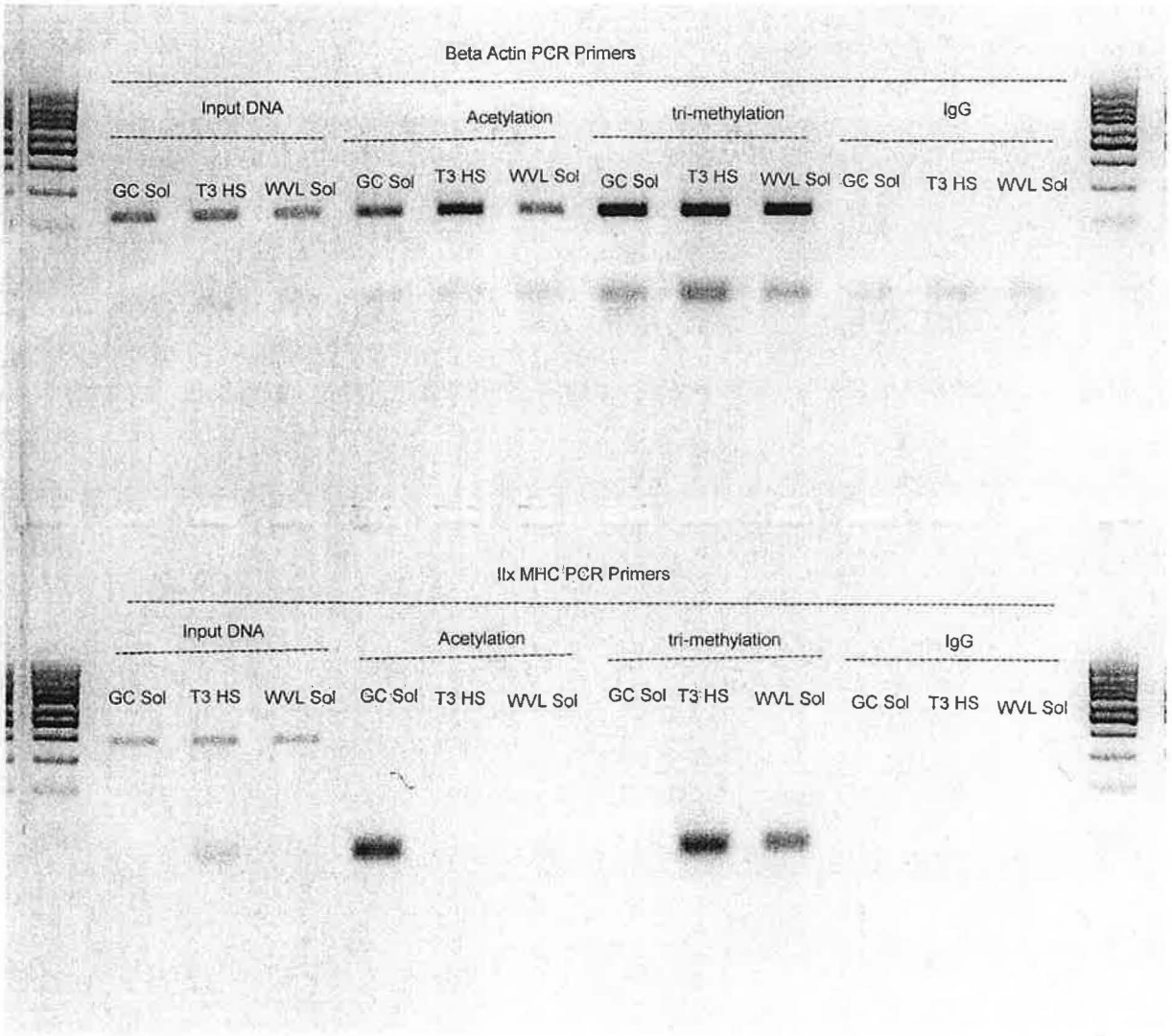


Friday Sept. 12, 2008 to Friday Sept. 19, 2008

Animal experiments to generate tissue for asnalysis.

Two groups of rats: Normal Control and Hindlimb suspension + Thyroid hormone treatment. Each group has 9 Sprague Dawley rats.

Animal Procedures.

Young adult female Sprague Dawley rats (Taconic farms, Germantown, NY) of ~ 150 grams body weight were used. Animals were assigned to two groups, a ground control (GC), and a 7d hind limb suspension and thyroid hormone treatment (HS+T3). The hind limb suspension (HS) model involved a tail traction method using a noninvasive casting procedure (see pictures of procedure). The base portion of rats' tails are prepared with a cast composed of SkinTrac skin traction strips and Tensoplast elastic adhesive bandages. The distal portion of the casting materials utilized a swivel harness system which is attached to a hook on the top of the cage. The hook is adjusted to allow only the front legs of the animal to reach the floor. Animals were anesthetized with ketamine: acepromazine: xylazine (50:1:4 mg/kg) for casting procedure. Thyroid hormone, treatment was given as an intraperitoneal injection of triiodothryonine (T3) at a dosage of 150μg/kg bw. T3 treatment was started on the second day after suspension, and was continued once daily. On day 7, the animals were euthanized approximately 6 hours after the T3 injection. The animals were euthanized with an overdose of sodium pentobarbital (100 mg/kg I.P.). This dose rapidly sedates the animal to a deep unconscious state. After the animal is unconscious, a pneumothorax is performed to induce cardiac arrest followed by skeletal muscle tissue removal. For the animals in the HS+T3 group, animals were sedated at the termination of the suspension, and did not exert any weight-bearing on their hindlimb before the muscles were taken out. These animal procedures were approved by the University of California Irvine Animal Care and Use Committee and are according to the guideline of the American Physilogical Society.

Monday Sept. 22, 2008

Order more rats for another set of experiments.

Thursday Oct. 2 to Thursday Oct. 9, 2008

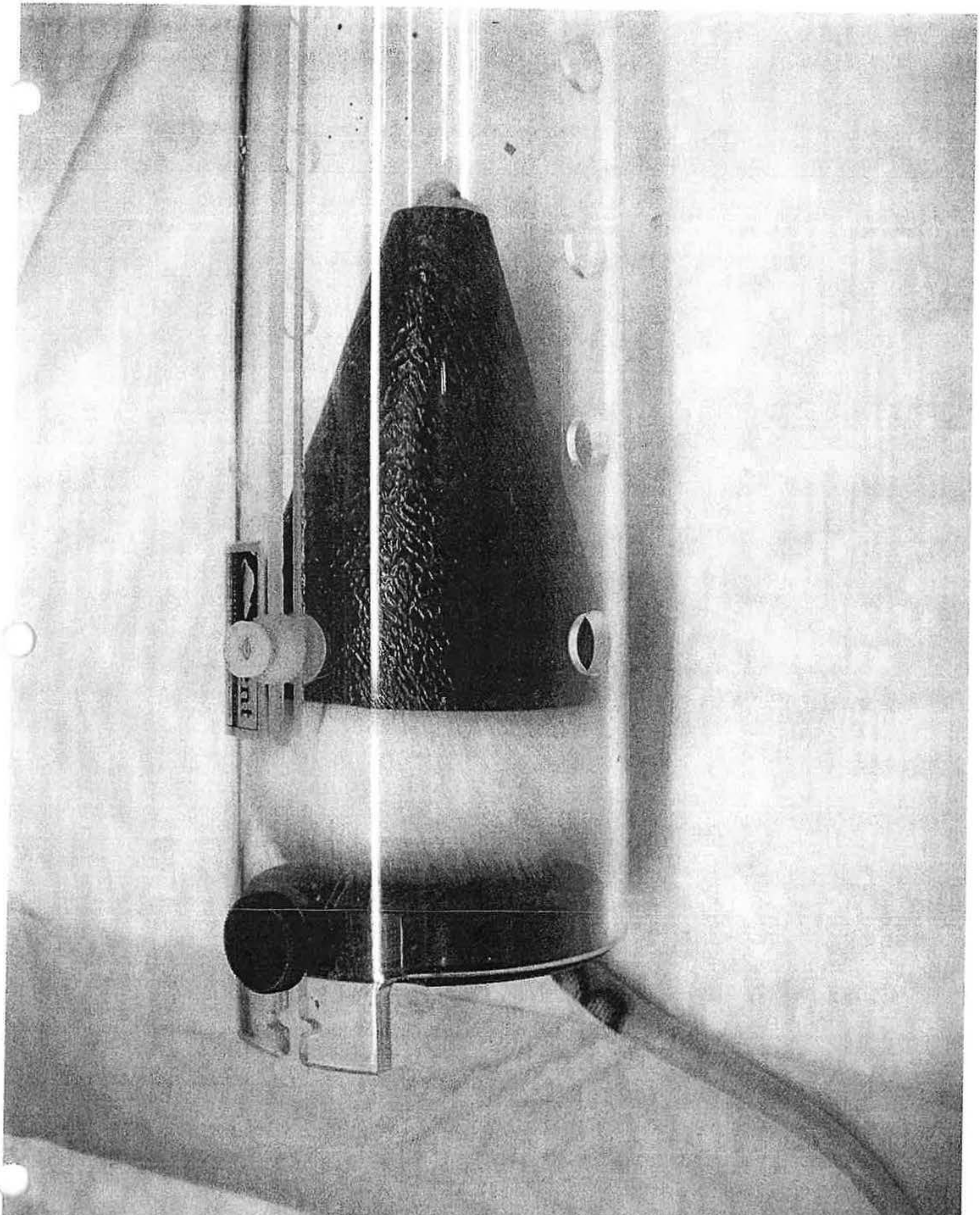
Perform another 7 day hind limb suspension / T3 procedure with 8 animals in the control and 8 animals in the experimental group.
Animal protocol and procedures are the same as above.

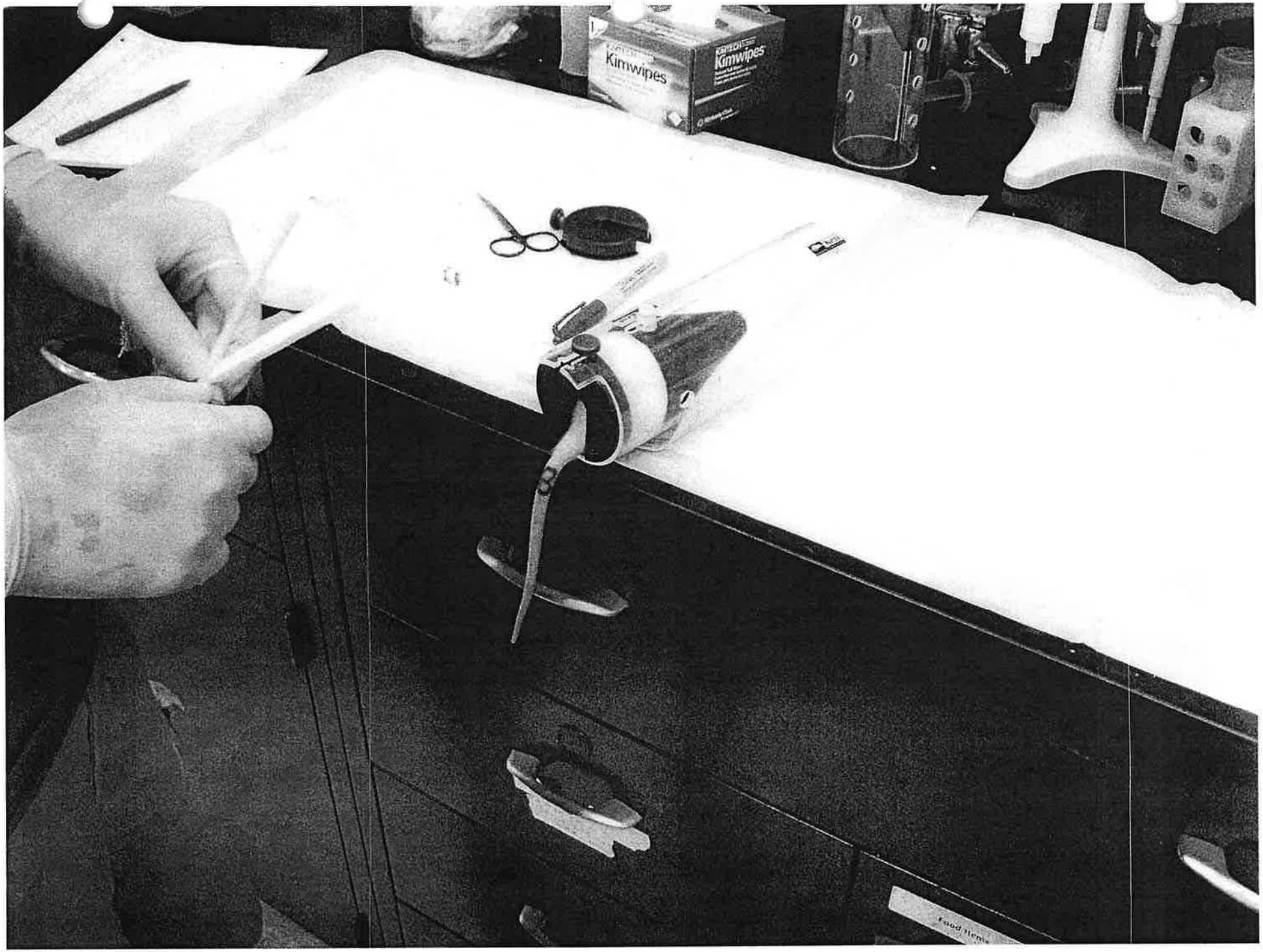
Animals euthanized on October 9. Muscles are taken from all animals, weighed and frozen at -80oC. The following muscles were taken:
Soleus, left and right leg
Plantaris, left and right leg
Medial Gastrocnemius, left and right leg
Vastus Intermedius, left and right leg.

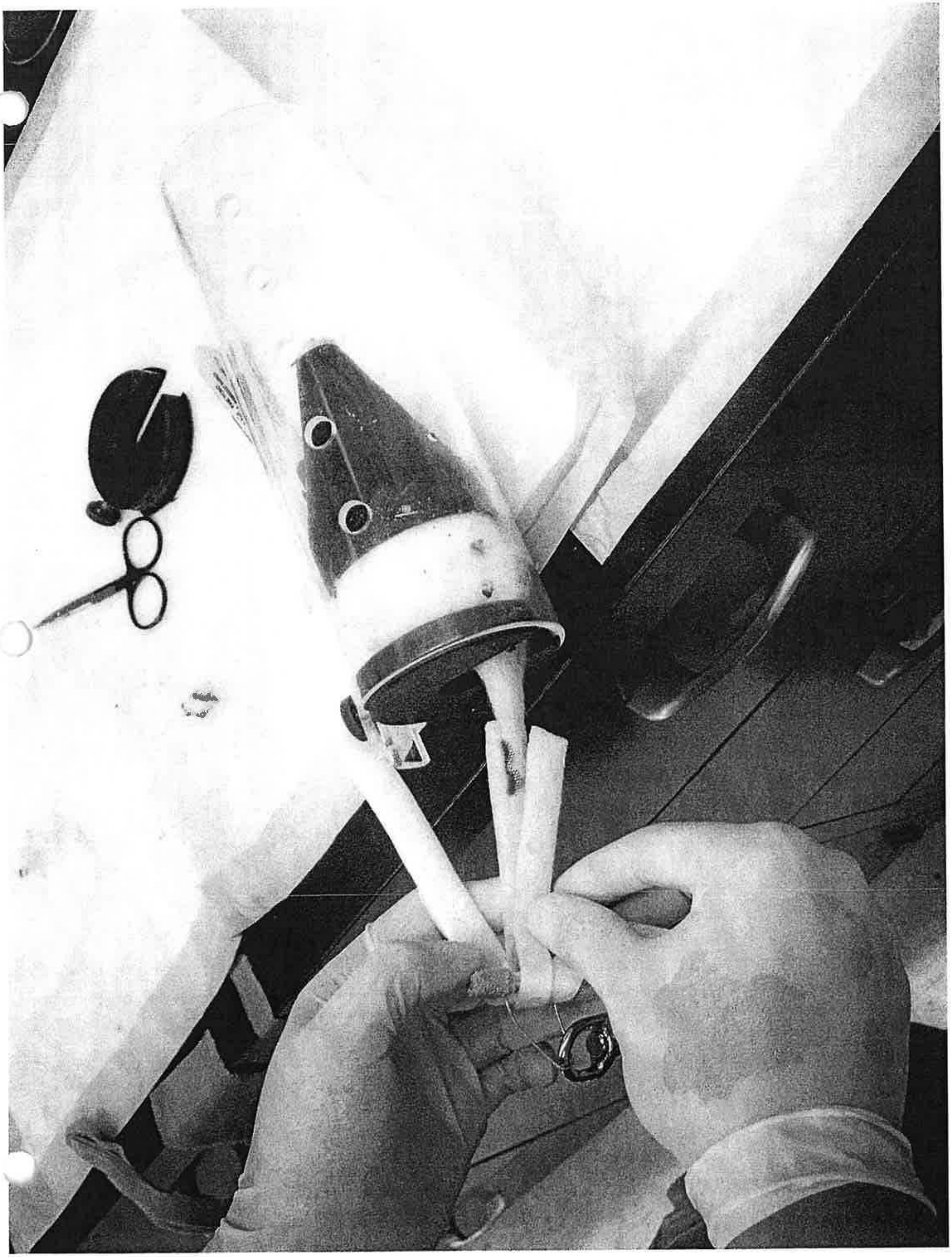
Friday Oct. 10, 2008

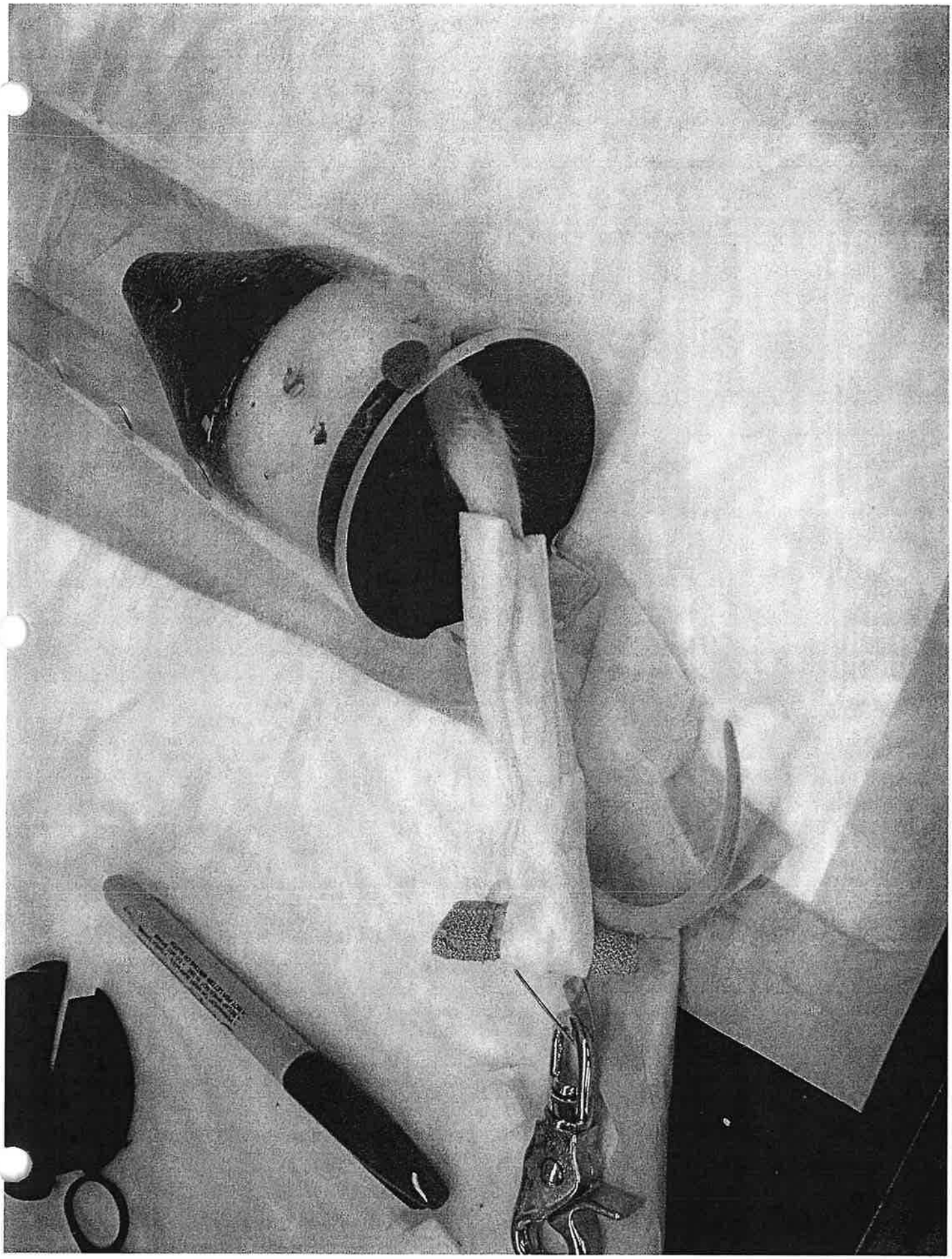
Organize tissues, data input and record taking

Rat Pictures




Kimwipes
Kimwipes



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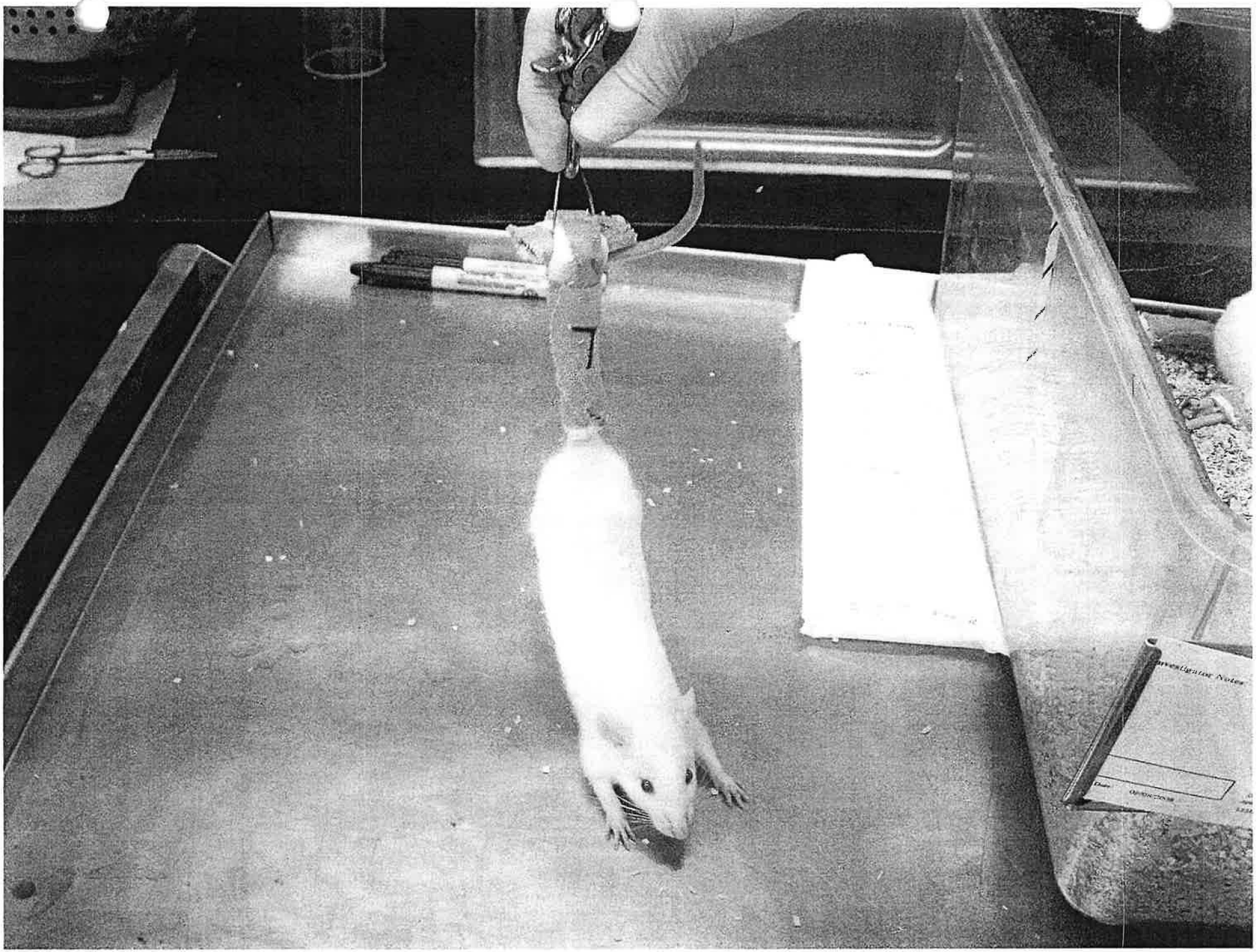

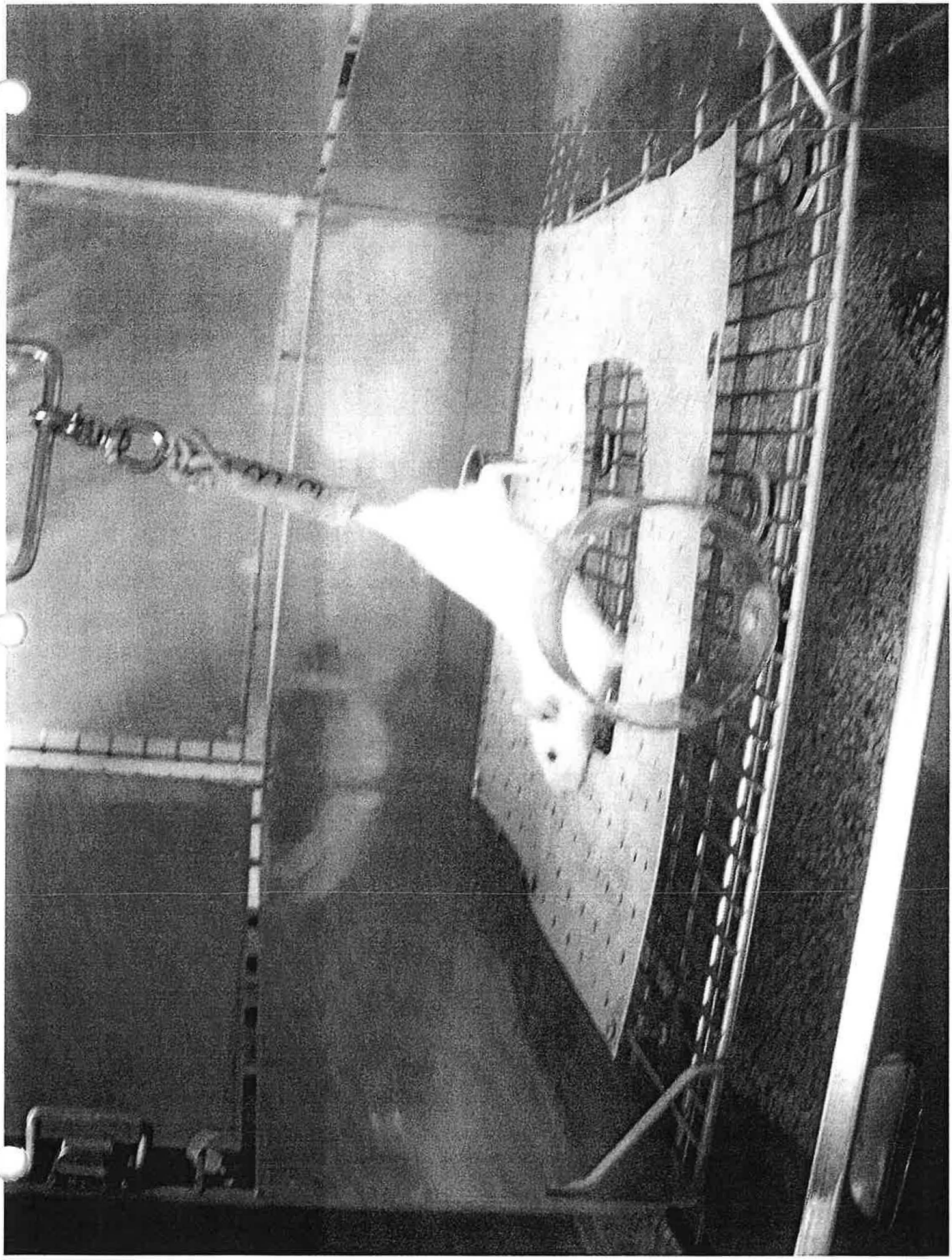

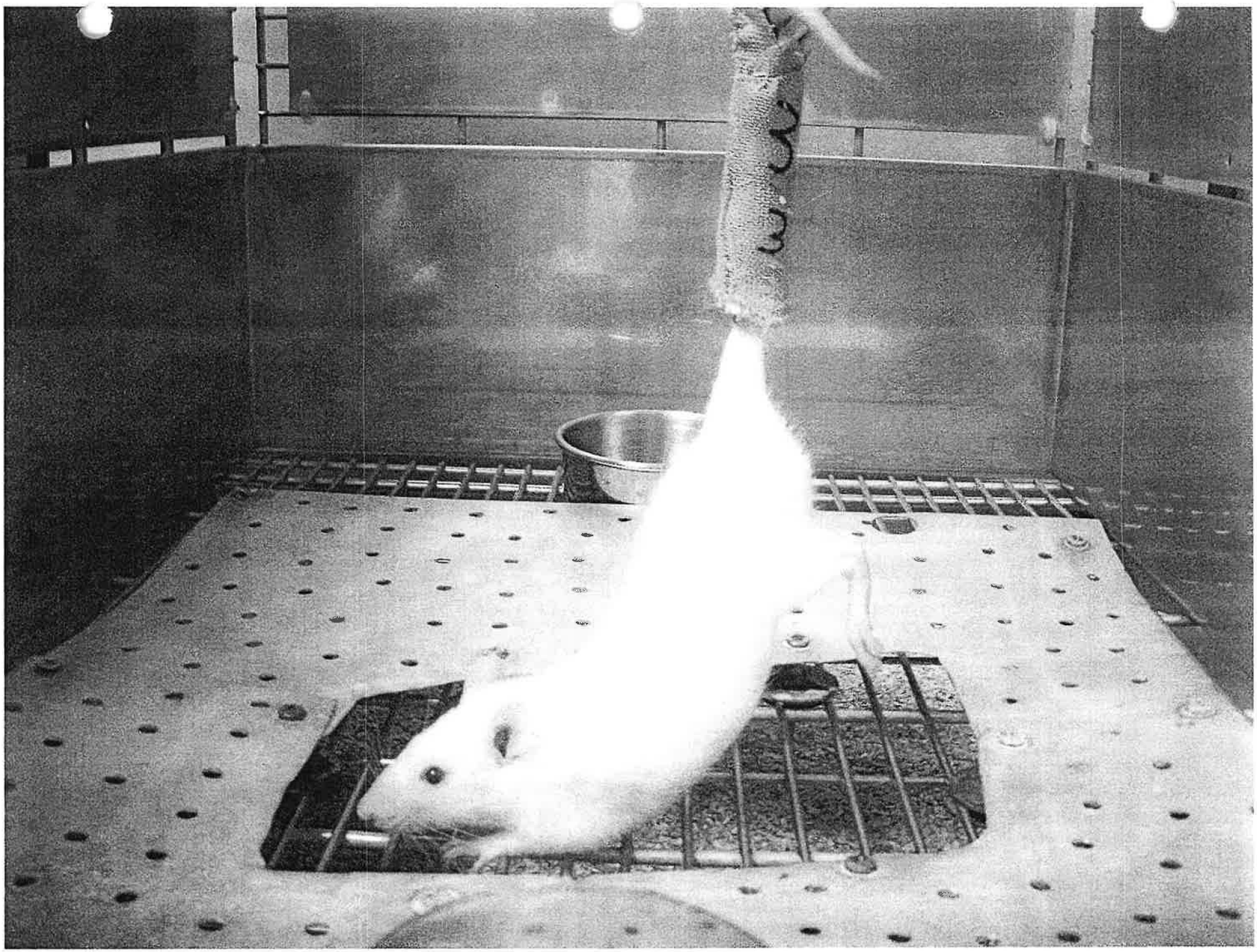

T3 HS vs. GC (n=8) (n=8) SEPT. 2008

9/18

	1 BW	2 VENT	3 SOL	4 PLAN	5 MG	6 VI	7 SOL	8 PLAN	9 MG	VI
			L					R		
1	168	548	69	170	368	52	68	173	375	52
2	165	526	65	162	342	57	74	152	356	63
3	177	572	68	197	401	39	71	203	414	50
4	168	518	70	172	376	51	74	185	377	45
GC 5	184	542	72	193	450	67	77	199	427	48
6	198	681	83	204	430	76	81	214	444	78
7	189	690	71	188	426	61	72	194	434	79
8	171	531	65	168	411	35	66	174	420	50
9										
10										
11	167		47	150	299	31	45	155	300	52
12	133		35	100	229	25	31	92	210	29
HS 13	160		42	112	253	31	35	117	253	27
14	164		45	157	272	24	44	143	266	38
15	150		35	122	264	20	29	125	253	35
16	131		30	91	209	41	30	98	214	43
17	145		44	121	298	40	40	124	280	25
*18	142		59	132	268		56	123	259	
19										
20										
21	* This rat got out of it's cast									
22	several times - insufficient extension									
23										
24										
25										
26	CAROLA: File is in									
27										
28	CAROLA \ CHIP \ Zoll. T3+HS Tissue									
29										
30										
31										

Studied carried out to collect tissue for CHIP assay to be performed by Carola

Animals were euthanized on 9-18-08

			Left	Left	Left	Left		Right	Right	Right	Right
GC	BW	Ventricles	Sol	Plan	MG	Vl		Sol	Plan	MG	Vl
1	168	548	69	170	368	52		68	173	375	52
2	165	526	65	162	342	57		74	152	356	63
3	177	572	68	197	401	39		71	203	414	50
4	168	518	70	172	376	51		74	185	377	45
5	184	542	72	193	430	67		77	199	427	48
6	198	681	83	204	430	76		81	214	444	78
7	189	690	71	188	426	61		72	194	434	71
8	171	531	65	168	411	35		66	174	420	50
	178	**576**	**70**	**182**	**398**	**55**		**73**	**187**	**406**	**57**

7day treatment

			Left	Left	Left	Left		Right	Right	Right	Right
HS + T3	BW	Ventricles	Sol	Plan	MG	Vl		Sol	Plan	MG	Vl
1	167	not taken!	47	150	299	31		45	155	300	52
2	133		35	100	229	25		31	92	210	29
3	160		42	112	253	31		35	117	253	27
4	164		45	157	272	24		44	143	266	36
5	150		35	122	264	20		29	125	253	35
6	131		30	91	209	41		30	98	214	43
7	145		44	121	298	40		40	124	280	25
8	142		59	132	268			56	123	359	
	149	**#DIV/0!**	**42**	**123**	**256**	**29**		**39**	**122**	**265**	**37**

 # 8 rat got out of its cast several times, not a good suspension. Should not be use for data collection

T_3 HS vs. GC OCT. 2008

10/9

	BW	VENT.	SOL (left)	PLAN	MG	VI	SOL (right)	PLAN	MG	VI
GC										
1	180	574	66	187	469	56	71	200	444	6
2	179	580	165	168	440	59	71	183	428	6
3	176	548	81	174	399	45	80	189	393	7
4	203	618	91	188	407	62	98	198	418	5
5	185	563	70	184	402	35	69	184	408	6
6	192	590	83	214	399*	29	82	183	385	6
7	191	578	86	200	465	93	81	204	469	6
8	184	615	66	194	408	79	62	188	413	6
9										
T_3 HS										
11	156	705	34	115	278	35	37	115	266	4
12	142	610	32	118	263	22	34	116	260	26
13	172	720	44	152	300	52	43	169	308	34
14	153	645	34	126	288	22	32	126	281	27
15	157	738	38	122	247	45	36	124	251	47
16	149	662	29	124	289	24	34	120	291	22
17	149	672	47	136	286	50	45	142	284	43
18	137	619	27	100	248	33	28	103	229	10
19										
20										
21										
22										
23										
24										
25										

*frozen weight

10-14-08

NC vs HS1T3 study

Tissue analysis Summary

Studied carried out to collect tissue for CHIP assay to be performed by Carola

Animals were euthanized on 10-9-08

GC	BW	Ventricles	Vent/BW		Left Sol	Left Plan	Left MG	Left VI		Right Sol	Right Plan	Right MG	Right VI
1	180	578	3.2	11-14-08	66	187	469	56		71	200	444	62
2	179	580	3.2	10-22-08	65	168	440	59		71	183	428	65
3	77	548	7.1	1) 2-5-09	81	174	399	45		80	189	393	73
4	203	618	3.0		91	188	407	62	11-21	98	198	418	59
5	185	563	3.0		70	184	402	35		69	184	408	69
6	192	590	3.1	2) 2-5-09	83	214	399	29		82	183	385	62
7	191	578	3.0	3) 2-5-09	86	200	465	83		81	204	469	62
8	184	615	3.3	10/13/2008	66	194	408	39		62	188	413	62
	174	**584**	**3.64**		**76.00**	**188.63**	**423.63**	**51.00**		**76.75**	**191.13**	**419.75**	**64.25**

7 day treatment HS + T3	BW	Ventricles	Vent/BW		Left Sol	Left Plan	Left MG	Left VI		Right Sol	Right Plan	Right MG	Right VI
1	156	705	4.5	11-14-08	34	115	278	35		37	115	266	46
2	142	610	4.3	10-22-08	32	118	263	22		34	116	260	26
3	172	722	4.2		44	152	300	52	3) 2-5-09	43	169	308	34
4	153	645	4.2	11-21	34	126	288	22	11-21	32	126	281	27
5	157	738	4.7	1) 2-5-09	38	122	247	45	1+2)	36	124	251	47
6	149	662	4.4	2) 2-5-09	29	124	289	24	11-21	34	120	291	22
7	149	672	4.5		47	136	286	50		45	142	284	43
8	137	619	4.5	10/13/2008	27	100	248	33	10/13/2008	28	103	229	16
	152	**672**	**4.43**		**34.00**	**122.43**	**273.29**	**33.29**		**34.86**	**124.71**	**269.43**	**31.14**

Marked in gray are tissue used for ChIP chromatin prep, also next to it the date used

Monday Oct. 13, 2008

Prepare samples for new ChIP assay
Use frozen muscle tissue from Oct. experiment Oct. 9 sac date)

For ground control **GC use Left Soleus # 8, 65 mg** of tissue

For hind limb suspension + T3 treatment sample **HS-T3 pool right and left Soleus # 8** (28 + 27 mg tissue), total of **55 mg** of tissue.

Prepare 1% Formaldehyde solution as follows:

to make 10 ml 1% formaldehyde		
37% formaldehyde	0.27	ml
10xPBS	1	ml
water	8.73	ml

Prepare necessary amount of 1xPBS solution and put on ice, to be used for different washes.
Need about 4.5 ml 1x PBS per sample.
When using PBS, always supplement with protease inhibitors
protease inhibitors: use leupetin, AEBSF, and aproteinin each are at 1000x stock solutions in the -80 C freezer.
Keep AEBSF and leupetin on dry ice, thaw and refreeze as necessary.
Keep aprotinin on ice and use as necessary.

Isolating and lysing cells from tissue
Mince tissue (small pieces) using a razor blade
Put minced tissue in 1.5ml tubes in ~1 ml cold PBS supplemented with protease inhibitors
Keep on ice until all pieces are minced
Drain cold PBS, add 1 ml 1% formaldehyde buffer (freshly made)
Incubate at room temperature for 10 minutes with mixing every few minutes
After 10 minutes incubation, add 110 ul 10x glycine (1.25M glycine) prepared in a 15 ml tube.
Incubate 5 minutes at room temperature
Change solution, take out the formaldehyde PBS, replace with 1 ml cold PBS (+protease inhibitors)
Repeat PBS wash once more (use PBS supplemented with protease inhibitor)
Add 19x cold PBS (plus inhibitors) to samples
Transfer to homogenizing pestle tube, and homogenize on ice, transfer to clean 1.5ml tube.

Spin down the homogenate at 1500g for 10 minutes in order to collect the cells as a pellet
Take out supernatant, use a pipet to leave a clean pellet (no liquid should be left)

Suspend the pellet in 400 ul lysis buffer (supplemented with protease inhibitors) in 1.5 ml tubes. Incubate 10-30 minutes on ice
Remove 5ul from the lysate to be used as unsheared DNA (high molecular weight)
Freeze lysate at -80oC over night.

Tuesday Oct. 14, 2008

Shearing the DNA with sonication:
Use the Sonics Vibracell, 130 watts ultrasonic from sonics and materials (VCX 130)
The converter is connected to a 2mm probe.
2mm probe is to process samples with volumes of 150ul to 5 ml.
Use the new probe that Clay ordered.

Sonication:
Put the samples on ice at all time to keep it cool
Immerse the probe tip in the sample
Sonicate using the following protocol:

amplitude	time	# of pulses
80% max	15 sec	10

25 sec rest on ice between pulses
(monitor tube temperature, as it can heat up quickly)
Set sonicator to do 2 pulses at a time. 2 pulses = 55 sec, with 25 sec rest set. Then vortex/mix sample, and return to ice, and start next sample. Rotate thru samples like this.
Make sure that all of the sample is in the lysis buffer, as it tends to stick to the sides of tube (use pipette tip to clears sides).
Have water in the ice, so that the tube is immersed in ice-cold temp, otherwise air pockets in ice around tube can heat up.
Sonication will solubilize the chromatin and break up the DNA
The goal is to find the condition that shears the DNA to 400-1000 bp size fragments
After sonication, spin the samples in a cold microfuge at 12,000g for 10 minutes to remove insoluble material.

Remove **10ul aliquot** of sheared DNA to analyze on agarose gel
Transfer the supernatant (containing soluble chromatin) to a fresh microfuge tube.
Freeze at -80 to be used later for the IP procedure.

Use the 10ul aliquot from above for DNA analyses: necessary to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.

To 10ul total sheared lysate, add 85ul nuclease free water, and 4ul 5M NaCl.
Incubate at 65oC **overnight** to reverse the DNA-protein crosslinking.

Wednesday Oct. 15, 2008

Continue DNA analysis to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.

Add 1 ul RNAse A (10ug/ul) to both samples and incubate 30 minutes at 37oC
Add 7ul (digestion buffer, see below) & incubate at 45oC for 2hours.
Digestion buffer=2ul 0.5M EDTA, 4ul 1M Tris-HCL pH 6.5, and 1ul proteinase K(10ug/ul), see below.
Load 40 µl on 2 % agarose gel with a 100 bp ladder with gel-green staining.

to 5 ul lysate, add 94 ul premix (NaCl 4, water, 90)
incubate at 65oC overnight

premix	**1x**
5M NaCl	4
H2O	90
	94

digestion buffer

premix:	**1x**
0.5M EDTA	2
1M Tris pH6.5	4
proteinase K	1
	7

Results of agarose gel are on next page.

There is too much high molecular weight DNA in my samples. Need to start over or re-shear the chromatin.

Compare pre-reshear and re-shear chromatin samples also on this gel.

Need an extra 8 15-second pulses with the sonicator.

File/Range: G:\Carola\10-6-08 GC vs HST3 Soleus Oct tissue.gel / 2057-11118 /Magnification - 3.70
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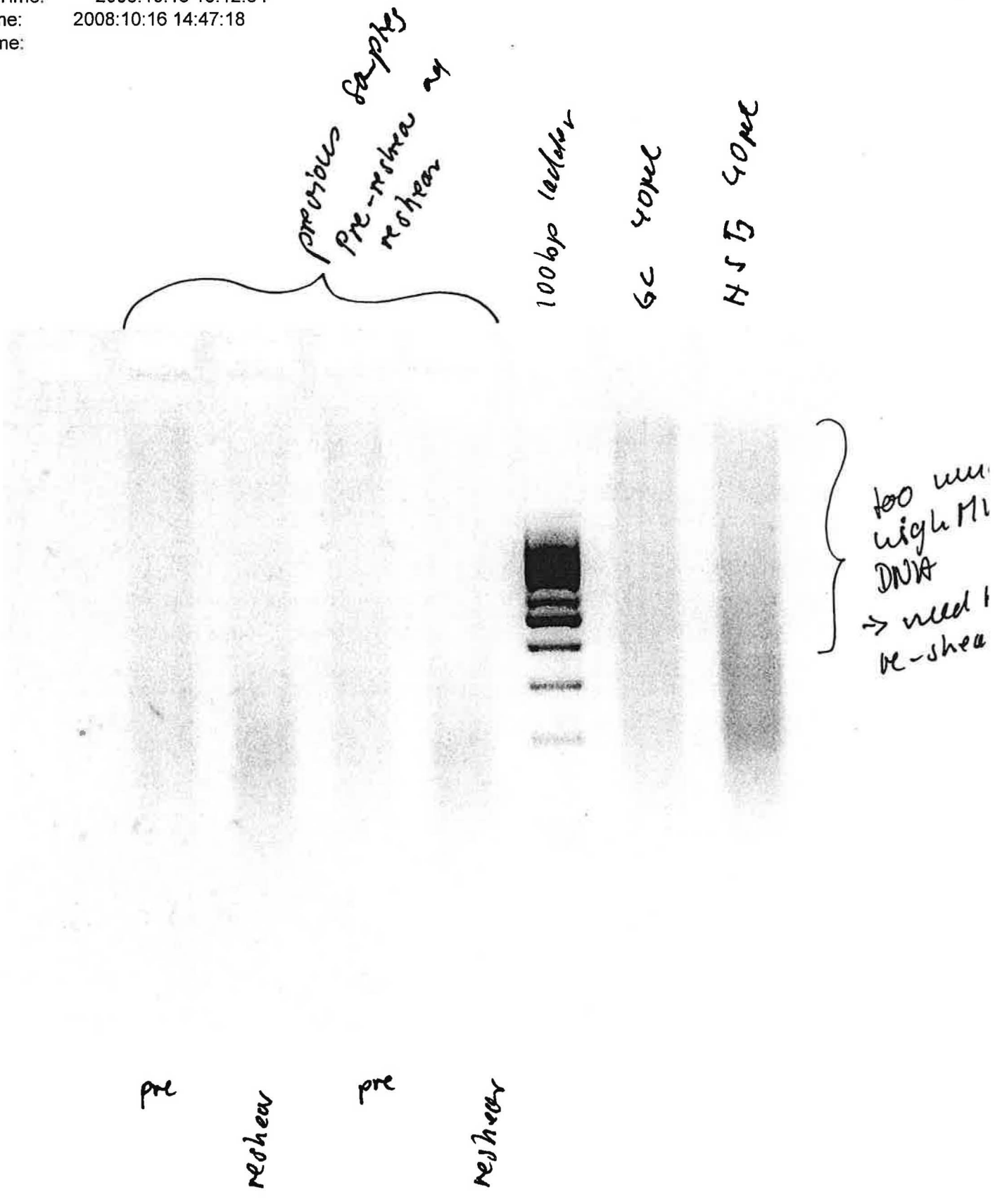
P Date/Time:

10-16-08



File/Range: G:\Carola\10-16-08 WMG Re-shearing from 3-10 and 4-30 tissue.gel / 2829-31867 /Magnification - 3.19
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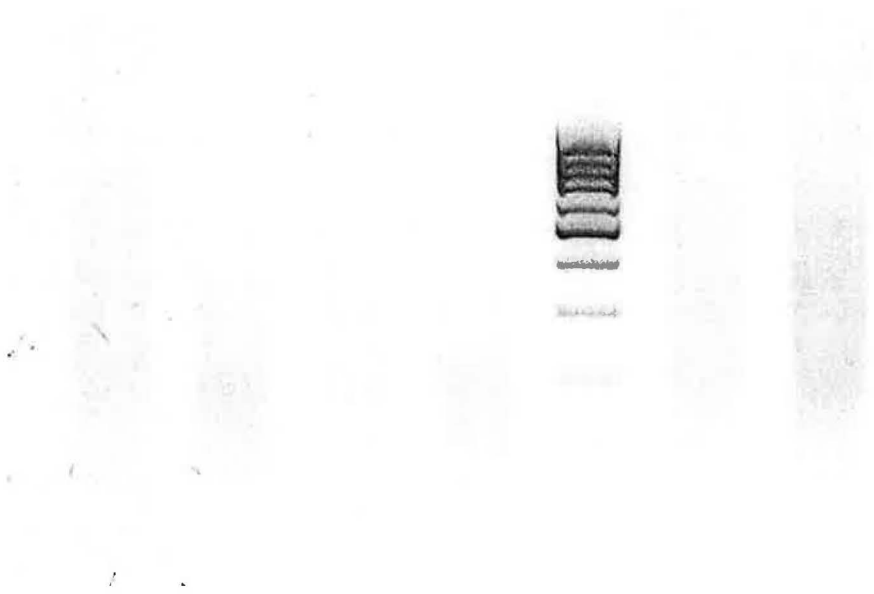
Image Name: G:\Carola\10-16-08 WMG Re-shearing from 3-10 and 4-30 tissue.gel
Image Comment:
Present Date/Time: 2008:11:04 15:01:39
Scan Date/Time: 2008:10:16 14:57:56
F ate/Time:

orig. shearing sample orig. shearing sample
re-sheared sample re-sheared sample
3-10 3-10 4-30 4-30 Carolas samples



WMG re-sheared another 10 pulses, same conditions as previous

80% ampl, 15 sec pulses

Note: 4-30 samples consist of combination of ~200ul each of 4-30 and 8-13 white muscles

File/Range: G:\Carola\10-16-08 Plan,Plan, GC HS Sol ChIP with pan-H3, AcH3, H3K4me3 (oldnew ab), PCR with IIb (1075, 1
User Name: Geldoc user
Image Name: G:\Carola\10-16-08 Plan,Plan, GC HS Sol ChIP with pan-H3, AcH3, H3K4me3 (oldnew ab), PCR with IIb (1075
Image Comment:
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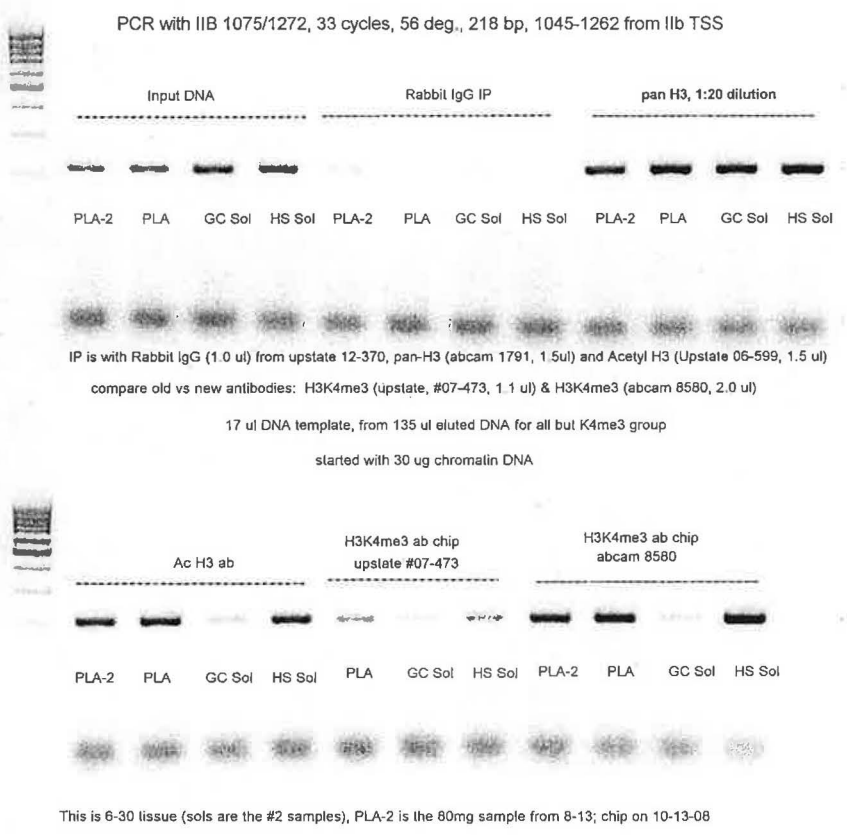
F ate/Time:



This is 6-30 tissue (sols are the #2 samples), PLA-2 is the 80mg sample from 8-13; chip on 10-13-08

Thursday Oct. 16, 2008

Prepare samples for quantitative DNA analysis with real time PCR machine.

Prepare 10 ml filtered TAE (1x) and add 1ul Sybr Green (10,000 x).

Dilute sample 1:40 with water (5 ul sample + 195 ul water).

Thaw DNA standards (o.5, 1, 2.5, 5, 10, 15, 20 and a water blank)

Add 50 ul of filtered TAE and 5 ul sample to each micro titer well. Set up in triplicates.

Use Stratagene real time PCR machine for plate read. Make sure machine is warmed up for 20 minutes before plate read.

Results for quantitative DNA analysis on next pages.

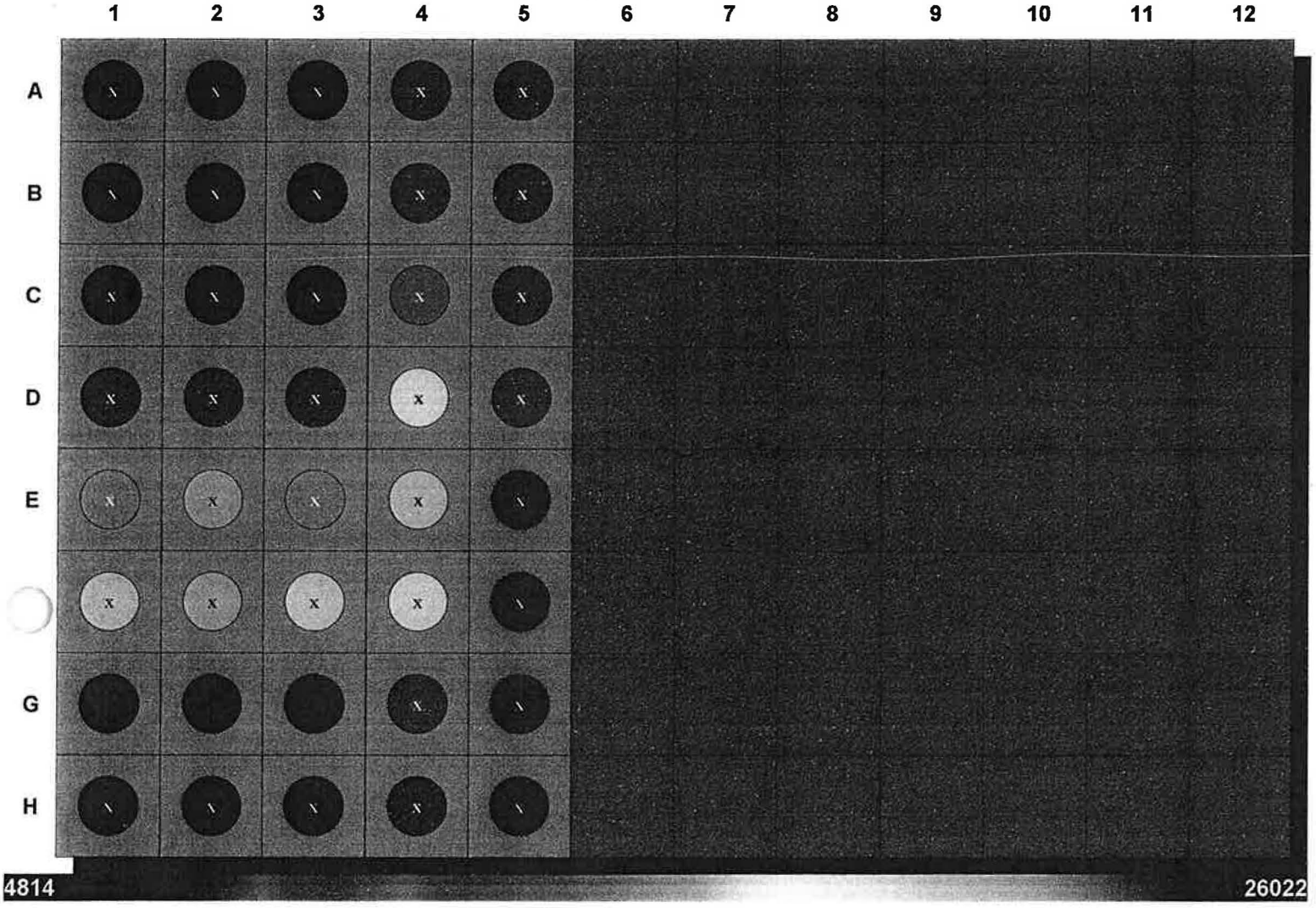
Friday Oct. 17, 2008

Analyze data from quantitative DNA analysis.
See Excel spreadsheet for data analysis.

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Fluorescence intensity values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 10-16-2008 GC vs HST3.mxp
Run date: October 16, 2008

Replicates: Treated individually (since no replicates in selection)



Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Plate sample values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 10-16-2008 GC vs HST3.mxp
Run date: October 16, 2008

Fluorescence : Rpre

Replicates: Treated individually (since no replicates in selection)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unknown 6821	Unknown 6610	Unknown 6575	Unknown 12274	Unknown 9511							
B	Unknown 6941	Unknown 6898	Unknown 7127	Unknown 11927	Unknown 10430							
C	Unknown 7824	Unknown 8100	Unknown 7886	Unknown 14431	Unknown 12215							
D	Unknown 9931	Unknown 10213	Unknown 9671	Unknown 18767	Unknown 11843							
E	Unknown 15145	Unknown 16270	Unknown 15160	Unknown 19958	Unknown 5104							
F	Unknown 19812	Unknown 20698	Unknown 19651	Unknown 19279	Unknown 4914							
G	Unknown 25383	Unknown 25853	Unknown 26022	Unknown 10711	Unknown 4861							
H	Unknown 6443	Unknown 6505	Unknown 6753	Unknown 10348	Unknown 4814							

10-17-08

SYBR green I, dilute 1:10,000 in filtered 1x TAE (same buffer as gel running buffer)

add 50 ul sybr green mix to well, 5ul of sample/standard

generally dilute sample 1:40

8-13-08 samples, read 8-18-08

using standards from 3-11

	ng/well/5ul				average	Net	predicted ng
water blank	0	6443	6505	6753	**6567**		
S0.5	0.5	6821	6610	6575	**6669**	102	1.2
S1	1	6941	6898	7127	**6989**	422	1.4
S2.5	2.5	7824	8100	7886	**7937**	1370	2.5
S5	5	9931	10213	9671	**9938**	3371	4.2
S10	10	15145	16270	15160	**15525**	8958	9.9
S15	15	19812	20698	19651	**20054**	13487	13.4
S20	20	25383	25853	26022	**25753**	19186	21.4
						r^2	**0.9833**

10 ul sheared DNA taken, in final volume of 112 ul

samples diluted 1:40 (5:200), measured 5ul in well

				average	Net		**ng/ul of original chromatin sample**
GC Sol	12274	11927	14431	**12877**	6310	6.01	**538.1**
HS Sol + T3	18767	19958	19279	**19335**	12768	9.95	**891.6**
				#DIV/0!	#DIV/0!	6.10	**547.0**

Well	Rpre
A1	6821
A2	6610
A3	6575
A4	12274
A5	9511
B1	6941
B2	6898
B3	7127
B4	11927
B5	10430
C1	7824
C2	8100
C3	7886
C4	14431
C5	12215
D1	9931
D2	10213
D3	9671
D4	18767
D5	11843
E1	15145
E2	16270
E3	15160
E4	19958
E5	5104
F1	19812
F2	20698
F3	19651
F4	19279
F5	4914
G1	25383
G2	25853
G3	26022
G4	10711
G5	4861
H1	6443
H2	6505
H3	6753
H4	10348
H5	4814

Monday Oct. 20, 2008

Re-shear chromatin samples with sonicator.

Give and extra four double pulses, i.e. an extra eight 15 second pulses.

Tuesday Oct. 21, 2008

Run DNA samples on 2% agarose gel

Results see next page. The DNA fragments are now too small.

Need to start over with fresh tissue

File/Range: G:\Captures\Capture_00143.tif / 2571-22873 /Magnification - 6.06
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Image Name: G:\Captures\Capture_00143.tif
Image Comment:
Present Date/Time: 2008:10:21 15:22:55
Scan Date/Time: 2008:10:21 15:02:56
P ate/Time:

Chromatin DNA

Re-shear Samples

Run on 2% agarose gel after rev. cross link and digestion w/ proteinase K

NC

HST3

4 extra cycles of 2 15 sec pulses @ 80%

⇒ total of 18 15 sec sonication pulses

DNA fragments are now too small

Wednesday Oct. 22, 2008

Prepare new samples for ChIP assay.
Use frozen muscle tissue from Oct. experiment (Oct. 9 sac date)

For ground control **GC use left Soleus # 2, 65 mg** of tissue

For hind limb suspension + T3 treatment sample **HS-T3 pool right and left Soleus # 2** (32 mg + 34 mg tissue respectively), total of **66 mg** of tissue.

Prepare 1% Formaldehyde solution as follows:

to make 10 ml 1% formaldehyde		
37% formaldehyde	0.27	ml
10xPBS	1	ml
water	8.73	ml

Prepare necessary amount of 1xPBS solution and put on ice, to be used for different washes.
Need about 4.5 ml 1x PBS per sample.
When using PBS, always supplement with protease inhibitors
protease inhibitors: use leupetin, AEBSF, and aproteinin each are at 1000x stock solutions in the -80 C freezer.

Isolating and lysing cells from tissue:
Mince tissue (small pieces) using a razor blade
Put minced tissue in 1.5ml tubes in ~1 ml cold PBS supplemented with protease inhibitors
Keep on ice until all pieces are minced
Drain cold PBS, add 1 ml 1% formaldehyde buffer (freshly made)
Incubate at room temperature for 10 minutes with mixing every few minutes
After 10 minutes incubation, add 110 ul 10x glycine (1.25M glycine) prepared in a 15 ml tube.
Incubate 5 minutes at room temperature
Change solution, take out the formaldehyde PBS, replace with 1 ml cold PBS (+protease inhibitors)
Repeat PBS wash once more (use PBS supplemented with protease inhibitor)
Add 19x cold PBS (plus inhibitors) to samples
Transfer to homogenizing pestle tube, and homogenize on ice, transfer to clean 1.5ml tube. Use brand new homogenizing pestle tube this time for best results (hopefully).

Spin down the homogenate at 1500g for 10 minutes in order to collect the cells as a pellet
Take out supernatant, use a pipet to leave a clean pellet (no liquid should be left)
Suspend the pellet in 400 ul lysis buffer (supplemented with protease inhibitors) in 1.5 ml tubes. Incubate 10-30 minutes on ice
Remove 5ul from the lysate to be used as unsheared DNA (high molecular weight)
Freeze lysate at -80oC over night.

Thursday Oct. 23, 2008

Shearing the DNA with sonication:
Use the Sonics Vibracell, 130 watts ultrasonic from sonics and materials (VCX 130)

Use the new probe that Clay ordered.

Sonication:
Put the samples on ice at all time to keep it cool
Immerse the probe tip in the sample
Sonicate using the following protocol:

amplitude	time	# of pulses
80% max	15 sec	14

25 sec rest on ice between pulses
(monitor tube temperature, as it can heat up quickly)
Set sonicator to do 2 pulses at a time. 2 pulses = 55 sec, with 25 sec rest set. Then vortex/mix sample, and return to ice, and start next sample. Rotate thru samples like this.
Make sure that the entire sample is in the lysis buffer, as it tends to stick to the sides of tube (use pipette tip to clears sides).
Have water in the ice, so that the tube is immersed in ice-cold temp, otherwise air pockets in ice around tube can heat up.
Sonication will solubilize the chromatin and break up the DNA
The goal is to find the condition that shears the DNA to 400-1000 bp size fragments
After sonication, spin the samples in a cold microfuge at 12,000g for 10 minutes to remove insoluble material.

Remove **10ul aliquot** of sheared DNA to analyze on agarose gel
Transfer the supernatant (containing soluble chromatin) to a fresh microfuge tube.
Freeze at -80 to be used later for the IP procedure.

Use the 10ul aliquot from above for DNA analyses: necessary to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.

To the set aside 10ul total sheared lysate from above, add 85ul nuclease free water, and 4ul 5M NaCl.
Incubate at 65oC **overnight** to reverse the DNA-protein crosslinking.

Friday Oct. 24, 2008

Run 40 ul of each sample on a 2% agarose gel together with 4 ul 100bp ladder.

Results see next page.

File/Range: G:\Captures\Capture_00176.tif / 2829--29558.000 /Magnification - 4.73
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Image Comment:
Present Date/Time: 2008:10:24 15:30:19
Scan Date/Time: 2008:10:24 15:10:41
P[illegible]ate/Time:

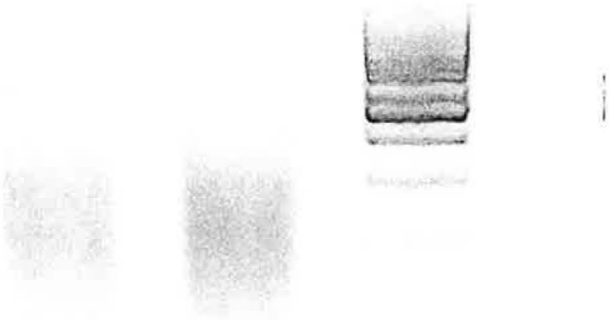
10-24-08

40 µl of sample for oligo DNA rev. protein crosslinking run on 2% agarose gel

Sheared lysate

GC HST3

100bp marker



328 ng/µl

569 ng/µl

Monday Oct. 27 & Tuesday Oct. 28, 2008

Quantitative DNA analysis using real time PCR machine.

Prepare 10 ml filtered TAE (1x) and add 1ul Sybr Green (10,000 x).
Dilute sample 1:40 with water (5 ul sample + 195 ul water).
Thaw DNA standards (o.5, 1, 2.5, 5, 10, 15, 20 and a water blank)
Add 50 ul of filtered TAE and 5 ul sample to each micro titer well. Set up in triplicates.
Use Stratagene real time PCR machine for plate read. Make sure machine is warmed up for 20 minutes before plate read.
Results for quantitative DNA analysis on next pages.

Data analysis on excel spreadsheet.
Calculate necessary amounts of sheared DNA for IP procedure.
Prepare solutions for IP procedure. Select antibodies to be used for procedure:

1: Rabbit IgG as control antibody
2. pan –H3
3. Anti-Histone H3 K4 me3 antibody and
4. Anti-Acetyl Histone H3 antibody

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Plate sample values
F:\Carola\ChIP\Quantitative Plate Read, 10-28-2008.mxp
Run date: October 28, 2008

Fluorescence : Rpre

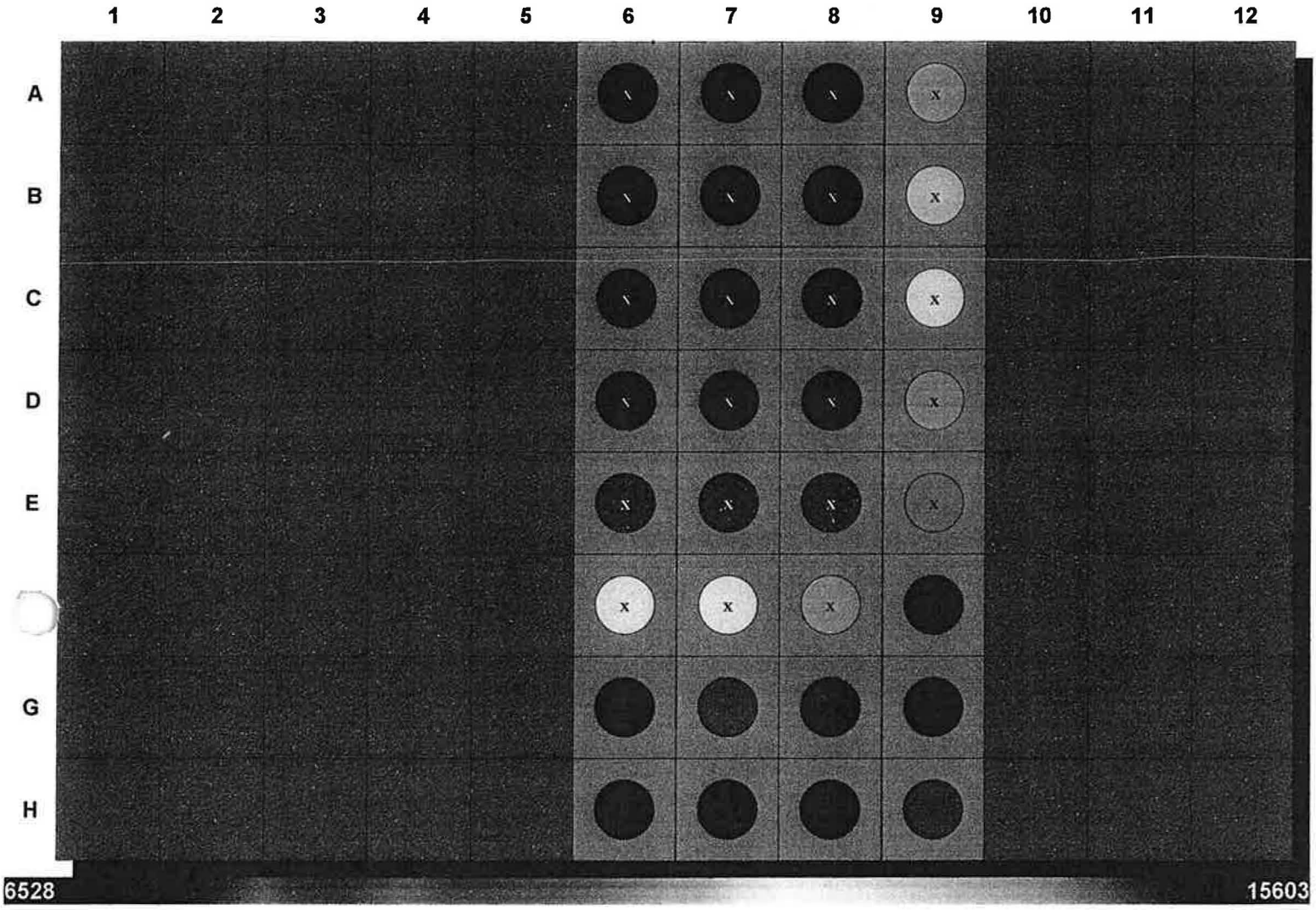
Replicates: Treated individually (since no replicates in selection)

	1	2	3	4	5	6	7	8	9	10	11	12
A						Unknown 6905	Unknown 6528	Unknown 6887	Unknown 11458			
B						Unknown 6805	Unknown 6742	Unknown 7007	Unknown 11616			
C						Unknown 7180	Unknown 6991	Unknown 7756	Unknown 11815			
D						Unknown 7505	Unknown 7338	Unknown 7508	Unknown 11276			
E						Unknown 8570	Unknown 8660	Unknown 8968	Unknown 13920			
F						Unknown 12353	Unknown 11866	Unknown 13540	Unknown 14780			
G						Unknown 14672	Unknown 14346	Unknown 15584	Unknown 14705			
H						Unknown 15603	Unknown 15305	Unknown 14978	Unknown 14383			

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Fluorescence intensity values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 10-28-2008.mxp
Run date: October 28, 2008

Replicates: Treated individually (since no replicates in selection)



10-28-08

10-28-08 DNA analysis

			ng DNA	ng DNA	ng DNA	ng DNA	ng DNA	ng DNA	ng DNA	sample 1	sample 2
		water	0.5	1	2.5	5	10	15	20	GC	T3HS
		6905	6805	7180	7505	8570	12353	14672	15603	11458	13920
		6528	6742	6991	7338	8660	11866	14346	15305	11616	14780
		6887	7007	7756	7508	8968	13540	15584	14978	11815	14705
										11276	14383
	Average	6773.333	6851.333	7309	7450.333	8732.667	12586.33	14867.33	15295.33	11541.25	14447
	net		78.00333	535.67	677.0033	1959.337	5813.003	8094.003	8522.003	4767.92	7673.67
SYBR green I, dilute 1:10,000 in filtered 1x TAE (same buffer as gel running buffer)											
add 50 ul sybr green mix to well, 5ul of sample/standard											
Sample diluted 1:40											

GC: 5813 ≙ 10 ng/5µl

4767.9 ≙ 8.2 ng/µl

× 40 = 328 ng/µl

T_3 HS: 8094 ≙ 15 ng/µl

7673.7 ≙ 14.22 ng/µl

× 40 = 569 ng/µl

30 µg

for 25 µg: 76.2 µl GC lysate 40.8 buffer 959

44 µl T_3 HS lysate 23.6 976

Wednesday Oct. 29, 2008

Begin ChIP procedure.

Wash 8 x 50µl protein A agarose (Perice) in 8 separate1.5 ml tubes with 500 µl ChIP dilution buffer (supplemented with protease inhibitors.
Spin at 1200g for 30 sec to pellet the agarose. Remove and discard supernatant.
Add chromatin and dilution buffer based on measurements made with tissue samples processed on Oct.23 (DNA analysis results Oct. 28).

This tissue prepped on 10-23-08

	Sample	muscle frozen weight	in mg
400 ul lysis buffer		GC Sol	65
80 % amplitude		HS + T3 Sol	66
15 sec pulse			
14 pulses			

Use 30µg of DNA for this ChIP

	ng/µl of original chromatin sample		for 30 µg
		µl of chromatin	µl buffer to yield 1ml
GC Sol	735.3	40.8	959
HS Sol + T3	1271.2	23.6	976

Prepare ~~three~~ four tubes for each muscle. Add the above amounts of chromatin solution and ChIP buffer to the washed agarose beads. Preclear chromatin for 45 min at 4 oC on rotator.
Spin at 1200g for 30 sec to pellet the agarose.
Collect the 1 ml supernatants into fresh 1.5 ml microtubes.
Remove 10 µl of the supernatant and **save at 4oC as input DNA** (one input DNA sample per muscle). For input DNA we need to save 1% of the total DNA used in each IP.

Add the following antibodies to the pre-cleared chromatin samples.
Four different antibodies for the two muscle chromatin samples (total of eight reactions):

1. Normal Rabbit IgG from Upstate (12-370) used 1.0 µl (1.0 µg).

2. Pan-H3 (abcam 1791), use 1.5 µl.

3. Anti-Histone H3 K4 me3 antibody (ab 8580), use 2.0 µl (~ 2 µg).

4. Anti-Acetyl Histone H3 antibody (Upstate #06-599), used 1.0 ul (~1 ug).

Incubate over night with antibodies. Tubes are in refrigerator on rotator.

Thursday Oct. 30, 2008

Wash another 8 x 50µl agarose beads with ChIP dilution buffer. Spin and discard supernatant. Add the washed beads to the chromatin antibody mixtures and incubate for another 2 hours in the refrigerator on rotator.
Pellet protein agarose by spinning at 1200 g for 40sec at 4oC.Discard the supernatant.
Wash the protein agarose-antibody chromatin samples by resuspending the beads in 1 ml of the cold buffers in the order listed
First use 1 ml Low salt Immune complex Wash buffer wash 10sec and spin, discard supernatant, then wash as listed:
For each wash, incubate for 15 minutes on a rotating platform followed by spin 1000g for 20sec at 4oc (supplementation with protease inhibitors is not necessary for the wash solutions.
Discard the supernatant after each wash
Wash buffer in the order to be used:

a Once wash with Low salt Immune complex Wash buffer
b Once wash with high salt Immune complex Wash buffer
c Once wash with LiCl complex Wash buffer
d twice wash with TE buffer

remove supernatant and procede to elution of the protein/DNA from the agaraose beads

Elution of Protein/DNA complexes
Bring 1M NaHCO3 solution to room temperature. Vortex at room temperature to dissolve precipitate
Set water bath to 65oC to use for reverse linking(next section)

Prepare elution buffer for all IP tubes as well as for input DNA tube (which was 1% of DNA in the IP)
For 10 tubes, make 11 x to have some extra

elution buffer	1x	6.5	11x	
20% SDS	10	65	110	
1M NaHCO3	20	130	220	+NaCl
sterile di Water	170	1105	1870	52
Sum	200	1300	2200	

For input DNA, add 200ul elution buffer and set aside at room tempertaure until reverse cross-linking
Add 100ul of elution buffer to each IP tube containing the agarose pellet

Mix by flicking the tube gently
Incubate at room temperature for 15 minutes
Pellet the agarose by brief centrifugation 1200 g for 40sec at 4oC)
Collect and save the supernatant into a new microfuge
Repeat the elution with another 100ul elution buffer.
Collect the supernatant and combine with the previous step 100ul, to make a total of 200ul eluate

Reverse crosslinks of Protein/DNA complexes to free the DNA

To all the tubes (input DNA and IP), add 8ul 5M NaCl
Incubate at 65oC overnight.

Friday, October 31 2008

RNA and Protein digestion:
To all tubes, add 1 ul RNAse A and incubate at 37oC for 30 minutes.
Add 13ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 2 hours.

digestion buffer

premix:	**1x**	**11x**
0.5M EDTA	4	44
1M Tris pH6.5	8	88
proteinase K	1	11
	13	143

DNA purification using spin columns

Use Qiagen miniprep columns

To each of the above tubes (input DNA and IP DNA) add 1 ml binding reagent"A"
Mix well.
A precipitate might form, this will not interfere with the procedure
Transfer 600ul of sample/Bind reagent A mixture to the column
Spin 30 sec at 12,000 g
Discard flow through
Add the remaining 600 ul to the column
Spin again and discard flow through
Add 750ul wash buffer of Qiagen to the column
Spin at 12,000 g
Discard flow through
Spin again for 12,000 g for 2min.
Discard the collection tube
Put the column in a new 1.5 ml collection tube
Add 135 ul warm water to elute the DNA off the column.
Spin at 12,000g for 2 minute (be careful to not zip the lids off the tubes.)

Eluate is pure DNA that can be analyzed by PCR
Use 12 µl of this DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples.

Freeze samples at -20 oC.

Monday Nov. 3, 2008

Set up PCR reactions with the new samples.
Use the beta MHC +1156 / +1441 primer set at 57 oC annealing and
The IIb 1075 F / 1272 R primer set also at 57oC annealing.

Total of 20 samples.
Each tube has a total of 25 µl plus mineral oil on top.

PCR mix is

PCR Reactions	**1x**
Water	6.1
10x PCR Buffer	2.5
10 mM dNTP	0.5
50 mM MgCl2	0.75
5+3' primer mix at 5 pmol/ul	3
Template (IP DNA/input DNA)	**12**
Biolase DNA Polymeras	0.15
SUM	25

Use 12 µl of eluted DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples (use 5µl less water per reaction for those samples).

30/33 cycles of

60 sec. 96 oC
45 sec. 57 oC
45 sec. of 72 oC

(Program # 33)
Use 2 different PCR machines:
33 cycles for trimethylation samples and 30 cycles for all other samples.

Run PCR products on 2% agarose gel.
Results on next page.

File/Range: G:\Carola\ChIP results 11-03-08 beta and IIb primers.gel / 1543-18210 /Magnification - 2.38
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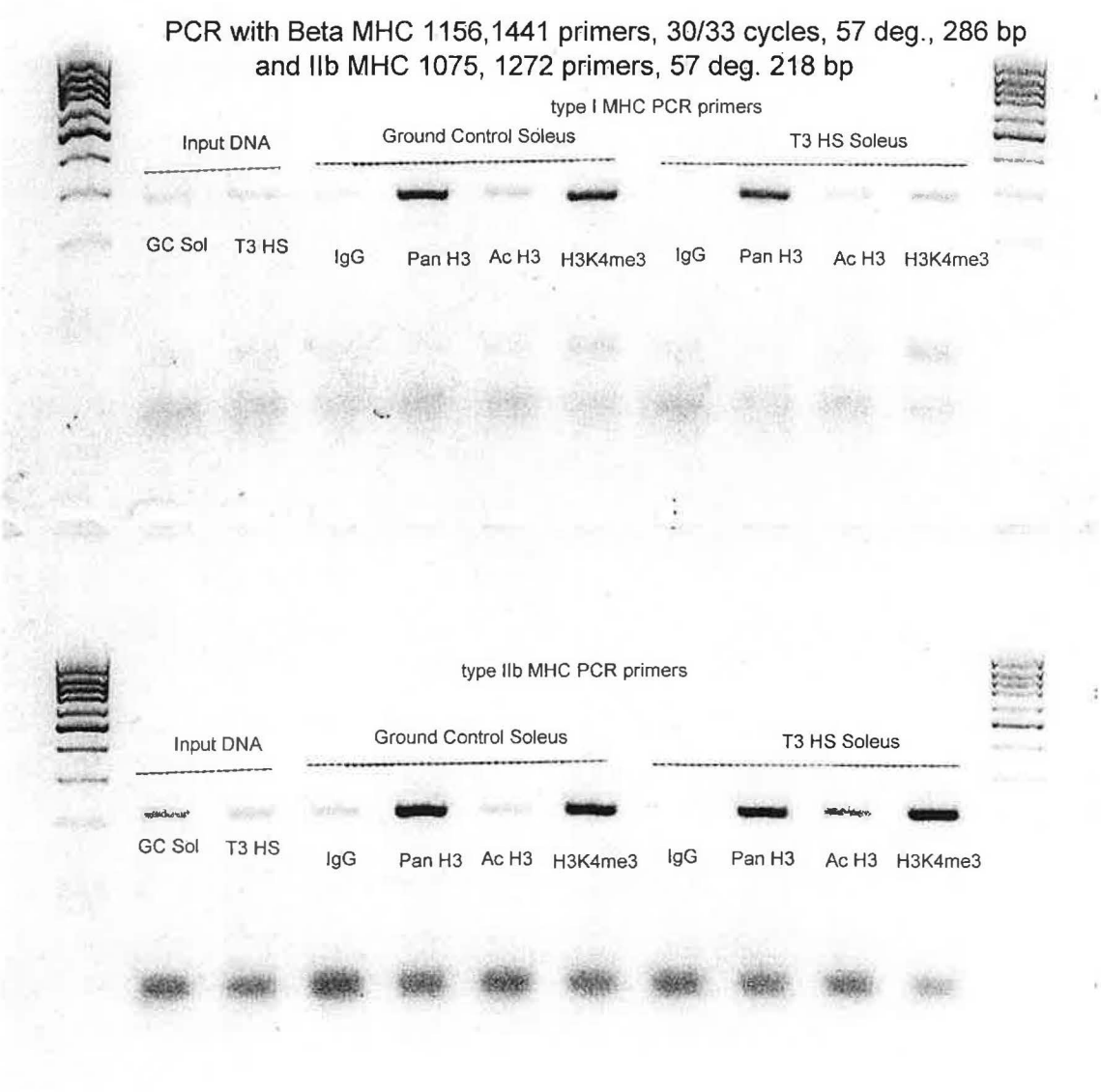
Present Date/Time: 2008:11:04 14:08:51
Scan Date/Time: 2008:11:03 15:58:06
F ate/Time:

Use 12 µl of eluted & purified DNA for each reaction except the trimethylation samples. use 17 µl eluted DNA for those



Tuesday Nov. 4, 2008

Set up more PCR reactions with same DNA samples:

Use the beta Actin +1517 F / 1741 R primer set at 56 oC annealing and
The IIx 5'end I 2 pre F + R primer set also at 56oC annealing.

Total of 20 samples.
Each tube has a total of 25 µl plus mineral oil on top.
Same PCR mix as yesterday (see above).

Use 12 µl of eluted DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples (use 5µl less water per reaction for those samples).

30/33 cycles of
60 sec. 96 oC, 45 sec. 56 oC, 45 sec. of 72 oC (Program # 33)
Use 2 different PCR machines: 33 cycles for trimethylation samples and 30 cycles for all other samples.

Run PCR products on 2% agarose gel.
Results and gel analysis on next page.

File/Range: G:\Carola\ChIP results 11-04-08 beta actin and IIx primers.gel / 2571-17283 /Magnification - 1.96
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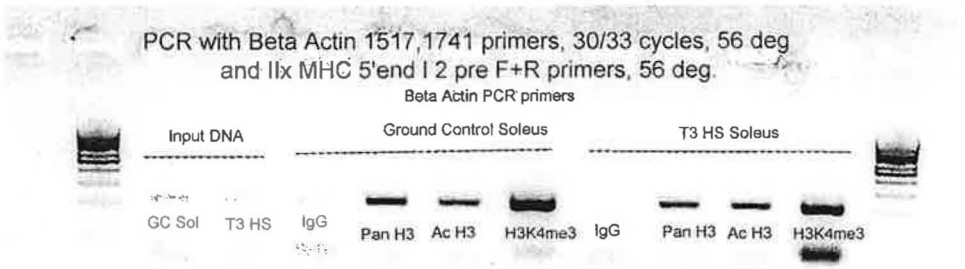
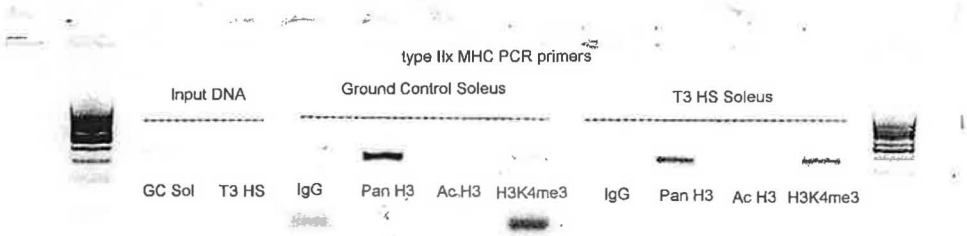
P ate/Time:





Thursday Nov. 6, 2008

Repeat PCR reactions with the same samples.

Use the beta MHC +1156 / +1441 primer set at 57 oC annealing and
The IIb 1075 F / 1272 R primer set also at 57oC annealing.
The beta Actin +1517 F / 1741 R primer set at 56 oC annealing and
The IIx 5'end I 2 pre F + R primer set also at 56oC annealing.

Total of 40 samples.
Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

32 cycles of
60 sec. 96 oC, 45 sec. 57 / 56 oC, 45 sec. of 72 oC (Program # 33)
Use 2 different PCR machines: 32 cycles for all samples.

Friday Nov. 7, 2008

Run PCR products on 2% agarose gel.
Results and gel analysis on next page.

Monday Nov. 10, 2008

Literature read on ChIP assay

Tuesday Nov. 11, 2008

Veteran's day Holiday

Wednesday Nov. 12, 2008

Literature read on ChIP assay

Thursday Nov. 13, 2008

Prepare for next ChIP assay round.

File/Range: G:\Carola\ChIP results 11-07-08 beta actin and IIx primers.gel / 1287-28130 /Magnification - 1.91
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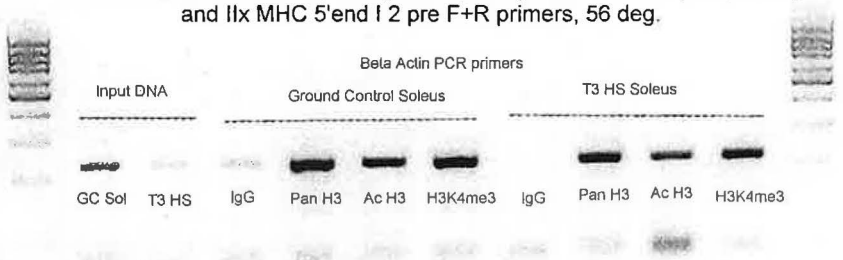
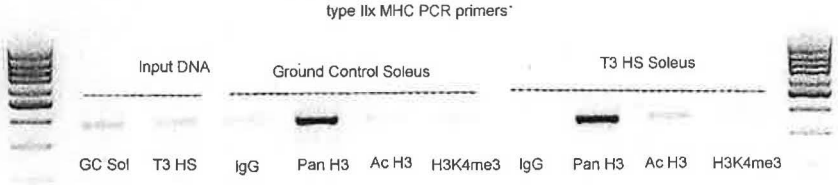
Image Name: G:\Carola\ChIP results 11-07-08 beta actin and IIx primers.gel
Image Comment:
Present Date/Time: 2008:11:12 13:45:25
Scan Date/Time: 2008:11:07 15:38:20
P ate/Time:

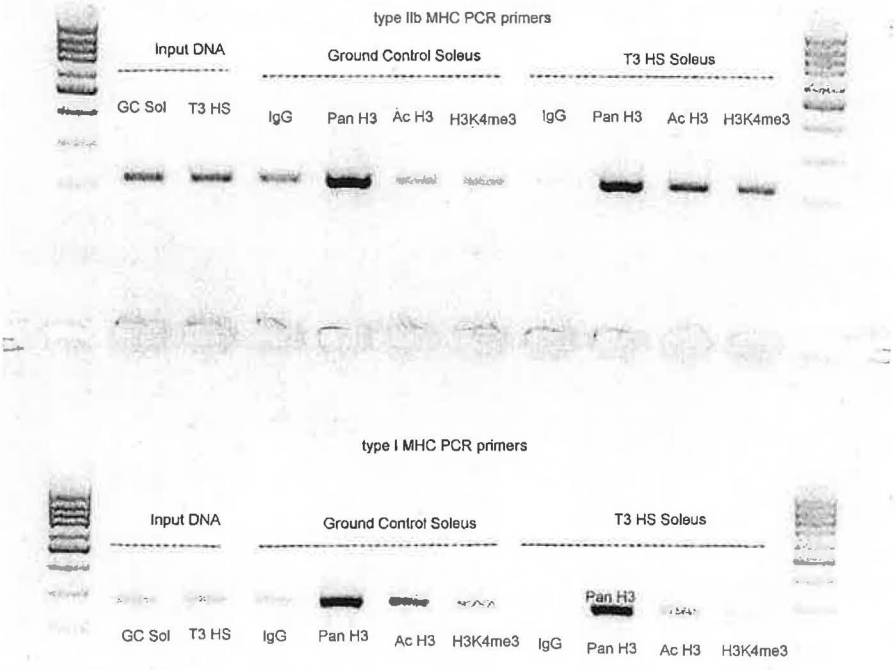
PCR with Beta Actin 1517,1741 primers, 32 cycles for all samples, 56 deg and IIx MHC 5'end I 2 pre F+R primers, 56 deg.





File/Range: G:\Carola\ChIP results 11-07-08 beta and IIb primers.gel / 1029-25878 /Magnification - 1.84
User Name: Geldoc user
Image Name: G:\Carola\ChIP results 11-07-08 beta and IIb primers.gel
Image Comment:
Present Date/Time: 2008:11:12 13:35:08
Scan Date/Time: 2008:11:07 15:30:18
P ate/Time:

PCR with Beta MHC 1156,1441 primers, 32 cycles for all samples, 57 deg., 286 bp
and IIb MHC 1075, 1272 primers, 57 deg. 218 bp



Friday Nov. 14, 2008

Prepare samples for new ChIP assay.
Use frozen muscle tissue from Oct. experiment (Oct. 9 sac date)

For ground control **GC use left Soleus # 1, 66 mg** of tissue

For hind limb suspension + T3 treatment sample **HS-T3 pool right and left Soleus # 1** (37 mg + 34 mg tissue respectively), total of **71 mg** of tissue.

Prepare 1% Formaldehyde solution as follows:

to make 10 ml 1% formaldehyde		
37% formaldehyde	0.27	ml
10xPBS	1	ml
water	8.73	ml

Prepare necessary amount of 1xPBS solution and put on ice, to be used for different washes.
Need about 4.5 ml 1x PBS per sample.
When using PBS, always supplement with protease inhibitors
protease inhibitors: use leupetin, AEBSF, and aproteinin each are at 1000x stock solutions in the -80 C freezer.

Isolating and lysing cells from tissue:
Mince tissue (small pieces) using a razor blade
Put minced tissue in 1.5ml tubes in ~1 ml cold PBS supplemented with protease inhibitors
Keep on ice until all pieces are minced
Drain cold PBS, add 1 ml 1% formaldehyde buffer (freshly made)
Incubate at room temperature for 10 minutes with mixing every few minutes
After 10 minutes incubation, add 110 ul 10x glycine (1.25M glycine) prepared in a 15 ml tube.
Incubate 5 minutes at room temperature
Change solution, take out the formaldehyde PBS, replace with 1 ml cold PBS (+protease inhibitors)
Repeat PBS wash once more (use PBS supplemented with protease inhibitor)
Add 19x cold PBS (plus inhibitors) to samples
Transfer to homogenizing pestle tube, and homogenize on ice, transfer to clean 1.5ml tube. Use brand new homogenizing pestle tube this time for best results (hopefully).

Spin down the homogenate at 1500g for 10 minutes at 4°C in order to collect the cells as a pellet
Take out supernatant, use a pipet to leave a clean pellet (no liquid should be left)
Suspend the pellet in 400 ul lysis buffer (supplemented with protease inhibitors) in 1.5 ml tubes. Incubate 10-30 minutes on ice.

Freeze lysate at -80°C over night.

Monday Nov. 17, 2008

Shearing the DNA with sonication:
Use the Sonics Vibracell, 130 watts ultrasonic from sonics and materials (VCX 130)

Sonication:
Put the samples on ice at all time to keep it cool
Immerse the probe tip in the sample
Sonicate using the following protocol:

amplitude	time	# of pulses
80% max	15 sec	12

25 sec rest on ice between pulses
(monitor tube temperature, as it can heat up quickly)
Set sonicator to do 2 pulses at a time. 2 pulses = 55 sec, with 25 sec rest set. Then vortex/mix sample, and return to ice, and start next sample. Rotate thru samples like this.
Make sure that the entire sample is in the lysis buffer, as it tends to stick to the sides of tube (use pipette tip to clears sides).
Have water in the ice, so that the tube is immersed in ice-cold temp, otherwise air pockets in ice around tube can heat up.
Sonication will solubilize the chromatin and break up the DNA
After sonication, spin the samples in a cold microfuge at 12,000g for 10 minutes to remove insoluble material.

Remove **10ul aliquot** of sheared DNA to analyze on agarose gel
Transfer the supernatant (containing soluble chromatin) to a fresh microfuge tube.
Freeze at -80 to be used later for the IP procedure.

Use the 10ul aliquot from above for DNA analyses: necessary to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.

To the set aside 10ul total sheared lysate from above, add 86ul nuclease free water, and 4ul 5M NaCl.
Incubate at 65oC **overnight** to reverse the DNA-protein crosslinking.

Tuesday Nov. 18, 2008
RNA and Protein digestion:
Add 1 ul RNAse A to both tubes and incubate at 37oC for 30 minutes.
Add 7ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 2 hours.

Run 40 ul of each sample on a 2% agarose gel together with 4 ul 100bp ladder.

Also, prepare samples for quantitative DNA analysis with real time PCR machine

Results and analysis see next pages.

File/Range: G:\Carola\DNA check 11-18-08.gel / 3085-16209 /Magnification - 3.39
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Image Name: G:\Carola\DNA check 11-18-08.gel
Image Comment:
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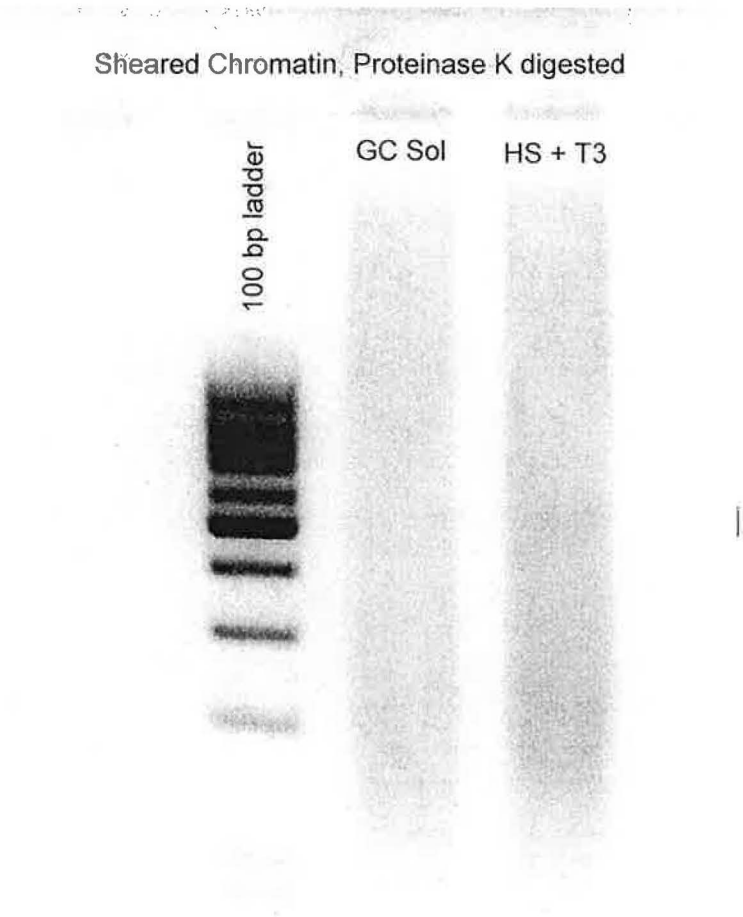
Scan Date/Time: 2008:11:18 15:29:28
P ate/Time:



These DNA samples were sheared with a total of 12 15 second pulses
DNA needs more shearing, try 6 more pulses.

														read 1	read 2
SYBR green I, dilute 1:10,000 in filtered 1x TAE (same buffer as gel running buffer)														6621	6539
add 50 ul sybr green mix to well, 5ul of sample/standard														6840	6808
	generally dilute sample 1:40													7090	7053
														13669	13446
11-18-08 samples														6995	7112
using standards from 3-11														6889	7006
														6980	7072
	ng/well/5ul							average						16365	15740
water blank	0	6539	6808	7053				**6800**	Net	predicted ng				7064	7158
S0.5	0.5	7112	7006	7072				**7063**	263	1.2				6994	7117
S1	1	7158	7117	7212				**7162**	362	1.4				7061	7212
S2.5	2.5	7374	7340	7411				**7375**	575	2.5				16223	15906
S5	5	7979	8943	8192				**8371**	1571	4.2				7218	7374
S10	10	14250	14676	11951				**13626**	6826	9.9				7299	7340
S15	15	16634	14985	15798				**15806**	9006	13.4				7281	7411
S20	20	18760	17523	18147				**18143**	11343	21.4				14715	14976
														7770	7979
									r^2	**0.9833**				8607	8943
10 ul sheared DNA taken, in final volume of 112 ul														8016	8192
samples diluted 1:40 (5:200), measured 5ul in well														15672	15372
						average	Net		**ng/ul of original chromatin sample**					13605	14250
														14109	14676
GC Sol		13446	15740	15906	14976	**15017**	8217	6.01	**538.1**					11398	11951
HS Sol + T3		15372	14398	13135	12731	**13909**	7109	9.95	**891.6**					14235	14398
														15755	16634
														14386	14985
														14939	15798
														12881	13135
														18049	18760
														16541	17523
														17445	18147
														12411	12731

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Plate sample values
F:\Carola\ChIP\Quantitative Plate Read, 11-18-2008-2.mxp
Run date: November 18, 2008

Fluorescence : Rpre

Replicates: Treated individually (since no replicates in selection)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unknown 6539	Unknown 6808	Unknown 7053	Unknown 13446								
B	Unknown 7112	Unknown 7006	Unknown 7072	Unknown 15740								
C	Unknown 7158	Unknown 7117	Unknown 7212	Unknown 15906								
D	Unknown 7374	Unknown 7340	Unknown 7411	Unknown 14976								
E	Unknown 7979	Unknown 8943	Unknown 8192	Unknown 15372								
F	Unknown 14250	Unknown 14676	Unknown 11951	Unknown 14398								
G	Unknown 16634	Unknown 14985	Unknown 15798	Unknown 13135								
H	Unknown 18760	Unknown 17523	Unknown 18147	Unknown 12731								

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Plate sample values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 11-18-2008.mxp
Run date: November 18, 2008

Fluorescence : Rpre

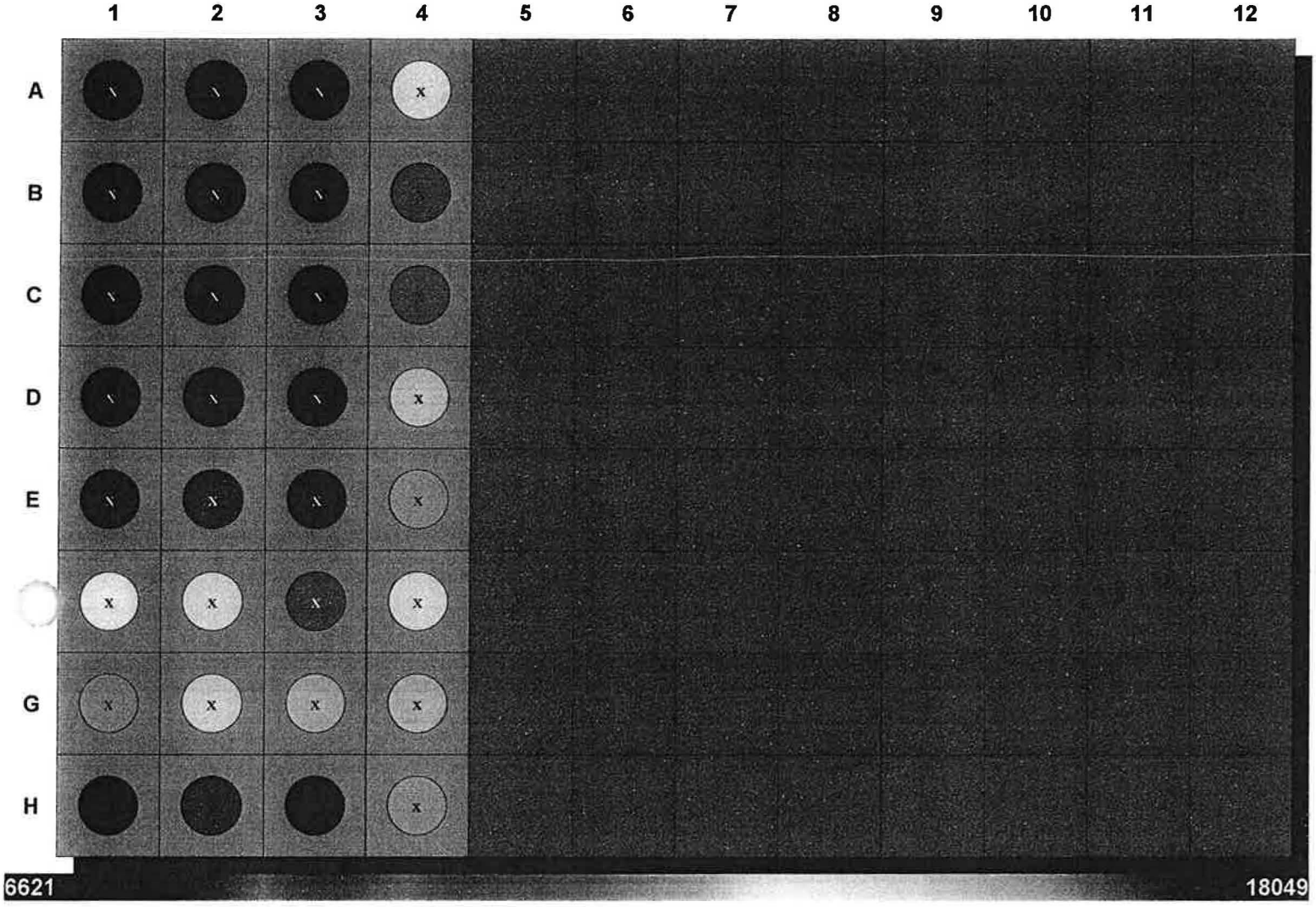
Replicates: Treated individually (since no replicates in selection)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unknown 6621	Unknown 6840	Unknown 7090	Unknown 13669								
B	Unknown 6995	Unknown 6889	Unknown 6980	Unknown 16365								
C	Unknown 7064	Unknown 6994	Unknown 7061	Unknown 16223								
D	Unknown 7218	Unknown 7299	Unknown 7281	Unknown 14715								
E	Unknown 7770	Unknown 8607	Unknown 8016	Unknown 15672								
F	Unknown 13605	Unknown 14109	Unknown 11398	Unknown 14235								
G	Unknown 15755	Unknown 14386	Unknown 14939	Unknown 12881								
H	Unknown 18049	Unknown 16541	Unknown 17445	Unknown 12411								

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Fluorescence intensity values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 11-18-2008.mxp
Run date: November 18, 2008

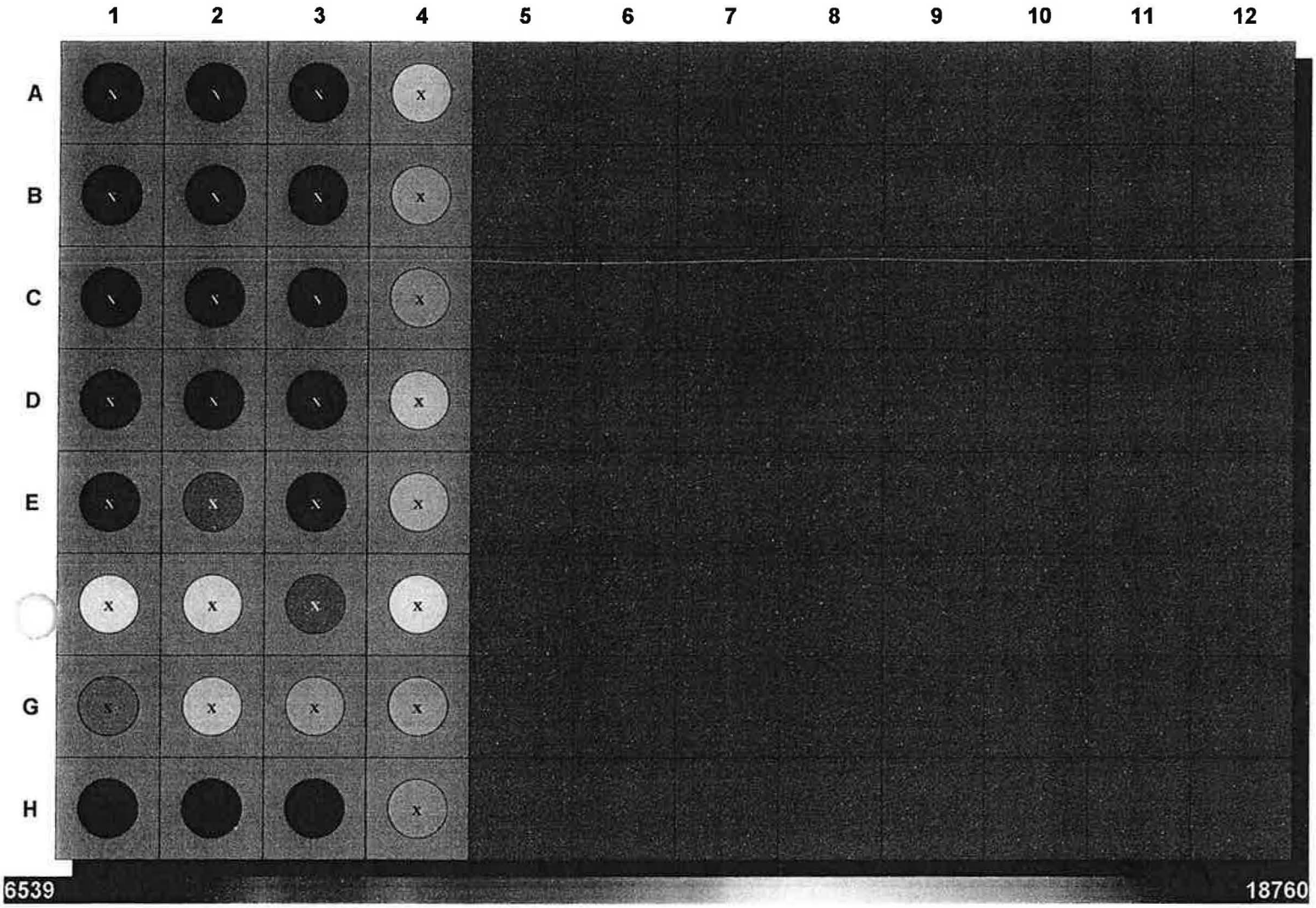
Replicates: Treated individually (since no replicates in selection)



Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Fluorescence intensity values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 11-18-2008-2.mxp
Plate date: November 18, 2008

Replicates: Treated individually (since no replicates in selection)



Wednesday Nov. 19, 2008

Since the Gel analysis showed that there was still too much high molecular weight DNA in the samples, it seems reasonable to re-shear the samples with an extra 6 15 second sonicator pulses.(same protocol as above).

After sonication take out another 10 ul of each sample and process for DNA analysis. Freeze the sheared lysate at -80 oC.

To the set aside 10ul total sheared lysate from above, add 86ul nuclease free water, and 4ul 5M NaCl.
Incubate at 65oC **overnight** to reverse the DNA-protein crosslinking.

Thursday Nov. 20, 2008
RNA and Protein digestion:
Add 1 ul RNAse A to both tubes and incubate at 37oC for 30 minutes.
Add 7ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 2 hours.

Run 40 ul of each sample on a 2% agarose gel together with 4 ul 100bp ladder.

Also, prepare samples for quantitative DNA analysis with real time PCR machine

Results and analysis see next pages.

File/Range: G:\Carola\DNA check 11-20-08.gel / 3085-18669 /Magnification - 4.36
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Image Name: G:\Carola\DNA check 11-20-08.gel
Image Comment:
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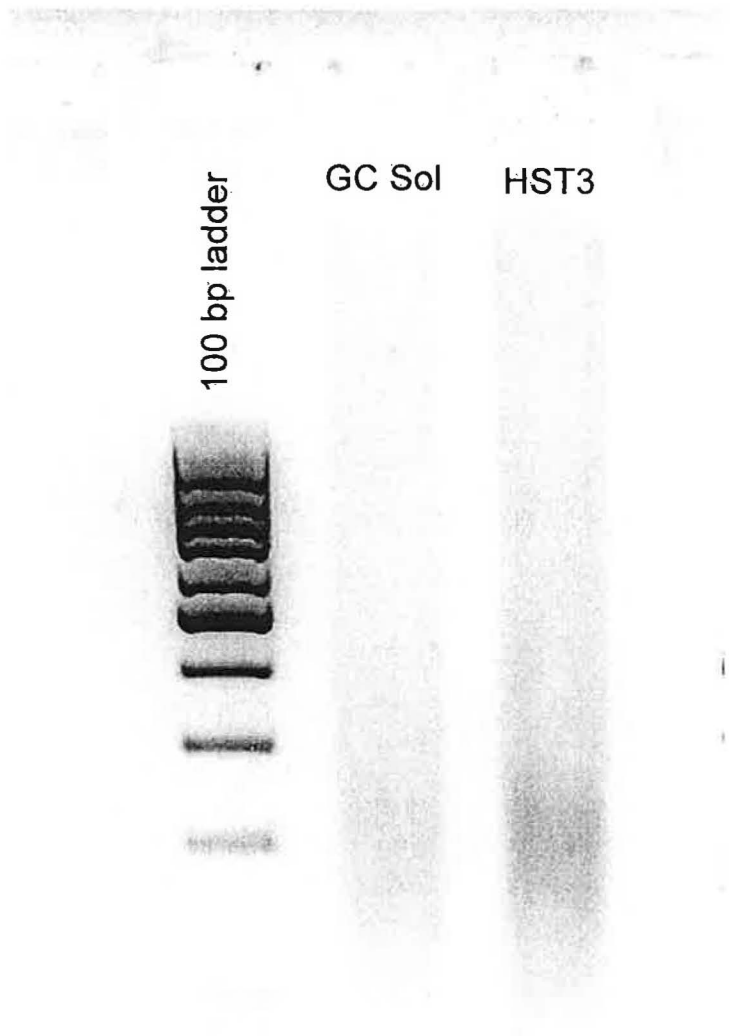
Scan Date/Time: 2008:11:20 15:12:56
Date/Time:



Re-Sheared Chromatin 11-20-08

total of 16 15sec pulses

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Plate sample values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 11-20-2008.mxp
Run date: November 20, 2008

Fluorescence : Rpre

Replicates: Treated individually (since no replicates in selection)

	1	2	3	4	5	6	7	8	9	10	11	12
A										Unknown 7451	Unknown 7444	Unknown 8288
B										Unknown 8060	Unknown 8393	Unknown 10230
C										Unknown 9048	Unknown 8349	Unknown 10416
D										Unknown 10251	Unknown 10024	Unknown 10272
E										Unknown 17128	Unknown 16054	Unknown 17353
F										Unknown 21272	Unknown 20399	Unknown 36597
G										Unknown 14255	Unknown 14602	Unknown 57734
H										Unknown 18059	Unknown 18717	Unknown 17642

													plate read 11-20-08	
SYBR green I, dilute 1:10,000 in filtered 1x TAE (same buffer as gel running buffer)													7451	
add 50 ul sybr green mix to well, 5ul of sample/standard													7444	
	generally dilute sample 1:40												8288	
													8060	
													8393	
11-18-08 samples													10230	
using standards from 3-11													9048	
													8349	
	ng/well/5ul							average					10416	
water blank	0	7451	7444					**7448**	Net	predicted ng			10251	
S0.5	0.5									1.2			10024	
S1	1	8060	8393					**8227**	779	1.4			10272	
S2.5	2.5	9048	8349					**8699**	1251	2.5			17128	
S5	5	10251	10024					**10138**	2690	4.2			16054	
S10	10	17128	16054					**16591**	9143	9.9			17353	
S15	15	21272	20399					**20836**	13388	13.4			21272	
S20	20									21.4			20399	
													36597	
									r^2	**0.9833**			14255	
10 ul sheared DNA taken, in final volume of 112 ul													14602	
samples diluted 1:40 (5:200), measured 5ul in well													57734	
						average	Net		**ng/ul of original chromatin sample**				18059	
													18717	
GC Sol			14255	14602		**14429**	6981	6.01	**538.1**				17642	
HS Sol + T3			18059	18717		**18388**	10940	9.95	**891.6**					

Std. 9143 ≙ 10 ng/5µl

GC 6981 ≙ 7.6ng/5µl = 1.52 ng/µl ×40 ×11 = 669 ng/µl (original sheared lysate)

HST3 10940 ≙ 12 ng/5µl = 2.4 ng/µl ×40 ×11 = 1056 ng/µl (original sheared lysate

For 30µg I need .669 µg ≙ 1µl
30 µg ≙ 45µl GC

dil: 1:40 and 1:11

1.056 µg ≙ µl
30 µg ≙ 28.4 µl

Friday Nov. 21, 2008

Prepare more tissue for next ChIP assay.
Use frozen muscle tissue from Oct. experiment (Oct. 9 sac date)

For ground control **GC use right Soleus # 4, 98 mg** of tissue.
Cut muscle in half and use 49 mg for each tissue prep.

For hind limb suspension + T3 treatment sample **HS-T3 use right and left Soleus # 4 and right Soleus # 6** (32 mg + 34 mg + 34 mg tissue respectively), total of **100 mg** of tissue.
Use right Sol # 4 + half of Left Sol # 4 for one tube and Right Sol # 6 + half of Left Sol # 4 for a second tissue prep.
Each tube starts out with 50 mg Soleus tissue. 4 tubes total: GC-1, GC-2, HST3-1, HST3-2

Prepare 1% Formaldehyde solution as follows:

to make 10 ml 1% formaldehyde		
37% formaldehyde	0.27	ml
10xPBS	1	ml
water	8.73	ml

Prepare necessary amount of 1xPBS solution and put on ice, to be used for different washes. When using PBS, always supplement with protease inhibitors
protease inhibitors: use leupetin, AEBSF, and aproteinin each are at 1000x stock solutions in the -80 C freezer.

Isolating and lysing cells from tissue:
Mince tissue (small pieces) using a razor blade
Put minced tissue in 1.5ml tubes in ~1 ml cold PBS supplemented with protease inhibitors. Keep on ice until all pieces are minced.
Drain cold PBS, add 1 ml 1% formaldehyde buffer (freshly made)
Incubate at room temperature for 10 minutes with mixing every few minutes
After 10 minutes incubation, add 110 ul 10x glycine (1.25M glycine) prepared in a 15 ml tube.
Incubate 5 minutes at room temperature
Change solution, take out the formaldehyde PBS, replace with 1 ml cold PBS (+protease inhibitors)
Repeat PBS wash once more (use PBS supplemented with protease inhibitor)
Add 19x cold PBS (plus inhibitors) to samples
Transfer to homogenizing pestle tube, and homogenize on ice, transfer to clean 1.5ml tube. Use the new homogenizing pestle tube this time for best results.

Spin down the homogenate at 1500g for 10 minutes at 4°C in order to collect the cells.
Take out supernatant, use a pipet to leave a clean pellet (no liquid should be left)
Suspend the pellet in 400 ul lysis buffer (supplemented with protease inhibitors) in 1.5 ml tubes. Incubate 10-30 minutes on ice.

Freeze lysate at -80°C over night.

Monday Nov. 24, 2008

Begin ChIP procedure with re-sheared chromatin (Nov. 19/20)

Wash 8 x 50µl protein A agarose (Perice) in 8 separate1.5 ml tubes with 500 µl ChIP dilution buffer (supplemented with protease inhibitors.
Spin at 1200g for 30 sec to pellet the agarose. Remove and discard supernatant.
Add chromatin and dilution buffer based on measurements made with tissue samples processed on Oct.23 (DNA analysis results Oct. 28).

This tissue prepped on 11-14-08

Sample	muscle frozen weight	in mg
400 ul lysis buffer	GC Sol	66
80 % amplitude	HS + T3 Sol	71
15 sec pulse		
12 pulses		

Re-sheared with an additional 6 pulses on Nov. 19.

Use 30µg of DNA for this ChIP

	ng/µl of original chromatin sample	µl of chromatin	for 30 µg µl buffer to yield 1ml
GC Sol	669	45	955
HS Sol + T3	1056	28.4	971.6

Prepare ~~three~~ four tubes for each muscle. Add the above amounts of chromatin solution and ChIP buffer to the washed agarose beads. Preclear chromatin for 45 min at 4 oC on rotator.
Spin at 1200g for 30 sec to pellet the agarose.
Collect the 1 ml supernatants into fresh 1.5 ml microtubes.
Remove 2x10 µl of the supernatant and **save at 4oC as input DNA** (two input DNA samples per muscle).
Add the following antibodies to the pre-cleared chromatin samples.
Four different antibodies for the two muscle chromatin samples (total of eight reactions):

1. Normal Rabbit IgG from Upstate (12-370) used 1.0 µl (1.0 µg).

2. Pan-H3 (abcam 1791), use 1.5 µl.

3. Anti-Histone H3 K4 me3 antibody (ab 8580), use 2.0 µl (~ 2 µg).

4. Anti-Acetyl Histone H3 antibody (Upstate #06-599), used 1.0 ul (~1 ug).

Incubate over night with antibodies. Tubes are in refrigerator on rotator.

Process Friday's tissue lysates (4 tubes).
Shearing the DNA with sonication:
Use the Sonics Vibracell, 130 watts ultrasonic from sonics and materials (VCX 130)
Sonication:
Put the samples on ice at all time to keep it cool
Immerse the probe tip in the sample
Sonicate using the following protocol:

amplitude	time	# of pulses
80% max	15 sec	12

25 sec rest on ice between pairs of pulses

Set sonicator to do 2 pulses at a time. 2 pulses = 55 sec, with 25 sec rest set. Then vortex/mix sample, and return to ice, and start next sample. Rotate thru samples like this. Make sure that the entire sample is in the lysis buffer, as it tends to stick to the sides of tube (use pipette tip to clears sides).
Have water in the ice, so that the tube is immersed in ice-cold temp, otherwise air pockets in ice around tube can heat up.

After sonication, spin the samples in a cold microfuge at 12,000g for 10 minutes to remove insoluble material.

Remove **10ul aliquot** of sheared DNA to analyze on agarose gel
Transfer the supernatant (containing soluble chromatin) to a fresh microfuge tube.
Freeze at -80 to be used later for the IP procedure.

Use the 10ul aliquot from above for DNA analyses: necessary to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.

To each set aside 10ul total sheared lysate from above, add 86ul nuclease free water, and 4ul 5M NaCl.

Incubate at 65oC **overnight** to reverse the DNA-protein crosslinking.

Tuesday Nov. 25, 2008

Continue DNA analysis to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.
Add 1 ul RNAse A (10ug/ul) to both samples and incubate 30 minutes at 37oC
Add 7ul (digestion buffer, see below) & incubate at 45oC for 2 hours.
Digestion buffer=2ul 0.5M EDTA, 4ul 1M Tris-HCL pH 6.5, and 1ul proteinase K(10ug/ul).
Load 40 µl on 2 % agarose gel with a 100 bp ladder with gel-green staining.
Gel results see next page.

File/Range: G:\Carola\DNA check 11-25-08.gel / 2315-24275 /Magnification - 2.65
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Image Name: G:\Carola\DNA check 11-25-08.gel
Image Comment:
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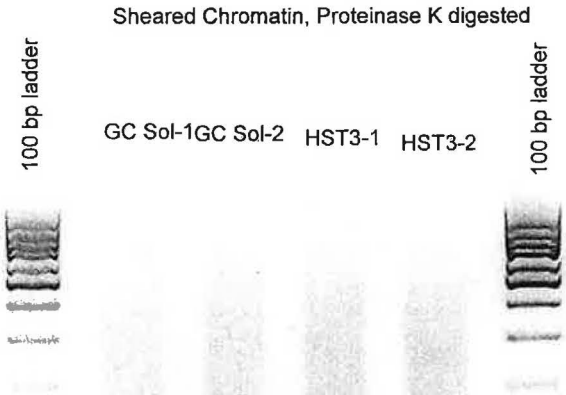
Scan Date/Time: 2008:11:25 15:33:24
P[illegible]ate/Time:

11-25-08



DNA looks good

File/Range: I:\Captures\Capture_00288.tif / 2315-17688 /Magnification - 5.78
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Image Name: I:\Captures\Capture_00288.tif
Image Comment:
Present Date/Time: 2008:11:25 14:22:40
Scan Date/Time: 2008:11:25 15:33:24
Date/Time:

GC-1 GC-2 HST3-1 HST3-2

Tuesday Nov. 25, 2008 - Continued

Continue ChIP procedure:
Wash another 8 x 50µl agarose beads with ChIP dilution buffer. Spin and discard supernatant. Add the washed beads to the chromatin antibody mixtures and incubate for another 2 hours in the refrigerator on rotator.
Pellet protein agarose by spinning at 1200 g for 40sec at 4oC.Discard the supernatant.
Wash the protein agarose-antibody chromatin samples by resuspending the beads in 1 ml of the cold buffers in the order listed
First use 1 ml Low salt Immune complex Wash buffer wash 10sec and spin, discard supernatant, then wash as listed:
For each wash, incubate for 15 minutes on a rotating platform followed by spin 1000g for 20sec at 4oc (supplementation with protease inhibitors is not necessary for the wash solutions.
Discard the supernatant after each wash
Wash buffer in the order to be used:

a	Once wash with Low salt Immune complex Wash buffer
b	Once wash with high salt Immune complex Wash buffer
c	Once wash with LiCl complex Wash buffer
d	twice wash with TE buffer

remove supernatant and procede to elution of the protein/DNA from the agaraose beads

Elution of Protein/DNA complexes
Bring 1M NaHCO3 solution to room temperature. Vortex at room temperature to dissolve precipitate
Set water bath to 65oC to use for reverse linking(next section)

Prepare elution buffer for all IP tubes as well as for input DNA tube (which was 1% of DNA in the IP)
For 10 tubes, make 11 x to have some extra

elution buffer	1x	6.5	11x	
20% SDS	10	65	110	
1M NaHCO3	20	130	220	+NaCl
sterile di Water	170	1105	1870	52
Sum	200	1300	2200	

For input DNA, add 200ul elution buffer and set aside at room tempertaure until reverse cross-linking
Add 100ul of elution buffer to each IP tube containing the agarose pellet

Mix by flicking the tube gently
Incubate at room temperature for 15 minutes
Pellet the agarose by brief centrifugation 1200 g for 40sec at 4oC)
Collect and save the supernatant into a new microfuge
Repeat the elution with another 100ul elution buffer.
Collect the supernatant and combine with the previous step 100ul, to make a total of 200ul eluate

Reverse crosslinks of Protein/DNA complexes to free the DNA

To all the tubes (input DNA and IP), add 8ul 5M NaCl
Incubate at 65oC overnight.
There are 12 tubes total: 4 input and 8 experimental

Wednesday, November 26, 2008

RNA and Protein digestion:
To all tubes, add 1 ul RNAse A and incubate at 37oC for 30 minutes.
Add 13ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 2 hours.

digestion buffer premix:	**1x**	**14x**
0.5M EDTA	4	56
1M Tris pH6.5	8	112
proteinase K	1	14
	13	182

DNA purification using spin columns
Use Qiagen miniprep columns
To each of the above tubes (input DNA and IP DNA) add 1 ml binding reagent"A"
Mix well.
A precipitate might form, this will not interfere with the procedure
Transfer 600ul of sample/Bind reagent A mixture to the column
Spin 30 sec at 12,000 g
Discard flow through
Add the remaining 600 ul to the column
Spin again and discard flow through
Add 750ul wash buffer of Qiagen to the column
Spin at 12,000 g
Discard flow through
Spin again for 12,000 g for 2min.
Discard the collection tube
Put the column in a new 1.5 ml collection tube
Add 135 ul warm water to elute the DNA off the column.
Spin at 12,000g for 2 minute (be careful to not zip the lids off the tubes.)
Eluate is pure DNA that can be analyzed by PCR
Use 12 µl of this DNA per 25 µl PCR reaction (use 17 µl of eluted DNA for trimethylated (H3 K4 me3) samples.
Freeze samples at -20 oC.

Thanksgiving Break Thursday and Friday, November 27/28, 2008

Monday, December 1, 2008
DNA analysis with muscle tissue lysates using Real Time PCR machine. Nov 21 tissue processing
Results and analysis see next pages.

Mx3000P

Monday Dec. 1

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Plate sample values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 12-1-2008.mxp
Run date: December 01, 2008

DNA analysis of muscle tissue lysates

Nov. 21 tissue processing

Fluorescence : Rpre

Replicates: Treated individually (since no replicates in selection)

Standards

donor V use data

	1	2	3	4	5	6	7	8	9	10	11	12
A					Unknown water 8110	Unknown 8024	Unknown 7626	Unknown GC-1 12180	Unknown 17023	Unknown H5T2-1 15980		
B					Unknown 0.5ng 11074	Unknown 8146	Unknown 8195	Unknown 13886	Unknown 15677	Unknown 16589		
C					Unknown 1ng 9511	Unknown 8020	Unknown 9534	Unknown 13689	Unknown 16301	Unknown 17421		
D					Unknown 2.5ng 8115	Unknown 8669	Unknown 8273	Unknown 13073	Unknown 16151	Unknown 16762		
E					Unknown 5ng 9789	Unknown 9554	Unknown 10010	Unknown GC-2 13609	Unknown 16821	Unknown H5T3-2 17309		
F					Unknown 10ng 16227	Unknown 15245	Unknown 15931	Unknown 13235	Unknown 13786	Unknown 18605		
G					Unknown 15ng 18926	Unknown 20305	Unknown 19007	Unknown 13602	Unknown 14428	Unknown 22474		
H					Unknown 20ng 21025	Unknown 21927	Unknown 22106	Unknown 13285	Unknown 17426	Unknown 18640		

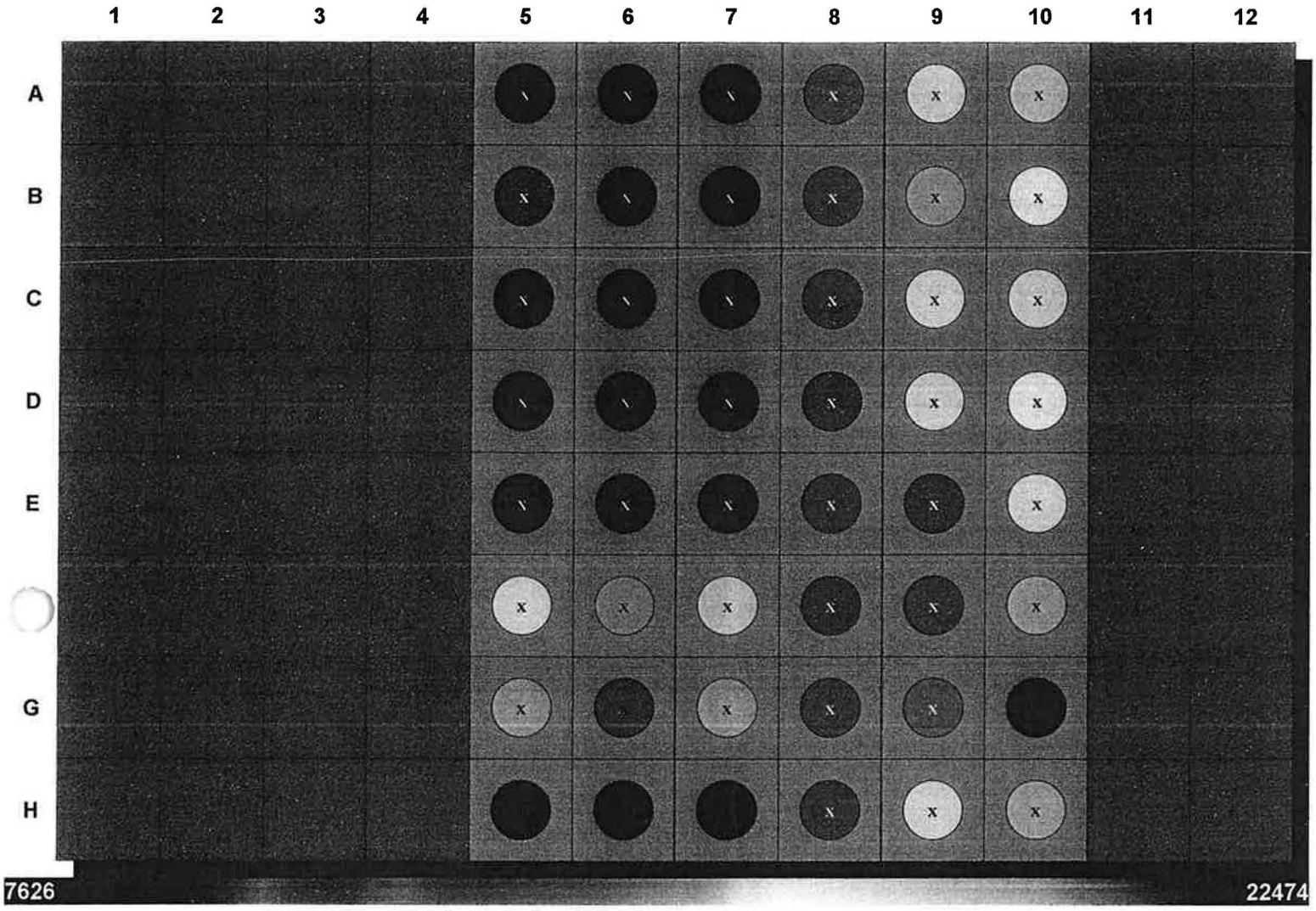
Sample diluted 1:40 into water

Use TAE buffer (10 ml) with Cybr green dye (1 µl @ 10,000x stock) 50 µl / well. Triplicates of standards, Quadruplets of samples (5 µl into 50 µl buffer per well).

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Fluorescence intensity values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 12-1-2008.mxp
Run date: December 01, 2008

Replicates: Treated individually (since no replicates in selection)



12-1-08

1-Dec-08

SYBR green I, dilute 1:10,000 in filtered 1x TAE (same buffer as gel running buffer)
add 50 ul sybr green mix to well, 5ul of sample/standard
generally dilute sample 1:40

11-21-08 samples
using standards from 3-11

	ng/well/5ul					average	Net	predicted ng
water blank	0	8110	8024	7626		**7920**	Net	predicted ng
S0.5	0.5		8146	8195		**8171**	251	1.2
S1	1	9511	8020	9534		**9022**	1102	1.4
S2.5	2.5	8115	8669	8273		**8352**	432	2.5
S5	5	9789	9554	10010		**9784**	1864	4.2
S10	10	16227	15245	15931		**15801**	7881	9.9
S15	15	18926	20305	19007		**19413**	11493	13.4
S20	20	21025	21927	22106		**21686**	13766	21.4
							r^2	**0.9833**

10 ul sheared DNA taken, in final volume of 112 ul
samples diluted 1:40 (5:200), measured 5ul in well

					average	Net		**ng/ul of original chromatin sample**
GC Sol-1	12180	13886	13689	13073	**13207**	5287		**590.4**
GC Sol-2	13609	13235	13602	13285	**13433**	5513		**615.6**
HS Sol + T3-1	15980	16589	17421	16762	**16688**	8768		**979.0**
HS Sol + T3-2	17309	18605		18640	**18185**	10265		**1146.2**

Std 7881 ≙ 10ng/5µl
GC-1 5287 ≙ 6.7 ng/5µl = 1.34 ng/µl × 40 × 11 = 590 ng/µl
HSTJ-1 8768 ≙ 11.13ng/5µl = 2.23 ng/µl × 40 × 11 = 979 ng/µl

.5896 µg = 1µl
30 µg = 51µl

.979 µg = 1µl
30 µg = 31µl

need 30 µg Chromatin per ChIP

Tuesday, December 2, 2008

Set up PCR reactions with the Nov. 26 samples.

Use the beta MHC +1156 / +1441 primer set at 57 °C annealing and
The IIb 1075 F / 1272 R primer set also at 57°C annealing.
The beta Actin +1517 F / 1741 R primer set at 56 °C annealing and
The IIx 5'end I 2 pre F + R primer set also at 56°C annealing.

Total of 48 samples.
Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

32 cycles of
60 sec. 96°C, 45 sec. 57 / 56 °C, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines: 32 cycles for all samples.

Run samples on two 2% agarose gels with 100bp ladder.

Results see next pages

For next time, run samples with same antibody side by side for better analysis.

File/Range: G:\Carola\Beta Actin and IIx Primer ChIP PCR results 12-02-08.gel / 2829--30805.000 /Magnification - 2.19
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Image Comment:
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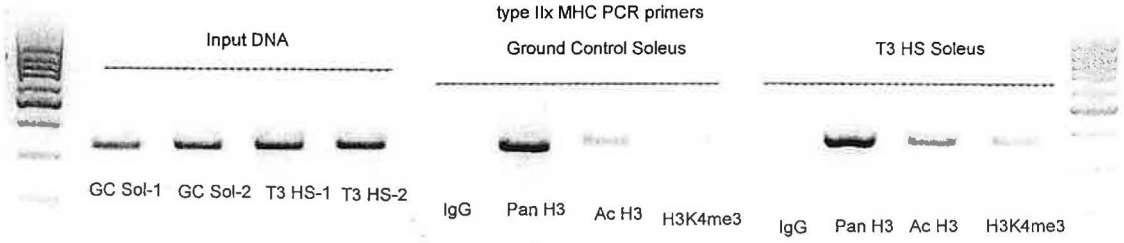
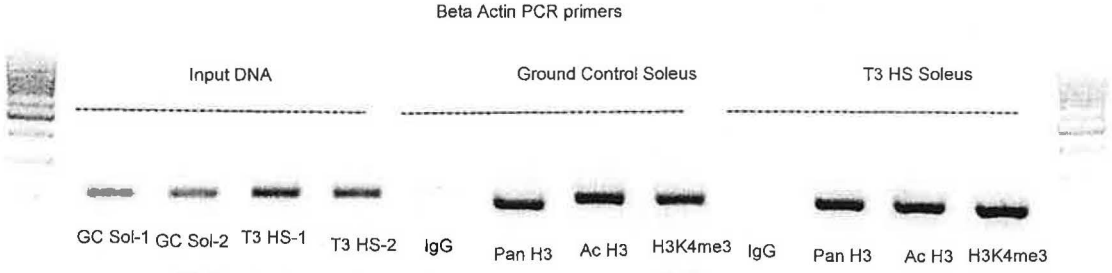
Scan Date/Time: 2008:12:02 15:46:16
P. ate/Time:

Tuesday Dec. 2

PCR with Beta Actin 1517,1741 primers, 32 cycles for all samples, 56 deg
and IIx MHC 5'end I 2 pre F+R primers, 56 deg.





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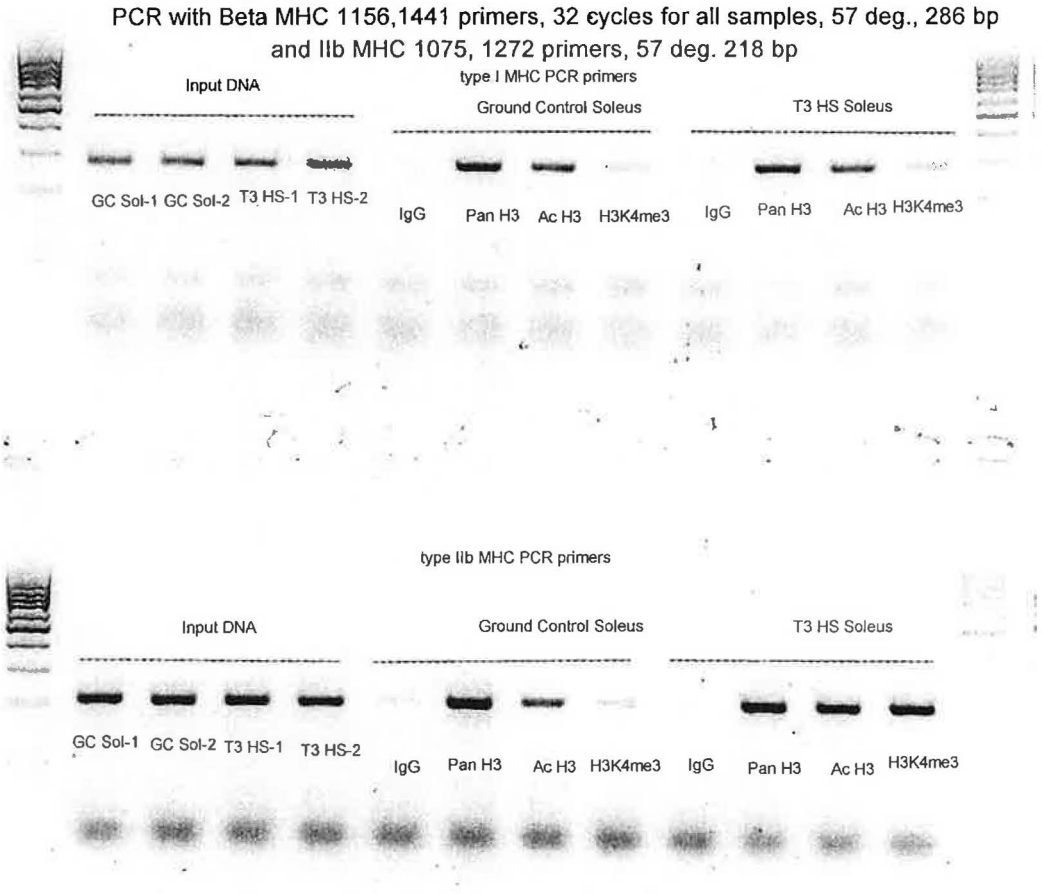
Dec. 2, 2008



Wednesday, December 3, 2008

Start new ChIP experiment with processed and analyzed (see Dec.1) Soleus GC-1 and HST3-1 chromatin lysate.
Wash 8 x 50µl protein A agarose (Perice) in 8 separate1.5 ml tubes with 500 µl ChIP dilution buffer (supplemented with protease inhibitors).
Spin at 1000g for 30 sec to pellet the agarose. Remove and discard supernatant.
Add chromatin and dilution buffer based on measurements made with tissue samples on Dec. 1.

This tissue prepped on 11-21-08

	Sample	muscle frozen weight in mg	
400 ul lysis buffer		GC Sol 1/2	49 x 2 = 98 mg
80 % amplitude		HS + T3 Sol 1/2	32 + 34 + 34 = 100 mg total
15 sec pulse			
12 pulses			

Use 30µg of DNA for this ChIP

	ng/µl of original chromatin sample	µl of chromatin	for 30 µg µl buffer to yield 1ml
GC Sol - 1	590	51	949
HS Sol + T3	979	31	969

Prepare four tubes for each muscle. Add the above amounts of chromatin solution and ChIP buffer to the washed agarose beads. Preclear chromatin for 45 min at 4 oC on rotator.
Spin at 1000g for 30 sec to pellet the agarose.
Collect the 1 ml supernatants into fresh 1.5 ml microtubes.
Remove 2x10 µl of the supernatant and **save at 4oC as input DNA** (one input DNA sample per muscle).
Add the following antibodies to the pre-cleared chromatin samples.
Four different antibodies for the two muscle chromatin samples (total of eight reactions):

1. Normal Rabbit IgG from Upstate (12-370) used 1.0 µl (1.0 µg).

2. Pan-H3 (abcam 1791), use 1.5 µl.

3. Anti-Histone H3 K4 me3 antibody (ab 8580), use 2.0 µl (~ 2 µg).

4. Anti-Acetyl Histone H3 antibody (Upstate #06-599), used 1.0 ul (~1 ug).

Incubate over night with antibodies. Tubes are in refrigerator on rotator.

Thursday, December 4, 2008

Continue ChIP procedure:
Wash another 8 x 50µl agarose beads with ChIP dilution buffer. Spin and discard supernatant. Add the washed beads to the chromatin antibody mixtures and incubate for another 2 hours in the refrigerator on rotator.
Pellet protein agarose by spinning at 1000 g for 40sec at 4oC.Discard the supernatant.
Wash the protein agarose-antibody chromatin samples by resuspending the beads in 1 ml of the cold buffers in the order listed
First use 1 ml Low salt Immune complex Wash buffer wash 10sec and spin, discard supernatant, then wash as listed:
For each wash, incubate for 15 minutes on a rotating platform followed by spin 1000g for 20sec at 4oc (supplementation with protease inhibitors is not necessary for the wash solutions.
Discard the supernatant after each wash
Wash buffer in the order to be used:

a	Once wash with Low salt Immune complex Wash buffer
b	Once wash with high salt Immune complex Wash buffer
c	Once wash with LiCl complex Wash buffer
d	twice wash with TE buffer

remove supernatant and procede to elution of the protein/DNA from the agaraose beads

Elution of Protein/DNA complexes
Bring 1M NaHCO3 solution to room temperature. Vortex at room temperature to dissolve precipitate
Set water bath to 65oC to use for reverse linking(next section)

Prepare elution buffer for all IP tubes as well as for input DNA tube (which was 1% of DNA in the IP)
For 10 tubes, make 11 x to have some extra

elution buffer	1x	6.5	11x	
20% SDS	10	65	110	
1M NaHCO3	20	130	220	+NaCl
sterile di Water	170	1105	1870	52
Sum	200	1300	2200	

For input DNA, add 200ul elution buffer and set aside at room tempertaure until reverse cross-linking
Add 100ul of elution buffer to each IP tube containing the agarose pellet.

Mix by flicking the tube gently
Incubate at room temperature for 15 minutes
Pellet the agarose by brief centrifugation 1000 g.
Collect and save the supernatant into a new microfuge
Repeat the elution with another 100ul elution buffer.
Collect the supernatant and combine with the previous step 100ul, to make a total of 200ul eluate.

Reverse crosslinks of Protein/DNA complexes to free the DNA

To all the tubes (input DNA and IP), add 8ul 5M NaCl
Incubate at 65oC overnight.
There are 10 tubes total: 2 input and 8 experimental

Friday, December 5, 2008

RNA and Protein digestion:
To all tubes, add 1 ul RNAse A and incubate at 37oC for 30 minutes.
Add 13ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 2 hours.

digestion buffer premix:	**1x**	**11x**
0.5M EDTA	4	44
1M Tris pH6.5	8	88
proteinase K	1	11
	13	143

DNA purification using spin columns

Use Qiagen miniprep columns

To each of the above tubes (input DNA and IP DNA) add 1 ml binding reagent"A"
Mix well.
A precipitate might form, this will not interfere with the procedure
Transfer 600ul of sample/Bind reagent A mixture to the column
Spin 30 sec at 12,000 g
Discard flow through
Add the remaining 600 ul to the column
Spin again and discard flow through
Add 750ul wash buffer of Qiagen to the column
Spin at 12,000 g
Discard flow through
Spin again for 12,000 g for 2min.
Discard the collection tube
Put the column in a new 1.5 ml collection tube
Add 135 ul warm water to elute the DNA off the column.
Spin at 12,000g for 2 minute (be careful to not zip the lids off the tubes.)
Eluate is pure DNA that can be analyzed by PCR
Use 12 µl of this DNA per 25 µl PCR reaction.
Freeze samples at -20 oC.

Monday, December 8, 2008

Set up PCR reactions with Friday's (12-5) samples (+ two input samples from previous experiment).

Use the beta MHC +1156 / +1441 primer set at 57 °C annealing and
The IIb 1075 F / 1272 R primer set also at 57°C annealing.
The beta Actin +1517 F / 1741 R primer set at 56 °C annealing and
The IIx 5'end I 2 pre F + R primer set also at 56°C annealing.

Total of 48 samples.
Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

33 cycles of
60 sec. 96°C, 45 sec. 57 / 56 °C, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines: 32 cycles for all samples.

Run samples on two 2% agarose gels with 100bp ladder.

Results see next pages.

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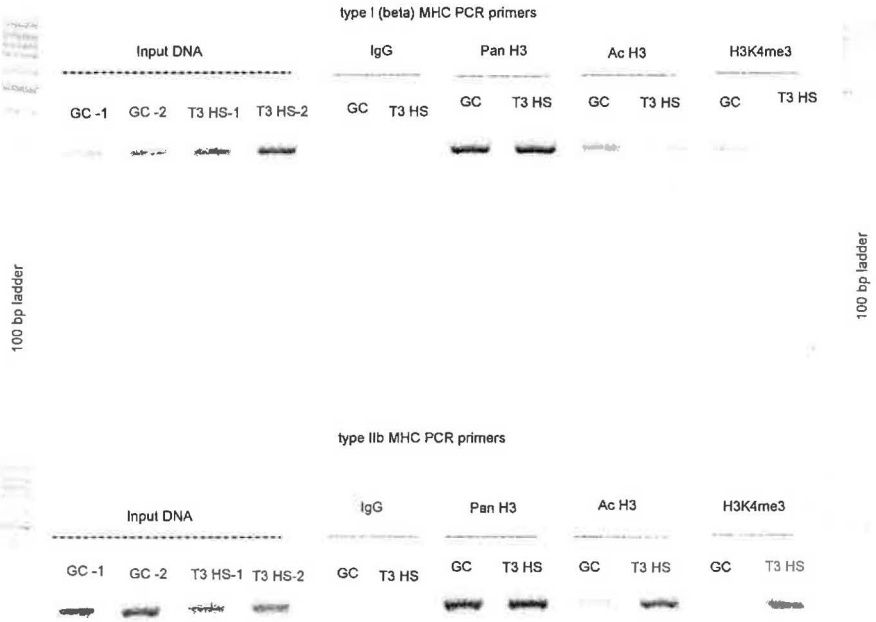
PCR with Beta MHC 1156,1441 primers, 33 cycles for all samples, 57 deg., 286 bp
and IIb MHC 1075, 1272 primers, 57 deg. 218 bp



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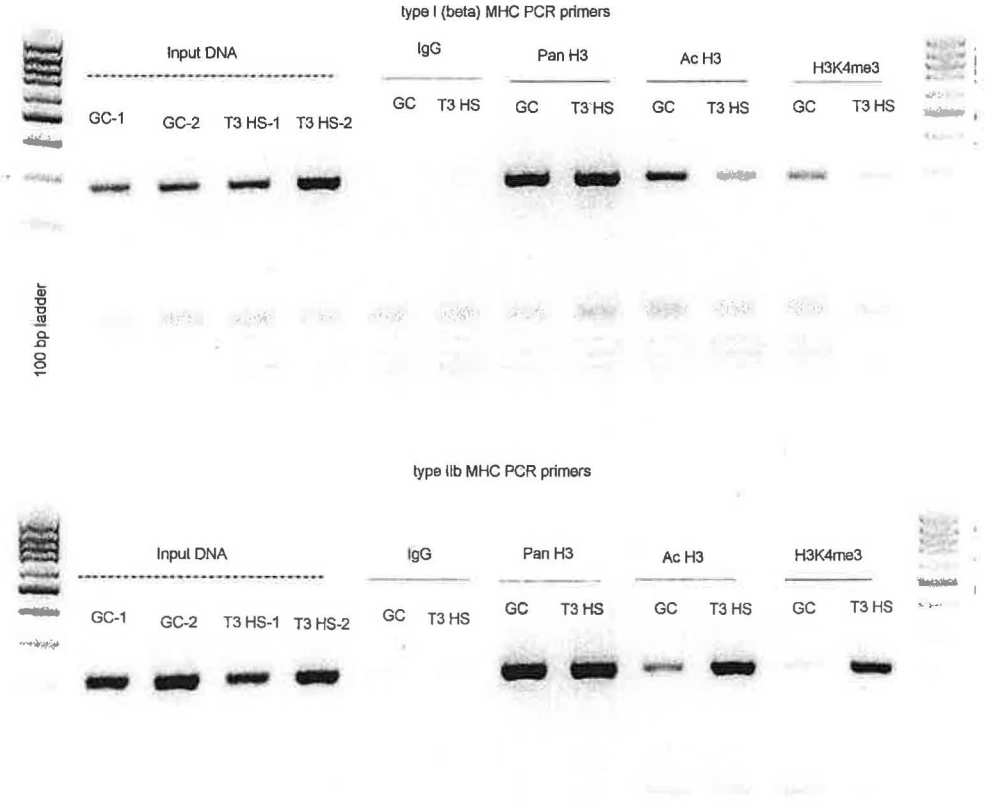
PCR with Beta MHC 1156,1441 primers, 33 cycles for all samples, 57 deg., 286 bp
and IIb MHC 1075, 1272 primers, 57 deg. 218 bp



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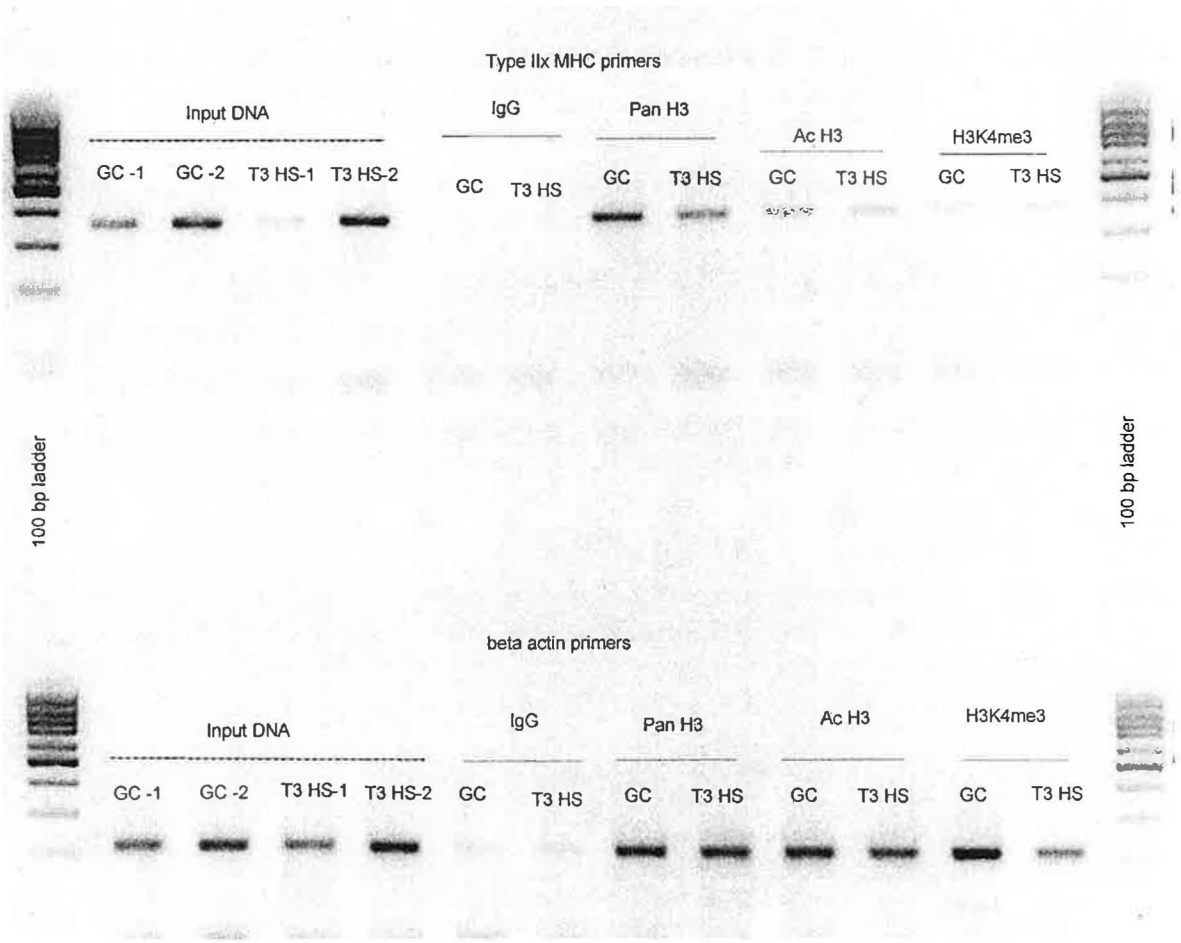

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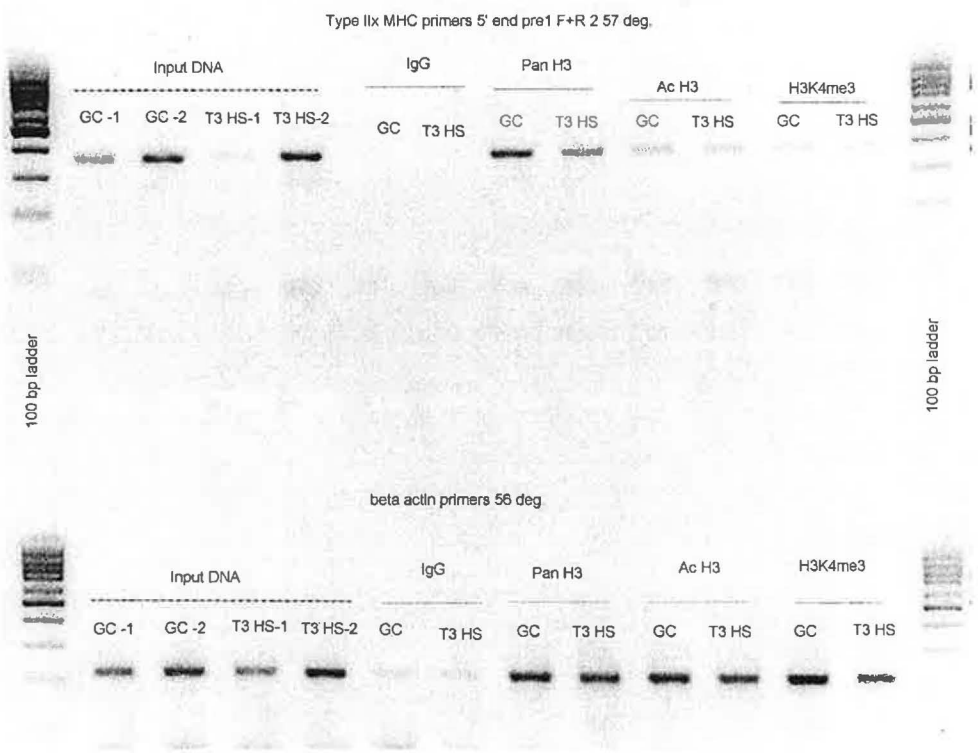

Thursday - Friday, January 22 & 23, 2009

Set up more PCR reactions with the12-5 samples (+ two input samples from previous experiment).

Use the beta MHC +1156 / +1441 primer set at 57 °C annealing and
The IIb 1075 F / 1272 R primer set also at 57°C annealing.
The beta Actin +1517 F / 1741 R primer set at 56 °C annealing and
The IIx 5'end pre 2 F + R 2 primer set at 57°C annealing.

Total of 48 samples.
Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

31/33 cycles of
60 sec. 96°C, 45 sec. 57 / 56 °C, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines:
33 cycles for the trimethylation samples.
31 cycles for all other samples

Run samples on two 2% agarose gels with 100bp ladder.

Results see next pages.

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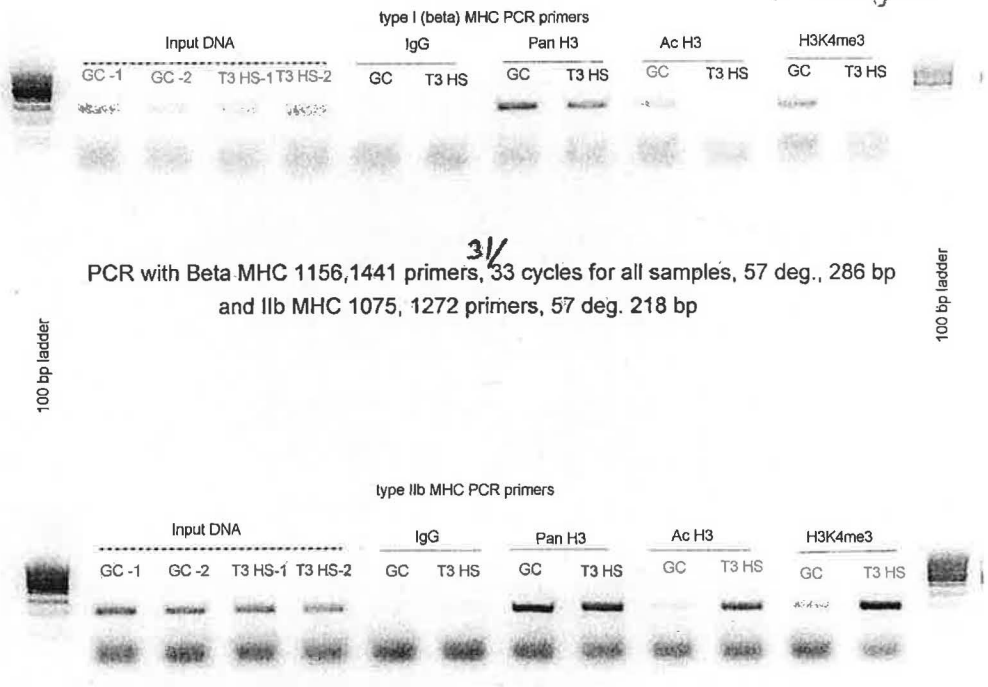
P Date/Time:

31 cycles

33 cycles



31

PCR with Beta MHC 1156,1441 primers, 33 cycles for all samples, 57 deg., 286 bp
and IIb MHC 1075, 1272 primers, 57 deg. 218 bp

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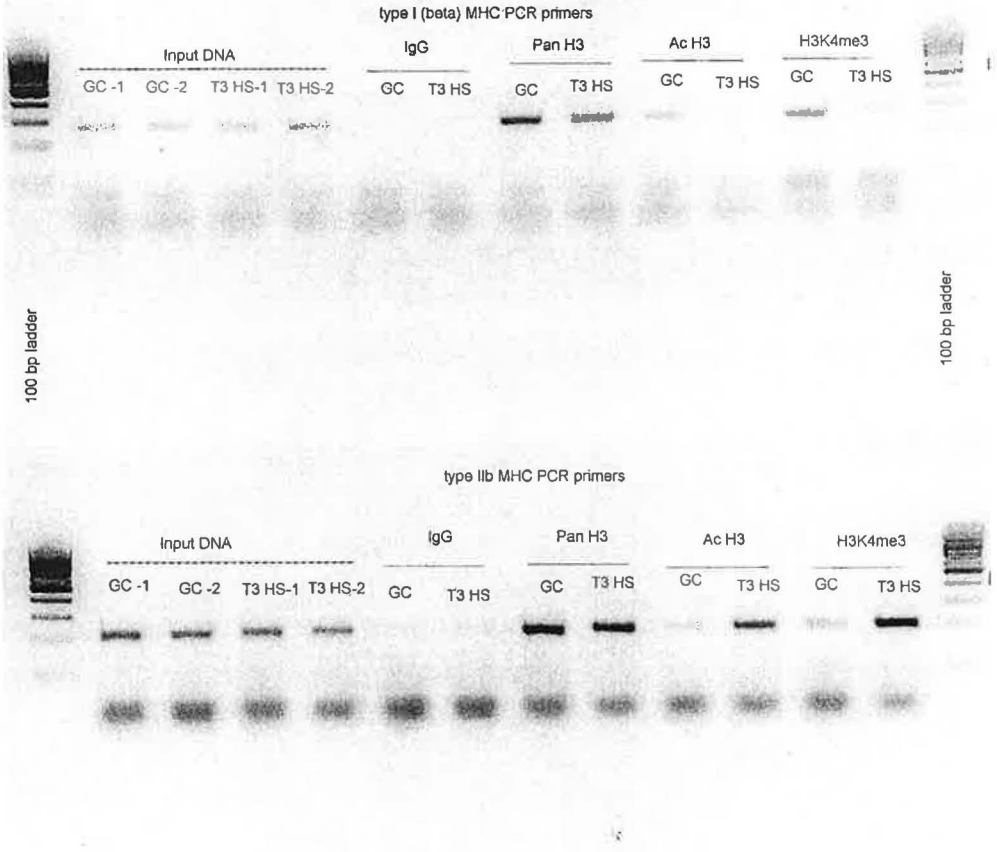
PCR with Beta MHC 1156,1441 primers, 33 cycles for all samples, 57 deg., 286 bp
and IIb MHC 1075, 1272 primers, 57 deg. 218 bp



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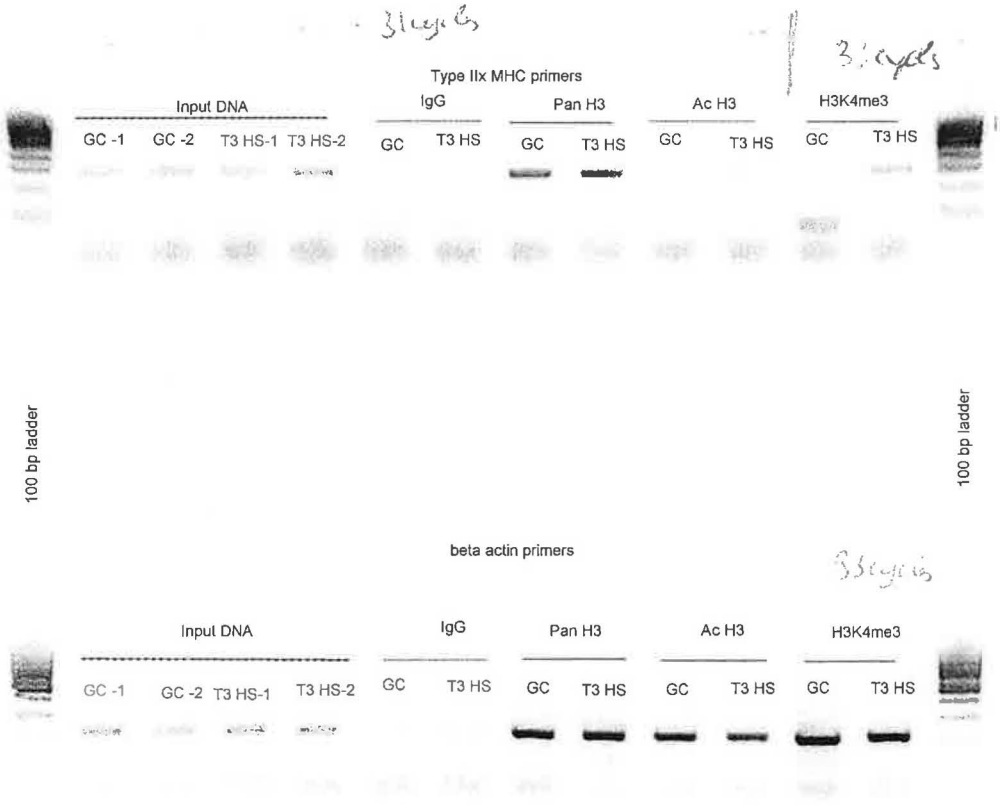

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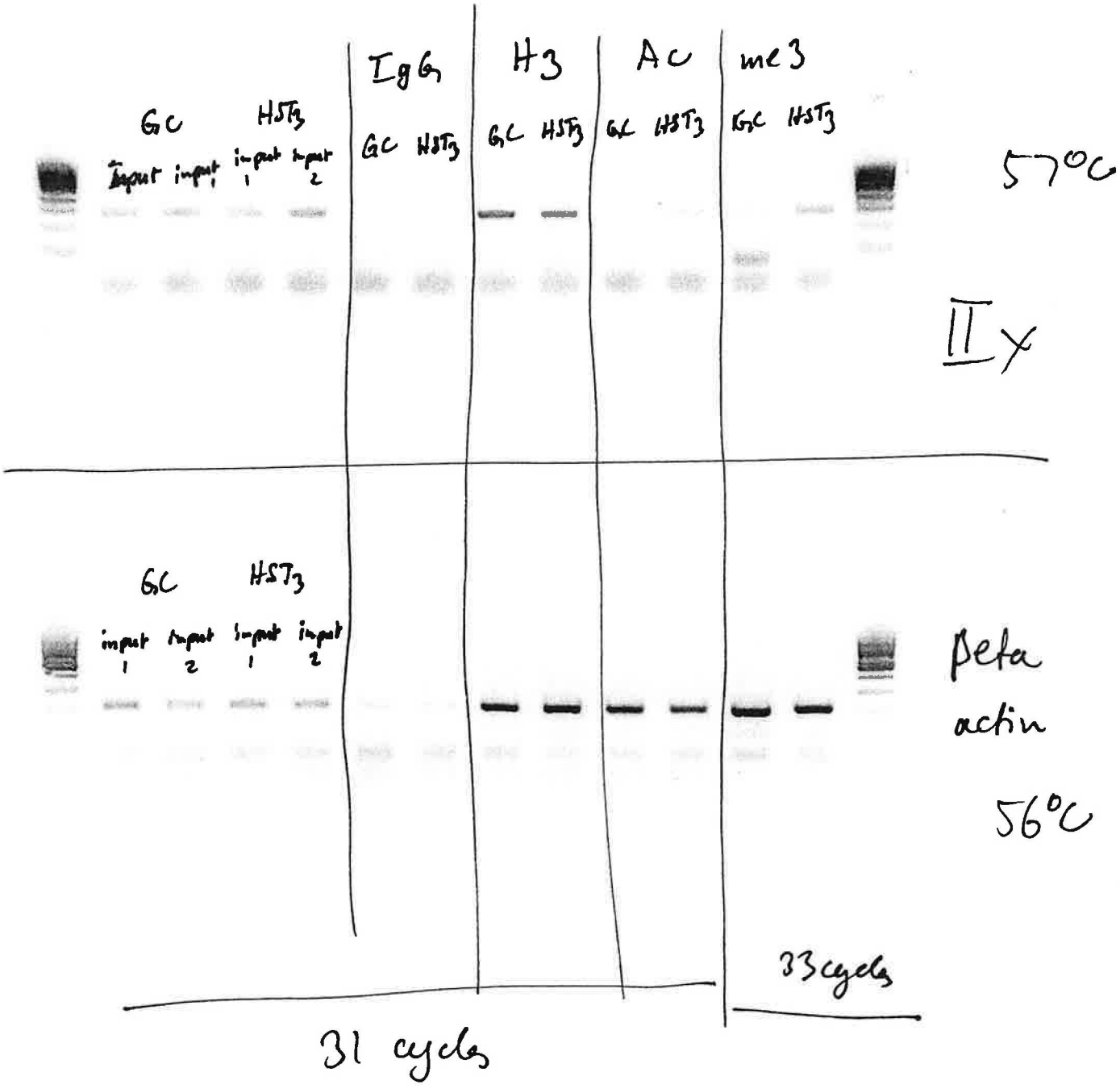

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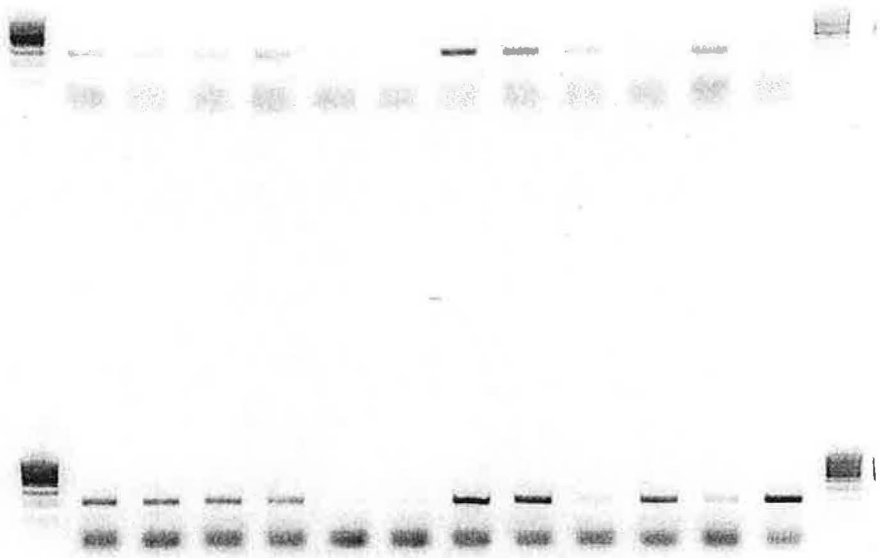

Wednesday and Thursday, January 28 & 29, 2009

Follow up PCR reactions with the 11-26 and 12-5 ChIP samples.

Use the beta MHC +1156 / +1441 primer set at 57 °C annealing and
The IIb 1075 F / 1272 R primer set also at 57°C annealing.

Total of 37 samples.
The 11-26 ChIP samples series has no input DNA sample for the type 1 MHC primer and only one input DNA sample for the II primer.
Next time skip the IgG samples if run out of PCRmix. Especially if I already know that the IgG sample is clean.
Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

31/33 cycles of
60 sec. 96°C, 45 sec. 57, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines:
33 cycles for the trimethylation samples.
31 cycles for all other samples

Run samples on two 2% agarose gels with 100bp ladder.

Gel analysis and results see next pages.

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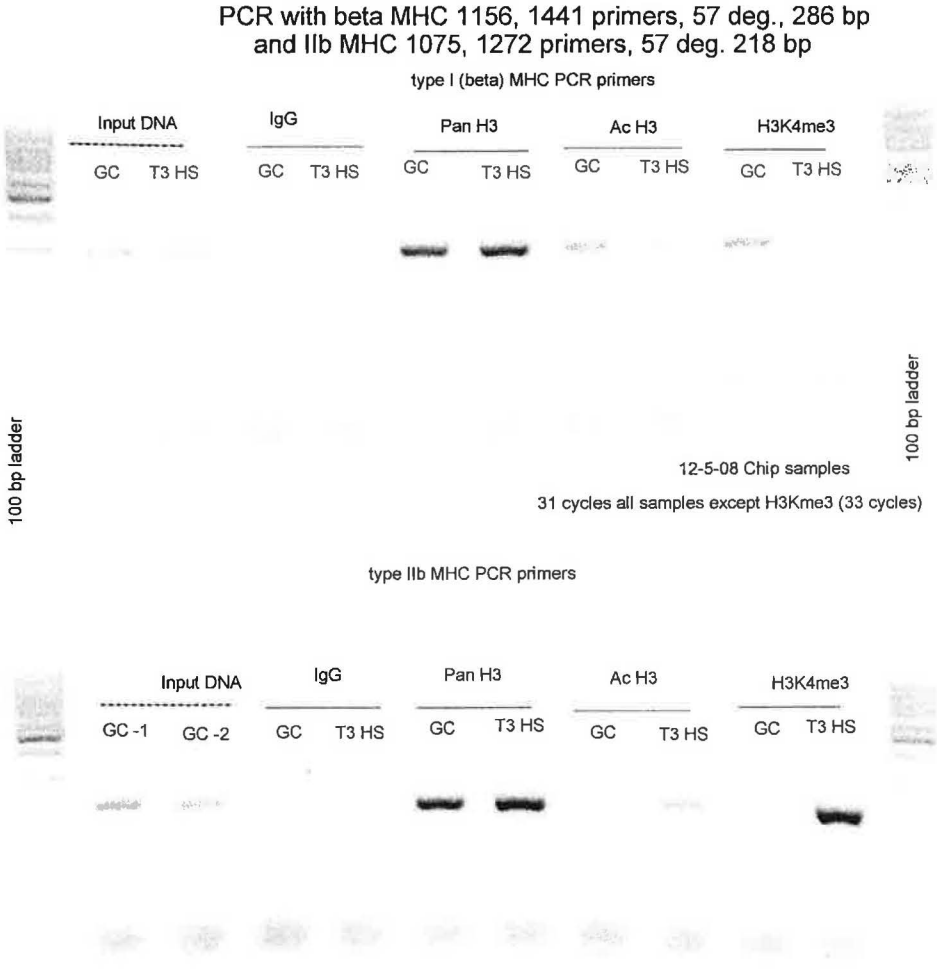

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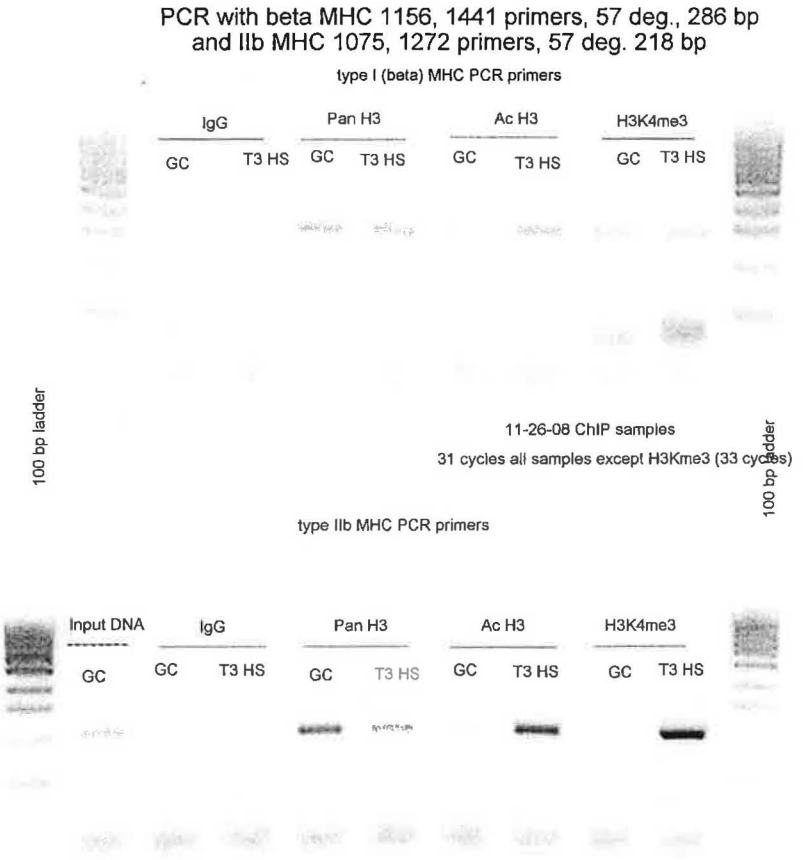

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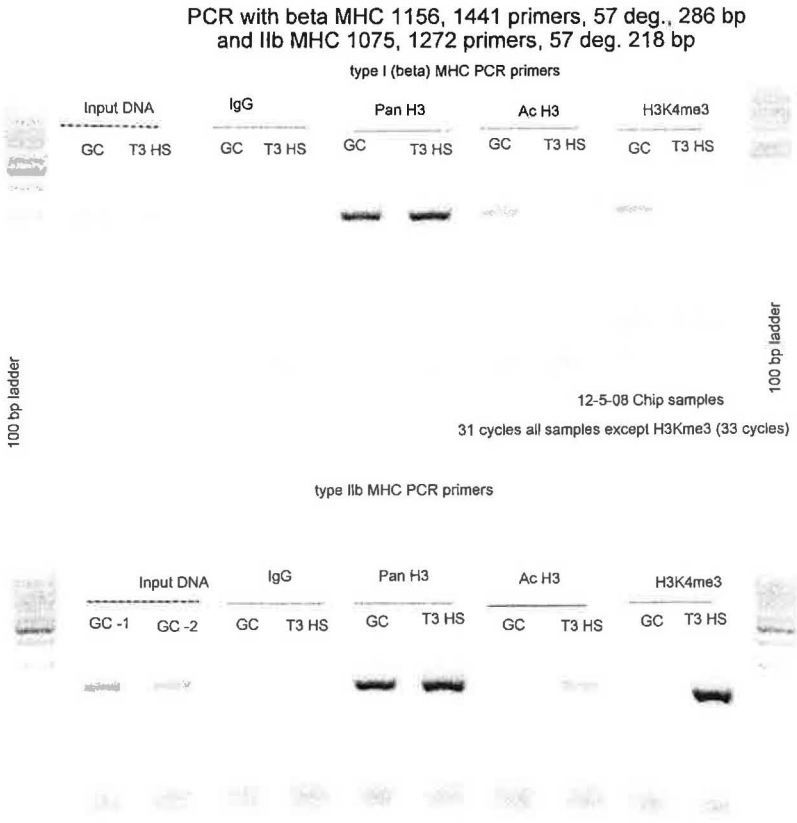

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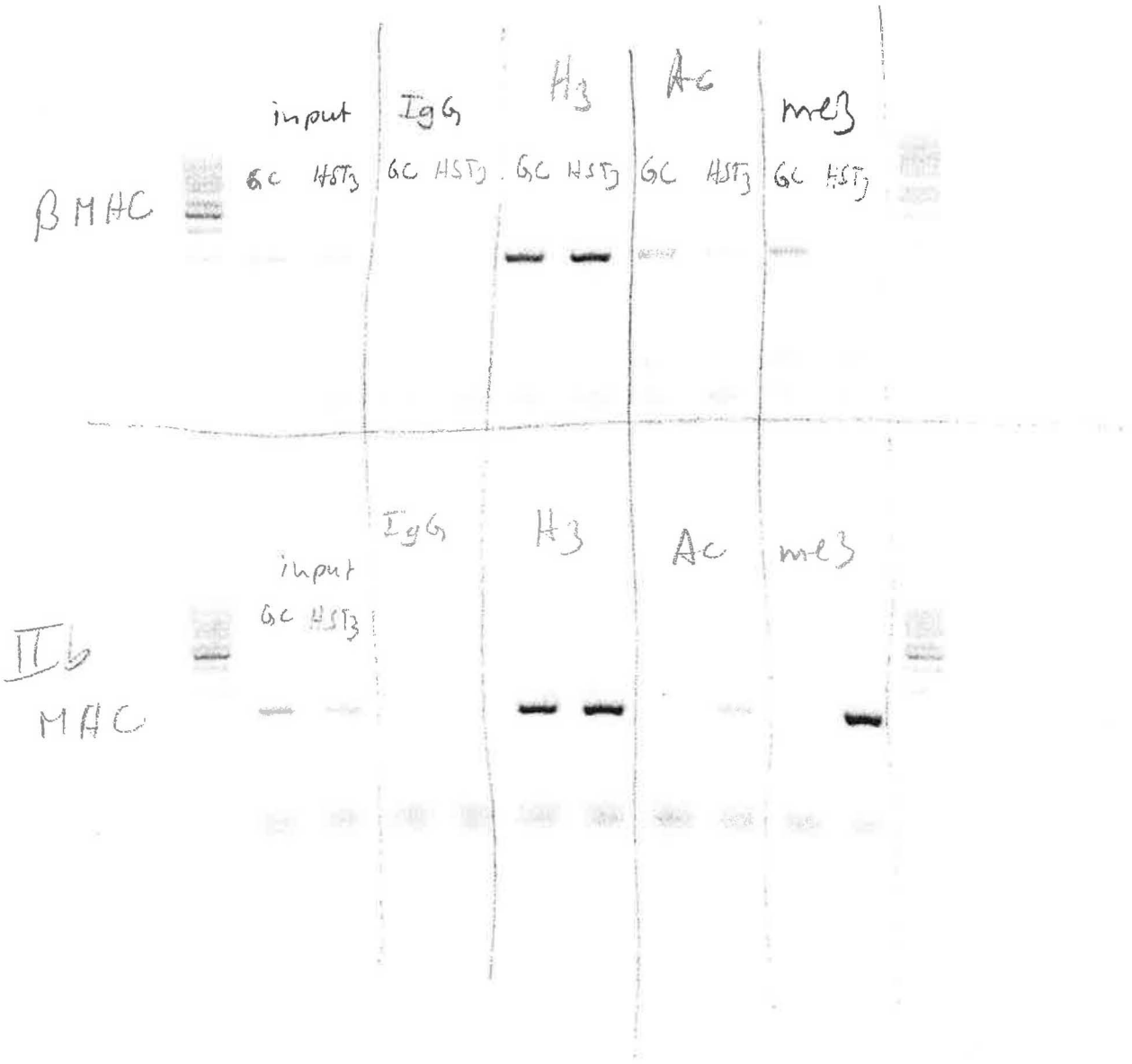
12-5 ChIP Samples



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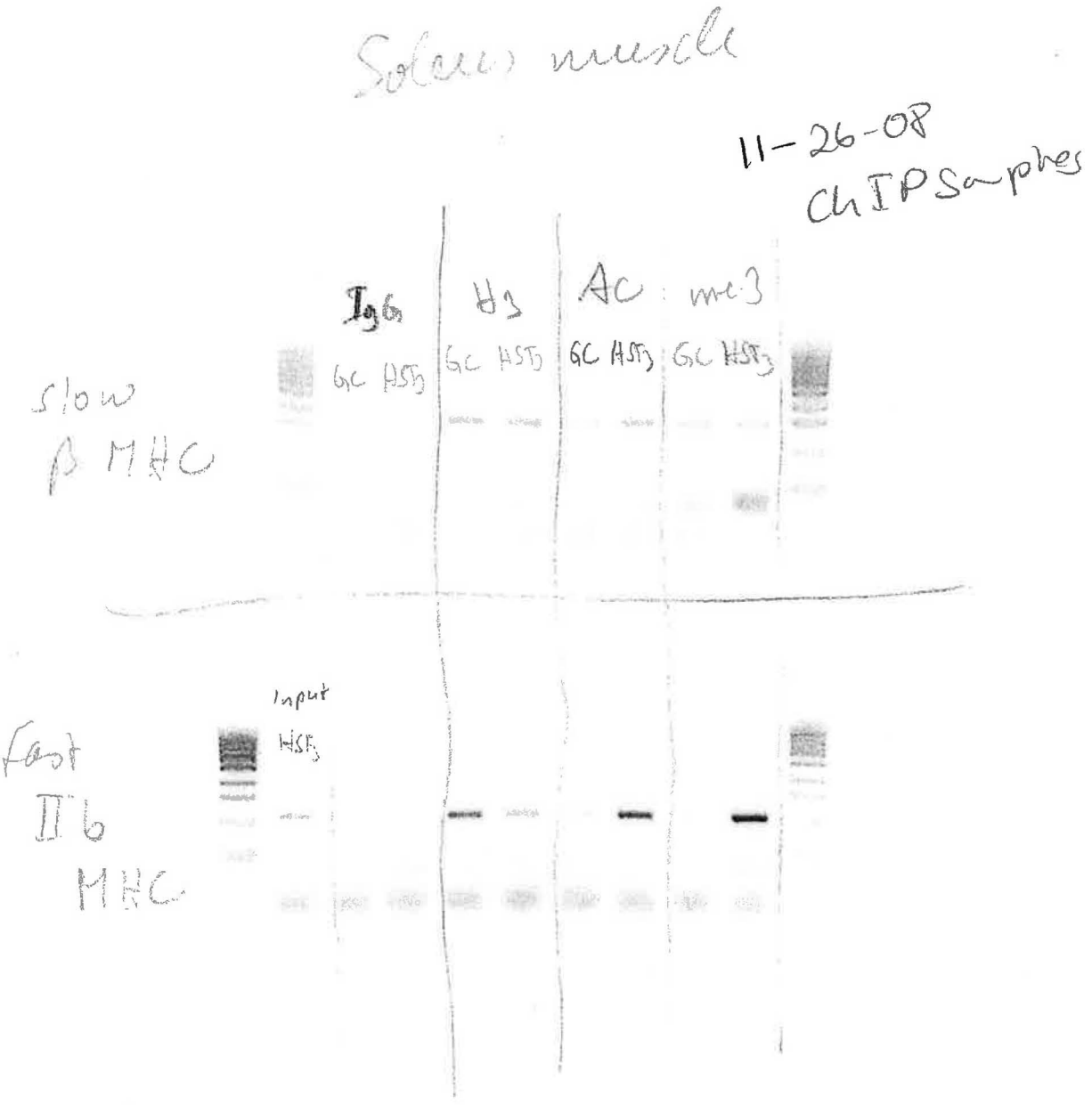
P ate/Time:



Friday, January 30, 2009

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Clay is writing a paperon his ChIP results and it would be good to include my data.
Densitometry analysis of my data is promising.

Monday and Tuesday, February 2 & 3, 2009

Follow up PCR reactions with the 9-8-08 and 11-26-08 ChIP samples.

Use the beta MHC +1156 / +1441 primer set at 57 °C annealing and
The IIb 1075 F / 1272 R primer set also at 57°C annealing.

Total of 36 samples.
The 11-26 ChIP samples series has a complete set of 20 reactions.
The 9-8-08 ChIP samples are missing the Pan H3 total histone acetylation antibody set. I did not use that antibody for that experiment. Total of 16 reactions in this set.

Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

31/33 cycles of
60 sec. 96°C, 45 sec. 57, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines:
33 cycles for the trimethylation samples.
31 cycles for all other samples

Run samples on two 2% agarose gels with 100bp ladder.

Gel analysis and results see next pages.

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Image Comment:
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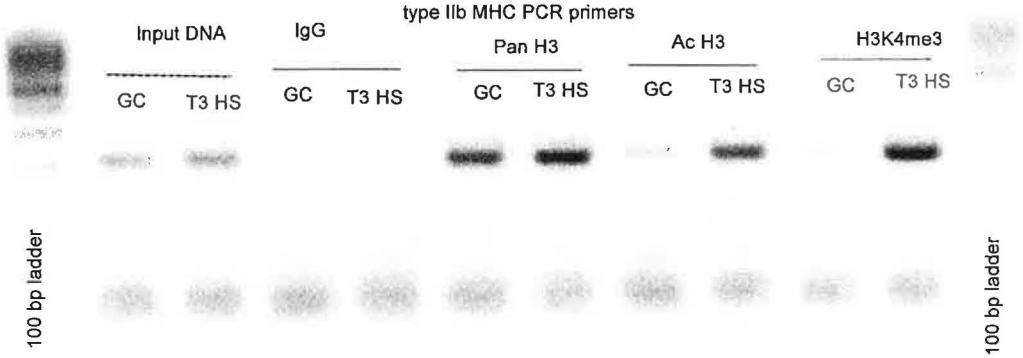
P[illegible]ate/Time:

PCR with beta MHC 1156, 1441 primers, 57 deg., 286 bp
and IIb MHC 1075, 1272 primers, 57 deg. 218 bp

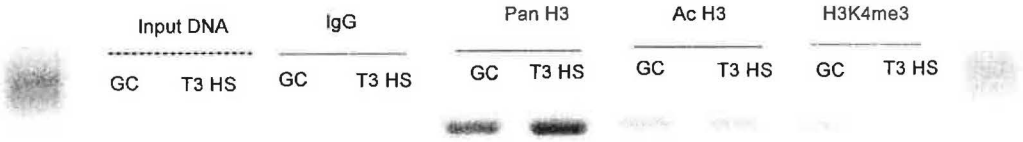
type IIb MHC PCR primers



11-26-08 Chip samples

31 cycles all samples except H3Kme3 (33 cycles)

type I (beta) MHC PCR primers



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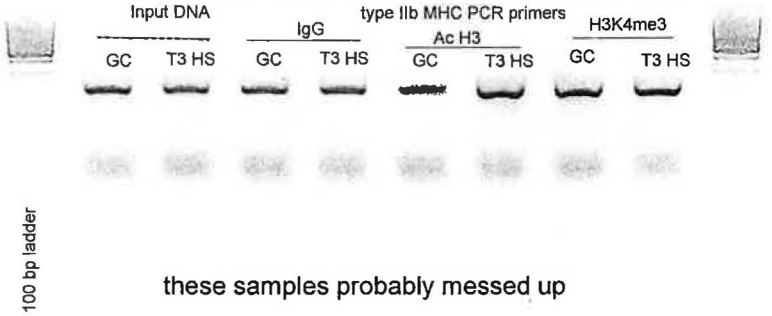
P: ate/Time:

PCR with beta MHC 1156, 1441 primers, 57 deg., 286 bp
and IIb MHC 1075, 1272 primers, 57 deg. 218 bp



these samples probably messed up

9-8-08 Chip samples

31 cycles all samples except H3K4me3, 31 cycles

type I (beta) MHC PCR primers

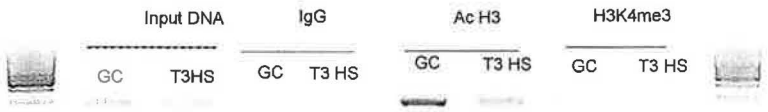


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Image Comment:
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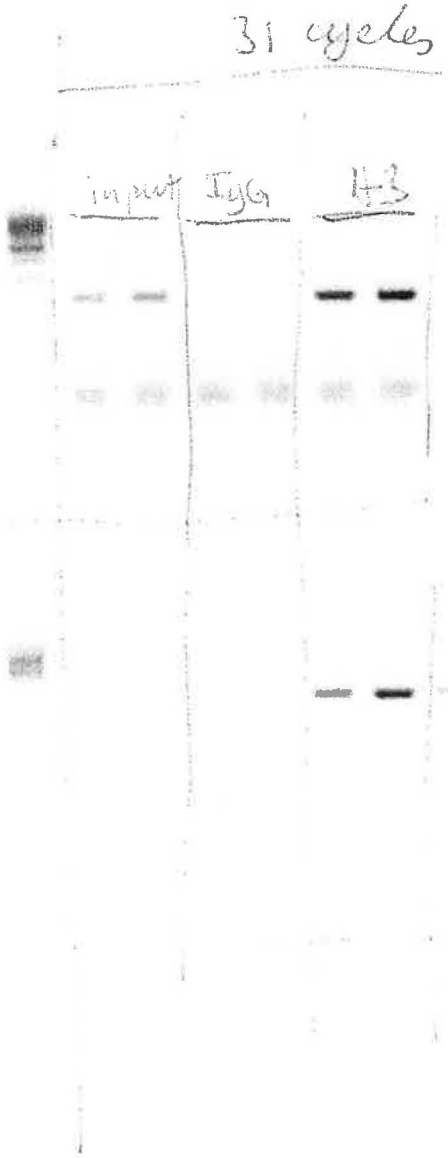
[illegible]ate/Time:

2-3

β 1156-1441
IIb 1075-1272

31 cycles 57°C
33 cycles

11-26 ChIP



PCR rxns β-red IIb-green
11-26 ChIP full set
10 samples (blue label)
Beta vs IIb primers 10

9-8-08 ChIP
8 samples beta vs IIb
No H3 samples

β MHC

File/Range: G:\Carola\ChIP results 2-3-09 beta and IIb primers 9-8-08 ChIP.gel / 2829--3600.000 /Magnification - 1.79
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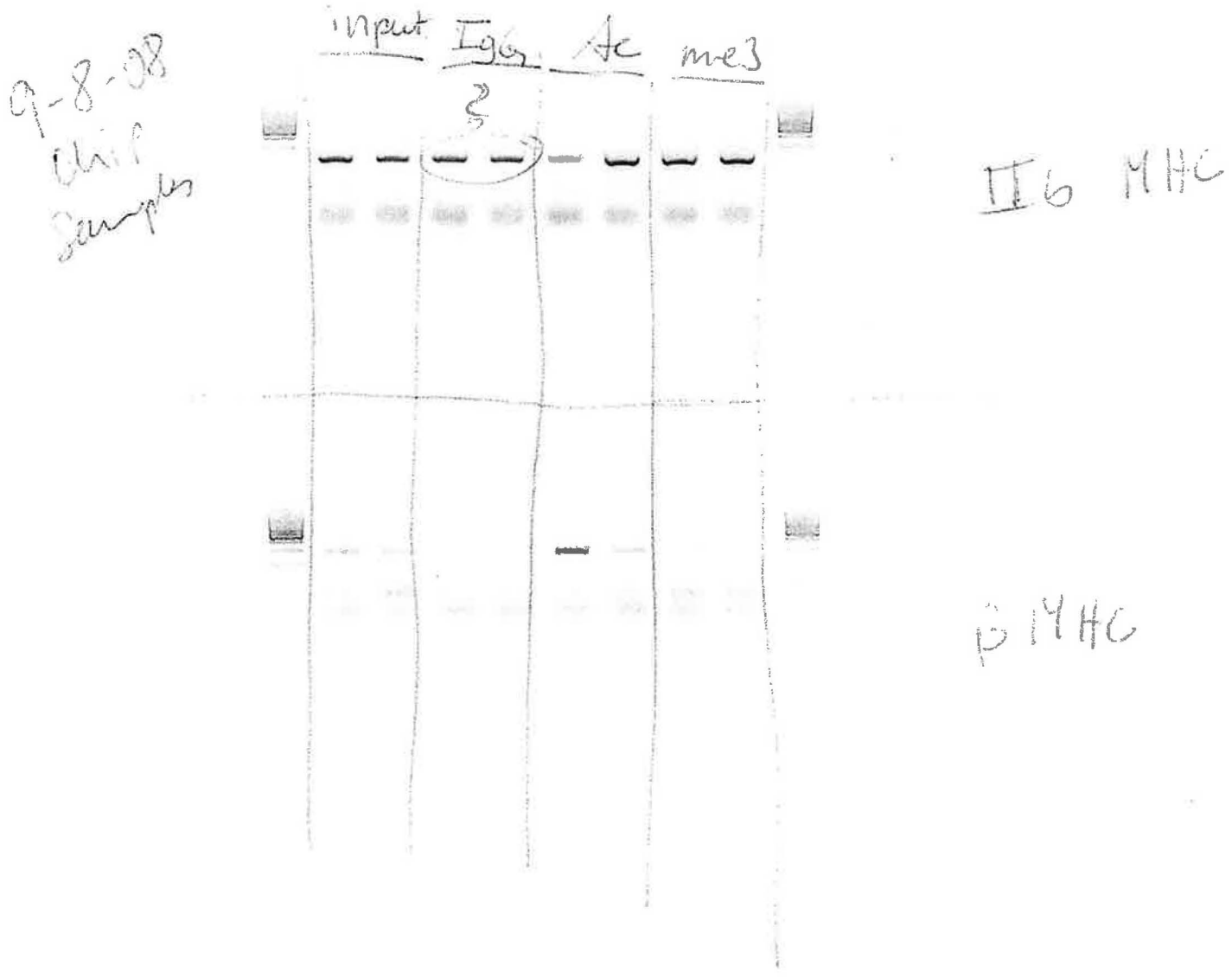
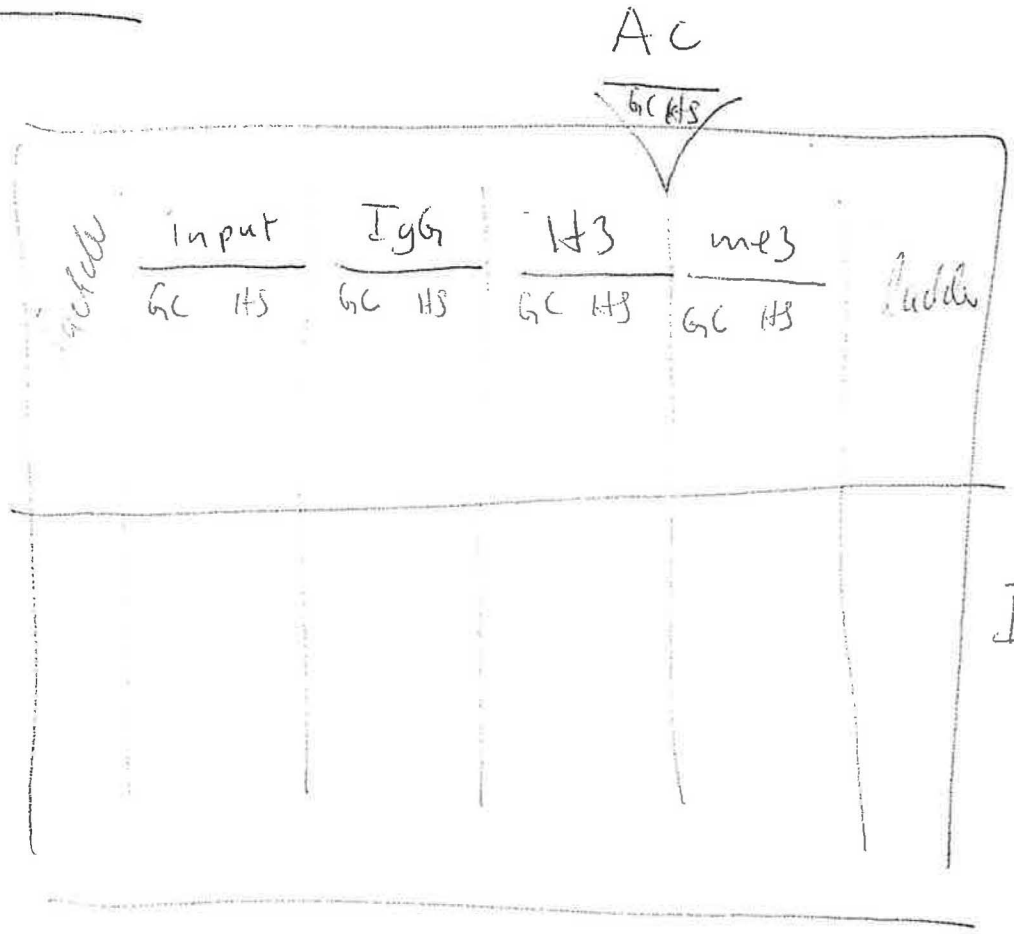
2-3



Feb 3 Gel

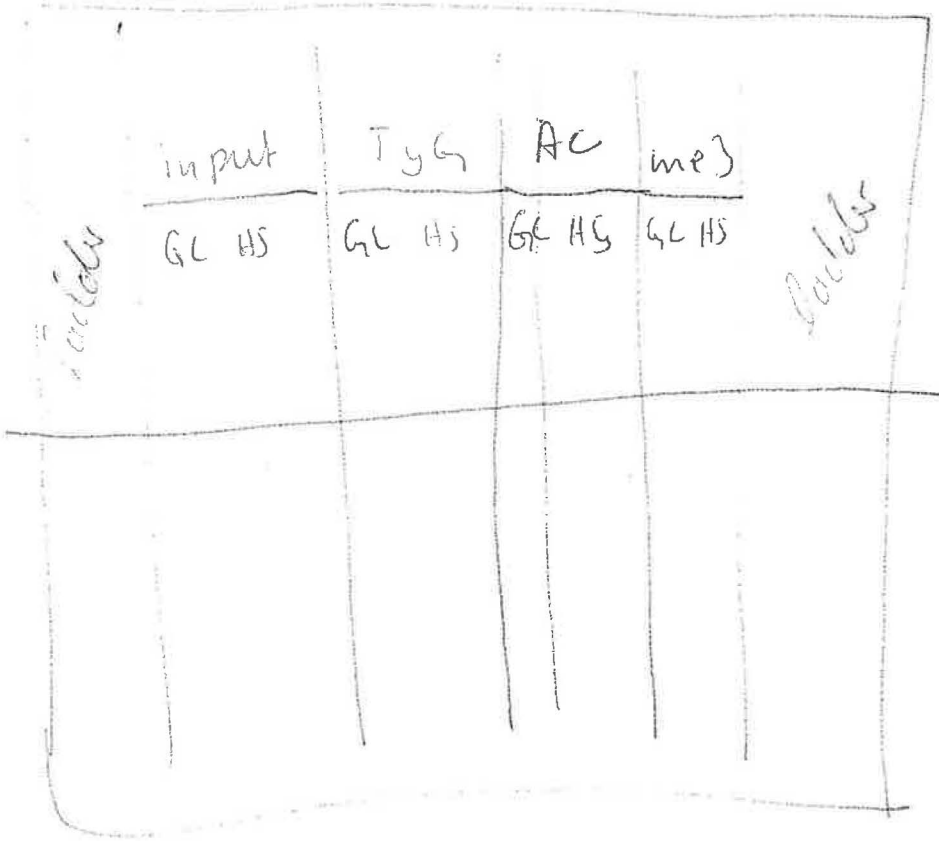
(1)

11-26-08 Chip



(2)

Chip 9-8-08



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Gel analysis and results see next pages.

Wednesday, February 4, 2009

Prepare for new ChIP procedures. Use three different pairs of ground control vs. Hindlimb suspended T3 treated soleus muscle.
This should give sufficient data to be included in Clay's paper.

Thursday, February 5, 2009

Use frozen muscle tissue from Oct. experiment (Oct. 9 sac date)
The ground control muscles are too big. Only 50 mg of muscle should be used for each essay. This makes it necessary to cut small pieces of the muscle off before using it in the experiment. The goal is to bring the muscle weight close to the desired ~50 mg weight.
For ground control **GC- A use left Soleus # 3, 81 mg – 28 mg = 53 mg of tissue**
GC- B use left Soleus # 6, 83 mg – 20 mg = 63 mg of tissue
GC- C use left Soleus # 7, 86 mg – 29 mg = 57 mg of tissue

For hind limb suspension + T3 treatment samples
HS-T3 - A use right Soleus # 5 (36 mg) + half of Left Soleus # 5 (19mg) for a total of 55 mg of tissue.
HS-T3 - B use left Soleus # 6 (29 mg) + half of Left Soleus # 5 (19mg) for a total of 48 mg of tissue.
HS-T3 - C use right Soleus # 3 (43 mg) by itself for a total of 43 mg of tissue.

Total of 6 tissue preps.

Prepare 1% Formaldehyde solution as follows:

to make 10 ml 1% formaldehyde		
37% formaldehyde	0.27	ml
10xPBS	1	ml
water	8.73	ml

Prepare necessary amount of 1xPBS solution and put on ice, to be used for different washes.
Need about 4.5 ml 1x PBS per sample.
When using PBS, always supplement with protease inhibitors
protease inhibitors: use leupetin, AEBSF, and aproteinin each are at 1000x stock solutions in the -80 C freezer.

Isolating and lysing cells from tissue:
Mince tissue (small pieces) using a razor blade
Put minced tissue in 1.5ml tubes in ~1 ml cold PBS supplemented with protease inhibitors
Keep on ice until all pieces are minced
Drain cold PBS, add 1 ml 1% formaldehyde buffer (freshly made)
Incubate at room temperature for 10 minutes with mixing every few minutes

After 10 minutes incubation, add 110 ul 10x glycine (1.25M glycine) prepared in a 15 ml tube.
Incubate 5 minutes at room temperature
Change solution, take out the formaldehyde PBS, replace with 1 ml cold PBS (+protease inhibitors)
Repeat PBS wash once more (use PBS supplemented with protease inhibitor)
Add 19x cold PBS (plus inhibitors) to samples
Transfer to homogenizing pestle tube, and homogenize on ice, transfer to clean 1.5ml tube. Use brand new homogenizing pestle tube this time for best results (hopefully).

Spin down the homogenate at 1500g for 10 minutes at 4°C in order to collect the cells as a pellet
Take out supernatant, use a pipet to leave a clean pellet (no liquid should be left)
Suspend the pellet in 400 ul lysis buffer (supplemented with protease inhibitors) in 1.5 ml tubes. Incubate 15 minutes on ice.

Shearing the DNA with sonication:
Use the Sonics Vibracell, 130 watts ultrasonic from sonics and materials (VCX 130)

Sonication:
Put the samples on ice at all time to keep it cool
Immerse the probe tip in the sample
Sonicate using the following protocol:

amplitude	time	# of pulses per sample
80% max	15 sec	10

Min. of 25 sec rest on ice between pulses
(monitor tube temperature, as it can heat up quickly)
Set sonicator to do 2 pulses at a time. 2 pulses = 55 sec, with 25 sec rest set. Then vortex/mix sample, and return to ice, and start next sample. Rotate thru samples like this.
Make sure that the entire sample is in the lysis buffer, as it tends to stick to the sides of tube (use pipette tip to clears sides).
Have water in the ice, so that the tube is immersed in ice-cold temp, otherwise air pockets in ice around tube can heat up.
Sonication will solubilize the chromatin and break up the DNA
After sonication, spin the samples in a cold microfuge at 12,000g for 10 minutes to remove insoluble material.

Remove **10ul aliquot** of sheared DNA per sample to analyze on agarose gel
Transfer the supernatant (containing soluble chromatin) to a fresh microfuge tube.
Freeze at -80 to be used later for the IP procedure.

Use the 10ul aliquot from above for DNA analyses: necessary to determine DNA concentration before proceeding, so that equivalent [DNA] used in all samples.

To each of the set aside 10ul total sheared lysate from above, add 86ul nuclease free water, and 4ul 5M NaCl.
Incubate at 65oC **overnight** to reverse the DNA-protein crosslinking.

Friday Feb. 6 2009

RNA and Protein digestion:
Add 1 ul RNAse A to each of the six tubes and incubate at 37oC for 30 minutes.
Add 7ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K] to each tube.
Mix and incubate at 45oC for 2 hours.

Run 40 ul of each sample on a 2% agarose gel together with 4 ul 100bp ladder.

Also, prepare samples for quantitative DNA analysis with real time PCR machine.

Prepare 10 ml filtered TAE (1x) and add 1ul Sybr Green (10,000 x).

Dilute sample 1:40 with water (5 ul sample + 195 ul water).

Thaw DNA standards (o.5, 1, 2.5, 5, 10, 15, 20 and a water blank)

Add 50 ul of filtered TAE and 5 ul sample to each micro titer well. Set up in triplicates.

Use Stratagene real time PCR machine for plate read. Make sure machine is warmed up for 20 minutes before plate read.

Results for quantitative DNA analysis on next pages.

Results and analysis see next pages.

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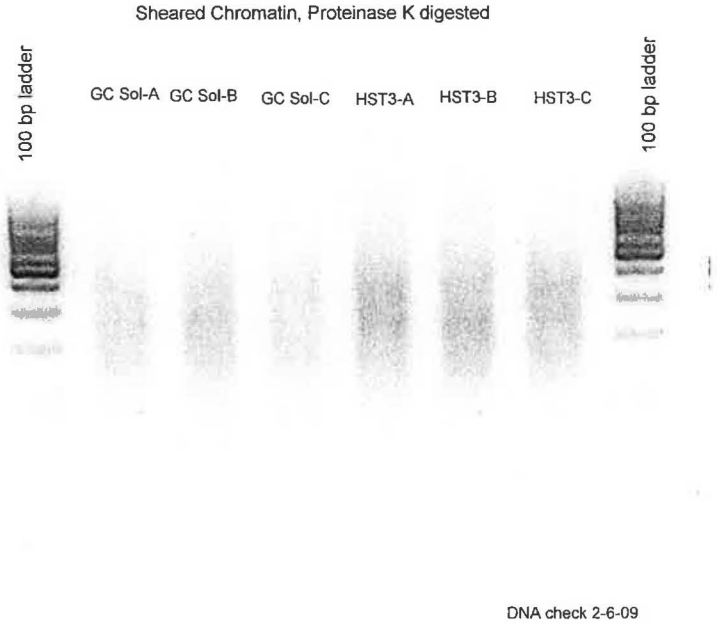


Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Plate sample values
C:\Program Files\Stratagene\Mx3000P\Storage\Quantitative Plate Read, 02-06-2009, 14Hr 02Min.mxp
Run date: February 06, 2009

Fluorescence : Rpre

Replicates: Treated individually (since no replicates in selection)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unknown 7512	Unknown 7179	Unknown 7661	Unknown [illegible]	Unknown 12211	Unknown 13145	GC-A					
B	Unknown 7312	Unknown 7652	Unknown 7074	Unknown 12806	Unknown 13783	Unknown 14574	GC-B					
C	Unknown 7271	Unknown 8055	Unknown 7056	Unknown 11259	Unknown 12522	Unknown 12587	GC-C					
D	Unknown 7107	Unknown 7032	Unknown 6726	Unknown 17296	Unknown 16827	Unknown 18627	HST3-A					
E	Unknown 7605	Unknown 7967	Unknown 7672	Unknown 19409	Unknown 16877	Unknown 17345	HST3-B					
F	Unknown 11866	Unknown 12291	Unknown 12294	Unknown 14276	Unknown 14861	Unknown 15307	HST3-C					
G	Unknown 15865	Unknown 14378	Unknown 15534	Unknown 12389	Unknown 13709	Unknown 14067	GC-A					
H	Unknown 19792	Unknown 19949	Unknown 18987	Unknown 5246	Unknown 5472	Unknown 5096						

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Plate sample values
C:\Program Files\Stratagene\Mx3000P\Storage\Quantitative Plate Read, 02-06-2009, 14Hr 08Min.mxp
Run date: February 06, 2009

Fluorescence : Rpost

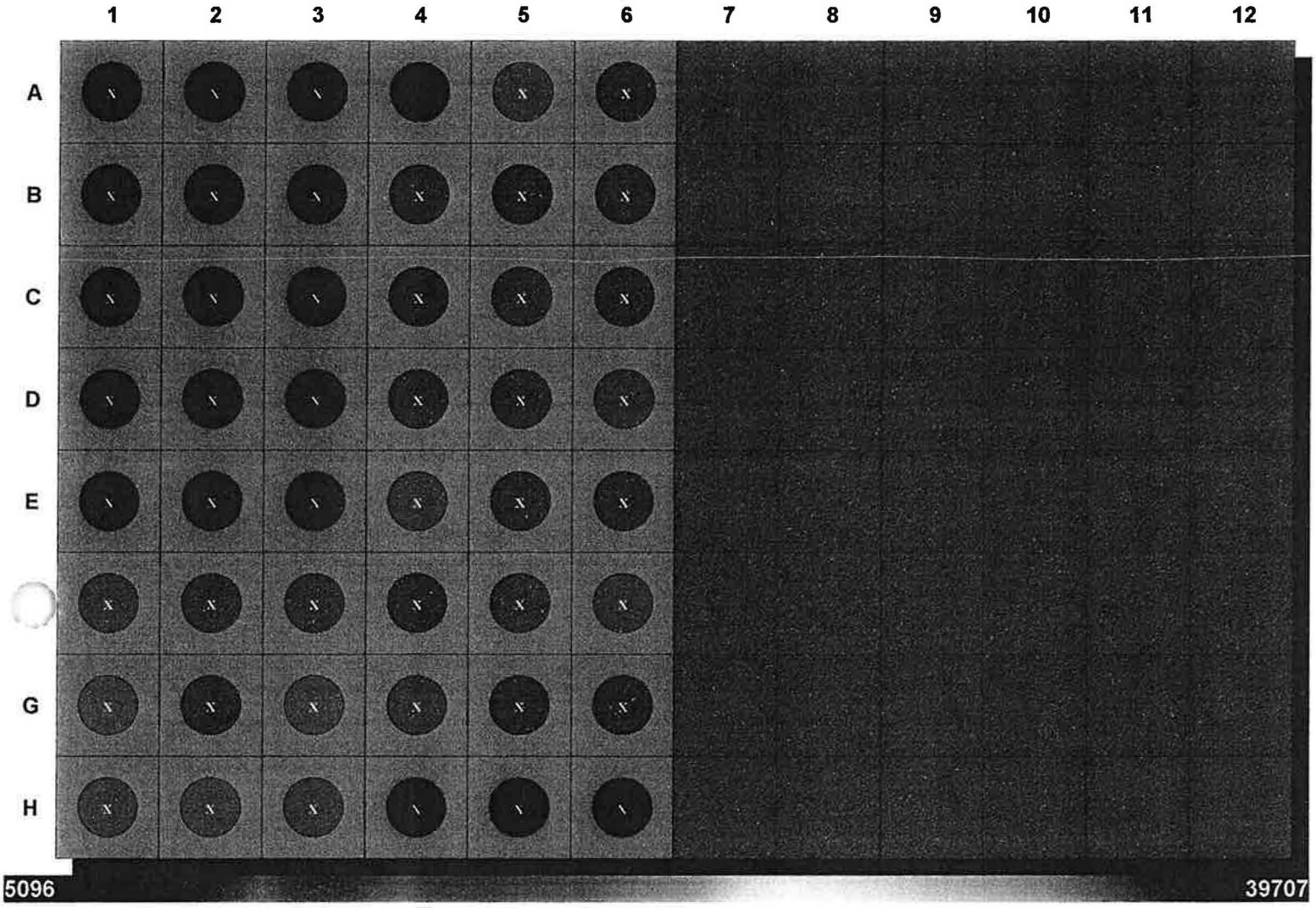
Replicates: Treated individually (since no replicates in selection)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unknown 7457	Unknown 7218	Unknown 7640	Unknown 41796	Unknown 12058	Unknown 12929						
B	Unknown 7267	Unknown 7680	Unknown 6965	Unknown 12507	Unknown 13348	Unknown 14012						
C	Unknown 7122	Unknown 8360	Unknown 6962	Unknown 10980	Unknown 12056	Unknown 12102						
D	Unknown 6966	Unknown 6972	Unknown 6578	Unknown 16854	Unknown 16165	Unknown 18169						
E	Unknown 7460	Unknown 7811	Unknown 7595	Unknown 18632	Unknown 16328	Unknown 16586						
F	Unknown 11702	Unknown 12023	Unknown 12071	Unknown 13777	Unknown 14502	Unknown 14827						
G	Unknown 15646	Unknown 14275	Unknown 15392	Unknown 12113	Unknown 13446	Unknown 13787						
H	Unknown 19760	Unknown 19926	Unknown 19078	Unknown 5080	Unknown 5343	Unknown 4957						

Mx3000P

Multiplex Quantitative PCR Systems
Quantitative Plate Read - Fluorescence intensity values
\\Server1\lab folder\Carola\ChIP\Quantitative Plate Read, 02-06-2009, 14Hr 02Min.mxp
[illegible] date: February 06, 2009

Replicates: Treated individually (since no replicates in selection)



2-9

Calculate DNA concentrations to be used for ChIP assays

SYBR green I, dilute 1:10,000 in filtered 1x TAE (same buffer as gel running buffer)
add 50 ul sybr green mix to well, 5ul of sample/standard
generally dilute sample 1:40

2-6-09 samples
using standards from 3-11

	ng/well/5ul				average			
water blank	0	7457	7218	7240	**7305**	Net	predicted ng	
S0.5	0.5	7312	7652	7074	**7346**	46	1.2	
S1	1	7271	8055	7056	**7461**	161	1.4	
S2.5	2.5	7107	7032	6726	**6955**	-345	2.5	
S5	5	7605	7967	7672	**7748**	448	4.2	
S10	10	11866	12291	12294	**12150**	4850	9.9	
S15	15	15865	14378	15534	**15259**	7959	13.4	
S20	20	19792	19949	18987	**19576**	12276	21.4	
						r^2	**0.9833**	

10 ul sheared DNA taken, in final volume of 112 ul
samples diluted 1:40 (5:200), measured 5ul in well

					average	Net		ng/ul of original chromatin sample
GC Sol-A		12211	13145	12389	13709	**12864**	5564	
GC Sol-B		12806	13783	14574	13348	**13628**	6328	
GC Sol-C		11259	12522	12587	12056	**12106**	4806	
HS Sol + T3 - A		17296	16827	18627	16854	**17401**	10101	
HS Sol + T4 - B		19409	16877	17345	16586	**17554**	10254	
HS Sol + T5 - C		14276	14861	15307	14502	**14737**	7437	

Need/ChIP
For 30µg DNA

[DNA]
Sheared Lysate

std 7959 ⇒ 15 ng/5µl

GC-A 5564 ⇒ 10.5 ng/5µl = 2.1 ng/µl × 40 × 11 = 923 ng/µl — 32.5µl
GC-B 6328 ⇒ 11.9 ng/5µl = 2.39 ng/µl × 40 × 11 = 1049 ng/µl — 28.6µl
GC-C 4806 ⇒ 9 ng/5µl = 1.81 ng/µl × 40 × 11 = 797 ng/µl — 37.6µl

HST3-A 10101 ⇒ 19 ng/5µl = 3.8 ng/µl × 40 × 11 = 1675 ng/µl — 17.9µl
HST3-B 10254 ⇒ 19 ng/5µl = 3.9 ng/µl × 40 × 11 = 1700 ng/µl — 17.6µl
HST3-C 7437 ⇒ 14 ng/5µl = 2.8 ng/µl × 40 × 11 = 1233 ng/µl — 24.33µl

Monday Feb. 9, 2009

Calculate DNA concentrations of all six sheared lysate samples.
Results in excel format on next page.
Prepare materials etc. for ChIP assays.

Tuesday Feb. 10, 2009

Start new ChIP experiment with processed and analyzed (see Feb. 9) Soleus GC-A and HST3-A chromatin lysate.
Wash 8 x 50µl protein A agarose (Perice) in 8 separate1.5 ml tubes with 500 µl ChIP dilution buffer (supplemented with protease inhibitors).
Spin at 1000g for 30 sec to pellet the agarose. Remove and discard supernatant.
Add chromatin and dilution buffer based on measurements made with tissue samples of 2-5.

This tissue prepped on **2-5-09**

	Sample	muscle frozen weight in mg	
400 ul lysis buffer		GC Sol A	53 mg
80 % amplitude		HS + T3 Sol A	36mg + 19mg = 55 mg total
15 sec pulse			
12 pulses			

* DNA conc. seem high as calculated w/ standard curve of 2-6-09
use 20% more

Use 30µg of DNA for this ChIP

	ng/µl of original chromatin sample		µl of chromatin for 30 µg	µl buffer to yield 1ml
GC Sol - 1	923*	(32.5)	40	960
HS Sol + T3	1675*	(18)	22	978

Prepare four tubes for each muscle. Add the above amounts of chromatin solution and ChIP buffer to the washed agarose beads. Preclear chromatin for 45 min at 4 oC on rotator.
Spin at 1000g for 30 sec to pellet the agarose.
Collect the 1 ml supernatants into fresh 1.5 ml microtubes.
Remove 2x10 µl of the supernatant and **save at 4oC as input DNA** (one input DNA sample per muscle).
Add the following antibodies to the pre-cleared chromatin samples.
Four different antibodies for the two muscle chromatin samples (total of eight reactions):

1. Normal Rabbit IgG from Upstate (12-370) used 1.0 µl (1.0 µg).

2. Pan-H3 (abcam 1791), use 1.5 µl.

3. Anti-Histone H3 K4 me3 antibody (ab 8580), use 2.0 µl (~ 2 µg).

4. Anti-Acetyl Histone H3 antibody (Upstate #06-599), used 1.0 ul (~1 ug).

Incubate over night with antibodies. Tubes are in refrigerator on rotator.

Wednesday Feb. 11, 2009

Continue ChIP procedure:

Wash another 8 x 50µl agarose beads with ChIP dilution buffer. Spin and discard supernatant. Add the washed beads to the chromatin antibody mixtures and incubate for another 2 hours in the refrigerator on rotator.

Pellet protein agarose by spinning at 1000 g for 40sec at 4oC.Discard the supernatant.

Wash the protein agarose-antibody chromatin samples by resuspending the beads in 1 ml of the cold buffers in the order listed

First use 1 ml Low salt Immune complex Wash buffer wash 10sec and spin, discard supernatant, then wash as listed:

For each wash, incubate for 15 minutes on a rotating platform followed by spin 1000g for 20sec at 4oc (supplementation with protease inhibitors is not necessary for the wash solutions.

Discard the supernatant after each wash

Wash buffer in the order to be used:

a	Once wash with Low salt Immune complex Wash buffer
b	Once wash with high salt Immune complex Wash buffer
c	Once wash with LiCl complex Wash buffer
d	twice wash with TE buffer

remove supernatant and procede to elution of the protein/DNA from the agaraose beads

Elution of Protein/DNA complexes

Bring 1M NaHCO3 solution to room temperature. Vortex at room temperature to dissolve precipitate

Set water bath to 65oC to use for reverse linking(next section)

Prepare elution buffer for all IP tubes as well as for input DNA tube (which was 1% of DNA in the IP)

For 10 tubes, make 11 x to have some extra

elution buffer	1x	6.5	11x	
20% SDS	10	65	110	
1M NaHCO3	20	130	220	+NaCl
sterile di Water	170	1105	1870	52
Sum	200	1300	2200	

For input DNA, add 200ul elution buffer and set aside at room tempertaure until reverse cross-linking

Add 100ul of elution buffer to each IP tube containing the agarose pellet.

Mix by flicking the tube gently

Incubate at room temperature for 15 minutes

Pellet the agarose by brief centrifugation 1000 g.

Collect and save the supernatant into a new microfuge

Repeat the elution with another 100ul elution buffer.

Collect the supernatant and combine with the previous step 100ul, to make a total of 200ul eluate.

Reverse crosslinks of Protein/DNA complexes to free the DNA

To all the tubes (input DNA and IP), add 8ul 5M NaCl
Incubate at 65oC overnight.
There are 10 tubes total: 2 input and 8 experimental

Thursday Feb. 12, 2009

RNA and Protein digestion:
To all tubes, add 1 ul RNAse A and incubate at 37oC for 30 minutes.
Add 13ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 2 hours.

digestion buffer premix:	**1x**	**11x**
0.5M EDTA	4	44
1M Tris pH6.5	8	88
proteinase K	1	11
	13	143

DNA purification using spin columns

Use Qiagen miniprep columns

To each of the above tubes (input DNA and IP DNA) add 1 ml binding reagent"A"
Mix well.
A precipitate might form, this will not interfere with the procedure
Transfer 600ul of sample/Bind reagent A mixture to the column
Spin 30 sec at 12,000 g
Discard flow through
Add the remaining 600 ul to the column
Spin again and discard flow through
Add 750ul wash buffer of Qiagen to the column
Spin at 12,000 g
Discard flow through
Spin again for 12,000 g for 2min.
Discard the collection tube
Put the column in a new 1.5 ml collection tube
Add 135 ul warm water to elute the DNA off the column.
Spin at 12,000g for 2 minute (be careful to not zip the lids off the tubes.)
Eluate is pure DNA that can be analyzed by PCR
Use 12 µl of this DNA per 25 µl PCR reaction.
Freeze samples at -20 oC.

Friday Feb. 13, 2009

Set up PCR reactions with Thursday's (2-12) samples (+ two input samples from previous experiment).

Use the beta MHC +1156 / +1441 primer set at 57 °C annealing **in duplicates**,
The IIb 1075 F / 1272 R primer set also at 57°C annealing and the
The beta Actin +1517 F / 1741 R primer set at 57 °C annealing

Total of 40 samples.
Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

31/33 cycles of
60 sec. 96°C, 45 sec. 57, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines: 31 cycles for all samples except the trimethylation samples that get 33 cycles.

Run samples on two 2% agarose gels with 100bp ladder.

Results see next pages.

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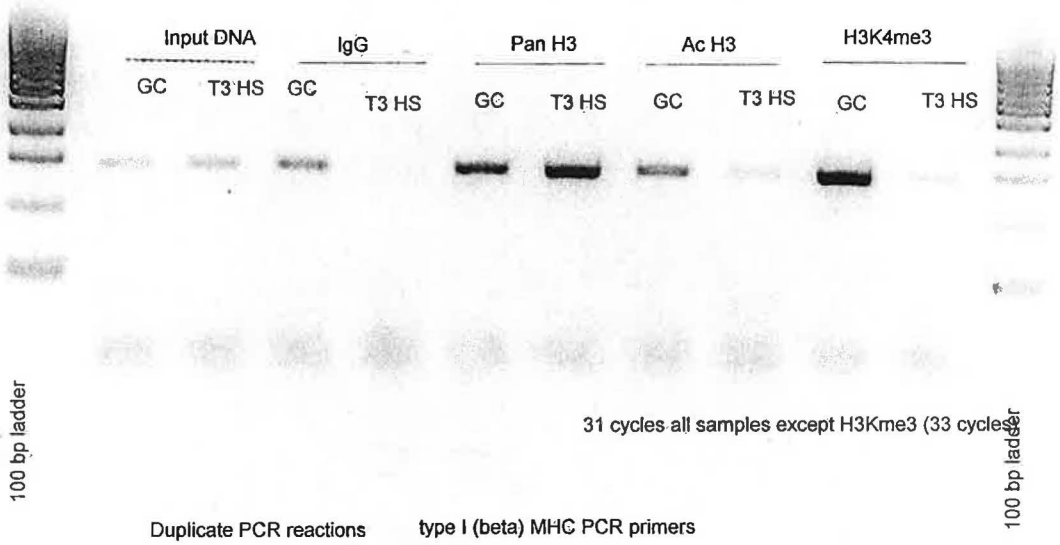
Image Comment:
Present Date/Time: 2009:02:17 13:46:33
Scan Date/Time: 2009:02:13 15:53:40
P ate/Time:

PCR with beta MHC 1156, 1441 primers, 57 deg., 286 bp

type I (beta) MHC PCR primers duplicate PCR reactions



Duplicate PCR reactions type I (beta) MHC PCR primers

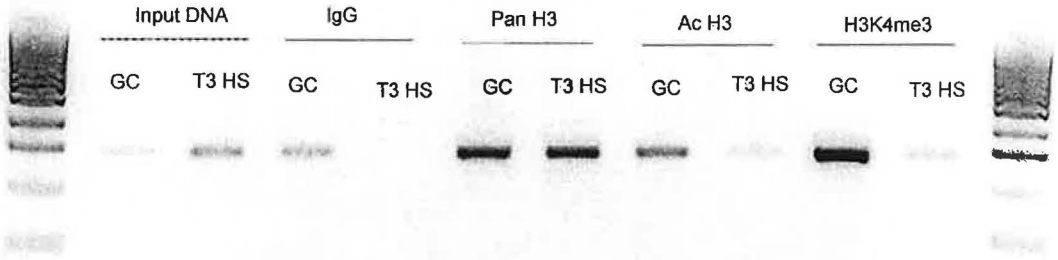


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Image Comment:
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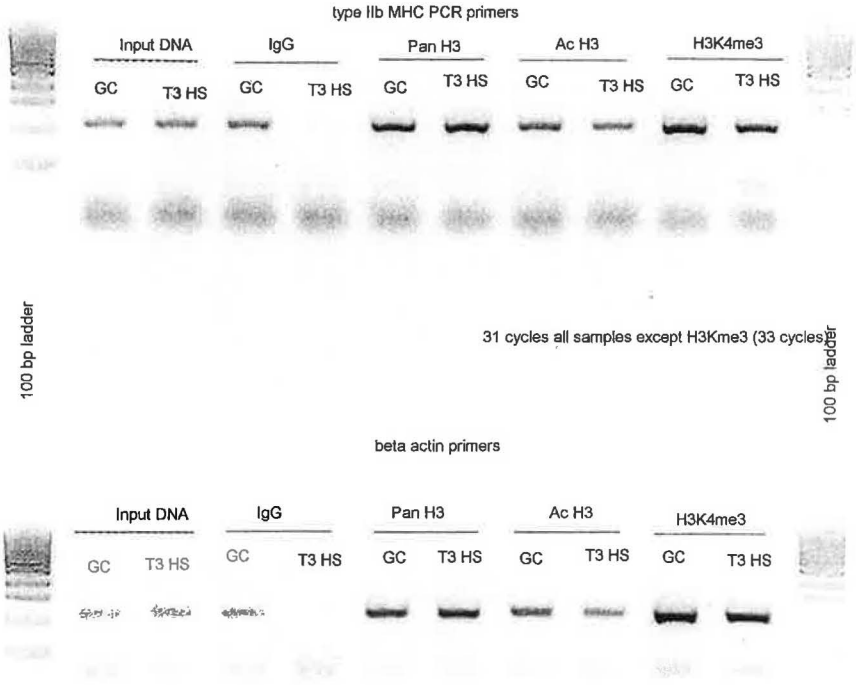
Scan Date/Time: 2009:02:13 15:55:53
P ate/Time:

PCR with IIb MHC 1075, 1272 primers, 57 deg. 218 bp
and Beta Actin 1517,1741 primers, 57 deg



Monday Feb. 16, 2009

President's Day Holiday

Tuesday Feb. 17, 2009

Start new ChIP experiment with processed and analyzed (see Feb. 9) Soleus GC-B, GC-C and HST3-B, HST3-C chromatin lysate.
Wash 16 x 50µl protein A agarose (Perice) in 16 separate1.5 ml tubes with 500 µl ChIP dilution buffer (supplemented with protease inhibitors).
Spin at 1000g for 30 sec to pellet the agarose. Remove and discard supernatant.
Add chromatin and dilution buffer based on measurements made with tissue samples of 2-5.

This tissue prepped on **2-5-09**

	Sample	muscle frozen weight in mg
400 ul lysis buffer	GC Sol B	63 mg
80 % amplitude	GC Sol C	57 mg
15 sec pulse	HS + T3 Sol B	29mg + 19mg = 48 mg total
12 pulses	HS + T3 Sol C	43 mg

Use 30µg of DNA for this ChIP

	ng/µl of original chromatin sample		for 30 µg
		µl of chromatin	µl buffer to yield 1ml
GC Sol - B	1049*	34	966
GC Sol – C	797*	45	955
HS Sol + T3-B	1700*	22	978
HS Sol + T3-C	1233*	30	970

Prepare four tubes for each muscle. Add the above amounts of chromatin solution and ChIP buffer to the washed agarose beads. Preclear chromatin for 45 min at 4 oC on rotator.
Spin at 1000g for 30 sec to pellet the agarose.
Collect the 1 ml supernatants into fresh 1.5 ml microtubes.
Remove 2x10 µl of the supernatant and **save at 4oC as input DNA** (one input DNA sample per muscle).
Add the following antibodies to the pre-cleared chromatin samples.
Four different antibodies for the two muscle chromatin samples (total of eight reactions):

1. Normal Rabbit IgG from Upstate (12-370) used 1.0 µl (1.0 µg).

2. Pan-H3 (abcam 1791), use 1.5 µl.

3. Anti-Histone H3 K4 me3 antibody (ab 8580), use 2.0 µl (~ 2 µg).

4. Anti-Acetyl Histone H3 antibody (Upstate #06-599), used 1.0 ul (~1 ug).

Incubate over night with antibodies. Tubes are in refrigerator on rotator.

Wednesday Feb. 18, 2009

Continue ChIP procedure:
Wash another 8 x 50µl agarose beads with ChIP dilution buffer. Spin and discard supernatant. Add the washed beads to the chromatin antibody mixtures and incubate for another 2 hours in the refrigerator on rotator.
Pellet protein agarose by spinning at 1000 g for 40sec at 4oC.Discard the supernatant.
Wash the protein agarose-antibody chromatin samples by resuspending the beads in 1 ml of the cold buffers in the order listed
First use 1 ml Low salt Immune complex Wash buffer wash 10sec and spin, discard supernatant, then wash as listed:
For each wash, incubate for 15 minutes on a rotating platform followed by spin 1000g for 20sec at 4oc (supplementation with protease inhibitors is not necessary for the wash solutions.
Discard the supernatant after each wash
Wash buffer in the order to be used:

a	Once wash with Low salt Immune complex Wash buffer
b	Once wash with high salt Immune complex Wash buffer
c	Once wash with LiCl complex Wash buffer
d	twice wash with TE buffer

remove supernatant and procede to elution of the protein/DNA from the agaraose beads

Elution of Protein/DNA complexes
Bring 1M NaHCO3 solution to room temperature. Vortex at room temperature to dissolve precipitate
Set water bath to 65oC to use for reverse linking(next section)

Prepare elution buffer for all IP tubes as well as for input DNA tube (which was 1% of DNA in the IP)
For 10 tubes, make 11 x to have some extra

elution buffer	1x	6.5	11x	
20% SDS	10	65	110	
1M NaHCO3	20	130	220	+NaCl
sterile di Water	170	1105	1870	52
Sum	200	1300	2200	

For input DNA, add 200ul elution buffer and set aside at room tempertaure until reverse cross-linking
Add 100ul of elution buffer to each IP tube containing the agarose pellet.

Mix by flicking the tube gently
Incubate at room temperature for 15 minutes
Pellet the agarose by brief centrifugation 1000 g.
Collect and save the supernatant into a new microfuge
Repeat the elution with another 100ul elution buffer.
Collect the supernatant and combine with the previous step 100ul, to make a total of 200ul eluate.

Reverse crosslinks of Protein/DNA complexes to free the DNA

To all the tubes (input DNA and IP), add 8ul 5M NaCl
Incubate at 65oC overnight.
There are 20 tubes total: 4 input and 16 experimental

Thursday Feb. 19, 2009

RNA and Protein digestion:
To all tubes, add 1 ul RNAse A and incubate at 37oC for 30 minutes.
Add 13ul digestion buffer [4ul 0.5M EDTA, 8ul 1M Tris-HCl, and 1ul proteinase K].
Mix and incubate at 45oC for 2 hours.

digestion buffer premix:	**1x**	**11x**
0.5M EDTA	4	44
1M Tris pH6.5	8	88
proteinase K	1	11
	13	143

DNA purification using spin columns

Use Qiagen miniprep columns

To each of the above tubes (input DNA and IP DNA) add 1 ml binding reagent"A"
Mix well.
A precipitate might form, this will not interfere with the procedure
Transfer 600ul of sample/Bind reagent A mixture to the column
Spin 30 sec at 12,000 g
Discard flow through
Add the remaining 600 ul to the column
Spin again and discard flow through
Add 750ul wash buffer of Qiagen to the column
Spin at 12,000 g
Discard flow through
Spin again for 12,000 g for 2min.
Discard the collection tube
Put the column in a new 1.5 ml collection tube
Add 135 ul warm water to elute the DNA off the column.
Spin at 12,000g for 2 minute (be careful to not zip the lids off the tubes.)
Eluate is pure DNA that can be analyzed by PCR
Use 12 µl of this DNA per 25 µl PCR reaction.
Freeze samples at -20 oC.

Friday Feb. 20, 2009

Set up PCR reactions with Thursday's (2-19) samples.

Use the beta MHC +1156 / +1441 primer set at 57 °C annealing,
The IIb 1075 F / 1272 R primer set also at 57°C annealing and the

Total of 40 samples (20 samples from B- set and 20 samples from C-set).
B samples are blue labeled and C samples are red.
Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

31/33 cycles of
60 sec. 96°C, 45 sec. 57, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines: 31 cycles for all samples except the trimethylation samples that get 33 cycles.

Run samples on two 2% agarose gels with 100bp ladder.

Results see next pages.

List of Primers used in ChIP DNA -PCR											
	Primerselect optimal Ta	use Ta	Tm	bp	pmol/ul	vial date		location on gene			
+1156 bMHC Fwd: GGCCTGGGCCTACCTCTTTATCC	57.5	58	60	286	32.6	3/2/2006		at +1156 beta gene			
+1441 bMHC Rev: TATTCAATTGGGGCACTCTTCGGGTGTAT			64.9		37.8	3/2/2006					
IIB 1075F: AGGGAATAAATGTTAACTTGTTGACACTGG	56.4	58	59.2	218	50	6/25/2007		at +1075 from IIb MHC gene TSS			
IIB 1272R: GGGGGCGGGGCTAATGAAGC			63.2		50	6/25/2007					
5end IIxpre Fwd2: TGCCACAGAAAGAGGGACGC	57	58	58.6	290	50.0	11/15/2007		1274/1563 vs IIx TSS			
5end IIxpre Rev2: CTGGCTGTGGTGTGGCTGAAA			57.9		50.0	11/15/2007					
beta Actin primers					pmole/ul						
+1517 bactin Fwd: CACGCCCTTTCTCAATTGTCTTTCT	55.8	58	58.8	225	50	9/2/2008		from intron 3			
+1741 bactin Rev: GGCCATTTATCACCAGCCTCATTAG			58.9		50	9/2/2008		from intron 3			
	Ta		Tm	bp	pmoles/ul	vial date					
IIa pre 1151 Fwd: CCTATCCCAAGGGCTTCAAGGTGAGA	58.7	58	63.3	306	50.00			stock at 50 pmole/mol			
IIa pre 1456 Rev: AGGGGCCCCAGATGCACATTACAC			63.7					so add 50 ul of each to 400 ul water			

Monday Feb. 23, 2009

Set up more PCR reactions with Thursday's (2-19) samples.

Use the beta MHC +1156 / +1441 primer set at 58 °C annealing,
The IIa pre 1151 F/1456 R primer set at 58 °C annealing,
The IIx 5' end Pre 2 F/R at 58 °C and
The IIb 1075 F / 1272 R primer set also at 58°C annealing and the

Total of 40 samples (all from C- set).

Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

31/33 cycles of
60 sec. 96°C, 45 sec. 58, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines: 31 cycles for all samples except the trimethylation samples that get 33 cycles.

Run samples on two 2% agarose gels with 100bp ladder.

Results see next pages.

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Image Comment:
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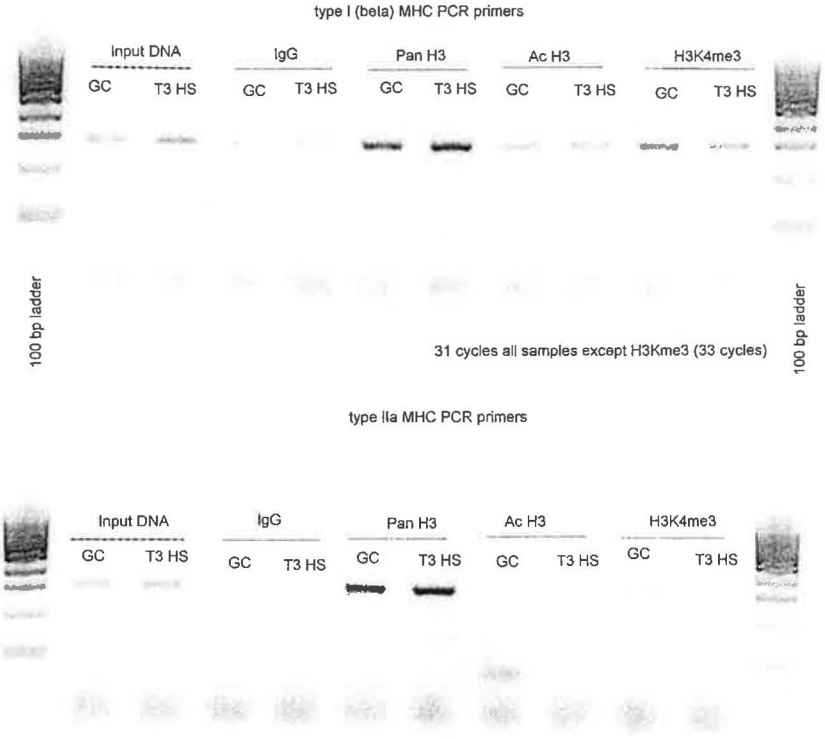
P ate/Time:

PCR with beta MHC 1156, 1441 primers, 58 deg., 286 bp
and IIa MHC 1151, 1456 primers, 58 deg.



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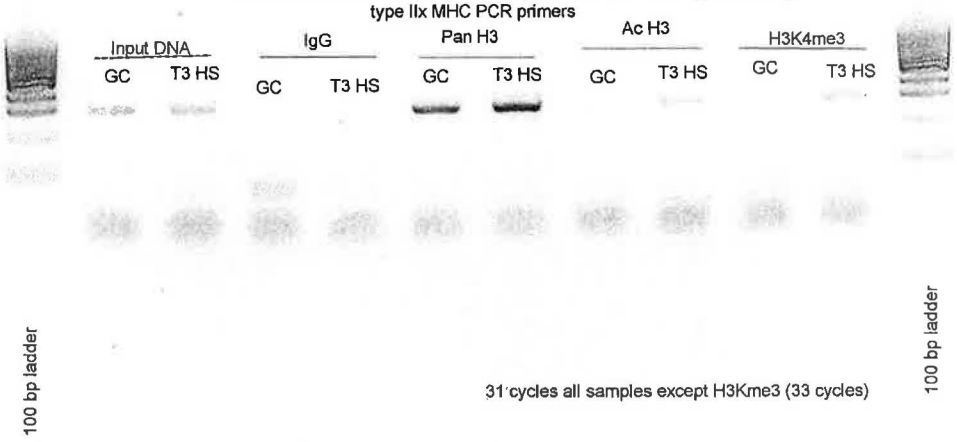
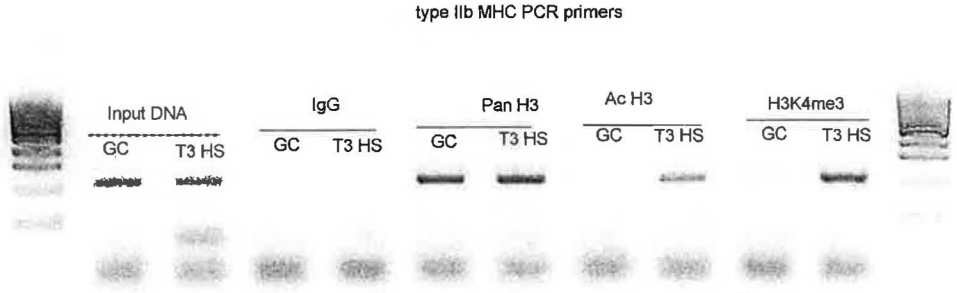
PCR with IIx 5' pre 2 F+R primers, 58 deg.
and IIb MHC 1075, 1272 primers, 57 deg. 218 bp





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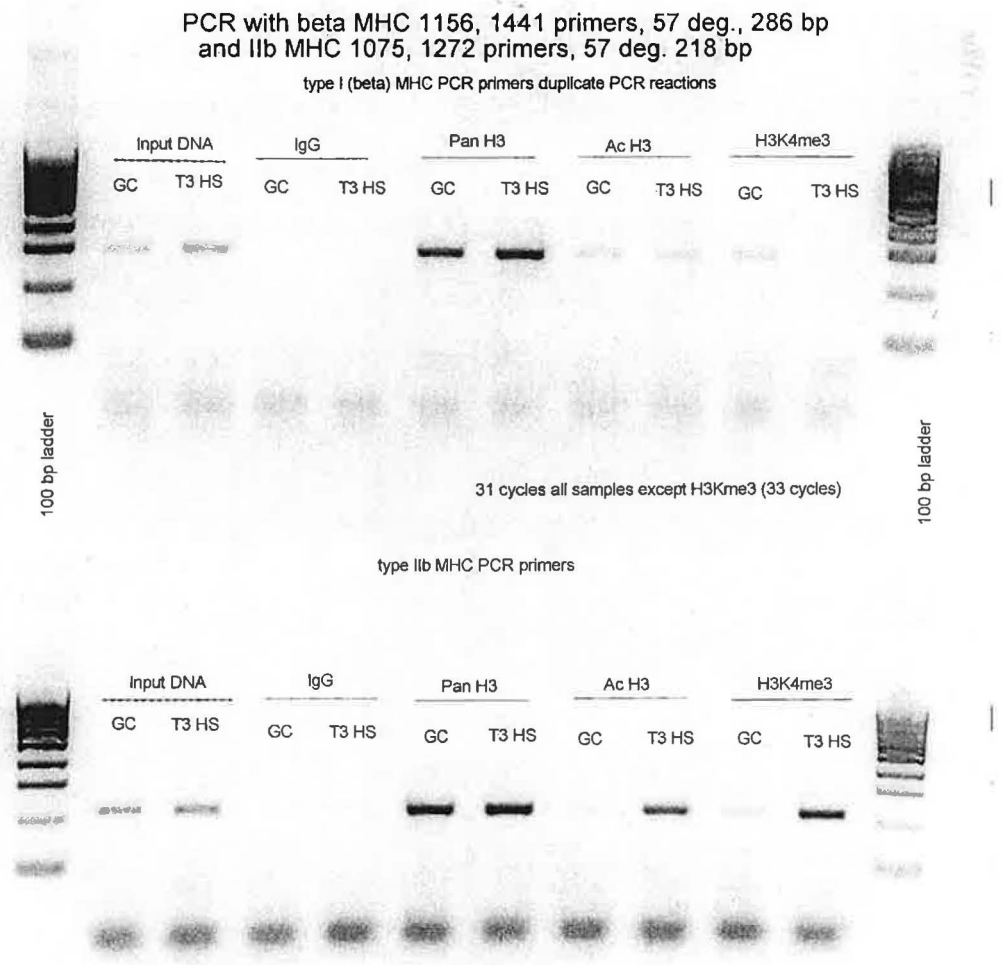

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[illegible]ate/Time:

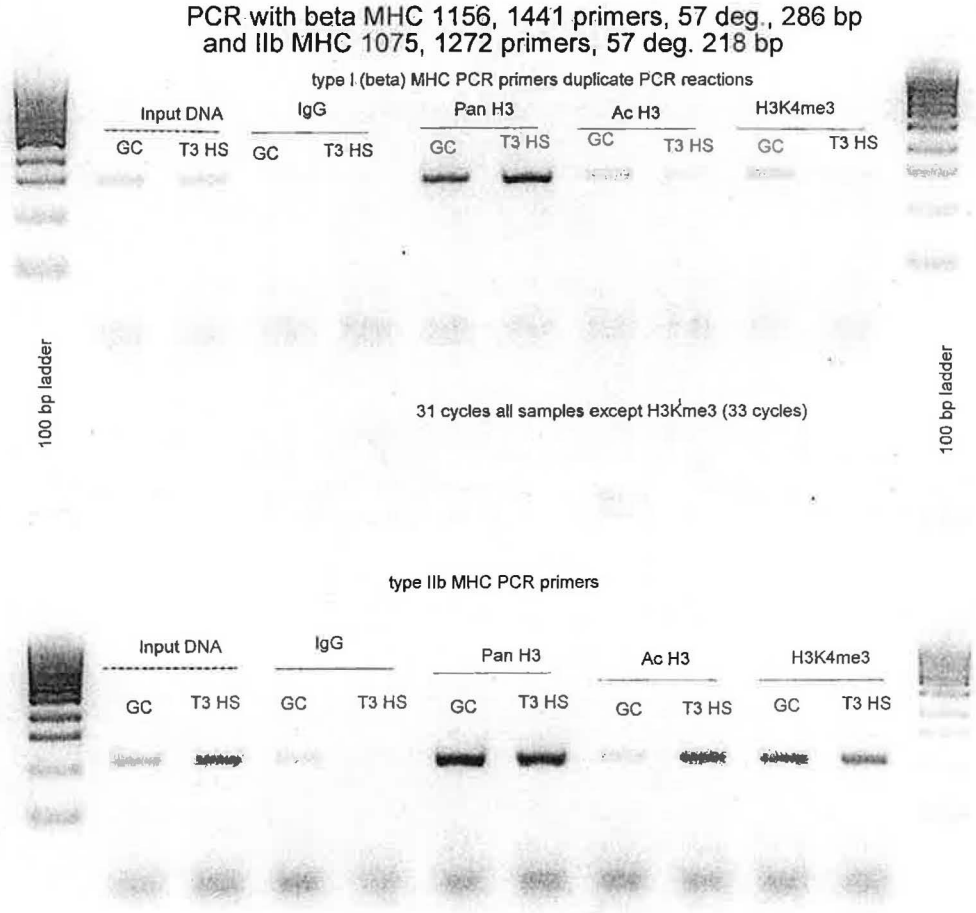

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Image Name: G:\Carola\2-05 tissue\ChIP results 2-13-09 beta actin and IIb primers.gel
Image Comment:
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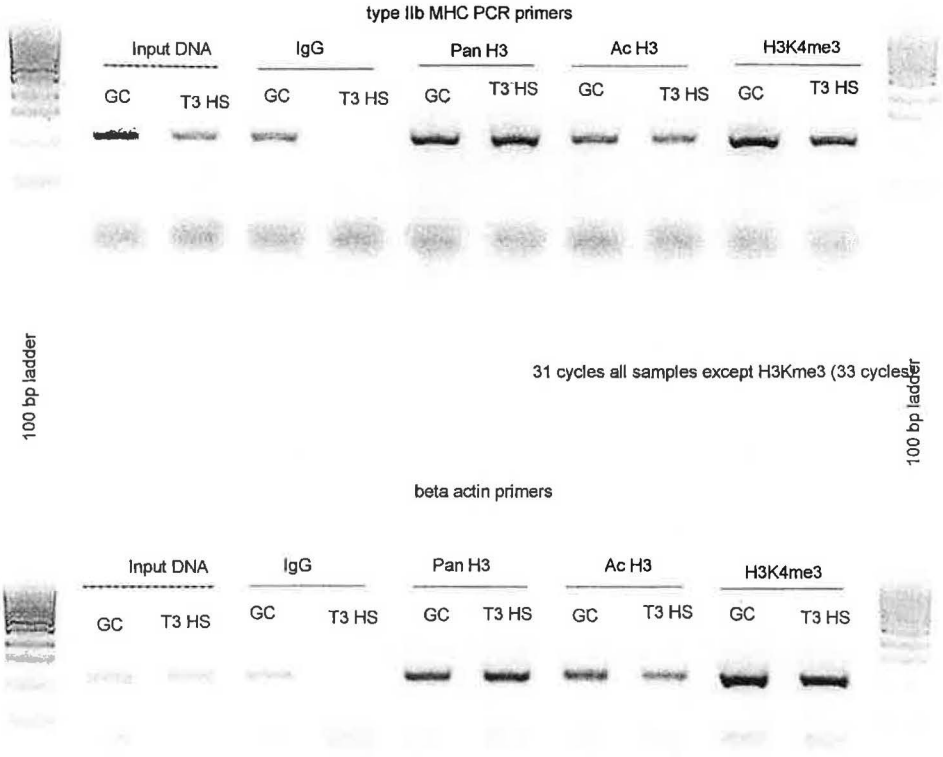
P. ate/Time:

PCR with IIb MHC 1075, 1272 primers, 57 deg. 218 bp
and Beta Actin 1517,1741 primers, 57 deg



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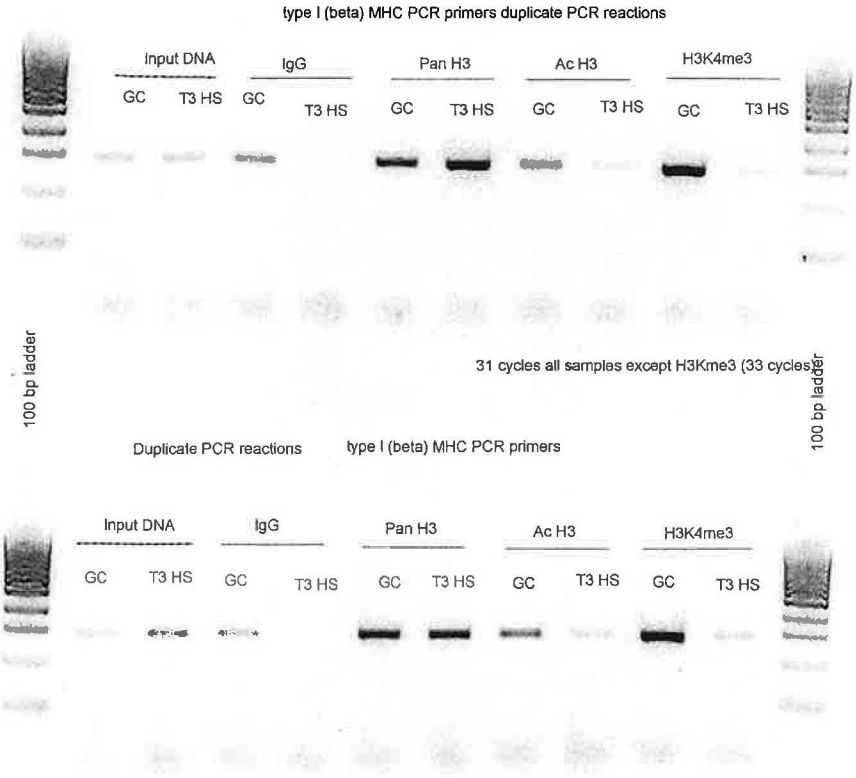
PCR with beta MHC 1156, 1441 primers, 57 deg., 286 bp



Tuesday Feb. 24, 2009

Set up more PCR reactions with the 2-19 samples.

Use the beta MHC +1156 / +1441 primer set at 58 °C annealing,
The IIa pre 1151 F/1456 R primer set at 58 °C annealing,
The IIx 5' end Pre 2 F/R at 58 °C and
The IIb 1075 F / 1272 R primer set also at 58°C annealing and the

Total of 40 samples (all from B- set).

Each tube has a total of 25 µl plus mineral oil on top.

Use 12 µl of eluted DNA per 25 µl PCR reaction. Same amount for all samples!

31/33 cycles of
60 sec. 96°C, 45 sec. 58, 45 sec. of 72 °C (Program # 33)
Use 2 different PCR machines: 31 cycles for all samples except the trimethylation samples that get 33 cycles.

Wednesday Feb. 25, 2009

Run samples on two 2% agarose gels with 100bp ladder.

Results see next pages.

File/Range: G:\Carola\2-05 tissue\ChIP results 2-25-09 betaMHC and IIa primers B-set.gel / 2829--26474.000 /Magnification
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Image Comment:
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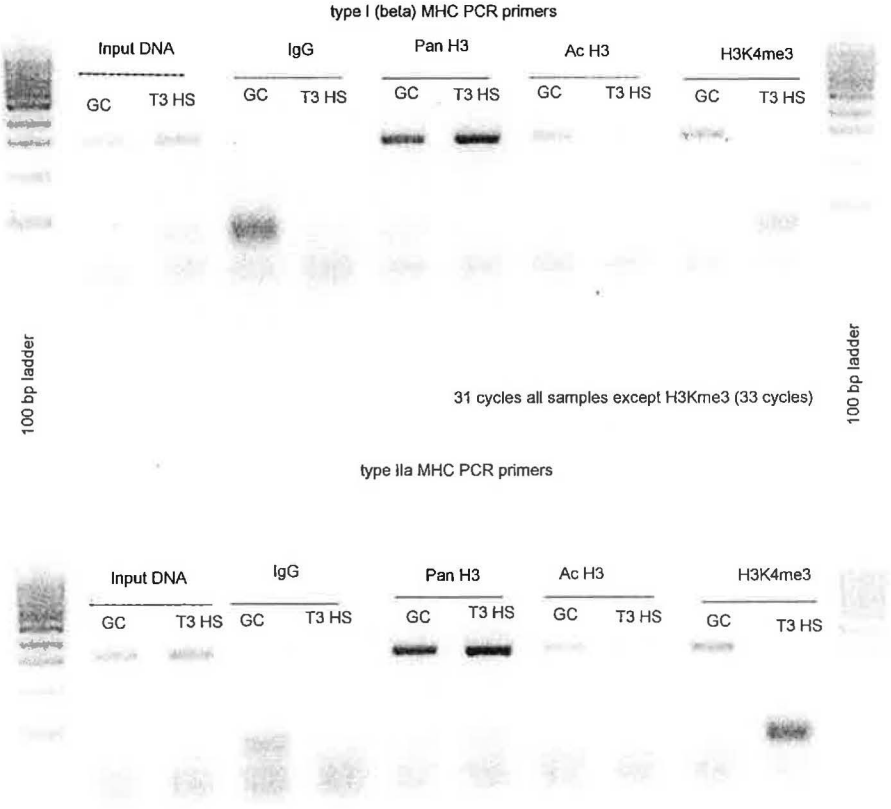
Print Date/Time:

PCR with beta MHC 1156, 1441 primers, 58 deg., 286 bp
and IIa MHC 1151, 1456 primers, 58 deg.



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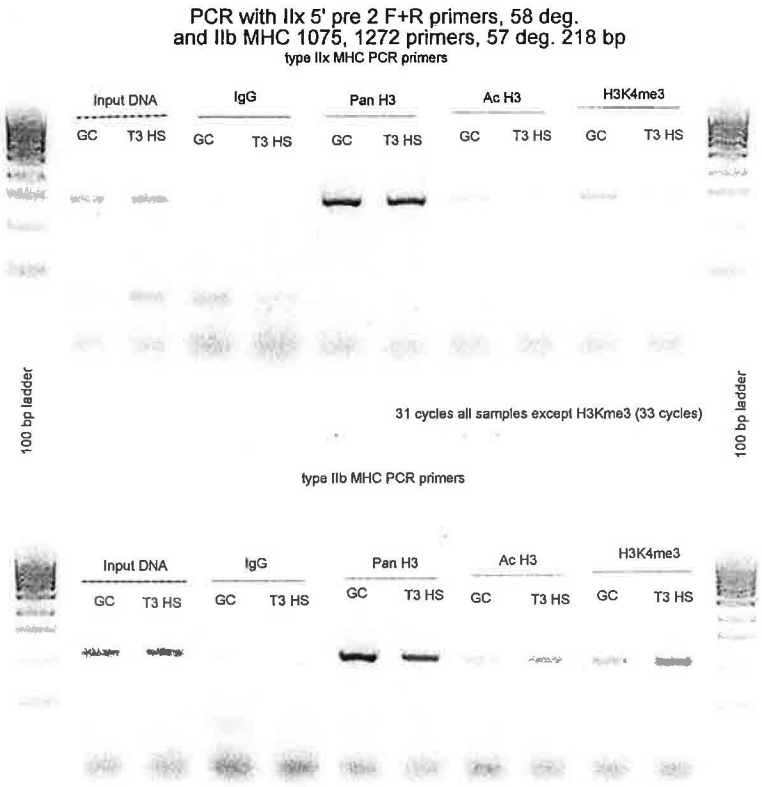
[illegible] Date/Time:



Carola's Data:

Tissue 8-13-08, ChIP 8-20, HS animals walked before sac.

ChIP results 8-25-08

Type I	Input	Input	Rab IgG	*H3 (Ave)*	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	2,401,094		37,358	*15,577,998*	863,776	5,230,116	6.47	0.34	**0.16**	2.16	**0.14**
T3+HS	2,401,094		66,894	*14,910,579*	-	3,934,936	6.18	(0.03)	**(0.01)**	1.61	**0.11**

9-08 ChIP samples

ChIP results 2-3-09 beta and IIb primers 9-8-08 ChIP

Type I	Input	Input	Rab IgG	*H3 (Ave)*	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	6,857,844		-	17,389,805	19,842,499	2,849,420	2.54	2.89	**1.16**	0.42	
T3+HS	5,165,159		-	17,992,287	7,186,612	2,230,452	3.48	1.39	**0.54**	0.43	

Tissue 10-22, ChIP 10-29 **good**

ChIP results 11-03-08 beta and IIb primers

Type I	Input	Input	Rab IgG	H3	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	1,093,586		729,954	9,728,646	1,907,364	8,285,426	8.23	1.08	**0.84**	6.91	**0.77**
T3+HS	1,086,620		-	7,520,544	1,250,817	2,657,150	6.92	1.15	**1.07**	2.45	**0.33**

11-16 tissue, ChIP started on 11-24 DNA was re-sheared on these samples, still didn't look very good.

Beta and IIb MHC ChIP PCR results 12-02-08

Type I	Input	Input	Rab IgG	H3	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	7,593,714	8,423,566	57,216	14,324,199	9,492,438	2,229,324	1.78	1.18	**0.83**	0.27	**0.95**
T3+HS	8,003,658	6,768,730	180,699	12,952,290	9,141,342	2,601,558	1.73	1.21	**0.95**	0.30	**1.18**

ChIP results 1-29-09 betaMHC and IIb primers 11-26 ChIP

Type I	Input	Input	Rab IgG	H3	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	not available	not available	7,880	10,541,268	3,320,354	5,885,372					
T3+HS			-	9,458,778	9,155,134	4,979,888					

ChIP results 2-3-09 betaMHC and IIb primers 11-26 ChIP

Type I	Input	Input	Rab IgG	H3	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	3,653,882		64,858	20,214,400	8,568,132	5,838,230	5.51	2.33	**0.81**	1.58	**1.18**
T3+HS	3,881,130		-	28,326,110	7,883,128	2,849,308	7.30	2.03	**0.50**	0.73	**0.39**

tissue prep on 11-21, CHIP on 12-5 **good**

ChIP results 12-08-08 beta and IIb primers

Type I	Input	Input	Rab IgG	H3	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	1,584	9,654,290	125,292	22,681,150	10,853,661	5,472	2.71	1.29	**0.97**	0.56	**0.99**

T3+HS	8,891,958	19,806,778	200,403	24,258,902	4,100,046	1,370,804	2.71	0.44	**0.19**	0.13	**0.14**

ChIP results 1-23-09 betaMHC and IIb primers, run gel longer

Type I	Input	Input	Rab IgG	H3	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	2,829,872	1,974,590	77,328	8,343,950	2,057,565	4,881,130	3.44	0.82	**1.00**	2.00	**0.97**
T3+HS	1,445,172	3,402,479	58,228	5,927,008	696,408	657,028	4.06	0.44	**0.45**	0.41	**0.17**

ChIP results 1-29-09 beta and IIb primers

Type I	Input	Input	Rab IgG	H3	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected Ac**	Net H3K4me3	**Corrected H3K4me3**
GC	5,092,906		259,890	21,144,315	8,221,306	9,328,412	4.10	1.56	**0.97**	1.78	**0.94**
T3+HS	4,372,832		151,286	23,790,951	3,318,590	2,696,258	5.41	0.72	**0.40**	0.58	**0.27**
									ave acH3		ave H3K4me3
									0.98		**0.96**
									0.35		**0.19**

Tissue prep on 2-5, Chip on finished on 2-12

ChIP results 2-13-09 betaMHC primers (duplicates)

Type I	Input	Rab IgG	H3	Ac H3	H3K4me3	Net H3	Net Ac H3	**Corrected**	Net H3K4me3	**Corrected H3K4me3**
GC	3,853,830	7,483,043	##########	9,923,976	28,220,203	4.10	0.63	**0.80**	5.38	**1.02**
T3+HS	4,552,774	134,080	##########	1,779,555	1,756,362	5.24	0.36	**0.30**	0.36	**0.04**
GC	2,982,533	6,308,497	##########	9,641,268	27,567,065	5.86	1.12	**1.28**	7.13	**1.22**
T3+HS	7,591,638	-	##########	2,551,430	3,394,750	2.20	0.34	**0.40**	0.45	**0.08**
ave	3,418,182	6,895,770	##########	9,782,622	27,893,634	4.87	0.84	**1.02**	6.14	**1.11**
ave	6,072,206	67,040	##########	2,165,493	2,575,556	3.34	0.35	**0.34**	0.41	**0.06**

Appendix E

Travel Activities
Selected Documentations

Tagung – Übernachtung Raum! Funktion

Erfolg ist zielgerichtetes, strukturiertes Handeln. Er basiert auf Know-how und Motivation. Und darauf, wie und wo Sie sich und Ihre Pläne, Überlegungen, Produkte präsentieren. Gehen Sie neue Wege. Verlassen Sie bekannte Pfade. Tagen Sie in technisch perfekter Umgebung und niveauvoll-legerem Ambiente. Dazu die außergewöhnliche Note Sport - bei uns! Schulen und bewegen Sie Ihre Mitarbeiter in unseren Räumen. Stellen Sie Ihr Angebot in gleichermaßen adäquater wie ungewöhnlicher Umgebung vor. Wir geben Ihrer Inspiration Raum!

20 Veranstaltungsräume für Tagungen, Kongresse oder Messen mit 10 bis 500 Teilnehmern stehen zur Verfügung. Dazu wahlweise folgende Ausstattung (und mehr):

- *Fernbedienbare Medienwände*
- *Mobile Medienschränke*
- *Großbildprojektoren, DVD-Player*
- *Video-HiFi-Projektion*
- *Flipcharts/Whiteboards/Tafeln*
- *Wandgroße Pinnwände*
- *Rednerpulte, mobile Trennwände*
- *Internet-Anschlüsse (Analog, ISDN, DSL, W-Lan)*

Übernachten Sie am Ende eines kreativen Tages in unserem Hotelbereich! 94 Gästezimmer (183 Betten), elegant als Einzel- oder Zweibettzimmer oder praktisch als Mehrbettzimmer, warten auf Sie.
Die Ausstattung: Dusche/WC, Fernseher und (tw.) Internetanschluss. Ein Teil unserer Zimmer ist behindertengerecht ausgebaut.
Welcome to our house!

TAGUNG/KONGRESS/MESSE/ÜBERNACHTUNG

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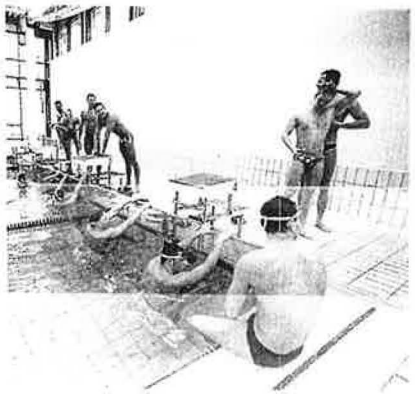
Sportschule und Bildungsstätt

des Landessportbundes Hessen e.V.

sportland hess



Sport: Ja! – und mehr ...

Es ist die außergewöhnliche Kombination, aus der die ungewöhnlichen Möglichkeiten entstehen. Sport und Bildung, Tagung und Restauration, Kongress und Hotellerie - unter einem, unter unserem Dach. Modern und mannigfaltig, großräumig und großzügig - das ist die „Sportschule und Bildungsstätte" des Landessportbundes. Eine Vielzahl sportlich-kreativer Möglichkeiten wartet auf Sie! All dies herrlich im Grünen, mitten im Frankfurter Stadtwald und trotzdem zentral. Sie sind herzlich eingeladen!

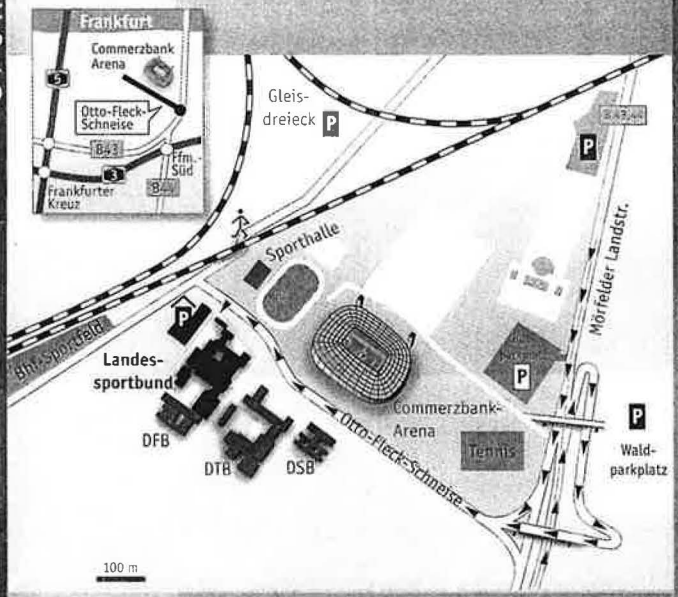
Sport ist unser Fach - in jeder Hinsicht. Und was den Raum für Ihren Bewegungsdrang betrifft, haben wir Einiges zu bieten. Sie finden bei uns:

- *zwei Großsporthallen*
- *eine Mehrzweckhalle*
- *Hallenbad mit wahlweise 4x50m oder 8x25m-Bahnen und*
- *Lehrschwimm- und Gymnastikbecken*
- *Trockenluftsauna, Dampfsauna*
- *Indoor- und Outdoor-Kletterwände*
- *Kraft- und Fitnessraum*
- *Diagnostikstrecke mit angegliederter Sprunggrube*
- *Motoraum, Tischtennis, Poolbillard etc.*

Dazu ausgebildete Sportpädagogen, die Sie (auf Wunsch) individuell betreuen. Wir bringen Sie in Bewegung!

SPORT

So erreichen Sie uns:



Mit dem Auto:

Von der Autobahn A3: Der Beschilderung „Frankfurt Süd" folgen. Nach Abfahrt Frankfurt Süd in Richtung Innenstadt. Rechts abbiegen - den Hinweisschildern „Stadion/Sportverbände" folgen. (Parkdeck vor Ort vorhanden!)

Aus der Innenstadt:

In Richtung „Stadion" (Frankfurt Süd) über die Kennedyallee und die Mörfelder Landstraße. Auf der rechten Spur bleiben (den Hinweisschildern „Stadion/Sportverbände" folgen). Hinter dem Stadion rechts in die Otto-Fleck-Schneise einbiegen. (Parkdeck vor Ort vorhanden!)

Die Haltestelle "Sportfeld" heißt jetzt "Stadion"

Mit der S-Bahn:

Die S 8 fährt ab Offenbach/ die S 9 ab Hanau über Frankfurt Süd -> Konstabler Wache -> Hauptwache -> Hauptbahnhof -> Niederrad -> bis „Sportfeld" und weiter über den Flughafen Frankfurt nach Wiesbaden. Vom Bahnhof „Sportfeld" aus sind es ca. 8 Minuten zu Fuß.

Sportschule und Bildungsstätte
des Landessportbundes Hessen e.V.
Otto-Fleck-Schneise 4
60528 Frankfurt am Main
Tel.: 069 [illegible]9-400
Fax: 069, [illegible]89-273
www.sport-in-hessen.de

Deutsche Gesellschaft für Kardiologie
– Herz- und Kreislaufforschung e. V.
GERMAN CARDIAC SOCIETY

WEITER- UND FORTBILDUNGS
AKADEMIE KARDIOLOGIE

Anerkannt nach § 10 der Fortbildungsordnung durch die Landesärztekammern Nordrhein (seit April 2001), Bayern (seit Juli 2003) und Westfalen-Lippe (seit Juni 2005)

Teilnahmebescheinigung

Herr/Frau Dr. Carola Wright
Titel/Name/Vorname

EFN

geboren am: 23. Dezember 1964 EFN.....
Datum

wohnhaft in: Schüttenhelmweg 51, 60529 Frankfurt /Main
Straße/Hausnr., PLZ, Ort

hat an der Fortbildungsveranstaltung zum Thema:

Herzinsuffizienz - Update 2009

Akademie-Veranstaltungsnummer: FGK - 09 - 06 - 06 - 24133

vom 06. Juni 2009 Beginn: 09:00 Uhr bis 06. Juni 2009 Ende: 17:00 Uhr

in Bad Nauheim teilgenommen.

Die Veranstaltung wurde von der Weiter- und Fortbildungs-Akademie *„Kardiologie“* bewertet, und ist im Rahmen der „Zertifizierung der ärztlichen Fortbildung“ unter der Voraussetzung der vollständigen Veranstaltungsteilnahme durch die Landesärztekammer Hessen mit 8 Fortbildungspunkt(en) anrechenbar.

Prof. Dr. Christian Hamm
Kerckhoff-Klinik GmbH
Ärztlicher Direktor

Bad Nauheim, 06.06.2009
Datum

Stempel/Unterschrift
Wissenschaftlicher Leiter

VNR *27606020090447300000*

Kategorie: A	Fortbildungspunkt(en): 8	Zusatzpunkte: 0

Wir möchten ausdrücklich darauf hinweisen, dass bei nicht aufgeklebtem LÄK-EFN-Barcode-auf der Teilnehmerliste die automatische Übermittlung der CME-Punkte durch den Veranstalter an den EIV nicht vorgenommen werden kann.
In diesem Fall müssen Sie diese Teilnahmebescheinigung direkt bei Ihrer Ärztekammer einreichen.

"Christina Reuschling"
<c.reuschling@kerckhoff-forschungs-gmbh.de>
05/28/2009 02:31 AM

To <CWright@MtSAC.edu>
cc
bcc
Subject [?SPAM?] Flyer Symposium "Herzinsuffizienz - Update 2009" am 6.6.2009

Sehr geehrte Frau Wright,
anbei sende ich Ihnen wie besprochen unseren Veranstaltungsflyer.
Wir freuen uns über Ihre Teilnahme.
Mit freundlichen Grüßen,
Christina Reuschling (Sekretariat Kerckhoff-Klinik
Forschungsgesellschaft mbH)

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00512 Einladung Symposium Update 2009_4.pdf

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13353 Berlin

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Universität
60590 Frankfurt

Zertifizierung

Die Veranstaltung wurde von der Weiter- und Fortbildungsakademie „Kardiologie" bewertet und zur Zertifizierung bei der Landesärztekammer Hessen eingereicht. Die vollständige Teilnahme wird voraussichtlich mit sieben Punkten anerkannt.

Die Veranstaltung steht unter der Schirmherrschaft der Deutschen Gesellschaft für Kardiologie.

Sponsoren

Wir danken folgenden Sponsoren für ihre Unterstützung:

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Teilnahmebedingungen

Keine Teilnahmegebühren. Anmeldung erforderlich (siehe Rückantwortfax anbei)

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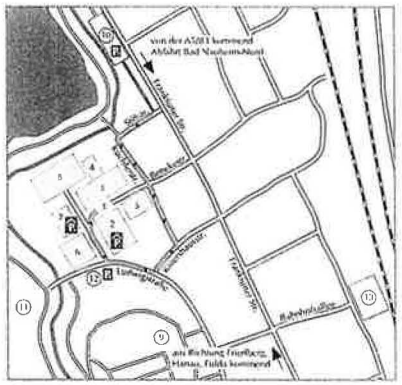
Anfahrt



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3 Herzchirurgie
4 Thoraxchirurgie
5 Gästehaus
6 Kerckhoff-Rehabilitations-Zentrum
7 Groedel-Institut
8 HELIOS William-Harvey-Klinik
9 Sprudelhof
10 Parkplatz Frankfurter Str.
11 Kurpark
12 Therme am Park
13 Bahnhof

P Parkplatz 1 (gebührenpflichtig)
P Parkhaus 2 (gebührenpflichtig)
P Parkhaus 3 (gebührenpflichtig)
P Parkplatz 4 (gebührenpflichtig)

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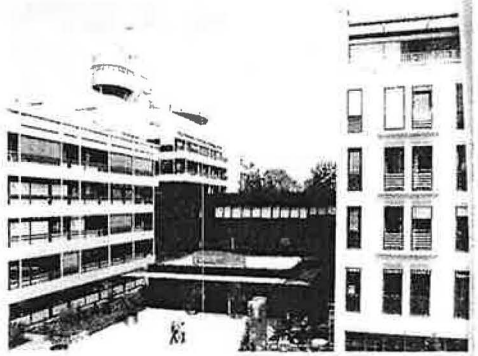

Symposium

Herzinsuffizienz – Update 2009

Samstag, 6. Juni 2009
9:00 – 17:00 Uhr

Herzinsuffizienz – Patientenseminar

Freitag, 5. Juni 2009
17:00 – 19:00 Uhr

Wissenschaftliche Leitung

Prof. Dr. med. Christian Hamm
Prof. Dr. med. Veselin Mitrovic

Liebe Kolleginnen und Kollegen,

wir möchten Sie herzlich zu unserem 8. Herzinsuffizienz-Symposium „Herzinsuffizienz – Update 2009" nach Bad Nauheim einladen. Dieses inzwischen jährlich gehaltene Symposium hat sich seit der ersten Veranstaltung vor 10 Jahren und nach den Erfolgen in den letzten Jahren zu einer bedeutenden Plattform der Herzinsuffizienz entwickelt.

Auch in diesem Jahr werden wir versuchen, Sie als Teilnehmer aktiv in den Symposiumsablauf einzubinden. Wir legen großen Wert auf Praxisnähe und die Möglichkeit der Umsetzung in der täglichen Klinikroutine und hoffen, durch eine Auswahl aktueller Themen und renommierter Referenten Ihren Erwartungen gerecht zu werden.

Innovative Lösungen für die Behandlung kardiovaskulärer Erkrankungen werden auch in diesem Jahr im Mittelpunkt unserer Fortbildung stehen. Trotz deutlicher Fortschritte in der modernen Diagnostik und Pharmakotherapie stellt die Herzinsuffizienz noch immer nicht nur eine große medizinische, sondern auch eine große sozioökonomische Herausforderung dar. Die Prognose ist nach wie vor ernst und häufig mit ungewissem Ausgang.

Eine leitliniengerechte Herzinsuffizienztherapie führt zu einer Verbesserung der Lebensqualität und einer Senkung der Mortalität von über 50%. Vor diesem Hintergrund kommen Therapieoptionen wie Herztransplantation und mechanische Unterstützungssysteme zunehmend zum Einsatz und mit der Betreuung solcher Patienten werden wir in Zukunft immer stärker in der täglichen Praxis konfrontiert werden. In diesem Symposium möchten wir Sie durch Auswahl aktueller Themen vorgetragen von kompetenten Referenten darin unterstützen, eine optimale Patientenversorgung zu gewährleisten.

Wie im letzten Jahr findet am Vorabend zur Veranstaltung ein Herzinsuffizienz-Patientenseminar statt. Wir möchten Sie schon jetzt bitten, die Information über den Ablauf des Seminars an interessierte und in Ihren Augen geeignete Patienten weiter zu geben und diese zur Teilnahme zu motivieren.

Wir freuen uns schon heute auf Ihr Kommen und verbleiben

mit freundlichen kollegialen Grüßen

Prof. Dr. med. C. Hamm
Ärztlicher Direktor
Kerckhoff-Klinik

Prof. Dr. med. V. Mitrovic
Med. Direktor Kerckhoff-Klinik
Forschungsgesellschaft mbH

PD Dr. med. M. Weber
Oberarzt der Abt. Kardiologie
Kerckhoff-Klinik

Dr. med. J. Sperzel
Oberarzt der Abt. Kardiologie
Kerckhoff-Klinik

Herzinsuffizienz – Update 2009

Programm

Samstag, 6. Juni 2009

9:00 - 9:05 Uhr
Begrüßung und Einleitung
C. Hamm, Bad Nauheim

9:05 - 9:20 Uhr
Plötzlicher Herztod
B. Lüderitz, Bonn

1. NICHT-ISCHÄMISCHE HERZINSUFFIZIENZ

Vorsitz: C. Hamm, Bad Nauheim,
B. Lüderitz, Bonn

9:20 - 9:35 Uhr
Neues in der Therapie der
systolischen Herzinsuffizienz
M. Böhm, Homburg/Saar

9:40 - 9:55 Uhr
Neues in der Therapie der
diastolischen Herzinsuffizienz
U. Hoppe, Köln

10:00 - 10:15 Uhr
Was ist in der Pipeline?
V. Mitrovic, Bad Nauheim

10:20 - 10:45 Uhr
Paneldiskussion
C. Hamm, B. Lüderitz, M. Böhm,
U. Hoppe, V. Mitrovic

10:45 – 11:15 Uhr
Pause

2. ISCHÄMISCHE HERZINSUFFIZIENZ

Vorsitz: V. Mitrovic, Bad Nauheim,
M. Böhm, Homburg/Saar

11:15 - 11:30 Uhr
Interventionelle und medikamentöse Therapie der
chronischen ischämischen Herzinsuffizienz
V. Schächinger, Frankfurt/Main

11:35 - 11:50 Uhr
Therapie der akuten Herzinsuffizienz und des kardiogenen
Schocks beim akuten Koronarsyndrom
A. Elsässer, Oldenburg

11:55 - 12:00 Uhr
Fallbeispiel
H. Nef, Bad Nauheim

12:00 - 12:20 Uhr
Paneldiskussion
V. Mitrovic, M. Böhm, V. Schächinger,
A. Elsässer, H. Nef

12:20 - 13:30 Uhr
Mittagspause

3. VALVULÄRE HERZINSUFFIZIENZ

Vorsitz: A. Elsässer, Oldenburg,
W.-P. Klövekorn, Bad Nauheim

13:30 - 13:45 Uhr
Pathophysiologie und Diagnostik der
valvulären Kardiomyopathie
C. Müller, Basel (CH)

13:50 - 14:05 Uhr
Operative Therapie bei
Herzklappenerkrankungen
M. Roth, Bad Nauheim

14:10 - 14:25 Uhr
Interventionelle Therapie bei
Herzklappenerkrankungen
M. Weber, Bad Nauheim

14:30 - 14:35 Uhr
Fallbeispiel
H. Möllmann, Bad Nauheim

14:35 - 14:55 Uhr
Paneldiskussion
A. Elsässer, W.-P. Klövekorn,
C. Müller, M. Roth,
M. Weber, H. Möllmann

14:55 - 15:15 Uhr
Kaffeepause

4. TERMINALE HERZINSUFFIZIENZ

Vorsitz: H.-R. Figulla, Jena, D. E. Birnbaum, Bad Nauheim

15:15 - 15:30 Uhr
Mechanische Herzunterstützung bei Akutversagen
H.-R. Figulla, Jena

15:35 - 15:50 Uhr
Wann ist wer für Herzersatz geeignet?
D. E. Birnbaum, Bad Nauheim

15:55 - 16:10 Uhr
Elektrostimulation bei Herzinsuffizienz
J. Sperzel, Bad Nauheim

16:15 - 16:20 Uhr
Fallbeispiel
H. Steiger, Bad Nauheim

16:20 - 16:45 Uhr
Paneldiskussion
H.-R. Figulla, D. E. Birnbaum,
J. Sperzel, H. Steiger

16:45 - 17:00 Uhr
Zusammenfassung, Verabschiedung
C. Hamm, Bad Nauheim

Herzinsuffizienz – Patientenseminar

Freitag, 5. Juni 2009

17:00 - 17:10 Uhr
Einleitung
V. Mitrovic, Bad Nauheim

17:40 - 17:50 Uhr
Stellenwert der Telemedizin in der Betreuung der
Herzinsuffizienz-Patienten
W. Ricken, Bad Nauheim

17:50 - 17:50 Uhr
Bewegungstherapie und andere Aktivitäten
M. Rauchhaus, Berlin

17:50 - 18:10 Uhr
Herztransplantation und Unterstützungssysteme
D. E. Birnbaum, Bad Nauheim

18:10 - 19:00 Uhr
Paneldiskussion
V. Mitrovic, W. Ricken,
M. Rauchhaus, D. E. Birnbaum

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23.4.2009

Teilnahmebescheinigung

Frau Carola Wright hat am 23. April 2009 an einer ganztägigen Veranstaltung der DBGS teilgenommen.

Themen der Veranstaltung waren die Durchführung von Belastungs-EKGs und die Messung von physiologischen Parametern (Lungenfunktionstests, Seh- und Hörfähigkeit etc.) im Rahmen von Sporttauglichkeitsuntersuchungen und Arbeitstauglichkeitsuntersuchungen.

Mit freundlichen Grüßen

U. Zie

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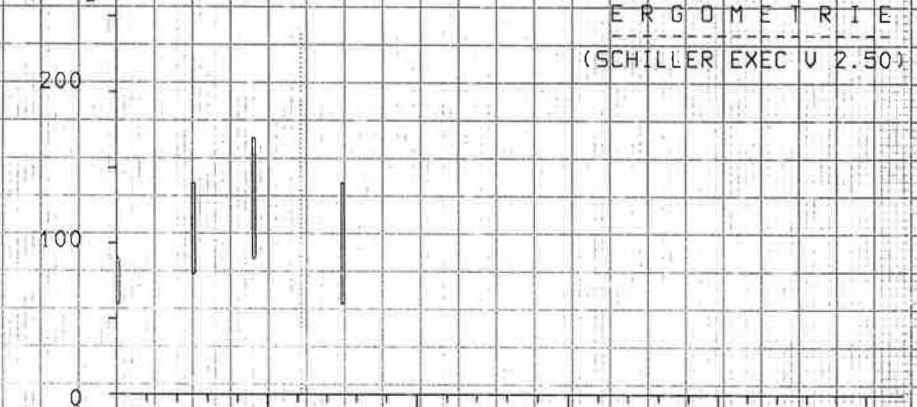
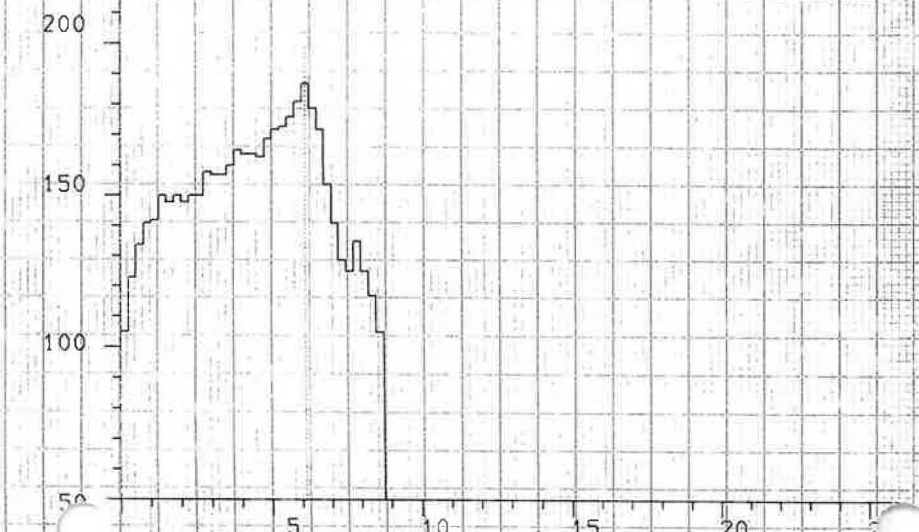
Fax: 069/265-25826

(mmHg) BLUTDRUCK



(W) LAST

(/min) HERZFREQUENZ



DB [illegible]undheitsService GZ Mitte

E R G O M E T R I E

(SCHILLER EXEC V 2.50)

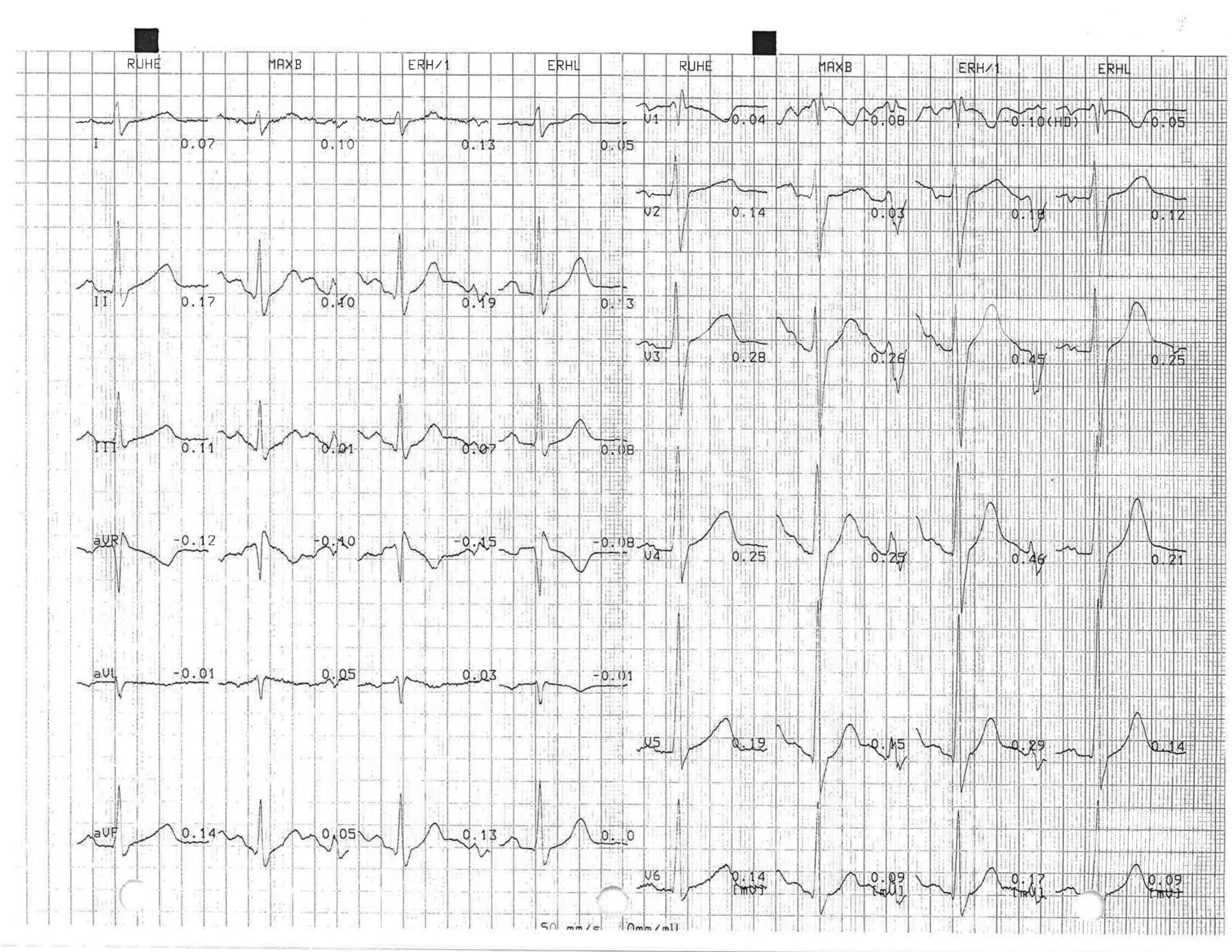
Grundlast:	100 W	Pat-Name :	
Laststufe:	25 W	PHILIP WRIGHT	
Stufendauer:	2 min		
		Pat-Nr. :	
Belastungsabbruch:	6:02		
Ende der Messung:	8:44	Geb. : 5.6.96	
		Alter : 12 Jahre	
Max. Herzfrequenz:	187/min	Gesch. : M	
	90 %	Grösse : 165 cm	
		Gewicht : 50 kg	
PWC max.:	175 W = 89%	BD: mmHg	
PWC150/170:	102/131 W	Med.:	
PWC rel.:	2.0/2.6 W/kg	Bem.:	

Max. BD: 170/ 90 mmHg
Max. BD(syst)*HF:29700

Abbruch wegen:
Erreichen der Ziel-HF

Do 23 APR 09 14:13:40

LAST	ZEIT	HF	BD	A	D	S
0	1:00	102	90/ 60	0	0	0
100	2:00	149	0/ 0	0	0	0
125	2:00	166	140/ 80	0	0	0
150	2:00	183	170/ 90	0	0	0
25	0:55	151	170/ 90	0	0	0
25	1:55	131	140/ 60	0	0	0
25	2:40	109	140/ 60	0	0	0
0	[illegible]		120/60			


RUHE
MAXB
ERH/1
ERHL
I
0.07
0.10
0.13
0.05
II
0.17
0.10
0.19
0.13
III
0.11
0.01
0.07
0.08
aVR
-0.12
-0.10
-0.15
-0.08
aVL
-0.01
0.05
0.03
-0.01
aVF
0.14
0.05
0.13
0.10
RUHE
MAXB
ERH/1
ERHL
V1
0.04
0.08
0.10(HB)
0.05
V2
0.14
0.03
0.18
0.12
V3
0.28
0.26
0.45
0.25
V4
0.25
0.25
0.46
0.21
V5
0.19
0.15
0.29
0.14
V6
0.14
0.09
0.17
0.09
50 mm/s
10mm/mV

Pat-Name :
PHILIP WRIGHT

Pat-Nr. :

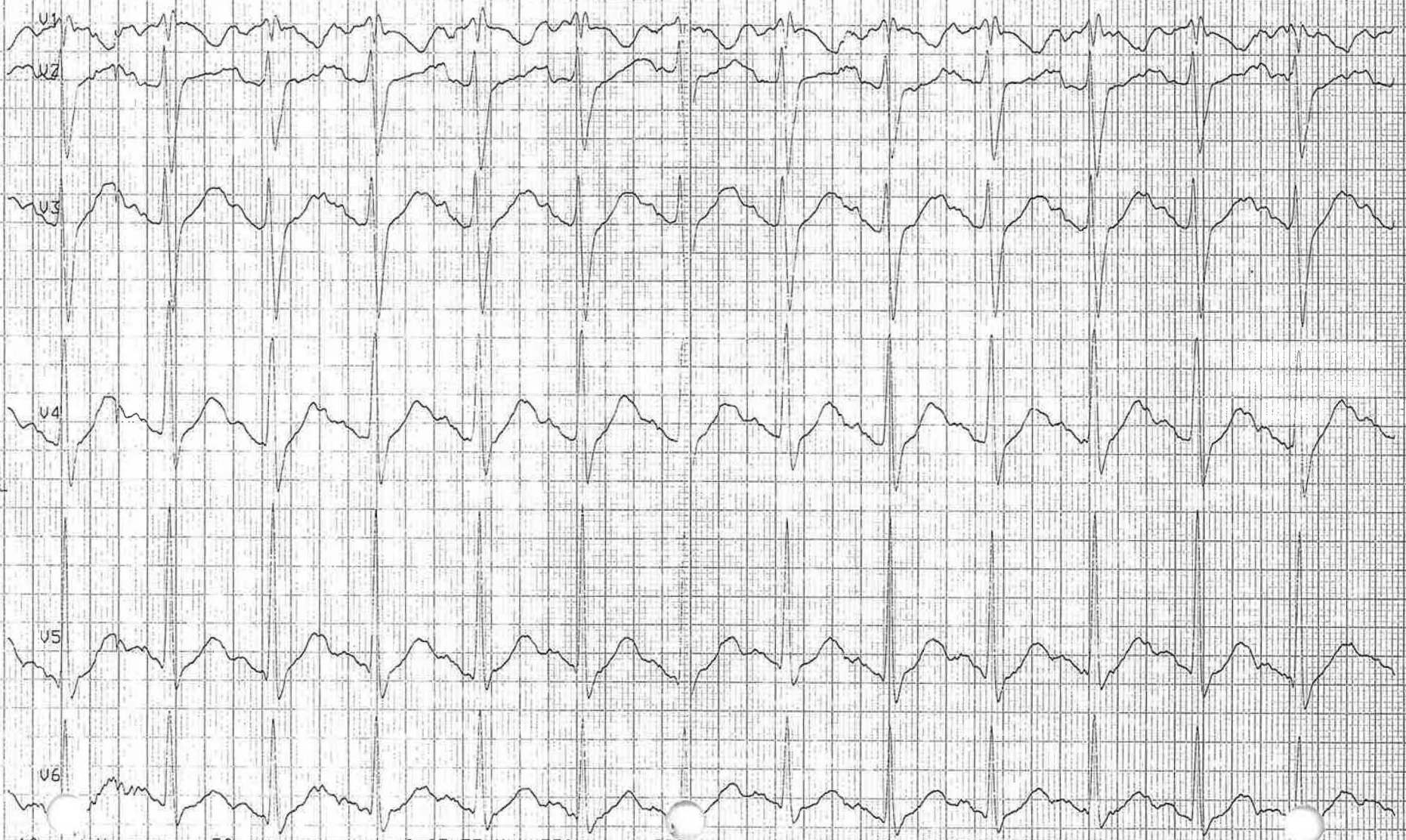
Geb. :5.6.96
Alter 12 Jahre
Geschl. : M
Grösse :165 cm
Gewicht :50 kg
BD: mmHg
Med.:
Bem.:

HF: 165/min
QRS: 103 ms
88 °
BD: 140 mmHg
080 mmHg

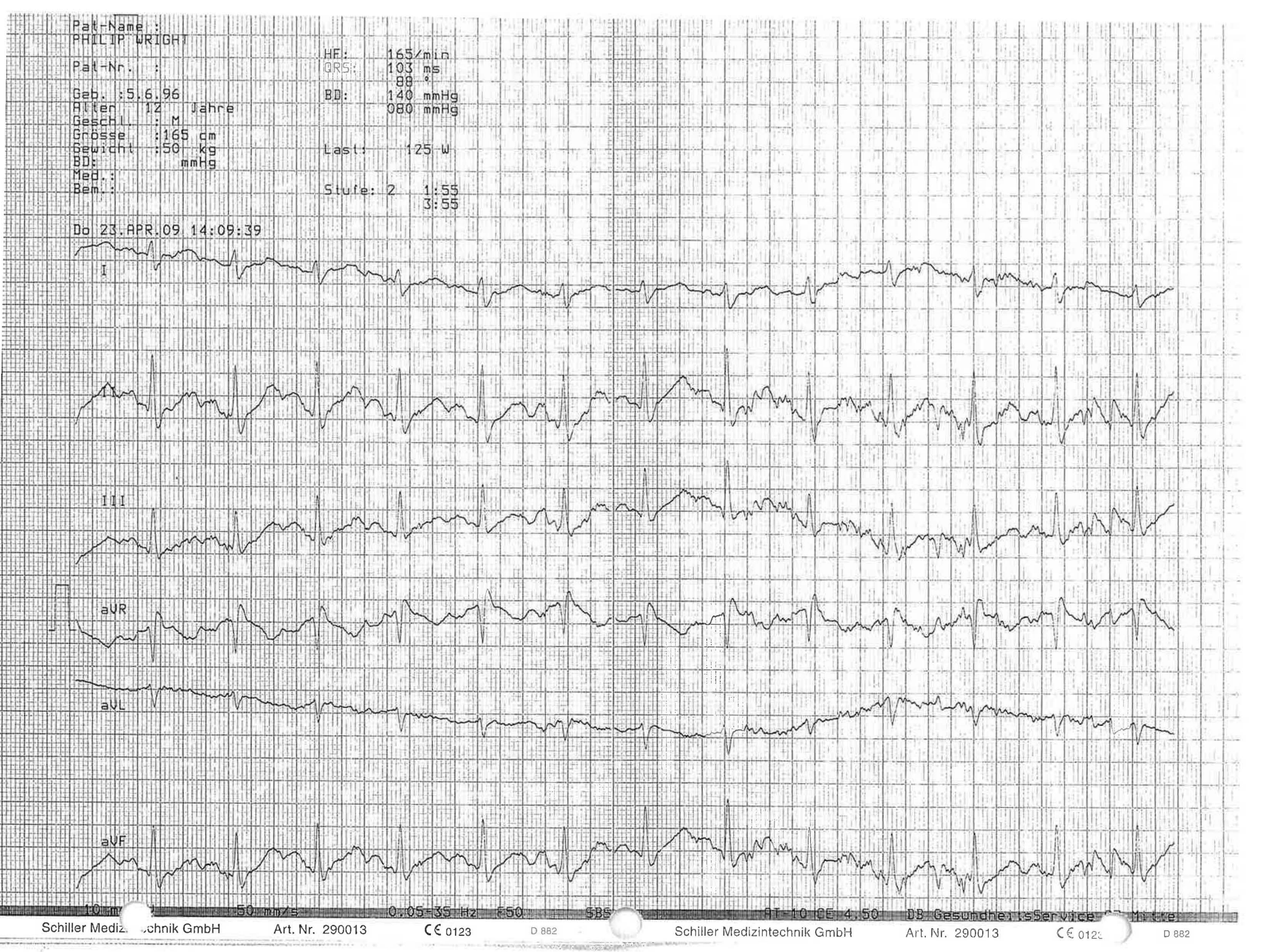
Last: 125 W

Stufe: 2 1:55
3:55

Do 23.APR.09 14:09:39



10 mm/mV 50 mm/s 0.05-35 Hz F50 SBS AT-10 DE 4.50 DB Gesundheitswesen



Schiller Medizintechnik GmbH Art. Nr. 290013 CE 0123 D 882

Pat-Name :
PHILIP WRIGHT

Pat-Nr. :

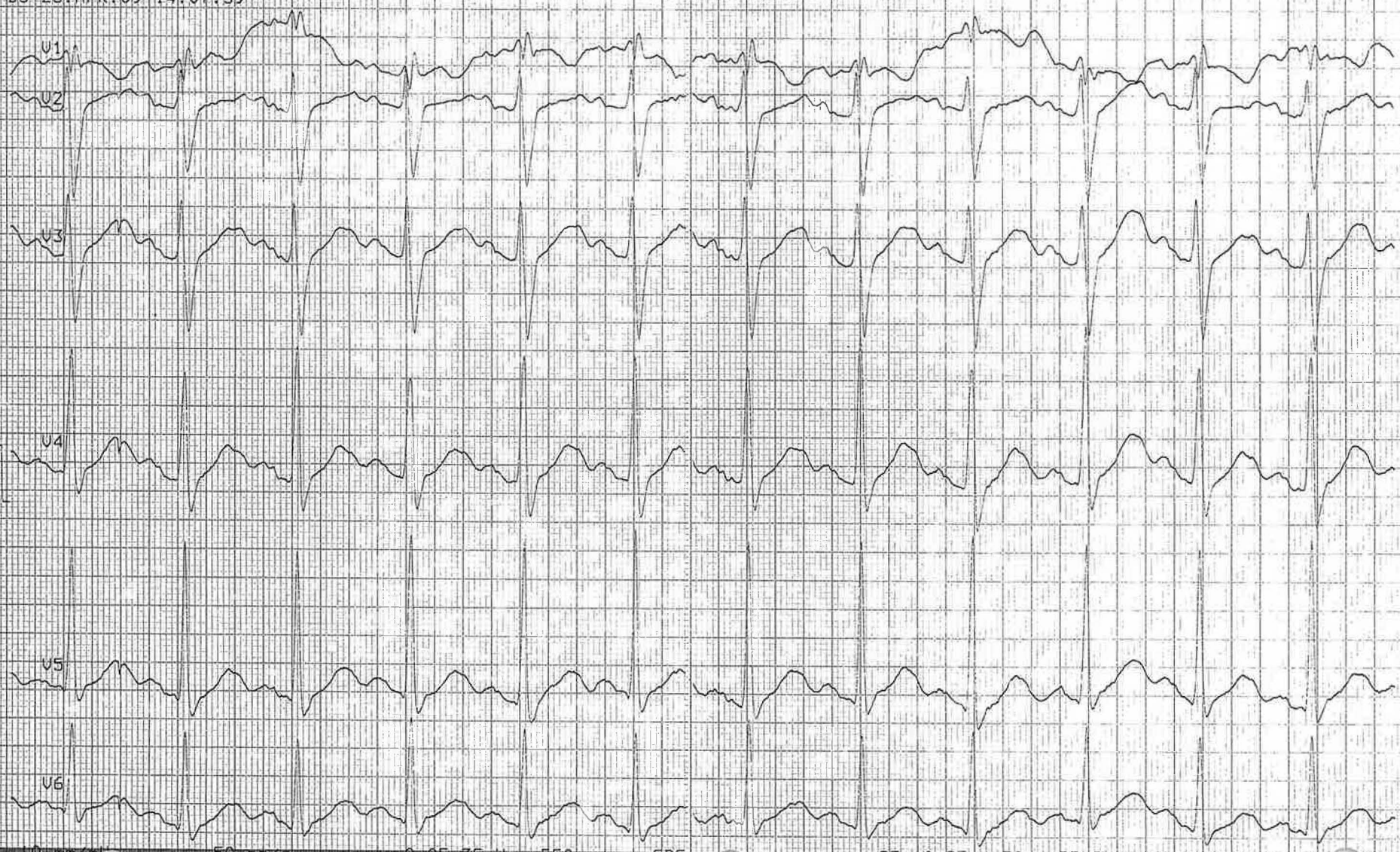
Geb. :5.6.96
Alter 12 Jahre
Geschl. : M
Grösse :165 cm
Gewicht :50 kg
BD: mmHg
Med.:
Bem.:

HF: 150/min
QRS: 100 ms
80 °
BD: 000 mmHg
000 mmHg

Last: 100 W

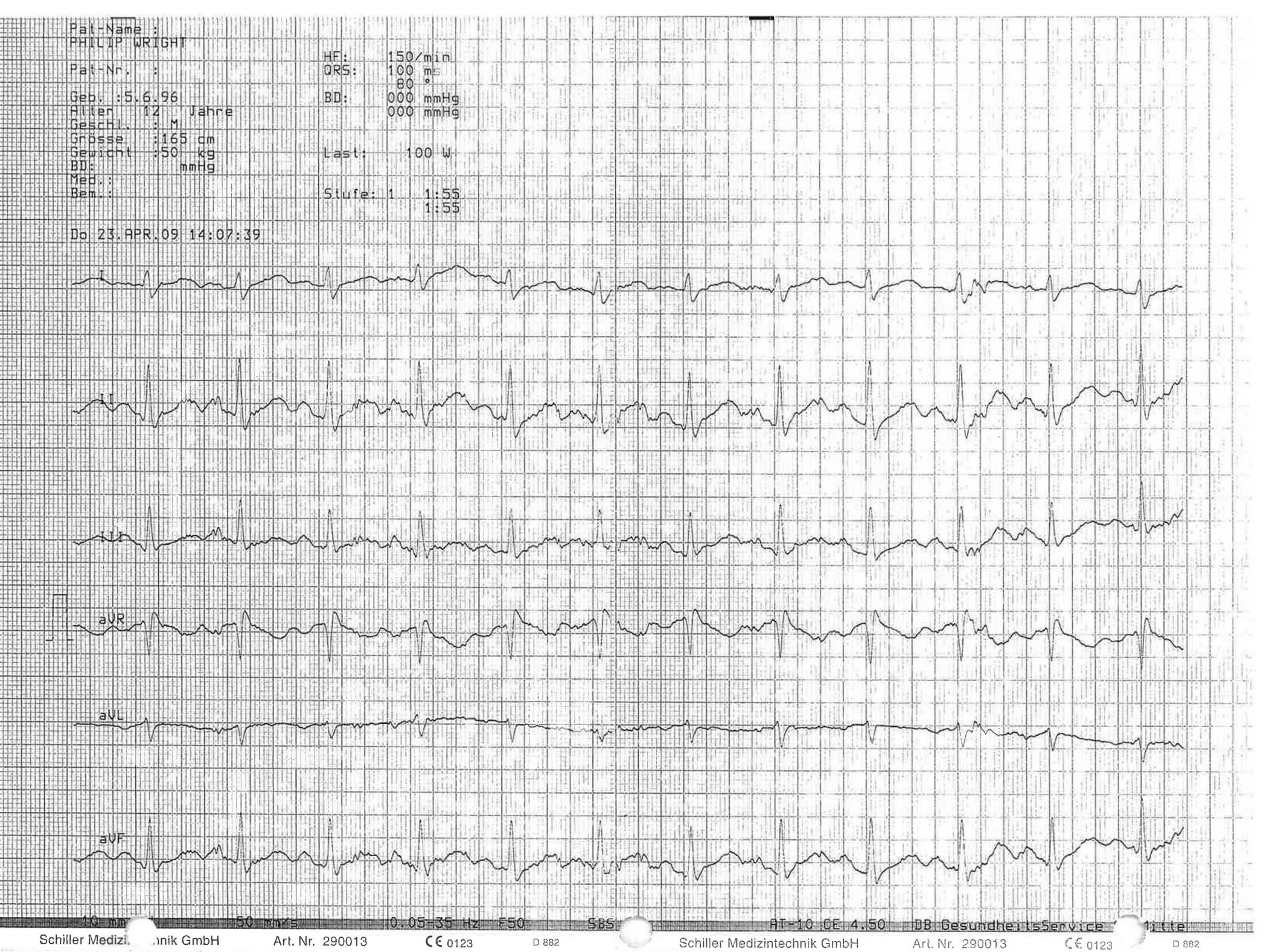
Stufe: 1 1:55
1:55

Do 23.APR.09 14:07:39



10 mm/mV 50 mm/s 0.05-35 Hz F50 SBS AT-10 CE 4.50 D3 Gesundheit sService GZ ltte

Schiller Medizintechnik GmbH Art. Nr. 290013 CE 0123 D 882



Pat-Name :
PHILIP WRIGHT

Pat-Nr. :

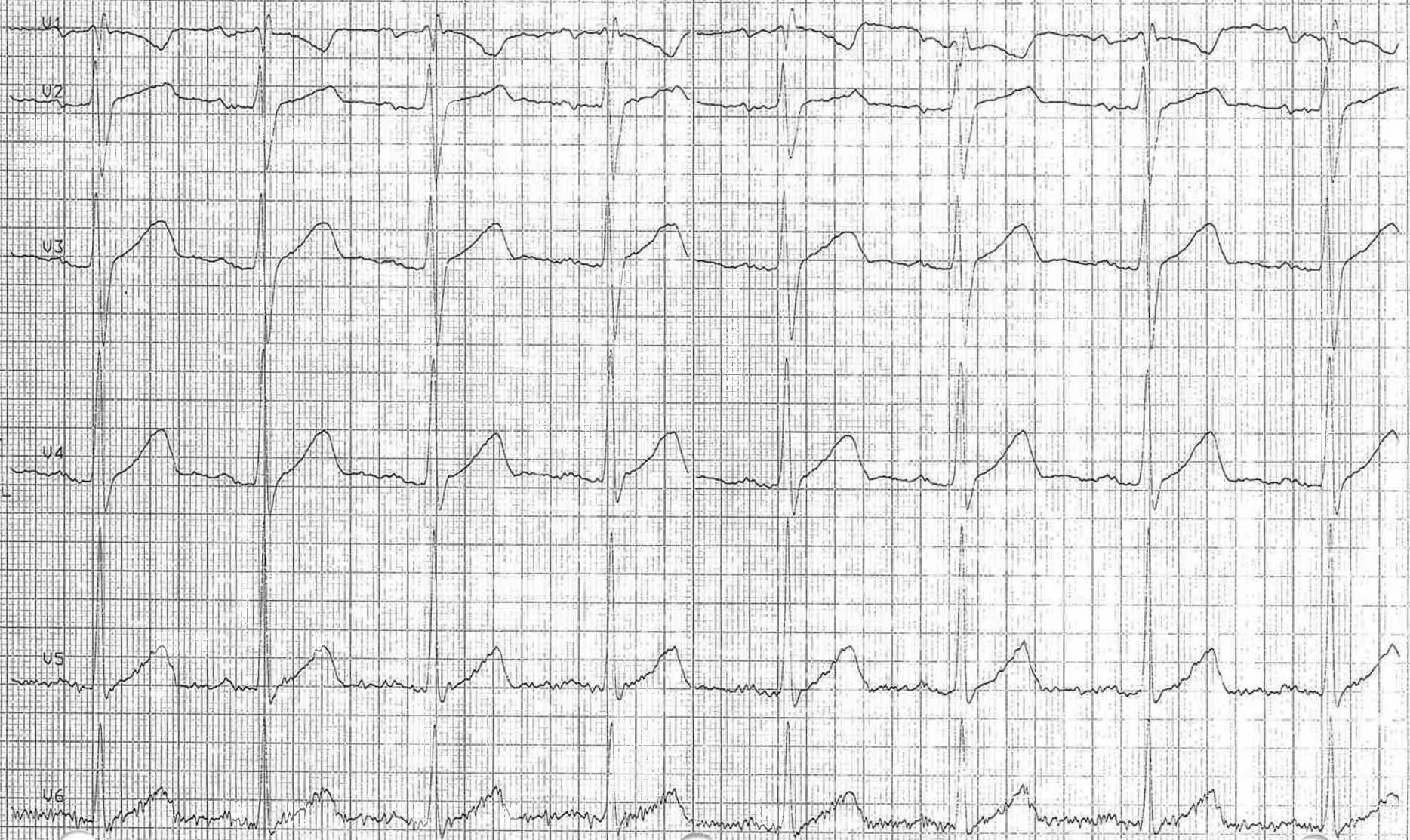
Geb. :5.6.96
Alter 12 Jahre
Geschl. : M
Grösse :165 cm
Gewicht :50 kg
BD: mmHg
Med.:
Bem.:

HF: 97/min
QRS: 103 ms
81 °
BD: 090 mmHg
060 mmHg

Last: 0 W

Stufe: P 0:55
0:00

Do 23.APR.09 14:05:37



10 mm/mV 50 mm/s 0.05-35 Hz F50 SB AT-10 DE 4.50 DB GesundheitsService Mitte

Schiller Medizintechnik GmbH Art. Nr. 290013 CE 0123 D 882

Schiller Medizintechnik GmbH Art. Nr. 290013 CE 0123 D 882

Pat-Name :
PHILIP WRIGHT

Pat-Nr. :

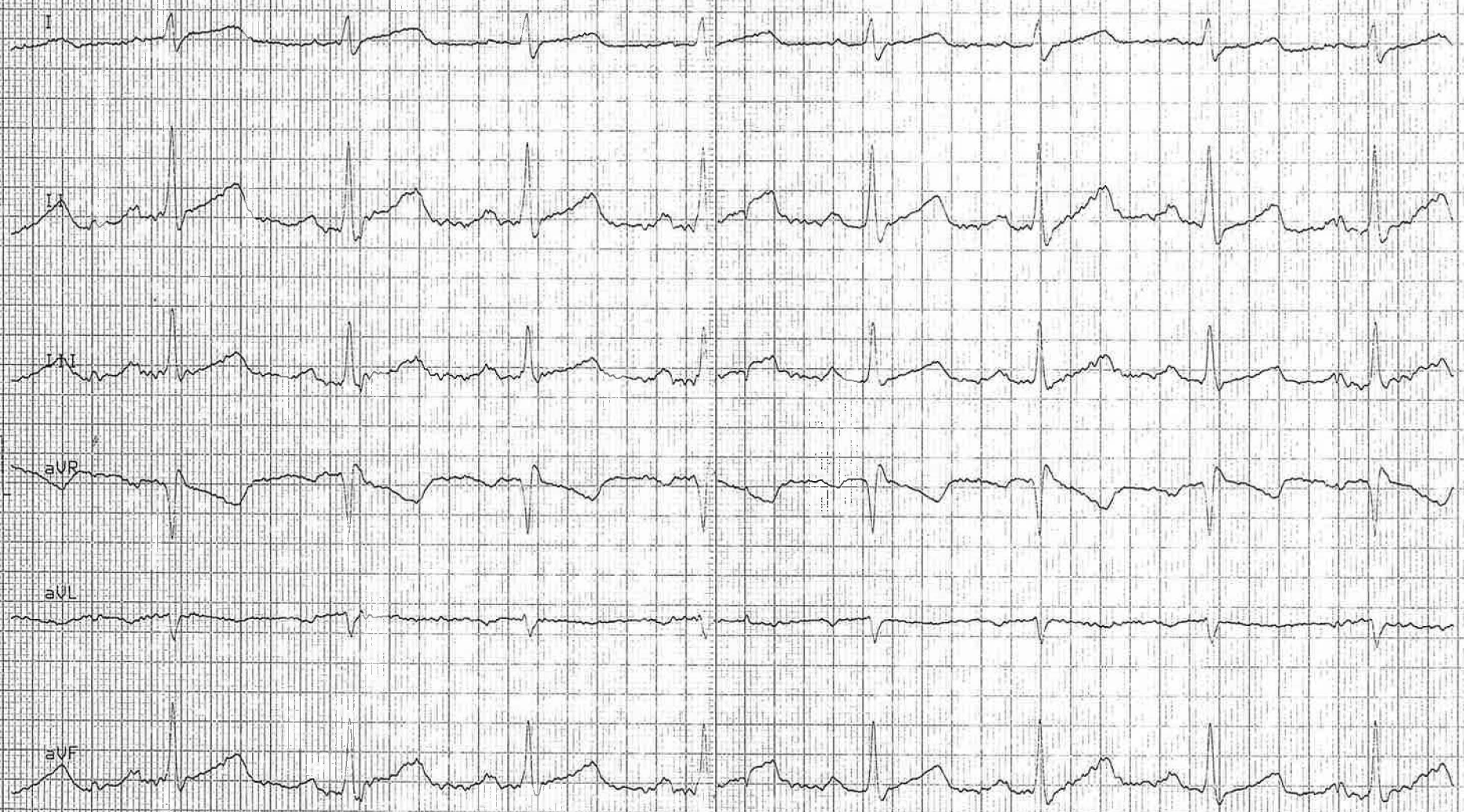
Geb. :5.6.96
Alter 12 Jahre
Geschl. : M
Grösse :165 cm
Gewicht :50 kg
BD: mmHg
Med.:
Bem.:

HF: 97/min
QRS: 103 ms
81 °
BD: 090 mmHg
060 mmHg

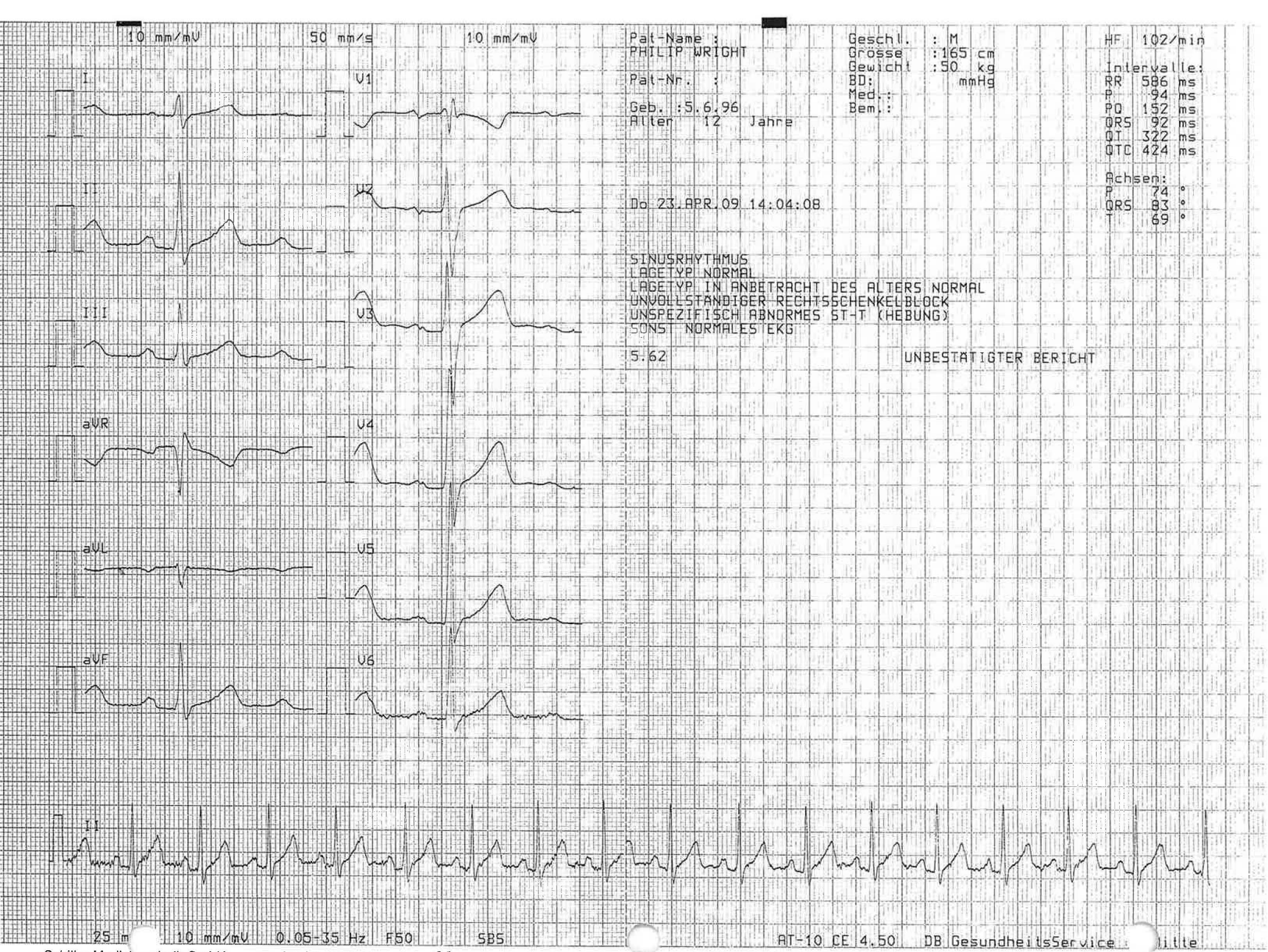
Last: 0 W

Stufe: P 0:55
0:00

Do 23.APR.09 14:05:37



10 mm/mV 50 mm/s 0.05-35 Hz F50 SBS RT-10 CE 4.50 DB GesundheitsService G tle


10 mm/mV
50 mm/s
10 mm/mV
I
II
III
aVR
aVL
aVF
V1
V2
V3
V4
V5
V6
II
Pat-Name :
PHILIP WRIGHT
Pat-Nr. :
Geb. :5.6.96
Alter 12 Jahre
Geschl. : M
Grösse :165 cm
Gewicht :50 kg
BD: mmHg
Med.:
Bem.:
HF 102/min
Intervalle:
RR 586 ms
P 94 ms
PQ 152 ms
QRS 92 ms
QT 322 ms
QTC 424 ms
Achsen:
P 74 °
QRS 83 °
T 69 °
Do 23.APR.09 14:04:08
SINUSRHYTHMUS
LAGETYP NORMAL
LAGETYP IN ANBETRACHT DES ALTERS NORMAL
UNVOLLSTANDIGER RECHTSSCHENKELBLOCK
UNSPEZIFISCH ABNORMES ST-T (HEBUNG)
SONST NORMALES EKG
5.62
UNBESTATIGTER BERICHT
25 m
10 mm/mV
0.05-35 Hz
F50
SB5
AT-10 CE 4.50
DB GesundheitsService
Schiller Medizintechnik GmbH
Art. Nr. 290013
CE 0123
D 882
Schiller Medizintechnik GmbH
Art. Nr. 290013
CE 0123
D 882

Pat-Name :
PHILIP WRIGHT

Pat-Nr. :

Geb. :5.6.96
Alter 12 Jahre
Geschl. : M
Grösse :165 cm
Gewicht :50 kg
BD: mmHg
Med.:
Bem.:

Do 23.APR.09 14:04:08

HF 102/min

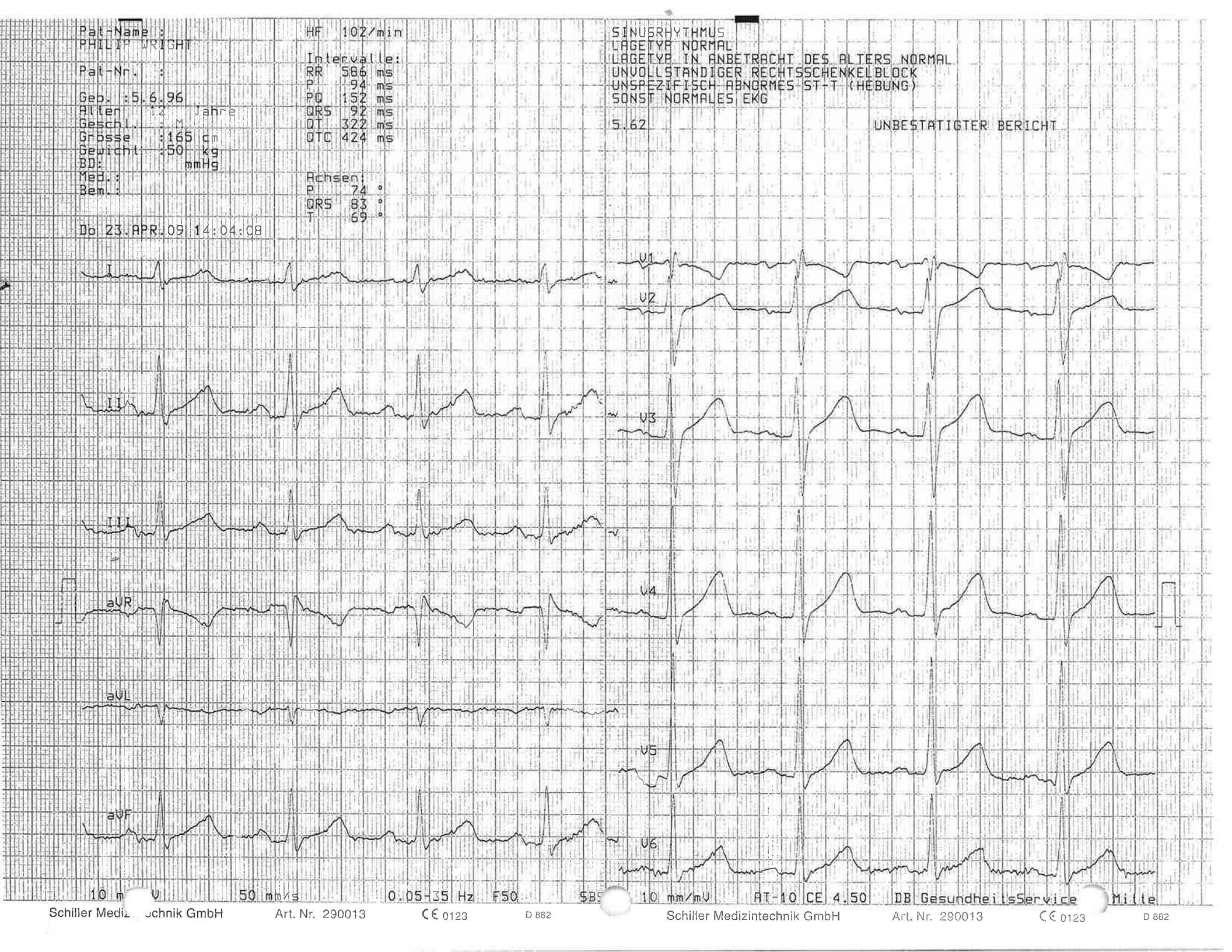
Intervalle:
RR 586 ms
P 94 ms
PQ 152 ms
QRS 92 ms
QT 322 ms
QTC 424 ms

Achsen:
P 74 °
QRS 83 °
T 69 °

SINUSRHYTHMUS
LAGETYP NORMAL
LAGETYP IN ANBETRACHT DES ALTERS NORMAL
UNVOLLSTANDIGER RECHTSSCHENKELBLOCK
UNSPEZIFISCH ABNORMES ST-T (HEBUNG)
SONST NORMALES EKG

5.62

UNBESTATIGTER BERICHT



10 mm/mV 50 mm/s 0.05-35 Hz F50 SBS 10 mm/mV AT-10 CE 4.50 DB GesundheitsService Mille

Schiller Medizintechnik GmbH Art. Nr. 290013 CE 0123 D 862

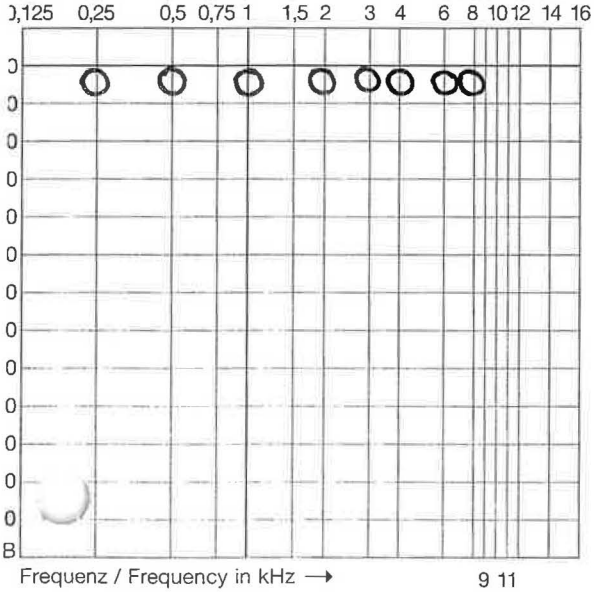
Schiller Medizintechnik GmbH Art. Nr. 290013 CE 0123 D 862

Tonaudiogramm

Name / Name	Geb. / Born	Prüfer / Examiner	Datum / Date
Pilz, Wrafft	1.6.06	Sch	23 Apr. 09
Adresse / Address		Kontrolle / Control	
		Kasse / Insurance	
Bemerkungen / Remarks			

Rechtes Ohr / Right Ear

LL / AC Vertäubung / LL / AC
KL / BC Masking KL / BC



WEBER
R M L

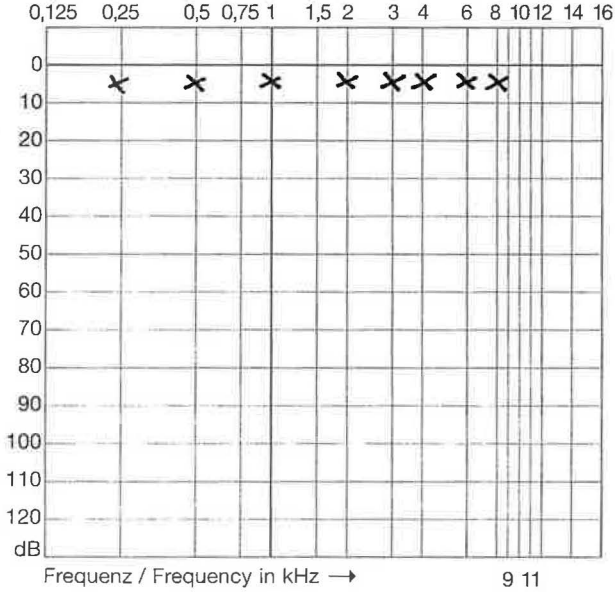
RINNE

Flü.Spr.
Zahlen (m)
Umg.Spr.

SISI

R	kHz	
	dB	
	Inkr.	
	%	
L	kHz	
	dB	
	Inkr.	
	%	

Linkes Ohr / Left Ear



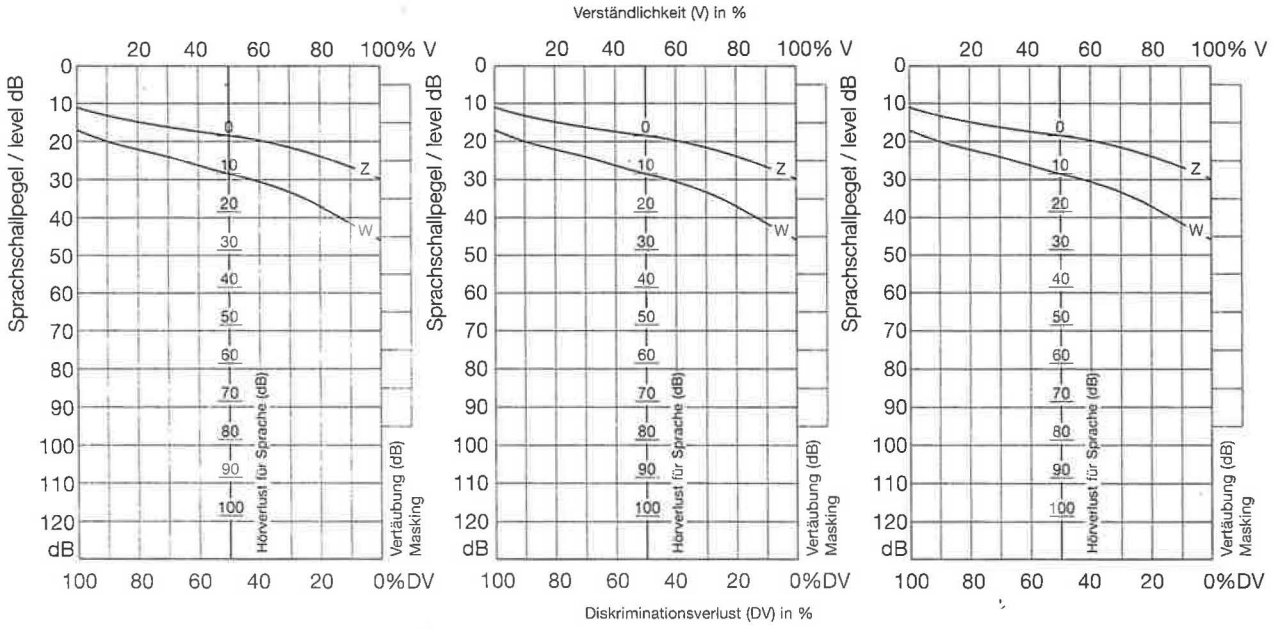
Sprachaudiogramm

Name / Name	Geb. / Born	Prüfer / Examiner	Datum / Date
Adresse / Address		Kontrolle / Control	
		Kasse / Insurance	
Bemerkungen / Remarks			

Rechtes Ohr / Right Ear

Freifeld / Freefield

Linkes Ohr / Left Ear

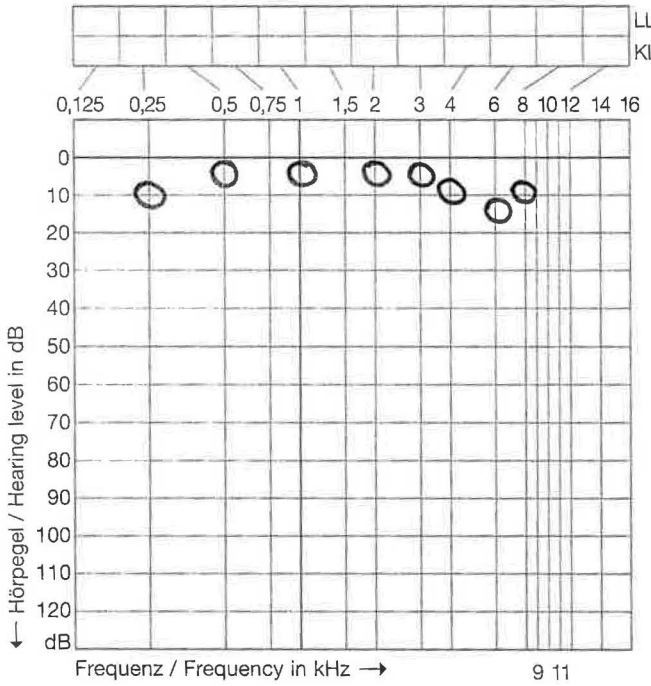


Tonaudiogramm

MAICO

Name / Name	Geb. / Born	Prüfer / Examiner	Datum / Date
Julia Wright	24.7.200	Sch.	23 Apr. 2009
Adresse / Address		Kontrolle / Control	
		Kasse / Insurance	
Bemerkungen / Remarks			

Rechtes Ohr / Right Ear



WEBER

R M L

RINNE

Flü.Spr.

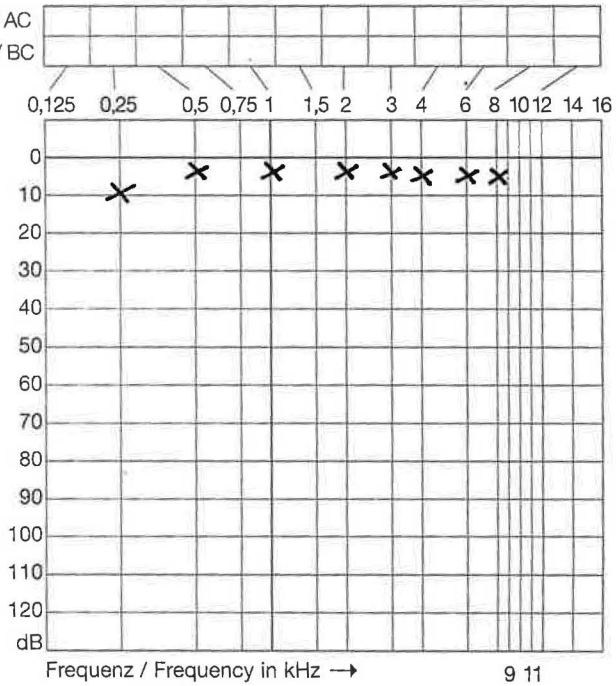
Zahlen (m)

Umg.Spr.

SISI

R	kHz	
	dB	
	Inkr.	
	%	
L	kHz	
	dB	
	Inkr.	
	%	

Linkes Ohr / Left Ear



Bestell-Nr.: 861002/6 10/03

Philip

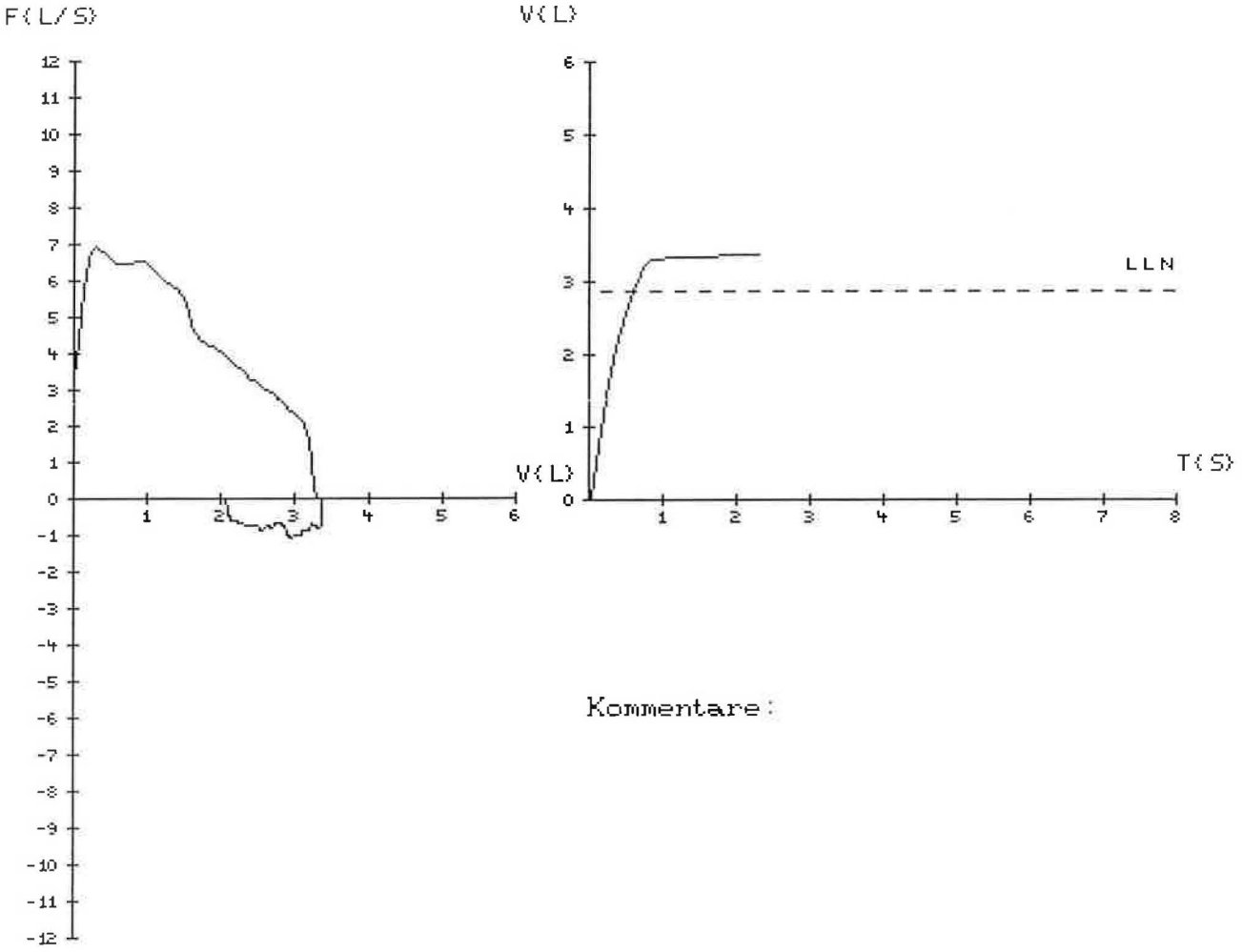
Vitalograph 2120

ID: P Testdatum: 23/04/2009 Zeit: 13:47:12
Alter: 12 Groesse: 165 cm Geschlecht: M Eth. Herkunft: Sonst
Geraete-ID: 08458 Kal.-pruefdatum: 14/08/2008

Testqualitaetsdaten:
Variabilitaet-FVC: 19 FEV1: 28
Anzahl der Tests: 3

Bester ERS Wert nach B.T.P.S - Polgar Sollwert (S/W 74320/2.08)

Index	Soll	Messwert	%
VC	3.61	4.02	111
FVC	3.61	3.39	94
FEV1	3.40	3.34	98
FEV1%	82	98	120
PEF	454	413	91
FEF25-75%	3.82	4.54	119



Kommentare:

Interpretation der Testergebnisse:
Normale Atemfunktion.

Unterschrift: _____

Julc

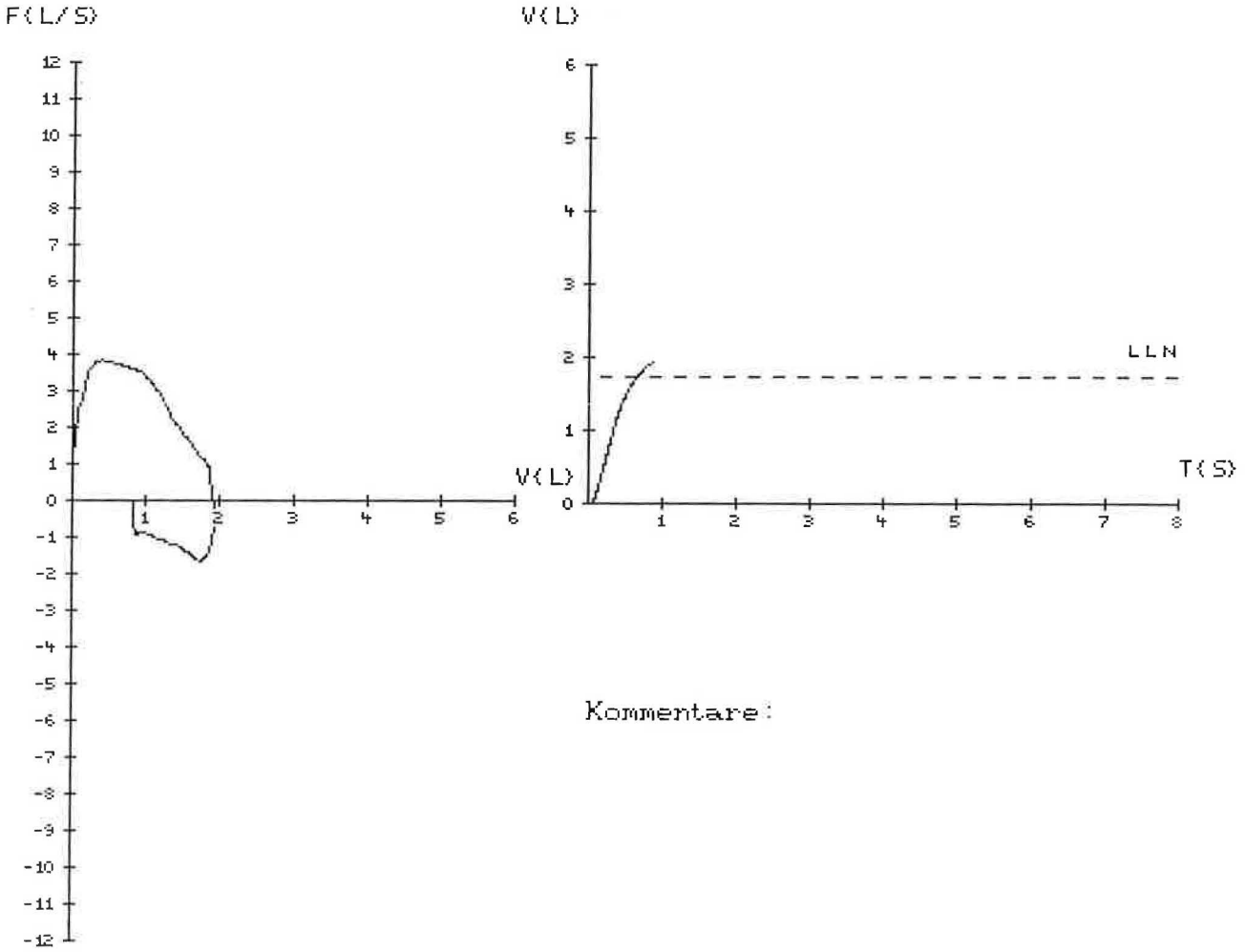
Vitalograph 2120

ID: J Testdatum: 23/04/2009 Zeit: 13:42:41
Alter: 9 Groesse: 140 cm Geschlecht: W Eth. Herkunft: Kaukasisch
Geraete-ID: 08458 Kal.-pruefdatum: 14/08/2008

Testqualitaetsdaten:
Variabilitaet-FVC: -- FEV1: 40
Anzahl der Tests: 3

Bester ERS Wert nach B.T.P.S - Polgar Sollwert (S/W 74320/2.08)

Index	Soll	Messwert	%
VC	2.16	1.99	92
FVC	2.16	1.94	89
FEV1	2.14	1.71	80
FEV1%	84	88	105
PEF	302	229	76
FEF25-75%	2.68	3.10	116

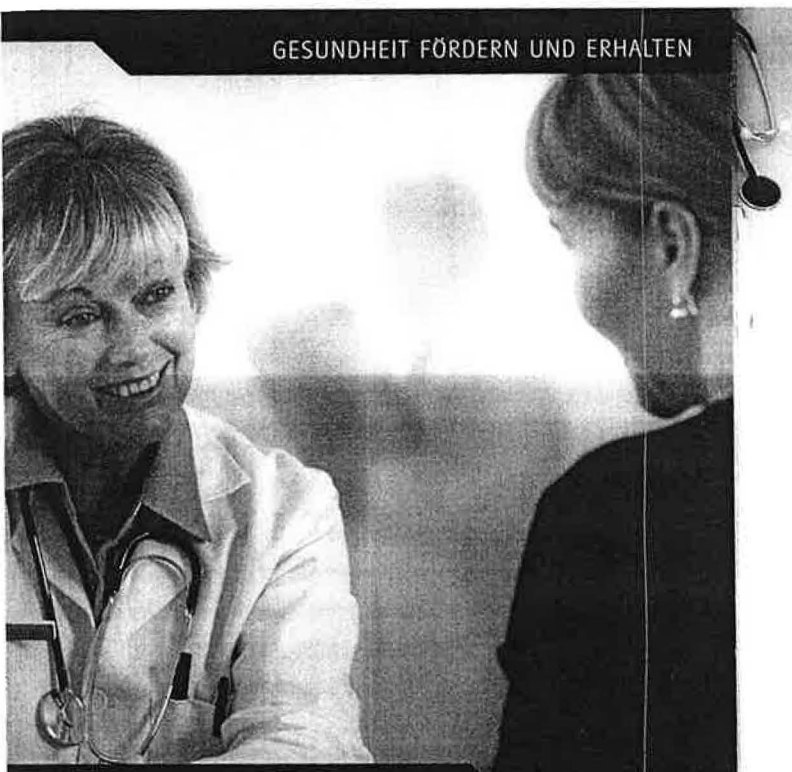


Kommentare:

Interpretation der Testergebnisse:
Normale Atemfunktion.

Unterschrift: _____

GESUNDHEIT FÖRDERN UND ERHALTEN



» Arbeits- und Verkehrsmedizin

Unterstützung durch Spezialisten

Unsere Arbeits- und Präventivmediziner unterstützen Unternehmen aller Branchen bei Einstellungs- sowie Tauglichkeitsuntersuchungen und führen arbeits- sowie sozialmedizinische Begutachtungen durch.

» Arbeitsmedizin

Zu den Leistungen gehören neben klassischen arbeitsmedizinischen Vorsorgeuntersuchungen und Beratung zu Arbeitsplatzergonomie zum Beispiel auch Reintegrationsmaßnahmen für leistungsgewandelte Mitarbeiter. In Anbetracht des demografischen Wandels ein Thema mit wachsender Bedeutung für viele Unternehmen.

» Verkehrsmedizin

In der Verkehrsmedizin haben wir durch unsere langjährige Erfahrung mit der Deutschen Bahn AG ein erprobtes Instrumentarium entwickelt, das den besonderen Anforderungen eines Verkehrsunternehmens gerecht wird.

Hier gilt es, physische und psychische Belastungen und Symptome frühzeitig zu erkennen, um Risiken zu vermeiden und so die Sicherheit aller Beteiligten zu gewährleisten.

» Gesundheitsmanagement

Als Dienstleister für umfassendes Betriebliches Gesu heits- und Risikomanagement implementieren wi Zusammenarbeit mit unseren Psychologen, Sozia dagogen und Sicherheitsingenieuren individuell auf Kundenunternehmen zugeschnittene Konzepte zur sunderhaltung und Gesundheitsförderung und ebnei den Weg zum gesunden Unternehmen.

Unsere interdisziplinär angelegten Konzepte garanti eine fachlich erstklassige Betreuung in allen Gesu heitsfragen. So können gesundheitliche Risikofakt früh erkannt und gegebenenfalls eliminiert werden.

Ein intensiver fachlicher Austausch und regelmäl Schulungen gewährleisten den hohen Wissensst unserer Mitarbeiter und stellen sicher, dass wir u re Kundenunternehmen stets nach aktuellen Stand beraten.

dbgs : GesundheitsService

Ein Unternehmen der IAS-Gruppe.

www.dbgs

Unsere Leistungen im Überblick

Integrierte Konzepte für Betriebliches Gesundheits- und Risikomanagement

Einstellungsuntersuchungen

Beratungs- und Unterstützungsfunktionen nach Arbeitssicherheits- (ASiG) und Arbeitsschutzgesetz (ArbSchG) sowie zahlreicher anderer Rechtsvorschriften (GefStoffV, BioStoffV etc.)

Reisemedizinische Untersuchungen

Check-up-Untersuchungen

Fehlzeitenmanagement/Reintegrationsberatung

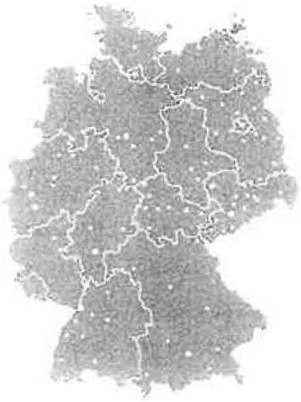
Tauglichkeitsuntersuchungen gemäß Tauglichkeitsrichtlinie DB AG und Fahrerlaubnis-Verordnung (FeV)

Umsetzung und Beratung bei der Anwendung der Eisenbahn-, Bau- und Betriebsordnung (EBO) und der Unfallverhütungsvorschriften (UVV)

Untersuchungen nach allen einschlägigen berufsgenossenschaftlichen Grundsätzen wie G 25 (Fahr-, Steuer- und Überwachungstätigkeit), G 37 (Bildschirmarbeitsplätze), G 20 (Lärm) u.v.m.

Mensch. Gesundheit. Mobilität.

140 Mal in Ihrer Nähe



Im Verbund mit der IAS Stiftung erhalten unsere Kunden an 140 Standorten in Deutschland rechtssichere Beratung und Begleitung durch Mediziner, Psychologen, Sozialarbeiter und Sicherheitsingenieure.

Ihre Spezialisten für

- Gesundheits- und Risikomanagement
- Arbeitsmedizin
- Verkehrsmedizin
- Arbeitssicherheit
- Psychologie
- Sozialberatung
- Gesundheits-Check-ups
- Akademie / Forschung

dbgs GesundheitsService GmbH

Gesundheitszentren:

04103 Leipzig Rosa-Luxemburg-Straße 12+14
Tel. (0341) 3195-200 Fax (0341) 3195-204

10785 Berlin Schöneberger Ufer 89-91
Tel. (030) 297-33340 Fax (030) 297-33339

30165 Hannover Vahrenwalderstraße 4
Tel. (0511) 286-1554 Fax (0511) 286-1975

50668 Köln Breslauer Platz 4
Tel. (0221) 139939-0 Fax (0221) 139939-15

60327 Frankfurt Mainzer Landstraße 181
Tel. (069) 265-25800 Fax (069) 265-25826

66165 Mannheim Augustaanlage 7-11
Tel. (0621) 300991-34 Fax (0621) 300991-40

80797 München Lothstraße 19
Tel. (089) 1308-1900 Fax (089) 1308-1973

Zentrale:

10785 Berlin Schöneberger Ufer 89-91
Tel. (030) 297-33316 Fax (030) 297-33390

Weitere Infos unter www.dbgs.eu

3123991150336 1 / 4.6.2009 12:21:30

d174

Verein Deutsche Turnfeste e.V.

TurnfestLIVE

Freitag, 05.06.2009

Tageskarte - Freitag

Messe Frankfurt
Ludwig-Erhard-Anlage 1, 60327 Frankfurt

Barverkauf von VVK: AD ticket - Turnfest 2009

Internationales Deutsches Turnfest 2009 Frankfurt am Main 30. Mai bis 5. Juni

wir schlagen brücken

DTB

HESSEN

Bundesministerium des Innern

Stadt Frankfurt am Main

messe frankfurt

KombiTicket

Gilt am Veranstaltungstag als Tageskarte im gesamten RMV-Gebiet.
Es gelten die gemeinsamen Beförderungsbedingungen und RMV-Tarifbestimmungen.
1. Klasse nur mit Zuschlag

11,00 €

d174

TurnfestLIVE

Freitag, 05.06.2009

Messe Frankfurt
Ludwig-Erhard-Anlage 1,
60327 Frankfurt

Tageskarte

11,00 €

www.turnfest.de

Reservix