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GÖTTINGEN MINIPIGS A/S

Newsletter 30
AUTUMN 2008

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DEAR READER,

I am pleased to announce that the construction of our third barrier is now well under way. When it is completed in mid 2009, this new building will increase our production capacity by around 80% and ensure the availability of high-quality Göttingen Minipigs from Ellegaard for years to come.

In planning this new building, we have focused on how to achieve:

- the best animal welfare possible,
- the best working environment for our staff,
- the lowest impact on the environment,
- a pleasing, well designed building to look at, and
- fine visibility sites where our visitors can see how the production of microbiologically-defined Göttingen Minipigs takes place – without having to enter the barrier.

Our animal technicians have been busy improving animal welfare conditions and making the new building an even better place to work. They submitted suggestions before the drawings were made and also after they had reviewed the drawings. Test models of newly designed pens and flooring were evaluated, both for the comfort of the minipigs but also to make sure the working environment of the new design is the best possible.

To minimize the use of resources the new building will be optimally insulated both in the roof, walls and floors. Pipes filled with coolant embedded in the concrete floor under the manure pits, will absorb heat from the manure and, using a heat pump, convert this heat into hot water, thus saving on heating oil. Building ventilation will be based on the CO2 content of the air in each animal room and other factors. Rooms with low animal density will have a lower need for ventilation, whereas rooms with higher animal density will have a higher CO2 content and thus require more ventilation. This ensures that the use of energy for heating and ventilation in animal rooms will be as efficient as possible.

When the new barrier building is completed, we look forward to showing you the new larger viewing windows in the concrete ceiling that allow for better views of our production process. As always you are warmly welcome to visit our facility.

One of our goals this year is to develop more background information about the Göttingen Minipig. This information will assist you in selecting the right animal model for your research. To achieve this we are financing an immunotoxicology study where background information about immunology in the Göttingen Minipig will be developed. The study results will be available later this year.

Background information on the clinical chemistry and haematology of the Göttingen Minipig will also be updated. Also, we are supporting the development of more background information in teratology and the use of Göttingen Minipigs in juvenile studies.

The more background information we can put at your disposal, the more secure you can feel that you are choosing the right animal model when buying Göttingen Minipigs. More background information will improve the Göttingen Minipig as an even better-documented research model – especially when regulatory authorities are evaluating your study. We hope these efforts will benefit your research and, if you have any suggestions as to where additional background information is needed, we will gladly assist you in developing this.

Jens Ellegaard
Managing Director

Minipigs
Biological products
Auxiliary equipment
Training & Courses

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For Göttingen Minipigs in North America please contact Marshall BioResources – infous@marshallbio.com

(http://www.ars.usda.gov/Services/docs.htm?docid=6065)

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ABSTRACT

Diverse genomics-based databases have developed to facilitate human genetic research and relevant rodent models. Current porcine gene databases, however, lack the nutritional and immunological orientation and comprehensive annotation to design effective molecular tools for comparative studies of relevant pig models. To address this need, a literature-based survey was conducted that first identified genes related to nutrition and associated with macro- and micronutrient metabolism, atherosclerosis, diabetes, and obesity. Also selected were genes related to immunology and associated with allergy and asthma; chemokines and cytokines and their receptors; dendritic, mast and NK cells; type I interferon (IFN)-induced proteins; inflammation; toll receptor signaling pathways; and B and T cell activation and development. The process identified 3,300 candidate genes or proteins used to select potential porcine homologues by searching multiple online sources of porcine gene information. We then developed real time PCR assays for 1,410 high priority genes of particular interest. The database also contains comprehensive information on antibody availability and published porcine gene and protein expression data. This database is unique among porcine gene databases in regard to linking gene expression to gene function, identification of related gene pathways, and integration with other porcine gene databases.

INTRODUCTION

Swine have been used extensively to model human anatomy and physiology, and particular aspects of nutrition and immunology. The organs of pigs are anatomically, histologically and functionally similar to human. In addition, sensory innervation and blood supply are comparable, and the gut micro flora colonization, diversity (except cellulolytic species) and enzymatic activity are similar (e.g., microbial butyrate generation). Finally, vitamin and mineral metabolism are very similar between the two species (except for aspects of carotenoid and vitamin C metabolism), and both species are omnivorous.

Pigs are naturally susceptible to infection with pathogens that are closely related or identical to those infecting humans including helminths *(Ascaris, Taenia, Trichuris, Trichinella, Schistosoma, Strongyloides)*, bacteria *(Campylobacter, E. coli, Helicobacter, Neisseria, Mycoplasma, Salmonella)*, protozoans (*Toxoplasma,Cryptosporidium)* and viri *(Coronavirus, Hepatitis E, Influenza, Nipah, Reovirus, Rotavirus)*. This suggests common immune strategies to control infection. We recently conducted a comprehensive examination of immune function (185 variables) between rodents, pigs and humans (H. Dawson et. al., unpublished). Overall, approximately 65% of the parameters examined were more similar between pigs and humans than mice, suggesting that evaluating immune function, particularly in response to nutritional manipulation in pigs, provides data that is more physiologically relevant to humans.

Despite these potential strengths as a model, the lack of annotated database for gene and protein expression data is a limiting factor for comparative analysis. Multiple online databases provide storage and retrieval of diverse, immunologically-related data (2-5, 7). All of these focus, however, on rodent or human immune-related genes or proteins. Several porcine databases currently online include, 1) The Computational Biology and Functional Genomics Laboratory of the Dana Farber Cancer Institute (DFCI, formerly based at The Institute for Genome Research) that maintains a pig EST database (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=pig) with Gene Ontology (GO) annotations (it is infrequently updated) (1); 2) The Porcine EST data Explorer (PEDE) database (http://pede.dna.affrc.go.jp/) (8) has recently added GO annotations (entries are reserved for the author's own data); 3) The AgBase database (http://www.agbase.msstate.edu/) accepts annotations of porcine genes, but the search interface and output are limited (6); and 4) the, NCBI includes Gene ontology data into curated entries, but does not provide such information for ESTs and HomoloGene or list swine as a reference species on other NCBI resources. As swine are an important model for human anatomy and physiology there is a critical need to curate and centralize a source of information for swine biomedical research tools to fully utilize the advantages of this animal model, as well as for the inherent interest in genomic-based tools for swine as an economically important

livestock species. To address this need, we created the Porcine Immunology and Nutrition (PIN) Database.

MATERIALS AND METHODS

A broad-based literature search was conducted for immunologically-related genes using the following terms; apoptosis, B cell development or activation, CD markers, chemokines, chemokine receptors, cytokines, cytokine receptors, dendritic cells, type I IFN-induced genes, inflammation, NFKB signaling pathway, toll receptor signaling pathway, T cell development or activation, Th1- and Th2-cell development. In addition, immunologically related genes associated with the susceptibility to or pathology of allergy, asthma, arthritis, atherosclerosis, and inflammation. The Gene Ontology Consortium's Community Annotation Wiki for Immunology (http://wiki.geneontology.org/index.php/Immunology) was searched for relevant information, and the Jackson Laboratory database of knockout mice phenotype was searched for genes leading to defects in immune phenotype when over or under expressed. Genes involved in the transport or metabolism of macronutrients, trace vitamins, and minerals were searched to provide information on common nutritional pathways, and nutritionally-related genes associated with the susceptibility to or pathology of atherosclerosis, diabetes, and obesity were identified. To date, this process identified 3,300 candidate genes. Genes belonging to several broad categories are listed in Table 2.

We then identified porcine homologues to human genes of interest by searching online sources of porcine gene information. Genbank (NR, EST, GSS, HTGS, and Trace Archive databases) was searched by discontiguous Megablast using human Reference Sequence Accession numbers. A similar search was conducted in the following databases using the human reference sequence, human Unigene number and/or HUGO gene symbols; DFCI/TIGR porcine EST, NIH Intramural Sequencing Center (NISC), Human UniGene and Swine UniGene, Pig EST Data Explorer (PEDE) and HomoloGene) databases. This process identified >2,800 corresponding pig sequences. We then developed real-time PCR assays for 1,400 of these genes making them cross reactive for a number of species including human, mouse, and bovine whenever possible. We also conducted a literature search for references related to the functional characterization of the human promoter regions of these genes to identify putative regulatory transcription factors for the corresponding porcine genes. A comprehensive search of catalog and published literature to identify antibodies to the corresponding proteins was added to the search. The current status and statistics are summarized in Table 2.

RESULTS AND DISCUSSION

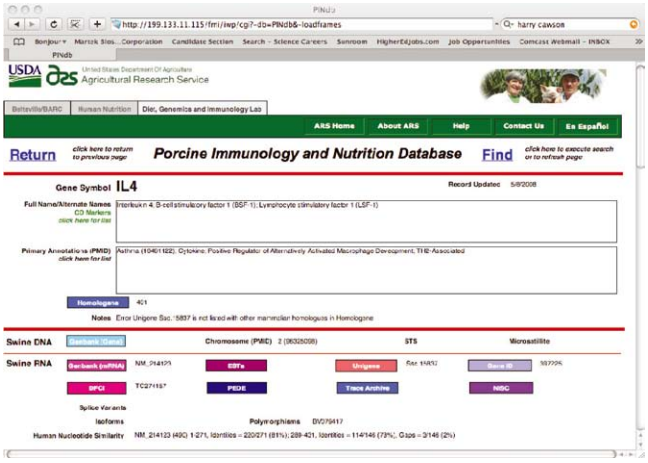
Table 1.
Major Nutritional and Immunological Annotations

Nutritional Annotations	**# of Genes**
Atherosclerosis	21
Insulin Receptor Signaling	54
Iron Metabolism or Transport	25
Lipid Metabolism	46
Obesity	83
Selenium	26
Vitamin D Metabolism or Transport	11
Vitamin A Metabolism or Transport	64
Zinc Metabolism or Transport	18
Immunological Annotations	**# of Genes**
Allergy/Asthma	170
Apoptosis	271
CD Marker	373
Chemokine/Chemokine Receptor	73
Cytokine/Cytokine Receptor	144
Dendritic Cell Associated	62
Inflammation	201
NFKB Signaling Pathway	138
Toll Receptor Signaling Pathway	78
T Cell Activation	84
Th1 Associated	113
Th2 Associated	124

Table 2.
Current Database Statistics Version 4.4 (07/28/2008)

- >3,300 Entries
- >2,800 Corresponding Pig Sequences Identified
  - MicroRNAs
  - RNA and RNA Splice variants
  - Antibody without RNA/DNA sequences
- 80 Searchable Fields
- Chromosomal Location
  - > 400 entries
- 1,410 Taqman Real Time PCR Assays
  - mRNA
  - microRNA
  - Antibody isotypes
  - TCR spectratypes
- SYBR Green Real Time PCR Assays
  - > 100 Published
- Gene Expression Data
  - > 300 entries
- 480 Separate Annotations for Gene Function Cross Referenced to Human Data
  - > 2,300 References
- Promoter Transcription Factor Mapping Cross Referenced to Human Data
  - > 400 entries
- Largest centralized repository for porcine antibody information
  - > 500 entries including negative antibody cross reactivity data.
- siRNA Constructs

Figure 1
Sample Database Entry



Our database is unique among databases targeting veterinary species in regard to linking of gene expression to gene function, identification of related gene pathways and integration with other porcine gene databases. In addition, it is the largest source of centralized antibody information for the pig. Figure 1 provides a screen shot of the entry for the gene interleukin-4 (IL4).

It is of significance that we discovered a large number of errors (about 10% of entries) in the publicly available sequence databases. These can be accessed by searching the Notes field using the word error (Figure 1). Most frequently, we encountered assemblies containing genomic DNA so that they were annotated as an unknown gene or an irrelevant gene from an unrelated species. The next most frequent error was incorrect or missing URL link outs. We also encountered a fair number human or bovine sequences listed as porcine. These data illustrate the importance of the hand-curation process.

The database has been consistently accessed by the scientific community with >20,000 accessions (around 400 per month) since it went online in March 2005. Approximately 45 labs worldwide routinely utilize the database, including ARS/USDA scientists doing work in four national programs (Food Safety, Human Nutrition, Animal Health, and Foreign and Exotic Diseases). It is referenced in at least 10 manuscripts and used as a resource for several reagent vendors (AbD Serotec, Biolegend). The database is listed as a resource on several genomics-related web pages including;

National Center for Biotechnology Information (NCBI) Home page for Pig Genome Resources
(http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/)

The International Veterinary Immunogenetics Website
(http://imgt.cines.fr/textes/IMGTveterinary/)

The NAGRP Pig Genome Coordination Program
(http://www.animalgenome.org/pigs/maps/index.html)

The USDA Animal Welfare Information Center
(http://awic.nal.usda.gov/nal_display/index.php?info_center=3&tax_level=2&tax_subject=170&topic_id=1493)

Online Resources for Immunology Research Using Comparative and Veterinary Animal Models
(http://www.animal.ufl.edu/hansen/Immunology_resources/VETIMMUNOLRESOURCES.htm)

The U.S. Veterinary Immune Reagent Network
(http://www.umass.edu/vetimm/swine/index.html).

Any database must be updated frequently in order to be useful. We anticipate expanding the content to include >4,000 entries, and will continue to develop new assays. We are currently including full GO and KEGG annotations for each entry as they are provided by the GO community, and intend to include full microarray cross referencing (Affymetrix, others). A database is only as useful as the accuracy of its content. We view our efforts as a community-based effort, and strongly encourage members of the porcine research community to assess the accuracy of the information in the database and to note information that may be incorrect or inadvertently omitted.

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LOOKING FOR PORCINE-SPECIFIC IMMUNE REAGENTS?

The first hurdle when setting up immune assays for pigs and minipigs is to identify appropriate immune reagents with specificity for the pig/minipig system.

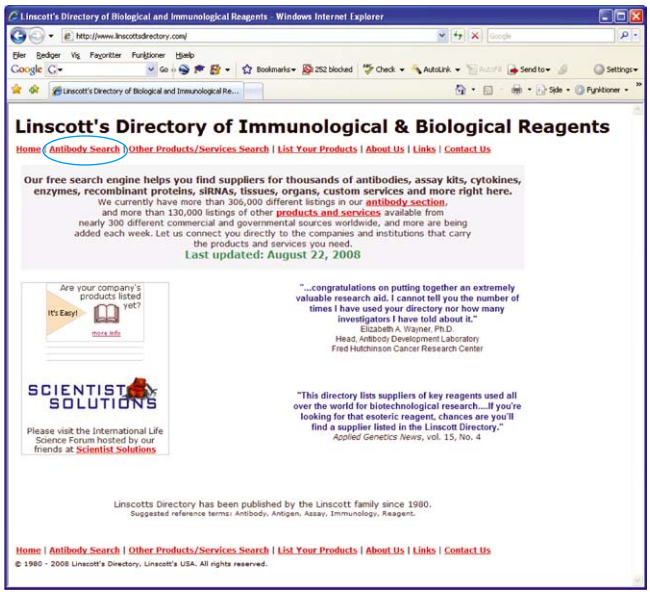
From an immunological point of view, domestic pigs and minipigs are expected to be similar, and thus the reactivity of the many immune reagents to domestic pig antigens is also expected to react in the minipig system. A simple control of the actual cross-reactivity between pig and minipig can easily be implemented in the initial experimental set-up, if needed.

Immune reagents can be found at Linscott's Directory website (http://www.linscottsdirectory.com). Select "Antibody Search" (circled in blue) and you will be directed to a world of various antibody reagents.

Typing the needed reagent in the search field, e.g. *Porcine IL10,* will take you to a total of 17 records, each identified by antigen specificity and available conjugates and include links to the supplier.

A search for *Porcine IgA* yields 25 records, *porcine IgG* yields 101 records, etc. Other search strategies can be used, such as defining host species, reactive species, application, etc.

Thanks to Mette Loftager, immunologist at LAB Research (Scantox), for this tip!



EMBRYOFOETAL DEVELOPMENT STUDIES

The Göttingen Minipig has been used for embryofoetal development studies since the mid nineties and offers a fine substitute to non-human primates. For fifteen years Ellegaard Göttingen Minipigs has been supplying a customer with animals (stillborn and malformed specimens, and animals that die shortly after birth). These animals have been thoroughly necropsied and the findings recorded. This has yielded a good empirical control database at the production facility. While such empirical control data cannot substitute a study's control group, it is valuable due to its insight into rare, yet naturally occurring deformities, which might otherwise have been interpreted as test-article related had this information not been available. This collaboration has since been expanded to include several companies, and the data obtained will be fed into a central registry. If you have any questions, please feel free to contact Niels-Christian Ganderup (ncg@minipigs.dk) for further details.

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HEALTH MONITORING REPORT

As previously described, a Health Monitoring Report is no longer attached to the newsletter. Instead, the biannual Health Monitoring Report will be posted on our website as soon as it is available. If you wish to be notified of this, or of important changes to our health status, please send an e-mail to ellegaard@minipigs.dk and type "HMR" in the subject field. This way we can make sure our customers receive important health-monitoring information from Ellegaard Göttingen Minipigs.

Thomas Bertelsen, LEO-Pharma
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Laust Peter Gade, Ellegaard Göttingen Minipigs

INTRODUCTION

Clicker training is training of an animal where a distinctive sound - the "click" - indicates to the animal exactly when it is performing the action or behaviour wanted by the trainer.
The use of the "click" is a form of operant conditioning, but the more common name "clicker training" is generally used because of the sound made by the mechanical device called a "clicker".
We have used clicker training to teach minipigs to follow a "target", i.e. to follow an object in order to direct the minipig from one location to another.
In dermatological experiments we have previously carried the minipigs from the pens to the procedure rooms where dosing and washing off is performed.
Now we use a moveable and automatic lift with a ramp.

MATERIALS

Animals
Göttingen minipig, female, 8-10 and 14-17 kgs.

Diet
SMP MOD (E) POLY SQC; Special Diets Services.

Bedding
"Staben kvalitetsstrøelse"; Staben, Sweden.
Autoclaved straw of grass from Scantox, Denmark.

Water
Ordinary tap water without additives, using drinking nipples.

Environmental enrichment
Biting chains.
Plastic balls.

Environment
Light cycle: 12/12 from 6 a.m. till 6 p.m.
Room temperature: 20 – 24°C.

Equipment
"Clicker"
Target
Electrical lifting cart: "Lyftvagn" 500 kg; Gerdmans.
55 x 85 cm stainless steel box with a 75 cm ramp.

PROCEDURE

Absolutely essential in the training is that the "click" is performed and the reward is given very consistently.
A **log-book** for each pig describes the actual training, the progress, any "unusual" events etc. The trainer also assesses and notes if the pig is ready for the next step.
A coding system describes if the pig is nervous, confident, takes rewards willingly etc.

Don't give rewards outside the training programme.

- Any edible item (apples, pasta, biscuits etc) will do as rewards! Make sure that the pieces are not too big; the pig must be hungry and interested in the reward.
- Appraisal and talking to the pig is very essential.
- If training of a new phase is started the following day, it is important to start with a repetition of the last known phase.
- If the pig becomes confused or unfocused, you take a break or go back to the preceding phases.
- During the training procedure the pig must be alone in the pen. As little external disturbance as possible should be assured.
- It is strictly forbidden to give rewards outside the training programme as this confuses the pig.
- If the pig can see all the rewards it easily gets overexited and unfocused.

The training is divided into twelve steps.
Building trust

Building trust by giving rewards through the bars

1. Rewards are given through the bars of the pen. "Click" when the pig shows interest or eats the reward.
2. The pig eats from the hand through the bars. "Click" when the pig takes the reward.

Training inside the pen - building trust and learning to follow target

Training inside the pen

3. Training is performed inside the pen. The pig eats from the hand. "Click" when the pig takes the reward.
4. Training is performed with the "target" in the pen. "Click" when the pig shows interest in the target and give a reward directly after.
5. Training is performed with the "target" in the pen. The pig must touch the target with the snout. "Click" only when touching and give a reward directly after.

6. Training is performed with the "target" in the pen. The pig must follow the moving target around in the pen. Start by only moving the target as little as the pig stills follows. Keep the target still and "click" when touching and give a reward directly after.

Training in the corridor – follow target to the ramp of the lift

Training in the corridor. Following the target.

7. Training in the corridor. Place the lift no longer than 2 meters away with the ramp down. Let the pig follow the target from the pen to the lift, taking small steps and letting the pig often touch the target. "Click" when touching and give a reward directly after.

Following the target to the lift ramp

8. The pig follows the target to the lift ramp. "Click" and reward when the pig reaches the start of the ramp and touches the target. The pig follows the target back to the pen. "Click" and reward when the pig gets to the pen and touches the target.

Training going up and down the ramp

Training to follow the target inside the lift box

9. Place the diet bowl (no diet yet) in the lift box, making sure it is visible for the pig. Let the pig follow the target a little of the distance up the ramp, if necessary make an intermediate step where only the front legs are on the ramp. Keep target still and "click" and reward when the pig touches the target. Repeat until the pig reaches the top of the ramp. The pig follows the target back to the pen. "Click" and reward when the pig gets to the pen and touches the target.

Training in the box of the lift

10. Place the diet bowl in the lift box and put the morning or afternoon ration of the daily diet in the bowl. Let the pig follow the target into the lift box. "Click" when it finds the diet. The pig follows the target back to the pen. "Click" and reward when the pig gets to the pen and touches the target.
11. As step 10, but close the ramp and raise/lower the lift while securing that the pig does not jump by placing your hand on its back. Let the pig get used to touching while it is eating. Place the bowl in the pen if there is residual diet.
12. As step 11, but where you accustom the pig to the procedures to be performed in the lift box.

CONCLUSIONS AND DISCUSSION

The training procedure requires spending a substantial amount of time initially, but it is our experience this pays off in the long term.
The previous procedure with catching and carrying the pigs made them more stressed, leading to a related potential negative impact on scientific results.
This fact and the impaired occupational health problems (ergonomics) also speak in favour of investing the time needed.

We have noticed that younger animals require more time in the trust building phase, but afterwards they are more willing to learn to follow the target, whereas older animals get faster confident with staff, but afterwards they tend to be more unwilling to learn to follow the target.

We acknowledge that companies and institutions that perform many studies with minipigs may have a more robust routine in learning the pigs the desired behaviour and actions.

ACKNOWLEDGEMENTS

Thanks to Copenhagen Zoo for helping us getting started.

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- Alexandra Kurland: Clicker Training for Your Horse. ISBN-13: 978-1890948351
- Melinda Johnson: Clicker Training for Birds (Getting Started). ISBN-13: 978-1890948153

MEETING CALENDAR

NAME	DATE	PLACE
Eurotox	5-8 October 2008	Rhodes, Greece
American College of Toxicology	9-12 November 2008	Tucson, Arizona, USA
LASA	19-21 November 2008	Scotland, UK
ICLAS	3-4 December 2008	Jerusalem, Israel
SOT ToxExpo	16-18 March 2009	Baltimore, Maryland, USA
IAT	31 March - 3 April 2009	Europe
Eurotox	13-17 September 2009	Dresden, Germany

NEW ARTICLES ABOUT MINIPIGS

- *Establishment and characteristics of Göttingen Minipig skin in organ culture and monolayer cell culture: relevance to drug safety testing.*
  Dame MK, Spahlinger DM, DaSilva M, Perone P, Dunstan R.
  In Vitro Cell. Dev. Biol. – Animal 44:245-252. JUN 2008

- *Observations on the microvasculature of bone defects filled with biodegradable nanoparticulate hydroxyapatite*
  Kilian O, Wenisch S, Karnati S, et al.
  Biomaterials 29(24-25): 3429-3437 AUG-SEP 2008

- *Trauma-shock-induced gut injury and the production of biologically active intestinal lymph is abrogated by castration in a large animal porcine model*
  Deitch EA, Senthil M, Brown M, et al.
  Shock 30(2): 135-141 AUG 2008

- *Evaluation of performance of a new algorithm for minipig ECG analysis*
  Olivier-Mercier V, Lacambre JB, Zitoun P
  Fundamental & Clinical Pharmacology 22(suppl. 2): 75-76 AUG 2008

- *Structural analysis of alpha-Gal and new non-Gal carbohydrate epitopes from specific pathogen-free miniature pig kidney*
  Kim YG, Gil GC, Harvey DJ, et al.
  Proteomics 8(13): 2596-2610 JUL 2008

- *Histological and modeling study of skin thermal injury to 2.0 mu m laser irradiation*
  Chen B, Thomsen SL, Thomas RJ, et al.
  Laser in Surgery and Medicine 40(5): 358-370 JUL 2008

- *Treatment of chronical myocardial ischemia by adenovirus-mediated hepatocyte growth factor gene transfer in minipigs*
  Yuan B, Zhang YR, Zhao Z, et al.
  Science in China Series C-Life Sciences 51(6): 537-543 JUN 2008

- *Successful cloning of the Yucatan minipig using commercial/occidental breeds as oocyte donors and embryo recipients*
  Estrada JL, Collins B, York A, et al.
  Cloning and Stem Cells 10(2): 287-296 JUN 2008

- *NBT-PABA test to assess efficiency and kinetics of substituted proteolytic enzyme action in pancreatic duct ligated minipigs*
  Mosseler A, Bergemann J, Becker C, et al.
  Journal of Animal Physiology and Animal Nutrition 92(3): 399-404 JUN 2008

- *Long-term bone tissue reaction to polyethylene oxide/polybutylene terephthalate copolymer (Polyactive (R)) in metacarpophalangeal joint reconstruction*
  Waris E, Ashammakhi N, Lehtimaki M, et al.
  Biomaterials 29(16): 2509-2515 JUN 2008

- *Experimental determination and allometric prediction of vitreous volume, and retina and lens weights in Gottingen minipigs*
  Shafiee A, McIntire GL, Sidebotham LC, et al.
  Veterinary Ophthalmology 11(3): 193-196 MAY-JUN 2008

- *Female mini-pig performance of Temporal Response Differentiation (TRD), Incremental Repeated Acquisition (IRA), and Progressive Ratio (PR) Operant Tasks*
  Ferguson S, Gopee N, Paule M, et al.
  Neurotoxicology and Teratology 30(3): 250-250 Meeting Abstract MAY-JUN 2008

- *Object preferences as environmental enrichment measures in the female mini-pig*
  Smith M, Gopee N, Howard P, et al.
  Neurotoxicology and Teratology 30(3): 250-250 Meeting Abstract MAY-JUN 2008

- *Plasmacytoid dendritic cells migrate in afferent skin lymph*
  Pascale F, Contreras V, Bonneau M, et al.
  Journal of Immunology 180(9): 5963-5972 MAY 2008

- *Osseointegration of a subperiosteal anchoring device in the minipig mandible*
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