Volume 2, Issue 1

PHENOGENOMICS
NEWSLETTER

Czech Centre for Phenogenomics

EMBO
Practical Course

Mouse genome engineering

10 – 20 July 2017 | Dresden, Germany

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REGISTRATION

Registration & abstract deadline
31 March 2017
Registration fee .....450 EUR

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http://meetings.embo.org/event/17-mouse-genome

#EMBO_MGE2017

EMBO
excellence in life sciences

Contents

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Jan Kucera
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Cover Image:
"Bamboo hair" in Klk5-/-Klk7-/-Sp5A135X/A135X mutant mouse at postanatal day 5.

Photo Credits
Christopher Chambers

Note to customers: As valued customers, we welcome your articles and feedback on the service you received. Please send all correspondence to ccc@phenogenomics.cz

The editorial team would like to thank the authors in this issue for their contribution.



Metabolism From Cells to Mouse

We would like to invite you to the workshop “Metabolism from cells to mouse”, co-organized by the Czech Centre for Phenogenomics (CCP) and HPST (authorized distributor of Agilent Technologies).

This unique workshop provides the introduction and practical hands-on of integrated metabolomics workflows, employing the techniques of cellular energy metabolism (**Agilent Seahorse XF**), detailed metabolomic analysis by **LC/MS Q-TOF** and the translation of cell culture results into a living mouse model using **indirect calorimetry**. Results from integrated experimental model, employing all the three techniques will be presented.

WORKSHOP OUTLINE:

Tue 25th April (9:00 - 17:00)

Lectures:

- Introduction into experimental workflow design
- Assay of cellular metabolism in real-time
- Mass spectrometry metabolomics workflows
- Indirect calorimetry technique

Centre of Phenogenomics facilities tour

Practical hands-on in groups

- Agilent Seahorse XFp
- Agilent LC/MS Q-TOF
- Indirect Calorimetry

Social event

Wed 26th April (9:00 - 14:00)

Practical hands-on in groups (continued)

Experimental results of the metabolomics study, discussion

Date: 25th - 26th April 2017

Location: Czech Centre for Phenogenomics
BIOCEV
Prumyslova 595
252 50 Vestec
Czech Republic

Registration: up to 20th April 2017

Course Fee: €50
(includes: coffeebreaks, lunches, social dinner, reagents & consumables for hands-on session, participant bag)

Please note the number of attendees is limited to **30 participants**.

For more information and registration visit:
http://hpst.cz/metabolomic-workshop

Organizers

Czech Centre for Phenogenomics

hpst

Agilent Technologies
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Message from Director

Dear Readers,

Now we are at the beginning of the second year of our operation in the new premises and I am very happy that activities of CCP are developing, establishing new techniques and methods and serving more and more users. Throughout the year we are organising very interesting courses and other educational activities in order to help the scientific community to employ new technologies and possibilities and achieve the goals.

In this issue of "Phenogenomics newsletter" you will read about several courses that we are organising in our new centre in Vestec or co-organizing and reports on past courses and free-of charge calls. You can also read featured articles and reviews, which bring information on behavioural research using the IntelliCage system, overview on technologies and services of the Cardiovascular Phenotyping Unit. Our In the spotlight article highlights the usage of the programmable nucleases to decipher role of the complex kallikrein proteases in the skin, especially in the mouse model of the Netherton syndrome. This syndrome represents one of the ***rare diseases***, which we in CCP are now able to reproduce in the animal models.

Regarding the educational activities, I would like to highlight three courses that we have organised. The first is the ***2nd Programmable nucleases Course***, which again attracted high interest and we are pleased to welcome the participants from various countries across the world. As part of the course we have an excellent team of tutors including Bernd Zetsche from Feng Zhang's lab (MIT), Lluis Montoliu and Francis Stewart. Staying with this revolutionary technology, CCP is also participating in '***Mouse genome engineering***' a practical course on CRISPR usage organized under EMBL by MPI-CBG in Dresden.

In addition to the genome editing technologies, we have also prepared a practical hands-on course '***Metabolism from cells to mouse***' introducing integrated metabolomics workflows, and employing the techniques of cellular energy metabolism (Agilent Seahorse XF) and detailed metabolomics analysis by LC/MS Q-TOF. The metabolomics analysis has been newly introduced into our portfolio of technologies and soon we well be able to offer this analysis as one of our services.

Last but not least, CCP established control quality system for new drugs development, accomplishing the establishment a good laboratory practice (GLP) mode for histopathology and biochemistry units; its extension to other units is planned. Thus, CCP has improved its capacity to foster preclinical and clinical research.

I hope you enjoy this issue of our newsletter and appreciate the work CCP is doing and the advanced technologies available at our centre.

Radislav Sedlacek

Anatomical Bases of Mouse Multimodal Imaging Course

Ivan Kanchev

Histopathology Unit

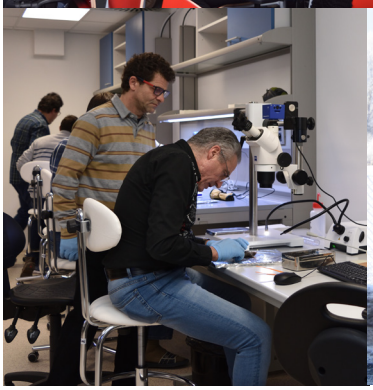
Studying the mouse anatomy can change the outcome of your research.

Mouse has become one of the most used models in the last century. The value of this particular rodent is extreme and allows scientists to dissect various disorders in a living animal rather than in controlled ex vivo model environment. Being a complex organism the mouse possesses all structures seen in mammals with of course reduced scale but still sharing structural complexity of a mammal.

Despite the fact that mouse was used in studying of general physiology for centuries mouse morphology is a recently developed field. It was not before 1965 when Margaret J. Cook published the first comprehensive mouse dissection anatomy. The microanatomy/histology of mice was done mainly by the toxicology pathologists and thanks to the contribution of R. Maronpot, C.H. Frith and J. Ward a lot of histology features of mice were revealed. Seen from that perspective, mouse morphology phenotyping is a new field and there is limited amount of knowledge in mouse anatomy, histology and imaging within the scientific community in the world.

In the Czech Centre for Phenogenomics we do understand the indispensable value of the multimodal structural mouse phenotyping. Therefore, we organized the first in the Czech Republic comprehensive course of Anatomical Bases of Mouse Multimodal Imaging having on board two world known experts in mouse anatomy: Jesus Ruberte Paris and Marc Navarro. The course consisted of detailed theory on mouse anatomy where the participants learnt the intimate mouse macrostructure not only on pure anatomical preparations but also in several imaging methods giving the ability to also show the modern methods of mouse imaging. The programme covered the main systems: muskoleskeletal, cardiovascular, respiratory, digestive, excretory, reproductive and nervous. Special emphasis was given to the differences of mouse strains and also to other rodents and human. Of course after that solid theory the participants had the possibility to check their newly acquired knowledge in practice. Every participant had the ability to conduct dissection of the discussed organs and systems under the guidance of the two lecturers. After every day of the course there was a wrap up where the studied systems were discussed.

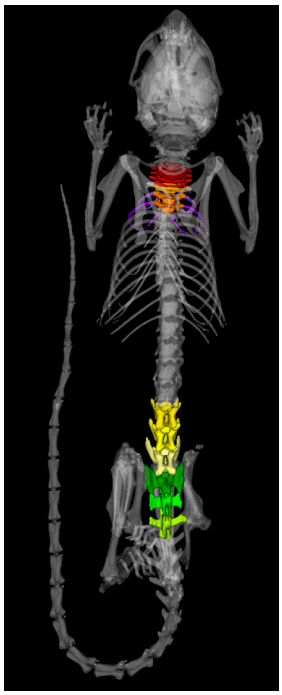
The course had participants from the Czech Republic, Italy, France and Spain. It really showed that knowledge of anatomy and the different methods for imaging of mouse body will definitely improve the outcome of the large scale mouse studies conducted nowadays. Therefore, we are looking forward to host another course with hope to share Jesus's and Marc's knowledge with even more people interested and working with mice.



Use of microCT technique for biological applications

Frantisek Spoutil

Bioimaging Unit



Segmentation of mouse skeleton with highlighted transition between cervical and thoracic vertebra on top, and lumbar and sacral vertebra on bottom. Scanned in SkyScan 1176 (Bruker microCT), voxel = 35 µm, segmentation in ITK-Snap (Yushkevich et al. 2006)

The Czech Centre for Phenogenomics in Vestec (CCP) hosted the second seminar dedicated to MicroCT techniques for biological applications. The seminar was held on the 2nd of February 2017 and attracted a large number of researchers from a broad spectrum of science disciplines from physics and engineering to biomedicine or palaeontology originating from institutions not only from Prague, but also from universities and institutes outside and also from Slovakia.

After the welcome from Jan Prochazka – Head of Phenotyping module and bioimaginng unit CCP, the introduction to microCT technique was in the hands of representatives of RMI, s.r.o, an exclusive distributer of Bruker microCT systems (former SkyScan) in the Czech Republic. The biggest star of the seminar was Phil L. Salmon, an application scientist of Bruker microCT, who explained not only the mechanisms of the microCT technique and differences between ex-vivo and *in-vivo* imaging, but also showed various applications and was eager to answer participants' questions about all the mentioned topics.

As our facility possesses SkyScan 1176, the valuable part of the seminar was the direct experience of our researches with the machine and merits and flaws of its application in particular projects. First, Jan Procházka, introduced CCP structure and goal to participants and highlighted some applications of microCT, which went on in the last year, e.g. skeleton morphology analysis, tooth enamel analysis, *in-vivo* arthrosis development, embryology or body composition analysis. Body composition was presented in detail by Nicole Chambers, head of the metabolism unit and František Špoutil, research assistant of bioimaging unit and main operator of SkyScan 1176 microCT, who are starting the project of validation of microCT application for body composition analysis for IMPC (International Mouse Phenotyping Consortium).

At the end of the seminar the participants could enjoy a demonstration of SkyScan 1176 of our facility, if interested in *in-vivo* imaging, or sample model of ScyScan 1275 provided by RMI for fast ex-vivo imaging. Both with valuable comments of Phil L. Salmon.

We are looking forward to hosting the 3rd seminar in spring 2018.

CCP Aids Publication Success

Nicole Chambers

Editor

On behalf of Phenogenomics newsletter, I would like to congratulate two groups from the Functional genomics research programme on their recently accepted publications.

Kasparek, P *et al*. KLK5 and KLK7 Ablation Fully Rescues Lethality of Netherton Syndrome-Like Phenotype. PLoS Genet. 2017 Jan 17;13(1).

Wald, T *et al*. Intrinsically disordered proteins drive enamel formation via an evolutionarily conserved self-assembly motif. Proc Natl Acad Sci U S A. 2017 Feb 14.

These publications showcase the technology and expertise available at the Czech Centre for Phenogenomcis and we are proud of the work achieved in these publications.

News In Brief

Successful applicants for free histopathology service

Sarka Suchanova

Histopathology Unit

The Czech Centre for Phenogenomics (CCP), Institute of Molecular Genetics AS CR offered support of researchers from academic institutions with a **free of charge service of histopathology unit**. Applications has been sent in the period during January 2017 as was announced in the Phenogenomics newsletter (Volume1, Issue 4, 2016, http://www.phenogenomics.cz/portfolio-view/phenogenomics-newsletter-volume-1-issue-4/). Provided services covered organ sampling and trimming, tissue processing, embedding, sectioning, H&E staining and slide scanning for 1 mice cohort **(max 15 mice, 5 organs per mouse)** or equivalent number of tissues**.**

We received a large number (11) of applications from 8 different institutions from CR and from abroad. All applications were assessed by our expert evaluation committee. A total of 5 histopatology projects from 5 different institutions **have been selected** based on their scientific merit and the professional level of the application.

According to our capabilities and capacity we plan to repeat the call: **FREE-OF-CHARGE HISTOPATHOLOGY SERVICE** in the second half of 2017.

Applicant	**Affiliation**	**Country**
Marcus Damme	Cristian-Albrechtes University, Kiel	Germany
Jiri Kohoutek	Veterinary Research Institute, Brno	Czech Republic
Regine Schneider-Stock	University Hospital & Friedrich-Alexander University, Erlangen-Nuremberg	Germany
Petr Sebo	Institute of Microbiology ASCR, Prague	Czech Republic
Marek Vrbacky	Institute of Physiology ASCR, Prague	Czech Republic

CCP - Free of charge mouse production service

Inken M Beck

Transgenic and Archiving Module

In autumn 2016, the Czech Centre for Phenogenomics opened a call for free of charge knockout mouse production. In total, five access units were available and following institutes were selected with one or two projects.

The mouse models will be produced by CCP on C57Bl/6N background and made available to the wider research community via the INFRAFRONTIER/ EMMA repository (https://www.infrafrontier.eu/).

Mouse lines produced by this service will be owned by CCP/IMG, but selected institutes can freely use the models for the project-specific research described in the evaluated proposals. Since CCP/IMG is member of the IMPC (http://www.mousephenotype.org/), we plan to phenotype all produced models according to IMPC standard pipeline.

Once models are produced, institutes will be notified and delivery of mice will be arranged. CCP thanks all research groups for sending their proposals!

Faculty/Department	**Institute**	**City**	**Project(s)**
Faculty of Science	Institute of Experimental Biology, Masaryk University	Brno	1 project
Faculty of Pharmacy in Hradec Kralove	Charles University	Hradec Kralove	1 project
Faculty of Science	Department of Zoology, Charles University	Prague, Vestec	2 projects
Laboratory of Mouse Molecular Genetics	Institute of Molecular Genetics ASCR	Prague, Vestec	1 project

Successful GLP Accreditation for CCP

Sarka Suchanova

CCP Management

CCP holds all the necessary components to foster preclinical and clinical research. It has dedicated and trained staff, expertise to work with animal models, adequate technology for analysis of various experimental procedures in animal models including the histopathological department, large capacity and up-to-date technology for cryopreservation of biomedical samples. In addition to above described effort, our CCP has established also activity for preclinical research important especially for biotechnological and pharmaceutical customers to test their compounds potentially suitable for treatment of human diseases. Preclinical testing on animals under the OECD (Organisation for Economic Co-operation and Development) GLP conditions (GLP – Good Laboratory Practice) with established control quality system is a key element of new drugs development. To accomplish this, we have established a ***Good Laboratory Practice (GLP) mode*** for selected phenotyping units of CCP. The GLP mode is currently restricted to histopathology and biochemistry unit and its extension to other units is planed.

CCP IMG is working as a "Test site" under "Test facility" **Institute of Physiology CAS** (Certificate of Good Laboratory Practice Ref. No.: sukls124283/2016).

Non-clinical testing of potential medicinal products including services under **OECD** (Organisation for Economic Co-operation and Development) **GLP** conditions (GLP – Good Laboratory Practice):

- Histopathology
- Biochemistry
- Hematology
- Bioanalytical testing

Other services for preclinical testing:

- Behavioural screening for neuroactive drugs
- Toxicity studies
- Cardiology diagnostic tests on animal models – electrocardiogram (ECG), blood pressure measurement, cardiac imaging (Echo)
- Bioimaging (MicroCT, radiography, whole body fluorescence imaging)
- Immunology, pharmacology on xenografts

FEATURED ARTICLE

INTELLICAGE – A NEW QUALITY OF BEHAVIOURAL RESEARCH

Agnieszka Kubik-Zahorodna

Neurobiology and Behavior Unit

Conventional behavioural models were designed and developed mostly for the benefit of fast and reliable screening of new psychoactive drugs. They are also broadly used in basic research elucidating biological mechanisms of behaviour and pathophysiology of Central Nervous System (CNS) diseases. With the help of genetic engineering techniques they shed light upon the genetic background of the brain function and its proper development. However, despite a long history of implementation, they possess two major disadvantages: 1) animals are tested individually out of their natural social context and 2) results are contaminated by variable, and largely uncontrolled, anxiogenic impact of human presence and handling during testing on the animal's performance and welfare.

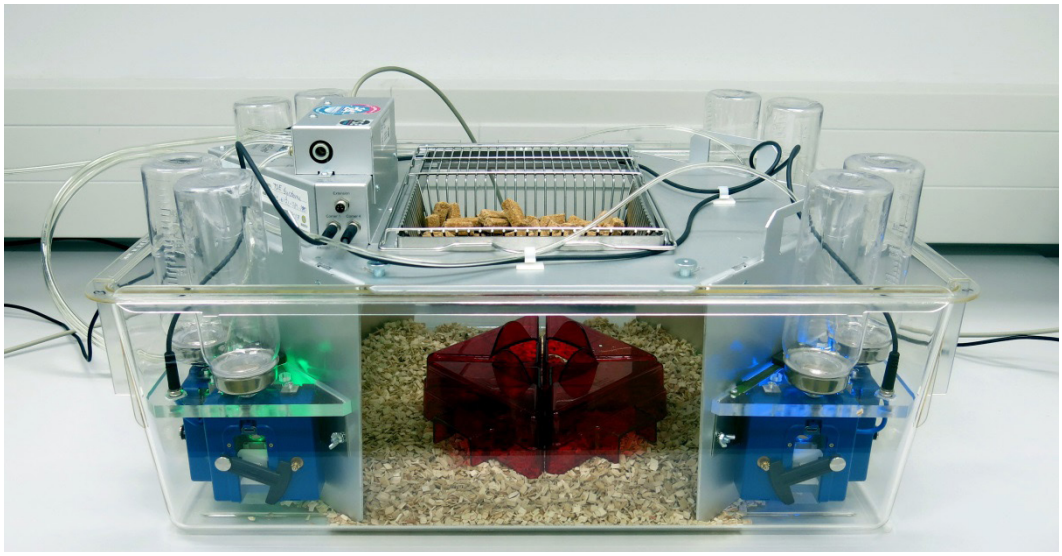


Figure 1. IntelliCage

IntelliCage components

At the turn of the century Hans-Peter Lipp designed and developed a computerized cage, he named IntelliCage (Fig. 1), to study spontaneous and cognitive behaviour of mice living in social groups without direct contact with an experimenter [1, 2]. IntelliCage is based on ability to recognize presence of a specific individual in one of four operant corners of the apparatus

IntelliCage behavioural tests:

- **Spontaneous behaviour**
  - Basic activity levels, circadian activity
  - Anxiety, neophobia and exploration
- **Spatial and temporal**
  - Stereotypical place preferences
  - Spatial preference and avoidance learning
  - Spatial reversal learning
  - Spontaneous alternation
  - Temporal conditioning
  - Temporo-spatial conditioning
  - Systematic patrolling schedules
  - Radial maze-like patrolling
- **Discrimination learning & preferences**
  - Visual discrimination
  - Gustatory discrimination learning
  - Spontaneous drug preference or avoidance
- **Memory**
  - Procedural memory
  - Habituation
  - Spatial short-term memory (working memory)
  - Visceral-gustatory memory
- **Social & others**
  - Competition rank orders
  - Approach-avoidance conflicts
- **Operant conditioning**
  - Procedural learning
  - Fix ration conditioning (motivation)
  - DRL (Differential reinforcement of low responding, response inhibition, timing)

Table 1. Examples of common programmable behavioural tests employed by IntelliCage.

and assign an animal to its performance. This is achieved by implanting the animals (mice) with small hypodermic transponders with unique codes, which are recognized by antennae build in each corner, which can only host one mouse at the time. Animal presence is co-detected by a temperature sensor. All corners are equipped with two door-guided openings to drinking bottles. Mice can either drink freely if the doors are programmed to be permanently open or have to perform a nose poke on a closed door in order to obtain liquid reward. In both cases animal's presence in the opening is detected by infrared light beams. The number and duration of water licks is obtained using a detector sensitive to changes in conductance. Beside sensors, the corners also contain so called "actors", which guide the animal behaviour. An air puff can be used as an aversive reinforcement stimulus. A sliding door controls access to appetitive reinforcement - water. Three LEDs in three different colours serve as conditioning stimuli (Fig. 2). The cage can house up to 16 mice for many days or weeks allowing undisturbed observation of their spontaneous behaviour and circadian rhythmicity in natural, social conditions. The system includes a computer, which can control maximum of eight IntelliCages. That allows testing of up to 128 mice simultaneously. Importantly, the system can be programmed to test the animals in a variety of programmable behavioural tasks in one carefully designed experiment.

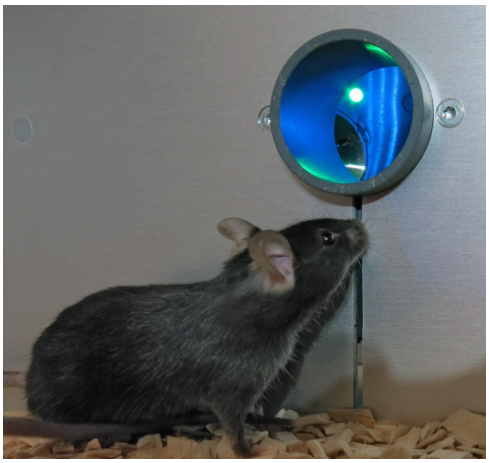


Figure 2. Operant corner opening.

IntelliCage functional specifications

The IntelliCage is designed for long-term, high-throughput investigation of cognitive abilities in laboratory mice. Animals perform variety of the behavioural tasks graphically programmed by a researcher (Fig. 3). A variety of simple or complex conditioning tasks are automatically initiated and controlled individually for each mouse. Data of every individual animal are simultaneously collected and analysed from the whole cohort (Fig. 4). The system also allows observing the development of animals' behaviour in time and after the completion of the experiment it provides an enormous amount of information about their cognitive abilities, hierarchical structure, anxiety, neophobia, exploration, stereotypical place preferences, and motivation[3-7]. Table 1, adopted from Lipp et al., 2005, presents an example of common programmable behavioural test employed by IntelliCage. The biggest advantages emerging from the IntelliCage is reducing human interaction with the animals during the experiment to the necessary minimum and the opportunity to study many aspects of rodent behaviour in more natural social groups. These virtues give rise to many other benefits. One overcomes the main obstacle in behavioural research, which is obtaining reproducible data across laboratories due to variations in the experimental conditions. Standardization of the laboratory

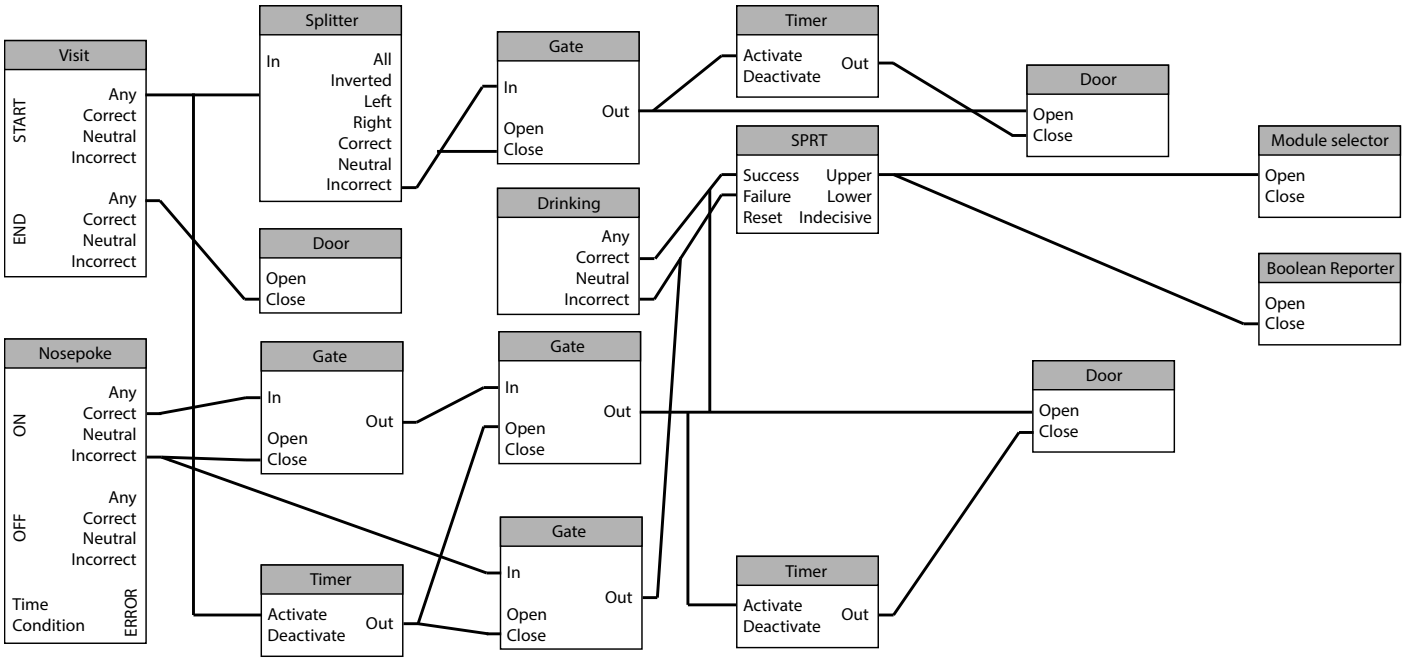


Figure 3. Example of graphical behavioural task program

environment, like using the same experimental utilities from the same cage, food, bedding supplier, housing conditions to the unified experimental set-ups, still is jeopardized by substantial source of the experimental "noise" that arise from an experimenter itself. Animals can sense differences between experimenters, traits that are practically impossible to standardise. Thus eliminating human factor increases quality and comparability of the data output [4]. Tests conducted in more natural social settings also provide ethologically more relevant data. Indisputably, it also improves animal welfare, minimizes investigator's effort, and reduces numbers of animals used in the study, which also reduces the costs of the experiments. Upgraded, and commercially available, new generation of IntelliCage allows scientists to study cognitive, emotional, and social aspects of behaviour in various genetic or developmental animal models of mental disorders such as autism, Huntington's disease, addiction, and many others [8-10]. It can also find its application in anxiety research by using unconditioned avoidance paradigm [5]. IntelliCage is a complementary approach to conventional behavioural tests and provides new quality of data collected in non anxiogenic experimental set-up. It is relatively simple and automated high-throughput screening tool that can find implementation in early stages of drug discovery and development or in phenotyping mutant mice [6]. It can capture a diversity of behavioural activities in socially-housed animals and deliver comparable and reproducible data. In Czech Centre for Phenogenomics (CCP) we implement a battery of conventional behavioural tests standardized or customized according to our clients' individual requirements. We also offer animal testing in IntelliCage using our standardized protocols and serve our help in design an experiment for specific study demands.

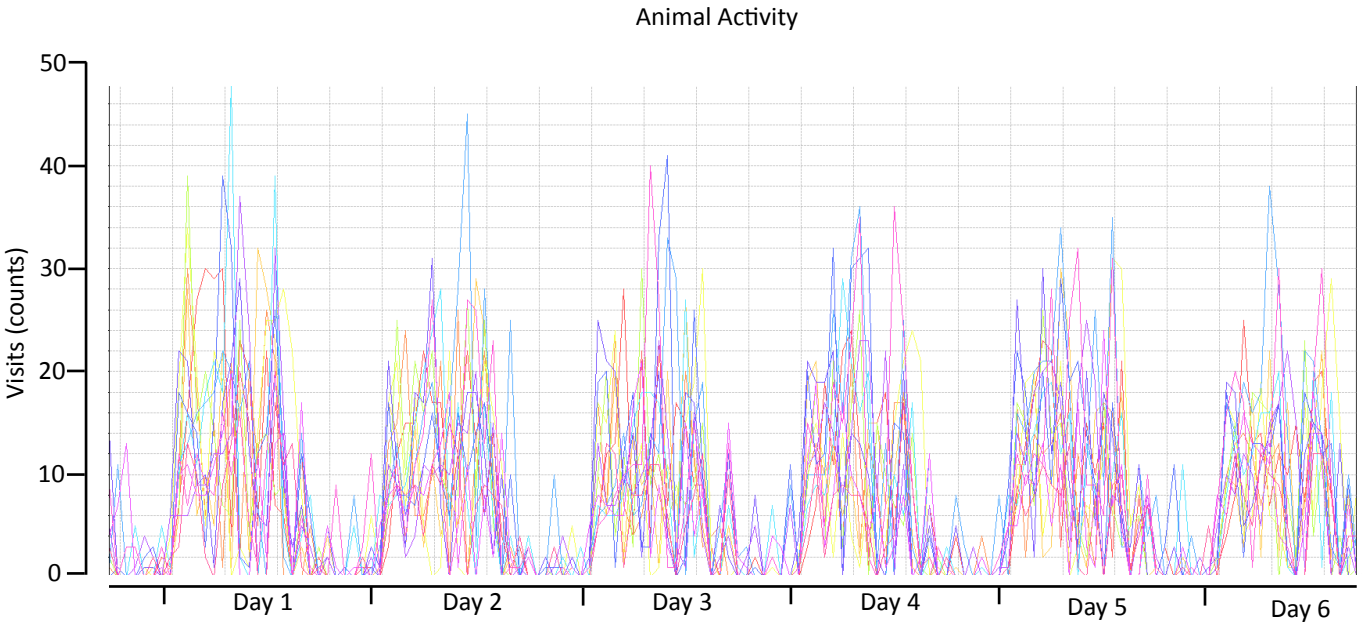


Figure 4. Graphical representation of mice circadian activity in IntelliCage determined by the number of corner visits.

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Triple KO using TALENS 'sheds light' on complex proteolytic pathway involved in Netherton Syndrome

Petr Kasparek

Functional Genomics Research Programme

Netherton syndrome (NS) is a rare and severe genetic disease that affects mainly skin and immune system. Newborns with NS show red, scaly and inflamed skin with a severe disruption of epidermal barrier [1,2]. Consequently, some of these children may die due to dehydration. Surviving patients often manifest itchy skin with a chronic inflammation, various food allergies and asthma. Characteristic feature of NS is a „bamboo hair" defect nicknamed after the similarity of patients´ hair to bamboo stalks with typical nodules and bumps on affected hair shafts [2].

By the beginning of 21st century it was found that NS is caused by mutations in the gene *SPINK5*, which encodes a protease inhibitor LEKTI [1,3]. This discovery linked NS symptoms to the unregulated protease activity in skin and possibly in other organs [4]. To develop a functional and specific therapy for NS, it is important to understand exactly which protease is responsible for the epidermal and immunological defects.

A crucial step in characterization of NS molecular pathogenesis was generation of *Spink5* deficient mice (Spink5$^{-/-}$) [5-7]. These mutant models closely mimicked the NS symptoms of human patients, including the skin barrier disruption that lead to early lethality of newborn Spink5$^{-/-}$ pups. Detail analysis of these mice revealed that the majority of proteases dysregulated in the absence of LEKTI belong to the family of kallikrein-related peptidases (KLKs) [5]. Especially KLK5 was considered a major player in NS as it is not only the direct inhibitory target of LEKTI, but it can also promote the proteolytic activation of KLK7, KLK14 and ELA2 – other proteases hyperactive in the skin of Spink5$^{-/-}$ pups [8,9]. Nevertheless, recent discoveries showed that the inactivation of KLK5 in Spink5$^{-/-}$ mice is not sufficient to rescue the lethal phenotype of the Spink5$^{-/-}$ pups, although it improves a number of their skin-defects [9]. This suggested that other proteases contribute to the lethality of NS mouse model in KLK5-independent manner. Assuming that also these enzymes likely belong to the KLK family, we speculated that generation of mouse mutants deficient in multiple KLK genes may further elucidate the complex proteolytic pathways that lead to NS pathology.

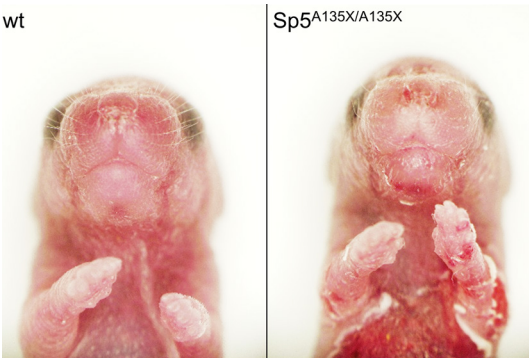

Fig1. **The phenotype of Sp5$^{A135X/A135X}$ newborn pups.** Sp5$^{A135X/A135X}$ show severe skin defects manifested by fragile, peeling epidermis.

However, generation of these models proved to be more complex due to the close genomic proximity of KLK genes which does not allow efficient generation of multiple-KLK deficient mice by conventional approaches (i.e. cross breeding of two independent gene-deficient strains).

This obstacle was recently overcome by rapid development of programmable nucleases (PNs), such as TALENs and CRISPR/Cas9 that revolutionized the field of mutant mice generation. Direct microinjection of PNs into mouse zygotes enables efficient generation of mutant mouse models significantly faster in comparison to the traditional strategies based on modification of embryonic stem cells [10]. This strategy allows rapid sequential targeting of several genes, making the generation of multiple-gene-deficient mice possible in a reasonable timeframe. Indeed, using TALEN technology we successfully prepared mice deficient for KLKs 5 and 7 (Klk5$^{-/-}$, Klk7$^{-/-}$) and also KLK5/7 – double deficient mutants (Klk5$^{-/-}$Klk7$^{-/-}$). Additionally, we applied TALENs to introduce a causative mutation known from NS patients into mouse genome. We showed that the mice homozygous for this "humanized mutation" of *Spink5* (Sp5$^{A135X/A135X}$) develop severe skin defects which recapitulate the phenotype of previously described Spink5$^{-/-}$ pups (Figure 1). However, in contrast to these gene-deficient models, Sp5$^{A135X/A135X}$ mutants carry only

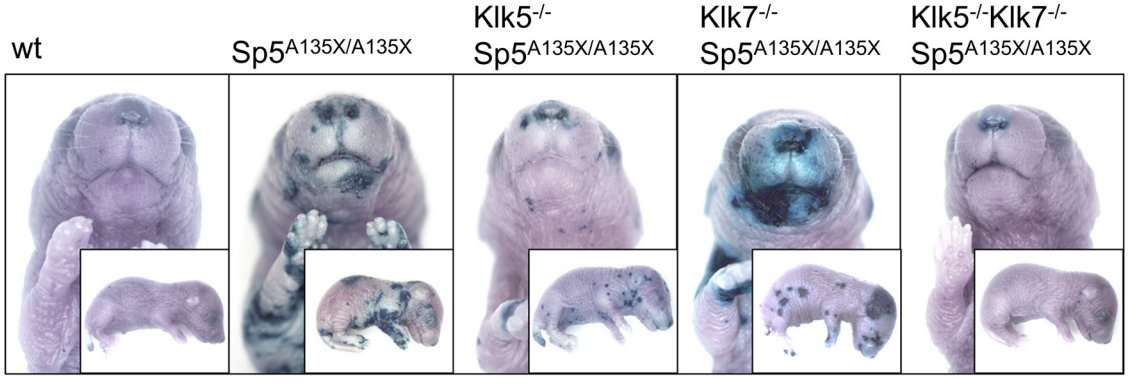

Fig2. **Disruption of epidermal barrier in Klk/Spink5 mutant pups.** Toluidine blue staining reveals the skin regions with disrupted barrier.

subtle modification of their genomic DNA, which is closer to the natural causes of genetic disorders such as NS, providing more physiological model of the disease.

Most importantly, we combined the KLK-deficient strains with $Sp5^{A135X/A135X}$ mice to identify the roles of these proteases in NS. Parallel analysis of four Klk/Spink5 mutant strains ($Sp5^{A135X/A135X}$, $Klk5^{-/-}Sp5^{A135X/A135X}$, $Klk7^{-/-}Sp5^{A135X/A135X}$ and $Klk5^{-/-}Klk7^{-/-}Sp5^{A135X/A135X}$) brought several novel findings on the molecular pathology of NS. First, we showed that single inactivation of KLK5 or KLK7 does not rescue the lethality of $Sp5^{A135X/A135X}$ newborn pups. Inactivation of KLK7 did not have any obvious effect on the survival of pups as $Klk7^{-/-}Sp5^{A135X/A135X}$ neonates were dying together with $Sp5^{A135X/A135X}$ approximately 12h after delivery. Consistently with the recent study by Furio et al. (9), the ablation of KLK5 prolonged the life of $Klk5^{-/-}Sp5^{A135X/A135X}$ pups. However, we showed that these mice die few days after delivery, typically at postnatal day 5 (P5). In striking contrast, simultaneous inactivation of both, KLK5 and KLK7, fully rescued the lethality of our NS model and the life-span of $Klk5^{-/-}Klk7^{-/-}Sp5^{A135X/A135X}$ was comparable to wild-type animals. We showed that the survival of Klk/Spink5 mutant mice is tightly associated with the integrity of their epidermal barrier. While the pups dying at P0 ($Sp5^{A135X/A135X}$ and $Klk7^{-/-}Sp5^{A135X/A135X}$) showed severe

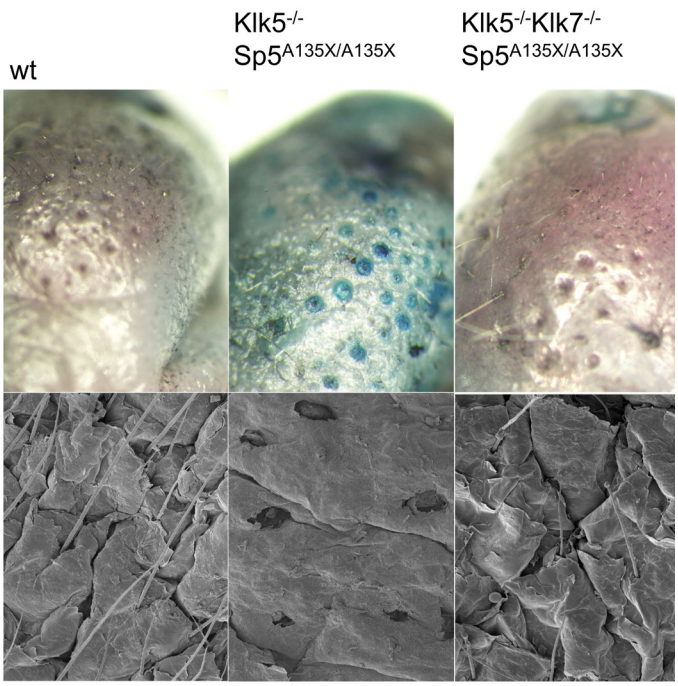

Fig3. **Activity of KLK7 leads to epidermal defects in the proximity of hair follicles.** Disruption of epidermal barrier in $Klk5^{-/-}Sp5^{A135X/A135X}$ can be visualized by toluidine blue staining of P5 pups (upper panel). Detail analysis by scanning electron microscopy revealed absence of hair shafts and epidermal "perforations" in dorsal skin of $Klk5^{-/-}Sp5^{A135X/A135X}$. Ablation of KLK7 (in $Klk5^{-/-}Klk7^{-/-}Sp5^{A135X/A135X}$) completely rescues these defects.

barrier defects immediately after delivery, $Klk5^{-/-}Sp5^{A135X/A135X}$ newborn pups manifested only minor barrier disruption at P0 (Figure 2). However, we observed that the activity of KLK7 in these pups gradually leads to the disruption of epidermal barrier in a close proximity of hair follicles, which may contribute to their death at P5 (Figure 3). $Klk5^{-/-}Klk7^{-/-}Sp5^{A135X/A135X}$ triple mutants did not reveal any major barrier defects at any time point. These experiments highlighted that KLK7 can be active in the absence of KLK5. This finding challenges the established view that KLK5 triggers the proteolytic activation cascade which eventually results in defective barrier. Our data clearly show that the hyperactivity of KLK7 alone can cause drastic damage resulting in compromised skin function. Furthermore, we show that KLK7 activity can trigger the cutaneous inflammation and differentiation defects – the features associated with NS that were previously attributed to the hyperactivity of KLK5.

The ability of $Klk5^{-/-}Klk7^{-/-}Sp5^{A135X/A135X}$ to survive the neonatal period provided unique opportunity to study LEKTI-deficiency in adult mice. Interestingly, we found that these triple mutant mice develop hair phenotype that strongly resembles the bamboo hair of Netherton syndrome patients (Figure 4). This suggests that although this pathology is triggered by the absence of LEKTI, it is not associated with the hyperactivity KLK5 or KLK7 hyperactivity. This observation not only elucidates

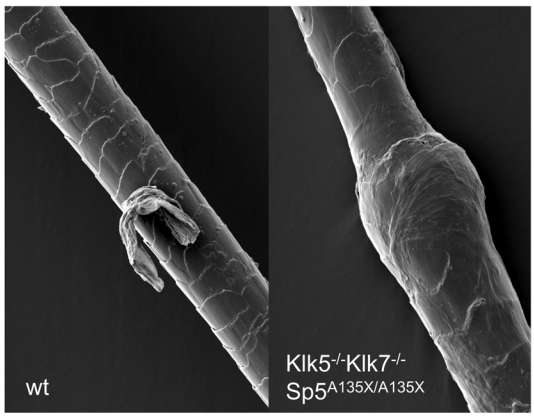

Fig4. Bamboo hair of $Klk5^{-/-}Klk7^{-/-}Sp5^{A135X/A135X}$ mutant mice.

the mechanisms leading to this cosmetic defect, but it also demonstrates that the LEKTI deficiency has the same functional consequences in mice and men. In summary, our study showed that both, KLK5 and KLK7 independently contribute to the epidermal defects associated with Netherton syndrome and both proteases should become targets for NS therapy. Importantly, we also showed that although the gene-deficient mouse models are indispensable tools to study the gene functions, in some cases the single- or double- gene deficiency may not be sufficient to provide a clear image of the complex situation *in vivo*.

Thankfully, in the era of programmable nucleases, the triple mutant animals can be generated rapidly and at a fraction of the cost to provide us valuable lessons on the mechanisms of severe diseases, such as Netherton syndrome. Nevertheless the animal holding and breeding capacity required for this project was also significant. Thanks to the large cage capacity available at CCP, we were able to maintain the large cohorts required to obtain the triple mutants.

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OVERVIEW OF SERVICES AVAILABLE

Silvia Petrezselyova

Cardiovascular Unit

The objective of the Cardiovascular Phenotyping Unit (CPU) is to provide investigators services to assess cardiovascular phenotypes in rodents, with current attention to mouse models. The mouse has become the preferred model for cardiovascular research for several reasons. Firstly, knowledge of the mouse genome and the availability of transgenic and knockout strains makes the mouse one of the most attractive models for research into the molecular basis of cardiovascular diseases. In addition, compared to other mammalian models, the mouse has also practical advantages, such as short pregnancy time, the ease of handling and low procedure costs. Because of two important limitations: the small size of the mouse heart and the structural differences with respect to human cardiovascular system, in future the CPU plans to expand cardiovascular screen also to rats.

The CPU uses validated approaches and state-of-the-art instrumentation that allow for sensitive screening of phenotypic variations. We use a variety of non-invasive *in vivo* techniques for the monitoring of physiological and bioelectrical variables (*e.g.* heart rate, ECG, respiration, blood pressure) in conscious animals. For imaging of the mouse heart and vascular system we use two-dimensional and M-mode High-Frequency Ultrasound (echocardiography), using the Vevo Imaging System. In this article we provide an overview of these methods and our facility services.

Electrocardiography (ECG)

The ECG is a primary diagnostic tool in human patients suffering from heart disease. In 1895, Einthoven first described the morphology of the human electrocardiogram [1]. Eighty-three years later, in 1968, Goldbarg and colleagues published the first detailed description of the mouse ECG [2]. Since then, many studies have been conducted to examine the patterns of activation and repolarization in the mouse heart and their relation to the ECG (for a review see [3]).

The CPU routinely performs recording of the murine cardiac electrical activity non-invasively through the animal`s paws using the ECGenie system (MouseSpecifics, Inc.). The size and spacing of disposable footplate electrodes facilitate contact between the electrodes and the paws to provide Einthoven lead II ECG in laboratory animals. For each animal, heart intervals and amplitudes are evaluated from continuous ECG recording after 5-min acclimation period. The CPU is equipped with ECG platforms and footplate electrodes of different sizes thus allowing ECG monitoring of mice and higher rodents, including newborn pups.

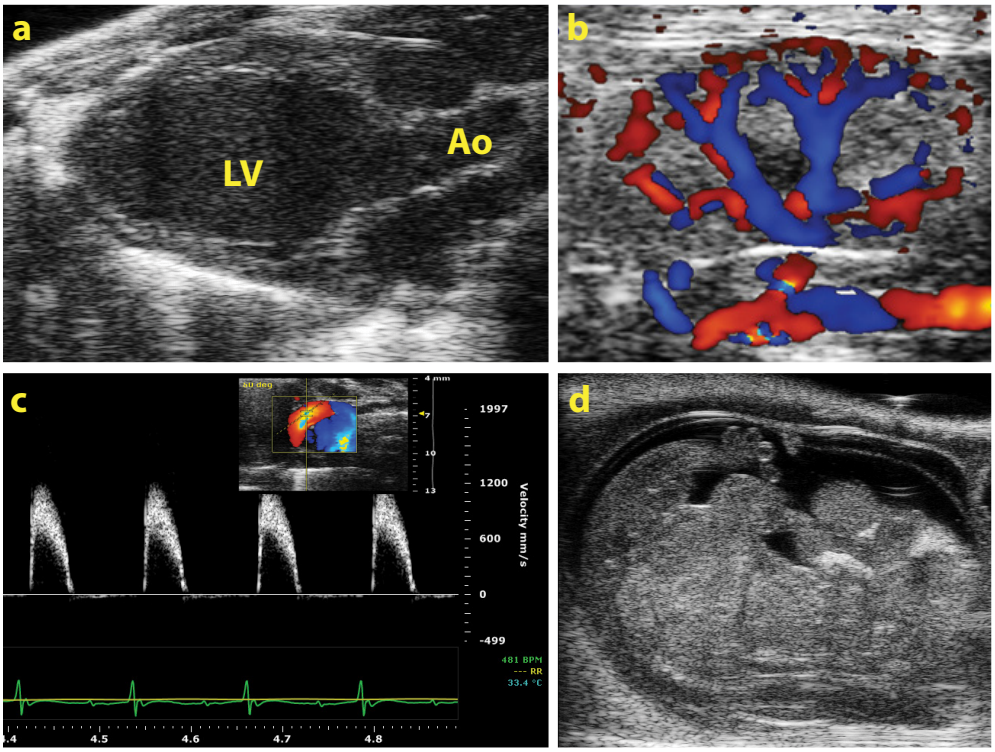

FIGURE 1: Two-dimensional (2D) ultrasound imaging. (a) – Representative B-mode echocardiographic image of an adult mouse heart. LV = left ventricle, Ao = aorta. (b) – Color Doppler ultrasound image shows renal arteries and veins. (c) – An example of PW Doppler recording across the transverse aortic arch. (d) – Representative B-mode image of embryonic day 15.5 fetus.

Blood pressure

The ability to monitor and record precise fluctuations in blood pressure in experimental animals is essential to research in human hypertension and other cardiovascular conditions. We provide accurate tail-cuff blood pressure measurement in mice using the CODA 8-channel High Throughput Non-Invasive Blood Pressure system (Kent Scientific). The CODA utilizes volume-pressure recording technology to detect changes in tail volume that correspond to systolic and diastolic blood pressures. Blood pressure measurements are made in conscious animals maintained in normal housing conditions with minimal handling and restraint of the animals. This reduces stress levels and physiological disturbances in blood pressure measurements over a longer period whereby data quality is improved. The CPU is equipped with two CODA

systems, thus the measurement can be done on up to 16 mice or rats simultaneously. The method enables accurate blood pressure phenotyping in rodents for linkage or mutagenesis studies, as well as for drug testing experiments requiring high-throughput blood pressure measurements.

Echocardiography

Murine echocardiography is a "gold standard" method to assess cardiovascular structure and function that has been adopted from human system [4]. The technology`s allure lies primarily in its versatility, non-invasive nature, and rapid real-time imaging capabilities. We use the Vevo 2100 system (Visualsonics), which offers excellent anatomical and soft tissue structural detail instantaneously and in real-time, and also permits dynamic vascular imaging. A comprehensive mouse echocardiography often includes:

- 2D long- and short-axis images of the heart
- M-mode images of the left ventricle and key structures
- Pulse-Wave Doppler recording of aortic and trans-mitral valve velocities
- Tissue Doppler recording of mitral annulus and left ventricle wall motion velocities

In the presence of wall motion abnormalities (*i.e*. after a myocardial infarction), more advanced analysis, including strain rate measurements, may be warranted. The CPU has three scanheads: 1) MS400: 28MHz with adjustable focal length providing an axial resolution of 55 µm – used in cardiac imaging in adult mice; 2) MS550S: 44 MHz with adjustable focal length providing an axial resolution of 40 µm – used in mouse vascular, abdominal, superficial embryonic imaging and small mouse cardiac; 3) MS250: 20 MHz – used in cardiac imaging of larger mice and rat, and imaging of large tumors (< 23 mm). Echocardiography is typically performed in anesthetized mice (the system contains an anesthesia device that uses isoflurane); however, echocardiography is possible to do in conscious mice as well.

General Echography

While the central focus of the CPU is cardiovascular research, the techniques that are employed may also be useful to investigators in other fields, such as cancer, neurobiology and developmental biology. The Vevo 2100 ultrasound system allows imaging of numerous internal anatomic structures other than the heart and vascular system, namely: abdominal (kidney, spleen, liver, larger abdominal vessels), pelvic (bladder, ovaries, prostate) organs and other (*e.g*. eye, testes), including tumors. For developmental studies, it is possible to monitor living mouse/rat embryos in uterus and follow the development of cardiac structures as well as changes in blood flow velocities in the heart and umbilical artery.

For many studies, multiple measurements can be coordinated with the other Units of the CCP: for example, non-invasive serial echocardiographic and blood pressure determinations during a period of high-fat feeding or other environmental stress, heart rate and ECG monitoring during challenge/stress tests, and histologic evaluation on sacrifice. For more information about the services we provide, visit our website: http://www.phenogenomics.cz/phenotyping/

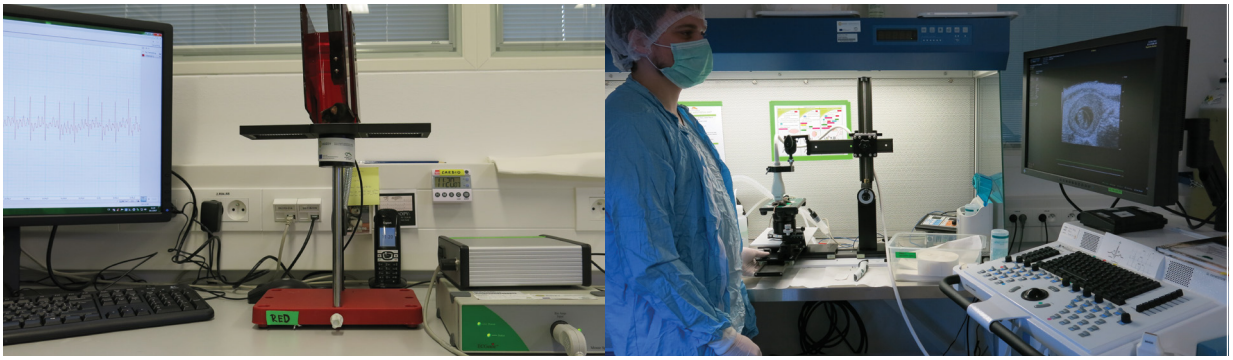

FIGURE 2: Equipment of the Cardiovascular Phenotyping Unit. Upper panel – Vevo2100 Ultrasound Imaging System (VisualSonics); bottom panel – ECGenie system (Mouse Specifics, Inc.).

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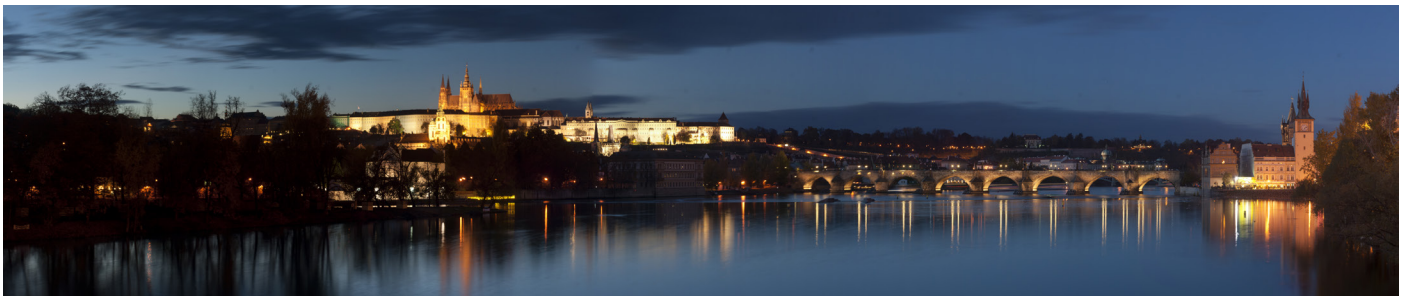
Careers



CCP comprises a young, multidisciplinary and international team. We believe in the personal and professional development of our staff and seek, where possible, to facilitate the attendance of relevant conferences and courses. We offer a competitive salary and various working contracts. Please click here for application details

Patient-Derived Xenograft specialist

The technology known as Patient-Derived Xenografts (PDXs) brings significant information tumor development and treatment and the successful applicant will establish and standardize the PDX technology in order to serve individual clinical groups to handle, archive and eventually treat tumor patients. Thus, the specialist will be involved in characterization of new PDX lines derived from patients with lymphoproliferative malignancies and solid tumors, new technologies for characterization of tumor xenografts including metabolomics, developing PDX models, developing a phenotyping pipeline for PDX samples, and establishing a biobank for PDX samples.

The successful applicant will have strong experience with animal work and surgery, should have a Ph.D. and/or education in veterinary or medical science and have a good proficiency in English. The successful applicant will also have excellent interpersonal, communication and organizational skills and be highly-motivated with the ability to work independently and as part of the multi-disciplinary team of CCP.

This position is available in the Czech Centre of Phenogenomics (hosted by the Institute of Molecular Genetics of the ASCR, v.v.i.) in BIOCEV campus (CCP building) in Vestec near Prague, Czech Republic. The position is available immediately as an initial fixed-term (2 years) contract, with longer-term extension possible upon demonstrated proficiency.

MALDI-IMAGING research operator

To further improve the scope of sample analysis, CCP is introducing the MALDI Imagining mass spectrometry technology, to effectively advance the standard histology and add a new molecular dimension to the analysis. The selected MALDI-IMAGING researcher/operator will develop protocols and technologies to detect new proteins and other molecules, will develop microbiome analysis, and will provide services to the research infrastructure and research community. The successful applicant will closely collaborate with other specialist of the CCP team, especially with groups of histopathology, clinical biochemistry and metabolomics.

The successful applicant will have a strong background and skillset in mass spectrometry, especially in MALDI, should have a Ph.D. in the relevant field of science and have a good proficiency in English. The successful applicant will also have excellent interpersonal, communication and organizational skills and be highly-motivated with the ability to work independently and as part of the multi-disciplinary team of CCP.

This position is available in the Czech Centre of Phenogenomics (hosted by the Institute of Molecular Genetics of the ASCR, v.v.i.) in BIOCEV campus (CCP building) in Vestec near Prague, Czech Republic. The position is available immediately as an initial fixed-term (2 years) contract, with longer-term extension possible upon demonstrated proficiency.

Research position (postdoc) in ubiquitin ligases or molecular biology

This project will focus on deciphering the function of selected E3 ubiquitin ligases identified following screening transgenic mouse models. The new research projects aims to characterize the biological role of the selected Ub ligases *in vivo*, their mechanism of action with particular interest in the description of their roles primarily in cancer and inflammatory diseases.

We are looking for an individual with a strong background and skillset in biochemistry and molecular biology willing to work also with animal models. Experience working with mice or willingness to be so trained is essential. You should have a Ph.D. in biology or biochemistry and have a good proficiency in English; experience in protein biochemistry will be advantageous.

The successful applicant will have excellent interpersonal, communication and organizational skills and be highly-motivated with the ability to work independently and as part of a multi-disciplinary team.

This postdoctoral research scientist position is available in the Laboratory of Transgenic Models of Diseases at the Institute of Molecular Genetics of the ASCR, v.v.i. located in BIOCEV campus (CCP building) in Vestec near Prague, Czech Republic.The position is available immediately as an initial fixed-term (2 years) contract, with longer-term extension possible upon demonstrated proficiency.

For more information or to apply for any of these positions, contact Mr Libor Danek (ccp@phenogenomics.cz). All applications should be made in English, include a letter of interest and a structured CV.

Upcoming Events

CITIM 2017 – CANCER IMMUNOTHERAPY & IMMUNOMONITORING International Congress in Prague

24th – 27th April 2017 | Prague, Czech Republic

http://www.canceritim.org/index.html

Metabolic disorders and liver cancer

23rd – 26th April 2017 | ES–Palma de Mallorca

EMBO Workshop

http://meetings.embo.org/events/17-brown-fat

BIOCEV-CCP Workshop: Metabolism From Cells to Mouse

25th – 26th April 2017 | Vestec, Czech Republic

http://hpst.cz/registration-metabolomic-workshop

Advances in transgenic animal models and techniques

11th – 12th May 2017 | Nantes, France

http://www.trm.univ-nantes.fr/

2nd European Advanced School for Mouse Phenogenomics

12th – 16th June 2017 | Alsace, France

https://advanced-school.phenomin.eu/

9th Workshop on Innovative Mouse Models

15th – 16th June, 2017| Leiden, The Netherlands

http://research.nki.nl/immworkshop/

Mouse genome engineering

10th – 20th July 2017 | Dresden, Germany

http://meetings.embo.org/event/17-mouse-genome

Single Cell Europe 2017

18th – 22nd September 2017 | Vestec, Czech Republic

http://www.singlecell2018.eu/

Journal Club

Marie-Christine Birling *et al*. Efficient and rapid generation of large genomic variants in rats and mice using CRISMERE. *Sci Rep*. Mar 7;7:43331. doi: 10.1038/srep43331 (2017).

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