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PHENOGENOMICS
NEWSLETTER

Czech Centre for Phenogenomics

The Czech Centre for Phenogenomics is hosted by the Institute for Molecular Genetics AVCR v.v.i.

IMG

PRE KLINIKY

Centre for Preclinical Testing

New services on preclinical testing

After obtaining the certificate of Good Laboratory Practice (GLP) in January 2017, the Centre for Preclinical Testing (CPT) commenced activities with the support of the Czech Academy of Science (CAS). The primary mission of the CPT is to perform preclinical testing of substances that have successfully passed through basic research, and thus to contribute towards the development of new pharmaceuticals to combat life-threatening diseases, including those currently difficult to cure.
The Institute of Physiology CAS performs a coordination role for the CPT, and testing is also performed by the Institute of Molecular Genetics CAS including National infrastructure CCP, the Institute of Animal Physiology and Genetics CAS, and the Institute of Biotechnology CAS
The CPT offers a broad portfolio of tests under Good Laboratory Practice (GLP).

Core services:

- **Toxicity studies**, including toxicokinetic studies of promising chemical or biological agents on model animals – rodents and non-rodents (test systems: mouse, nude mouse, rat, guinea pig, rabbit, minipig) in compliance with ICH and OECD guidelines.
- **Bioanalytical, hematological and biochemical testing** of samples taken from animals during toxicity studies (determination of active substance in plasma or other biological matrices.
- **Development and validation of bioanalytical methods** for various test systems and biological matrices**.**
- **Determination of metabolites** in tissues and biological matrices (blood, plasma, urine)
- **Histopathological evaluation** of tissues from animals used in toxicity studies
- **Pharmacological studies on xenografts (nude mice)** with various cancerous cell lines, including Patient Derived Xenografts (PDX)
- **Cardiology diagnostic tests on animal models** – electrocardiogram (ECG), blood pressure measurement, cardiac imaging (Echo)
- **Synthesis, characterization and certification** of chemical substances with therapeutic potential, development of formulations for drug application

CPT facilities working under GLP regulations have established quality system, which is regularly inspected internally by Quality assurance unit and by National Authority and by our customers. The quality of our services is top priority for all our team members

The CPT offers its services to customers from both the academic and commercial sectors. Testing is carried out by recognised experts with long-term experience. The great advantages are the coordinated approach, flexibility, and the comprehensive nature of the offered services. You can find out more about the scope of CPT activities on the website: www.prekliniky.cz.

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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.

Message from the director

Dear Readers,

This issue of our 'Phenogenoms newsletter' brings you information about new technologies, workshops that we are offering as well as job opportunities at CCP.

We are organizing two workshops that will take place in autumn 2018. The first workshop 'Mouse versus Human comparative morphology' will be led by Jesús Ruberte París, the author of the book 'Morphological Mouse Phenotyping: Anatomy, Histology and Imaging' and will provide an introduction to comparative morphology, practical histology and imaging to participants. The second workshop is entitled 'Programming in R: Basics and Graphs', which is dedicated to scientists who wish to learn the basics of data manipulation and visualization using the free, powerful programming language R.

This newsletter will present two new technologies that are now establishing to provide a more comprehensive service portfolio; both technologies will advance certain types of imaging, although the technology base is completely different. Imaging has become a very important aspect of modern biology, as often images speak a thousand words.

The first instrument that enhances our imaging options is a new micro-CT scanner, which demonstrates the highest resolution scanning (3 µm Voxel size) and we can, for instance, reconstitute whole embryos with superb detail. The increased resolution comes at a cost though as the X-ray source has to be much harsher, and doesn't allow the imaging of live animals. The scanned volume is also smaller, which means that whole adult mice can't be scanned at this resolution, however organs or embryos can be scanned in outstanding quality and resolution.

The second device, a MALDI imager, instead uses mass spectrometry and will provide analytical data that cannot be obtained by any other technology. This device can scan sections of tissue samples and acquire MS spectra at a resolution of 4 µm, allowing MS imaging at a cellular scale. This opens up a whole new range of possibilities as one can determine the distribution of proteins, metabolites and drug products at a cellular level, even when no specific labelling method is available.

I also invite you to visit the career page, which announces several open positions at CCP, upcoming events and our recommended journal club pages.

Radislav Sedlacek

Phenogenomics Newsletter sees 2 years of publications

Nicole Chambers

In December 2015 the Czech Centre for Phenogenomics (CCP) launched its quarterly newsletter PHENOGENOMICS NEWSLETTER (PGN). With an aim to showcase the state of the art facility established at CCP, the newsletter features overviews of the services available to individual researchers including; archiving of transgenic lines and housing and monitoring of animals in digitally ventilated cages. Various units from the phenotyping module regularly showcase technical developments including InteliCages in the behaviour unit, Q-TOF LC-MS in the Metabolomics unit, ZEISS Axio Scan.Z1 slide scanner for both bright-field and fluorescence imaging in the histopathology unit and many more.

The newsletter also provides a valuable platform to advertise and report various courses and free calls to both the national and international scientific community. Calls such as 'Free histopathology services' and 'Generation of transgenic model' saw both national and international applications. Courses (either hosted or co-hosted by CCP) advertised in PGN also generate applications.

In December 2015 PGN launched to 140 external readers and more than 500 internal readers. Thanks to individual web subscribers and the increased number of researchers using various facilities available at the centre the current number of subscribers stands at over 200 external readers and is continually growing.

Programming in R: Basics and Graphs

This short intensive course is intended for scientists who wish to learn the basics of data manipulation and visualization using the free, powerful programming language R. The course is suitable for those who have no prior knowledge of programming and likewise for those with basic skills in programming. Those who wish to refresh their skills are welcome.

Venue

- **Time**: 20 - 21 September 2018
- **Location**: Czech Centre for Phenogenomics, Průmyslová 595, Vestec
- **Price**: 1200 CZK including refreshments during breaks. Participants should bring their own laptop and are encourage to also bring their own data to be plotted.

Course Outline

Day 1

- R studio: installing, packages, library, R environment set up
- Read data into R
- Base R: different data type, subsetting data
- Descriptive statistics
- Loops, if and else statements
- Package Tidyverse

Day 2

- R Markdown
- Package Ggplot2: all different graphs including export of graphs for publications
- Creating heatmaps
- Package Plotly: interactive graphs
- Time for your data

Applications

- A short motivation letter should be sent on Vendula.Novosadova@img.cas.cz before 14th July. Participants will be chosen according their motivation letter.

MALDI-MSI: A SHORT HISTORICAL AND TECHNICAL EXCURSION

Lukas Kucera

In this article, an historic overview of the development of mass spectrometry is presented, from the early days of the technique to the state of the art MALDI imaging.

A little more than a century ago, Wilhelm Wien observed that positively charged particles have curved trajectories in a strong magnetic field. More so, he was also able to use the trajectories to calculate their mass, and thus, mass spectrometry was born. The method was further refined by many other physicist especially after the second world war when the top physicist of that time where released from the development of nuclear bombs for the Manhattan project.

Until 1948, solely a magnetic field was used to manipulate particle trajectories in mass spectrometry. However, the use of a magnetic field had considerable drawbacks: the range of masses that could be analysed at one time was very narrow and there was quite low upper mass limit. This led Cameron, together with Eggers to optimize the technology in 1948 by replacing the magnetic field with an electric field, and they coined the new instruments Time-Of-Flight mass spectrometers (TOF-MS). This electric field accelerates the particles in a tube until the particles reach a detector at the end of the tube; because the acceleration is inversely proportional to the mass of the particle, it allows the mass of the particle to be determined by measuring the 'time of flight' of the particle in the tube. Within seven years of transitioning to TOF mass spectrometers, the newest generation of the instruments had reached resolutions powerful enough to distinguish distinct isotopes of one element.

Around the same time as Cameron and Eggers developed TOF-MS, another big step towards MALDI imaging was taken, when scientist discovered that molecules could be ionized and ejected from a surface by bombarding that surface with accelerated electrons or ionized atoms. The advantage of this Secondary Ion Mass Spectrometry (SIMS), as the method was called, is that the energy involved in such ionization is much smaller than by the other particle ionization methods, which makes the method suitable for the ionization of high mass bio-molecules without excessive fragmentation. Another big advantage is that with this method you can scan a surface and thus generate a mass spectrometry image. This was first done by Castaign and Slodzian in 1962, who wanted to investigate the consistency of the composition of metal alloys. So they scanned the surface an alloy sample and plotted the composition for each point they ionized, thus generating the first mass spectrometry image. It took another 8 years of development to be able to do that on biological samples.

The last important step towards the development of MALDI imaging was the discovery in the early 1980's that some compounds when mixed with a sample are capable to transfer the energy of a laser pulse to an analyte to ionize it without fragmenting or chemically altering the molecule of interest. These compounds are generally called matrices and the ionization method is called 'Matrix-Assisted Laser Desorption Ionization' or MALDI. In the early years of MALDI, the used matrices allowed for the ionization of small molecules only (to about 1000 dalton), but by the end of the 80's Tanaka and Fenn managed to refine the technique and develop matrices which

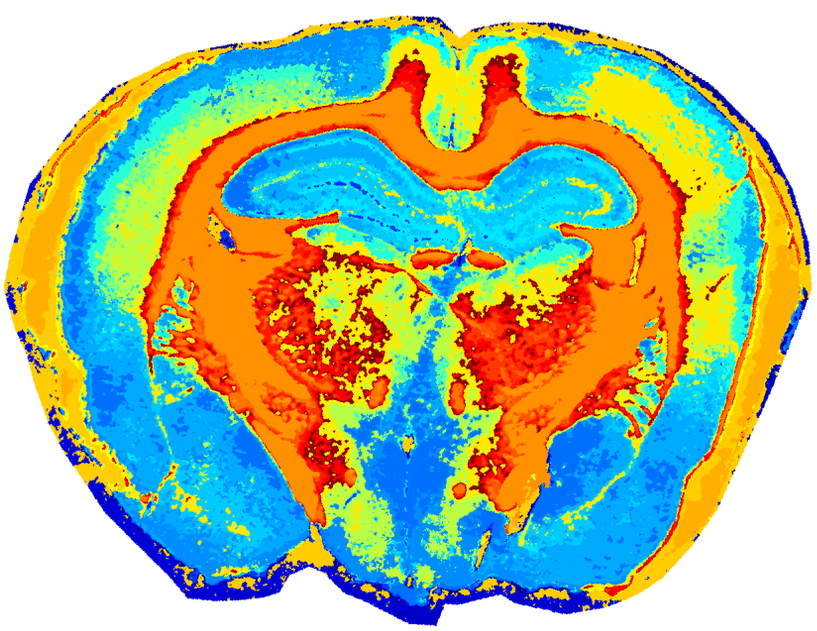


Figure 1: Segmentation MALDI image of a rat brain. The colours represent MALDI spectrum similarity, the more similar the colour the more similar the MALDI spectrum is.

allowed the ionization of large bio-molecules with molecular weight of up to 100000 daltons. They were awarded the Noble Price in Chemistry in 2002 for this achievement.

With the MALDI we now have all the pieces of the puzzle to get to MALDI imaging as we know it now. It was Caprioli who first put all these methods together in 1997 and published the first paper about MALDI imaging. But the MALDI-TOF instrument at that time were not very sensitive, had low resolution and a limited mass range that could be analyzed. Additionally the spatial resolution was poor and the results not very reproducible. It took another 20 years to further develop the sample preparation and instrumentation before the method could be considered reliable and robust.

Sample preparation appeared to be critical in achieving this robustness and reliability. Many new matrices were developed in the first decade of the 21st century and nowadays matrices can be chosen to suit specific analyte classes for measurement in both negative and positive mode. Also the application of the matrix to the sample needed to be optimized as large matrix crystals can lead to delocalization of the analyte during its extraction from the tissue to the matrix and thus lowering of the special resolution of the image. The finest crystal size is now achieved by automated sublimation of the matrix on the sample.

Also the mass spectrometers evolved a lot in the last 2 decades and they now achieve a 1000 times higher resolution compared to the early MALDI imaging machines and are able to detect analytes in femto-mole quantities. The speed of acquisition of MALDI imaging devices was also greatly increased thanks to lasers which can now achieve a 10 kHz fluency. With these modern machines imaging of a sample at sub-cellular resolution can be done in a few minutes or many images with lower resolution can be made in 1 day which makes MALDI imaging suitable for high throughput screening. An example of such images are shown in Figure 1 and Figure 2, which was captured at a 10 µm resolution.

From the beginning of mass spectrometry imaging, research has been focused on biological samples, from simple observations of tissue composition to the current search for disease biomarkers. With simultaneous progress in the computer hardware, software and statistical methods we are another step closer to personalized medicine. Some prove of concept experiment showed that MALDI imaging can be used to monitor drug penetrance to target tissue during the treatment for example. Other experiments showed that with the help of sophisticated algorithms it can also be used for the classification of type and stage of disease and to predict the response to therapy.

Currently, we are still far away from daily use in the clinic. But the method is evolving rapidly and could become a useful tool in clinical environments and not only in research field. Time will tell…

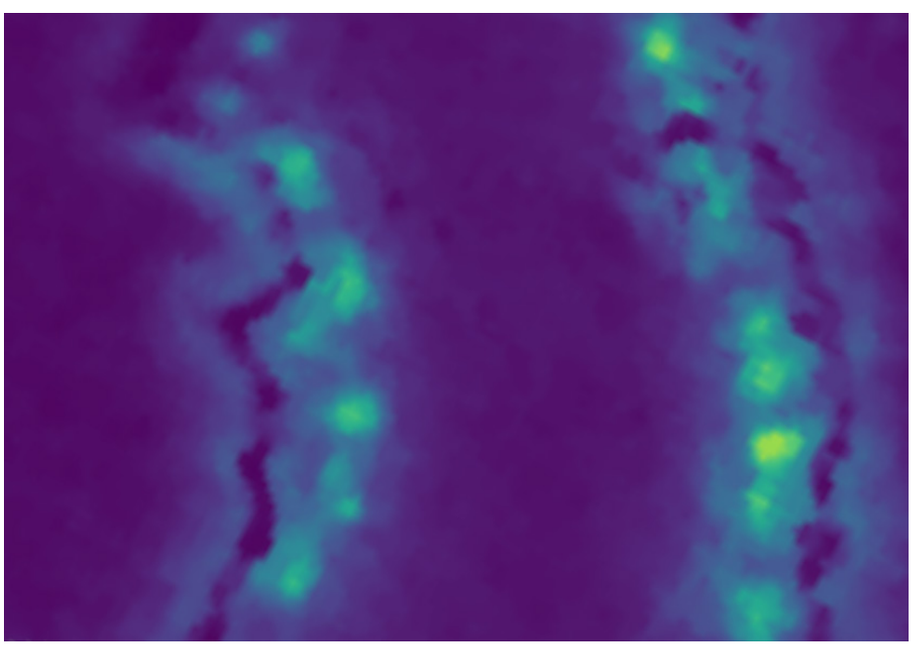


Figure 2: MALDI-TOF analysis of skin. The color represents the density of molecules with a m/z of 774 +/- 40 MDa.

Handling of samples for metabolomics: Freezing and thawing is an issue

Karel Chalupsky

Metabolomics is the study of metabolites in biological samples. A metabolome can be defined as a chemical fingerprint that represents the small compounds that are the result of all metabolic processes in the body at a specific time. The metabolome contains information about the metabolic state of the organism; it reflects changes in gene expression, translation, protein modification, and environmental influences. Additionally, in vertebrates, the metabolome contains products from the gastrointestinal system, a microbiome of bacteria, and thus the products that sometimes cannot be synthetized by the host organism. In this case, the production of such metabolites is completely independent of the genome of the organism in which it they live. Analysis of the metabolome can provide critical insights into all of the processes and interactions of the metabolic pathways within the body as a whole.

Since analysis of metabolome is very complex procedure and usually cannot be done in same day that the blood samples were collected, it is important to establish a system for sample preservation in order to continue analysis. To determine optimal conditions, we studied three different stages of sample handling (fresh, frozen, and refrozen), in order to demonstrate the change of results when using the same method of analysis. We have analyzed metabolome from six C57BL6N male mice on Agilent 6545 iFunnel Q-TOF using C18 Zorbax column with increasing gradient of 5 % methanol to 100% over 25 minutes with 50 mmol/l NH4F using both polarities. We have found that molecular features (mzRT pairs) are highly expressed among the mice in fresh and frozen samples, but completely lost (or in some cases present but with very low intensity values) among refrozen samples. Our data suggests that a plasma sample can withstand a single freezing event and storage at -80˚C at least for two weeks without changing or reducing the number of detected molecular features, which would allow the plasma leftover from the regular IMPC screen to be used for metabolomics. Furthermore, we identified that some features were lost in refrozen samples (Figure 1). Our next task will be to extend the storage time so that plasma from single animal can be separated into several aliquots and then analyzed on same column after different lengths of time stored at -80˚C.

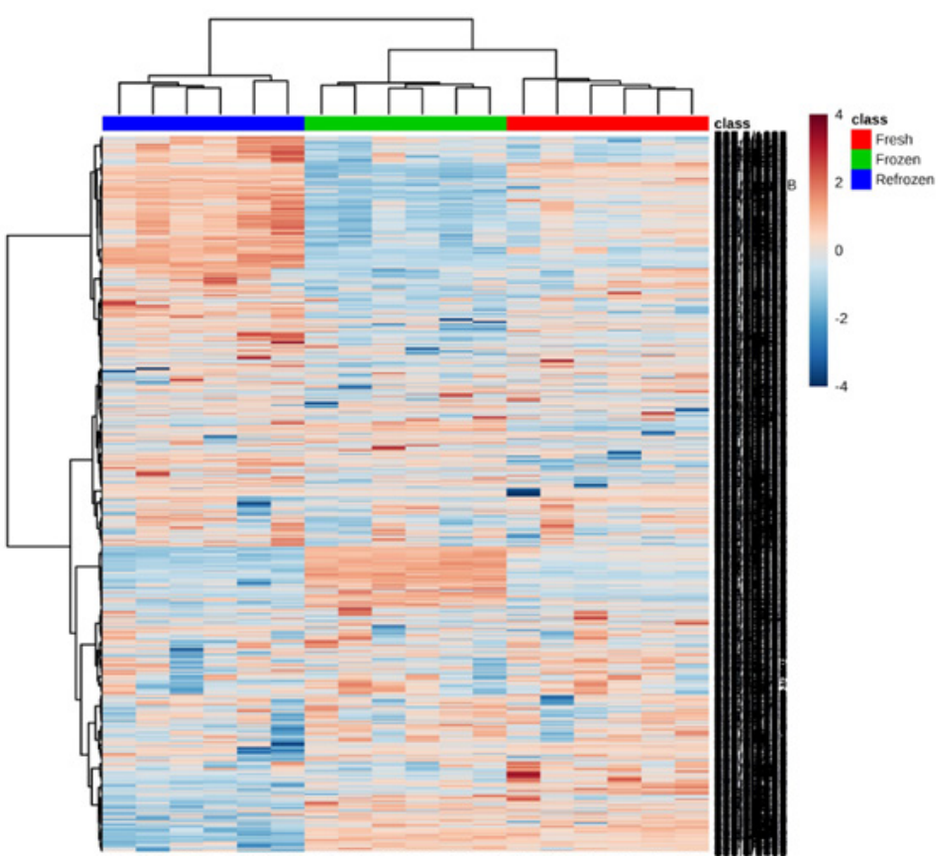
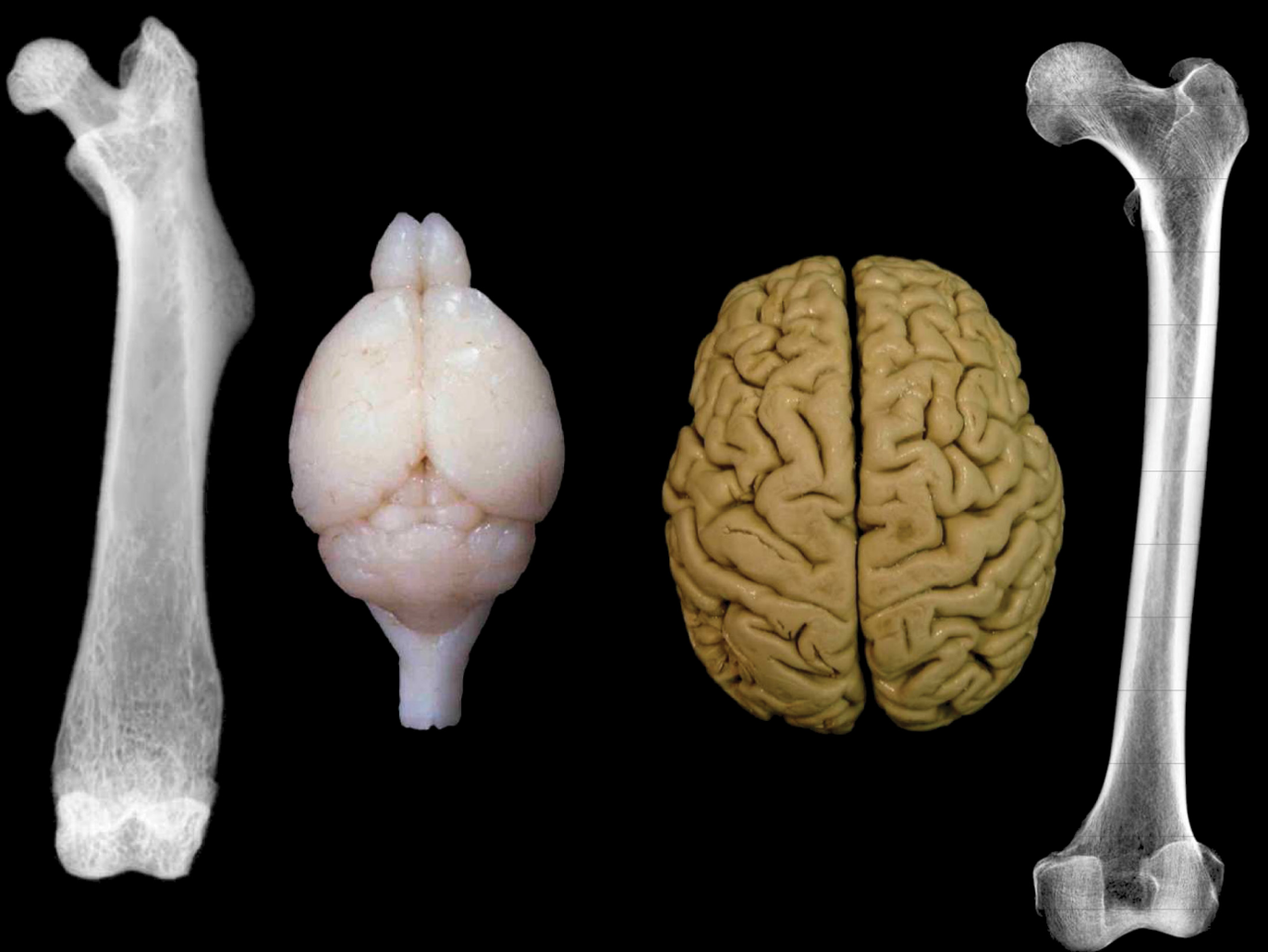


Figure 1: Heatmap showing the differences between fresh, frozen and refrozen samples

Course on Comparative morphology

3-7 September 2018
Czech Centre for Phenogenomics, Vestec

Mouse vs Human



With Prof Dr Jesus Ruberte
author of Morphological Mouse Phenotyping

UAB
Universitat Autònoma de Barcelona

Czech Centre for Phenogenomics

INFRAFRONTIER

Zygote electroporation (ZEN), a simple technique to generate transgenic mouse

Irena Jeníčková, Head of the ES-cell manipulation unit

Petr Kašpárek, Head of the targetting unit

The generation of transgenic mice relies on several essential techniques which require expensive equipment and highly skilled technical staff. Namely these techniques include isolation of embryos from donors, delivery of transgenic tools (nucleic acid, nuclease) via pronuclear microinjection (PNI) into the embryos, and transfer of the embryos to recipients. Primarily, a pronuclear microinjection needs to be accomplished on an adequately equipped micromanipulator, this takes time, as only a single embryo can be injected at a time. This is why there have always been attempts to develop other, more simple techniques (e.g. liposomal transfection, virus infections, electroporation, and sperm mediated gene transfer) for the delivery of the construct into the embryo. Nevertheless, until recently, only PNI remains as the reliable and widely used technique to deliver the construct into the embryo.

In addition, until the development of programmable nucleases for mouse transgenesis, only random integration of transgenes were possible via microinjection of whole plasmids (BACs). Now, with the new possibilities of programmable nucleases, we can perform targeted knock-outs and knock-ins. The injection mixtures have changed. There is no need for injection of long constructs (BACs, ˜ 120kb), the targeting construct is rather a mixture of smaller agents (RNA and DNA). The change in the targeting strategy has re-opened the door for other techniques to efficiently deliver the construct into developing embryos.

Zygote electroporation (ZEN)

Zygote electroporation (ZEN) is a new developing technique for the delivery of targeting constructs into the embryos, which is recently becoming very popular in transgenic facilities all over the world. Zygote electroporation does not require a micromanipulator, it is less invasive, and technically simple. A quantity of embryos can be electroporated at a time instead of individual microinjection. Moreover, a key advantage of ZEN seems to be in the fact that the development of embryos is much higher than after PNI. The technique was demonstrated for the first time by Grabarek et al., (2002; 1) who established basic parameters for efficient delivery of nucleic acids into isolated oocytes and zygote by electroporation. Later, ZEN was applied in other laboratories, and it was widely presented during the ISTT conference in Prague (March 2016). The application of ZEN was also part of the First Nuclease Workshop organised before the conference by Czech Centre for Phenogenomics (lecturer Prof. Haoyi Wang, Figure 1).

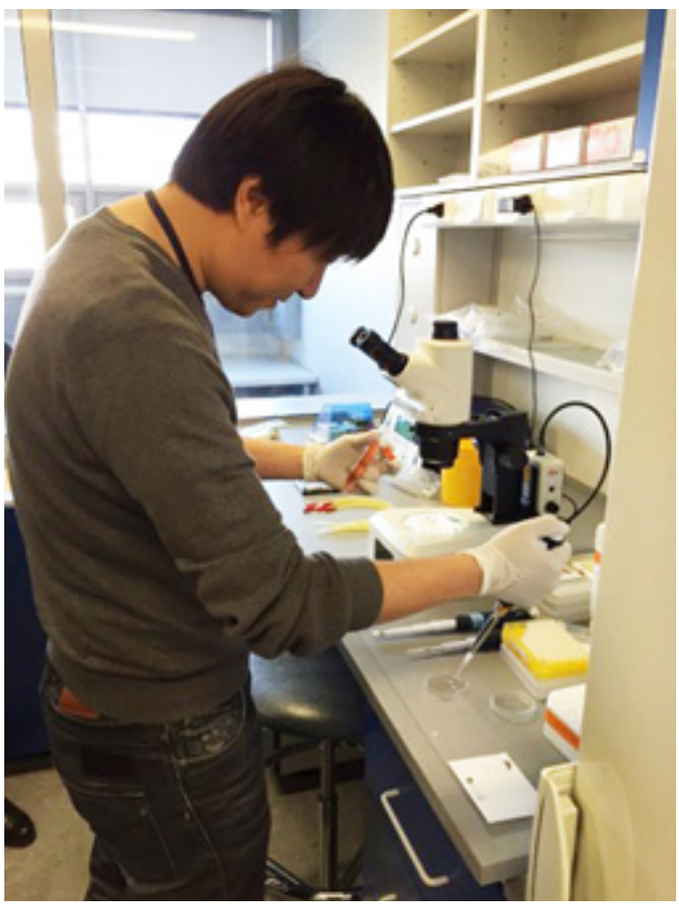


Figure 1: Prof. Haoyi Wang demonstrating the simplicity of the zygote electroporation (ZEN) during First Nuclease Workshop organised by Czech Centre for Phenogenomics

During electroporation, electrical pulses generate transient pores in the plasma membrane through which the construct can enter the cell. In contrast to somatic cells or bacteria, the mammalian embryo is surrounded with a protein layer called zona pellucida (ZP). The targeting construct must therefore penetrate not only the plasma membrane of the embryo, but also the zona pellucida. To facilitate the delivery of the construct via ZP, the embryos are treated shortly with Tyrode´s Acidic Solution. The solution weakens the zona pellucida and allows enhanced uptake of exogenous DNA into the embryo. Complete removal of ZP is detrimental for embryonic development. However, with the optimisation of the ZEN, some laboratories have shown that the construct can also be successfully delivered without any treatment of ZP (2).

The embryos are electroporated in a small amount

of medium (10-20µl), usually in the mixture of Opti-MEM media and targeting constructs. About 20-50 zygotes can be electroporated at once in the cuvette. The pulses are lower in comparison to somatic cells or bacteria (30-50V), but more pulses are applied with a short break (2-9 pulses, 100ms interval). The application of more pulses leads to higher DNA uptake, but it is followed by lower embryonic development. The setting used in our Transgenic Unit is typically 30V, 2 pulses, 3ms duration with a 100ms interval (3).

Zygote electroporation requires a higher concentration of construct than what is common for PNI. Typically used concentrations are 600 ng/µL for Cas9 mRNA, 300ng/µL for sgRNA, and 1000 ng/µL of oligo DNA. Besides, the Cas9 can be delivered as a protein (8-16µM RNPs), meaning there is no need to electroporate RNA. The Cas9 protein is now commercially available from commercial suppliers.

The first successful electroporation was performed on BTX or CUY21/BEX machines. These machines enable usage of a special platinum chambers. Nevertheless, successful zygote electroporation can be accomplished with any electroporation machine which has square wave pulses with the option of low voltage using the classical 0.1-cm electroporation cuvettes. For example, the Gene Pulser XCell electroporator (BioRad) is used in our Transgenic Unit.

The efficiency of ZEN is high (sometimes even as many as 80% of embryos contain the mutation). It is no problem to generate indels or deletions up to 2.2kb with electroporation (4). Regarding insertion, the LoxP sites and transgene of about 200 bp were successfully integrated (4, 5).

Zygote electroporation is not solely limited for the laboratory mouse. It has been successfully used in many other species including rats (2, 6). What is more, we consider that it can be also applied in those mouse strains which are more sensitive to manipulations and the survival rate after PNI is low.

The whole technique can be further optimised with the right timing of electroporation. For example, Hashimoto et al. (2016) electroporated IVF zygotes, shortly after fertilisation, to mediate targeting before the first replication occurs. This lead to more balanced targeting and the elimination of the mosaicism (7). Moreover, conditional knock-out mice were produced with sequential electroporation (8).

ZEN in Transgenic Unit

In our Transgenic Unit we have first compared the efficiency and developmental rate after PNI and ZEN. In accordance with other researchers, we have seen much higher and more stable development after ZEN then after PNI. The ratio of mutated embryos from those which developed up to blastocyst (indels/exdels) was higher for PNI; however, when we calculated the total number of embryos surviving the procedures, efficiency of ZEN and PNI is comparable or even higher (Figure 2). Additionally, we have checked the different concentrations of constructs and usage of protein vs. mRNA for Cas9. The efficiency increased with higher concentrations of sgRNAs and Cas9 mRNA and above all, the highest efficiency was reached with the ZEN using Cas9 protein (Figure 3).

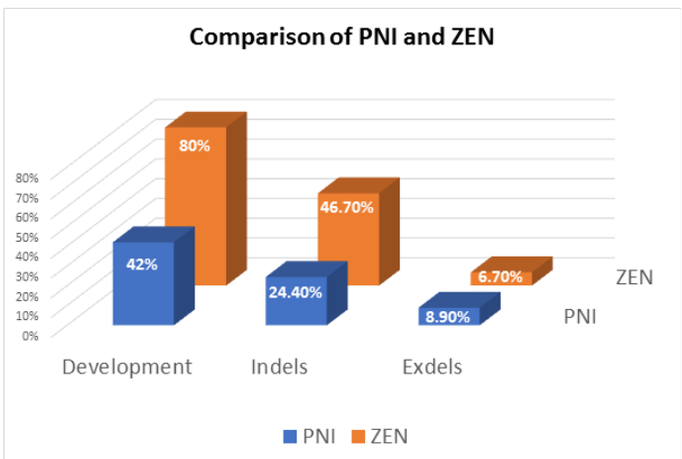


Figure 2: Comparison of development and efficiency of PNI and ZEN. There is much better development of embryos after ZEN than PNI (80 % vs. 42 %). The efficiency of mutations (indels/exdels) in ZEN is comparable (exdels) with PNI or higher (indels). % of indels/exdels was counted from the total no. of cultivated embryos after PNI and ZEN, respectively. For both, PNI and ZEN, protein for Cas9 (IDT) and synthetic RNA (syntG, IDT) were used. PNI: 100ng/ ul Cas9 protein, 100ng/ ul crRNA+trRNA; ZEN: 110ng/ ul Cas9 protein, 200ng/ ul crRNA+trRNA.

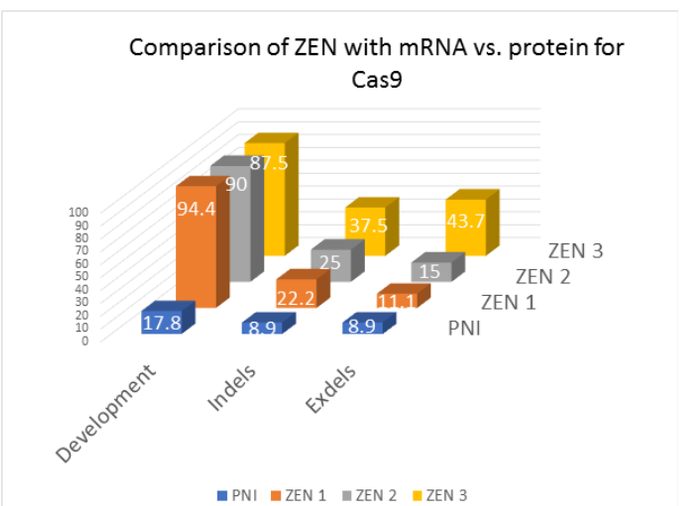


Figure 3: Efficiency of ZEN under various conditions of the construct mixture. ZEN 1: 4 x 25ng/µl sgRNA, 100ng/µl mRNA for Cas9; ZEN 2: 4x 100ng/ µl sgRNA, 100ng/ µl mRNA for Cas9; ZEN 3: 4x100ng/ µl sgRNA; 400ng/ µl Cas9 protein. The efficiency of mutation in ZEN can be improved with higher concentration of the construct (ZEN 1 and ZEN 2). The ZEN with protein for Cas9 had higher efficiency then ZEN with mRNA (ZEN 2 and ZEN 3). Control PNI: 4 x 25ng/µl sgRNA, 100ng/µl mRNA for Cas9

From our experience with ZEN, we appreciate mainly the simplicity and high efficiency of the technique. What is more, selecting zygotes with expanded pronuclei is not required for ZEN, and we can use all the isolated embryos and cultivate them in an incubator. On the following day, only the embryos that have successfully developed into the 2-cell stage are transferred into pseudo-pregnant recipients (Figure 4). This minor procedure modification enables us to select only strong and viable embryos for transfer and thus minimises loss of embryos and unnecessary work.

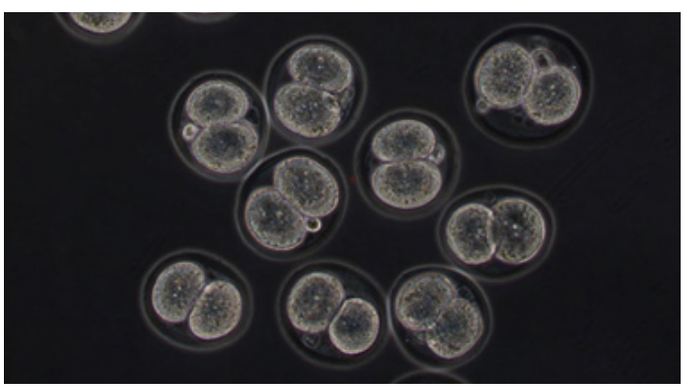


Figure 4: Well-looking 2-cell stage embryos developed after zygote electroporation are transferred into recipients.

Prospects of PNI and ZEN

It could seem that, with the development of zygote electroporation, the older techniques such as pronuclei microinjection, are not needed any more. However, it would be a rash and wrong decision to eliminate all the micromanipulators. A more complicated targeting using longer constructs still need to be carried out via direct injection into the pronucleus. It might be that the electroporation does not immediately transfer construct into the zygote nucleus and therefore the chance of DNA integration in the genome is low. The longest successful target integration with electroporation so far is still quite small (up to 200 bp), in comparison to PNI where even a 7.1 kb long transgene was integrated using the CRISPRs (9).

Above the scope of ZEN, electroporation has much wider potential. Using CUY21/BEX Co.,Ltd.,Tokyo, Japan as an example, the zygotes were successfully targeted in-vivo. The targeting construct was first injected into the oviduct containing the embryos and then the special electrode mediated electroporation of the construct into the embryos directly in the oviduct (a technique called GONAD). Upon completion, the donor mouse gave birth to the transgenic animals (10, 11). This technique eliminated many steps of the complicated procedure, in particular the isolation of the embryos and embryo transfers.

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Featured Service

In Phenotype Analysis: 'Seeing is Believing'

Jan Prochazka

Head of the phenotyping module

The Bioimaging unit is an integral parts of systematic phenotyping pipelines at CCP. It is designed and developed to provide comprehensive image data to support results from all other phenotyping units, and carry out individual imaging tasks requested by the research community either locally from Czech Republic or abroad. To aid the contribution to phenotype analysis and solving of individual PI requests, the Bioimaging unit also has its own technology development programme with a focus on developmental phenotype analysis, cancer models and metabolism research applications. Our technology platform is based on in-vivo imaging systems such as Bruker Xtreme and SkyScan 1176 for microCT scanning, however we can also provide imaging support for embryology phenotyping pipelines, which are based on ex-vivo microCT imaging, tissue clearing procedures and macroscopic analysis of embryos. One very important aspect of our imaging workflow is the image accent analysis procedure, where every member of Bioimaging has access to PC stations equipped with full software packages for microCT data analysis, optical in-vivo imaging and other 3D data sets including Imaris. This has been continuously expanding with our collaboration with the Bioinformatics unit.

In-vivo microCT for comprehensive phenotype analysis

MicroCT technology provides significant advantages in the analysis of skeleton morphology, bone structure and body composition phenotypes. Our imaging pipeline allows the generation of all data required in one scanning event with a spatial resolution of 35µm voxel size, with semi-automated software allowing further data processing.

Firstly, the entire skeleton is separated for 3D morphological evaluation, this approach can reveal changes in bone morphology in much higher detail then standard 2D x-ray projections (Figure 1).

The same data set is then used for segmentation analysis of body composition, based on differential x-ray absorption of lean and fat tissues. Using 3D matrices for body composition gives the advantage of easily accessing detail of the localisation of adipose tissue within the body, This provides more biologically relevant information such as comparing subcutaneous and visceral deposition of adipose tissue (Figure 2).

In addition to this, we are also able to obtain data from

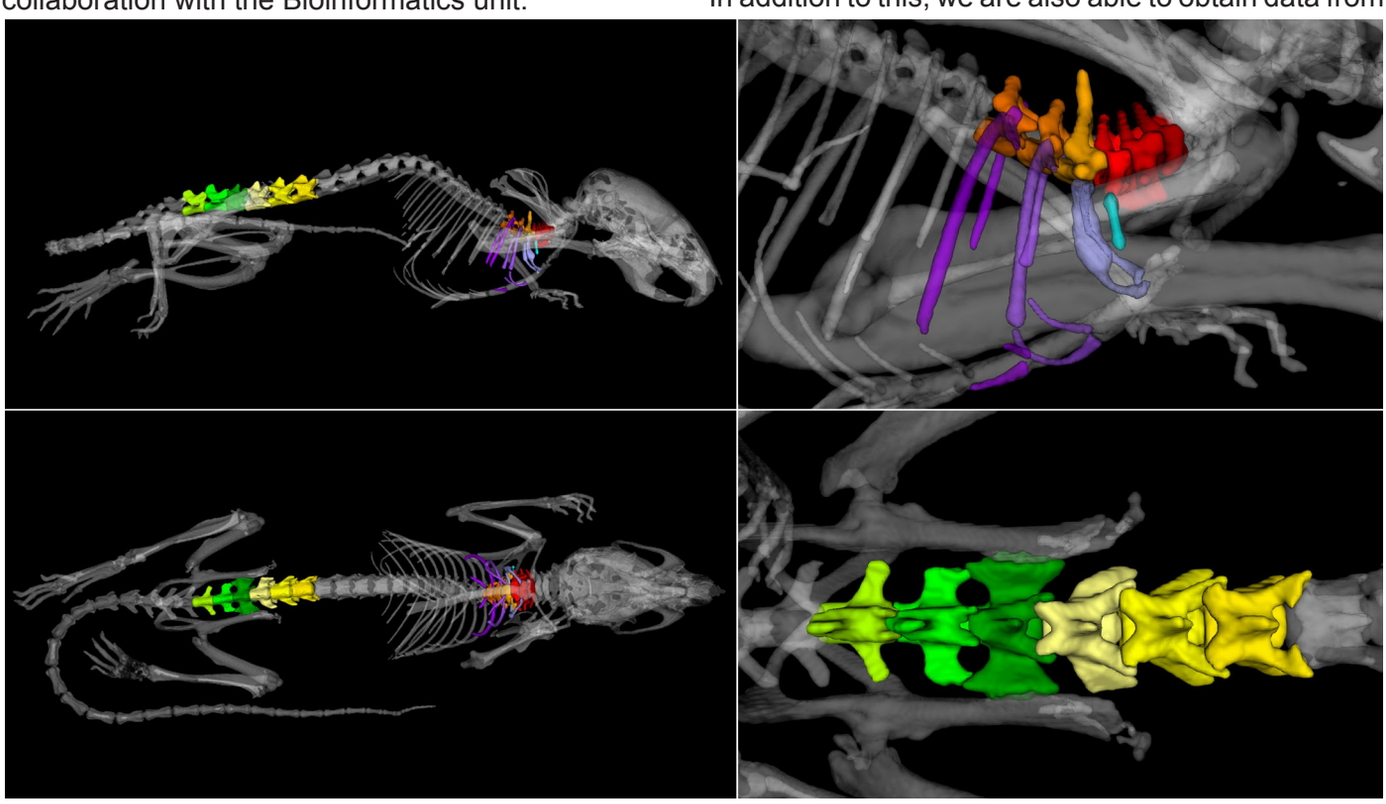


Figure 1: Detailed analysis of the mouse skeleteon, anomalies are highlighted by pseudo-colours.

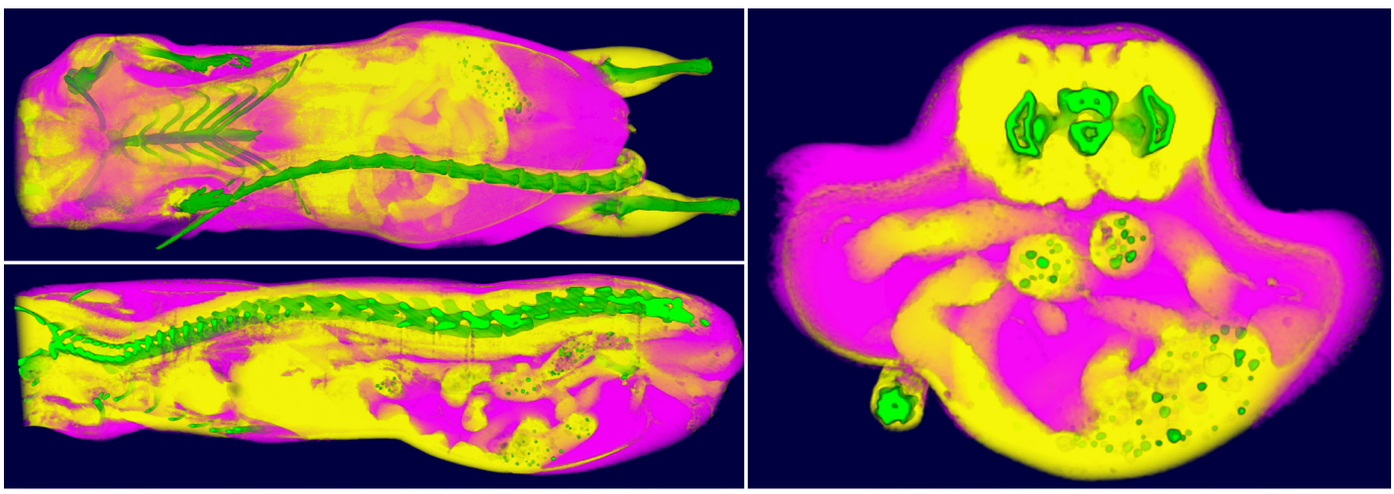


Figure 2: Body composition analysis: skeleton: green, lean body mass: pink, fat: yellow

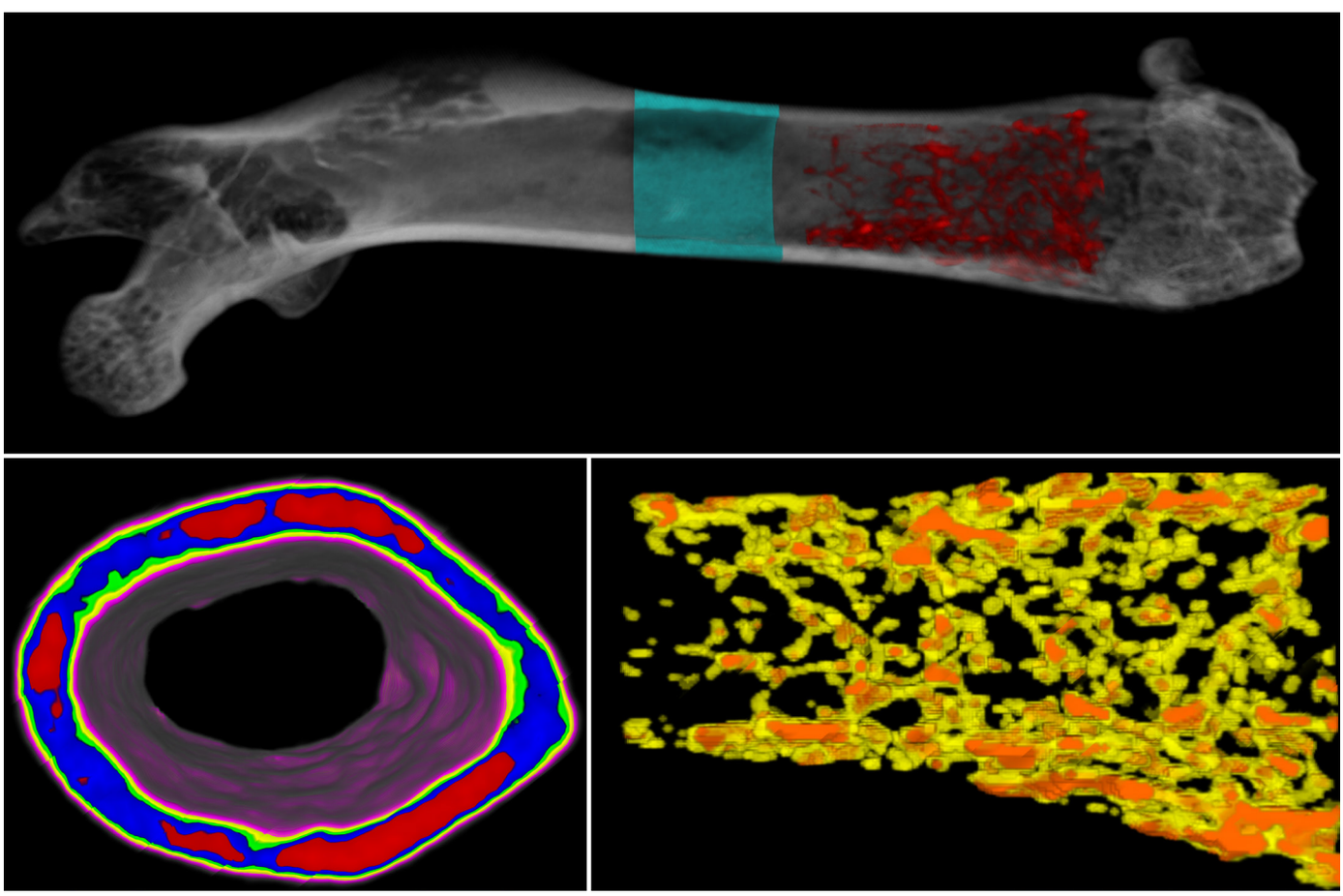


Figure 3: Analysis of mineralised tissue. Top image: virtual section through X-ray μCT of mouse femur with 2 highlighted areas for bone analysis: cortical bone in blue, and trabecular bone in red. Bottom Left image: virtual section through cortical bone of mouse femur. Pseudo-colours correspond to levels of signal from X-ray μCT, and thus to bone mineralization. Colors are ordered from the least dense to most dense from yellow > green > blue > red. Bottom Right image: Segmentation of trabecular bone from mouse distal femur X-ray μCT. Two levels of mineralization are overlapping: low (yellow) and high (orange).

mineralised tissues by alteration of x-ray absorption in bones and teeth. Mineralisation defects can be identified rapidly and scanning resolution is sufficient for the supplementary evaluation of individual bone structures to better pin-point mineralisation defects in bones (e.g. trabecular, cortical or general mineralisation problems, Figure 3).

The sensitivity of body composition analysis was cross-correlated with a metabolic screen under high

fat diet challenge conditions. A pilot experiment provided very promising results, stressing the importance of evaluating the distribution of adipose tissue that microCT based body composition analysis allows. In summary, in-vivo microCT scans provide a valuable source of data for morphology and body composition phenotype analysis, and allows many more detailed additional tests from an original 3D matrix without the need to repeat the scanning of cohorts.

Imaging of soft tissue

Contrary to mineralised tissues, the imaging of soft tissue by microCT is more demanding and needs the use of special contrast agents. Contrast agents are usually based on salts of heavy metals and need sufficient time to fully penetrate tissues. The potential of contrast agents was unveiled not long ago, but since then several have been discovered. As standard in Bioimaging unit we use three contrast protocols for their ability to enhance x-ray absorption in soft tissues of several organs. Lugol's solution is able to increase visibility of glycogen-rich cells and for example can be confidently used for detailed

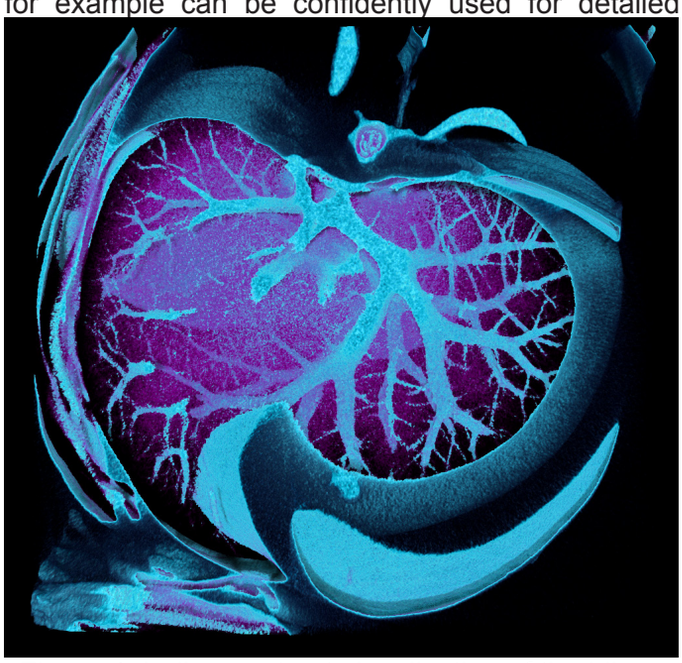


Figure 4: Iodine contrast visualisation of the bile-ducts

imaging of liver structure. PTA (phosphotungstic acid) based contrast protocol can be used for visualisation of connective tissue, and thus increasing contrast in collagen rich tissues such as blood vessels. Finally PMA (phosphomolybdenic acid) shares similar properties as PTA, however it also has an affinity for lipids and supplies a slightly different absorption pattern. But other contrast agents can also be used upon request, like iodine, which can be used for the visualization of the bile-ducts in the liver (Figure 4).

Imaging of cancer models

In vivo imaging provides unique opportunities to study and experimentally assess the behaviour of cancer cells in the mouse model by non-invasive and truly quantitative ways. In our unit we have established protocols for inoculation and further imaging experiments with multiple cancer model cell lines with either bioluminescence or fluorescence reporters. The big advantage of whole body imaging in cancer models is the detection of metastatic growth, which could be released from primary tumours, and therefore their invasive behavior can by directly quantified (Figure 5).

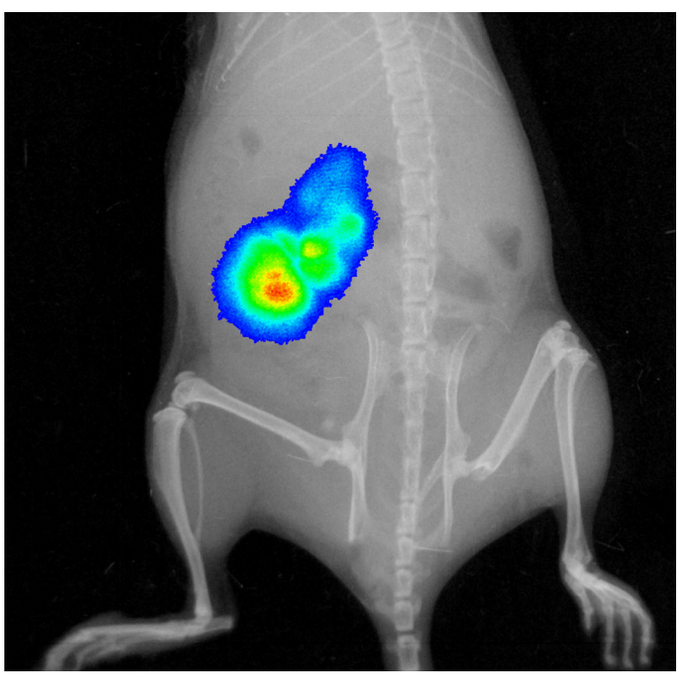
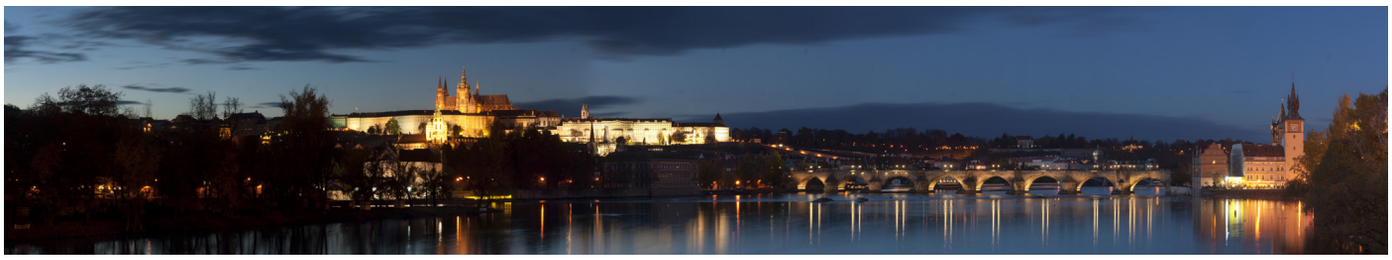


Figure 5: Imaging of tumor development, colour scale shows tumor density

Development of Imaging unit in 2018

The Bioimaging unit is following the current state of development in the area of macroscale imaging in-vivo and ex-vivo and keep all our users updated in order to provide the best solution for their projects. To achieve this we invested in the purchase of new a microCT device for ex-vivo scanning with spatial resolution from 0,3um. This imaging modality will be launched in mid-March. Also, the imaging of soft tissues and embryonic tissues in 3D is under rapid development, as we are preparing tissue clearing pipeline base on clarity technology with an increased high resolution 3D imaging on our macroscope. A significant part of technological development is carried by the Bioinformatics unit to provide high-throughput analysis of 3D tomographic data in the area of body composition analysis.

With the increasing number of imaging modalities and data processing throughput, we aim to host more complex imaging projects and look forward to accepting new challenges this year.



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 visit http://www.phenogenomics.cz/jobs/ for application details.

Pathologist (rodent pathology)

To advance and further improve services of our histopathology lab we are seeking experienced pathologist who will be responsible for analyses and descriptions of mouse and rat tissue samples, especially:

- to provide expertise in the pathology of genetically-engineered mouse (GEM) and rat models
- to provide full pathology analysis including complete gross- and histopathological evaluation supplied with image-based report, digital images, and recommendations
- to perform phenotype investigation and characterization together with histology-lab managing scientist; this includes necropsy, macroimaging, tissue sample collection, supervision of histological processing, histopathological evaluation, digital photomicrographs, and consultations.
- to follow and implement GLP rules and manage work of lab technicians
- to drive his/her own research projects and actively participate within the other projects of the centre.

Successful applicant should have DVM or MD (or equivalent advanced degree in relevant field) and relevant research and/or hands-on experience. Capability to work in English speaking environment is a must, previous experience with SOPs for GLP is an asset.

Head of Embryonic stem cell manipulation and transgenesis

The Transgenic and Archiving Module of the CCP (www.phenogenomics.cz) is seeking a motivated post-doc or experienced senior specialist to join our international team and lead the subunit of embryonic stem cell manipulation and transgenesis.

Your key responsibilities are:

- organization and supervision of our ESC laboratory
- ESC manipulation and culture, back-up freezing
- optimizing and improving the use of ESC for genome targeting by classical techniques and programmable nucleases (CRISPR/Cas)
- organization and supervision for validation of generated ESC clones (PCR, Southern blotting, karyotyping)
- assistance in general lab management, administrative tasks including communication with users and overviewing work of technical staff
- support the team in rodent handling and operation (embryo transfer into foster mice and rats)

You should possess a PhD in biology, molecular biology, biotechnology, or related subject and must have experience in cell culture (mouse ESC culture is an advantage), general laboratory work, and organization. Qualified experienced person without PhD can be accepted as well. Experience in rodent handling as well as knowledge in rodent genetics and transgenic technologies would be advantageous. Candidates must be fluent in English, have good interpersonal communication skills, and be able to work independently as well as part of a team. If you are an organized and responsible person, have a keen perception and like to establish new techniques in the field of rodent transgenesis, you are welcome to apply for this job.

The position is available immediately as an initial fixed-term contract (negotiable up to three years) with longer-term extension possibility 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.

Careers

Senior Bioinformatician/Data Analyst

For the exciting work in analysis of large complex data sets with the bioinformatics team of CCP. At the position you will be responsible for:

- Analysis of metabolomics and proteomics data (using different clustering and machine learning methods)
- Developing of methods for image analysis (using deep learning)
- Conducting individual research projects to increase the knowledge base of CCP

The best candidate should:

- be creative and able to find unusual new solutions
- be willing to learn new things and to pass on the knowledge to others
- have very good knowledge of deep learning methods
- have provable experience with image analysis and segmentation
- know at least one programming language (Java, R, Python, C ++)
- be fluent in English (written and spoken)
- have good communication skills
- be capable of working in team and also on a self-contained basis
- possess PhD in bioinformatics, statistics, mathematics or related subject and/or have relevant track record in the field. Basic knowledge of cluster computing or previous experience within biology or biomedicine field is a plus.

The position is available immediately as an initial fixed-term contract (one year) with longer-term extension possibility 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

Mouse vs Human Comparative Morphology

Essentials for accurate interpretation of Precision Medicine models

3rd - 7th September 2018 | Vestec, Czech Republic

Programming in R: Basics and Graphs

Basic data handling and visulaization in R

20th-21st September 2018 | Vestec, Czech Republic

Infrafrontier Stakeholders meeting

3rd-4th December 2018 | Munich Germany

Epigenetics and Epigenetic Inheritance Symposium

5th December 2018 | Munich, Germany

Journal Club

1. Gregorova, S. et al. Modulation of Prdm9-controlled meiotic chromosome asynapsis overrides hybrid sterility in mice. eLife 2018;7:e34282 (2018)
2. Yao, X. et al. Tild-CRISPR Allows for Efficient and Precise Gene Knockin in Mouse and Human Cells. Develop. Cell Volume 45, Issue 4, p526–536.e5 (2018)
3. Zeraati, M. et al. I-motif DNA structures are formed in the nuclei of human cells. Nature Chemistry 10: 631–637 (2018)
4. Charpentier, M et al. CtIP fusion to Cas9 enhances transgene integration by homology-dependent repair. Nature Com. 9: 1133 (2018)
5. Ohtsuka, M. et al. i-GONAD: a robust method for in situ germline genome engineering using CRISPR nucleases. Gen. Bio. 19:25 (2018)
6. Miura, H. et al. Easi-CRISPR for creating knock-in and conditional knockout mouse models using long ssDNA donors. Nature Protocols 13: 195–215 (2018)

Czech Centre for Phenogenomics

Delivering and Characterizing Research Models

IMG

The Czech Centre for Phenogenomics is hosted by the Institute of Molecular Genetics AVCR v.v.i.