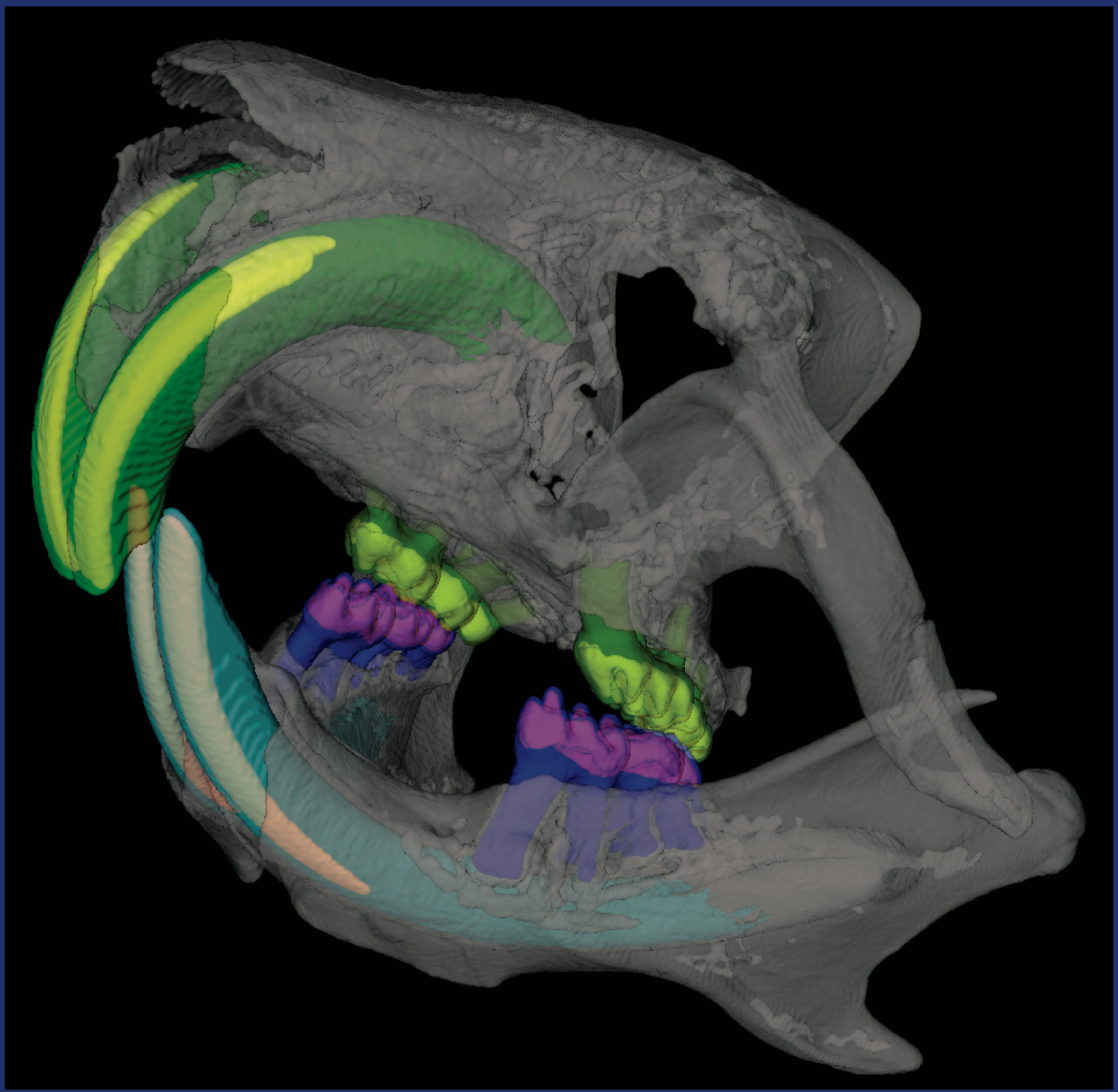
Volume 2, Issue 2

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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Cover Image:
Skull of a mouse: 3D segmentation of microCT data (SkyScan 1176, Bruker) with colorized teeth and tooth enamel (ITK-Snap, Yushkevich et al.)

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.

The Czech Centre is hosted by the Institute of Molecular Genetics of the ASCR, v. v. i.

Czech Centre for Phenogenomics

Overview of our services

Transgenic and Archiving Module

Model generation and transgenesis
Reanimation and rederivation
Archiving

Animal Facility Module

SPF breeding
Import and export of animals
Contract breeding

Phenotyping Module

Comprehensive standardized screens
Histopathology
Embryology
Biochemistry and hematology
Bioimaging
Neurobiology and behaviour
Immunology
Metabolism
Cardiovascular function
Lung function
Vision
Hearing

For further information please visit: www.phenogenomics.cz

Message from Director

Dear Readers,

It is my pleasure to send you the latest issue of "Phenogenomics newsletter". Inside you will find reports and news about CCP's activities and also articles about supported research and newly established technologies.

The news section features reports from the INFRAFRONTIER project meeting, IMPC annual meeting, BIOCEV annual conference, and a report from our second '*Programmable nuclease course*' at CCP. This hand-on course profited very much from excellent speakers-scientists who are not only at the forefront of genome editing who also move it. Among all I would like to mention here Bernd Zetsche from Broad Institute who gave updating lecture about Cpf1 nucleases and show participants how to design and employ this nuclease tool, Francis Stewart who provided the update to homologous recombination and Lluis Montoliu who gave us information about historical and current developments in the field.

In this issue, we are also describing three services, genotyping, auditory and metabolomics. Due to the increasing usage of CRISPR technology, we had to stress the importance of genotyping for successful management of experimental animal colonies and especially for quality control of the targeting. Specially featured are articles about the auditory screen laboratory that is focused on testing of fundamental hearing functions in mice and the newly established metabolomics platform whose powerful analyses will support our customers.

Last but not least, this issue also reports about a research project supported by CCP scientists, which together with scientist from Microbiology institute in Prague discovered how tooth enamel, the hardest and most resistant tissue in our body, is created. The results, published in Proc. Natl. Acad. Sci. U S A (2017; T. Wald, at al.), detail how the most abundant proteins in enamel protein matrix, amelogenin and ameloblastin are interacting via three tyrosil motif (Y/F-x-x-Y/L/F-x-Y/F). This motif was identified during this study and CCP generated a mouse mutant which showed that this is critical for the self-assembly capacity in process of formation protein multimers.

As CCP continues to develop and expand, new positions are available at our centre. I therefore invite you to review the careers page which has a selection of positions currently available.

I hope you enjoy reading this issue of our Phenogenomics Newsletter.

Radislav Sedlacek

2ND PROGRAMMABLE NUCLEASE COURSE AT CCP

Inken M. Beck

Transgenic and Archiving Module

Figure 1. Participants and tutors arrive for the start of the course.

After our 1st programmable nuclease course in March 2016 in frame of the TT2016 conference in Prague, we prepared the 2nd course from 3-7 April 2017, this time in our new centre in Vestec. On board we had a co-organizing institution, ICS phenomin, from Strasbourg (http://www.phenomin.fr/) and as before, the course took place under INFRAFRONTIER umbrella (https://www.infrafrontier.eu/).

16 participants from Europe and Asia, with a wide range of experience, came together to learn basic and advanced strategies to use programmable nucleases for genome editing, in which we focused on Cas9 and Cpf1 nuclease targeting. In addition to lectures, the focus lay on hands-on work in the lab and using databases to understand all steps important for design, evaluation and preparation of CRISPRs. Participants also had the possibility to submit individual projects in advance that were analyzed and discussed during the course.

We had extraordinary support from Marie-Christine Birling, Guillaume Pavlovic (both phenomin-ICS, Strasbourg), and Lluis Montoliu (CNB, Madrid, Spain), who instructed, gave lectures and stayed during all five course days!

In the lab participants gained insight into guideRNA validation and testing their activity. This was done by a cell-based assay and completely *in vitro* using Cas9 protein digest. For preparation of sgRNA, participants performed template preparation, *in-vitro* transcription, RNA purification and concentration measurement. To identify resulting mutations after given CRISPR targeting, participants screened for indel mutations using native PAGE and high-resolution capillary electrophoresis system.

The course had assigned time for three parallel hands-on sessions, which focused on a) pronuclear injection - to show one option to deliver nucleases into mouse embryos, b) digital PCR - as genotyping option after CRISPR targeting and c) bioinformatics - to get a taste about different options of targeting possibilities and online available nuclease design tools. During the bioinformatics session, individual projects were analyzed and discussed one-on-one. Lectures giving an overview of nuclease features and their application for generating deletions and integrations into the genome facilitated the practical sessions.

Figure 2. Pictures from the course including guest lectures, practical sessions and one-on-one discussions with experts.

Dynamic support during the bioinformatics session was given by Bernd Zetsche (Broad Institute, USA) who also gave an updating lecture about Cpf1 nucleases, a protein that shows RNase and DNase activity; with Cas9 the only two proteins known so far with this activity. Two further guest speakers, Qingzhou Ji (MilliporeSigma) who presented technical application of CRISPR tools and Francis Stewart (TU Dresden, Germany) who talked about relationship between homology arm lengths and CRISPR targeting efficiency, completed the long course days.

In addition, an extensive summary about course lectures written by Lluis Montoliu can be accessed here: http://www.phenogenomics.cz/2017/04/report-from-2nd-programmable-nucleases-course-april-2017/

With this, CCP cordially thanks sponsors and all speakers and instructors for their time, effort and enthusiasm spending at this course!

The preparation of good blood samples

Karel Chalupsky

Biochemistry and Hematology Unit

We are now serving a larger community of scientists who use rodents as their experimental model. This opening of our services brings one common problem – sample preparation. In this article, we would like to highlight a few common issues. Nevertheless, the consistency of sample handling is crucial, therefore variations in collection technique, time of sampling and time to freezer should be minimised. These procedures should never change during a study. In our experience, beside study design, it all starts with anaesthesia and blood withdrawal. Ketamine based anaesthesias usually have an effect on glucose homeostasis and later measured values are higher than values measured in conscious animals or animals anaesthetized with isoflurane. Another common problem frequently observed is extremely high values of hemolysis (fig1). In such samples, measurement of potassium or activity of lactate dehydrogenase make no sense. Also in hemolytic samples, false positive results indicating increased concentration of cytokines as IL-6 can be observed. For these reasons hemolysis in plasma should not be present if you want to maximize the quality of data output and perform experiments according to three Rs with no additional repeats because of low plasma quality. Last but not least proper labelling of propylene tube is essential. Adhesive-on labels tend to detach when frozen in -80C and therefore these labels should not be used for long term storage or shipping of samples. When precious samples from complex and extensive studies arrive at our centre, it is our aim to process these samples as quickly and robustly as possible. Having 'non-labelled' samples or good or poor quality, not only slows down our processing, but in the worst case can lead to us not being able to process the samples.

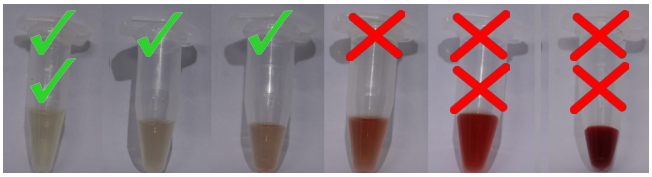


Figure 1. Examples of plasma samples exhibiting no hemolysis (far left) and extremely high values of hemolysis (far right).

INFRAFRONTIER2020 Kick-Off Meeting Report

Michael Hagn

INFRAFRONTIER GmbH

On the 24th and 25th of April 65 members of INFRAFRONTIER gathered to kick-off the new EU Horizon 2020 funded INFRAFRONTIER2020 project. The INFRAFRONTIER Research Infrastructure integrates European Mouse Clinics and the European Mouse Mutant Archive (EMMA) with the common goal to ensure access to mouse models for basic research of human health and disease, and to translate this knowledge into therapeutic approaches for the benefit of the European society.

The expanded INFRAFRONTIER2020 network, coordinated by the INFRAFRONTIER GmbH, includes 3 SMEs and is strategically responding to the challenge of ensuring the long-term sustainability of the infrastructure. Towards achieving this goal aligned objectives were defined which are 1) the development of business models and a stable legal framework; 2) raising awareness of the INFRAFRONTIER Research Infrastructure; 3) providing bespoke services aligned with user demands; 4) promoting best practices in mouse phenogenomics; and 5) enhancing robustness of the INFRAFRONTIER IT infrastructure and use of the EMMA strain resource.

INFRAFRONTIER2020 will 1) enhance the sustainable operation of the INFRAFRONTIER infrastructure; 2) foster innovation, and 3) address major societal challenges in human health by customised service pilots supporting research into common and rare diseases. A sustainable INFRAFRONTIER infrastructure will also ensure the quality of deposited mice and support the reproducibility of biological results. Outreach efforts will raise awareness of resources and services and facilitate sustainable engagement with industry and global consortia such as the International Mouse Phenotyping Consortium (IMPC).

INFRAFRONTIER2020 Kick-Off Meeting Report

Continued...

The Kick-Off meeting provided all participants with an overview of overall project objectives, project structure, key deliverables, and outlined major project activities in 2017. Among these is the launch of new service pilots covering CRISPR/Cas9 technology to generate rodent disease models, new secondary phenotyping services, access to an ageing phenotyping pipeline, and the derivation of germ-free mice. Information and application forms for open Trans-national Access service calls will be available on the INFRAFRONTIER portal at https://www.infrafrontier.eu/resources-and-services/infrafrontier-open-calls. Furthermore, the outline of the first open INFRAFRONTIER Annual Stakeholder Meeting was presented which will be held in Athens on November 14-16. The meeting will be jointly organised with the International Mouse Phenotyping Consortium (IMPC) and focus on advances in Crispr/Cas9 technology and the contribution of mouse resources to Personalised Medicine. INFRAFRONTIER2020 consortium members further engaged in a series of workshops at the Kick-Off meeting focussing on communications and outreach, defining user requirements for re-engineering the IT infrastructure, and on sharing latest advances in cryopreservation technologies.

The Kick-Off meeting saw the successful launch of a new INFRAFRONTIER project that will be instrumental for the sustainable operation of the INFRAFRONTIER infrastructure, and that will pilot attractive new services for the INFRAFRONTIER user community.

BIOCEV days 2017 Conference

Petr Solil

BIOCEV

On June 19-20th BIOCEV held its annual conference in Vestec. BIOCEV days 2017 had over 179 participants and more than 40 posters. The opening of the conference saw welcome addresses by BIOCEV director Pavel Martásek and IMG director Petr Dráber. Research groups from all 5 research programmes gave presentations of the exciting and ground breaking research conducted here at BIOCEV and showcased the exceptional collaborative work done by our core facilities. Guest speakers included Jiří Zavadil (WHO International Agency for Research on Cancer, Lyon, FRANCE) and Jean-Michel Camadro (Jacques Monod Institute - Paris Diderot University). During the poster session more than 40 posters were presented and the relaxed atmosphere allowed students and senior research members to interact and discuss scientific projects which continued during the BBQ. The BIOCEV days conference was well attended and we look forward to hosting the 3rd BIOCEV conference in 2018.

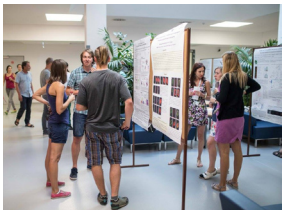
Pavel Martásek, BIOCEV Director

Petr Dráber, IMG Director

Jiří Zavadil

Jean-Michel Camadro

Conference attendees



Poster session

Jan Konvalinka, Vice-Rector for Research, Charles University

CCP Supports 2nd orbis pictus lecture at TT2017

Nicole Chambers

Editor

The Czech Centre for Phenogenomics is proud to support the 2nd orbis pictus lecture presented as part of the 14th Transgenic Technologies Meeting 2017. The orbis pictus lectures were introduced as part of the 13th Transgenic Technologies Meeting which was held in Prague 2016. Due to its tremendous success, the International Society for Transgenic Technologies have included a second orbis pictus lecture as part of the TT2017 meeting which will be held in Salt Lake City, Utah.

The Orbis pictus lecture will be given by Martin Cohn from the University of Florida, USA.

ISTT

Czech Centre for Phenogenomics

TT2017

October 1-4, 2017
Salt Lake City, Utah, USA
Snowbird Resort

IMPC Annual Meeting Report

Jan Procházka

Phenotyping Module & Bioimaging Unit

On May 9-11, the annual meeting of IMPC was held in the heart of Nanjing, China and was organised by the Model Animal Research Center (MARC). The meeting was well organized and brought many interesting talks from Chinese colleagues. The enthusiasm in IMPC achievement was obvious from everybody. New ideas and approaches for standardized targeting were discussed, results in increasing efficiency of gene targeting within individual IMPC members were compared, and promising new strategies were suggested. There were many enjoyable talks and from a personal point of view Henrik Westerberg's talk showing automatization of phenotype analysis in microCT scans of embryos in regular screens was just amazing. Beside many interesting talks and targeting new aims in phenotyping procedures we enjoyed the tremendous hospitality of MARC and enjoyed tour of their modern facility as well as roof based bar.

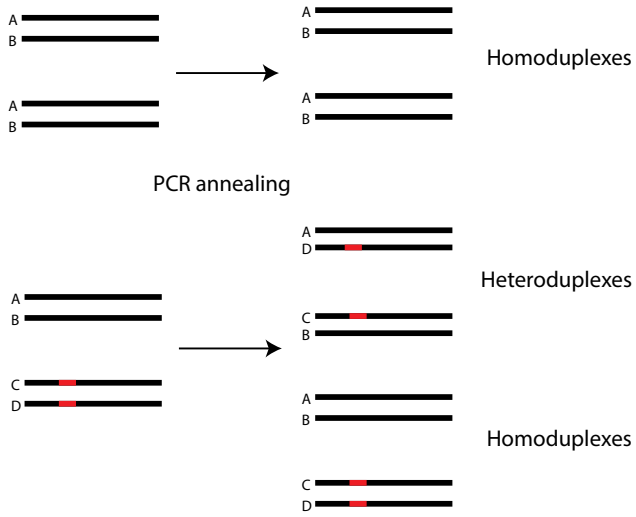
TRANSGENIC RESEARCH STARTS WITH ACCURATE GENOTYPING

Jana Kopkanova

Genotyping Unit

Genotyping is the process of determining differences in the genetic make-up (genotype) of an individual by examining the DNA sequence using biological assays and comparing it to another individual's sequence or a reference sequence. It reveals the alleles an individual has inherited from their parents. Traditionally genotyping is the use of DNA sequences (Fig 1) to define biological populations by use of molecular tools. It does not usually involve defining the genes of an individual. This is due to current technological limitations, so far almost all genotyping is partial. Means that only a small fraction of the individual's genotype is determined, such as with Genotyping By Sequencing (GBS) or RADseq. New mass-sequencing technologies promise to provide whole genome genotyping (or whole genome sequencing) in the future.

Current methods of genotyping include restriction fragment length polymorphism identification (RFLPI) of genomic DNA, random amplified polymorphic detection (RAPD) of genomic DNA, amplified fragment length polymorphism detection (AFLPD), polymerase chain reaction (PCR), DNA sequencing, allele specific oligonucleotide (ASO) probes, and hybridization to DNA microarrays or beads. Genotyping is important in research of genes and gene variants associated with disease.



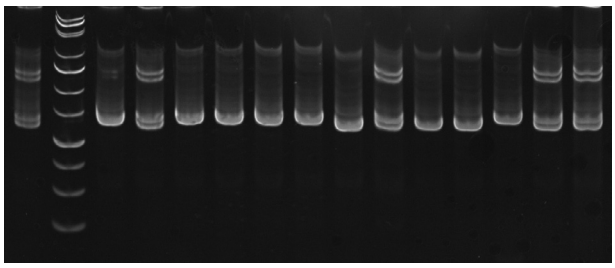


Figure 2. Heteroduplexis identified on native PAGE

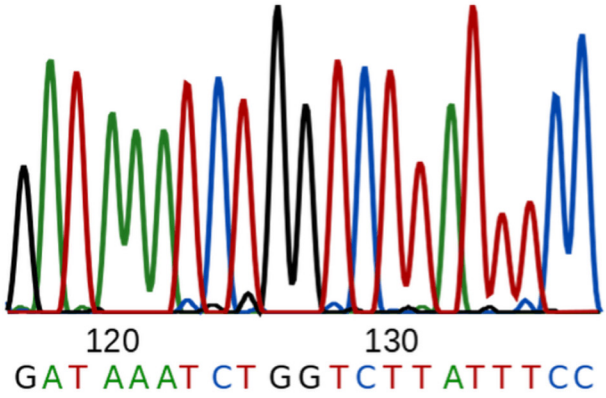


Figure 1. Example genomic DNA chromatogram.

The most commonly used methods in research are PCR, sequencing and enzymatic digestion. PCR is an easy and cheap tool to amplify a specific segment of DNA, useful for such purposes as the diagnosis and monitoring of genetic diseases, identification of criminals (in the field of forensics), and studying the function of a targeted segment of DNA. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, so the DNA template is exponentially amplified. Because the primers are key components for successful PCR, it is necessary to design them properly following the common rules (e.g. length of primers, melting temperature of primers, content of GC and AT bases) Another important point is the position of the primers, it is highly recommended that the primers overlapping regions carrying the mutation or region where occurs integration or deletion, preferentially that the mutation is in the middle of the PCR product.

In the past research focused on determination of differences between 2 populations to identify the mutations causing diseases. Recently, heavily using CRISPR/Cas9 system, we are producing mutations in the gene of interest to make the transgenic models to study function of this gene. Using the CRISPR/Cas9 system we are able to make insertion/deletion (indels) mutations in gene to make frame shift and inactivate this gene, or make exon deletion to inactivate the gene or integrate foreign DNA into gene (for example GFP for easy screening). As the transgenic technologies expand, the genotyping became "daily breed" for all researchers.

So how to identify the mutations, especially the small indels (usually few bases integrations or deletions)? Sequencing is the logical option, but can be very expensive when you are producing thousands of animals per year. And very often it can happen that only small number of animals carry the mutation. Another possibility is T7 endonuclease digestion. T7 endonuclease I (T7EI) recognizes and cleaves non-perfectly matched DNA. This method is easy, but has some limitations. Robust and clean PCR amplification is critical, so nested PCR is highly recommended. It is important to use correct amount of T7EI and run the digestion for optimal time. If too much T7EI is used or the digestion is too long, more non-specific digested fragments will occur. Another method based on heteroduplexis is native polyacrylamide gel electrophoresis (native PAGE). It is an easy and very cheap method. Heteroduplex DNA complex make additional bands above the major wild type band (Fig 2). With this method a lot of animals can be screened quickly and then select only animals where targeting occur and send them for sequencing to define exact mutations. Another advantage of this method is that from G1 generation band pattern is same for all animals with same mutations, so there is no need to sequence the animals further and this bring economical savings.

The genotyping unit, part of TAM, is well established and equipped. Because transgenic and archiving module consist of targeting part, production and genotyping part, we can offer the researchers that we will make the project from scratch, means that we will design the targeting strategy, do the pronuclear injection, optimize the PCR in advance, so we are ready to screen the animals as soon as possible, send positive ones for sequencing to determine the exact mutations, select the optimal animals and send them to costumer or breed them further and establish the colony with the defined mutation. We can also offer customer specific services, for example isolation of DNA only if genotyping is established in customer's lab.

GENOTYPING UNIT OF TAM OFFERS:

- DNA isolation using phenol/chloroform/ isoamylalcohol or DNA extraction using DirectPCR extraction solution from mouse/rat tails or ear punches
- PCR, long range PCR, digital droplet PCR
- Agarose and native PAGE gels, Dynex capillary electrophoresis
- Preparation of samples for sequencing
- Primers design and validation
- Final protocols preparation
- Consultation and troubleshooting

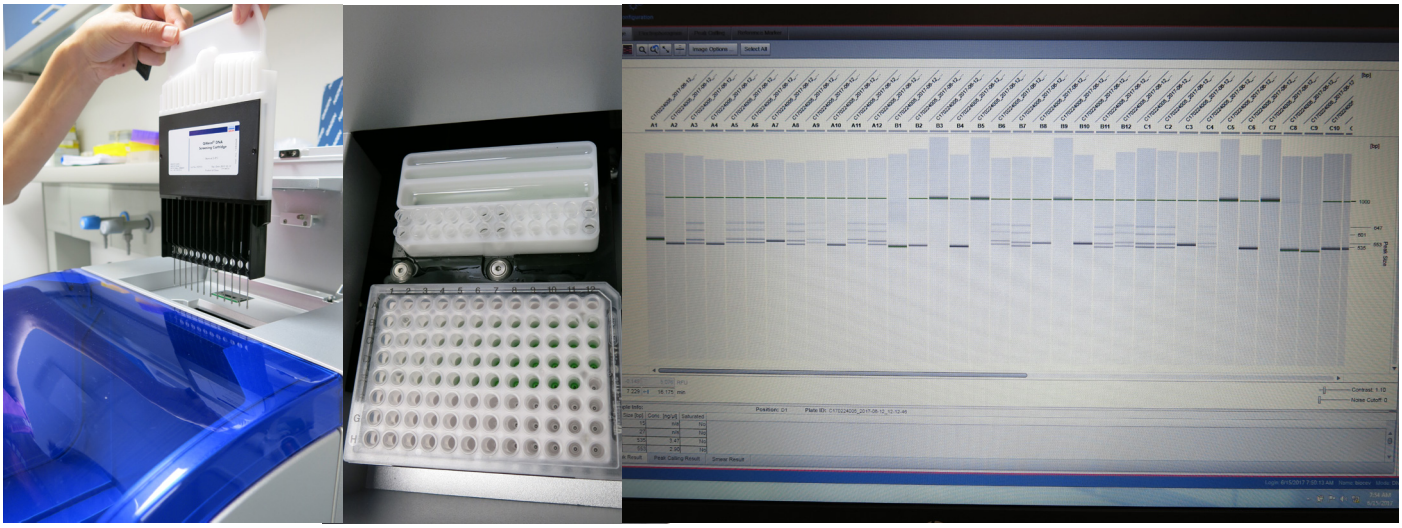


Figure 3. The QIAxcel Capillary electrophoresis device and data readout example.

INTRINSICALLY DISORDERED PROTEINS DRIVE ENAMEL FORMATION VIA AN EVOLUTIONARILY CONSERVED SELF-ASSEMBLY MOTIF

Jan Procházka

Bioimaging Unit

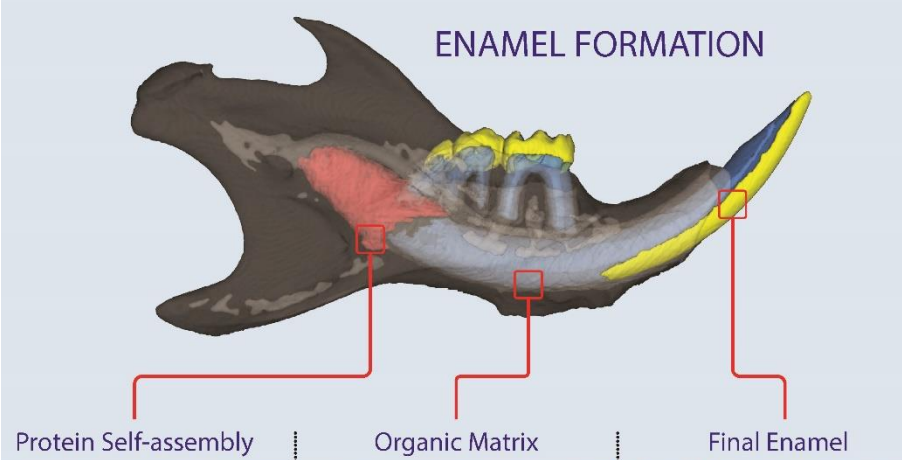


Figure 1: MicroCT scan of adult mouse mandible. The microCT and subsequent segmentation highlights the areas of enamel development in model of ever-growing rodent incisor. Purple: cervical loop area where stem cells and renewal of ameloblasts takes place together with secretion and self-assembly of enamel matrix proteins, blue: formation of organic matrix and its subsequent mineralization, yellow: maturated enamel.

Tooth enamel is the hardest and most resistant tissue in our body. It consists of 98% inorganic minerals, in particular hydroxyapatite, the other 2% is water and organic material. However, the crystalline structure of enamel is very complex in contrast to hydroxyapatite found naturally in the wild. It is precisely organised in complexity of arranging individual crystals within the individual bundles. Crossing the individual bundles with each other is achieved with a precise micrometer spacing, giving the tooth enamel its unique physico-mechanical-chemical properties (Fig 1). The formation of enamel begins with the secretion of structural proteins from specialized cells called ameloblasts. These structural proteins organize themselves into a complex three-dimensional structure, followed by secretion of the mineral components for very precisely controlled crystallization process. Cleavage and removal of the original structural proteins clears the way for crystal growth. As a result, the "only" crystalline hydroxyapatite remains, arranged in a unique complex structure, characterized by its high resistance to both physical and chemical influences and, in the case of humans, remains for the duration of the life of the individual. Synthesis of a similar material comparable to tooth enamel has not been achieved so far. Therefore, the understanding of enamel formation is an important step in the possibility of design and technological preparation of similar materials in-vitro. As humans only have 2 enamel formation events in their life, investigating this is almost impossible using human tissue. This work requires the use of models which have continuously growing teeth, and for this reason we chose the mouse model, which continuously growing incisors in each of the upper and lower jaws.

Together with colleagues from Institute of microbiology Czech Academy of Science we tackled the central question of how mineralized tooth enamel is formed and the role of enamel matrix protein in this formation. From another mineralization processes in body, the formation of bones, we have learnt that the mineralization matrix is composed via self-assembly of collagen I, however very little is known about the molecular mechanism which governs the establishing of enamel mineralization matrix in teeth.

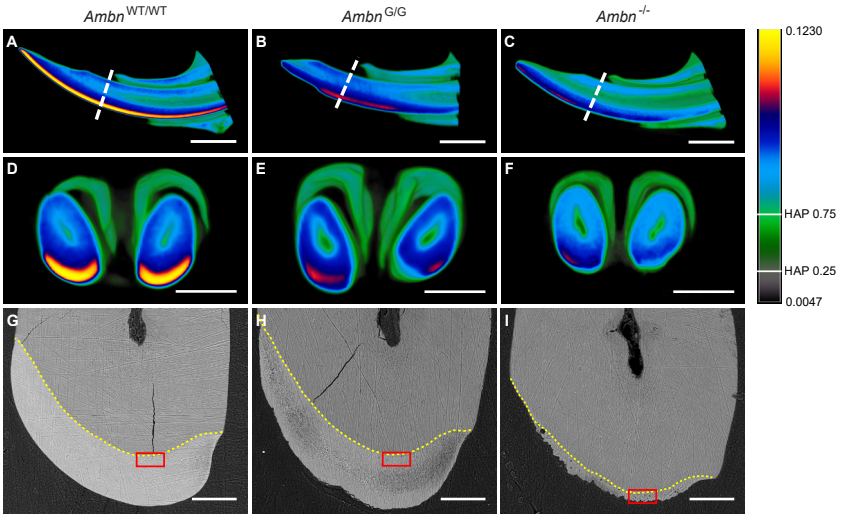


Figure 2: Analysis of tooth mineralization phenotypes. A-F tomographical section through lower incisor. Pseudo colours show the level of mineralization – the warmer the more mineral is deposited in tissue. G-I – electron-optical imaging of sections from lower incisors show that develop the enamel with the comparable thickness as WT mice, however the mineralization status is severely altered.

Molecular and human genetic studies identified that intrinsically disordered enamel matrix proteins, unrelated to collagen, are critical for the development and structural integrity of matured enamel. Their absence or malfunction always leads to severe enamel deficiencies resulting in the symptoms of *Amelogenesis imperfecta*.

The most abundant proteins in enamel protein matrix are two intrinsically disordered proteins Amelogenin and Ameloblastin. By mutation analysis in vitro three tyrosil motif (Y/F-x-x-Y/L/F-x-Y/F) has been identified in both proteins as critical for the self-assembly capacity in process of formation protein multimers.

However biological role of such protein multimer formation was still enigmatic. In order to characterize the phenomena of enamel matrix protein self-assembly during formation of enamel, CCP with the great experience in

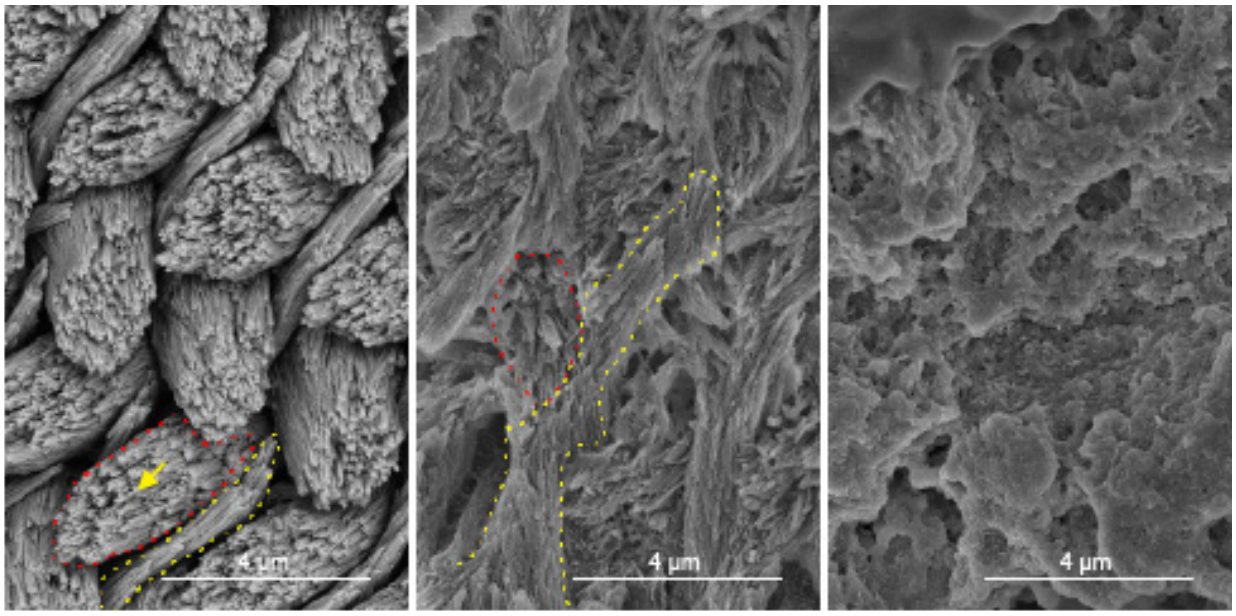


Figure 3: High resolution scanning electron microscopy was used for analysis of enamel ultrastructure. Mutated Amb (Amb$^{YLF-GGG}$) (B) showed dramatic effect of mutation on organization of enamel prisms compared to WT enamel (A), however still hydroxzapatite crystals showed certain level of polarization compared to AmbnKO enamel, where only amorphic deposition was observed.

mouse model preparation and characterization came to scene. At first we generated a transgenic mouse model harbouring a triple-glycine substitution (G-x-x-G-x-G) in the three key residues in self-assembly motif in ameloblastine (Y-x-x-L-x-F). The big advantage was use of programmable nucleases TALENs, which rapidly accelerated generation of mouse model with desired mutation. The strength of CCP in characterization of novel mouse models provided ideal knowledge and technology platform for analysis of mineralization defects in enamel formation. The whole body microCT scanning help to identify alteration in enamel structure immediately and helped focus analysis towards higher resolution (Fig 2).

We found that ultrastructure of enamel was severe damaged by substitution mutation and hydroxyapatite crystals, which are supposed to from nicely oriented bundles in enamel prisms were almost entirely missing (Fig 3). Such a striking phenotype led us to further analysis of organization of enamel matrix proteins. Confocal imaging of enamel matrix protein network provided first evidence of importance of self-assembly property in ameloblastin for formation of regular protein scaffold. The mutated ameloblastin mouse showed very irregular enamel matrix organization, however all other tested properties of ameloblast were indistinguishable from controls.

Thus, our research contributed to biomineralization field by bringing new evidences, how is self-assembly of enamel proteins important for building enamel protein matrix, which structure is then critical for regulation of crystallization process and opens further perspectives for our research.

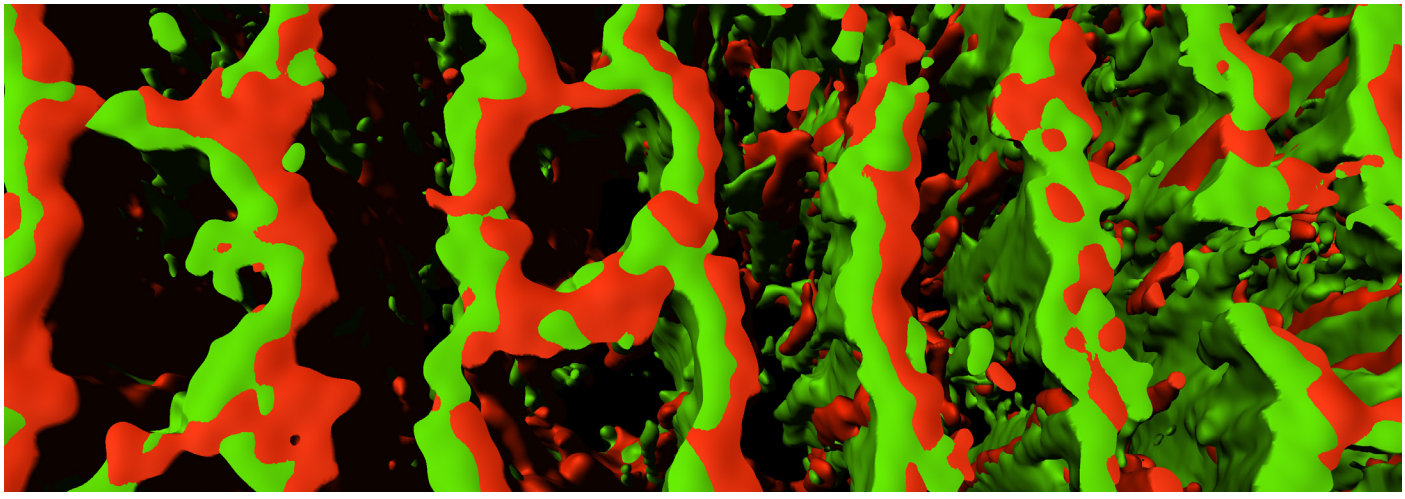


Figure 4: Super-resolution microscopy (3D SIM) of Amelogenin (green) and Ameloblastin (red) in formed enamel protein matrix. The highly organized structure of enamel matrix protein is evident with inner spaces which will in further development serve as basis for enamel prisms. Such organized structure was lost in mutated ameloblastin and only disorganized protein deposition was observed.

AUDITORY TESTS AT CCP

Jiri Lindovsky

Hearing Unit

The auditory screen laboratory is focused on testing of fundamental hearing functions in mice. It is concerned primarily with electrophysiological tests of the auditory part of the nervous system using evoked potentials technique, but we can measure otoacoustic emissions, too, which inform us about the correct function of the cochlea. The methods are non-invasive in principle, however in animals, unlike in the human clinical practise, the general anaesthesia must be used in order to prevent movements. The obtained data may be used for evaluation of peripheral and central changes resulting from a genetic modification, aging, exposition to noise or other pathologies.

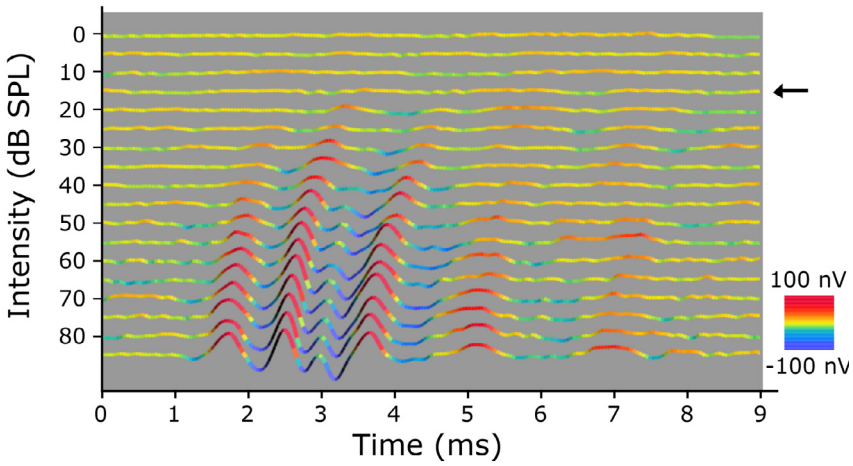


Fig. 1. Auditory Brainstem Response. The animal was stimulated by a pure tone of 24 kHz frequency, 5 ms duration and intensities ranging between 0 - 85 dB SPL. Each curve represents an average of 600 repetitions. For the sake of clarity the signal amplitudes are color-coded and the recordings are ordered according to the increasing stimulus intensity. The lowest sound intensity that evoked a visible response is referred to as the hearing threshold for the respective frequency, marked with an arrow.

Hearing is generally recognised as one of the main senses. On the other hand, we usually start appreciating its role in the social and emotional aspects of life only when we are already losing it, for example due to aging, tinnitus or after an illness or injury. Then, we discover how hearing is important not only for the overall space orientation with respect to things we do not directly see, but that owing to voice communication we are in everyday contact with people and information. Sound always arises as a result of some activity therefore to hear also means to know what is actually going on around us. We may expect that ears are similarly important also for mice which have very well developed sensitivity mainly to high frequency sounds, they are active preferably at night, live a social life and use vocalizations for communication.

Hearing is not only those two ears on the sides of our head, however. Above all, it is the nervous system that analyses and interprets the information entering through the ears. A huge amount of tricky computation is done by the auditory part of the brain without our notice just to deliver to us such common percepts like the sound of an approaching car, understanding of a continuous melody or comprehension of speech. It is fair to say, though, that the gateway which supplies the brain with the acoustic information - the inner ear, cochlea - is quite sophisticated and delicate structure, too. It is definitely not just a simple passive detector.

Speaking about the auditory system we usually distinguish two parts:

The periphery: the outer, middle and inner ear with the cochlea that contains the organ of Corti with hair cells. It transforms air pressure changes into mechanical vibrations of liquids in the cochlea and thus also of the inner hair cells which code these vibrations as changes of their membrane potential. This information is further transmitted onto the endings of the acoustic portion of the eighth cranial nerve. Apart from the inner row of hair cells there are three rows of outer hair cells in the cochlea that are also sensitive to vibrations. Changes of their membrane potential, nevertheless, are not passed on to the nerve. They are linked via a positive feedback with their own active movements by which the original vibrations get amplified. Outer hair cells hence work as an active electromechanical preamplifier. Loss of function of the outer hair cells leads to weakening of the auditory sensitivity by 40 - 60 dB[1].

The centre. Nerve pathways and nuclei that analyse various features of the information flow sent from the periphery: frequency content, temporal structure, amplitudes, phase, binaural, emotional context, etc. In addition to the afferent stream leading from the brainstem through the thalamus to the temporal cortex, there are several efferent pathways, too. Their modulatory effect points back to lower structures and reaches even the function of the periphery[2,3].

Alterations in hearing may arise from dysfunction in the auditory periphery as well as in any of the central pathways. In our laboratory we use two fundamental methods the first of which, recording of otoacoustic emissions, aims at the peripheral function, whereas the second, sound-evoked responses of the brainstem, says something about the central sound processing.

Distorsion Product Otoacoustic Emissions

The term otoacoustic emissions (OAE) may be translated as sounds emanating from the ear. Except for the sound that enters the ears it is possible, using a sensitive microphone, to detect also sounds leaving the ears. They are not some sort of reflections or echo, however. The sound emitted by ear is produced by active vibrations of the outer hair cells in the cochlea as a result of their stimulation by the incoming sound wave. It is a sign of their mechanical amplification function. At some circumstances, in newborns for example, OAE may appear spontaneously as well. But for experimental and diagnostic purposes OAE are usually elicited by acoustic stimuli. The most common variant of this technique is known as distortion product otoacoustic emissions (DPOAE). During DPOAE measurement a small probe consisting of a microphone and two independent loudspeakers is inserted

in the outer ear canal. The ear is stimulated simultaneously by two tones of different frequencies f1 and f2. As a consequence of non-linear character of the cochlea OAE arise not only at those frequencies used but also at many other difference and summative frequencies, so called distortion products. The most prominent one of them is emitted at the frequency F = 2*f1 - f2. This frequency is recorded by the microphone whereas all others are filtered out in order to increase the signal-to-noise ratio. Diminished or missing DPOAE are a mark of damaged or missing outer hair cells. It is commonly seen in older age, after exposition to loud noise or ototoxic antibiotics and is observed in patients as well as in laboratory mice. Certainly, the physiological status of the outer hair cells may be changed in mutants with developmental dysfunctions[4]. In our laboratory we record DPOAE with a setup based on Tucker-Davies RZ6 system with Etymotic low-noise microphones and BioSig software.

Auditory brainstem responses

Activity of brain structures, most importantly the formation and advancement of action potentials in neurons, manifests on the head surface as electric field oscillations which may be detected using low-impedance electrodes. Such a recording is referred to as an electroencephalogram, EEG. If a mouse is stimulated repeatedly by a short (milliseconds) sound and we average the recorded surface potentials, we shall obtain a typical signal. Its exact shape will depend on the position of the recording electrodes and the stimuli used, nevertheless, during the first 2 - 7 ms it will most probably contain a series of distinct waves – the auditory brainstem response (ABR, Fig. 1). ABR reflects activation of the first several nerve structures which successively participate in the acoustic information analyses: the cochlear nerve, the cochlear nucleus, the superior olivary complex, the nuclei of the lateral lemniscus and the inferior colliculus[5]. Each of these structures contributes to the response waveform, the higher in the processing hierarchy, the later (i.e. with a longer latency) its respective peaks appear in the signal of ABR. Hence, the ABR peaks' shape, latencies and amplitudes may help to infer conclusions about the function of individual parts of the auditory brainstem[6]. If we record a bit longer, more than 10 ms, we may observe additional waves in the signal. These come from even further portions of the auditory pathway, mainly the brain cortex[5]. Interesting information is achieved if stimulation by pure tones is used, rather than by broadband sounds like clicks or noises. It is possible then to estimate how the hearing is sensitive to individual frequencies. We can, for example, easily demonstrate that during aging people (as well as mice) lose the sensitivity primarily for the highest sound frequencies[7].

Our stimulation equipment is placed in a 6 m^3 custom-made sound-attenuated anechoic chamber (Fig. 2) that allows to select from two major stimulation arrangements. We may play the sound from a distance and ensure a homogeneous and reliable sound level all around the animal (open-field condition), or we may move the sound source closer or even into the ear canal of the animal. This letter arrangement (closed-field condition) is accompanied with principal difficulties related to complex acoustics of small-volume spaces, on the other hand it enables independent stimulation of each ear with different stimuli. The frequency range of the setup covers all bands needed for experiments in mice, the theoretical limit is at 100 kHz. This is important, because laboratory rodents commonly hear and communicate in ultrasonic frequencies. We use the newest Tucker-Davis Technologies RZ6 system with some custom adaptations and BioSig programme in combination with own visualization software tool.

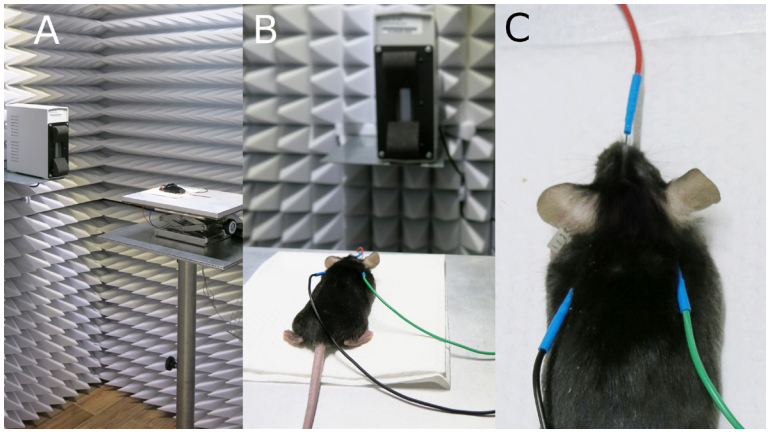


Fig. 2. The anechoic chamber. A) Overview of the chamber. A high-frequency load speaker points at a table with a custom-made heating pad and an anaesthetised mouse. B) In this arrangement the speaker creates a reliable acoustic field covering the whole heating pad where up to 4 animals may be recorded simultaneously, if needed. C) Subcutaneous needle electrodes placed for the auditory brainstem recording. The active electrode on the top of the head, the reference and grounding electrodes behind the left and right bulla acoustica, respectively.

In addition to our own methods which are still open to further development and individual adaptations regarding the techniques as well as the possibilities of analysis, we keep in close contact with the CCP behavioural unit where other methods are run related to hearing. Namely the acoustic startle response together with the prepulse inhibition can be interesting ways of testing of the auditory system in awake behaving animals.

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METABOLOMICS: ANOTHER 'OMICS' OR SOMETHING MORE?

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What is metabolomics?

Metabolomics, one can say is like another "omics" besides genomics, transcriptomics, proteomics which is considered in biological systems (Fig 1). However, it is a little bit different from other omics. Metabolomics analysis provides information about the immediate state of organism including aspects from all omics presented in the organism. For example, your DNA is transcribed into RNA but not all genes get transcribed so there is more unused information in DNA than in RNA and again not all RNA is translated into functional protein. Once you get the protein, it can be modified on several levels such as glycosylation, phosphorylation etc. which will change its activity and turnover

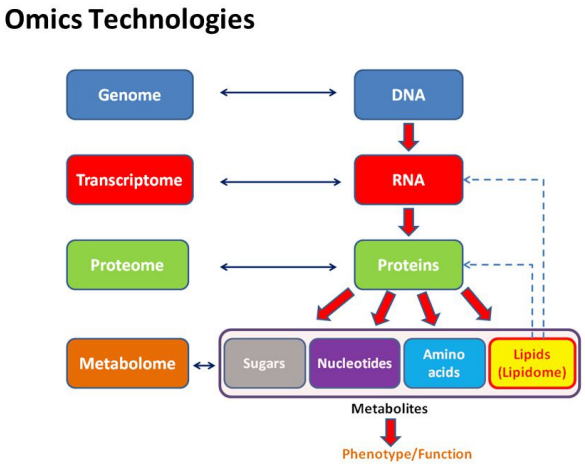


Fig 1. Schematic overview of Omics technologies

in the organism. Moreover, not all genome presented in human or rodent body is host origin. In the last decade, huge progress was made in microbiome research. So far about 1000 bacteria strains were found in the human gastrointestinal tract and only 60 of them can be cultured in the best laboratories. For all these reasons, analysis of metabolites present in an organism will give the best overview what is going on because it is the only omics technology, which covers the whole complexity of living organism and in fact, it is partially independent of the genome and transcriptome and proteome, which are usually considered alone. Additionally, environmental stimuli is another gene-free factor which can be analyzed only by metabolomics.

With such complexity comes another problem, how to measure and analyze the vast amount of data? Major tools used are mass spectrometry coupled to gas or liquid chromatographic separation techniques, or NMR. None of these methodologies can screen all metabolites in a single step as different metabolites e.g. volatile metabolites cannot be detected by liquid chromatography but only by gas chromatography and also different separation and extraction techniques will be used for lipophilic and lypophobic metabolites.

With acquired data comes another problem how to process and interpret data. Metabolomics data are usually large sets of points and unfortunately, you always get data like from RNA chip. But how valid, which filter should be used, and how to distinguish between noise and real signal depends fully on operator. Recently two leading companies Bruker and Agilent have brought their own software solution which use very similar work flow: firstly, they filter data by setting up threshold over signals, secondly they work with statistics models such as principal components analysis so they reduce amount of data in batches which are processed. If somebody read it up to here

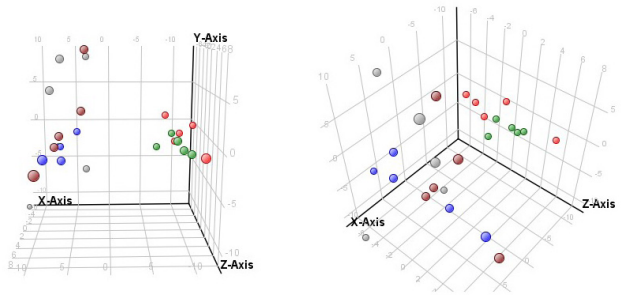


Fig 2. Principal components analysis (PCA)

please send me an email. Principal components analysis, where grouping between control and modified condition should appear (see Fig 2), is followed by identification of metabolic features which are different within experimental groups. Using databases like Metlin, which contains over 64,727 structures, is necessary. Once your hit feature is identified, it is put in metabolite map. All related- and close metabolites are selected

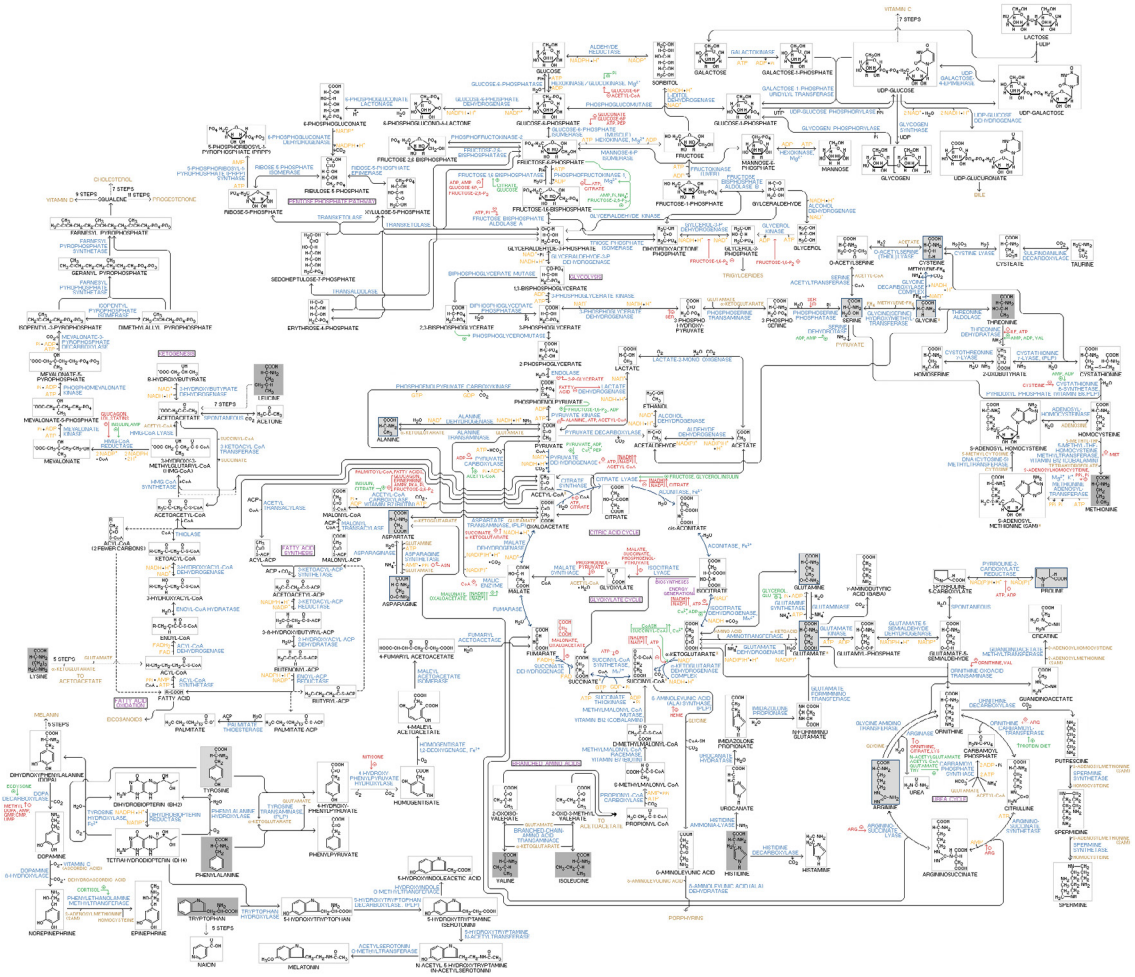


Fig 3. Example KEGG view with compound data on a global and overview map

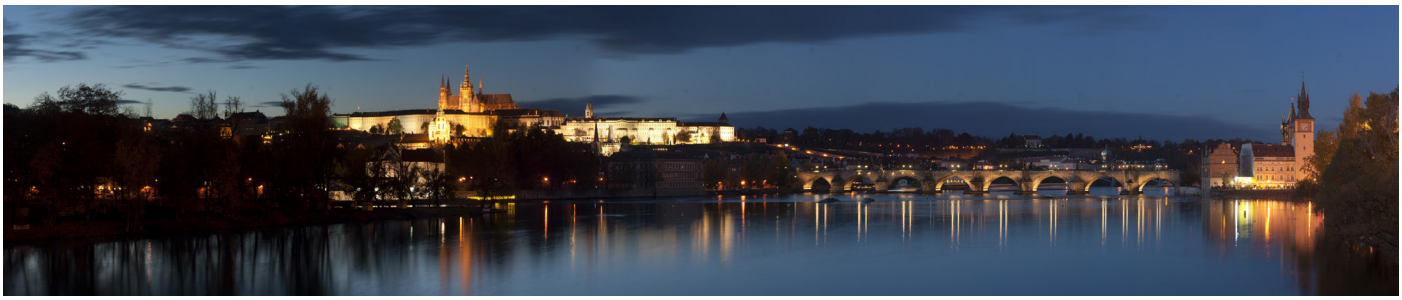
and screened again in your raw data sets. This approach can help you find more hits in a particular metabolic pathway, since other metabolites in this pathway can be filtered out by your statistical methods used in first step. Thus this second analysis is quite important in order to find relevant and significant changes within your experimental groups.

Applications of metabolomics

Metabolomic data mining can be done with several approaches. The first is targeted metabolomics, where you know what you are looking for. Here you have several advantages; you know the compounds you are interested in, you know their chemical properties and sometimes you can get standards or even labeled standards. This analysis is in fact quantification of your compounds of interest in the biological matrix. The second approach is non-targeted metabolomics. Here, large sets of data are analyzed by statistical methods as described above. The aim is to find out and identify differences between experimental groups. Another approach is to study metabolic fluxes and reveal the structure of metabolic pathways and networks using labeled compounds. Labeled compounds, primarily heavy hydrogen- deuterium and carbon 13, are used in biological systems and enable the unambiguous tracing of heavy elements through complex metabolic pathways (Fig 3).

As alterations in metabolites are so closely linked to the phenotype of an organism, here at CCP we are incorporating the metabolomics approach in our regular screening pipeline. Using the LC/MS/Q-TOF (Agilent technologies) we are developing the analysis in plasma, urine and stool samples. Our overall task is to determine gene function in our mice and now combined with all other techniques available at CCP we are adding metabolite profiles.

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

Head of Bioinformatics Unit

The Czech Centre for Phenogenomics is currently seeking an experienced biostatistician / bioinformatician to lead recently opened Bioinformatics unit.

The ideal candidate should have at least 3 years' experience in multidisciplinary research, deep knowledge of statistical methods, proficiency in at least two computer languages, excellent communication skills, GTD minset and strong publication record. Previous experience in, genetic databases (Ensembl, UCSC, MGI), data analytical skills (R or Python) and advanced machine learning (e.g. deep neural networks) is advantageous. Previous experience with mouse genetics, metabolomics or other omics is also desirable.

The Czech Centre for Phenogenomics offers state of the art research equipment and a stimulating, multidisciplinary environment encompassing all aspects of mouse molecular genetics (from mutant generation to complex phenotyping). Moreover, CCP is also developing strong link to medical research. We offer opportunities for career advancement and a competitive salary. The position is available immediately for an initial 1 year fixed-term contract, with further longer-term extension. The place of work is Vestec u Prahy (Czech Centre for Phenogenomics – campus BIOCEV).

Pathologist (rodent pathology)

To advance the services of our histopathology lab we are seeking an 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.

BIOINFORMATICIAN / BIOSTATISTICIAN

The recently opened Bioinformatics unit is currently seeking a Bioinformatics Analysts. The primary responsibility will be to provide statistical and analytical expertise to research scientists. This will be complemented with the ability to create and develop mathematical tools for the analysis of big data sets obtained from metabolomics, proteomics, and other omics. This focus will form part of an independent research project aiming to design new computational methods and biostatistics. The ideal candidate is a recent graduate (BSc level or above) in bioinformatics, biology, mathematics or related scientific field. Knowledge of genetics or previous experience in a multidisciplinary environment is desirable. You should be proficient in at least one programming language (ideally Python or R). Experience with Unix based OS, command line tools, cluster computing and bioinformatics tools and databases (Ensembl, UCSC, MGI, IGV) is advantageous. You must have strong communication skills and the ability to cooperate with non-mathematicians. This position is available as a full-time or part-time contract.

We offer opportunities for career advancement and a competitive salary. The position is available immediately for an initial 1 year fixed-term contract, with further longer-term extension. The place of work is Vestec u Prahy (Czech Centre for Phenogenomics – campus BIOCEV).

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

The Genomics of Common Diseases

6-9 September 2017 | Cambridge, UK

https://coursesandconferences.wellcomegenomecampus.org/events/item.aspx?e=654

Mitochondira, apotosis and cancer

16–18 September 2017 | Bled, Slovenia

EMBO Workshop

http://meetings.embo.org/event/17-mito-cancer

The 5th Helmholtz-Nature Medicine Diabetes Conference

September 17-19, 2017 | Munich, Germany

http://www.nature.com/natureconferences/hmgu2017/index.html

Single Cell Europe 2017

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

http://www.singlecell2018.eu/

14th Transgenic Technology Meeting

October 1-4, 2017 Salt Lake City, USA

http://www.tt2017.org/

Metabolomics bioinformatics for life scientists

5–9 February 2018 | Cambridge, UK

EMBO Practical Course

http://events.embo.org/coming-soon/index.php?EventID=pc18-10

Journal Club

1. Bak RO and Porteus MH. CRISPR-Mediated Integration of Large Gene Cassettes Using AAV Donor Vectors. *Cell Reports* **20**, 750–756 (2017)
2. Karp NA., *et al*. Prevalence of sexual dimorphism in mammalian phenotypic traits. *Nature Communications*. (2017) DOI: 10.1038/ncomms15475
3. Kuscu C., et al. CRISPR-STOP: gene silencing through base-editing-induced nonsense mutations. *Nature Methods* **14** (7) (2017)
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