Issue 3, Summer 2016

PHENOGENOMICS
NEWSLETTER

Czech Centre for Phenogenomics

Czech Centre for Phenogenomics

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Reanimation and rederivation
Archiving

Animal Facility Module

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Import and export of animals
Contract breeding

Phenotyping Module

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Embryology
Biochemistry and hematology
Bioimaging
Neurobiology and behaviour
Immunology
Metabolism
Cardiovascular function
Lung function
Vision
Hearing

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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 ccp@phenogenomics.cz

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

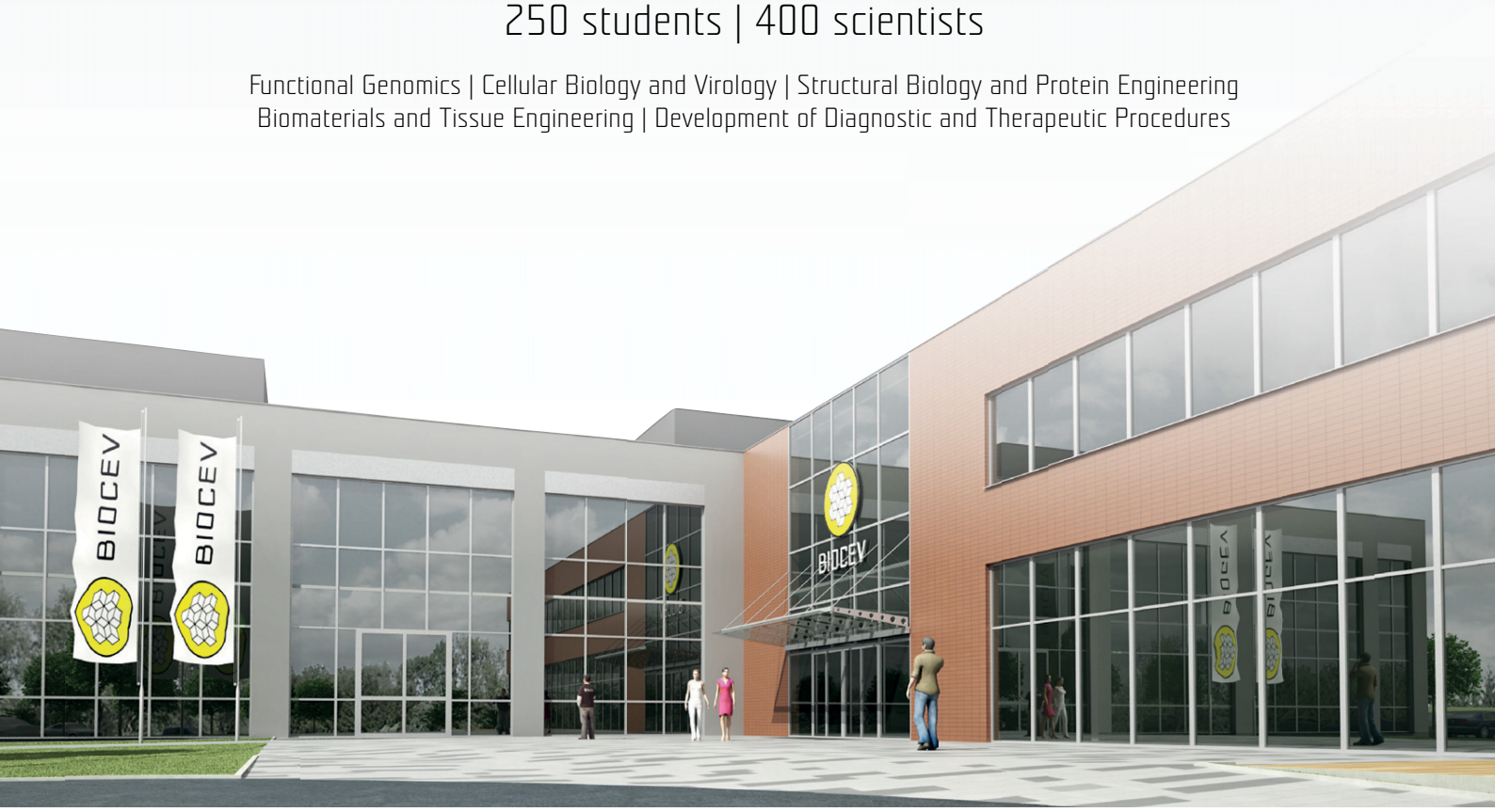
BIOCEV

Biotechnology and Biomedicine Centre
of the Academy of Sciences and Charles University in Vestec

excellent science in favour of modern society

5 research programmes | 6 core facilities | 54 research teams
250 students | 400 scientists

Functional Genomics | Cellular Biology and Virology | Structural Biology and Protein Engineering
Biomaterials and Tissue Engineering | Development of Diagnostic and Therapeutic Procedures



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Message from the Director

Dear Readers,

Following the hive of activity associated with hosting the 13th Transgenic Technologies meeting, the Czech Centre for Phenogenomics has been very active 'fine tuning' the services offered by our facility as well as organising future events aimed at the scientific community. The highlights of our activities have been included in this issue of our 'Phenogenomics newsletter'. Events such as the opening ceremony of our host institution - BIOCEV and our seminars and workshops can be found in our news section. Our featured section gives detailed reports on three specialized units of CCP as well as our work as the Czech node of the European Mouse Mutant Archive (EMMA). I would like to draw your attention to the featured article from our Immunology Unit, which details its work focused on cancer immunotherapy using whole tumour cell-based, as well as dendritic cell-based cellular vaccines used in murine models. Our Immunology Unit performs these studies as part of contract research, which aims to provide proof-of-principle studies towards novel immunotherapeutic modalities using different murine models for solid cancers, as well as different kinds of immunotherapies.

As part of our open access research service I am pleased to include two detailed announcements in this issue. Firstly, CCP will host a morphological-anatomical hands-on course '***Anatomical bases of Mouse Multimodal Imaging***'. This 5 day course will be led by Jesús Ruberte París, one of the authors of the recent book 'Morphological Mouse Phenotyping', and will provide comprehensive information about mouse morphology, anatomy, and histology.

The second highlight of the upcoming period is the call for '***Mouse production service***'. CCP will generate 5 knockout mouse models free of charge. Projects and new mouse models should be focused on genes that have not been used to generate mouse models within IKMC/IMPC (www.mousephenotype.org). More details about these calls can be found in this issue as well as on the CCP website.

I am inviting you to take some time to read this issue of our newsletter, visit our website, and learn more about CCP's services, activities, accomplishments, expertise and events.

I look forward to working with you on your research projects.

Radislav Sedláček

News In Brief

BIOCEV Officially Opens

From the left - Pavel Martásek (BIOCEV Director), Tomáš Zima (Rector of Charles University), Miloš Petera (Governor of the Central Bohemian Region), Pavel Bělobrádek (Deputy Prime Minister for Science, Research and Innovation), Kateřina Valachová (Minister of Education, Youth and Sports), Jiří Drahoš (President of the Czech Academy of Sciences) and Tibor Švec (Mayor of Vestec)

Petr Solil,
BIOCEV PR Manager

The opening ceremony, which was attended by significant figures both from the Czech Republic and abroad, including Deputy Prime Minister for Science, Research and Innovation Pavel Bělobrádek, the Minister of Education, Youth and Sports Kateřina Valachová, President of the Central Bohemian Region Miloš Petera, President of the Czech Academy of Sciences Jiří Drahoš, Rector of Charles University Tomáš Zima and Director of the Institute of Molecular Genetics (IMG) Václav Hořejší, was followed by tours of selected BIOCEV workplaces. In the afternoon, the two-day international conference opened and was attended by major representatives from both Czech and foreign science and research bodies.

The Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec (BIOCEV) was developed with the help of significant financial aid from the European Union. Currently, 56 research groups under 5 synergic research programmes are focused on obtaining a more detailed understanding of organisms at the molecular level. The results of their work are oriented towards applied research and the development of new medical procedures to combat severe health problems. The end results of BIOCEV's research work include drugs targeted at the exact location of damaged metabolism and protein and tissue engineering. The center employs more than 390 researchers and technicians. Almost one-third of them come from abroad, such as from Australia, Canada, France, Ukraine, Poland and Germany. BIOCEV's research teams have published more than 320 research outputs, including articles in prestigious international journals (such as the Cell, Molecular Cell, Nature Communication and Gastroenterology and others).

Pavel Bělobrádek
Deputy Prime Minister for Science, Research and Innovation

Václav Hořejší,
Director of IMG

Pavel Martásek
BIOCEV Director

Charles River and The Jackson Laboratory - Seminar Tour 2016

Jan Honetschläger
Head of Animal Facility Module

On Thursday the 19th of May 2016 Peter Kelmenson of the Jackson Laboratories gave a series of informative talks at the BIOCEV Centre for Phenogenomics. The feedback on the presentations was very positive, with over 75 participants in attendance for the first presentation alone. Below is a short description of each lecture:

Key Differences among B6 Substrains and the Research Impact

The most widely used inbred strain, C57BL/6 (B6), has several substrains such as the B6J and the B6N and many assume them to be genetically identical. However, there are genetic and phenotypic differences between them that can confound interpreting and reproducing results.

Comparing Models for Obesity and Diabetes Research

Multiple inbred mouse models serve as powerful tools to study the genetics, underlying mechanisms, and therapies for human type 2 diabetes. In this seminar, participants learned about the strengths and weaknesses of the most popular and emerging strains to assist with mouse model selection.

Introduction to Humanized Mice for Cancer Immunotherapy

The mammalian immune system has developed surveillance mechanisms that can detect cancerous cells; however successful tumor cells have evolved strategies to evade detection. Current therapeutic strategies focus on improving cancer cell recognition and tumor elimination. Mouse models have been instrumental in the development of these therapies, including both immunocompetent and immunodeficient mice.

For further information on JAX™ Mice please contact AFM ccp-afm@img.cas.cz

FREE-OF-CHARGE TRANSGENIC SERVICES:

GENERATION OF KNOCKOUT MOUSE

The Czech Centre for Phenogenomics (CCP), Institute of Molecular Genetics ASCR (CCP-IMG) will support researchers with a **free of charge mouse production service**. A total of **5 knockout mouse models** will be produced as part of this service and will be made available to the wider research community via the INFRAFRONTIER/EMMA repository.

The **service** provided covers the production of a minimum of two heterozygous mice carrying the targeted gene of choice. Only projects, which consider genes that have not been converted into a mouse model within the IMPC (International Mouse Phenotype Consortium) will be considered; this information can be found at http://www.mousephenotype.org/. The models could be generated from the corresponding validated gene-targeted ES cell clone(s) or alternatively by using programmable nuclease technology.

COSTS:

The service of CCP within this call is free of charge. This includes the purchase of ES cell clones from IKMC repositories, the nuclease design and testing. Shipment costs of the produced live mice to the customer's facility are not included and must be paid by the selected customer.

SPECIAL CONDITIONS AND OWNERSHIP:

Mouse lines produced by this CCP free of charge service will be owned by CCP/IMG, however the user can freely use the models for his/her project-specific research as described in the evaluated proposal. The mouse line will be phenotyped according to the IMPC standard pipeline and archived in public EMMA/INFRAFRONTIER repository. After a grace period after the mouse line was produced and provided to the customer, the line will be made freely available for the research community according to the conditions of the EMMA/INFRAFRONTIER repository (https://www.infrafrontier.eu/).

ELIGIBILITY:

the user must work in an academic institution and must select a gene that has not been converted into a mouse model within the IMPC.

SELECTION PROCEDURE:

Service requests for free of charge access to this CCP service will be subject to a review procedure, which will be initiated after the call is closed. All applications will be treated with strict confidentiality. The review will be based on short descriptions of the projects involving the mouse mutants that are generated by the service. Members of the CCP Evaluation Committee will assess all requests. Applications will be evaluated mainly on **1)** scientific merit, **2)** soundness of submitted proposal, and **3)** access of applicants to transgenic facilities. IKMC ES cell clones or any genes that are assigned for active mouse production in IMPC (www.mousephenotype.org) will not be considered. Only one access unit will be granted to a principal investigator for this call. Applicants will be informed on the outcome of the evaluation within 3 weeks after the end of the call**.**

To apply for this call or for more information, please visit our website www.phenogenomics.cz

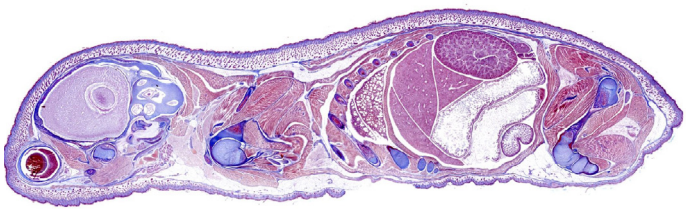
IMG

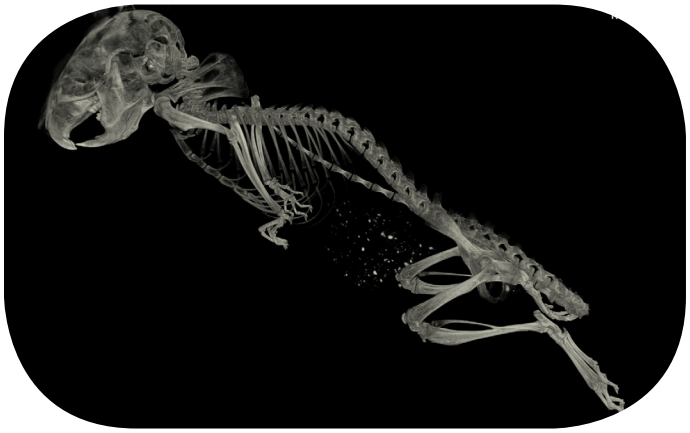
Czech Centre for Phenogenomics

Anatomical Bases of Mouse Multimodal Imaging

Czech Centre for Phenogenomics

This intensive course is organised by the Czech Centre for Phenogenomics and will focus on deeper understanding of the mouse anatomy.





Course Leader

Jesús Ruberte París

Center for Animal Biotechnology and Gene Therapy (CBATEG), Universitat Autònoma de Barcelona, Spain

Prerequisite

Applicants should have a basic to intermediate knowledge of rodent anatomy

Course Details

Location

Czech Centre for Phenogenomics
Prumyslova 595
252 50 Vestec
Czech Republic

Duration

5 days

Tuition Fee

€ 1500

Date

16th - 20th January 2017

Application Details

Applications for this course should include:

- A letter of motivation
- Short CV (1 page)
- A reference letter

Deadline for applications

31st October 2016

Applications should be sent to
ccp@phenogenomics.cz

For more details visit www.phenogenomics.cz

The Meeting with Tasmanian Tiger

Jan Prochazka & Frantisek Spoutil
Bioimaging Unit

Tasmanian Tiger specimens housed in original glass holder.

Our Bioimaging unit was honoured to take part in an examination of one of the world's most unique specimens for vertebrate zoology: newborns of *Thylacinus cynocephalus*, better known as Tasmanian wolf or Tasmanian tiger. These specimens were refound recently in the collections of the Faculty of Science, Charles University in Prague and represent the youngest known pups of this fascinating marsupial, whose last specimen died in 1936.

The Tasmanian tiger was the only recent member of the family Thylacinidae, a sister group of the family Dasyuridae with animals like Tasmanian devil (*Sarcophilus harrisii*), and quolls (*Dasyurus*), but due to astonishing ecological convergence it strongly resembled a small wolf with shorter legs, thick tail, and stripes over its back. It was a carnivore hunting other Tasmanian marsupials and had the important role as the top predator in the ecosystem of this island. However, the arrival of humans proved to be a bad omen for marsupial carnivores. Firstly, it probably lost the competition with the dogs accompanying the first men colonizing New Guinea and Australia, and secondly, much later, the species was made extinct in Tasmania (last shot in wild in 1930) as they learned to prey on sheep and other smaller livestock of farmers. And then the marvelous example of convergent evolution was lost.

To study the Tasmanian tiger, we are restricted to very old museum specimens if we want to study anatomy or development. As all these specimens are extremely rare, usage of nondestructive methods are needed, and computed microtomography (µCT) is one of them. Fortunately, the possibility of SkyScan 1176 (Bruker) suited for whole body imaging in CCP bioimaging unit is suited for scanning large specimens. Thus the imaging chamber is big enough to handle the whole glass jar with four specimens. Nevertheless, the scanning of Tasmanian tiger's pups will be challenging in many ways: we are going to obtain data from specimens through glass and stored in a liquid, which we can only supposed to be ethanol, for more than a hundred years. We are also not sure, how such a long storage can affect the specimens in terms of suitability for µCT scanning. For us, imaging of such challenging projects is extremely interesting and helps us to move the limits of imaging forward.

INFRAFRONTIER Industry & Innovation Workshop

Michael Raess
General INFRAFRONTIER management

On 28 and 29 June the INFRAFRONTIER Industry & Innovation Workshop took place in Munich. 88 participants from Europe, North America, Asia and Australia, among them 23 representatives from industry, discussed technological innovations, how biopharma uses mammalian models in drug discovery and how the resources and services provided by the INFRAFRONTIER Research Infrastructure and the International Mouse Phenotyping Consortium (IMPC) can support translational research. In two panels the 'Impact of CRISPR technology' and the 'Impact of rodents as models of human diseases' were explored, taking particularly the issue of animal welfare into account. The workshop provided ample opportunity to initiate new collaborations and new alliances between industry and academia. In addition, eleven suppliers were able to present their goods and services at 11 booths in the exhibition area.

Lluis Montoliu leading a panel discussion

FEATURED ARTICLE

CELL IMMUNOTHERAPY OF CANCER USING MURINE EXPERIMENTAL MODELS.

Milan Reinis, PhD
Head of Immunology Unit

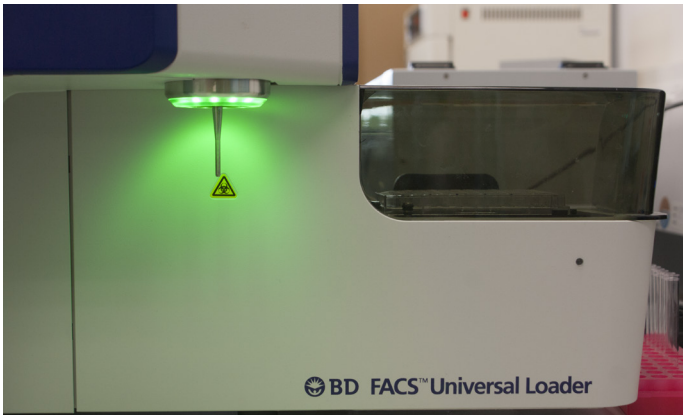
Cancer immunotherapy is considered to be a promising tool that can be integrated with standard anti-tumour modalities, such as surgery, chemotherapy and irradiation. The first clinical use dates back to the 19th century, when William B Coley treated cancer patients with a bacteria lysate (Coley toxin)[1]. Various immunotherapeutic approaches, especially those based on cytokine or antibody treatments, have been well established throughout the years[2]. However, cancer immunotherapy is still paradoxically considered to be an emerging and novel technology. After many years of an intensive effort and a lot of scepticism, new immunotherapeutic drugs have been recently introduced to clinical practice, namely vaccines and check-point inhibitors. We also now better understand the role of the immune system in cancer progression and therapy. Indeed, over the last several years we can talk about a renaissance of tumour immunology[3]. Cell therapy or cellular vaccines have been intensively studied as tools for cancer immunotherapy. Sipuleucel-T (APC8015, Provenge; Dendreon Corp)[4] was the first cellular vaccine based on dendritic cells and was approved by FDA for clinical practice in 2010. Interestingly, DC-based therapy of prostate cancer has also been studied in clinical trials in the Czech Republic[5]. Further, a number of whole tumour cells therapies that employ established allogeneic tumour cell lines engineered to produce cytokines that provide strong activation signals to the immune system have been developed and tested, unfortunately with limited success[6].

The immunology unit at CCP, has experience with whole tumour cell-based, as well as dendritic cell-based cellular vaccines used in murine models. We have evaluated the capability of several murine tumour cell lines engineered to produce various cytokines (IL-2, IL-12, GM-CSF) to inhibit the tumour growth in mice in the settings of combined immunotherapy with either surgery or chemotherapy[7]. Interestingly, irradiated cellular vaccines served in our experiments as a source of continuously releasing cytokine in the vicinity of the growing tumour rather than a source of tumour-associated antigens. A significant difference in the efficacy of the vaccines based on the same lineage as the growing tumour and those derived from a distinct tumour cell line of the same genetic background as a growing tumour was not observed[8]. We have also shown that cell therapy using IL-12-producing cells repaired the absence of cytotoxic and proliferative responses of tumour infiltrated leukocytes after chemotherapy[9]. IL-12-producing cellular vaccine also displayed additive effects against MHC class I-deficient tumours when combined with 5-azacytidine, DNA methyltransferase inhibitor that increased MHC class I expression on tumour cells and sensitized them to specific immune responses[10].

Dendritic cells, key players in immune response activation, are professional antigen-presenting cells that link innate and adaptive immune responses. DC can be generated *in vitro* and used for immunization or immunotherapy[11]. The clinical trials using DC vaccines started in 1996, and so far a number of clinical trials have been performed world-wide. Typical autologous DC-based vaccine preparation starts from their precursors (monocytes in humans or bone marrow cells in mice). Immature DC are prepared, loaded with relevant tumour antigens and subjected to subsequent maturation. DC maturation is mediated through activation of pattern-recognition signalling pathway and mature DC effectively present antigen in the context of MHC molecules. They also provide additional necessary activation signals by co-stimulatory molecules and cytokine production. For antigen loading, various strategies have been developed. Immature DC can be pulsed with tumour cells inactivated by their lysis (ultrasonic treatment, repeated freeze-thaw), lethal irradiation or other methods before mixing them with DC. Alternative strategies involve loading with peptides, protein, DNA or RNA transfection. In our laboratory, we have experience mainly with the tumour cell- and peptide-loaded DC. We have evaluated these preparations in various murine tumour models, including MHC class I-deficient tumours as examples of tumours that escaped anti-tumour immunity[12,13].

Prostate cancer is considered to be one of the most promising targets for the DC-based therapy. Recently, we have focused on the proof-of-principle studies of combined chemoimmunotherapy of prostate cancer, using either mice transplanted with syngeneic tumour cell TRAMP-C2[14] or transgenic orthotopic model TRAMP mice[15]. The experiments have been performed in collaboration with the SOTIO a.s. company that supported us by a research grant. In this study, we employed high hydrostatic pressure (HHP) to inactivate tumour cells before their use for DC antigen loading. HHP has been previously shown to induce immunological cell death in several human tumour cell lines. HHP-treated cells co-cultured with DC were able to induce monocyte-derived DC maturation, and, finally, T cell activation *in vitro*. These findings justify HHP as an important tool for tumour cell inactivation/killing before their use for DC pulsing[16].

Our objective was to evaluate DC-based vaccines pulsed with HHP-inactivated tumour cells in murine models as a suitable tool for prostate cancer immunotherapy. We have demonstrated immunogenicity of the HHP-treated tumour cells in mice, as well as the therapeutic potential of the DC vaccines loaded with antigen by co-culture with HHP-treated tumour cells[17]. As expected, HHP was able to induce immunogenic cell death of tumour cells and HHP-treated cells induced stronger immune responses in mice immunized with these tumour cells, as compared to irradiated tumour cells, standardly used for DC antigen loading.



Further, we have demonstrated immunogenicity and DC co-cultured with HHP-treated tumour cells and matured by a TLR 9 agonist. In a therapeutic setting, this vaccine combined with docetaxel chemotherapy significantly inhibited the growth of TRAMP-C2 tumours.

Further, DC-based vaccines pulsed with HHP-inactivated tumour cells were also effective in reducing prostate cancer growth in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model when used alone or in the combination with docetaxel[18]. This clinically relevant TRAMP model mimics well humans carcinoma as it develops and progresses through all stages of carcinogenesis similarly to humans.

Our Immunology Unit, as an integral part of the Czech Centre for Phenogenomics, can offer various services in the field of mouse immunology and cancer (immuno)therapy. Our laboratory is endowed with an expertise in proof-of-principle studies focused on novel immunotherapeutic modalities, using different murine models for solid cancers, as well as different kinds of immunotherapies. We are also interested in analysis of immunological consequences of chemotherapeutic interventions. Our laboratory is equipped with necessary up-to date instruments, including flow cytometer BD FACSVerse™, ELISA reader, plate washer, gentleMACS Dissociator etc. We offer mouse immunophenotyping by spleen cell flow cytometry analysis, as well immune response monitoring. We would be happy to share our skills and we are open for possible collaborations in the field.

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TISSUE ORGANOIDS – STEP FORWARD FROM CELL TO BODY

Jolana Tureckova, PhD

Molecular and developmental biology nowadays requires many different approaches in order to elucidate complex mechanisms of mammalian development and tissue regeneration and homeostasis. Over recent decades, embryonic stem cells (ESCs) have been derived from the epiblast and expanded in vitro. Later on, inducible pluripotent stem cells (iPSCs) cultures were established originating in almost any mature cell type in our bodies. These milestones made possible differentiation of various pluripotent stem cell populations into somatic cells in vitro. However, until recently, there has been a lack of an in vitro model suitable for studying tissue patterning and morphogenesis. The recent development of 3D culture systems has made it possible to recapitulate partially mammalian morphogenesis in vitro. Many attempts have been made to employ either stem cells or different tissues in mini-organ self-reconstructing process in vitro and some appeared to be successful (Fig.1). Based on those advances, now it is clear that stem cells and some tissues have the capability to generate organoid structures in culture as a parallel to their in vivo counterparts.

An organoid is a 3D structure, in which cells spontaneously self-organize into properly differentiated functional cell types and progenitors, and which resemble their *in vivo* counterpart and recapitulate at least some function of the organ.

Lately, conversion of culture systems from 2D into 3D has allowed the development of organoids to model tissues of all three germ layers in origin, i.e. ectoderm, endoderm and mesoderm and the number of reports grows amazingly. Among organs of the ectodermal origin, organoids from retina, pituitary, cerebrum and inner ear have been produced so far[1]. Of endoderm, especially stomach, both intestines, pancreas, liver and lung organoids have been successfully made. Out of the mesodermal parts, cardiac muscle organoids have been presented up to date. Prerequisite for successful production of such structures is placing either stem cells or a tissue parts into 3D media usually containing a mesh of extracellular proteins serving as a scaffold and an anchor.

In our laboratory, we have established cultures of different parts of gastrointestinal tract so far. As already mentioned, organoids can be made either from single stem cell or from part of a given tissue. Although such a system does not contain mesenchymal cells, it uses specific media conditions to meet the niche requirements. As an example, small intestinal enteroids can be prepared both ways, i.e. either from sorted Lgr5 positive precursor or from plating whole freshly isolated intestinal crypts. Plating entire crypts is more advantageous since the procedure does not require preceding stem cell population sorting and forming organoids takes less time. On the other hand, production of organoids out of sorted cells have brought valuable findings concerning molecular basis of stemness in different tissues. For instance, this has been the case of observation that Sox9 positive cells are necessary for self-renewal of colonic crypts[2].

Another way of intestinal organoid culture starts with seeding entire intestinal extracts from murine fetus (obtained from fetuses between embryonic days 15 and 18). Such ex vivo system enables answering contextual questions regarding development and maturation process of the entire intestinal tube as it grows and stratifies. In this setup, explants form mostly spheroids without buds instead of typical organoids (see Fig.2). According to Mustata *et al.*[3], this kind of spheroids presents very useful model for studying differentiation molecular sequences occurring during morphogenesis of fetal intestine. The authors found out that neonatal progenitors express connexin 43 while postnatal precursors are Lgr5 positive instead. They also showed that growth of both fetal and adult enteroids is Lgr5 independent yet without Lgr4 undoable. Indeed, intestine in Lgr4 knockout mice displays considerably decreased amount and differentiation status of Paneth cells, i.e. the cells responsible for controlling homeostasis of the intestinal crypt. This finding coming from organoid studies is fundamental since both Lgr proteins had been considered

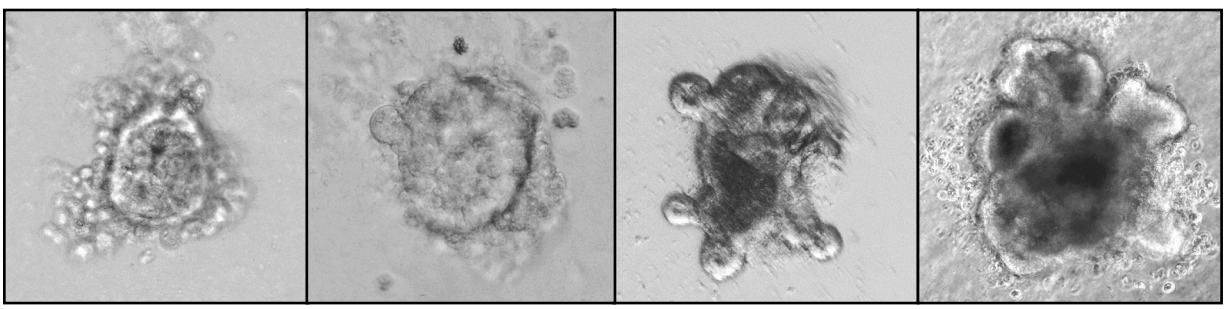

Figure 1. Enteroids (from left to right): day 1, day 2, day 3 and day 4 in culture

as equally functional intestinal stem cell markers before. In addition to bringing important answers about ontogenesis, the system of producing spheroids out of fetal intestine might be an excellent tool elucidating context of recently documented interconversion of the various adult intestinal stem cell types in epithelial regeneration[4].

Last but not least, a specific category of organoids is spheroids formed out of tumorous tissue or cells. This special condition was designed by Sato *et al*.[5]. This technique employs seeding sorted cancer stem cells in the basal lamina mimetics, Matrigel. In recent years, development of targeted therapies against tumors brings opportunities for patients to receive a more personalized approach. Such a strategy is necessary especially in cases of drug resistant tumors since they can become even more aggressive than before treatment. Hence, primary culture of intestine-derived cancer cells may represent appropriate model to predict the drug response of individual tumor subsets in experimental animals or in human patients.

Organoids or tumor spheroids might present a precious tool also for human predictive biomedicine and enable us to monitor the growth of the patient's tumor and its response to a given treatment. The system has several advantages, first, since both organoids and spheroids can be derived from just one sorted stem cell, small biopsies are sufficient as a source of material to grow in vitro. Second, the starter cells become immobilized in Matrigel and so their clonal healthy or colorectal cancer (CRC) organoids can be tracked on real time basis. Third, the expansion efficiency of organoids is up to 1000 times per month with passaging ability for several years without signs of senescence. The latter makes them suitable for multiple biochemical, metabolic or drug testing analyses performed at the same time as well as they serve as "living biobanks" for any kind of genetic monitoring including deep sequencing. The mentioned benefits have been broadened by a possibility to genetically modify organoids using DNA transfections or infection of viral particles bearing the desired gene sequence. In addition, also CRISPR/Cas9 system has recently been used to correct genetic mutations in CFTR gene causing cystic fibrosis[6]. Thus, besides opportunities listed above, organoid culture system also opens an entirely new field in experimental medicine focused on grafting chemically or genetically modified organoids into injured epithelia. This all makes organoid cultures unprecedented tool bridging limited cell culture outcomes and whole organism homeostasis.

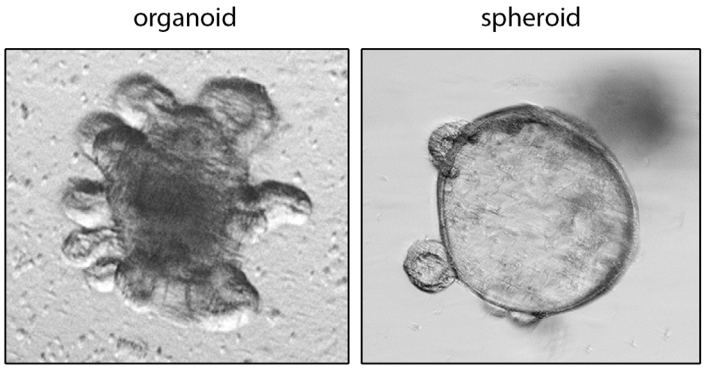

Figure 2. Budding structure of an organoid compared with the budless structure of a spheroid.

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HISTOPATHOLOGY, PAST, PRESENT & FUTURE

Peter Makovický & Ivana Švecovà
Histopathology Unit

Veterinary pathology – the diagnosis of disease based on the gross, microscopic and molecular examination of organs, tissues and whole bodies, is one of the oldest fields of veterinary medicine. It is believed to focus on pathology analysis, however this is only partially true. It is an interdisciplinary medical field, with veterinary pathologists playing an integral role in the diagnostic process. Their work significantly contributes to other clinical fields, including the prognostic process, therapy, and also a spectrum of preventative measures.

Veterinary pathology can be divided into 3 main categories: Necropsy, biopsy and clinical cytology.

Necropsy, a post-mortem examination to determine cause of death or the changes produced by disease, is one of the oldest and most noted disciplines in pathology. Necropsies allow the identification of cause of death and the knowledge gained from such procedures can be used to expand the understanding of various diseases. Whilst this method is performed post mortem, the information gathered can be used to build a complex picture of the disease, its effect and progression, and therefore the information can be used to further develop the specificity of preventative medicine.

Biopsy, the removal of parts of living tissue to discover the presence, cause, or extent of a disease, is a progression from necropsy. Unlike necropsies, the results of this examination can be used directly for diagnosis and also treatments as well as developing knowledge used for preventative medicine.

Clinical cytology, is a branch of pathology that studies and diagnoses diseases on the cellular level.

It is widely known that genetics plays a role in many illnesses and, therefore, a lot of effort is being applied in this area of therapy in modern medicine[1,2]. Here at the Czech Centre for Phenogenomics (CCP), we believe it is necessary to know more about the functions of individual genes and understand their roles as this provides insights into the pathophysiology of disease.

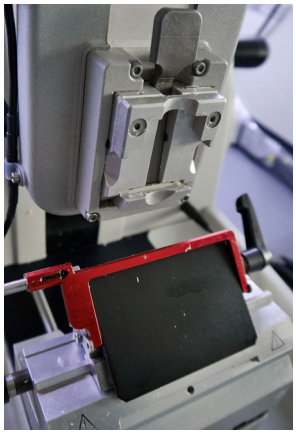
The Histopathology laboratory at CCP was established as a comprehensive research service. It is fully equipped and the state of the art equipment is comparable to other top European veterinary histopathological laboratories. Many activities are performed by modern semi-automated machines. The work, which in the past lasted a month, can now be completed in one week, by strengthening the use of histopathology techniques as diagnostic tools. Modern machines have reduced the time interval for samples processing and almost eliminated the effect of human influences during processing of the samples. This will greatly reduce the time interval from sampling to the final diagnosis, or final histopathological report. Currently, we offer a complex service in the following areas: Clinical cytology, biopsy and necropsy. Our clinical cytology service identifies pathological changes within isolated cells obtained by the aspiration of tissues or liquids, paracentesis of body cavities, smears, imprints, lavage, and examination of cerebrospinal fluid. Our biopsy, is primarily aimed at the diagnostic evaluation of tissue obtained by clinical process. Our necropsy service is focused on the histological investigation of selected samples from laboratory animals.
The major part of our work is still our histological service with basic staining, including broad histochemical and immunohistochemical procedures, which increases the level of the diagnostic profile. The work includes wider possibilities of morphometric analysis and morphometric evaluation of examined material. Currently, the main histopathological evaluation of samples (internal and external) are taken during necropsy.

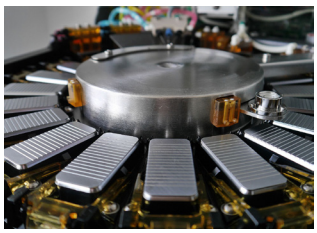
Besides classical immunohistochemical methods examining the protein expression, valuable cytogenetic techniques e.g. in situ hybridization are being developed. These methods allow detection of specific sequences directly in fixed sections. There are several methods of in situ hybridization which differ according to the signal detection (fluorescent or chromogenic). We are currently optimizing a new method of chromogenic in situ hybridization, according to assay of Advanced Cell Diagnostic patented signal amplification. This method is automated with the use of Ventana Discovery Ultra system. It is a very useful method due to its high sensitivity and ability to detect a signal even in partially degraded material. It is also possible to map signal in individual cells, directly in dissected tissue. The greatest advantage of this method is that RNA extraction, which destroys a tissue context of gene expression measurement, is not required.

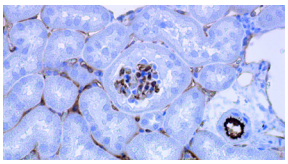
Specific signals are targeted due to a significantly supressed background noise from nonspecific hybridization. The strategy is in a ZZ probe design, which means a double Z series of probe, able to hybridize to target mRNA sequence. The robustness of the method is ensured by the up to 20 target probe pairs, each spanning 40 to 50 nucleotides along the target RNA molecule. The probe can be labelled fluorescently, visualised by epifluorescent microscope or conjugated to an Fast Red with alkaline phosphatase or horseradish peroxidase with DAB (3,3 diaminobenzidine), both for chromogenic reactions[3].

Our laboratory uses chromogenic labelling with DAB, which has the big advantage, the ability to examine the slides under a standard bright-field microscope, which is similar to IHC procedure. The presence of the certain sequence in mRNA is visible as a spot in cells, the result is scored according to a number of spots per cell. This method is very sensitive as it is possible to detect only one or two copies of specific nucleic acid sequence. Therefore, it is a unique method, applicable to the detection of viral agents and early stage cancer growth. The actual pathology here undoubtedly still holds an important place[4,5]. Firstly in the diagnostic role as it supports or refutes the findings, including detailed descriptions documenting these changes. Furthermore, in application and therapeutic level.

For a detailed description of our services and to organise an individual consultation visit our website http://www.phenogenomics.cz/phenotyping/







The state of the art equipment available at CCP (from left to right) Leica microtome, Ventana Benchmark special stains, Ventana Symphony - automated H&E stainer and coverlipper, kidney CD31 stain.

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Featured Service

Vision Screen

Barbora Antosova
Head of Vision Screen

The International Mouse Phenotyping Consortium (IMPC) was established to identify the physiological function of every gene in the mouse genome. This was made possible due to the extensive work done by the International Knock-out Mouse Consortium (IKMC), which systematically generated KO models for every mouse gene (over 20, 000). The vision screen is part of the Phenotyping Module of the Czech Centre for Phenogenomics (CCP). The vision unit specialises in the detection of various abnormalities including mouse eye morphology and eye physiology, which could affect both the quality and accuracy of vision. Our screen is currently focused on the mouse eye, however, we plan to broaden our screen to include the analysis of rat vision.

Here at CCP, we screen mutant mice lines according to IMPC guidelines, as a part of primary screen. Moreover, we can also offer other services, which are available to the scientific community, that are not included within the primary screen.

The main purpose of tests that are included in primary screen based on IMPC guidelines is to detect abnormalities in eye morphology. This includes optical coherence tomography and Scheimpflug Imaging. Currently the vision unit is equipped to perform optical coherent tomography and Scheimpflug imaging will also be available soon (as soon as the required equipment will be obtained).

In addition to services included in IMPC primary screen, the vision unit also aims to detect abnormalities in physiology of vision using electroretinography and virtual vision tests. The main advantage of these services is that the analysis of eyes and vision is performed *in vivo*. This enables the progression of a specific phenotype to be monitored, thus allowing the correct interpretation of the selected gene's function.

Highlighted here are services that vision unit will be able to provide at the end of 2016 based on available equipment.

Optical Coherence Tomography *(part of primary screen)***.** This non-invasive imaging procedure is used to examine the posterior part of the eye (retina and retinal blood vessels). An anaesthetised animal is placed on a platform and the spectral domain OCT, integrated with confocal scanning laser ophthalmoscopy (cSLO), is used to produce a detailed cross-sectional image of the retina and retinal blood vessels. This approach enables us to detect and analyze a wide range of mouse retinal pathologies, including changes in retinal thickness and layering, and in retinal vessel number or localisation. It is also possible to assess dynamic processes like edema formation or retinal degeneration.

Equipment: Spectralis OCT system (Heidelberg Engineering Inc., Heidelberg, Germany

Scheimpflug Imaging *(part of primary screen).* This test is used for *in vivo* imaging of the anterior eye (cornea and lens) and quantitative determination of lens transparency. It is ideal for studying cataract formation and can be used in longitudinal studies.

Electroretinography *(part of customised screen).* The electroretinogram (ERG) is a non-invasive diagnostic test to evaluate the function of various retinal cell populations in response to a light stimulus. The ERG can provide important diagnostic information on a variety of retinal disorders, and can also be used to monitor disease progression.

Equipment: RETI-port/scan 21 model RETIanimal (Roland Consult Stasche & Finger GmbH, Germany)

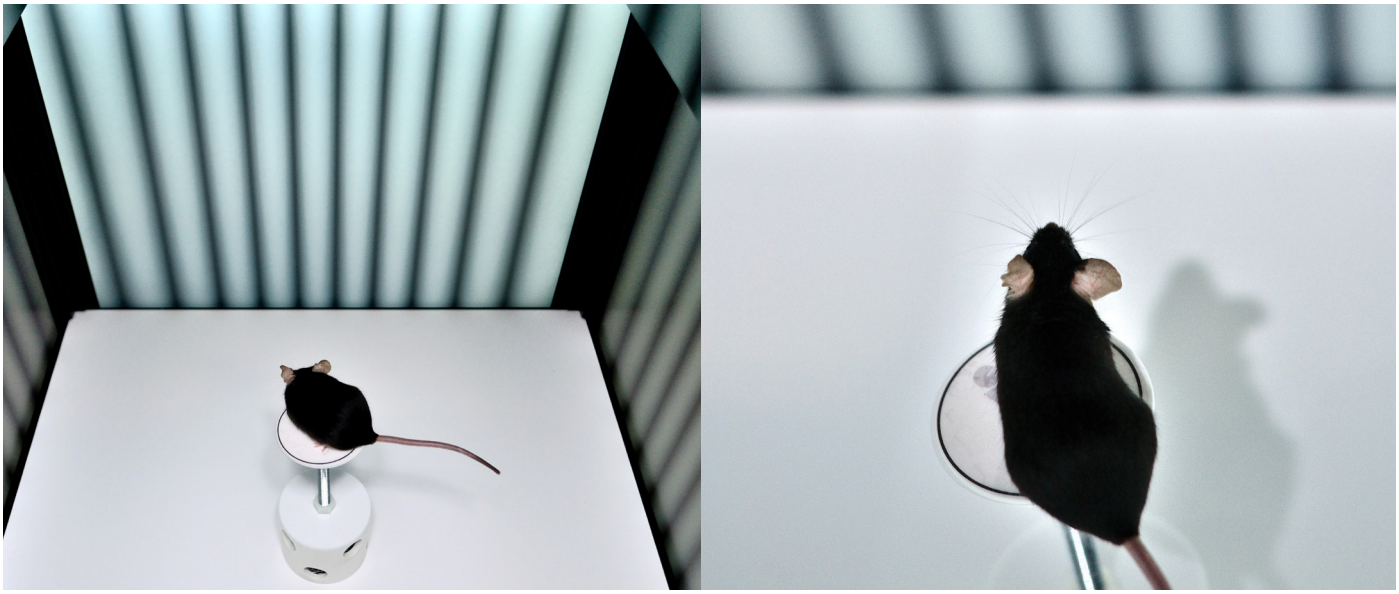
Spectralis OCT image of a wild-type mouse eye. Left: Image of fundus. Right: High resolution OCT scan of retina.

Virtual vision test *(part of customised screen).* This behaviour test provides a non-invasive functional analysis of visual performance in mice. The OptoMotry© system[1,2] uses the tracking of optokinetic head and neck movements, that are reflexive in the mouse for the screening of functional vision. By changing of threshold of spatial frequency, contrast, and motion of the grating, we are able to determine the visual acuity ("clarity of vision") of the tested animal. The advantage of this test is that animals with no previous exposure to the task can be tested and the measurements can be repeated regularly. This method can provide a powerful test of visual performance in genetically modified and pharmacologically treated mice.

Equipment: OptoMotry system (CerebralMechanics Inc., Canada)

A common output of described assays provides scientists a complex picture of eye morphology and function. For customised projects, the analysis can be focused on a specific test(s) and therefore on specific properties of eye and vision. Such a service can provide more detailed information about certain parameters of eye or vision.

For more information about the services we provide, visit our website http://www.phenogenomics.cz/phenotyping/



OptoMotry System. Mouse stands on an elevated platform in the epicenter of the arena and tracks the grating with reflexive head and neck movements.

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EMMA: The European Mouse Mutant Archive

Necessity and role of the European mouse repository

Inken M. Beck
Head of Transgenic and Archiving Module

The Czech Centre for Phenogenomics/ Institute of Molecular Genetics is partner of INFRAFRONTIER, a European non-profit research infrastructure for the development, systemic phenotyping, archiving and distribution of mammalian models[1]. INFRAFRONTIER (https://www.infrafrontier.eu) offers different services; among these the cryopreservation and distribution of mouse models under the European Mouse Mutant Archive (EMMA). The EMMA network consists of 16 members in 13 countries and is funded by institutional partners, national research programs and by programs of the European Commission. The Transgenic and Archiving Module (TAM) of the Czech Centre for Phenogenomics (CCP) represents the Czech node of EMMA.

The archive contains over 5000 mutant mouse lines and is one of the largest mouse repositories worldwide. The lines are cryopreserved as frozen embryos and/or sperm. These mutants carry targeted, transgenic, induced and other types of mutations. A major part of the repository consists of strains donated by individual researchers. In addition, EMMA has built up major collections from large-scale projects (e.g. IKMC/ IMPC). The collection includes more than 250 Cre driver lines, Flp deleter lines and lines using TET expression system.

CBS(TM) visotubes and goblet with High security straws for cryopreservation archiving.

The generation of a mouse model means to have developed a tool to study specific subjects with potential and hope to answer research questions, but it can generate also unwanted problems. In times of programmable nucleases, the generation of genetically engineered models has become easy and rapid as never before and often several founder animals are produced by applying one nuclease mixture in just one injection round. To run studies with all generated lines is practically and financially not feasible. After experiments are performed mouse lines are not in use anymore and space in animal facilities is often limited. Researchers who are generating the mice often have no possibility to maintain them and to breed them appropriately. Mice are susceptible to pathogens that can influence the phenotype, and reduced breeding performance can lead to the loss of a line. To keep models on a specific background is essential when expecting a certain described phenotype. To deposit mouse models to a repository (e.g. to EMMA) can circumvent problems and reduces these risks.

EMMA's primary objective is to establish and manage a unified repository for maintaining medically relevant mouse mutants and making them available to the scientific community. Strain submission can be done by the owner of a mouse strain or by a third party with permission to donate it to an archiving repository. While submitting a line to EMMA the owner keeps full intellectual property rights, which means any existing MTA will maintain its validity. EMMA developed and implemented SOPs for quality control, archiving and distribution of frozen material and live mice. All archived material and live mice are specified pathogen free (SPF) in compliance with FELASA guidelines (http://www.felasa.eu/). The archiving services are free of charge, only shipment costs for mice or frozen material to the archiving center must be paid by the depositing laboratory. The submitted line will be evaluated by an external committee and archived by one of the EMMA facilities, e.g. in our archiving module in Prague. Submitted mouse lines are freely available to the research community. If necessary, the owner can request a grace period during which, that the line is not available to order, but can be archived.

To deposit a mouse model to EMMA/ INFRAFRONTIER means

- To receive high quality cryopreservation free of charge
- Intellectual property rights are secured
- To contribute to the progress and development of the scientific community
- To archive and obtain models under SPF (Felasa) conditions
- To increase the visibility of a mouse line and trigger potential collaborations

The INFRAFRONTIER website is EMMA's central interface to the research community where strains can be searched for and ordered. The models are distributed to qualified laboratories solely for research purposes only when existing MTA is fully executed by the owning and requesting institution. Cryopreserved strains may be exported as frozen material or rederived upon request. Popular strains actively bred can be provided in short time.

Cryopreservation storage tanks

Besides archiving and distribution, training and development is another key objective of EMMA. During the year, hands-on cryopreservation courses are offered that focus on sperm and embryo cryopreservation, as well as operation techniques and transport and storage solutions for frozen and refrigerated material. Thanks to improved sperm freezing methods especially on C57Bl/6 background[2] that were adapted to EMMA's SOPs, more strains are archived using spermatozoa, which needs fewer animals for preserving one line compared to embryo freezing. Time and mice used for quality control was also reduced by establishing blastocysts genotyping[3].

Due to optimized shipping solutions, EMMA is shipping more and more frozen material than live mice. Overall, to cryopreserve a line, less mice are used, which supports for the principles of the 3Rs: replacement, reduction and refinement, and is in compliance with EU directive 2010/63/EU **on the protection of animals used for scientific purposes**.

EMMA and other international mouse mutant archives like JAX and MMRRC in North America and RIKEN and CARD in Asia are interconnected via the International Mouse Strain Resource (IMSR, http://www.findmice.org/). EMMA is the primary mouse repository here in Europe and offers high quality standards for the archiving and distribution of mouse models.

So, as the CRISPR revolution is speeding up the process to generate transgenic mice, repositories such as EMMA are fast becoming a necessity, not only to safeguard lines from pathogens and genetic drift, but also to relieve the financial burden associated with maintaining a transgenic line.

USEFUL LINKS:

EMMA Respository Interface
https://www.infrafrontier.eu

International Mouse Strain Resource, IMSR
http://www.findmice.org/

Federation of International Mouse Resources
http://www.fimre.org/

Nomenclature guides
http://www.informatics.jax.org/mgihome/nomen/index.shtml

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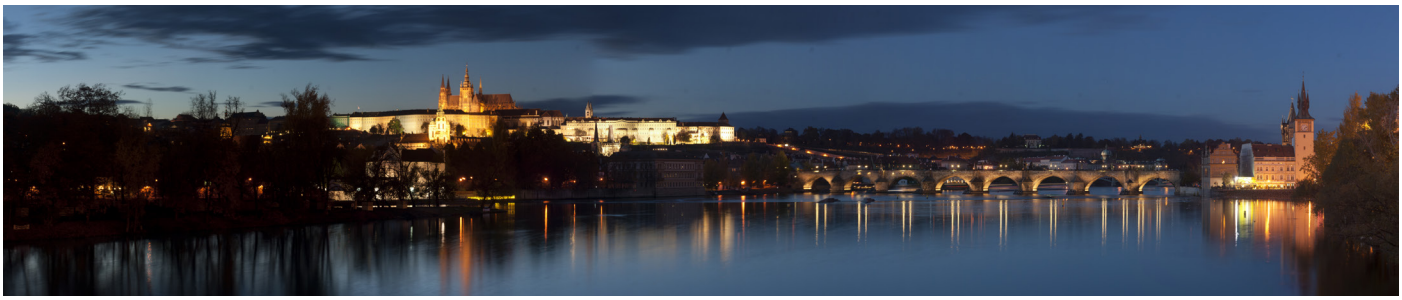
Careers



CCP comprises a young, multidisciplinary and international team. We believe in the personal and professional development of our staff and seek, where possible, to facilitate the attendance of relevant conferences and courses. We offer a competitive salary and various working contracts. Please visit www.phenogenomics.cz for current vacancies.

Deputy Director

The Deputy Director will work with the Director, heads of modules and units, and other senior staff members to ensure efficient and effective delivery of the professional services and co-lead Phenotyping module.
Together with the director, the successful candidate will work with the multi-disciplinary and international team to establish and expand the services for the domestic and international scientific community. This will include strategic planning, grant proposal preparation, and management of scientific/research cooperation. The Deputy Director will also work with staff within the centre to monitor overall activity and will be responsible for developing and implementing performance policies.

The successful candidate should have extensive research management experience including generating funding, generating and implementing policies and should be able to communicate at all levels within the centre. Excellent communication skills in English and Czech (written and spoken) are also essential. A proven track record in physiology or related field would be advantageous, but is not essential.

Application should include CV mentioning relevant professional achievements, list of publications, cover letter and 3 letters of recommendation.

Technical Assistants

We are seeking talented and motivated technical assistants to work as part of our Phenotyping Module.

Candidates should be highly motivated, well organized and capable of working as part of a team. Candidates should also possess a BSc in biological sciences (or related field) and have a good command of English (spoken and written). Previous rodent handling experience would be advantageous but is not essential.

Successful candidates will work primarily in one unit, however, there will be opportunities for cross training with other units within the Phenotyping Module.

Applications for this position should be made in English and should include a motivation letter, CV and two references. The positions are available immediately, and applications should be sent to Libor Danek

Junior research position in phenotyping pipeline

The open position is focused on the standardized phenotyping of generated transgenic lines with aim to reveal and annotate unknown functions of genes from systematic production of KO lines within International Mouse Phenotyping Consortium.

The current open position is for vision screen & research unit. The workflow of the unit consists of standardized measurement focused on eye morphology and function. The junior researcher will also have the opportunity to carry out their own research project on eye physiology, morphology, or development areas.

The Czech Centre of Phenogenomics offers state of the art research equipment and a very stimulating, multidisciplinary environment encompassing all aspects of mouse molecular genetics (from mutant generation to complex phenotyping). The attendance of relevant scientific meetings and conferences is also encouraged.

IT specialist / Database manager

We are seeking a qualified and motivated database manager to maintain and develop our MausDB software. This database is a web-based CGI application which is built on Linux, Apache, MySQL and Perl (LAMP). This database employs Perl with some additional R (www.r-project.org) scripts. The successful candidate will support the specific needs of the application, including data handling, scripting, creating templates, setting up workflows, fixing errors and educating users. He/she will also strive to develop the software in collaboration with our partners in Munich.

The position is available immediately for a fixed-term of 1 year with the possibility for long-term employment.

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

Cell Symposium: 10 years of iPSCs

25th - 27th September, Berkeley, USA

http://www.cell-symposia-ipscs.com/

INFRAFRONTIER-I3 / Mouse Metabolic Phenotyping Training Course

10th - 13th October, Munich, Germany

https://www.infrafrontier.eu/

EMBO | EMBL Symposium — Organoids: Modelling organ development and disease in 3D culture

12th - 15th October 2016, Heidelberg, Germany

http://www.embo.org/events

2016 IMB Conference: Epigenetics in Development

20th - 22nd October 2016, Mainz, Germany

http://www.imb.de/2016conference

Precision Genome Engineering (A2)

January 8 - 12, 2017, Breckenridge, Colorado, USA

http://keystonesymposia.org/index.cfm?e=web.Meeting.Program&meetingid=1461

Journal Club

1. Stappenbeck TS, Virgin HW. Accounting for reciprocal host-microbiome interactions in experimental science. *Nature.* **534**, 7606 (2016)

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