Issue 1 / Winter 2015

PHENOGENOMICS
NEWSLETTER

13TH TRANSGENIC TECHNOLOGY MEETING

CCP WHO WE ARE & WHAT WE DO

HISTOPATHOLOGY IN ACTION

TRANSGENIC AND ARCHIVING MODULE

Czech Centre for Phenogenomics

Czech Centre for Phenogenomics

IMG

13th Transgenic Technology Meeting (TT2016)
Prague, Czech Republic

Practical training courses

The Czech Centre of Phenogenomics is delighted to sponsor 3 hands on workshops:

1. Cryopreservation and Archiving (16th – 18th March 2016)
2. Programmable Nucleases (CRISPR/Cas9) Transgenesis (16th – 18th March 2016)
3. Fish Transgenesis (23rd – 25th March 2016)

These workshops have been designed to allow participants to gain practical experience in the most recent techniques and speak to leading experts in the field of transgenesis and cryopreservation.

Application dead line: 15th January 2016

Announcement of acceptance: 25th January 2016

Applicants need to be registered at the TT2016 conference. Participation on the course is subject to a selection process. Applications must be accompanied by a short CV, letter of motivation (max 200 words), and a short letter of support.

Accommodation details and other particulars will be communicated to selected applicants.

Applications should be sent to the following email address:

tt2016-workshops@guarant.cz

For more information and specific course details visit our webpage:
http://www.transtechsociety.org/tt2016/scientific-programme/workshops/

charles river

eppendorf

ZEISS

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IMPRINT

Editorial team
Radislav Sedlacek *(Director)*
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PRINTER: AMOS TYPOGRAFICKE, PRAHA 4

Note to customers: As valued customers, we welcome your articles and feedback on the service you received. Please send all correspondence to nicole.chambers@img.cas.cz
The editorial team would like to thank the authors in this Issue for their contribution.

Cover photo: Main entrance of the CCP building (photo taken by Nicole Chambers)

13TH TRANSGENIC TECHNOLOGY MEETING

March 20–23, 2016 | Prague, Czech Republic

Invited Speakers

Tomomi Aida *(Japan)*
Jeff Batton *(USA)*
Richard R. Behringer *(USA)*
Ethan Bier *(USA)*
Thomas Boehm *(Germany)*
Aurora Brønstad *(Norway)*
Mary E. Dickinson *(USA)*
Denis Duboule *(Switzerland)*
Nicholas Gale *(USA)*
Charles A. Gersbach *(USA)*
F. Kent Hamra *(USA)*
Ralf Kühn *(Germany)*
K.C. Kent Lloyd *(USA)*
Robin Lovell-Badge *(UK)*
Yonglun Luo *(Denmark)*
René Maehr *(USA)*
Francisco J.M. Mojica *(Spain)*
Lluis Montoliu *(Spain)*
Andras Nagy *(Canada)*
Masato Ohtsuka *(Japan)*
Konstantin Severinov *(USA)*
John Schimenti *(USA)*
Didier Stainier *(Germany)*
Michelle Stewart *(UK)*
Toru Takeo *(Japan)*
Haoyi Wang *(China)*
Ron Weiss *(USA)*
Eckhard Wolf *(Germany)*
Bernd Zetsche *(USA)*
and others

Hands-on Workshops

Mouse cryopreservation workshop – **March 16–18, 2016**
CCP programmable nucleases (CRISPR/Cas9) Transgenesis Course – **March 16–18, 2016**
Zebrafish genome editing workshop – **March 23–25, 2016**

Key Dates

Application for ISTT registration awards – **December 15, 2015**
Abstract submission deadline – **December 15, 2015**
Awards to be communicated by – **January 14, 2016**
Application deadline for Workshops – **January 15, 2016**
Early Bird registration fee deadline – **January 15, 2016**
Workshops announcement of acceptance – **January 25, 2016**

Meeting Secretariat

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ISTT
Czech Centre for Phenogenomics
IMG
BIOCEV

www.tt2016.org

Message from the director

Dear Readers,

I am pleased to introduce our inaugural issue of “Phenogenomics newsletter”, the new quarterly newsletter of the Czech Centre for Phenogenomics (CCP). This issue coincides with moving into our custom built premises in the BIOCEV campus, a major milestone in the development of our research infrastructure. While I write these words, our staff are busy installing equipment in the new building to continue our service to the research community. It is an exciting time and represents a milestone for preclinical research in the Czech Republic.

CCP, now one of the largest European research infrastructures for supporting genetically-modified rodent (mouse and rat) research, is hosted by the Institute of Molecular Genetics and partly embedded in the BIOCEV-project (Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec*). We provide the international research community with a full spectrum of genetic engineering services. These include the generation of gene modified mouse models of diseases, cryopreservation (archiving) and distribution including import and export, advanced phenotyping and imaging modalities as well as specific pathogen free (SPF) animal housing and husbandry. Although CCP has established a comprehensive portfolio of services, we strive to further enlarge the service portfolio - so that the domestic and international community use the service for high impact research and applications.

The New Year will bring another significant event to our organization as we have the honour of hosting the 13th Annual Meeting of the International Society of Transgenic Technologies, a meeting which will bring to Prague, leading international experts in genetic engineering. Associated with the meeting will be three hands-on workshops enabling researchers to gain important practical skills in cryopreservation and genome editing, both in mice and in fish.

This first issue generally highlights our infrastructure, services, technologies as well as our involvement in coordinated international research efforts. Future issues will include news related to recent and new technologies and services as well as relevant scientific news. It will include commentary and opinion from our in-house team of scientific experts on a wide range of subjects, as well as editorials, commentaries and interviews from our growing list of clients and partners.
We look forward to working together to further improve the quality your scientific research.

Radislav Sedlacek
Director of the Czech Centre for Phenogenomics

*** CCP is funded by:**

- BIOCEV - Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University“ (CZ.1.05/1.1.00/02.0109) from the European Regional Development Fund and state budget of Czech Republic

- Higher quality and capacity for transgenic models (CZ.1.05/2.1.00/19.0395) from the European Regional Development Fund and state budget of Czech Republic

- Infrafrontier-CZ/Czech Centre for Phenogenomics as a national centre of “The European infrastructure for phenotyping and archiving of model mammalian genomes”: Integration of the Czech national centre into international network (LM 2011032) by Ministry of Education, Youth and Sports of the Czech Republic

-INFRAFRONTIER-I3 Development of mouse mutant resources for functional analyses of human diseases - Enhancing the translation of research into innovation (Project reference: 312325) by Horizon 2020

- IPAD-MD - Research Infrastructures for Phenotyping, Archiving and Distribution of Mouse Disease Models - Promoting International Cooperation and User Engagement to Enhance Biomedical Innovation (Project reference: 653961) by Horizon 2020

CCP WHO WE ARE & WHAT WE CAN DO FOR YOU

A brief review of CCP organisation

The Czech Centre for Phenogenomics (CCP)/ Infrafrontier-CZ is the only site in the Czech Republic with expertise and capacity for large-scale genetically-modified model rodent generation and advanced phenotyping. This expertise in combination with high breeding capacity under specific pathogen free (SPF) conditions and cryo-archiving and recovery services, secures Czech and international customer access to services and expertise at a level comparable to leading research institutions in Europe.

The generation of genetically modified rodents is a technologically demanding and costly procedure. Prior to 2008, the year when we set up the first transgenic core facility in the Czech Republic, Czech scientists had to employ centers abroad. Thus, CCP addresses the needs of Czech science to secure capacity in an era when global capacity (especially for SPF housing) is under severe constraint, and where broader systems-level analyses are increasingly required for publication in high-impact factor journals. We are continually building upon our good track record of supporting the research community.

CCP has established (and is continuing to develop) a broad range of technologies that are divided in three basic areas: animal model generation, housing and breeding, and phenotyping.

TAM (Transgenic and Archiving Module)
CCP has established a comprehensive technology portfolio that is fully comparable with any world-class laboratory in this specific area. Moreover, we have invested lots of effort into the development of new technologies, specifically the technology of “programmable nucleases” such as “TALEN and CRISPR/Cas9” assisted gene targeting and genome editing, which substantially improved our services in custom-tailored targeting projects, saving cost and time. Technologies provided by TAM include embryonic stem (ES) cell derivation and manipulation, development of targeting strategies and tools, generation of transgenic animals including conditional knock-out and knock-in models, genotyping, cryo-archiving, export/import/distribution, consultancy support, and administrative support (GMO licenses).

AFM (Animal Facility Module)
The animal facility is based on the newest developments in housing and breeding of mice and rats. We have one of the most progressive animal facilities with regards to logistics, versatility and consideration for animal health. Technologies employed include housing/ breeding animals in individually ventilated cages (IVC), barrier system and one-way flow of material and animals, a health monitoring system, and advanced software-assisted management of animal facility operations.

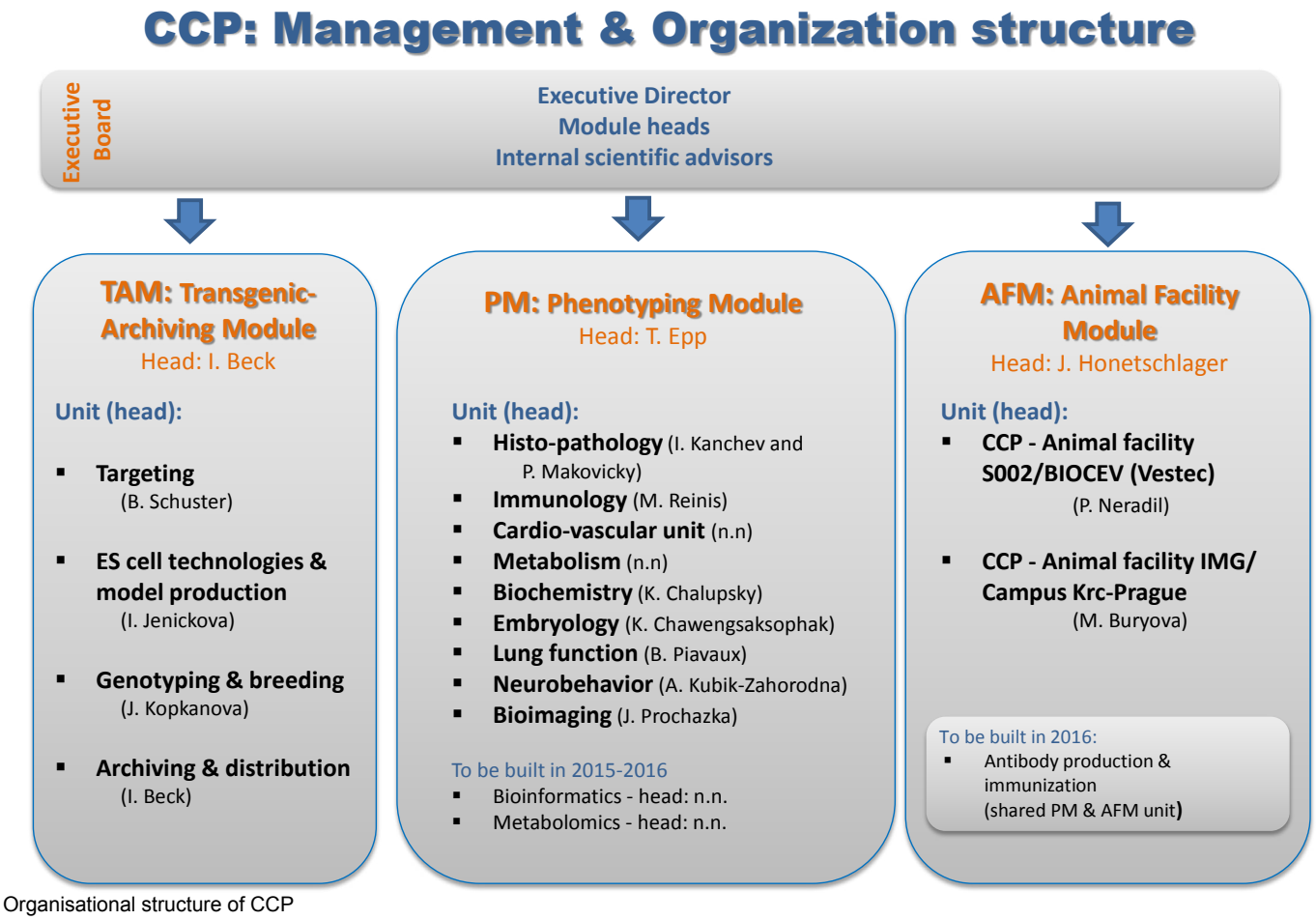
PM (Phenotyping Module)
The phenotyping module constitutes a compendium of technologies and expertise for investigating major biological systems. The technologies accompanying specific services are grouped into units: 1) histopathology (provides comprehensive services in processing of tissue samples including imaging and histologic evaluations), 2) metabolism, 3) biochemistry and hematology, 4) cardiovascular function, 5) lung

function, 6) embryology, 7) Bioimaging, and 8) neurobiology and behaviour. Each of these units with their specific sets of technology creates a unique collection of expertise in a single location. In total, available technologies enable the collection of more than 400 parameters for each phenotyped animal; the portfolio of services and parameters is expected to develop further.

Technology development

We have adopted the revolutionary application of programmable nucleases including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas RNA-guided nucleases for the faster and more efficient generation of genetically modified animals compared to "conventional" methodologies employing ES cells. These site-specific nucleases can be designed to cleave genomic DNA at preselected sites, which significantly increases the frequency of homologous recombination (HR) events in the targeted locus. Importantly, programmable nucleases can facilitate targeted mutagenesis in zygotes where the frequency of HR between exogenous DNA and intact chromosomal loci is too low to be employed for efficient gene modification without these new molecular tools.

We have established this new technology and developed additional tools and methodologies to further improve its efficiency. CCP can thus offer a comprehensive service using this new technology: the only thing that we need is your gene and a description of the mutation or type of targeting desired.



Organisational structure of CCP

Global Goals

The International Mouse Phenotyping Consortium (IMPC) was launched in September 2011 with the vision to 'produced a comprehensive encyclopaedia of mammalian gene function.' In 2014 CCP/IMG joined this global consortium. As an institutional member of IMPC, CCP is dedicated to the goal of 'systematic genome-wide phenotyping project of knock-out mice in order to provide the wider research community with a lasting resource of mammalian gene function.' Along with the other 17 members, CCP contributes to IMPC's mouse production using ES cell resources and CRISPR-based targeting strategies. All mouse lines generated at CCP under the IMPC will be archived and open for distribution via Infrafrontier/ EMMA.

More information can be found at
www.mousephenotype.org/

'One of the most important tools at our scientific disposal in understanding mammalian gene function is the laboratory mouse.' IMPC

CCP & MERCK COLLABORATION

Björn Schuster, PhD
Head of Targeting Unit

Since the early 1980s mouse genome editing has been used as a way of studying gene function (Gordon J et al 1981). Whilst mouse transgenesis made great progress thanks to the work of Frank Ruddle (Gordon JW et al 1980) and Mario Capecchi (Thomas KR et al 1987), the discovery and application of enginerable nucleases such as ZFNs and TALENs and recently the CRISPR/Cas9 technology has revolutionized genome editing. This technology has made custom genome modifications in cells or animals more efficient, reliable and less time consuming compared to conventional methods. In this system, a short guide RNA (gRNA) directed to the genomic target site together with the Cas9 endonuclease mediate a DNA double strand break at a specific genomic sequence. This site is then available for subsequent repair by non-homologous recombination or it can serve as insertion site for exogenous DNA by homologous recombination.

Now, with this new technology, customized genomic alterations can be achieved more consistently and confidently in as little as six weeks to model human conditions in rodents. Thus, employing the latest developments in gene editing the Transgenic Module at CCP helps to accelerate the pace of translational research allowing scientists to get more reproducible outcomes in their experiments in a fraction of time.

In January 2015, CCP entered into a new collaboration with Sigma-Aldrich Corporation (now part of the MERCK group) as industrial partners (www.sigma.com/crisprs). As part of the collaboration, Sigma-Aldrich will provide the Transgenic Module at CCP with Sigma CRISPR technology, including reagents, experimental design consultation and dedicated gene editing bioinformaticians. This collaboration will allow our module to achieve and maintain the highest standards and to provide the best services to the International scientific community.

We look forward to assisting you with your next project.

For a comprehensive list of our services or to request a customised quote visit our dedicated page
www.phenogenomics.cz/model_generation_services/

References:
Gordon, J.; Ruddle, F. (1981). "Integration and stable germ line transmission of genes injected into mouse pronuclei". Science 214 (4526): 1244–6.
Gordon, J.W., Scangos, G.A, Plotkin, D.J., Barbosa, J.A. and Ruddle F.H. (1980). "Genetic transformation of mouse embryos by microinjection of purified DNA". Proc. Natl. Acad. Sci. USA 77 (12): 7380–7384.
Thomas KR, Capecchi MR (1987). "Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells". Cell 51 (3): 503–12.

Animal facility - new cages

Jan Honetschläger
Head of Animal Facility

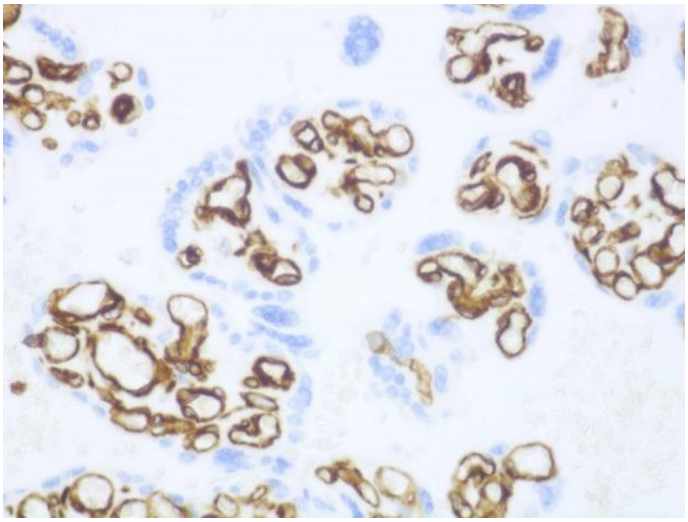
The CCP animal facility contains 5 individual, fully separated breeding and experimental barrier areas. Each barrier includes modern devices like big volume steam sterilizers and H_2O_2 chambers , air and wet personal areas and pass through boxes, modern and eco-friendly HVAC (heating, ventilating and air conditioning) technology. All of these important devices help to keep the “clean” side of the SPF barrier. The animals are protected again at the cage level with individually ventilated cages and with state-of-the-art digital ventilated cages. These cages significantly increase the animal welfare level and animal facility efficiency, but they are also useful for research as they increase the detection of strange behavior (sick or wounded animals), reduce animal stress and provide continuous information about animal activity. The hub of the animal facility is washing area equipped with modern semi-automated tunnel cage washer, bottle washer and rack washer including waste disposal and bedding dispensing vacuum system to increase the in-house biosecurity.

GM 500 IVC cages

CCP HISTOPATHOLOGY IN ACTION

Ivan Kanchev & Peter Makovicky
Heads of Histopathology Unit

The histopathology unit was established in 2012 as an integral part of CCP, focusing on mouse pathologic anatomy and histopathology evaluation. Additional activities in the early days of the unit were included validation and establishment of novel methods including special stains and immunohistochemistry.



Placenta - Immunohistochemistry for Vimentin

Initially the unit consisted of two technicians and one morphologist and used the already established histology laboratory at IMG. In 2014 the unit relocated to a new laboratory in Vestec and also received its new equipment. Today, the team consists of four technicians and two morphologists. We are equipped with state-of-the art instruments enabling us to deliver a total histology solution: from necropsy, through to standard and special stains, immunohistochemistry, completed with analysis and reporting. The mouse organ sampling is standardized according to the International Mouse Phenotyping Consortium guidelines (https://www.mousephenotype.org/impress/protocol/181/7).

Necropsies and organ collection are performed on a dedicated necropsy platform with the ability for image documentation. Processing is done on the latest generation automatic vacuum tissue processor (Leica ASP6025), allowing full control of each step of processing and automatic turnover of the reagents. The microsections are done by four technicians operating fully motorized microtomes and cryostats.

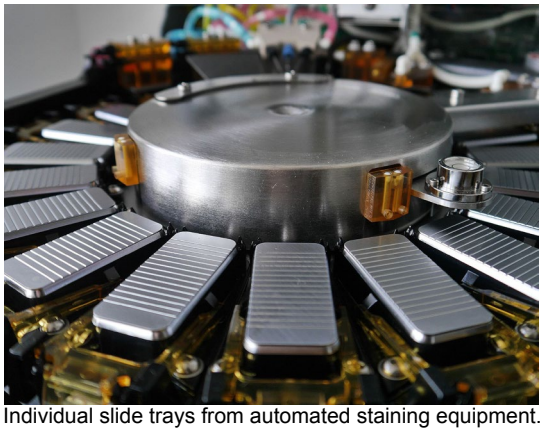
Cryostat

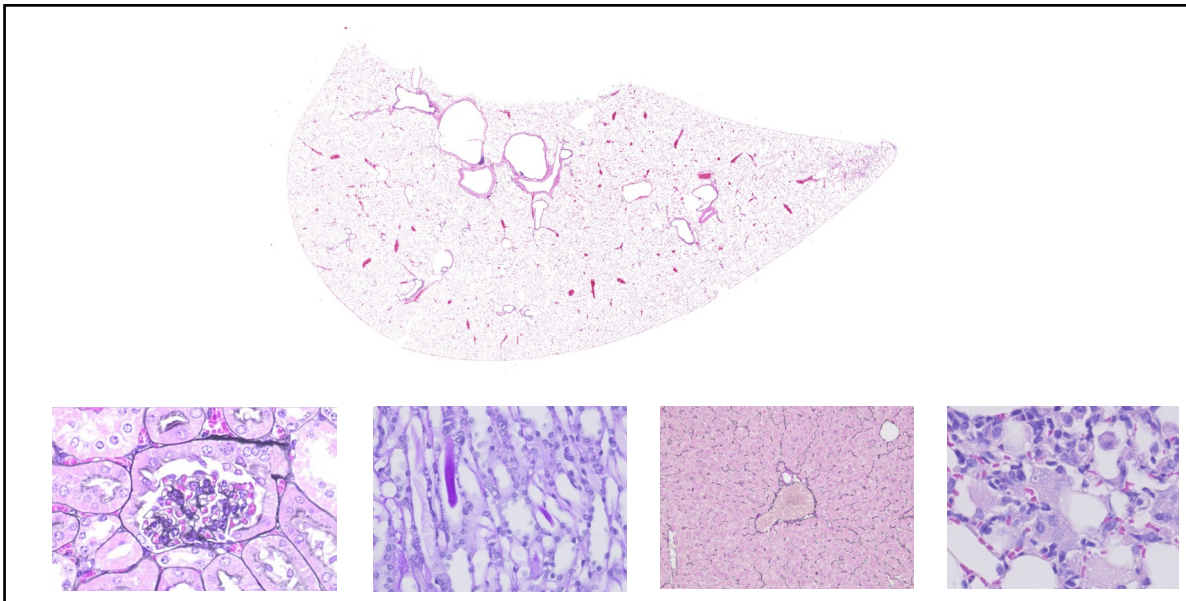
All staining platforms are automated, eliminating the risks of sample cross-contamination and inappropriate results.

Our work can be divided into 2 parts: 1- Internal support for IMG research scientists and 2- Support for external customers. The department has experience in experimental diseases investigation using established procedures. Each sample is specified by a unique request ID therefore allowing tracking of the samples and most importantly, accurate processing of the samples.

Going forward, the histopathology department seeks to maintain the internal support for IMG researchers and work closely with external customers and therefore facilitating the understanding of gene function. The laboratory also offers traineeships, including technical traineeships for people working in veterinary histopathology. The department also plans to develop educational activities that might be suitable for industry and academic institutions. Our mission is to deliver quality and innovation, therefore we regularly introduce novel techniques and analytical methodology.
For a comprehensive list of our services or to request a customised quote visit our dedicated page www.phenogenomics.cz/phenotyping/

Fully automated H&E slide staining


Individual slide trays from automated staining equipment.



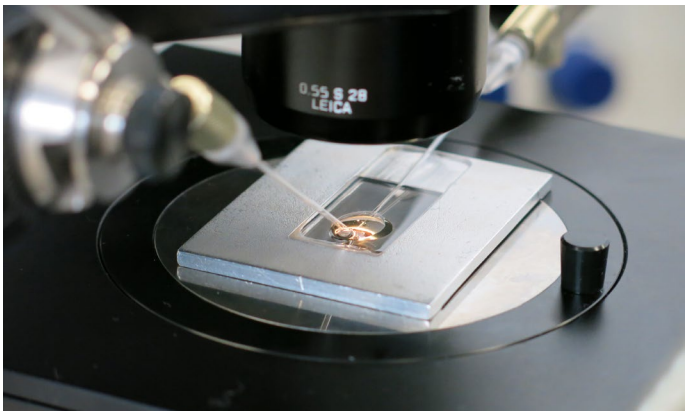
Top: Rat lung full scan. Bottom panel (left -right): Mouse Kidney - Jones stain, Kidney collective ducts Hyaline cylinders - PAS reaction, Liver Triad - Retcular stain, Lung Pneumocystis spp infection.

FEATURED MODULE

MODEL GENERATION & ARCHIVING UNDER ONE ROOF

Inken M. Beck
Head of Transgenic and Archiving Module

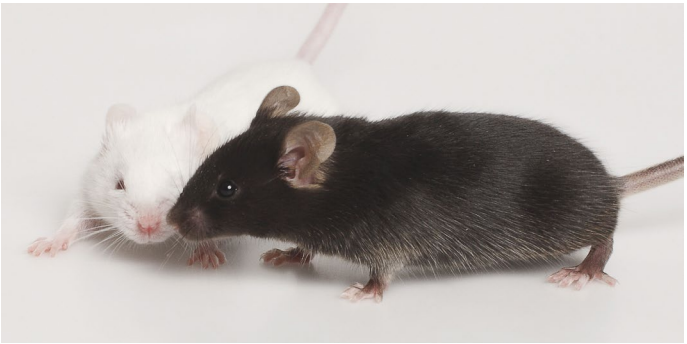
The Transgenic and Archiving Module (TAM) of the CCP, Czech Centre for Phenogenomics (http://www.phenogenomics.cz/), is combining mouse model generation, archiving and distribution under one umbrella. Our unit was established in 2008/2009 under the supervision of Radislav Sedláček. With a small set of equipment and with low experience in embryo handling techniques we started with three technical assistants and tried our first embryo implantations. Transgenic core facilities in Dresden, Germany, and Kuopio, Finland, supported us as we developed standard techniques. To generate classical transgenic models we focused on PNI (pronuclear injection) in the beginning and subsequently strengthened our work with mouse embryonic stem cells (ESCs) and their injection into blastocysts to produce chimeric mice. From the beginning we performed also cryopreservation of mouse embryos and spermatozoa for archiving purposes.



Initially models were generated for research groups of the IMG, but as the only transgenic facility in Czech Republic, requests for model production and cryopreservation from external customers increased quickly. In 2013/2014 we included a targeting and genotyping unit in our module, which increased our workforce to its currently level of twelve including three heads overviewing and organizing the archiving, genotyping and targeting work.

Transgenic generation

We offer a comprehensive customized service for model production using programmable nucleases which starts with a consultation with the investigator to specify the research need with the aim to fit best the researcher's expectations. The mouse model production process includes targeting design, nuclease activity testing in vitro using a mouse cell culture based assay and injections into fertilized oocytes to produce the founder generation. Once founder mice are confirmed by genotyping we can breed to the next generation in order to confirm germ line transmission of a specific mutation. Whilst the C57Bl/6N is used as the standard genetic back ground, we are able to generate models on various backgrounds.



OUR SERVICE COMPRISES:

- Classical plasmid and BAC transgene generation using PNI (pronuclear injection)
- Mouse model generation using programmable nucleases (TALENs, CRISPR/Cas9)
- Mouse model generation using ES cells
- Embryo and sperm cryopreservation, and reanimation of strains from frozen material
- Ovary transplantation
- Rederivation/ cleaning of mouse strains
- Genotyping service
- Import/ Export arrangements (together with the animal facility module)

Murine embryonic stem cells (ESCs)

For mouse model generation using embryonic stem cells, we accept ESC clones generated by individual labs as well as clones coming from large repositories as KOMP (Knockout Mouse Project, UC Davis, USA) and EuMMCR (European Mouse Mutant Cell Repository, Helmholtz Zentrum, Munich, Germany). We routinely inject into 8-cell stage embryos using laser-assisted techniques to open the embryo's surrounding zona pellucida. To culture ES cells before injection standard as it allows us to observe cell proliferation and get an overall impression of each specific clone. Each cell clone will be archived in our ESC library and undergoes a quality control that includes mycoplasma screening and chromosome count. Only "happily" grown cells free of pathogens and with correct number of chromosomes can be pluripotent and possess the potential to result in chimeras and germ line transmission.

In the CCP we can offer to inject ESCs into embryos from black- (C57Bl/6) and white-haired (B6(Cg)-Tyr/J) strains in order to have the opportunity to use ES cells originated from various strain backgrounds.

All embryos generated in our module are transferred into pseudo-pregnant foster mice housed in a clean barrier of the animal facility. All barriers in the facility are monitored according to Felasa guidelines, Federation of European Laboratory Animal Science Associations (http://www.felasa.eu/). This allows us to be confident of the health status of the models we provide to investigators.

Archiving

The cryopreservation and archiving of research models is as important as their production. In order to protect strains from accidental loss, pathogen contamination and genetic drift, to reduce animal housing cost, and to efficiently distribute them around the world, it is important to cryopreserve these valuable genetic resources.

Sperm cryopreservation is the most widely used method of choice for C57Bl/6 strains (probably the most widely used background). Thanks to technical improvements and studies on mouse sperm freezing during last 10 years, stable methods for cryopreservation and reanimation have been developed (Ostermeier et al., 2008; Takeo et al, 2011; Guan et al, 2014).

IMG has been a partner of Infrafrontier/EMMA (https://www.infrafrontier.eu/) since 2010. EMMA (European Mouse Mutant Archive) is a non-profit repository for the collection, archiving and world-wide distribution of relevant mutant strains essential for biomedical research and was established to create capacity for well-coordinated archiving and world-wide distribution. This repository has 16 partners and CCP officiate as the Czech node of EMMA. Submission and archiving of mouse models is free of charge and individual research models as well as those coming from large scale production (IMPC) can be found in the archive. For every frozen strain the quality control is essential in order to be able to reanimate the strain as required. Only when the quality control is passed is the strain fully archived. Of course, alongside EMMA archiving our module offers separate cryopreservation of lines for individual use as well.

Featured module

Furthermore, our module provides services for rederivation, means cleaning of pathogen infected strains. Contamination of a mouse colony with bacteria, viruses or parasites can affect research results and phenotypes, thus, we prefer and recommend to work with specified pathogen-free animals that are available from our production site. Regardless of the amount of samples, our genotyping service helps to identify a specific mutation in short time in order to plan further breeding or experiments in advance. We are open to run full genotyping service or only to isolate DNA and send it to the researcher.

The services from our module are flexible and can be arranged individually for the researcher's need. In the CCP customers have the fantastic opportunity to have model production, archiving and phenotyping consolidated under one umbrella.

For a comprehensive list of our services or to request a customised quote visit our dedicated page www.phenogenomics.cz/model_generation_services/

References

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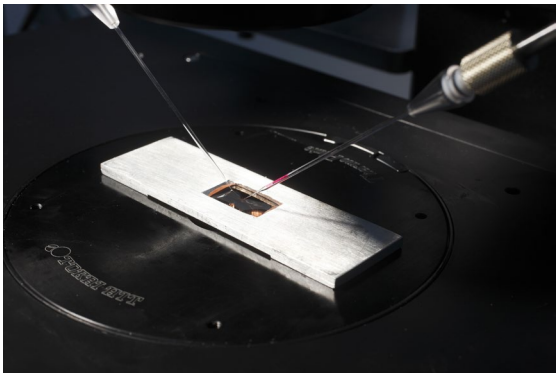
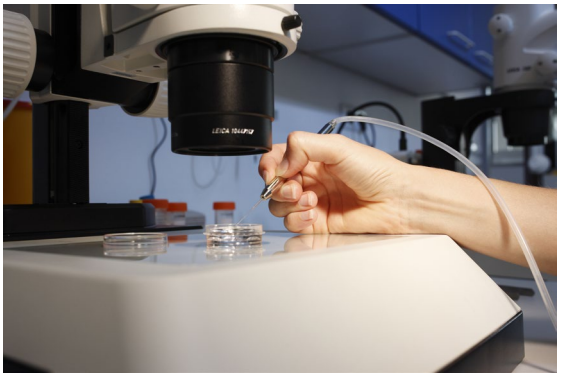
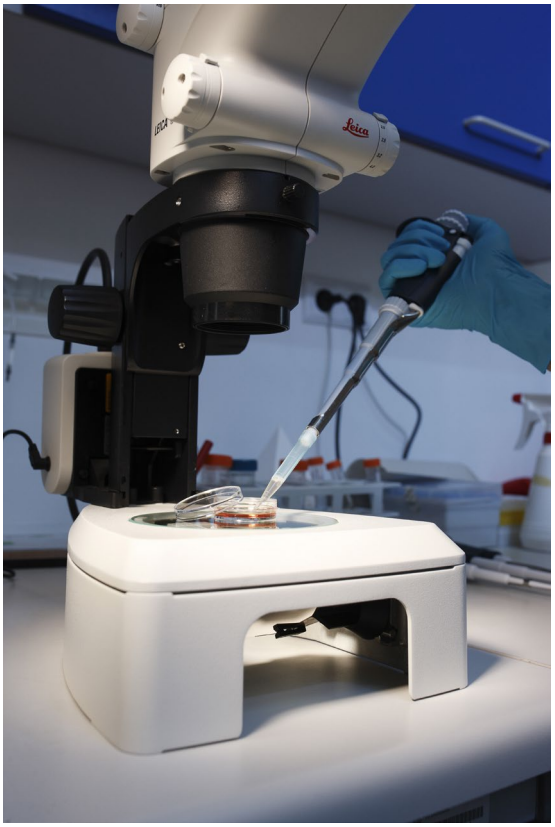
Guan M, Bogani D, Marschall S, Raspa M, Takeo T, Nakagata N, Fray M. In vitro fertilization in mice using the MBCD-GSH protocol. Curr Protoc Mouse Biol. 2014 Jun 16;4(2):67-83. PMID: 25723919







Up coming Events

13th Transgenic Technology meeting 2016 20th - 23rd March 2016, Prague CR
http://www.transtechsociety.org/tt2016/

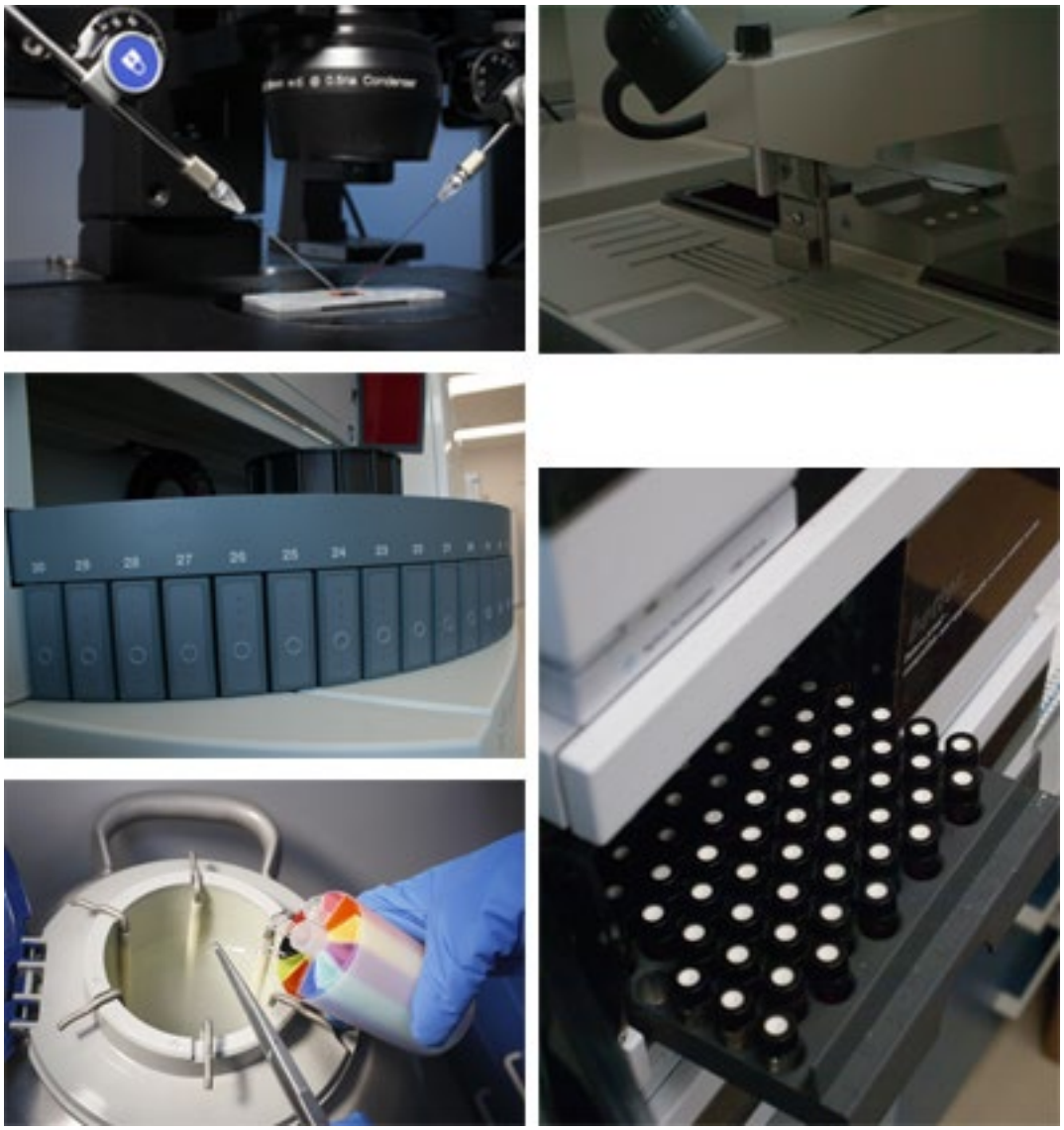
13th Transgenic Technology nuclease and Cryo workshop 16th - 18th March 2016, Prague
http://www.transtechsociety.org/tt2016/scientific-programme/workshops/

13th Transgenic Technology Fish transgenesis course 24th - 25th March 2016, Prague
http://www.transtechsociety.org/tt2016/scientific-programme/workshops/

13th Transgenic Technology Meeting 20th - 24th March 2016, Prague
http://www.transtechsociety.org/tt2016/

IEEE Internationals symposium in Biomedical imaging 2016 13th - 16th April 2016, Prague CR
http://www.biocev.eu/en/event/ieee-international-symposium-on-biomedical-imaging-2016/

Genetic Engineering of Mammalian Stem Cells 11-23 April 2016
Application and Bursary Deadline: 6 November
https://registration.hinxton.wellcome.ac.uk/events/item.aspx?e=538



THE CZECH CENTRE FOR PHENOGENOMICS

CCP IS HOSTED BY BIOCEV & IMG

BIOCEV

IMG