Issue 2, Spring 2016

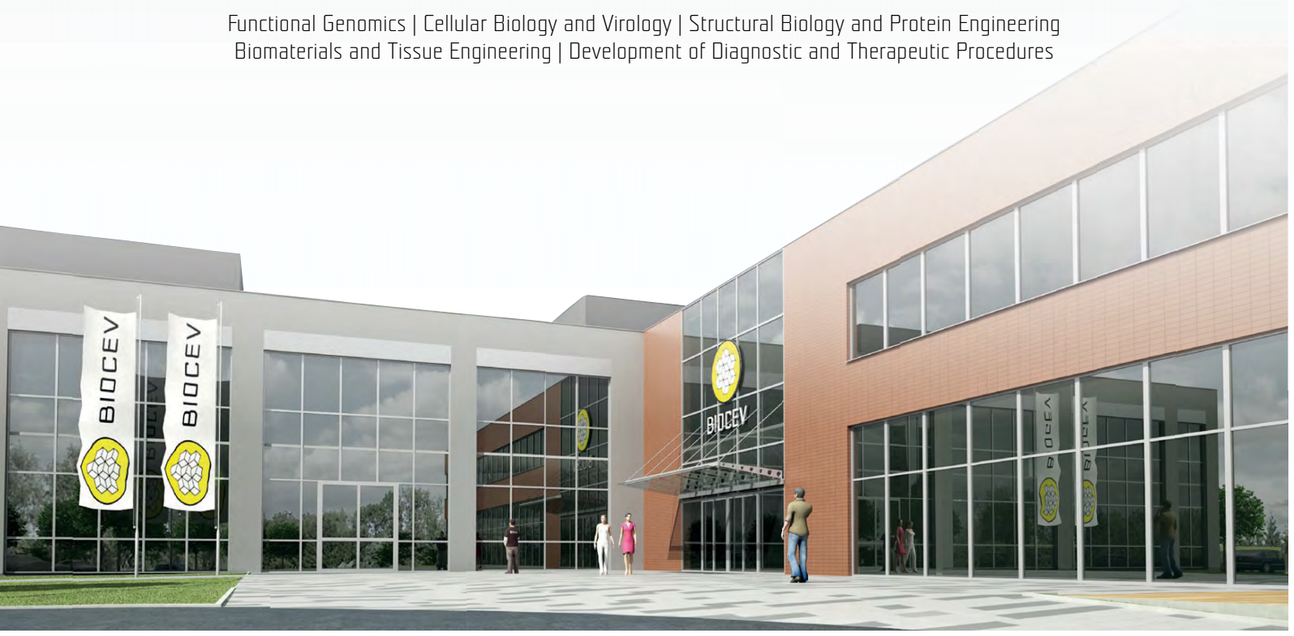
PHENOGENOMICS
NEWSLETTER

TT2016 Report

Universal Genome Modification

Embryology Unit

Czech Centre for Phenogenomics



Contents

Editor
Nicole Chambers

Editorial team
Inken M. Beck
Karel Chalupsky
Kallayanee Chawengsaksophak
Trevor Epp
Ivan Kanchev
Agnieszka Kubik-Zahorodna
Peter Makovicky
Benoit Piavaux
Jan Prochazka
Milan Reinis
Radislav Sedláček

Printers: AMOS typografické, Praha 4

Cover Image: Artists illustration of the precision of the Cas9 cut. Illustration by Lydia Rafferty-Weekes

Photo Credits
Nicole Chambers
Agnieszka Kubik-Zahorodna
Lluis Montoliu
Marcello Raspa
Guarant International

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.

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 the Director

Dear Readers,

I would like to introduce this issue of "Phenogenomics newsletter" which is dedicated to the Transgenic Technology meeting (TT2016) and the newest revolutionary technology of design nucleases. Although CRISPR/Cas technology is still very young, it is being widely used in labs across the world, almost as routinely as PCR. These designer nucleases offer new solutions for faster and more cost effective genome editing approaches both *in vitro* and *in vivo*.

The TT2016 meeting (organised by the Czech Centre for Phenogenomics), brought together over 600 researchers, transgenic core facility managers and technicians and focused on the latest advances and applications in transgenic technologies. During this meeting, I realised that the research community is hungry for new information and training in nuclease technology. Indeed, the interest in our '*CCP programmable nucleases (CRISPR/Cas9) Transgenesis Course'* was more than 3 times over the course capacity. I am also aware of the new challenges facing the core facilities, as they seek to find ways to cope with the ever expanding demand to generate and house novel transgenic models. This demand brings with it not only the need for larger breeding and maintenance facilities, but also the demand for expert personnel capable of applying this technology as part of a service. So, while model generation of transgenic models may be getting easier, faster and more precise, there is a greater need for bioinformatics, accurate and fast genotyping and QC of the generated models. This is one of the main reasons our centre will run another training course in the autumn.

I hope that as you read this issue, you will appreciate the advances being made in this field and also see how the Czech Centre for Phenogenomics is aiding researchers and core facility staff in the generation and maintenance of their transgenic models.

Radislav Sedláček

Director of the Czech Centre for Phenogenomics

CCP Programmable Nucleases (CRISPR/Cas9) Transgenesis Course Report

Nicole Chambers
Editor

The CCP Programmable Nucleases (CRISPR/Cas9) Transgenesis course was organized within the framework of the TT2016 meeting. On Wednesday 15th March 2016 at 8:30am the bus carrying the course participants arrived at the main entrance of the host institute IMG (Institute of Molecular Genetics). Radislav Sedláček welcomed the participants and, following a brief history of both IMG and CCP, presented an opening lecture on the pros and cons of mouse transgenesis. The participants then got stuck in with their 'hands on' training. The hands on sections of the course included CRISPR validation using different cell transfection reagents and confirming transgenic mutations in founder mice. However, the highlight of the course was the ability of the participants to plan a targeting strategy for their individual locus of interest, have 'hands on' microinjection training by Dr. Ronald Naumann (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden) and Dr. Sandra Stobrawa (Eppendorf AG, Germany), as well as being able to learn (and try out) the new and developing zygote, electroporation of nucleases technique from the pioneering scientist Dr. Haoyi Wang (Institute of Zoology, Chinese Academy of Sciences, China).

In addition to the practical sessions of the course, participants had lectures from leading scientists in the field of gene editing. Dr. Lluis Montoliu (National Centre of Biotechnology (CNB-CSIC), Spain) gave an exploratory overview of the CRISPR/Cas field and Bernd Zetsche (Broad Institute, USA) explored the newest alternatives to Cas proteins. Both lectures were opened to all IMG staff as their relevance was not only applicable to course participants. Other course lectures included a sponsored lecture from Merck representative Dr. Caroline Beckett, and a guest lecture from Dr. Petr Svoboda (Institute of Molecular Genetics, Czech Republic).

In addition to the lectures and practical sessions, participants also had the opportunity to sample the local beers and traditional Czech cuisine during the two course dinners. This proved a great opportunity for participants to speak to the tutors in an informal setting and also to network with other participants.

It would be remiss of me not to mention the tremendous support from the sponsors of the course who provided material as well as expert tutors to facilitate the excellent quality of the course.

CCP programmable nucleases (CRISPR/Cas9) Transgenesis course group photo with participants, tutors and technical staff.

Course Sponsors

Czech Centre for Phenogenomics · charles river · eppendorf · MERCK · OLYMPUS Your Vision, Our Future · ZEISS

Images from top left clockwise: Martin Fray & Ronald Naumann; Participants trying microinjections, participants trying zygote electroporation of nucleases; course meal; participants and course leader - Bjoern Schuster; course tutor Haoyi Wang demonstrating the ZEN technique.

Mouse Cryopreservation Workshop report

Inken M. Beck
Head of Targeting & Archiving Module

In frame of the TT2016 meeting we held a Mouse Embryo and Spermatozoa Cryopreservation Workshop from 16th-18th March 2016 at the Institute of Molecular Genetics ASCR. The workshop was coordinated by Martin Fray (MRC Harwell, UK). With the increasing adoption of programmable nucleases more and more strains are produced in a short period of time. This places ever increasing pressure on cryopreservation workflows, which are necessary for their archiving and distribution of this increasing number of lines. With this focus, 14 participants from 10 different countries came to Prague and were trained by an experienced team consisting of 14 persons originating from EMMA (European Mouse Mutant Archive) nodes in Harwell (UK), Madrid (Spain), Munich (Germany) and Prague (Czech Republic).

The participants, with varying experience in the field of cryopreservation, were trained in the newest mouse spermatozoa cryopreservation and *in vitro* fertilization (IVF) techniques. Different storage and shipping solutions for mouse spermatozoa were demonstrated. Furthermore, embryo vitrification using straws and the thawing was taught. A non-surgical embryo transfer method (NSET) was shown by Barbara Stone (ParaTechs, USA) and participants had a chance to test this device in mice. Full protocols and methods were provided to all participants.

The hands-on sections were enriched with lectures about cryopreservation techniques, animal welfare, and recent innovations in mouse super ovulation and cold transportation. The talks were held by well-known researchers, namely Lluis Montoliu (National Centre of Biotechnology (CNB-CSIC), Spain), Marcello Raspa (Italian National Research Council, Italy) and Toru Takeo (Kumamoto University, Japan). Infrafrontier services and achievements were demonstrated by Susan Marschall (Helmholtz Zentrum München, Germany), and Soren Knudsen updated about dry shipper services offered by the company Cryoport.

The workshop was sponsored by Infrafrontier (Munich, Germany) and we received generous support from companies Eppendorf and Zeiss. The course organizers and participants enjoyed three long days at the IMG with intense work on cryopreservation and had a traditional Czech dinner, including Czech beer, in a small restaurant in Prague.

With this I want to express my gratitude to all course tutors and lecturers for their time and energy they spent into this course. Thank you!

Mouse Embryo and Spermatozoa Cryopreservation Workshop group photo with participants, tutors and technical staff.

Course Sponsors

Czech Centre for Phenogenomics · charles river · eppendorf · INFRAFRONTIER mouse disease models · ZEISS

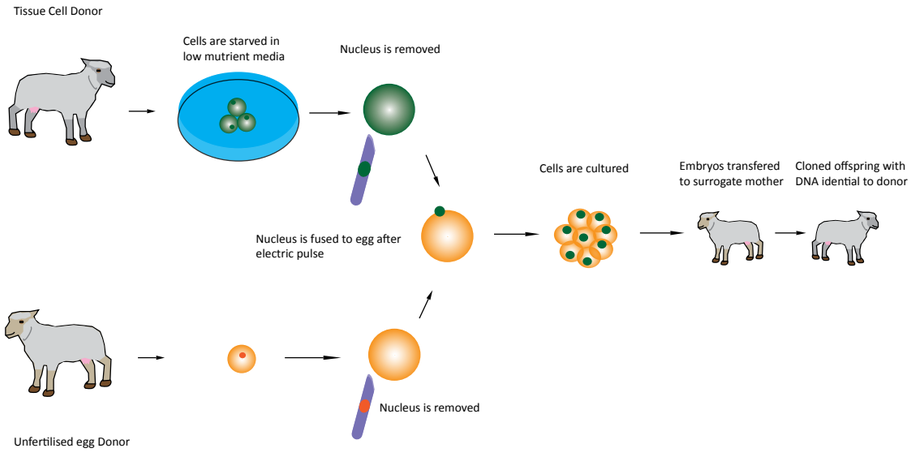
Images from top left clockwise: Lecture from Toru Takeo; Radislav Sedláček and Martin Fray (course leader); hands on experience in oocyte isolation; participants during a lecture; course dinner; participants and tutors Mo Guan and Toru Takeo; lab demonstration; demonstration by Mo Guan; Thank You & Goodbye in participants' and tutors' native language.

UNIVERSAL GENOME MODIFICATION NOW POSSIBLE WITH CRISPR-CAS TECHNOLOGY

Lluis Montoliu
Research Scientist
National Centre for Biotechnology (CNB-CSIC) and Biomedical Research Networking Centre on Rare Diseases (CIBERER-ISCIII), Madrid, Spain

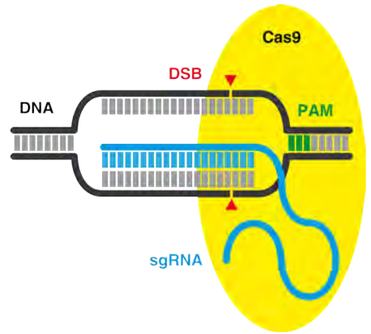
Researchers using vertebrates as experimental animal models have traditionally envied mouse geneticists for their extensive toolbox, envisaging almost any possible genome modification. Most of this capacity was brought about thanks to the pioneer establishment of embryonic stem (ES) cells from mouse blastocysts in 1981, and their subsequent use for targeting genetic alterations driven by homologous recombination events. This was a phenomenal feature that was acknowledged 26 years later with the 2007 Nobel award to Evans, Smithies and Capecchi. Disappointingly, and in spite of numerous attempts, the isolation of functionally equivalent ES cells from other species has mostly failed, with the exception of rat ES cells, which were reported in late 2008[1]. Porcine ES cells have repeatedly been announced but, to date, not proven to recapitulate all features found in mouse ES cells. Hence, scientists using non-rodent animal models had to adapt their genome modification projects to current available methods, including pronuclear microinjection, lentivirus and somatic cell nuclear transfer (SCNT) approaches.

Somatic cell nuclear transfer (SCNT) scematic diagram



SCNT boosted genome modification capacities in livestock. After the pioneer experiments reported in sheep by members of the Roslin Institute the SCNT technique[2] was successfully replicated in other species, including pigs, cattle and goats. SCNT strategies were largely based on the use of fetal/embryonic fibroblasts, which were kept in culture for a limited number of doublings while the targeted insertion of gene modifications, through homologous recombination, was assessed and selected. The selected recombinant cell clones were used as nuclei donors for reconstituting metaphase II-oocytes or zygotes, depending on the protocol and the species, which had been previously enucleated. Eventually these reconstructed embryos had to be transferred to suitable foster females of the corresponding species with the hope that some would develop to term and result in new-borns carrying the expected genomic modification. Logically, these were technically-demanding methods, only available to a few laboratories around the world.

The entire scenario changed when the CRISPR-Cas tools became available in early 2013. These heterologous compounds, originally derived from acquired immune defence systems that are active in bacteria and archaea, were shown to operate as RNA-guided DNA endonucleases in mammalian cells and organisms. The CRISPR-Cas strategy relies on directing a double strand break (DSB) at precise genome locations, hence triggering endogenous DNA repair mechanisms. In the absence of heterologous DNA template the default repair mechanism (non-homologous end-joining, or NHEJ) will progress by deleting and adding nucleotides, searching for



micro-homologies to seal the scar, and eventually leading to small IN-sertions or DEL-etions (INDELs), thus usually resulting in gene disruption. In the presence of heterologous DNA molecules with homologies to sequences at either side of the DSB, the homology-directed repair (HDR) mechanism will take over and result in the substitution of target sequences by donor DNA sequences, thus leading to gene edition. Not only small and large insertions or deletions are possible but also knock-in strategies, gene substitution experiments, inversions, duplications and many other genome modifications are now feasible through the use of CRISPR-Cas tools.

The essential compounds of the CRISPR-Cas system are small guide RNA molecules (gRNA) and nucleases (Cas-like, mostly Cas9, derived from *S. pyogenes*, although many other variants are being characterized). These compounds appear to operate in all animals tested to date. In 2013, the first animals whose genome was edited by CRISPR-Cas were mice[3] and zebrafish[4], but soon the technique was extended to rats, pigs, cattle, non-human primates, dogs, sheep, goats, rabbits, … among other vertebrates where these innovative tools have been successfully applied. None of these species, except mice and rats, had bona-fide ES cells established, although for several, robust SCNT procedures were regularly applied for genome modification purposes. The CRISPR-Cas tools made it all possible, providing the technical approach required to edit animal genomes at will, without involving ES cells, without requiring complex nuclear-transfer experiments, simply by microinjection of small RNAs into fertilized oocytes or early embryos.

The universal application of CRISPR-Cas technology, valid for all animal species where cells or early embryos are available and can be manipulated, has restored the equilibrium among the different animal experimental models. Thanks to CRISPR-Cas tools, mice are no longer unique for genome editing experiments or for the generation of animal models of human disease. However, due to their high reproductive rate and ease of maintenance, mice will continue to be one of the best mammalian species to assess gene function.

References

1. Buehr, M. *et al*. Capture of authentic embryonic stem cells from rat blastocysts. *Cell* **135**(7), 1287-98 (2008).
2. Wilmut, I. *et al*. Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**(6619), 810-3 (1997).
3. Wang, H. *et al*. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell* **153**(4), 910-8 (2013).
4. Hwang, WY. *et al.* Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol*. **31**(3), 227-9 (2013).

Photograph of Lluis Montoliu is courtesy of Ines Poveda

FOCUS, PERSEVERANCE, SUCCESS

Nicole Chambers
Phenogenomics Editor

Dr Pablo Ross was awarded the 4th ISTT Young Investigator Award at the 13th Transgenic Technologies meeting held in Prague, Czech Republic. Dr Ross graciously received the award from inGenious Targeting Laboratory representative Thomas Zeyda (Director of Technology Integration). With over 500 participants in the Meridian hall, Dr Ross described his surprise at receiving the award (as the society has long focused on small animal models) and conveyed his deep-felt honour and sense of responsibility.

Dr Pablo Ross presented with his ISTT young investigator award. People from left to right: Thomas Zeyda, Pablo Ross and Jan Parker-Thornburg

Dr Ross obtained his D.V.M. in 1999 from La Plata National University (Argentina) followed by his M.S. from Mar del Plata National University (Argentina) in 2002. After moving to the USA, Dr Ross was awarded his Ph.D. in Animal Science from Michigan State University in 2007 and is currently Associate Professor at University of California, Davis. His work focuses on livestock embryo technologies and his major achievement was elucidating the conditions for the successful maintenance of iPS/ES-like cells in livestock species[1].

The field of livestock transgenics has made slow progress compared with the small animal model transgenics field. The main difference being the inability to successfully culture livestock species embryonic stem cells (ESCs). The work of Dr Ross' lab looked to remedy this problem. He tried old techniques again in alternative culture conditions in an attempt to culture bovine ESCs. Five conditions were tested and four didn't work, but one did! The derived cells had high derivation efficiency and expressed SOX2 & OCT4 – all characterized markers of pluripotency. These cells were able to form embryoid bodies and teratomas and were amenable to complex genetic modification. This substantial breakthrough in the large animal model world, together with the CRISPR/Cas explosion means that 'the boundaries are our imagination and the possibilities are endless'. Dr Ross described that 'for the first time in the history of transgenic and food animals for agriculture we're seeing industries and bio companies very interested in developing this technologies.' No longer is it the 'crazy scientist' trying to show the possibilities, but now , the commercial companies are coming and saying 'we want to do this because this (we think) is the next step towards providing sufficient food for a growing population.' And not too soon! With the global population currently standing at 7.4 billion there is growing concern that with an increasing population comes an increased demand for food and medical treatments. Another aspect of Dr Ross' work looks at the ability to generate and maintain human-animal chimeras. Whilst this topic has drawn a lot of media attention, the prospects for the medical community are also significant. Current demand for various organs far exceeds the supply, resulting in a rise in organ trafficking and the ever growing black market for organ purchases. One of the potential solutions for this is more research into the alternatives to human-human transplantation[2].

The rate of development in this field is breath-taking and the potential and applications of this technology is limitless. The revolution in programmable technology (namely the CRISPR/Cas advances) have levelled the playing field. CRISPRs have made large animal model generation as efficient as mouse and have begun to integrate everything together. Now both fields are using the same technology and deploying it in the same way and using all the same approaches. Any development made in a mouse is directly applied to a cow or pig and Dr Ross hopes it will also be vice versa, that things that are developed in pigs or cows are also applied to mouse.'

So, from one of Argentina's most important national universities whose motto is 'Pro Scientia et Patria' (For Science and the Motherland) to one of the top public universities in the USA, UC Davis, Dr Ross has continued to push scientific boundaries and explore novel and sustainable ways to deal with some of this planets future problems. His message to budding researchers is one of perseverance and exploration. 'Keep an open mind when doing your research and follow your instincts. Many colleagues will seek to discourage you from following a particular path, but follow your gut instincts. Also, think outside the box, and try your ideas out. But most of all, be prepared for failure! This is part of learning and development and many researchers are discouraged when their work does not produce the expected results. So, follow your instincts with a lot of dedication and hard work. Get ready to get up & keep going and things don't always turn out the way you expect.'

Truly Dr Ross is an inspiration to young researchers seeking to advance scientific knowledge, develop novel technologies and contribute to the sustainability of life on this planet.

References

1. Soto, DA, Ross, PJ. Pluripotent stem cells and livestock genetic engineering. *Transgenic Research* [Epub ahead of print] 2016.

2. Bulletin of the World Health Organization, Volume 85, Number 1, January 2007, 1-84

LARGEST TT2016 MEETING

Jan Parker - Thornburg
ISTT President

The opening night of TT2016 was momentous with promises of good friends and good science. There were welcomes, an opening lecture from Andras Nagy, followed by a succulent buffet with wine, friends, colleagues and music. Over the next three days, we heard many excellent talks—talks that encompassed the use of transgenic technologies, and especially CRISPR/Cas9 technology. We heard about methods to ameliorate muscular dystrophy, to humanize large animals for xenotransplantation, to make swine resistant to an endemic disease, and to examine fertility in humans. We discussed technologies that would target the epigenome, that would examine non-coding areas of the genome, that would recapitulate immune syndromes in ES cells, and that would allow us to assess phenotypic changes in embryonic lethal mutant mice using imaging. We learned about the history behind the CRISPR/Cas9 system, and newer CRISPR systems in development. We discussed ethics, non-injection technologies, new injection technologies, and methods of generating more oocytes in mice. There were seventeen abstracts chosen for full presentation, examining technological developments (including changing mouse strain by nuclear substitution), large CRISPR/Cas9 initiatives, and transposon-mediated transgenesis. And, the number of poster submissions, and the quality of the posters was excellent. On the last day, the ISTT Young Investigator Award (sponsored by inGenious Targeting Laboratory) was given to Pablo Ross based on his work developing ES cells in farm animals.

There were a number of new initiatives at TT2016. We had *Orbis pictus* lectures—lectures designed to use pictures and clear descriptions to demonstrate answers to a problem. Richard Behringer gave an excellent, encyclopedic presentation of methods of producing genetically modified animals in a vast variety of species. Later, Thomas Boehm described how lymphoid organs developed throughout evolution to the point where vertebrates now have a thymus. Also, for the first time, we had concurrent sessions. Delegates needed to choose whether to hear about ethics in animal use, or new injection and superovulation technologies. Overall, the scientific program was exceptional!

The social program was also amazing. Delegates enjoyed the opening buffet and live music. However, it was the Gala Dinner that proved to be the high point of the social program. The Žofin Palace was full with partygoers. The food was wonderful, and the music was fantastic as well. Overall, TT2016 can be considered as one of the best TT meetings ever, and I am proud, as ISTT President, that we helped to host such a wonderful meeting. Thanks so very much to the organizers—Radislav Sedláček, Inken Beck and Nicole Chambers. Due to their amazing work, the ISTT has again had a successful TT meeting! We look forward to seeing you again in Salt Lake City, Utah, USA for TT2017.

TT2016 awards

4th Young Investigator Award	Pablo J Ross (*University of California, Davis, CA, USA)*
Full Travel Award	Melissa Larson (*University of Kansas Medical Center, Kansas City, USA)*
	Sara Ortica (*Paris-Saclay Institute of Neuroscience, Paris, France)*
Registration Awards	Samy AlGhadban (*University of Leicester, UK)*
	Katharina Boroviak (*Wellcome Trust Sanger Institute, Cambridge, UK)*
	Carme Cucarella (*Instituto de Biomedicina de Valencia, Spain)*
	Paul Devenney (*MRC IGMM at the University of Edinburgh, UK)*
	Peter Major (*NAIK, Hungary*)
	Ana Nóvoa (*Fundação Calouste Gulbenkian Instituto Gulbenkian, Portugal)*
	Karolina Piotrowska-Nitsche (*Transgenic Mouse/Gene Targeting Core Facility, Atlanta, USA)*
	Lucas Pitt (*Malaghan Institute of Medical Research, NZ)*
	Selin Yagcioglu (*Istanbul University Faculty of Veterinary Medicine, Turkey)*
Poster Awards	Vera Jansen (*Caeser Research Center, Bonn, Germany)*
	Charles-Etienne Dumeau (*Wellcome Trust-MRC Stem Cell Institute, UK)*
	Hiromi Miura (*Tokai University, Isehara, Japan)*


2016
THE ART OF SPERM GENETICS
2016
ISTT
TT2016
TT2016 TRANSGENIC TECHNOLOGY MEETING
MEETING PROGRAM
Services – Europe


TYDHO-KEPLER
REGISTRATION
POSTER AREA
SPEAKER'S READY ROOM
MIXROOM
EXIT
horizon
COSMO BIO CO.,LTD.

PROVIDING SOLUTIONS FOR EMBRYOLOGICAL QUESTIONS

Kallayanee Chawengsaksophak
Head of Embryology Unit

"The student of Nature wonders the more and is astonished the less, the more conversant he becomes with her operations; but of all the perennial miracles she offers to his inspection, perhaps the most worthy of admiration is the development of a plant or animal from its embryo."

T.H. Huxley, *Darwiniana* (1896).

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, which systematically generated KO models for every mouse gene (over 20,000). However approximately 30% of these KO lines are predicted to be embryonically lethal (die during embryonic or perinatal periods) and therefore cannot be used in the adult phenotyping pipelines. The physiological roles of these genes must therefore be identified/studied during embryonic development. The ability to study the various stages of embryonic development opens the potential to characterise the function of genes during embryonic growth, differentiation and organogenesis[1]. Due to this fact the IMPC included embryogenesis to its phenotyping pipeline[2]. This work, whilst technically challenging, can identify genes involved in developmental pathways[3] and also shed light on genes for which not much is known.

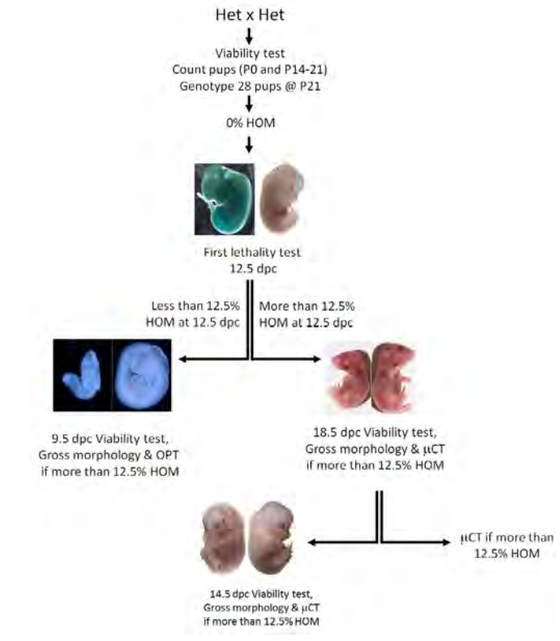


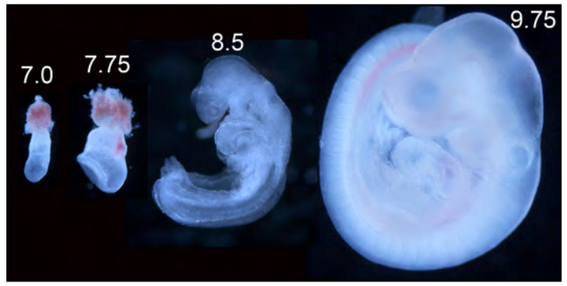
Figure 1. Embryology unit work flow chart to screen embryonically lethal KO lines

Investigation into embryonically lethal lines is also of clinical significance. Currently in the Czech Republic, the incidence of prenatally diagnosed birth defects has increased[4]. A similar trend is also observed across Europe on a whole, with approximately 1:40 births showing developmental anomalies and birth defects[5]. When investigating the clinical relevance of these lethal lines, one should also remember that whist the homozygous KO may be embryonically lethal, the heterozygous lines may be viable (even up to adulthood), but display some clinical phenotype. This brings into context the need to combine the detailed analysis of embryonic development of both homozygotes and heterozygotes of embryonically lethal lines.

Here at CCP, the embryology unit screens embryonically lethal lines according to IMPC guidelines (Fig 1). We also offer services, which are available to the scientific community on a cost-recovery basis. Highlighted here are some of the standardised services we offer as well as customised projects, which can be tailored to specific requirements.

Embryo dissections (mice and rats). We can dissect embryos and extraembryonic tissues at any stage of development, including the technically advanced, 4.5 days post coitum (dpc), postimplantation stage. We can also perform embryonic organ dissections. Recent examples include isolations of para-aortic splanchnopleura (9.5 dpc), aorta-gonad-mesonephros (AGM) (10.5 dpc) and embryonic pancreas (14.5 dpc), usually for biochemical analyses or *ex-vivo* organ culture.

Stem cell derivation (mice only). The derivation of stem cell lines of embryonic origin provides a valuable tool to study developmental potency, lineage specification, and differentiation. We offer services to isolate several murine stem cell types, including embryonic stem cells (ESCs), primary mouse embryonic fibroblasts (MEFs), epiblast stem cells (EpiSCs), extraembryonic endoderm cells (XEN), and trophoblast stem cells (TSCs). Currently, we also offer the generation of induced pluripotent stem cells (iPSCs).



Fresh preparation of dissected mouse embryos at various stages of embryo development.

Effect of Genetic Modifications (mice and rats): The first goal in our analysis is to determine the gestational timing of death. Usually, this can provide informative clues as to the underlying causes, especially if they coincide with essential developmental milestones such as implantation, gastrulation, the establishment of the cardiovascular system, or the establishment of the chorioallantoic placenta. Once the timing has been established, mutant embryos are imaged with a stage-appropriate modality (e.g. optical projection tomography, micro-computed tomography, confocal microscopy) and subjected to standard histological processing and analysis. The output is a descriptive analysis of the observed morphological changes accompanied by appropriate photo documentation (Fig 2).

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

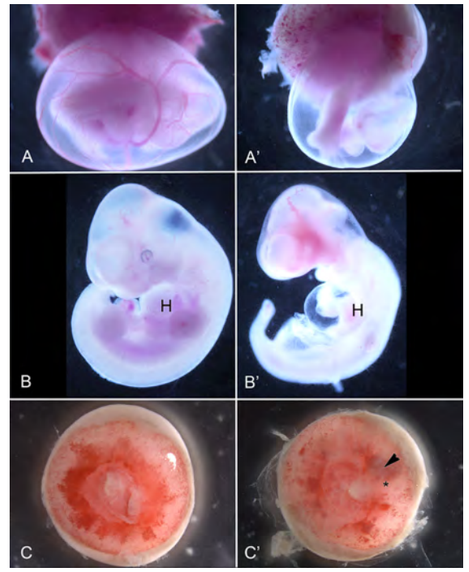
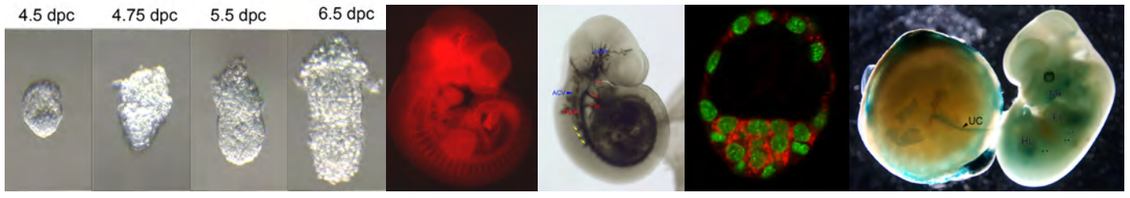


Figure 2. A montage of a fresh preparation of mouse embryos at 10.5 dpc showing the vascular defect of mutant (A'-C') compared to wild-type (A-C). A, A' embryo inside yolk sac attached to the placenta in toto. The mutant embryo (B') exhibits haemorrhage in the developing head, the heart chambers are atrophic with distended pericardium membrane and cardiac looping. C' placenta of mutant embryo showed haemorrhage (black arrow head), defective vascular arborization in the mutant placenta (*) compared to wild-type (C).

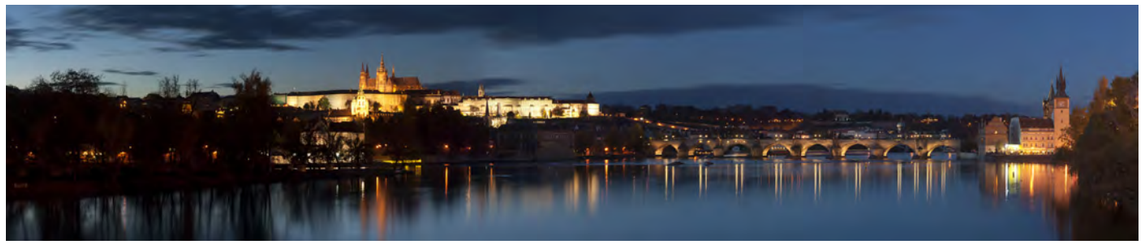


Images (left to right): Fresh preparation of dissected mouse embryos from 4.5 to 6.5 dpc; Fluorescence wholemount immunohistochemistry of mouse embryo at 9.75 dpc showing expression of CD31 (PECAM), a pan-endothelial maker; India ink injection through the heart of mouse embryo at 9.5 dpc to trace vascular circulation in developing mouse embryo. Dorsal aorta (DA), Anterior cardinal vein (ACV), Head vascular plexus (HVP), * showed pharyngeal arch artery (PAA) I, II and II. Yellow arrows showed intersomitic vessels; Fluorescent in situ hybridization showing mRNA expression of Pou5f1 (Oct4), the pluripotency master gene regulator in the cytoplasm of the inner cell mass (red) of 3.5 dpc blastocyst. Green is the pseudo colour of DNA staining dye to mark nuclei; Beta-galactosidase staining of a mouse embryo at 11.5 dpc and its placenta. Prominent blue colour depicted gene expression in umbilical cord (UC), forelimb (FL) and hindlimb (HL), maxilla. Stars show gene expression in nipple placodes.

References

1. Adams, D. *et al.* Bloomsbury report on mouse embryo phenotyping: recommendations from the IMPC workshop on embryonic lethal screening. *Disease Models & Mechanisms* **6**(3), 571-579 (2013).
2. Brown, SD. & Moore, MM. Towards an encyclopaedia of mammalian gene function: the International mouse Phenotyping Consortium. *Disease Models & Mechanisms* **5**, 289-292 (2012).
3. Cox, BJ. *et al*. Phenotypic annotation of the mouse X chromosome. *Genome Research* **20** (8) 1154-64 (2010).
4. Gregor, V. *et al.* [Prenatal diagnostics of birth defects in the Czech Republic--a fifteen-year period]. *Ceska gynekologie* **77**(5) 437-44.
5. Dolk, H. *et al.* The prevalance of congenital anomalies in Europe. *Advances in experimental medicine and biology* **686**, 349-364 (2010).

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

Deputy Director

The Czech Centre for Phenogenomics (CCP) is currently seeking a Deputy Director to serve as part of the management team and co-leading a Phenotyping Module.

CCP, is one of the large national infrastructures located in the municipality of Vestec south of the Capital city of Prague in the Czech Republic. Set on the campus of the newly constructed BIOCEV, CCP is a state of the art facility and combines the generation, phenotyping and archiving of rodent strains. As a member of the International Mouse Phenotyping Consortium (IMPC) and INFRAFRONTIER, CCP is dedicated to the goal of 'systematic genome-wide phenotyping project of knock-out mice in order to provide the broader research community with a lasting resource of mammalian gene function.'

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.

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 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 would be advantageous, but is not essential.

The closing date for applications is June 15, 2016 with the first round of interviews scheduled to be held July 2016.

For more details click here

Cardiovascular unit leader

The cardiovascular screen is an integral part of the comprehensive phenotyping platform of the Czech Centre for Phenogenomics (CCP) and will deal with mouse and rat models. We are seeking an outstanding expert whose main task will be to efficiently run and further develop primary and secondary phenotyping screens and build a service unit for researchers that use mice and rats as models.

Besides ensuring service commitments, successful candidate will be encouraged to pursue their own research projects in the field of cardiovascular physiology.

For the position we expect applicants to have PhD degree (or equivalent) degree in relevant field with provable research experience and publications. Good command of English is required, other languages are an asset. Valid certificate for laboratory animal handling will be considered advantageous.

Starting date is negotiable, but the position is available immediately for with one-year fixed term period. After this period, the successful researcher will have an opportunity to get a long-term appointment. The place of work is Prague (IMG) and Vestec u Prahy (BIOCEV centre; since winter 2015).

For more details click here

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 click here.

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

Upcoming Events

15th International Congress of Histochemistry and Cytochemistry (ICHC 2016)

19th - 22nd June 2016, Istanbul, Turkey

http://www.ichc2016.com/

The Allied Genetics Conference (TAGC)

13th - 17th July, Florida USA

http://www.genetics2016.org/index.htm

12th International Congress of Cell Biology

21st - 25th July, Prague, Czech Republic

http://www.iccb2016.org/

Cell Symposium: 10 years of iPSCs

25th - 27th September, Berkeley, USA

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

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

12th EFIS-EJI Tatra Immunology Conference

3-7th September 2016, Štrbské Pleso,Slovakia

https://tatra.img.cas.cz/home.html

Journal Club

1. Beura, LK. *et al*. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* **532**, 512–516 (2016)
2. Fonfara, I. *et al*. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. *Nature* **532**, 517-521 (2016)
3. Gao, F. *et al*. DNA-guided genome editing using the Natronobacterium gregoryi Argonaute. *Nature Biotechnology* [Epub ahead of print] (2016)
4. Komor, AC. *et al*. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* [Epub ahead of print] (2016)
5. Nelles, DA. *et al*. Programmable RNA Tracking in Live Cells with CRISPR/Cas9. *Cell* **165**(2). 488-496 (2016)
6. Qin, W. *et al*. Generating Mouse Models Using CRISPR-Cas9 Mediated Genome Editing. *Current Protocols in Mouse Biology* **6(1)**, 39-66 (2016)

Delivering...

... Your Research Model

Czech Centre for Phenogenomics