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IMG Institute of Molecular Genetics of the ASCR, v. v. i.
INVESTMENTS IN EDUCATION DEVELOPMENT

The Czech Centre for Phenogenomics: Design, Generation, and Archiving of your Mutant Mouse Strain

Czech Centre for Phenogenomics

Maja Sabol, PhD & Zuzana Ileninová, PhD

The presentation is supported from the project OP EC CZ 1.07/2.3.00/30.0050 "Phenoimage"

INVESTMENTS IN EDUCATION DEVELOPMENT

What is CCP?

Large national infrastructure for supporting genetically-modified rodent (mouse and rat) research

Why?

- to produce a **comprehensive 'encyclopedia' of gene function**
  - identify causative factors of human diseases
  - Identify novel targets for therapeutic intervention
- to produce **high-quality data**
  - scientific reference catalog,
  - comprehensive meta-analyses

How?

- standardized procedures and pipelines
- quality control measures and cross-validation
- membership in global networks

The presentation is supported from the project OP EC CZ 1.07/2.3.00/30.0050 "Phenoimage"

European Research Infrastructure for phenotyping and archiving of model mammalian genomes

INFRAFRONTIER mouse disease models

It includes:

- Mouse Clinics from Europe and Canada
- EMMA Archiving and Distribution Nodes
- European Bioinformatics Institute (EMBL-EBI)

The European Mouse Mutant Archive

EMMA mouse repository

- non-profit repository for the collection, archiving (via cryopreservation) and distribution of relevant mutant strains essential for basic biomedical research
- Now part of Infrafrontier

International Mouse Phenotyping Consortium

IMPC

- 18 research institutions and 5 national funders
- broad-based, systematic genome-wide phenotyping project of knockout mice
- long-lasting resource of mammalian gene function information

International Mouse Phenotyping Resource of Standardized Screens

IMPReSS

- standardized phenotyping protocols
- definitions of the phenotyping Pipelines and mandatory and optional Procedures and Parameters
- data collected by international mouse clinics

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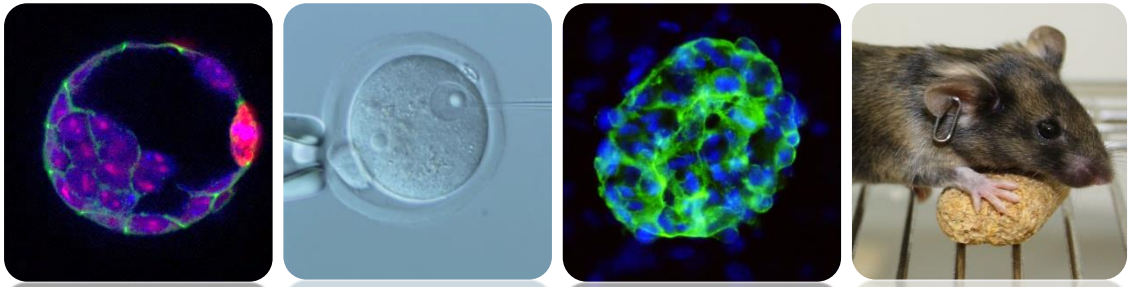
Basic facts

- unique in the Czech Republic, large national infrastructure
- 7200 m^2, 24 mil. €
- 2 locations: Vestec (20 000 cages) and IMG-Prague (10 000 cages), mouse+rat
- one of 5 in Europe, and of 12 in the world
- International centre, open access



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Our mission

comprehensive, systematic and standardized screening of rodent phenotypes

cost-effective, demand-driven support of the research community

The presentation is supported from the project OP EC CZ 1.07/2.3.00/30.0050 "Phenoimage"

CCP: Management & Organization structure

Executive Board

Executive Director
Module heads
Internal scientific advisors

TAM: Transgenic-Archiving Module

Head: I. Beck

Unit (head):

- **Targeting** (B. Schuster)
- **ES cell technologies & model production** (I. Barnetova)
- **Genotyping & breeding** (J. Kopkanova)
- **Archiving & distribution** (I. Beck)

PM: Phenotyping Module

Head: T. Epp

Unit (head):

- **Histo-pathology** (I. Kanchev)
- **Immunology** (M. Reinis)
- **Cardio-vascular unit** (M. Durik)
- **Metabolism** (J. Polak)
- **Biochemistry** (K. Chalupsky)
- **Embryology** (K. Chawengsaksophak)
- **Lung function** (B. Piavaux)
- **Neurobehavioral** (A. Zahorodna-Kubik)

To be built in 2015-2016

- **Dysmorphology & whole body imaging** (J. Prochazka)
- Bioinformatics - head: n.n.
- Reproduction - head: n.n.

AFM: Animal Facility Module

Head: J. Honetschlager

Unit (head):

- **CCP - Animal facility S002/BIOCEV (Vestec)** (P. Neradil)
- **CCP - Animal facility IMG/ Campus Krc-Prague** (M. Buryova)

To be built in 2016:

- Antibody production & immunization - head: n.n. (shared PM & AFM unit)

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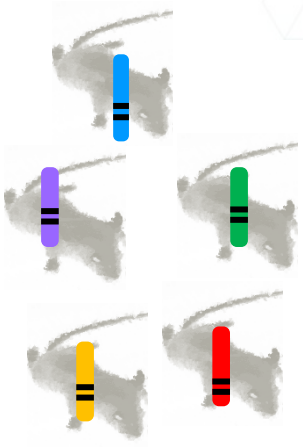
Phenogenomics

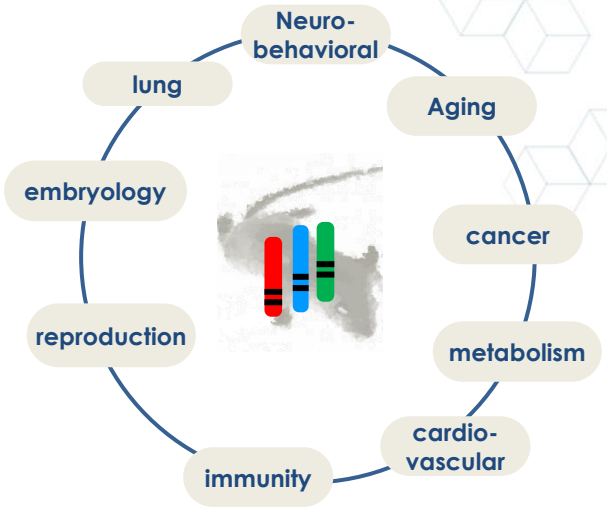
Systematic phenotyping of animal models for annotation of gene function

genes to knock-out
(informative mutation)



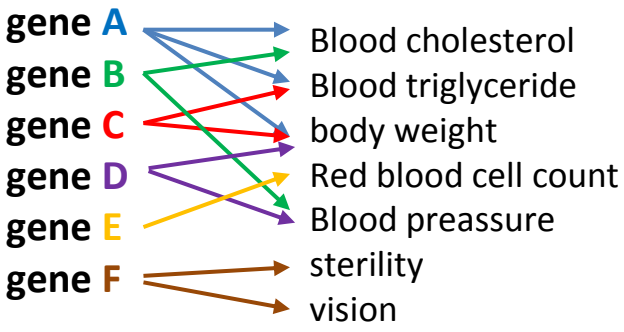
potential relevance to human diseases

comprehensive & large-scale phenotyping



more than 400 standardized parameters

Phenotype: deciphering functions of individual genes



Encyclopaedic database on gene function

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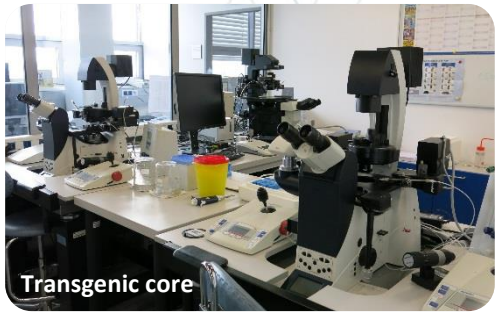
INVESTMENTS IN EDUCATION DEVELOPMENT

Czech Centre for Phenogenomics activities



Targeting:
design and generation of tools

Generation, archiving and distribution of transgenic models

Animal husbandry
& administration of the projects

Phenotyping
- systemic primary
- individual & secondary

Biomedical community

Top-down
Large scale projects:

IMPC & INFRAFRONTIER

Bottom-up

Individual user research projects

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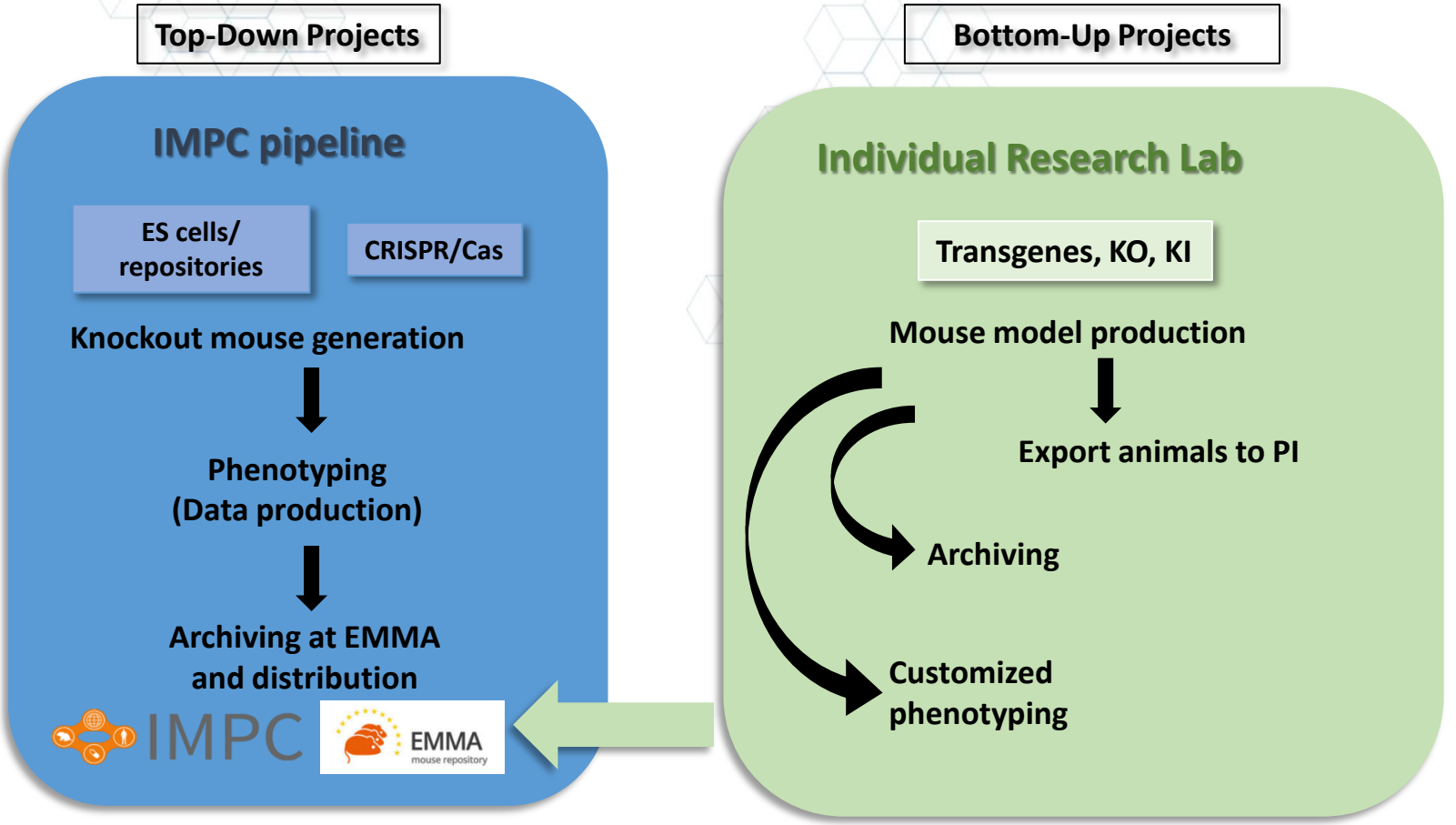
INVESTMENTS IN EDUCATION DEVELOPMENT

Mouse production, archiving and genotyping at the CCP



The goal of the International Mouse Phenotyping Consortium (IMPC) is to discover functional insight for every gene by generating and systematically phenotyping 20,000 knockout mouse strains.

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INVESTMENTS IN EDUCATION DEVELOPMENT

1. Transgenic-archiving module

Inken M. Beck, PhD
Head of Transgenic and archiving module

TARGETING

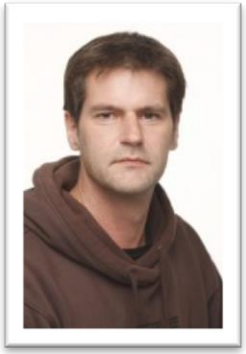
ES CELL TECHNOLOGIES & MODEL PRODUCTION

GENOTYPING AND BREEDING

ARCHIVING & DISTRIBUTION

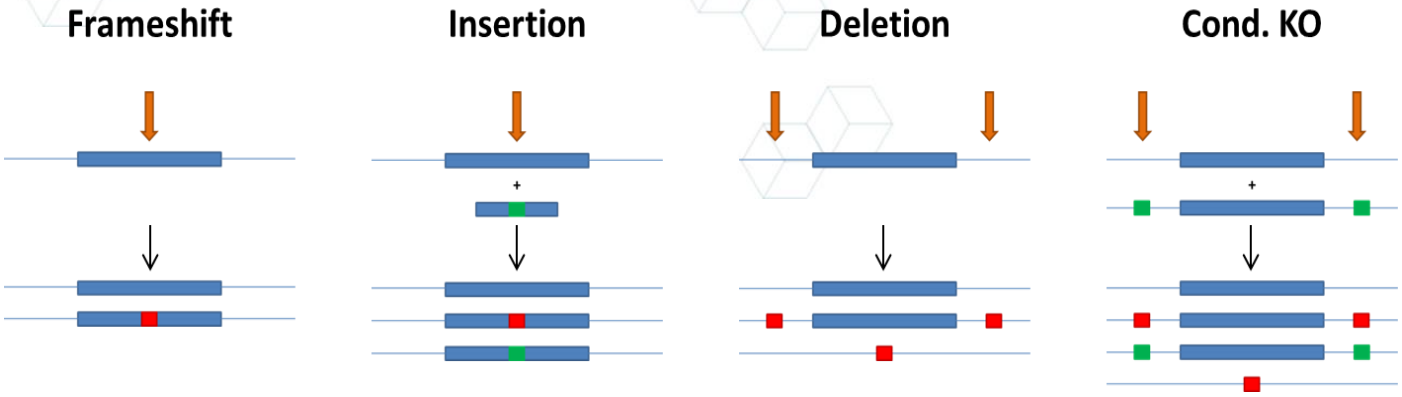
The presentation is supported from the project OP EC CZ 1.07/2.3.00/30.0050 "Phenoimage"

1.a. TARGETING



Björn Schuster, PhD
head of Targeting unit

Targeting using programmable nucleases (TALENs, CRISPR/Cas9)



- **Generation of TALENs targeting ROSA26 locus**
- **Developed reporter system to test functionality of CRISPRs and TALENs in vitro**

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INVESTMENTS IN EDUCATION DEVELOPMENT

highlight

Mouse model (KO) production for IMPC in 2015: 50 novel genes
CRISPR – assisted, non-conditional
Cooperation with SIGMA-Aldrich - the only facility in Europe

Sigma-Aldrich and the Institute of Molecular Genetics, Czech Center for Phenogenomics, Establish CRISPR Core Lab Partnership

ST. LOUIS – January 27, 2015 – Sigma-Aldrich Corporation's (NASDAQ: SIAL) Research business unit, which supports scientific research by supplying products, services and solutions, today announced it has entered into a new gene editing partnership with the Institute of Molecular Genetics (IMG) at the **Czech Center for Phenogenomics (CCP)** in Prague. Under the partnership, Sigma-Aldrich will provide the Transgenic Module at IMG/CCP with Sigma CRISPR technology, including reagents, experimental design consultation and dedicated gene editing bioinformaticians. The partnership is intended to accelerate academic services dedicated to transgenic animal development using CRISPR technology.

"**We are pleased to partner with the Czech Center for Phenogenomics at the IMG to advance their mission to become the leading European transgenic animal supplier**," said Sean Muthian, Ph.D., MBA, Director of Strategic Marketing and Collaborations at Sigma-Aldrich. "The Sigma-Aldrich CRISPR Core Lab Partnership helps accelerate the pace of translational research by removing the design and production hurdles with CRISPR or zinc finger nucleases, allowing scientists at the transgenic facility to focus on developing animal models that reflect the human condition."

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1.b. ES CELL TECHONOLOGIES & MODEL PRODUCTION

- **ES cells injections, ES cell culture, karyotyping**
- **Pronuclear Injections (PNI)**
- **Programmable nucleases (TALENs, CRISPR/Cas9)**

Irena Jeničkova, PhD
Head of ES cell manipulation and transgenesis

Equipment: Microscope with DIC, 2 micromanipulators incl. capillary holders, DNA injection pump
Ideal fragment size: 2-20kb, BAC constructs are much larger
DNA concentration: 1-3 ng/ul in microinjection buffer (7.5 mM Tris, 0.15 mM EDTA pH7.4)

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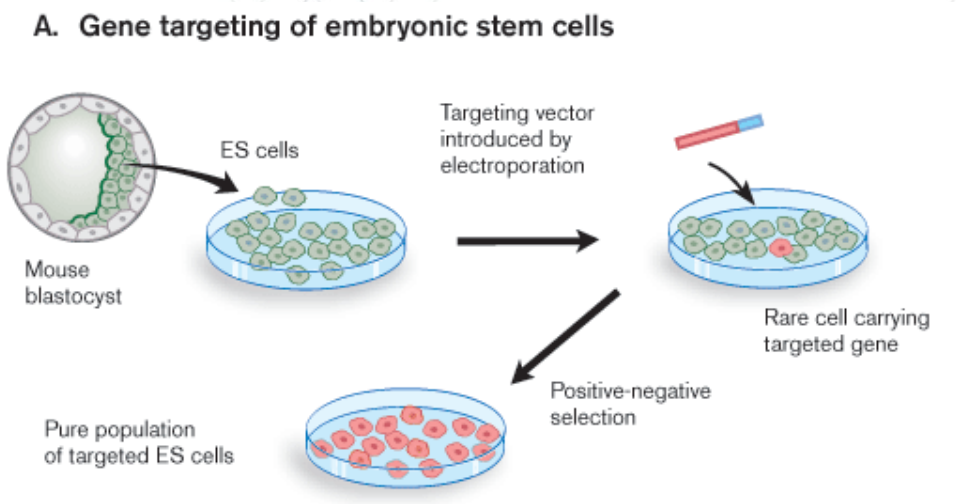
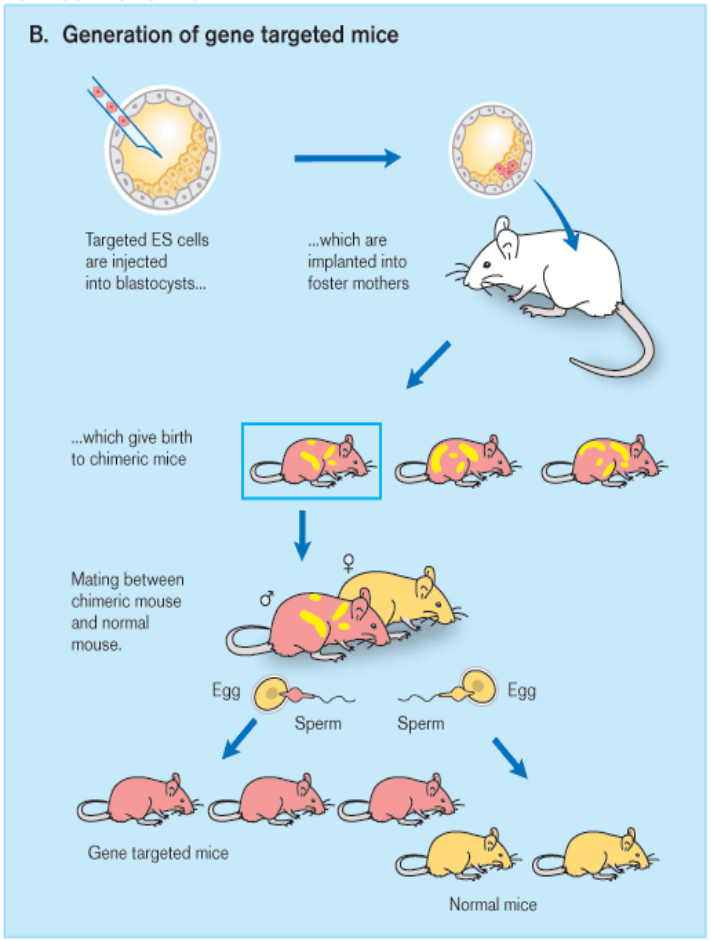
Generation of transgenic mice using ES cells





https://www.partners.org/researchcores/transgenic/transgenic_BWH.html

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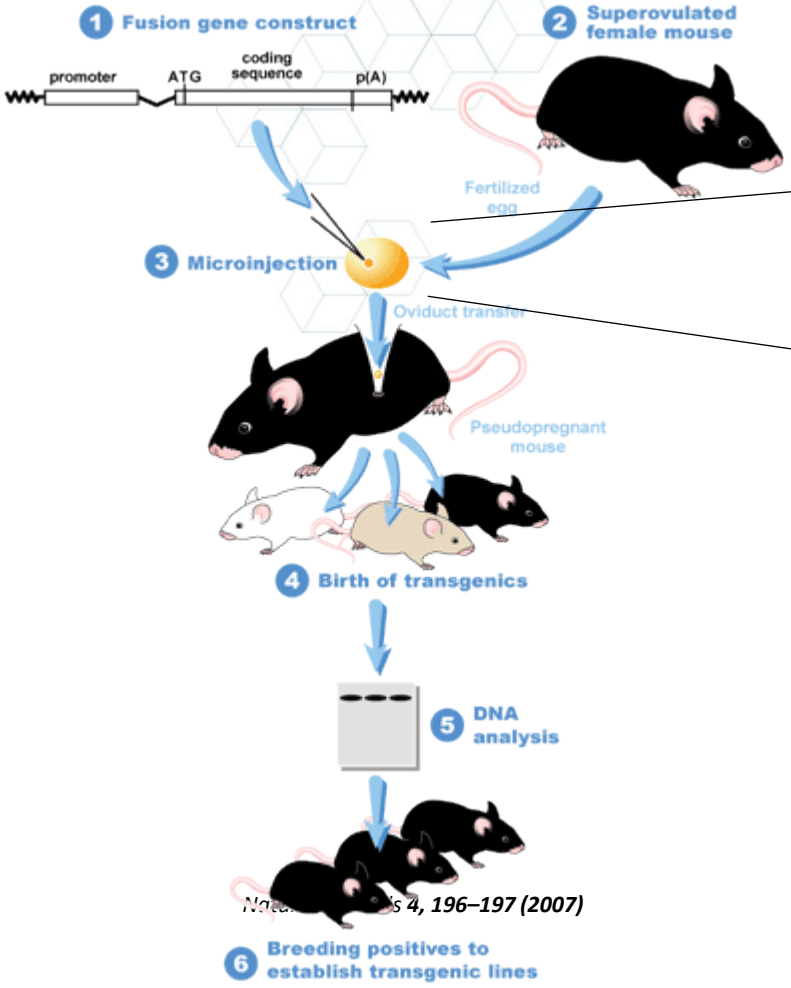
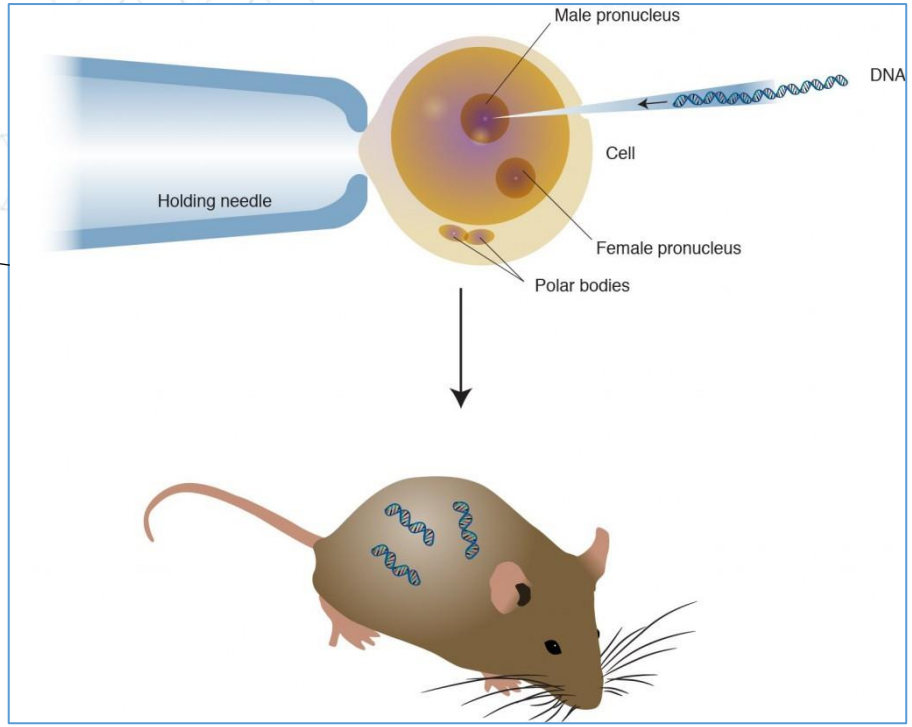
Rapid generation of transgenic mouse

Pronuclear injection of TALENs and CRISPRs





Nat... ...s 4, 196–197 (2007)

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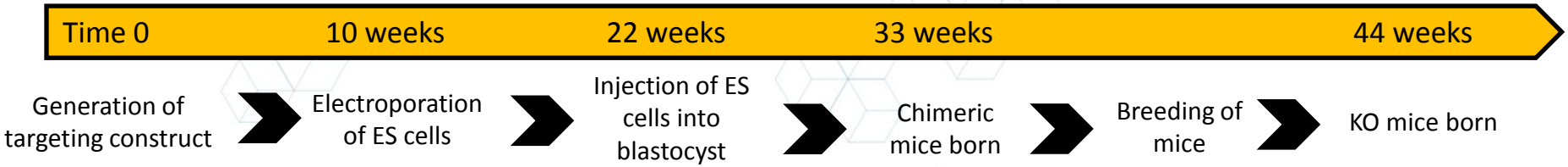
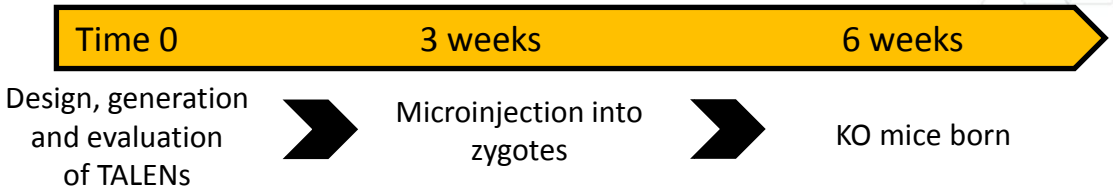
KO mouse generation by HR in ES cells vs TALEN technology:





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INVESTMENTS IN EDUCATION DEVELOPMENT

Production pipeline for 2015.

Gene Targeting using ES cells			Gene Targeting using CRISPR/Cas9	
Gene name	GLT, archived tm1a and tm1b		Gene name (cooperation with Sigma-Aldrich) in progress	
Farp1	EMMA		Asb4	Fam60a
Btbd3	EMMA		Rhobtb1	1810011H11Rik
Klk14	EMMA		Cep170b	2510009E07Rik
Klhl5	tm1a	in progress	Rnf186	1810041L15Rik
Eci2	EMMA		Trim9	Tmem60
Mex3b	EMMA		March8	Fam83h
Klk5	EMMA, tm2b		Trabd2b	Fam134a
Rnf121	EMMA		1700026D08Rik	Lamtor4
Papd7	EMMA, tm1a		Mzb1	Fam172a
Trim15	EMMA		Tmem47	Tmem132b
Nub1	EMMA	in progress	Fam64a	Fam126a
Wdsub1	EMMA		Edrf1	Sbspon
Stk26		in progress	Tmem196	Lppr5
Tmem216		in progress	Coa6	Tmem132c
Tmem237		in progress	1700017N19Rik	Brinp3
Hepacam2		in progress	1700026D08Rik	Fam71f1
Ndufs5		in progress	3425401B19Rik	Tmem88
Fmr1nb			Fam84B	Tmem150b
Gpatch2			Cluh	Tmem62
Lpp			3110002H16Rik	Fam155a
Pcp4l1			0610009O20Rik	Nwd2
Shox2			Fam69c	Tmem240
Strn4			Cipc	Tmem55b
Abcg8			2210018M11Rik	Strip2
Angptl3			Fam102a	

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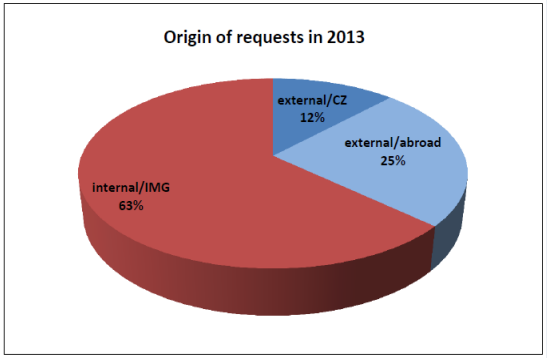
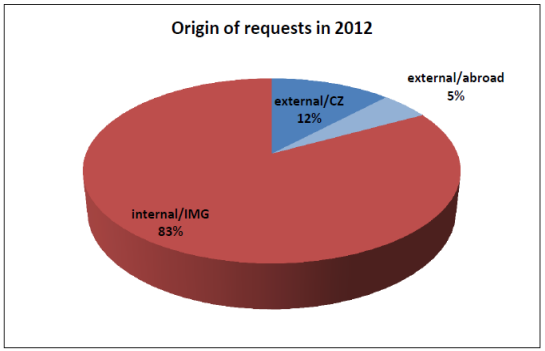
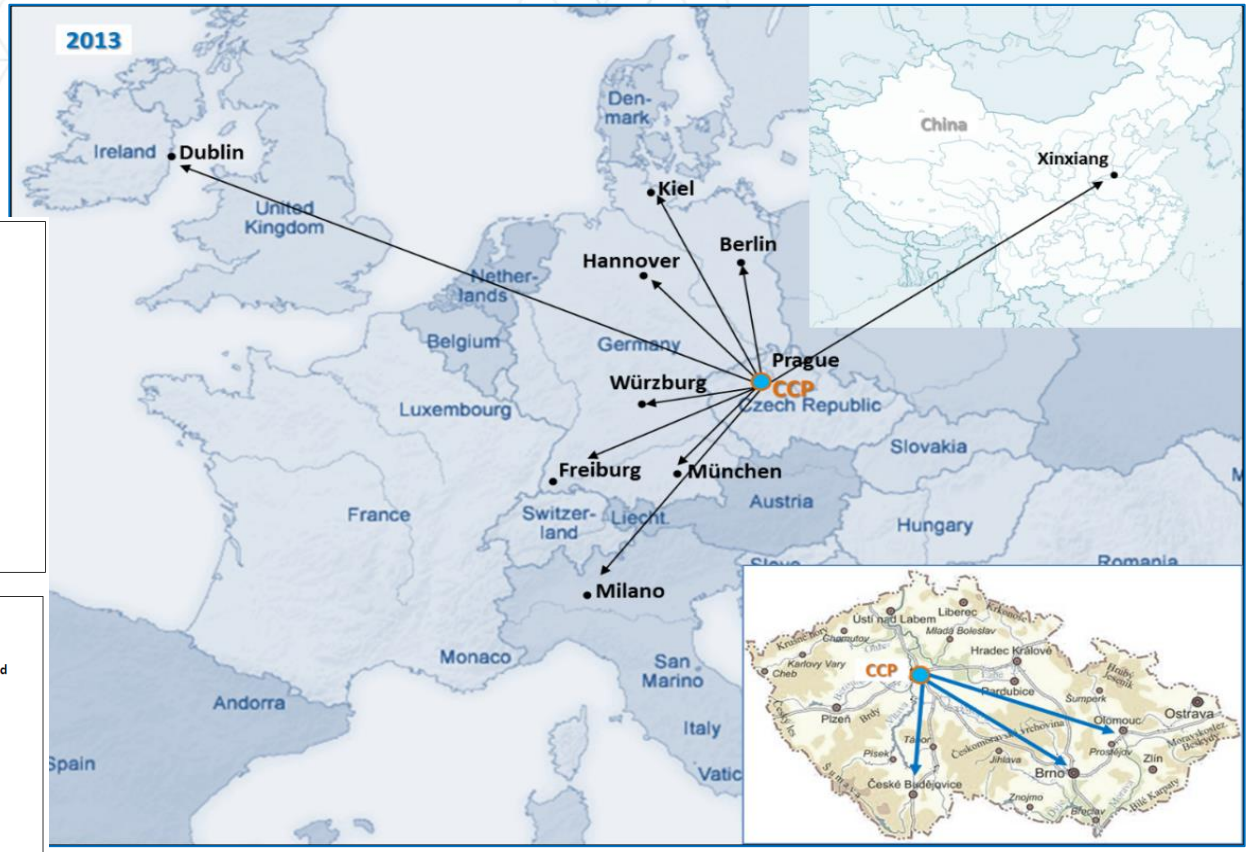
Mouse model generation service







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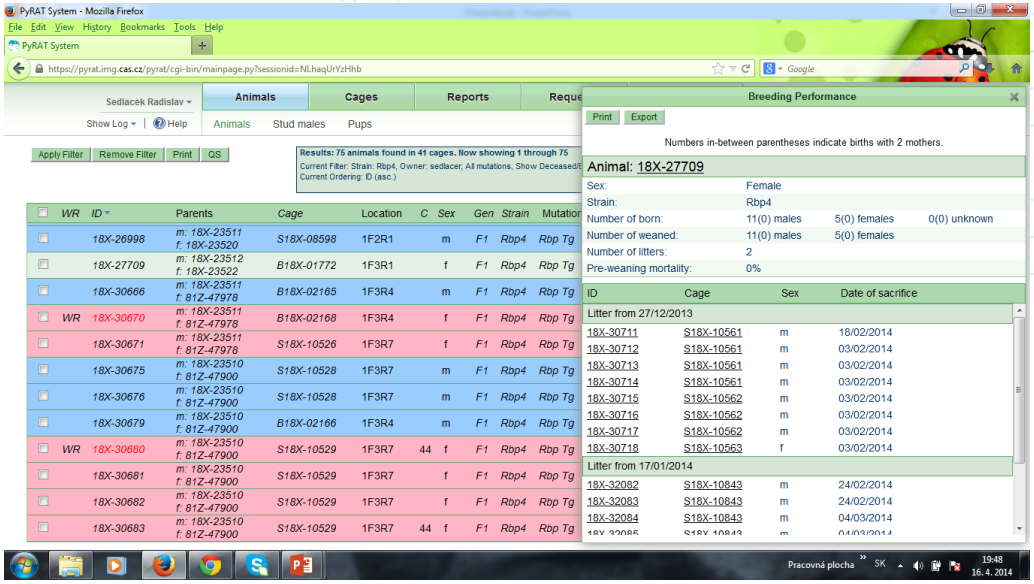
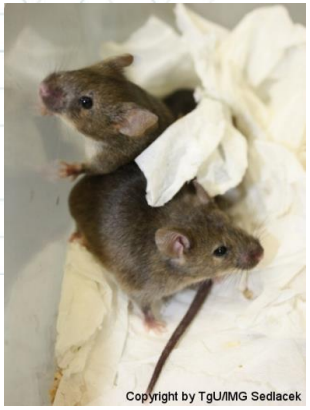
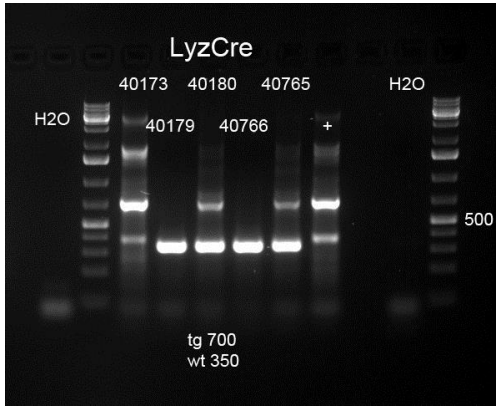
1.c. GENOTYPING AND BREEDING

Jana Kopkanova, MEng.
Head of Genotyping unit







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IMG BIOCEV

Czech Centre for Phenogenomics

1.d. ARCHIVING AND DISTRIBUTION

For individual requests and for the European Mouse Mutant archive (EMMA)

- When mouse strain is not longer needed for experiments or backup
- To reduce costs for animal housing
- To safe time for mouse colony organization
- To have a safe and controlled storage of your mouse strain over decades

Inken M. Beck, PhD
Head of Archiving and distribution

Cryopreservation of mouse embryos

- For mouse lines that necessarily need specific mating scheme
- For fast and unproblematic recovery without using IVF

Cryopreservation of mouse spermatozoa

- For mouse lines that can easily be recovered by using WT donor oocytes (IVF)
- For lines that are kept as WT x Mutant crossing (classical transgenic mice)
- For lines that can be crossed Het x Het to obtain Homozygous mice

+ cleaning/rederivation of mouse lines via IVF or mating

EMMA

Archiving at EMMA is free of charge service
Strain will be made available for the community and you still retain owner of the strain

INFRAFRONTIER mouse disease models

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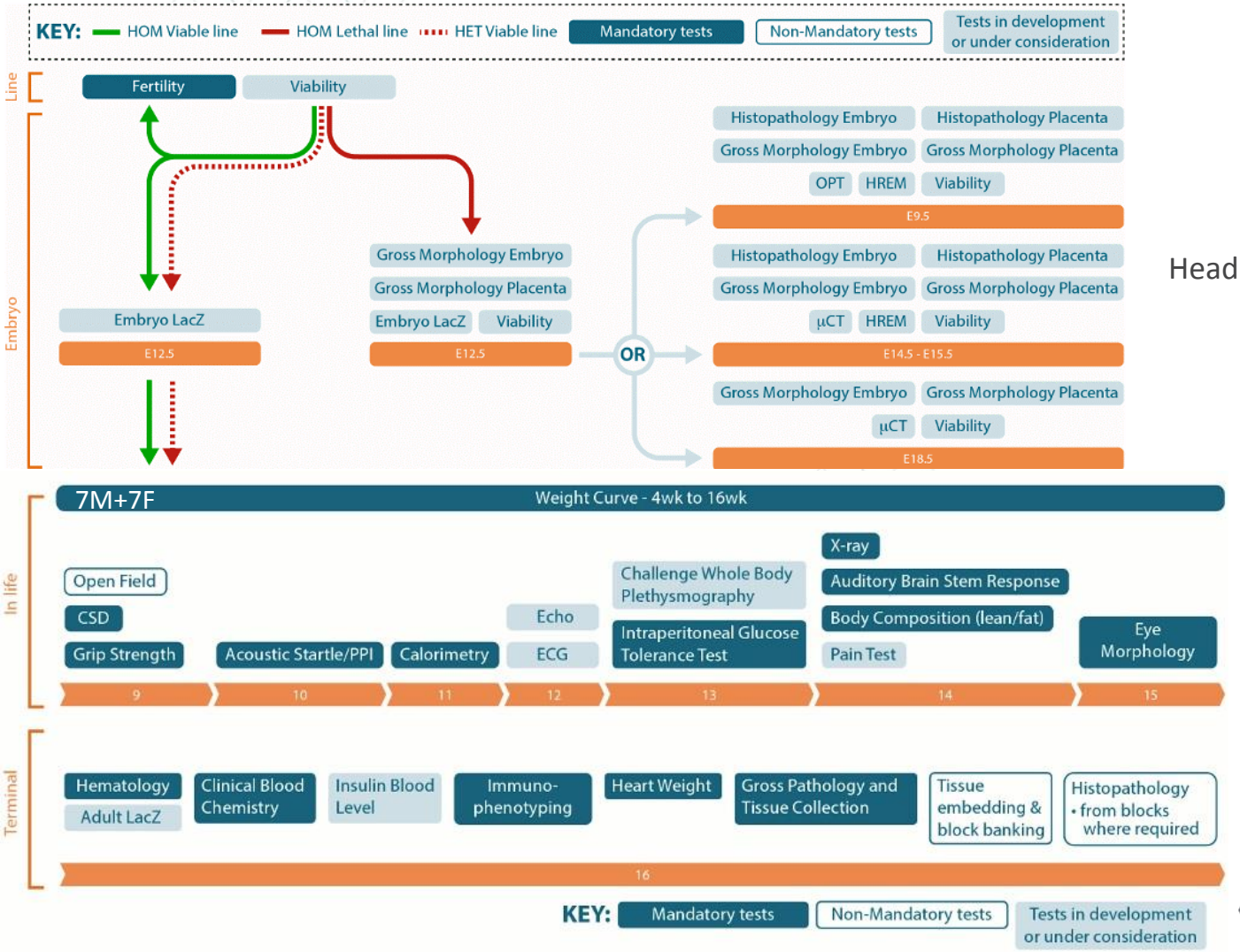
IMG BIOCEV

INVESTMENTS IN EDUCATION DEVELOPMENT

2. PHENOTYPING MODULE



Trevor Epp, PhD
Head of Phenotyping module

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INVESTMENTS IN EDUCATION DEVELOPMENT

Phenotyping units

- BIOCHEMISTRY AND HEMATOLOGY
- HISTOPATHOLOGY
- EMBRYOLOGY
- NEUROBIOLOGY AND BEHAVIOR
- METABOLISM
- IMMUNOLOGY
- LUNG FUNCTION
- CARDIOVASCULAR SYSTEM
- DYSMORPHOLOGY AND WHOLE BODY IMAGING
- (BIOINFORMATICS)
- (REPRODUCTION/ METABOLOMICS)

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2.a. Embryology

Kallayanee Chawengsaksophak, Ph.D.
Head of Embryology

- Analyze strains that exhibit prenatal lethality or subviability
- systematically examine and score embryonic and organ development relative to established morphological and molecular milestones
- identify the time window and likely causative factors leading to embryonic death

METHODOLOGY

- Immunohistochemistry and In Situ Hybridization (section and whole-mount)
- Alcian Blue/Alizarin Red staining of cleared embryonic skeletons
- Late gestation vascular anatomy (india ink)
- Beta-galactosidase staining
- Microcomputed tomography (coming soon)
- High resolution episcopic microscopy (coming soon)

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2.b. Neurobiology and behaviour

Agnieszka Kubik-Zahorodna Ph.D.
Head of Neurobiology and Behaviour

PRIMARY SCREEN

- Modified SHIRPA and dysmorphology evaluation
- Open Field animal emotionality and affect
- Grip Strength neuromotor abilities
- Acoustic Startle and PPI sensorimotor gating
- Auditory Brainstem Response
- Eye Morphology evaluation

OPTIONAL AND/OR SECONDARY SCREEN

- Animal emotionality and affect
- Cognitive functions
- Neuromotor abilities
- Sensimotor gaiting
- Pain sensitivity
- IntelliCage
- Eye screen
- Auditory screen

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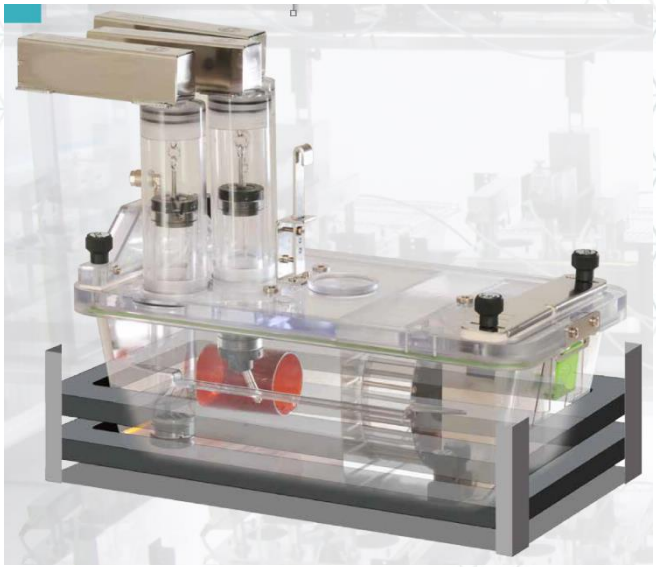
2.c. Metabolism

PhenoMaster (TSE)

- Indirect gas calorimetry
- Feeding & drinking
- Body weight
- Activity
- Exercise
- Telemetry

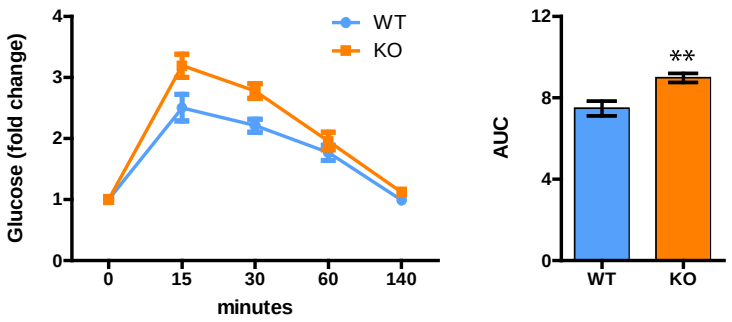
i.e. blood pressure, heart rate,

core body temperature…

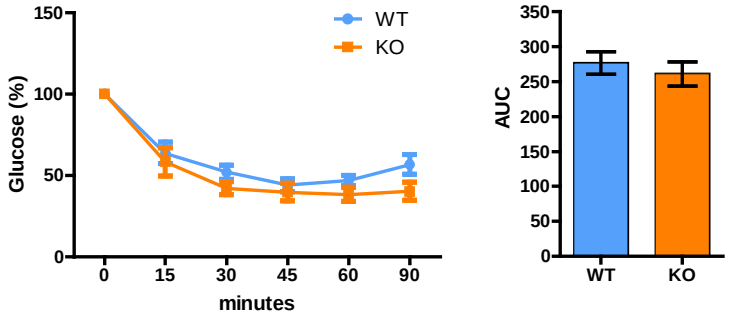


Jan Polak, MD, PhD
Head of Metabolism

Glucose Tolerance Test (GTT)



Insulin Tolerance Test (ITT)



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2.d. Lung function

Benoit Piavaux, PhD
Head of Lung function

screening of lung-function phenotypes

- dynamic resistance and compliance using plethysmography
- 'forced oscillation technique'
- challenges with or without aerosol or injection
- longitudinal studies with repeated lung-function measurements in the same animal

Lung pathophysiology in models of pulmonary diseases

- asthma, pulmonary emphysema, pulmonary fibrosis or acute lung injury
- tailor made services, custom interventions (compound administration, surgery, …)

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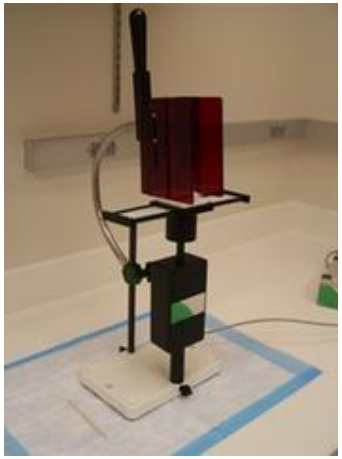
2.e. Cardiovascular system

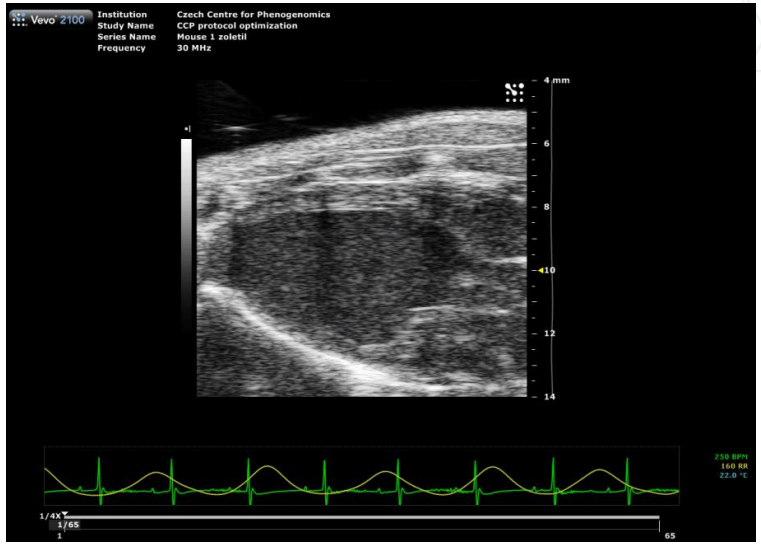
Primary screening

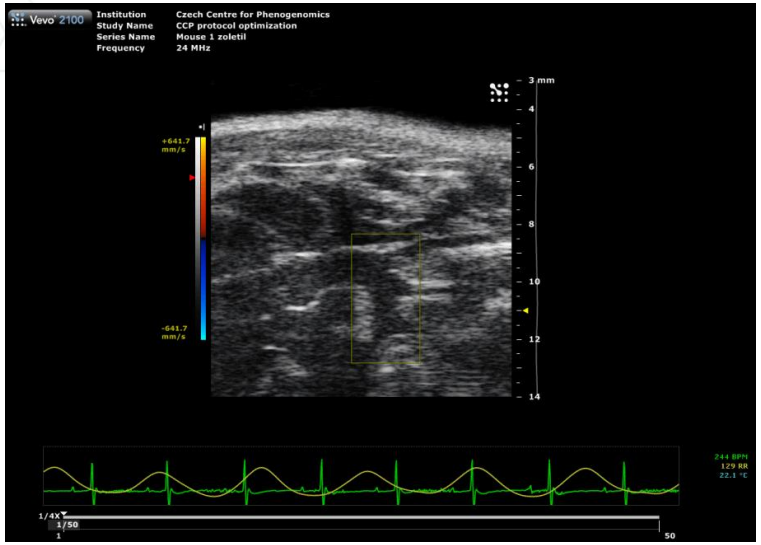
- Transthoracic echocardiography
  - Morphological abnormalities and systolic function of the heart
- Electrocardiography
  - assessment of rhythm abnormalities

Secondary screening

- Advanced emodynamic assessment by echocardiography (doppler flow)
  - Diastolic function, pulmonary and aortic flow
- Conscious restrained blood pressure measurement using tail-cuff







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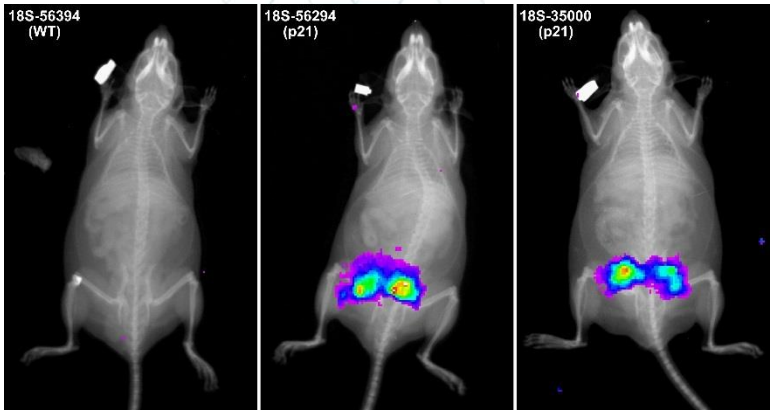
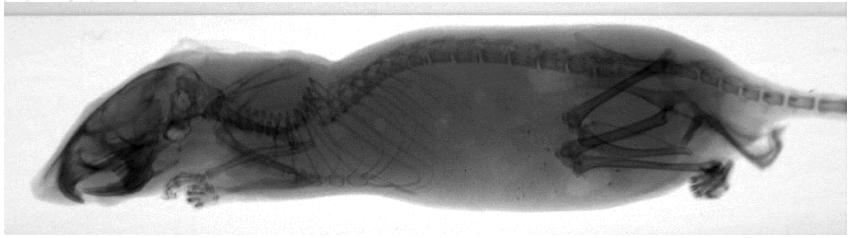
INVESTMENTS IN EDUCATION DEVELOPMENT

2.f. Dysmorphology and whole body imaging

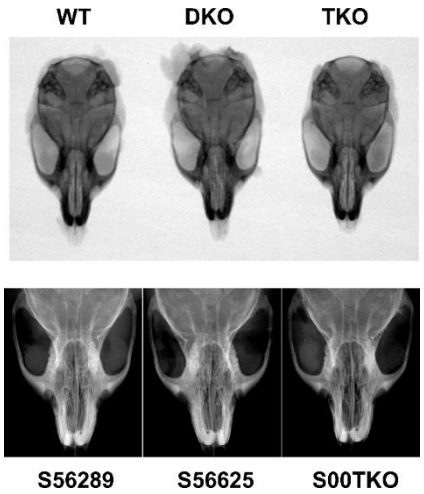
Jan Prochazka, PhD
Head of Dysmorphology & Whole-Body Imaging



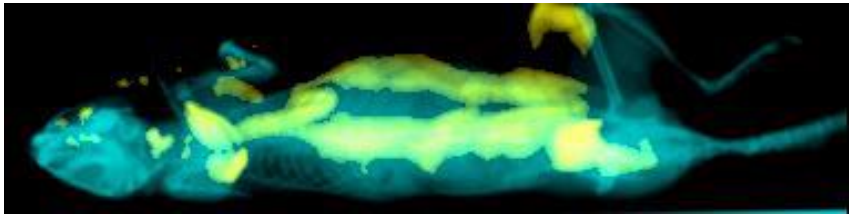
Bioimaging – luciferase expression in vivo



3D X-ray imaging of whole mouse



detailed analysis of morphology of nasal bones by high resolution X-ray



co-detection of X-ray imaging (blue) and fluorescence (labelling muscles in yellow)

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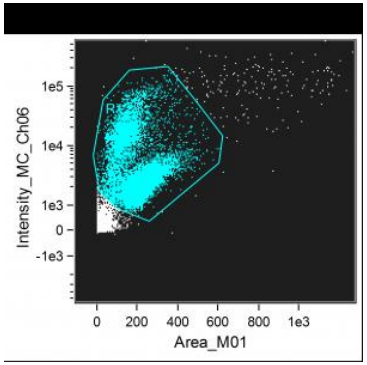
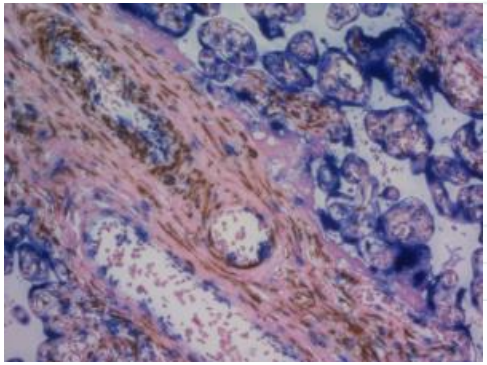
2.g. Immunology

Milan Reiniš, PhD
Head of Immunology





revealing of the etiopathogenic mechanisms of the immunological diseases using the transgenic mouse model

METHODOLOGY

- ELISA
- Microbead based assays
- Flow cytometry
- Imunohistochemistry
- In situ hybridization

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2.h. Biochemistry and hematology

Karel Chalupský, Ph.D.
Head of Biochemistry

ELECTROLYTES	Na, K, Cl, (LIH) -hemolysis
METABOLITES	Glucose, Creatinine, Lactate, Triglycerides, Cholesterol, HDL, LDL, Urea, Bilirubin, Mg, P, Ca, Fe, Uric acid, Glycerol, Unsaturated fatty acids, Bile acids
ENZYMES	ALP, ALT, AST, LDH, Amylase, Lipase
PROTEINS	Total, Albumin and multiplex analysis
HEMATOLOGY	Red blood cell count, white blood cell count, platelet count, hemoglobin, hematocrit and the complete differential white blood cell count

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Multiplex assays

Limit of sample volume for classical Elisa
60ul of serum/ plasma up to 32 simultaneous assays

Commercial bead assays

CD40L	IL-2	IL-18	MCP-1
Eotaxin	IL-3	IL-1β	M-CSF
bFGF	IL-4	IL-21	MIG
G-CSF	IL-5	IL-22	MIP-1α
Ghrelin	IL-6	IL-23 p19	MIP-1β
GIP	IL-9	IL-25	MIP-2
GLP-1	IL-10	IL-27 p28	MIP-3α
Glucagon	IL-12 (p40)	IL-31	PAI-1
GM-CSF	IL-12 (p70)	IL-33	PDGF-BB
GM-CSF	IL-13	Insulin	RANTES
ICAM-1	IL-15	KC	Resistin
IFN-γ	IL-17A	Leptin	TNF-α
IL-1α	IL-17F	LIF	VEGF

CCP bead assays

Amphiregulin
Betacellulin
HGF
sIL6R

Custom bead assays

Available upon demand

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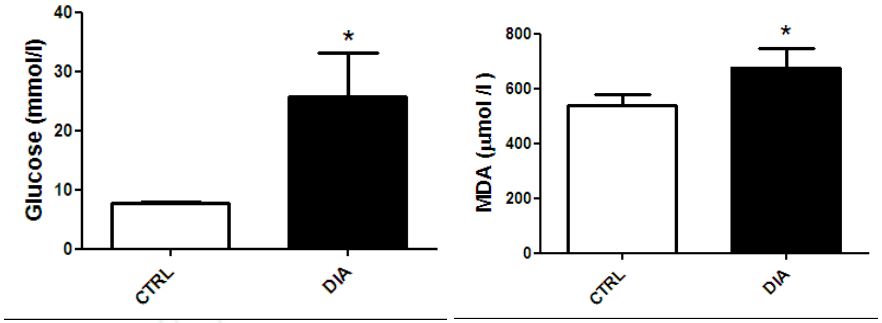
Oxidative stress

Detection of free radicals and their adducts

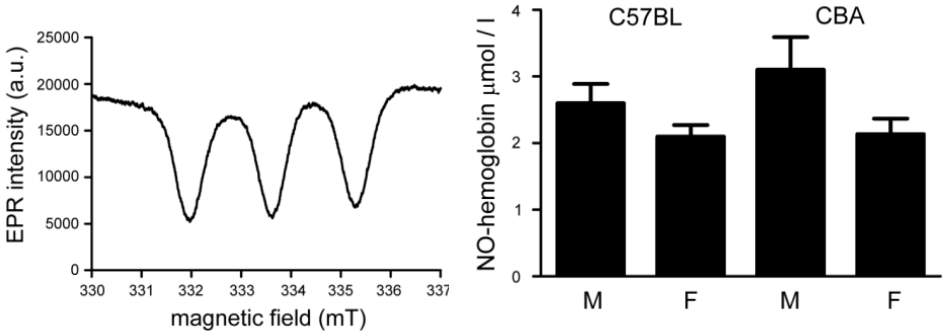
HPLC

ROS	malonyldialdehyde oxyethidium
RNS	tetrahydrobiopterin nitrotyrosine



Electron spin resonance

NO and superoxide in blood and tissues
Differences in mouse strains



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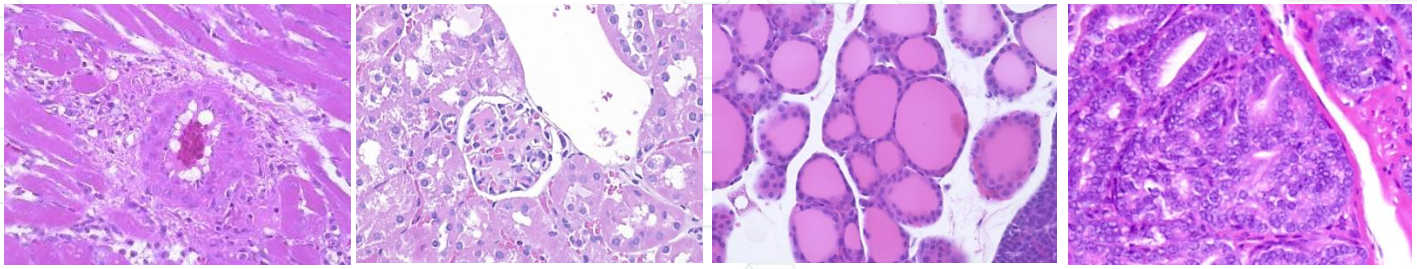
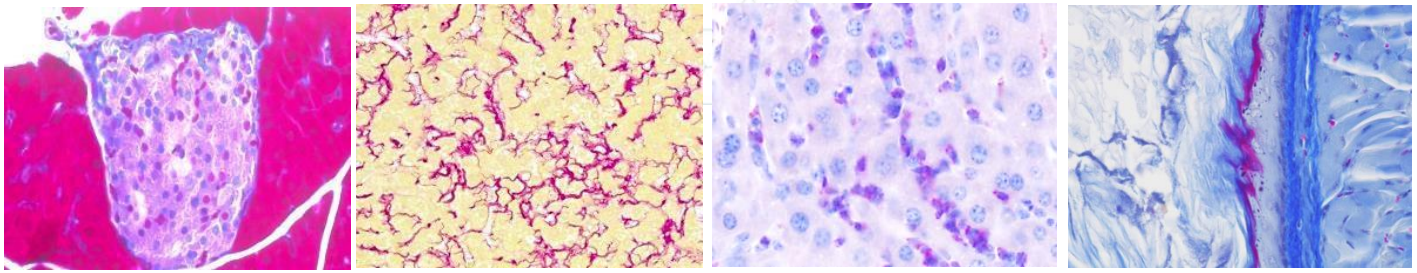
2.i. Histopathology

Ivan Kanchev, MVSc, DVM
Head of histopathology

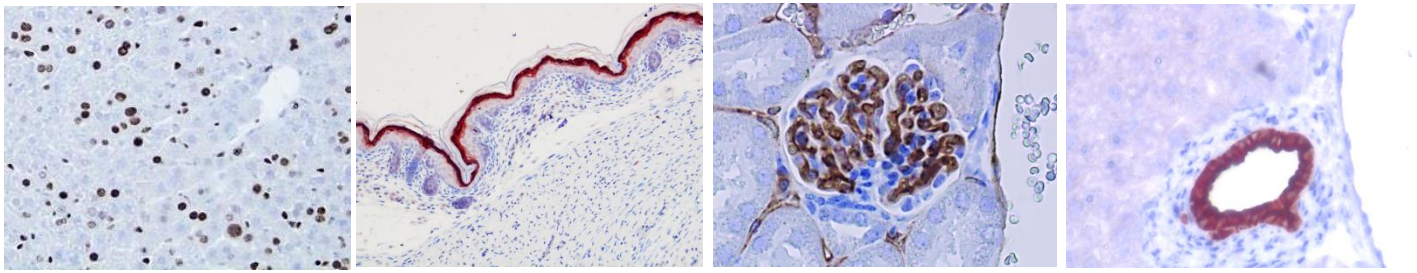
Morphology



Special Stains



IHC



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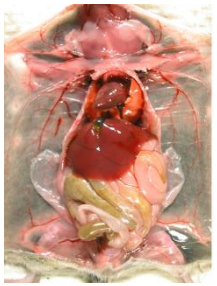
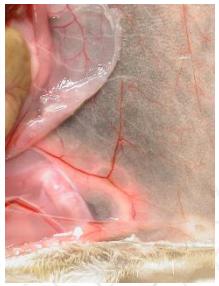
Gross pathology

STANDARD AND SPECIAL STAINS

Method	Performance
HE	A
PAS	M+A
PAS+Diastase	A
PAS+Alcian Blue	A
Chromotrope 2R- Aniline blue	M
AZAN	M
Reticulin	A
Giemsa	M
NASDCL	M
Massons Trichrome	M+A
Jones Methenamine Silver	A
Grocott Silver Impregnation (GMS)	A
Pricrosirius red	M
Pricrosirius red trichrome	M
Congo Red	A

IMMUNOHISTOCHEMISTRY

Target (Clone)	Species	Performance
Ki67 (Tec3)	Mouse	M
Ki67 (SP6)	Mouse	M
Glucagon (Poly)	Mouse	M
Insulin (Poly)	Mouse	M
CK19	Mouse	M
F4/80	Mouse	M
Pax5	Mouse & Rat	M
PCNA	Mouse	M
Caspase 3	Mouse & Human	M
CD31 (MM1)	Mouse	M
Glutamine Synthetase (Poly)	Mouse	M
aSMA (1A4)	Rat	M
Vimentin	Mouse & Rat	M
panCK (AE1+AE3)	Rat	M
eYFP (poly)	Mouse	M
HMW CK (Poly)	Rat	M

OTHER SERVICES: tissue microarrays

The presentation is supported from the project OP EC CZ 1.07/2.3.00/30.0050 “Phenoimage”

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Phenotyping Module
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- Ivan A. Kanchev, MVSc, DVM *(head, histopathology unit)*
- Karel Chalupsky, PhD *(head, biochemistry unit)*
- Kallayanee Chawengsaksophak, PhD *(head, embryogenesis unit)*
- Agnieszka Kubik-Zahorodna, PhD *(head, neurobehavioral unit)*
- Milan Reinis, PhD *(head, immunology unit)*
- Benoit Piavaux, PhD*(head, lung function unit)*
- Jan Polák, MD, PhD *(head, metabolic unit)*
- Jan Procházka, PhD *(head, dysmorphology & whole-body imaging unit)*

Transgenic and Archiving Module
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  Irena Placerova, MEng.
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  Marketa Rynekrova *(deputy head for IMG animal facility)*

CCP administrators
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OP EC projects

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Thank you for your attention

http://www.phenogenomics.cz/

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INVESTMENTS IN EDUCATION DEVELOPMENT

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