INFRAFRONTIER

mouse disease models

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Consiglio Nazionale delle Ricerche
Monterotondo (Italy)

EMMA (EUROPEAN MOUSE MUTANT ARCHIVE)

Generazione, monitoraggio, nomenclatura
di modelli murini geneticamente modificati

EMMA-Infrafrontier Network

CNR/IBC
Istituto di Biologia Cellulare, Monterotondo, Italy

CNRS/CDTA
Centre de Distribution, de Typage et d'Archivage animal, Orléans, France

MRC/MGU
Mammalian Genetics Unit, Harwell, UK

Karolinska Institutet
Karolinska Institutet, Stockholm, Sweden

FCG/IGC
Instituto Gulbenkian de Ciência , Oeiras, Portugal

HMGU/IEG
Institute of Experimental Genetics, Munich, Germany

EMBL/EBI
European Bioinformatics Institute, Hinxton, UK

GIE-CERBM/ICS
Institut Clinique de la Souris, Illkirch/Strasbourg, France

Sanger Institute
Wellcome Trust Sanger Institute, Hinxton, UK

CNB/CSIC
Centro Nacional de Biotecnología, Madrid, Spain

Fleming
Biomedical Sciences Research Center Al. Fleming, Athens, Greece

OULU
University of Oulu, Oulu, Finland

BIAT
Vetmeduni Vienna, Biomodels Austria, Vienna, Austria

IMG
Institute of Molecular Genetics, Prague, Czech Republic

TAU
Tel Aviv University, Tel Aviv, Israel

NKI
Netherlands Cancer Institute, Netherlands, Amsterdam

Il modello di EMMA - Infrafrontier

I modelli mutanti vengono ricevuti e controllati geneticamente

Criopreservati come embrioni e gameti in azoto liquido

Distribuiti in condizioni sanitarie SPF (Specific Pathogen Free)

TRANSFER FROM MOUSE TO HUMAN

INFRAFRONTIER

MOUSE STUDIES CONTRIBUTED TO **MORE THAN 30** NOBEL PRIZES IN MEDICINE AND PHYSIOLOGY SINCE 1905.

22.500 Mouse protein-coding genes

The genetic and physiological **characteristics of mice resemble those of humans**

17.000 of these genes are similar

20.500 Human protein-coding genes

Many symptoms of human conditions & diseases **can be replicated** in mice

Therefore, the mouse is **a preferred model organism** for scientific research.

Mouse studies help to **understand diseases in humans** and to develop treatments for them.

Identification of **histocompatibility antigens** with practical implications on treating infections and **improving organ transplantation** in humans

Nobel Prize 1980

Development of a **treatment for blood cancer** (acute promyelocytic leukaemia APL), improving **treatment regimes for breast cancer**

Discovery how **prions cause and transmit neurodegenerative diseases**, identification of the **biological principles underlying common types of dementia** such as Alzheimer's

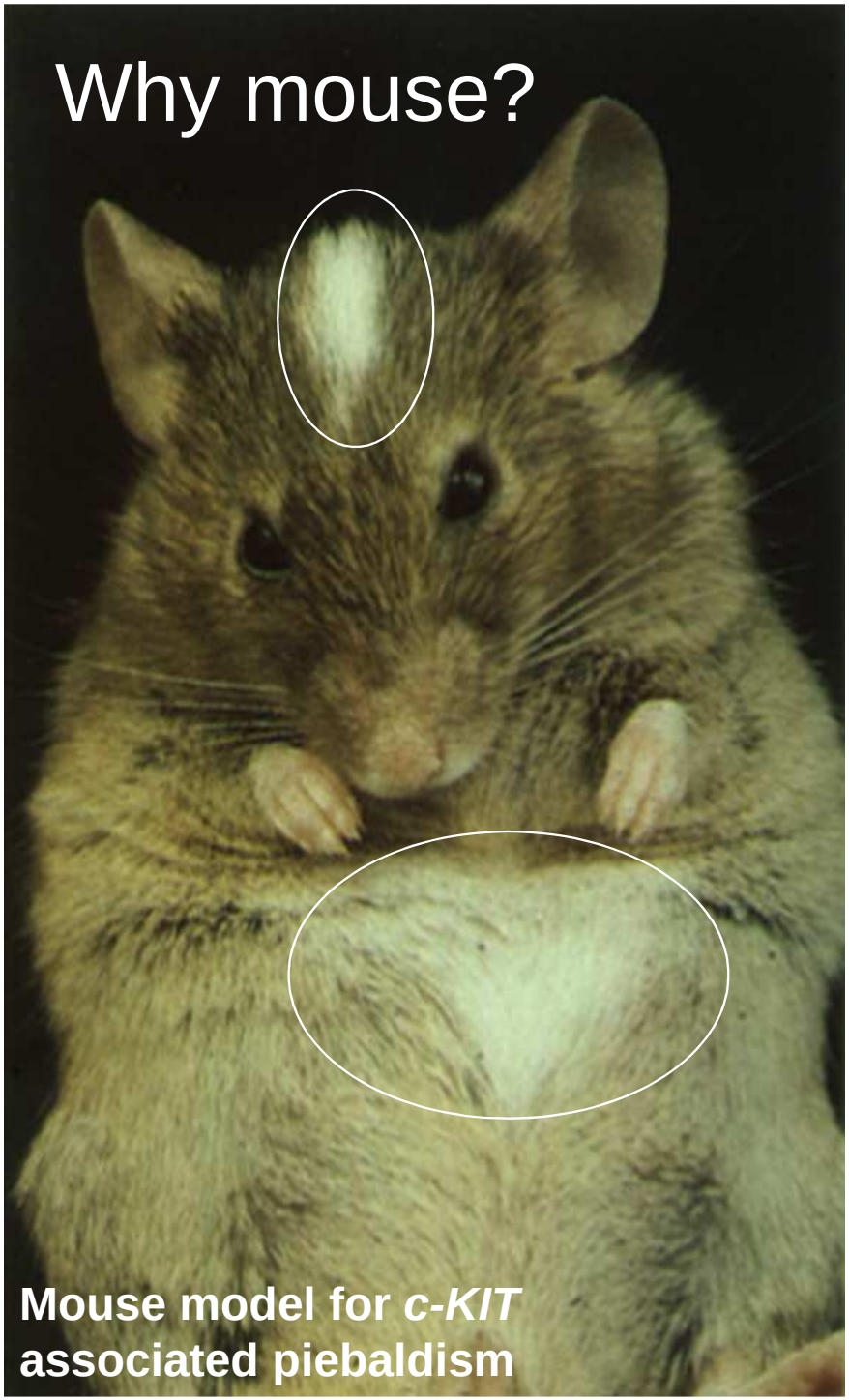
Nobel Prize 1997

Identification of key principles for the activation of the immune system provides new avenues for **battling infections, cancer, and inflammatory diseases**

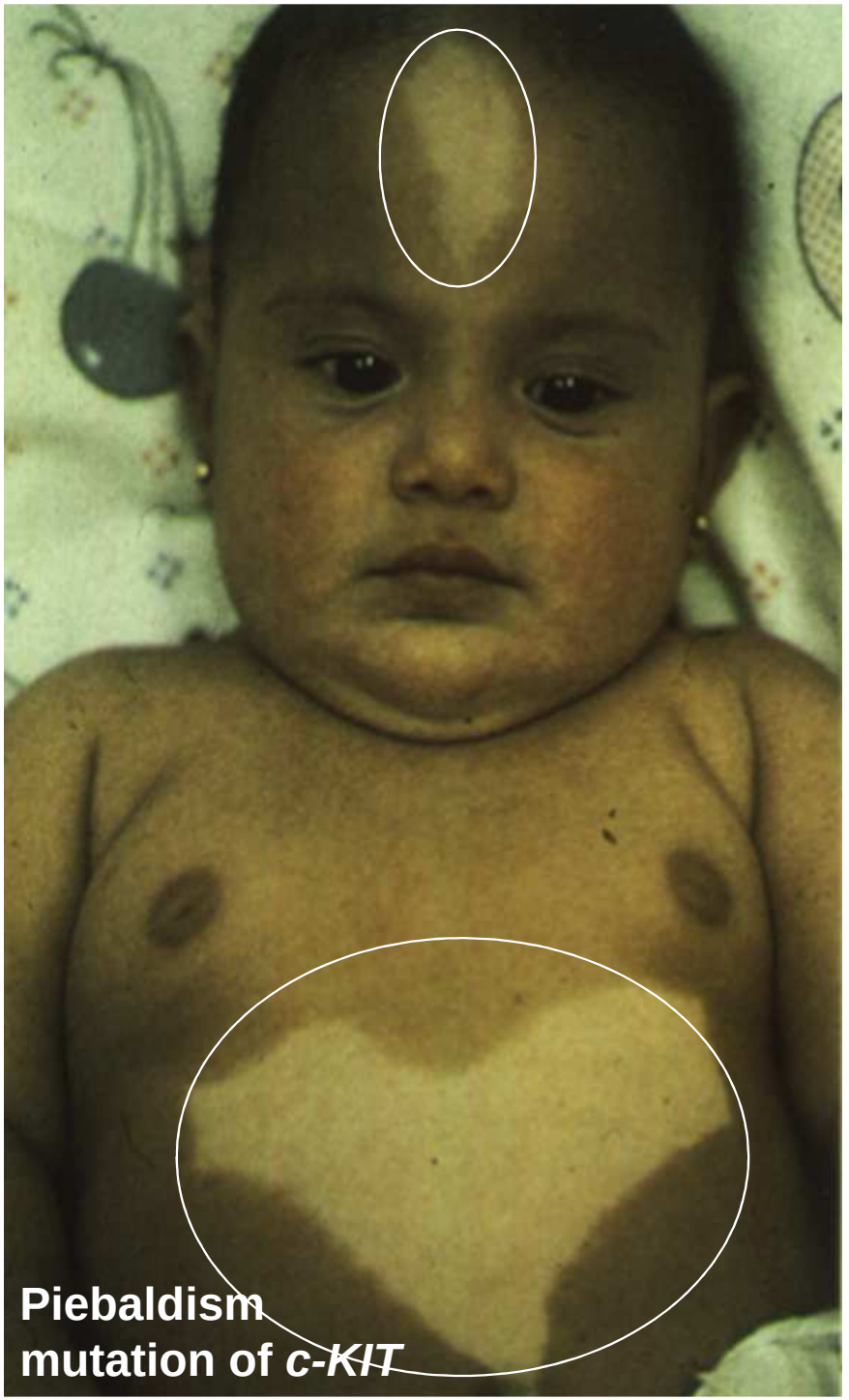
Nobel Prize 2011

Source: National Center for Biotechnology Information; MGI-Mouse Genome Informatics; AnimalResearch.info

Why mouse?



Mouse model for *c-KIT* associated piebaldism



Piebaldism mutation of *c-KIT*

Some Historical Milestones

1942 – Mouse Embryo Transfer (Fekete & Little)
- **1953 – DNA double-helix structure (Watson & Crick)**
- 1960 – mRNA discovery
- 1964 – Genetic Code
- 1969 – Isolated first gene (Harvard Med School)
- 1970 – Restriction Enzymes, Reverse Transcriptase, Gene Synthesis
- **1981 – Culture pluripotential mouse ES from embryos**
- **1982 – Transgenic mouse (Brinster & R. Palmiter)**
- 1983 – PCR (K. Mullis)
- 1984 – Germ-line chimeras (blastocyst injection of mouse ES)
- 1987 – Human Genome Project launched (HUGO)
- **1987 - KO mouse by homologous recombination in ES (Capecchi)**
- 1988 – Transgenic mouse patented in USA
- 1989 – KO mouse: germ-line transmission of targeted allele
- 1990 – PCR is already “popular”
- 1996 – Dolly cloned sheep: somatic nuclear transfer from adult cells (Roslin Inst.)
- **2001 – HUGO Human genome completed**
- **2002 – Mouse genome completed**
- **2008- EUCOMM--the European conditional (Cre-Lox) mouse mutagenesis program**
- **2012- CRISPR/Cas-mediated gene editing**
- **2013- Infrafrontier and International Mouse Phenotyping Consortium IMPC**
- **2020- IMPC the Italian Mouse Clinic at Monterotondo**

Genetic Engineering Technologies

Transgenesis (Tg)

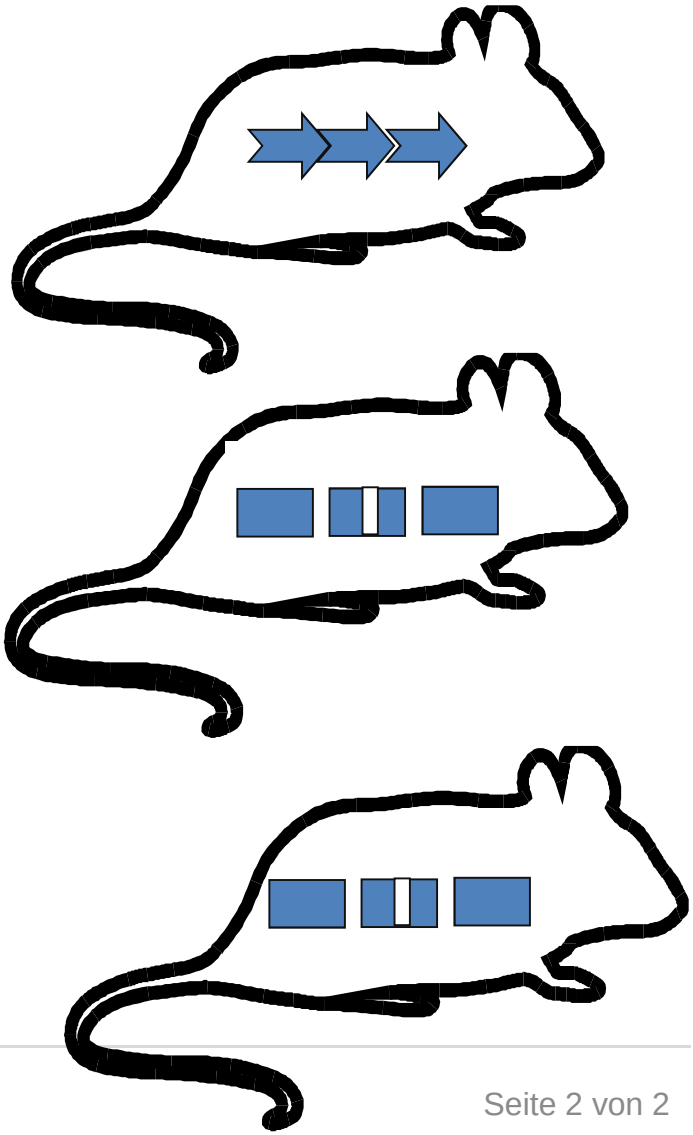
- Adding new genetic material

Homologous Recombination and Target Mutation (Tm)

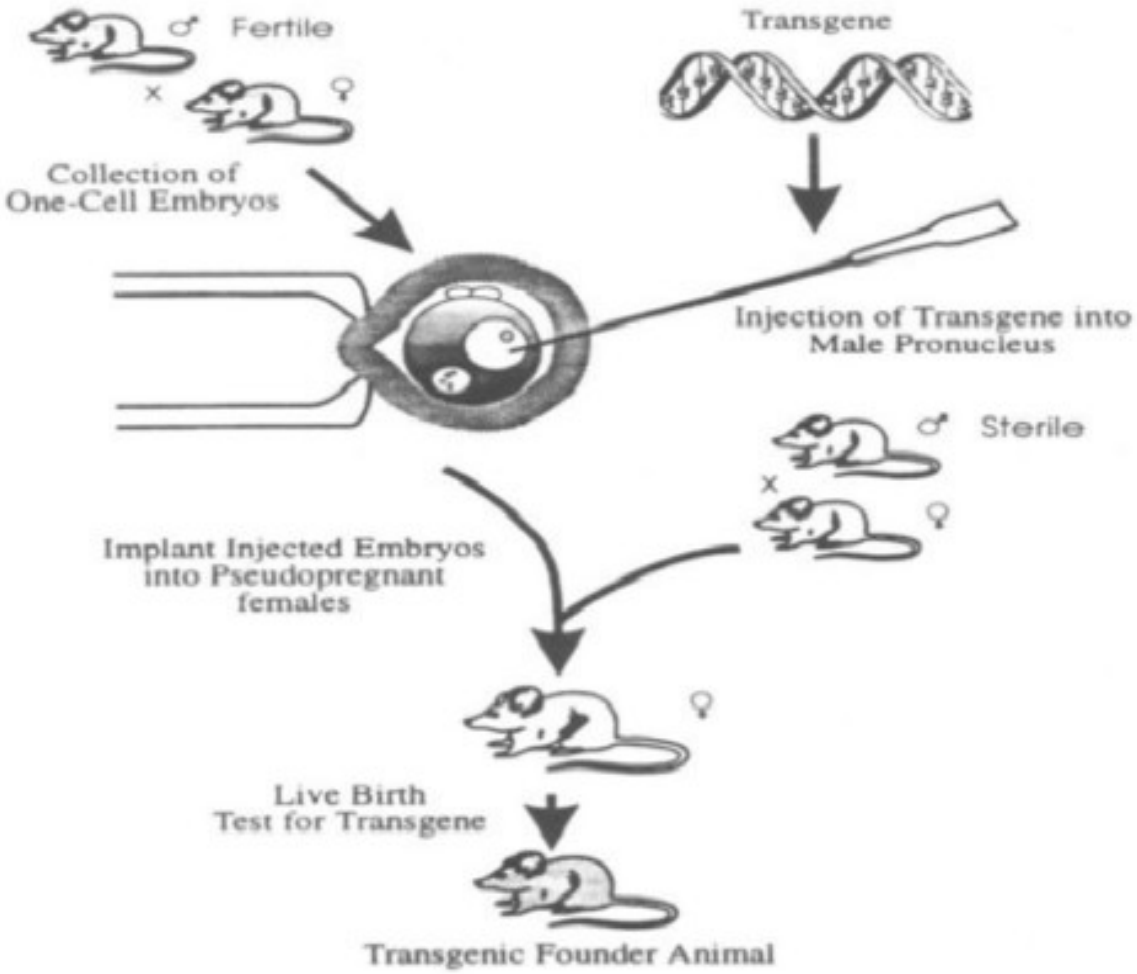
-Targeting a specific gene using ES cells

Endonuclease Mediated Mutation (Em)

- Targeting a specific gene using Crisp/Cas



TRANSGENIC MOUSE (Tg)



Unpredictable features linked to Transgenesis

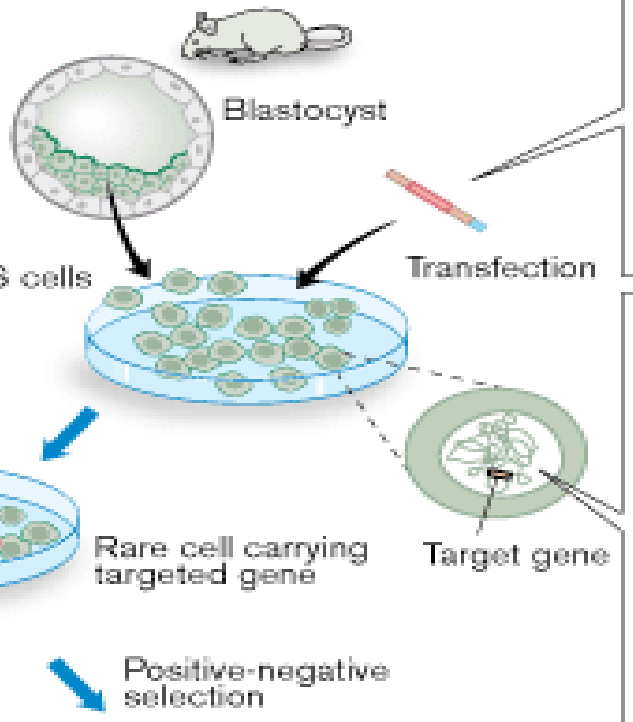
- Disruption (functional KO) of endogenous gene
- Misregulation of endogenous gene (expression profile)
- Misregulation of transgene (expression profile)
- Unstability of insertion site in successive generations
- Variegation of transgene expression (uneven profile within a tissue)
- Silencing of transgene expression in successive generations
- Mosaicism and reduced transmission to F1
- Multiple integrations and segregation > F1
- Embryolethality

- Immunity defects (consequences on housing hygiene and HM)
- Behavioral alterations (could be subtle defects)
- Reproductive defects
- Other defects

General strategy for gene targeting in mice

Step 1 Gene targeting in ES cells

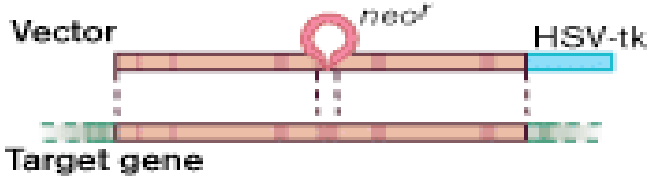
1. ES cell culture
Embryonic stem (ES) cells are cultivated from mouse pre-implantation embryos (blastocysts).

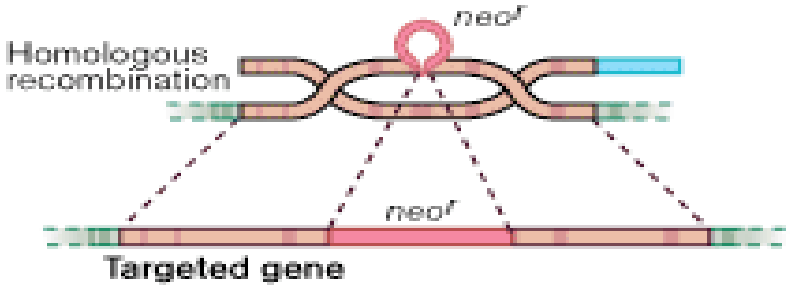


4. Proliferation of targeted ES cell
Selection for presence of *neo*^{r} and absence of HSV-tk enriches targeted ES cells.

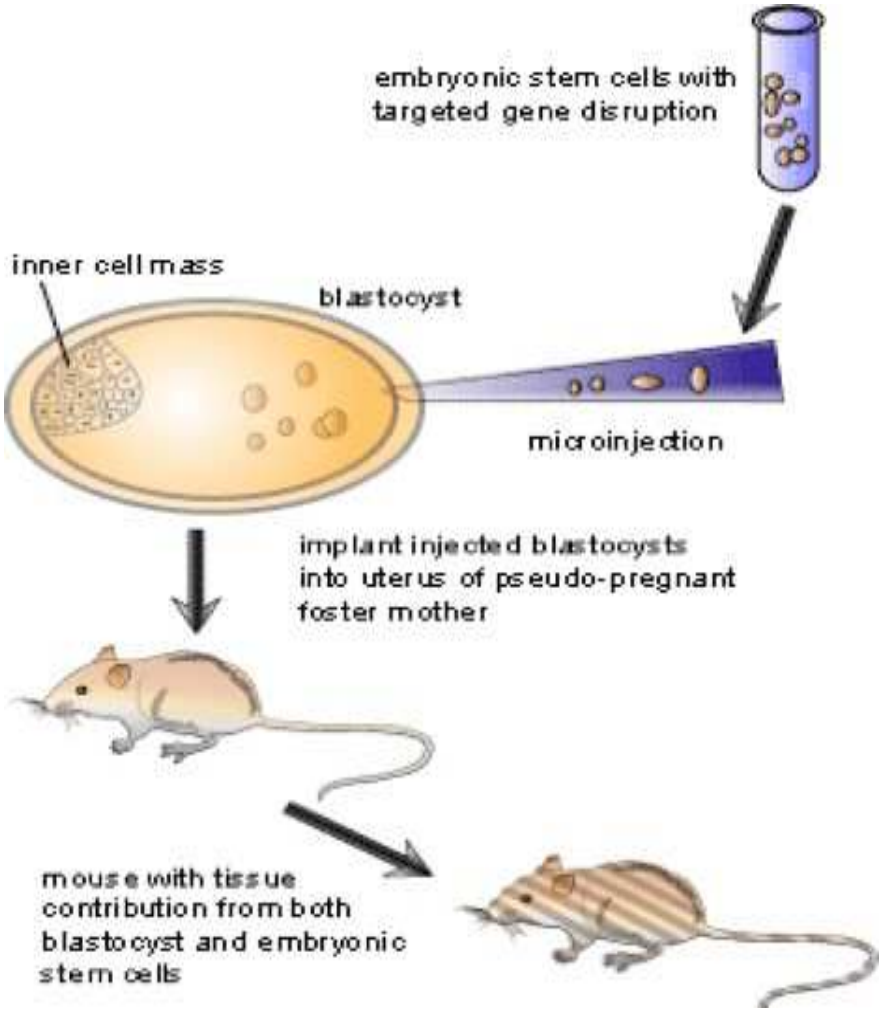
2. Construction of targeting vector
The vector contains pieces of DNA that are homologous to the target gene, as well as inserted DNA which changes the target gene and allows for positive-negative selection

3. ES cell transfection
The cellular machinery for homologous recombination allows the targeting vector enables the target vector to find and recombine with the target gene.





TARGET MUTATION (KNOCK OUT and KNOCK IN)

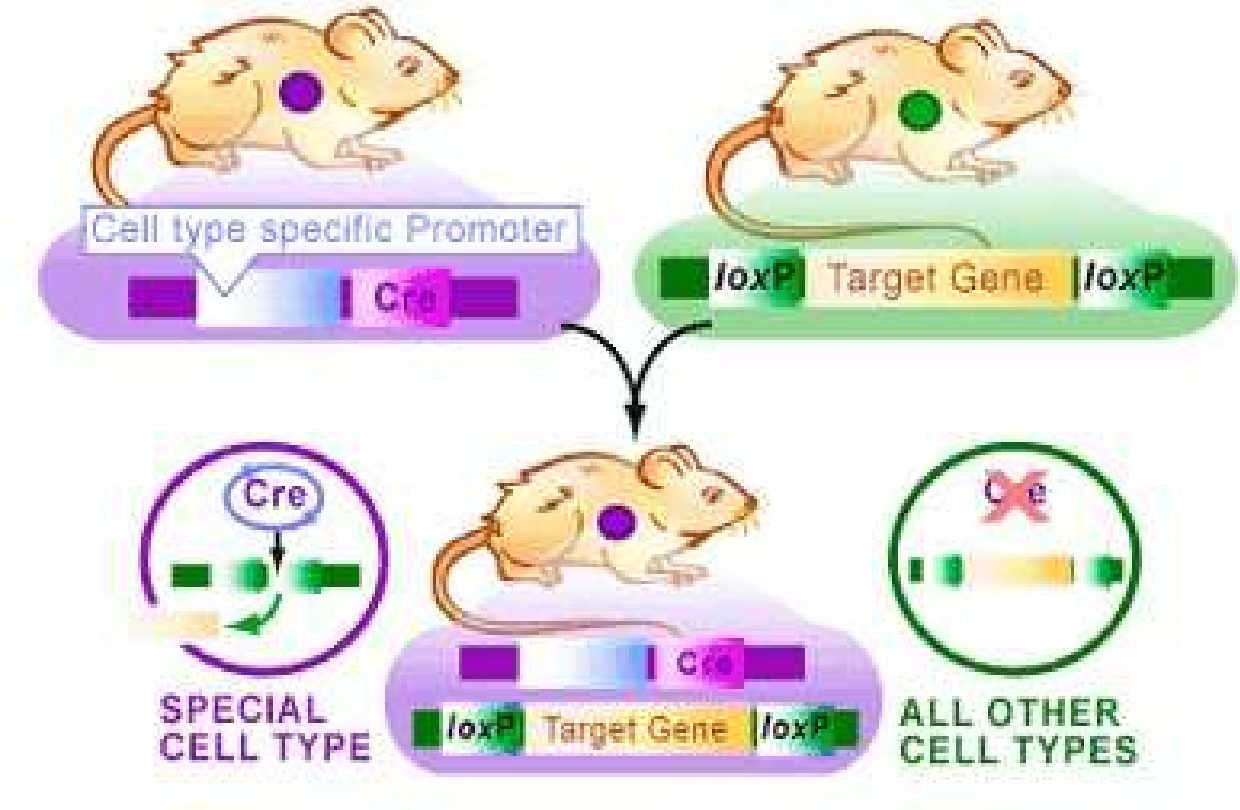


CONDITIONAL MUTAGENESIS

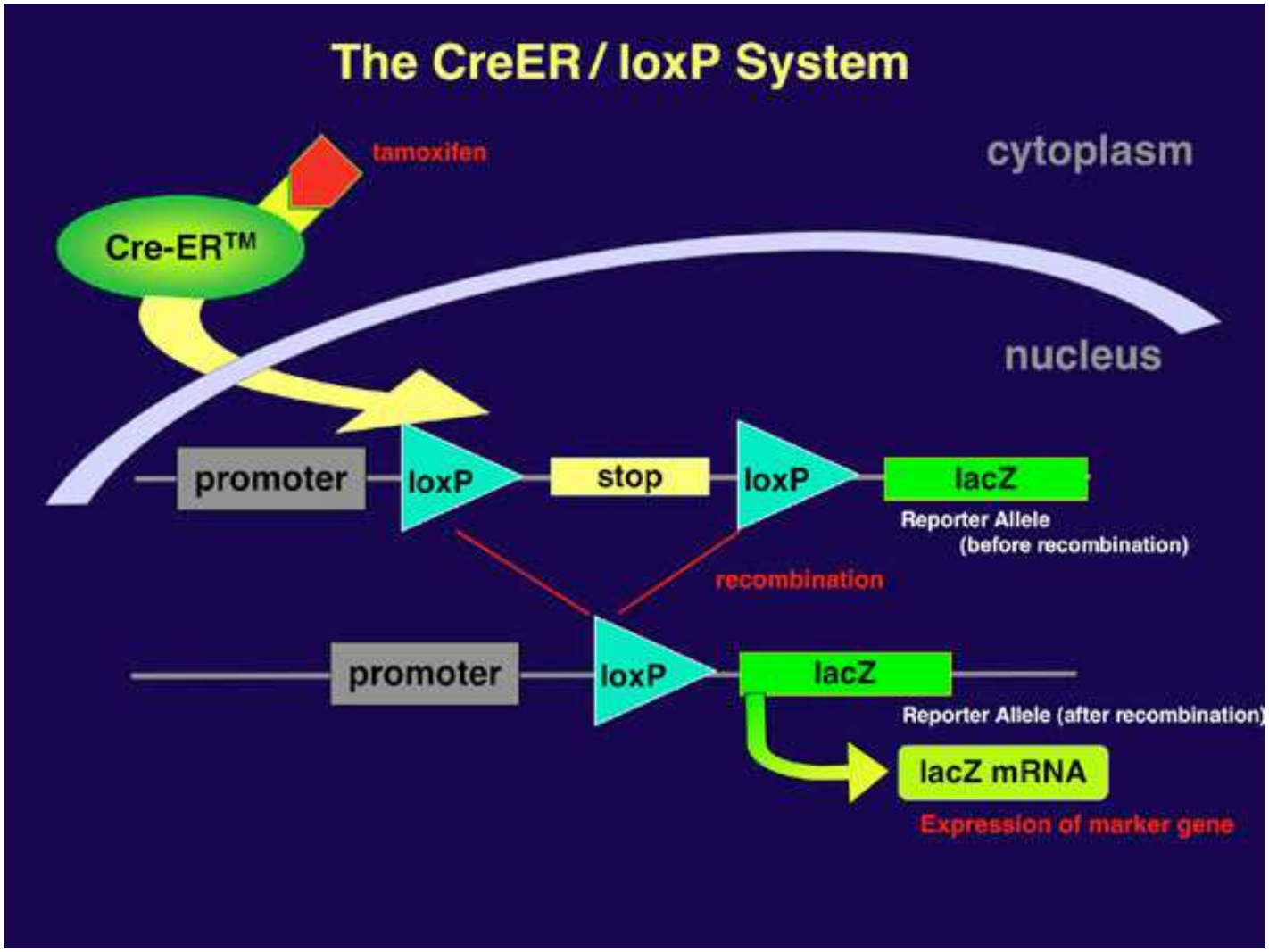
Multiple strategies are being developed to topologically and / or temporally restrict mutations of genes in transgenic mice, knock out, knock in and knock down models from year 2000.

They rely on the use of site-specific recombinases, of heterologous origin, that promote deletion of targeted genomic DNA fragments, thereby inactivating the gene of interest, and variations of these systems. The most popular is the Cre/lox system. The Cre recombinase acts on your element when it is flanked by loxP sites. The Flp recombinase acts on your element when it is flanked by FRT sites.

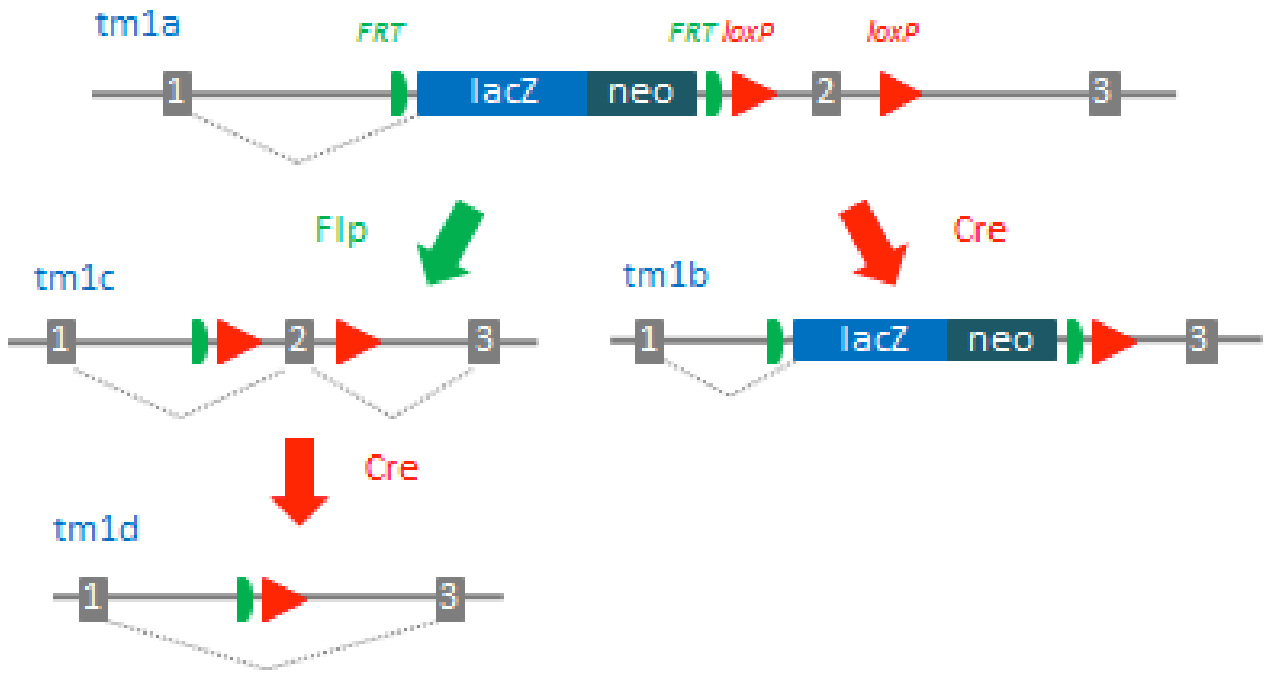
CONDITIONAL KNOCK OUT



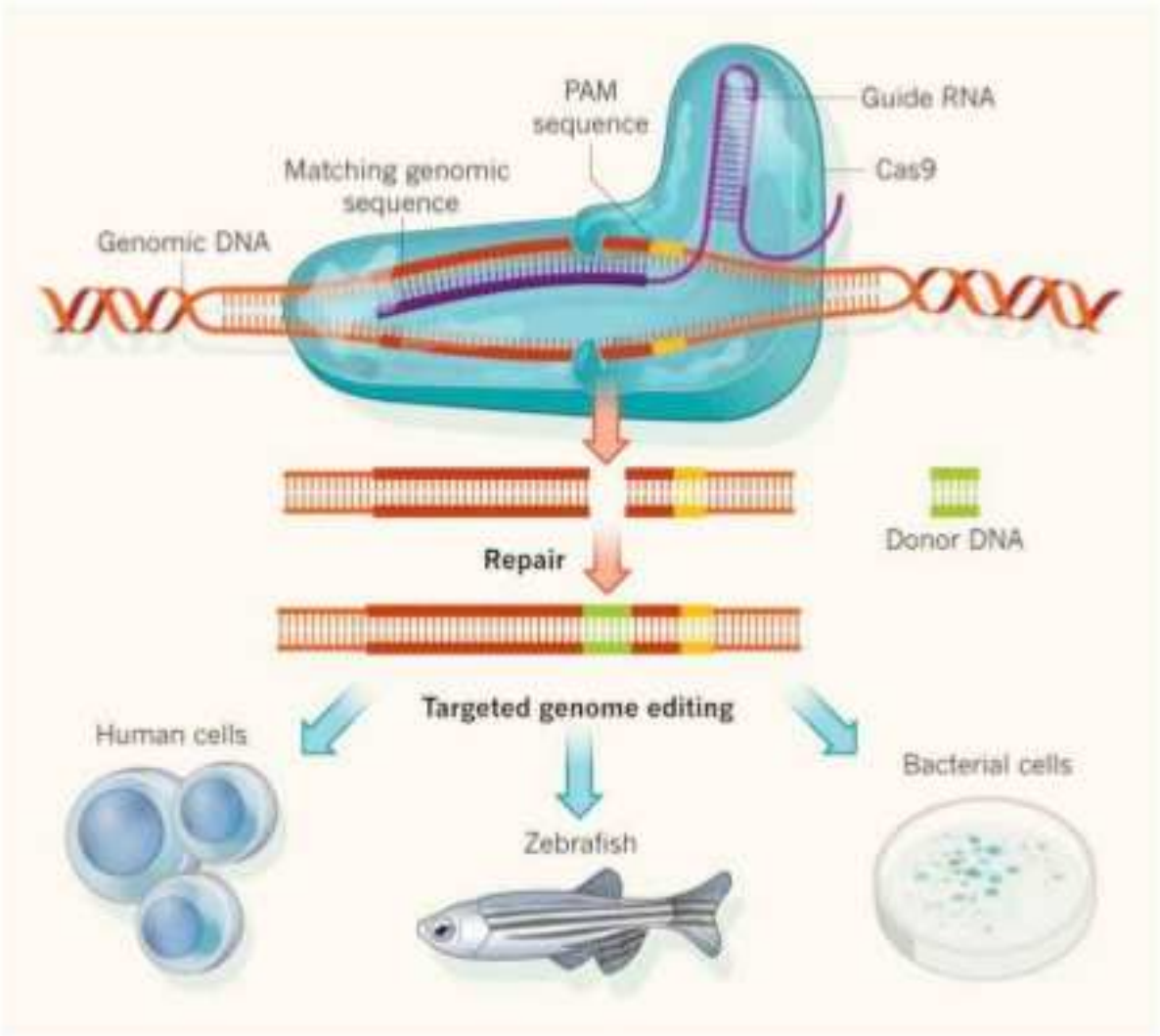
TAMOXIFEN INDUCIBLE CRE EXPRESSION



Knockout-first allele: Promoterless selection cassette



Crispr Cas-9 editing tool



1909 C.C. Little begins developing inbred strains
defined genetic background to study
complex trait, tumor transplant acceptance
genetic component?
map tumor transplantation genes?

- Genetically identical
- DBA- first inbred strain, now at > ~F250 Other common strains: AKR/J, BALB/cJ, C57BL/6J
- Coisogenic strains- spontaneous mutations give rise to strains that are genetically identical except at the mutated locus.
- Congenic strains- are artificially derived by breeding and include extraneous genetic material from the donor parent.

Inbred Strains

Advantages

- Genetic and Phenotypic uniformity
- Well Characterized
- Most of standard inbred strains >200 generations

Disadvantages

- Not as robust (smaller, lower reproductive performance, shorter lifespan)
- Strain-specific characteristics
- Expensive

Uses

- Widely used in all types of research (immunology, cardiovascular biology, neurobiology etc.)
- Models for human disease
- Background for mice with targeted mutations

Genetically Modified Mice can be compared experimentally with Wild-Type inbred strain, as control, if the line is on a pure genetic background

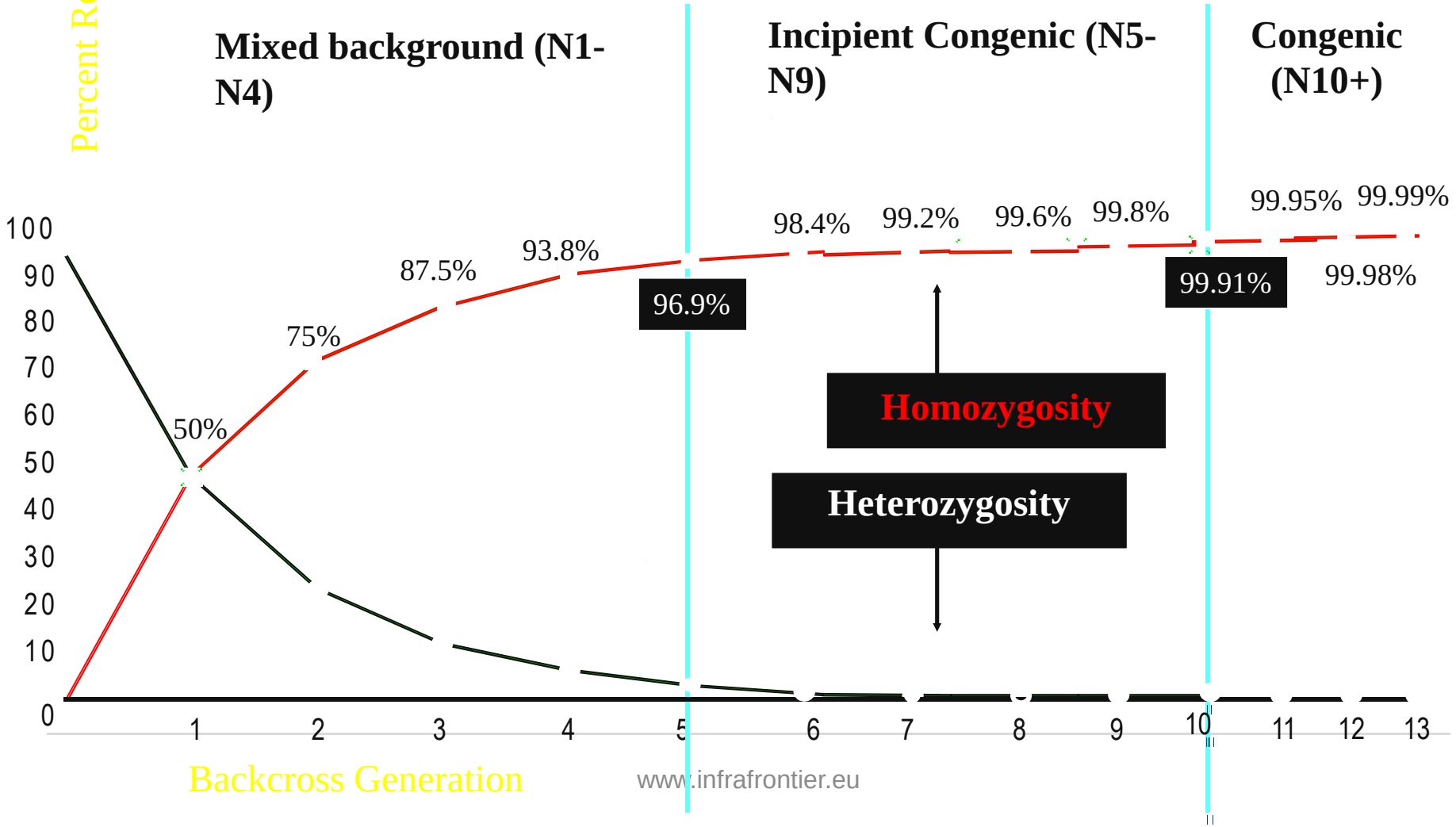
The line has been created from C57BL/6N ES Cells and maintained on B6N background

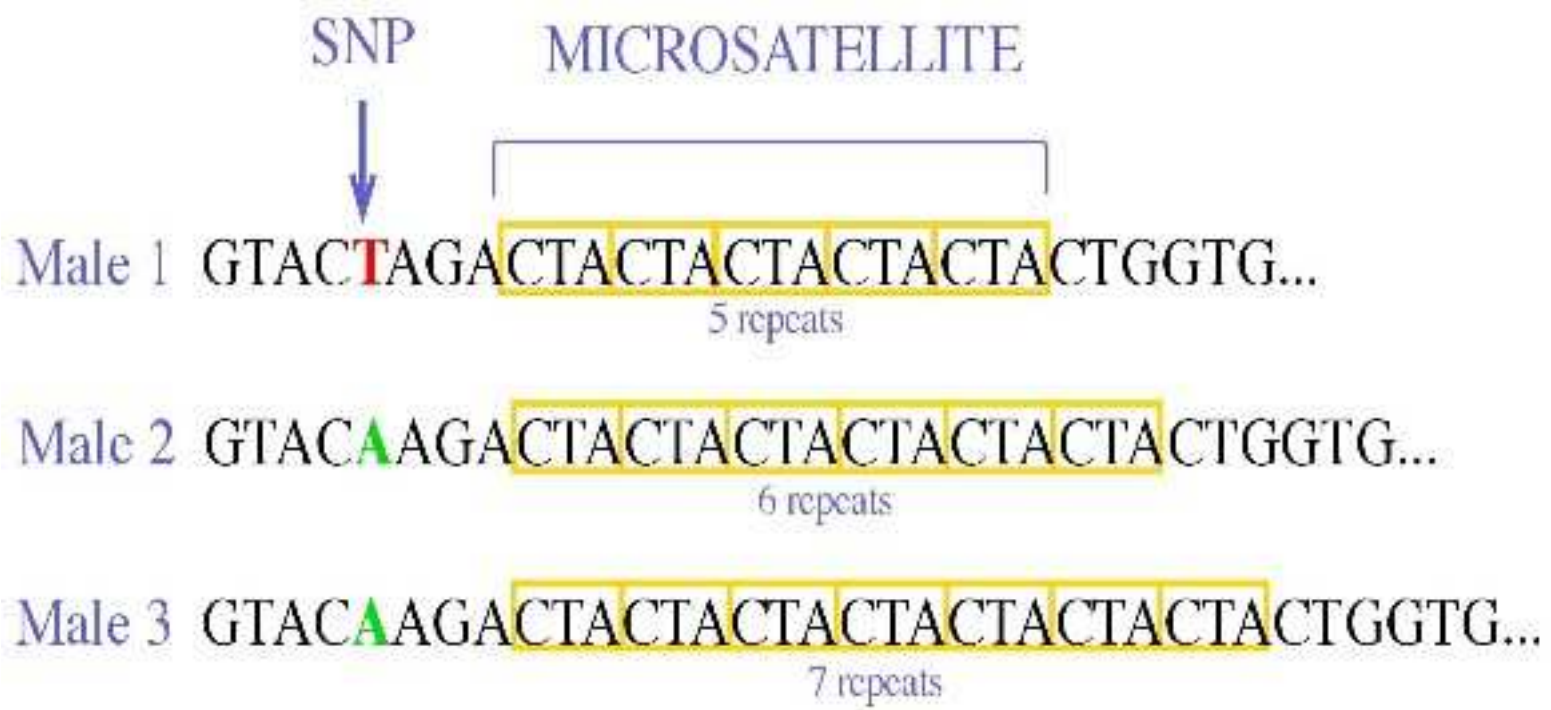
The line has been created from another ES line (e.g. 129 ES Cells) then Backcrossed on B6N background for more than 10 generations

It is important to maintain a record of each crossing of your line to know if you are working on a pure or a mixed background

Statistical Percentage of the Recipient Genome with Increasing Generations of Backcrossing

Backcrossing




SNP
MICROSATELLITE
Male 1 GTACTAGACTACTACTACTACTACTGGTG...
5 repeats
Male 2 GTACAAGACTACTACTACTACTACTACTGGTG...
6 repeats
Male 3 GTACAAGACTACTACTACTACTACTACTACTGGTG...
7 repeats

B6;129P2 - *Apoa1*^{tm1Unc}/J

B6.129P2-*Apoa1*^{tm1Unc}/J

B6N-*Apoa1*^{tm1Unc}/J

- B6;129P2: mix of C57BL/6 and 129P2 (from ES cell line)
- B6.129P2: backcrossed to C57BL/6 for ≥5 generations
- ES cells **C57BL/6N** and maintained on B6N (Pure background)
- *Apoa1*: targeted gene (Apolipoprotein a1)
- *tm1*: first targeted allele mutation of *Apoa1* gene in this lab
- *Unc:* lab registration (ILAR code) for Univ. of North Carolina (or scientist)
- J where the strain is mantained (The Jackson)

EM:00323 (EMMA ID) B6.129P2-Gjb6^{tm1Kwi}/Cnrm

Provider : Klaus WILLECKE

Strain type Targeted Mutant Strains : Knock-out

Genetic description : Exon2, including the whole open reading frame of Cx30 (Gjb6), is homologously replaced by the open reading frame of LacZ. The selection marker gene (Neo) is maintained in the genome.

Phenotypic description : Deafness.

References :

Connexin30 (Gjb6)-deficiency causes severe hearing impairment and lack of endocochlear potential.; Teubner Barbara; Michel Vincent, Pesch Jörg, Lautermann Jürgen, Cohen-Salmon Martine, Söhl Goran, Jahnke Klaus, Winterhager Elke, Herberhold Claus, Hardelin Jean-Pierre, Petit Christine, Willecke Klaus; 2003; Hum Mol Genet; 12; 13-21; 12490528

C57BL/6N-Adgrd1 $^{tm1a(EUCOMM)Wtsi}$/Cnrm

C57BL/6N-Adgrd1 $^{tm1b(EUCOMM)Wtsi}$/Wtsi

C57BL/6N-Abcb5$^{em1(IMPC)Wtsi}$/Wtsi

C57BL/6N-Ap2a2$^{em1(IMPC)Wtsi}$/Wtsi

STOCK-Tg1 (ACTB-Neurog3)**2** ^{BzaI}/Orl

B6.129-Map4$^{Gt(pGT1.8geo)2Pgr}$/Cnrm

- tg1: first transgenic mutation
- em1: first endonuclease mutation (Crisp/cas9 or other endonucleases)
- Stock Complex Background, Gt Gene Trap

CRE LOX CROSSES

Cre deletion generates an heritable allele

B6-Tg1 (cre deleter) ^{Cnrm} X B6-*Apoa1*$^{tm1(fl/fl)Unc}$

=

B6-*Apoa1*$^{tm1.1(KO)}$ NEW STRAIN

Cre deletion does not generate an heritable allele (somatic event)

B6-Tg1 (Sox10 cre) ^{Cnrm} X B6-*Apoa1*$^{tm1(fl/fl)Unc}$

=

B6-*Apoa1*$^{tm1(fl/fl)Unc}$ B6-Tg1 (Sox10 cre) ^{Cnrm} The Strain carries both mutations

Strain Names Based on Origin

- SJL: Swiss, Jim Lambert
- NZW: New Zealand White
- NZB: New Zealand Black

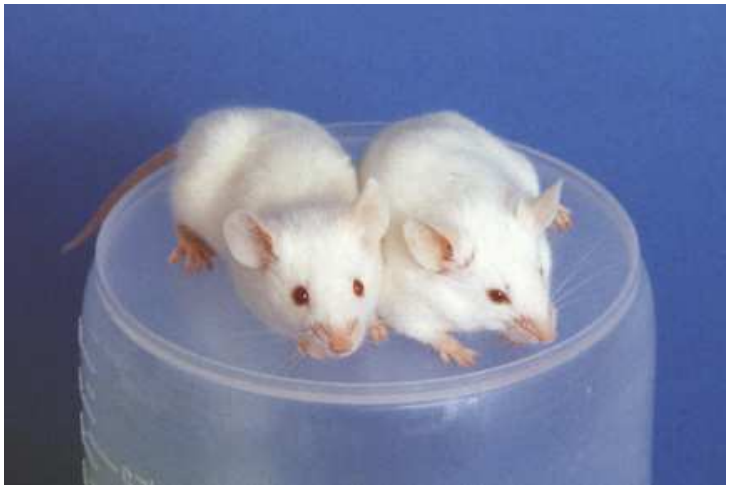
• Strain Names Based on Phenotype

NOD: Nonobese Diabetic

DW: Dwarf *Pit1*^{dw}

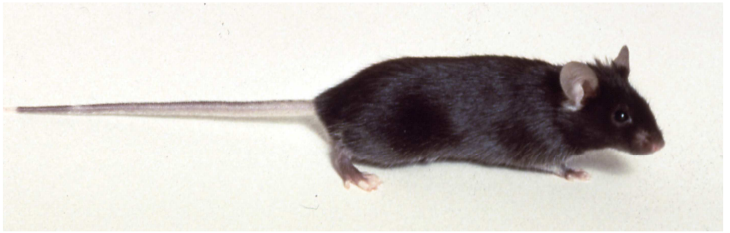
NU: Nude

NOD/LtJ NON/LtJ



Strain Names Based on Coat Color

C57BL: *a/a*
Coat Color: nonagouti (black)



C57BR: *a/a Tyrp1^{b}/Tyrp1^{b}*
Coat Color: brown

C57L: *a/a Tyrp1^{b}/Tyrp1^{b} ln/ln*
Coat Color: brown leaden (gray)

SITI WEB UTILI ALLA NOMENCLATURA ED ALLA RICERCA DI MODELLI MUTANTI

www.jax.org (The Jackson Laboratories)

www.informatics.jax.org (MGI)

www.mousephenotype.org (IMPC)

www.Infrafrontier.eu (EMMA/Infrafrontier)

www.informatics.jax.org/mgihome/nomen/inc.shtml (Nomenclature Committee)

www.findmice.org (IMSR)

National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes

- CORRIGENDUM of 24 January 2013 -

Working document on genetically altered animals

Brussels, 23-24 January 2013

The Commission established two Expert Working Groups (EWG) (to develop common format for statistical reporting and for the assessment of severity of procedures) to facilitate the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. All Members States and main stakeholder organisations were invited to nominate experts to participate in the work.

The EWG for the statistical reporting met several times in 2011. During their work it became apparent that some further understanding was needed as to how genetically altered animals are to be considered. To seek some clarity to some of the questions, the first meeting of the Severity Assessment EWG focused on the genetically altered animals in its meeting in December 2011.

The consensus reached for the understanding of how genetically altered animals are authorised and covered by the statistics is detailed in this document. The document is the result of the work of the different EWGs, discussions with the Member States as well as legal input from the Commission. It was endorsed by the National Competent Authorities for the implementation of Directive 2010/63/EU at their meeting of 22 - 23 March 2012, followed by the endorsement of the GA welfare assessment scheme (incorporated in the Annex) at their meeting of 11-12 July 2012. A corrigendum to the Annex was endorsed on 24 January 2013.

Key Elements of a GA Rodent Welfare Assessment Scheme

Include animals of representative age groups

- soon after birth, around weaning and again following sexual maturity*)
- a minimum of 7 males and 7 females sampled from more than one litter
- data from a minimum of two breeding cycles (from F2 onwards)
- comparisons made wherever possible with similar non GA animals.

*) and at additional time points as considered appropriate by a prospective review of the potential impact of the gene alteration e.g. where there is an age dependent onset of disease

CRITERIA	WHAT TO LOOK FOR
Overall Appearance	Is the animal morphologically 'normal'? Are there any malformations or any other indicators that the phenotype has been affected? For example skeletal deformity or hydrocephalus.
Size, conformation and growth	Are there any deviations from expected size or growth curve?
Coat condition	Is there any piloerection, areas of fur loss, loss of whiskers, barbering? Is the skin / fur in good condition?
Behaviour – **Posture, gait, activity and interactions with the environment**	Do they exhibit the full repertoire of behaviours appropriate for the strain/species, including social interactions, grooming, walking, running, digging, climbing? Are these normal? Is the animal hunched or reluctant to move? Is movement impaired or is there any difficulty with orientation? Any signs of rigidity or tremors? Any abnormal activity levels? Prolonged inactivity could indicate chronic stress or depression (anhedonia) and/or sickness/pain, particularly if linked with a hunched posture and/or rough or unkempt coat. Unusual activity, such as hyperactivity, could indicate stereotypy or other behavioural abnormality.
Clinical signs	For example - nasal or ocular discharge, swollen or closed eyes; increased respiratory rate; dyspnoea; seizures/twitches/tremors; increased vocalisation with handling; overgrown teeth; presence of tumours, neurological or musculoskeletal abnormalities. Is metabolism impaired, for example, increased or decreased food or water intake, excessive urination? Consistency of faeces.
Relative size	Any unusual changes in size of the animals should be noted, and comparisons made within the litter. It may be helpful to generate a growth curve for the line.
Numbers	Where death occurs, it is important to maintain accurate records such that any pre- or post-weaning losses can be investigated. Where appropriate (e.g. higher than anticipated mortality rate), post mortem examinations should be carried out to help determine the cause of death. A review of fertility can also be helpful in assessment of whether or not the modification is having an effect e.g. conception rates; abortions; stillbirths.

Working Party Report

Laboratory Animals
2017, Vol. 51(6) 573–582

DOI: 10.1177/0023677217718863
journals.sagepub.com/home/lan

Guidelines on severity assessment and classification of genetically altered mouse and rat lines

Anne Zintzsch[1,2], Elena Noe[1,3], Monika Reißmann[1,4], Kristina Ullmann[1,3], Stephanie Krämer[1,2,5], Boris Jerchow[1,6], Reinhart Kluge[1,5], Claudia Gösele[1,2], Hannah Nickles[1,3], Astrid Puppe[1,7] and Thomas Rülicke[8]

Abstract

Genetic alterations can unpredictably compromise the wellbeing of animals. Thus, more or less harmful phenotypes might appear in the animals used in research projects even when they are not subjected to experimental treatments. The severity classification of suffering has become an important issue since the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. Accordingly, the breeding and maintenance of genetically altered (GA) animals which are likely to develop a harmful phenotype has to be authorized. However, a determination of the degree of severity is rather challenging due to the large variety of phenotypes. Here, the Working Group of Berlin Animal Welfare Officers (WG Berlin AWO) provides field-tested guidelines on severity assessment and classification of GA rodents. With a focus on basic welfare assessment and severity classification we provide a list of symptoms that have been classified as non-harmful, mild, moderate or severe burdens. Corresponding monitoring and refinement strategies as well as specific housing requirements have been compiled and are strongly recommended to improve hitherto applied breeding procedures and conditions. The document serves as a guide to determine the degree of severity for an observed phenotype. The aim is to support scientists, animal care takers, animal welfare bodies and competent authorities with this task, and thereby make an important contribution to a European harmonization of severity assessments for the continually increasing number of GA rodents.

Keywords

animal welfare, genetically altered animals, harmful phenotype, severity assessment, severity classification

Date received: 27 February 2017; accepted: 6 June 2017

CRITERIA	WHAT TO LOOK FOR
Overall Appearance	Is the animal morphologically 'normal'? Are there any malformations or any other indicators that the phenotype has been affected? For example skeletal deformity or hydrocephalus.
Size, conformation and growth	Are there any deviations from expected size or growth curve?
Coat condition	Is there any piloerection, areas of fur loss, loss of whiskers, barbering? Is the skin / fur in good condition?
Behaviour – **Posture, gait, activity and interactions with the environment**	Do they exhibit the full repertoire of behaviours appropriate for the strain/species, including social interactions, grooming, walking, running, digging, climbing? Are these normal? Is the animal hunched or reluctant to move? Is movement impaired or is there any difficulty with orientation? Any signs of rigidity or tremors? Any abnormal activity levels? Prolonged inactivity could indicate chronic stress or depression (anhedonia) and/or sickness/pain, particularly if linked with a hunched posture and/or rough or unkempt coat. Unusual activity, such as hyperactivity, could indicate stereotypy or other behavioural abnormality.
Clinical signs	For example - nasal or ocular discharge, swollen or closed eyes; increased respiratory rate; dyspnoea; seizures/twitches/tremors; increased vocalisation with handling; overgrown teeth; presence of tumours, neurological or musculoskeletal abnormalities. Is metabolism impaired, for example, increased or decreased food or water intake, excessive urination? Consistency of faeces.
Relative size	Any unusual changes in size of the animals should be noted, and comparisons made within the litter. It may be helpful to generate a growth curve for the line.
Numbers	Where death occurs, it is important to maintain accurate records such that any pre- or post-weaning losses can be investigated. Where appropriate (e.g. higher than anticipated mortality rate), post mortem examinations should be carried out to help determine the cause of death. A review of fertility can also be helpful in assessment of whether or not the modification is having an effect e.g. conception rates; abortions; stillbirths.

Additional considerations for assessment in Neonatal animals

CRITERIA	WHAT TO LOOK FOR
Colour of pups (for neonate only)	Do any pups show evidence of abnormal skin colour (e.g. anaemia, poor circulation)
Activity of pups (for neonate only)	Any abnormal activity, e.g. reduced wriggling? Righting reflex intact?
Milk spot (for neonate only)	Do any pups fail to show presence of a milk spot? Any evidence of mis-mothering?
Litter	Litter sizes; litter homogeneity; development and growth of pups

WELFARE ASSESSMENT

Animali Neonati

Additional considerations for assessment in Neonatal animals

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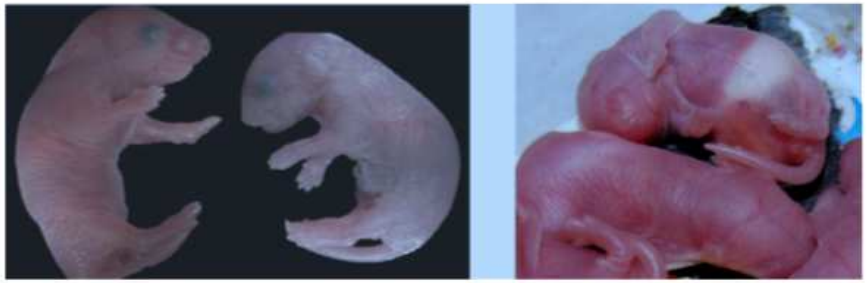
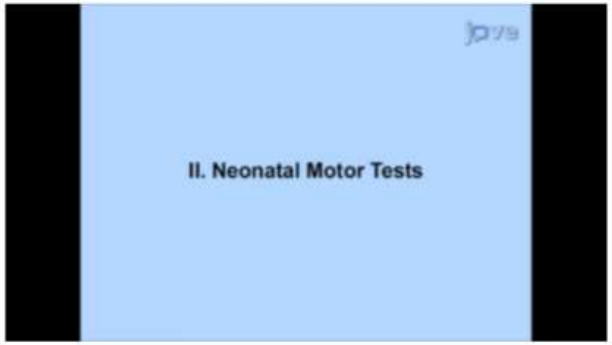
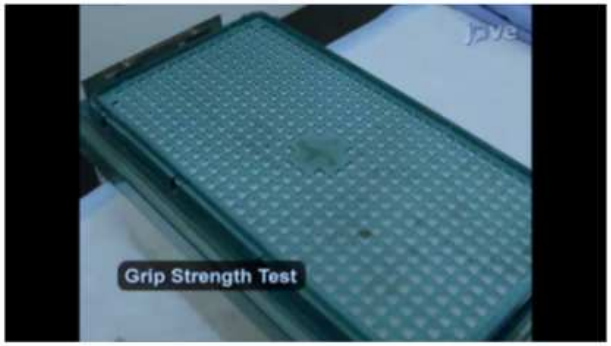






A Battery of Motor Tests in a Neonatal Mouse Model of Cerebral Palsy, Feather-Schussler, Danielle N., and Tanya S. Ferguson , *Journal of Visualized Experiments : JoVE* 117 (2016): 53569. *PMC.* Web. 24 Sept. 2018.

BASIC WELFARE ASSESSMENT OF NEW LINES

- Breeding of lines with a potential harmful phenotype
- Harmful phenotype: equivalent or higher than that caused by the introduction of a needle with good veterinary practice
- Generation of a new line → until the line is established: minimum of 2 generations
- 7 M & 7 F
- Neonates, at weaning, adult every 2/3 months → line-specific
- Develop line-specific Score Sheets for basic welfare assessment

Table 1. Major categories of systemic and behavioural disorders and diseases.

1. Lethal factors
2. Behavioural disorder
3. Alterations of the skin and the coat
4. Alterations of the sensory organs
5. Diseases of the nervous system
6. Diseases of the immune system
7. Cardiovascular and haematological diseases
8. Diseases of the respiratory tract
9. Diseases of the digestive system
10. Metabolic diseases
11. Reproductive diseases
12. Tumour diseases
13. Renal diseases
14. Alterations of the locomotor system

Annex 2

"Assessment of newborn litter"*

*at the latest during the first cage change

Location[1]:	Husbandry system[2] :
Owner:	Origin[3]:
Line (international designation): Only needs to be specified after publication of the line _____ Line (internal designation):	Current particularities[4]:_____ _____ _____
Designation of the altered gene(s):	Genetic background of the line:
Expected phenotype: (brief description)	

Dam no.:	Sire no.:	Litter born on:	Generation:
Number born:	Date of assessment:	Assessor:	

Colour of pups	□ normal	□ Abnormalities (please specify, e.g. pale)	
Activity of pups	□ normal	□ Abnormalities (please specify, e.g. conspicuous restlessness)	
Size, development of pups	□ homogeneous	□ not homogeneous	Weight □ normal □ reduced □ increased
Milk spot	□ present	□ not present	
Care by dam	□ normal	□ Abnormalities (please specify, e.g. neglect, cannibalism)	
Other conspicuous signs			

[1] Institute and room	[4] e.g.: noise due to construction site, sanitation activities, relocation of rooms etc.
[2] e.g.: IVC, conventional cage, filter top, isolator etc. state hygiene status where applicable	
[3] Name of breeder, external institution etc.	

Annex 3

"Assessment of litter on weaning"

Location[1]:	Husbandry system[2] :
Owner:	Origin[3]:
Line (international designation): Only needs to be specified after publication of the line! Line (internal designation):	Current particularities[4]:
Designation of the altered gene(s):	Genetic background of the line:
Expected phenotype: (brief description)	

Dam no.:	Sire no.:	Litter born on:	Generation:
Number born:	Number weaned:	Difference born/weaned:	

Animal number								
Weaning date								
Sex								
Body weight								
Conspicuous signs[5] Please use letters (see footnote)!								
Animal number								
Weaning date								
Sex								
Body weight								
Conspicuous signs[5] Please use letters (see footnote)!								

Conspicuous signs prior to weaning	Date:			
	Conspicuous signs[5]:			

[1] Institute and room

[2] e.g. IVC, conventional cage, filter top, isolator etc. state hygiene status where applicable

[3] Name of breeder, external institution etc.

[4] e.g.: noise due to construction site, sanitation activities, relocation of rooms etc.

[5] a = No conspicuous signs
b = Areas of fur loss
c = Runt
d = Bite wounds
e = Microphthalmia
f = Abnormal teeth
g = Hydrocephalus
h = Other (please state)

Annex 4

"Assessment of individual animal"

First assessment at the age of 2 months, then every 3 months*

*in the event of conspicuous signs, the examination intervals are to be reduced

Line (internal designation):	Line (international designation): only needs to be specified after publication of the line!	Owner:	Husbandry system:

Animal no.:	from litter on:	Generation:	Sex:	Genotype:

In the case of conspicuous signs, please enter the relevant letter!
(see code at bottom of table)

Multiple conspicuous signs may be selected!

Date								
Signature of assessor								
	normal	conspicuous	normal	conspicuous	normal	conspicuous	normal	conspicuous
Nutritional status (1)								
Posture (2)								
Behaviour and motor function (3)								
Coat and orifices (4)								
Reaction to handling (5)								
Other (6)								
Weight (g)								

Date								
Signature of assessor								
	normal	conspicuous	normal	conspicuous	normal	conspicuous	normal	conspicuous
Nutritional status (1)								
Posture (2)								
Behaviour and motor function (3)								
Coat and orifices (4)								
Reaction to handling (5)								
Other (6)								
Weight (g)								

(1) Nutritional status:
- a = Emaciated
- b = Overweight
- c = Dehydrated

(2) Posture:
- a = Crooked
- b = Cowering

(3) Behaviour and motor function:
- a = Segregation
- b = Apathetic
- c = Stereotypes
- d = reduced Motion
- e = Paralysis
- f = Spasms

(4) Coat:
- a = Ruffled
- b = Dirty

Orifices:
- c = Red tears
- d = Diarrhoea/Discharge

(5) Reaction to handling:
- a = Aggressive
- b = Timid
- c = Apathetic

(6) Other:
- a = Tumors
- b = Skin inflammation
- c = Injuries
- d = Cannibalism
- e = Vocalisations
- f = Rectal prolapse
- g = Other (please specify)

Date of death and particularities during autopsy:

Final assessment of genetically altered lines

Institution and address: _____

Street: _____ Postcode: _____ Town: _____

Assessed line (international designation): Only needs to be specified after publication of the line!	Assessed line (internal designation):
_____	_____

Description of genetic alteration(s) leading to harm if not yet described in databases:

_____

Husbandry system of assessed animals: _____

Gene loci and genotype: _____

Assessed animals

Total number: _____ of which female: _____ and male: _____
Average assessment period (weeks) _____ ±stand. dev.: _____
Average no. of assessments per animal: _____ ±stand dev.: _____

Conspicuous signs in terms of:	Occurred:	Number of animals affected	Conspicuous signs in terms of:	Occurred:	Number of animals affected
Nutrional status	Yes ☐ No ☐		Tumor	Yes ☐ No ☐	
Posture	Yes ☐ No ☐		Skin changes	Yes ☐ No ☐	
Reaction to handling	Yes ☐ No ☐		Injuries	Yes ☐ No ☐	
Coat/Orifices	Yes ☐ No ☐		Cannibalism	Yes ☐ No ☐	
Behaviour	Yes ☐ No ☐		Rectal prolapse	Yes ☐ No ☐	
Motor function	Yes ☐ No ☐		Other:		

Female animals:

Average number of pregnancies	Average rearing losses (difference born – weaned ±stand. dev.)	Colony index (average number of offspring of females per unit of time)

Final assessment: *(if necessary, please use extra sheet)*
The harm burdens are classified as none ☐ mild ☐ moderate ☐ severe ☐.

Reasons: *(comprehensible description of the characteristics of the harm)* _____

_____

The described harm occurred from an age of _____ weeks with a frequency of _____% of the examined animals.

In the event of harm, it is recommended that offspring of this line will be killed at an age of ____weeks if this is not contrary to the purpose of the project. The following refinement measures are recommended to reduce the potential harm: _____

_____

_____

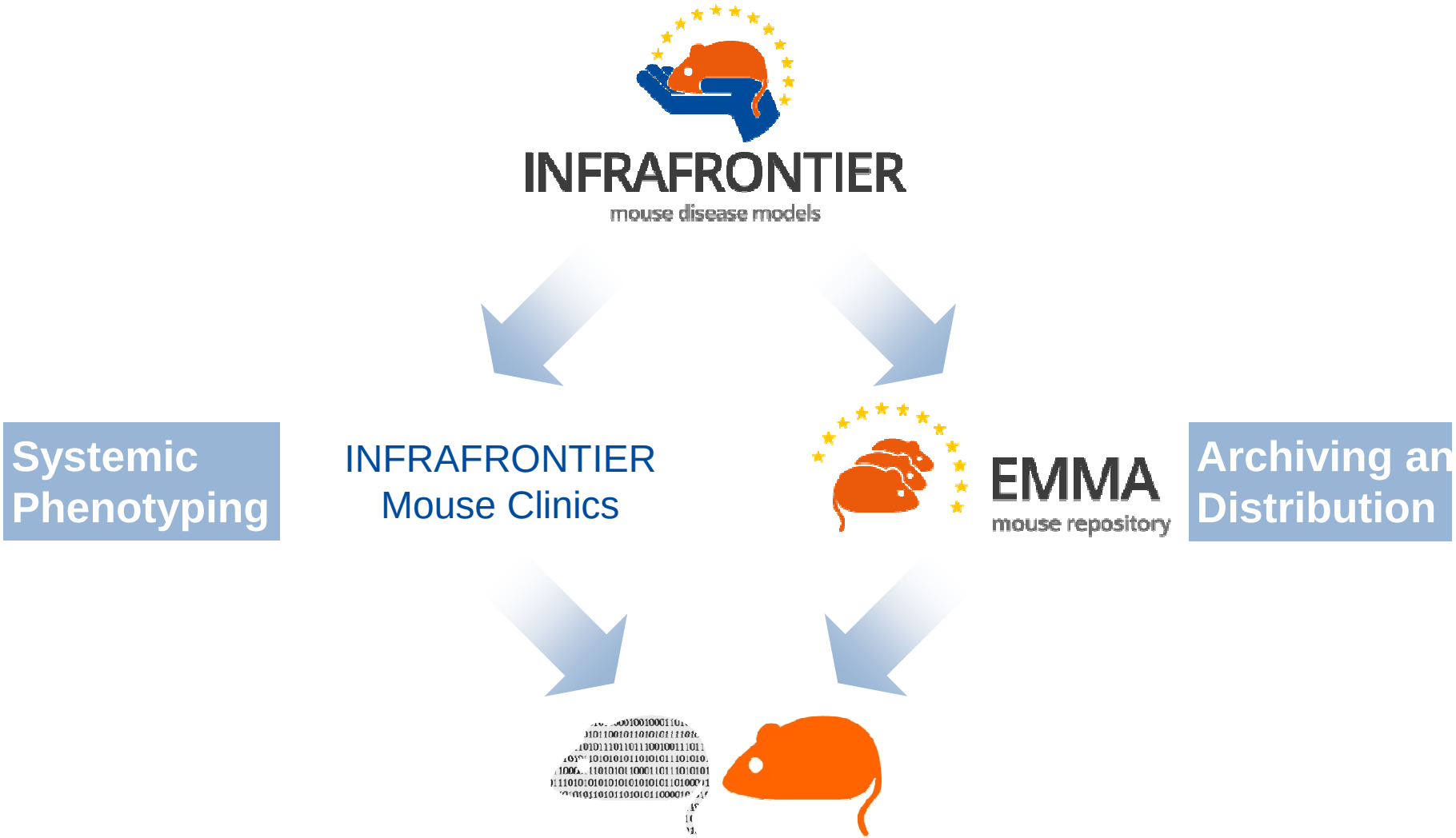
Where appropriate, members of animal welfare committee involved in the assessment:

_____

_____

Place: _____ Date: _____ Noted: _____
(Project manager and animal welfare officer)

INFRAFRONTIER Research Infrastructure



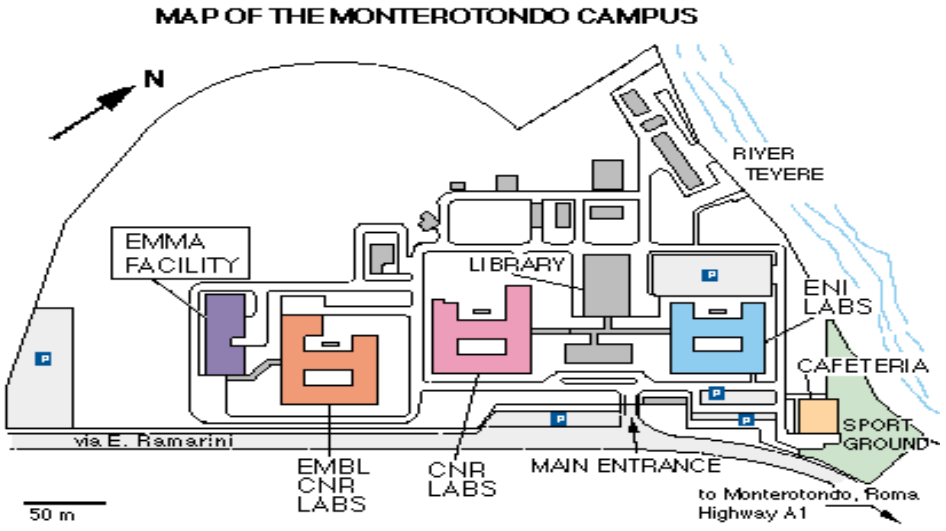
Access to scientific platforms, data and mouse models

In vivo Research Infrastructures - Campus 'A. Buzzati
Campus A. Buzzati-Traverso' - CNR di Monterotondo

The ITALIAN MOUSE CLINIC

The EMMA-INFRAFRONTIER Biobank & Biorepository

CNR-Campus International Development



Infrafrontier & International Mouse Phenotyping Consortium (IMPC) Activities

Molecular and Cellular Biology

Generation of new GA lines and archiving

Breeding and Colony Management

Phenotyping and *in vivo* experiments

Ex-vivo testing and Pathology

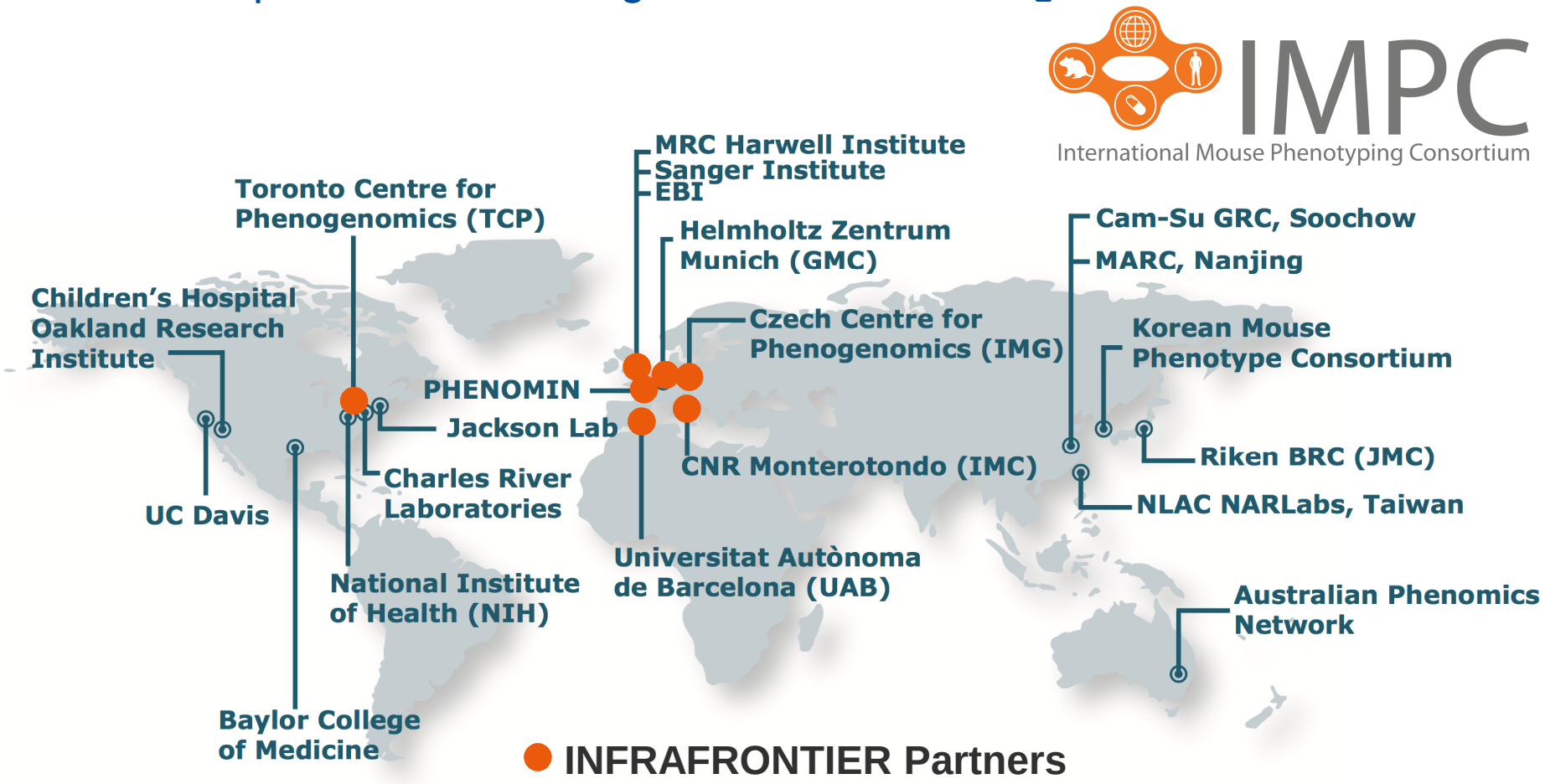
Gene or Phenotype

Translational studies

Project management

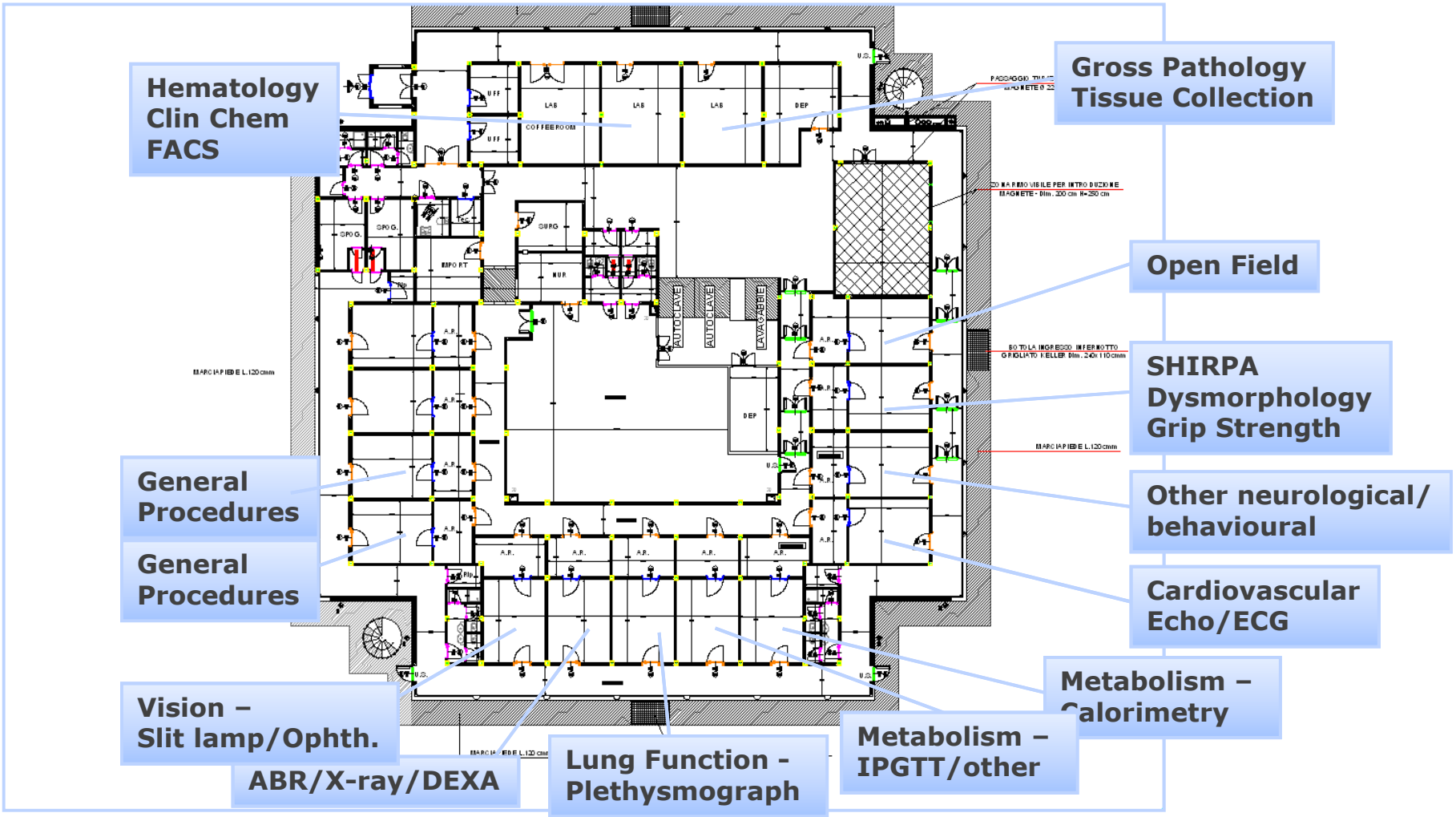
International Mouse Phenotyping Consortium

- Creating 20.000 Knock Out strains on a single background
- Characterizing each through a standardized phenotyping protocol
- Open access to data and resources
- First comprehensive catalogue of mammalian gene function



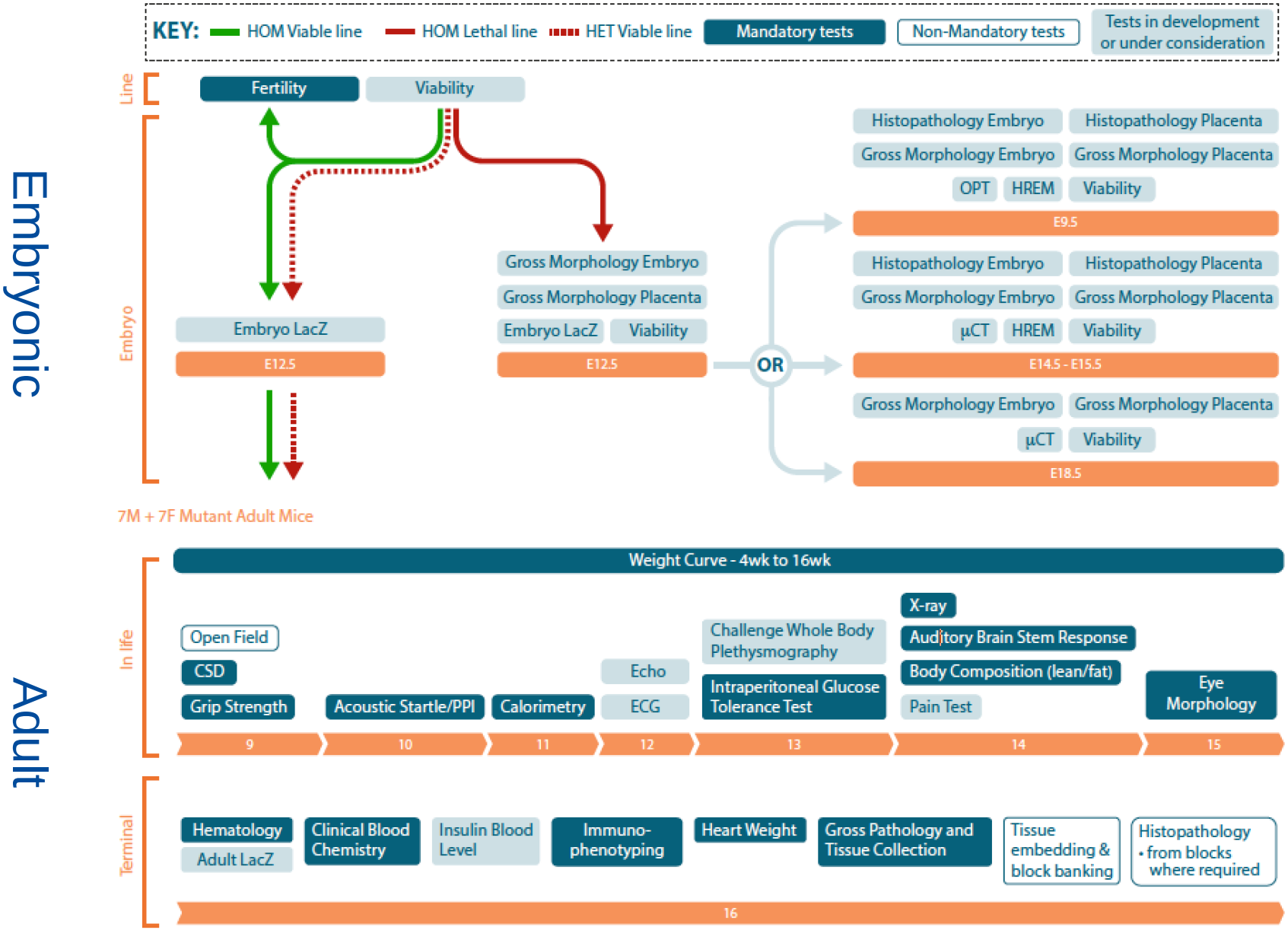
The Italian Mouse Clinic

IMPC
International Mouse Phenotyping Consortium



IMPC phenotyping

International Mouse Phenotyping Consortium



nature genetics

Disease model discovery from 3,328 gene knockouts by The International Mouse Phenotyping Consortium

Meehan et al. Nature Genetics 26 June 2017

The pipeline measures a total of 509 phenotyping parameters that encompass diverse biological and disease areas including neurological, behavioral, metabolic, cardiovascular, pulmonary, reproductive, respiratory, sensory, musculoskeletal and immunological parameters. Standardized protocols developed by the IMPC and automated statistical analysis are used to decrease phenotypic variance across the centers.

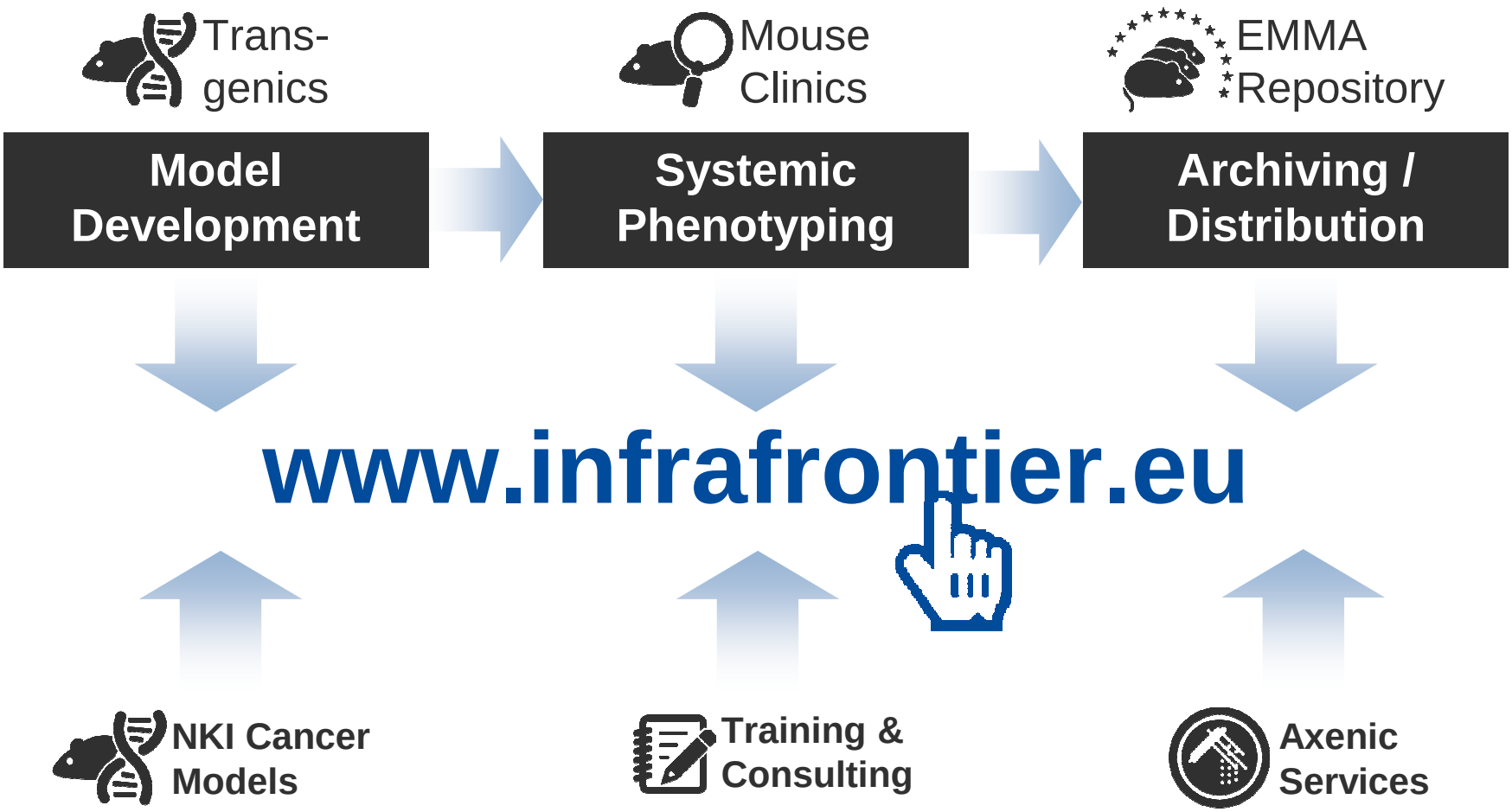
Identified new mouse models for human mendelian disorders with a known genetic basis

Uncovered candidate disease-associated genes for human mendelian rare disease by phenotypic similarities with OMIM and Orphanet database

Identified new mouse disease models involving genes with little or no previous functional annotation.

IMPC adheres to the ARRIVE guidelines for reproducibility of animal-model experiments, including making all data publicly available.

INFRAFRONTIER Resources and Services



Distributed research infrastructure – one face to the customer