Czech Centre for Phenogenomics

Phenotyping

The genomes of humans, mice and other species have been completely sequenced, yet the knowledge of genome sequences as such does not shed light on questions concerning the functions of these sequences. In order to describe biological functions of a gene, informative genetic modifications are introduced into the genes...

Learn more →

CCP: model generation services

Genetically modified mouse models have become a key tool in basic and biomedical research. The ability to engineer the mouse genome has greatly transformed biomedical research in the last decade.

Learn more →

Research

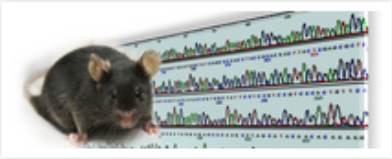
The research program is focused onto functional genomics using genetically engineered models and is closely connected to the infrastructure of Czech Centre for Phenogenomics that provides the project indispensable core facilities

Learn more →

Czech Centre for Phenogenomics

OP EC Projects

The Education for Competitiveness Operational Programme Projects

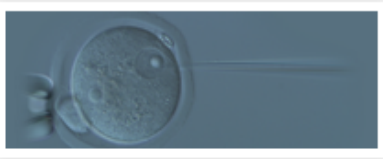


Phenogenomics

ECOP CZ.1.07/2.3.00/20.0102

Founding of an expert team for the Centre for Phenogenomics

Vytváření expertního týmu centra fenogenomiky

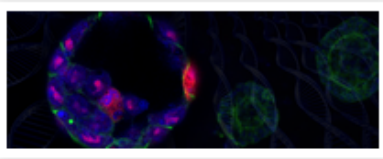


Transgenesis

ECOP CZ.1.07/2.3.00/30.0027

Founding the Centre of Transgenic Technologies

Tvorba Centra transgenních technologií



PhenoImage

ECOP CZ.1.07/2.3.00/30.0050

Founding the expert platform for phenotyping and imaging technologies

Vytváření expertní platformy fenotypických a zobrazovacích technologií

esf european social fund in the czech republic

EUROPEAN UNION

MINISTRY OF EDUCATION, YOUTH AND SPORTS

OP Education for Competitiveness

INVESTMENTS IN EDUCATION DEVELOPMENT

// Search our website

Search

// Contact us

Institute of Molecular Genetics of the ASCR, v.v.i.
Videnska 1083
Prague 4, CZ-142 20
Czech Republic

+420 241 063 137

+420 224 310 955

libor.danek@img.cas.cz

// Latest news

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

Introduction to gene targeting and genome editing

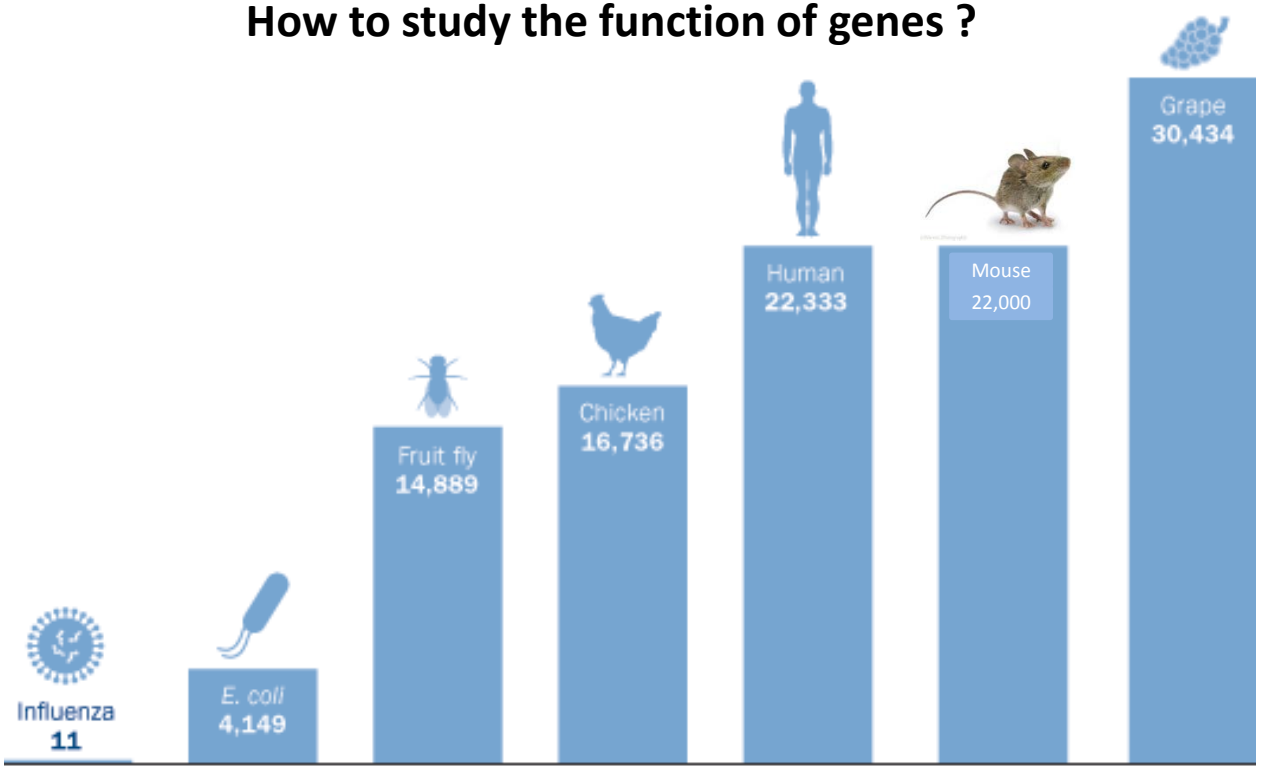
Slavomír Kinský, PhD

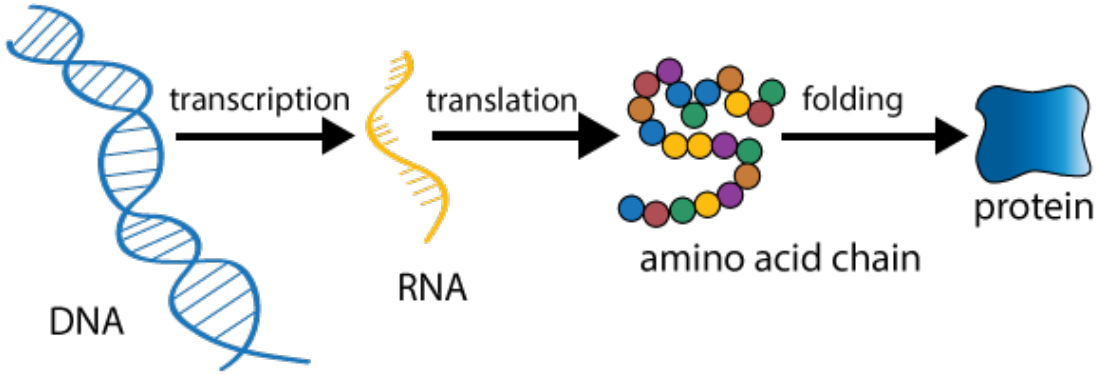
Czech Centre for Phenogenomics

Institute of Molecular Genetics of the ASCR, v.v.i.

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027
"Founding the Centre of Transgenic Technologies"

How to study the function of genes ?

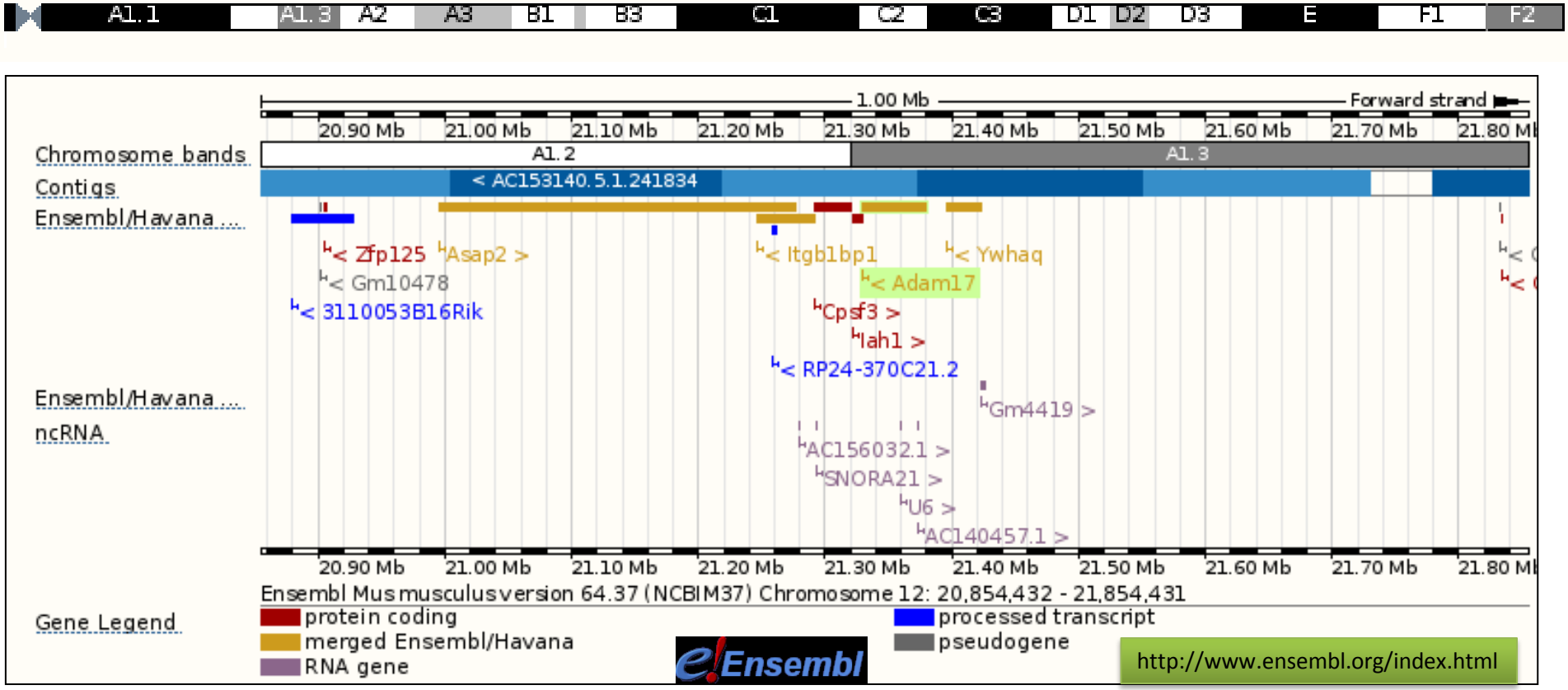




The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

.....we know identities of genes, their sequences and organization in genomes.....

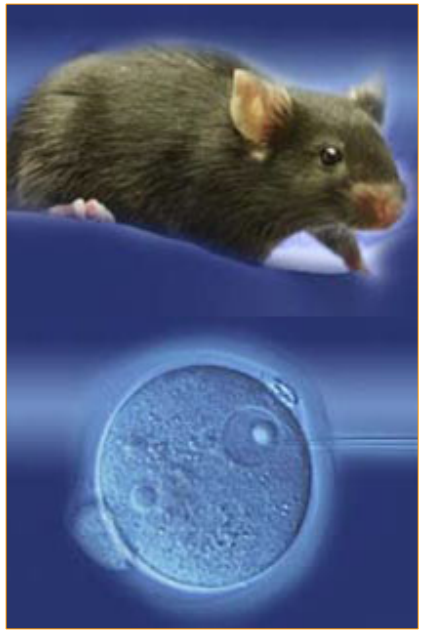
chromosom 12



The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

How to create mouse models of human diseases

Generation of transgenic mice



The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

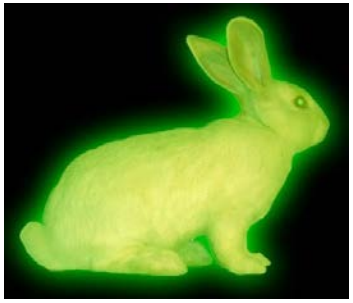
Animal transgenic models

1974 *Rudolf Jaenisch* ... First transgenic mouse

1989 first „knock-out“ mouse... *M.R.Capecchi, M.J. Evans, O. Smithies* (Nobel price 2007)

Rosie; 1997

Freitas et al. 2007



Houdebine et al. 2000

Menchaca et. al 2013

Sasaki et al. 2009

Kim et al. 2011

Wongsrikegao et al. 2011

Park et al. 2001

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

Nobel price for physiology/medicine in 2007

Mouse model publications – models of human diseases

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

Photo: U. Montan

Mario R. Capecchi

1/3 of the prize

USA

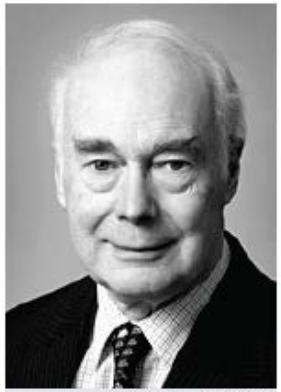


Photo: U. Montan

Sir Martin J. Evans

1/3 of the prize

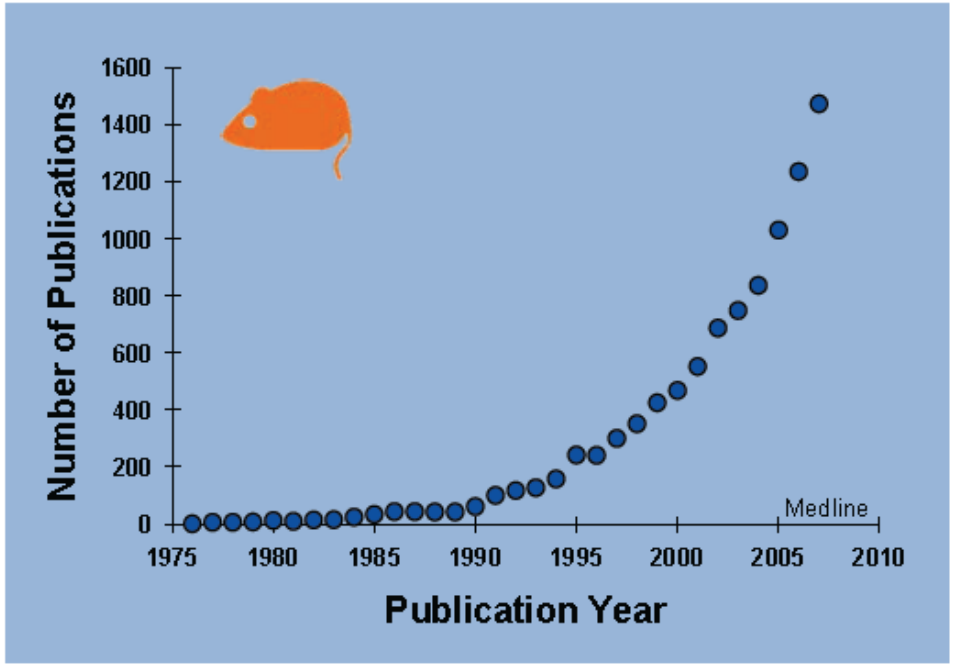
United Kingdom

Photo: U. Montan

Oliver Smithies

1/3 of the prize

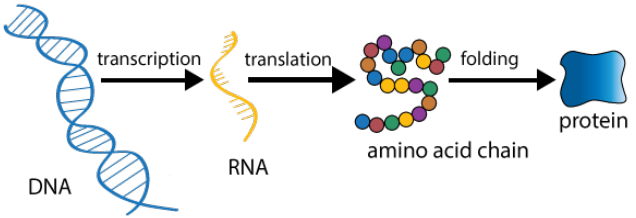
USA



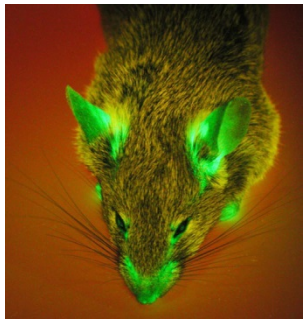
M. Räß, Infrafrontier

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

Gene targeting:

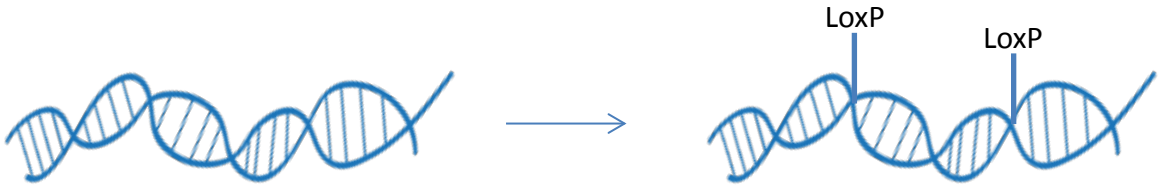


a) Knock-out mutants



b) Knock-in mutants

c) Conditional mutants



- Tissue specific or developmental specific mutants

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

Example of mouse knock-in mutant

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

Why mouse?

Functions of individual genes should be studied in complexity of whole organisms

Mouse genome became the 2nd mammalian genome, which was completely sequenced .....

Number of genes in both (mouse, human) is about.....20,000 -22,000.

Mouse and human differ in only several hunderts genes……

Additional advantages of the mouse model:

- Fysiology of human and mouse is very similar; pathology of many diseases is reproducible
- Mouse breeding is economical and relatively easy
- Mouse breeding is efective: large litters & short generation time

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

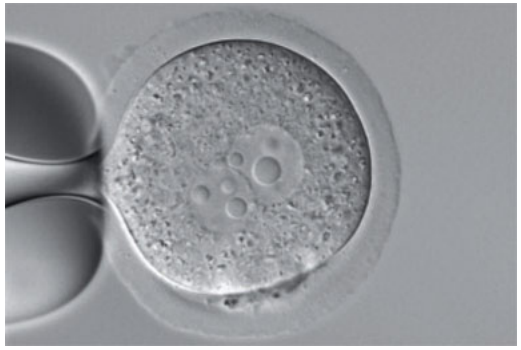
How to create transgenic mouse

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

How to create transgenic mouse

1. Injection into pronuclei (PNI)

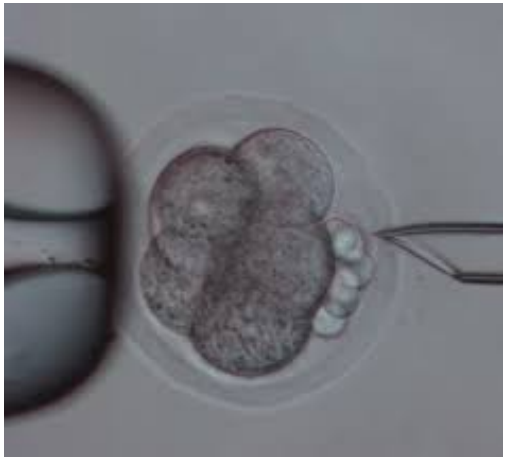
>>>> trangenesis by injection of DNA into fertilized oocytes



2. Injection of ES cells into developing embryo

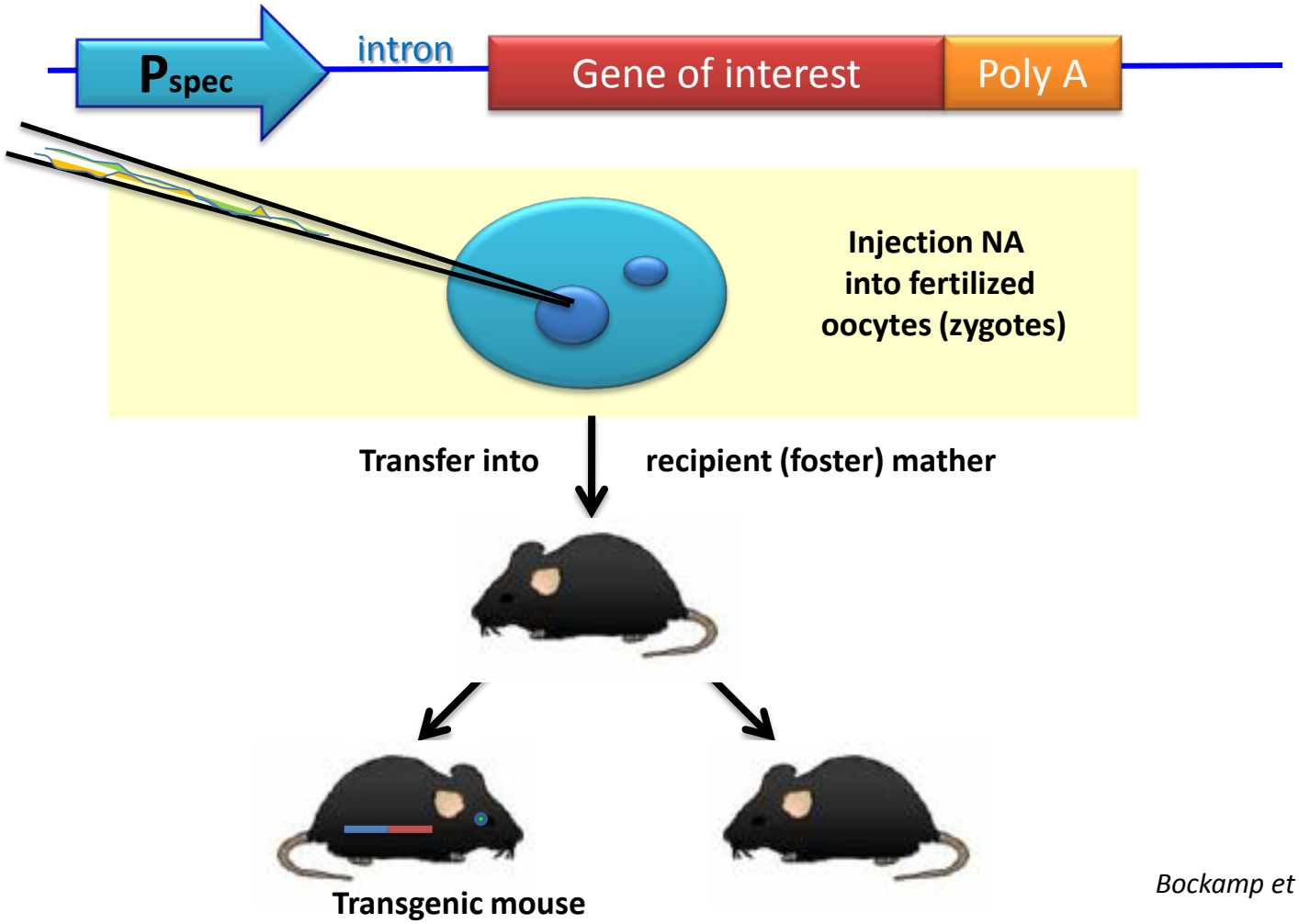
>>>> gene targeting by homologous recombination in Embryonic stem cells

Micromanipulator



The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

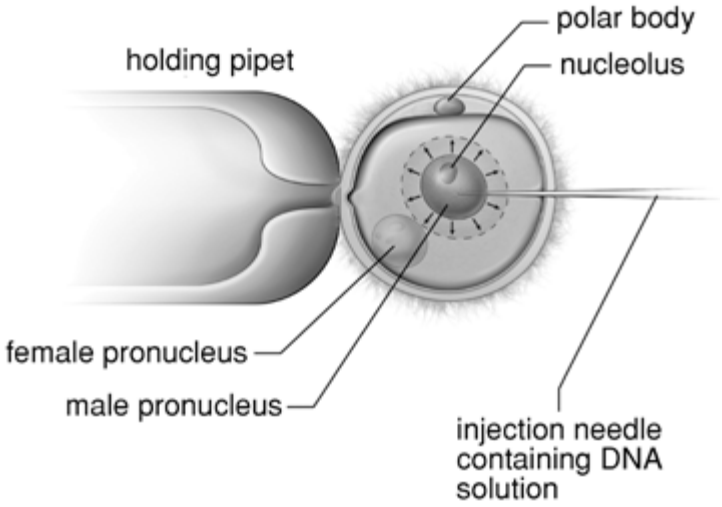
I. Pronuclear microinjection
generation of transgenic mouse with random insertion

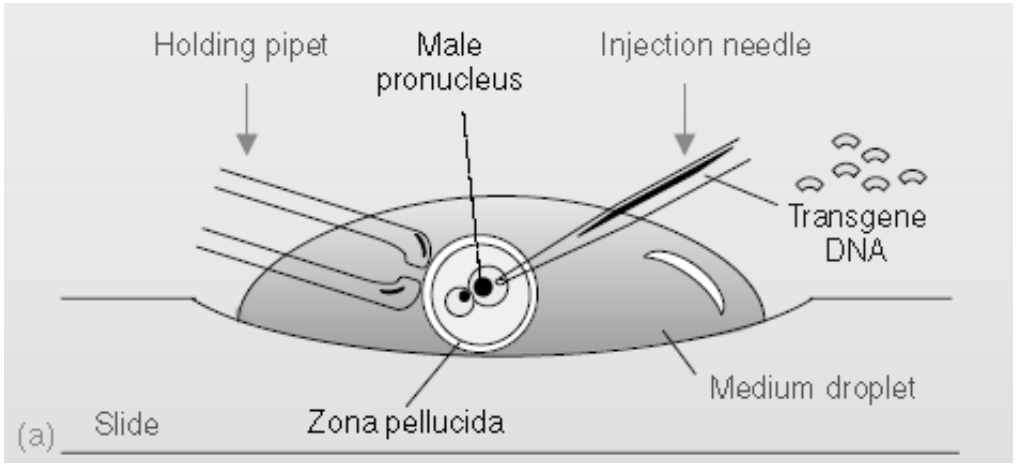


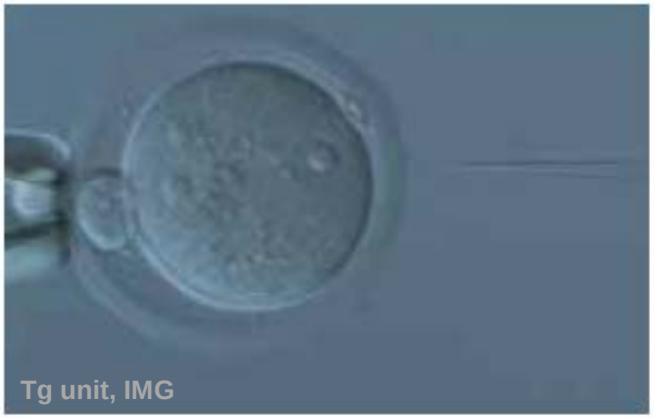
Bockamp et al., 2002, adapted

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

Pronuclear microinjection (PNI)







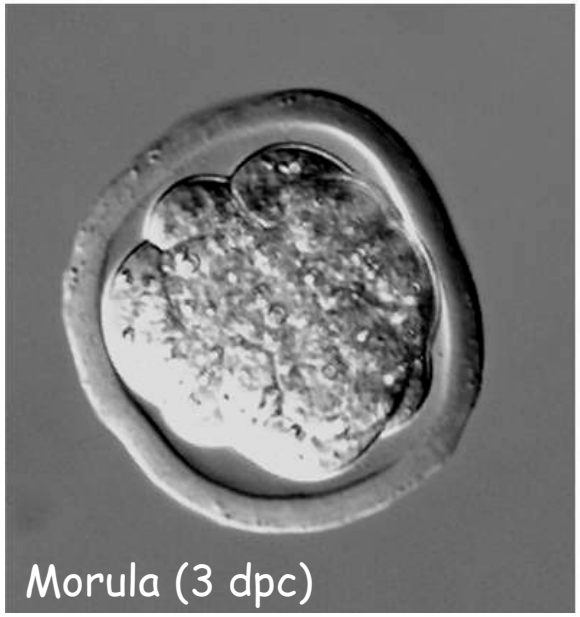
The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

Early development of mouse embryo


Zygota (0,5 dpc)


2- buněčné embryo (1,5 dpc)

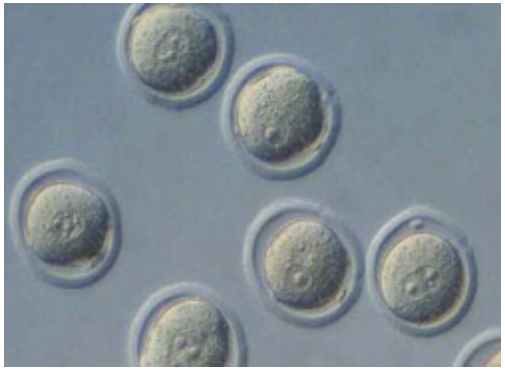

8- buněčné embryo (2,5 dpc)

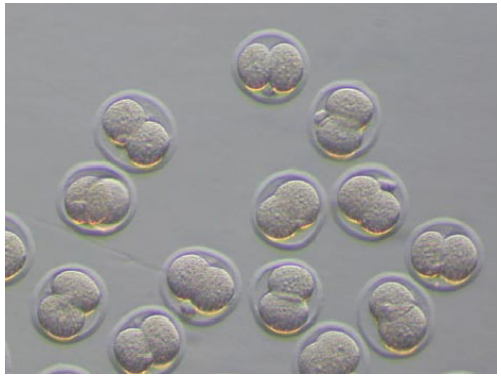

Morula (3 dpc)


Blastocysta (3,5 dpc)


Blastocysta (4,5 dpc)

Pronuclear microinjection (PNI) and generation of transgenic mouse





Adult mouse (7-8 weeks)

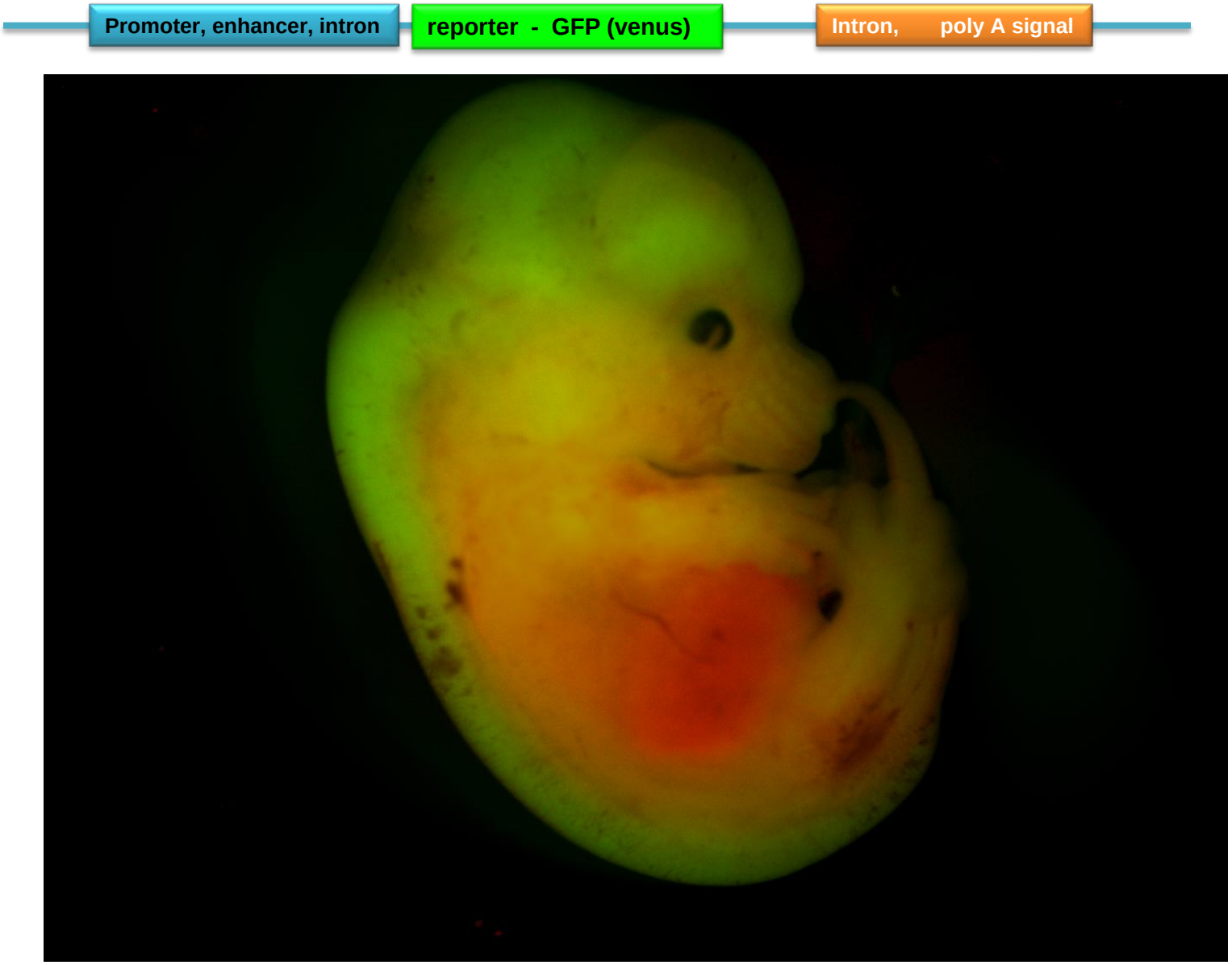
transfer into foster mother

In 3 week – newborn

In next 3 weeks – Weaning of pups

I. Pronuclear microinjection

example



I. Pronuclear microinjection
II. random insertion

- gene is present in every cell - transferred into germ line

- expression is often controlled by associated regulatory elements – the expression is influenced by the insertion place
- generally, the expression must not be achieved in every cell
- ! Carefull evaluation of founder - line selection

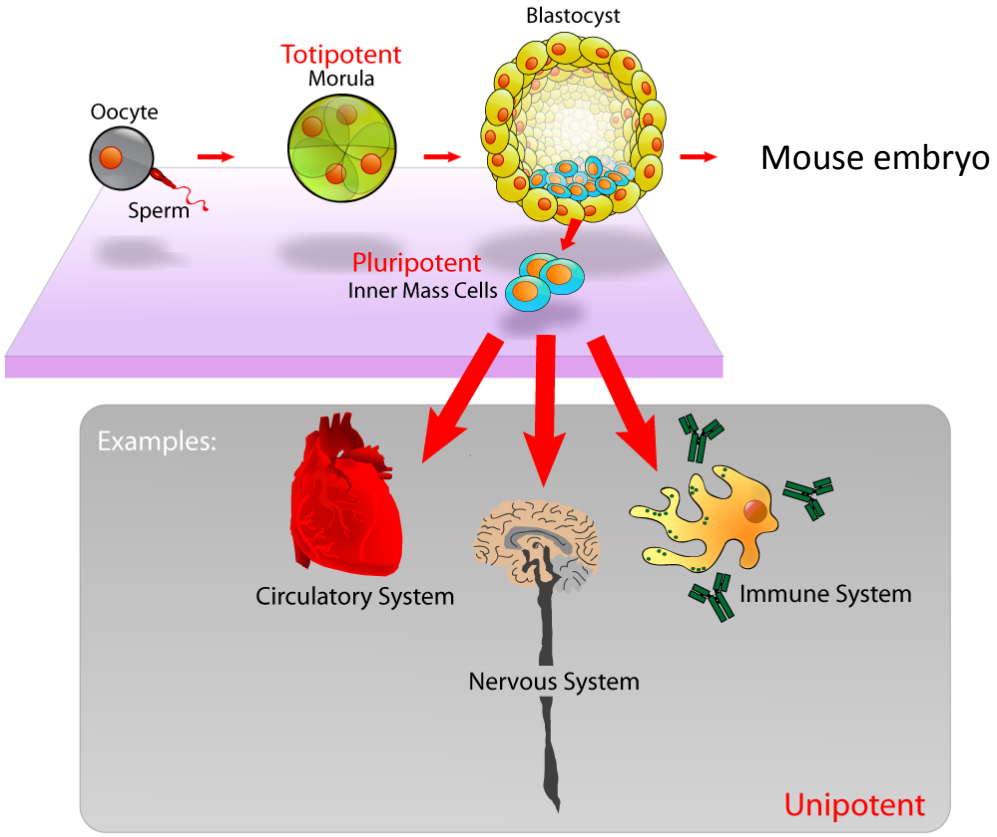
The expression of some transgenes can be dependent on the place of insertion

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

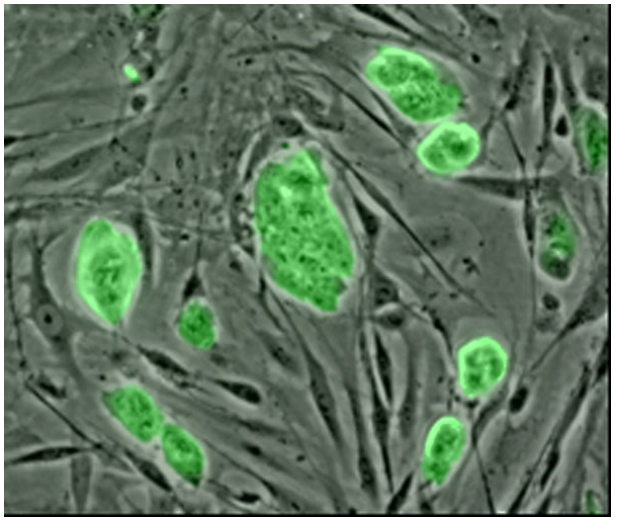
How to create transgenic mouse by using of ES cells

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

Embryonic stem cells (**ES cells**) are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage preimplantation embryo.



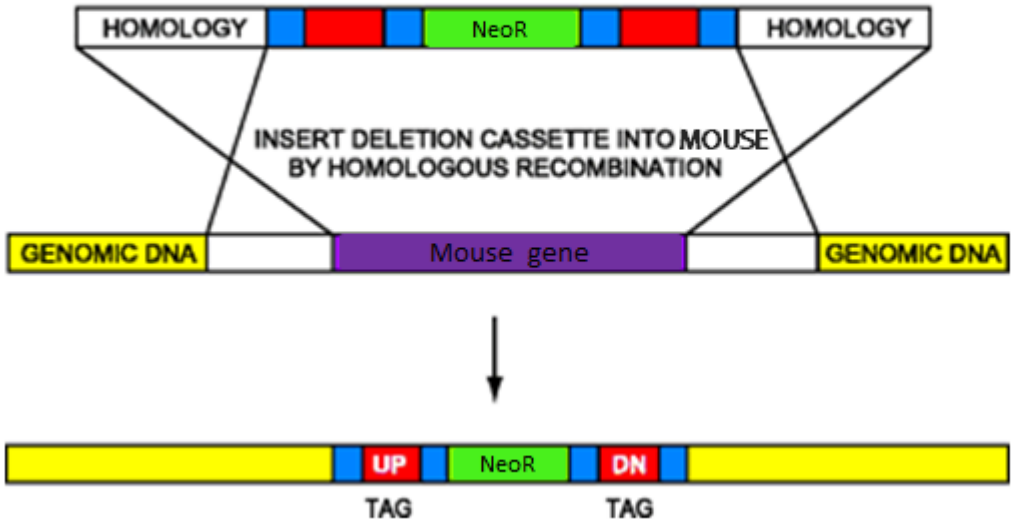
Mouse embryo



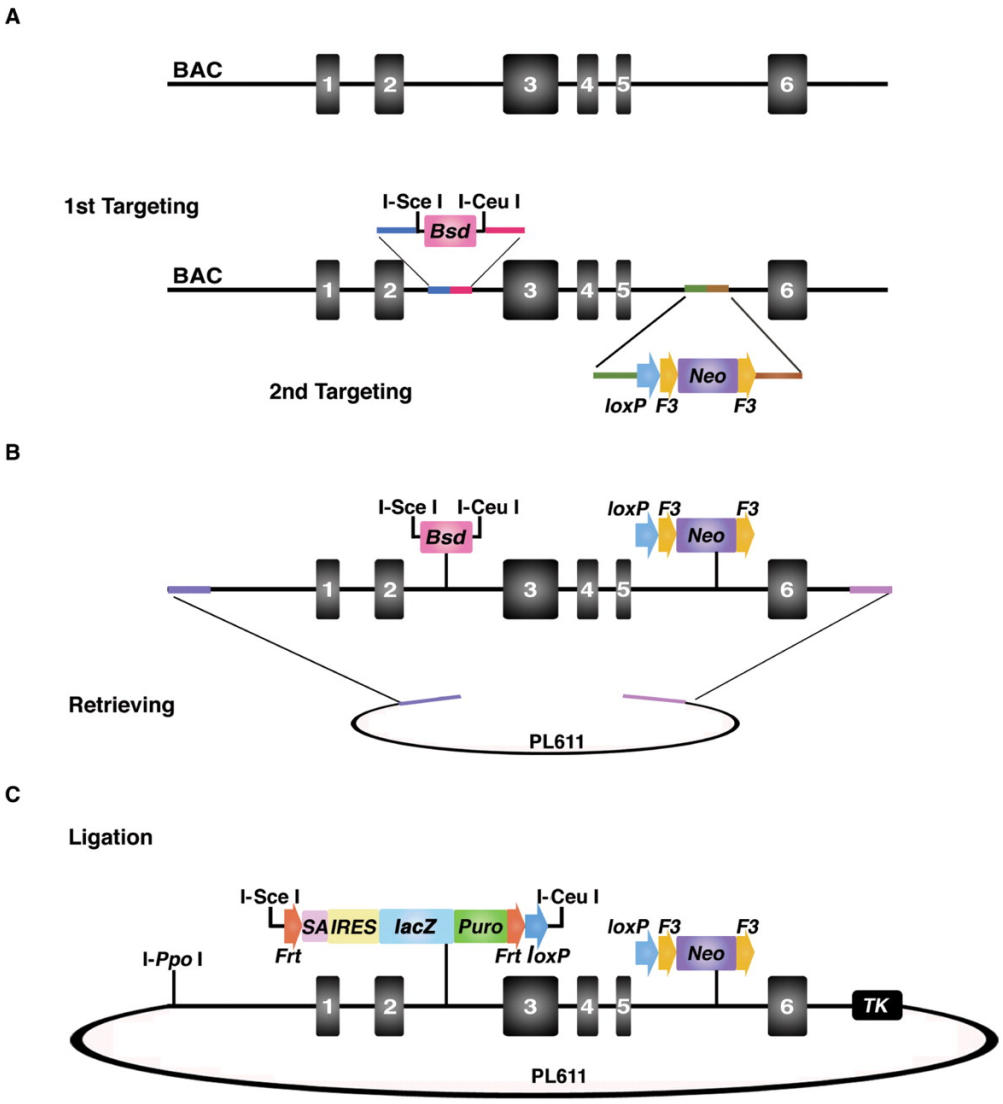
The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

Generation of mutants by deletion cassete - by DNA cloning

Cassette with **Long homology arms** –
which are difficult to construct

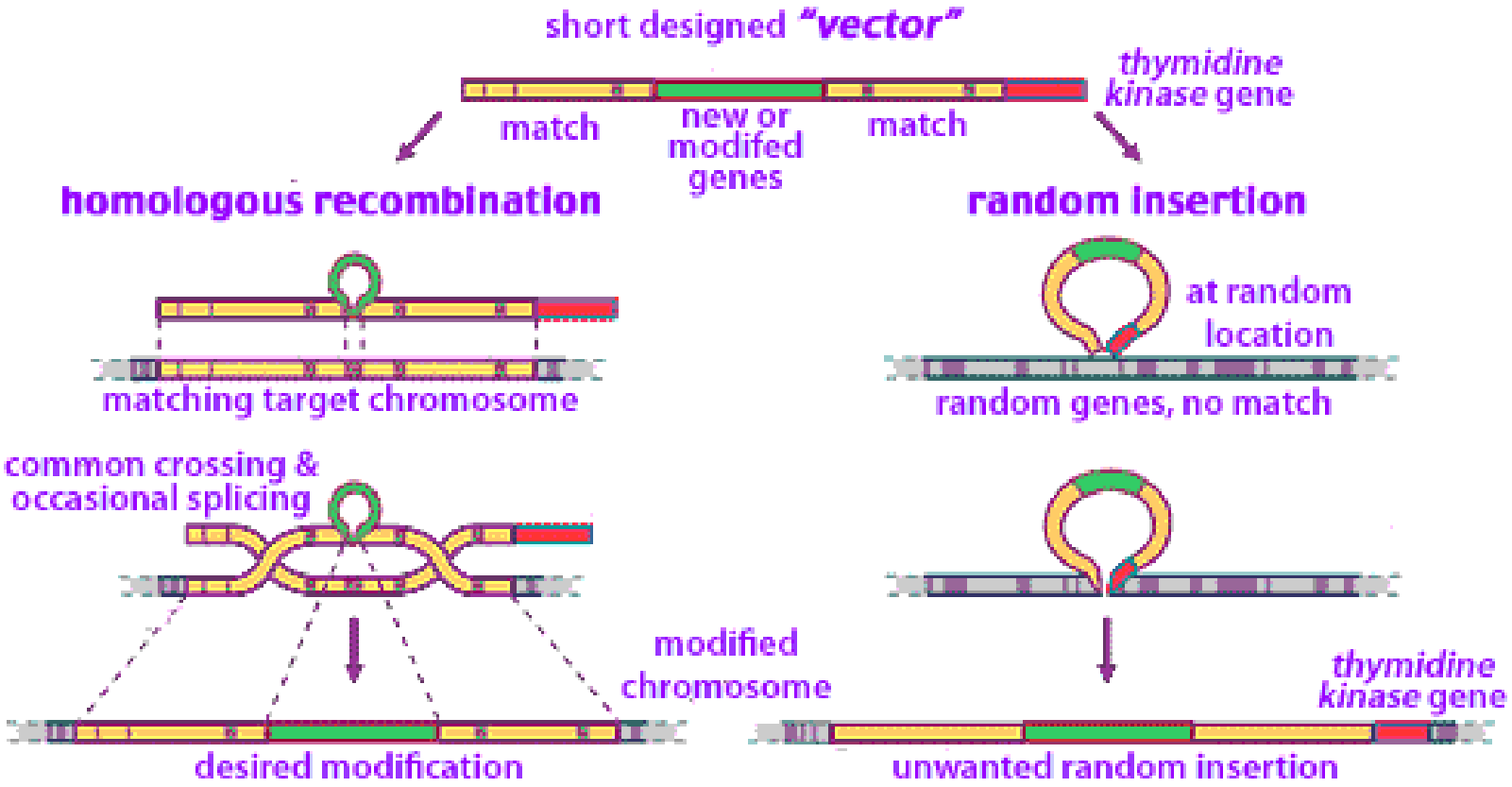


Transgenic Mouse Production by BAC (Bacterial Artificial Chromosomes) Transgenes



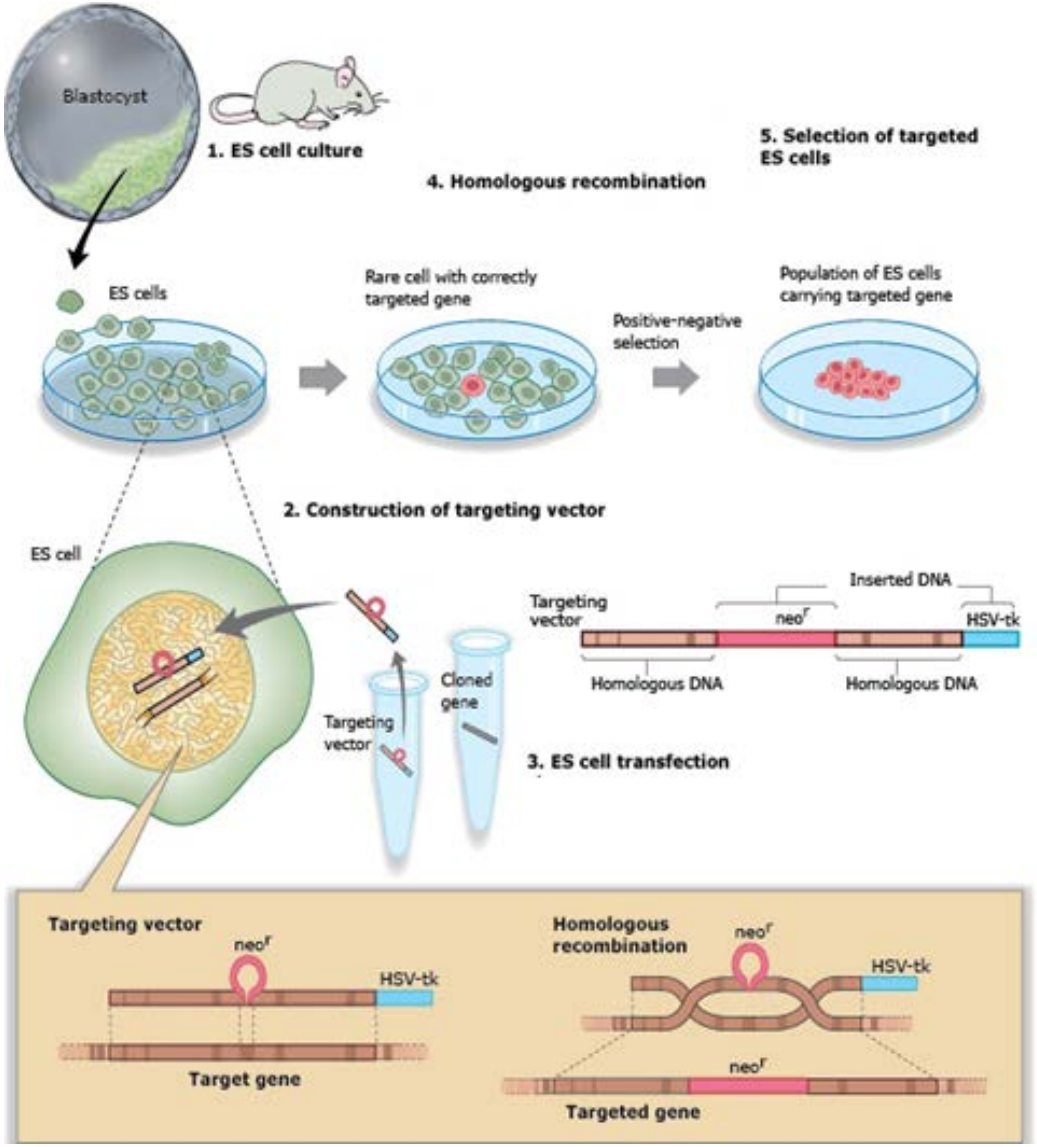
The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

Homologous recombination is always necessary for integration of vector DNA in proper genomic locus

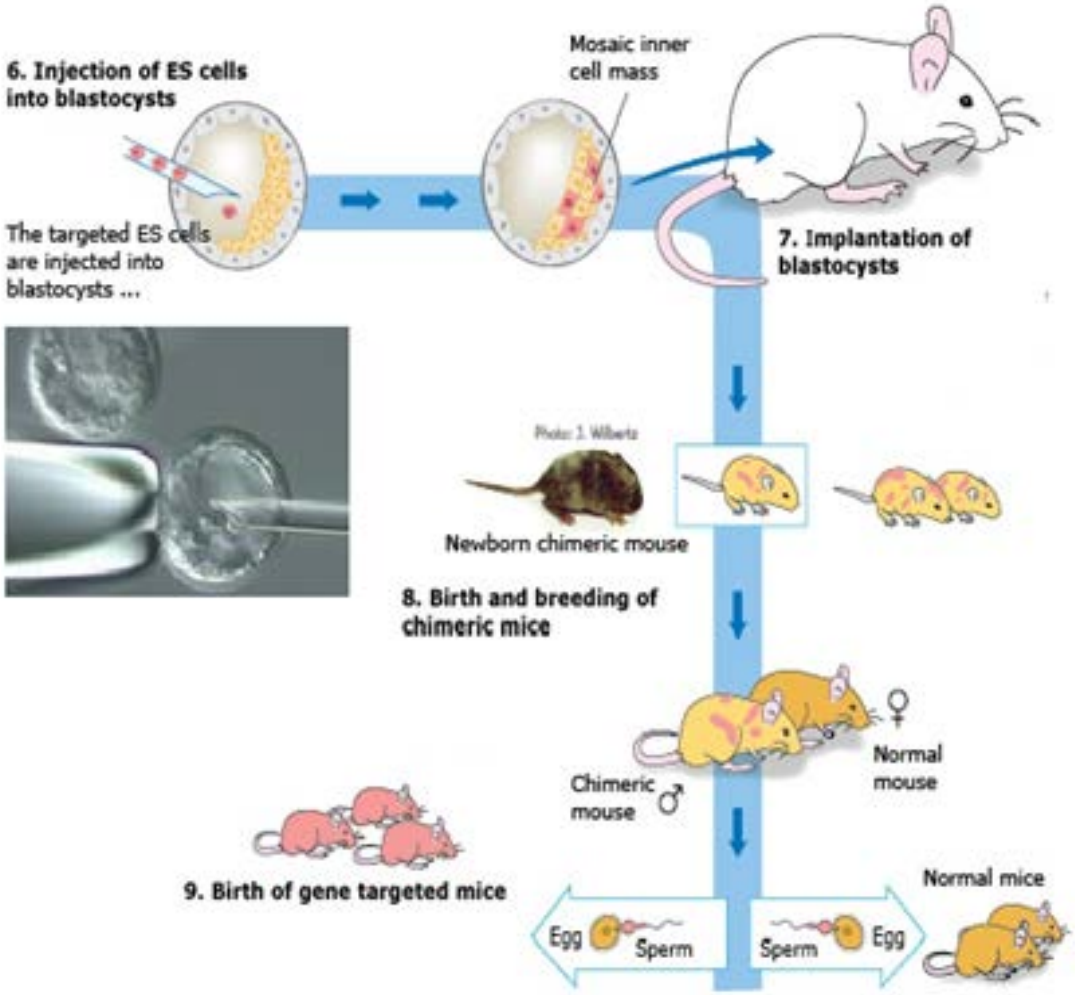


The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

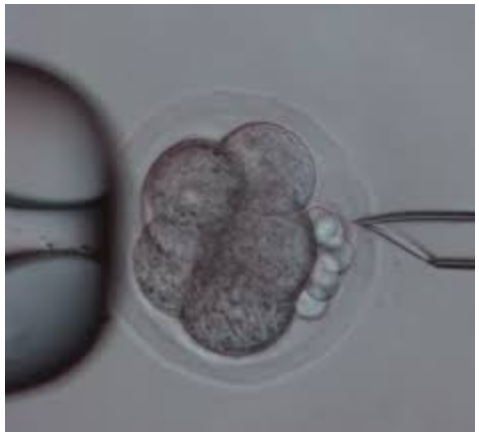
Embryonic stem cells & homologous recombination

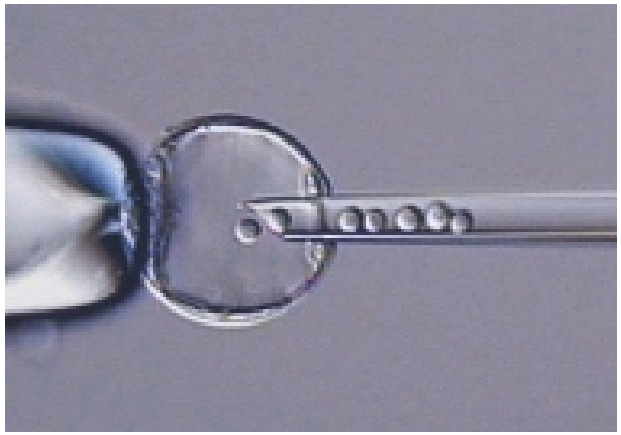


Embryonic stem cells & homologous recombination



Transgenic models from ES cells (pluripotent cells)





Injection in 8-cells embryo stage

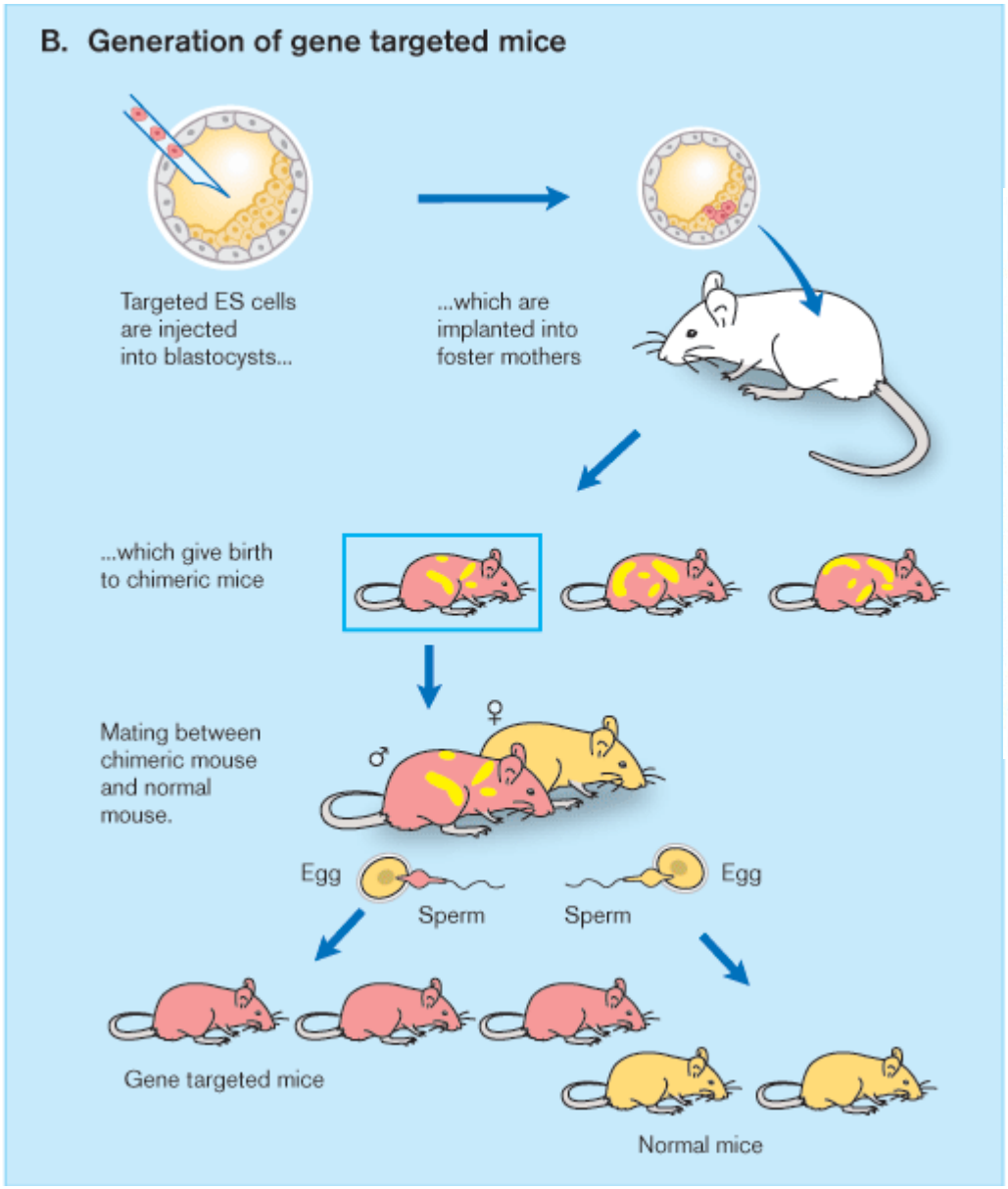
or

into blastocyst

transfer into foster mother

... In 3 week **chimeric** mouse is born

Chimeric mouse and generation of transgenic mouse



black:
No germ line transmission

Chimera light brown - founder:
Strong possibility for trasnmission

Chimera brown/black- founder:
Weak possibility for transmission

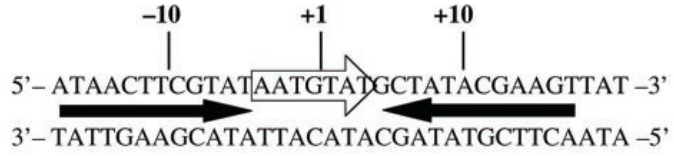
ES cell derived
from 129 Strain

How to create conditional mutant mouse

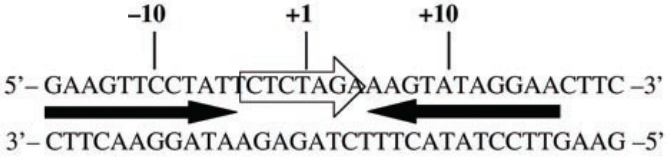
- Mutation of gene is
  a) tissue specific
  b) induced by tamoxifen (in particular time)

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

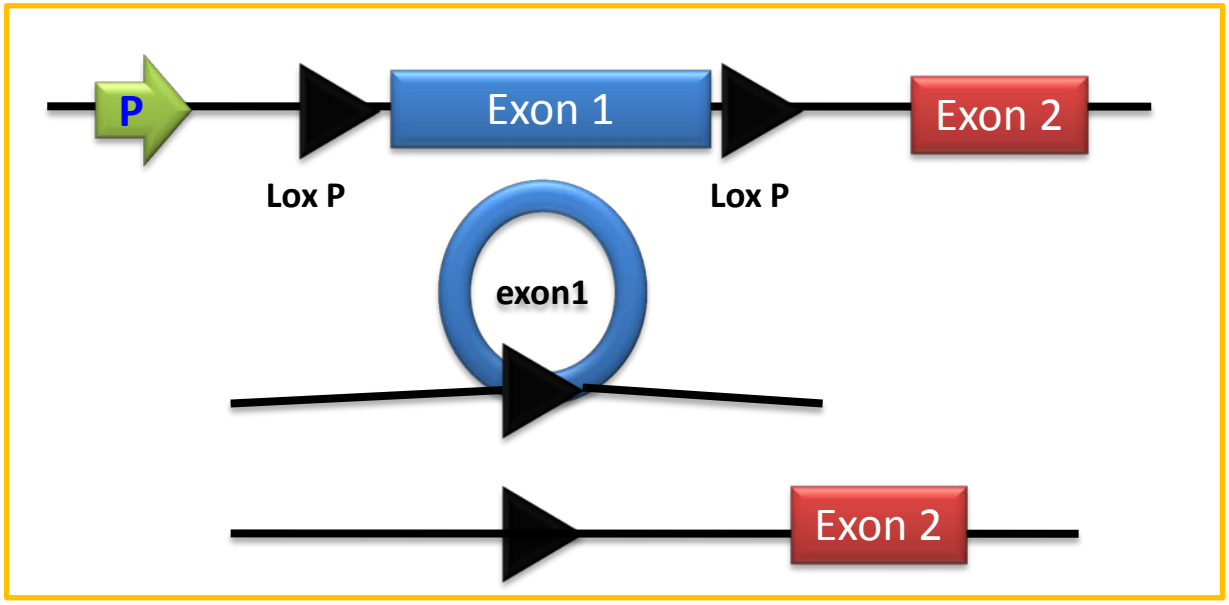
Conditional gene targeting in ES cells



loxP site / Cre recombinase

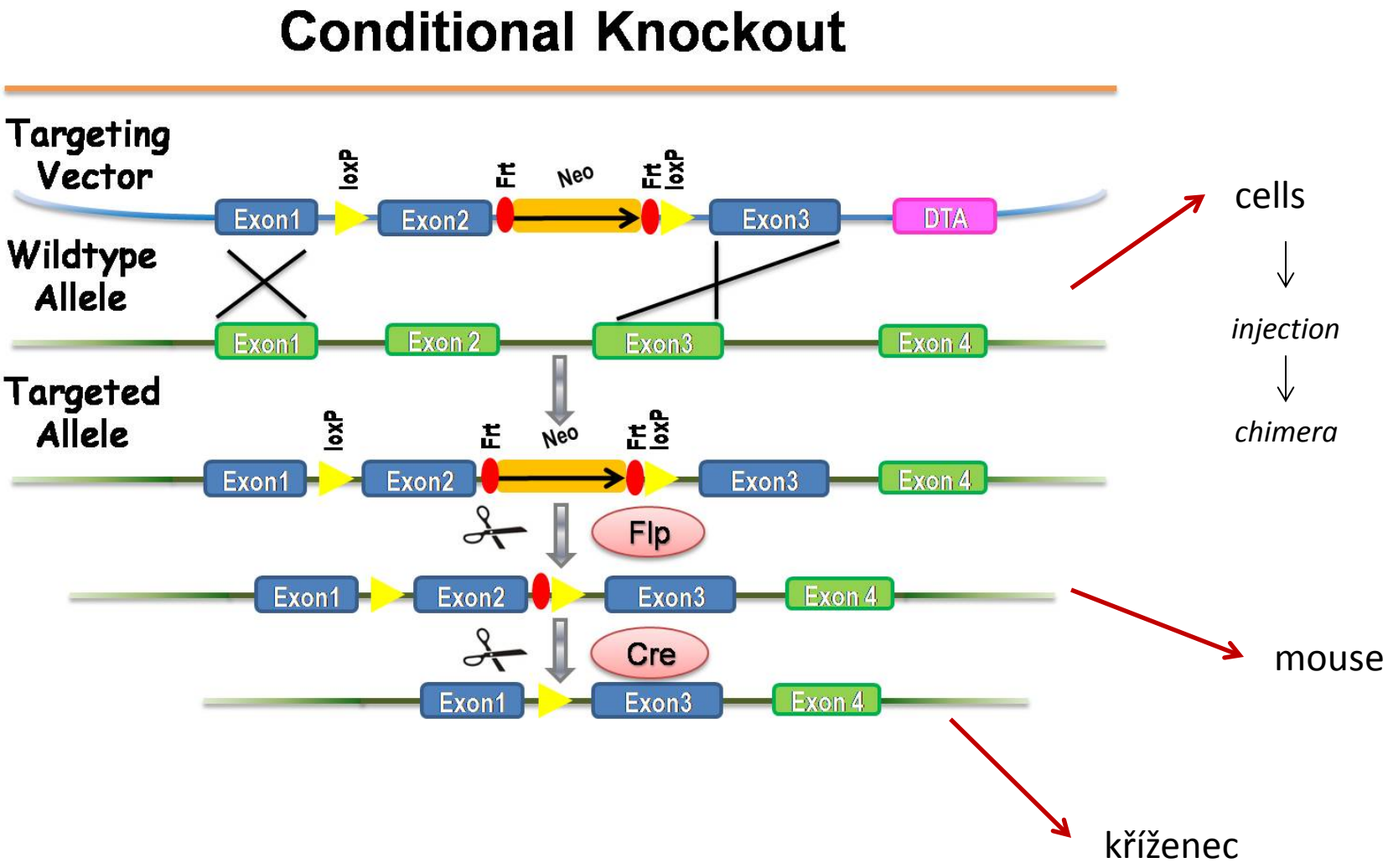


Frt site / Flp recombinase



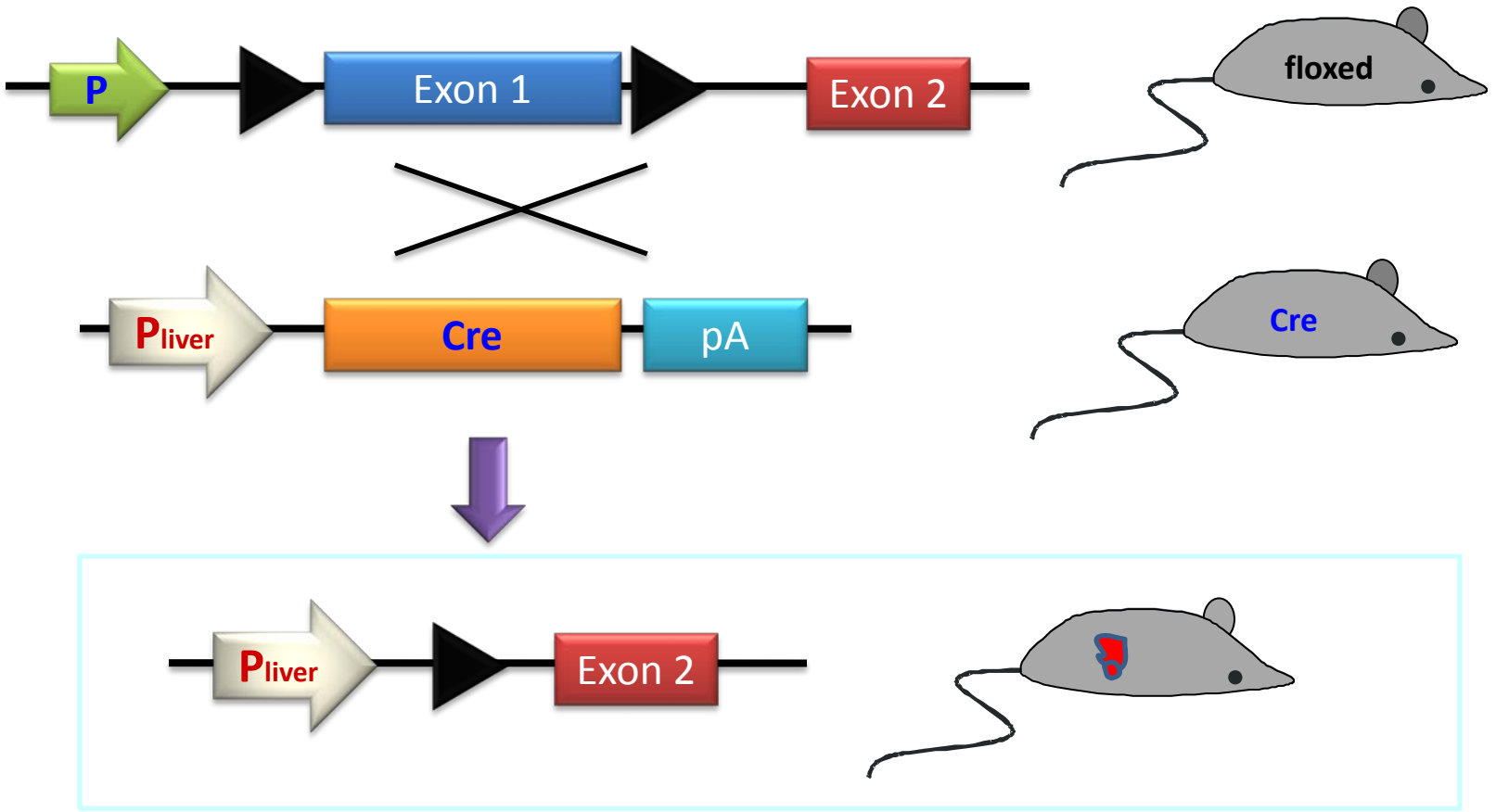
The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

Conditional knock-out mouse



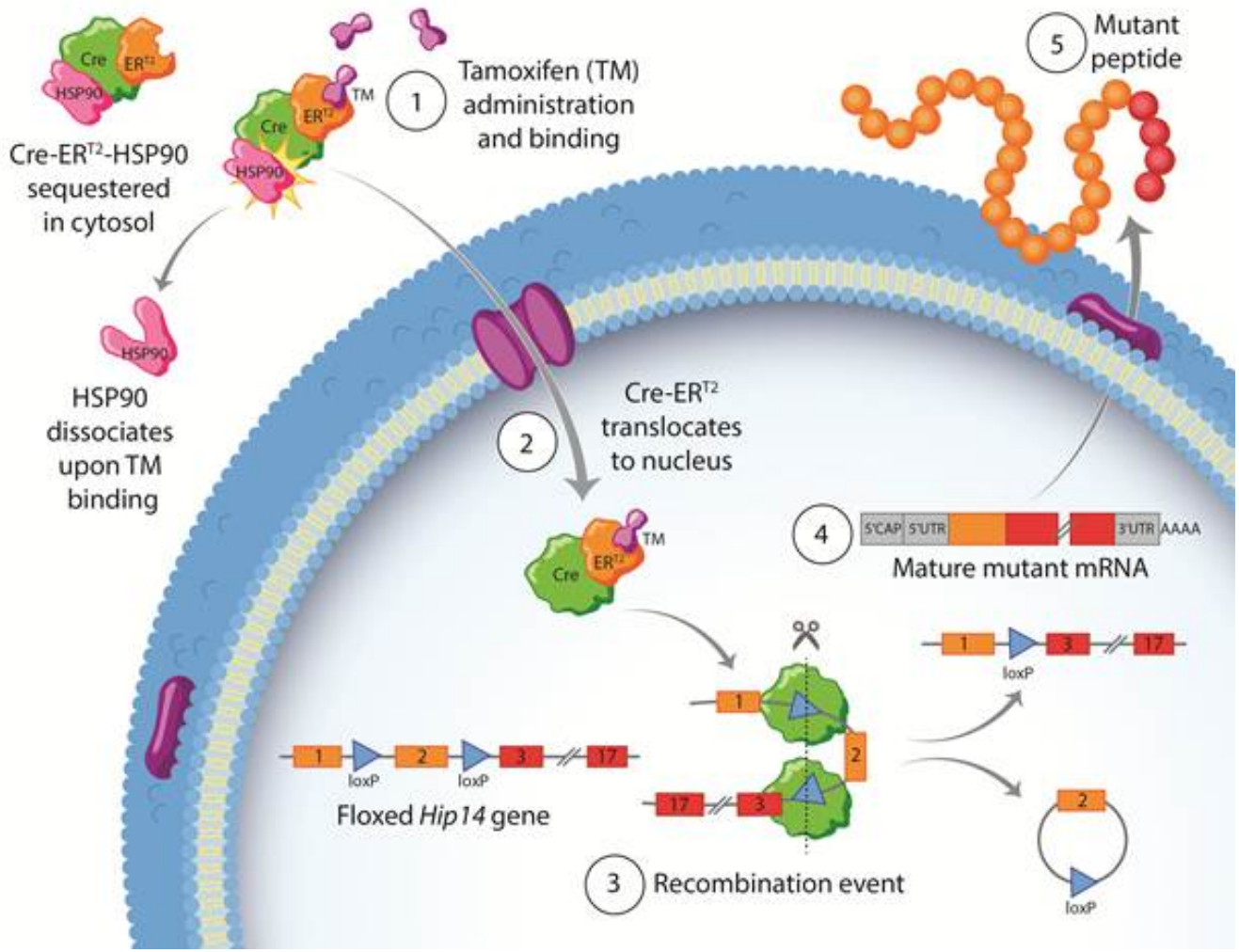
The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

Conditional gene targeting in ES cells



The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

Tamoxifen-inducible Cre–loxP system



New tools to target genes and genomic DNA

Zinc-finger, TAL-efector nucleases, CRISPR/Cas9 system

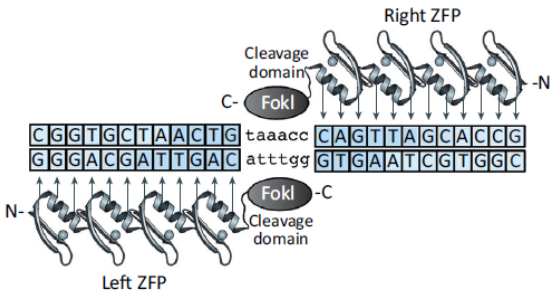
The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

programmable nucleases

-mediated gene modifications

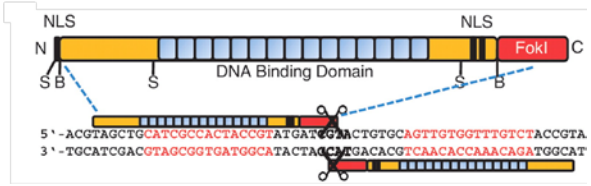
Zinc Finger Nucleases

- Cys2-His2 zinc finger domain
- Artificial arrays of 3-6 Zinc Fingers (9 – 18 bp)
- C-terminal fusion with endonuclease (FokI) – ZFN



Transcription Activator-Like Effectors nucleases (TALENs)

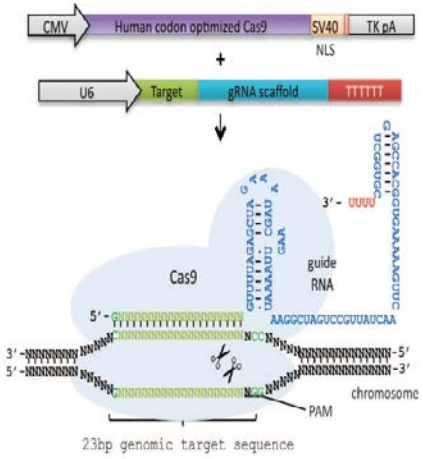
- Central Repeat Domain (CRD) responsible for DNA binding
- CRD consisting of 34aa highly homologous repeat modules
- DNA specifity determined by aminoacids 12 and 13 of each repeat
  – repeat variable diresidues (RVDs)



Modular assembly allows efficient and low-cost generation of TALEN vectors

CRISPR/Cas9 system

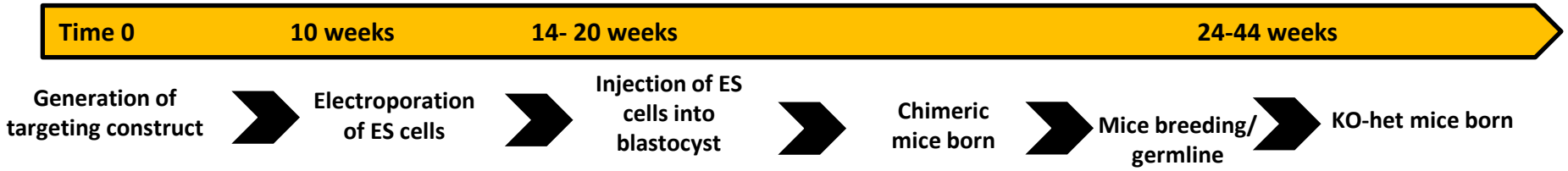
- interspaced short palindromic repeats (CRISPR) systems
- CRISPR RNAs (crRNAs) in complex with CRISPR-associated (Cas) proteins



Mali et al., Science 2013

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

KO mouse generation by homologous recombination in ES cells vs TALEN technology



The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 “Founding the Centre of Transgenic Technologies”

Available online at www.sciencedirect.com
ScienceDirect
Journal of Genetics and Genomics 41 (2014) 7–19
JGG
REVIEW

CRISPR/Cas9 and Genome Editing in *Drosophila*

Andrew R. Bassett*, Ji-Long Liu*

MRC Functional Genomics Unit, University of Oxford, Department of Physiology, Anatomy and Genetics, South Parks Road, Oxford OX1 3QX, United Kingdom

Received 25 November 2013; revised 10 December 2013; accepted 11 December 2013
Available online 18 December 2013

ABSTRACT

Recent advances in our ability to design DNA binding factors with specificity for desired sequences have resulted in a revolution in genetic engineering, enabling directed changes to the genome to be made relatively easily. Traditional techniques for generating genetic mutations in most organisms have relied on selection from large pools of randomly induced mutations for those of particular interest, or time-consuming gene targeting by homologous recombination. *Drosophila melanogaster* has always been at the forefront of genetic analysis, and application of these new genome editing techniques to this organism will revolutionise our approach to performing analysis of gene function in the future. We discuss the recent techniques that apply the CRISPR/Cas9 system to *Drosophila*, highlight potential uses for this technology and speculate upon the future of genome engineering in this model organism.

KEYWORDS: *Drosophila melanogaster*; CRISPR; Cas9; Genome engineering; Targeted mutagenesis

Development, Growth & Differentiation
The Japanese Society of Developmental Biologists
Develop. Growth Differ. (2014) **56**, 46–52
doi: 10.1111/dgd.12110

Review Article

Gene targeting technologies in rats: Zinc finger nucleases, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeats

Tomoji Mashimo*

Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho Sakyo-ku, Kyoto, 606-8501, Japan

The laboratory rat has been widely used as an animal model in biomedical science for more than 150 years. Applying zinc-finger nucleases or transcription activator-like effector nucleases to rat embryos via microinjection is an efficient genome editing tool for generating targeted knockout rats. Recently, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated endonucleases have been used as an effective tool for precise and multiplex genome editing in mice and rats. In this review, the advantages and disadvantages of these site-specific nuclease technologies for genetic analysis and manipulation in rats are discussed.

Key words: clustered regularly interspaced short palindromic repeats, genome-editing, rats, transcription activator-like effector nucleases, zinc-finger nucleases.

Introduction

Genetically modified animals that have been altered using gene targeting technologies are used as experimental models to perform functional analyses or various tests in biomedical research. In particular, knockout (KO) animals can help in understanding how knockout mice with spatial or temporal control of genetic inactivation, are widely used. Gene targeting technologies have become critical tools for understanding gene functions including the genetic basis of human diseases.

Until recently it was difficult to produce mammalian KO animals other than mice using gene targeting tech-

CRISPR-mediated direct mutation of cancer genes in the mouse liver

Wen Xue, Sidi Chen, Hao Yin, Tuomas Tammela, Thales Papagiannakopoulos, Nikhil S. Joshi, Wenxin Cai, Gillian Yang, Roderick Bronson, Denise G. Crowley, Feng Zhang, Daniel G. Anderson, Phillip A. Sharp & Tyler Jacks

Affiliations | Contributions | Corresponding author

Nature **514**, 380–384 (16 October 2014) | doi:10.1038/nature13589
Received 18 February 2014 | Accepted 17 June 2014 | Published online 06 August 2014

The study of cancer genes in mouse models has traditionally relied on genetically-engineered strains made via transgenesis or gene targeting in embryonic stem cells[1]. Here we describe a new method of cancer model generation using the CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins) system *in vivo* in wild-type mice. We used hydrodynamic injection to deliver a CRISPR plasmid DNA expressing Cas9 and single

BRIEF COMMUNICATIONS

TALEN-mediated precise genome modification by homologous recombination in zebrafish

Yao Zu[1–3], Xiangjun Tong[1,3], Zhanxiang Wang[1], Du Liu[1], Ruochuan Pan[1], Zhe Li[1], Yingying Hu[1], Zhou Luo[1], Peng Huang[1], Qian Wu[1], Zuoyan Zhu[1], Bo Zhang[1] & Shuo Lin[2]

We report gene targeting via homologous recombination in zebrafish. We co-injected fertilized eggs with transcription activator–like effector nuclease mRNAs and a donor vector with long homologous arms targeting the *tyrosine hydroxylase* (*th*) locus, and we observed effective gene modification that was transmitted through the germ line. We also successfully targeted two additional genes. Homologous recombination in zebrafish with a dsDNA donor expands the utility of this model organism.

Gene targeting by homologous recombination can precisely modify the genome and has been widely used to study gene function and introduce mutations of interest in mice[1]. Recently, zinc-finger nuclease and transcription activator–like effector nuclease (TALEN) technologies have been developed to generate site-specific DNA double-strand breaks (DSBs), resulting in unpredictable gene mutations when the DSBs are erroneously repaired by nonhomologous end joining[2–5]. In zebrafish, various targeted mutagenesis strategies, including the use of zinc-finger nucleases and TALENs, have been established[6–8]. More recently, TALEN-mediated gene editing using ssDNA oligonucleotides (ssODNs) has also been reported[9,10]. However, homologous recombination using a long dsDNA donor, for knock-in of large DNA fragments, has yet to be achieved in the zebrafish.

Targeted gene modification mediated by spontaneous homologous recombination occurs at a very low frequency. DSBs generated by targeted nucleases stimulate homologous recombination dramatically in fruit flies, rats, mice and maize[11–14]. We therefore reasoned that TALENs may be useful for targeted knock-in through homologous recombination in zebrafish as well.

We constructed a TALEN pair targeting exon 4 of the zebrafish *th* gene. We selected a 47-base-pair (bp) site including a restriction site for BccI using the targeting software TALE-NT[15,16] (**Fig. 1a**) and used the 'unit assembly' method[4] for construction of the transcription activator–like effector repeats. To evaluate the efficiency of the generated TALENs, we PCR-amplified a 297-bp genomic DNA fragment containing the target site from injected or control embryos and then digested the amplified DNA with BccI. In embryos injected with TALEN mRNAs, ≥70% of the DNA fragments remained intact after digestion (**Fig. 1b**). Indeed, sequencing of the intact fragments showed that different indel mutations had occurred (**Fig. 1c**).

For gene targeting by homologous recombination, we sequenced parental animals and used only parents whose *th* locus was identical to each other and to that of the targeting construct (**Supplementary Results**), as precise homology may affect the efficiency of homologous recombination[17]. On the basis of the parental sequence, we constructed several targeting donor vectors in which a part of exon 4 of *th*, including the TALEN binding sites, was replaced by *EGFP* flanked by homologous arms of different lengths (**Supplementary Table 1**). We injected each donor and the TALEN mRNAs into zebrafish embryos. Most injected

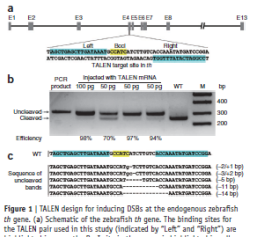


Figure 1 | TALEN design for inducing DSBs at the endogenous zebrafish *th* gene. (**a**) Schematic of the zebrafish *th* gene. The binding sites for the TALEN pair used in this study (indicated by "Left" and "Right") are highlighted in cyan; the BccI site in the spacer is highlighted in yellow. E, exon. (**b**) Gel showing BccI digestion of PCR products amplified from pooled genomic DNA of three embryos injected with TALEN mRNAs. The uncleaved and cleaved PCR products are indicated. WT, wild type; M, marker; pg, picograms. (**c**) Representative sequencing results of the uncleaved PCR fragments, revealing different indel mutations in the TALEN target site.

[1]Key Laboratory of Cell Proliferation and Differentiation of Ministry of Education, College of Life Sciences, Peking University, Beijing, China. [2]Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, Los Angeles, California, USA. [3]These authors contributed equally to this work. Correspondence should be addressed to S.L. (shoulin@ucla.edu) or B.Z. (bzhang@pku.edu.cn).

RECEIVED 26 SEPTEMBER 2012; ACCEPTED 15 JANUARY 2013; PUBLISHED ONLINE 24 FEBRUARY 2013; DOI:10.1038/NMETH.2374

BRIEF COMMUNICATIONS

Genetic engineering of human pluripotent cells using TALE nucleases

Dirk Hockemeyer[1,4], Haoyi Wang[1,4], Samira Kiani[1], Christine S Lai[1,2], Qing Gao[1], John P Cassady[1,2], Gregory J Cost[3], Lei Zhang[3], Yolanda Santiago[3], Jeffrey C Miller[3], Bryan Zeitler[3], Jennifer M Cherone[3], Xiangdong Meng[3], Sarah J Hinkley[3], Edward J Rebar[3], Philip D Gregory[3], Frodor D Urnov[3] & Rudolf Jaenisch[1,2]

Targeted genetic engineering of human pluripotent cells is a prerequisite for exploiting their full potential. Such genetic manipulations can be achieved using site-specific nucleases. Here we engineered transcription activator–like effector nucleases (TALENs) for five distinct genomic loci. At all loci tested we obtained human embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) clones carrying transgenic cassettes solely at the TALEN-specified location. Our data suggest that TALENs employing the specific architectures described here mediate site-specific genome modification in human pluripotent cells with similar efficiency and precision as do zinc-finger nucleases (ZFNs).

Nat. Biotechnol. **29**, 731–734 (2011); doi:10.1038/nbt.1927
First published in *Nature Biotechnology* 29, 731–734 (2011); doi:10.1038/nbt.1927

Gene targeting of human pluripotent cells by homologous recombination is inefficient, which has impeded the use of human ESCs and iPSCs in disease models. To overcome this limitation, we and others have shown that ZFNs can be used to modify the genomes of ESCs and iPSCs[1–3]. ZFNs can be engineered to induce a double-strand break precisely at a predetermined position in the genome[4]. The double-strand break can be repaired through nonhomologous end-joining to drive targeted gene disruption or through the homology-directed DNA repair pathway using an exogenous donor plasmid as a template. Depending on the donor design, this repair reaction can be used to generate large-scale deletions, gene disruptions, DNA addition[5] or single-nucleotide changes[5].

Recent work on transcription activator–like effectors (TALEs) provides an alternative approach to the design of site-specific nucleases[6]. Natural TALEs are transcription factors used by plant pathogens to subvert host genome regulatory networks[6]. The DNA-binding domain of TALEs is unusual. Multiple units of ~34 amino acids (called TALE repeats) are arranged in tandem, their sequence nearly identical except for two highly variable amino acids that establish the base-recognition specificity of each unit[7,8]. Each individual domain determines the specificity of binding to one DNA base pair in the TALE recognition sequence, and therefore arrays of four different repeat units are sufficient to generate TALEs with novel DNA recognition sites[7,8]. Nucleases based on such engineered TALE domains have been shown to target endogenous genes in transformed human cells[9,10]. Here we evaluate the use of TALENs for genetic engineering of endogenous loci in human ESCs and iPSCs.

We designed TALENs targeting the *PPP1R12C* (the AAVS1 locus), *OCT4* (also known as *POU5F1*) and *PITX3* genes at precisely the same positions as targeted earlier by ZFNs[2]. TALEN expression constructs and corresponding donor plasmids bearing homologous sequences were introduced into ESCs (line WIBR3)[11] and iPSCs (line C1)[12] by electroporation (**Supplementary Fig. 1** and **Supplementary Tables 1** and **2**). Southern blot analysis was used to identify correctly targeted clones.

We targeted *PPP1R12C* with a gene trap approach (expressing puromycin (Puro) from the endogenous gene; **Fig. 1a,b** and **Table 1**) or with an autonomous selection cassette (puromycin expressed from the PGK-promoter (**Fig. 1**, **Table 1** and **Supplementary Figs. 1–3**). Targeting efficiency was high and similar to that with ZFNs[2]: 50% of the clones were targeted in one or both alleles and carried no randomly integrated transgenes (**Fig. 1b**, **Table 1** and **Supplementary Fig. 2**). Similarly, an SA-Puro-CAGGS-eGFP transgene was highly expressed from this locus (**Fig. 1a** and **Supplementary Fig. 3a,b**). Notably, such targeted cells remained pluripotent based on analysis of marker expression and of teratomas. (**Supplementary Fig. 3c–e**). Cells of all germ layers in teratomas expressed eGFP, indicating that TALEN- as well as ZFN-mediated targeting of *PPP1R12C* results in robust transgene expression in pluripotent as well as in differentiated cells (**Supplementary Fig. 3a,b,d,e**).

OCT4 was targeted using three different donor plasmids, resulting in expression of puromycin and an OCT4-exon1–eGFP fusion protein under control of the endogenous *OCT4* promoter. The first two donor plasmids were designed to integrate a splice acceptor–eGFP-2A-Puro cassette into the first intron of *OCT4*, whereas the third donor generated an in-frame fusion of exon 1 with the eGFP-2A-Puro cassette (**Supplementary Fig. 4**). Targeting efficiency in ESCs and iPSCs was 70–100% as determined by Southern blot analysis and DNA sequencing of single cell–derived clones (**Table 1** and **Supplementary Figs. 1** and **4**).

We also targeted the first exon of *PITX3*, which is not expressed in human pluripotent cells, and found that ~6% of drug-resistant clones carried the transgene solely at the *PITX3* locus as revealed by Southern blot analysis (**Table 1** and **Supplementary Figs. 1** and **5**). Notably, in one clone the transgene had integrated into both alleles of *PITX3*.

To target the C terminus of OCT4, which has not been previously targeted by ZFNs, we generated TALENs directed against sequences flanking the stop codon of *OCT4* using two donor plasmids: the last *OCT4* codon was either fused in frame with an eGFP-PGK-Puro construct or with eGFP preceded by a 2A sequence (2A-eGFP-PGK-Puro; **Fig. 1c** and **Table 1**). After excision of the Lox P–flanked PGK-Puro cassettes, either a C-terminal OCT4-eGFP fusion protein or a separately translated eGFP protein will be expressed under the control of endogenous *OCT4* (ref. 13). Southern blot analysis showed that 2–10% of single cell–derived ESC and iPSC clones carried the transgene cassette at the *OCT4* locus (**Fig. 1d,e** and **Table 1**). Cre-mediated excision of the PGK-

[1]The Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA. [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA. [3]Sangamo BioSciences, Inc., Richmond, California, USA. [4]These authors contributed equally to this work. Correspondence should be addressed to R.J. (jaenisch@wi.mit.edu).

Received 11 March; accepted 28 June; published online 7 July 2011; doi:10.1038/nbt.1927

Nucleic Acids Research Advance Access published April 14, 2011
Nucleic Acids Research, 2011, 1–11
doi:10.1093/nar/gkr218

Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting

Tomas Cermak[1], Erin L. Doyle[2], Michelle Christian[1], Li Wang[2], Yong Zhang[1,3], Clarice Schmidt[2], Joshua A. Baller[1,4], Nikunj V. Somia[1], Adam J. Bogdanove[2,*] and Daniel F. Voytas[1,*]

[1]Department of Genetics, Cell Biology & Development and Center for Genome Engineering, 321 Church Street SE, University of Minnesota, Minneapolis, MN 55455 [2]Department of Plant Pathology, 351 Bessey Hall, Iowa State University, Ames, IA 50011, USA, [3]Department of Biotechnology, School of Life Sciences and Technology, University of Electronic Science and Technology of China, Chendu 610054, China and [4]Biomedical Informatics and Computational Biology Program, University of Minnesota Rochester, 111 South Broadway, Rochester, MN 55904, USA

Received March 8, 2011; Revised March 23, 2011; Accepted March 24, 2011

ABSTRACT

TALENs are important new tools for genome engineering. Fusions of transcription activator-like (TAL) effectors of plant pathogenic *Xanthomonas spp.* to the FokI nuclease, TALENs bind and cleave DNA in pairs. Binding specificity is determined by customizable arrays of polymorphic amino acid repeats in the TAL effectors. We present a method and reagents for efficiently assembling TALEN constructs with custom repeat arrays. We also describe design guidelines based on naturally occurring TAL effectors and their binding sites. Using software that applies these guidelines, in nine genes from plants, animals and protists, we found candidate cleavage sites on average every 35 bp. Each of 15 sites selected from this set was cleaved in a yeast-based assay with TALEN pairs constructed with our reagents. We used two of the TALEN pairs to mutate *HPRT1* in human cells and *ADH1* in *Arabidopsis thaliana* protoplasts. Our reagents include a plasmid construct for making custom TAL effectors and one for TAL effector fusions to additional proteins of interest. Using the former, we constructed *de novo* a functional analog of AvrHah1 of *Xanthomonas gardneri*. The complete plasmid set is available through the non-profit repository AddGene and a web-based version of our software is freely accessible online.

INTRODUCTION

Transcription activator-like (TAL) effectors are a newly described class of specific DNA binding protein, so far unique in the simplicity and manipulability of their targeting mechanism. Produced by plant pathogenic bacteria in the genus *Xanthomonas*, the native function of these proteins is to directly modulate host gene expression. Upon delivery into host cells via the bacterial type III secretion system, TAL effectors enter the nucleus, bind to effector-specific sequences in host gene promoters and activate transcription (1). Their targeting specificity is determined by a central domain of tandem, 33–35 amino acid repeats, followed by a single truncated repeat of 20 amino acids (Figure 1a). The majority of naturally occurring TAL effectors examined have between 12 and 27 full repeats (2). Members of our group and another lab independently discovered that a polymorphic pair of adjacent residues at positions 12 and 13 in each repeat, the 'repeat-variable di-residue' (RVD), specifies the target, one RVD to one nucleotide, with the four most common RVDs each preferentially associating with one of the four bases (Figure 1a) (3,4). Also, naturally occurring recognition sites are uniformly preceded by a T that is required for TAL effector activity (3,4). These straightforward sequence relationships allow the prediction of TAL

*To whom correspondence should be addressed. Tel: +1 612 626 4509; Fax: +1 612 626 2600; Email: voytas@umn.edu
Correspondence may also be addressed to Adam J. Bogdanove. Tel: +1 515 294 3421; Fax: +1 515 294 9420; Email: ajbog@iastate.edu

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027 "Founding the Centre of Transgenic Technologies"

Introduction to gene targeting and genome editing

esf european social fund in the czech republic
EUROPEAN UNION
MINISTRY OF EDUCATION, YOUTH AND SPORTS
OP Education for Competitiveness
IMG Institute of Molecular Genetics of the ASCR, v. v. i.
INVESTMENTS IN EDUCATION DEVELOPMENT

Thank you for your attention

Slavomír Kinský, PhD

Czech Centre for Phenogenomics

Institute of Molecular Genetics of the ASCR, v.v.i.

The presentation is supported from the project OP EC CZ.1.07/2.3.00/30.0027
"Founding the Centre of Transgenic Technologies"

1. Organizmy, které byly geneticky modifikovány vnesením jednoho nebo více cizorodých genů se nazývají:
a) transgenní
b) ligační
c) inzerční

2. Jakým způsobem NENÍ možné vytvořit transgenní myš?
a) použitím DNA metyláz a restrikčních endonukleáz
b) injikací transgenu do pronuklea (PNI)
c) injikací geneticky pozměněných embryonálních buněk do vyvíjejícího se embrya (zygoty)

3. Embryonální kmenové buňky jsou pluripotentní. Co to znamená?
a) jsou to potomci totipotentních buněk a mohou produkovat jakékoliv typ buňky kromě buňky totipotentní
b) mohou produkovat pouze jediný typ buněk
c) jsou to buňky schopné intenzivního dělení

4. Která z uvedených metod vám umožní zkonstruovat transgenní myš rychleji?
a) injikací konstruktu nesoucí transgen do myšího oocytu
b) použití modifikovaných myších embryonálních buněk na vytvoření chiméry

5. Co se rozumí pod pojmem knock-in mutace?
a) cílená delece několika nukleotidů pomocí homologní rekombinace
b) inzerce protein-kódující DNA sekvence procesem homologní rekombinace v definovaném místě genomu
c) transverze protein-kódující DNA sekvence procesem nehomologní rekombinace

6. Co se rozumí pod pojmem kondicionální mutace?
a) mutace vedoucí ke translokaci části chromozomu
b) inzerce DNA sekvence kódující fluorescenční protein
c) mutace, kdy je možné modifikaci sledovaného genu vyvolat kdykoli během života zvířete v předem definovaných tkáních

7. Které z nasledujících tvrzení o embryonálních kmenových buňkach jsou správne?
a) embryonální kmenové buňky jsou diferencované buňky vyizolovány z pozdejšího stádia vývinu embrya
b) embryonální kmenové buňky jsou pluripotentní kmenové buňky nacházející se ve vnitřní buněčné mase raného embrya ve stadiu tzv. blastocysty
c) embryonální kmenové buňky nejsou schopné obnovy poškozené nebo opotřebované části a udržovat homeostazi organizmu

8. Které z uvedených tvrzení o homologní rekombinace NENÍ správné:
a) umožňuje inaktivovat nebo nahradit endogenní kopii genu transgenem
b) je proces uplatňující se při opravě dvouřetězcových zlomů DNA
c) umožňuje integraci transgenní molekuly nespecificky, t.j. kdekoli v genomu

9. Která z nasledujících možností představuje embryonální vývoj od nejrannejšího po nejstarší stadium embrya?
a) morula → blastula → gastrula → zygota
b) zygota → morula → blastula → gastrula
c) zygota → blastula → gastrula → morula

10. Co je to chiméra z pohledu genetických manipulací?
a) vyhynulý živočich z doby pravěku
b) organizmus, který se vyvinul z embryonálních buňek pocházejících ze 2 různých zdrojů v laboratoři
c) organizmus, který vznikl z buňek nebo genů pocházející z 2 a více různych druhů živočichů