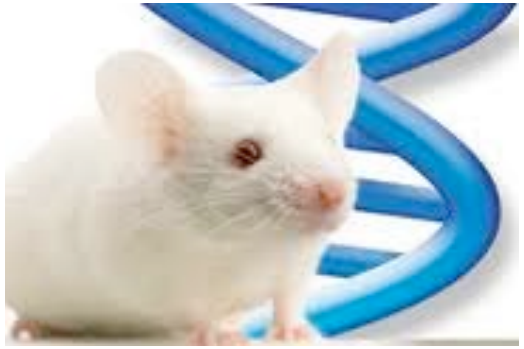
Dr Fabien DELERUE

UNSW AUSTRALIA

Never Stand Still | Medicine

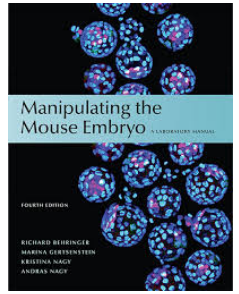
MANIPULATING THE MOUSE EMBRYO: FROM ES CELLS TO GENOME EDITING

Transgenic Animal Unit - Mark Wainwright Analytical Centre
Dementia Research Unit - School of Medical Sciences



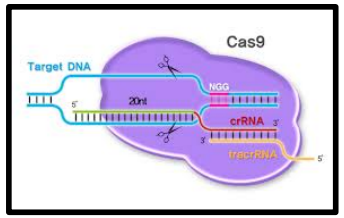
Overview

Mouse embryology

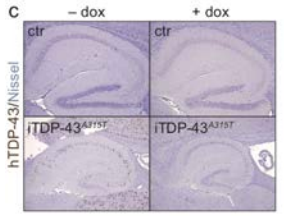


Microinjection and its applications

Genome editing

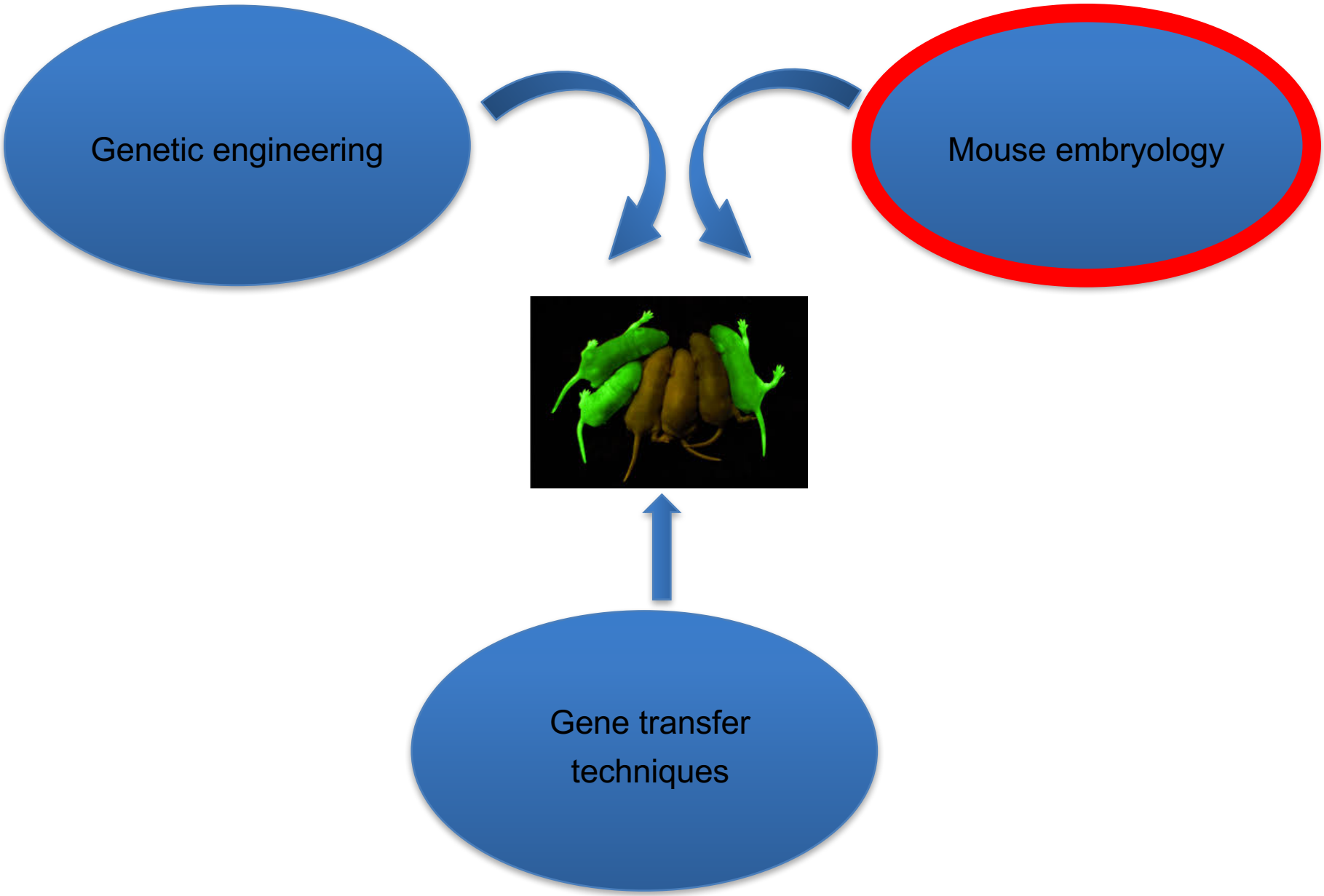


Mouse models generated



Beyond mouse models: gene therapy?

Genome manipulation in mice



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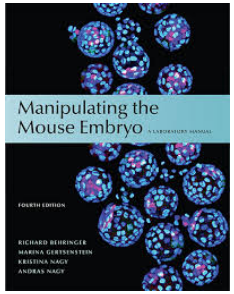
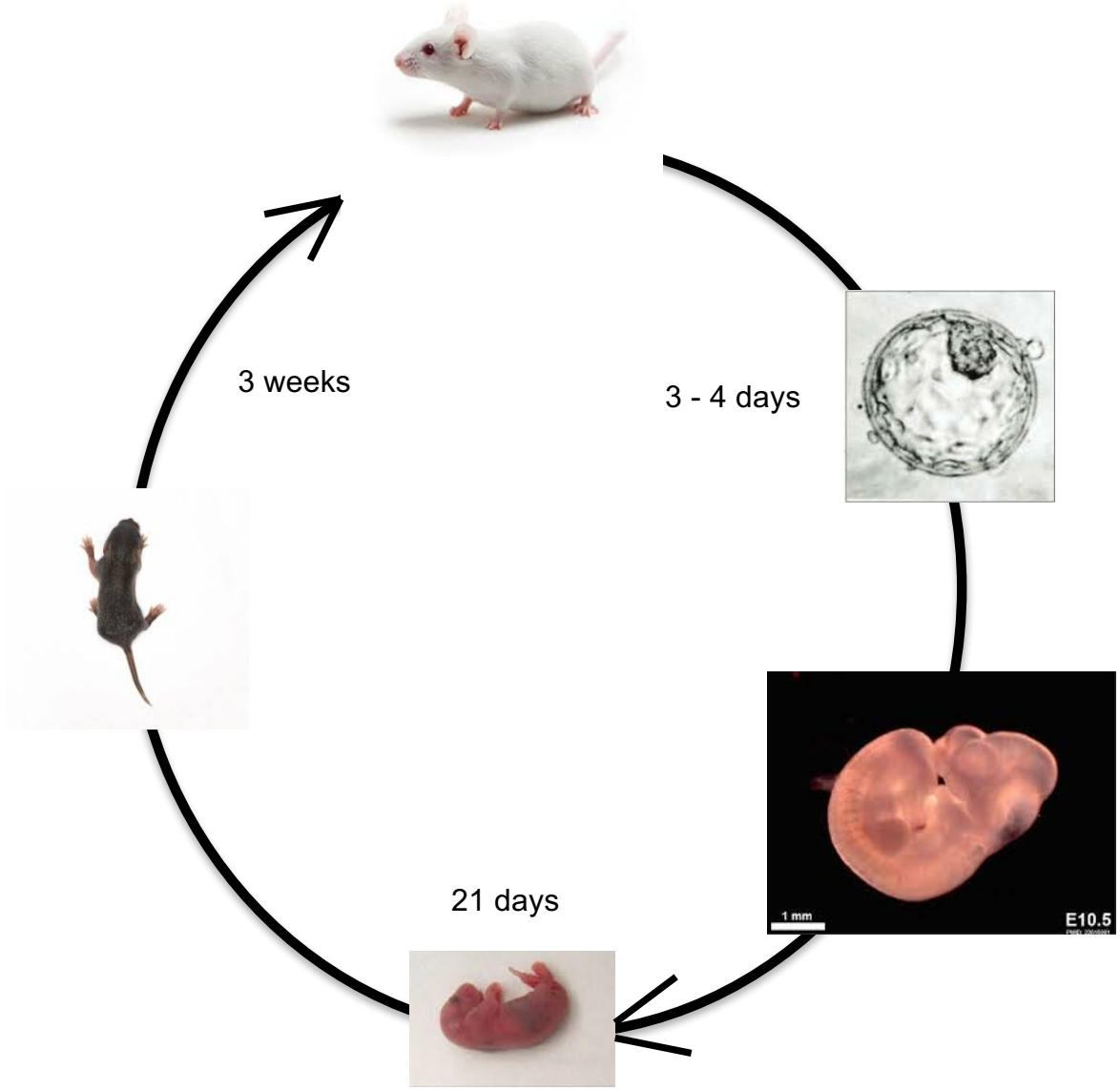


Genome manipulation in mice

Mouse embryology



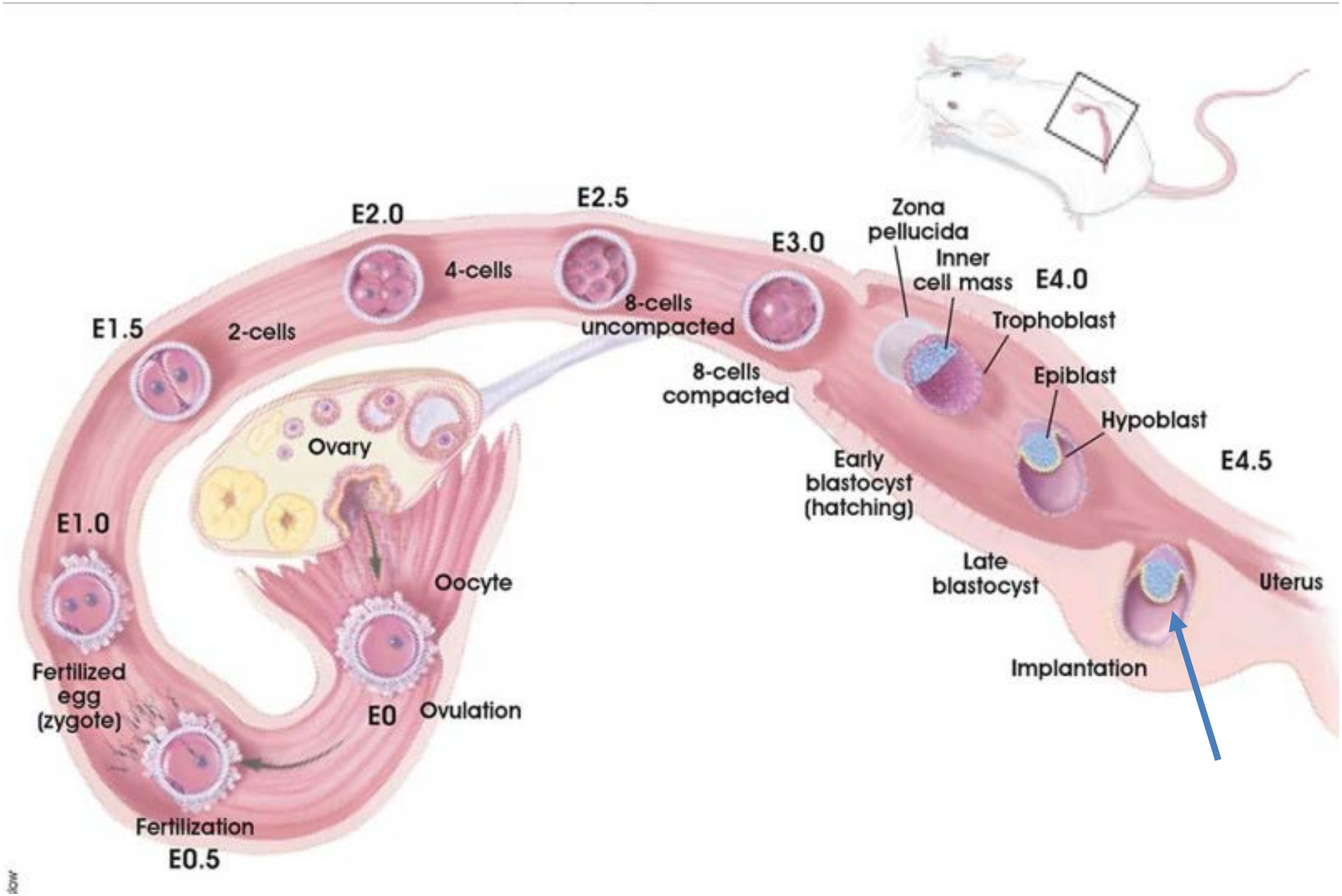
Advantages of the mouse:

- Easy to breed
- “Fast” development
- Good litter size
- Genome entirely sequenced in 2002

Mouse is the most commonly used animal model

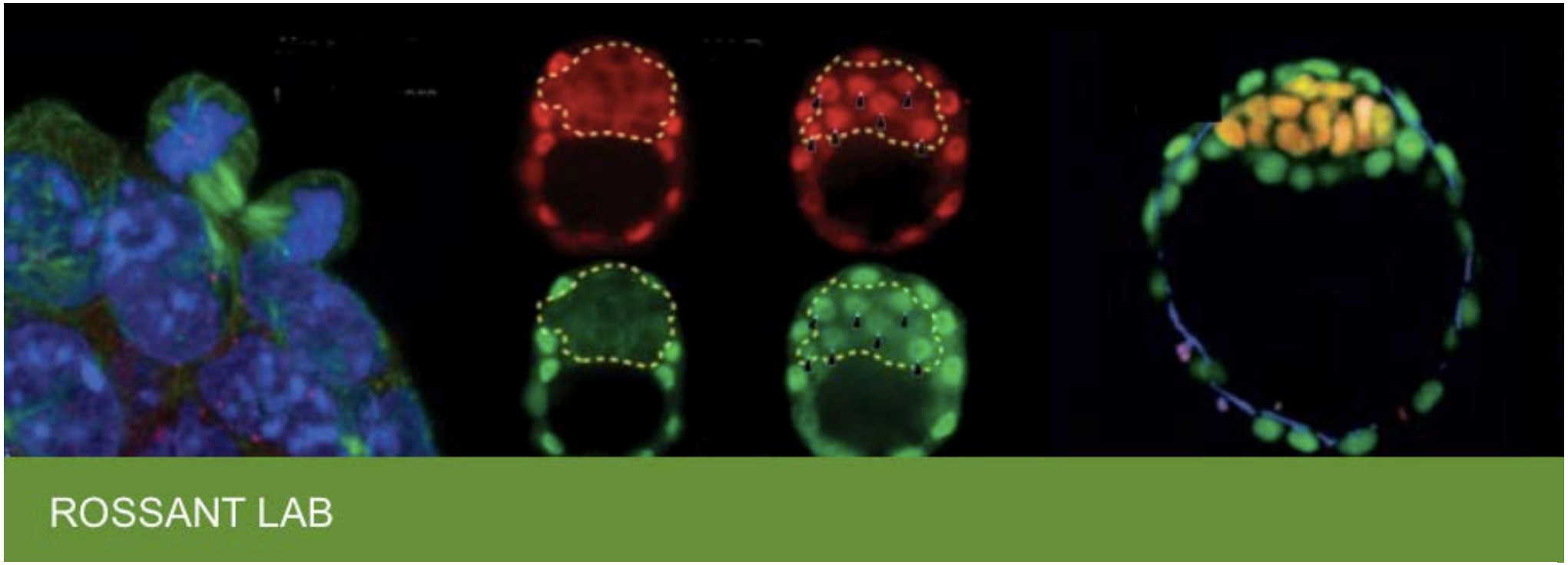
Genome manipulation in mice

Mouse embryology



Genome manipulation in mice

Mouse embryology



Current projects

- Developmental timing and molecular characterization of commitment to inner cell mass and trophectoderm lineages
- Timing and commitment of epiblast and primitive endoderm cell fate

Prof Janet Rossant – Stem Cell research

Genome manipulation in mice

Mouse embryology

PLOS ONE

RESEARCH ARTICLE

Superovulation Using the Combined Administration of Inhibin Antiserum and Equine Chorionic Gonadotropin Increases the Number of Ovulated Oocytes in C57BL/6 Female Mice

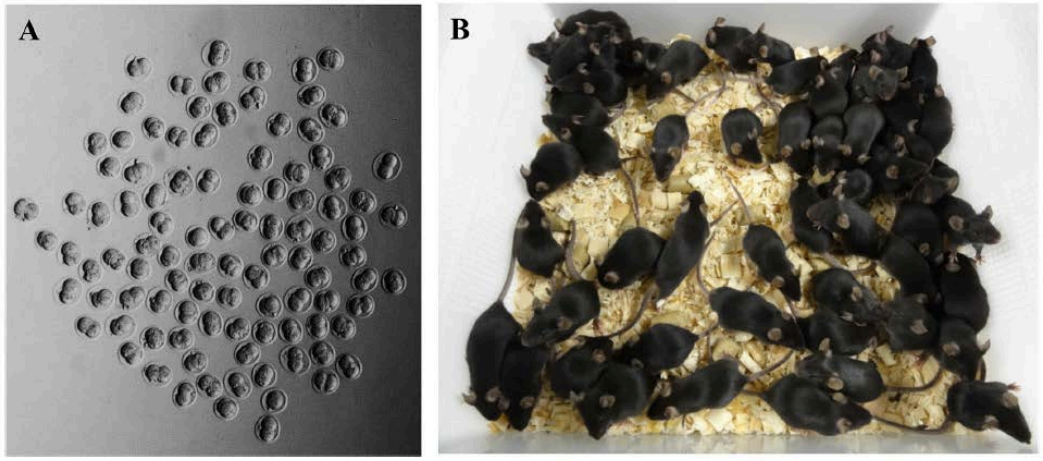
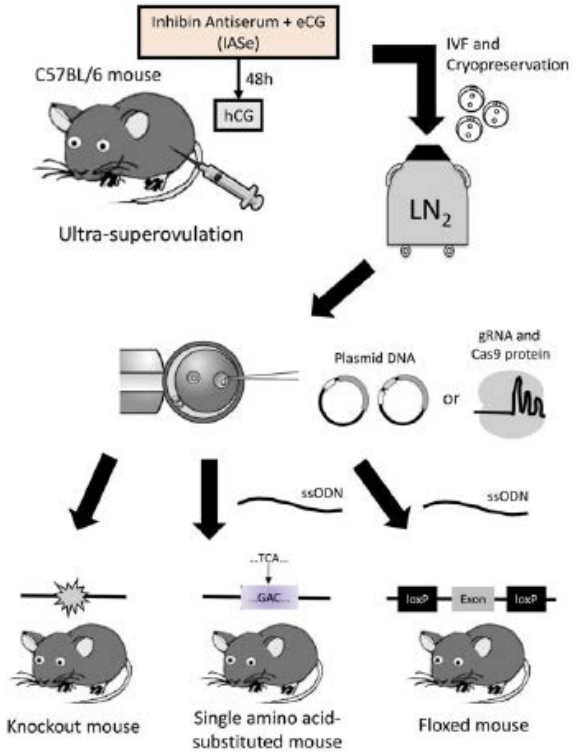


Fig 2. Production of 2-cell embryos (A) and live pups (B) from single female mice superovulated using IASe (0.1 mL IAS and 3.75 IU eCG).

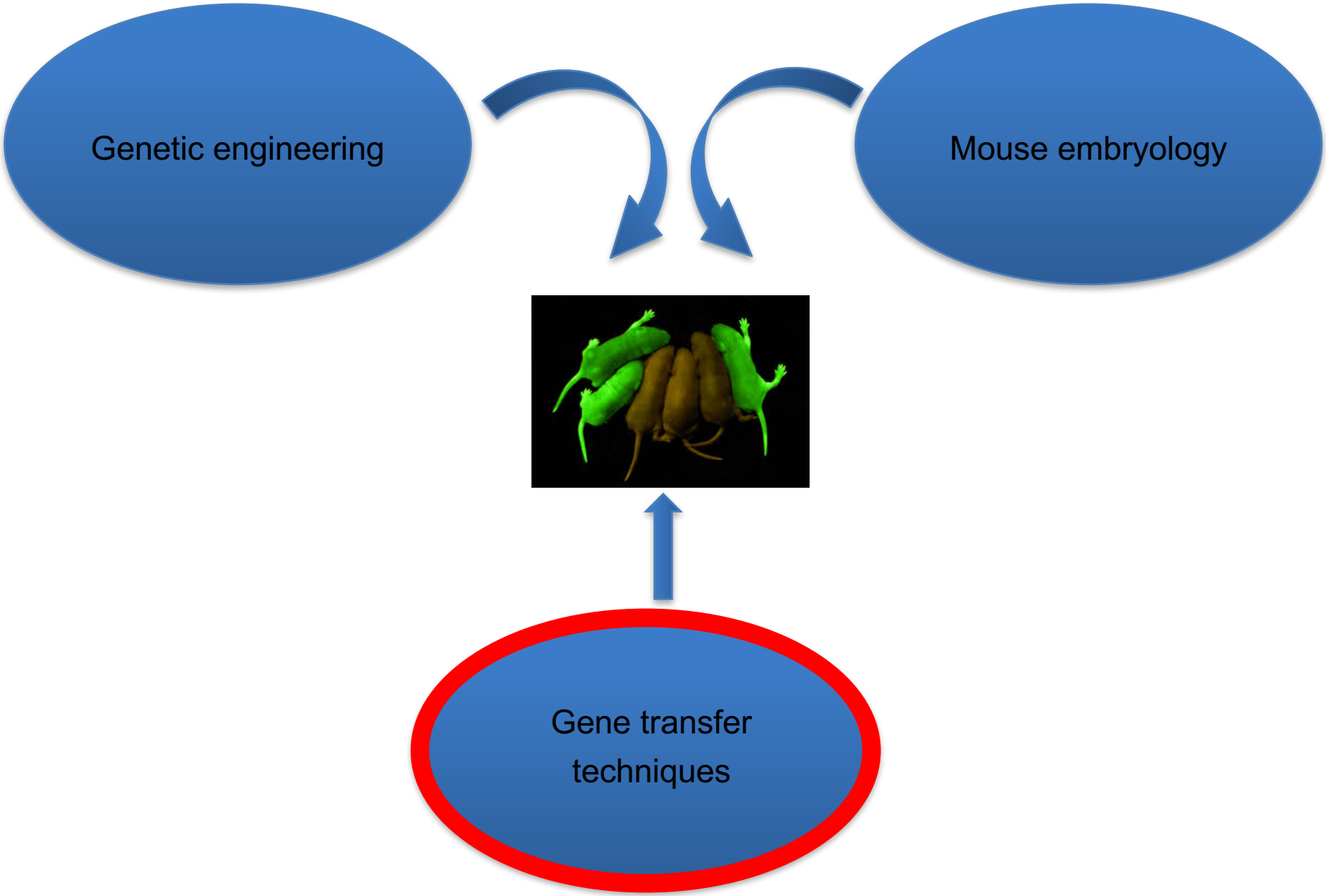
METHODS & TECHNIQUES

Ultra-superovulation for the CRISPR-Cas9-mediated production of gene-knockout, single-amino-acid-substituted, and floxed mice



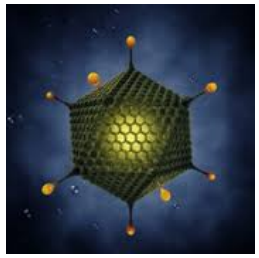
Prof Naomi Nakagata – CARD

Genome manipulation in mice



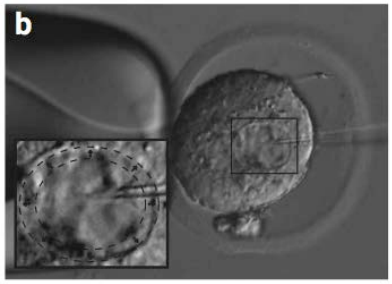
Genome manipulation in mice

Gene transfer



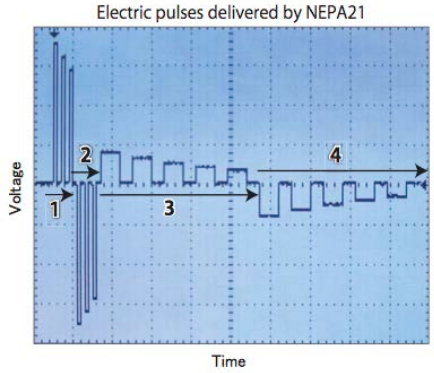
Viral delivery

Jaenisch R. *Proc. Natl. Acad. Sci. USA (1976) 73:1260-1264*



Microinjection

Ittner L.M. & Gotz J. *Nat Protoc. (2007) 2(5):1206-15.*

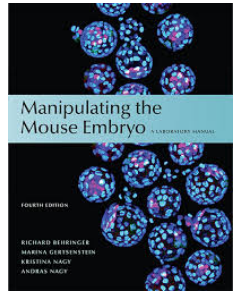


Electroporation

Kaneko T. *et al. Sci Rep. (2014) Oct 1;4:6382*

Overview

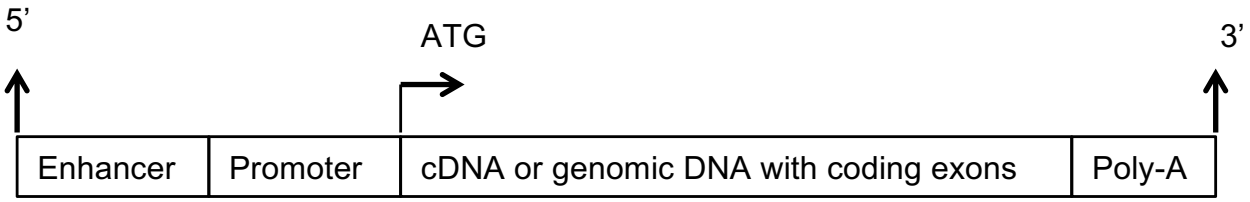
History of genome manipulation in mice



Microinjection and its applications

Genome manipulation in mice

Genetic engineering



ATG: beginning of the transcriptional reading frame

↑ rare restriction enzyme recognition site

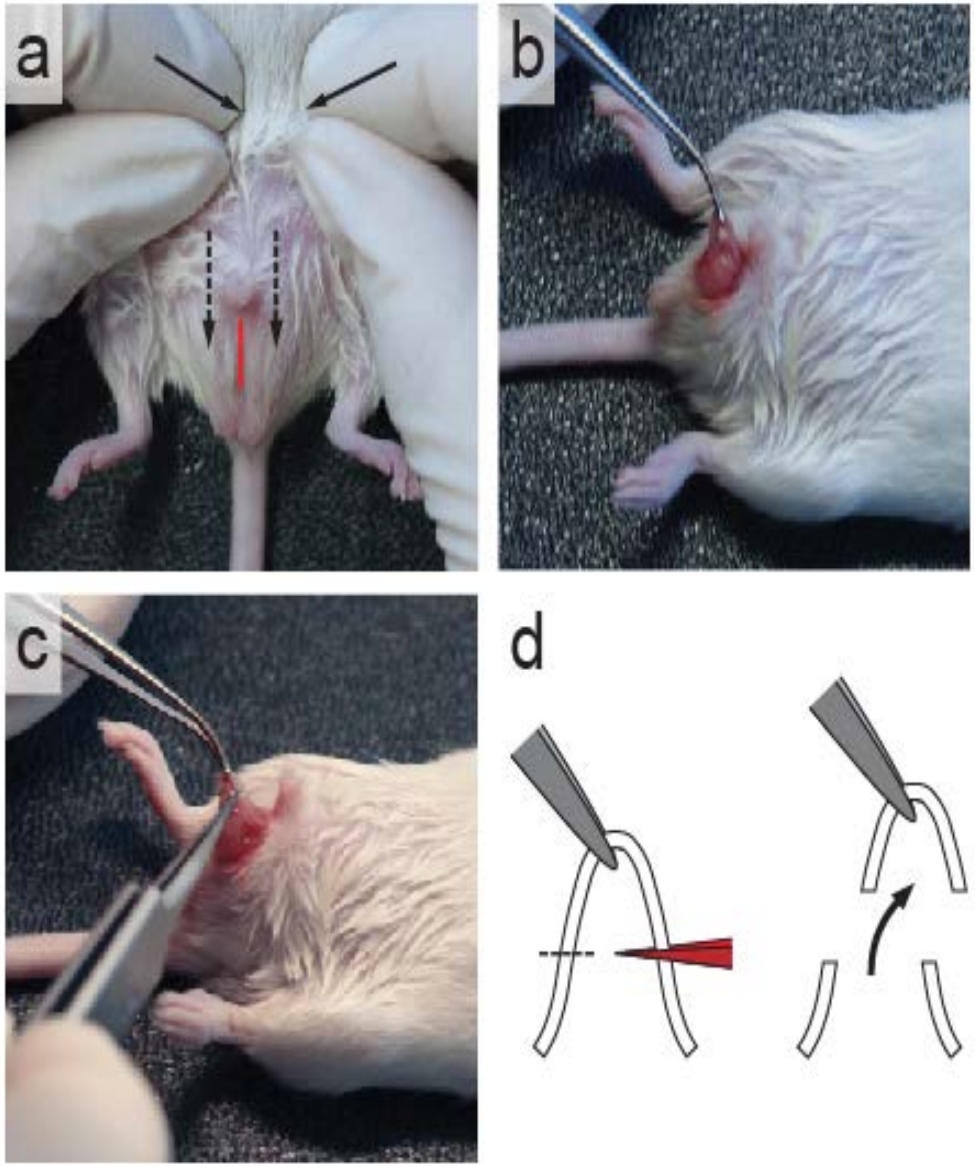
Schematic layout of a typical transgene construct

Adapted from Auerbach AB. *Acta Biochem. Pol. 2004;51(1):9-31. Review*

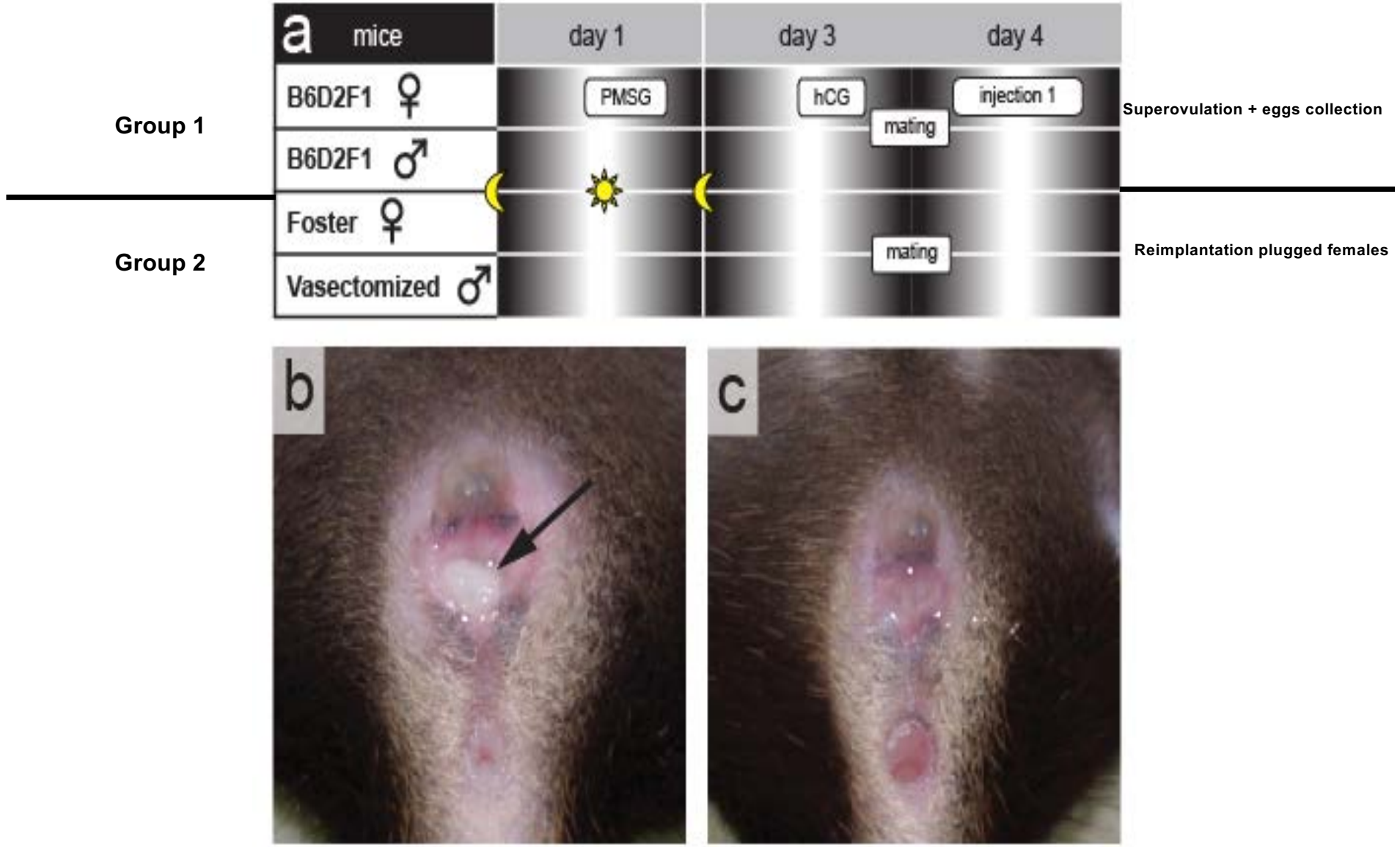
1980s “Era of Recombinant DNA technology”

Microinjection and its applications

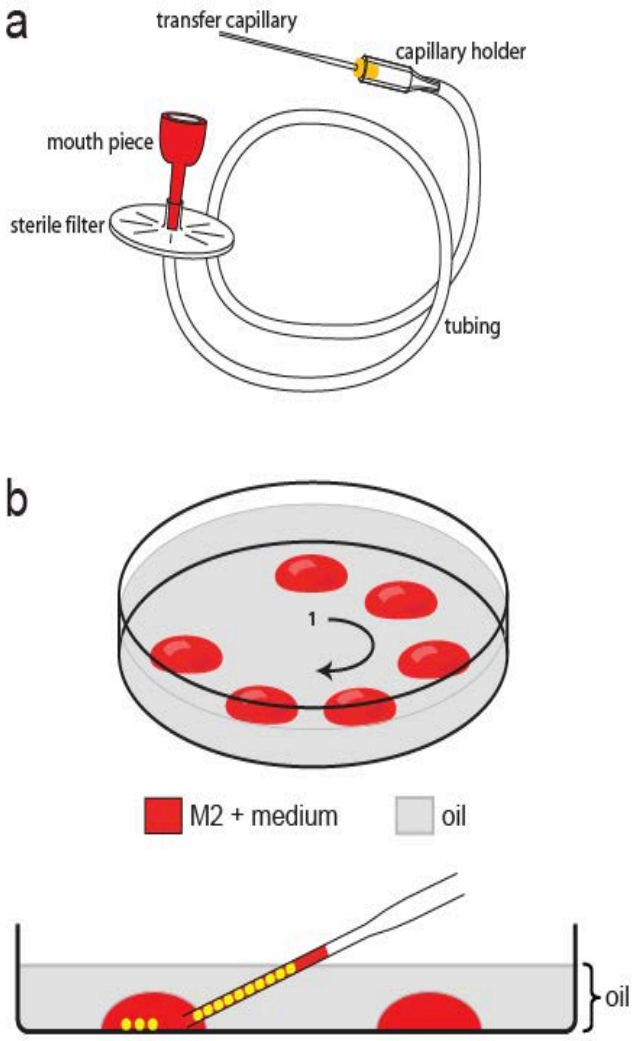
Vasectomy

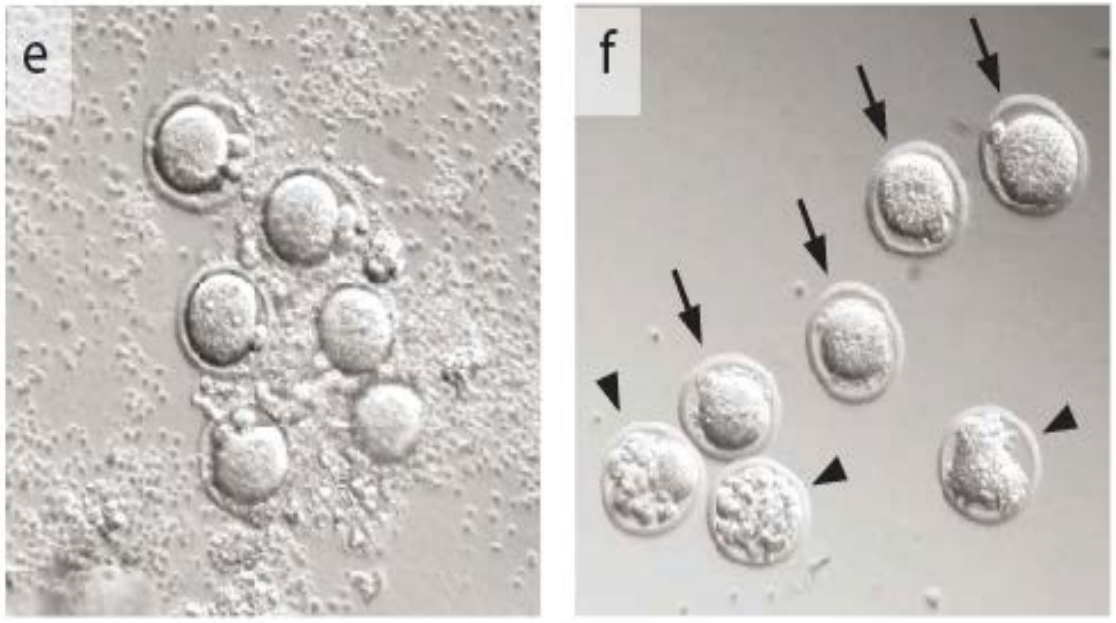


Microinjection and its applications



Microinjection and its applications





Collection and Purification (hyaluronidase) of the eggs
(incubator 37C – 5% CO_2)

Microinjection and its applications

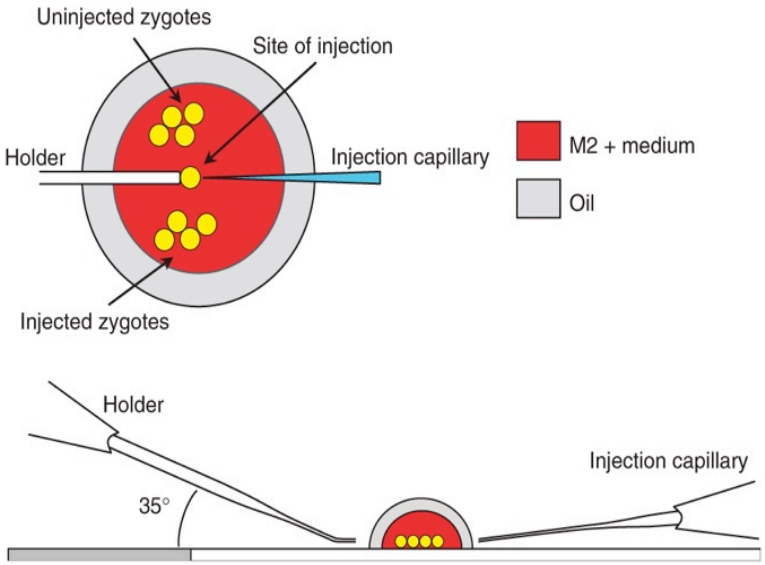
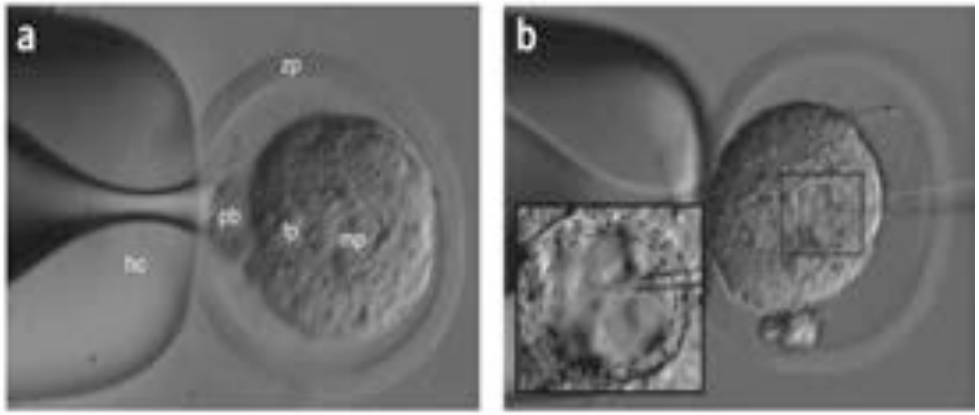


Figure 5 | Arrangements of zygotes, holder and injection capillary on injection stage.

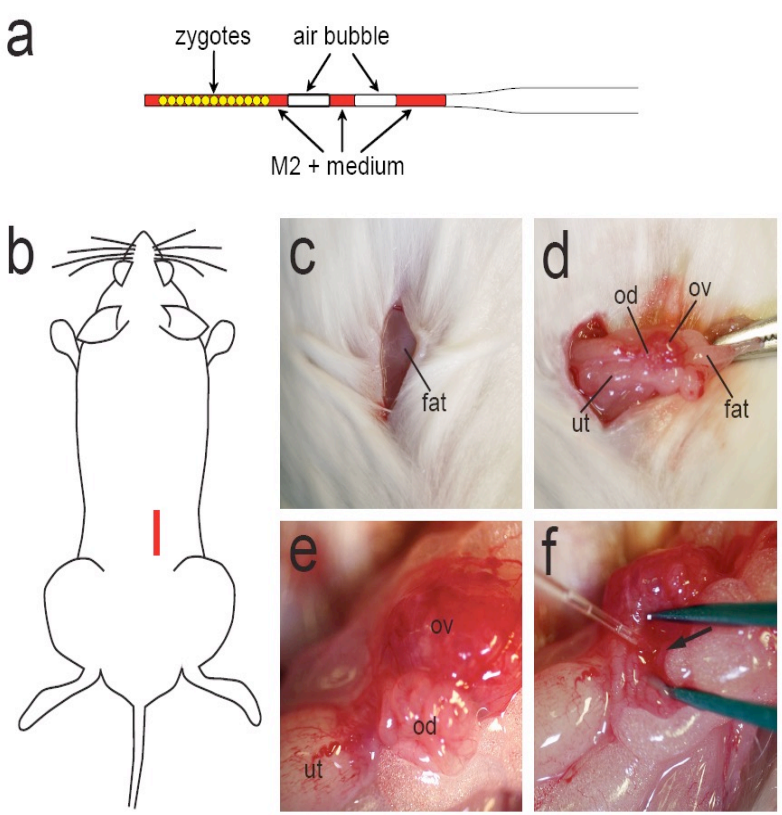


Setting up the injection chamber (inverted microscope)

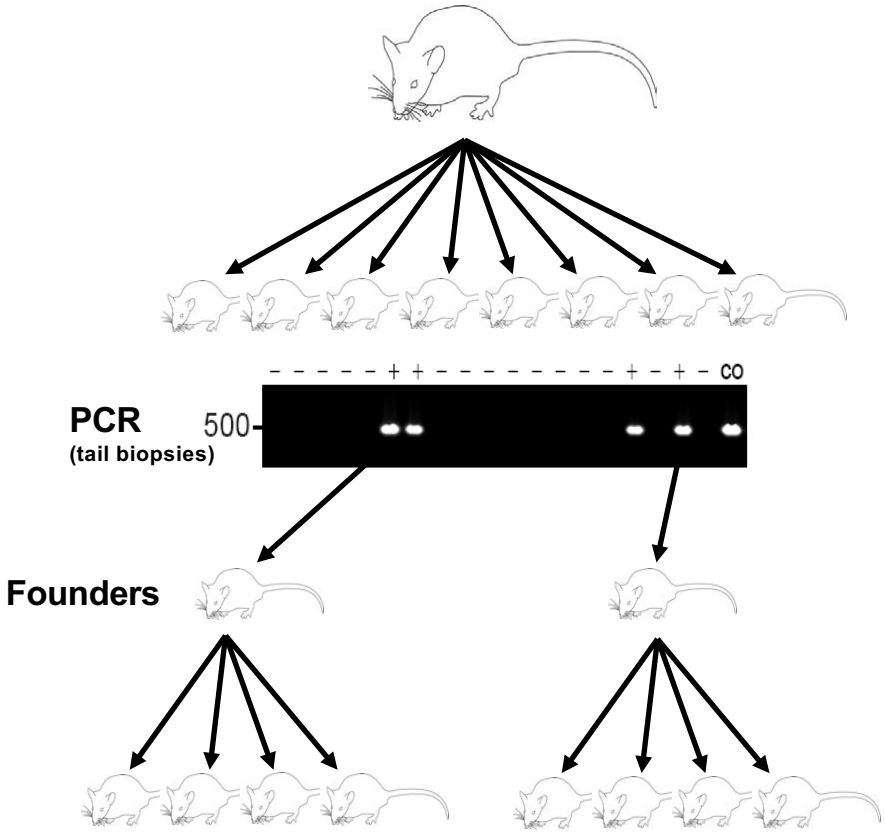
DNA (or RNA, or protein) is injected into one pronucleus

Microinjection and its applications

Reimplantation (plugged fosters)



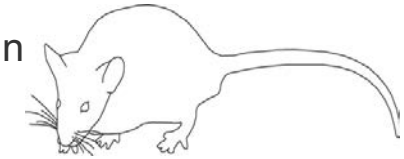
Establishing transgenic lines



Ittner & Goetz, *Nat. Prot. 2007*
Delerue & Ittner, *Jove 2017*

Microinjection and its applications

New transgenic strain



1.

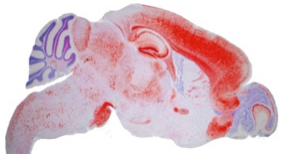
2.

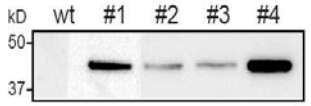
3.

4.

5.

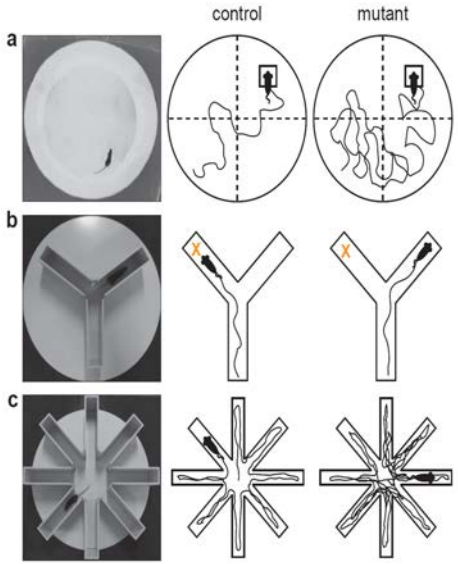
Expression analysis
Histology
Biochemistry



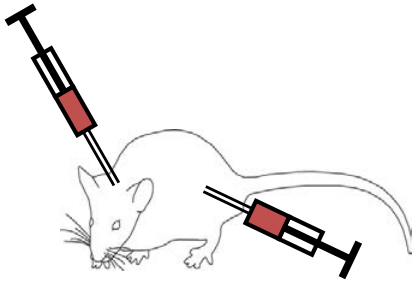


Cell culture

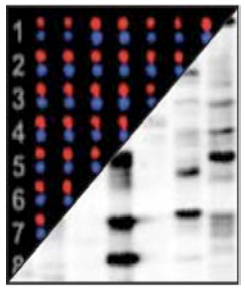
Behaviour testing



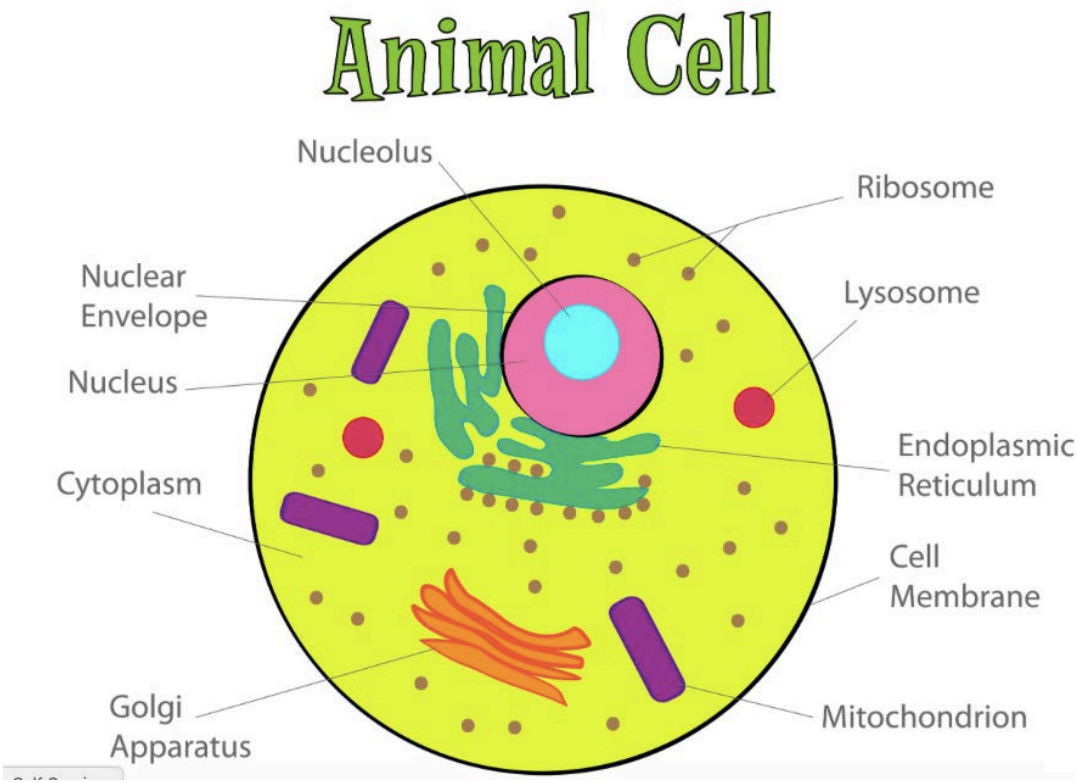
Treatment trials

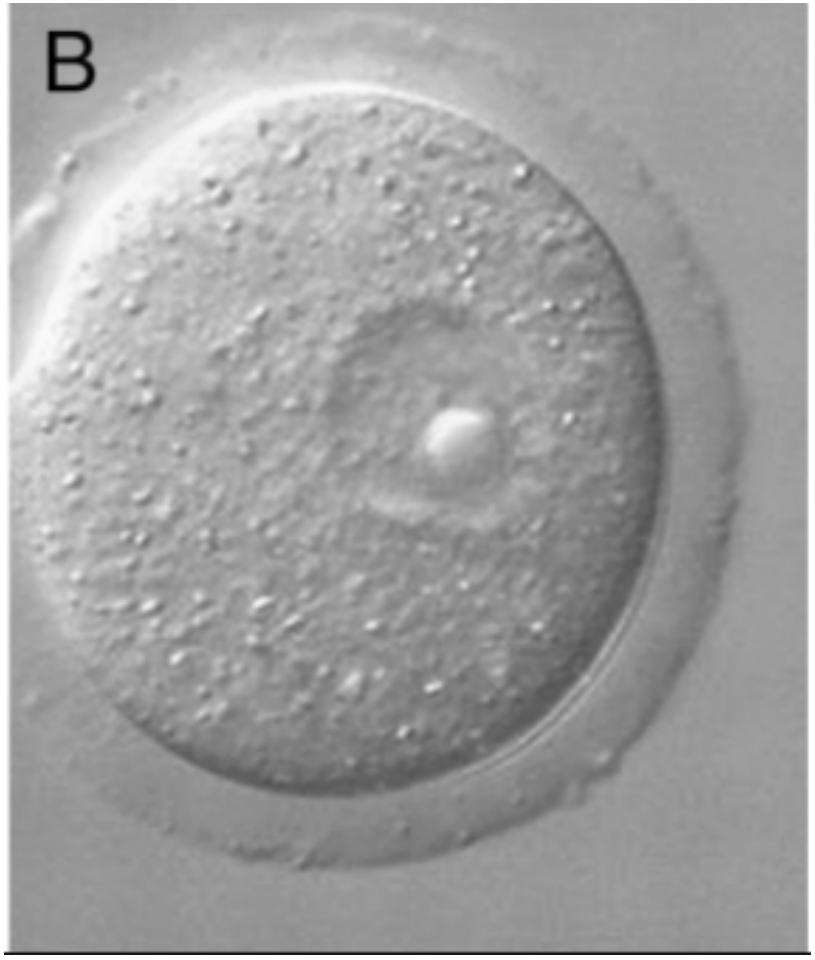


Candidate screening



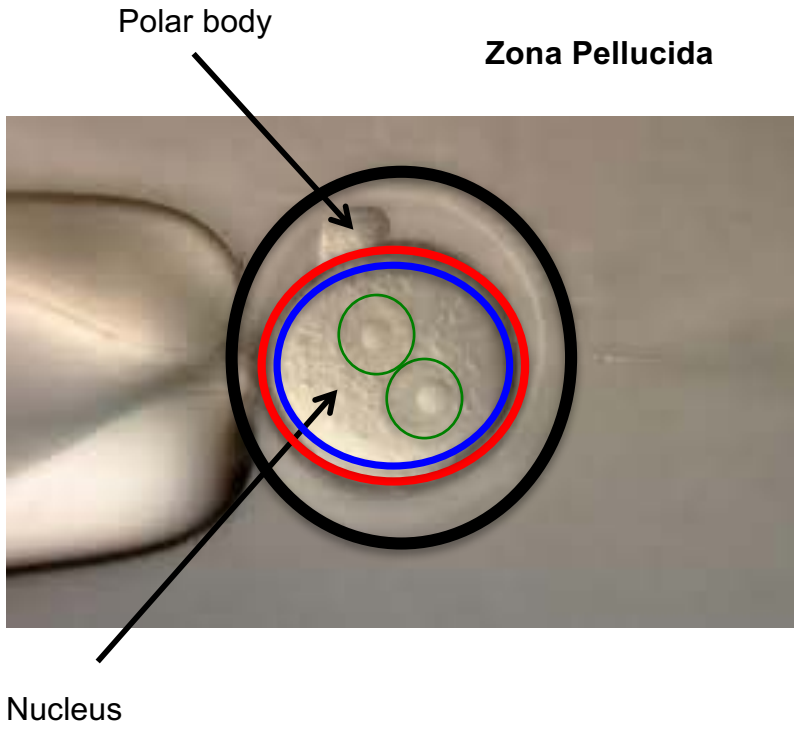
Microinjection and its applications





Oocyte organisation

Microinjection and its applications



Cell membrane (oolemma)

Nuclear envelope

Pronuclear membranes

Oocyte organisation

Microinjection and its applications

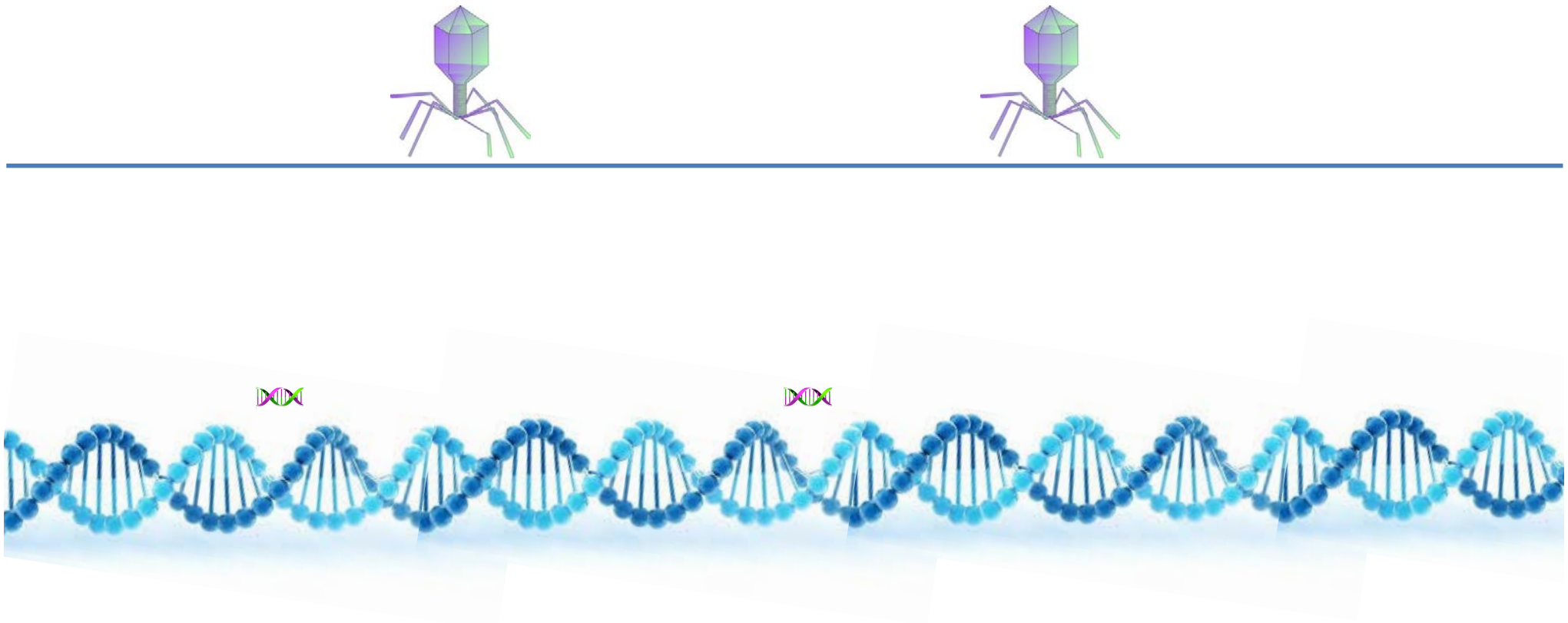
Perivitelline (subzonal) injection of
viral particles

Microinjection and its applications

Subzonal injection of viral particles

Multiple insertion sites – one copy per site

Microinjection and its applications



Subzonal injection of viral particles

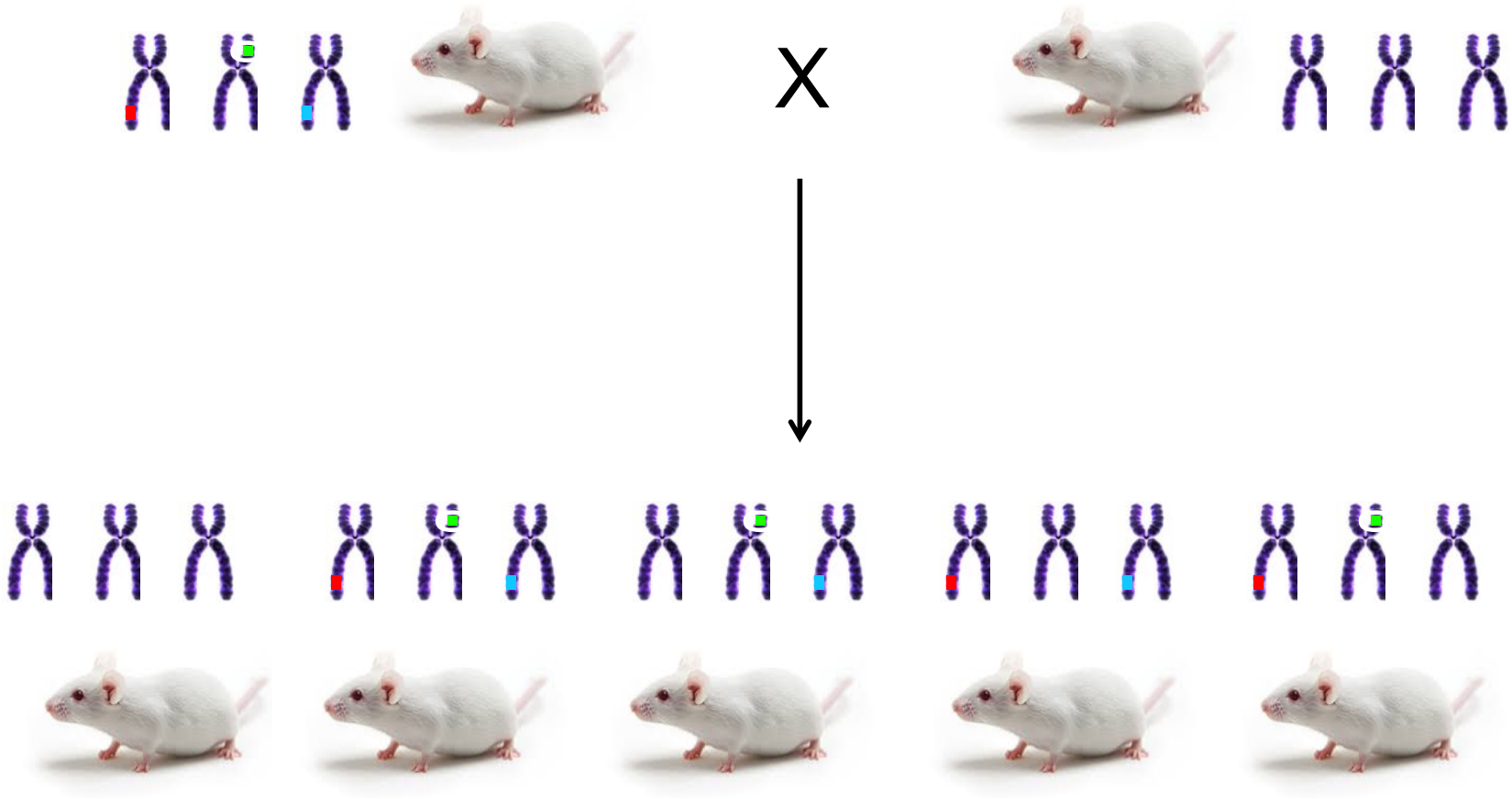
Multiple insertion sites – one copy per site

Microinjection and its applications

Subzonal injection of viral particles

Multiple insertion sites – one copy per site

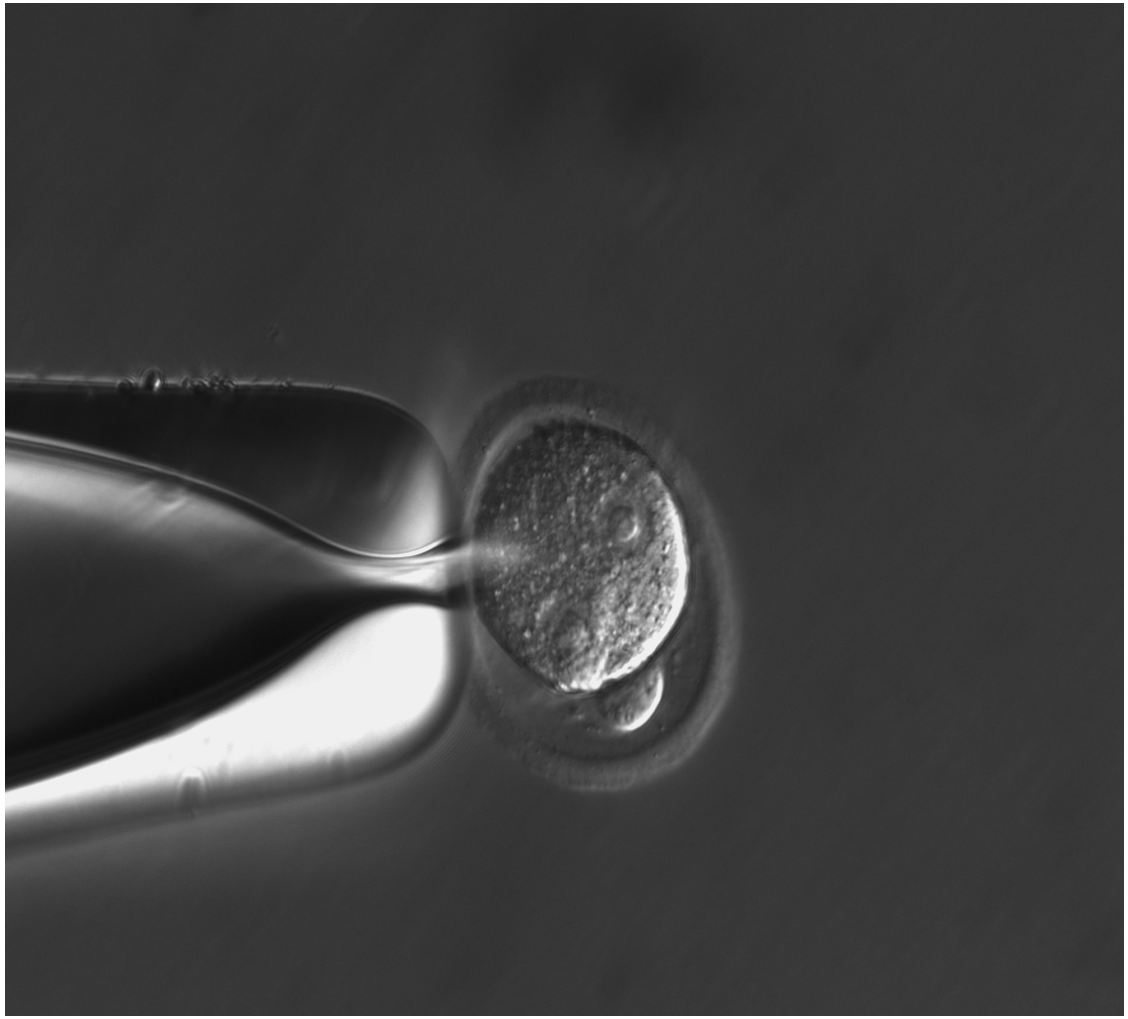
Microinjection and its applications



Subzonal injection of viral particles

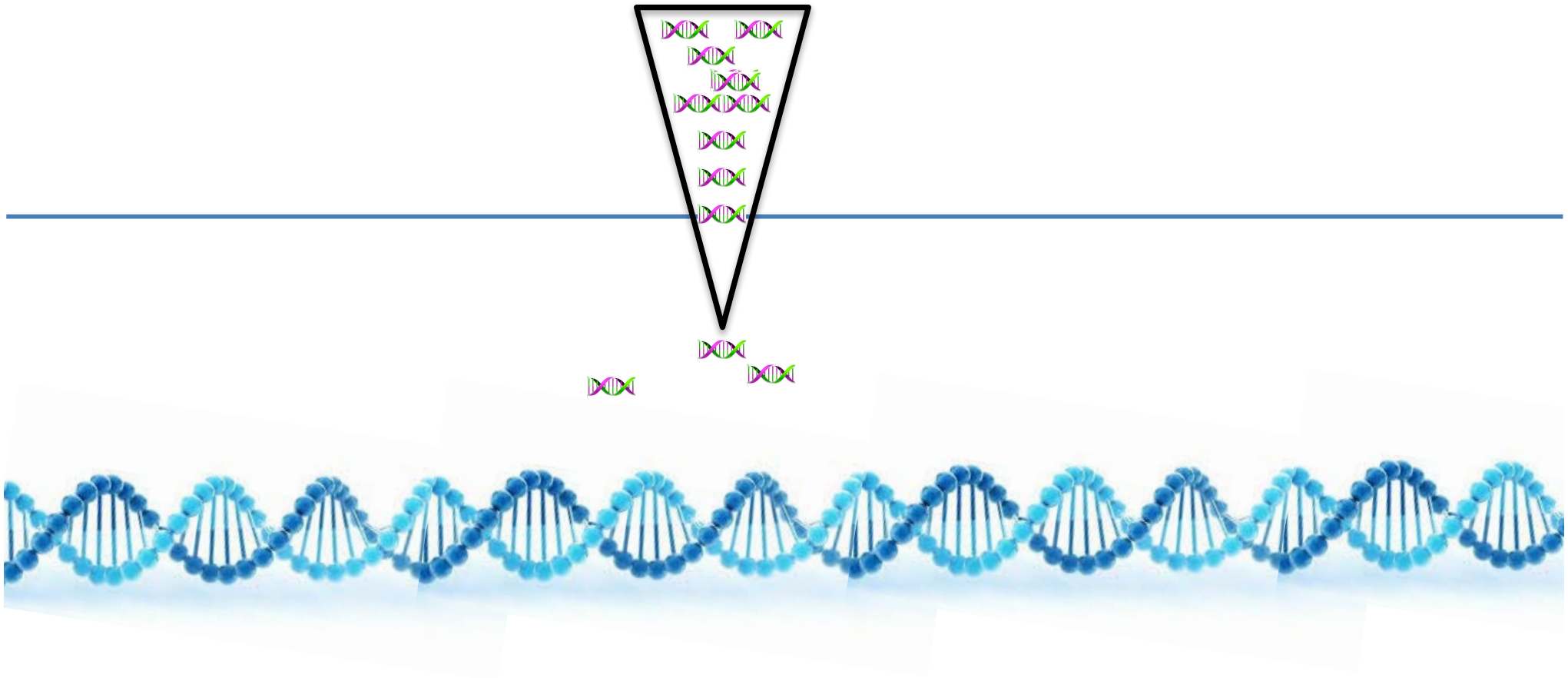
Multiple insertion sites – variable expression among individuals

Microinjection and its applications



Pronuclear injection of transgenes

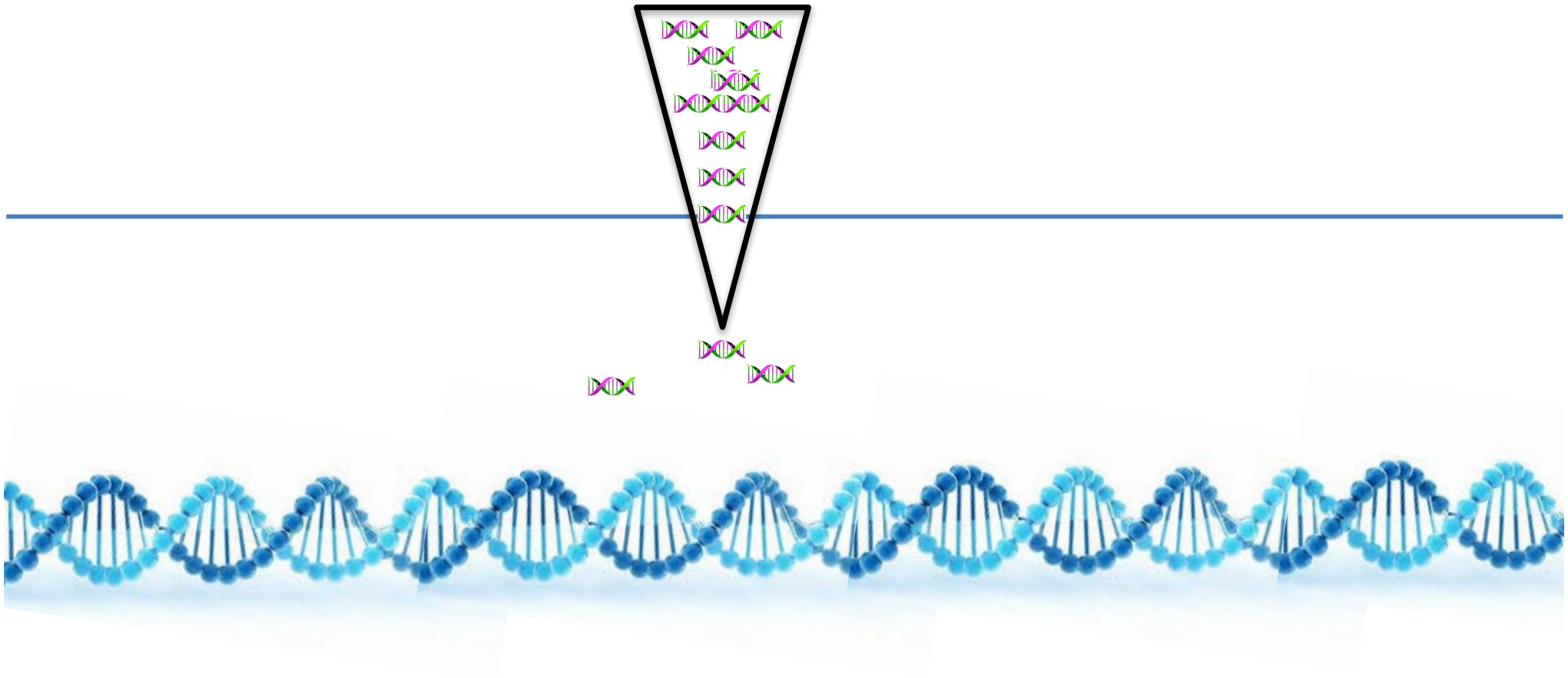
Microinjection and its applications



Pronuclear injection of transgenes

One insertion site – one or multiple copies (concatemere)

Microinjection and its applications



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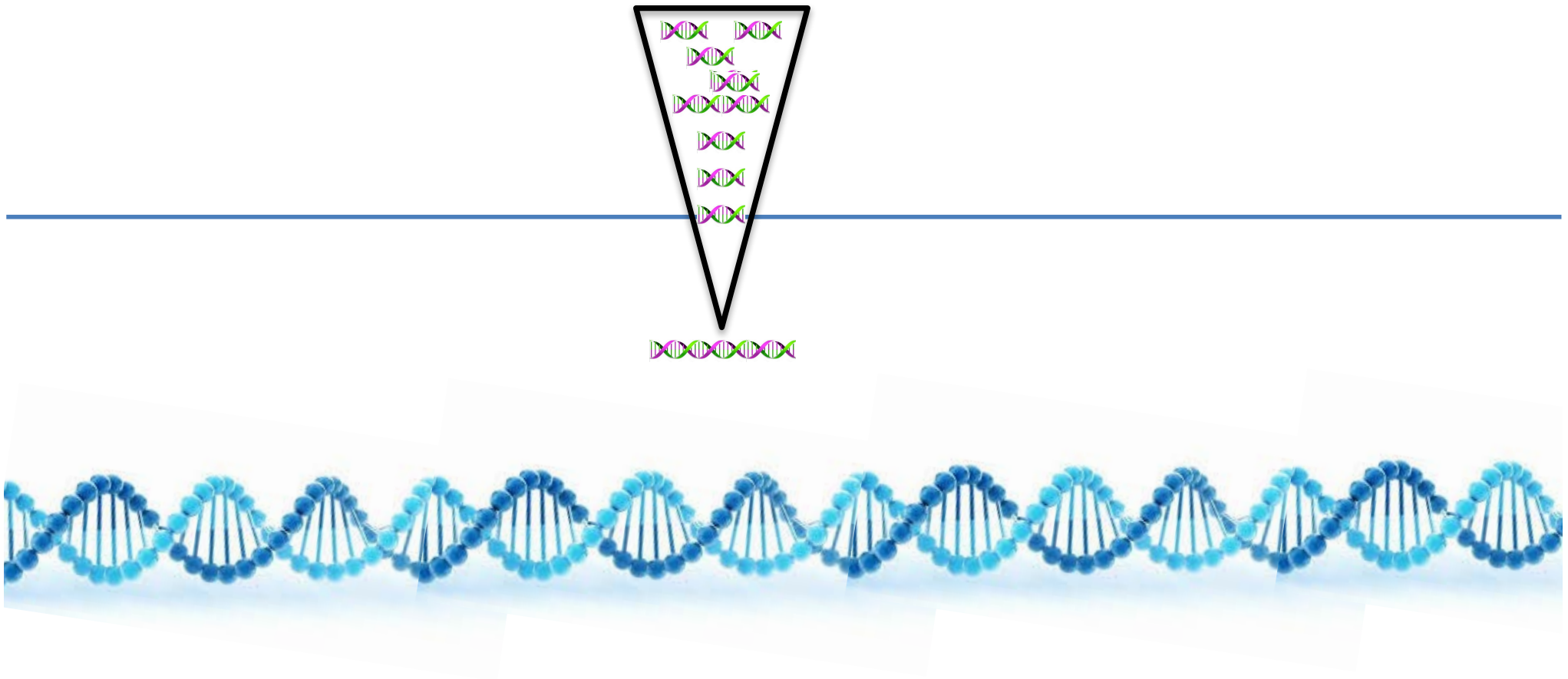

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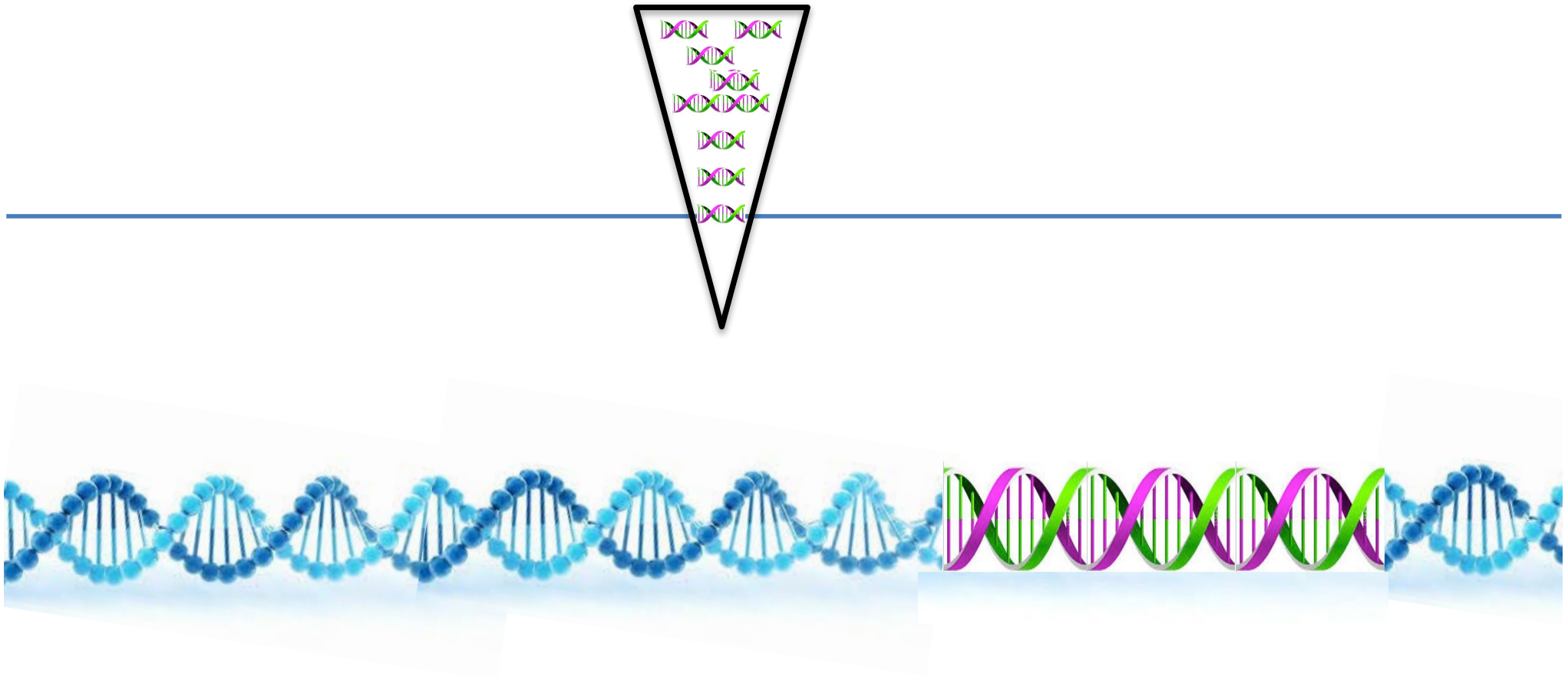

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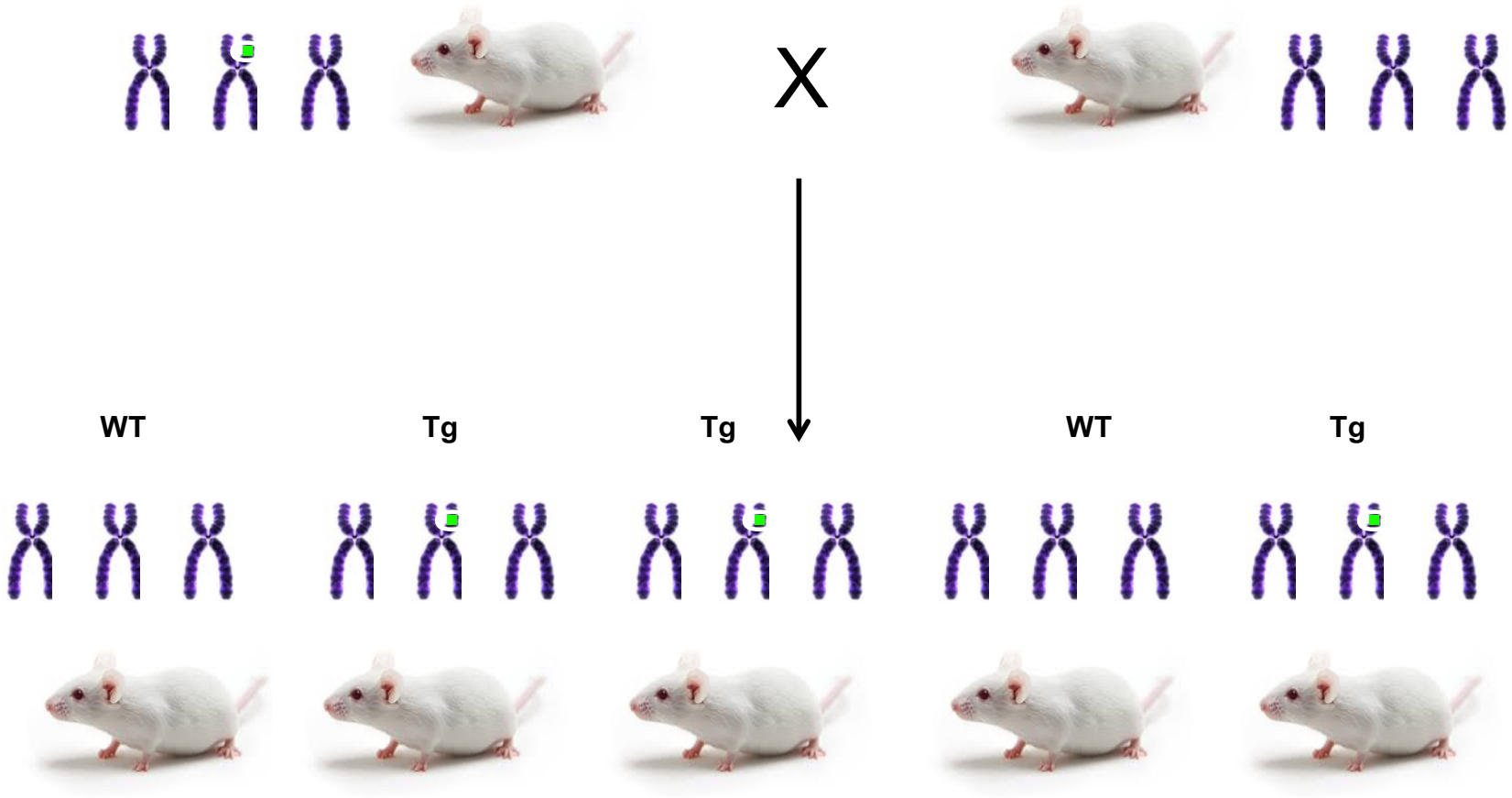
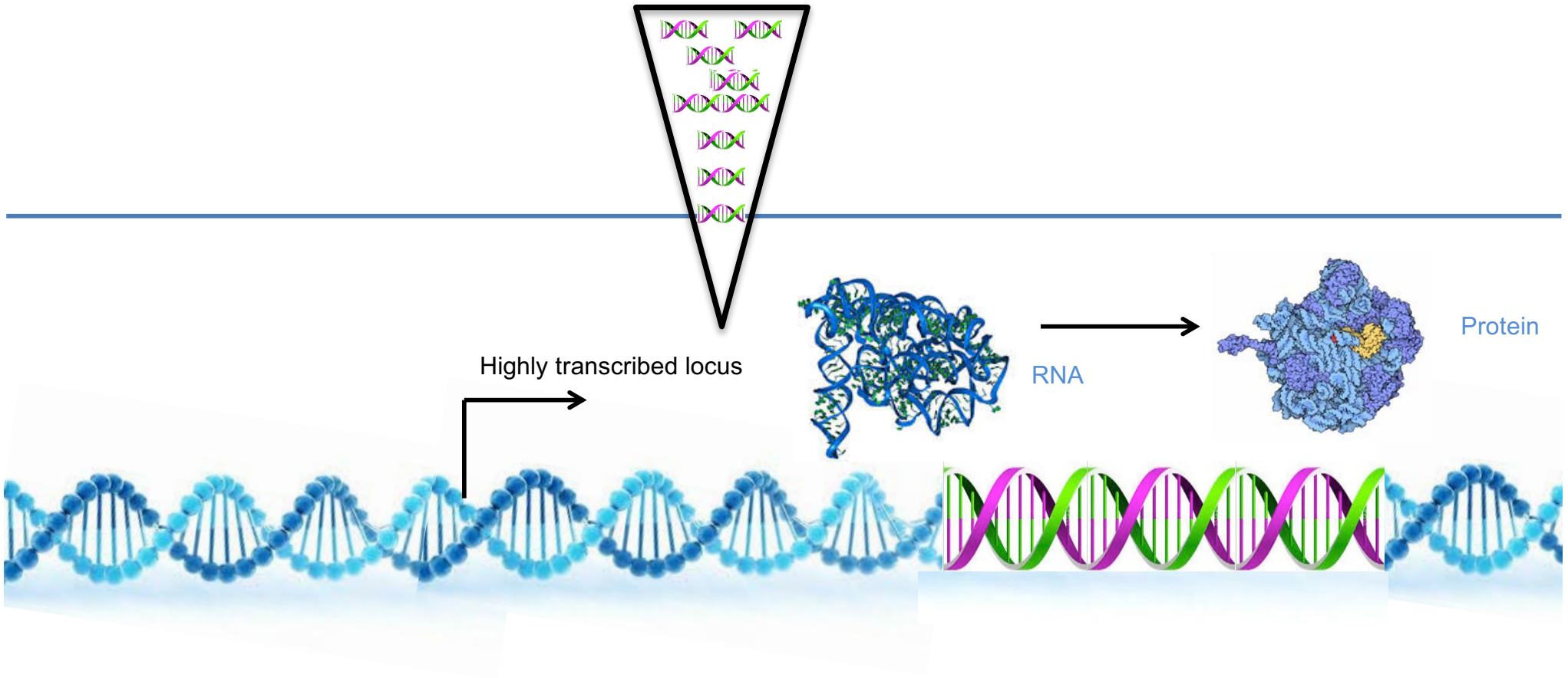
Microinjection and its applications



Pronuclear injection of transgenes

One insertion site – one or multiple copies

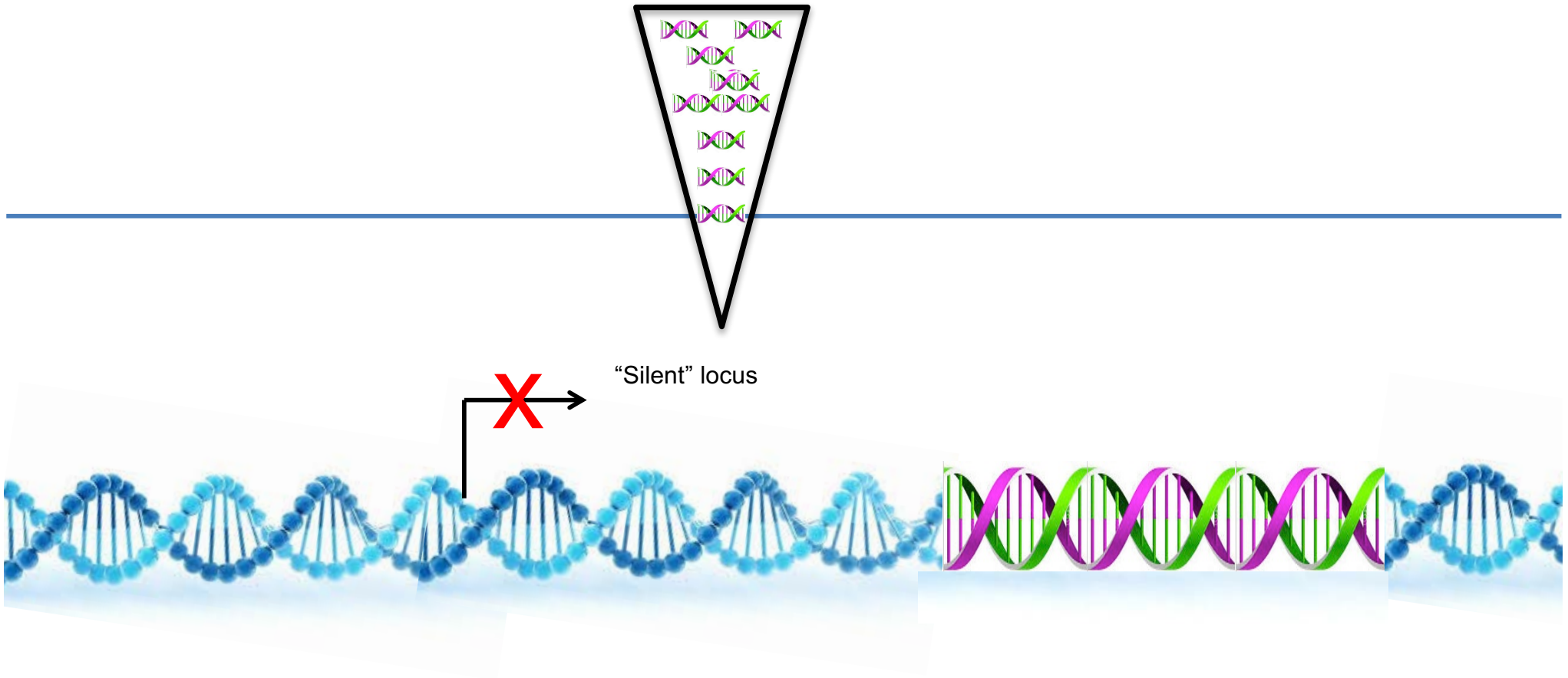
Microinjection and its applications



Pronuclear injection of transgenes

“Position” effect

Microinjection and its applications



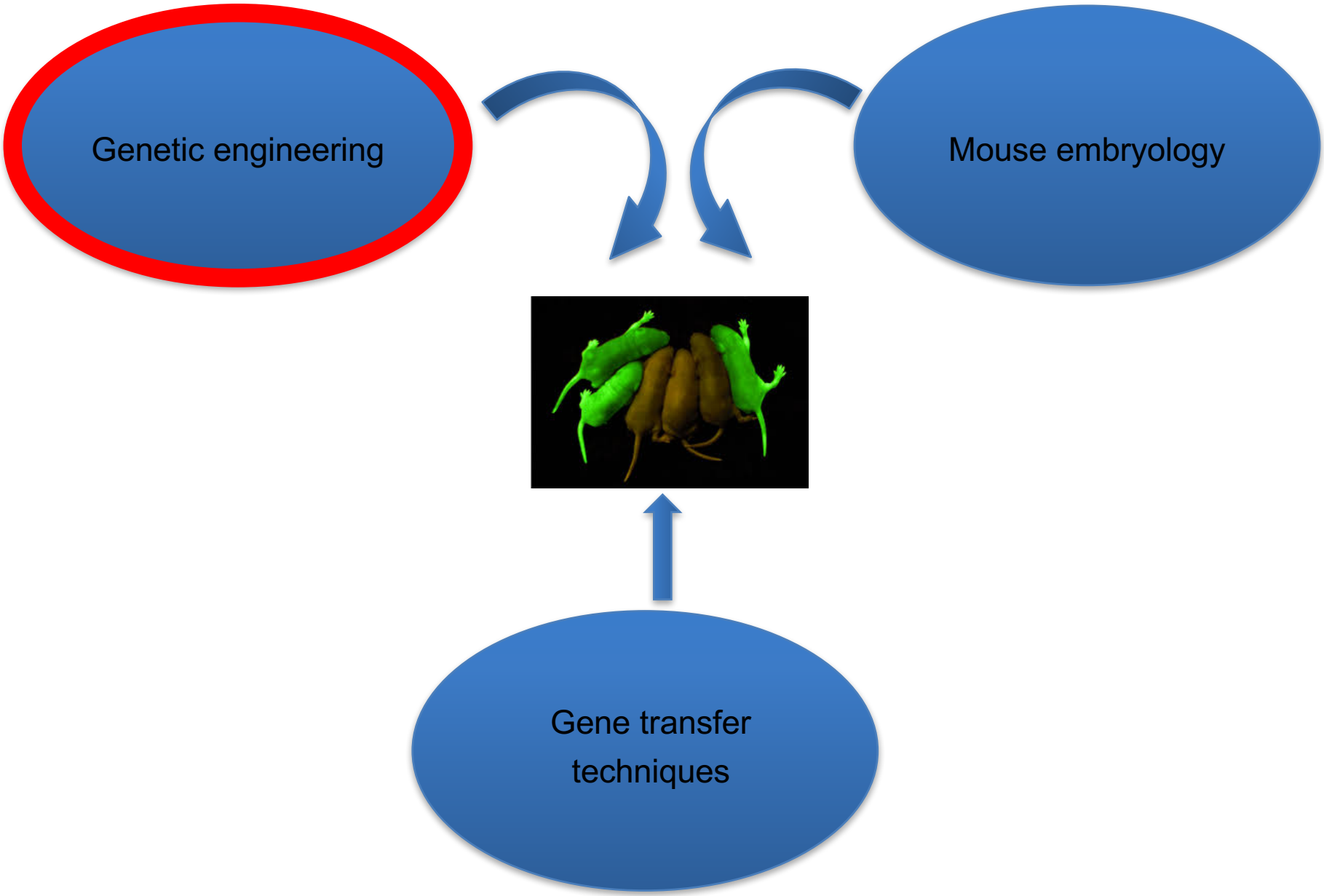
Pronuclear injection of transgenes

“Position” effect

Microinjection and its applications

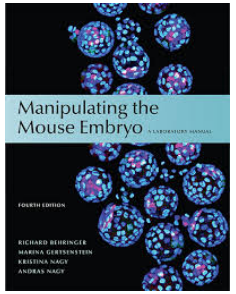
Cytoplasmic injection of RNA
(endonucleases)

Genome manipulation in mice



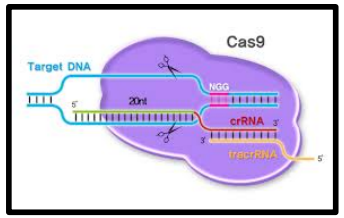
Overview

History of genome manipulation in mice



Microinjection and its applications

Genome editing



Genome editing

Gene targeting using ES cells

MARIO'S TRANSGENIC TECHNOLOGY "Knocks Out" Nobel Prize

Mario R. Capecchi, Ph.D., of the University of Utah, has won the 2007 Nobel Prize in Physiology or Medicine. Capecchi shares the prize with Oliver Smithies of University of North Carolina, Chapel Hill and Sir Martin Evans of Cardiff University in the UK.

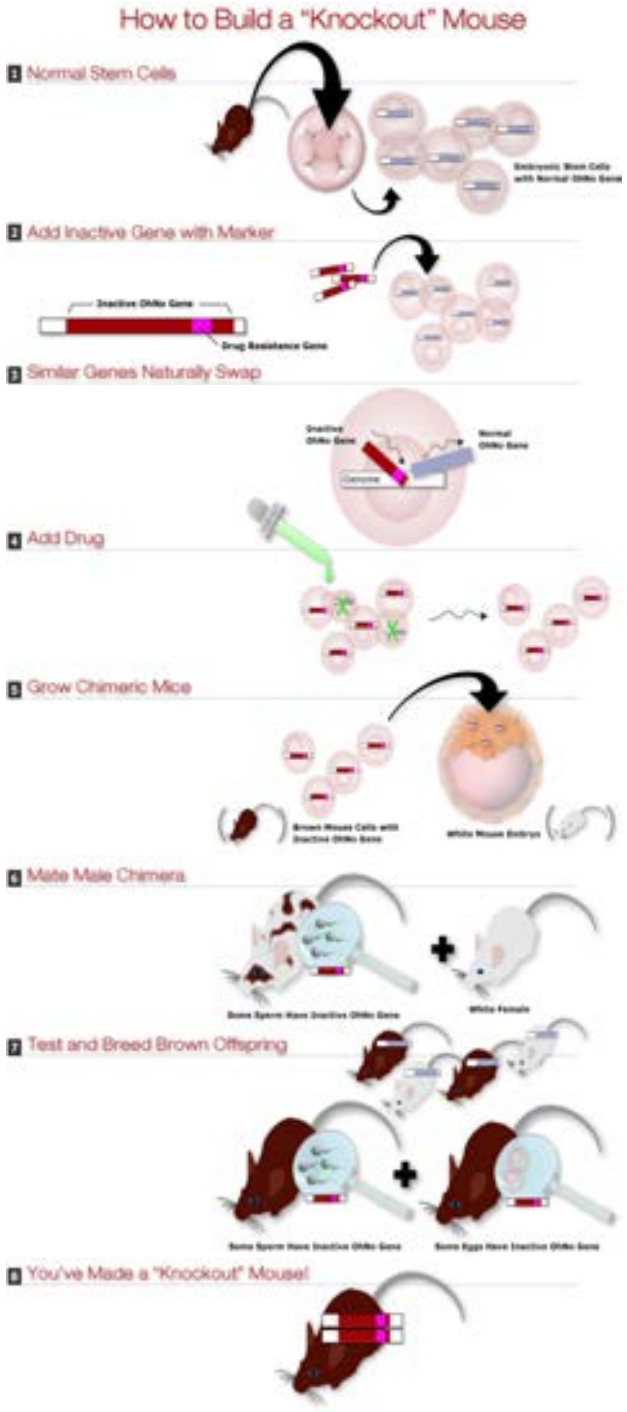
The prize recognizes Capecchi's pioneering work on "knockout mouse" technology, a gene-targeting technique that has revolutionized genetic and biomedical research, allowing the creation of animal models for hundreds of human diseases.

Watch a video of The University of Utah Nobel Press Conference

Genome editing

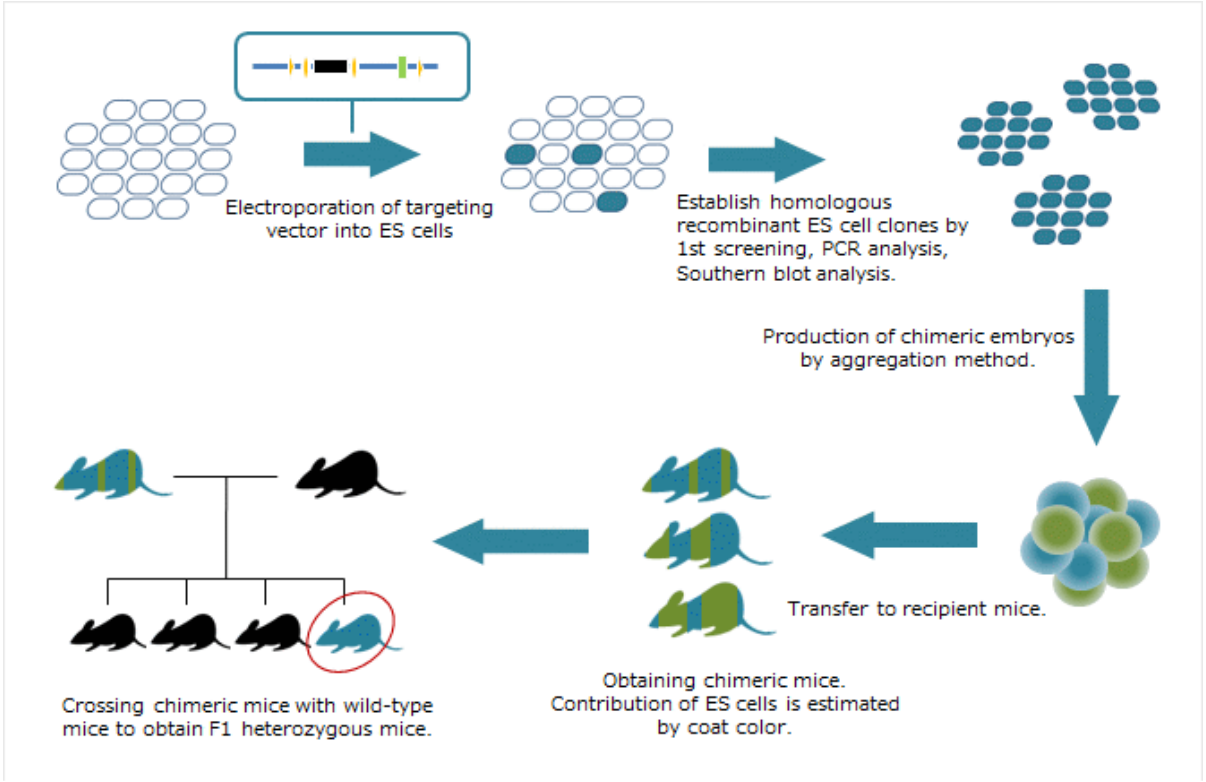
- Targeted integration
(Knock-In) e.g Rosa26 safe Harbor

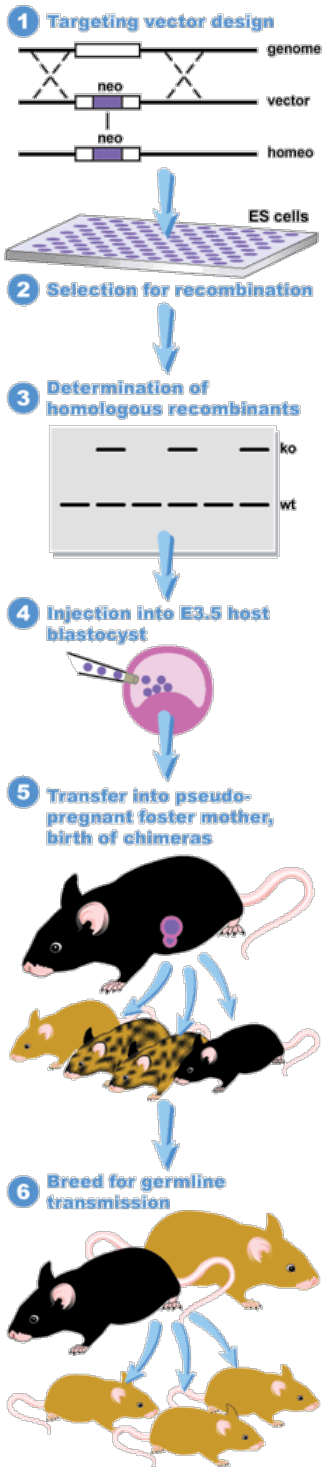
- Targeted deletion (Knock-Out)



Genome editing

Targeted transgenesis using ES Cells





Major drawbacks:

Natural Homologous Recombination (=HDR) is a VERY rare event (1/1.000.000th)

Germline transmission is not guaranteed

This is an ADDITIVE technology

Genome editing

Gene targeting using ES cells

Drawbacks:

ES cell lines not available for all species (rats)

ES cells do not always propagate to the germline

ES cell culture is technically challenging

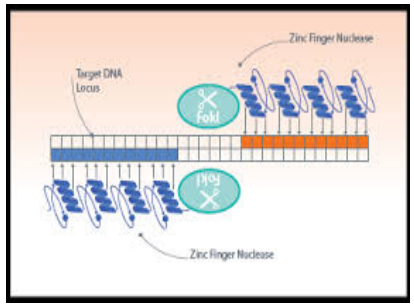
Time Line = 12 – 18 months

Price (>$50,000)

Genome editing

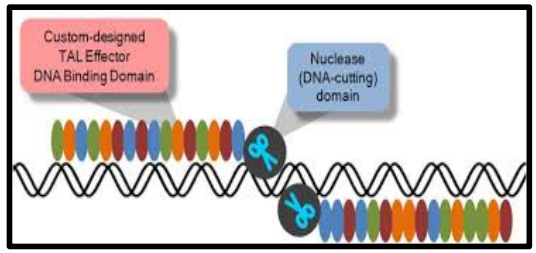
Gene targeting using nucleases

Engineered nucleases =
Molecular scissors able to
precisely target and cut
a defined genomic sequence



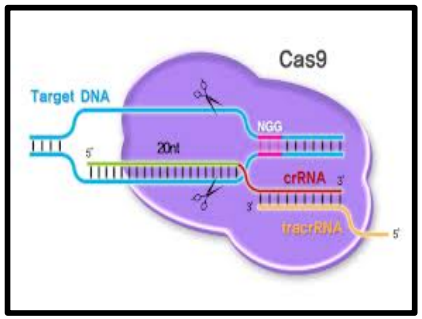
1 Zinc Finger Nuclease
(ZFN)

Accuracy in pinpointing the individual
letters of 3 billion “base pairs” =
correcting a single misspelled word in a
23-volume encyclopedia



2 Transcription
activator-like
effector nuclease
(TALEN)

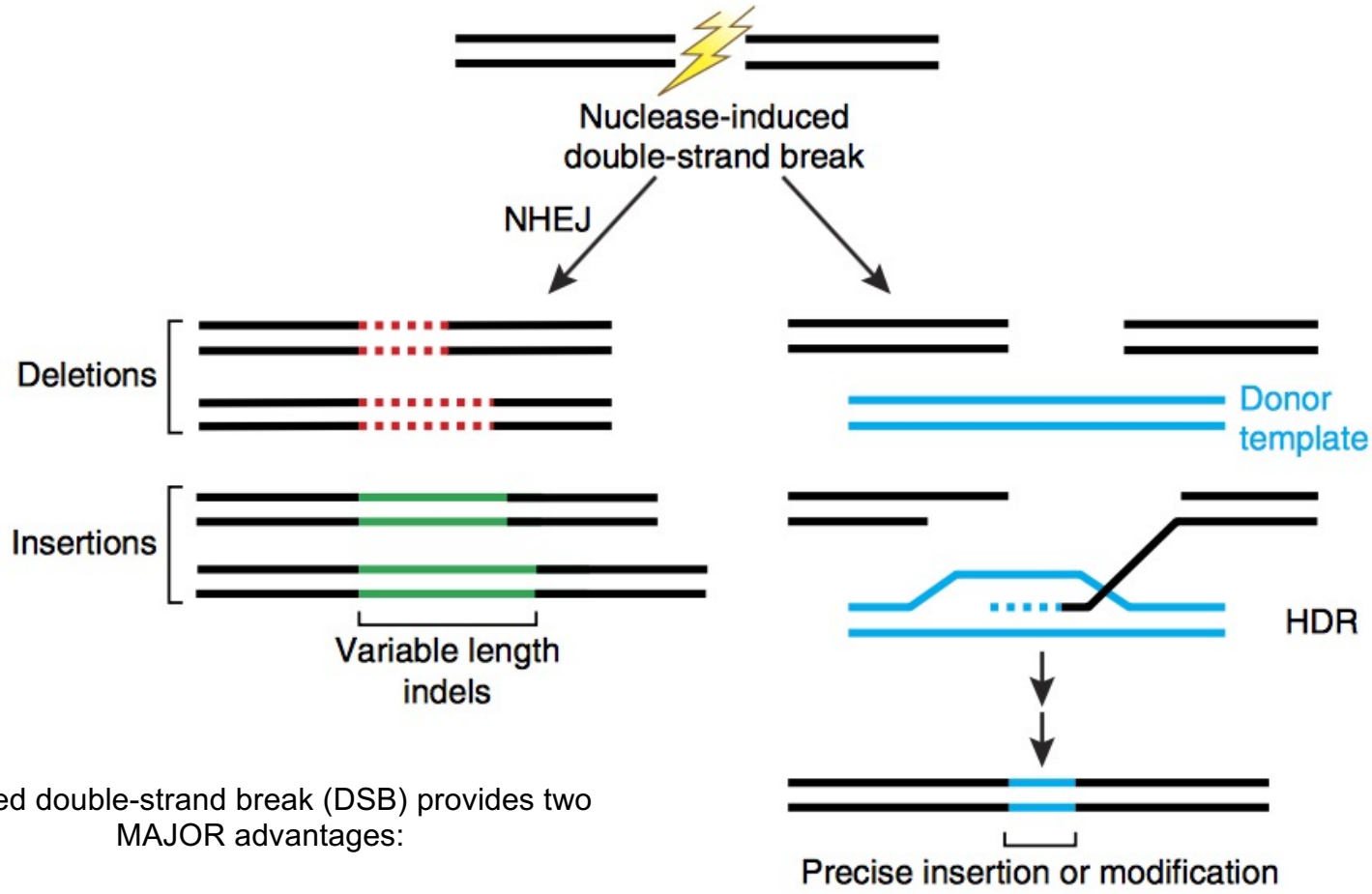
(*Nature* Method of the year 2011,
Science Breakthrough of the year 2015)



3 CRISPR/Cas9
(Clustered Regularly
Interspaced Short
Palindromic Repeats)

Genome editing

Targeted transgenesis using engineered endonucleases



The induced double-strand break (DSB) provides two MAJOR advantages:

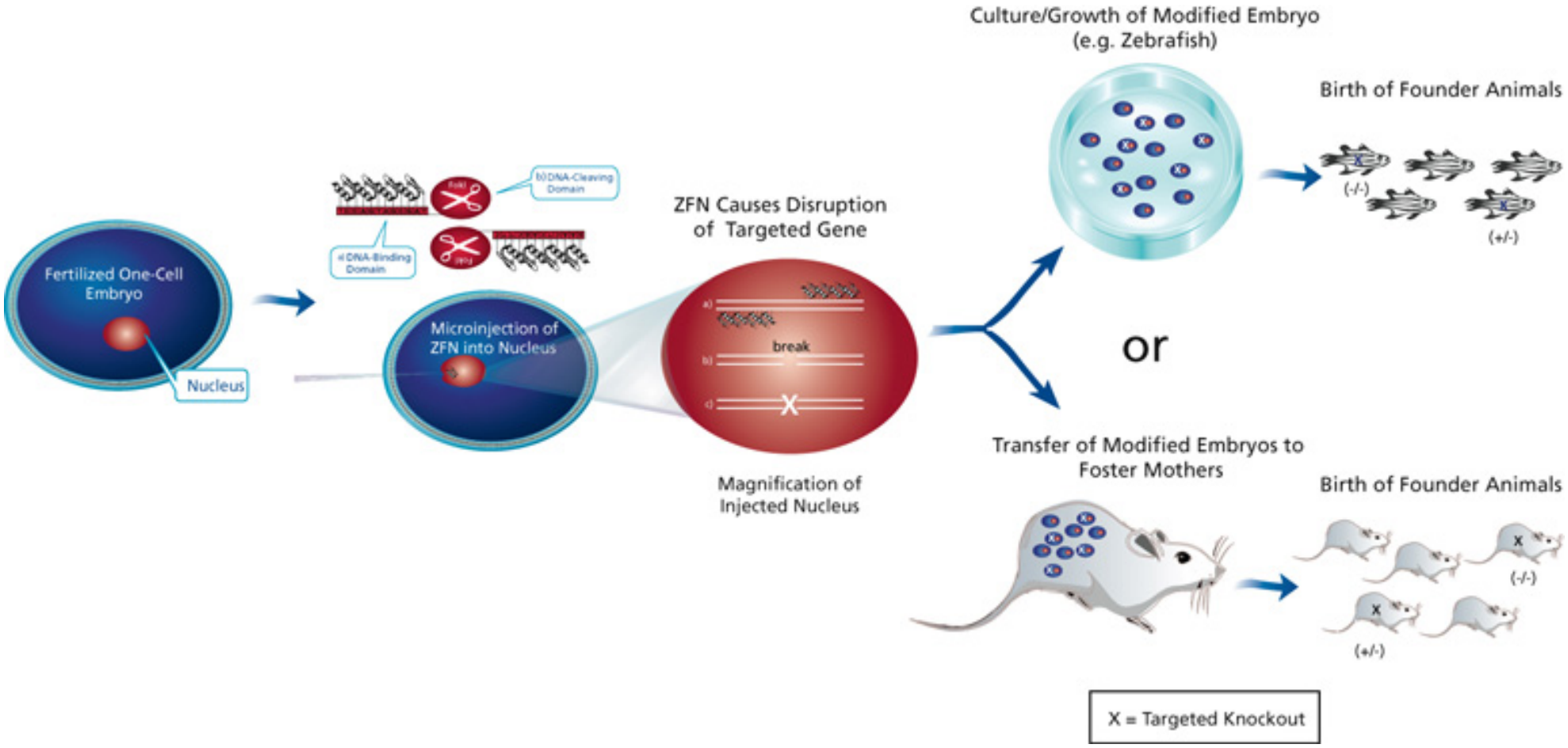
- Can be non-additive (KO)
- Stimulates cell-repair mechanisms: drastic increase in HDR events (1/100th). HDR frequency decreases as size of insert increases.

Sander & Joung Nat. Biotech. 2014

Allows for ES cells free gene targeting

Genome editing

Gene targeting using nucleases

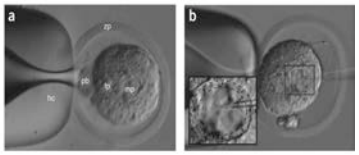


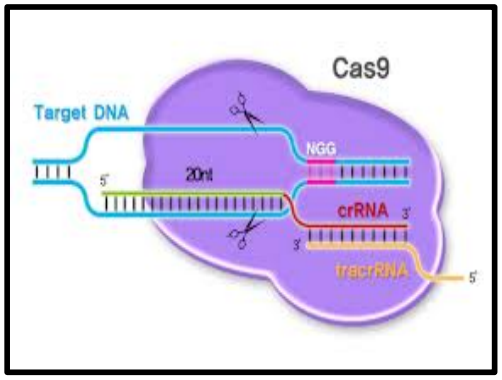
To date, NO species has been reported to be resistant to CRISPR genome editing

Genome editing

Gene targeting using nucleases

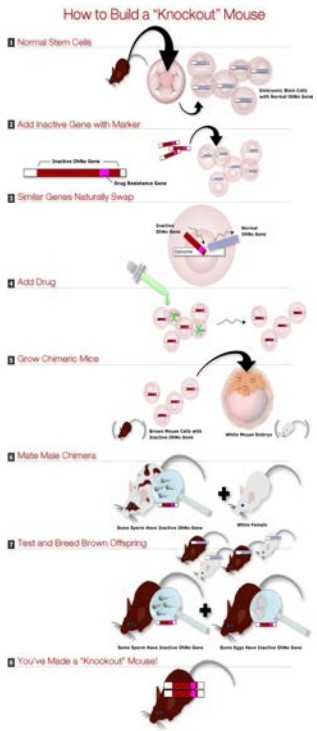
6 weeks



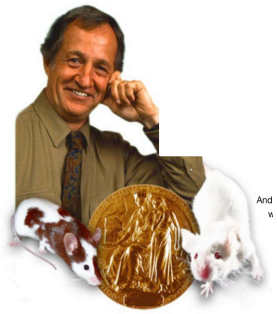


CRISPR

Disruptive Technology!

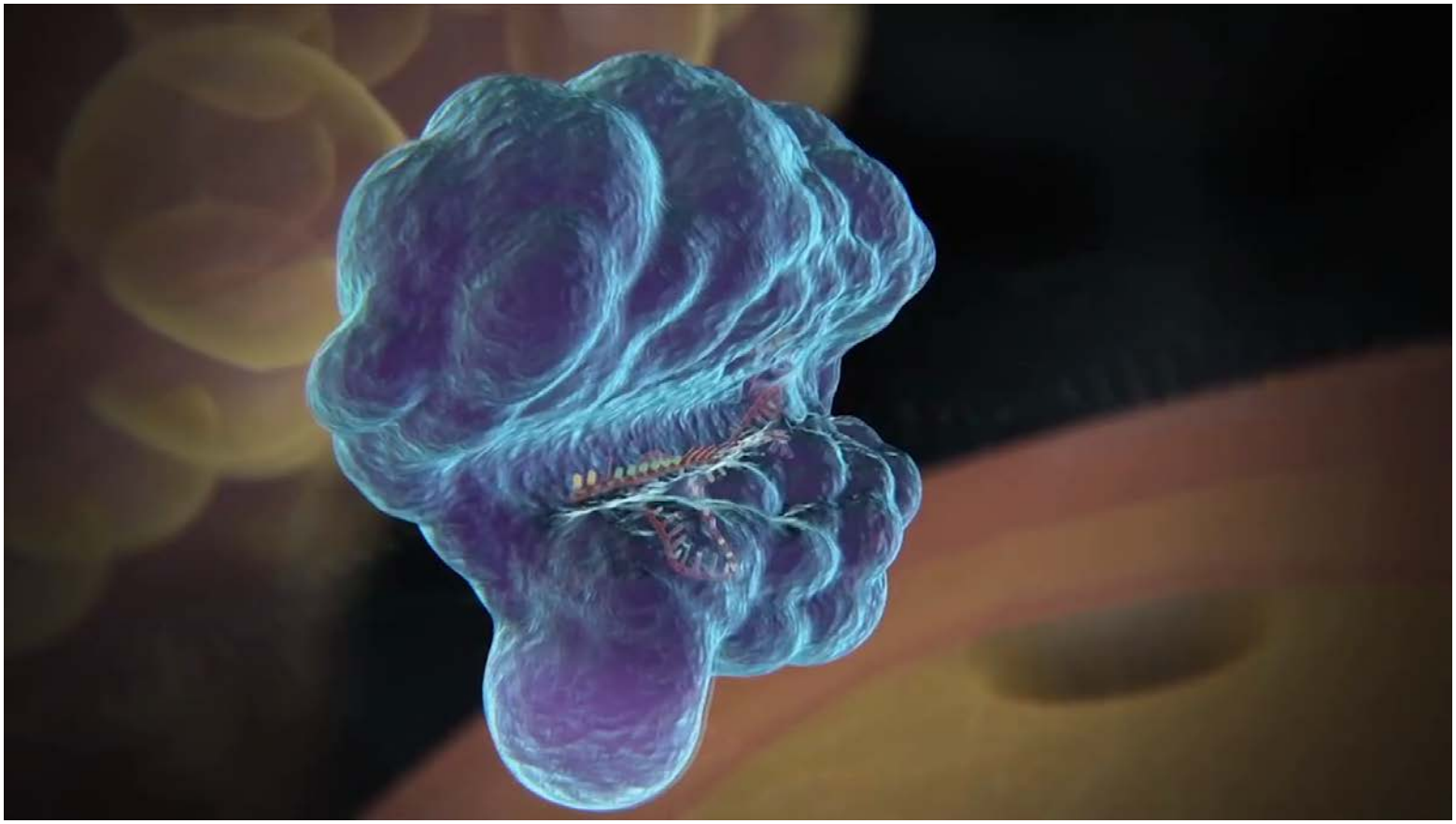


1-2 years



Genome editing

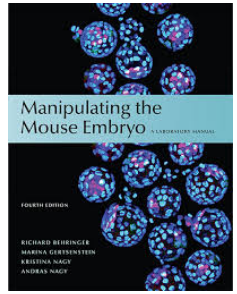
Gene targeting using nucleases



CRISPR/Cas9 in action

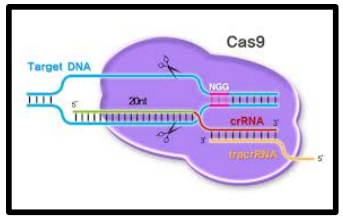
Overview

History of genome manipulation in mice

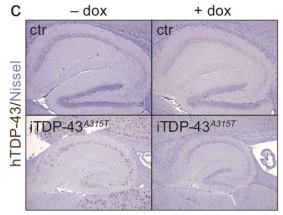


Microinjection and its applications

Genome editing



Mouse models generated

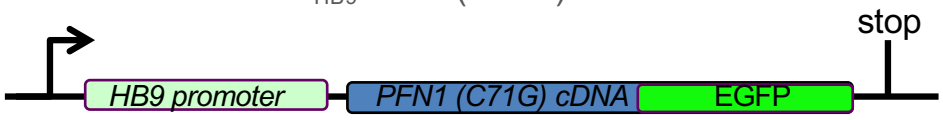


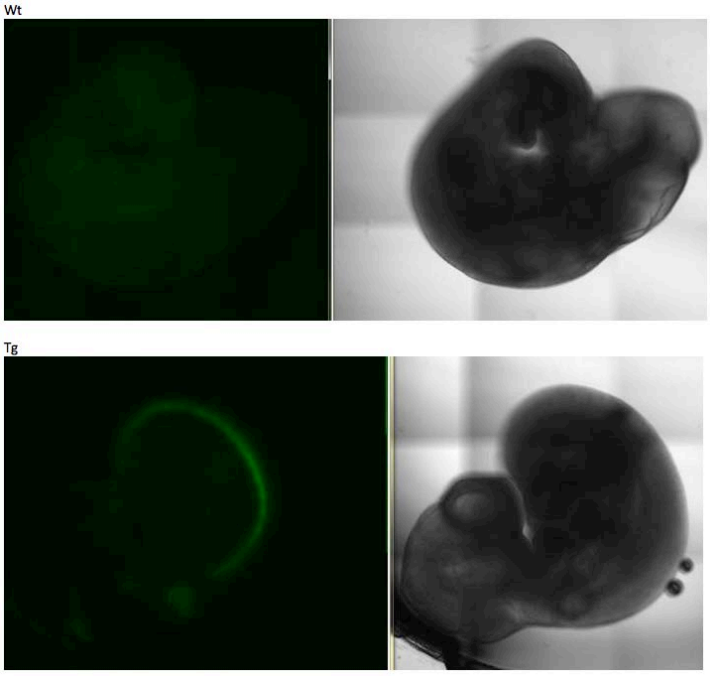
Mouse models generated

Tissue specific (motorneurons) of PFN1 (ALS)

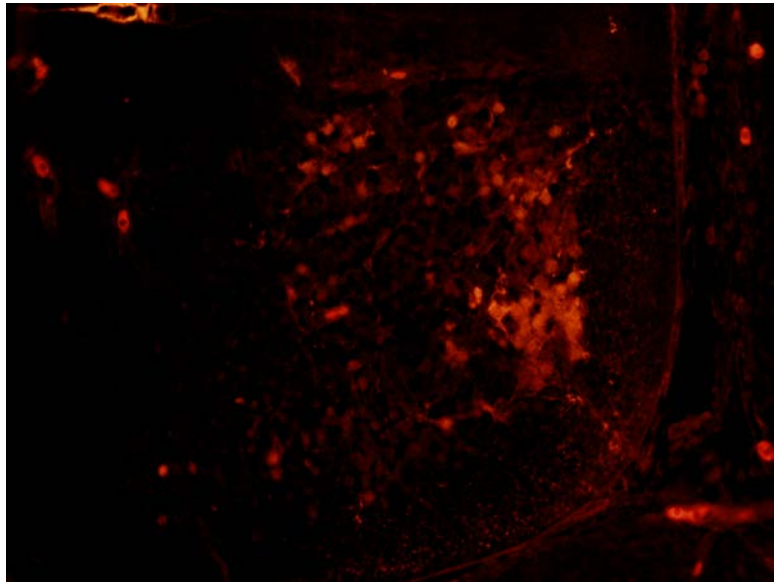
M. Brettle - unpublished

P_{HB9}-PFN1(C71G)V5-EGFP





EGFP



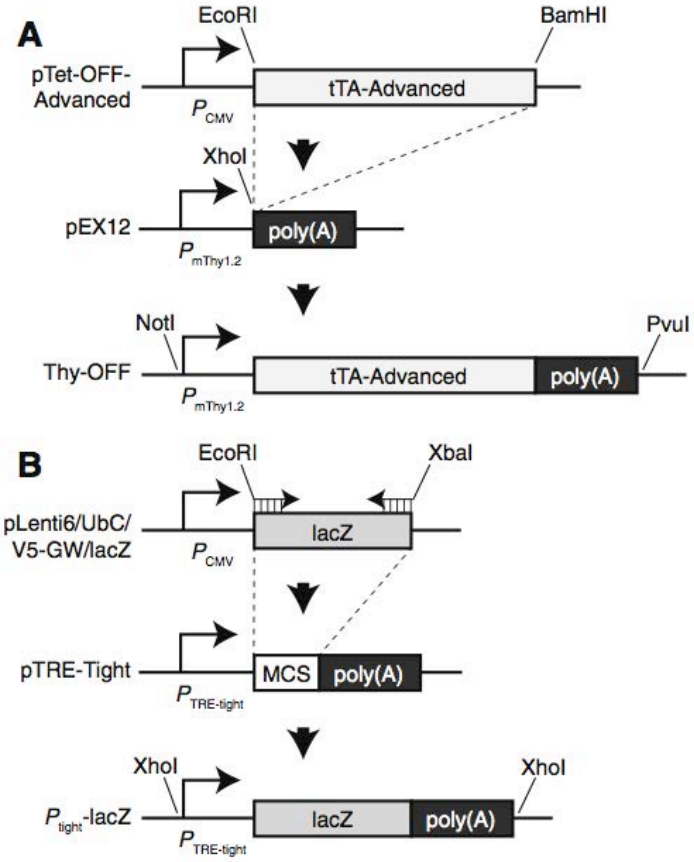
V5

WT Tg

Clasping
Body weight reduction
Tremor

Mouse models generated

Delerue F. *et al. Transg. Res. (2014) Apr;23(2):225-33*



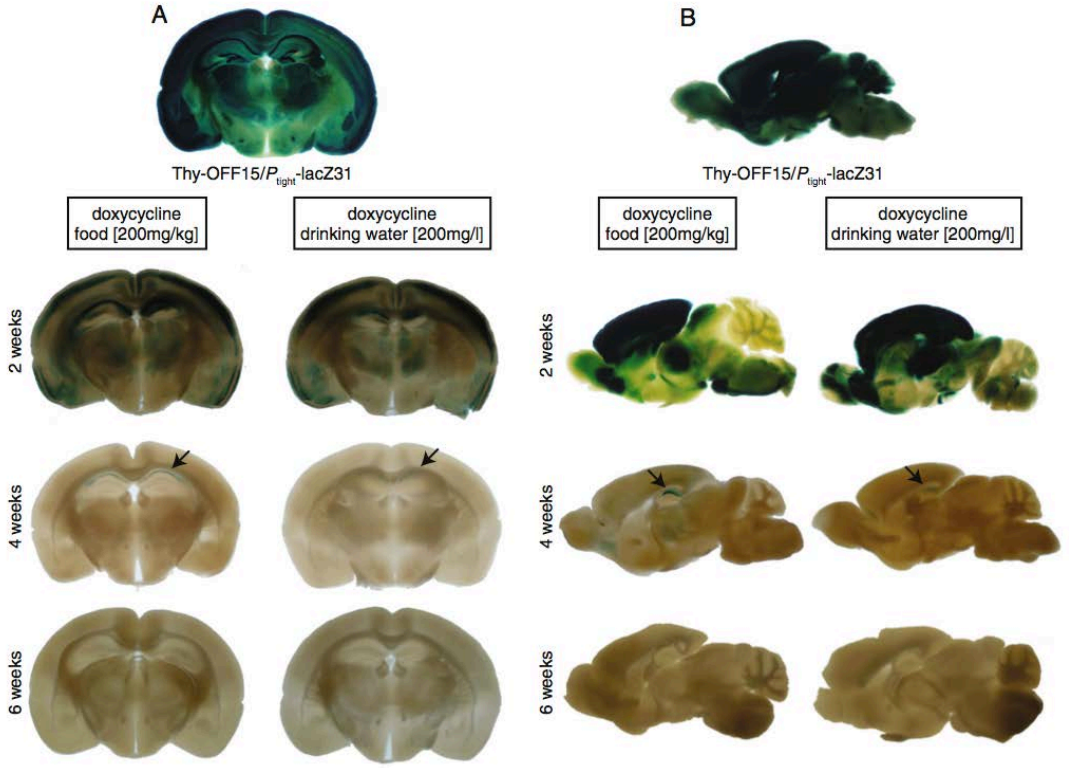
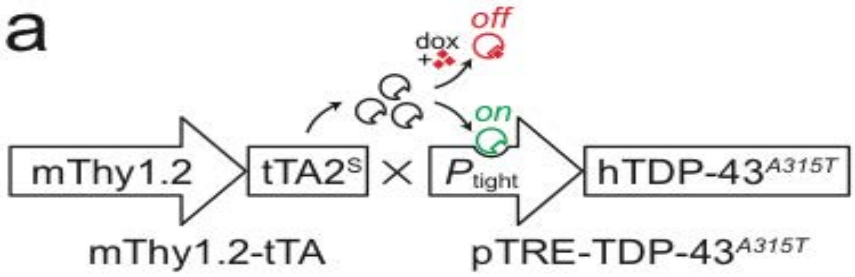
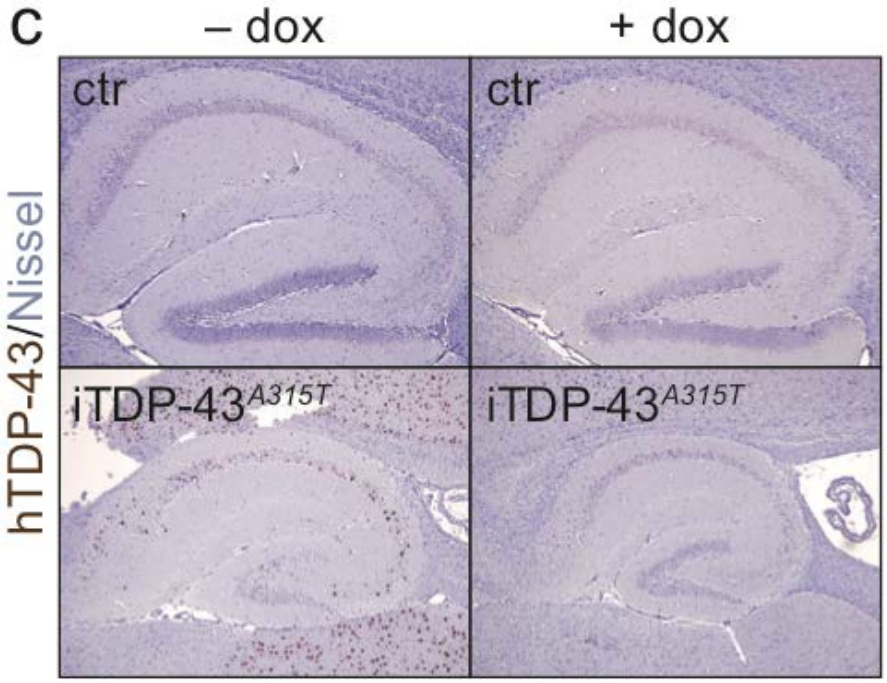


Fig. 5 Tight doxycycline-induced suppression of transgene expression in vivo. Thy-OFF15/P_{tight}–lacZ31 mice that present with strong neuronal transgene expression, as indicated by X-Gal staining of coronal (**a**) or sagittal (**b**) brain sections, were treated for up to 6 weeks with doxycycline ad libitum added to their regular diet (200 mg/kg—left panels), or to the drinking water (200 mg/l—right panels). Both delivery methods resulted in pronounced reduction of transgene expression within 2 weeks and only residual expression in hippocampal CA1 neurons (*arrows*) 4 weeks after treatment commenced. There was no detectable X-Gal staining after 6 weeks of doxycycline treatment, suggesting complete transgene suppression

Mouse models generated

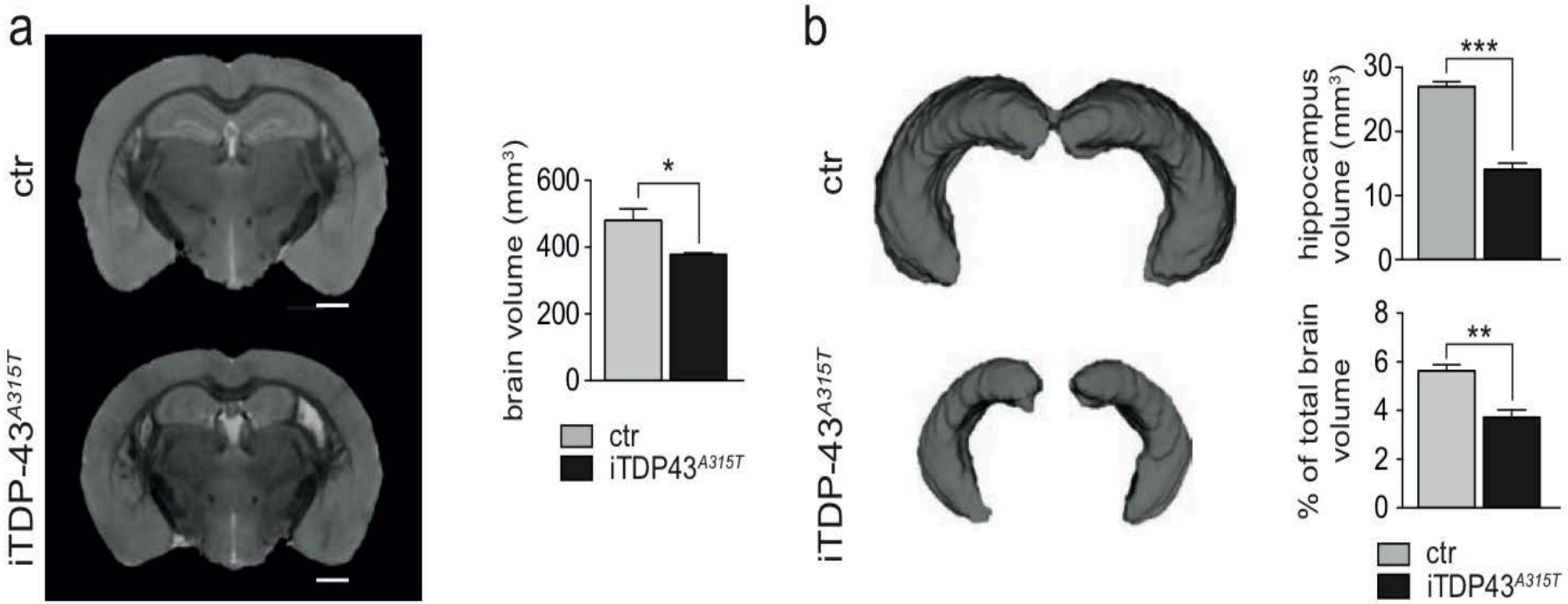
Ke Y. *et al. Acta Neuropathol. 2015 Nov;130(5):661-78*





Mouse models generated

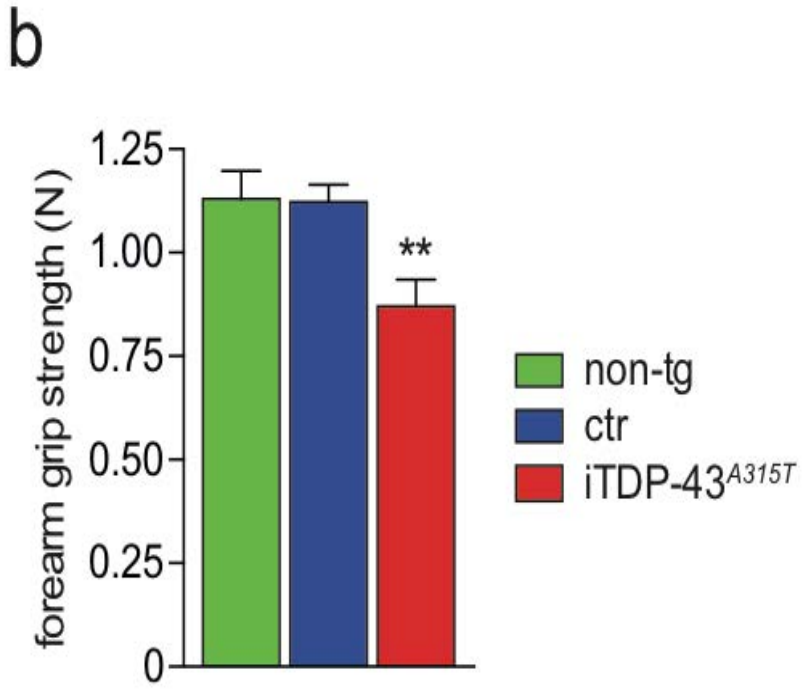
Ke Y. *et al. Acta Neuropathol. 2015 Nov;130(5):661-78*



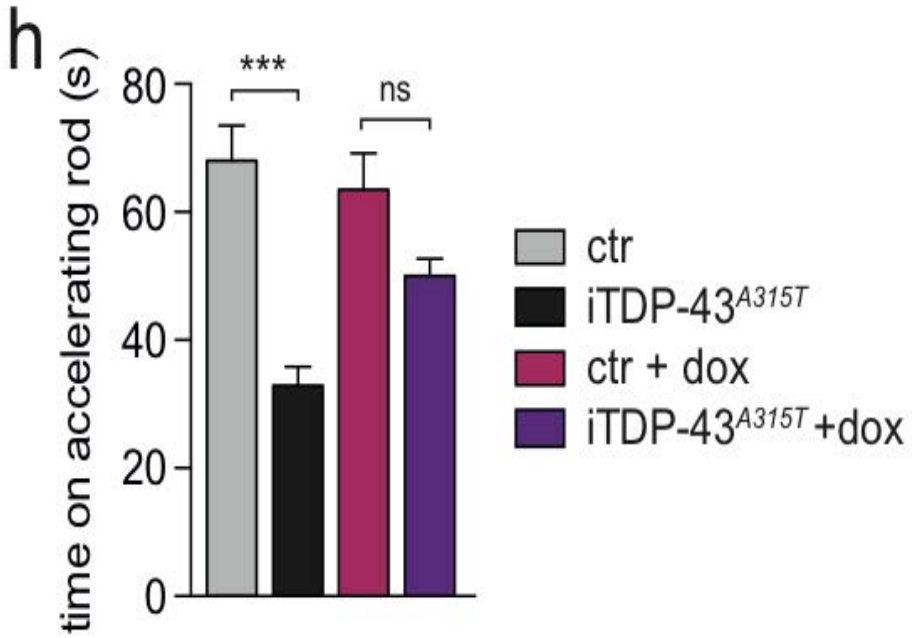
Widespread neurodegeneration

Mouse models generated

Ke Y. *et al. Acta Neuropathol. 2015 Nov;130(5):661-78*



Muscle weakness



Reversible phenotype

Mouse models generated

Multiplex CRISPR/Cas9 genome editing (using two guides)

F. Delerue - unpublished

Figure 1. Tyrosinase gene and sgRNA placement. A representation of the WT B6 *Tyr* (NM_011661.4) coding sequence with the five exons (numbered) and flanking untranslated regions in gray. The region of interest for genome editing is enlarged. The SNM resulting in an eye and coat color change is indicated (*) and corresponds to nt 230 with reference to the translation start site. The amino acids encoded are shown below the DNA sequence and the critical "DDRE" motif for *Tyr* function is boxed. Four sgRNAs were designed flanking or including this site with indicated orientations (guides A, B, C, and D). The sgRNA binding sites on the homologous DNA are indicated in red, with the PAM sites underlined.

Mouse models generated

Multiplex CRISPR/Cas9 genome editing (using two guides)

F. Delerue - unpublished

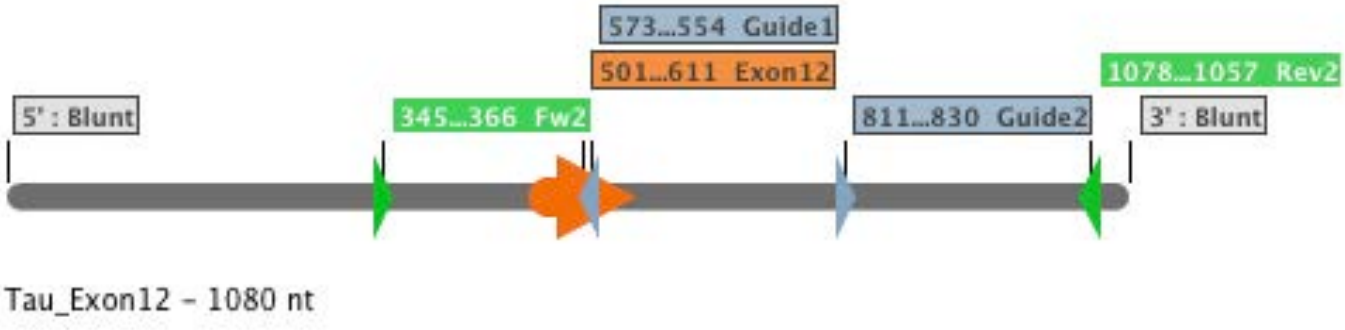
Injection	Concentration (Cas9-G1-G2)	embryos reimplanted	Live pups (dead)	Edited (homo)
Pronuclear	5 - 5 - 5 ng/ul	152	7 (5)	2 (2★)
Cytoplasmic	100 - 50 - 50 ng/ul	122	8 (0)	8 (8)

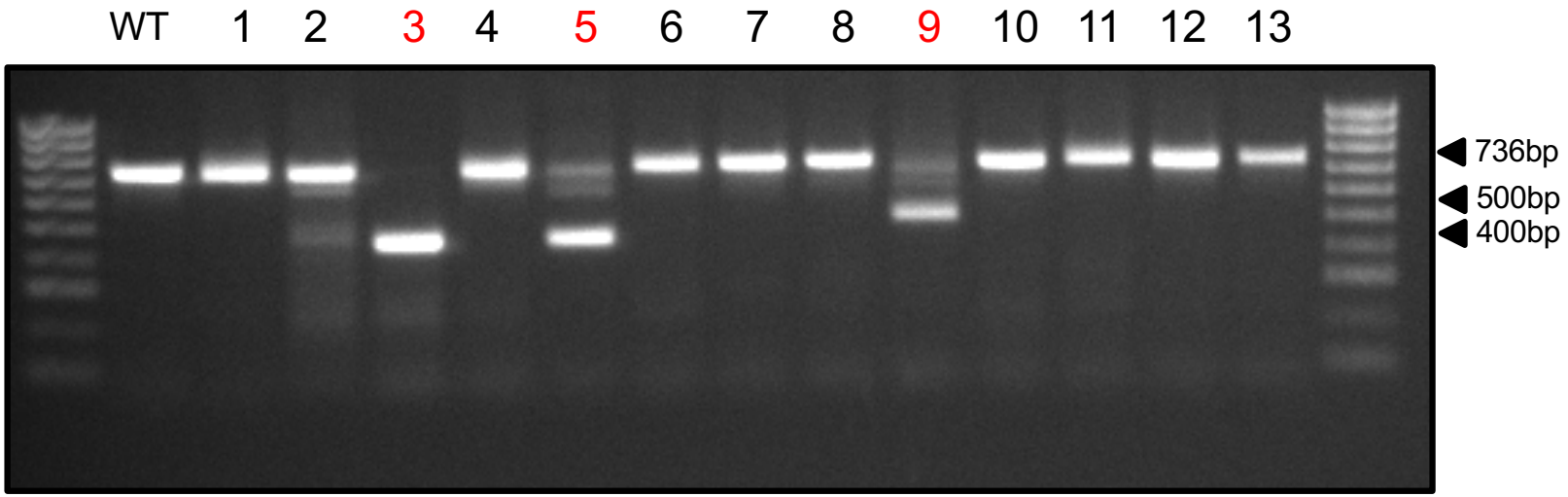
Mouse models generated

Multiplex CRISPR/Cas9 genome editing (using two guides)

D. Tan - unpublished

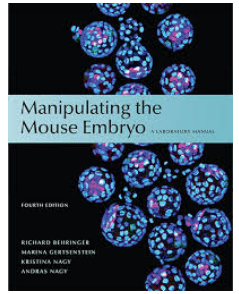
Investigating the C-terminal tail of tau





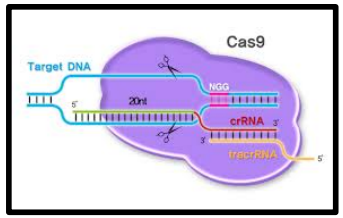
Overview

History of genome manipulation in mice

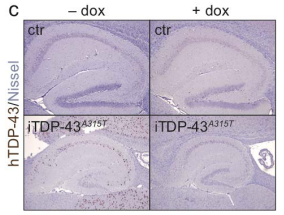


Microinjection and its applications

Genome editing



Mouse models generated



Beyond mouse models: gene therapy

Beyond mouse models

Technology

Chinese Scientists Cure Wheat Fungal Infection by Gene Editing

By *Jayalakshmi K*

July 22, 2014 08:19 BST

Powdery mildew is a fungal infection that affects the wheat crop. REUTERS

The gene-deletion trick is particularly tough in wheat because the plant has a hexaploid genome, that is, it has six copies of each of its seven chromosomes. Multiple genes must be disabled or the trait will not be changed.

Nat Biotechnol. 2014 Sep;32(9):947-51. doi: 10.1038/nbt.2969. Epub 2014 Jul 20.

Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew.

Wang Y[1], Cheng X[2], Shan Q[3], Zhang Y[3], Liu J[3], Gao C[3], Qiu JL[4].

Disruption of 6 alleles in one go

Beyond mouse models

NEWS GENETICS

Gene editing makes pigs safer for human transplants

CRISPR/Cas9 method disables viruses that make organs hazardous

BY TINA HESMAN SAEY 4:38PM, OCTOBER 12, 2015

LIFE SAVERS? New gene-editing methods may make pig organs safe for human transplant.

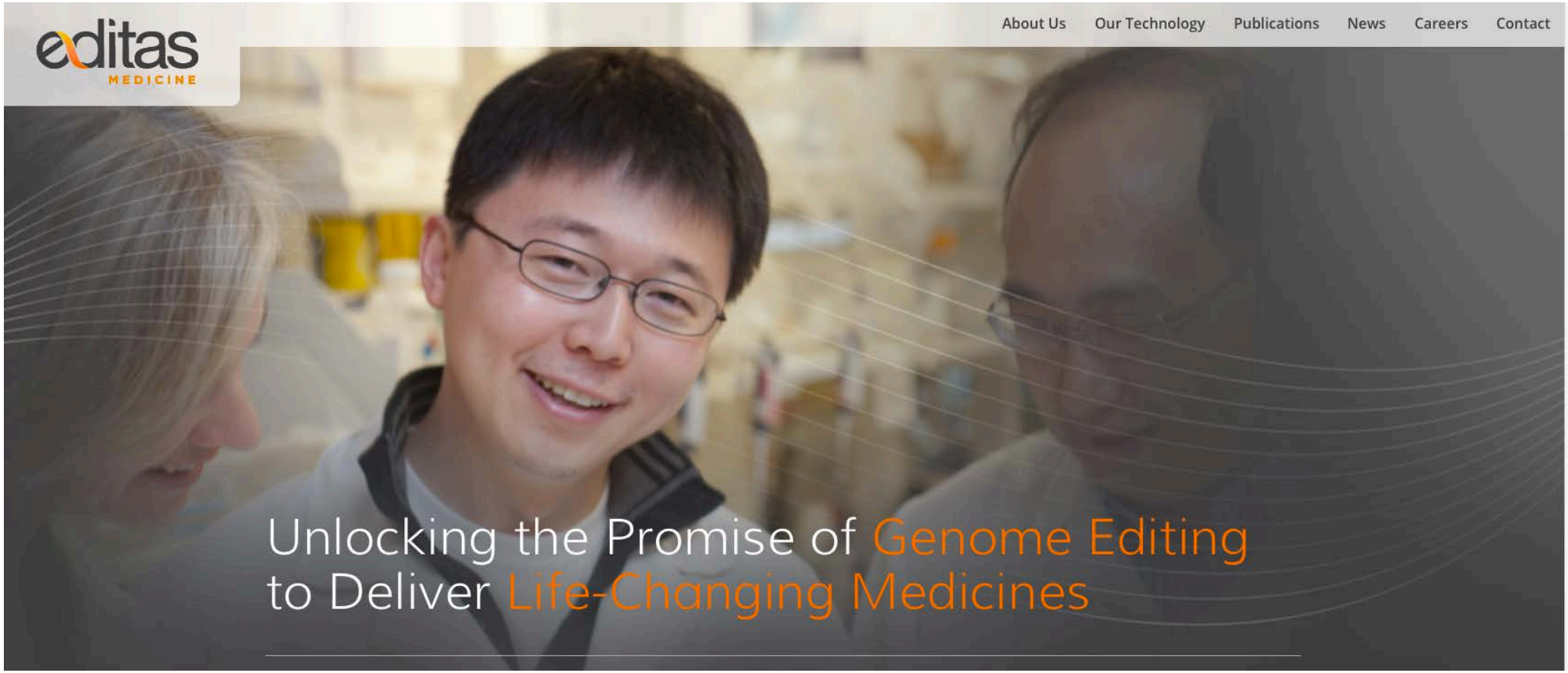
Science. 2015 Nov 27;350(6264):1101-4. doi: 10.1126/science.aad1191. Epub 2015 Oct 11.

Genome-wide inactivation of porcine endogenous retroviruses (PERVs).

Yang L[1], Güell M[2], Niu D[3], George H[4], Lesha E[4], Grishin D[4], Aach J[4], Shrock E[4], Xu W[5], Poci J[4], Cortazio R[4], Wilkinson RA[6], Fishman JA[6], Church G[1].

Disruption of 62 copies in one go

Beyond mouse models: gene therapy?



Beyond mouse models: gene therapy?

ZFN

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ORIGINAL ARTICLE

BRIEF REPORT

Long-Term Control of HIV by *CCR5* Delta32/Delta32 Stem-Cell Transplantation

Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S., Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D., Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D., Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D., and Eckhard Thiel, M.D.

N Engl J Med 2009; 360:692-698 | February 12, 2009 | DOI: 10.1056/NEJMoa0802905

We transplanted stem cells from a donor who was homozygous for CCR5 delta32 in a patient with acute myeloid leukemia and HIV-1 infection. The patient remained without viral rebound 20 months after transplantation and discontinuation of antiretroviral therapy. This outcome demonstrates the critical role CCR5 plays in maintaining HIV-1 infection.

Timothy Brown

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ORIGINAL ARTICLE

Gene Editing of *CCR5* in Autologous CD4 T Cells of Persons Infected with HIV

Pablo Tebas, M.D., David Stein, M.D., Winson W. Tang, M.D., Ian Frank, M.D., Shelley Q. Wang, M.D., Gary Lee, Ph.D., S. Kaye Spratt, Ph.D., Richard T. Surosky, Ph.D., Martin A. Giedlin, Ph.D., Geoff Nichol, M.D., Michael C. Holmes, Ph.D., Philip D. Gregory, Ph.D., Dale G. Ando, M.D., Michael Kalos, Ph.D., Ronald G. Collman, M.D., Gwendolyn Binder-Scholl, Ph.D., Gabriela Plesa, M.D., Ph.D., Wei-Ting Hwang, Ph.D., Bruce L. Levine, Ph.D., and Carl H. June, M.D.

N Engl J Med 2014; 370:901-910 | March 6, 2014 | DOI: 10.1056/NEJMoa1300662

Comments open through March 12, 2014

HIV RNA became undetectable in one of four patients who could be evaluated. The blood level of HIV DNA decreased in most patients.

SANGAMO Sangamo BioSciences

Beyond mouse models: gene therapy?

TALEN

2.4K

DAILY NEWS 5 November 2015

Gene editing saves girl dying from leukaemia in world first

Layla is doing well so far
Sharon Lees/GOSH

Layla was diagnosed with acute lymphoblastic leukaemia when she was just three months old, and all conventional treatments failed. She was too small and too sick for collection of her own T-cells, so she received T-cells from a donor with two types of gene modifications:
- KI of a CARD19 receptor gene (directs T-Cells against leukemic cells)
- KO of a gene involved in transplant rejection

She’s recovered and receives no further treatment so far.

cellectis

Beyond mouse models: gene therapy?

CRISPR

Protein Cell. 2015 May;6(5):363-72. doi: 10.1007/s13238-015-0153-5. Epub 2015 Apr 18.

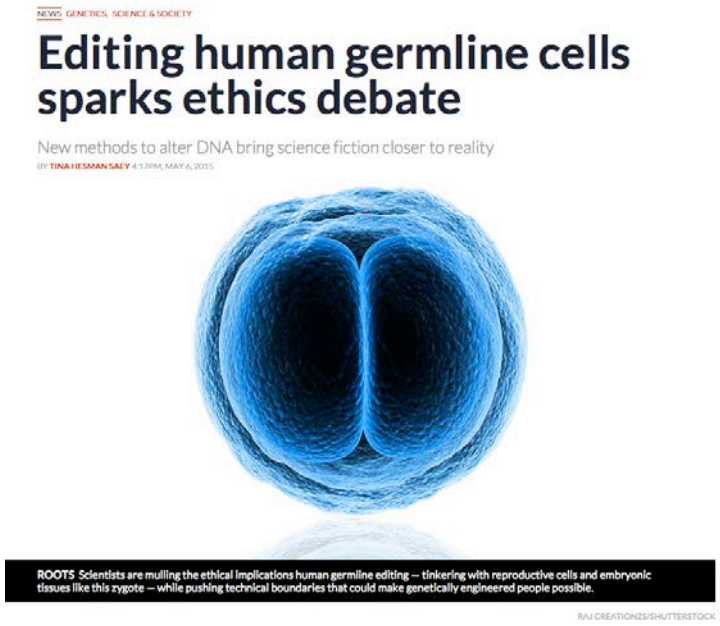
CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes.

Liang P[1], Xu Y, Zhang X, Ding C, Huang R, Zhang Z, Lv J, Xie X, Chen Y, Li Y, Sun Y, Bai Y, Songyang Z, Ma W, Zhou C, Huang J.

J Assist Reprod Genet. 2016 Apr 6. [Epub ahead of print]

Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing.

Kang X[1], He W[1], Huang Y[1], Yu Q[1], Chen Y[1], Gao X[1], Sun X[1], Fan Y[2].



NEWS GENETICS, SCIENCE & SOCIETY

Editing human germline cells sparks ethics debate

New methods to alter DNA bring science fiction closer to reality

BY TINA HESMAN SAEY 4:17PM, MAY 6, 2015

ROOTS Scientists are mulling the ethical implications human germline editing — tinkering with reproductive cells and embryonic tissues like this zygote — while pushing technical boundaries that could make genetically engineered people possible.

RAJ CREATIONZS/SHUTTERSTOCK

Nature. 2015 Mar 26;519(7544):410-1. doi: 10.1038/519410a.

Don't edit the human germ line.

Lanphier E, Urnov F, Haecker SE, Werner M, Smolenski J.

Beyond mouse models: gene therapy?

CRISPR

ARTICLE

doi:10.1038/nature23305

Correction of a pathogenic gene mutation in human embryos

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

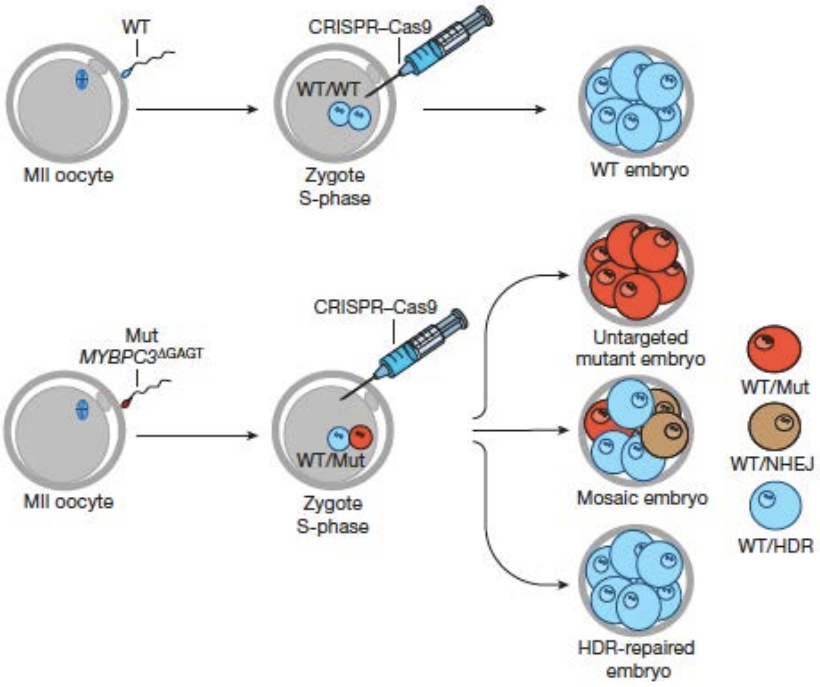
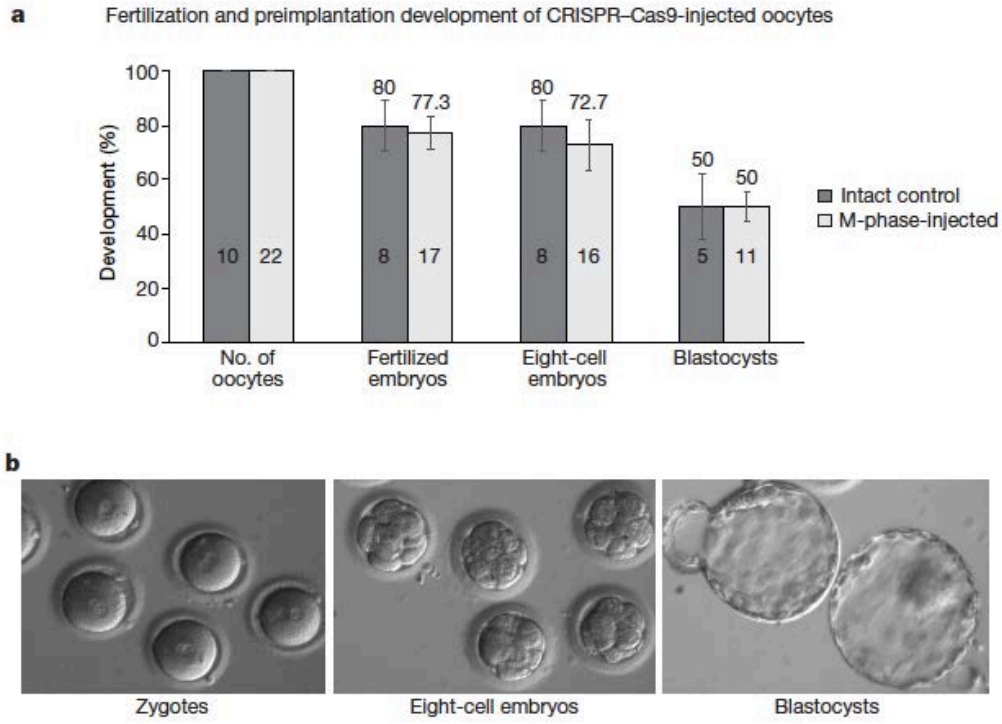


Figure 1 | Gene correction in S-phase-injected human embryos.



Transgenic Animal Unit

TAU

TRANSGENICANIMALUNIT

Welcome to the Transgenic Animal Unit (TAU). Our facility is part of the Mark Wainwright Analytical Centre, and we operate within the Biological Research Center (BRC) in the Wallace Wurth Building.

We generate customised genetically modified mice for medical research purposes, and can offer this service on a collaborative basis to UNSW and external researchers. We use cutting edge technologies (e.g. engineered endonucleases such as ZFN and CRISPR) for genome editing, and aim at developing sophisticated animal models for the study of human diseases.

Please contact us to discuss your project needs.

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Authorised by the Managing Director of Mark Wainwright Analytical Centre, UNSW CRICOS Provider Code 00098G, ABN 57 195 873 179
Page last updated: Wednesday 16 December 2015

Mark Wainwright Analytical Centre, UNSW, Sydney, NSW, 2052, Australia | Email: analytical@unsw.edu.au

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