



MACQUARIE
University

MANIPULATING THE MOUSE EMBRYO: FROM ES CELLS TO GENOME EDITING

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Senior Lecturer

Dementia Research Centre, Faculty of Medicine and Health Sciences

UNSW - 16 October 2019

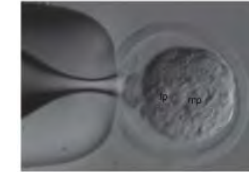


GENOME EDITING at MACQUARIE (GEM)

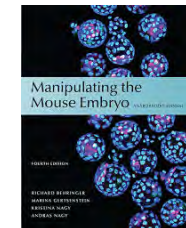


OVERVIEW

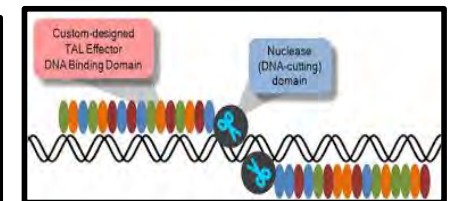
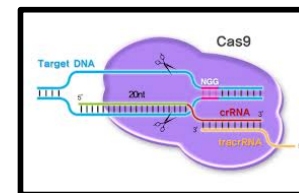
GEM: structure / organization & services



Manipulating the mouse genome



Genome editing



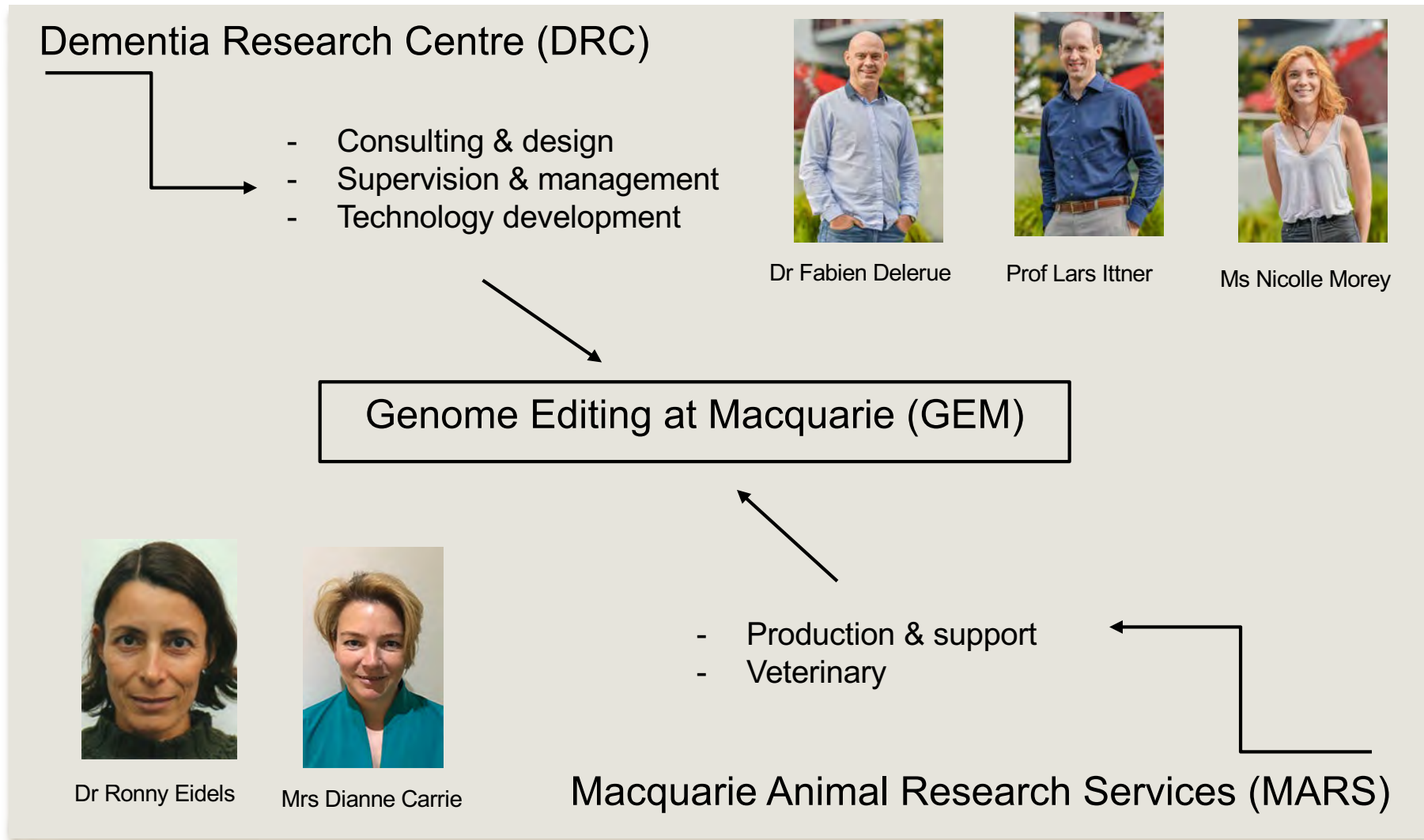
Clinically relevant models
across species



GENOME EDITING at MACQUARIE (GEM)



ORGANISATION



GENOME EDITING at MACQUARIE (GEM)



SERVICES



Assisted Reproductive Techniques

- Superovulation
- IVF
- Reimplantation
- Artificial insemination
- Cryopreservation/rederivation
- Importations
- Ethics
- Aggregation chimeras



Genome Editing

- Pronuclear/cytoplasmic injections
- In vitro/in situ electroporation
- Gene targeting (KO, KI, Point mutations...)
- Overexpression (random integration)
- Non conventional backgrounds
- Sequencing / Genotyping
- (Phenotyping)

Genome Editing at Macquarie (GEM)

Macquarie Researchers > External > International (and commercial)

GENOME EDITING AT MACQUARIE (GEM)

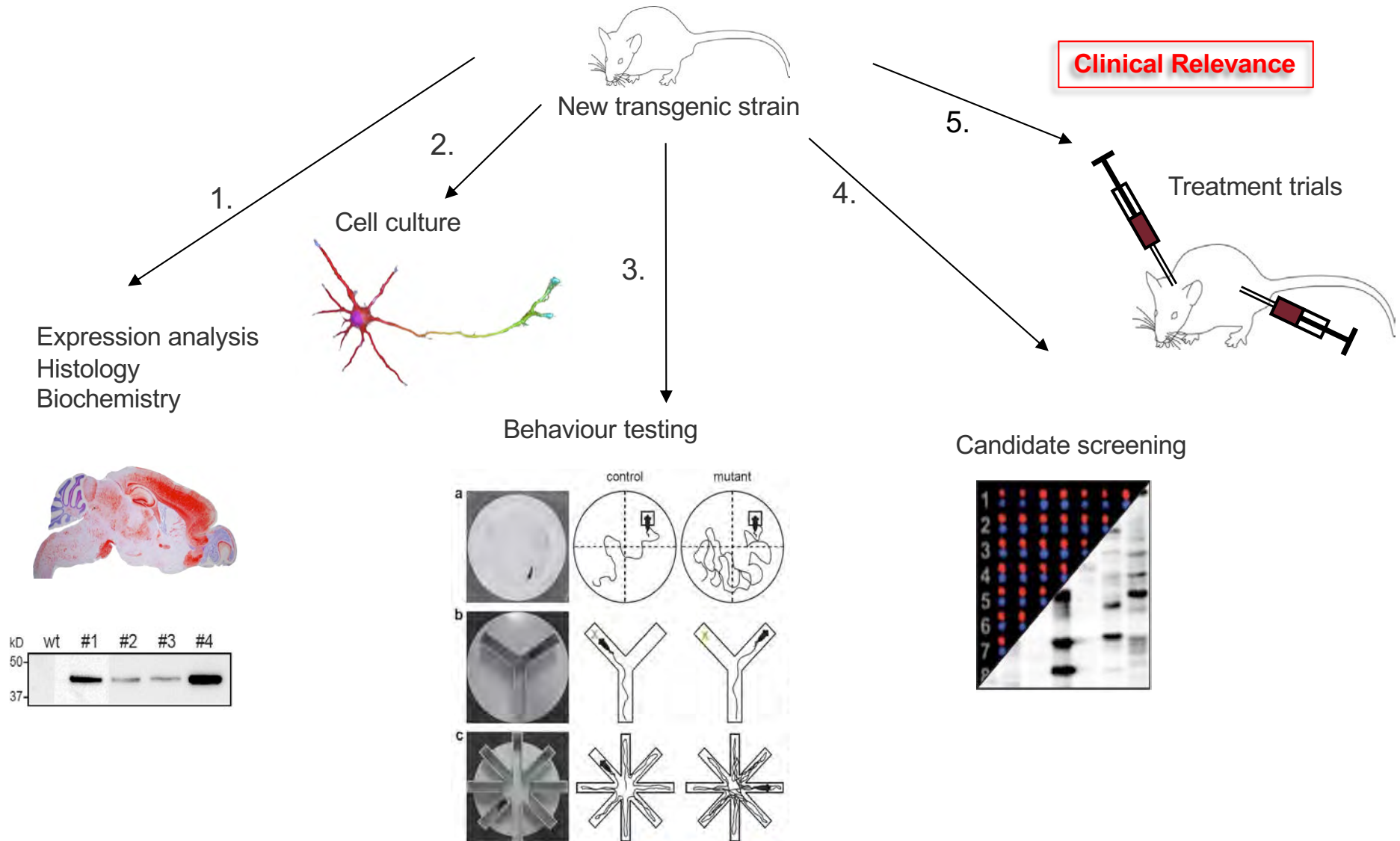


WEBSITE



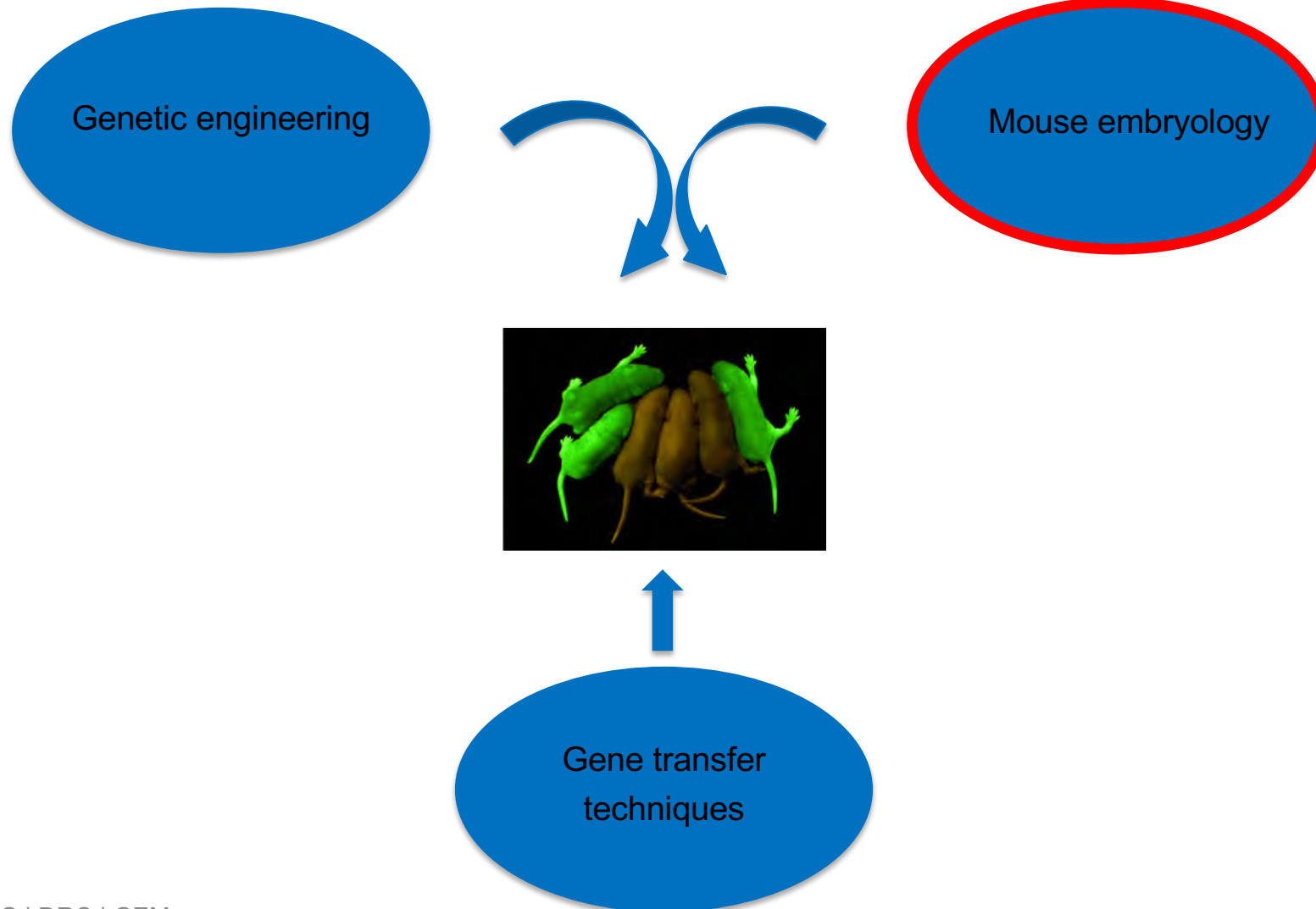
GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE



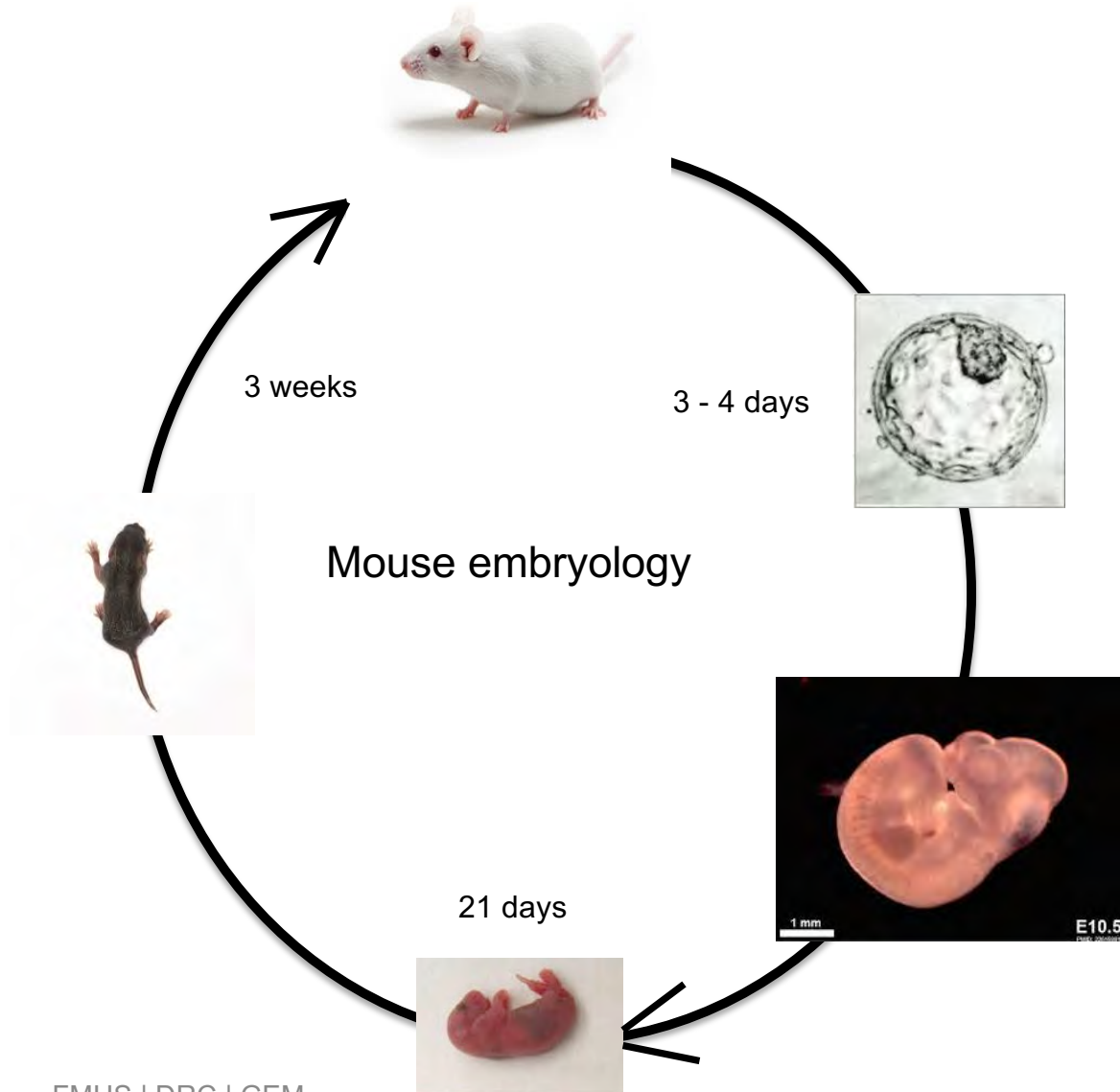
GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE



GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE



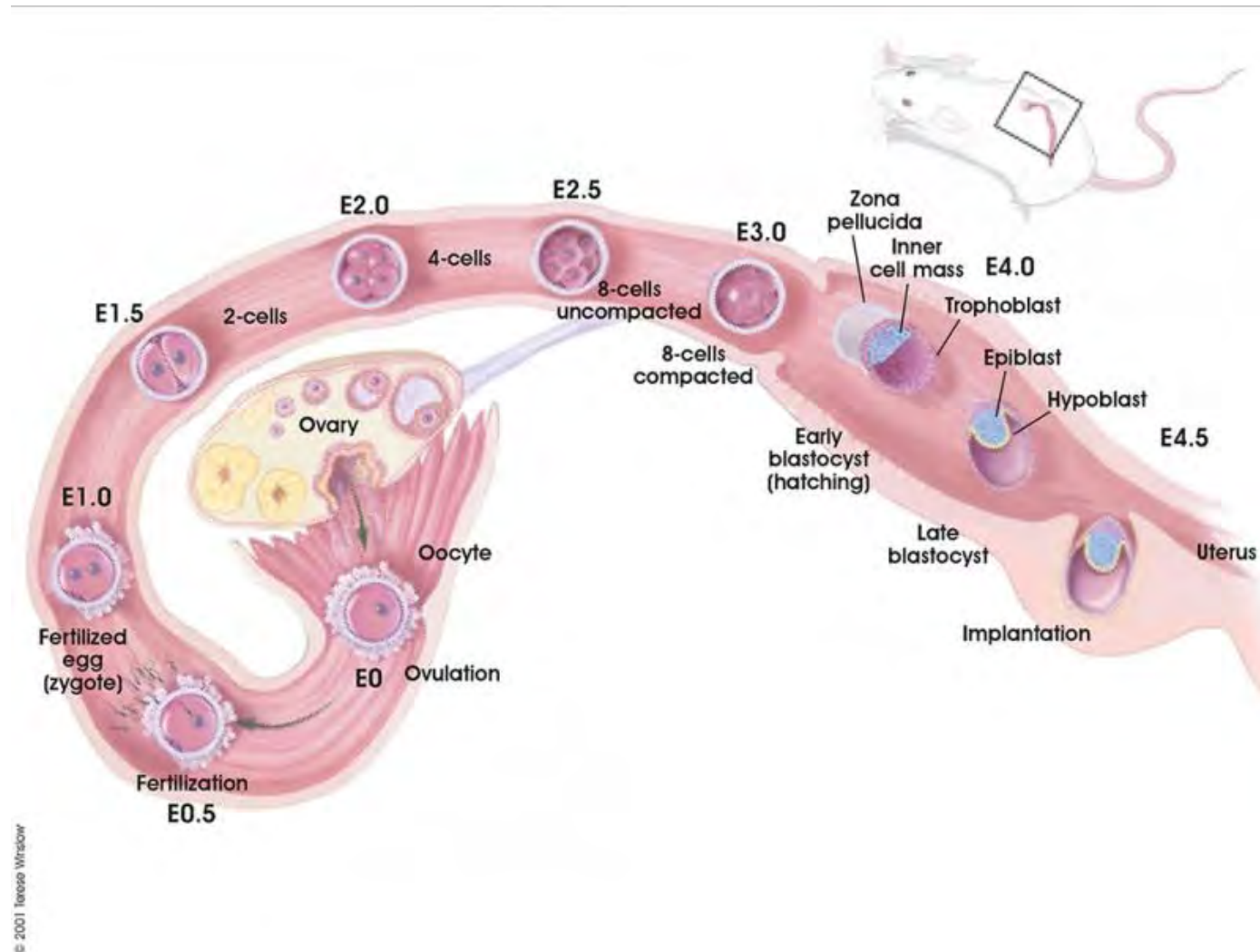
Advantages of the mouse:

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

Mouse is the most commonly used animal model

GENOME EDITING AT MACQUARIE (GEM)

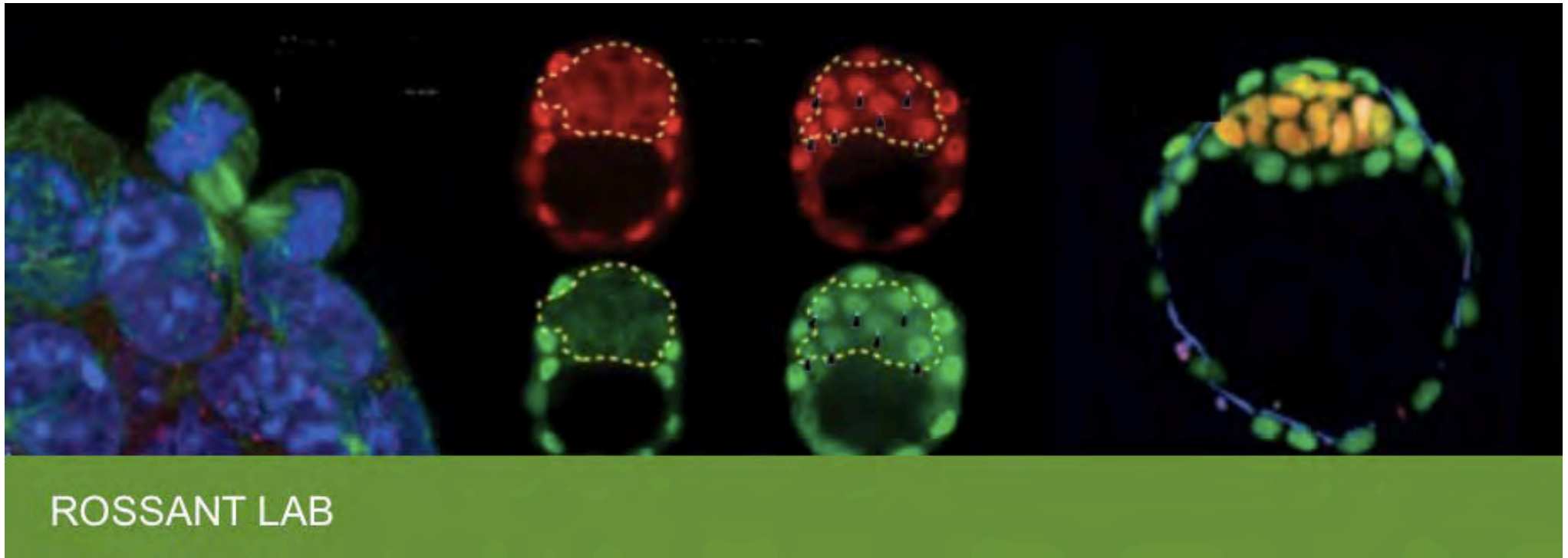
GENOME MANIPULATION IN MICE




GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE



Efficient generation of targeted large insertions by microinjection into two-cell-stage mouse embryos

Bin Gu^{1,3}, Eszter Posfai^{1,3} & Janet Rossant^{1,2} 

GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE

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

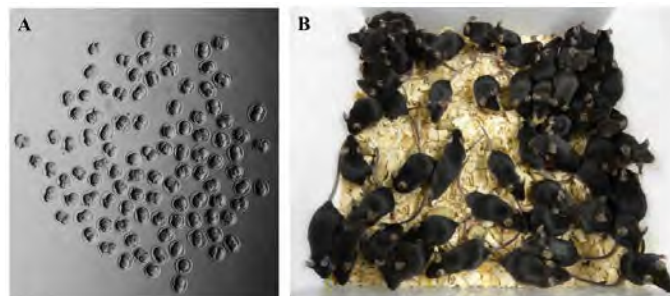
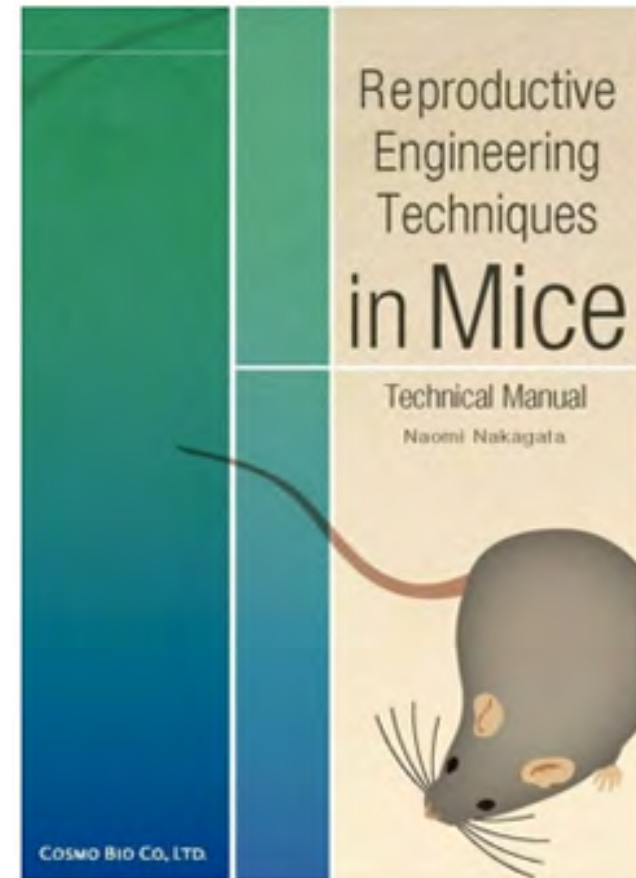


Fig 2. Production of 2-cell embryos (A) and live pups (B) from single female mice superovulated using IASe (0.1 mL IAS and 3.75 IU eCG).



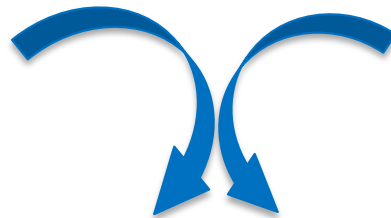
Prof Naomi Nakagata – CARD

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GENOME MANIPULATION IN MICE

Genetic engineering



Mouse embryology

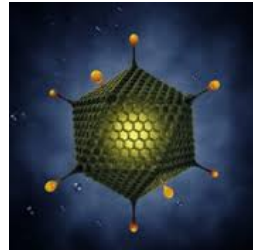


Gene transfer techniques

GENOME EDITING AT MACQUARIE (GEM)

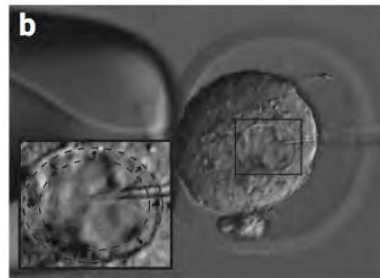


GENOME MANIPULATION IN MICE



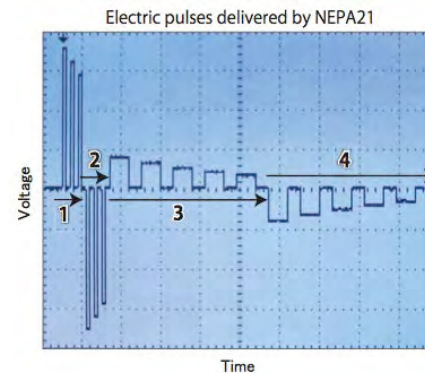
Viral delivery

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



Microinjection

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



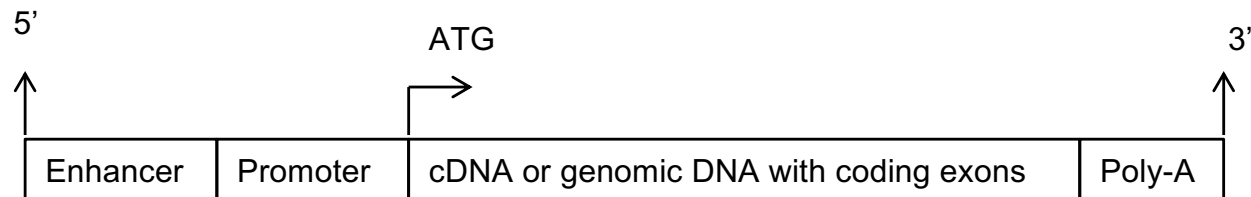
Electroporation

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

GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE



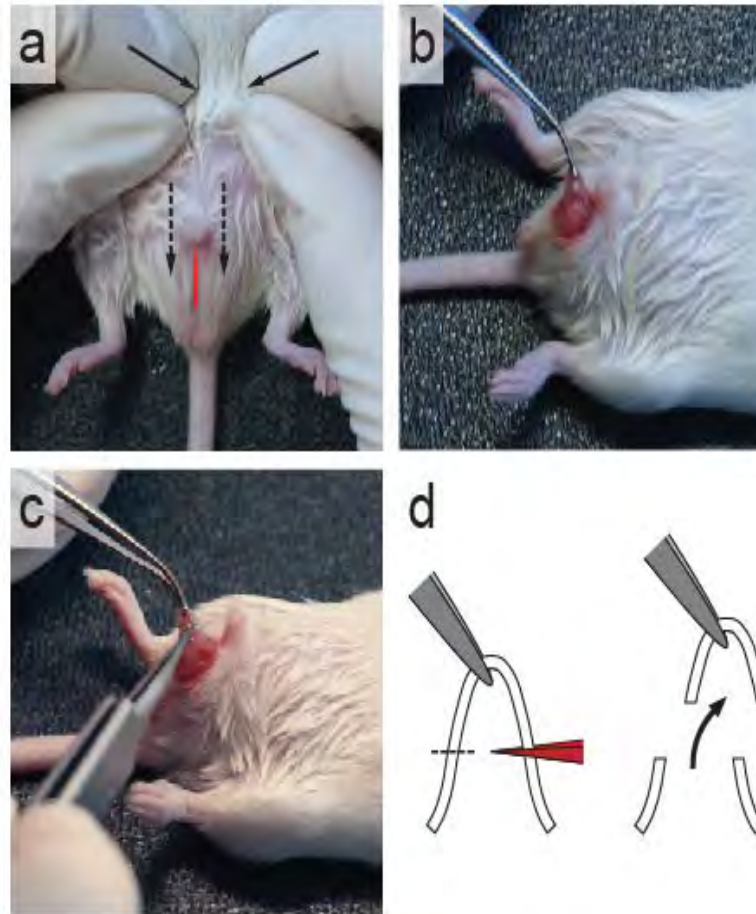
Schematic layout of a typical **transgene** construct (Adapted from Auerbach AB. *Acta Biochem. Pol.* 2004;51(1):9-31. Review)

1980s “Era of Recombinant DNA technology”

GENOME EDITING AT MACQUARIE (GEM)

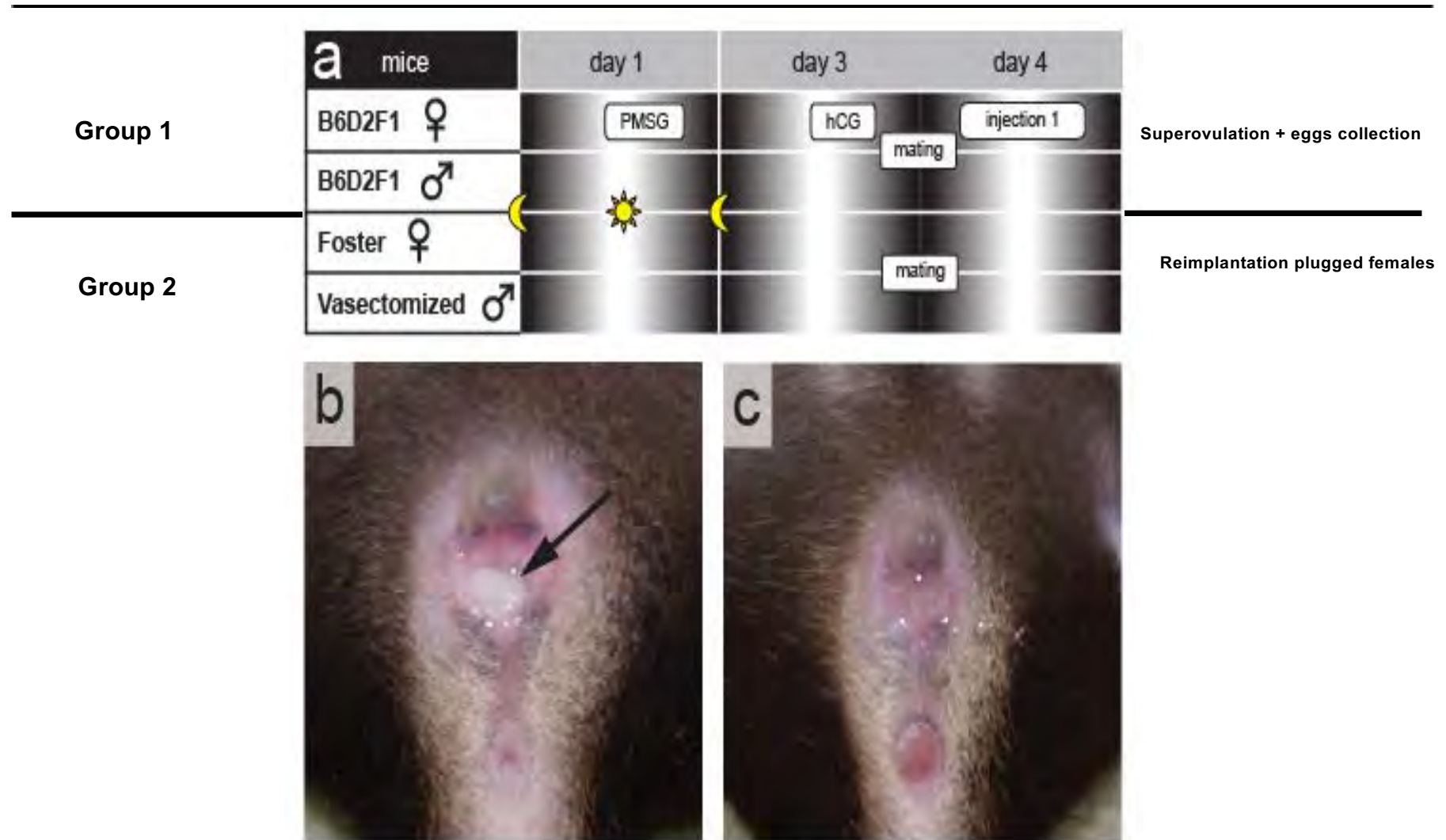
GENOME MANIPULATION IN MICE

Vasectomy



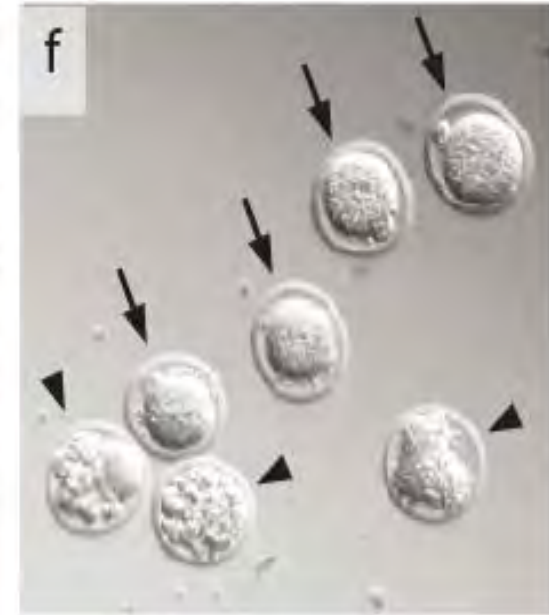
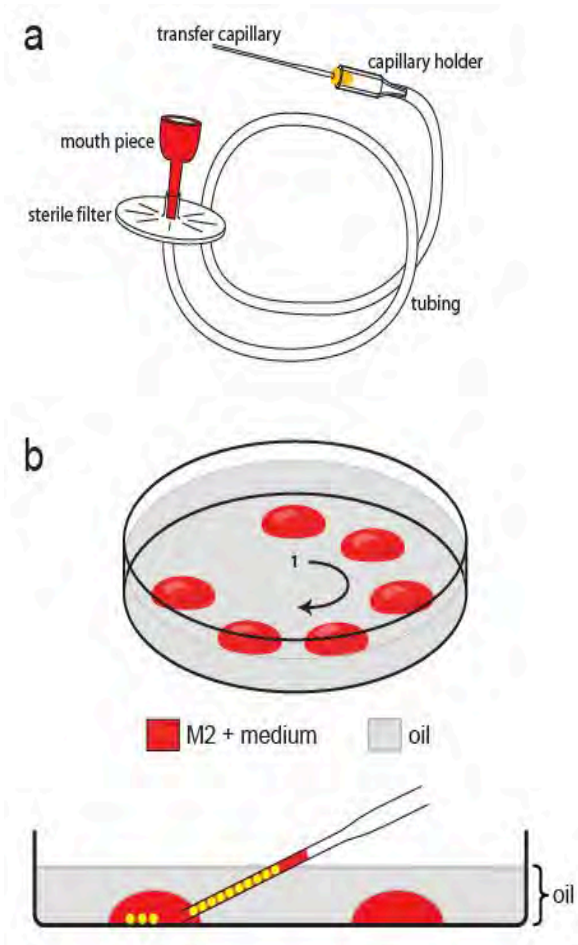
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GENOME MANIPULATION IN MICE



GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE



Collection and Purification (hyaluronidase) of the eggs
(incubator 37C – 5% CO₂)

Microinjection and its applications

GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE

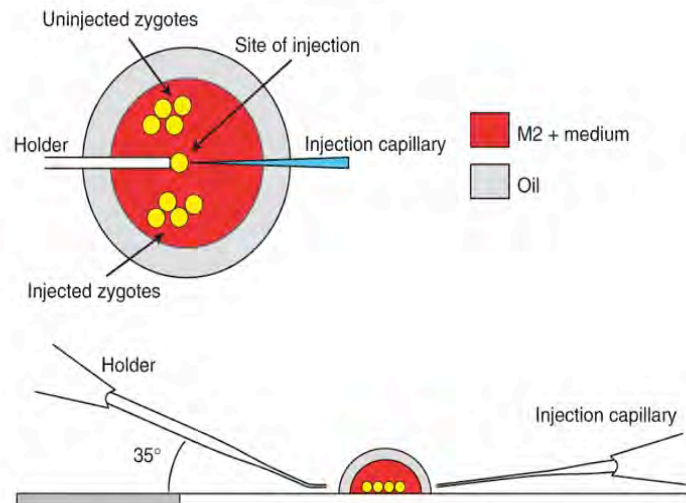
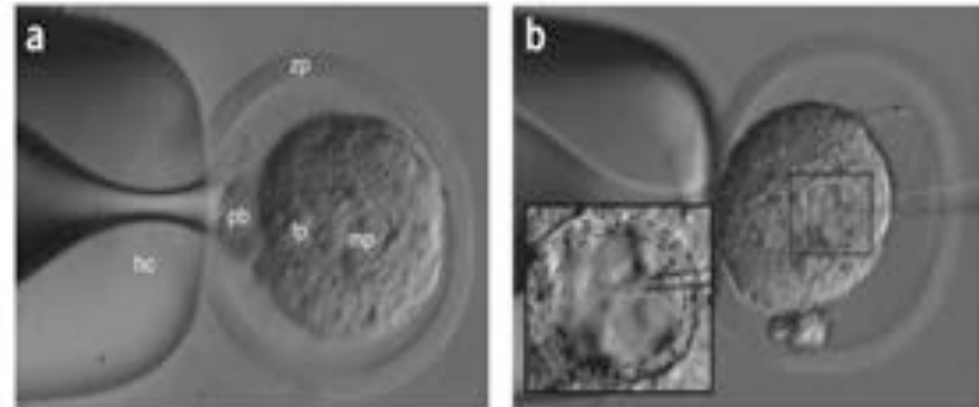


Figure 5 | Arrangements of zygotes, holder and injection capillary on injection stage.



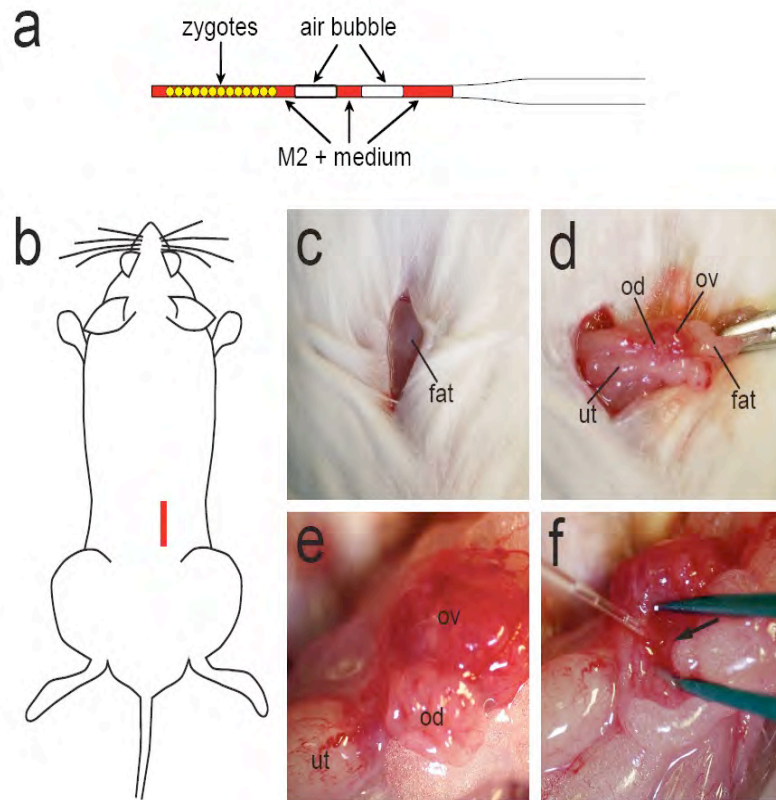
Setting up the injection chamber
(inverted microscope)

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

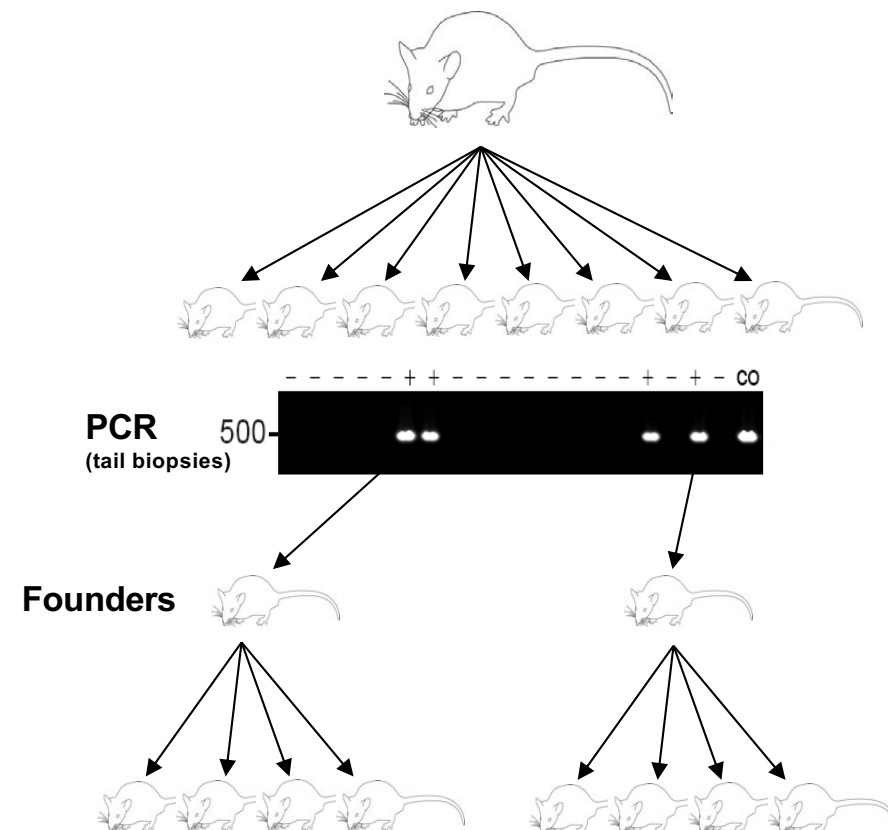
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GENOME MANIPULATION IN MICE

Reimplantation (plugged fosters)



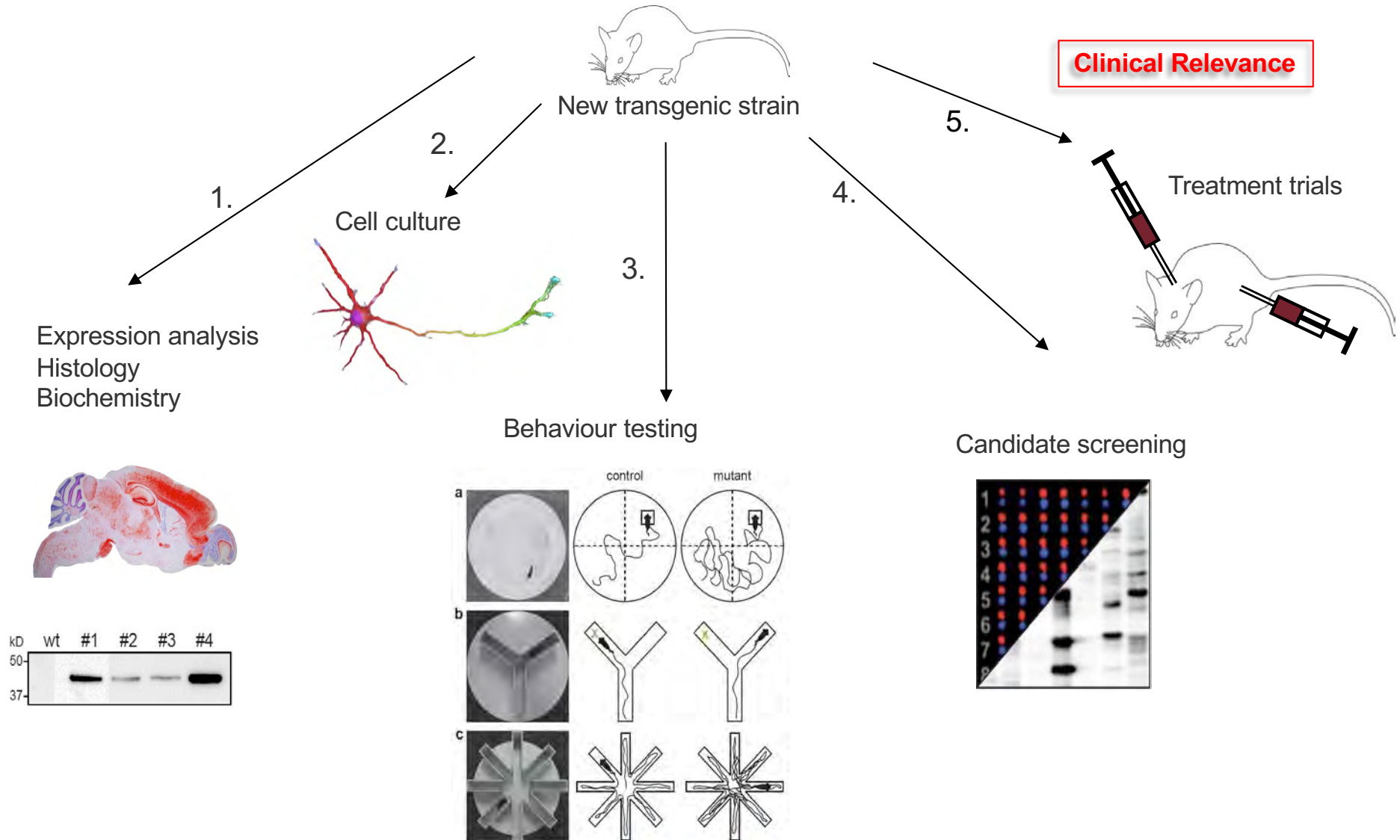
Establishing transgenic lines



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

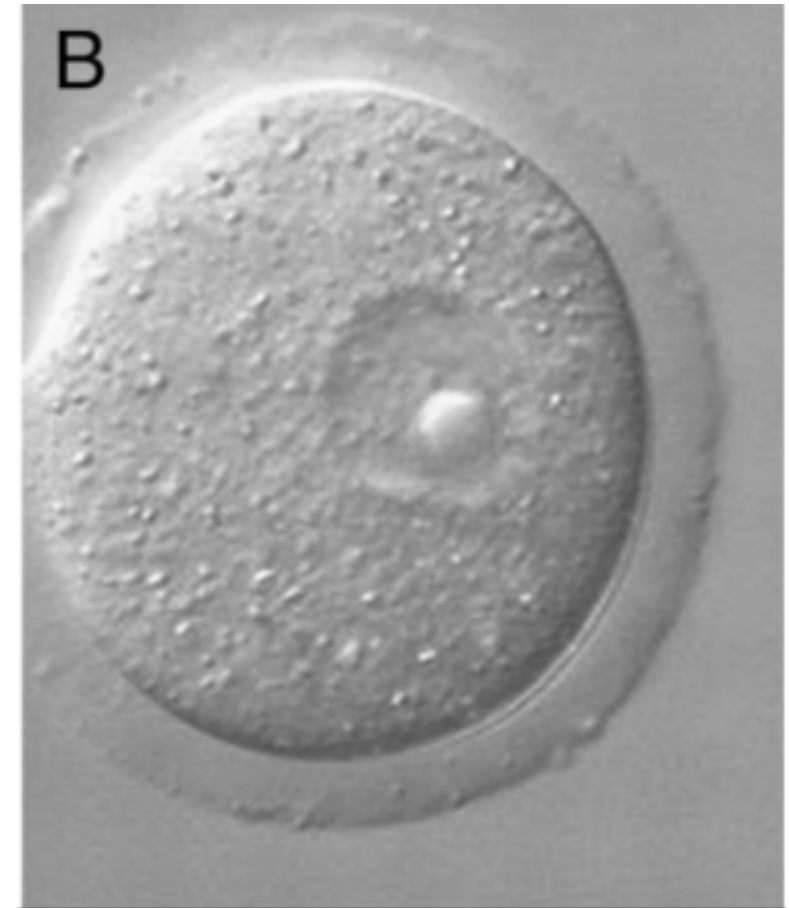
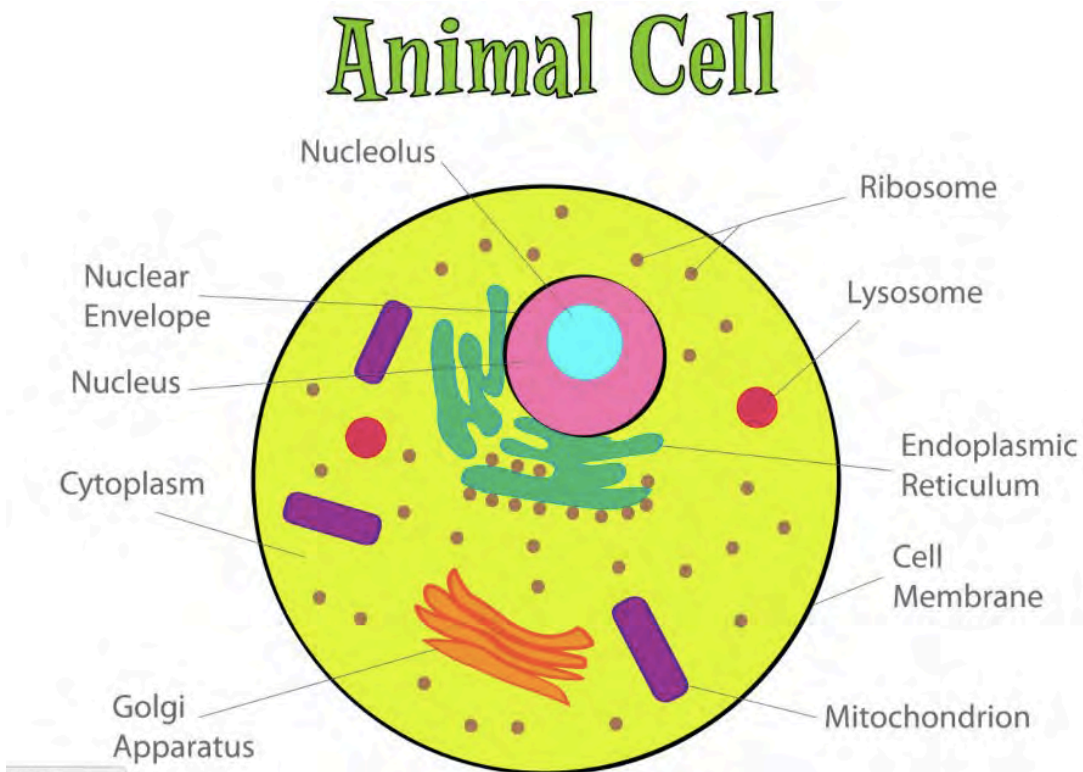
GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE



GENOME EDITING AT MACQUARIE (GEM)

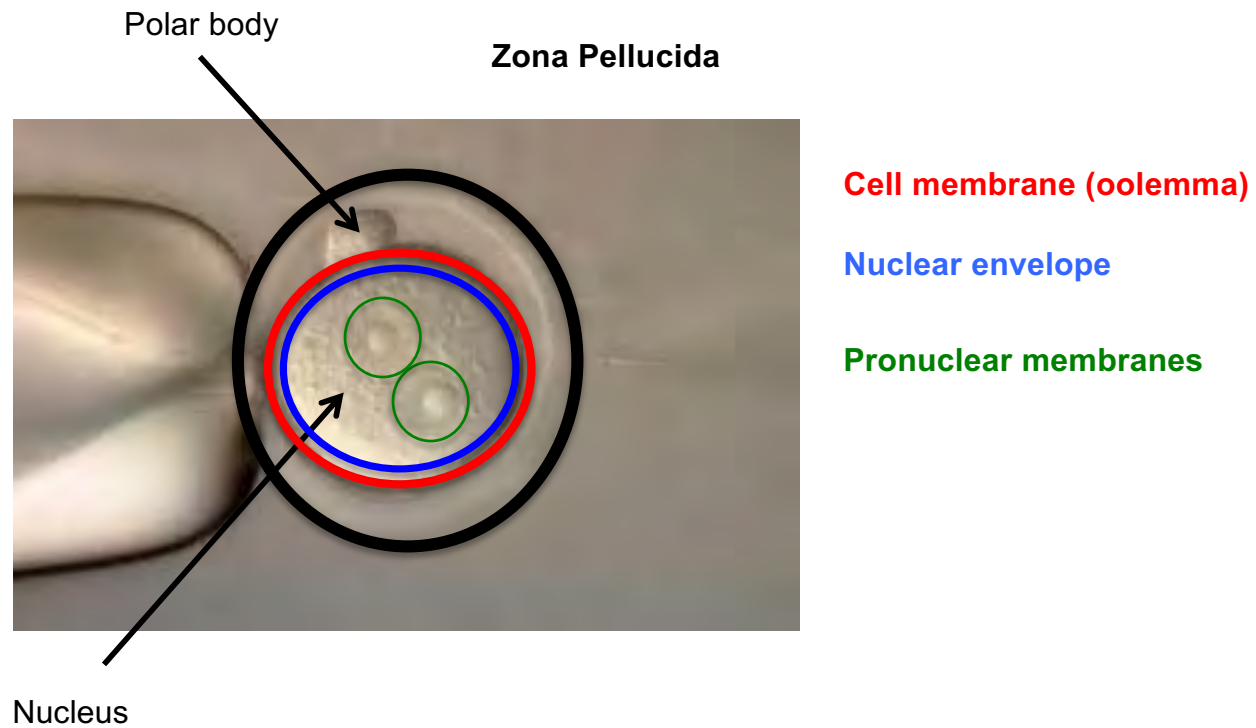
GENOME MANIPULATION IN MICE



Organisation of the zygote (= 1C embryo)

GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE



Organisation of the zygote (= 1C embryo)

GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE

Microinjection and its applications

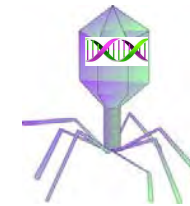
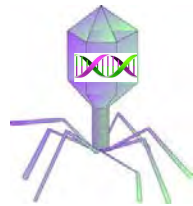


Perivitelline (subzonal) injection of viral particles

GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE



Subzonal injection of viral particles

Multiple insertion sites – one copy per site

GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE



Subzonal injection of viral particles
Multiple insertion sites – one copy per site

GENOME EDITING AT MACQUARIE (GEM)



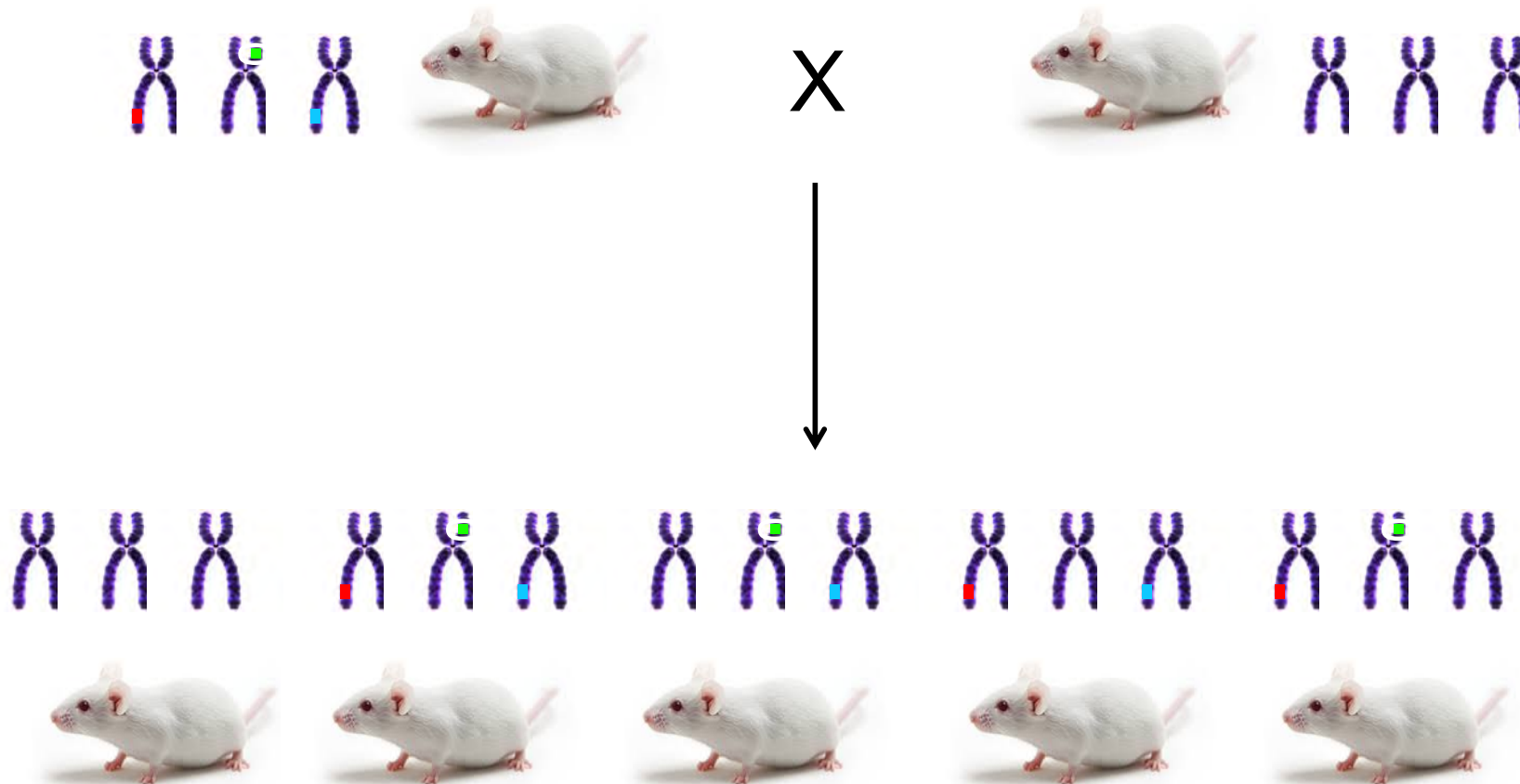
GENOME MANIPULATION IN MICE



Subzonal injection of viral particles
Multiple insertion sites – one copy per site

GENOME EDITING AT MACQUARIE (GEM)

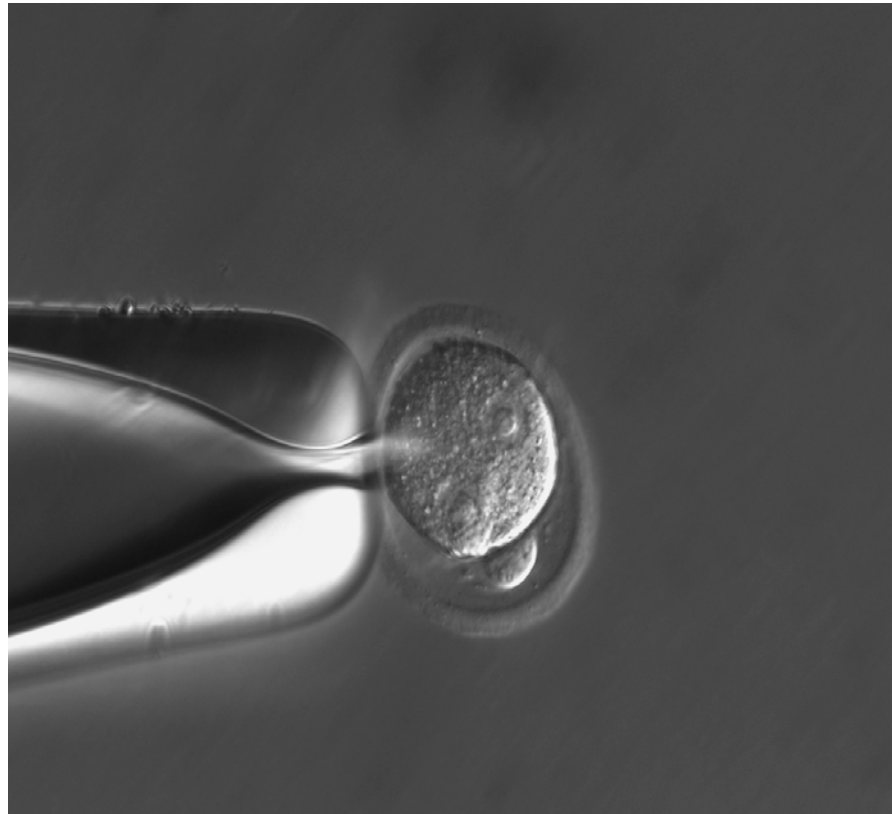
GENOME MANIPULATION IN MICE



Subzonal injection of viral particles
Multiple insertion sites – one copy per site

GENOME EDITING AT MACQUARIE (GEM)

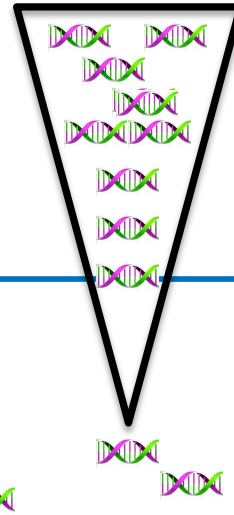
GENOME MANIPULATION IN MICE



Pronuclear injection of transgenes

GENOME EDITING AT MACQUARIE (GEM)

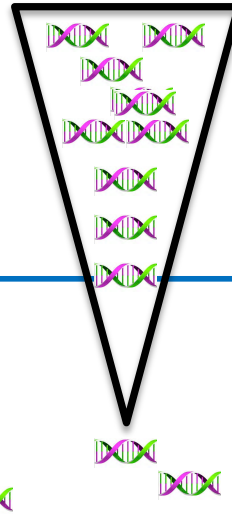
GENOME MANIPULATION IN MICE



Pronuclear injection of transgenes
**One insertion site – one or multiple copies
(concatemere)**

GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE

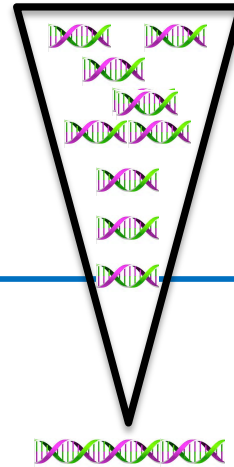


Pronuclear injection of transgenes
**One insertion site – one or multiple copies
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GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE

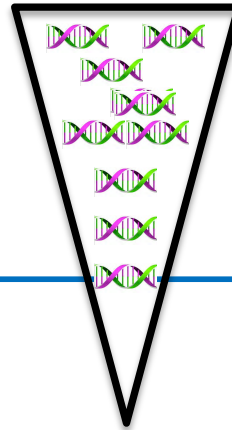


Pronuclear injection of transgenes
**One insertion site – one or multiple copies
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GENOME EDITING AT MACQUARIE (GEM)



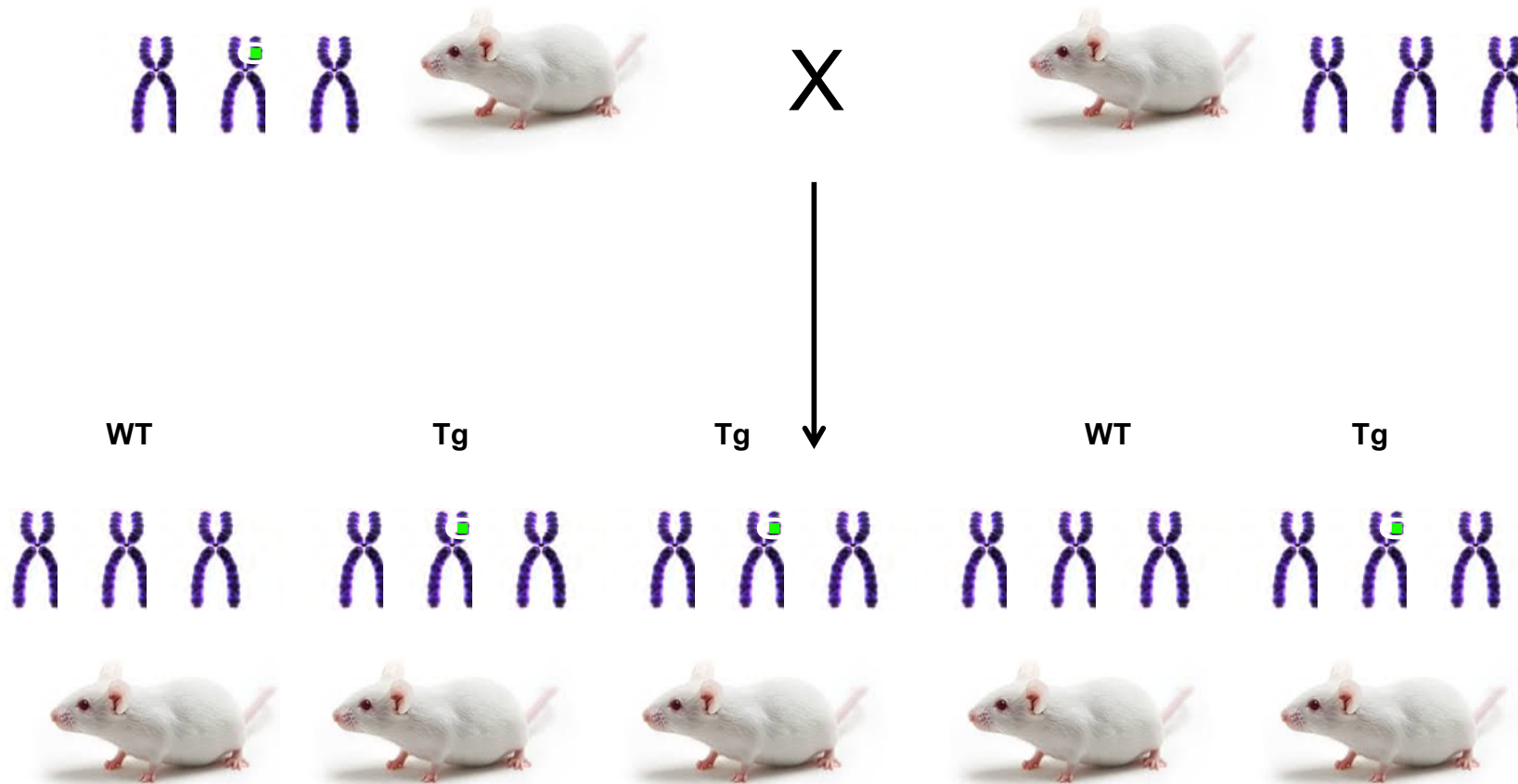
GENOME MANIPULATION IN MICE



Pronuclear injection of transgenes
**One insertion site – one or multiple copies
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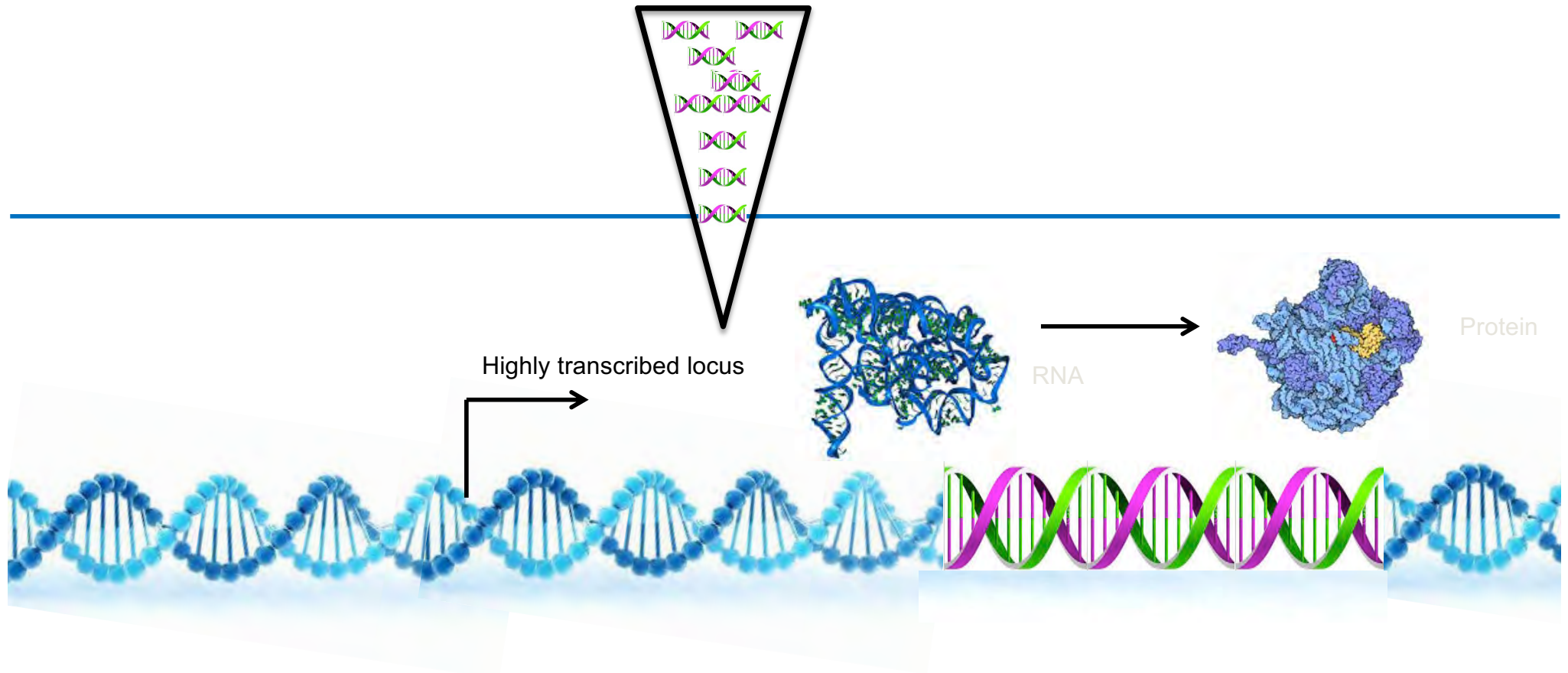
GENOME MANIPULATION IN MICE



Pronuclear injection of transgenes
**One insertion site – one or multiple copies
(concatemere)**

GENOME EDITING AT MACQUARIE (GEM)

GENOME MANIPULATION IN MICE

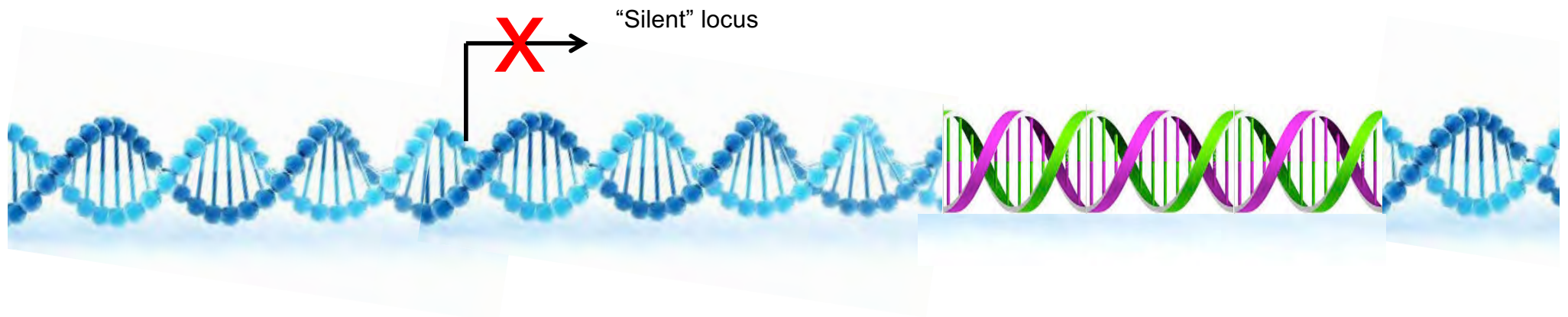
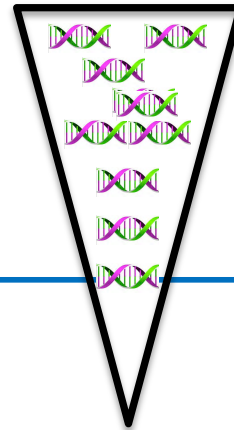


Pronuclear injection of transgenes
“Position” effect

GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE



Pronuclear injection of transgenes
"Position" effect

GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE

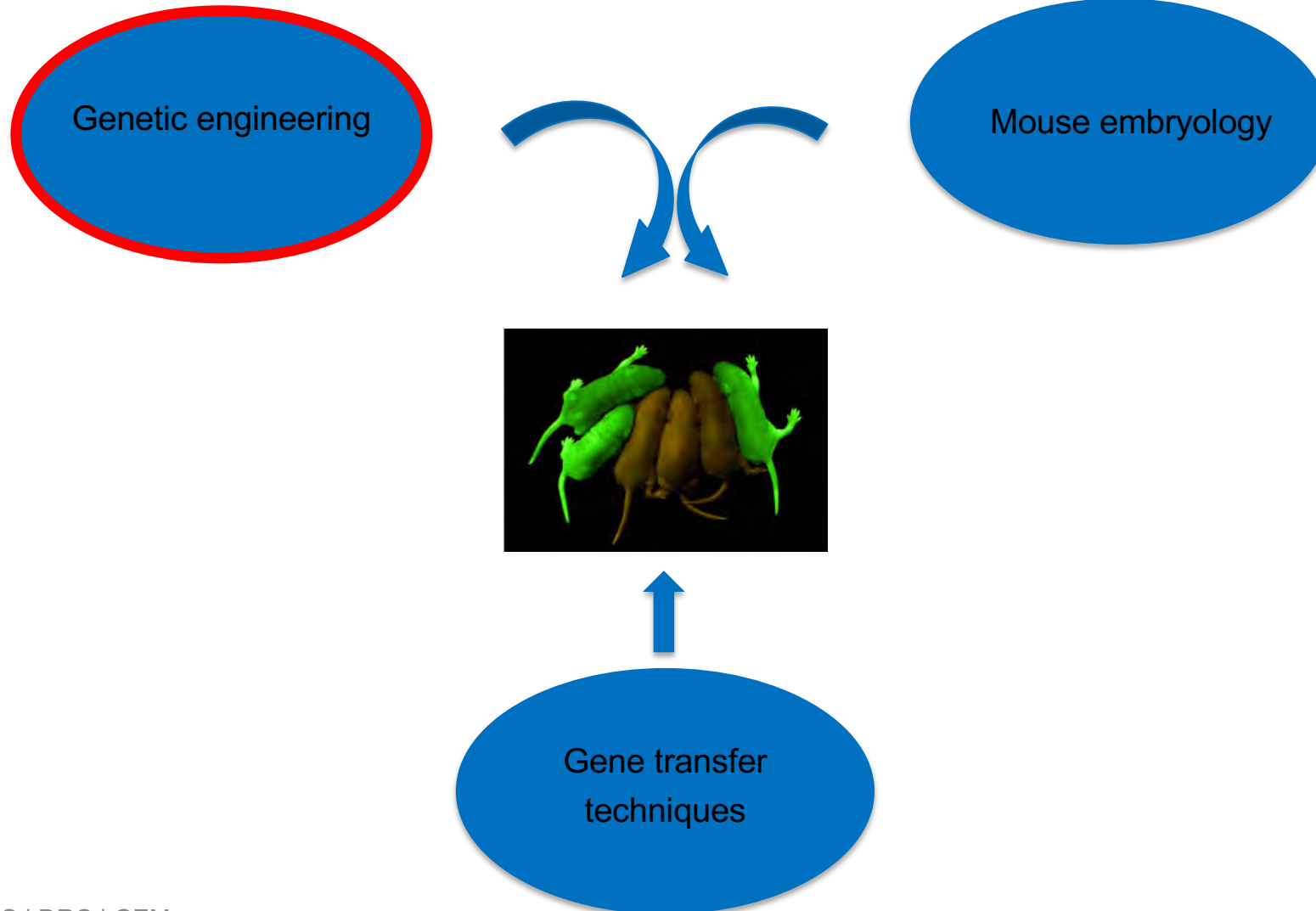


Cytoplasmic injection of RNA
(endonucleases)

GENOME EDITING AT MACQUARIE (GEM)



GENOME MANIPULATION IN MICE



GENOME EDITING AT MACQUARIE (GEM)



GENOME EDITING



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.

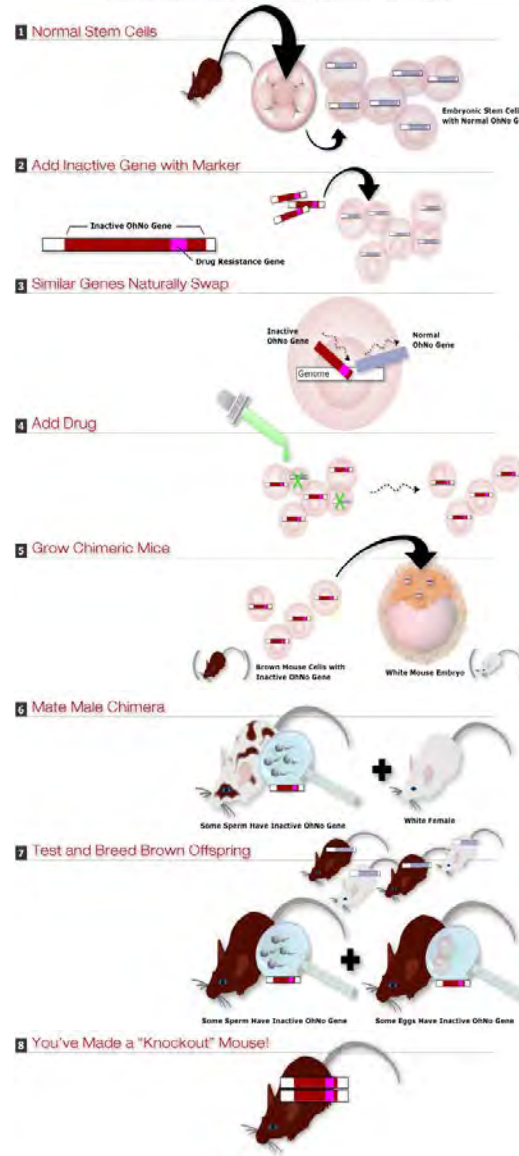


[Watch a video of The University of Utah Nobel Press Conference](#)

GENOME EDITING AT MACQUARIE (GEM)

GENOME EDITING

How to Build a "Knockout" Mouse

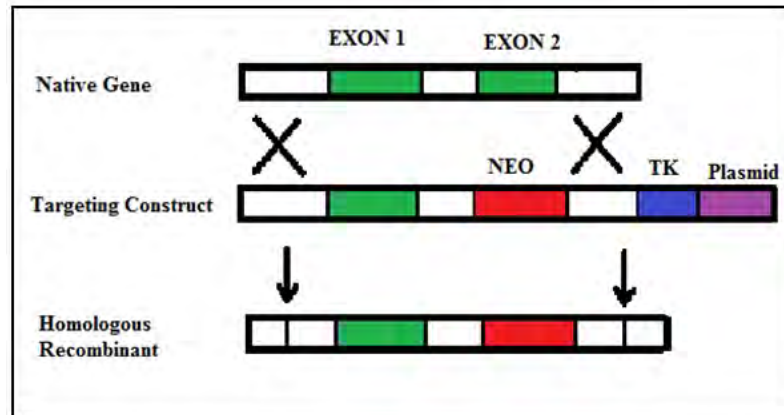


- Targeted integration
(Knock-In) e.g Rosa26 safe Harbor

- Targeted deletion (Knock-Out)

GENOME EDITING AT MACQUARIE (GEM)

GENOME EDITING

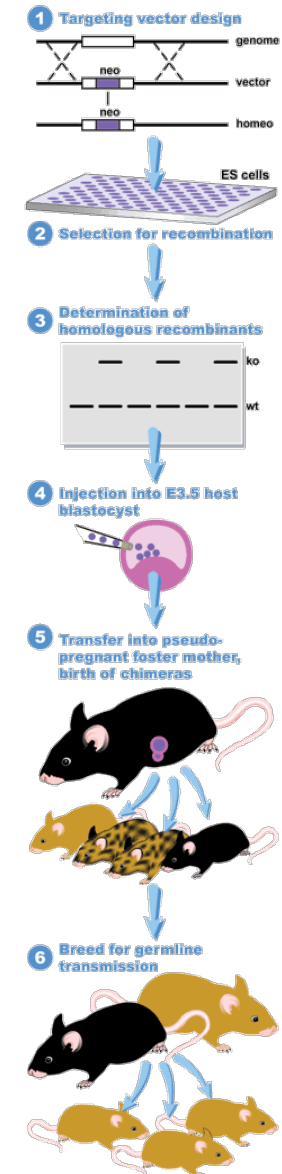


Homologous Recombination (HR)
Homologous Direct Repair (HDR)

Major drawbacks:

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

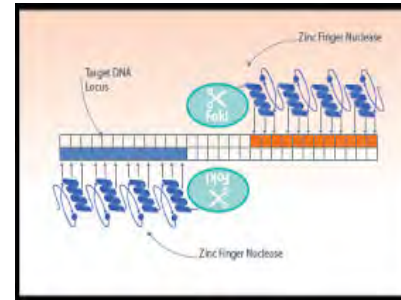
Germline transmission is not guaranteed



GENOME EDITING AT MACQUARIE (GEM)

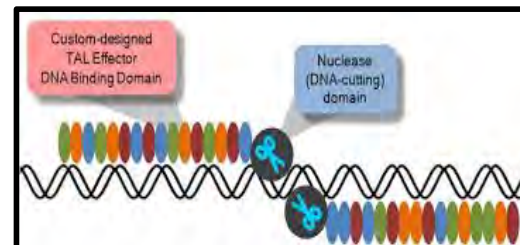
GENOME EDITING

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



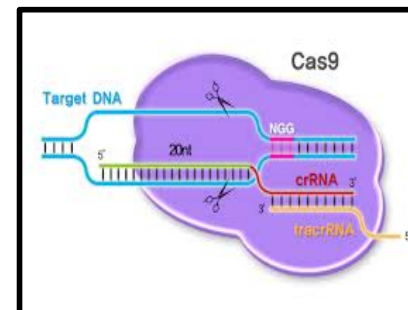
1 Zinc Finger Nuclease
(ZFN)

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)

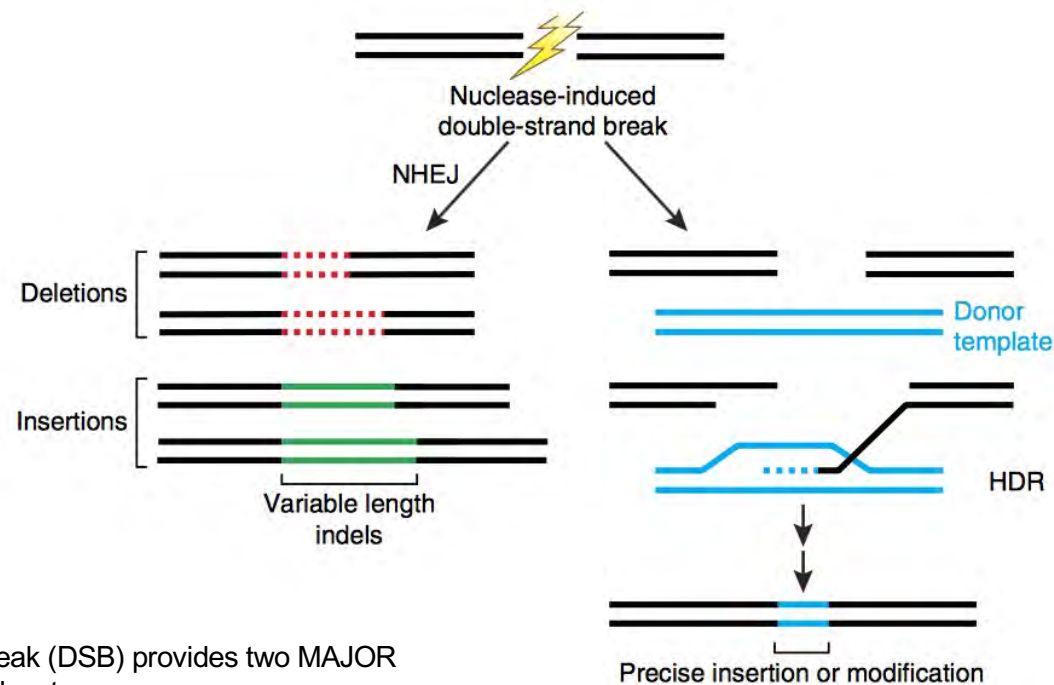


3 CRISPR/Cas9
(Clustered Regularly
Interspaced Short
Palindromic Repeats)

GENOME EDITING AT MACQUARIE (GEM)

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.

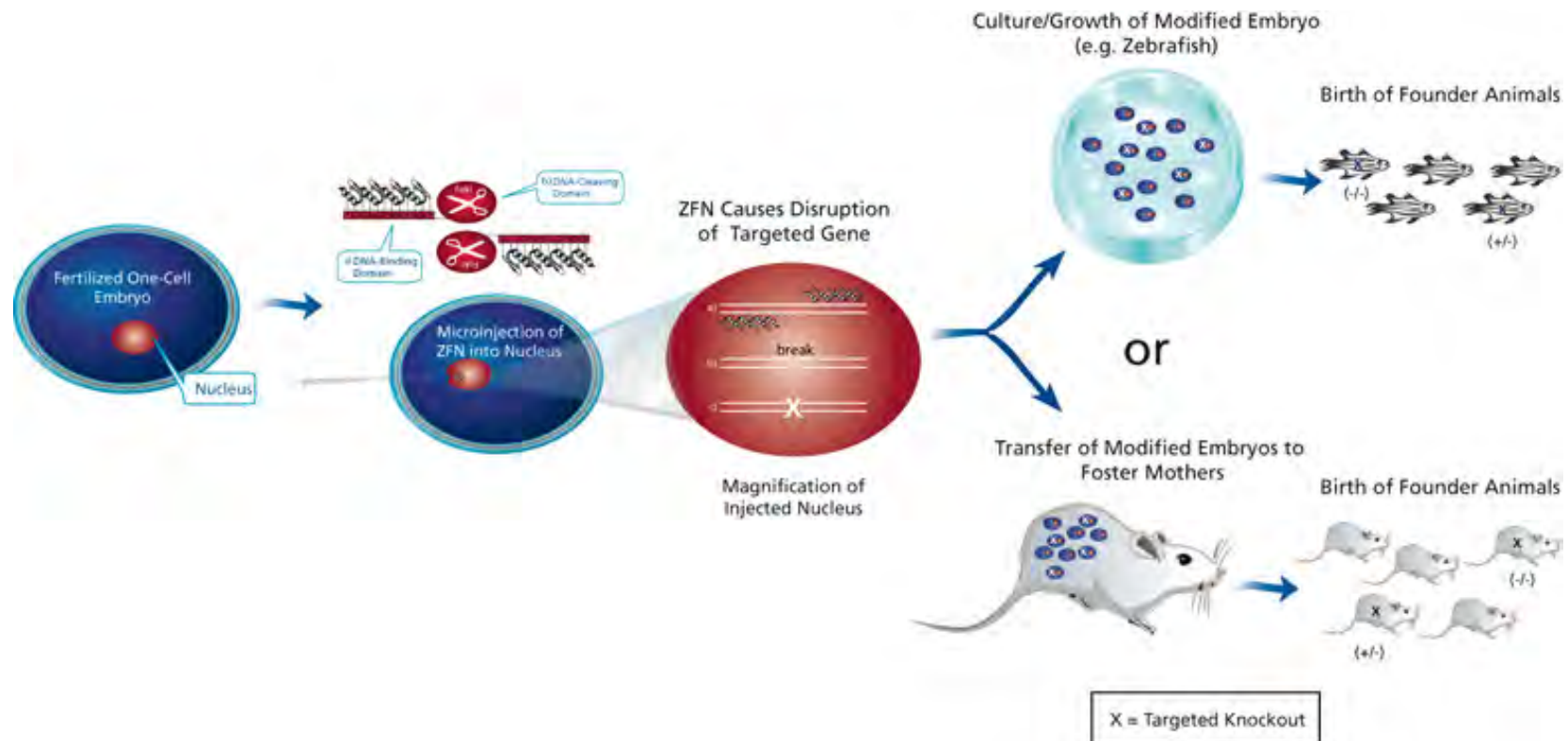
Sander & Joung Nat. Biotech. 2014

Allows for ES cells free gene targeting

GENOME EDITING AT MACQUARIE (GEM)

GENOME EDITING

Gene targeting using nucleases



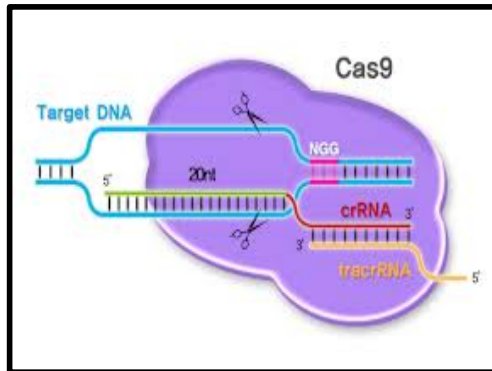
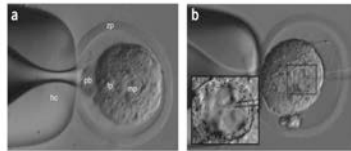
To date, NO species has been reported to be resistant to CRISPR genome editing

GENOME EDITING AT MACQUARIE (GEM)

GENOME EDITING

Gene targeting using nucleases

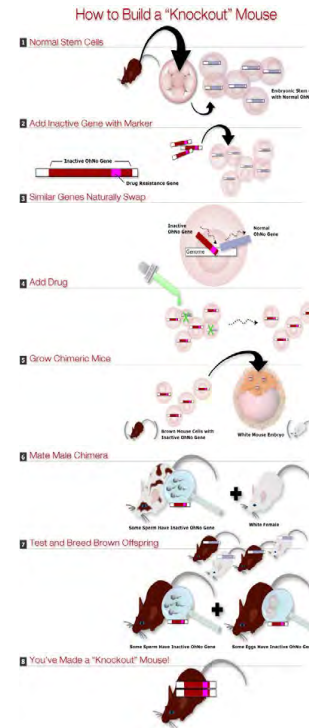
6 weeks



CRISPR
Disruptive Technology!



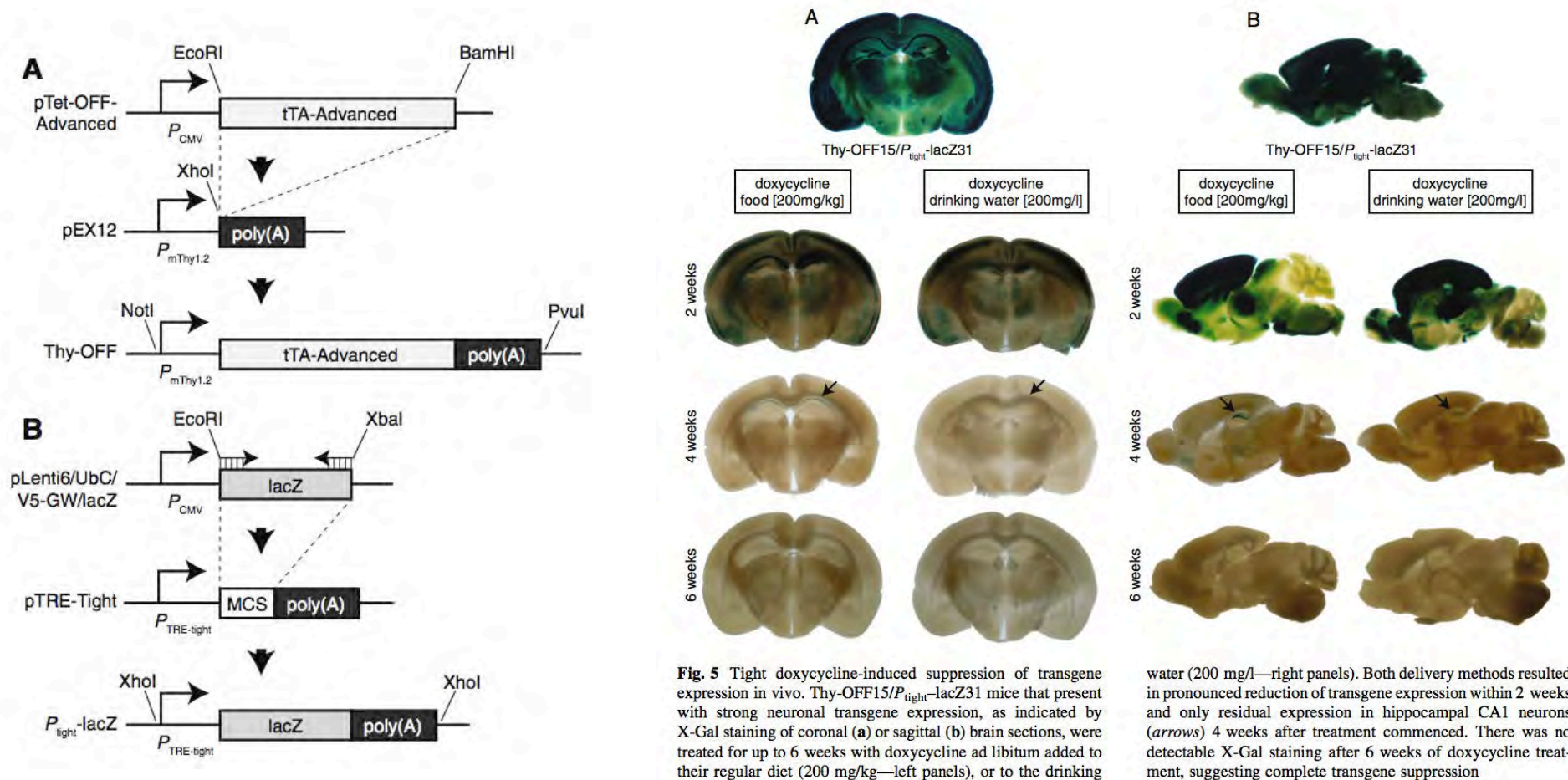
1-2 years



GENOME EDITING AT MACQUARIE (GEM)

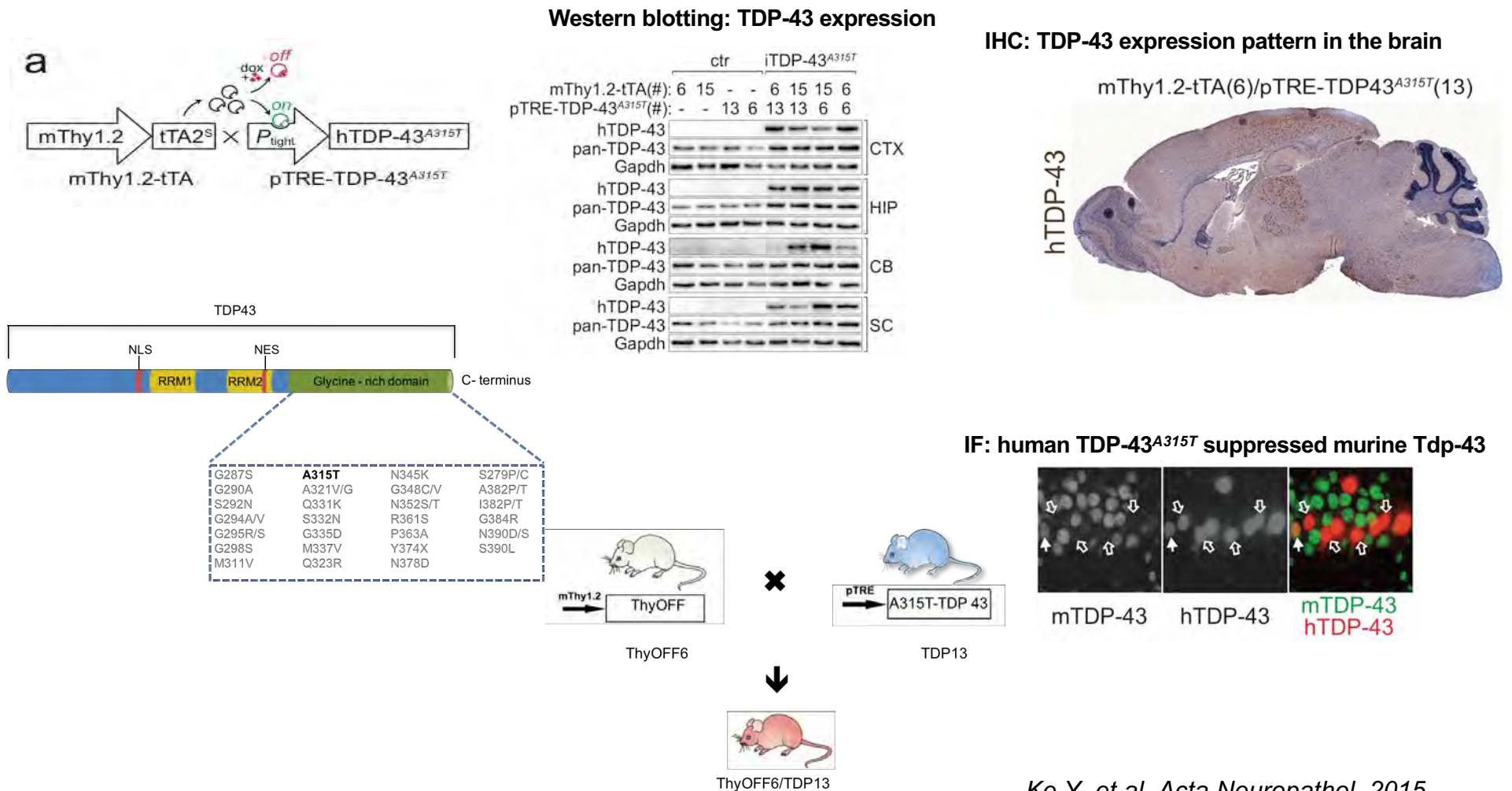
ANIMAL MODELS

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



ANIMAL MODELS

Inducible TDP-43 transgenic mouse model of FTD and ALS (*iTDP-43^{A315T}*)

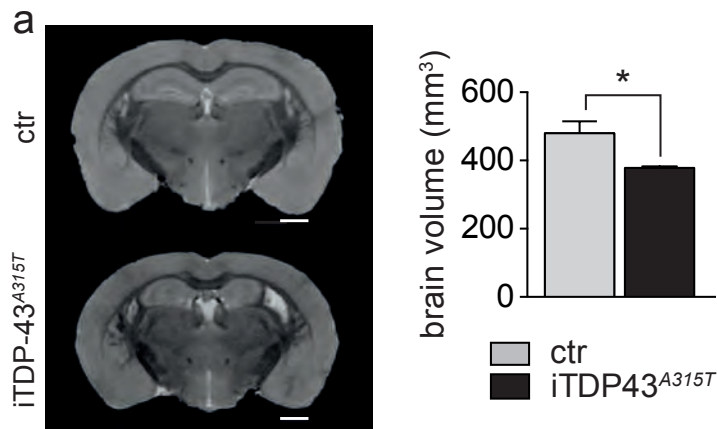


Ke Y. et al. Acta Neuropathol. 2015

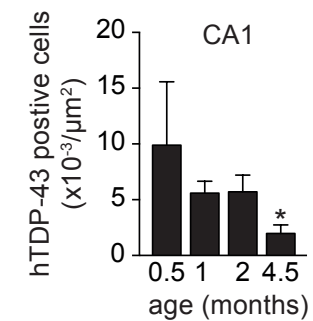
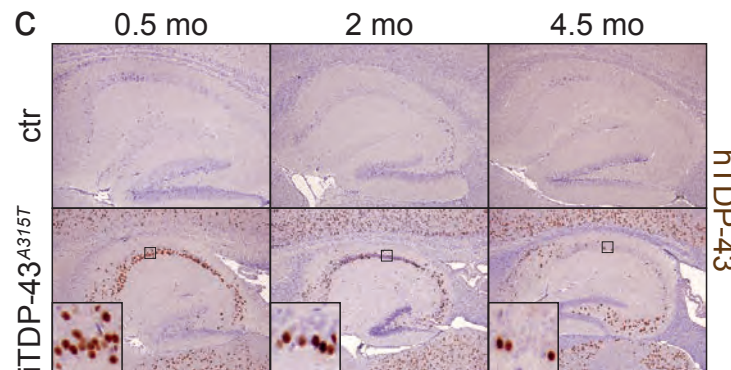
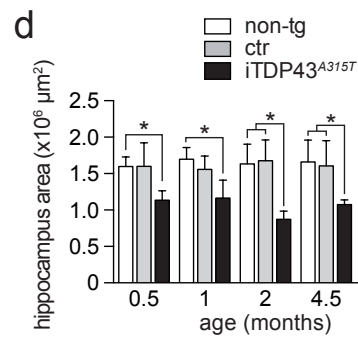
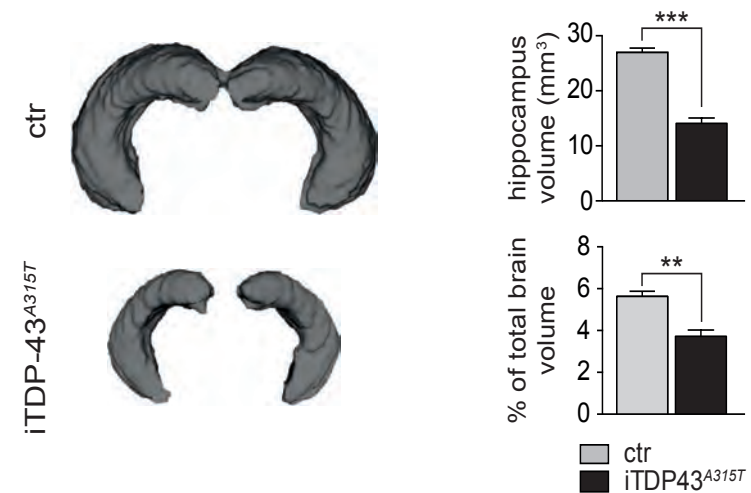
ANIMAL MODELS

Global brain and hippocampal atrophy in iTDP-43^{A315T} mice

9.4T MRI @ 6 months of age



9.4T MRI – 3D hippocampus segmentation
@ 6 months of age

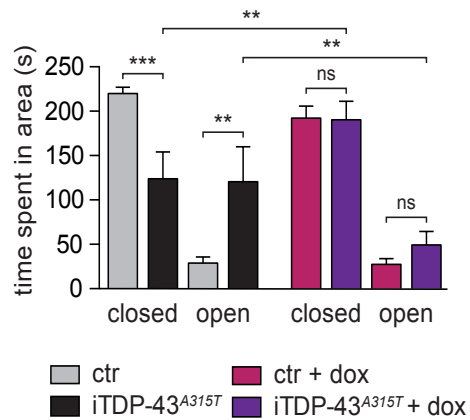


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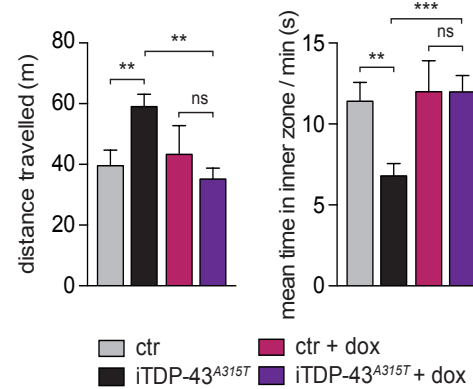
ANIMAL MODELS

Functional improvements after short-term suppression of transgene expression in iTDP-43^{A315T} mice

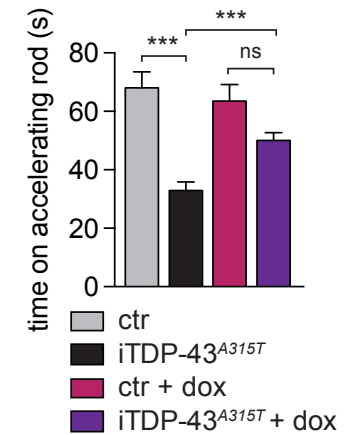
EPM: TDP-43^{A315T} depletion reverts disinhibition



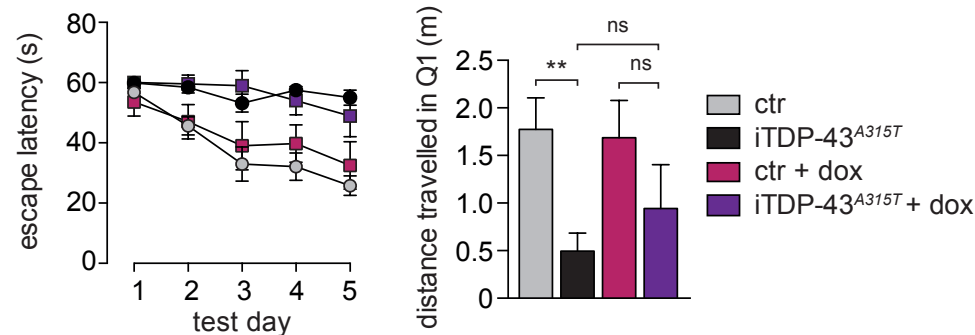
OF: TDP-43^{A315T} depletion reverts hyperactivity and abnormal exploration



Rota-Rod: TDP-43^{A315T} depletion improves motor deficits



MWM: TDP-43^{A315T} improves memory consolidation



GENOME EDITING AT MACQUARIE (GEM)

CLINICALLY RELEVANT MODELS

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)

Tyrosinase KO – Model of albinism



GENOME EDITING AT MACQUARIE (GEM)



MACQUARIE
University

CLINICALLY RELEVANT MODELS

Alzheimer / Parkinson's disease

Generation of a New Tau Knockout (tau Δ ex1) Line Using CRISPR/Cas9 Genome Editing in Mice.

J Alzheimers Dis. 2018

Accelerated aging exacerbates a pre-existing pathology in a tau transgenic mouse model.

Aging Cell 2017

Site-specific phosphorylation of tau inhibits amyloid- β toxicity in Alzheimer's mice.

Science 2016

Single Nucleotide Variants (SNVs) Define Senescence-Accelerated SAMP8 Mice, a Model of a Geriatric Condition.

J Alzheimers Dis. 2013

Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models.

Cell 2010

Sodium selenate mitigates tau pathology, neurodegeneration, and functional deficits in Alzheimer's disease models.

PNAS 2010

Experimental diabetes mellitus exacerbates tau pathology in a transgenic mouse model of Alzheimer's disease.

PLoS One 2009

ALS (= MND)

Short-term suppression of A315T mutant human TDP-43 expression improves functional deficits in a novel inducible transgenic mouse model of FTLN-TDP and ALS.

Acta Neuropathol. 2015

Developmental expression of mutant PFN1 in motor neurons impacts neuronal growth and motor performance of young and adult mice.

Front. Mol. Neurosci. 2019

Rare genetic disorders

Macrothrombocytopenia

Mutations in Tropomyosin 4 underlie a novel form of human macrothrombocytopenia.

J Clin Invest. 2017

Leukodystrophy

In vivo characterization of the aspartyl-tRNA synthetase DARS: Homing in on the leukodystrophy HBSL.

Neurobiol Dis. 2017

Canavan disease

Uncoupling N-acetylaspartate from brain pathology: implications for Canavan disease gene therapy.

Acta Neuropathol. 2018

GENOME EDITING AT MACQUARIE (GEM)



CLINICALLY RELEVANT MODELS



```

Seq_1 1 -----TCCACCGGCCTCACCTTCA
Seq_2 3241 CTCGGGGCCAAGCTGGCCTTTAAGCGCGGGGGCGCGCCTCACCTCCACCGGCCTCACCTTCA

Seq_1 20 TGGACCGCGCCGGCTCCCTCTACTGGCCCATGTCGCCCTTCCTGTCC-TGCACCACCC
Seq_2 3301 TGGACCGCGCCGGCTCCCTCTACTGGCCCATGTCGCCCTTCCTGTCC-TGCACCACCC

Seq_1 79 GCGCCAGCAGCACTTTGAGTTACAACGGGACCACGTCCGGC-----
Seq_2 3361 GCGCCAGCAGCACTTTGAGTTACAACGGGACCACGTCCGGCCTACCCAGCCACCCCATGC
    
```

c946del

GENOME EDITING AT MACQUARIE (GEM)



CLINICALLY RELEVANT MODELS

A screenshot of the Mission Massimo Foundation website. The header includes the logo "Mission Massimo Foundation" with a colorful dot pattern, and social media icons for Facebook, LinkedIn, Twitter, and YouTube. A navigation bar contains links for HOME, ABOUT, LEUKOWHAT, INITIATIVES, SPACE, PRESS, PARTNERS, and SUPPORT. The main content area is titled "MASSIMO" and contains a personal message from Massimo. The message reads: "Thanks for dropping past and visiting my website. My name is Massimo and I have a really rare (like one in a gazillion) genetic condition, with a long funny sounding name, called **Leukodystrophy**. All of a sudden, way back in July 2009, I started to lose some basic skills like crawling, cruising, sitting and even talking. I had to have all these yucky tests with needles and go into a huge loud tunnel machine to take pictures of inside my head. It was a pretty scary time but a group of extraordinary people from around the world worked tirelessly to figure out what the hell was going on and to help me overcome some of these challenges. So... if you haven't met someone like me before here's the low down. First, some technical stuff. The word Leukodystrophy comes from the Greek roots *leuko* (white), *dys* (lack of) and *troph* (growth). Leukodystrophy refers to a group of rare genetic conditions affecting the myelin of the central nervous system." To the right of the text is a photograph of a young boy, Massimo, wearing a red NASA-style space suit with a "COMMANDER" patch.

Mission Massimo Foundation

HOME ABOUT LEUKOWHAT INITIATIVES SPACE PRESS PARTNERS SUPPORT

MASSIMO

Thanks for dropping past and visiting my website.

My name is Massimo and I have a really rare (like one in a gazillion) genetic condition, with a long funny sounding name, called **Leukodystrophy**. All of a sudden, way back in July 2009, I started to lose some basic skills like crawling, cruising, sitting and even talking. I had to have all these yucky tests with needles and go into a huge loud tunnel machine to take pictures of inside my head. It was a pretty scary time but a group of extraordinary people from around the world worked tirelessly to figure out what the hell was going on and to help me overcome some of these challenges.

So... if you haven't met someone like me before here's the low down. First, some technical stuff. The word Leukodystrophy comes from the Greek roots *leuko* (white), *dys* (lack of) and *troph* (growth). Leukodystrophy refers to a group of rare genetic conditions affecting the myelin of the central nervous system.

A photograph of a young boy, Massimo, wearing a red space suit with a NASA logo and a "COMMANDER" patch. He is smiling and looking towards the camera.A screenshot of an ABC News article. The header shows the "ABC NEWS" logo, the location "Sydney, NSW", and a "Change" button. A navigation bar includes links for "Just In", "Politics", "World", "Business", "Analysis", "Sport", "Science", "Health", "Arts", and "F". Below the navigation bar are social media sharing options for Print, Email, Facebook, Twitter, and More. The main headline reads: "Massimo Damiani's family determined to finish mission and find cure for rare disease leukodystrophy". Below the headline, it says "Australian Story By Kristine Taylor" and "Updated 3 Dec 2018, 9:43pm".

ABC NEWS LOCATION: Sydney, NSW Change

Just In Politics World Business Analysis Sport Science Health Arts F

Print Email Facebook Twitter More

Massimo Damiani's family determined to finish mission and find cure for rare disease leukodystrophy

Australian Story By Kristine Taylor

Updated 3 Dec 2018, 9:43pm

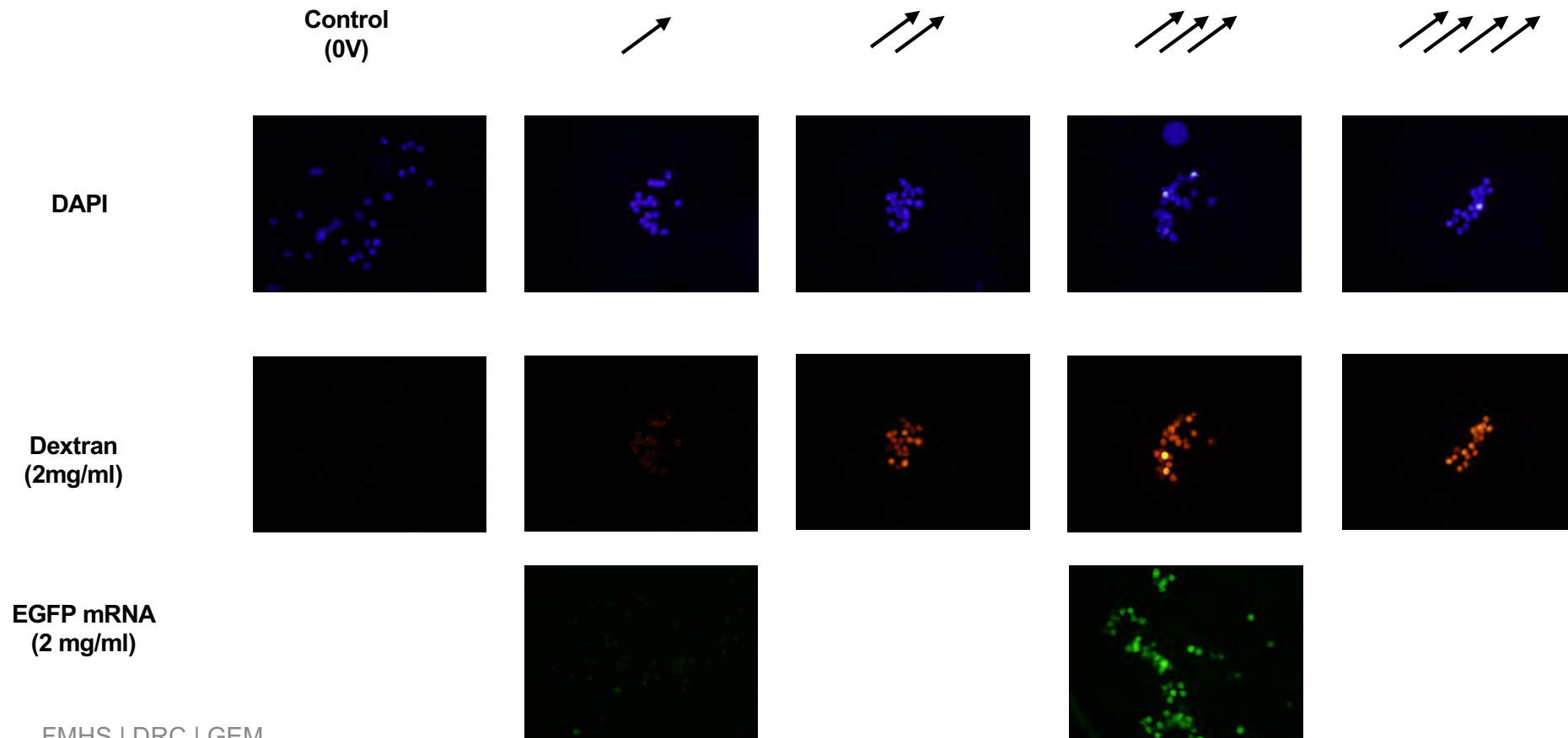
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CLINICALLY RELEVANT MODELS ACROSS SPECIES



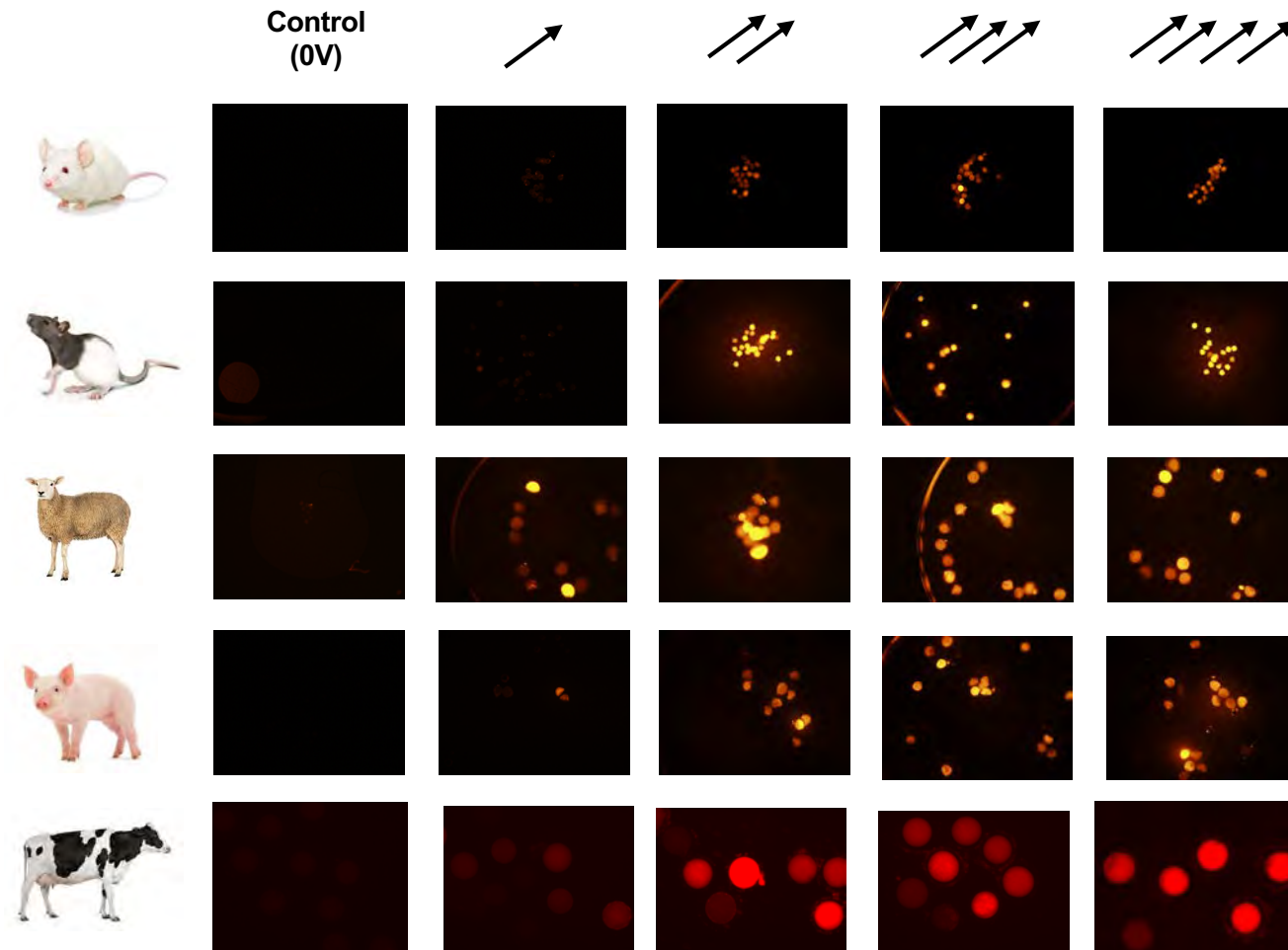
MICE



GENOME EDITING AT MACQUARIE (GEM)



CLINICALLY RELEVANT MODELS ACROSS SPECIES



GENOME EDITING AT MACQUARIE (GEM)

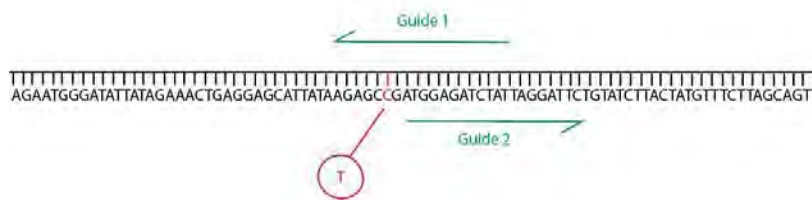


CLINICALLY RELEVANT MODELS ACROSS SPECIES

CLN7 (Batten): up to 45% KI (Point Mutations)



CLN7 gene - Batten disease (neuronal ceroid lipofuscinoses)



#	Group	Dvt	Sequencing
CLN7_1	Control	Early	WT
CLN7_2	Control	Early	WT
CLN7_3	Control	Early	WT
CLN7_4	Control	Early	WT
CLN7_5	Control	Early	WT
CLN7_6	Control	Early	WT
CLN7_7	Control	Early	WT
CLN7_8	Control	Expanded	WT
CLN7_9	Guide 1	Morula	KI
CLN7_10	Guide 1	Early	KI
CLN7_11	Guide 1	Early	
CLN7_12	Guide 1	Early	KI
CLN7_13	Guide 1	Early	
CLN7_14	Guide 1	Early	
CLN7_15	Guide 1	Early	
CLN7_16	Guide 1	Early	
CLN7_17	Guide 1	Expanded	
CLN7_18	Guide 1	Expanded	KI
CLN7_19	Guide 1	Expanded	
CLN7_20	Guide 1	Expanded	
CLN7_21	Guide 1	Expanded	
CLN7_22	Guide 1	Expanded	KI
CLN7_23	Guide 1	Expanded	
CLN7_24	Guide 1	Hatched	
CLN7_25	Guide 1	Hatched	
CLN7_26	Guide 1	Hatched	KI
CLN7_27	Guide 1	Hatched	
CLN7_28	Guide 1	Hatched	
CLN7_29	Guide 1	Early	KI
CLN7_30	Guide 1	Expanded	
CLN7_31	Guide 1	Hatched	KI
CLN7_32	Guide 1	Hatched	
CLN7_33	Guide 1	Hatched	
CLN7_34	Guide 2	Early	KI
CLN7_35	Guide 2	Early	
CLN7_36	Guide 2	Early	KI
CLN7_37	Guide 2	Hatched	KI
CLN7_38	Guide 2	Hatched	
CLN7_39	Guide 2	Hatched	KI
CLN7_40	Guide 2	Hatched	
CLN7_41	Guide 2	Early	KI
CLN7_42	Guide 2	Early	
CLN7_43	Guide 2	Early	
CLN7_44	Guide 2	Early	KI
CLN7_45	Guide 2	Early	
CLN7_46	Guide 2	Early	
CLN7_47	Guide 2	Early	KI
CLN7_48	Guide 2	Expanded	
CLN7_49	Guide 2	Expanded	KI
CLN7_50	Guide 2	Expanded	KI
CLN7_51	Guide 2	Expanded	
CLN7_52	Guide 2	Expanded	
CLN7_53	Guide 2	Expanded	
CLN7_54	Guide 2	Expanded	
CLN7_55	Guide 2	Expanded	
CLN7_56	Guide 2	Hatched	
CLN7_57	Guide 2	Hatched	KI
CLN7_58	Guide 2	Hatched	KI
CLN7_59	Guide 2	Hatched	KI
CLN7_60	Guide 2	Hatched	
CLN7_61	Guide 2	Hatched	KI
CLN7_62	Guide 2	Morula	

Control

Guide 1

Guide 2



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 Lee¹, Keiichiro Suzuki³, Amy Koski¹, Dongmei Ji¹, Tomonari Hayama¹, Riffat Ahmed¹, Hayley Darby¹, Crystal Van Dyken¹, Ying Li¹, Eunju Kang¹, A.-Reum Park², Daesik Kim⁴, Sang-Tae Kim², Jianhui Gong^{5,6,7,8}, Ying Gu^{5,6,7}, Xun Xu^{5,6,7}, David Battaglia^{1,9}, Sacha A. Krieg⁹, David M. Lee⁹, Diana H. Wu⁹, Don P. Wolf¹, Stephen B. Heitner¹⁰, Juan Carlos Izpisua Belmonte^{3,§}, Paula Amato^{1,9,§}, Jin-Soo Kim^{2,4,§}, Sanjiv Kaul^{10,§} & Shoukhrat Mitalipov^{1,10,§}

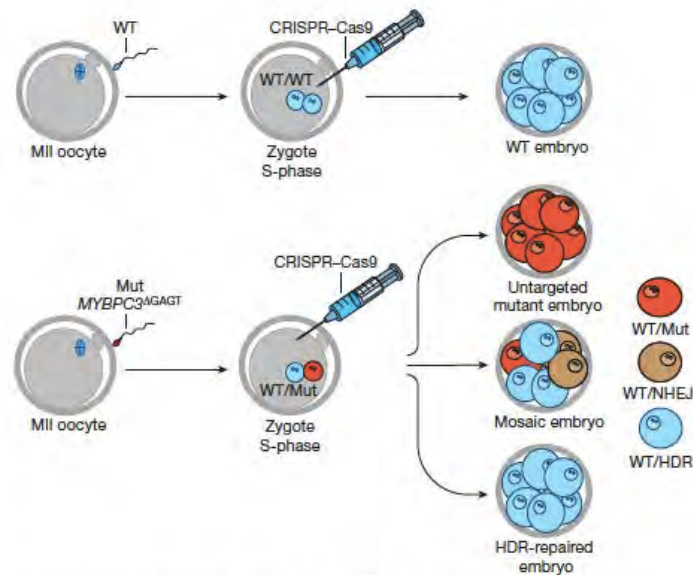
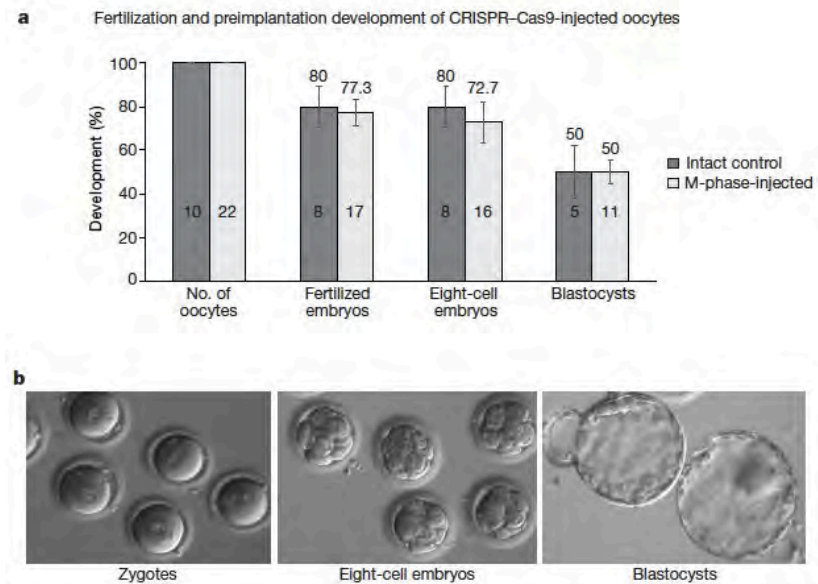


Figure 1 | Gene correction in S-phase-injected human embryos.





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Dr Kate Kinlan
Dr Yann Gambin



Prof Imke Tammen
A/Prof Chris Gruppen
Prof Glenda Halliday
Dr Nicolas Dzanko
Dr Anthony Ashton



Dr Shondra Miller



Prof Charles Long



Prof Dianne Fatkin



A/Prof Estelle Sontag



Prof Mark McKeage

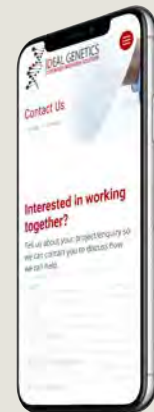


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