Generation of Genetically Engineered Rodent Models

TO CRISPR OR NOT TO CRISPR ?

That is the question !

Jimmy MANCIP

À CHAQUE ETAPE DU PROCESSUS

TO CRISPR OR NOT TO CRISPR, THAT IS THE QUESTION:

Questions to Answer Regarding Intellectual Property (IP), Technical Issues, & the 3Rs

Does CRISPR allow for the generation of relevant models?

Is it compatible with the goals of the 3Rs?

CRISPR/Cas9 IP/license constraints: how does it impact your project?

CHARLES RIVER
CUSTOM RODENT MODEL GENERATION SERVICES

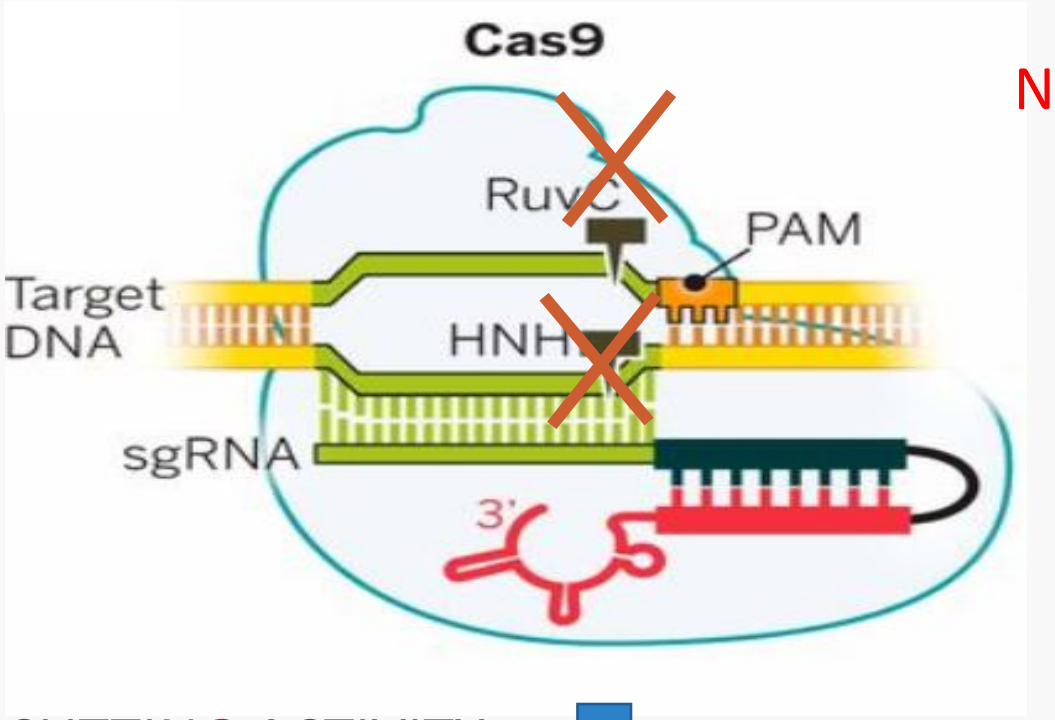
Charles River's global partnerships for *in vivo* model creation:

Phenomin ICS, IMPC member and leader in the field of mouse & rat model creation and phenotyping.

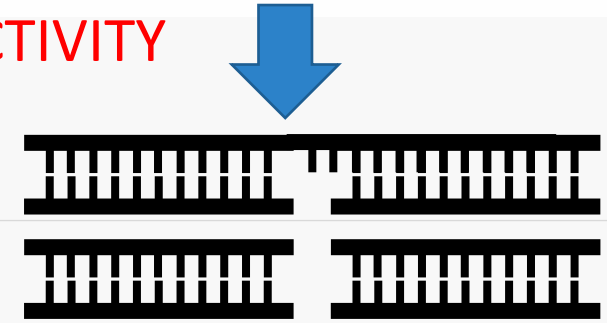
Mirimus, a leader in RNAi interference technology specializing in the creation of customized genetically engineered mouse models.

Laboratory Animal Resource Center, University of Tsukuba, one of Japan's largest production institutes for genetically modified mice.

STREPTOCOCCUS PYOGENES CAS9
A REMARKLY FLEXIBLE TOOL



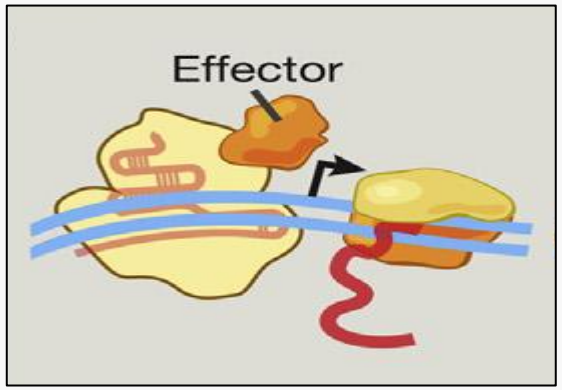
CUTTING ACTIVITY



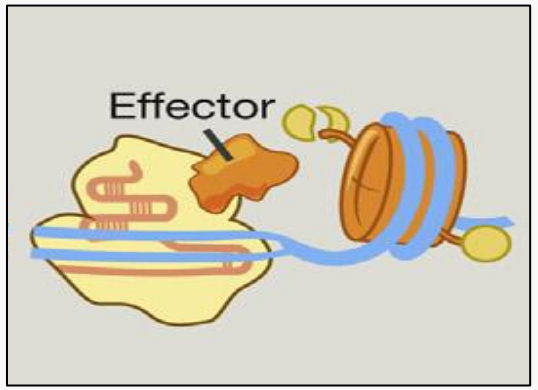
NON CUTTING ACTIVITY

deadCas9 protein

- Mutations in RuvC1 and HNH nuclease domains
- dCas9 retains the ability to target specific sequences through the sgRNA and PAM.



Transcriptional Control



Epigenetic Modulation

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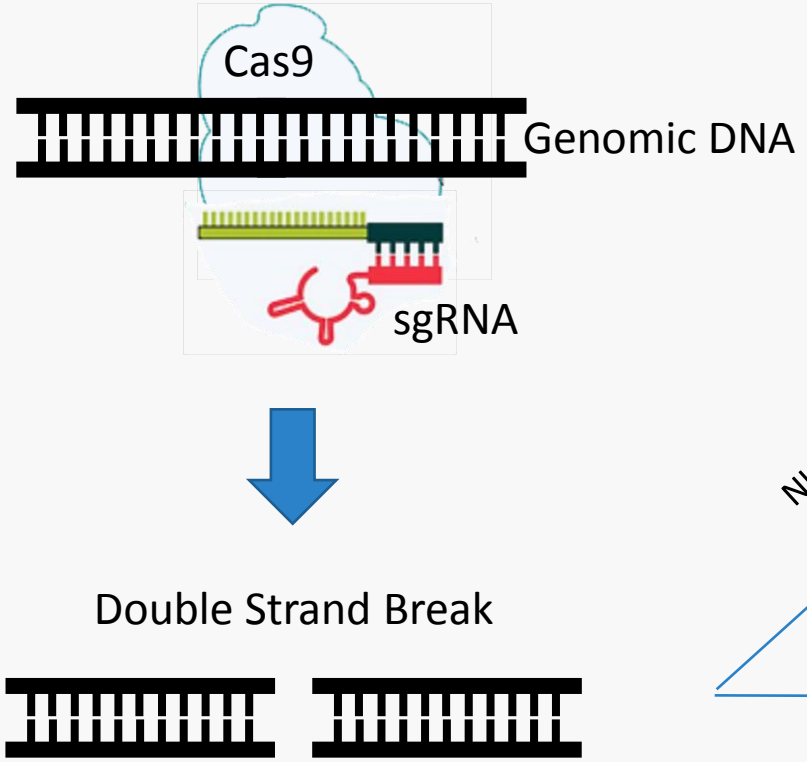
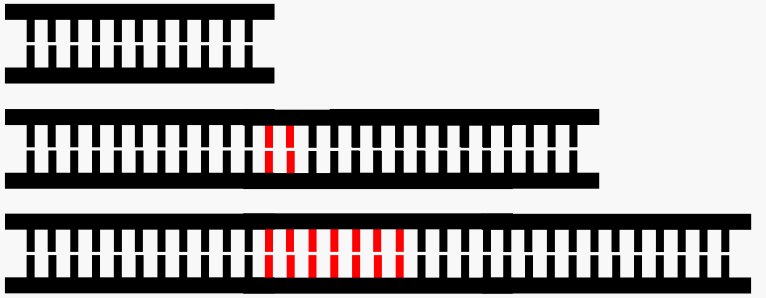
CRISPR/CAS9 FOR GENE EDITING

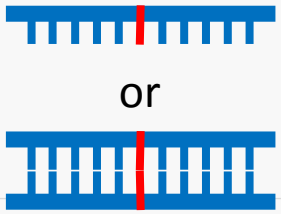


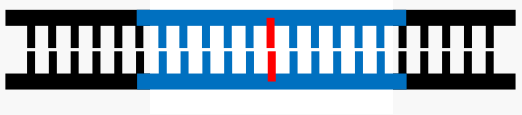
NHEJ : Non homologous end-joining repair



Gene disruption by small insertions or deletion

Homologous Directed Repair





Precise insertion at target locus

SYSTEM IS SO EFICIENT THAT IT CAN BE USED IN PREIMPLANTATION EMBRYOS

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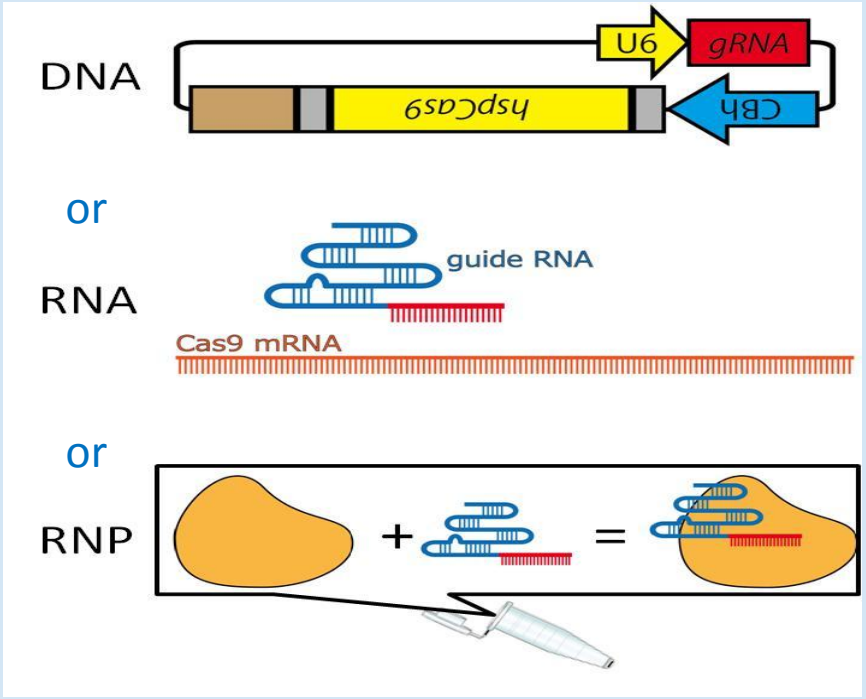
ADAPTATION OF CRISPR-CAS9 FOR RODENT MUTANT PRODUCTION

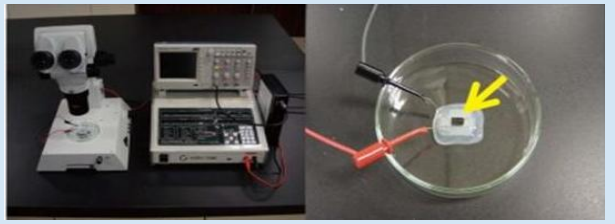
MATERIAL TO INJECT



DELIVERY ROUTE

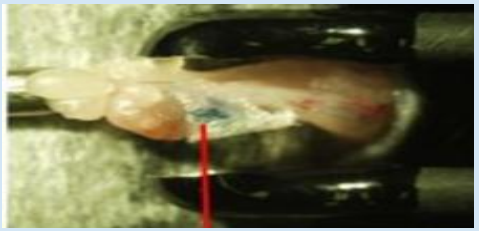
Microinjection

or



Electroporation
in vitro

or



Electroporation
in vivo (GONAD)

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CRISPR/CAS9 GENE EDITING IN EMBRYOS
THE NEW GOLD STANDARD FOR RODENT MODEL GENERATION ?

CRISPR

QUICK

EASY

INEXPENSIVE

'Any idiot can do it.' Genome editor CRISPR could put mutant mice in everyone's reach

By Jon Cohen | Nov. 3, 2016 , 10:00 AM

https://www.sciencemag.org

Is mouse embryonic stem cell technology obsolete?

William C Skarnes

Genome Biol. 2015; 16(1): 109.
Published online 2015 May 27. doi: 10.1186/s13059-015-0673-6

Thus, the end of mouse ES cell technology now seems inevitable.

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CRISPR/CAS9 PERFORMANCE FOR RODENT MODEL PRODUCTION

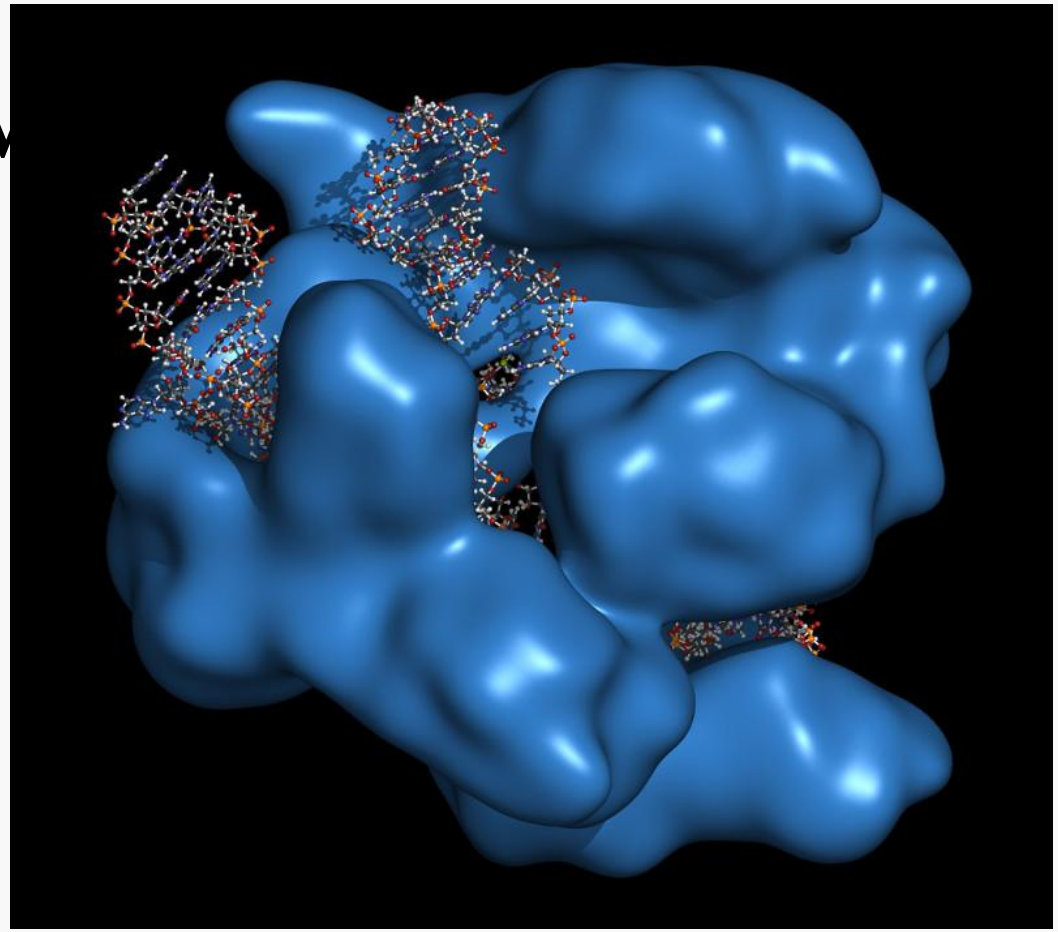
- **ADVERTIZED CRISPR "BONUSES" SHOULD BE CAREFULLY VERIFIED !**
- **ONLY FACTS MATTER !**
- **THE TECHNOLOGY SHOULD BE ASSESSED FROM VARIOUS ANGLES**

- MODEL RELEVANCY & TIMELINES
- 3Rs
- INTELLECTUAL PROPERTY

TO CRISPR OR NOT TO CR

Model Relev

- Species ?
- Genetic backgrounds ?
- Model types ?
- Additional mutations ?
- Timelines ?



MODEL RELEVANCY

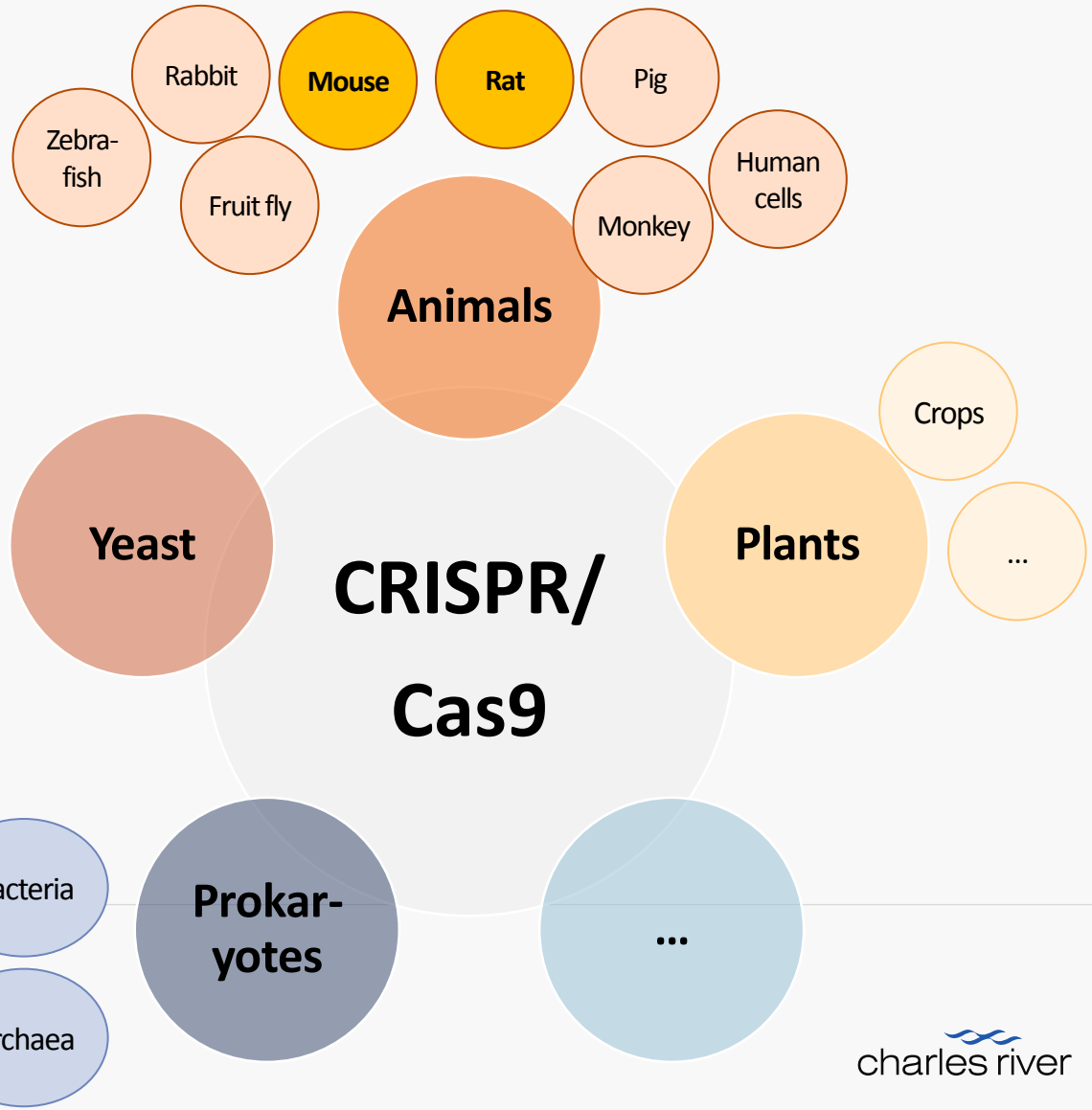
EXTENDING TO A LARGE PANEL OF SPECIES

Not an exhaustive list

The only limits of CRISPR/Cas9 genome editing are the ability to make the system enter germinal cells

RAT FOCUS

- Most efficient tool today to generate rat models
- *In vitro* (microinjection and electroporation) as well as *in vivo* CRISPR/Cas9 gene editing (GONAD technology) have been successful in rats
- In rats, the technology suffers from same limitations as in mice



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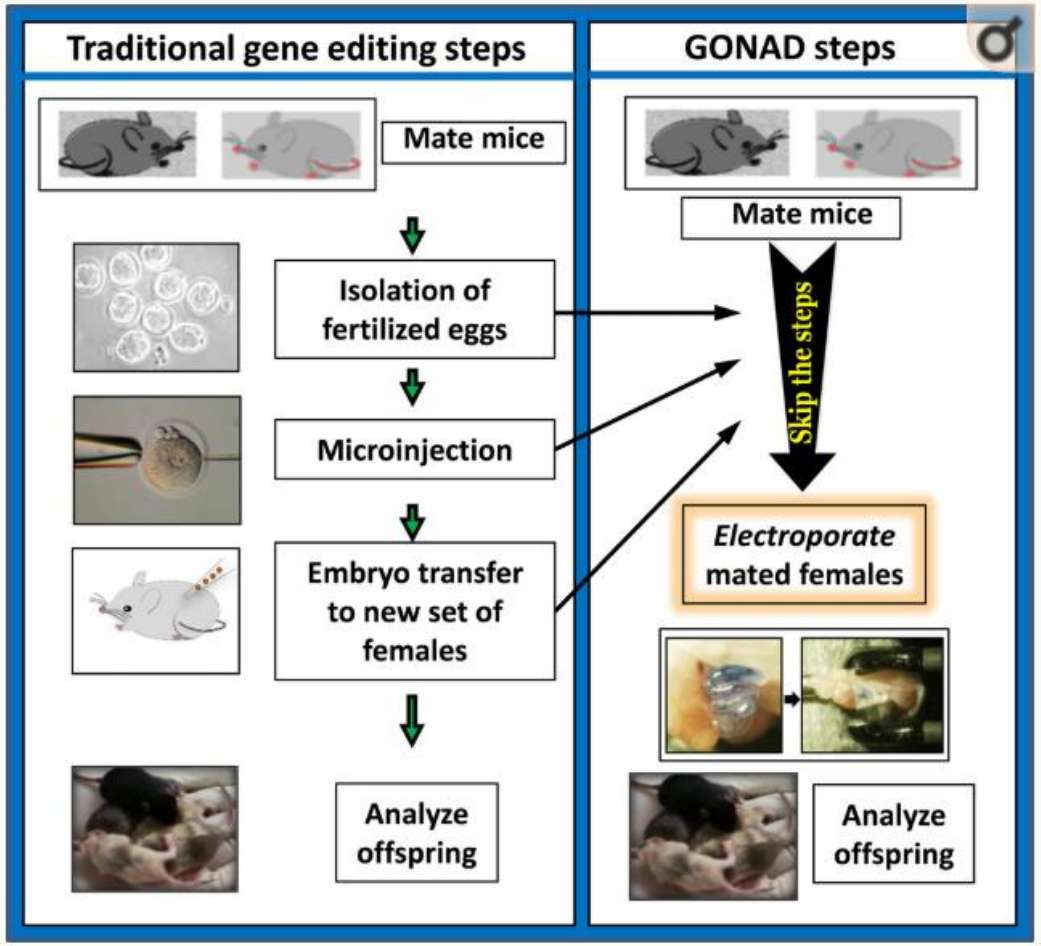
CRISPR/CAS9 GIVES DIRECT ACCESS TO A WIDE RANGE OF GENETIC BACKGROUNDS AND STRAINS

- Any strain from which pre-implantation embryos can be isolated and manipulated *in vitro* is amenable to a CRISPR gene editing approach
- *In vivo* CRISPR gene editing (injection of CRISPRCas9 into oviducts of mated female) may even extend the technology to « difficult » strains
- Wide range of mouse or rat strains can thus be used, allowing the generation of models on defined genetic backgrounds without a backcross step
  - ✓ Diverse inbred genetic background
  - ✓ Mutant strains
  - ✓ Disease model strains



Gurumurthy et al. Curr Protoc Hum Genet. ; 88: 15.8.1–15.8.12.

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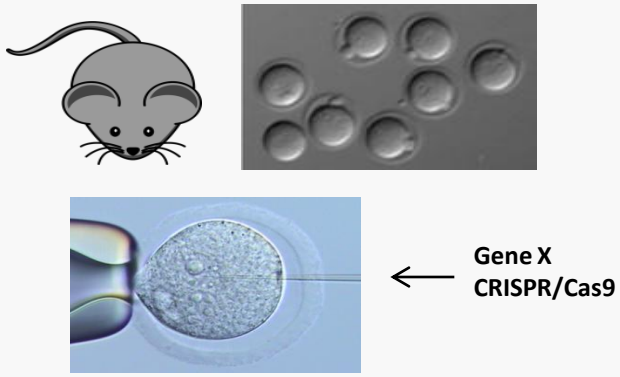
GENE EDITING IN ANIMAL DISEASE MODELS

NOD/ShiLtJ



NOD/ShiLtJ
Gene X KO

CRISPR/Cas9-mediated gene editing for 10 genes delivered by microinjection

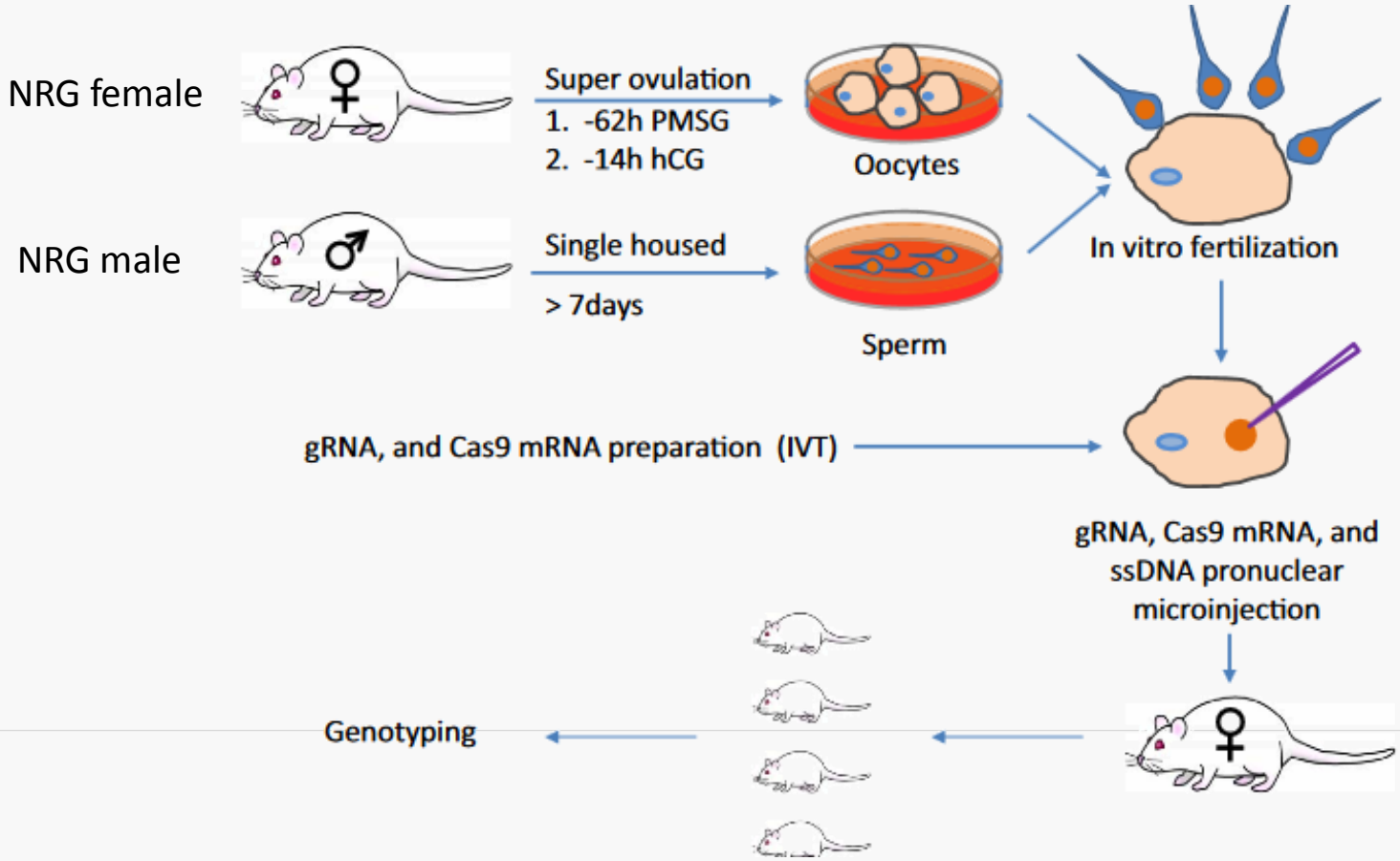
	Microinjection (NOD/ShiLtJ)			
Target gene	**Embryos transferred**	**Mice born**	**Mutant mice**	**Mutant percentage (%)**
Cd69	59	23	0	0
Cd226	61	19	4	21
Clec16a	57	0	NA	NA
Cyp27b1	64	23	12	52
Fut2	64	25	7	28
Ormdl3	62	19	17	89
Rgs1	62	18	8	44
Tlr7	66	22	6	27
Tlr8	60	15	1	7
Tnfsf9	61	21	15	71
Total	616	185		Live birth rate 30%

Qin *et al.* Genetics 2015

NOD/ShiLtJ: Non Obese diabetic, polygenic model for type 1 diabetes

CRISPR/CAS9 GENOME EDITING IN NON-STANDARD GENETIC BACKGROUNDS

Getting fertilized eggs or embryos from NRG mice through natural mating is difficult due to lack of plugging and fertilization by NRG males



Fah KO on immunodeficient NOD-SCID-IL2RgammaC-null (NRG) mutant

Li *et al.* 2016 Scientific reports

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MODEL RELEVANCY

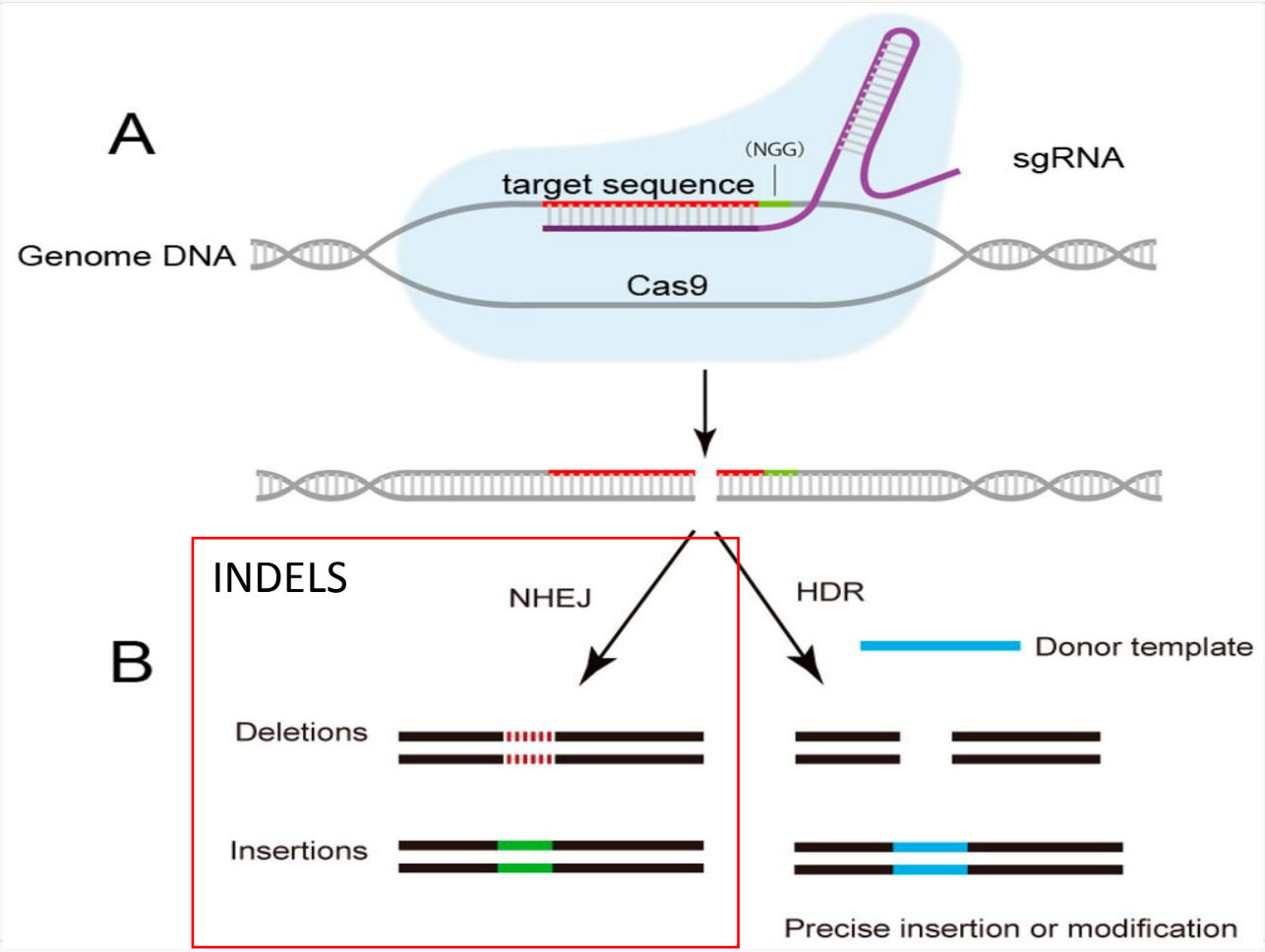
GENE EDITING EFFICIENCY

Perfect seek and destroy tool !

Very high efficiency !

Type of generated mutations depends mainly on repair mechanisms of the cell -> we have poor control !

INDELS are the most frequent observed mutations



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CRISPR/CAS9 STILL SHOWS OBVIOUS LIMITATIONS FOR KIN MODELS

- CRISPR/Cas9 in mouse & rat embryos works extremely well for the generation of simple alleles such as constitutive knock-out and knock-in using donor DNA less than 2-3kb (SNPs/tags, small reporter molecules)

- In mice, it is not the technology of choice for the introduction **in embryos** of complex modifications relying on homologous recombination for larger regions (ex: paired loxP site and large cDNAs/transgenes)

- In rats, there are no other options (ES cell) so still worth trying complex Kin models but with potential risks of failure and extended timelines and budget !

- Promising recent data about Easi-CRISPR technology (long single stranded DNA) indicate that CRISPR limitation towards some Kins models may be overcome in the future

ALTERNATIVE APPROACH: CRISPR/CAS9 IN ES CELL

- CRISPR/Cas9 boosts HR in ES cells: 100% targeting success whatever the locus (Phenomin-iCS)
- **Up to 40 kb fragment replacement achieved (Phenomin-iCS)**
- **Opens new possibilities for humanization of large genomic regions in mice**

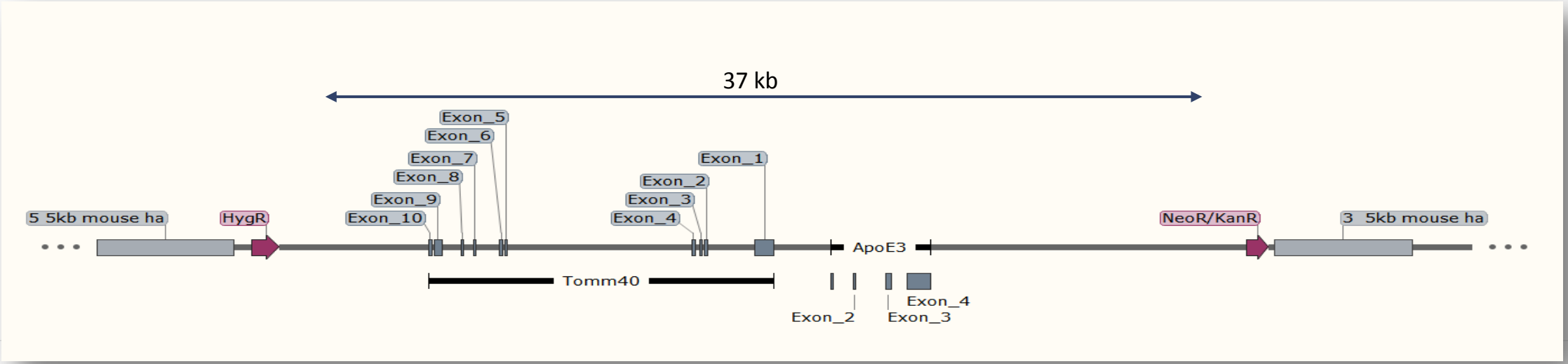
Project	DNA matrix	positives clones by LR-PCR/clones screened	positives clones by LR-PCR/clones screened	% positive
		without CRISPR/Cas9	+ CRISPR/Cas9	
1	linear vector	0/543	16/372	4.3%
2	circular vector	0/586	78/141	55.3%
3		0/586	46/186	24.7%
4		0/1560	26/158	16.5%
5		0/85	165/186	88.7%
6		ND	131/159	82.4%
7		ND	44/93	47.3%
8		ND	154/163	94.5%
9		0/29	13/36	30.5%
10		0/333	15/93	16.1%

Rescue of failed projects with the help of CRISPR/cas9

PHENOMIN-ICS unpublished results

CASE STUDY: HUMANIZATION OF LARGE LOCUS IN MICE

- ✓ Replacement of ApoE mouse gene by ApoE2, ApoE3 and ApoE4 human variants including Tomm40 gene
- ✓ Full replacement of a 37 kb sequence achieved in C57BL/6N ES cells using Neo/Hygro + CRISPR/Cas9 selection
- ✓ 4 variants achieved (5.2%±0.9% positive/screened clones)



PHENOMIN-ICS unpublished results

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MODEL RELEVANCY

ADDITIONAL MUTATIONS – OFF TARGET EVENTS

Off target mutations (cut outside target sequence)

- ✓ Rare when CRISPR/cas9 is used *in vivo* - mouse and rat and other species too…
- ✓ Proper experiment design reduces the risk
- ✓ Line breeding to segregate additional mutations further reduces the risk

RISK is very limited for *in vivo* models if proper experiment design is followed

No unexpected CRISPR-Cas9 off-target activity revealed by trio sequencing of gene-edited mice

Vivek Iyer, Katharina Boroviak, Mark Thomas, Brendan Doe, Laura Riva, Edward Ryder, David J. Adams

Exome sequencing in the knockin mice generated using the CRISPR/Cas system

Kazuo Nakajima, An-a Kazuno, John Kelsoe, Moe Nakanishi, Toru Takumi & Tadafumi Kato

Nat Methods. 2015 Jun;12(6):479. doi: 10.1038/nmeth.3408.

Off-target mutations are rare in Cas9-modified mice.

Iyer V[1], Shen B[2], Zhang W[3], Hodgkins A[1], Keane T[1], Huang X[2], Skarnes WC[1].

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MODEL RELEVANCY

ADDITIONAL MUTATIONS AT TARGET LOCUS

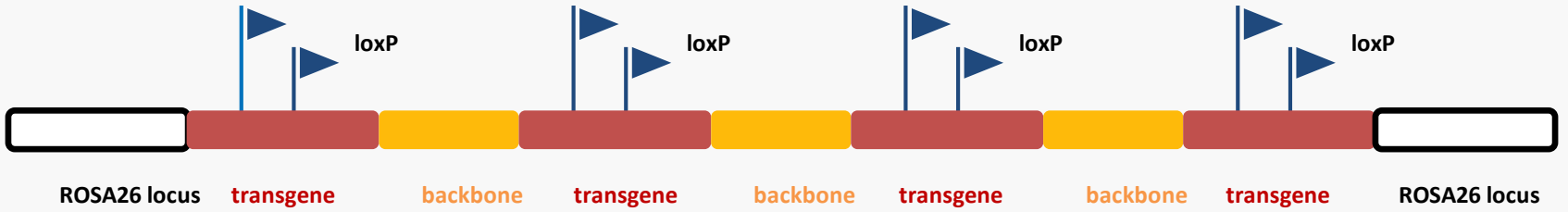
Unwanted mutational events at the target locus can frequently be observed !

- Double DNA strand break at target locus following CRISPR/Cas9 and cell repair mechanism can be at the origin of a series of additional mutational events
- *In vivo* genetic screening strategy should be highly relevant for detection of all types of mutational events
- Mosaicism in founder mice (F0) adds extra genetic complexity rendering identification of target and additional mutations challenging

ADDITIONAL MUTATIONS AT TARGET LOCUS

Multiple integration at target site - CASE STUDY (Phenomin-ICS)

- ROSA26 locus offers an open chromatin region well suited for expressing cDNAs
- ROSA26 has been extensively used in ES cells
- Case study: CRISPR/Cas9 was used to target ROSA26 locus with homologous recombination vector



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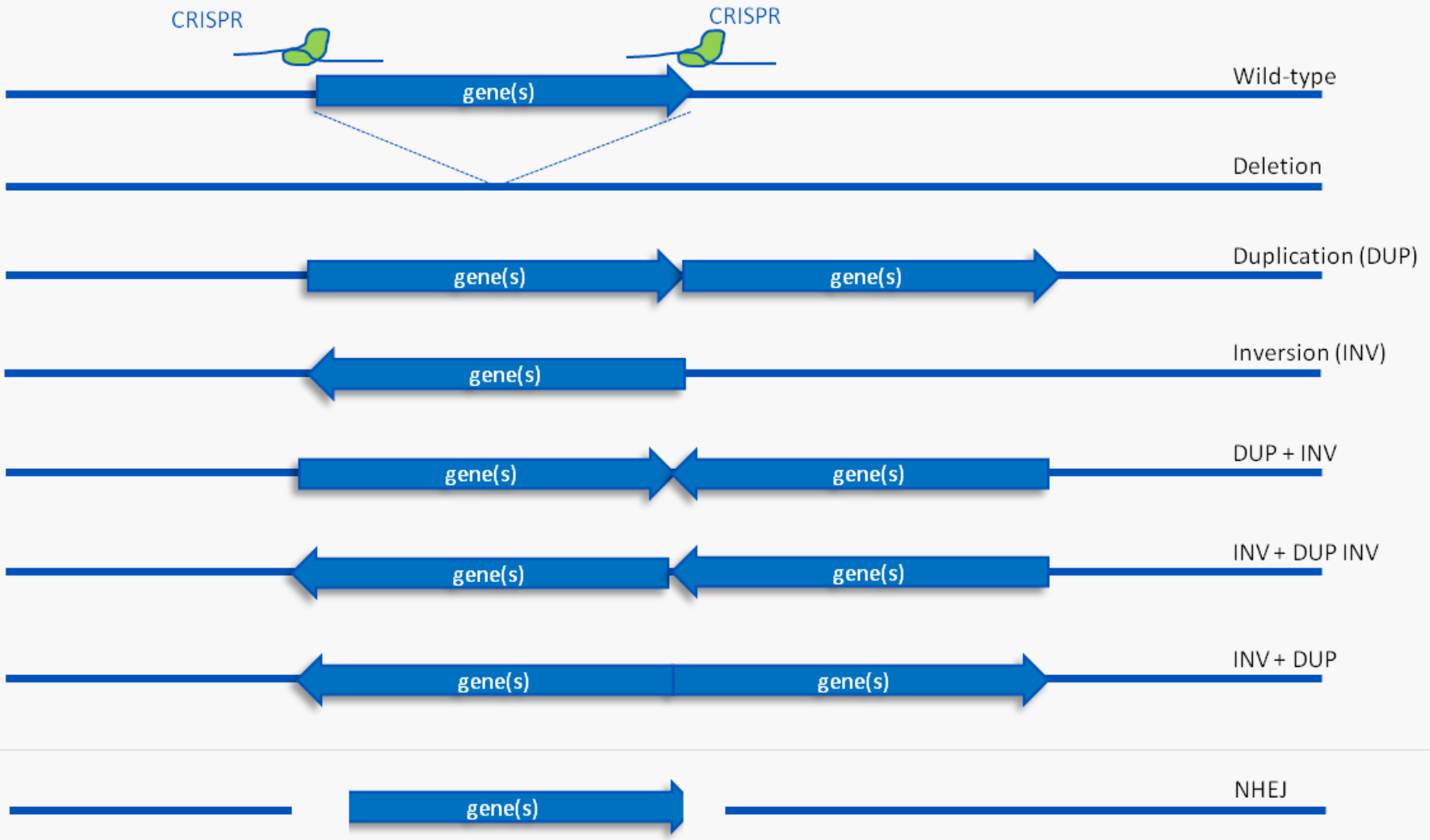
ADDITIONAL MUTATIONS AT TARGET LOCUS

LARGE GENOMIC REARRANGEMENTS



CRISPR/Cas9 is generating structural variation at target locus

Observed frequently in mice and rat

Can be used to generate relevant models of human diseases

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UNDERSTANDING HUMAN STRUCTURAL VARIATIONS LEADING TO DISEASES

✓ Examples of syndromes in Human

- Viable trisomy
  - Trisomy 21
  - Trisomy 18
  - Trisomy 13
  - Klinefelter syndrome (XXY)

- Microdeletions and microduplications
  - 1p36 microdeletion syndrome
  - 1p21.1 recurrent microdeletion and microduplication syndrome
  - 3q23 microdeletion and microduplication syndrome
  - 7q11.23 duplication syndrome and Williams-Beuren syndrome
  - Angleman syndrome /Prader-Willi syndrome
  - 16p11/2-12.2 microdeletion syndrome
  - 16p13.11 recurrent microdeletion and microduplication syndrome
  - Potock-Lupski syndrome, Smith-Magenis syndrome

HOW GENOME EDITING MAKES IT POSSIBLE TO GENERATE MODELS OF STRUCTURAL VARIATION DISEASES

SCIENTIFIC REPORTS

Article | OPEN | Published: 07 March 2017

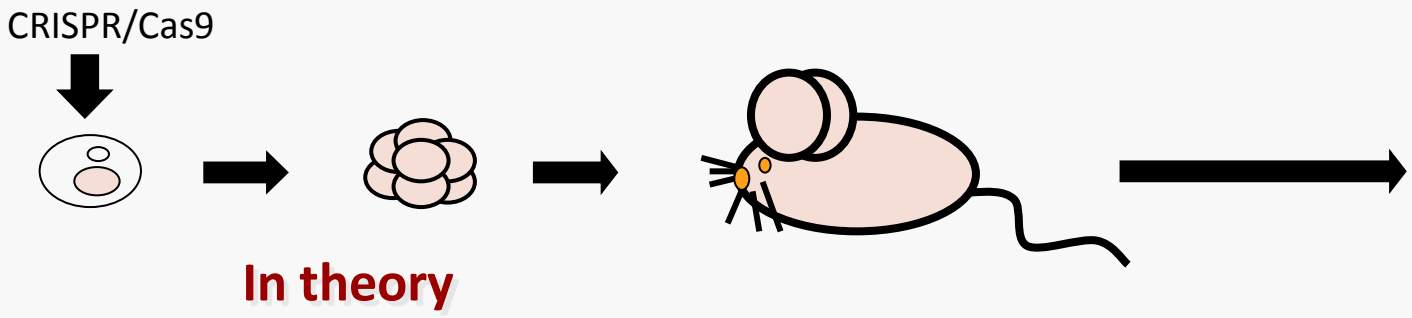
Efficient and rapid generation of large genomic variants in rats and mice using CRISMERE

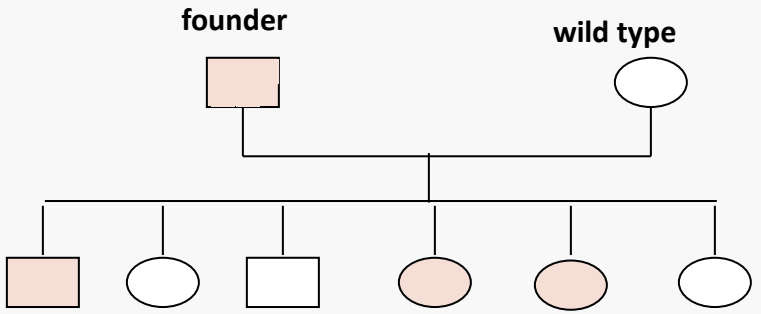
Marie-Christine Birling, Laurence Schaeffer, Philippe André, Loic Lindner, Damien Maréchal, Abdel Ayadi, Tania Sorg, Guillaume Pavlovic & Yann Hérault

Scientific Reports **7**, Article number: 43331 (2017) | Download Citation

MOSAICISM LEADS TO COMPLEX GENOTYPES IN F0 GENERATION

THEORY (frequently advertised): One-step generation of mice





F1 are heterozygotes of the same allele

Cell

Volume 153, Issue 4, 9 May 2013, Pages 910–918

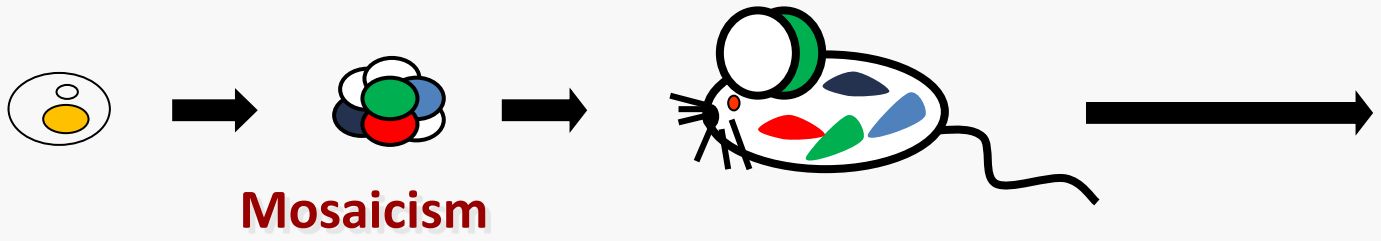
Resource

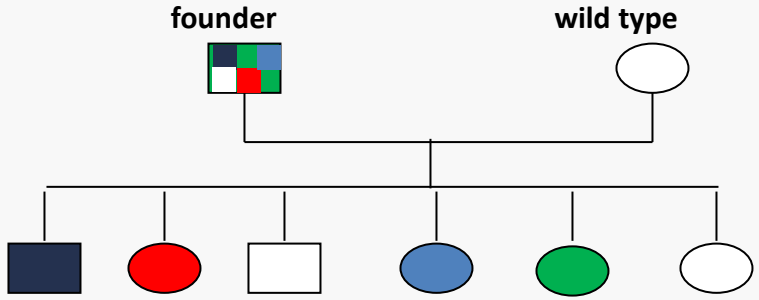
One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering

Haoyi Wang[1, 6], Hui Yang[1, 6], Chikdu S. Shivalila[1, 2, 6], Meelad M. Dawlaty[1], Albert W. Cheng[1, 3], Feng Zhang[4, 5], Rudolf Jaenisch[1, 3],

MOSAICISM & SERIES OF ALLELIC VARIANTS

REALITY: mice & rats born from injection of CRISPR/Cas9 are mosaic





F1 heterozygote of different alleles

Tyr locus mutation

- Completely albino and a wide range of pigmentation mosaic founders were generated
- Deep sequencing showed that most founders had >2 new mutant alleles

Yen *et al.*, 2014, Dev Biol

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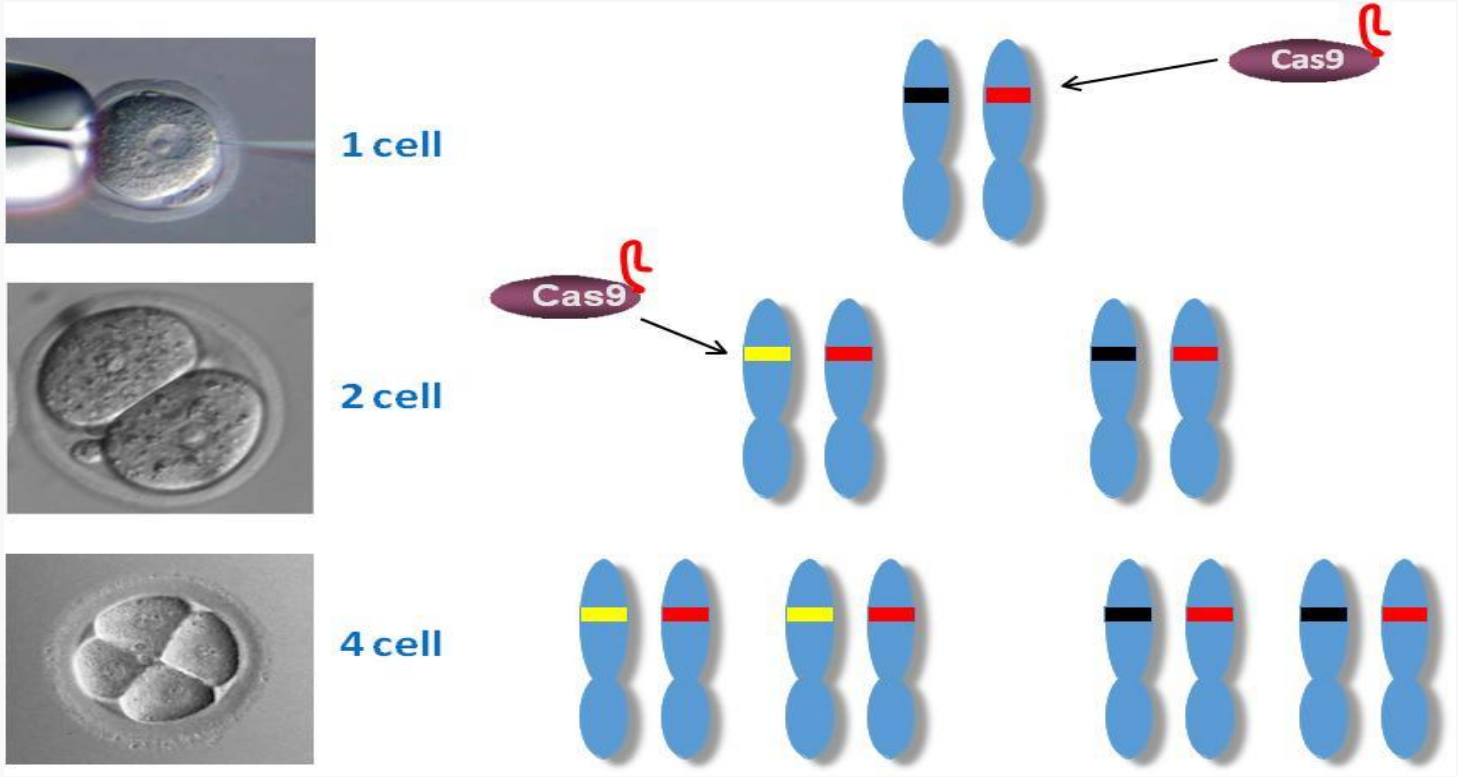
CAS9-INDUCED MOSAICISM IN FOUNDER ANIMALS



http://jackson.jax.org/rs/444-BUH-304/images/LT0071_CRISPR_whitepaper_WEB.pdf

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MOSAICISM IMPACT ON MODEL DEVELOPMENT

- Many **different allele variants** can be found in one single founder
- F0 are not established lines
  - ✓ Presence of several alleles in same animal
  - ✓ No phenotyping at F0 stage
- Breeding to F1 generation is still mandatory
  - ✓ To be included in budget and timelines
  - ✓ Breeding to F1 generation is still mandatory

MODEL RELEVANCY

TIMELINES

Ex: constitutive KO or SNP mutation models

ES	WEEKS		CRISPR
VECTOR	10	4	gRNA +/- ssODN
RECOMBINANT CLONES	10		
INJECTION	10	10	INJECTION
CHIMERA BREEDING	11	11	FOUNDERS BREEDING
HOMOZYGOUS GENERATION	11	11	HOMOZYGOUS GENERATION
	52	**36**	

- About 30% timeline reduction for the generation of basic modified alleles with CRISPR/cas9 approach
- What about complex mutations, large Kins ?

Advantage of CRISPR/Cas9 approach tends to vanish as complexity of model increases but for structural variants and copy number variation models

3Rs

- Replace
- Refine
- Reduce

RODENT MODEL GENERATION VIA CRISPR/CAS9 AND THE 3RS

3Rs

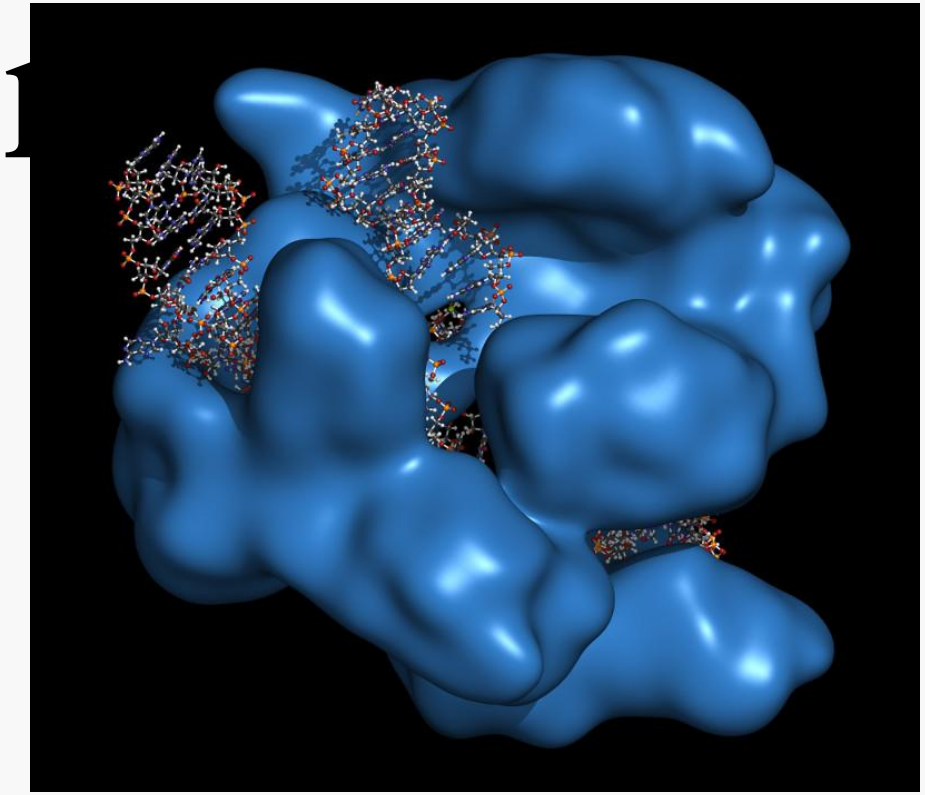
1. **Replacement:** methods which avoid or replace the use of animals in research
2. **Reduction**: use of methods that enable researchers to obtain comparable levels of information from fewer animals, or to obtain more information from the same number of animals.
3. **Refinement:** use of methods that alleviate or minimize potential pain, suffering or distress, and enhance animal welfare for the animals used.

REDUCE

Major contributions of CRISPR/Cas9 to the 3Rs:

- Access to wider range of genetic backgrounds and strains -> abolish the need for backcrossing the generated line to genetic background of interest
  - ✓ 400 mice are used on average during a standard backcross (10 generations)
  - ✓ 200 for accelerated backcross (5 generations, SNPs selection of breeders)
- Very high germ line transmission of mutation
  - ✓ CRISPR: 3-4 founders are sufficient
  - ✓ ES: at least 6-8 chimeric males (from 2 independent clones) are required

Intellectual Pr



- Historic battle…
- Current landscape
- Potential impact on your *in vivo* research

A BATTLE BETWEEN ACADEMIC RESEARCH INSTITUTIONS

US appeals court -> lost

Berkeley's disclosure of using CRISPR-Cas9 in prokaryotes did not prevent Broad from patenting its use in eukaryotes

Vilnius patent provides CRISPR Therapeutics and Intellia with complementary rights to inventions claimed by the earliest developers in the discovery and application of CRISPR/Cas9 technology.

US20140068797A1
US Application

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Inventor: Jennifer A. DOUDNA, Martin Jinek, Emmanuelle Charpentier, Krzysztof Chylinski, James Harrison Doudna Cate, Wendell Lim, Lei Qi

Current Assignee : Universitaet Wien , University of California

Original Assignee: Universitaet Wien, University of California

Priority date : 2012-05-25 May 2012

Family: US (22) EP (2) JP (2) KR (2) CN (2) CA (1) DE (3) DK (2) ES (2) GB (3) WO (1)

Date	App/Pub Number	Status
2013-03-15	US13842859	Pending
2014-03-06	US20140068797A1	Application

US8697359B1
US Grant

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Inventor: Feng Zhang

Current Assignee : Massachusetts Institute of Technology , Broad Institute Inc

Original Assignee: Massachusetts Institute of Technology, Broad Institute Inc

Priority date : 2012-12-12 December 2012

Family: US (7) EP (2) JP (1) KR (1) CN (2) CA (1) DK (1) ES (1) WO (1)

Date	App/Pub Number	Status
2013-10-15	US14054414	Active
2014-04-15	US8697359B1	Grant

US9637739B2
US Grant

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Inventor: Virginijus {hacek over (S)}ik{hacek over (s)}nys, Giedrius Gasiunas, Tautvydas Karvelis

Current Assignee : Vilniaus Universitetas

Original Assignee: Vilniaus Universitetas

Priority date : 2012-03-20

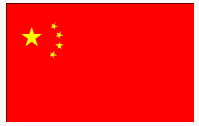
Family: US (5) EP (1) CN (1) CA (1) WO (1)

Date	App/Pub Number	Status
2013-03-15 March 2012	US14385241	Active
2015-02-12	US20150045546A1	Application
2017-05-02	US9637739B2	Grant
Show 3 more applications		
2017	US15834578	Pending

CRISPR IP CURRENT LANDSCAPE

- On 10 September 2018, the US Court of Appeals for the Federal Circuit held the use of CRISPR-Cas9 in eukaryotes, as claimed by the Broad Institute of MIT and Harvard in Cambridge, Massachusetts, was patentable over Doudna's and Charpentier's use of CRISPR-Cas9 *in vitro*
- This means both the Broad group and the Berkeley group can separately license their technologies in the US

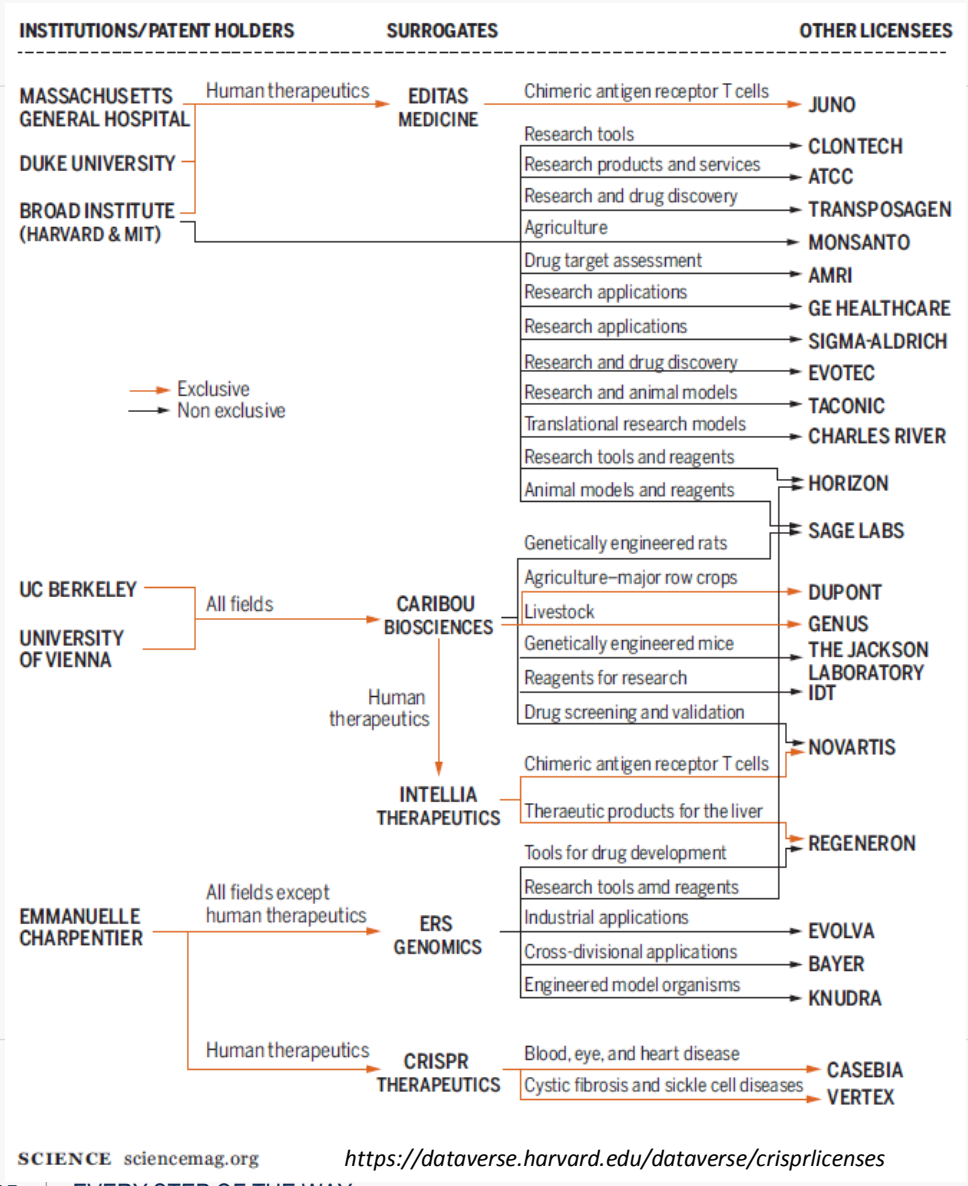
- The European Patent Office revoked the patent that was granted to the Broad group, leaving only the patents awarded to the Berkeley group
- The awarded patents cover the use of the CRISPR technology in both prokaryotic and eukaryotic cells/organisms
- Opposition proceedings at the European Patent Office have just begun…It is still possible that Broad may be awarded patents as well



- The Berkeley group was also awarded patent protection in China
- it is still possible that Broad may be awarded patents there as well

These conflicting decisions are further complicated by a set of interlocking license agreements from the inventors' biotech companies, with a great deal of uncertainty playing out in the global commercial sector for CRISPR

CRISPR-CAS9 LICENSING AGREEMENTS



SCIENCE sciencemag.org *https://dataverse.harvard.edu/dataverse/crisprlicenses*

Several layers:

1. **Patent holders**

1. **"Surrogate" companies** have been granted exclusive licenses:
   - Editas Medicine*
   - Caribou Biosciences
   - ERS genomics
   - CRISPR Therapeutics*

3. **Spin-out companies**

Intellia Therapeutics* with exclusive license from Caribou Biosciences: focus on their own R&D activities in human therapy, and specific out-licensing in certain areas.

** Editas Medicine, CRISPR Therapeutics and Intellia Therapeutics are publicly registered in the NASDAQ Stock Market*

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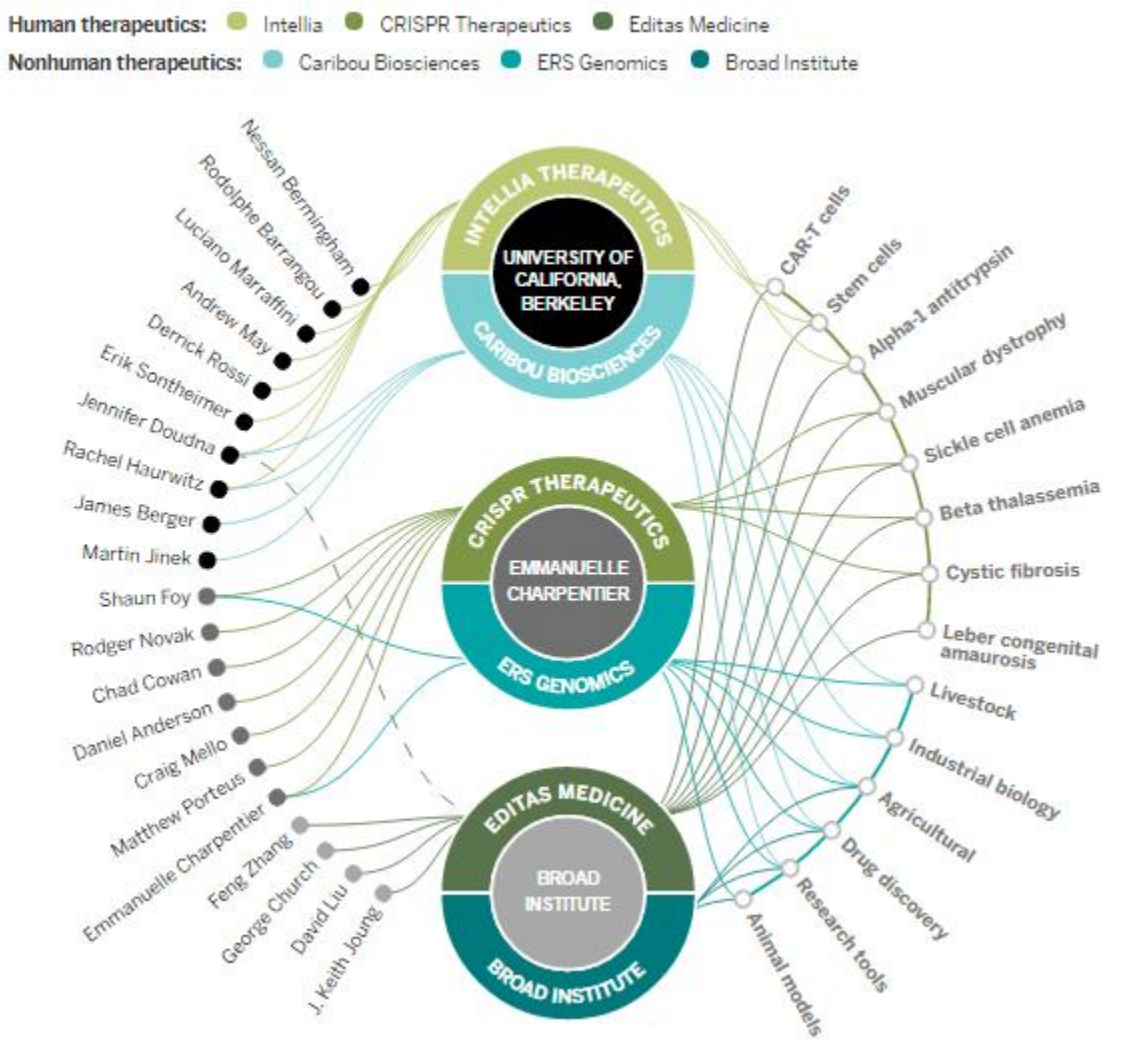
APPLICATION AREAS



3 different commercialization areas of CRISPR/Cas9 patents:

1. CRISPR/Cas9 used in medical applications with focus on human therapeutics and drug discovery
2. Agriculture and food applications
3. **Research tool applications, cell line and animal models**

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CRISPR IP – IMPACT ON IN VIVO RESEARCH

ACADEMIC & NON PROFIT ORGANIZATION	COMMERCIAL (FOR-PROFIT) ORGANIZATION
CRISPR tools, knowledge, methods and other IP for genome-editing freely available to the academic and non-profit community	Non-exclusive license granted for use in commercial research Non-exclusive license granted to sell tools and reagents for genome editing
No financial impact if all work performed in house and no profit application of research **Financial impact if work is outsourced to profit organization and if profit application is considered**	**Impact on model development costs and related services due to royalties** **Infringement risks**

INFRINGEMENTS RISKS

Patent landscape is complex, constantly changing, with several main actors

IP management may be complex for small size companies or academic institutions

Potential consequences of infringement

- To be obliged to stop all uses of the invention and to pay for past uses
- To have to destroy all material made with the use of the patent
- To pay damages to the patent owner (or exclusive licensee)
- To pay all costs of the lawsuit
- To have very deleterious publicity (information is public and infringer may be obliged to post it on its website)

Make sure you are covered when developing in house or out-sourcing services to be able at later stages to operate your model

Alternative technologies may replace CRISPR/Cas9 to generate your rodent model thus securing your IP environment

IMPACT ON FINANCIALS

MODEL DEVELOPMENT & ASSOCIATED RESEARCH/DEVELOPMENT

From www.technologyreview.com - December 27, 2016 12:08 PM

Royalties usually apply to development of licensed product (custom mouse or rat model) and all related services using the licensed product:

- Propagation services (breeding, feeding and health monitoring…)
- Embryology services (rederivation, cryopreservation…)
- Genotyping
- Quarantine to support health monitoring
- Obtention of samples (blood, urine…)
- TOX studies

… not exhaustive list…

- **Impact on global project budget (and not only on mouse/rat model generation step) is to be evaluated**
- **Alternative model creation technologies may replace CRISPR/Cas9 thus reducing global project cost**

CRISPR/CAS9 - OUR IP POSITION

- **Freedom to operate** for ES cell models in Europe
- CRISPR/Cas9: secure your project with both **ERS Genomics Limited & Broad Institute** in Europe, Japan & US
- Our offering allows clients to work with one company to develop ***in vitro* models** and cell lines and later place them into ***in vivo* studies**

Charles River Adds ERS License to CRISPR/Cas9 Service Offering

WILMINGTON, Mass.--(BUSINESS WIRE)--Dec. 6, 2017-- Charles River Laboratories International, Inc. (NYSE: CRL) today announced the expansion of its CRISPR/Cas9 service offering with the addition of a license from ERS Genomics Limited. The license, coupled with the existing license through the Broad Institute of MIT and Harvard, allows Charles River to offer custom *in vivo* and *in vitro* genome editing.

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Conclusion

STRATEGIES FOR RODENT MODEL GENERATION

TO CRISPR OR NOT TO CRISPR ?

CRISPR/Cas9 embryo-mediated gene editing is highly relevant for:

- Rat models
- Mouse and rat models on specific genetic backgrounds/strains: model relevancy, 3Rs, cost
- Generation of structural variants/copy number models (CRISMERE technology)
- Quick and inexpensive generation of basic mutation models: constitutive KO, small Kins

…when IP issues (IP fees and infringement risks) are not problematic

STRATEGIES FOR RODENT MODEL GENERATION

TO CRISPR OR NOT TO CRISPR ?

Alternative strategies to CRISPR/Cas9 in embryos are worth considering when:

- Need for models, even basics, and would like to retain freedom to operate the model wo IP fees & infringement risks
- Kin models involving fragments above 2-3kb: use of ES cells is advised to secure project budget and timelines
- Large humanization in mouse -> CRISPR in ES cell is a very good option

Generation of Genetically Engineered Rodent Models

TO CRISPR OR NOT TO CRISPR ?

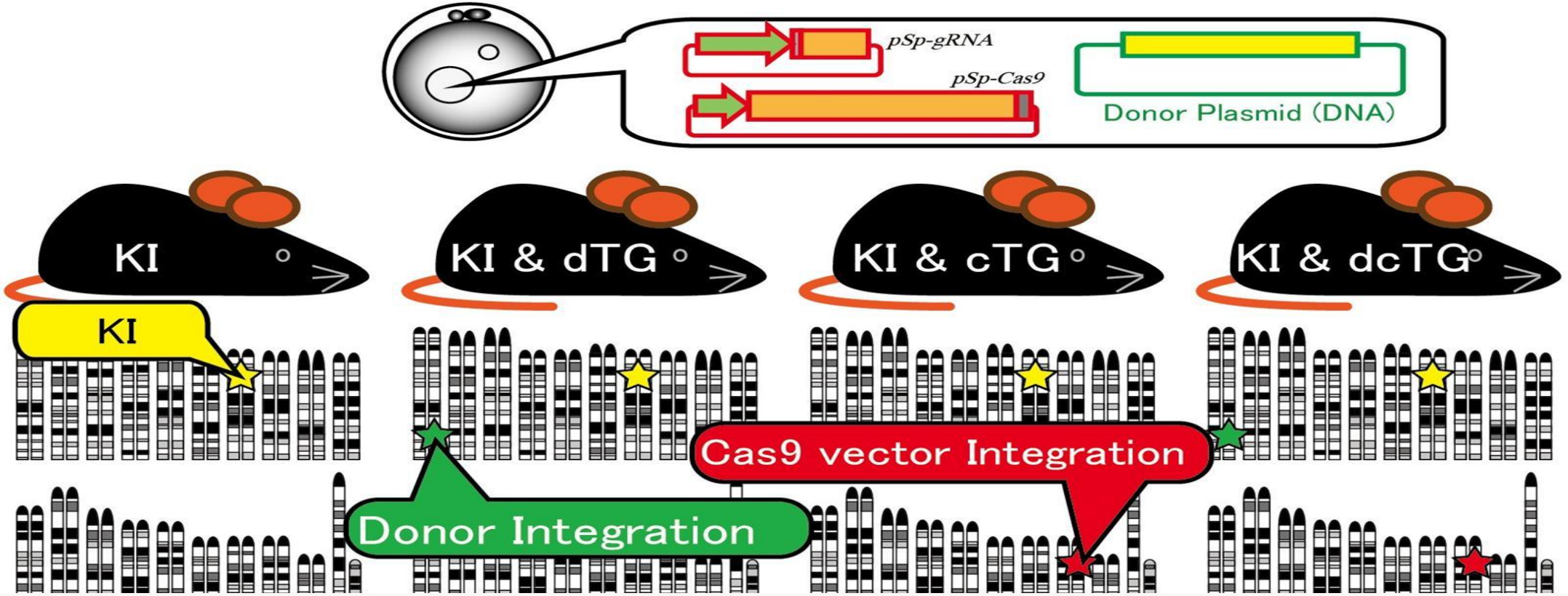
That is the question !

Dr. Jean Cozzi

À CHAQUE ÉTAPE DU PROCESSUS

ADDITIONAL MUTATIONS - OFF TARGET LOCUS

RANDOM INSERTION EVENTS IN KNOCK-IN MODELS



Unpublished data from Charles River Japan

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OFF-TARGET ssODN DONOR INTEGRATION

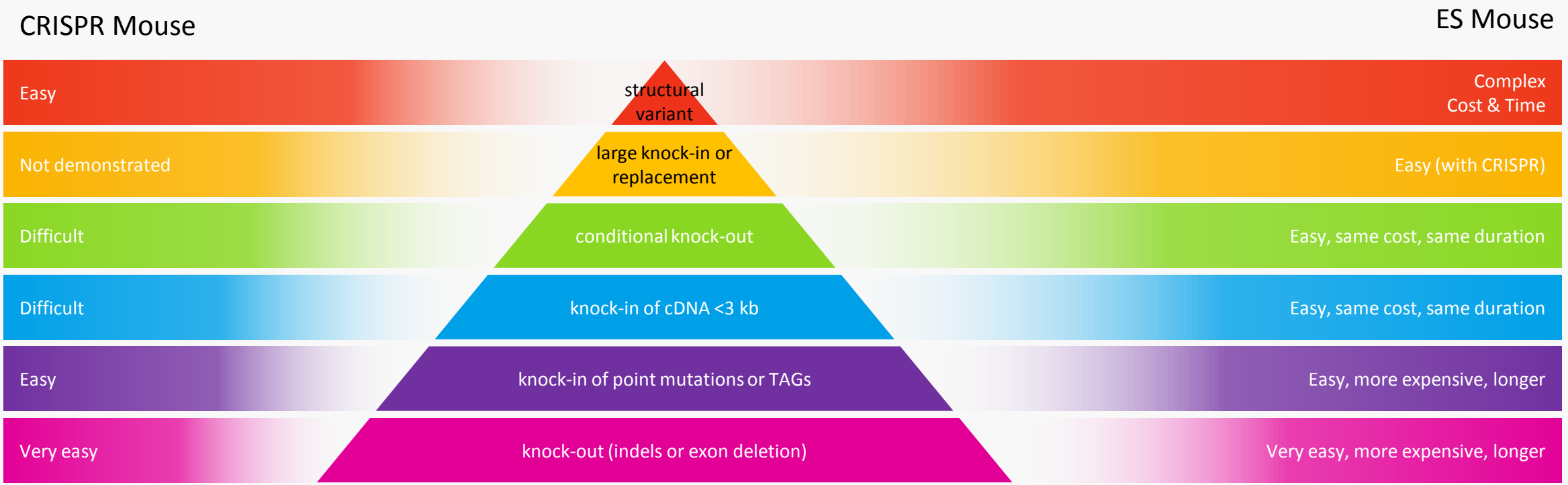
Gene targeted	Species	Number of F0 screened	Number of F0 with on-site integration	Number of F0 with NHEJ	Number of F0 with random integration of ssDNA	% random integration
Gene #1	Mouse	42	1	4	6	14%
Gene #2	Mouse	30	0	2	4	13%
Gene #3	Rat	31	3	6	1	3%
Gene #4	Mouse	29	3	3	1	3%
Gene #5	Mouse	46	4	8	4	9%

ssODN= single stranded oligodeoxynucleotide

PHENOMIN-ICS unpublished results

MODEL RELEVANCY
GENE EDITING EFFICIENCY

ES cell vs CRISPR/Cas9 in embryos



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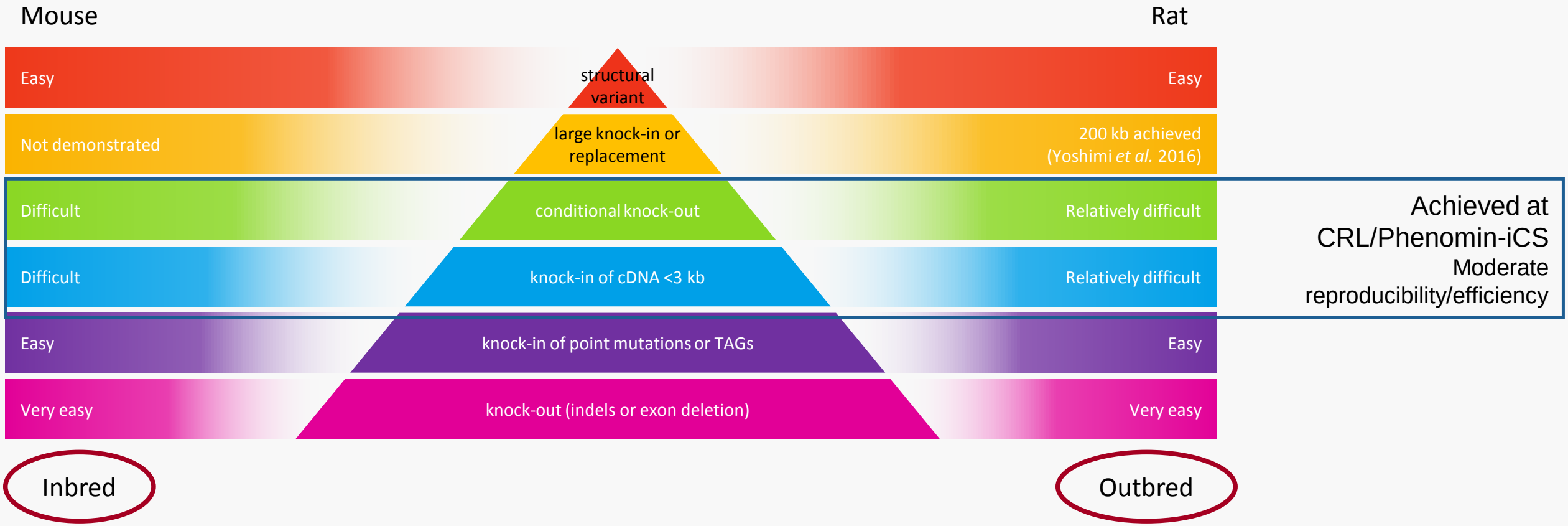
MODEL TYPES
GENE EDITING EFFICIENCY

Mouse vs. Rat



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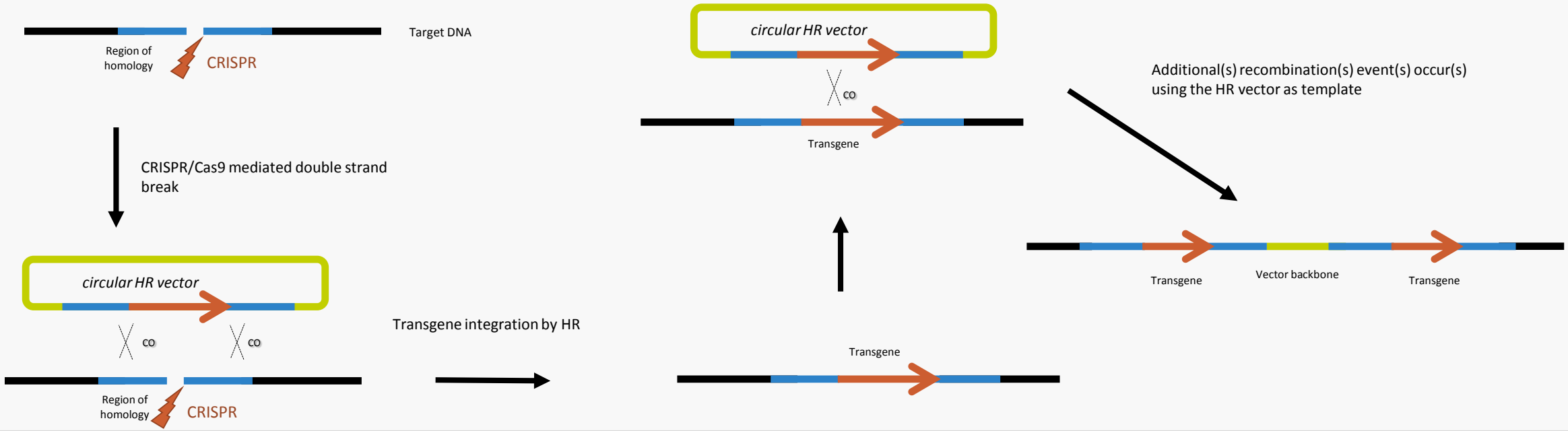
MULTIPLE TRANSGENE INSERTION AT TARGET SITE

PROPOSED MECHANISM

Integration of the transgene by homologous recombination (HR) followed by multiple integration of the vector by one crossing over



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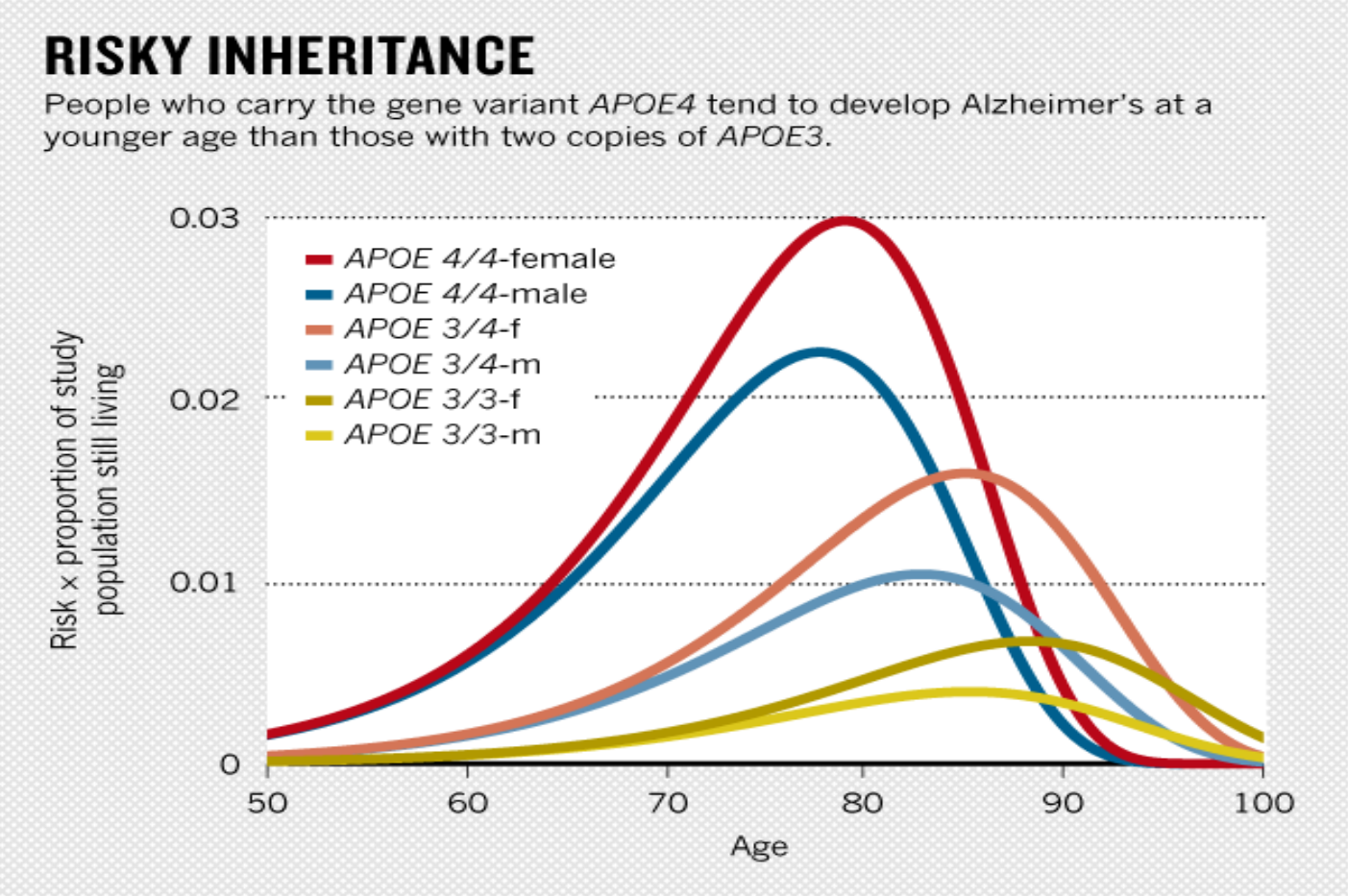
CASE STUDY: HUMANIZATION OF LARGE LOCUS IN MICE



Replacement of ApoE mouse gene by:

ApoE2, ApoE3 and ApoE4 human alleles including neighbour orthology

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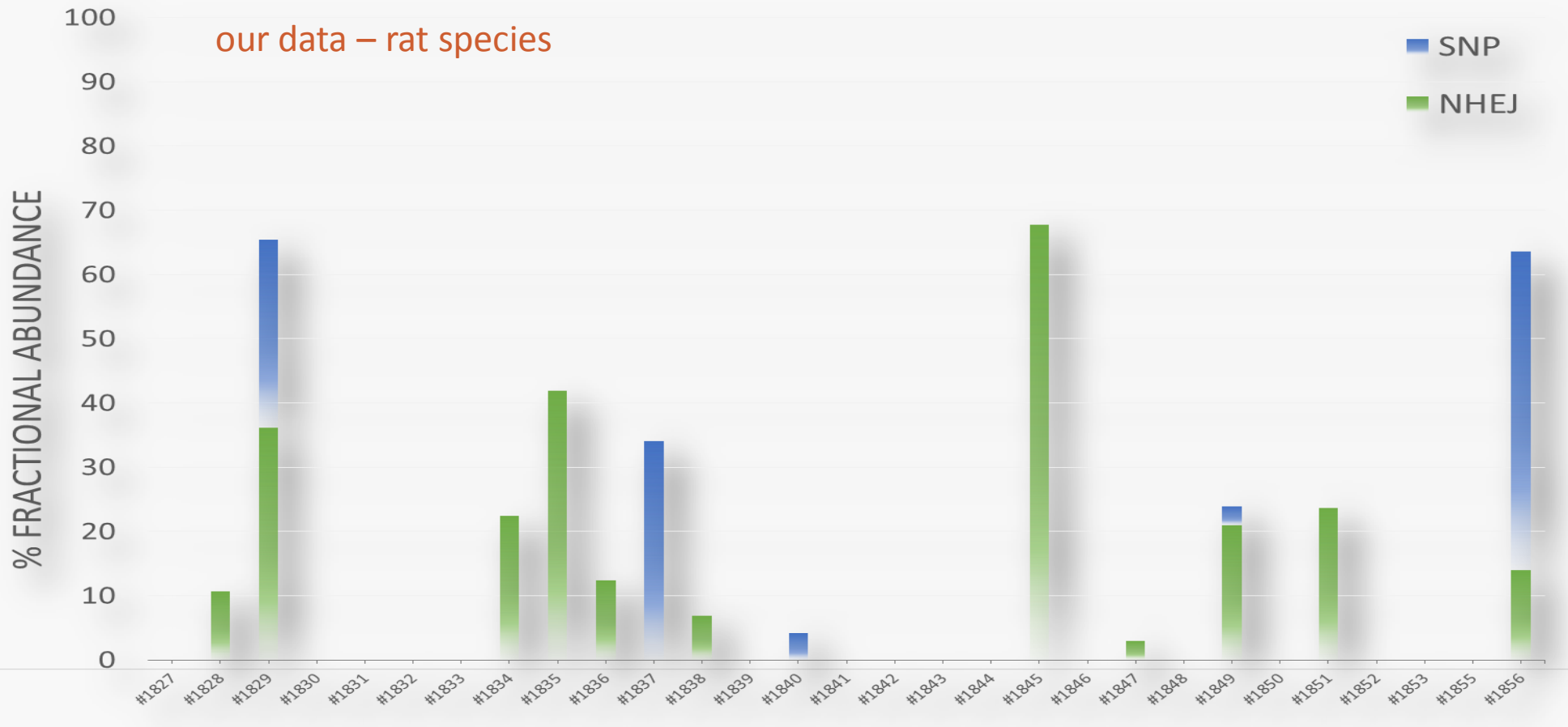
UNDERSTANDING HUMAN STRUCTURAL VARIATIONS LEADING TO DISEASES

- ✓ **>60 000 SVs** were discovered in human (Huddleston and Eichler, 2016)
  - pathological SVs are mostly **not known**
- ✓ One of the most common cause in **morbidity and mortality** in human population
- ✓ SVs likely play a major role in very various diseases not only restricted to neuronal disorders (Conrad et al., 2010; Fanciulli et al., 2007; McCarroll and Altshuler, 2007; Wu and Hurst, 2016) but also including cancers (Taki & Taniwaki, 2006)

MOSAICISM & SERIES OF ALLELIC VARIANTS

Droplet digital genotyping (quantitative) of Sprague Dawley point mutants F0 founders



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