BNORC Transgenic Core

Mouse Genetic Engineering made “Easi”

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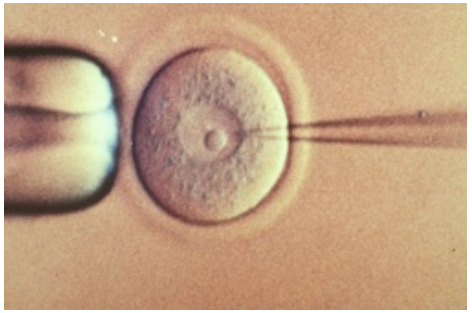
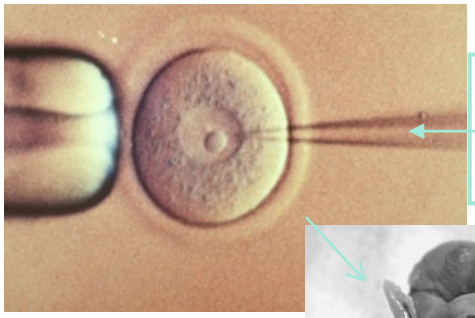


Two general types of mouse genetic engineering

1. Addition of exogenous DNA to the mouse genome – a.k.a. "Transgenics".

Transgene | Promoter that Drives Expression | Protein Coding Sequence



Trans-gene

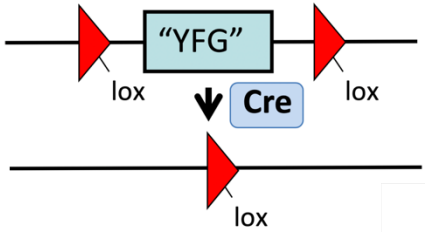
Ubiquitously active promoters
Tissue-specific promoters:
white fat, brown fat,
muscle, β-cell, etc.

Anything
Constitutively active,
Dominant negative,
etc., etc., etc.

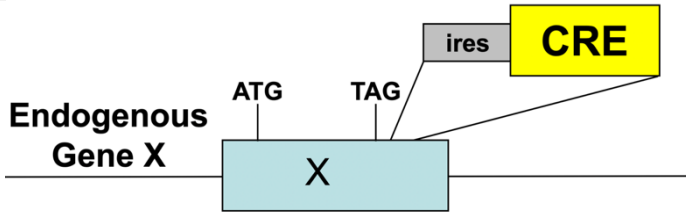
2. Targeted manipulation of the mouse genome – a.k.a. "Gene Targeting".

a) Endogenous gene is made nonfunctional – "global gene knockout"
b) Endogenous gene is subtly altered – i.e. mimic a human point mutation, etc.

c) Endogenous gene is "loxed" for doing tissue-specific knockouts

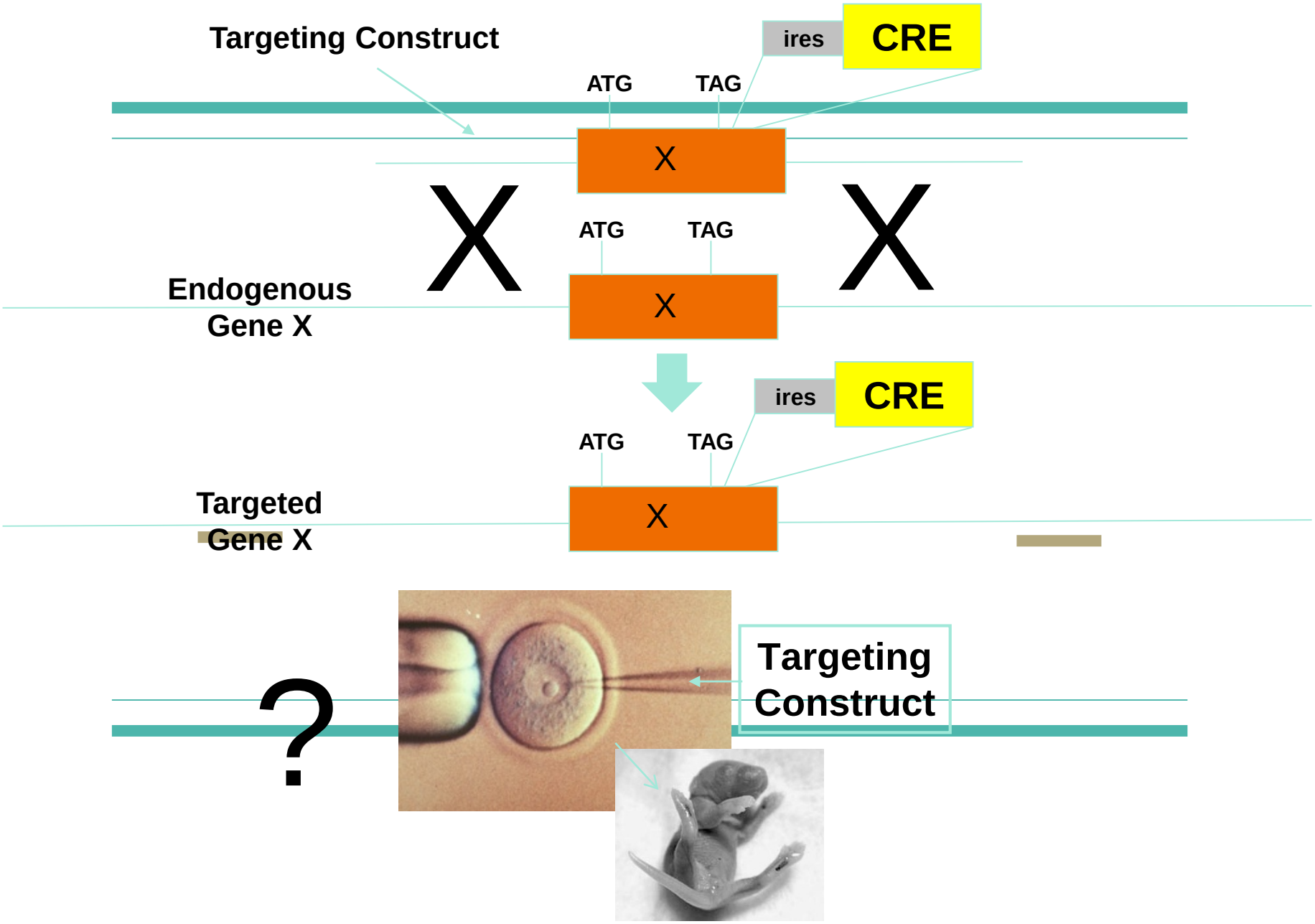


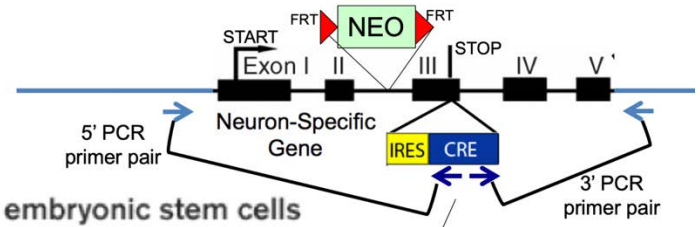
d) Endogenous gene is modified to drive expression of a protein (anything): i.e. Cre Recombinase



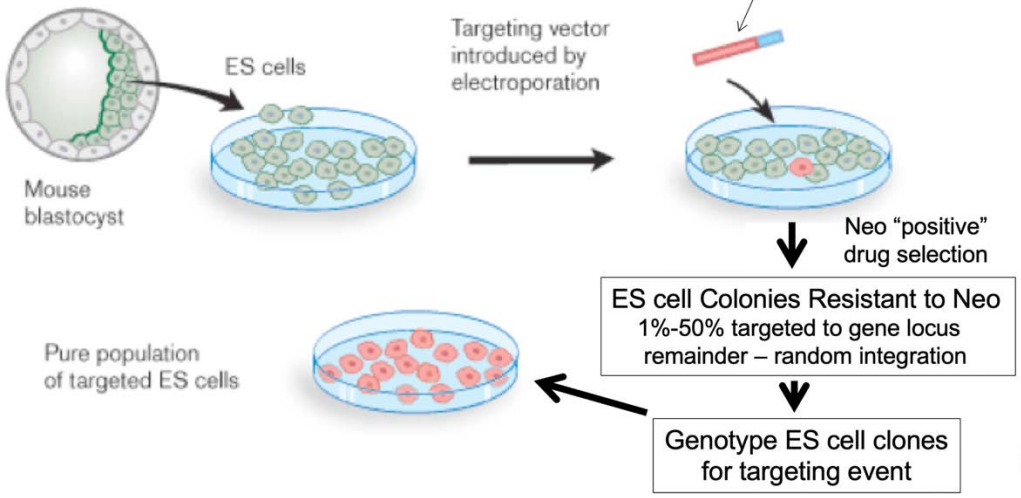
"ires-Cre Knockin Mice"

Gene Targeting – key role of Homologous Recombination





A. Gene targeting of embryonic stem cells



The Nobel Prize in Physiology or Medicine 2007

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


Photo: U. Montan

Mario R. Capecchi

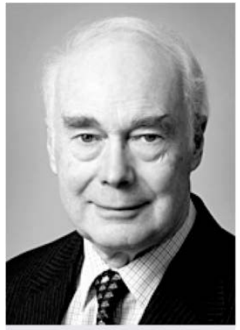


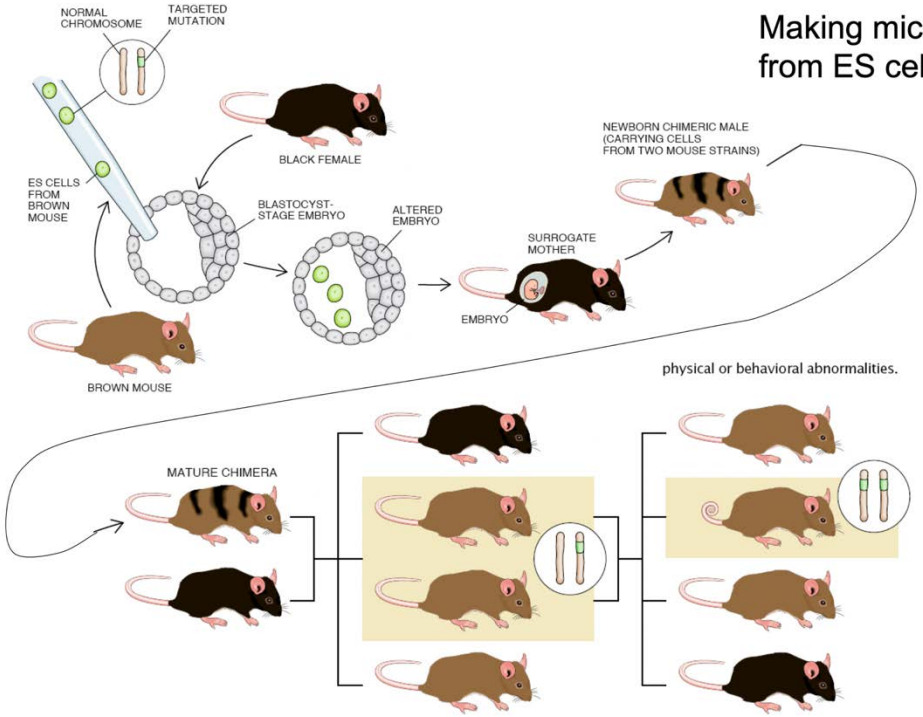
Photo: U. Montan

Sir Martin J. Evans

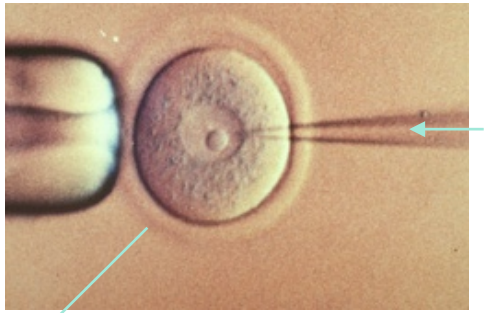

Photo: U. Montan

Oliver Smithies

Making mice from ES cells



Easi-CRISPR: rapid, easy Gene Targeting



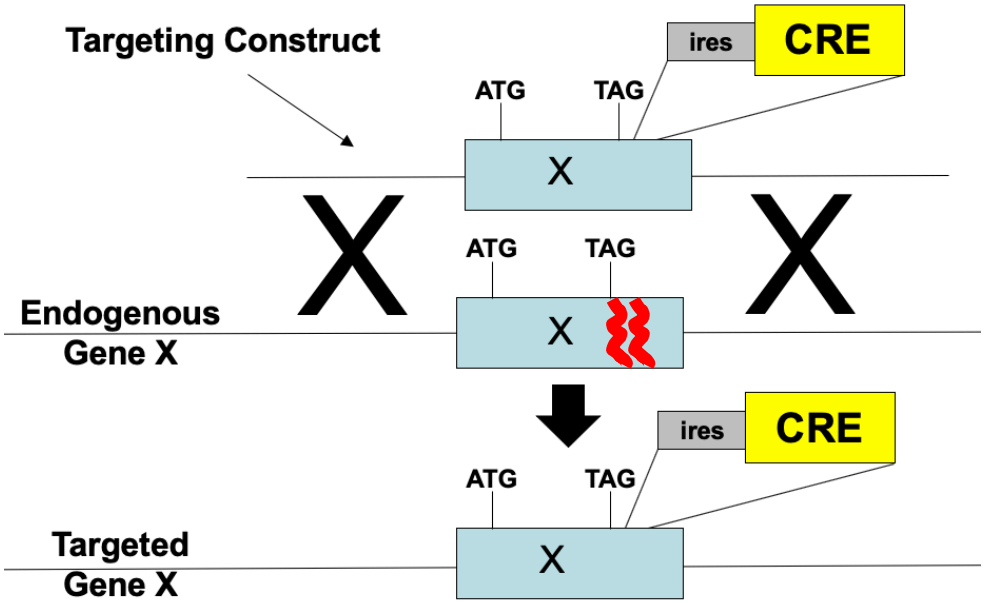
Targeting Construct and CRISPR "reagents"

1. **CRISPR** is used to create **break** in DNA:

↑'s rate of homologous recombination (HR).

2. A **ssDNA** targeting construct is used:

- no random integration.
- ↑'s rate of HR.



Easy mouse gene targeting with Easi-CRISPR

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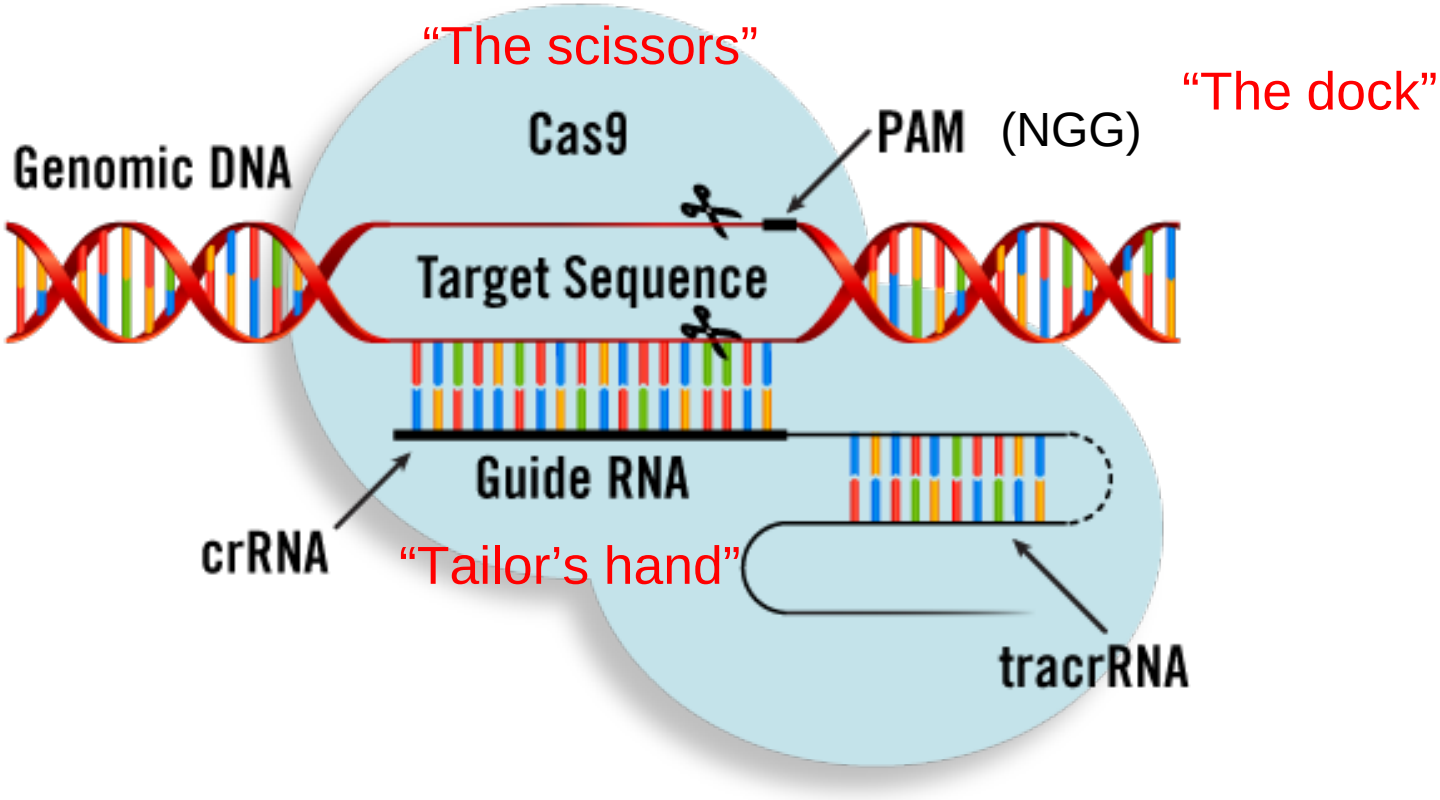
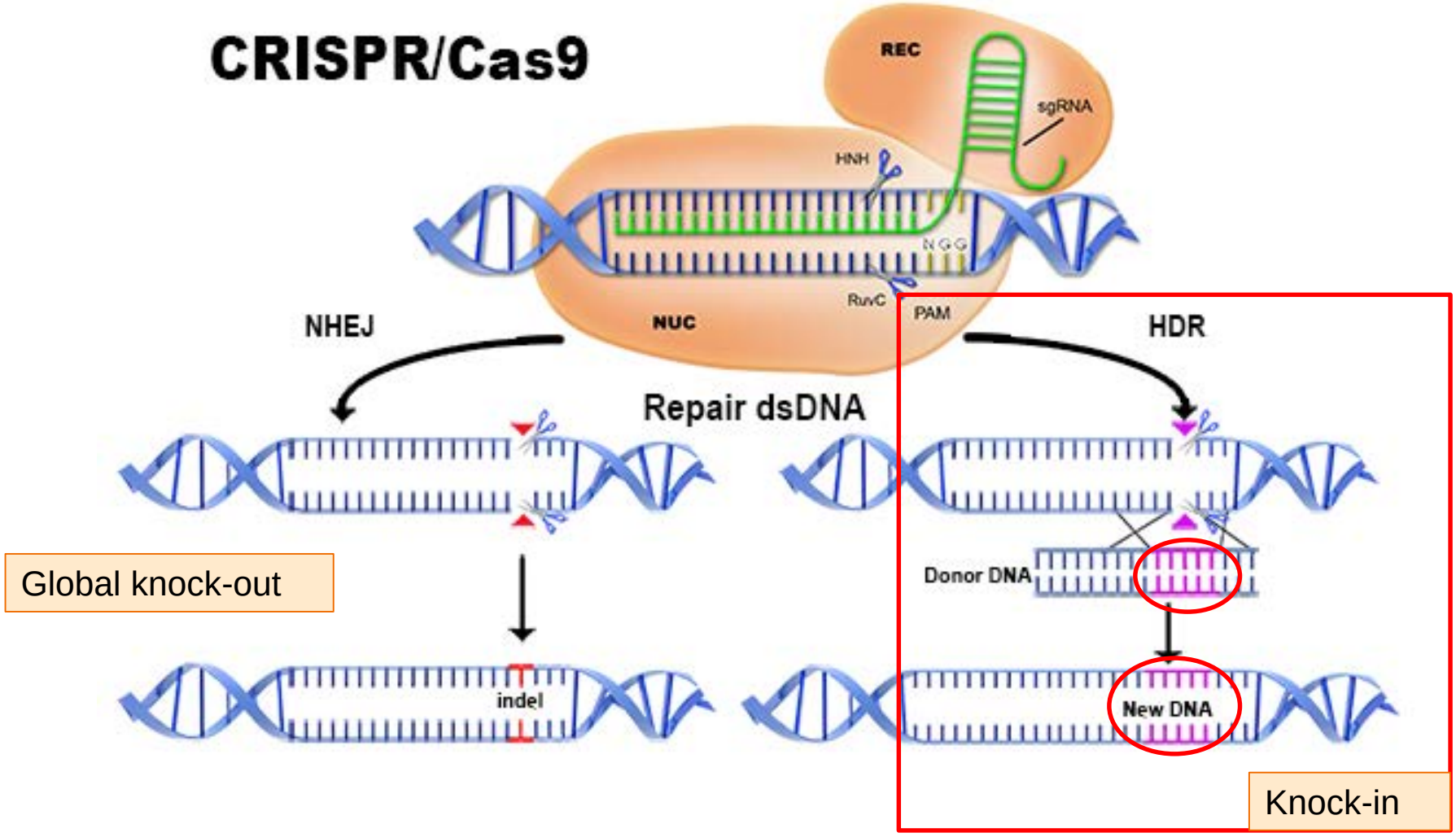
An overview of CRISPR technology

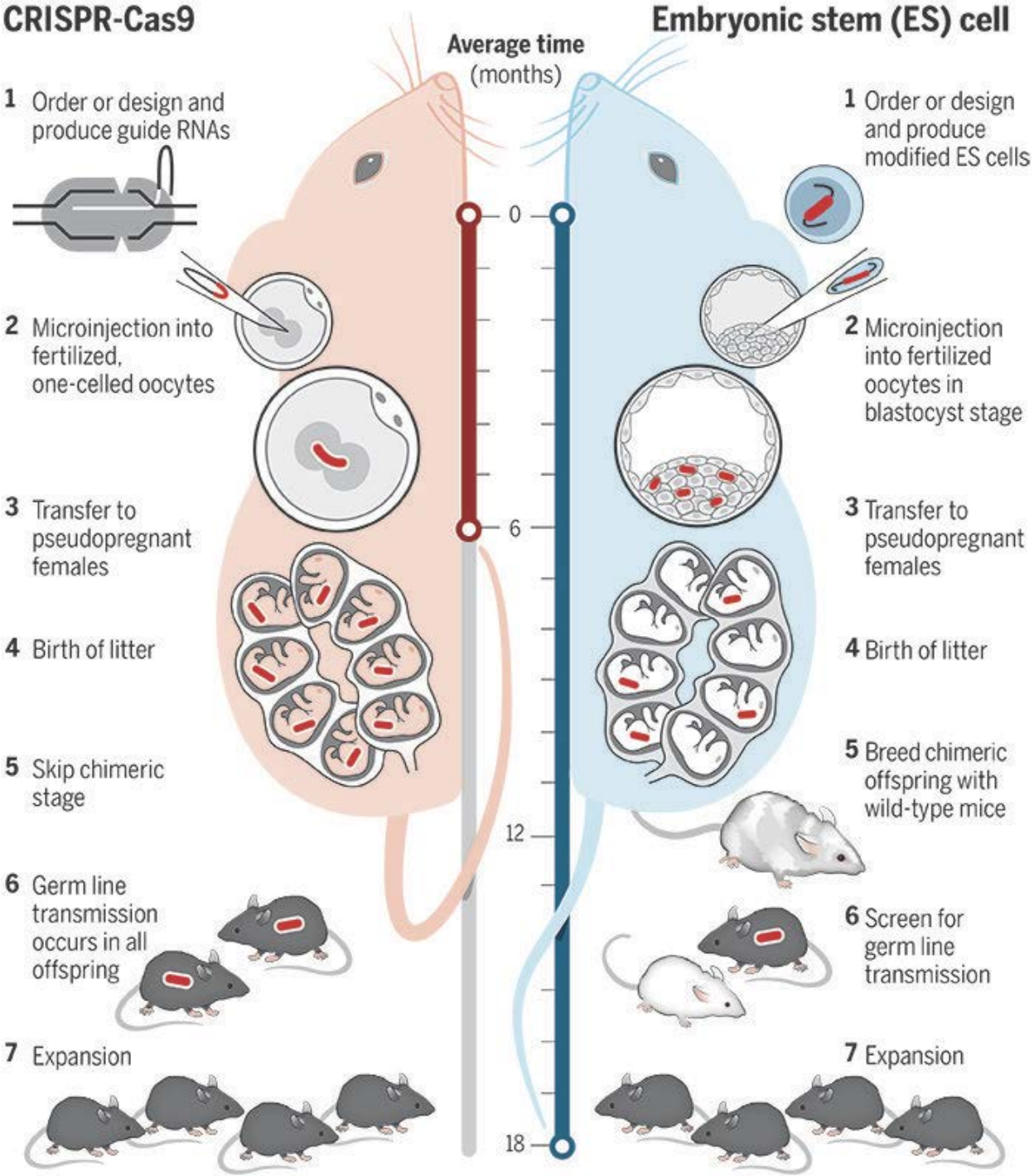
A brief overview of CRISPR technology

To create double-stranded DNA break



Working with DSB





CRISPR/CAS9 method greatly shortens the timeline

K. Sutliff/ *Science*

Easi-CRISPR

Quadros *et al. Genome Biology* (2017) 18:92
DOI 10.1186/s13059-017-1220-4

Genome Biology

RESEARCH Open Access

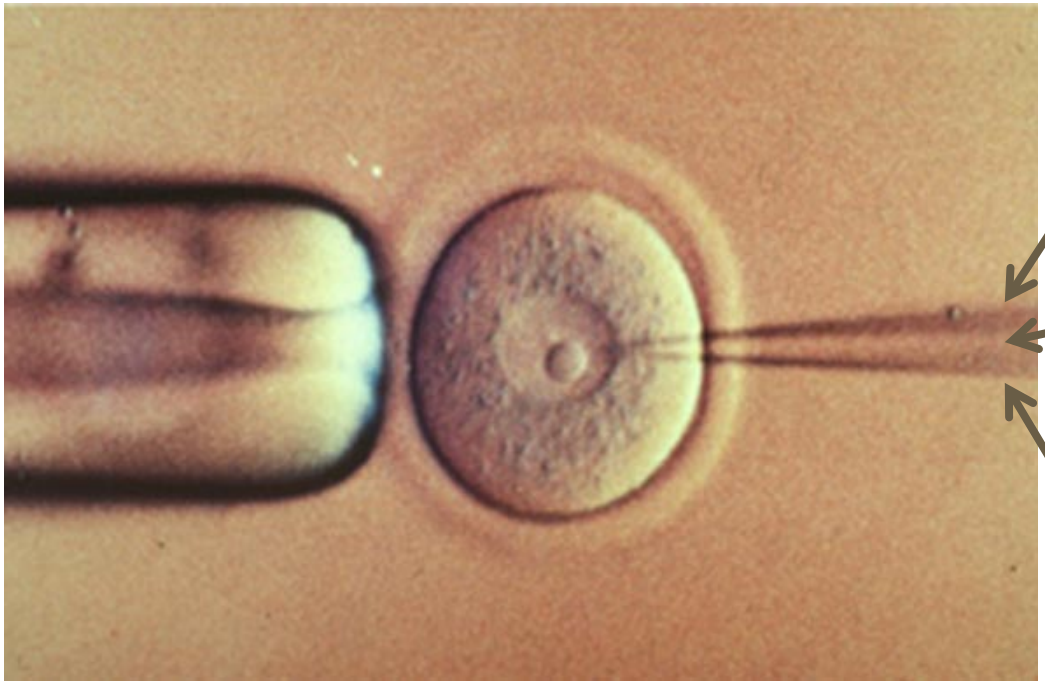
Easi-CRISPR: a robust method for one-step generation of mice carrying conditional and insertion alleles using long ssDNA donors and CRISPR ribonucleoproteins

Rolen M. Quadros[1†], Hiromi Miura[2,3†], Donald W. Harms[1], Hisako Akatsuka[2,4], Takehito Sato[4], Tomomi Aida[5,6,7], Ronald Redder[8], Guy P. Richardson[9], Yutaka Inagaki[3,10,11], Daisuke Sakai[10,12], Shannon M. Buckley[13,15], Parthasarathy Seshacharyulu[14], Surinder K. Batra[14,15], Mark A. Behlke[16], Sarah A. Zeiner[16], Ashley M. Jacobi[16], Yayoi Izu[17], Wallace B. Thoreson[18], Lisa D. Urness[19], Suzanne L. Mansour[19*], Masato Ohtsuka[2,3,10*] and Channabasavaiah B. Gurumurthy[1,20*]

Comparison with conventional CRISPR

	Conventional	Easi-CRISPR
Targeting construct form	Plasmid (dsDNA)	ssDNA
Targeting construct availability	Cloning	Chemical synthesis
Homology arm length	> 2.5 kb	100 nt
Genotyping	Long range PCR / Southern blot (difficult)	Regular PCR (easy)
Success rate	< 10%	25% (and counting)

Easi-CRISPR Reagents



CAS9 protein

Targeting construct

sgRNA design: in silico

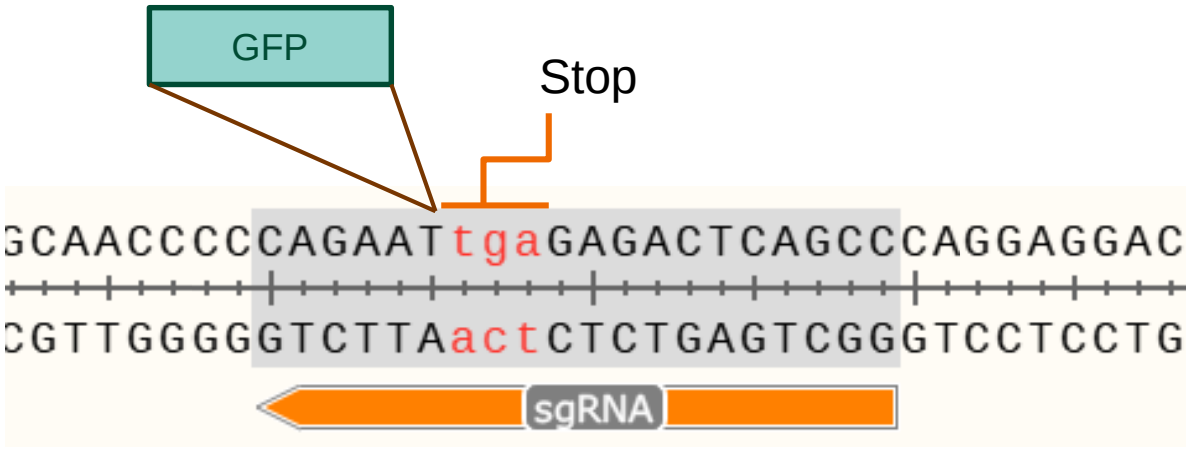
- Institutional sites: Broad, MIT, crispor.org
- Commercial sites: IDT, Synthego, etc.
- Cloud-based bioinformatics sites: Benchling.com

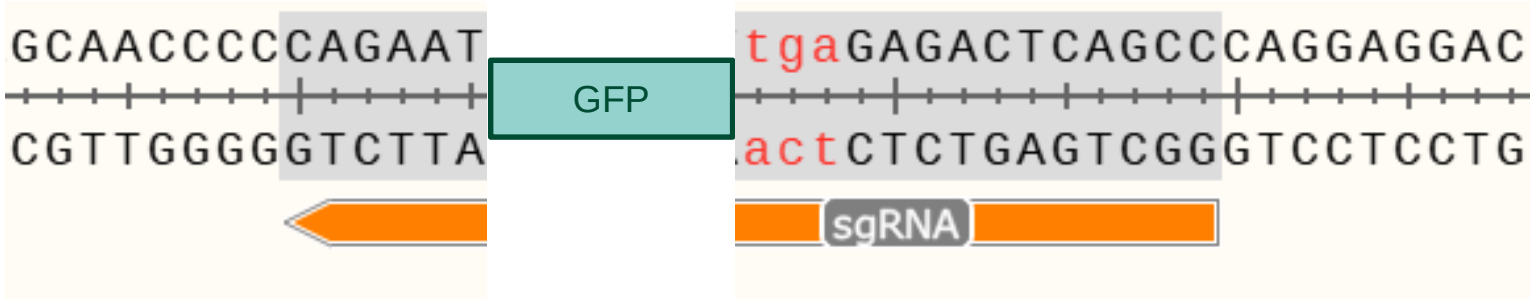
Online predicting algorithms are based on analysis of 3000+ sgRNAs (Doench et al. 2014 and 2016)

Criteria of a good sgRNA

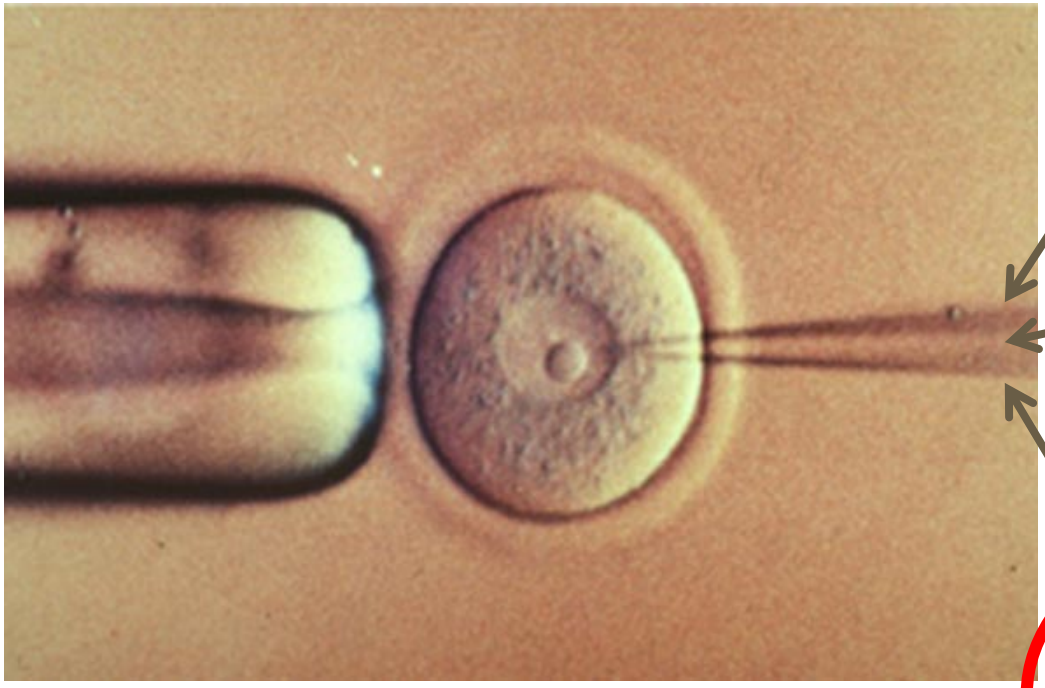
- Efficacy or on-target score – cuts where it should
- Specificity or off-target score – does not cut where it shouldn't
- Close to the insertion site

The ideal sgRNA site: contains the insertion site



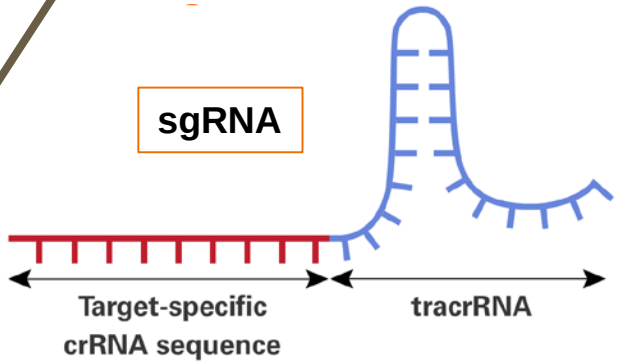


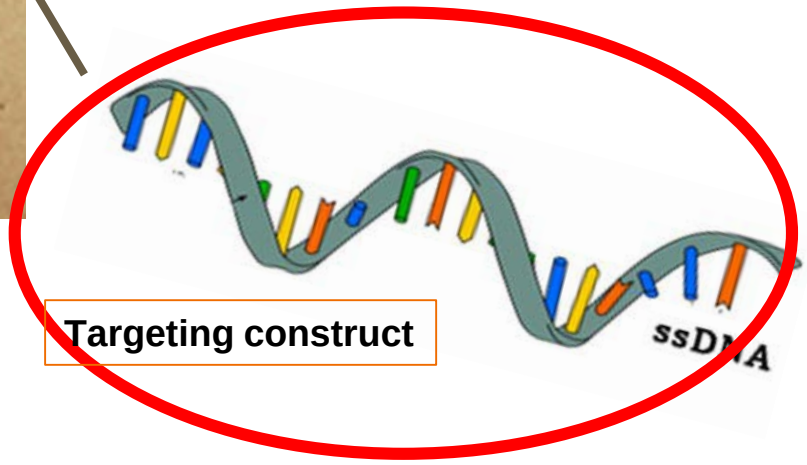
Easi-CRISPR Reagents



CAS9 protein

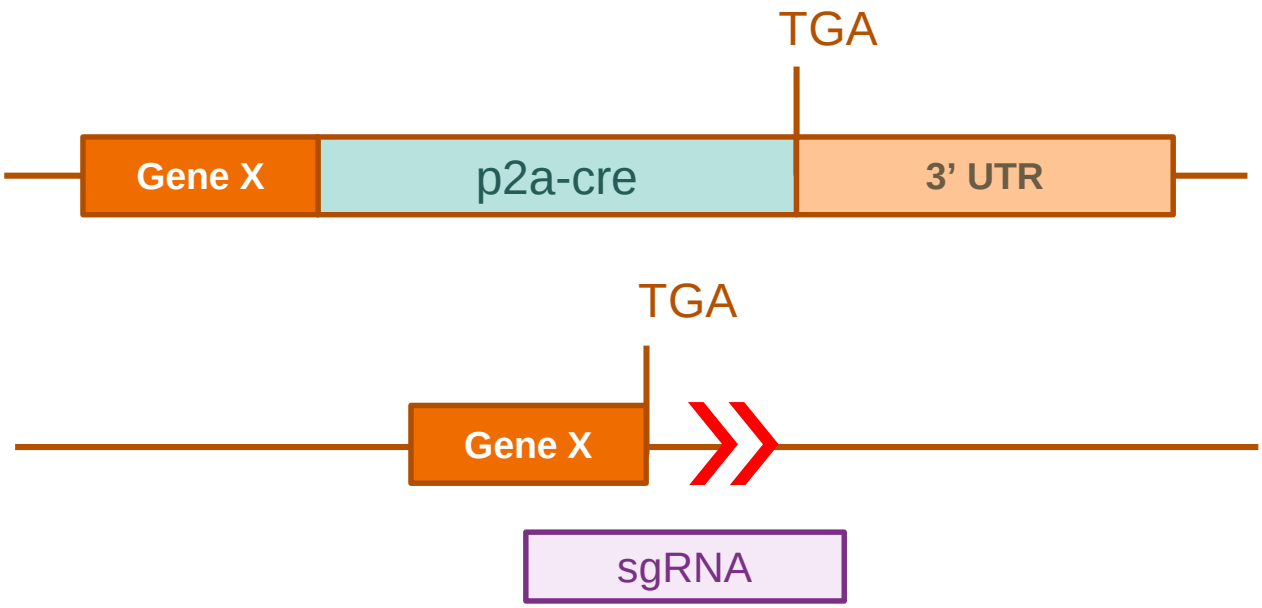
sgRNA



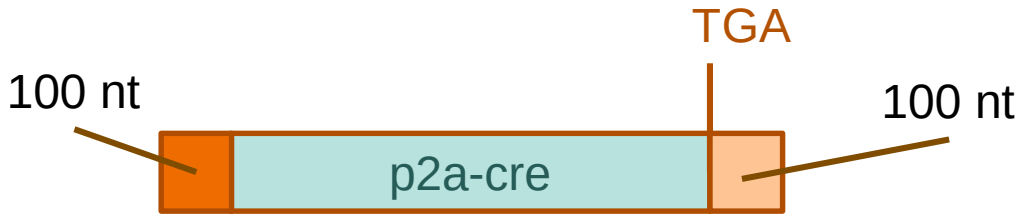


Targeting construct

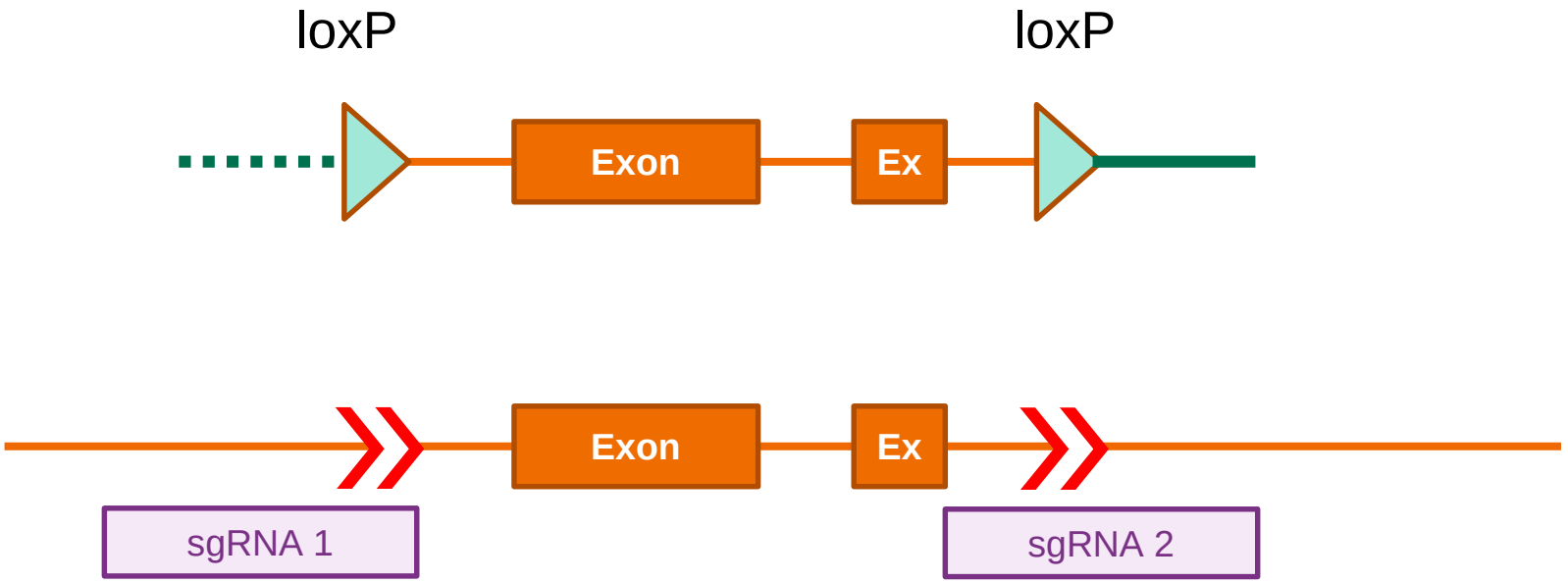
Project design (I) – reporter or driver



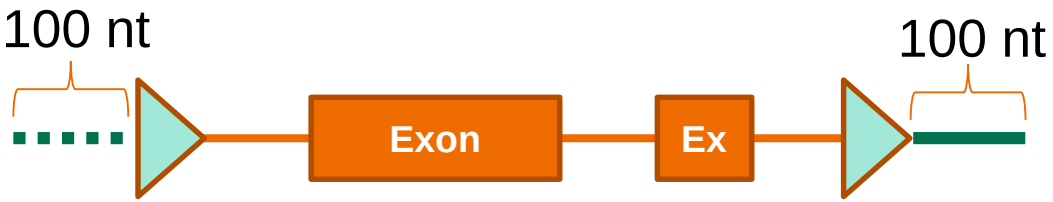
Targeting Construct:



Project design (II) – floxed exon(s)



Targeting construct:



Easi-CRISPR limitation: template availability

1. size: 2kb (IDT), 5kb (Genewiz)
2. Sequence composition: GC content, repetitive sequence, etc.
3. Cost: 90 cents/nt
4. Time: ~ 1 month

Easi-CRISPR Workflow

1. Contact us
2. Design sgRNA and targeting construct
3. Order the reagents
4. Send the reagents to us

   4 weeks later…

5. Pick up the tail snips and genotype
6. Breed the founder(s)

Project summary

Summary

- Turn-around time: 3 months or sooner (from reagents to founder)
- Average success rate (Easi-CRISPR): 25% (71% the highest)
- Number of successful projects : 50+ within 2.5 years
- Including 4 co-injections: two targeting constructs with one sgRNA
- **We provide free consultation on strategy and design**

Questions and comments?

Thank you!!

Joel Lawitts, PhD
Director, Transgenic core