CRISPR-Cas9 Mediated Mouse Model Creation and Transcription Regulation

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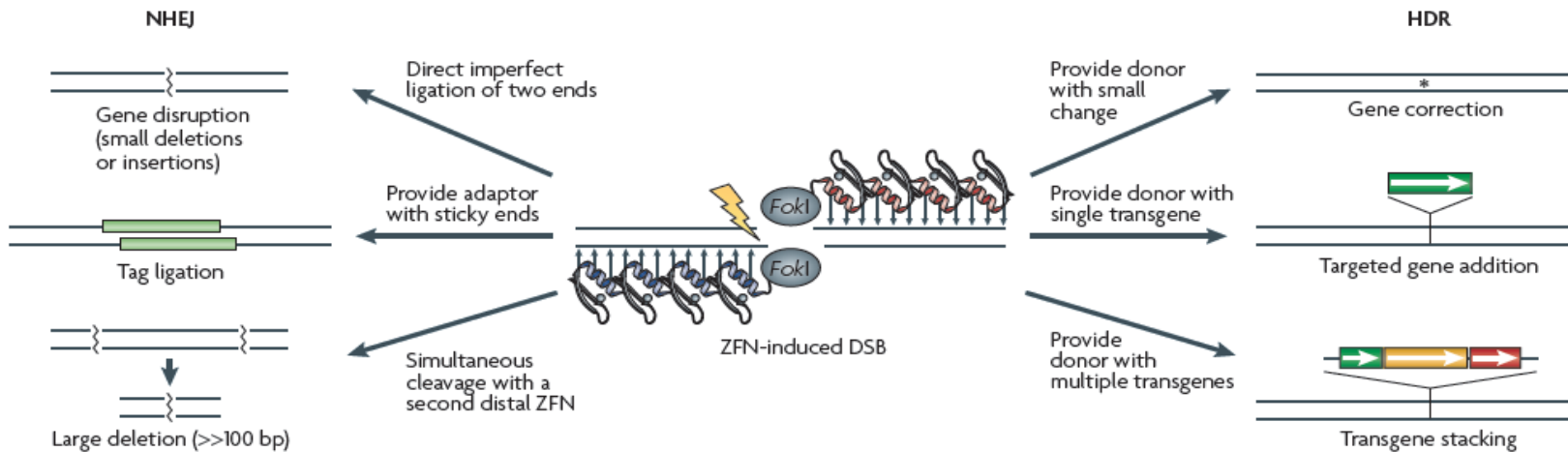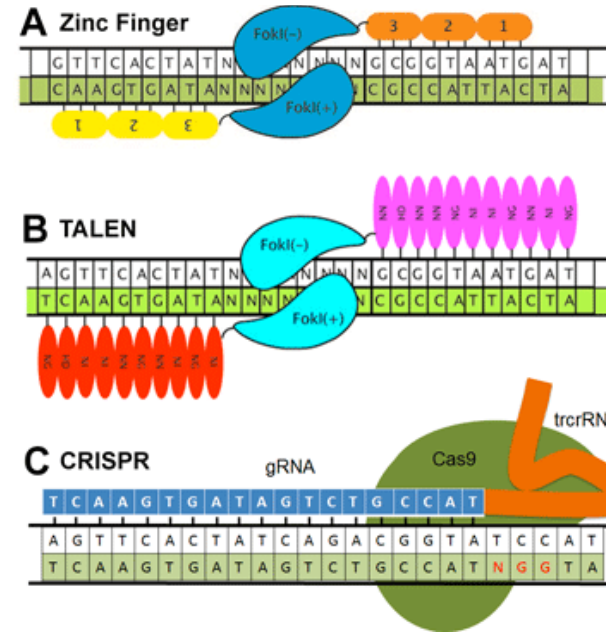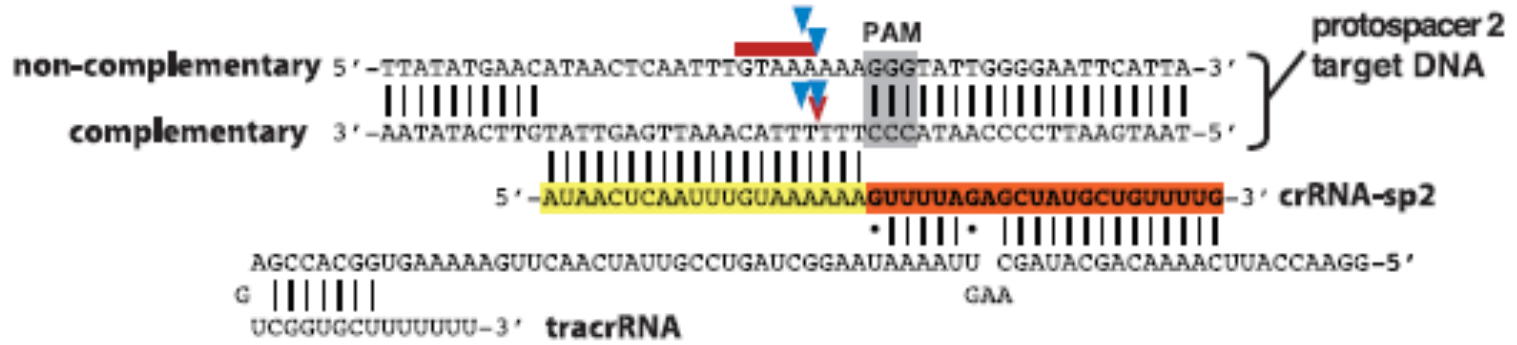# Precision Medicine

Genome Editing

- Zinc-finger Nuclease (ZFN)

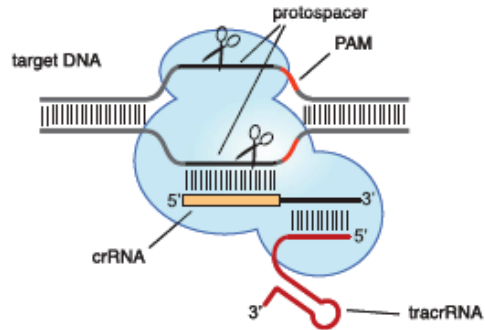- Transcription Activator–Like Effector Nuclease (TALEN)

- CRISPR-Cas9

Urnov et al., *Nat Rev Genet* 2011

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
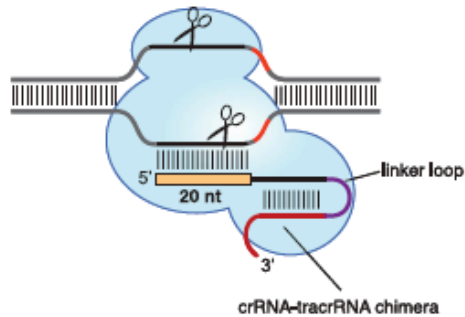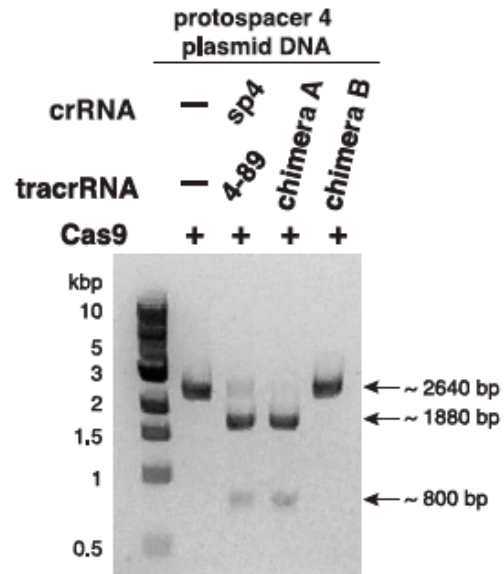Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

Jinek et al., 2012
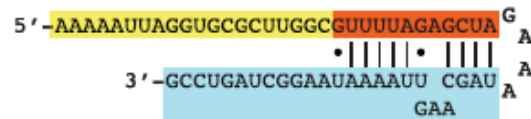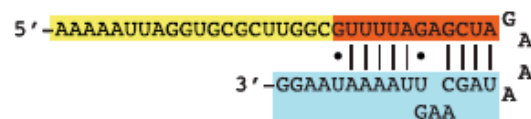
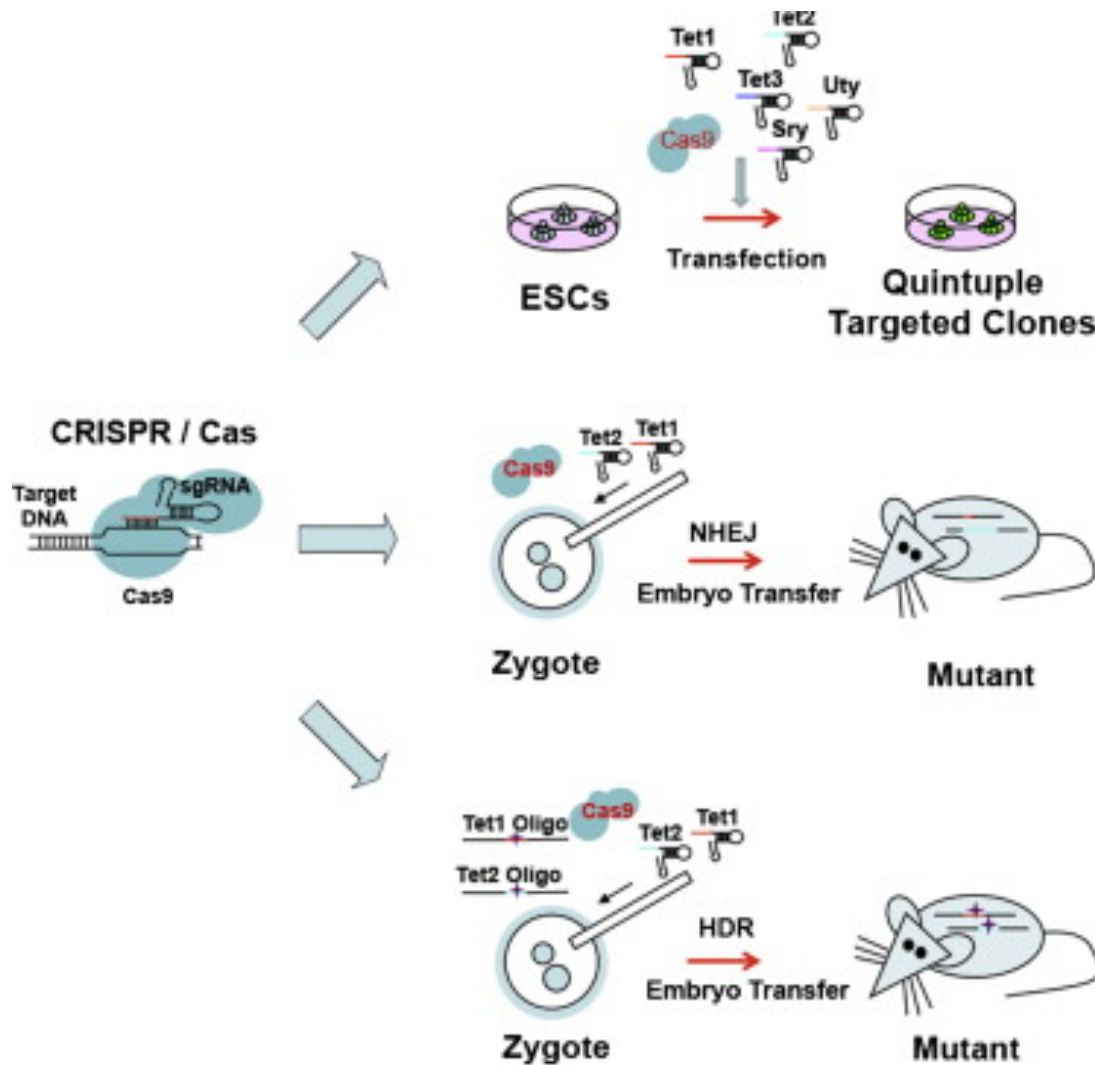# Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,[1,2]* F. Ann Ran,[1,4]* David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,[1,5] Luhan Yang,[1,3,5] Kevin M. Esvelt,[2] John Aach,[1] Marc Guell,[1] James E. DiCarlo,[4] Julie E. Norville,[1] George M. Church[1,2]*
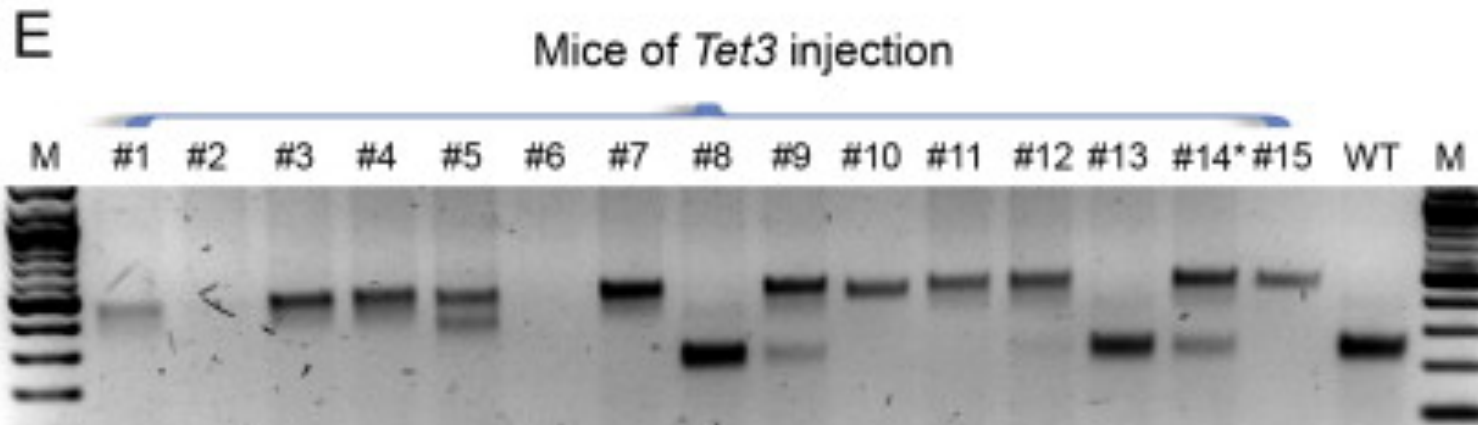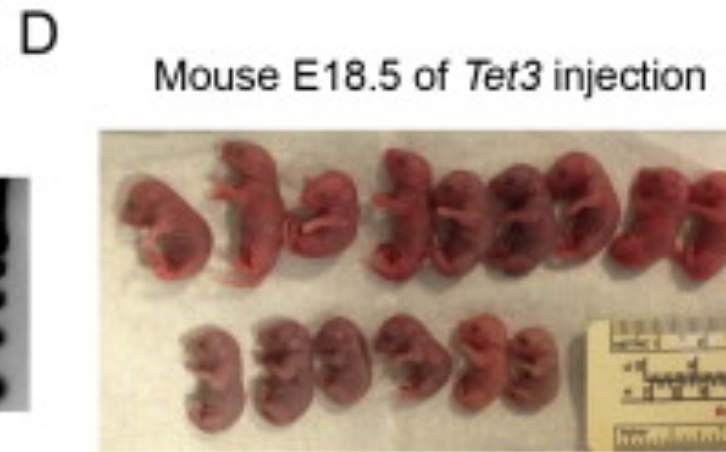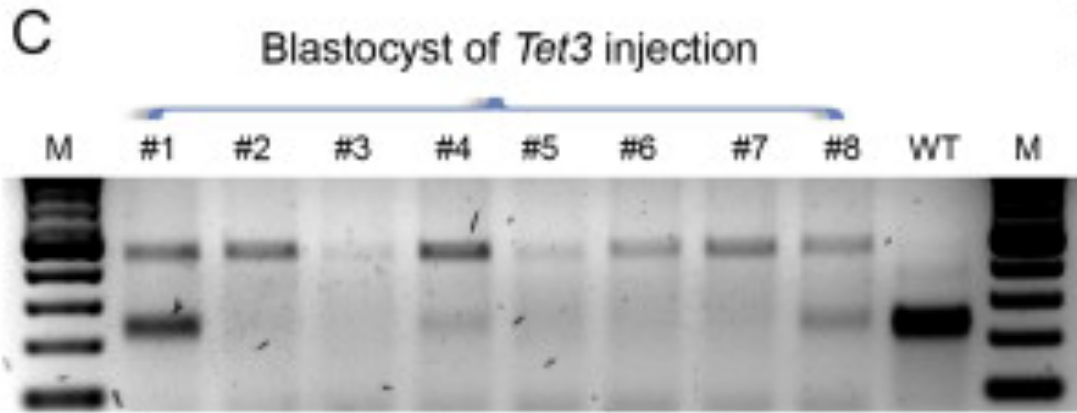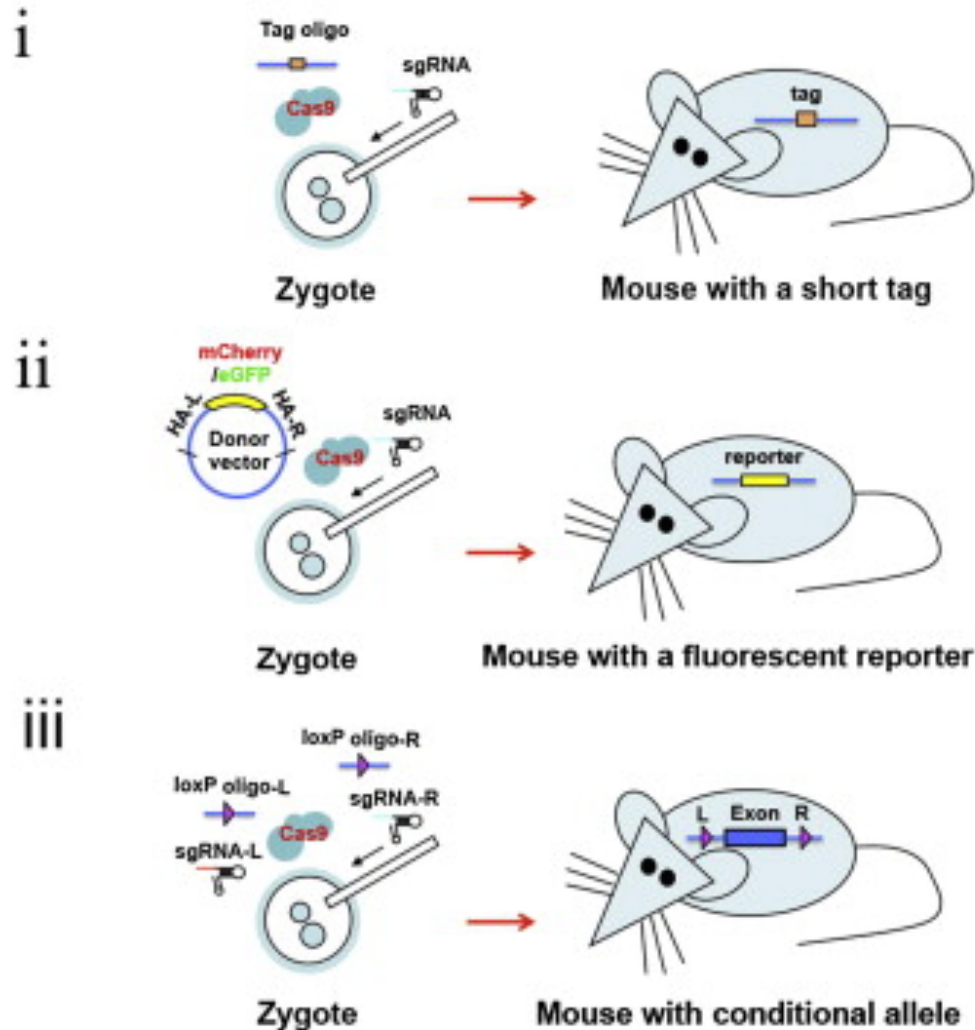
CRISPR mediated mouse model generation

Phenotype in Founder Animals
Tet3 Mutants with Neonatal Lethality

C — Blastocyst of *Tet3* injection

D — Mouse E18.5 of *Tet3* injection

E — Mice of *Tet3* injection

Wang, Yang and Shivalila et al. 2013

CRISPR mediated mouse model generation

CRISPR-mediated Genome Editing:
Faster Timeline, Lower Cost, More Flexibility

	Conventional Gene Targeting	CRISPR-based Gene Knockout	CRISPR-based Gene Knock in
Timeline	**40 weeks**	**6 weeks**	**8 weeks**
Cost – Model Creation	$15,000	$3,000	$7,000
Cost - Breeding to GLT/NEO Excision	$20,000	$4,000	$4,000
Flexibility	**129**	**any strain**	**any strain**
	C57BL/6		
Cost – Strain Change	$13,000	$0,0	$0.0
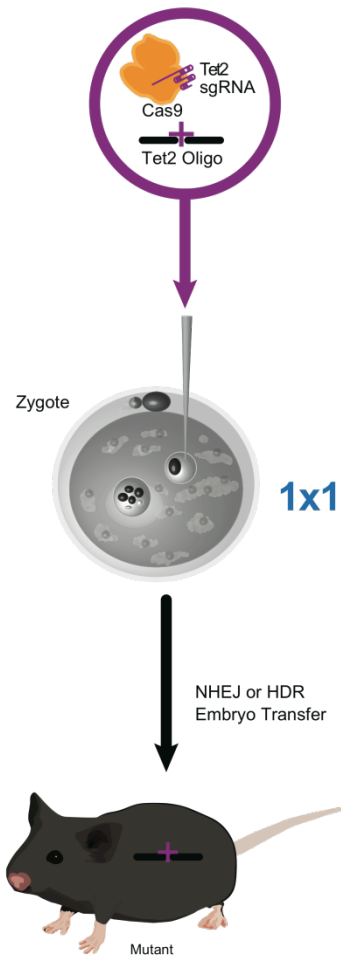
Zygote Electroporation of Nuclease (ZEN)

- Electroporation parameters
- Electroporation solution
- CRISPR concentration

ECM 830 Square Wave Electroporation System
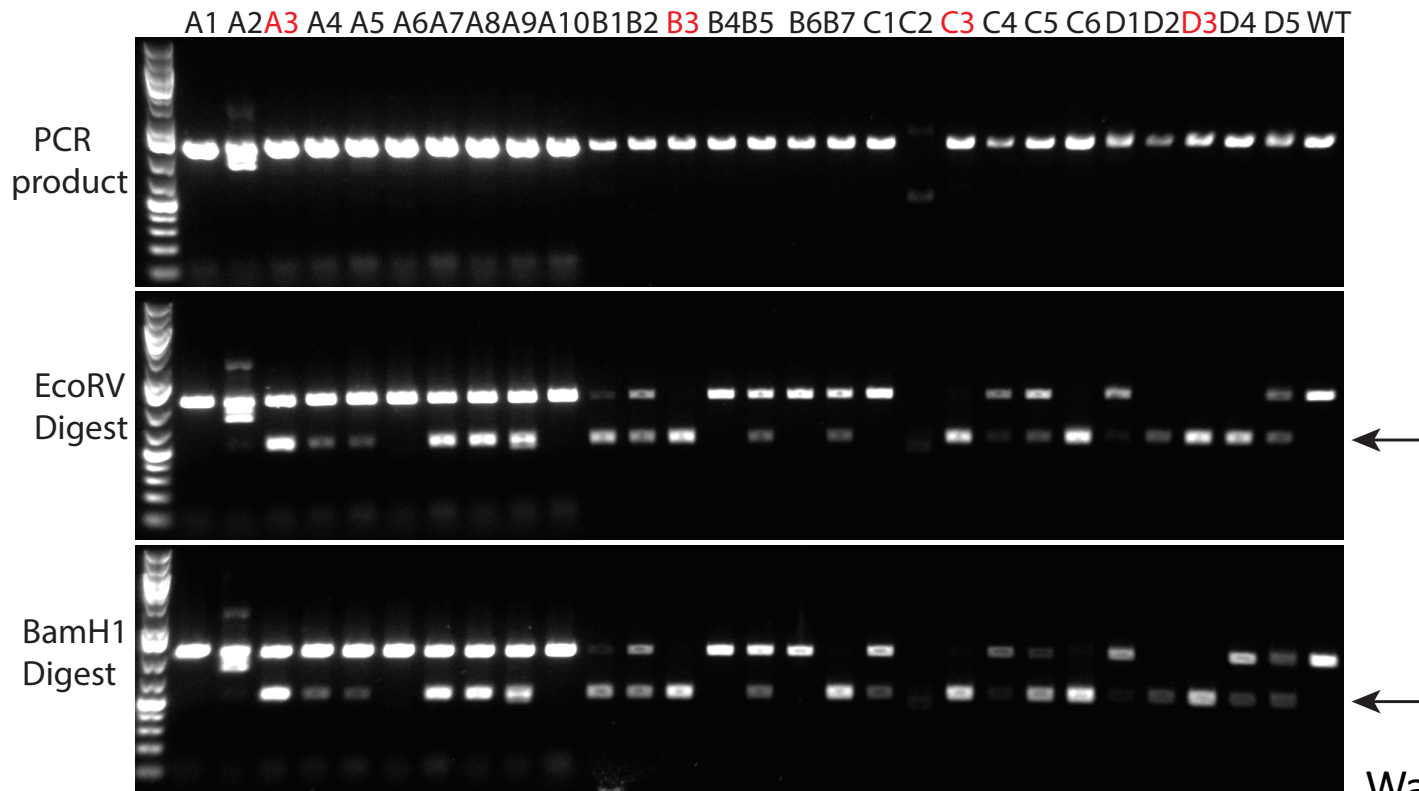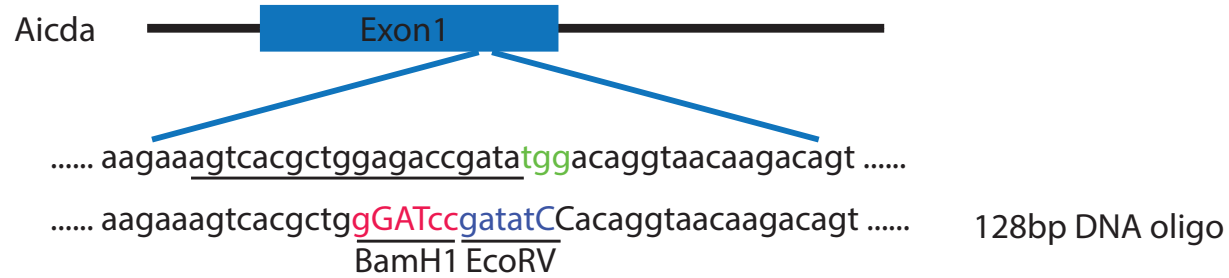## (with 1 mm electroporation cuvette, Model 610, P/N 45-0124)
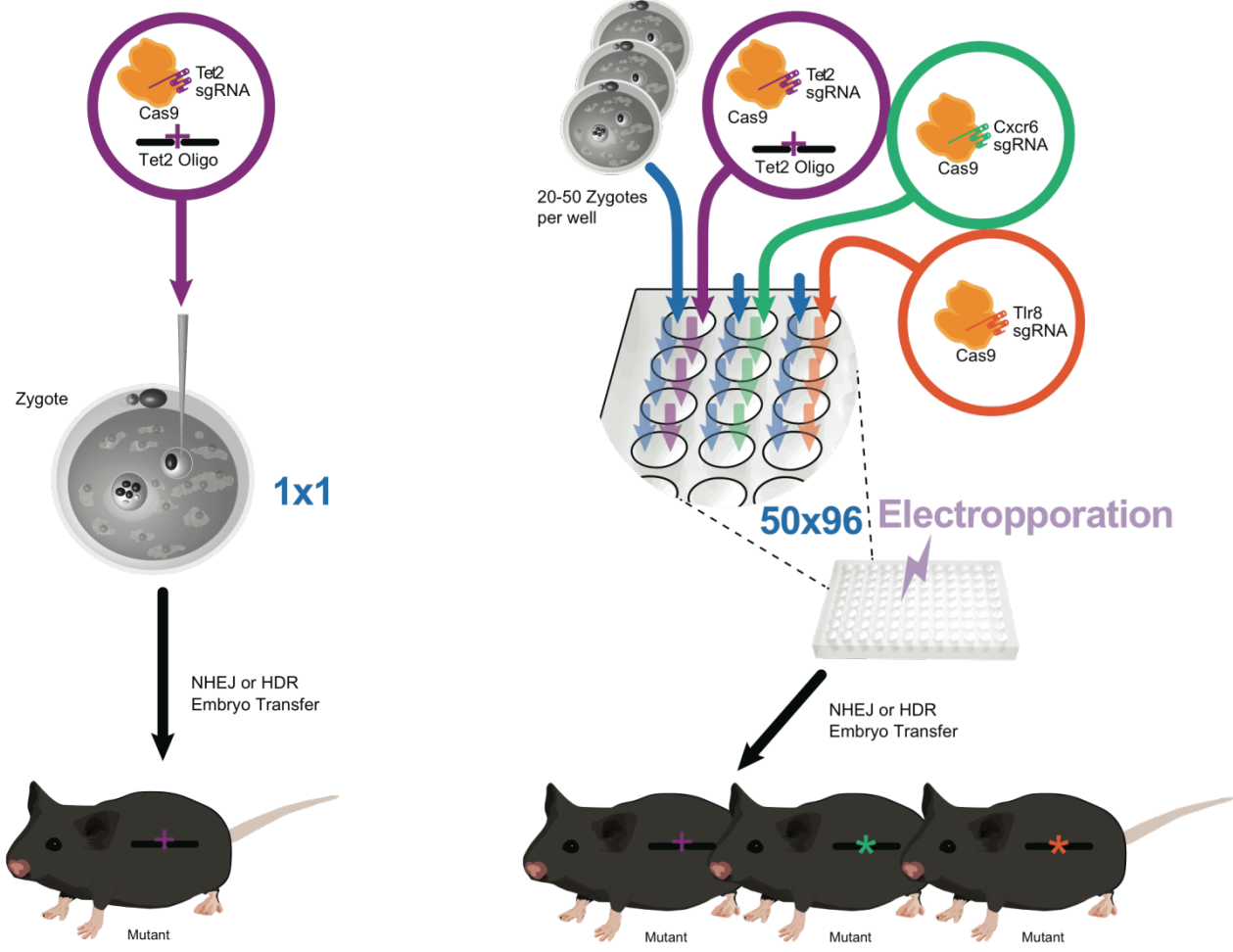

FEATURES

- A wide range of voltages from 5 to 3000 V
- Finer voltage discrimination
- Pulse durations from 10 µsec to 10 sec
- Arc Quenching
- Digital display of actual voltage and pulse length delivered

ZEN Improvement

Aicda KI using Cas9 protein

Wang et al. , 2016

CRISPR-mediated Genome Editing in Mice, High Throughput.

Qin et al. , *Genetics* 2015

Generating mouse models in high throughput

- Quickly screen candidate genes in founder animals

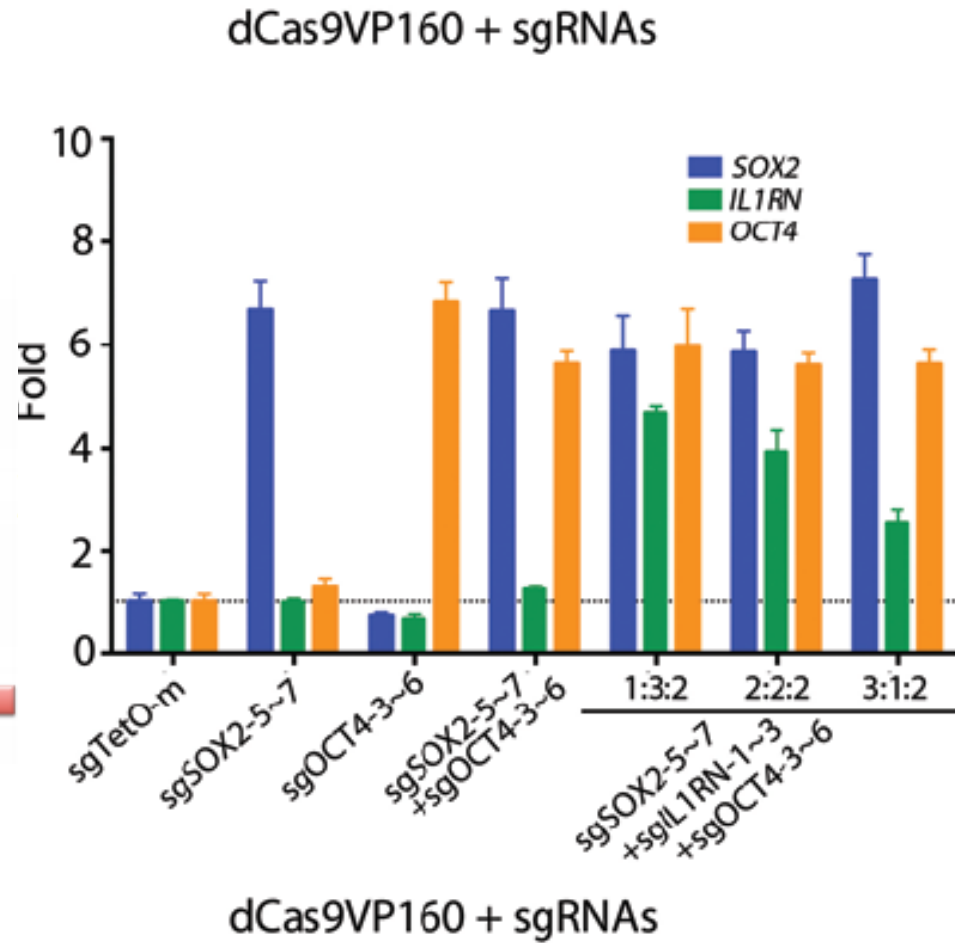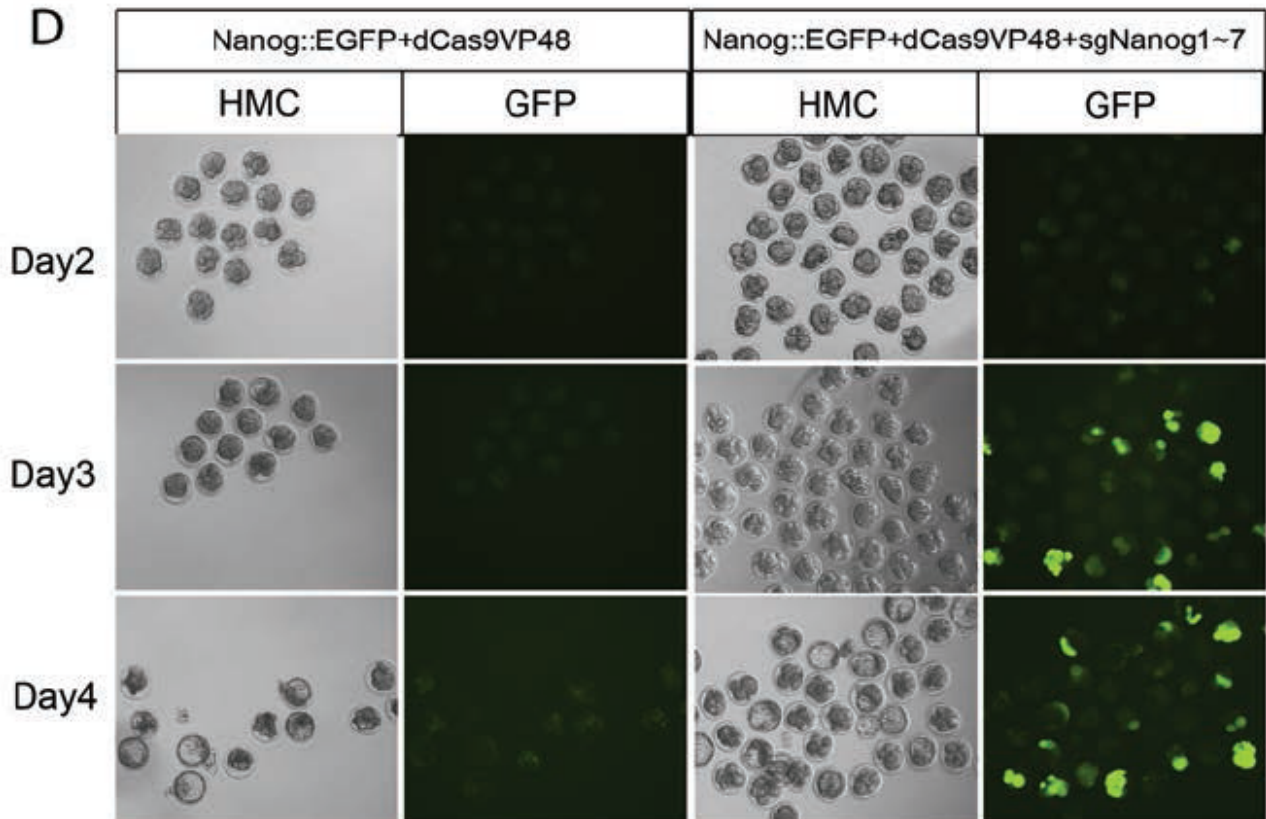- Generate human genetic variant in mouse ortholog
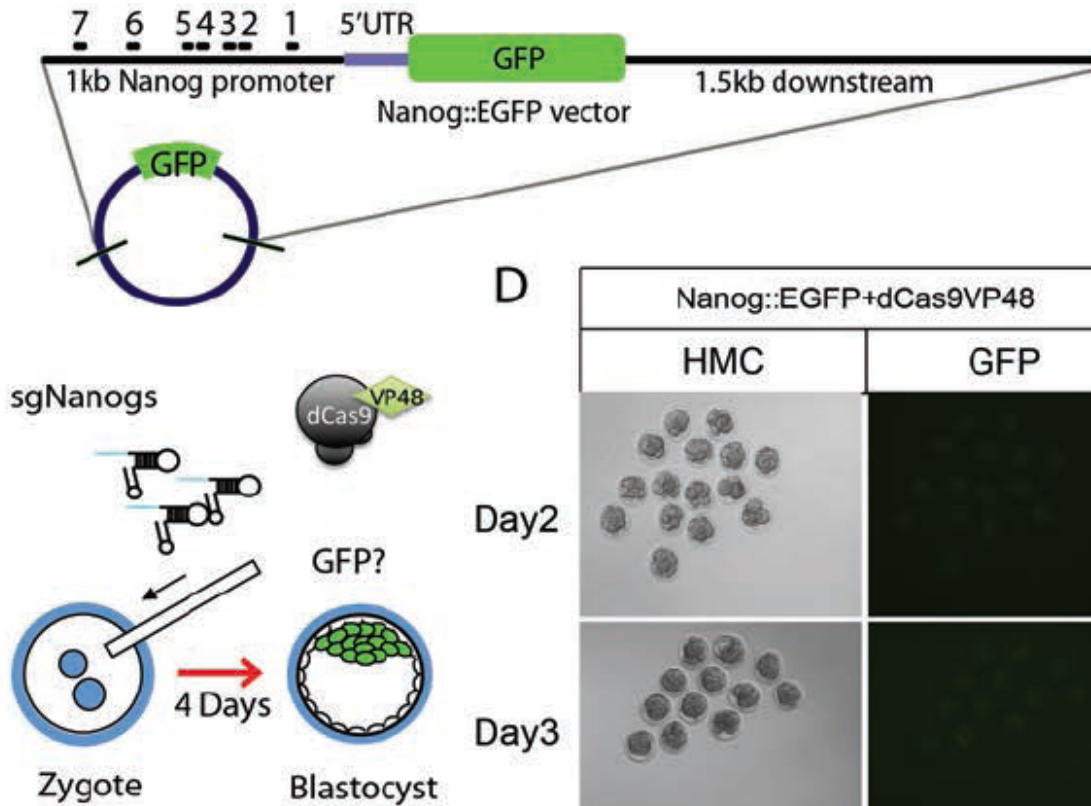
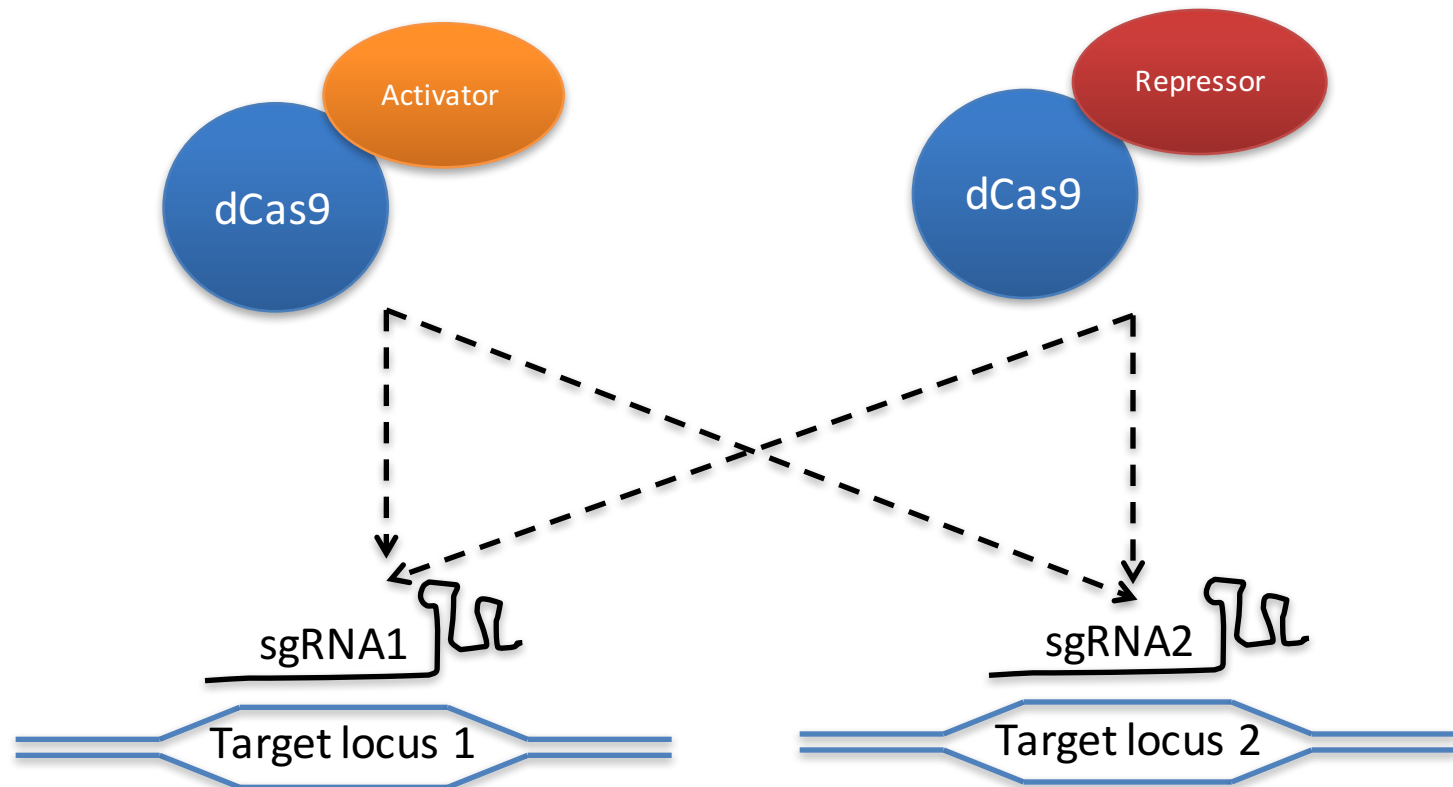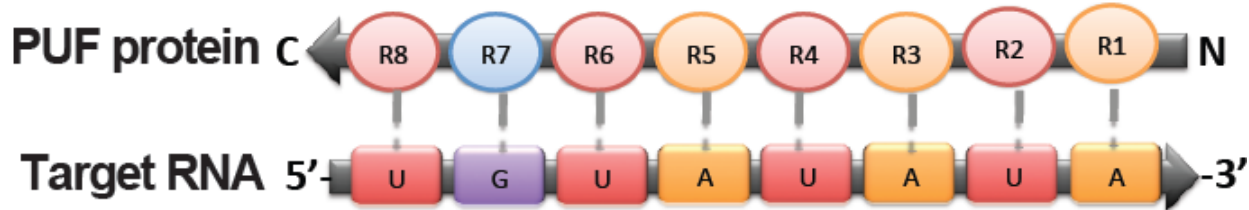# Different Flavors of Cas9

WT
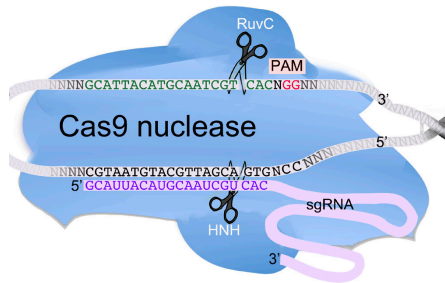
CRISPR-on
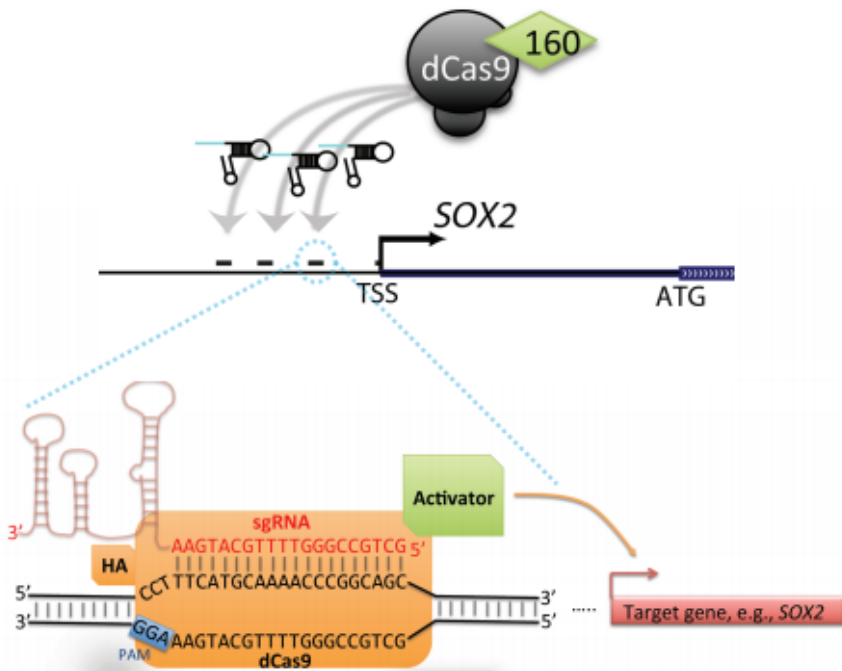
(Cheng et al, 2013)

CRISPR-on

(Cheng, 2013)

sgRNA-dCas9 fusions Cross-react

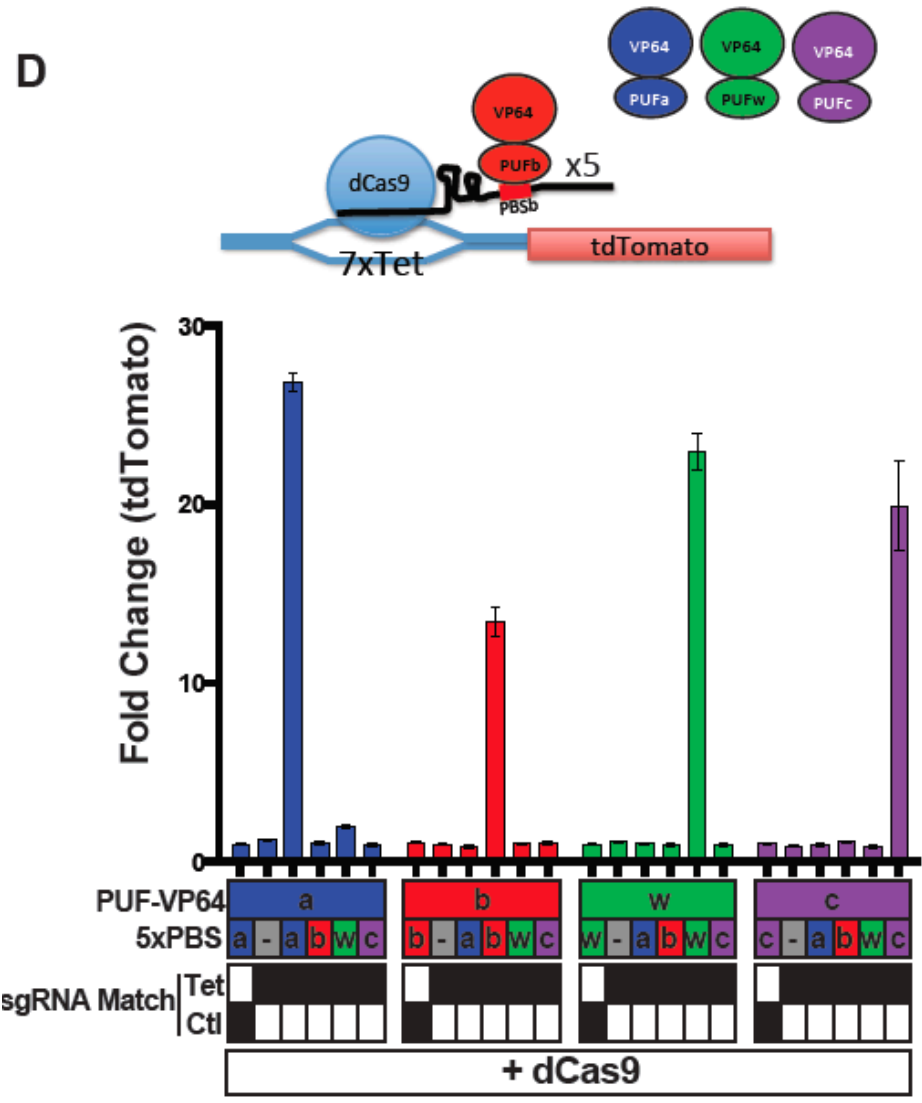- We cannot use the multiple sgRNA to direct different dCas9 fusions to different loci

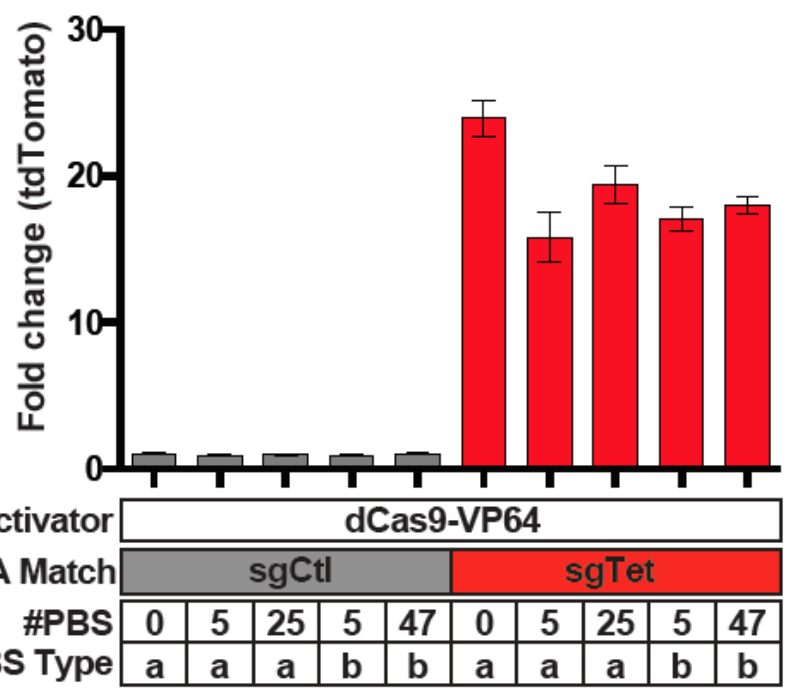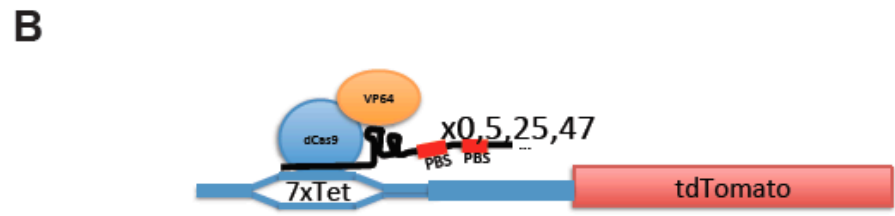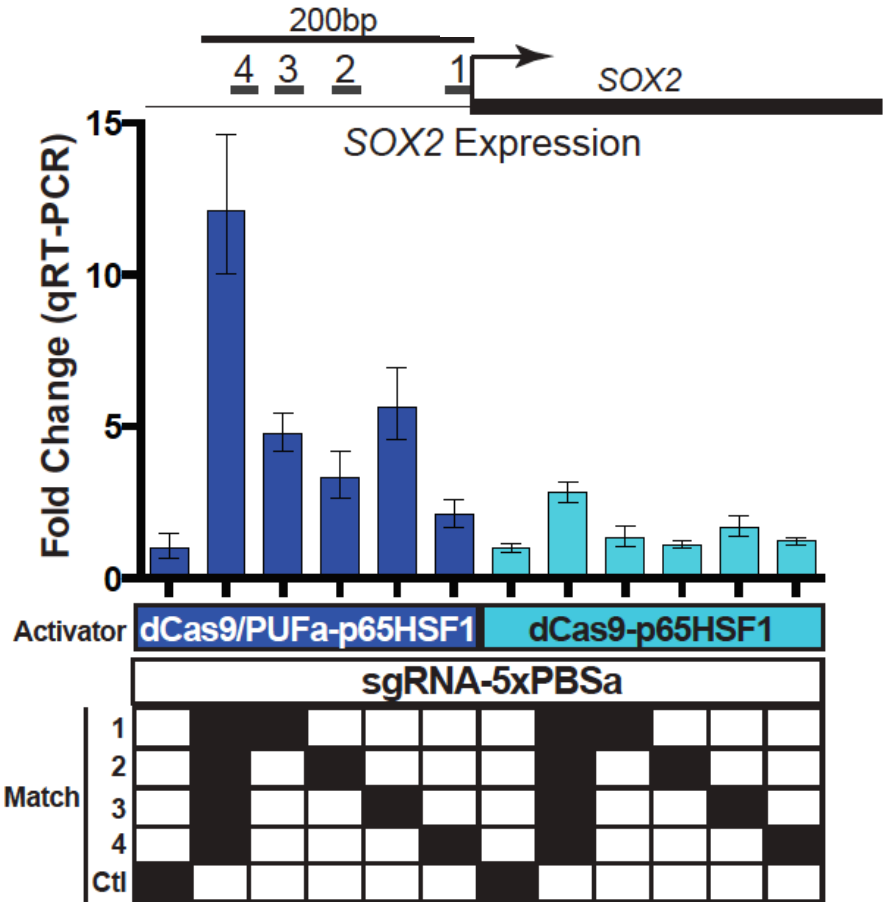# Casilio = <u>Cas</u>9 + Pum<u>ilio</u>
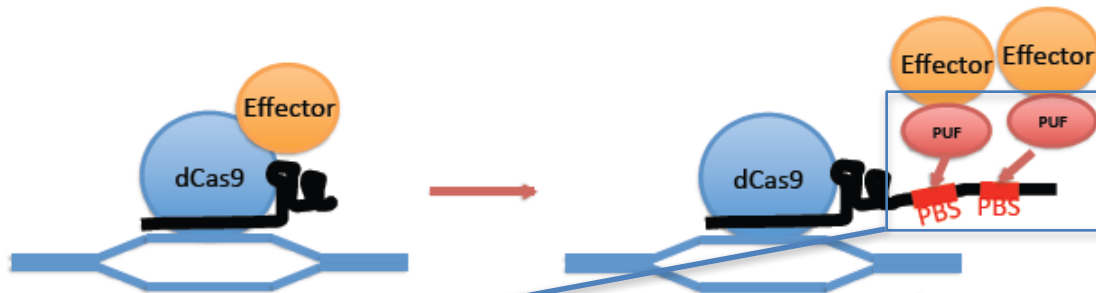
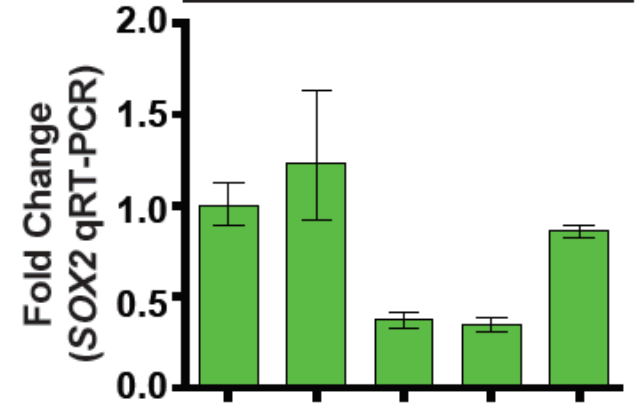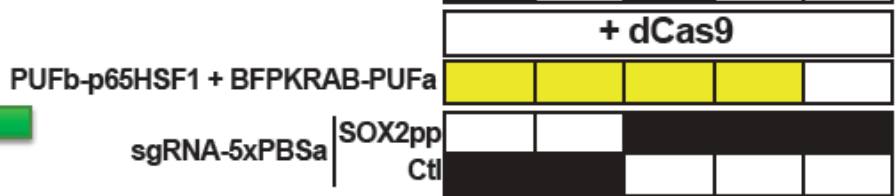| PUF Type	PBS Type	Binding Seq
PUFa	PBSa	UUGAUgUA
PUFb	PBSb	UugAUAUA
PUFw	PBSw	UGUAUAUA
PUFc	PBSc	UugAUgUA

Casilios are orthogonal without significant cross-reaction
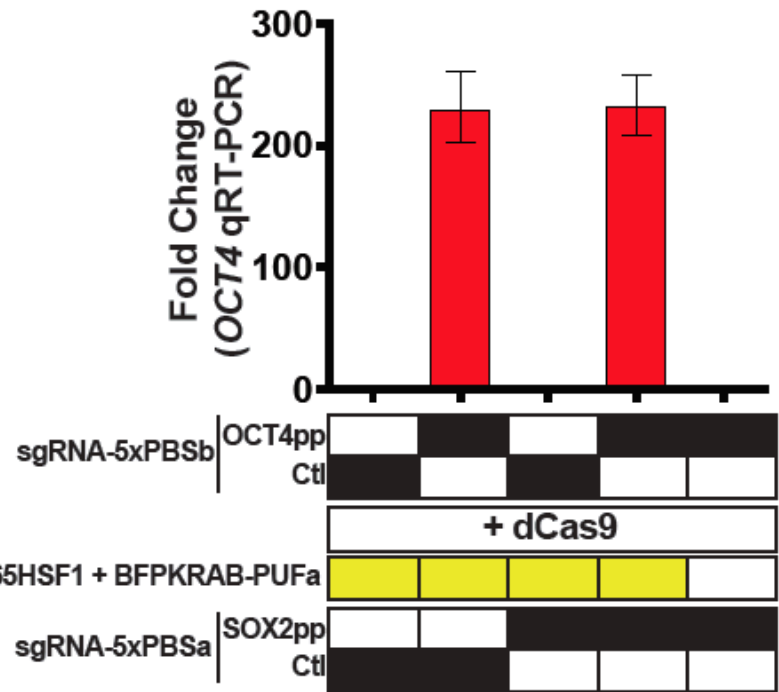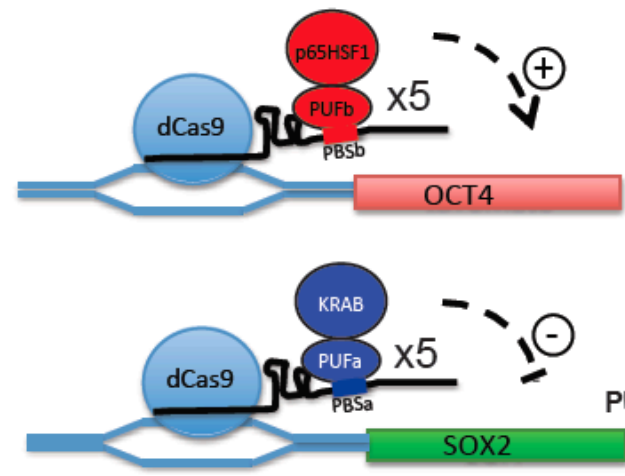
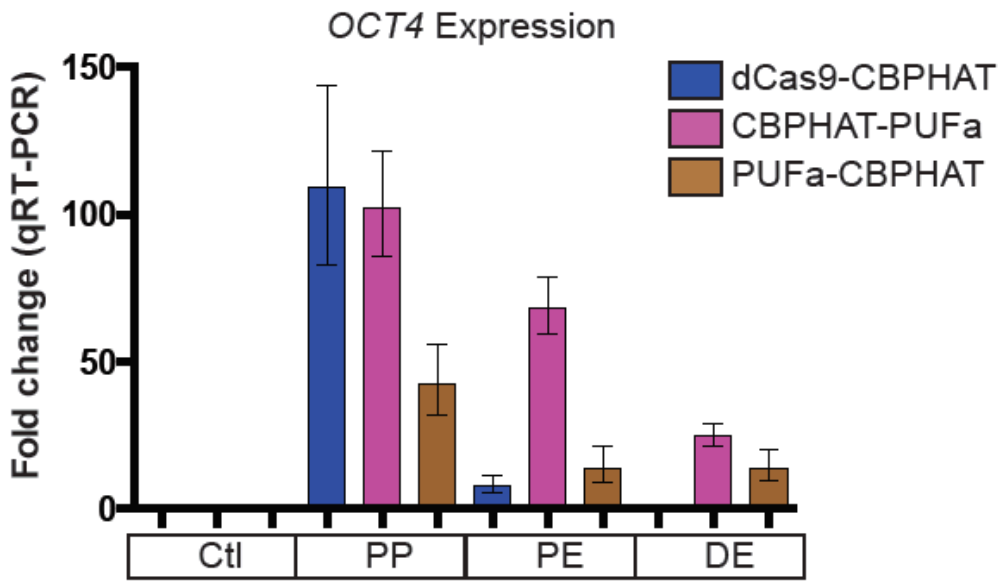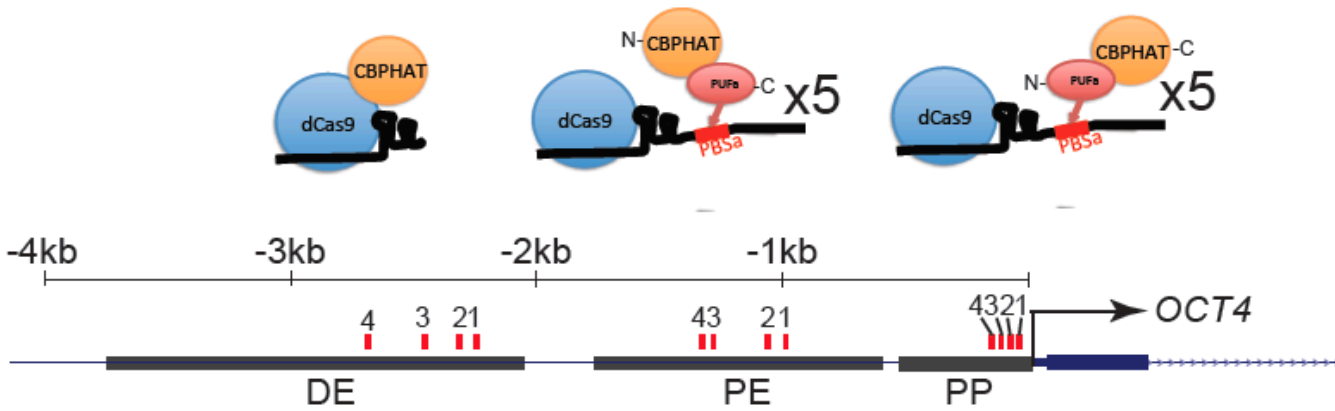# Casilios activate endogenous genes more efficiently than dCas9 direct fusion
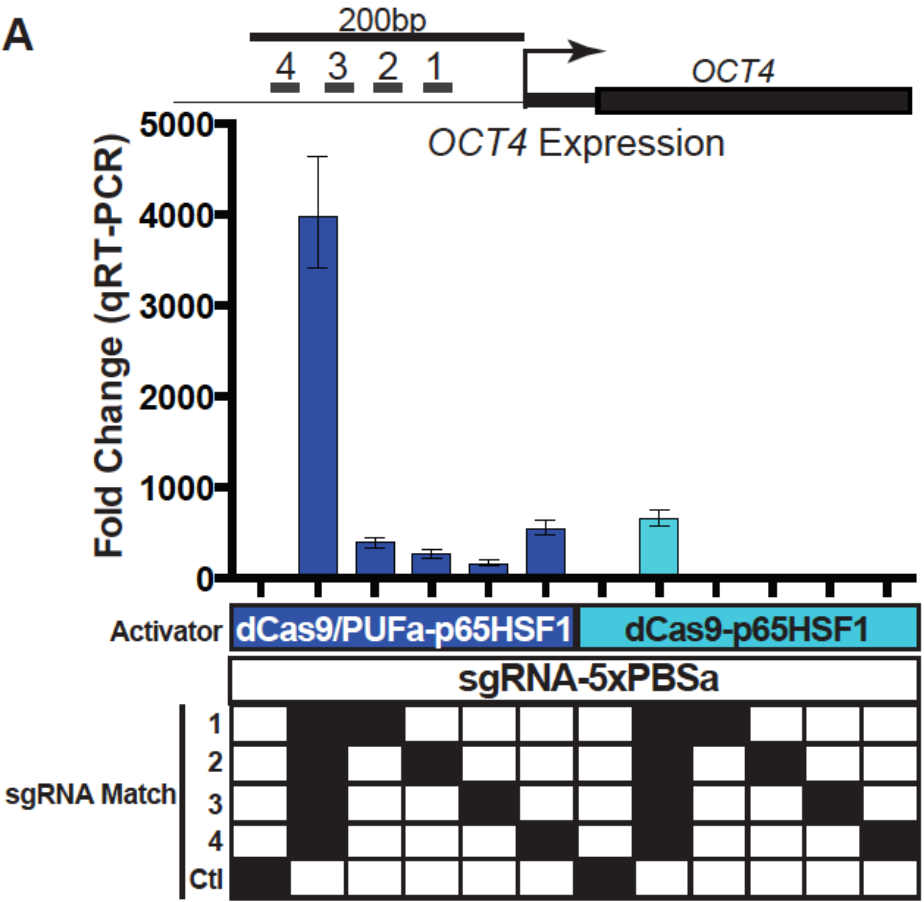
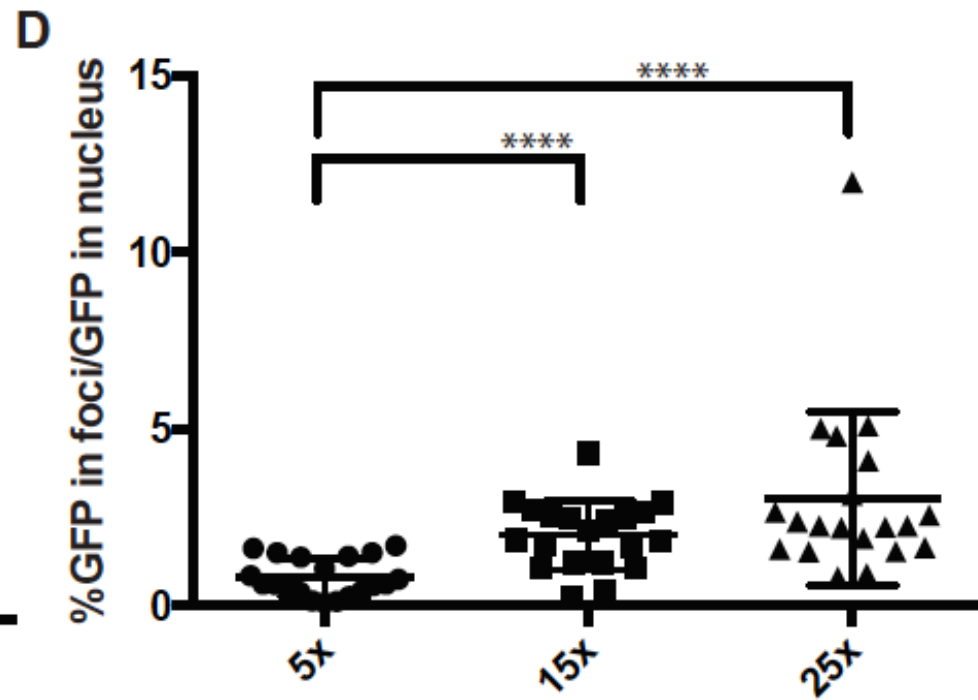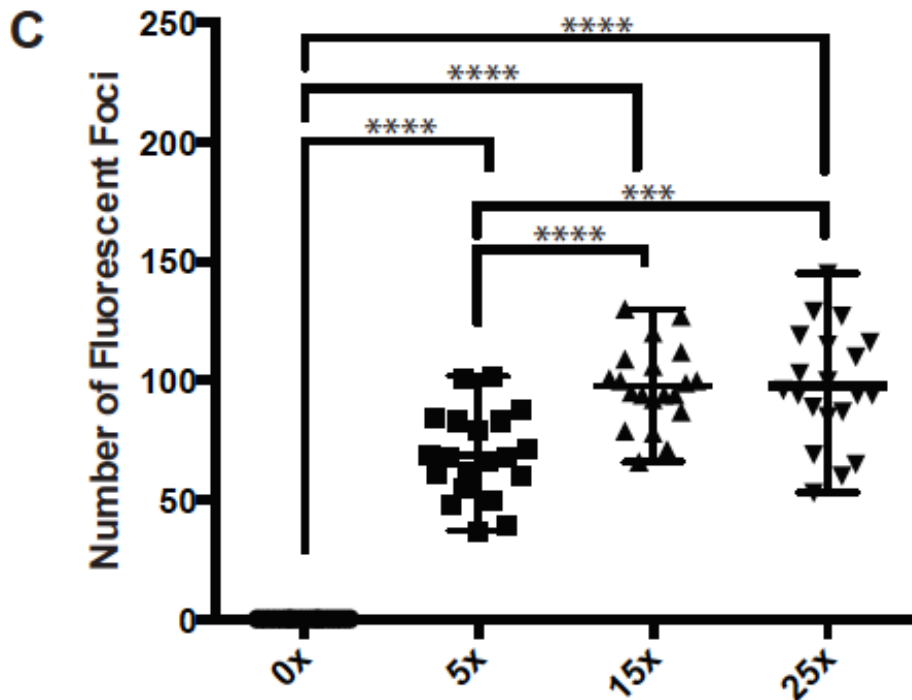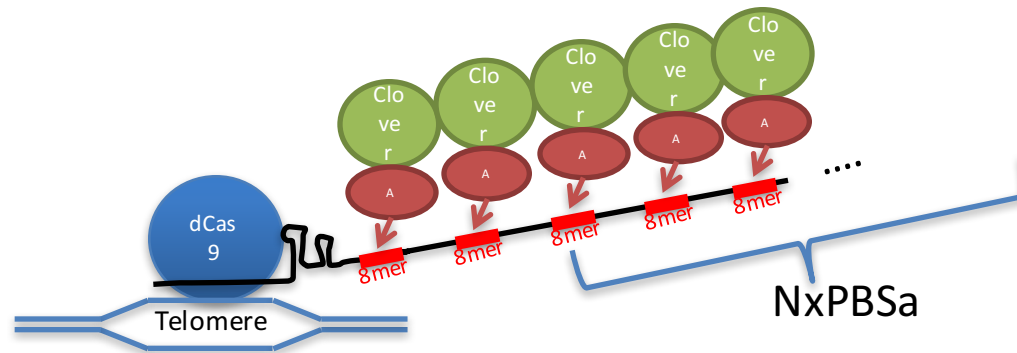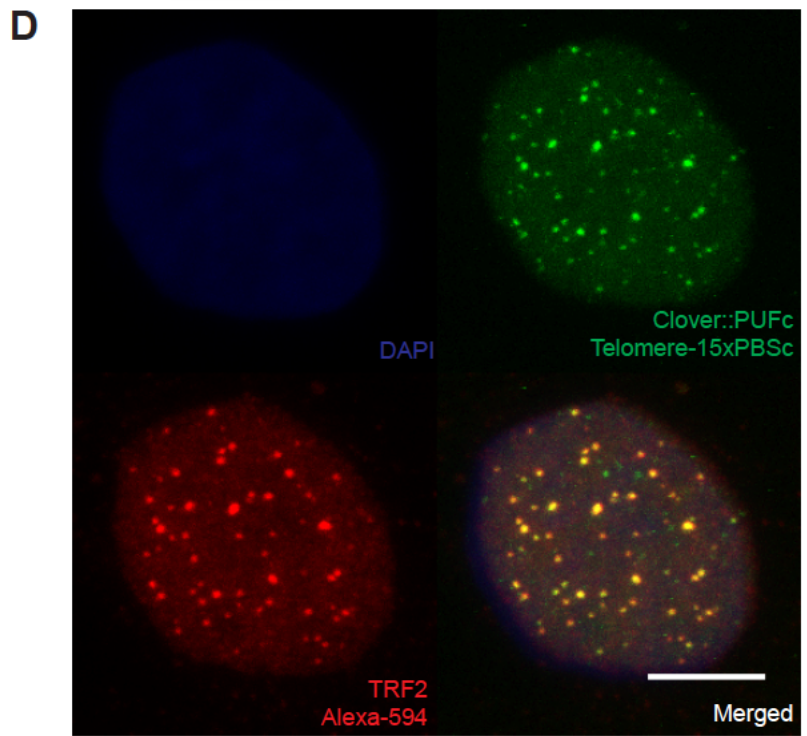# Casilios simultaneously activate and repress separate endogenous genes
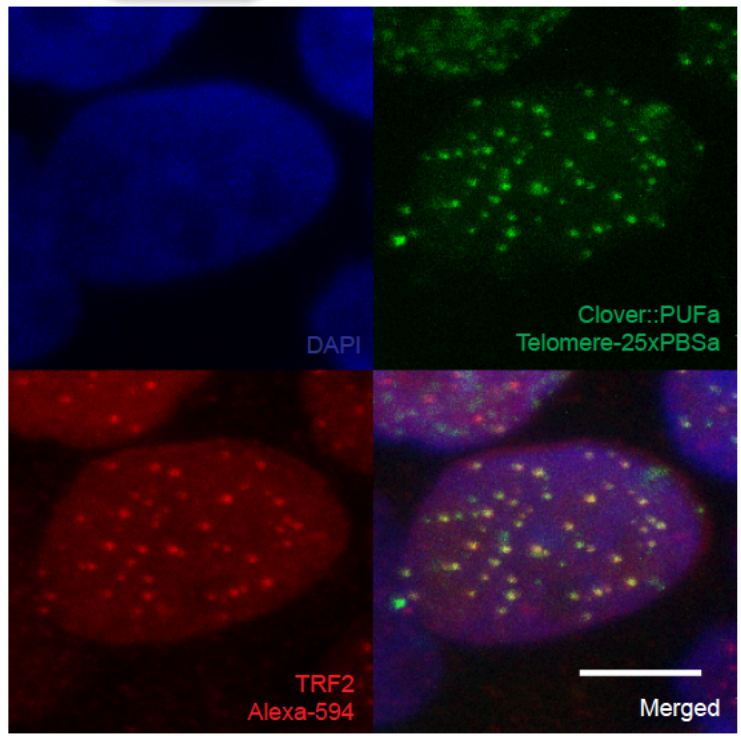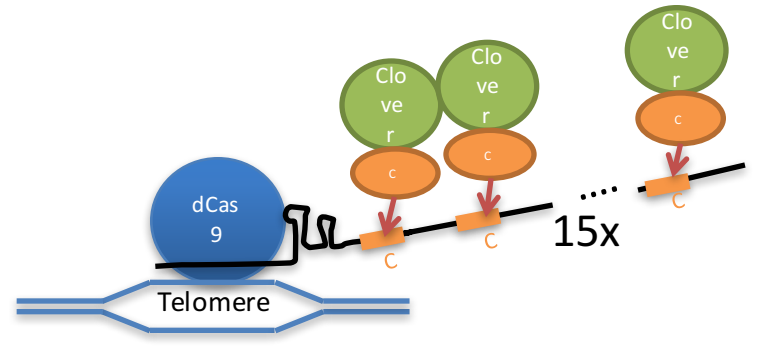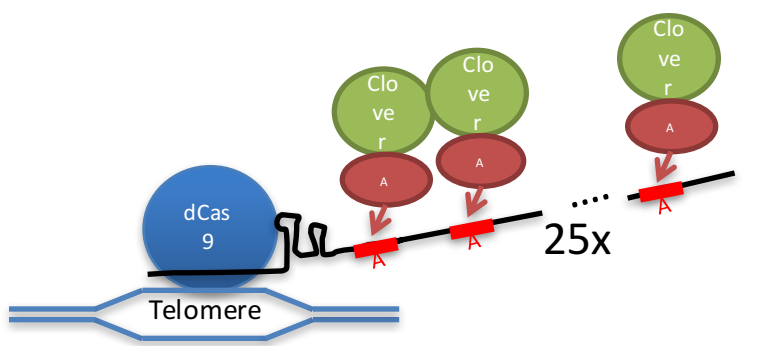
Casilios recruit HAT to enhancers activating gene expression
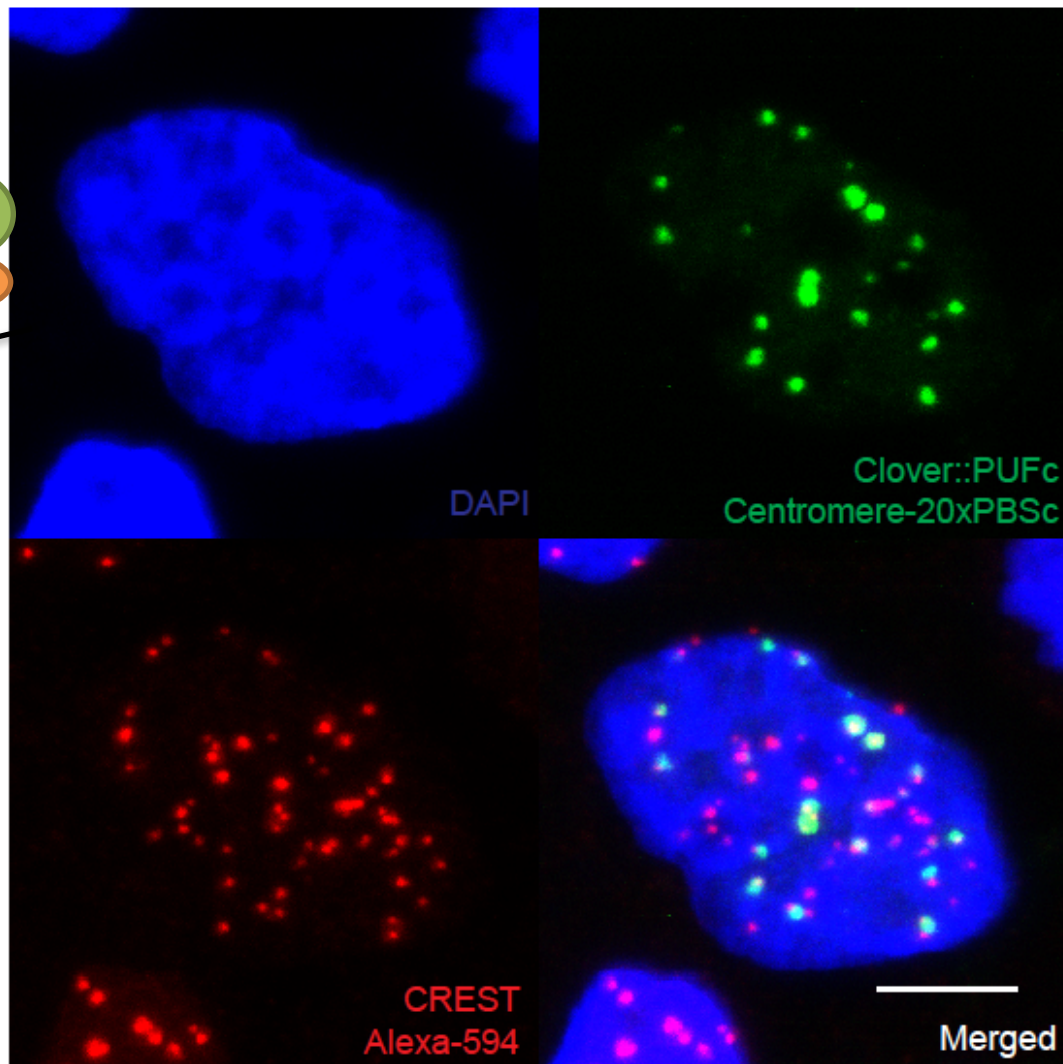
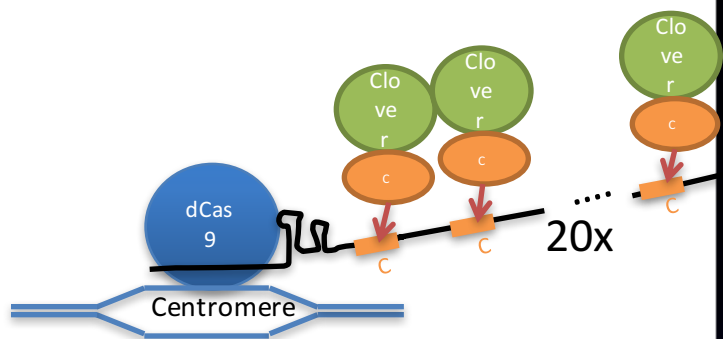# Polymerization of Clover by Casilio improves imaging

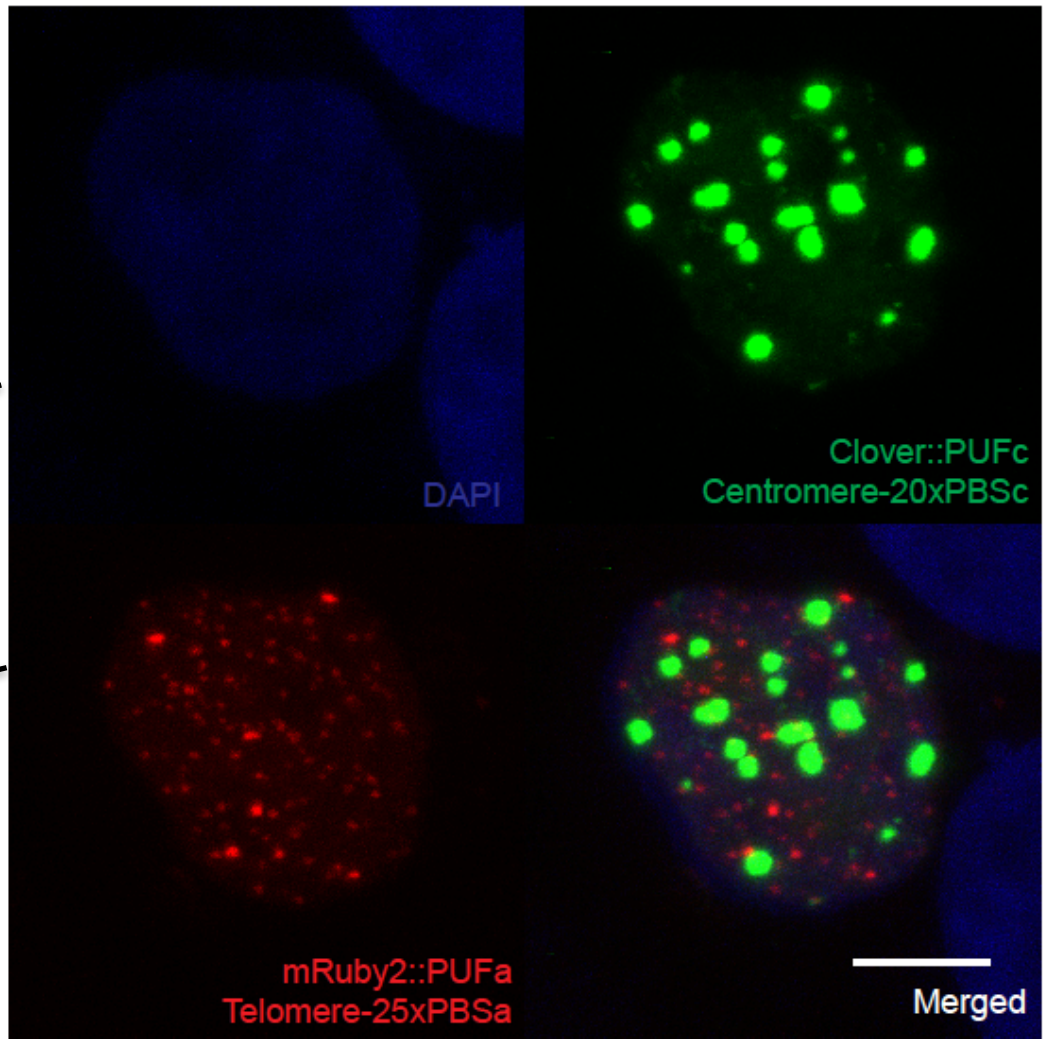# Casilio isotypes can achieve labeling using cognate sgRNA-PBS

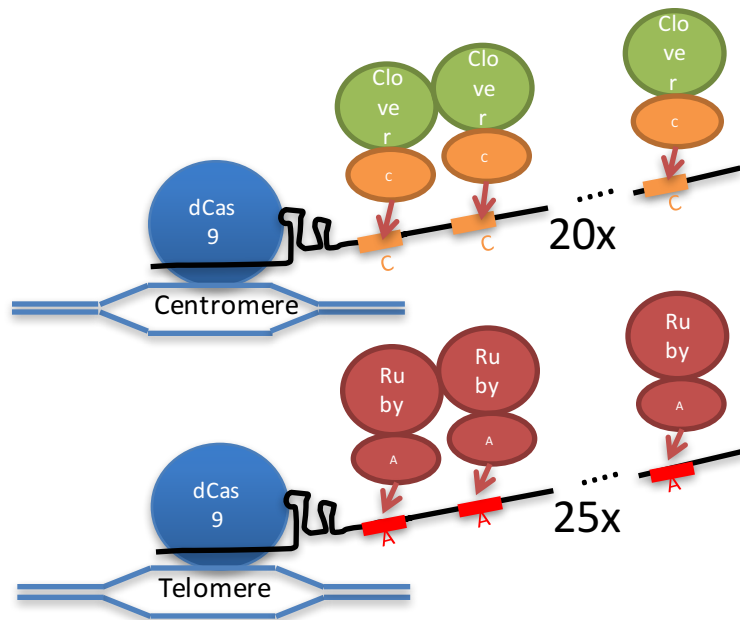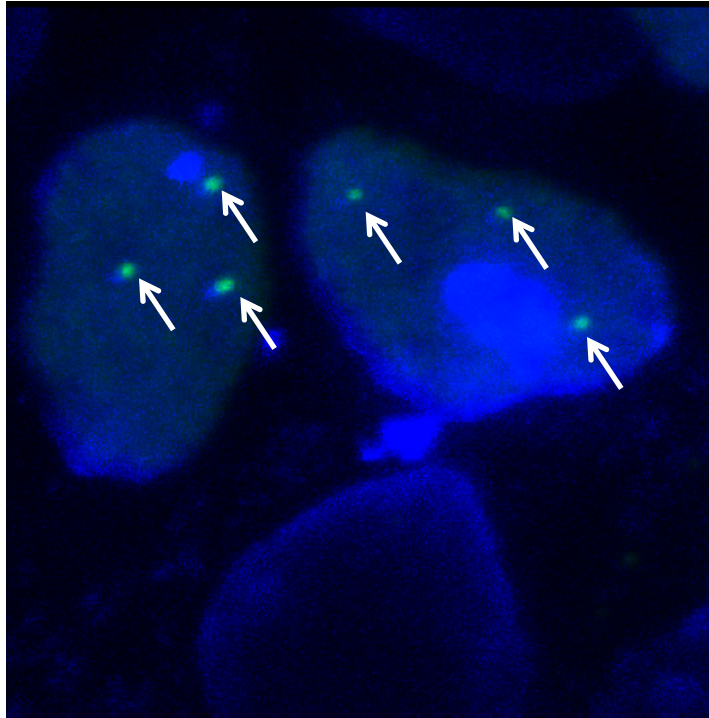# Labeling of Centromeres

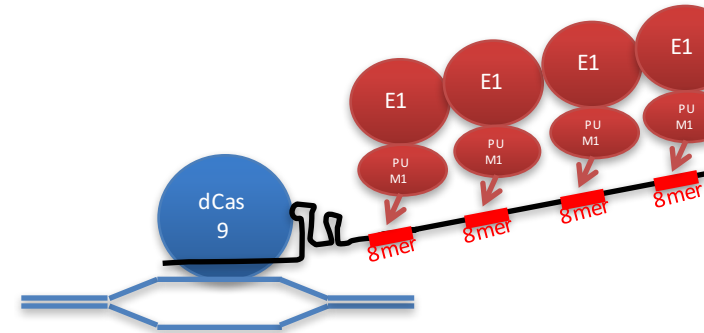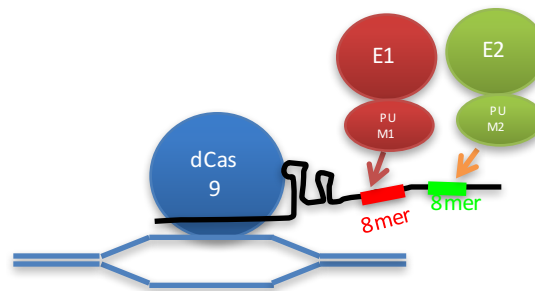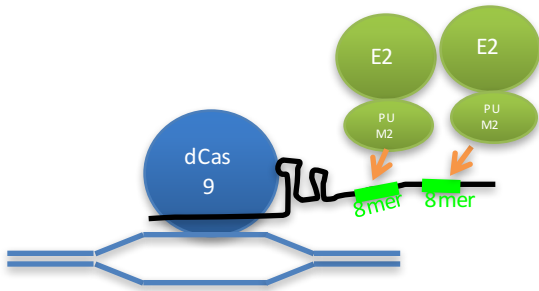# Simultaneous labeling of Centromeres and Telomeres

Improving Non-repeat labeling?

Preliminary data: Casilio allows labeling of non-repeat region with 7 sgRNA-15xPBS32 targeting MUC4 locus. Representative confocal microscopy image of the MUC4 labeling. Need IF-FISH to confirm

Casilio applications
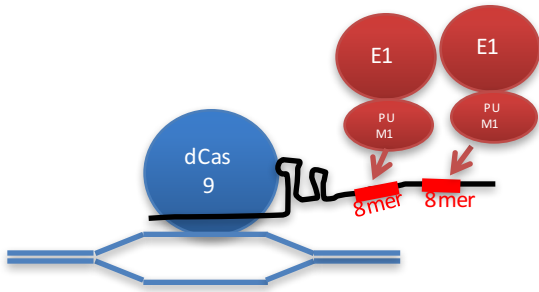
Multiplexing

Complex formation

Polymerization

Gene Editing as Therapeutics

- Identify a list of actionable diseases and genes
- Could we developed a relatively cost-effective model for the therapeutic development for rare diseases
- How do we evaluate the risk/benefit

Acknowledgements

- Genetic Engineering Technologies (GET) at JAX
  - **Wenning Qin**
  - **Peter Kutny**
  - **Stephanie Dion**
  - Michael DaCosta
  - Bill Buaas

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  - Rob Taft
  - Shannon Byers
  - Charles Longstaff

- My lab at JAX
  - **Wenbo Wang**
  - Yingfan Zhang
  - **Albert Cheng**
  - Nathaniel Jillette

Acknowledgements

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  - **Qi Zhou**
  - State Key Laboratory of Stem Cell and Reproductive Biology
  - My lab members in Beijing