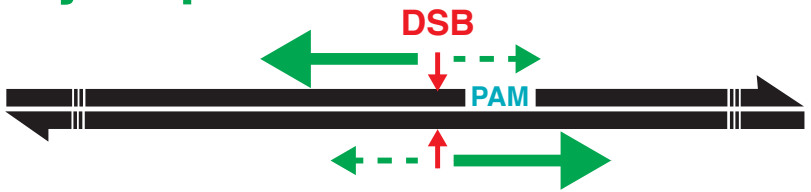
Improving the efficiency of precise Cas9-mediated genome editing

Behnom Farboud[1], Aaron F. Severson[2], and Barbara J. Meyer[1]

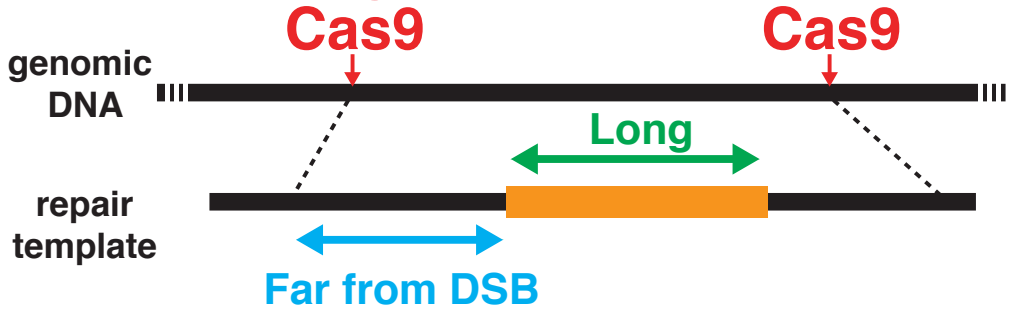
[1]HHMI, University of California, Berkeley and
[2]Cleveland State University

Strategies to improve efficiency of Cas9 editing

1) **Polarity: repair relative to PAM**

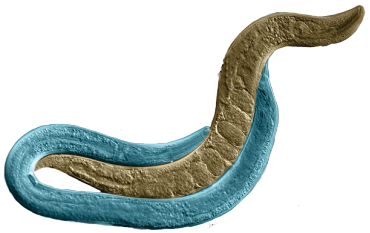


2) **Two DSBs: Long inserts, far from DSB**



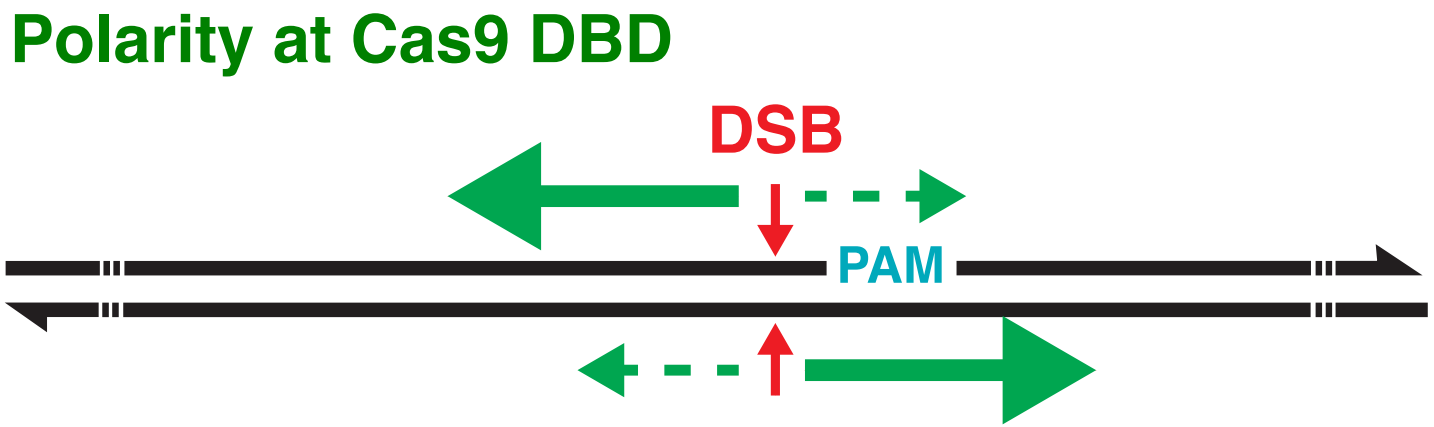
3) **Male vs. hermaphrodite DNA targeting efficiencies differ**

Cas9 +

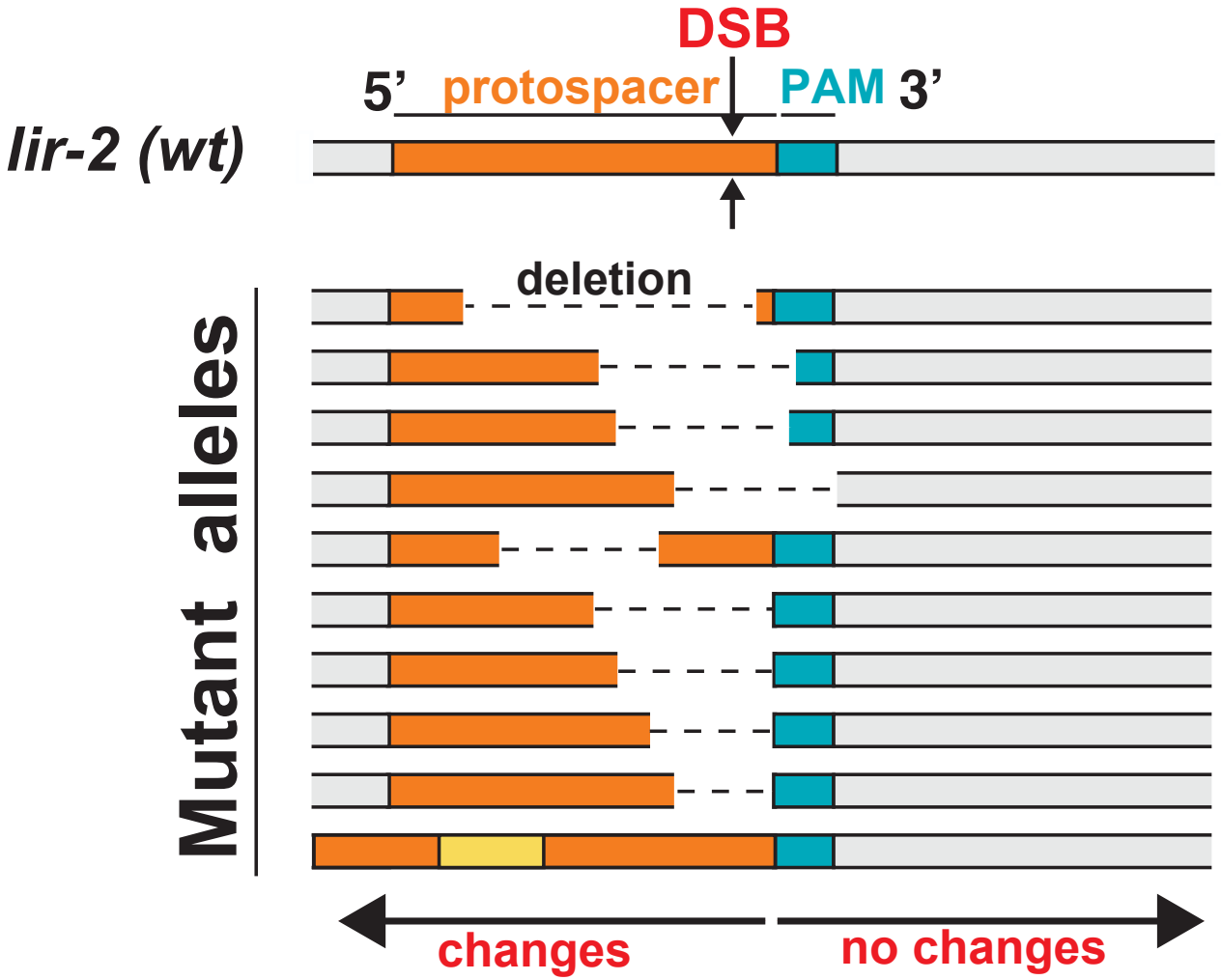


4) **New co-conversion markers efficient across species**

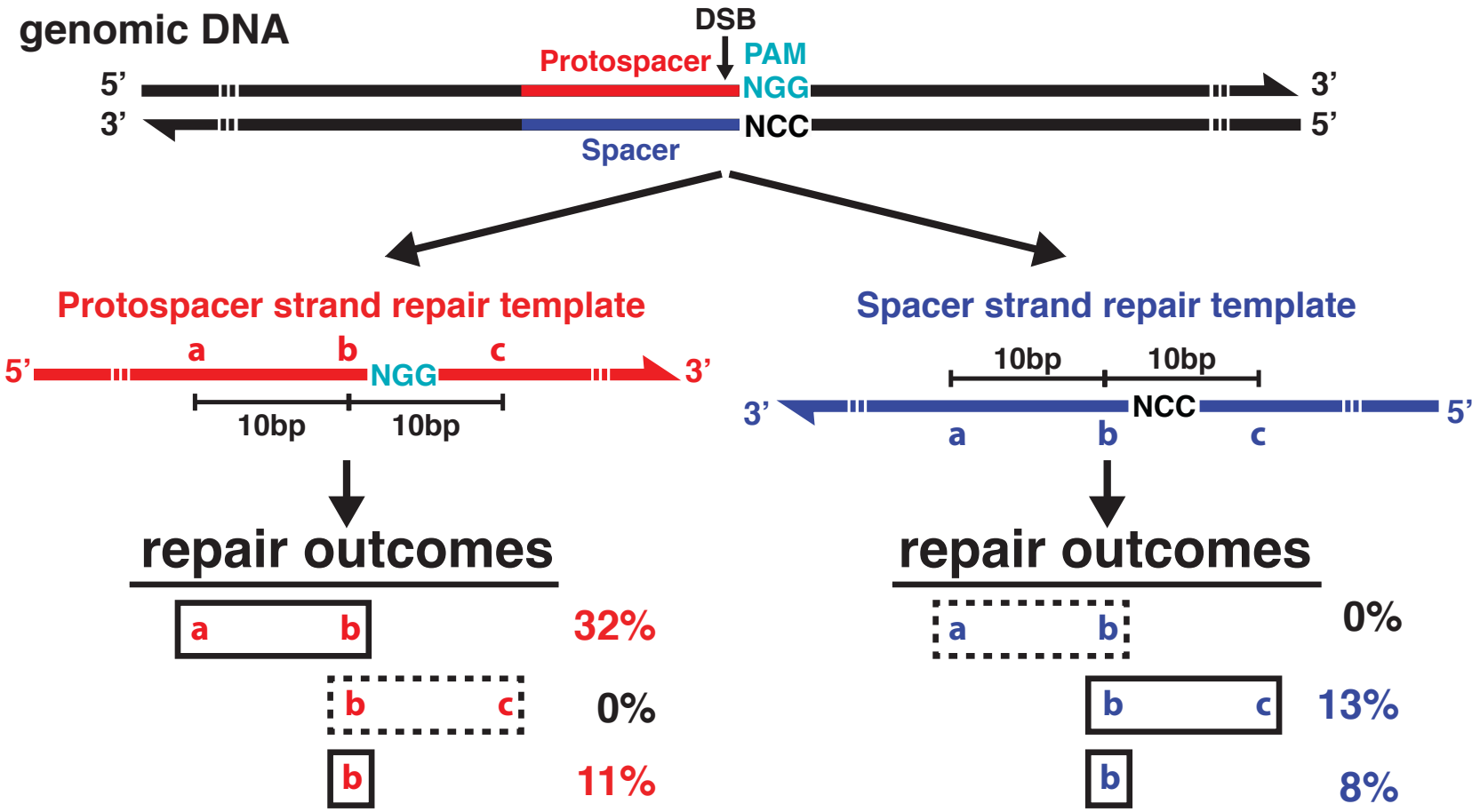
Repair outcomes for imprecise repair and HDR are influenced by polarity at Cas9 DSB



Polarity at Cas9 DSB: 3' of PAM protected after Cas9 cleaves



ssDNA repair strand choice dramatically affects repair outcomes



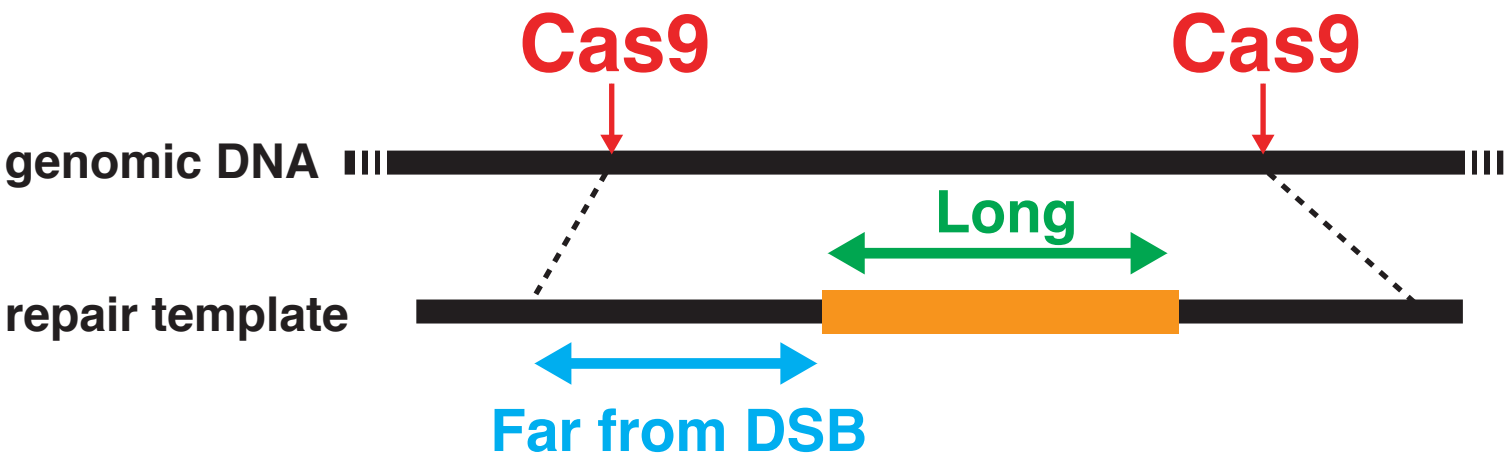
(% of co-converted animals)

Consistent across 4 loci, 8 repair templates

To incorporate changes using ssDNA templates:

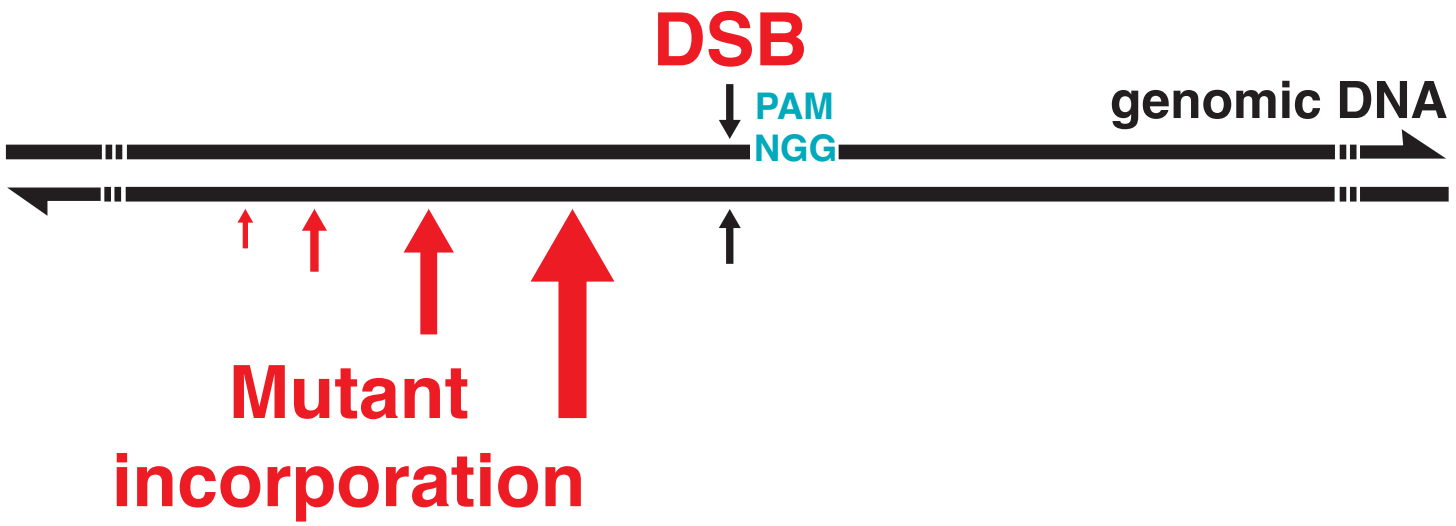
- Use protospacer strand for changes 5’ of PAM
- Use spacer strand for changes 3’ of PAM

Use Two Cas9 DSBs to incorporate long insertions or changes distant from DSB

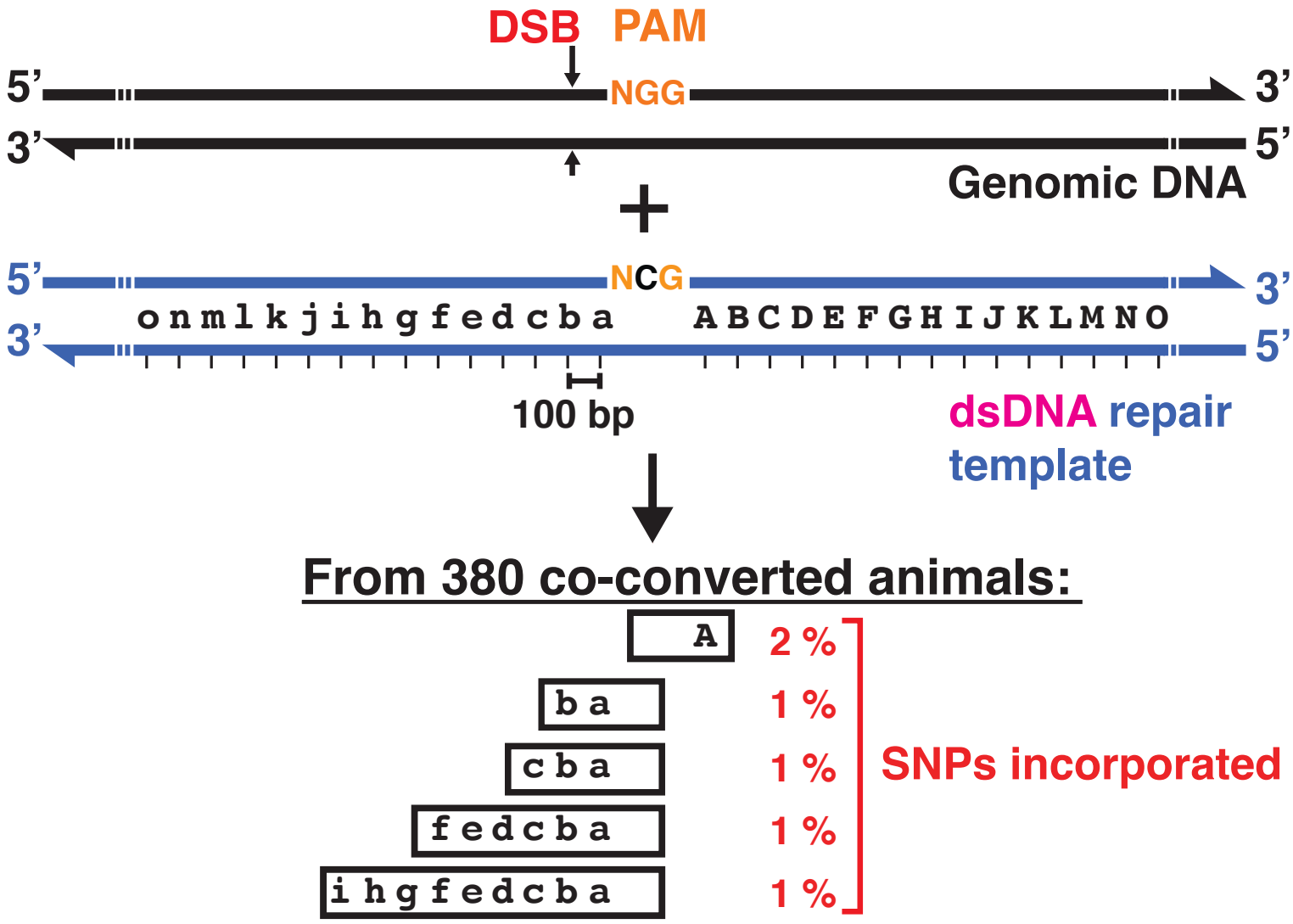


HDR is less efficient further from the Cas9 DSB

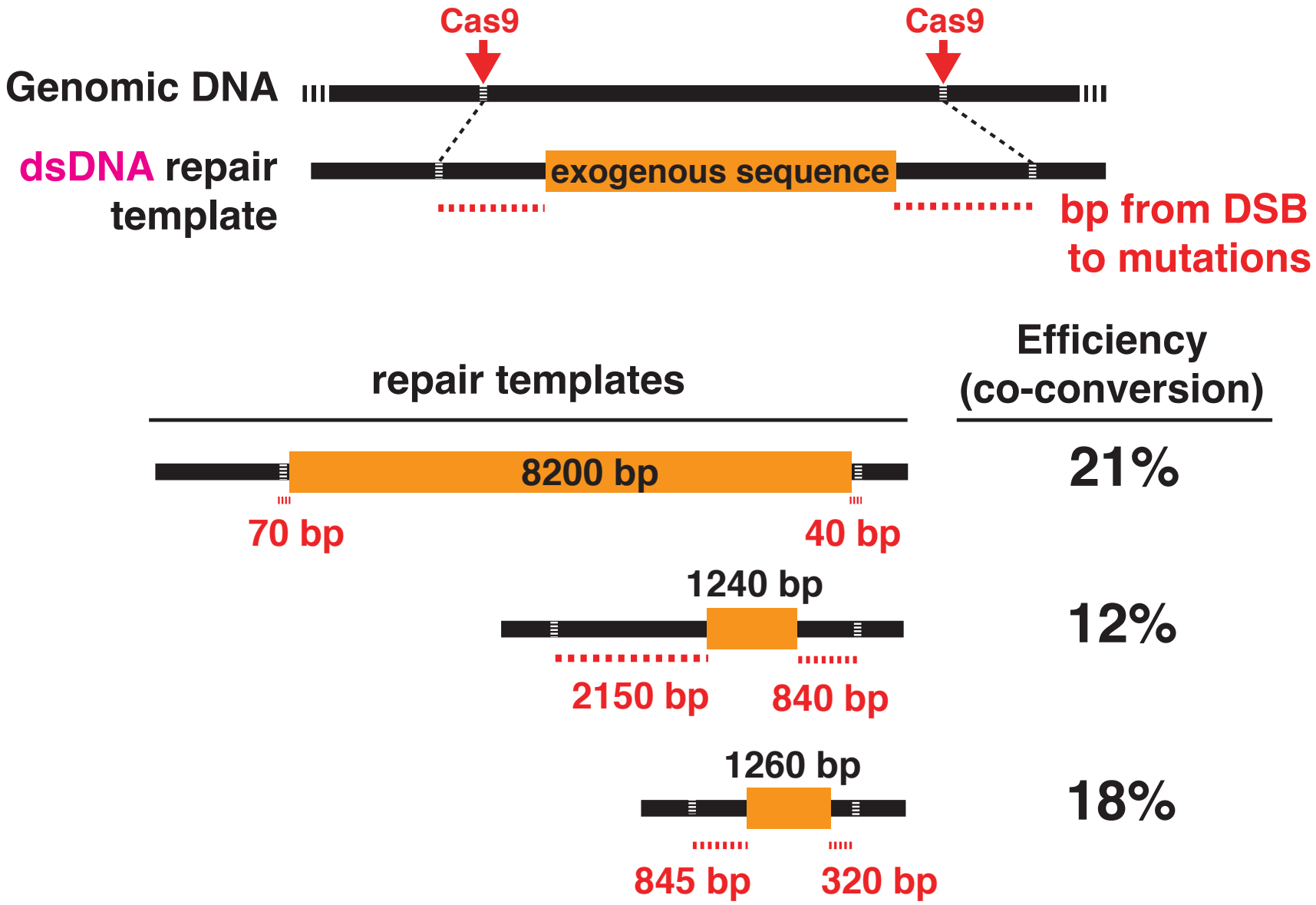
HDR efficiency $\propto \frac{1}{\text{bp from DSB}}$



Conversion tracts are longer 5’ of PAM, but efficiency is low



Efficient incorporation of long inserts using two Cas9 DSBs



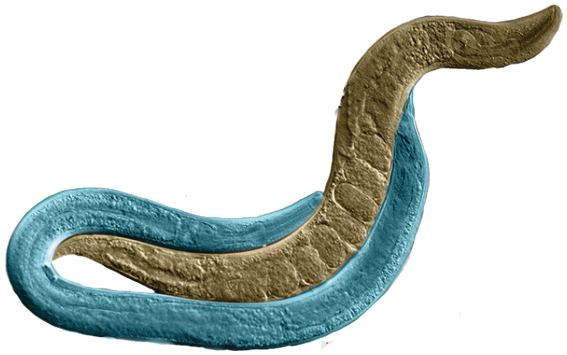
Use two flanking Cas9 sites to:

- incorporate long inserts

OR

- to insert sequences far from Cas9 DSBs

Cas9 +



Mating can increase Cas9 mutagenesis rates, because parental origin of target can bias Cas9 efficiency

Mating can increase Cas9 efficiency

Targetable alleles	Protospacer	PAM
sex-1 (G)	GGATGAGAATCTGACAAA**G**G	TGG
sex-1 (A)	GGATGAGAATCTGACAAA**A**G	TGG

Targeting A allele only (no repair template):

		% co-conversion
Maternal target control	♀A (self)	31%
Paternal target	♀G x ♂A	90%

Is it the parental origin of DNA or the act of mating that's responsible for the increased Cas9 efficiency?

DNA from MALE sperm is more susceptible to editing

Targeting A allele only (no repair template):

		% co-conversion
Maternal target control	A ♀ (self)	31%
Paternal target	G ♀ x A ♂	90%
Maternal target with mating	A ♀ x G ♂	35%

3 of 4 loci show this paternal DNA bias

Parental origin of target, rather than mating alone, can bias efficiency of Cas9 mutagenesis

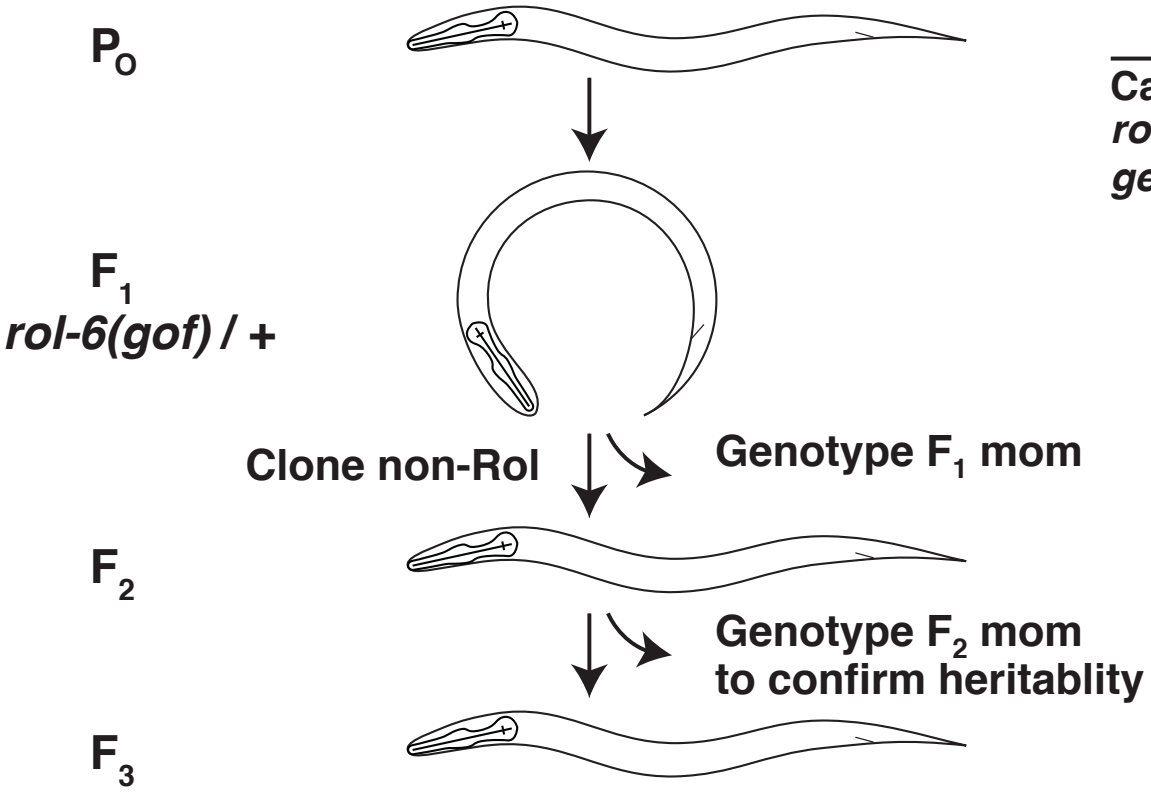
New co-conversion markers for editing in multiple nematode species

- ***ben-1 III***
- ***zen-4 IV***

rol-6 II* and *dpy-10 II* were not effective co-conversion markers in *C. briggsae

Co-CRISPR/co-conversion simplifies screening

Experimental Strategy: **target gene of interest + reference gene (*rol-6*)**

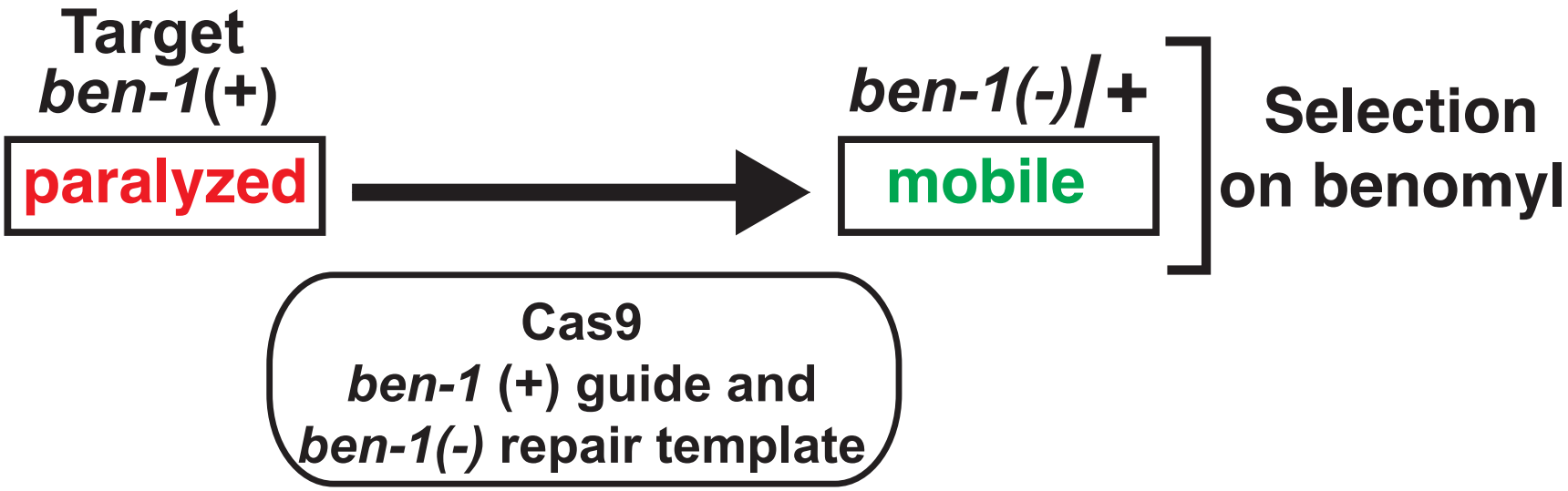


Inject:

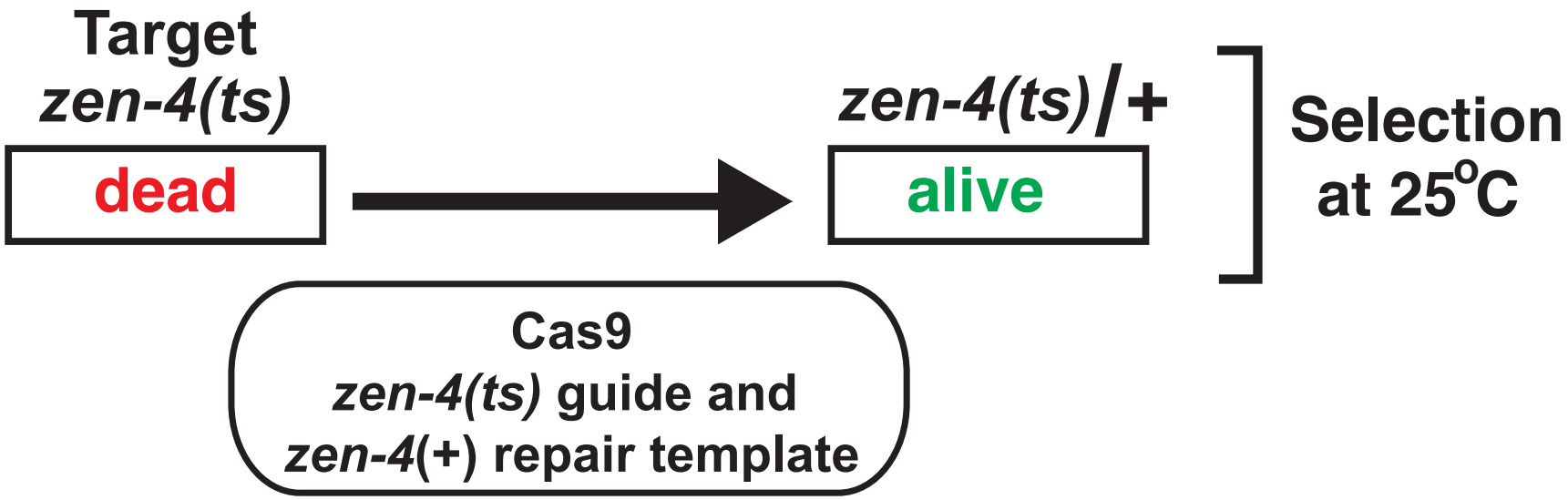
Cas9
***rol-6* guide + *rol-6 (gof)* oligo**
***gene X* guide + *gene X* oligo**

Kim, H., *et al.*, (2014). *Genetics* 197: 1069-1080.
Arribere, J. A., *et al.*, (2014) *Genetics* 198: 837-846.
Ward, J. D. (2014). *Genetics*. 199: 363-77.

ben-1 co-conversion phenotype: paralyzed vs. mobile



zen-4 co-conversion phenotype: dead vs. alive



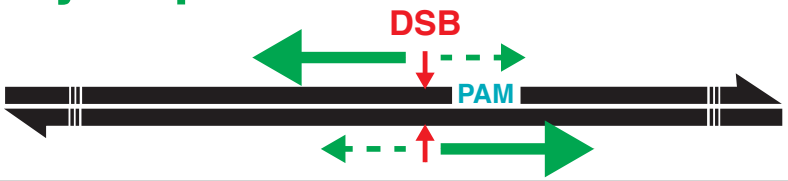
ben-1 and *zen-4*:

– permit robust screening or selection

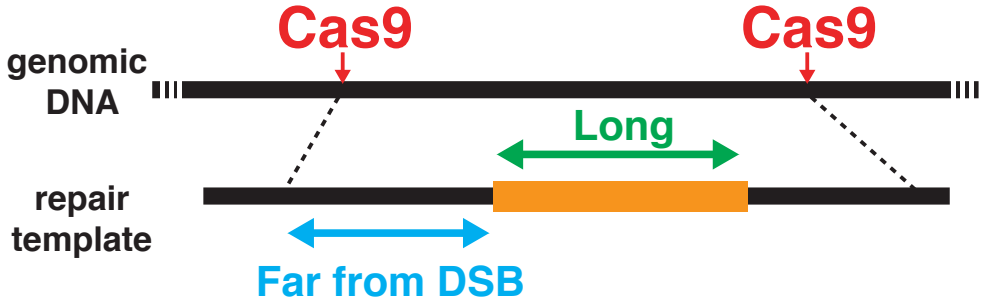
– Conserved among nematodes,
these genes are widely applicable
co-conversion markers

Strategies to improve efficiency of Cas9 editing

1) **Polarity: repair relative to PAM**

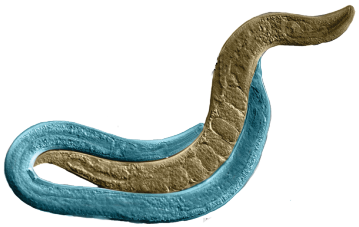


2) **Two DSB: Long inserts, far from DSB**



3) **Male vs. hermaphrodite DNA targeting efficiencies differ**

Cas9 +



4) **New co-conversion markers efficient across species**

Acknowledgments

Barbara Meyer

Erika Anderson
Qian Bian
Katjusa Brejc
Nick Fuda
Kristina Krassovsky
Denise Lapidus
Ed Ralston
Beth Rockmill
Deborah Stalford
Satoru Uzawa

Aaron Severson (Cleveland State University)

hhmi

NIH