sculpting evolution

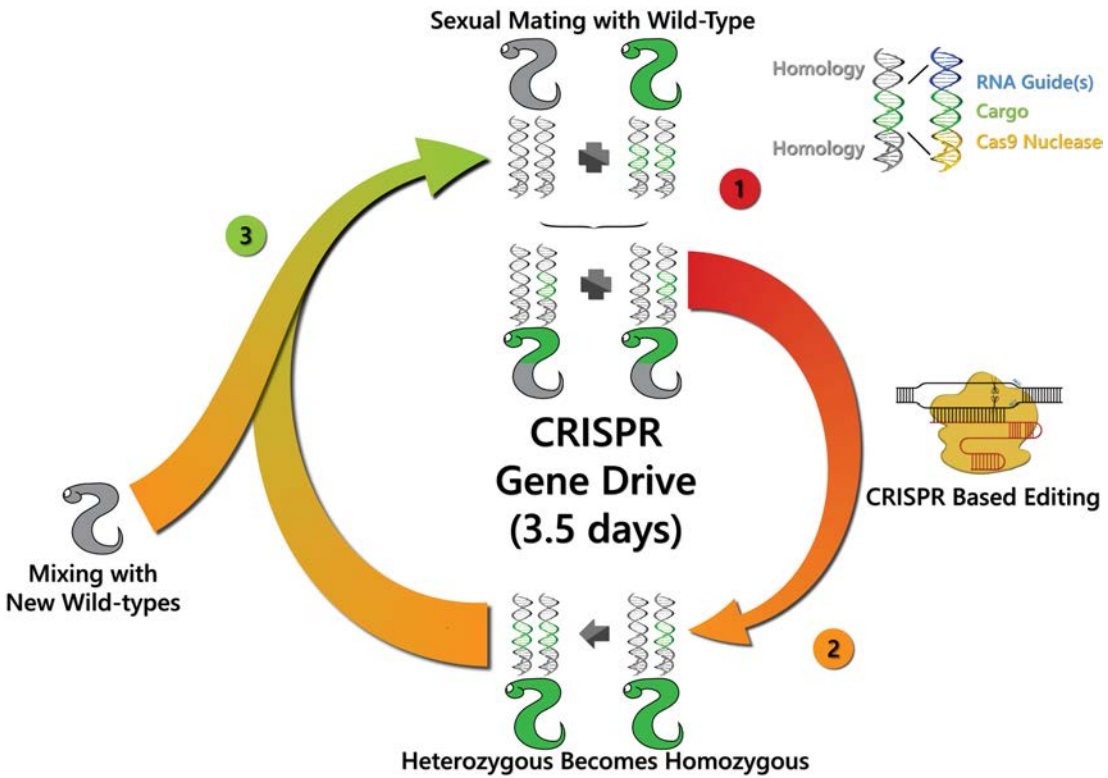
Exploring evolutionary and ecological engineering

Generating the tools to enable LbCas12a editing in *C. elegans*

Stephen Von Stetina

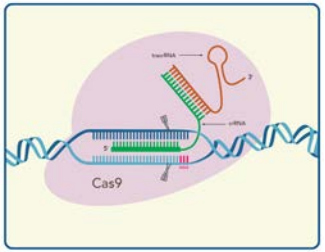
mit media lab

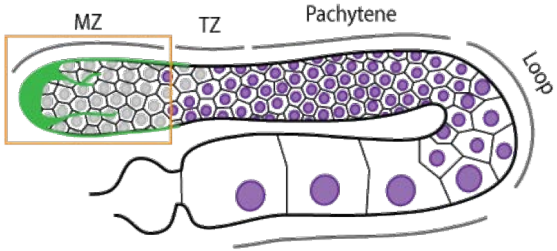
Using *C. elegans* as a model for CRISPR gene drive



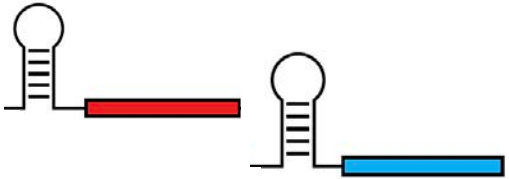
Things required for a gene drive in worms

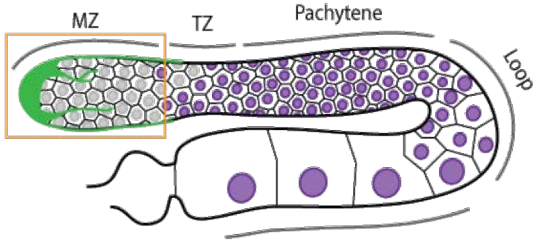
Germline Licensed (Meiosis-Restricted) Nuclease





Highly active, germline-expressed guide RNAs



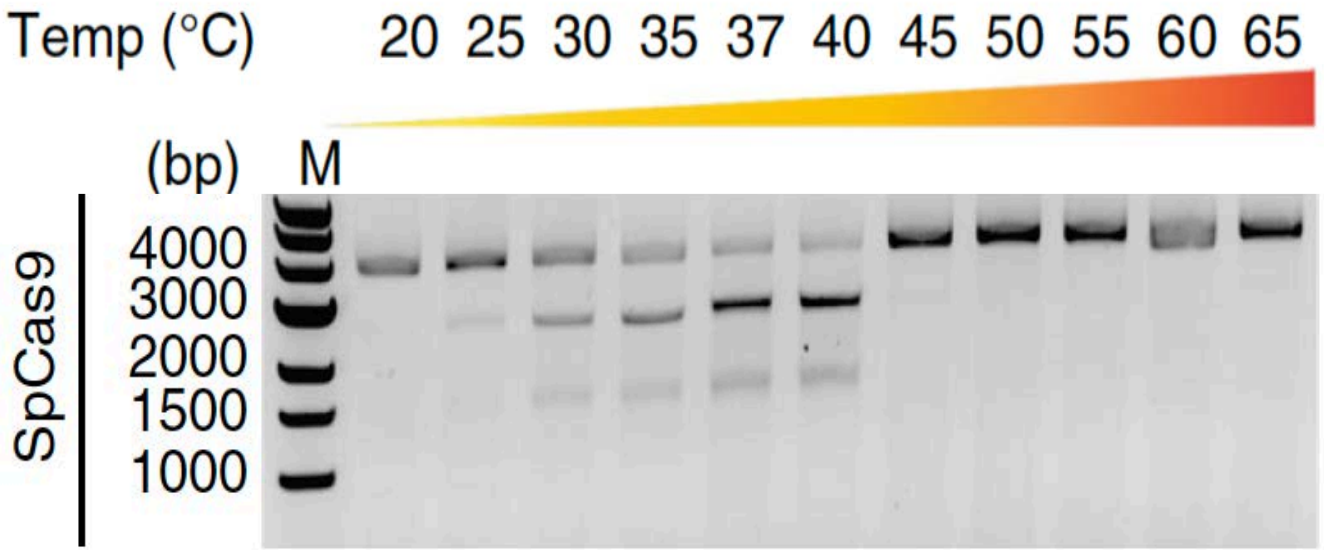


Obligate male-female mating

Poster 487C
Sunday 4-5pm

Schedl 1998; Katju et al 2008; Barrangou 2015; Narbonne et al 2016; wormatlas.org; IDT.com

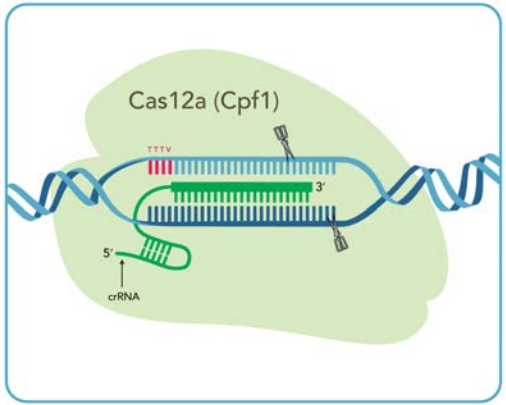
In vitro data suggest Cas9 has low activity at worm rearing temperatures



Mougiakos et al 2017

Is there a lower-temp nuclease?

- Cas12a/Cpf1 from *Lachnospiraceae bacterium ND2006* (Zetsche et al 2015)

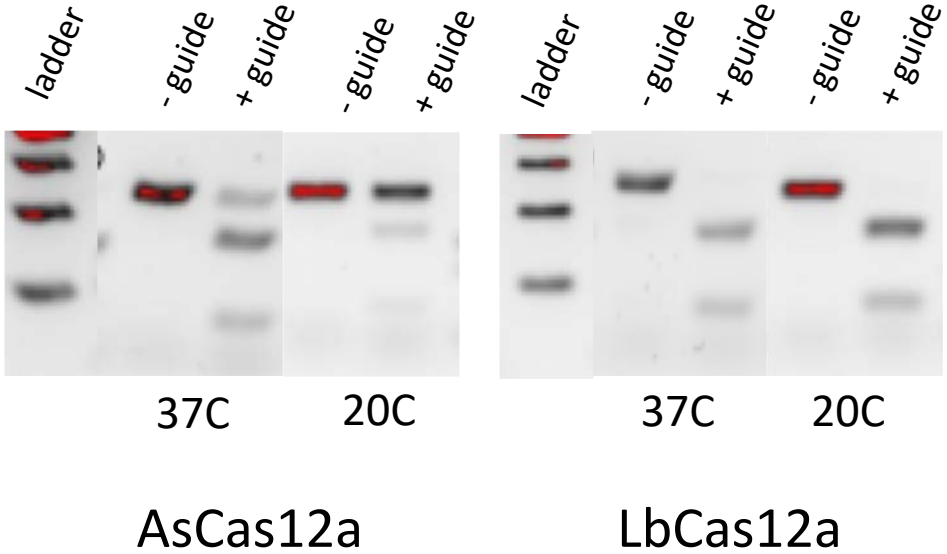


- Reportedly active from 16-48C
- Shorter guide RNA
- Has RNAse-activity, can process its own guide arrays
  - useful for multiplexing
- WT targets TTTV PAMs
- RR mutant has broader specificity, uses TYCV PAMs (Gao et al 2017)
- AsCas12a successfully used by Korswagen group (Ebbing et al 2017)
  - Less active at worm-rearing temps than LbCas12a

LbCas12a as active *in vitro* at 20C as at 37C

Ashton Strait

Mariah Avila



Reagents for LbCas12aRR in *C. elegans*

- UC-Berkeley generated LbCas12aRR purified protein for us
  - we have successfully used RNPs to edit genome
- We likely have generated a single-copy LbCas12a
  - Need to test for germline-licensing
- We have developed an LbCas12a *spe-9* coCRISPR strategy and identified active guide(s) for use with *dpy-10* coCRISPR.
- We have developed a screening strategy to enrich for large-fragment insertions – compatible with Cas9 and LbCas12a

dpy-10 guide induces deletions

Loss of FP strategy for large insertions

Most of the constructs we wish to integrate are >5kb

- Have very low % success rate for these large constructs
- Designed this strategy to enrich for CRISPR integrations by HDR over NHEJ repair

Inserted FP cassette flanked by optimal target sequence

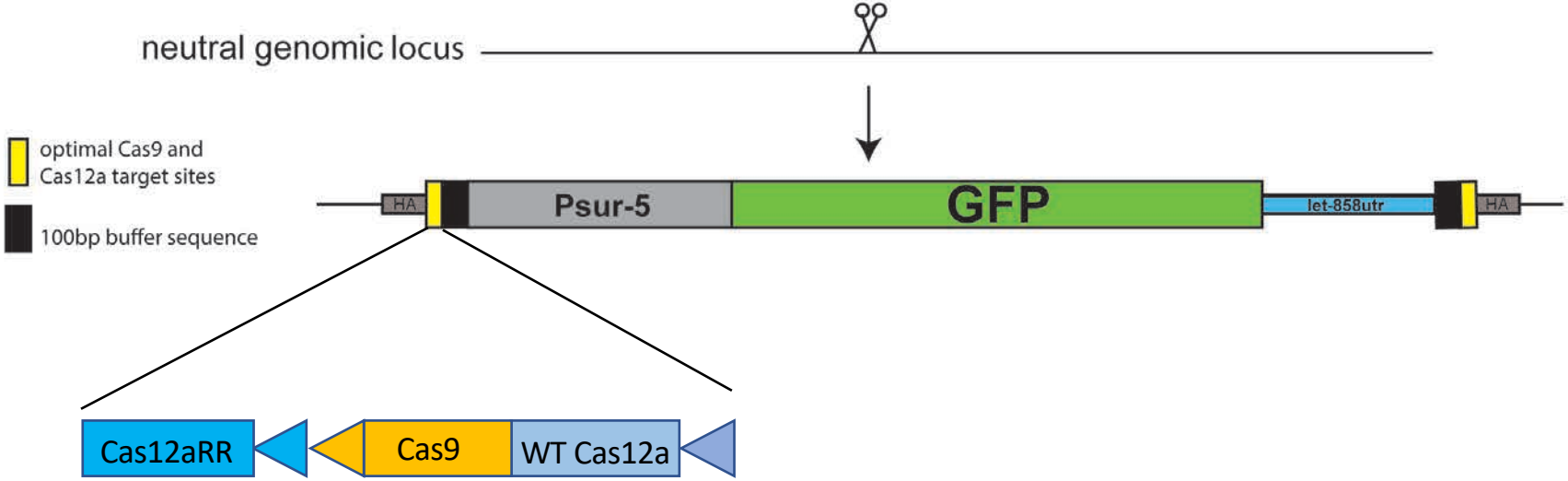
- Cas9 guides from Meyer lab's recent paper (GGNGG)
- Cas12a guides from CINDEL/CRISPR-DT database

Use optimal guides to cut FP cassette out, knock GOI in

- Designed such that NHEJ should not affect FP expression, so loss of fluorescence only associated with loss of FP and HDR repair

Loss of FP strategy for large insertions

- Step 1: Insert FP cassette into neutral locus

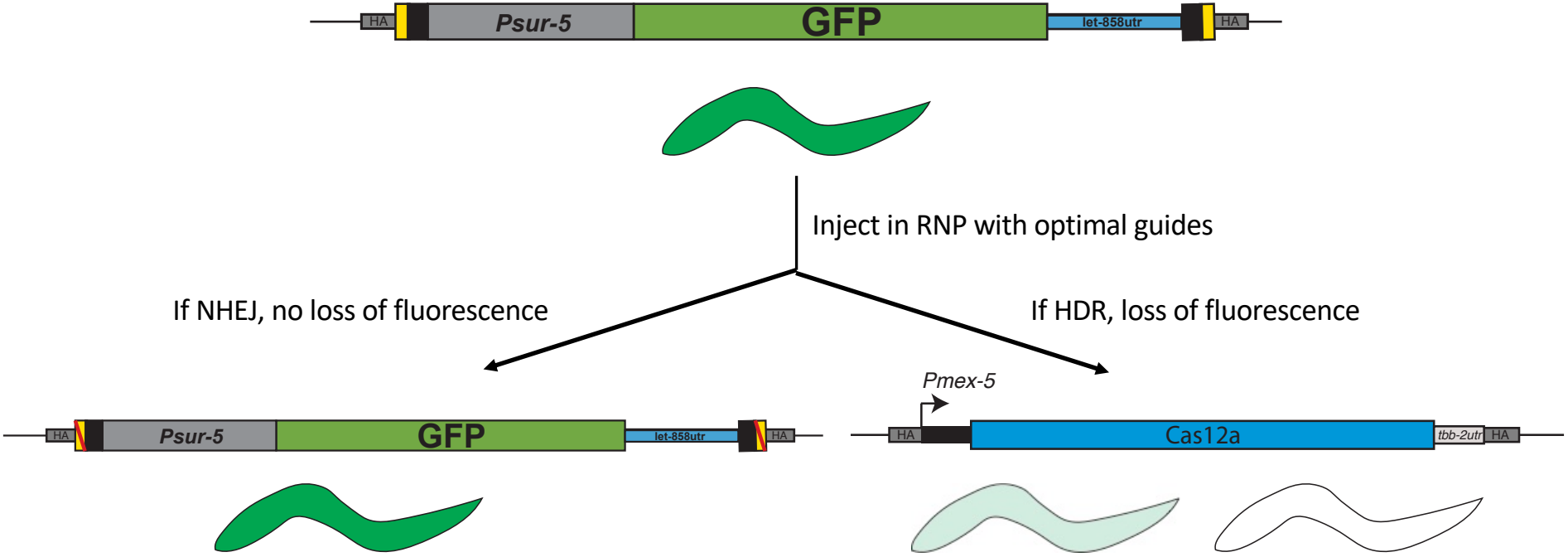


Cas9 PAM “out”, Cas12a PAM “in”

Kim et al *Nat Meth* 2017; Zhu and Liang *Bioinformatics* 2019; Farboud, Severson and Meyer *Genetics* 2019

Loss of FP strategy for large insertions

- Step 2: Cut with highly active guides, screen for loss of FP to indicate HDR events



Results

Injection 1

- 80 worms injected
- Picked 38 F1s with dim/no FP
- Genotyped 18 that had F2s with no mVenus
    - 39% (7/18) were positive for insertion by genotyping SWPCR

Injection 2

- 80 worms injected
- Picked 17 F1s with dim/no FP
- Genotyped 8 that had F2s with no mVenus
    - 37.5% (3/8) were positive for insertion by genotyping SWPCR

Acknowledgments

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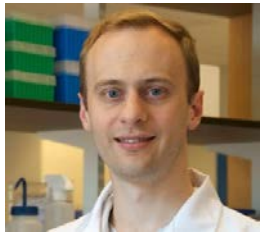
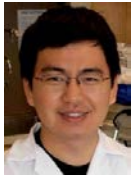


Kevin Esvelt, PI



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HIVE BIOSYSTEMS

High-throughput nematode transgenesis

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