Creation of *odd-skipped* fluorescent reporter strains in *C. elegans* via CRISPR/Cas9 genome editing

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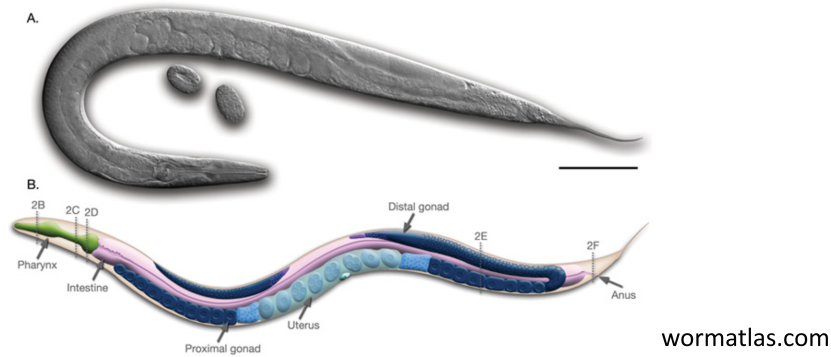
Introduction

- ***C. elegans*** – microscopic nematodes; model organism
  - Easy visualization due to transparency
  - Easy to grow/maintain
  - Entire genome has been sequenced[1]



- ***Odd-skipped*** gene family –
  - *Odd-skipped related* genes in mammals
  - *Odd-1* and *odd-2* expressed in gut of *C. elegans*[2]
  - *Odd* gene array-based[3] reporter strains exist but express inconsistently.

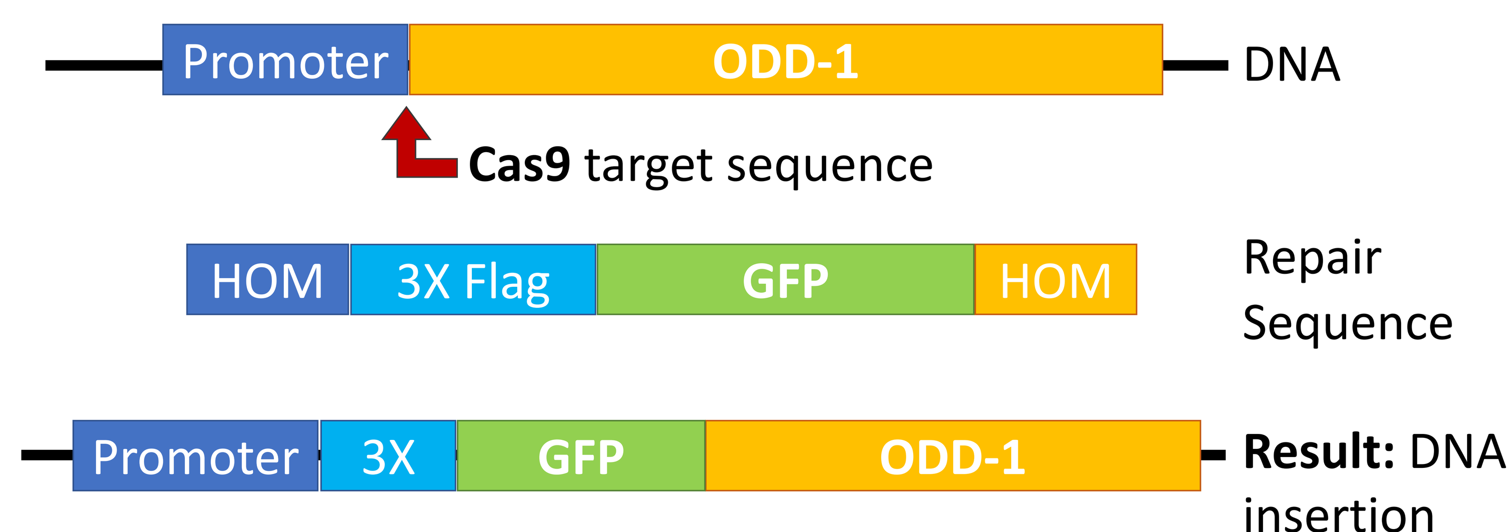
- **CRISPR/Cas9** system used for **genetic engineering**[4]
  - CRISPR - clustered regularly interspaced short palindromic repeats

GOAL: Make reporter strains with fluorescence insertions at the beginning of the *odd-1* and *odd-2* genes

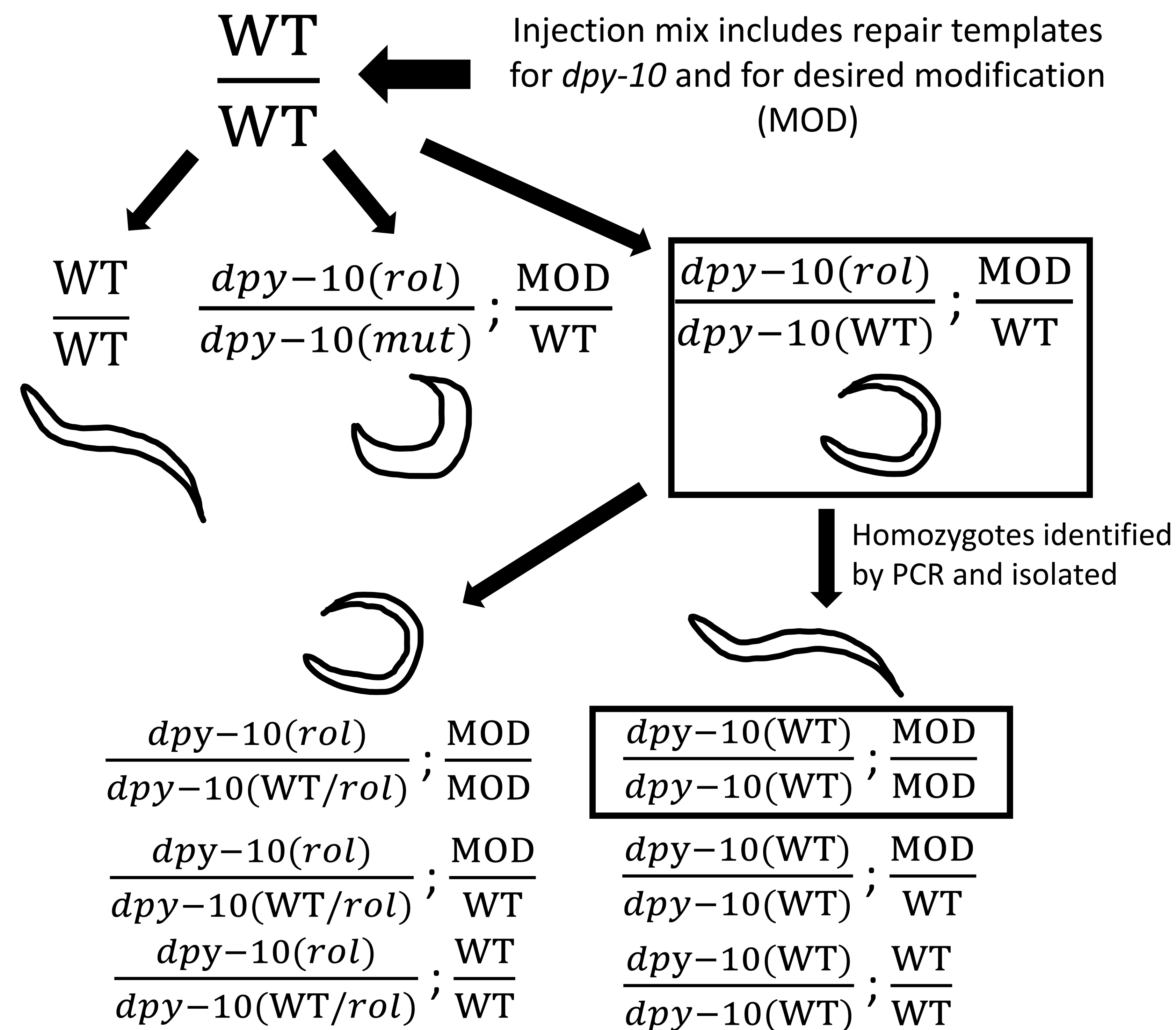
Methods: CRISPR/Cas-9

- Modified **guide-RNA** directs Cas9 to target sequence in DNA[4]
  - Both delivered via plasmid in the injection mix
- Cas9 (an endonuclease) makes double-strand breaks in DNA

- The repair sequence includes:
  - Fluorescent protein (and epitope tag, if desired)
  - Homology regions on both sides matching sequence around the double-strand break (HOM)
    - Either short homology (40 bp) or long homology (500 bp)



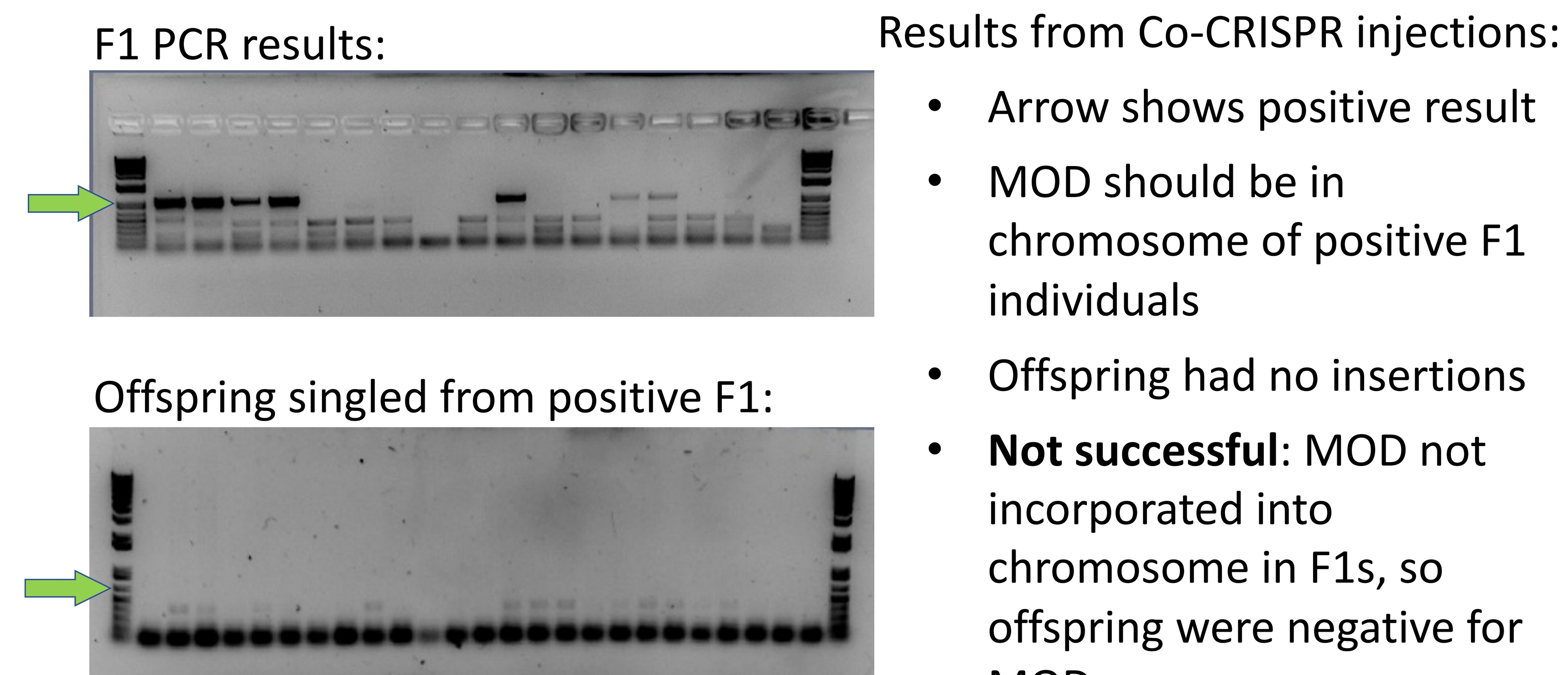
Methods: Co-CRISPR



(Figure adapted from Dickinson and Goldstein, 2016)

- Allows screening by phenotype: screen for non-dumpy rollers in F1 and for wild type in F2[5]
- Desired modification (MOD) is either ***odd-2::mCherry*** or ***odd-1::GFP***
- >150 worms were injected and screened using this method

Results



Results from Co-CRISPR injections:

- Arrow shows positive result
- MOD should be in chromosome of positive F1 individuals
- Offspring had no insertions
- **Not successful**: MOD not incorporated into chromosome in F1s, so offspring were negative for MOD

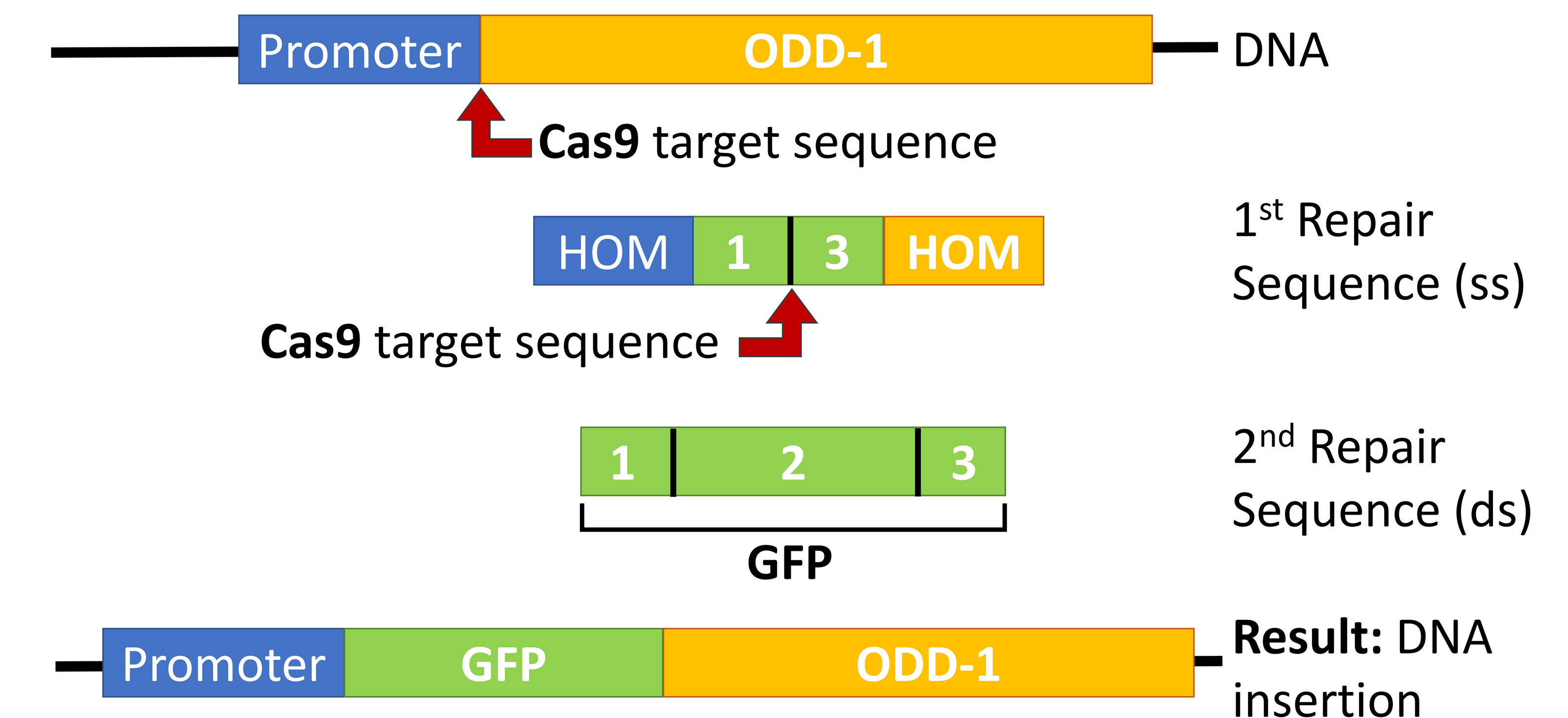
Future Directions

Approaches for increasing efficiency:

- Using single-stranded repair templates
- Using ribonucleoprotein complexes (RNPs) = Cas9 protein + guide RNA

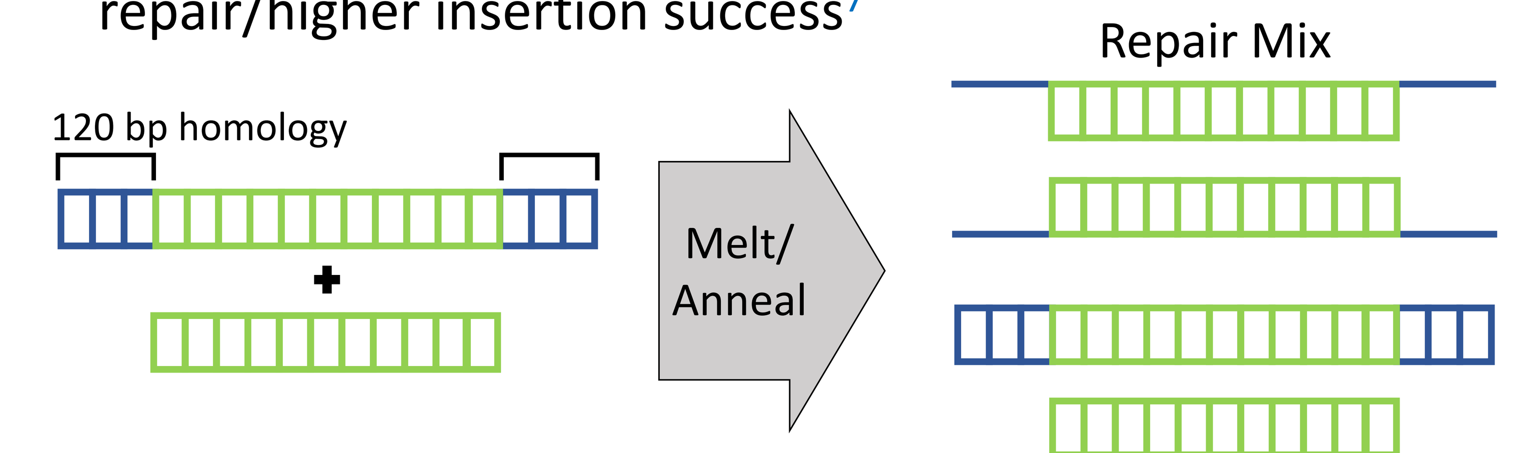
Nested CRISPR strategy:

- Higher success rate than original CRISPR/Cas9 strategy[6]
- 1st insertion (single-stranded) contains beginning and end of the MOD and homology from DNA
  - Uses user-designed sgRNA
- 2nd insertion (double-stranded) uses whole MOD as repair sequence
  - Beginning and end of MOD act as homology
  - Uses known/tested sgRNA for GFP



Hybrid CRISPR strategy:

- Melting/annealing of repair sequences makes "free ends"
- 50% of repair mix is partially single-stranded DNA = better repair/higher insertion success[7]



Acknowledgments/References

Acknowledgments

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References

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