Workshop II
phiC31-mediated single copy insertion

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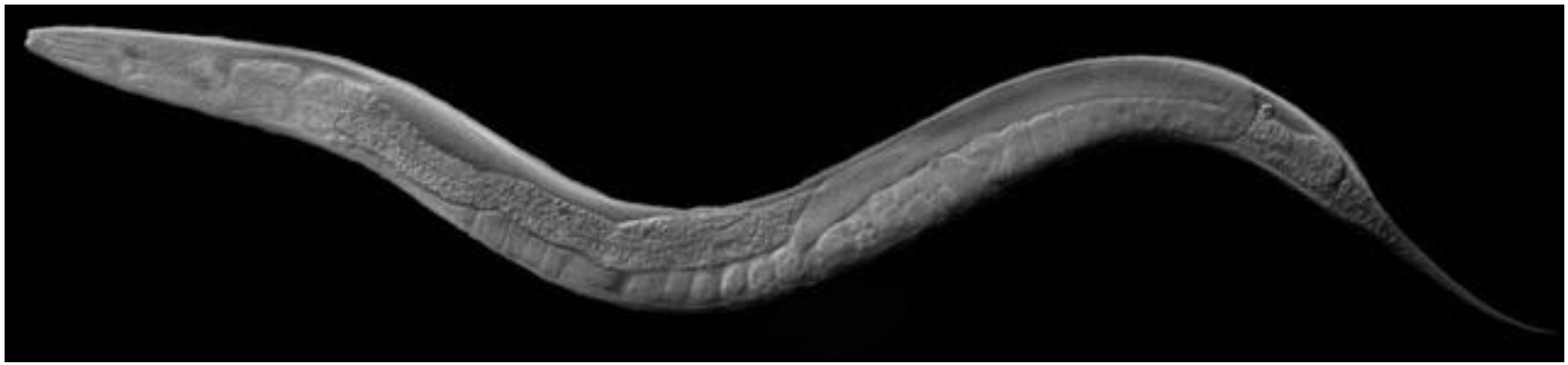
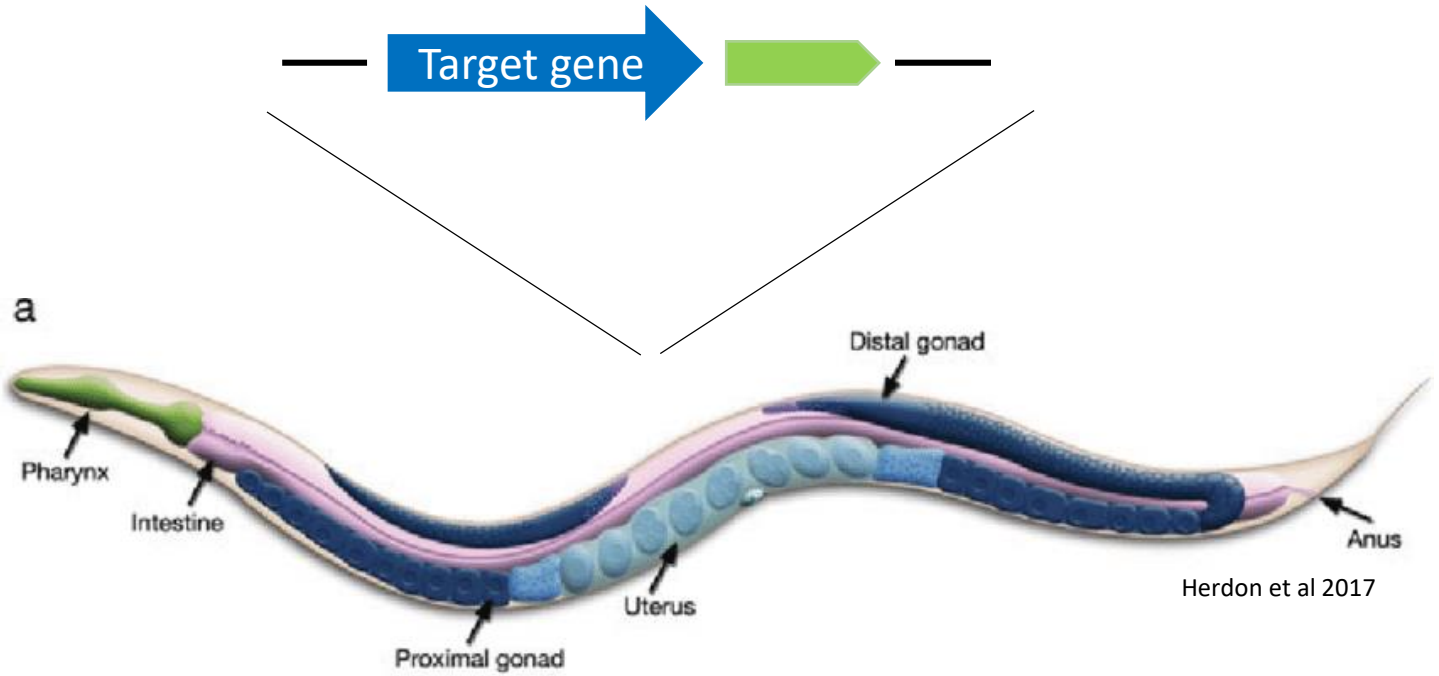


Collaboration with Shih-Peng Chan

Goal: Single copy insertion into *C. elegans*

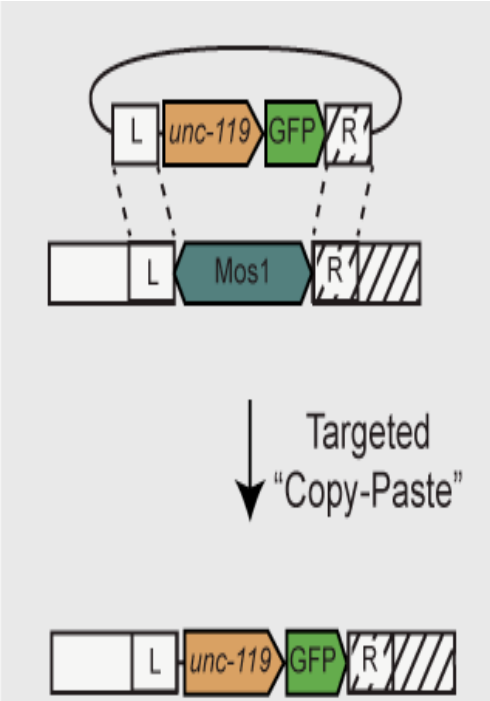


Herdon et al 2017

Goal: Single copy insertion into *C. elegans*

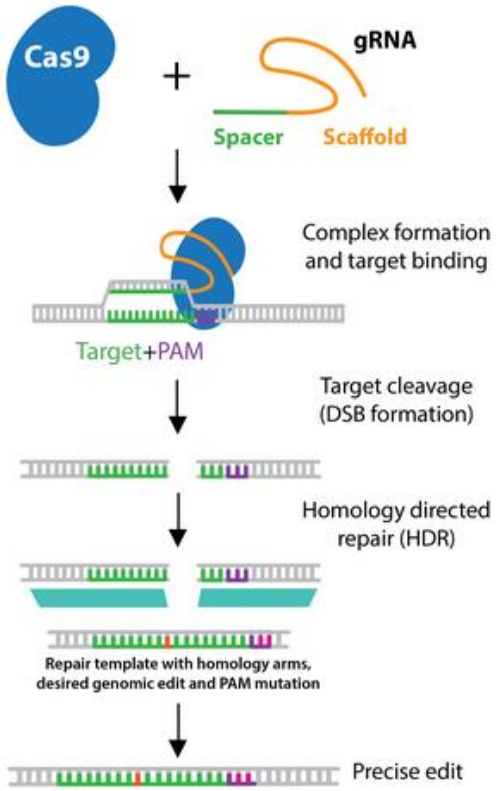
Repair mechanism

MosSCI



Limited insertion size
Low efficiency
Limited sites

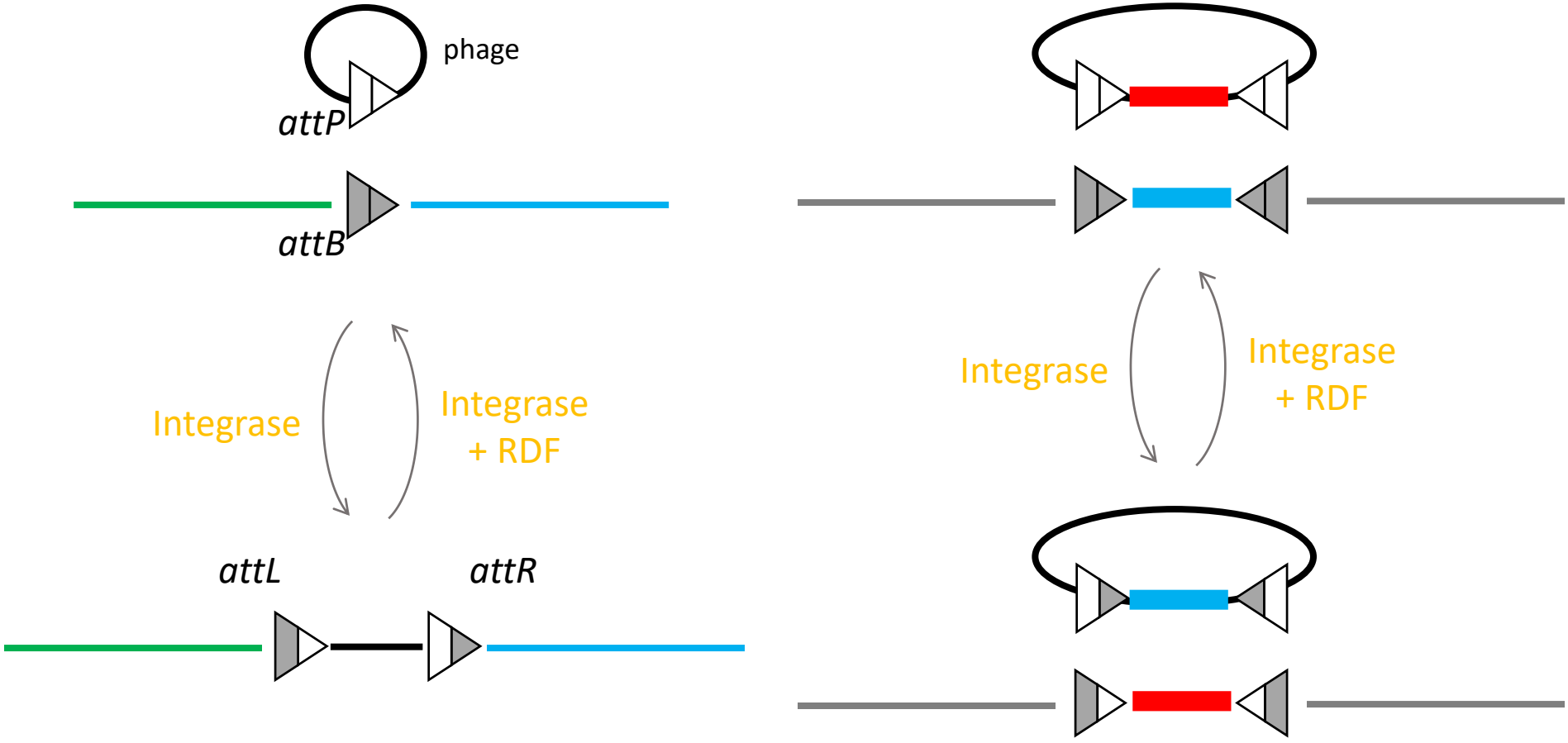
CRISPR/Cas9



Limited insertion size
Efficiency variable (0.4~12%)
Many sites

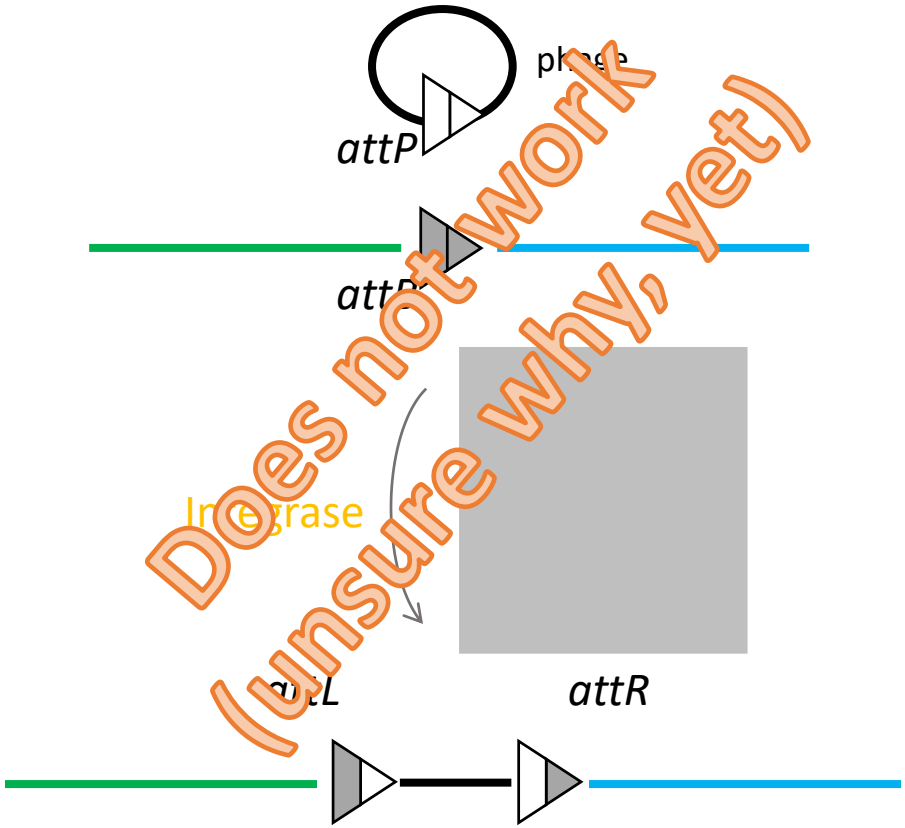
phiC31 integrase and RDF mediate site-specific recombination

Conceptually like Gateway Cloning

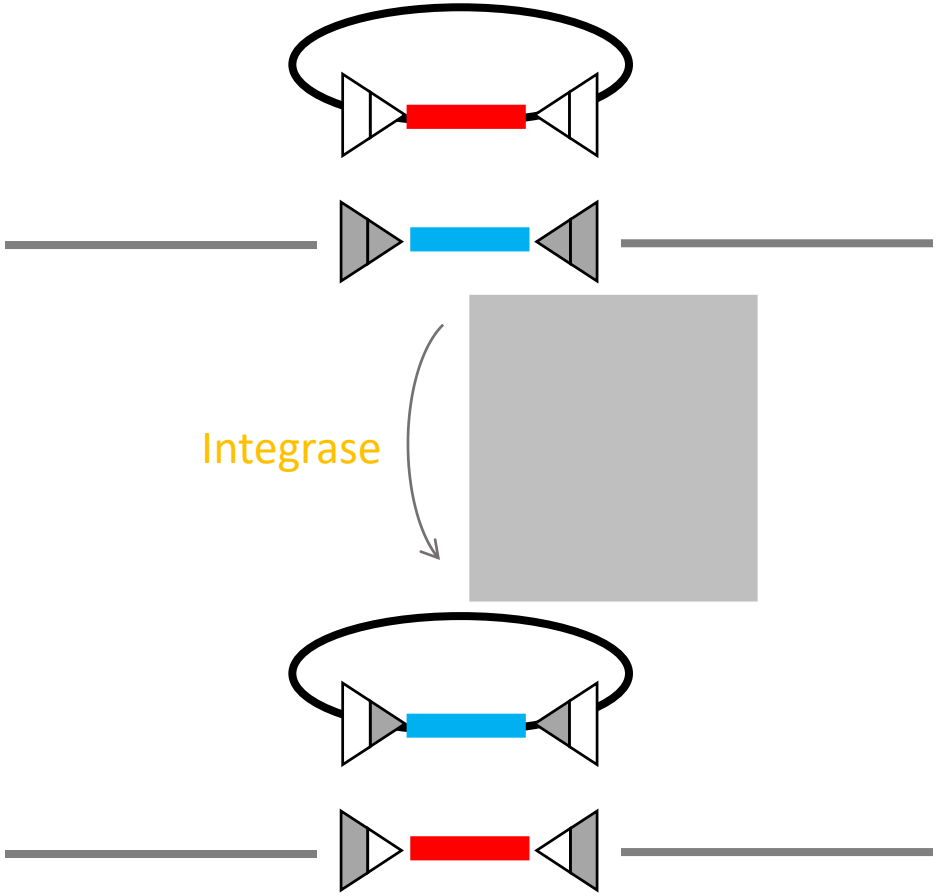


Works in mammals, zebrafish, *Drosophila*, and others

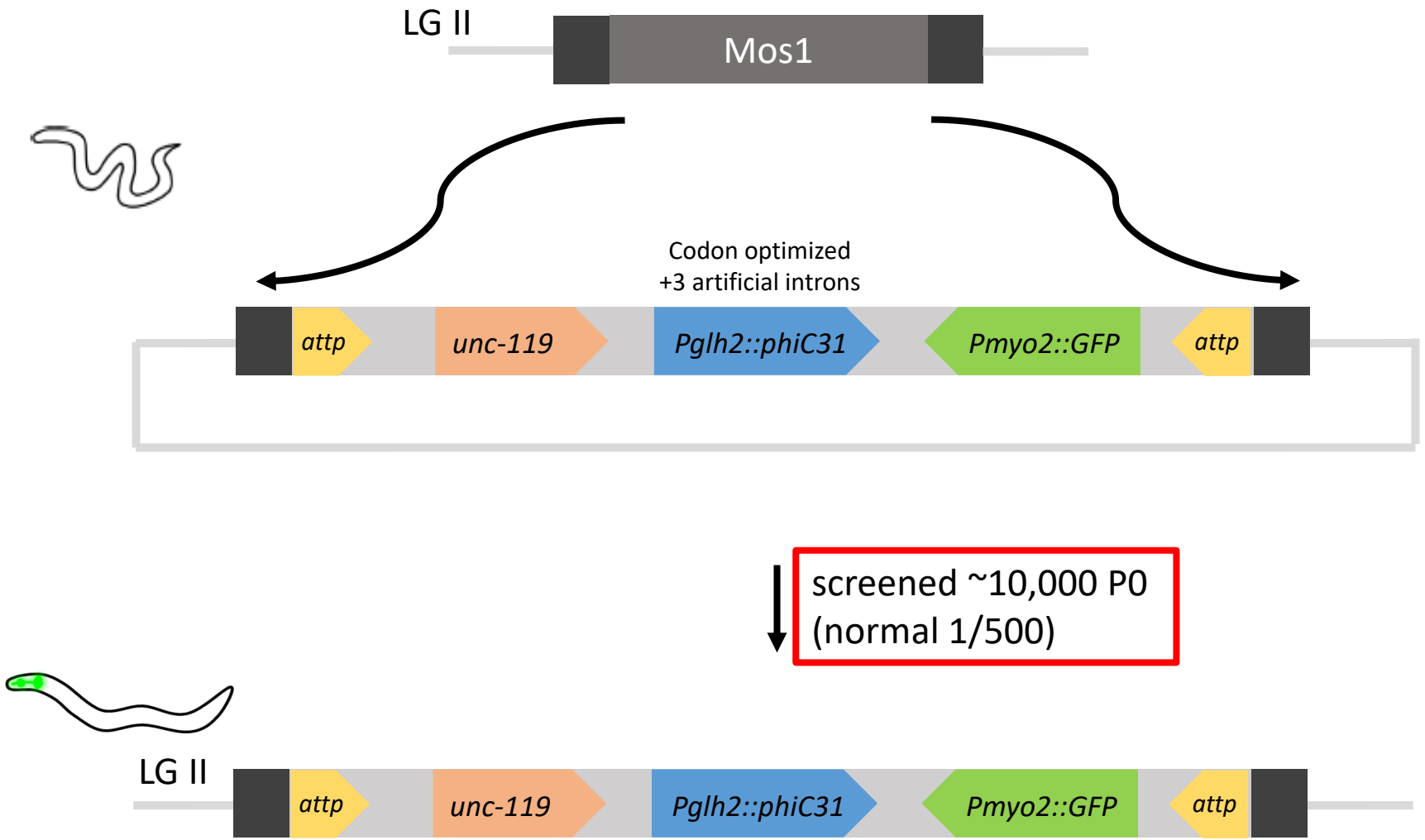
phiC31 integrase and RDF mediate site-specific recombination



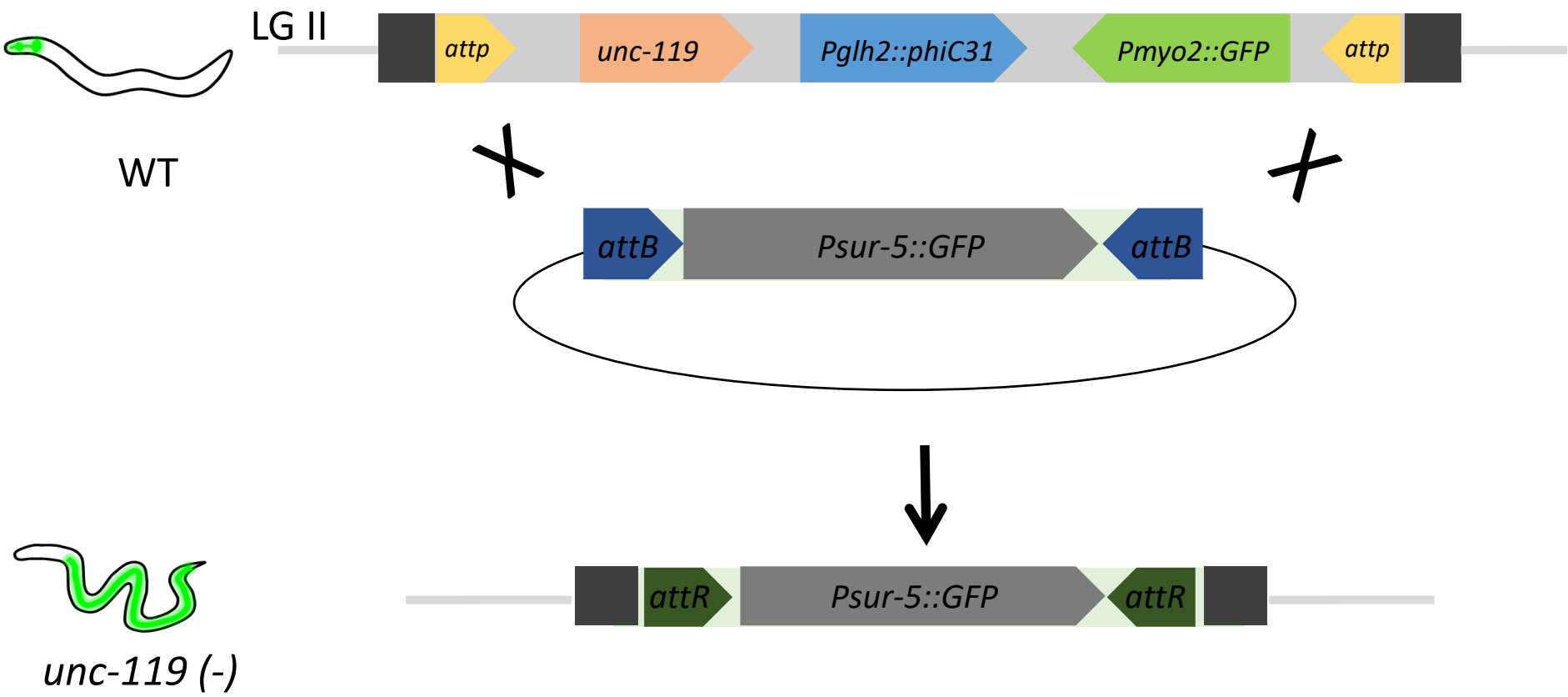
Does not work (unsure why, yet)



Creation of *phiC31* integrase system – using MosSCI



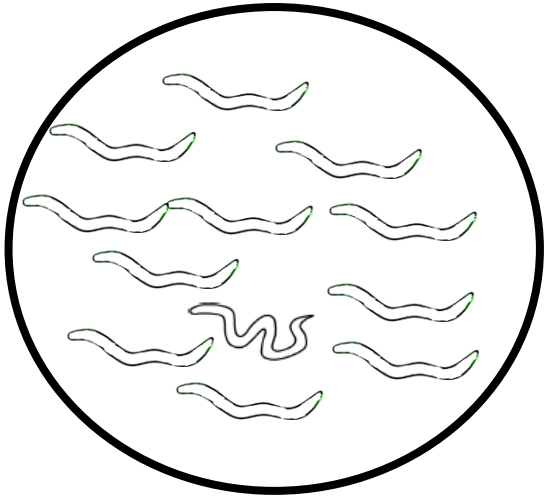
Preliminary test of *phiC31* integrase system



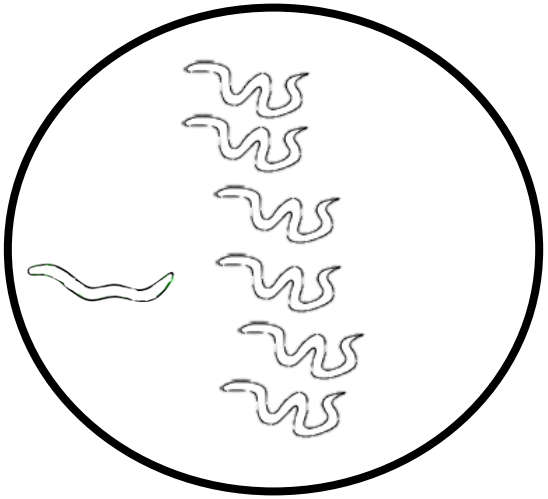
Injected P0	Transgenic F1	Potential integrants	Successes
26	28	2	2

Screening for Uncs is hard

WT



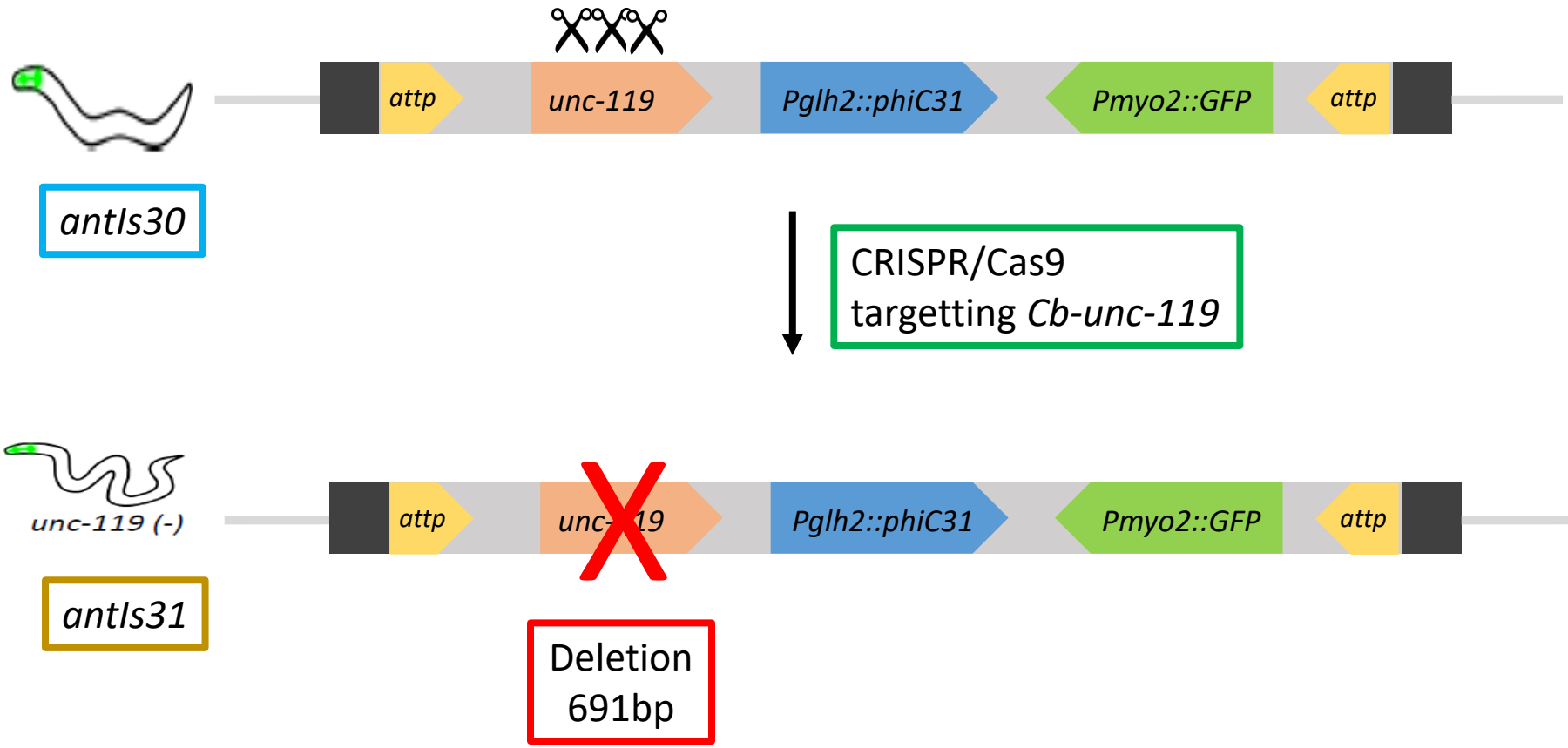
unc-119 (-)



Hard to find that one *unc-119 (-)* worm

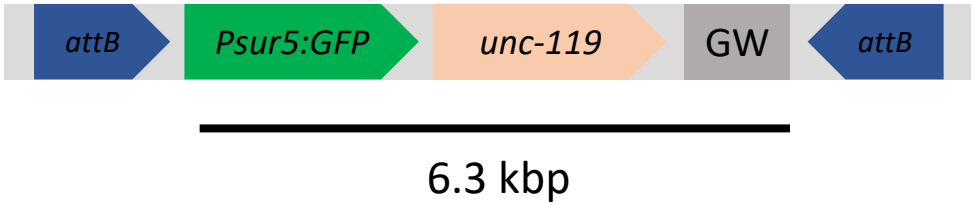
Easy to find that one WT worm

Improved strain



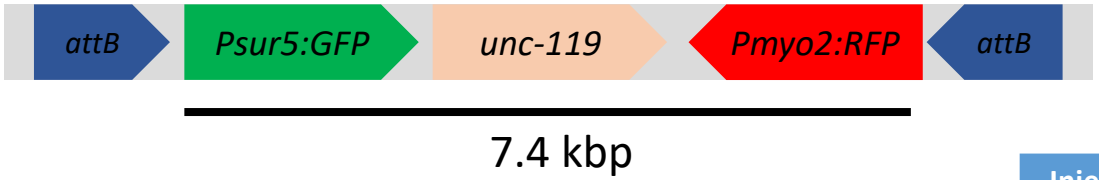
Validation / proof of principle

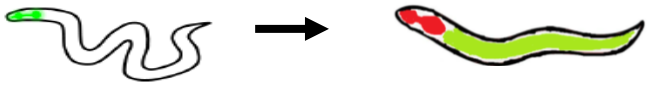
pBRC_double_attB_donor



Injected P0	Transgenic F1	Potential integrants	Successes
165	104	6	4

pBRC_double_attB_mcherry

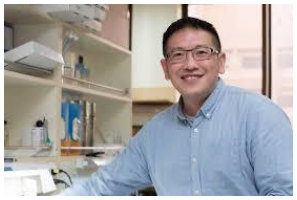


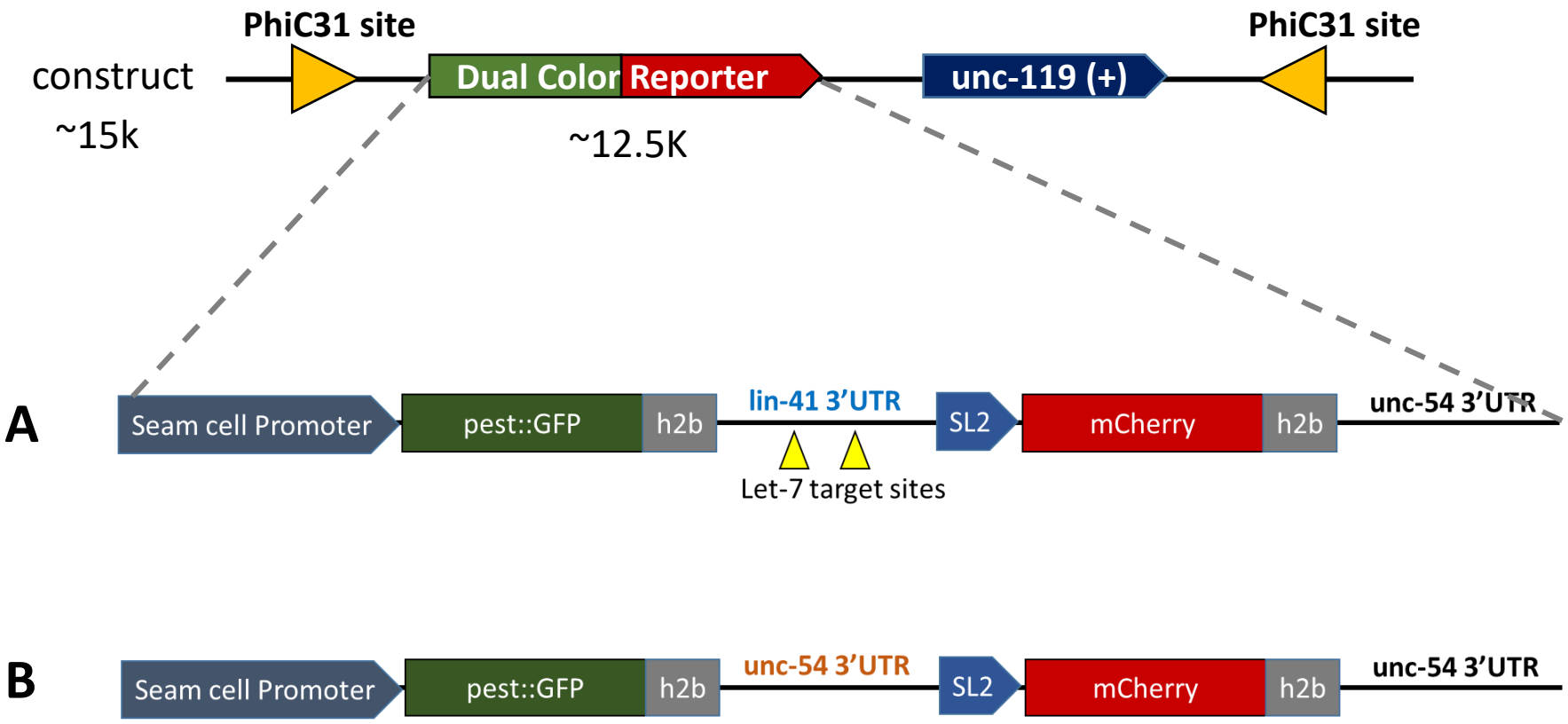


Injected P0	Transgenic F1	Potential integrants	Successes
180	80	5	3
47	112	25	19

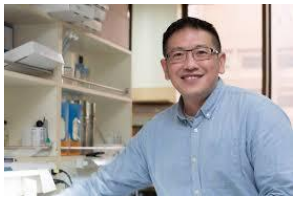
Plus more examples from Ou, Hsueh, and Chan labs

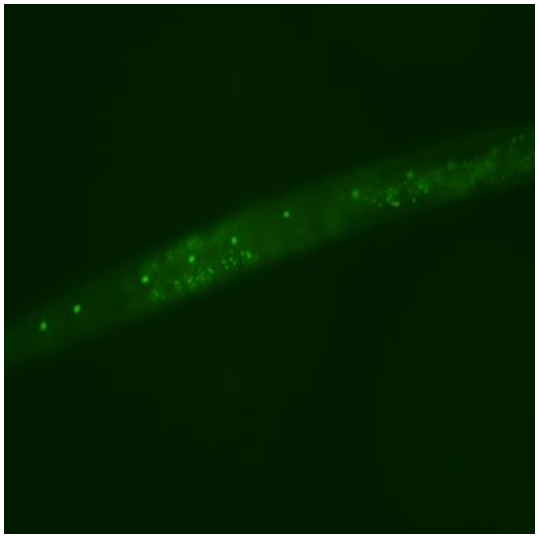
The design of dual color reporter

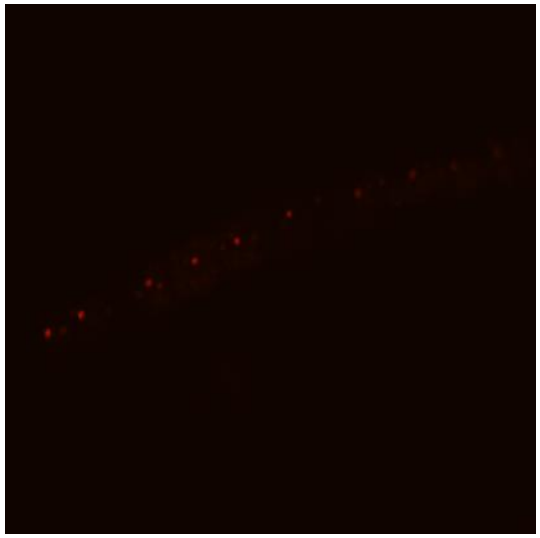


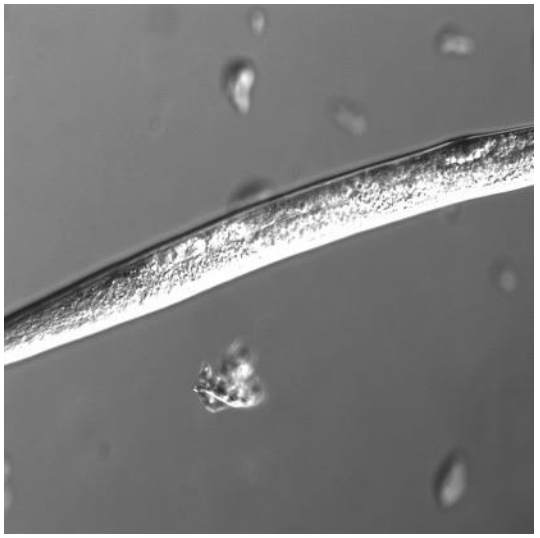


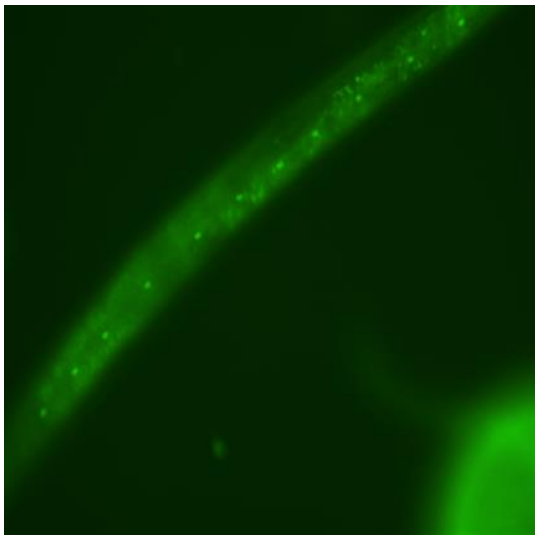
7PW L4

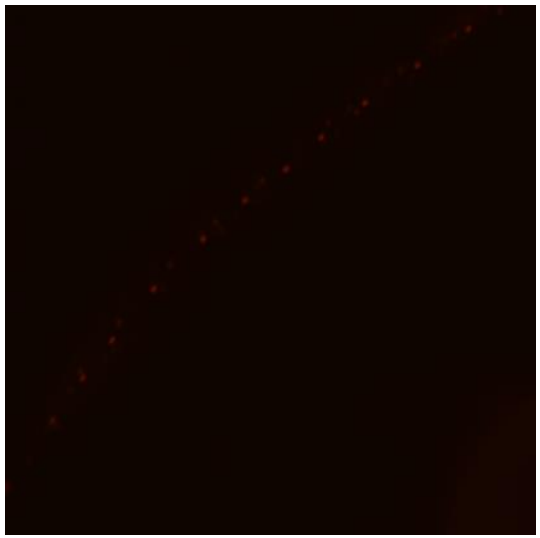


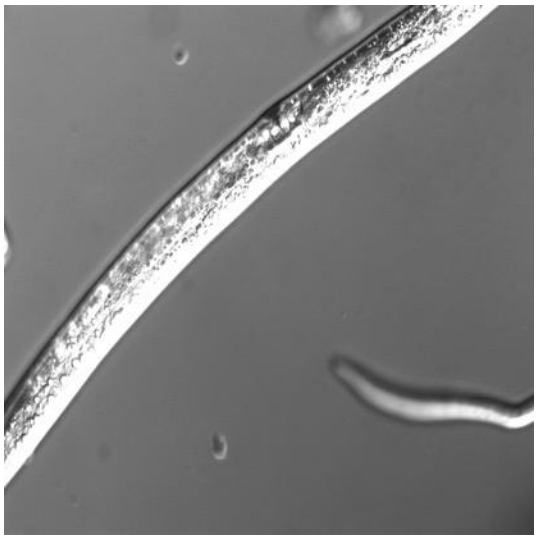










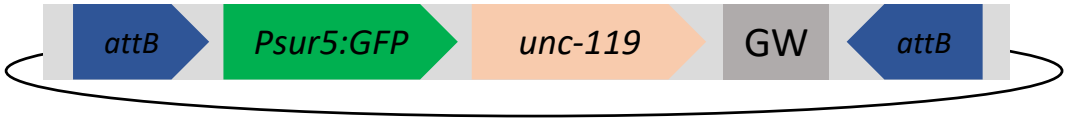


Our current protocol

Our current protocol

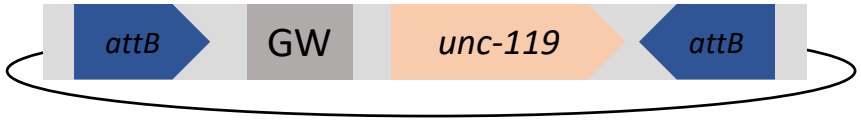
1. Choose vector and insert your gene
   Gateway cloning (or RE)

pBRC_double_attB_donor



GFP as marker

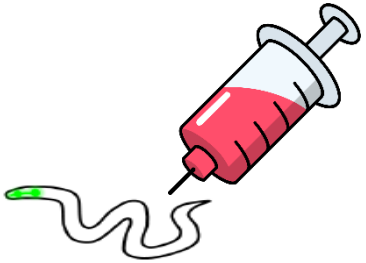
P5-5_pCG150_phiC31_v2



Your own GFP/RFP marker

Our current protocol

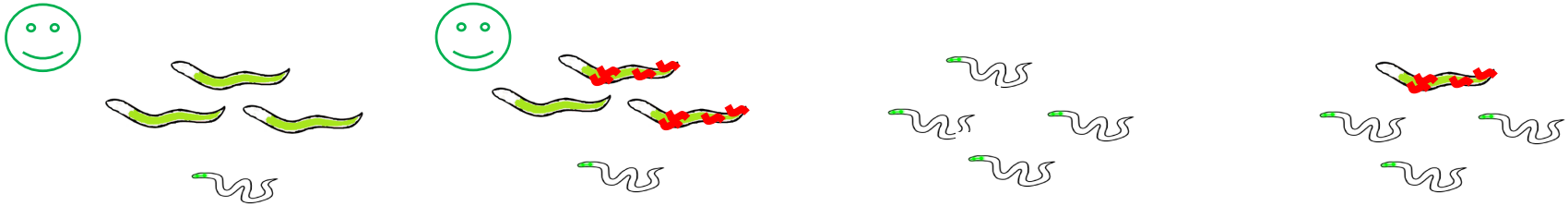
2. Inject ~20+ P0's with construct + co-injection marker (e.g., *myo-3::RFP*)



Make sure you get the "gonad flush"

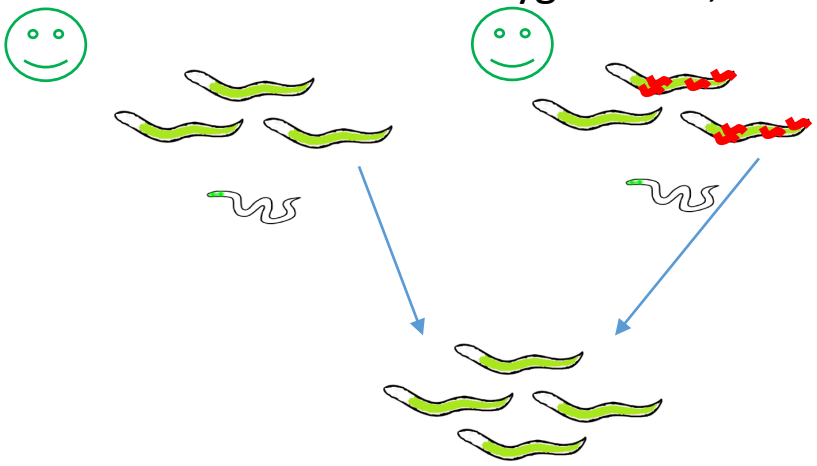
3. Pick WT F1s and construct marker (e.g., *sur-5::GFP*) and co-injection marker (e.g., RFP)
Ex array and/or heterozygous insertion

4. Look for segregating ~3/4 and loss of co-injection marker



Our current protocol

5. Pick several for homozygous line, confirm loss of Ex array by phenotype

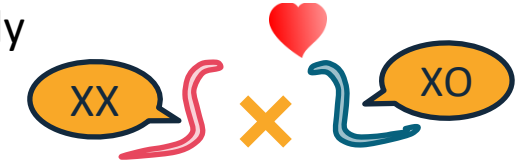


6. Extract DNA

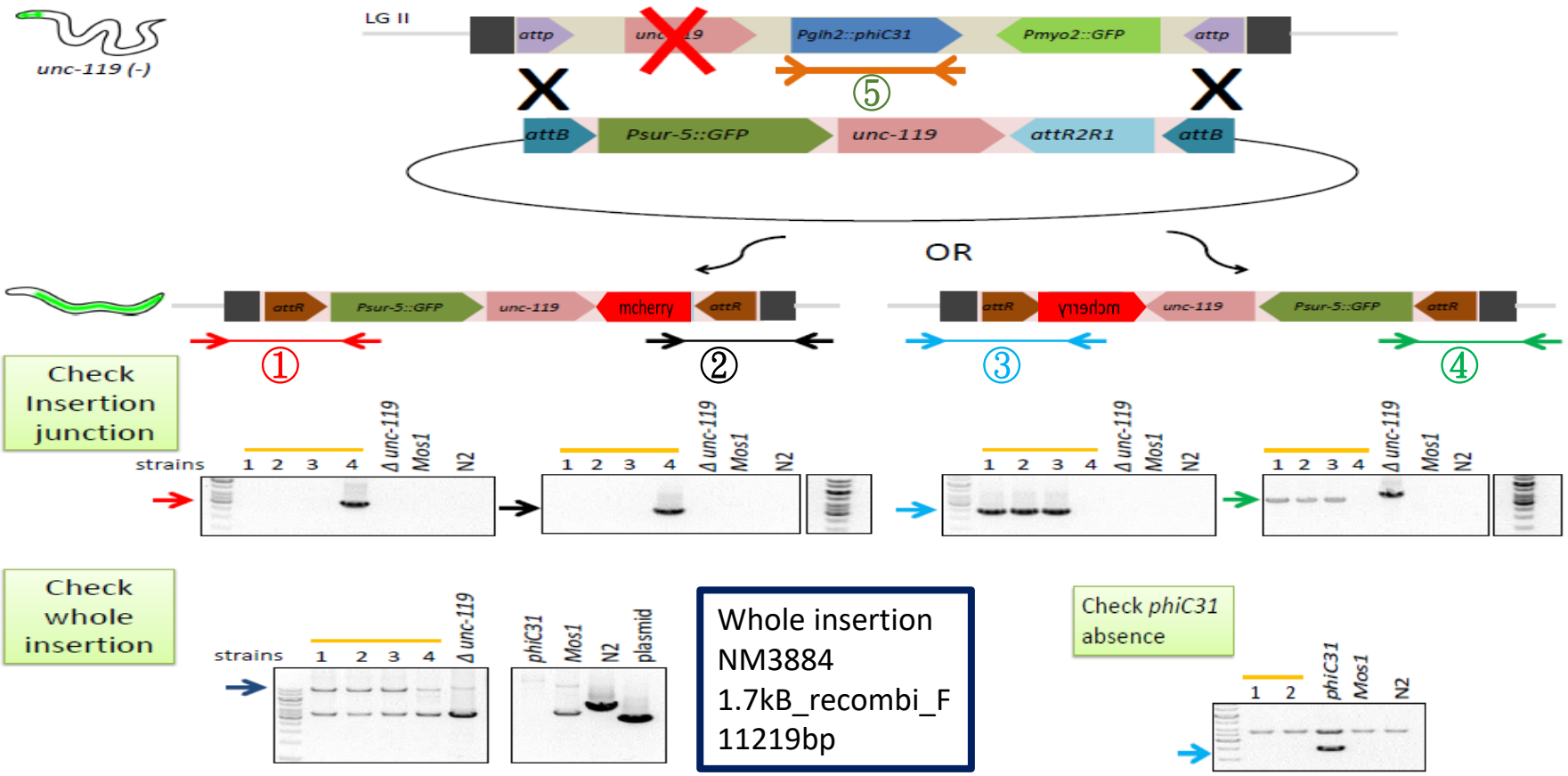
medium quality for short PCR tests
high quality for long PCR tests
(not sure about very large insert yet, Oxford Nanopore?)

More details next slide

7. Backcross ideally



pBRC_doubleattB_mcherry integration validation



① NM3884 GFPfusionREV_primer 2926 bp

② mcherry_F 1.7kB_recombi_F 3637 bp

③ NM3884 mcherry_F 3133 bp

④ GFPfusionREV_primer 1.7kB_recombi_F 3430 bp

⑤ phiC31_43.1_seq_F phiC31_43.1_seq_R 2452 bp

Summary and miscellaneous final thoughts (I)

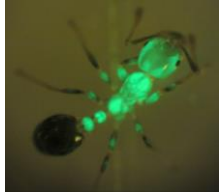
- Developed phiC31 system for RMCE (recombination-mediated cassette exchange)
- Should be easier and simpler to get single copy insertions, say for quick tests of rescue
- We think it is easier than Crispr/Cas9 or MosSCI, especially for “longer” insertions (10kb+)
- Larger constructs allows more experiments (long gene, long promoter, or “genome engineering”)
- Since recombination, should be more robust than MosSCI and CRISPR/Cas9
- We have used this to invert ~ 8Mb region on LG 4 (attB x attP)
- But have so far failed with a BAC (bacterial artificial chromosome, ~130 kb)

Summary and miscellaneous final thoughts (II)

Issues:

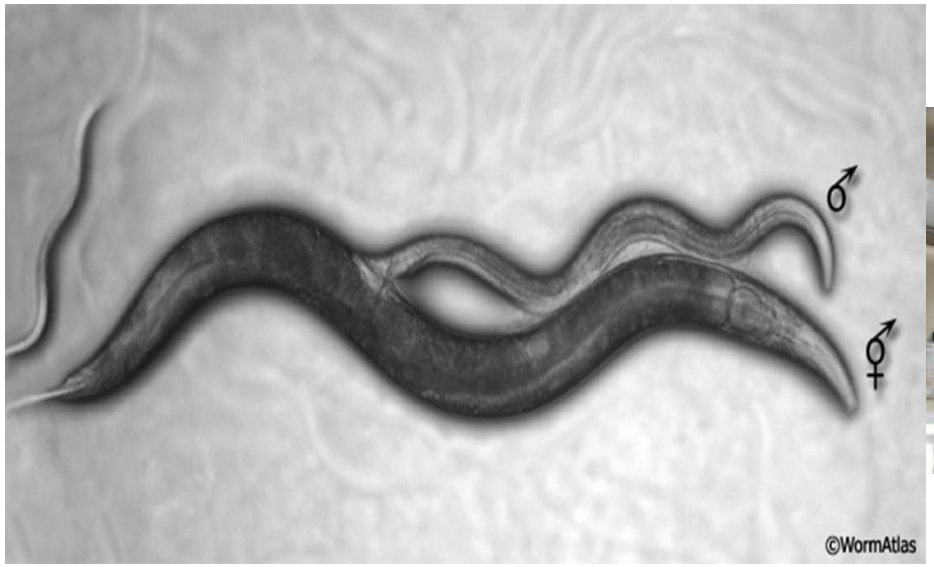
- Only 1 docking site (so far)
- Not as flexible as CRISPR/Cas9
  - But remember this uses “repair” to insert DNA
- Plasmid with single attB into a single attP site → we get complex insertions
- So we only trust the double attB/double attP approach with plasmids
- When insertion happens is unclear
  - Immediately as original plasmid or after plasmids are part of Ex array?
- phiC31 strain may be unstable and/or slightly deleterious
  - We lost the strain through some type of large-ish deletion (after about 1 year on bench)
- Injecting Unc-119 strains harder than WT

THANKS FOR YOUR ATTENTION

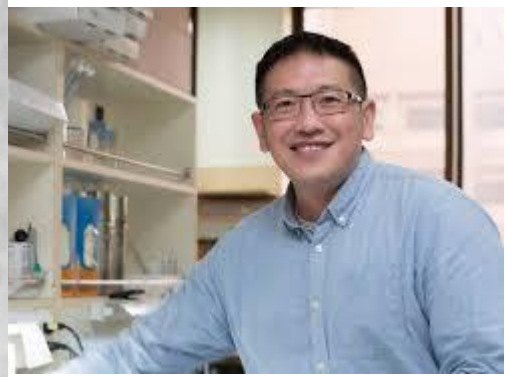


Emily Fang-Jung Yang
Tiffany Chang

Ni-Chen Chang
Chia-Yi Kao
Chih-Chi Lee
Yu-Ching Huang
Rong-Jan Hsu



Shih-Peng Chan



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