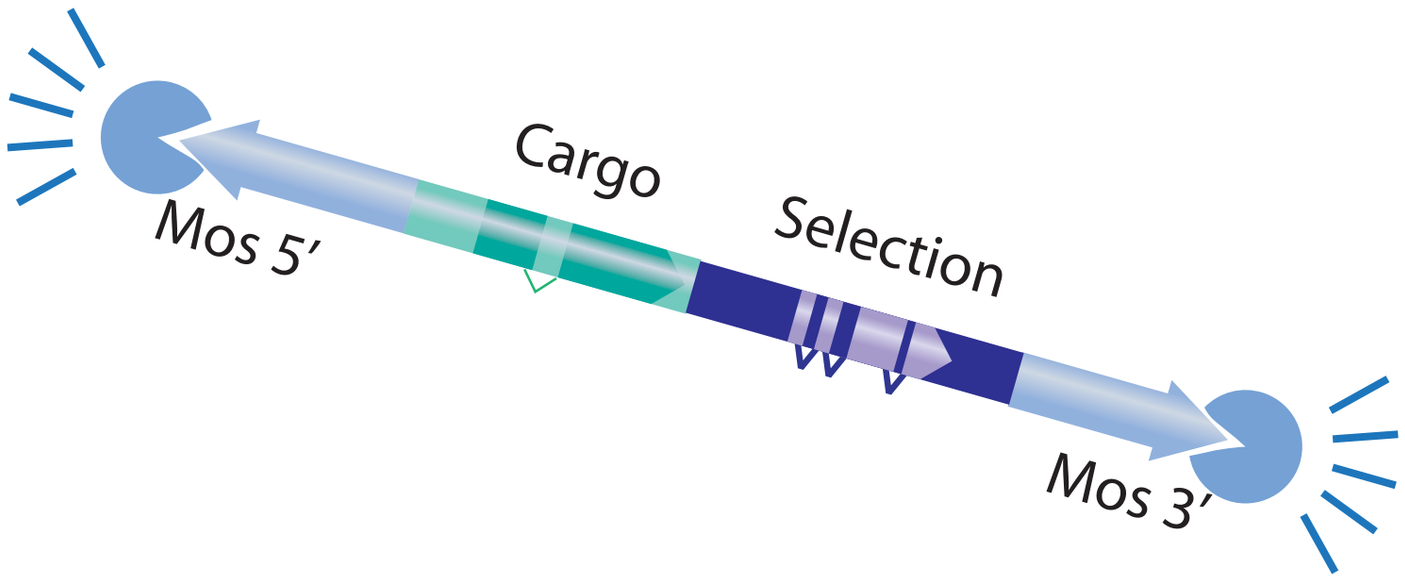
Hopping transgenes and universal MosSCI insertion sites into genomes



Christian Frøkjær-Jensen
Postdoctoral fellow
HHMI, University of Utah

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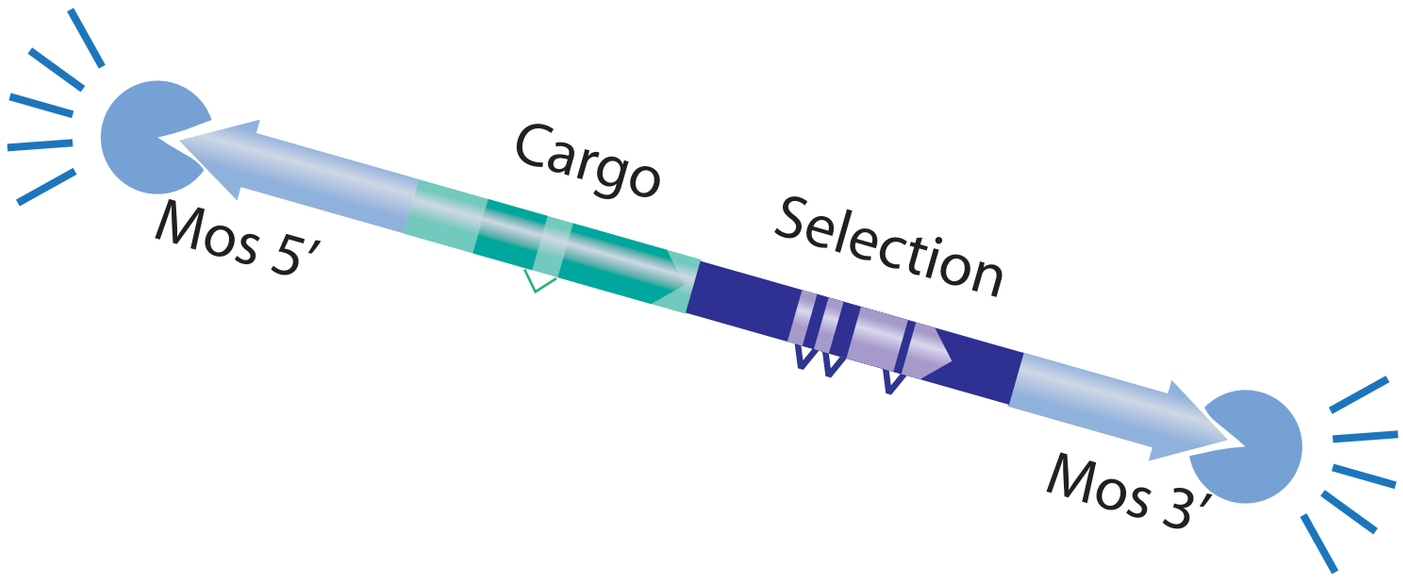

ApE
(A plasmid Editor)

Christian Frøkjær-Jensen
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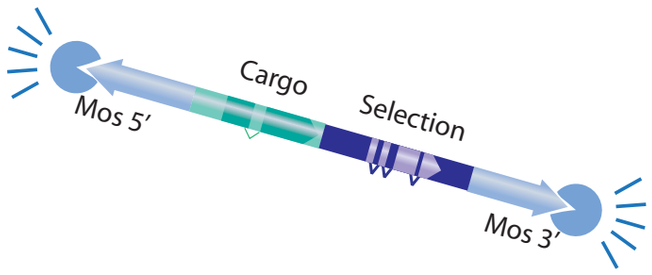
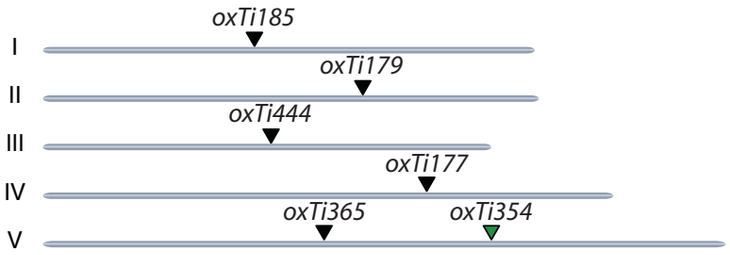
Wayne Davis

Methods to engineer the genome based on Mos1



1. MiniMos transposon

Hopping transgenes into the genome



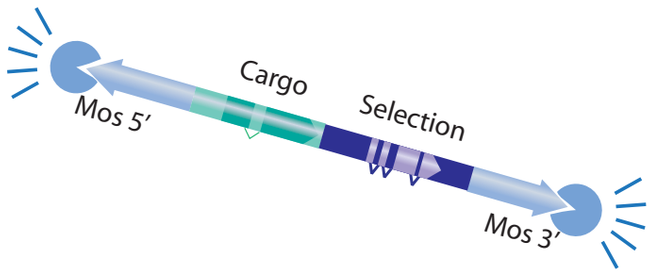
2. Universal MosSCI sites

Targeted insertion at different sites across the genome

3. wormbuilder.org

Reagents, strains and troubleshooting advice

Methods to engineer the genome based on Mos1



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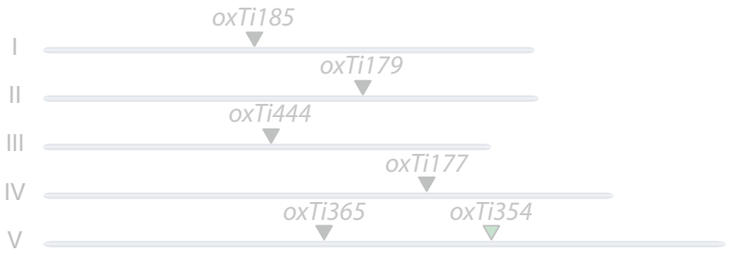

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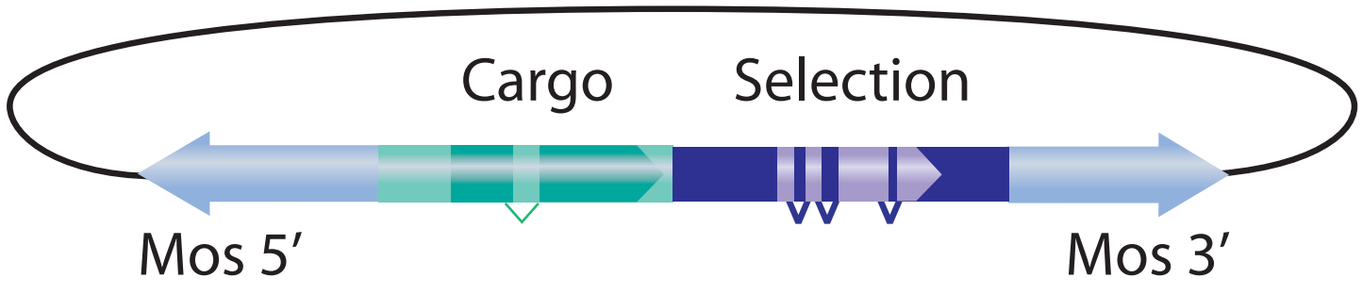

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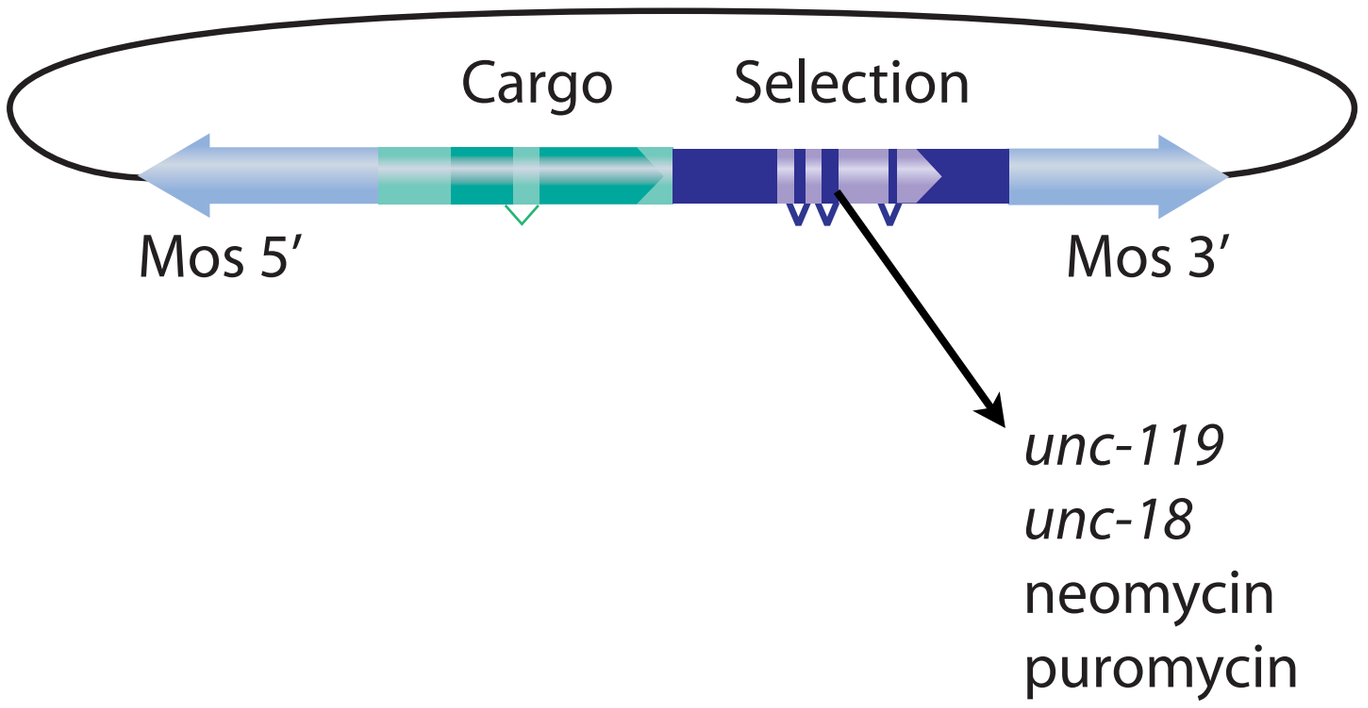
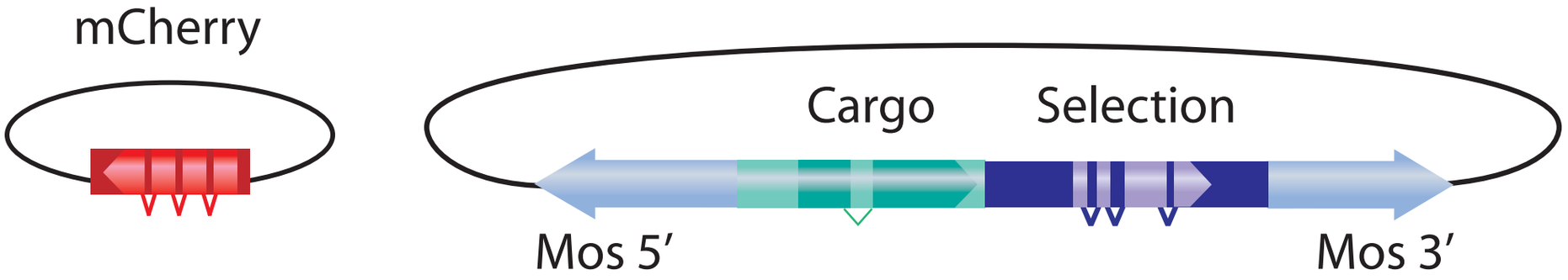


mCherry co-injection plasmid marks array

Injected DNA



Genomic DNA

Transposase mobilizes Mos1 with cargo

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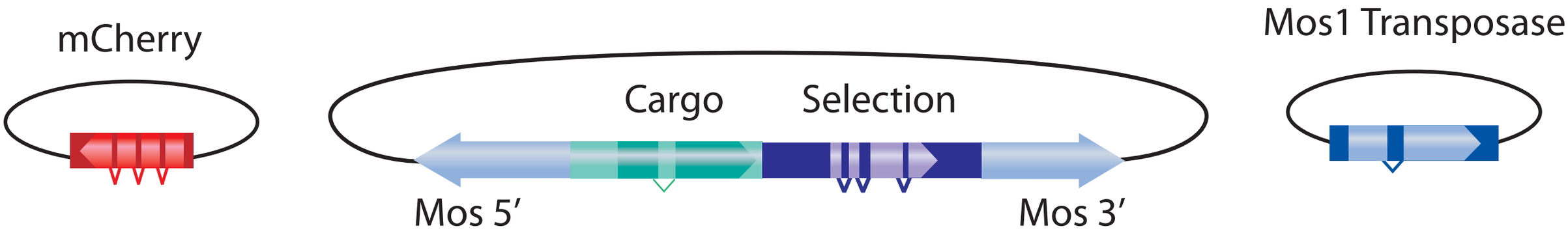

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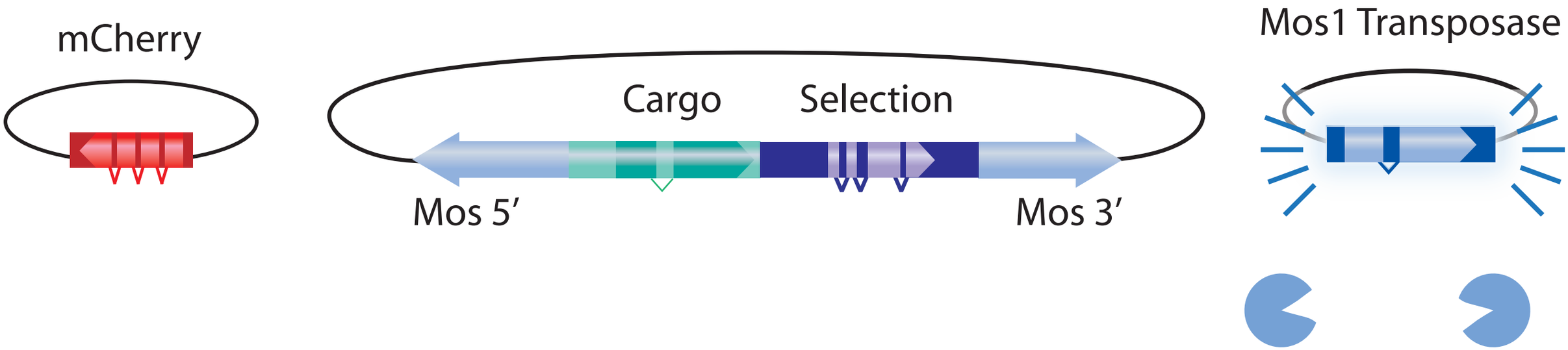

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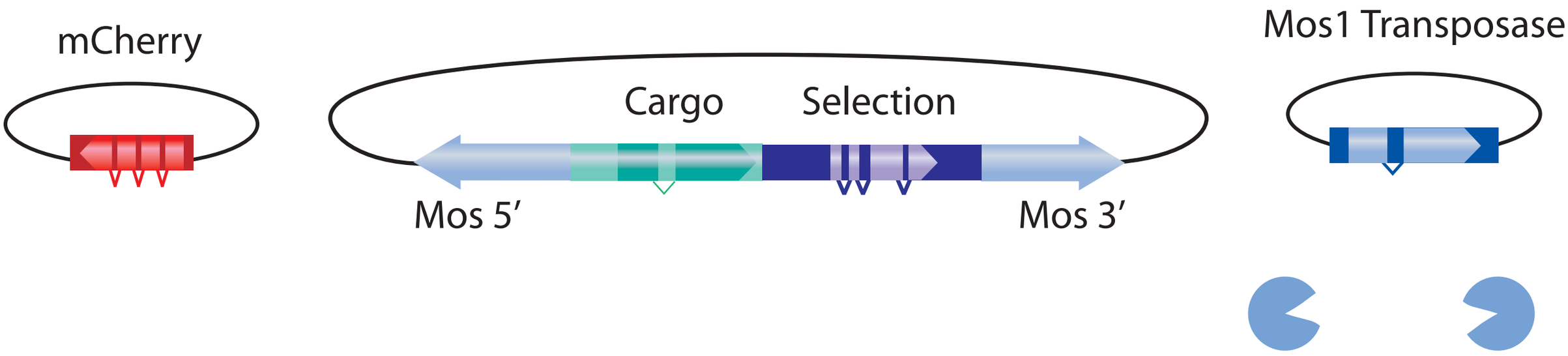

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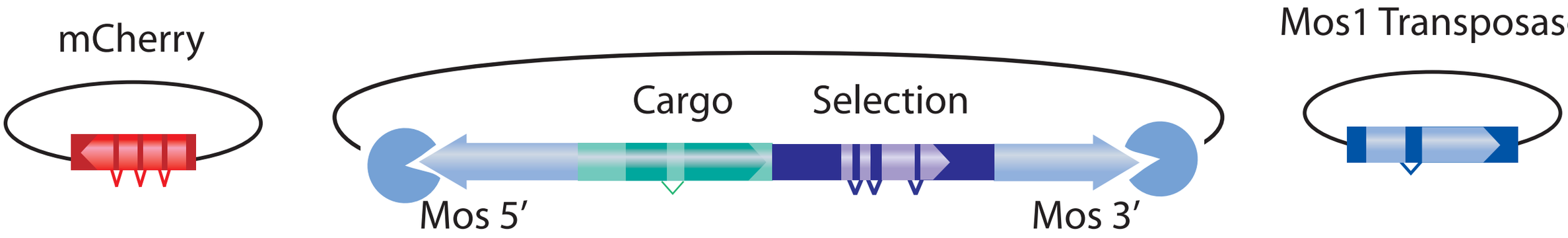
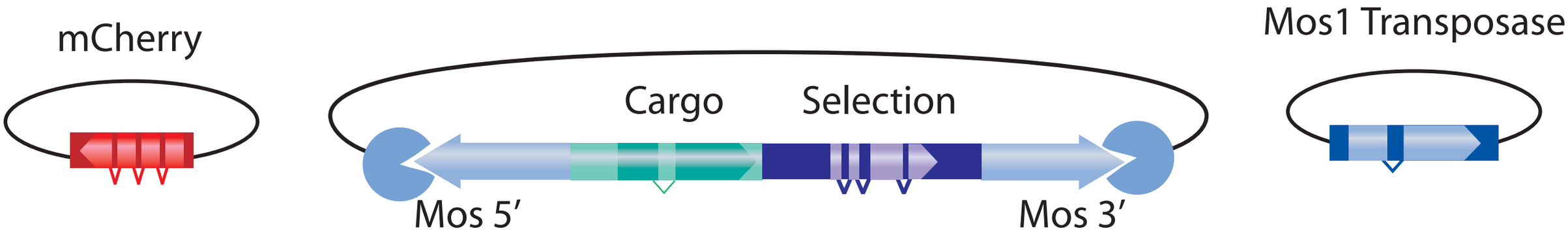


Genomic DNA

60 % insertion frequency

Injected DNA



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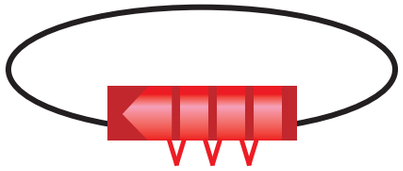
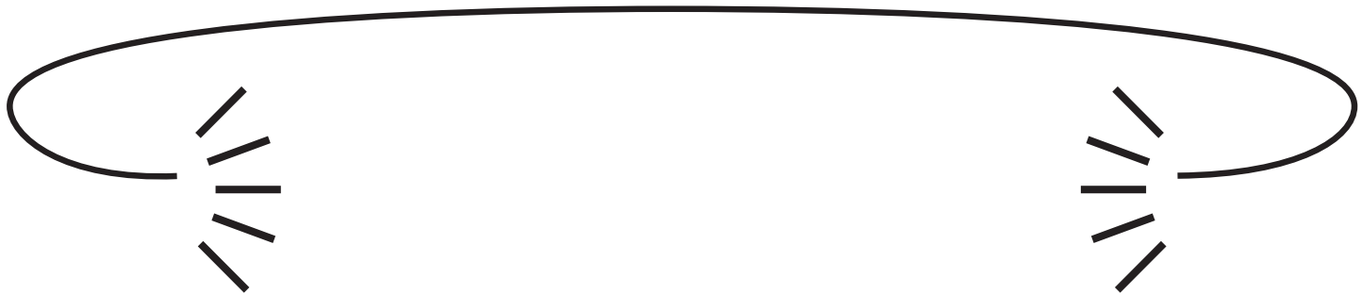
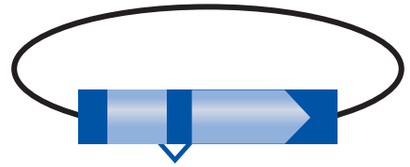
mCherry





Mos1 Transposase



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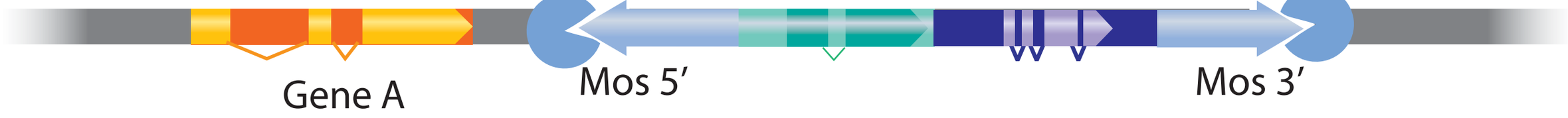

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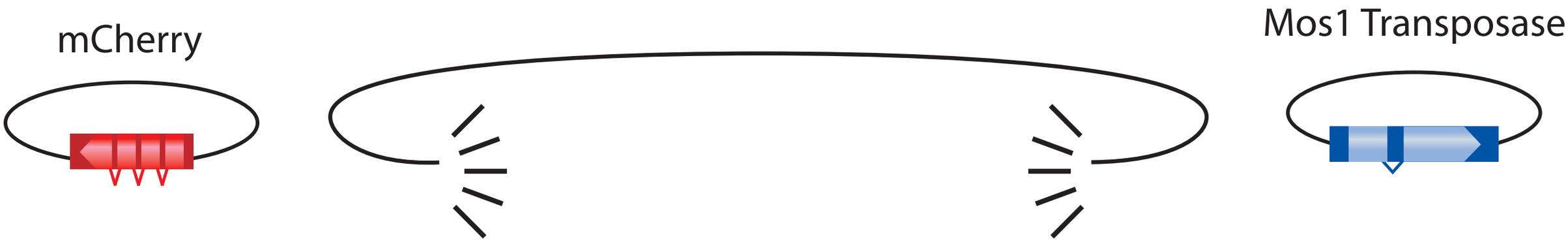

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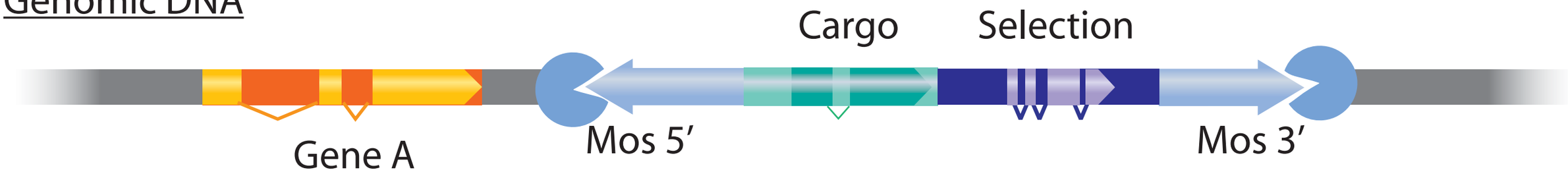

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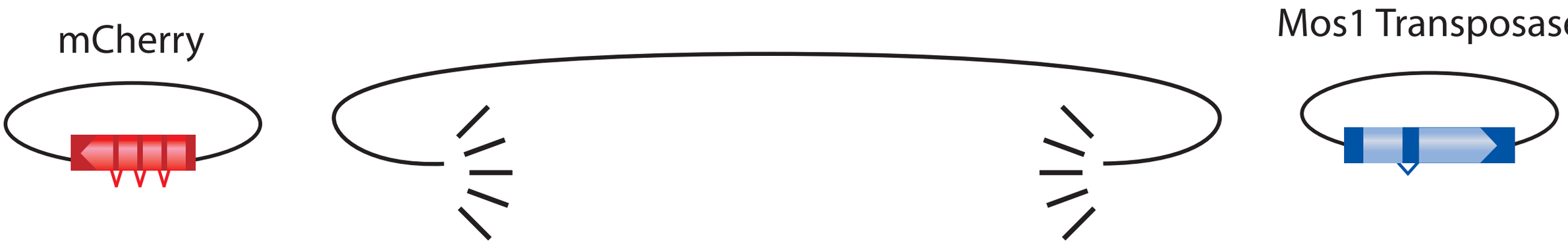

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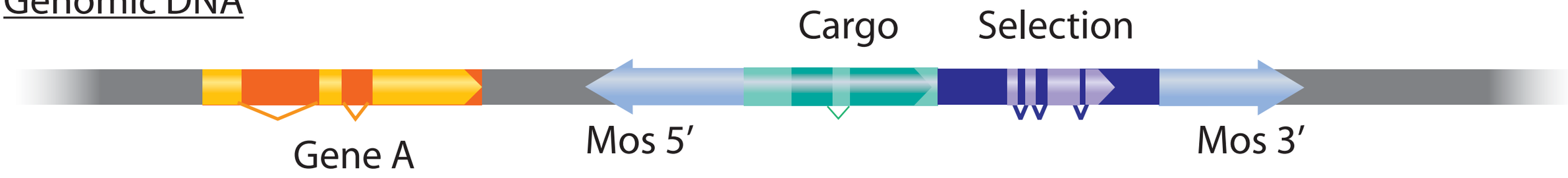

Extrachromosomal array is unstable and lost

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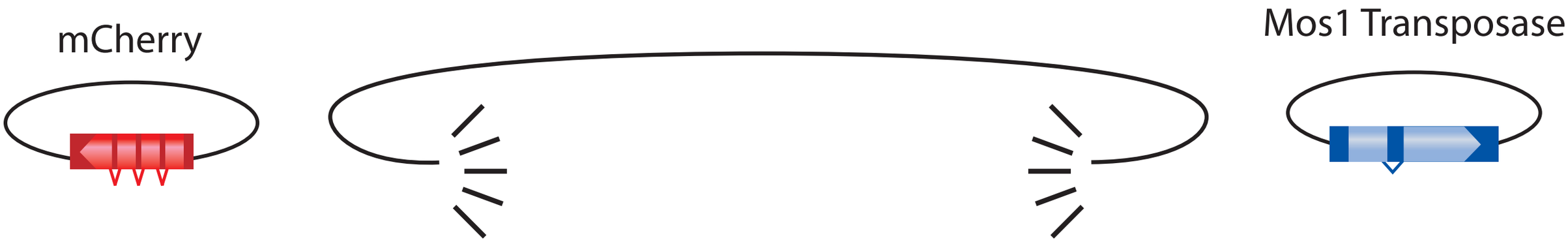

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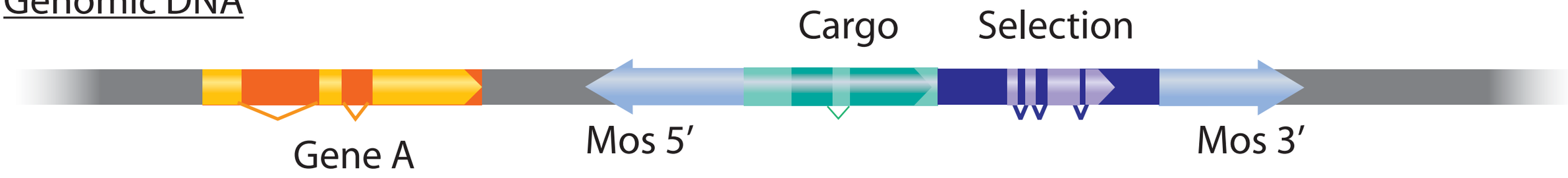

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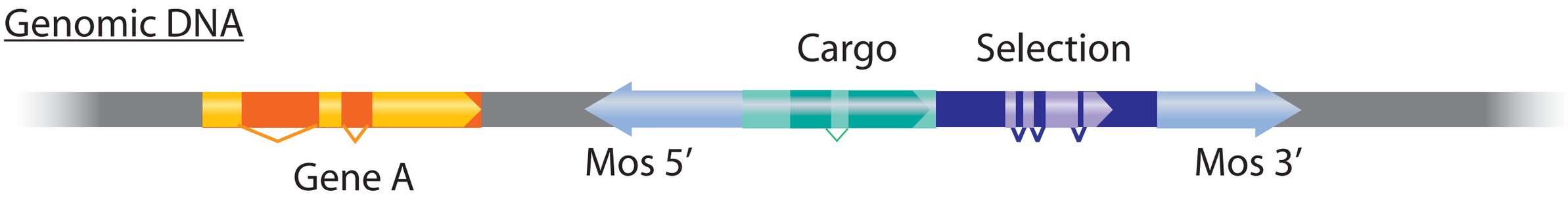
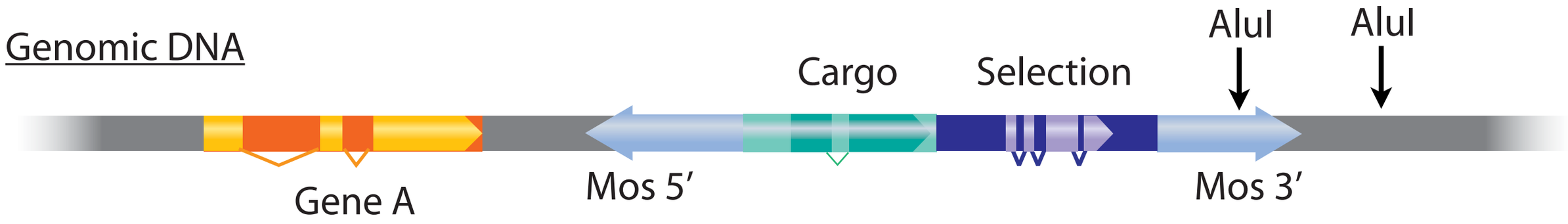


Insertion site can be determined by PCR



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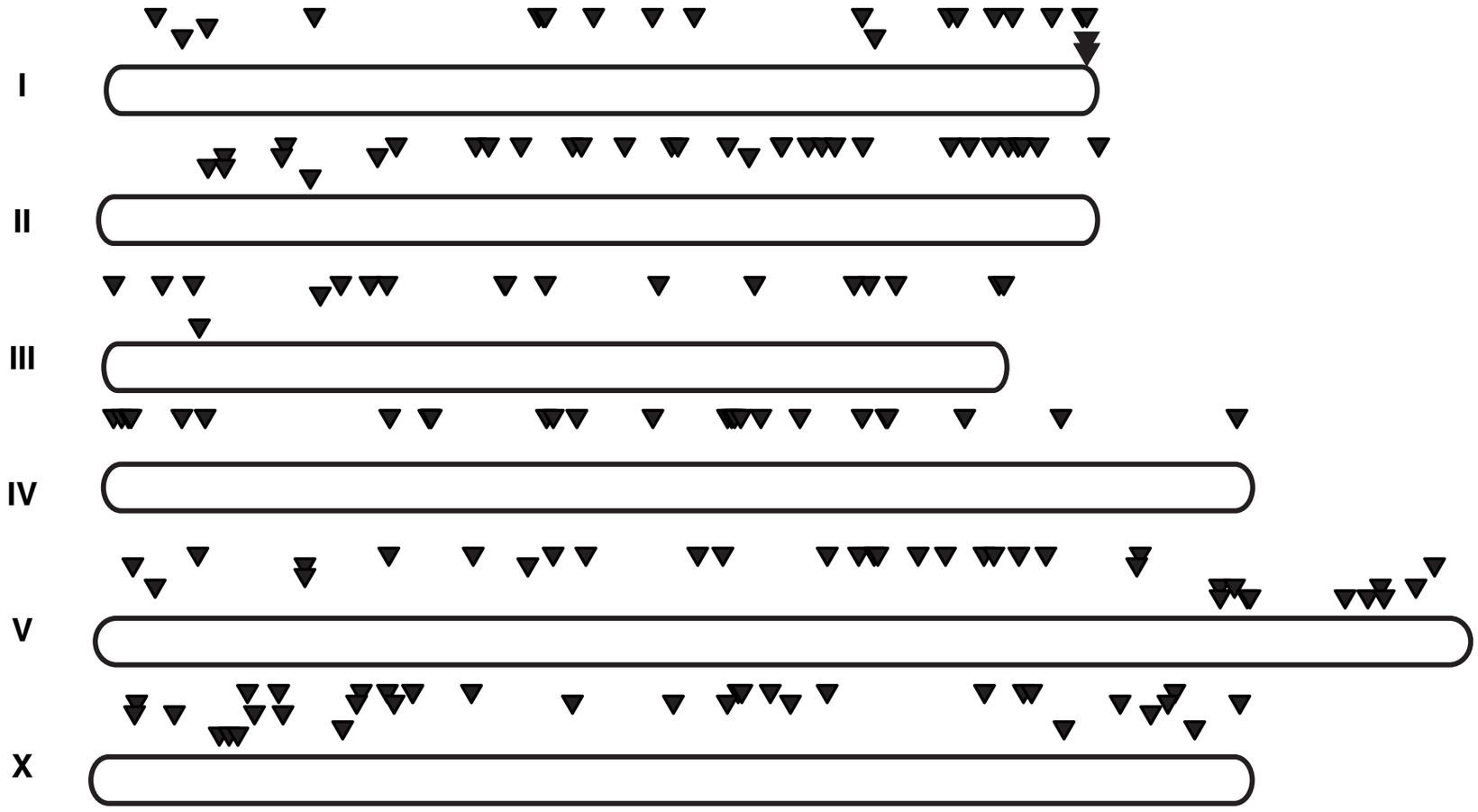
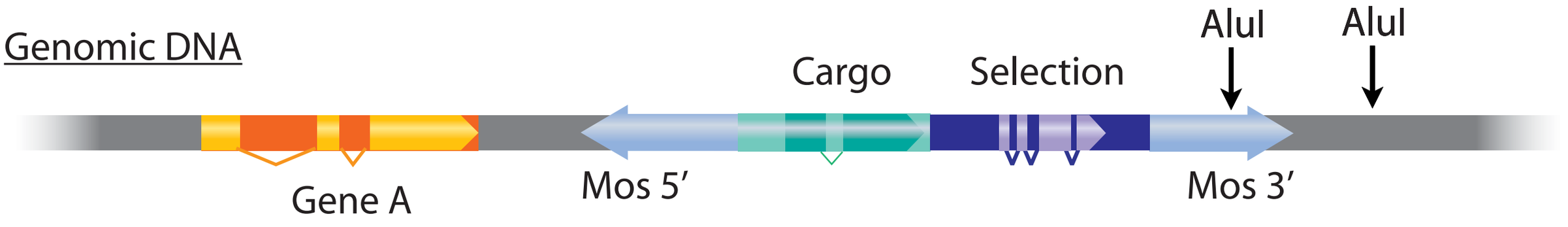
P*eft-3*:tdTomato:H2B insertions





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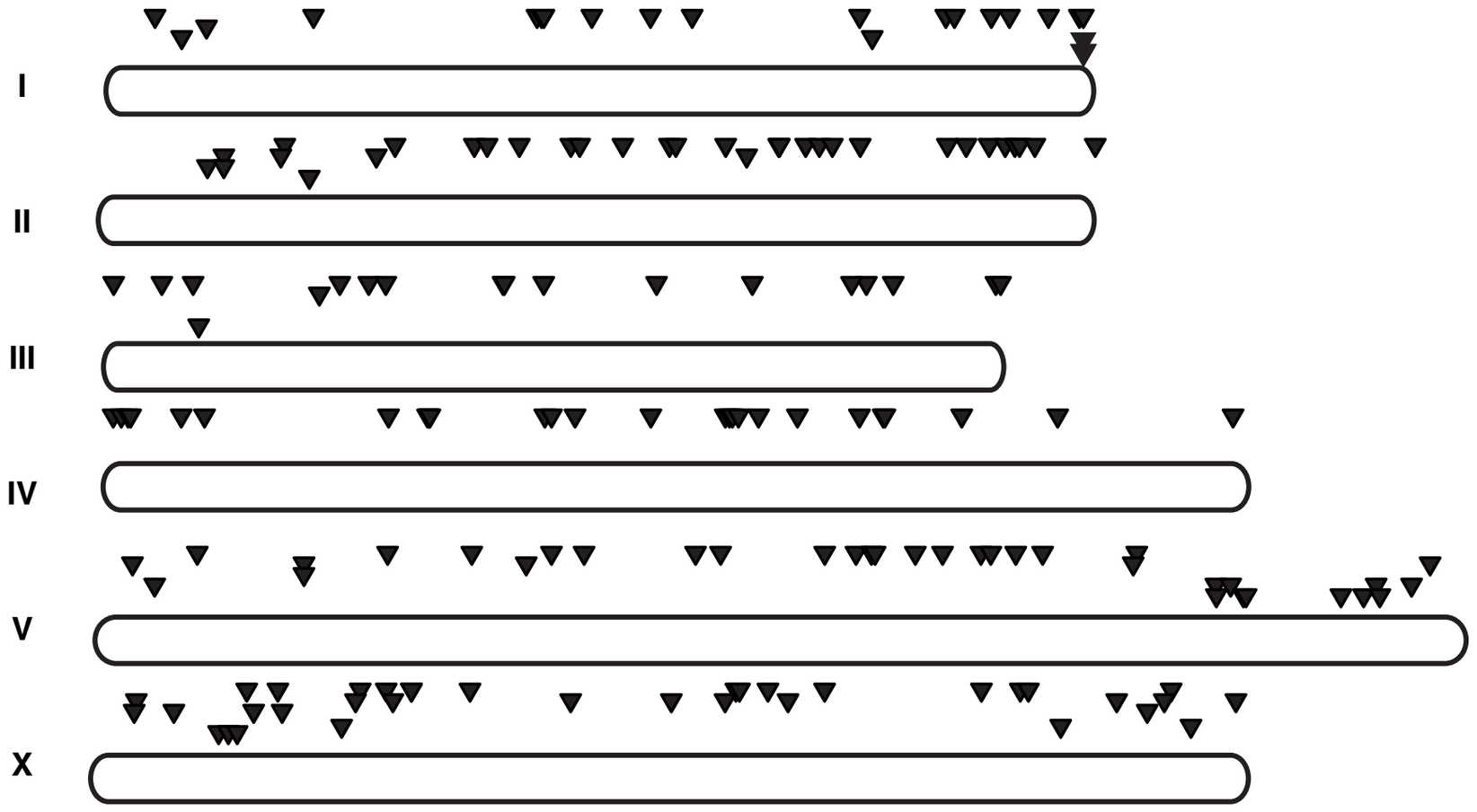

<u>These insertion are useful for</u>:

1) Moving mutations around (e.g. building doubles and triples)
2) Outcrossing mapped mutations
3) Mapping new mutations (mapping strains with 3x fluorophores)

Most strains deposited at the CGC

Insertion frequency and fidelity is high

Experiment: Inject a mix of three different miniMos elements (tdTomato:H2B, mCherry, GFP:H2B)

Injected animal	#1	#2	#3	#4	#5	**Total**
Singled F1s (rescued)	24	45	40	18	29	**156**

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Insertions	5	5	1	1	6	**18**
Insertions from non-rescued	0	1	0	0	1	**2**

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Multiple fluorophores	0	0	0	0	0	**0**

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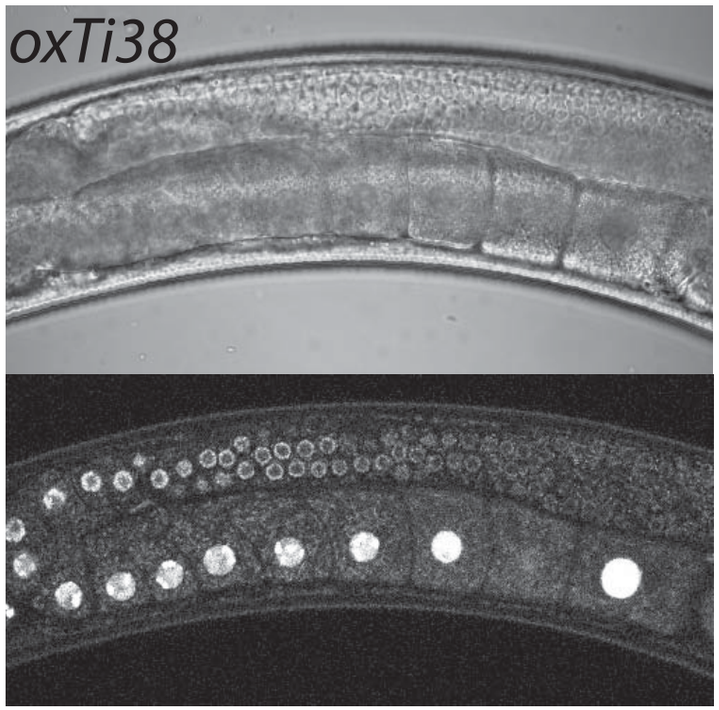
1) Multiple insertions are generated pr. injected worm (1-7 insertions/P0 injected)

2) Each strain carries a single transgene insertion

3) The fidelity of insertion is high - no non-fluorescent insertions

Approx. 50% of insertions are fluorescent in germline

P*pie-1*:GFP:H2B



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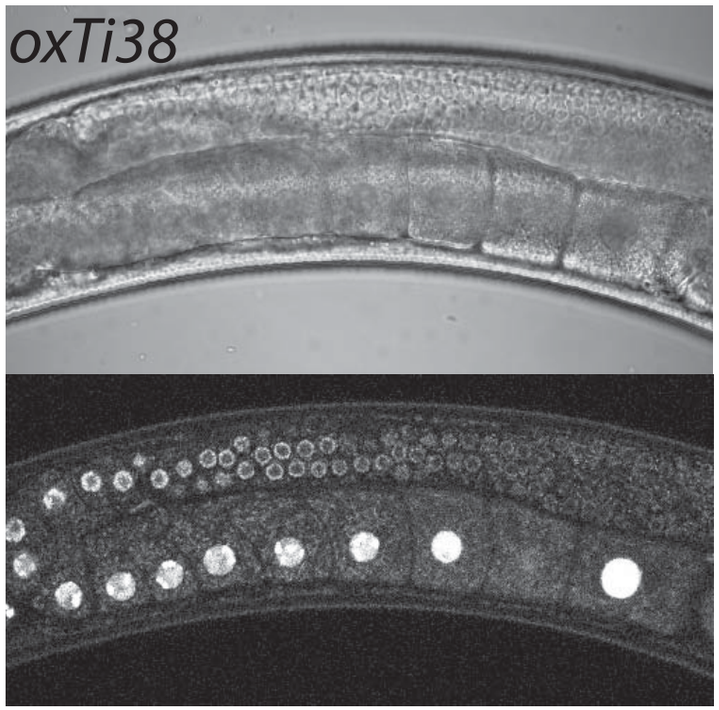

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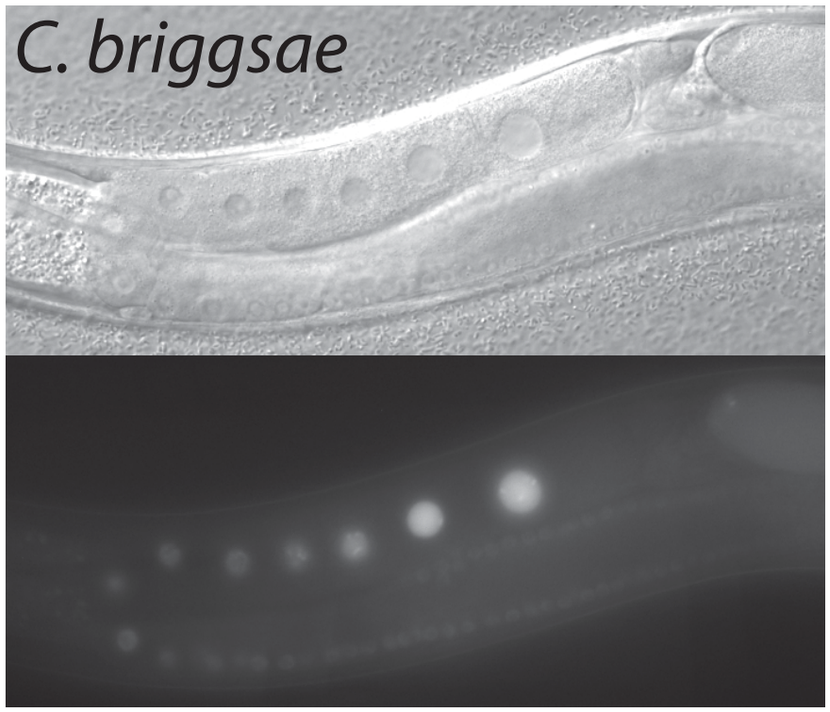
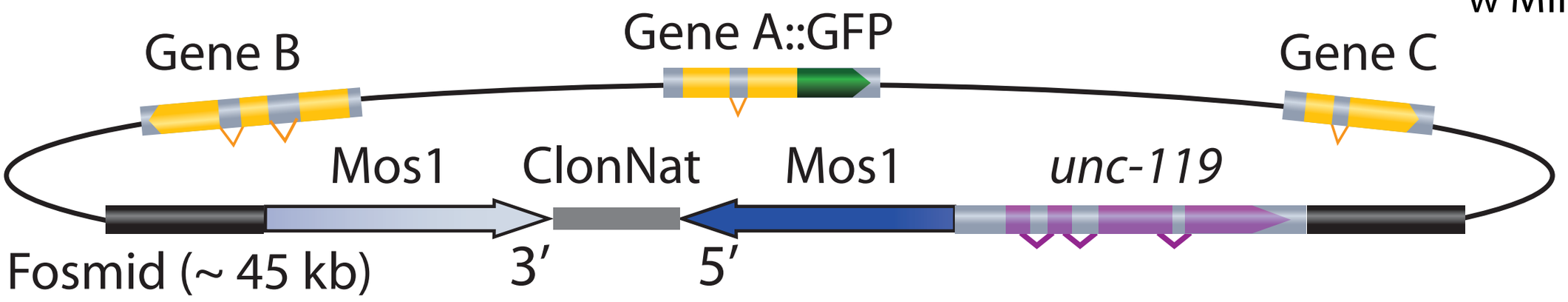


Minimos can carry large transgenes

w Mihail Sarov



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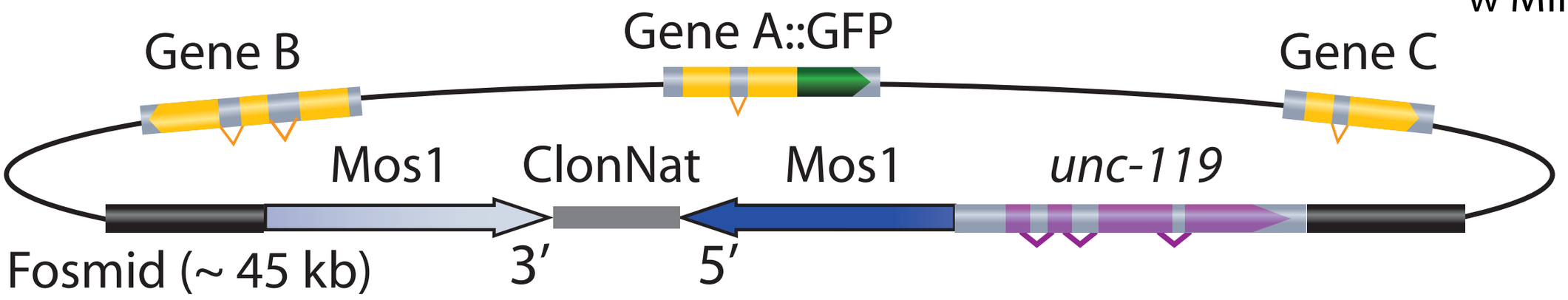
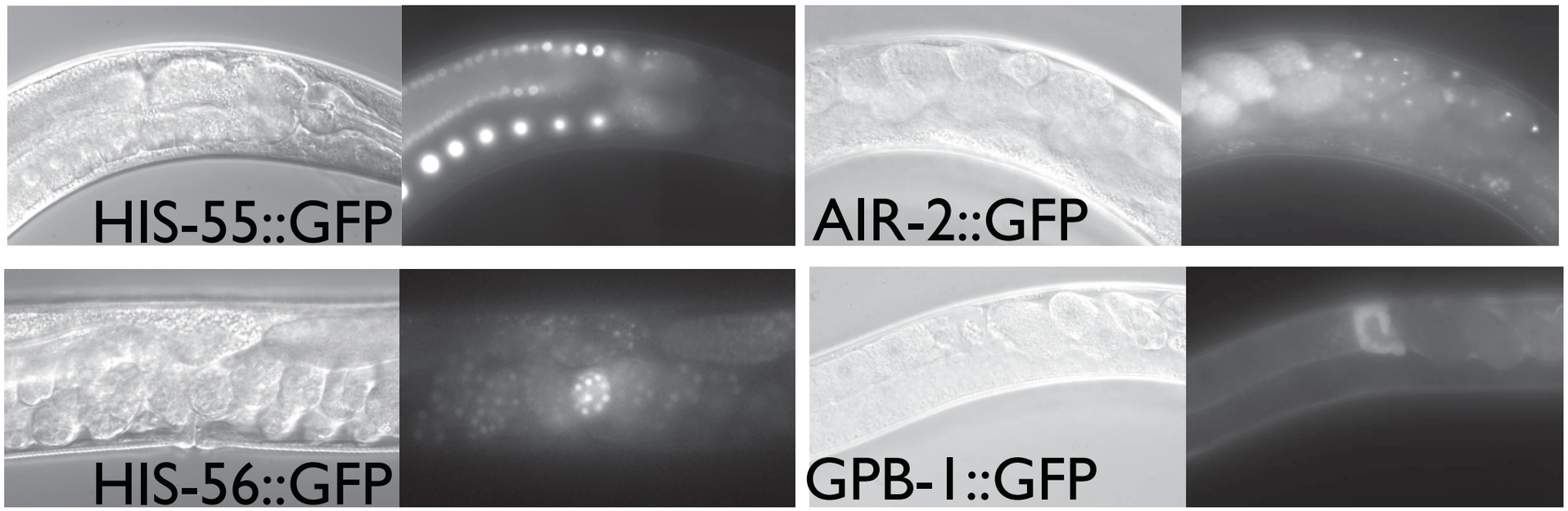
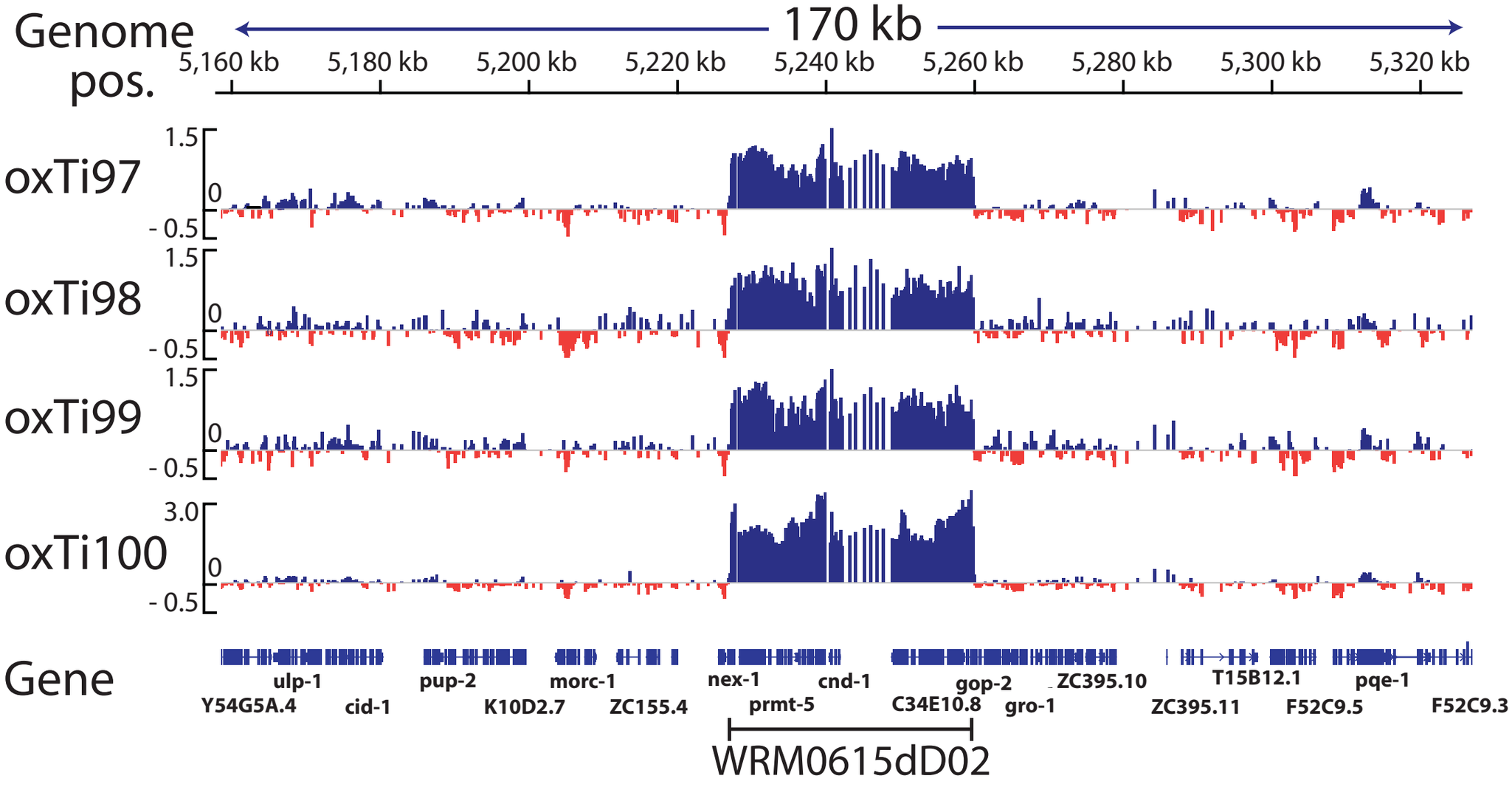


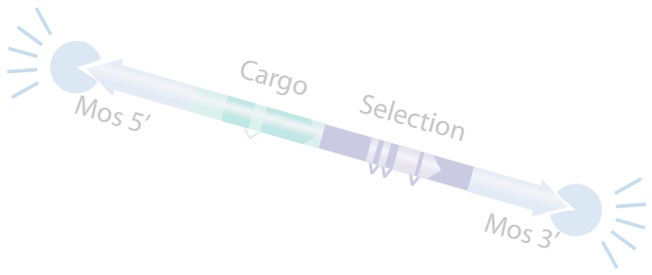


Fully intact fosmids are inserted

w Moerman lab



Methods to engineer the genome based on Mos1



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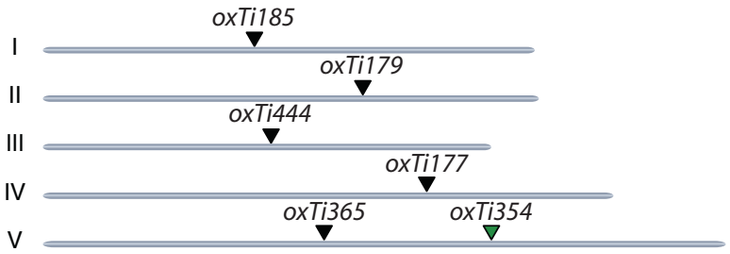

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Targeted insertion at different sites across the genome

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Reagents, strains and troubleshooting advice

1. Generate universal landing site

chromosomal DNA

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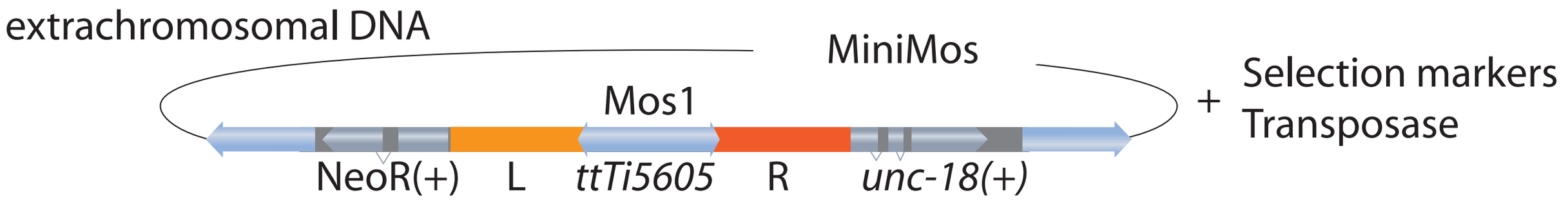

chromosomal DNA

1. Generate universal landing site

extrachromosomal DNA

MiniMos

\+ Selection markers
Transposase

insertion

chromosomal DNA

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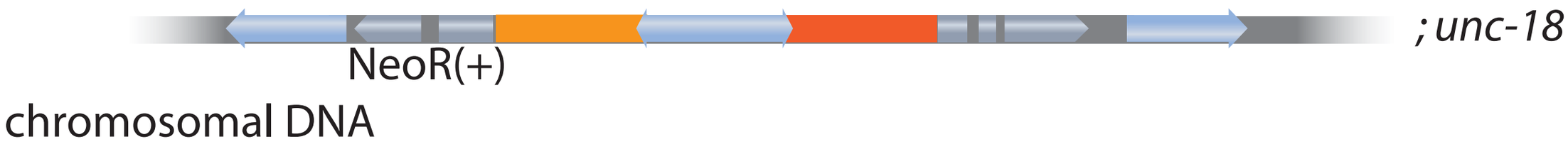
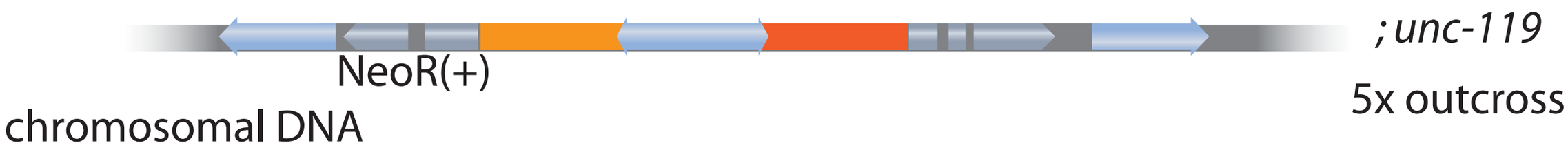
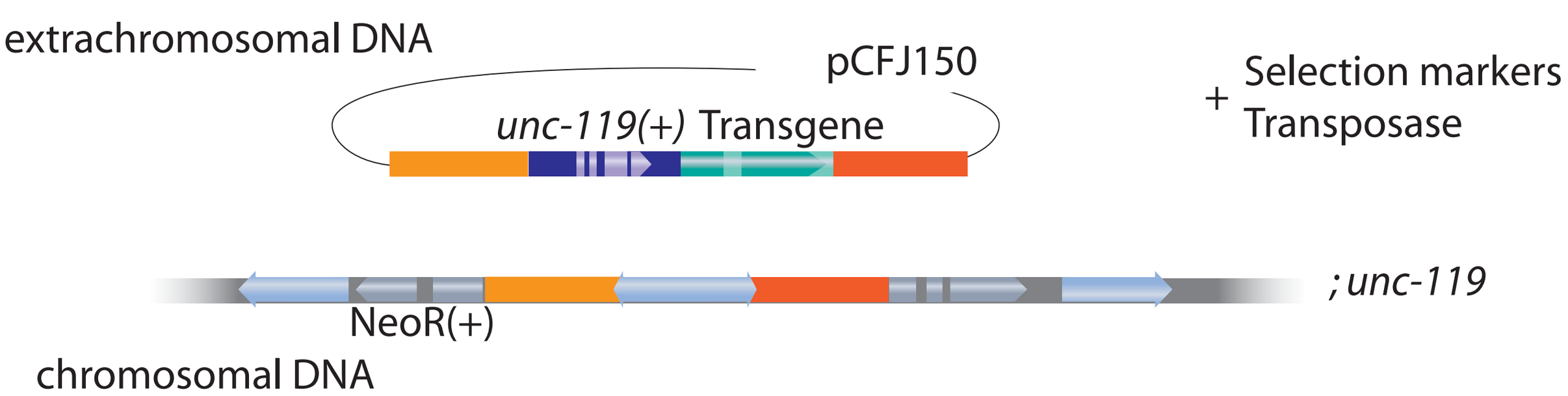


2. Cross into *unc-119* background



3. Inject pCFJ150 based targeting vector



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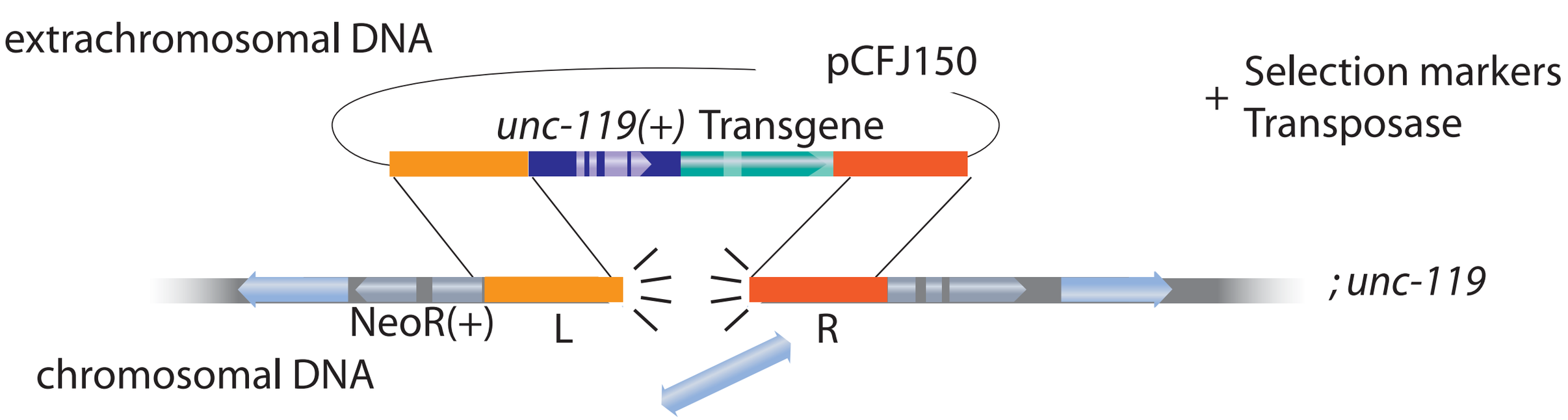

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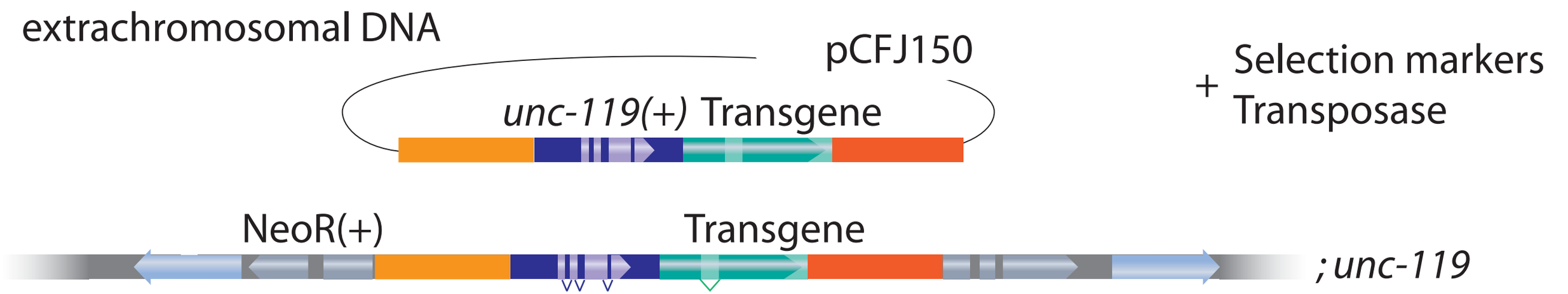

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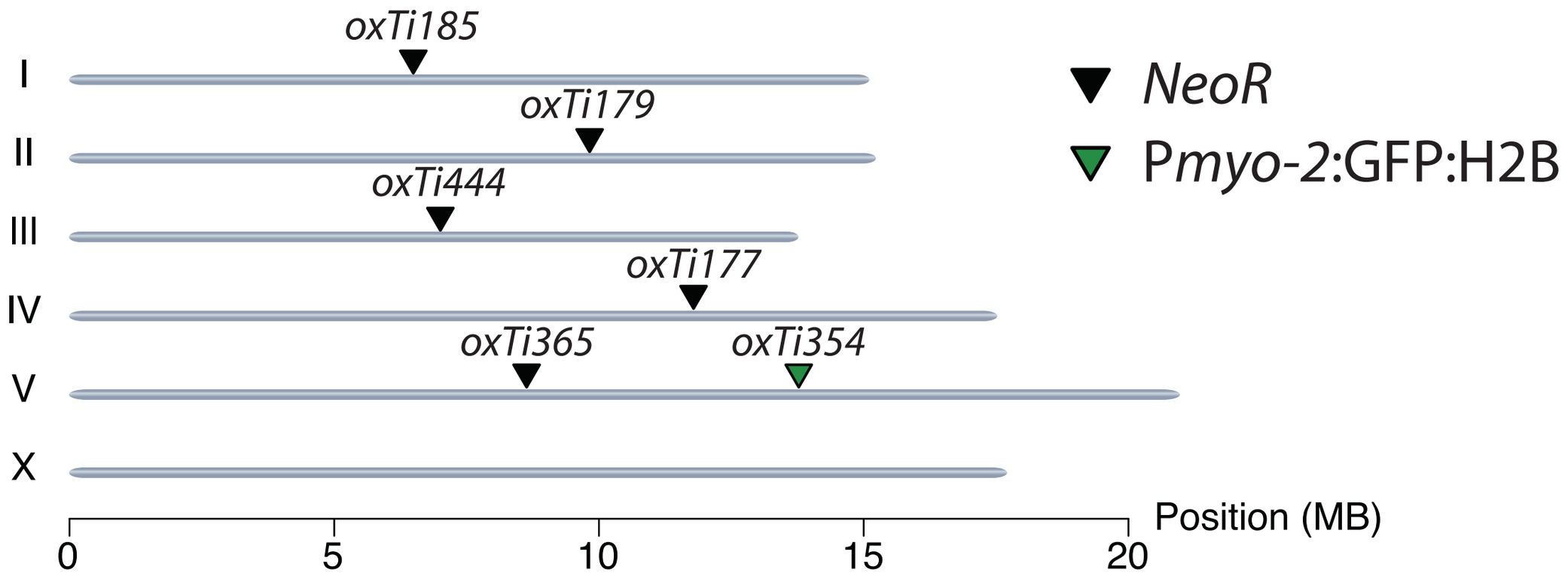
Standard insertion sites



5x outcrossed
Germline fluorescence verified
Available at the CGC

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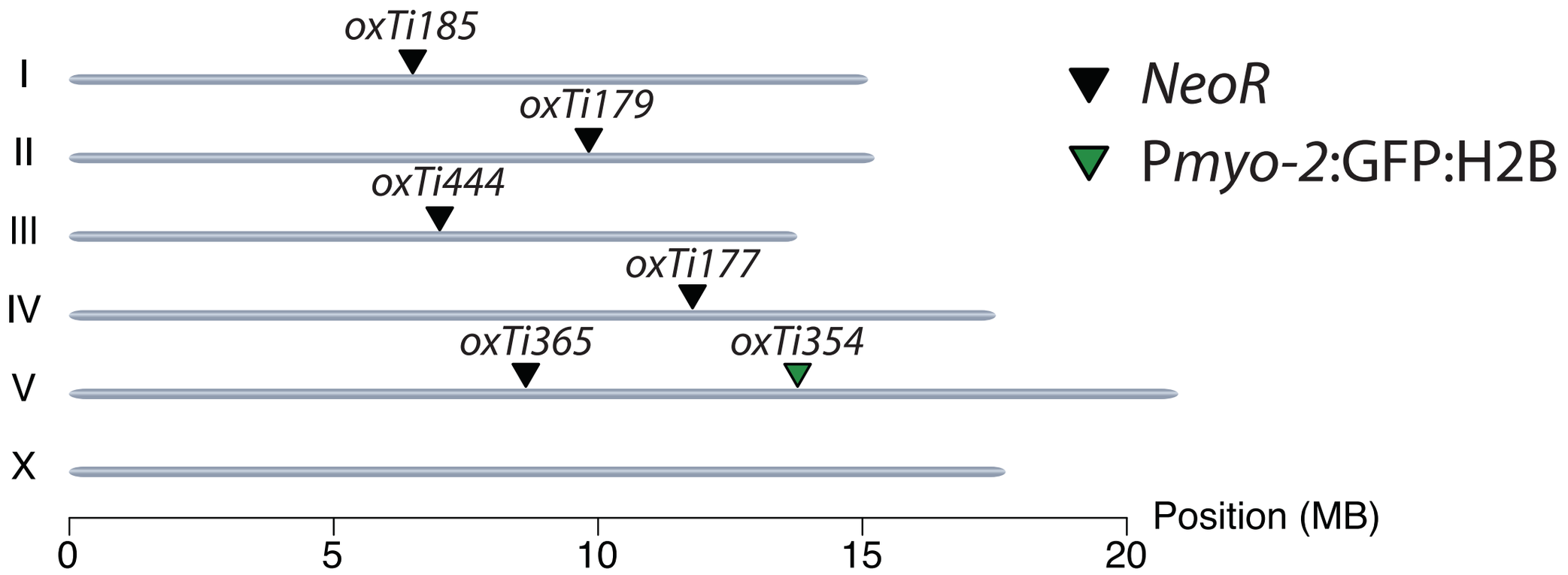

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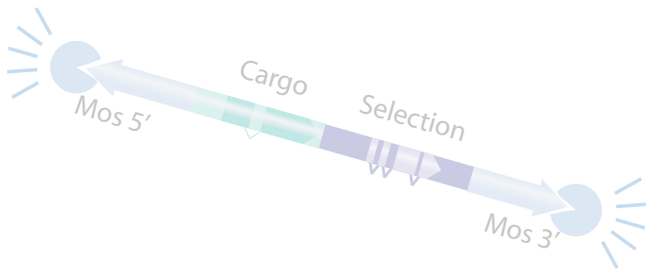
Available at the CGC

<u>Advantages</u>:
- A single targeting plasmid is compatible with many insertion sites
- Backwards compatible with constructs for Chr. II site (*ttTi5605*)
- MosSCIs can be followed by Neomycin resistance or P*myo-2* fluorescence

Methods to engineer the genome based on Mos1



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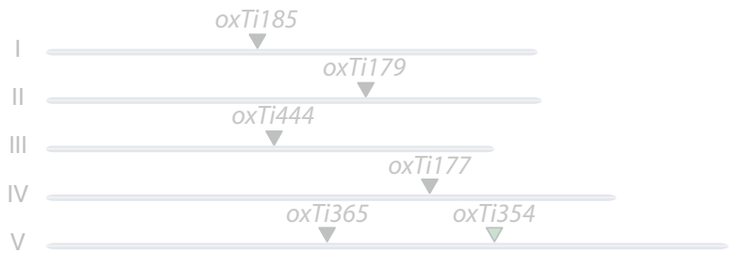

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Reagents, strains and troubleshooting advice

MosSCI | Universal MosSCI | MosDEL | miniMos | Reagents | Feedback | About

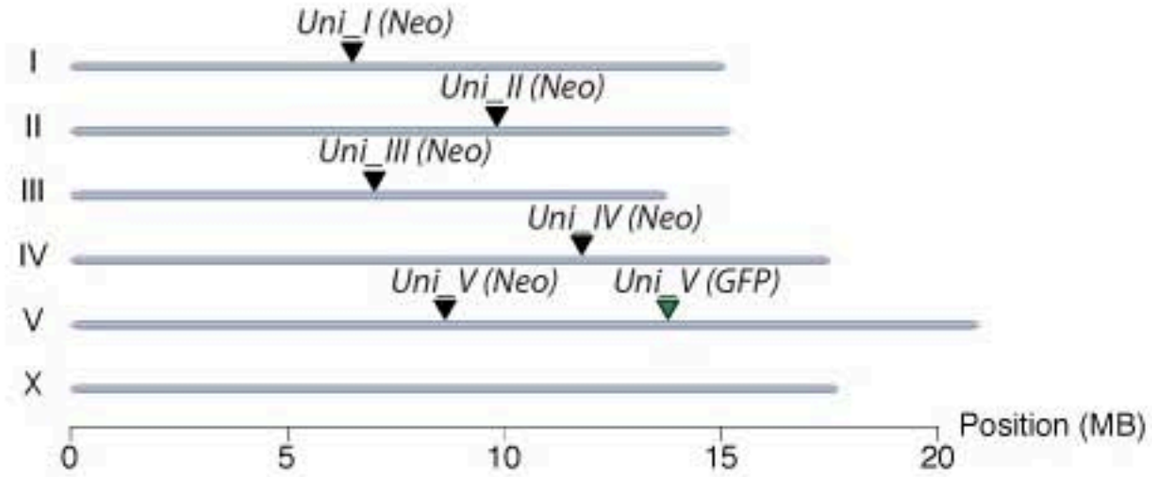
- About MosSCI
- Protocol
- Plasmids
- Insertion strains
- Bright MosSCI inserts
- Comments/FAQ

ıted Single Copy Insertion (mosSCI)

a single copy of a transgene into a well-defined location in the *C. elegans* genome. The method has the

- Transgenes can be expressed at levels close to endogenous gene expression
- Transgene insertions are stable
- Transgene expression is possible in the germline.

Universal insertion sites



All universal MosSCI insertion sites are targeted by vectors for the *ttTi5605* locus. These vectors include pCFJ150 and pCFJ350.

Insertion site	Locus	Co-insertion marker	Genetic position	Genomic position (WS190)	Genomic environment	Strain	Germline expression
unc-119							
UniI_I (Neo)	*oxTi185*	NeoR + *unc-18*(+)	I:1.17	I: 6,503,678	Intergenic	EG8078	Yes
Uni_II (Neo)	*oxTI179*	NeoR + *unc-18*(+)	II:1.73	II: 9,833,502	In ZK938.3	EG8079	Yes
Uni_III (Neo)	*oxTi444*	NeoR + *unc-18*(+)	III:-0.85	III: 7,014,336	In *lgc-38*	EG8080	Yes
UniI_V (Neo)	*oxTi177*	NeoR + *unc-18*(+)	IV:7.43	IV: 13,048,924	In *scl-10*	EG8081	Yes

MosSCI | Universal MosSCI | MosDEL | miniMos | Reagents | Feedback | About

- About reagents
- Fluorescent marker strains
- LacO insertions

Home › Reagents › Fluorescent marker strains

Use search box to limit strains to chromosomes (LG1, LG2 etc) or fluorophores (GFP, mCherry, tdTomato).

Please request strains from the CGC.
Strain column has link to the strain info at the CGC.

Show 10 entries

Search:

Chr.	Genetic pos.	Position (WB230)	Promoter	Fluorophore	Allele	Strain	Insertion marker(s)
LG1	I:-18.63	657,302	P*eft-3*	tdTomato:H2B	*oxTi550*	EG8004	*cb-unc-119(+)*
LG1	I:-16.97	1,070,957	P*eft-3*	tdTomato:H2B	*oxTi648*	EG7831	*cb-unc-119(+)*
LG1	I:-14.67	1,455,659	P*eft-3*	tdTomato:H2B	*oxTi638*	EG7832	*cb-unc-119(+)*
LG1	I:-4.48	3,116,556	P*eft-3*	tdTomato:H2B	*oxTi559*	EG7833	*cb-unc-119(+)*
LG1	I:1.23	6,585,618	P*eft-3*	tdTomato:H2B	*oxTi556*	EG7835	*cb-unc-119(+)*
LG1	I:1.28	6,656,763	P*eft-3*	tdTomato:H2B	*oxTi587*	EG7836	*cb-unc-119(+)*
LG1	I:1.29	6,692,324	P*eft-3*	tdTomato:H2B	*oxTi712*	EG7837	*cb-unc-119(+)*
LG1	I:2.07	7,436,394	P*eft-3*	tdTomato:H2B	*oxTi718*	EG7838	*cb-unc-119(+)*
LG1	I:2.75	8,346,811	P*eft-3*	tdTomato:H2B	*oxTi623*	EG7839	*cb-unc-119(+)*
LG1	I:3.35	8,993,439	P*eft-3*	tdTomato:H2B	*oxTi590*	EG7840	*cb-unc-119(+)*

Edit

Showing 1 to 10 of 158 entries

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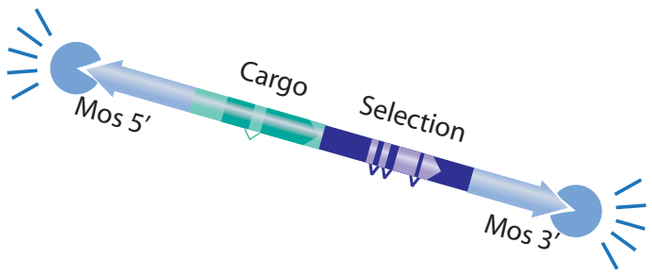
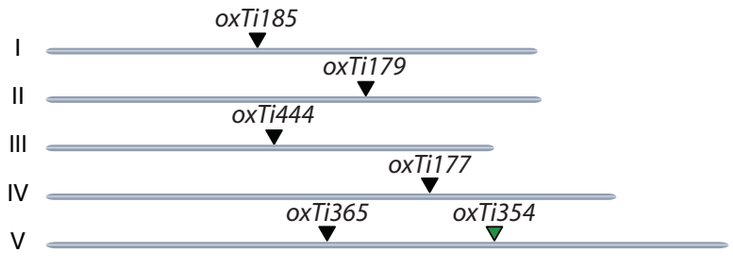
Methods to engineer the genome based on Mos1



1. MiniMos transposon

- High efficiency
- High fidelity
- Versatile: *C. elegans*, *C. briggsa*e and natural isolates
- High frequency of germline expression
- Can carry large transgenes



2. Universal MosSCI sites

- All insertion sites compatible with one targeting vector
- Easier to follow insertion in crosses
- Strains available at CGC

3. www.wormbuilder.org

- Protocols & Reagents
- Search for Universal insertion sites or bright markers
- Troubleshooting advice

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HHMI HOWARD HUGHES MEDICAL INSTITUTE

NATIONAL INSTITUTES OF HEALTH

Saturday 3:30 - 5:00 pm
Poster 1216C
MiniMos and Universal MosSCI sites

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