Single/Low-copy integration of transgenes by UV/TMP

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Aims of the Method

- To obtain single/low-copy transgenic animals, which enable us to express genes without overexpression.
- To express genes at the germ cells.
- To obtain parental strains for conditional Knock-Out (See our poster 1217A in this evening).

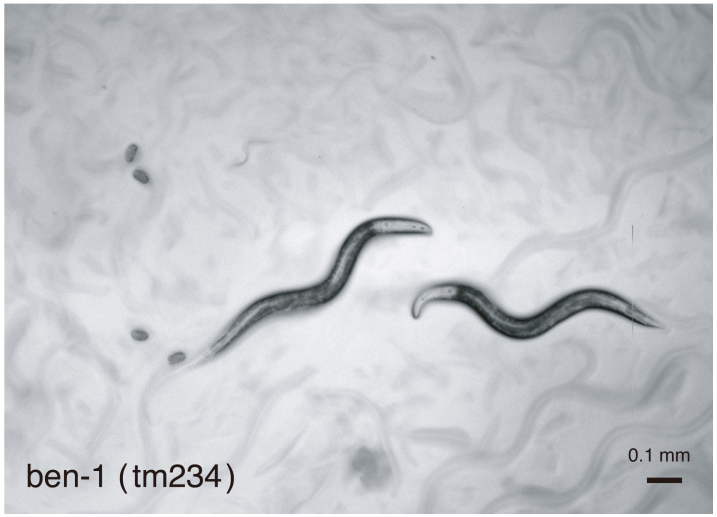
Comparison between multi-copy and single/low-copy integration methods

	Multi-Copy	Single/Low-Copy
Mutagen	UV (245 nm) from a Cross-linker for nylon membranes	TMP and UV (365 nm) from a hand monitor for agarose gel
Possible Copy Number (rough estimate)	50-1,000	1 – around 50
Selection	Single Culture of marker positive animals to find alleles with transmission rate of 100%	Positive (Let phenotype) and Negative (benomyl-sensitivity) selection; wait and see!
References	Mitani, 1995	Kage-Nakadai *et al.*, 2012

Method Summary

- Generation of extra-chromosomal multi-copy transgenic animals with DNA fragments to be integrated and positive/negative selection marker plasmids.
- Examination of selection before integration.
- UV/TMP treatment of multi-copy transgenic animals.
- Cultivation of animals on selection media.
- Examination of the copy number by PCR etc.

The *ben-1* mutation as a marker



- Easy to isolate: Wild-type animals are Dpy, Unc, and Gro, while *ben-1* mutants are paradoxically, completely resistant when raised on the selection (benomyl) media.
- Single locus: *ben* mutations have been mapped on a single locus.
- Small gene size: The coding region of *ben-1* gene (a *β*-tubulin) is about 3.3 kb in length on the genome, and appropriate for subcloning in a plasmid.

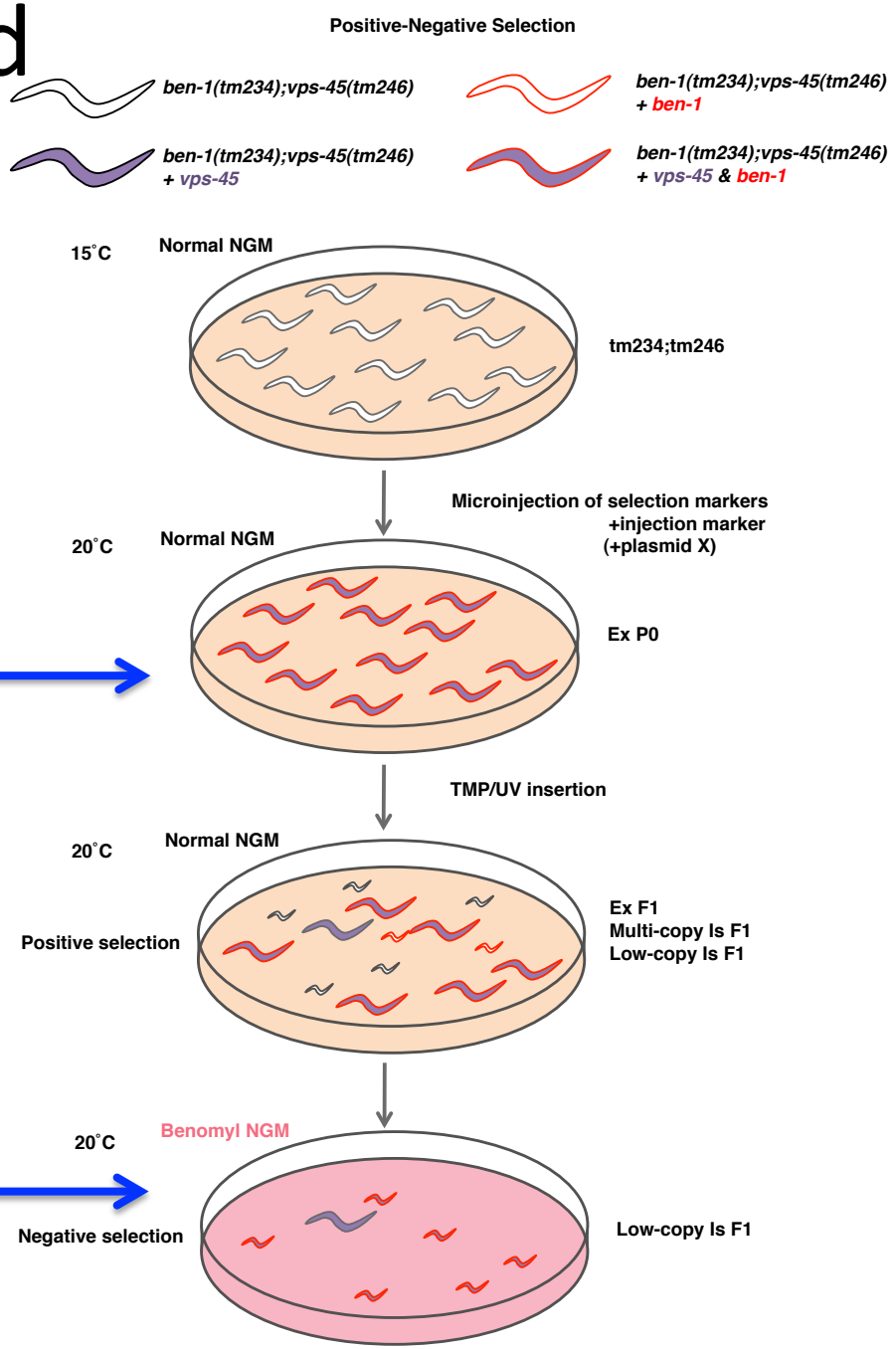
Outline of the method

Positive selection= rescue of a ts lethal phenotype of *vps-45*

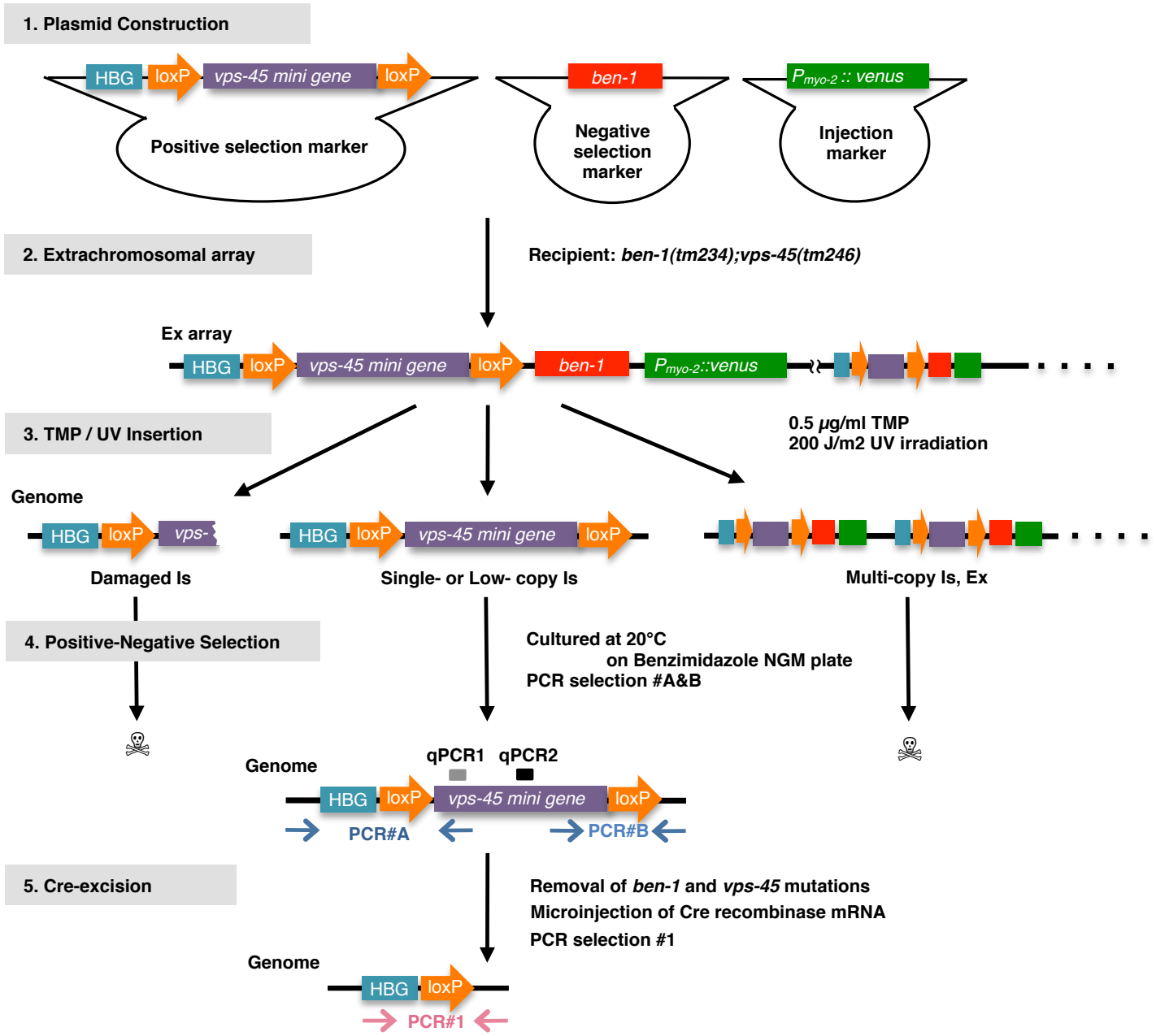
Negative selection=rescue of benomyl-sensitive phenotype

You can obtain a large amount of parent animals at this step very easily!

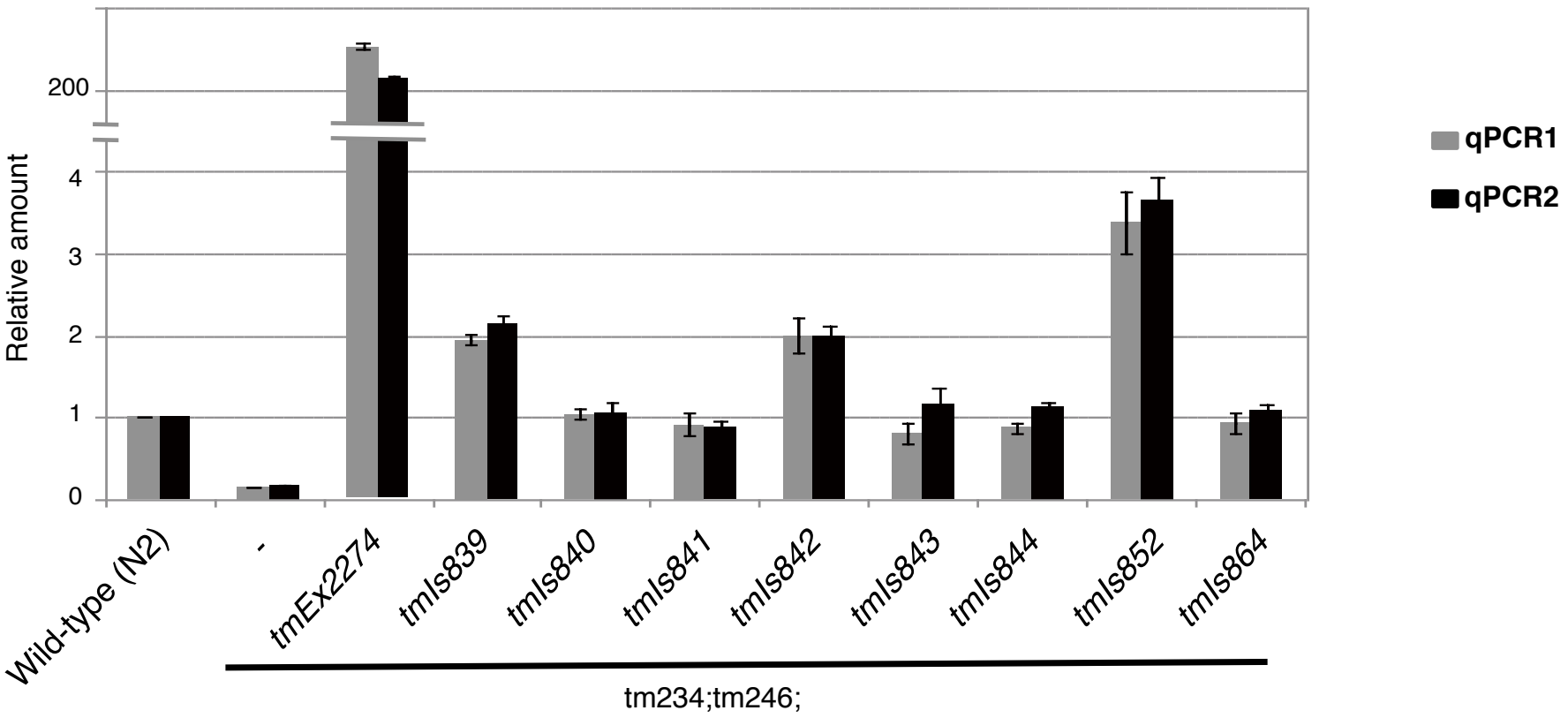
You can obtain many integrant strains by just waiting!



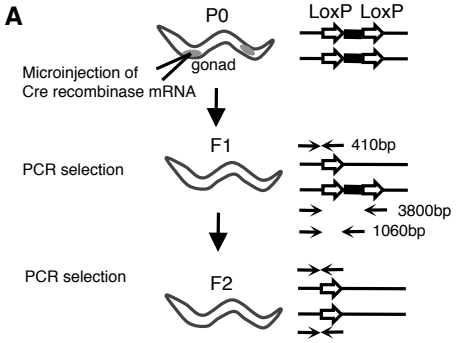
Plasmid Construction and Selection Strategy



Copy number analyses of the isolated transgenic strains



We could excise the single-copy transgenes by Cre recombinase treatment in the gonad



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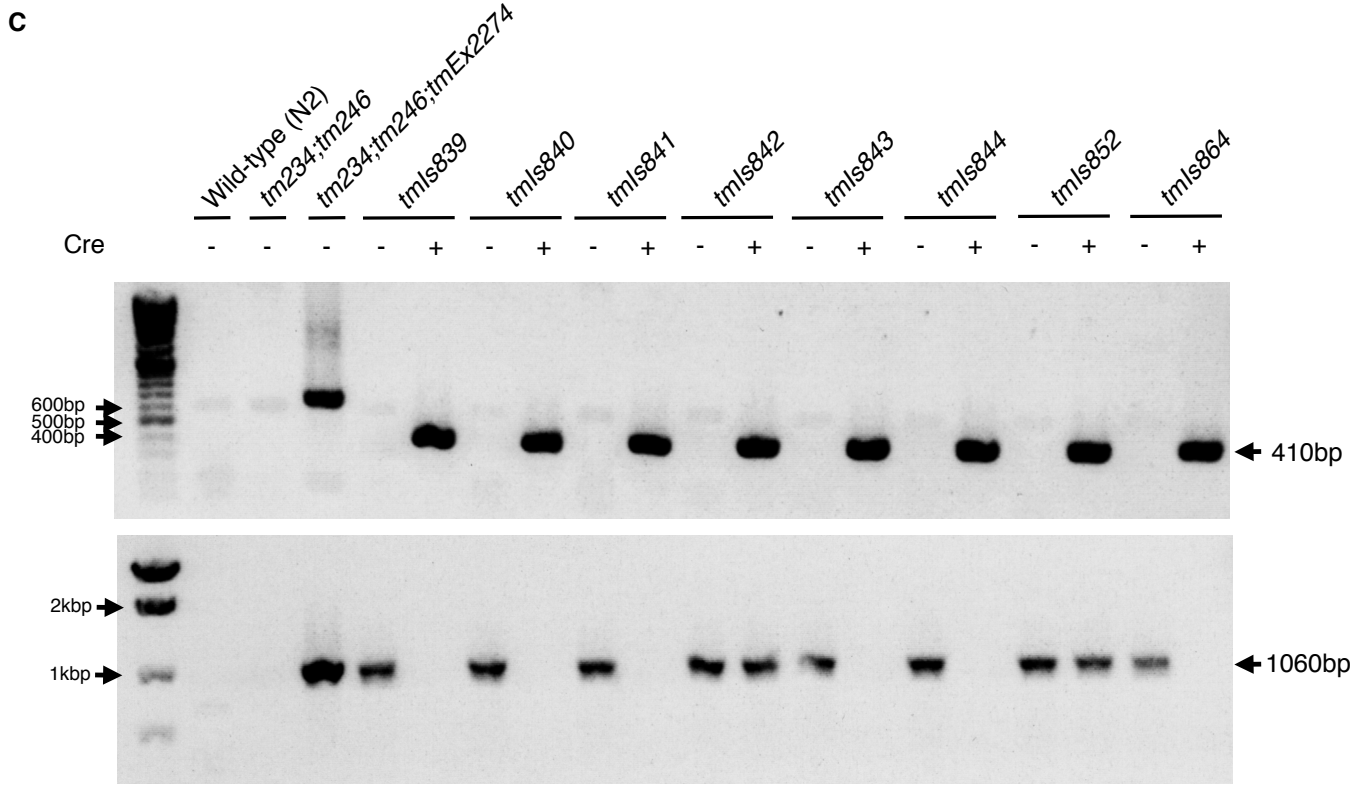
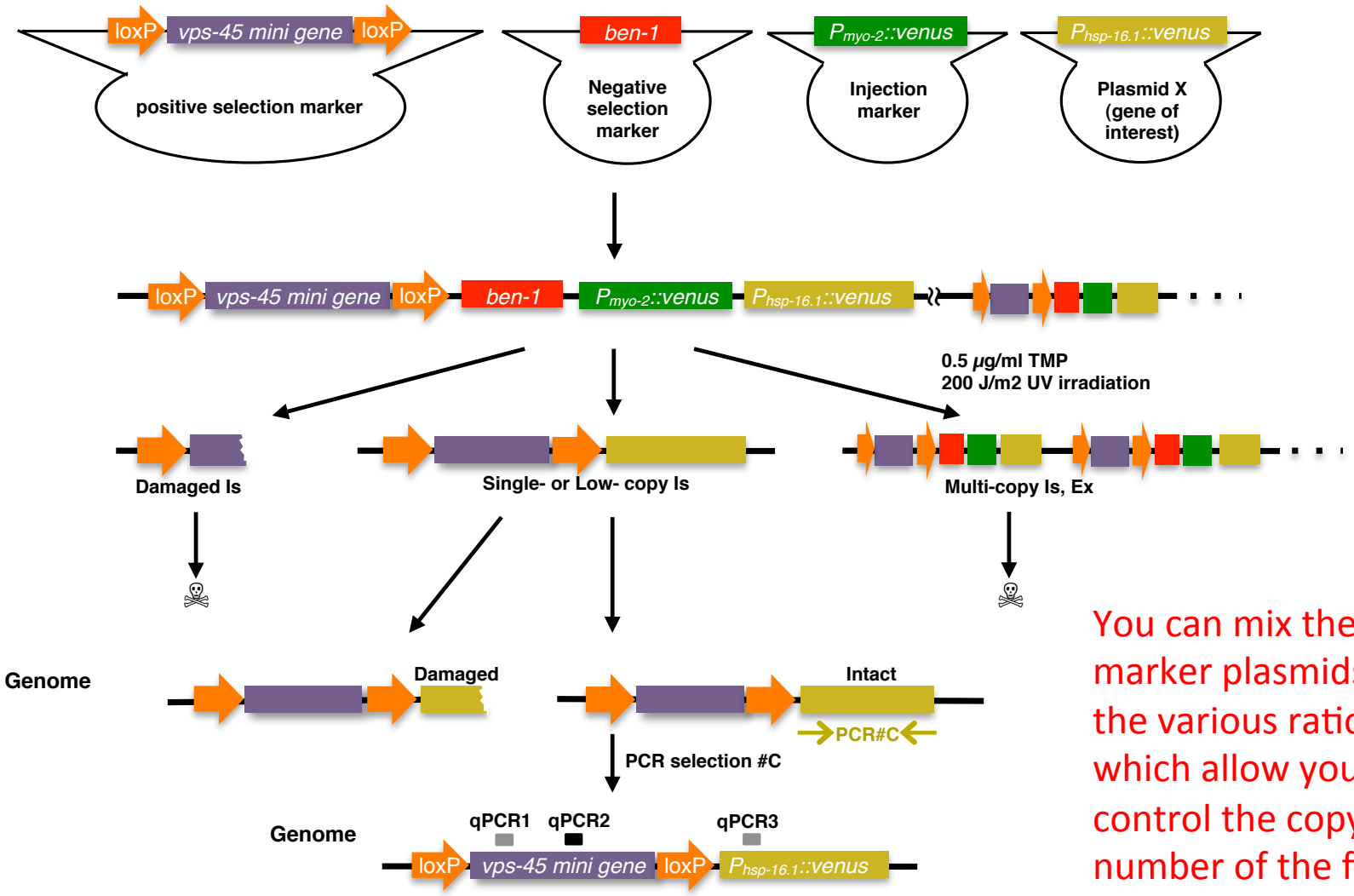
Line	Positive/total F1	Frequency (%)
N2 Cre	0/16	0
tmIs839 Mock	0/16	0
tmIs839 Cre	12/90	13
tmIs840 Cre	1/90	1
tmIs841 Cre	4/90	4
tmIs842 Cre	6/90	7
tmIs843 Cre	6/90	7
tmIs844 Cre	5/90	6
tmIs852 Cre	16/112	14
tmIs864 Cre	7/32	22

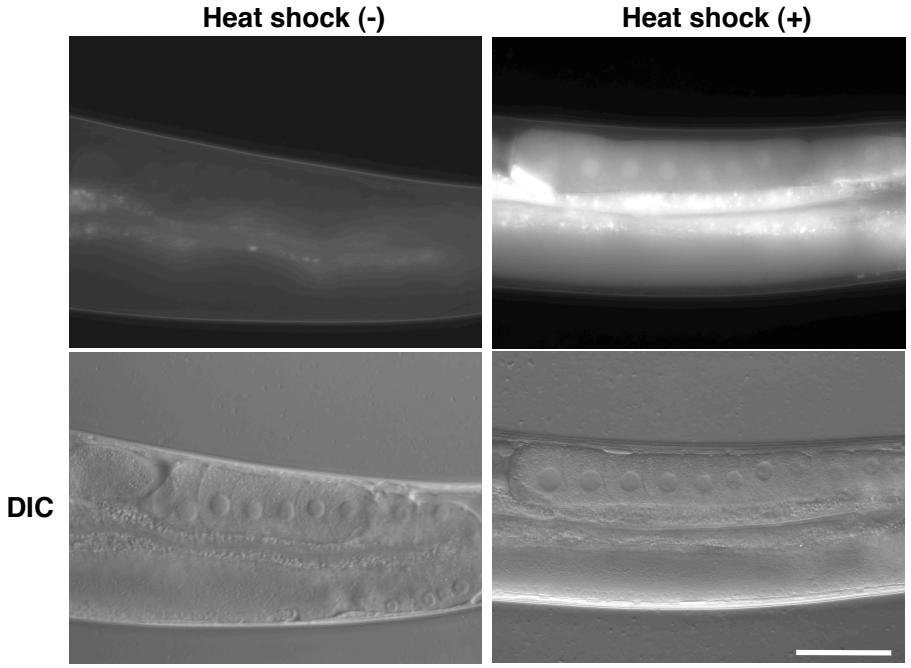


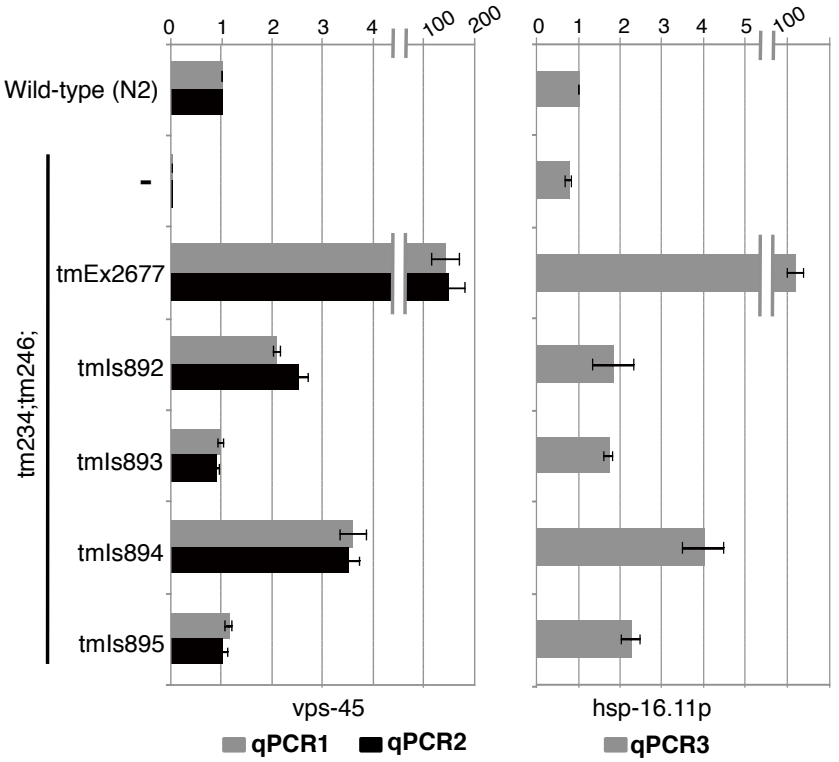
Co-injection of another gene of interest can create single-copy transgenic strains



You can mix the marker plasmids at the various ratio, which allow you to control the copy number of the final transgenic strains.

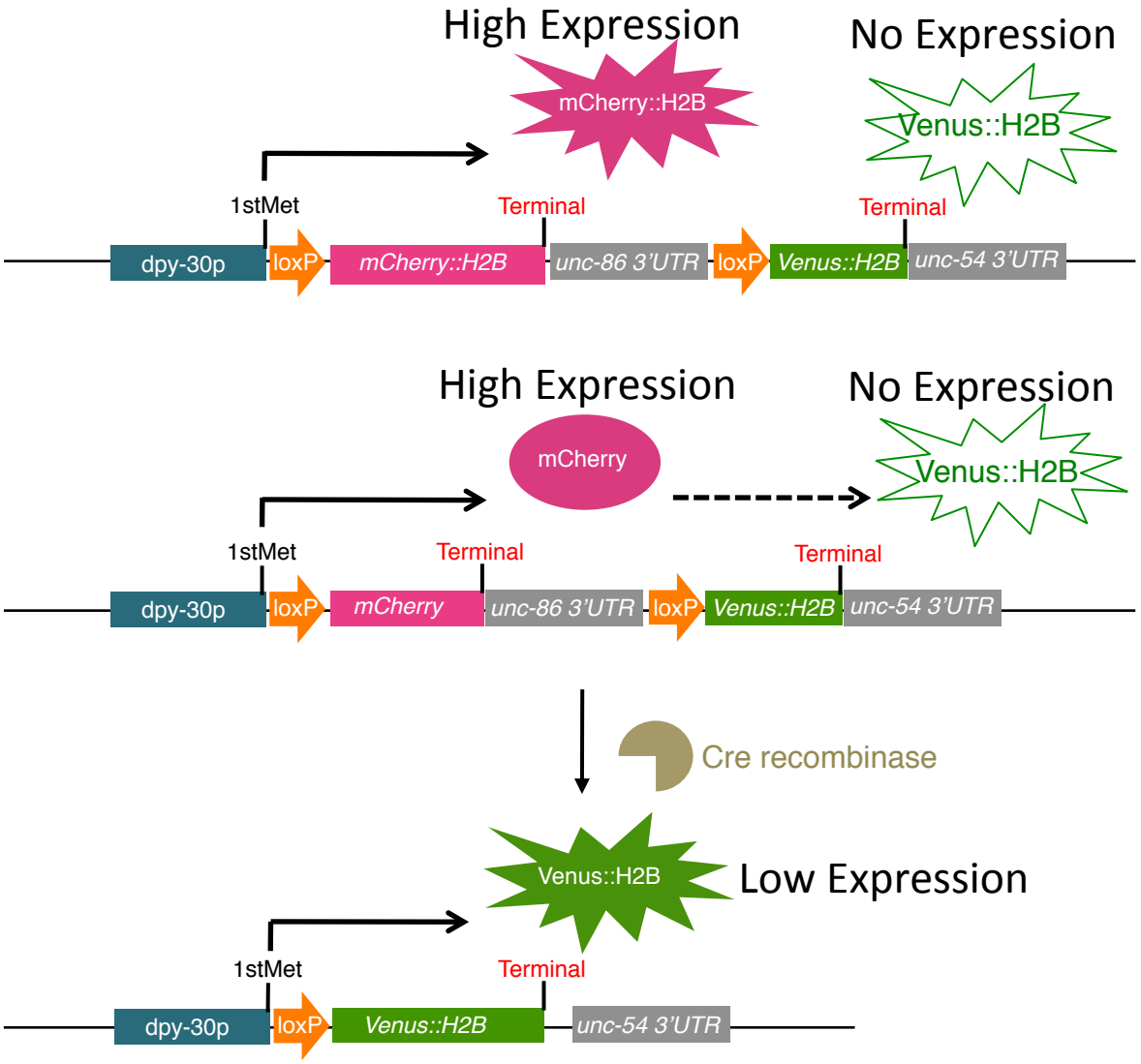
Single/low copy transgenes can express Venus in the germ cells.





Frequency: 4 single/low-copy Is strains from 16,000 P0 animals (0.025%)

Toxic genes can be transgenic



Transgenic Outcome

Ex---P0: 29, F1: 0

Ex----P0: 36, F1: 63, F2: 9

Single/Low Copy Is---
P0: 40,000 , Is: 6 (0.015%)

After Integration, low-copy transgenes are not toxic anymore.
You can express the Venus-H2B at a lower level.

Summary

- Our method is based on a two-step selection.
- The first step is based on popular multiple-copy transgenics (Ex).
- The second step can be easily scaled up and the probability of integration is about 0.02% of P0 animals (5,000 P0 animals treated with UV/TMP for one functional transgenic strains).
- Germ-cell expression is available.
- No new expensive equipment is necessary.
- Plasmid construction is easy because we only mix independent small plasmids but not targeting vectors.
- Overexpression-resistant genes can be transgenic.
- Cre-expressing transgenics are now being prepared for representative promoters for conditional KO, which can be combined with appropriate pre-existing mutants (Please visit our poster poster 1217A this evening).