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

<table>
<thead>
<tr>
<th></th>
<th>Multi-Copy</th>
<th>Single/Low-Copy</th>
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<tbody>
<tr>
<td><strong>Mutagen</strong></td>
<td>UV (245 nm) from a Cross-linker for nylon membranes</td>
<td>TMP and UV (365 nm) from a hand monitor for agarose gel</td>
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<td><strong>Possible Copy Number (rough estimate)</strong></td>
<td>50-1,000</td>
<td>1 – around 50</td>
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<tr>
<td><strong>Selection</strong></td>
<td>Single Culture of marker positive animals to find alleles with <strong>transmission rate of 100%</strong></td>
<td><strong>Positive</strong> (Let phenotype) and Negative (benomyl-sensitivity) <strong>selection</strong>; wait and see!</td>
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<tr>
<td><strong>References</strong></td>
<td>Mitani, 1995</td>
<td>Kage-Nakadai <em>et al.</em>, 2012</td>
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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 (benomyyl) media.
- Single locus: *ben* mutations have been mapped on a single locus.
- Small gene size: The coding region of *ben-1* gene (a \( \beta \)-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 \textit{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!
1. Plasmid Construction

2. Extrachromosomal array

3. TMP / UV Insertion

4. Positive-Negative Selection

5. Cre-excision

Plasmid Construction and Selection Strategy
Copy number analyses of the isolated transgenic strains

Wild-type (N2)

- tmEx2274
- tmls839
- tmls840
- tmls841
- tmls842
- tmls843
- tmls844
- tmls852
- tmls864

Relative amount

qPCR1
qPCR2

tm234;tm246;
We could excise the single-copy transgenes by Cre recombinase treatment in the gonad.
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.

Heat shock (-)  Heat shock (+)

DIC

Wild-type (N2)

tmEx2677
tmIs892
tmIs893
tmIs894
tmIs895

vps-45

qPCR1  qPCR2  qPCR3

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).