Determining Transgene Copy Number and Number of Integration Sites in Transgenic Mice

Fig. 1 Transgene Concatemer Integrated onto Chromosome

Note that the restriction enzyme cuts once in the transgene. The restriction enzyme cuts will produce DNA fragments the same size as the transgene, 3 Kb in this example. Note that the probe overlaps the restriction enzyme cut site. In this example, the probe will hybridize to unique 5' and 3' DNA fragments of unpredictable size. Probes to one side of the restriction enzyme cut site can also be used, in this case either the unique 5' or 3' fragment, but not both will hybridize to the probe.

Copy Standards For Southern Blot

Calculations:
Assumption: the Haploid content of a mammalian genome is $3 \times 10^9$ bp
Assumption: you will run 10 micrograms of tail DNA per lane on the Southern gel

Since the transgenic mice are hemizygous:

\[
\text{mass of transgene DNA} = \frac{N \text{ bp transgene DNA}}{3 \times 10^9 \text{ bp genomic DNA}} \times \text{5 microgram genomic DNA}
\]

Example: for a 5480 bp transgene insert or plasmid

\[
\text{mass of transgene DNA} = \frac{5480 \text{ bp cloned DNA}}{3 \times 10^9 \text{ bp genomic DNA}} \times \text{5 micrograms genomic DNA}
\]

mass of transgene DNA = \( (\frac{5480 \text{ bp cloned DNA}}{3 \times 10^9 \text{ bp genomic DNA}})\times (\frac{5 \mu\text{g genomic DNA}}{3 \times 10^9 \text{ bp genomic DNA}}) \) or

\[
\text{mass of transgene DNA} = \frac{5480 \text{ bp cloned DNA}}{3 \times 10^9 \text{ bp genomic DNA}} \times 5 \mu\text{g genomic DNA}
\]

mass of transgene DNA = 9.15 picograms

Thus, for 1 copy: add 9.15 pg of transgene DNA to 10 micrograms tail DNA

<table>
<thead>
<tr>
<th>Copies</th>
<th>mass of DNA (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.15</td>
</tr>
<tr>
<td>5</td>
<td>45.8</td>
</tr>
<tr>
<td>10</td>
<td>183.0</td>
</tr>
<tr>
<td>25</td>
<td>228.8</td>
</tr>
<tr>
<td>50</td>
<td>457.5</td>
</tr>
</tbody>
</table>

Digest the tail DNA for Southern analysis then inactivate the restriction enzyme.
Phenol extract and precipitate DNA or place heat labile enzymes at 60°C for 20 minutes.
Then add the transgene insert DNA (not the entire plasmid) to the digested DNA.
Remember to save one lane for genomic DNA only with no spike (0 copies of the transgene).
For an example of copy standards in Southern blots, refer to:

Note that Mouse 2 has an unusually strong signal for the transgene and that three unique bands can be seen. This is typical of integration in two chromosomes. Since different integration sites can give different expression levels and patterns breeding Mouse 2 means that the pups will need to be analyzed by Southern to determine which integration site they inherit.

Note that mouse 3 has only one visible unique band. This may be because the second band is too small and ran off the gel or it is too big to transfer efficiently to the Southern membrane.

Band intensity comparison suggests that Mouse 1 copy number is 5-10 and Mouse 3 copy number is 10-25. Mouse 2 integration site copy numbers will be determined in its offspring. Phosphor image data can provide more quantitative results.