

Quantification of Transgene Copy Number

Your assay for transgenic founders must be able to detect the transgene at a single copy per genome.

This is done by running your detection assay on wildtype mouse DNA that has been spiked with graduating amounts of your transgene. That is, you will run your assay on DNA samples containing the equivalent of your transgene at 0.1, 0.5, 1, 3, 10, and 30 copies per haploid genome. Your assay must be able to detect the transgene at 0.5 copies per genome, because transgenic founders are hemizygous.*

Calculating the amount of DNA to use

The haploid DNA content of the mouse is about 3×10^6 kbp. Thus, you can determine the dilution factor of your transgene into control mouse DNA to arrive at one copy per haploid genome by dividing the length of your transgene (in kb) by 3×10^6 .

$$\text{Amount of DNA to add} = \frac{\text{Length of your transgene (kbp)}}{3 \times 10^6} \times \text{DNA per assay}$$

Thus, if

Your transgene is 9 kbp in length

Your assay uses 10 μg of DNA

Then you would add 30 μg DNA for one copy-equivalent per haploid genome.

You would then set up your dilution series with the following amounts of transgene DNA per 10 μg wildtype mouse DNA:

Copy Number	Transgene DNA added (μg)
0.1	3
0.5	15
1	30
3	90
10	300
30	900

**Note*

Transgenes typically integrate as head-to-tail concatemers. If your assay is a Southern blot, then you can run the control dilution series with your samples and use the relative band intensity to estimate actual copy number.

You can also use the dilution series and a quantitative PCR assay for estimating copy number.