

Preparation of Copy Standards for PCR Genotyping

(This single-copy screening protocol is adopted from Transgenic Animal Model Core, University of Michigan: <http://www.med.umich.edu/tamc/spike.html>)

The purpose of spiking tail DNA with a known amount of transgene DNA is to produce copy standards. These are used to verify the sensitivity of your PCR genotyping assay and to establish transgene copy number in Southern blot analysis. We recommend that your PCR detect transgene DNA at the single copy level.

Calculation of Copy Number Standards

Assumption: the Haploid content of a mammalian genome is 3×10^9 bp
Assumption: you have 10 micrograms of DNA to spike

Since the transgenic founder mice are heterozygous:
$$\frac{\text{mass of transgene DNA}}{5 \text{ micrograms genomic DNA}} = \frac{N \text{ bp transgene DNA}}{3 \times 10^9 \text{ bp genomic DNA}}$$

Example: for a 2,740 bp transgene construct

$$\frac{\text{mass of transgene DNA}}{5 \text{ micrograms genomic DNA}} = \frac{2,740 \text{ bp cloned DNA}}{3 \times 10^9 \text{ bp genomic DNA}} \quad \text{or}$$
$$\text{mass of transgene DNA} = \frac{(2,740 \text{ bp cloned DNA}) \times (5 \mu\text{g genomic DNA})}{3 \times 10^9 \text{ bp genomic DNA}} \quad \text{or}$$

mass of transgene DNA = 4.567 picograms

Thus, to prepare a 1 copy standard: add 4.6 pg of transgene DNA to 10 micrograms tail DNA

10 copy	45.7 pg
50 copy	228 pg
100 copy	457 pg

For use as a PCR standard, use the single copy spiked DNA as a substrate to test the PCR assay you devised for genotyping transgenic mice.

For use in Southern blot analysis, digest the tail DNA as you would for Southern analysis, and add the transgene DNA just before you load your gel. For an example of copy standards in Southern blots, refer to Camper, SA. (1987) Research applications of transgenic mice. Biotechniques 5:638-650.