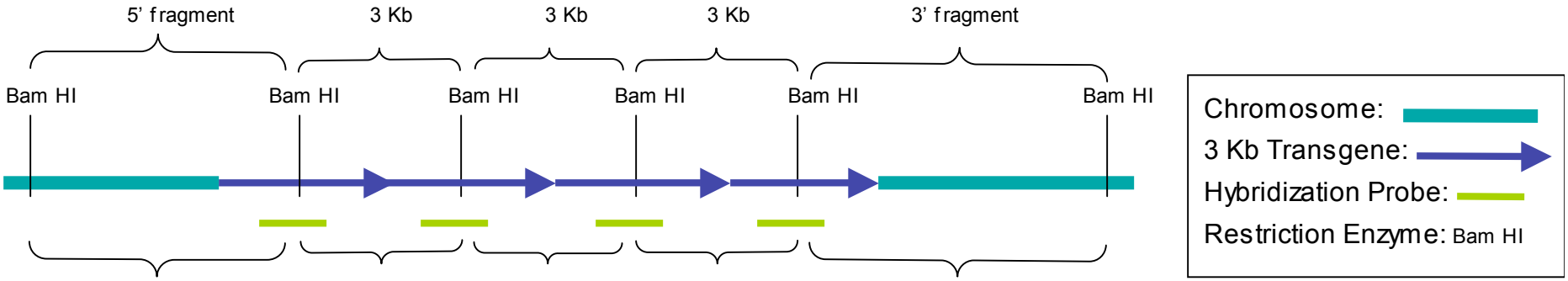
# Determining Transgene Copy Number and Number of Integration Sites in Transgenic Mice




## 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.

Thom Saunders. University of Michigan Transgenic Core. http://www.med.umich.edu/tamc

# Copy Standards For Southern Blot

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

Since the transgenic mice are hemizygous:

$$\frac{\text{mass of transgene DNA}}{\text{5 microgram genomic DNA}} = \frac{\text{N bp transgene DNA}}{\text{3 X 109 bp genomic DNA}}$$

Example: for a 5,480 bp transgene insert or plasmid

$$\frac{\text{mass of transgene DNA}}{\text{5 micrograms genomic DNA}} = \frac{\text{5,480 bp cloned DNA}}{\text{3 X 109 bp genomic DNA}} \quad \text{or}$$

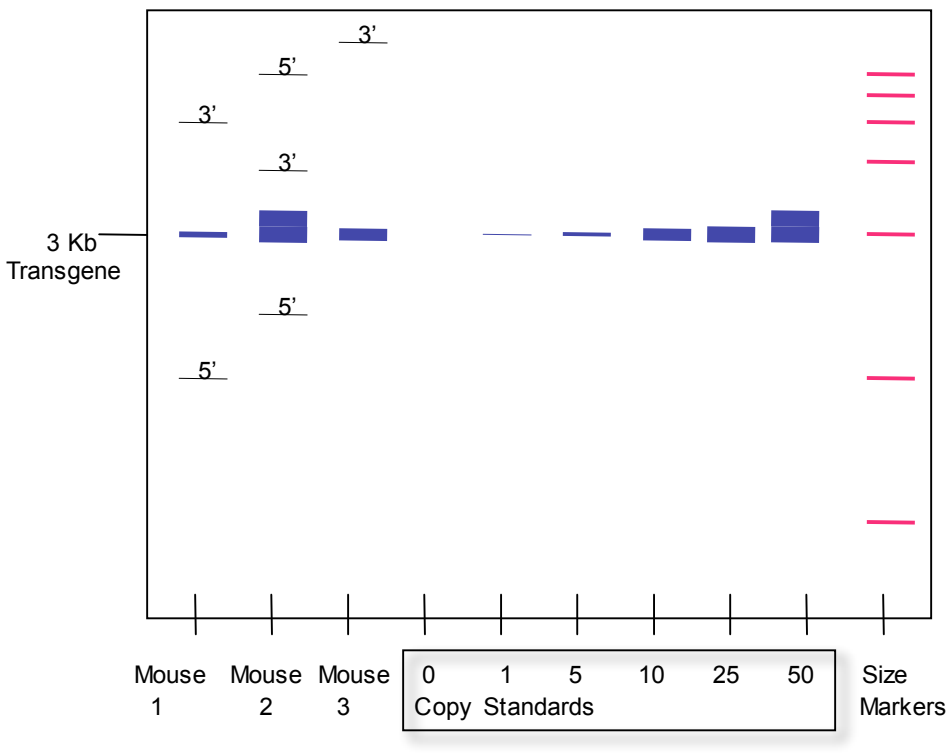
$$\text{mass of transgene DNA} = \frac{\text{(5,480 bp cloned DNA) X (5 } \mu\text{g genomic DNA)}}{\text{3 X 109 bp genomic DNA}} \quad \text{or}$$

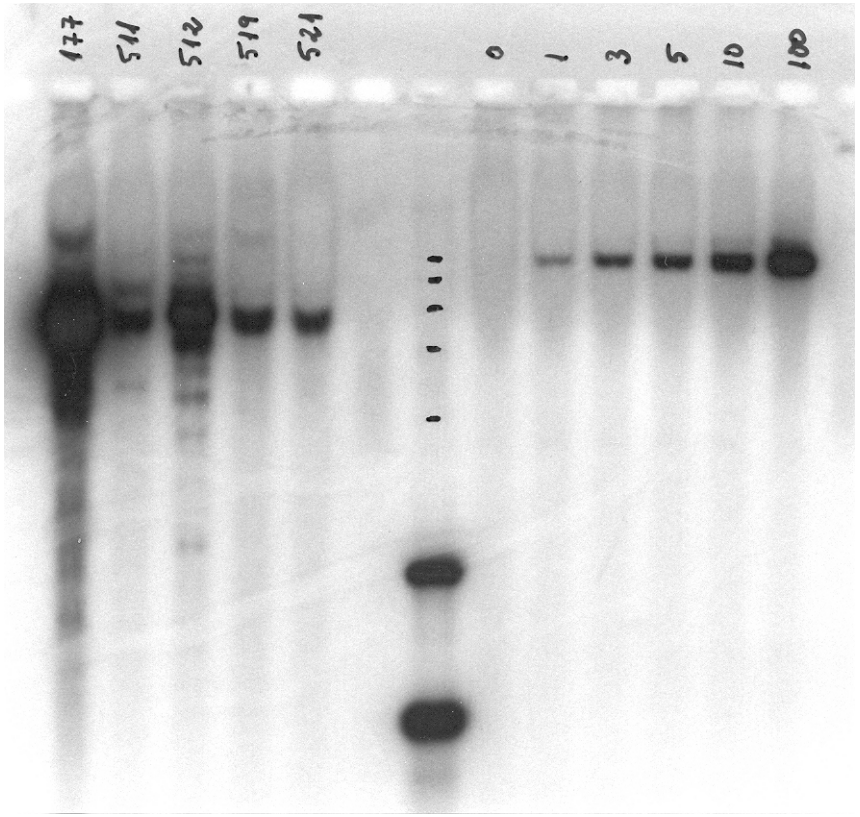
mass of transgene DNA = 9.15 picograms

Thus, for 1 copy: add 9.15 pg of transgene DNA to 10 micrograms tail DNA
5 copies 45.8 pg
10 copies 183.0 pg
25 copies 228.8 pg
50 copies 457.5 pg

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:
Camper SA. 1987. Research applications of transgenic mice. Biotechniques 5, 638–650.

# Interpretation of Southern Blot Data




Film image kindly provided by Ricardo Pardal

Note that Mouse 2 has a 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.

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