

How many do I need to do?

Background:

A researcher is trying to develop a strain of "transgenic" mice, by introducing an altered gene (transgene) into the genome. In order to breed true, the animals must be made to be homozygous, i.e. to have two copies of the introduced gene. Molecular biology techniques can detect whether the transgene is present in an individual animal (without having to sacrifice the animal), but cannot distinguish a hemizygote, with one copy of the gene, from a homozygote. This difference can only be detected by breeding strategies. Time and resources are pressing.

First generations:

A copy of the transgene is injected into the pronucleus of a newly fertilized ovum, prior to fusion with the male pronucleus. Thus all animals that develop from these zygotes can at most have one copy of the gene, from the ovum. After birth, screening is performed to detect these "positive" animals, called founders. After sexual maturation, all founders are bred to normal "wild type" animals, to ensure that the transgene has been incorporated in such a way as to be heritable. Pairs of positive (hemizygous) animals in this F1 generation are then bred to each other. By Mendelian genetics, the distribution of F2 offspring should be 1:2:1, homozygous transgenic : hemizygous transgenic : homozygous normal. The homozygous normal animals are not used. The question is, how to tell the homozygous transgenic mice (the desired endpoint) from the hemizygous?

F2 breeding:

All positive F2 animals are bred to wild type. Possible F3 genotypes are as follows: (by Mendelian genetics)

- Hemizygous (which comprise 2/3 of the F2 animals used) x wild type = 50:50, hemizygous : normal
- Homozygous (which comprise 1/3 of the F2 animals used) x wild type = all hemizygous

That is, while only half of the offspring from a Hemi x WT pair will be positive when screened, all of the offspring of a Homo x WT pair will be positive.

The question:

How many F3 offspring from a particular pairing does the researcher have to screen before declaring the positive parent as homozygous? Note: as soon as an offspring is screened as negative, one knows the parent must have been hemizygous. The point is check the least number of offspring and do as few repeated breedings as possible to detect homozygous animals.

(T) = transgene, (-) = normal

Founder breeding:		Founder	x	wild type	
		T-	x	--	
F1 generation:		T-	:	--	
		50	:	50	
F1 breeding: (positives only)		F1	x	F1	
		T-	x	T-	
F2 generation:	TT	:	T-	:	--
	1	:	2	:	1
F2 breeding: (positives only)		F2	x	wild type	
		(TT, T-)	x	--	
		1	:	2	
F3 generation:	Situation 1:	from TT	x	--	
				all T- (positive)	
	Situation 2:	from T-	x	--	
				half T- (positive)	
				half -- (negative)	

The question: How many consecutive positive offspring have to be screened to convince one that Situation 1 has occurred, and that the positive F2 parent is the desired homozygote TT?