JUSTIFICATION OF ANIMAL NUMBERS

The committee's primary requirement in this section is a description of how many animals are being used for each distinct portion of the protocol.

In all cases, the numbers used should reflect:

- Understandable experimental design
- Statistical adequacy relative to the measurements being taken
- Animal use based on experimental design and not on budget, personnel, or time constraints
- A good-faith effort to minimize the numbers of animals used

The following guidelines are to be applied

Experimental hypothesis testing
For studies with controls and other comparative groups, list all of the categories in a flow-chart fashion, describing the number of animals to be used in each for the entirety of the study during the three-year period (not year by year). The priority in this case is to demonstrate the study's experimental design and the breakdown of animal requirements within it. Statistical explanations for a chosen sample size should be kept brief, specifying which test is to be used. The committee is aware that values are chosen to optimize statistical power and only needs to know the projected animal numbers.

Example of hypothesis test using mice ordered for this purpose:

To test the effect of Drugs X and Y on motor responses, we need 8 mice per group (based on Smith et al., 1999).

Drug X: 8 mice; Drug X + blocker: 8 mice
Drug Y: 8 mice; Drug Y + blocker: 8 mice
Control: 8 mice

Total = 40 mice

Pilot studies and characterization
For pilot or exploratory studies, the Committee acknowledges that all such numbers are estimates, subject to change. The researcher should state the anticipated maxima for number of animals used per sample, which should correspond to those utilized in similar published studies or as advised by a consulting colleague; provide references. The priorities in this case are (a) to state a hard quantitative maximum and (b) to cite why observing a given variable (protein extraction, behavioral observation, etc) is expected to require a given number of animals.

Example of a pilot study using mice ordered for this purpose:

To characterize the role of Protein A on brain stem development, we anticipate that 9 mice per group will be sufficient to generate reliable tissue data (based on Jones 2001 and consultation with Dr. Wong).

Protein A, dosage 1: 9 normal mice at 3 ages = 27 mice
Protein A, dosage 2: 9 normal mice at 3 ages = 27 mice
Protein A, dosage 1: 9 knockout mice at 3 ages = 27 mice
Protein A, dosage 2: 9 knockout mice at 3 ages = 27 mice

Total = 108 mice

Breeding and self-sustaining colonies

If the proposal includes breeding females for purposes of generating phenotypes and/or sustaining a laboratory colony, the above guidelines apply and the following clarifications are also required.

1. Whether pups born will serve as breeding individuals for colony maintenance
   - The anticipated number of pups and the size of the colony must be included
2. Whether pups (P_0-P_{21}) are utilized as sources of data
   - If all are data sources, then estimated numbers of pups on hand and the total anticipated must be included
   - If only a subset of pups born are utilized as data sources, then the calculation of experimental pups from those to be culled must be explained, and the estimated numbers of pups to be utilized must be included

In Section 13, both estimated numbers of pups born and numbers of pups utilized (including maturation and further use or breeding) should be included. In all cases, the total number of animals described in Section 13 should accord with the tables in Section 4. A discrepancy at this level constitutes grounds for the committee to require a revision.

The committee is aware that all such numbers are estimates and recommends higher estimates rather than lower to provide for unanticipated adjustments during the study.

Example: hypothesis test including breeding

We are generating 2 different DNA constructs. We will obtain approximately 3 transgenic lines per construct, for a total of 6 transgenic lines. We need at least 2 breeding pairs of the XYZ transgenics for each line, for a total of 12 breeding pairs (24 mice). They will generate litters of approximately 8 mice, for a total of 96-100 mice per line.

Approximately 25% of the pups produced will be be positive for the transgene. 12 of the positive transgenic animals and 12 nontransgene mice will be used for behavioral studies (see below). 25 of the nonpositive animals will be euthanized. The rest of the animals (50) will be maintained throughout years 2-3 of the study for breeding. The breeding colony will be maintained at 100 mice per year.

In order to test the effects of gene Q expression on grooming behavior, the following numbers of mice are required:

Transgenic line	*Positive*	*Negative (control)*
1	*12*	*12*
2	*12*	*12*
3	*12*	*12*
4	*12*	*12*
5	*12*	*12*
6	*12*	*12*

Total = 144

Example: pilot/characterization including breeding

Estimated numbers of chimeric mice required for muscle regeneration experiments:

Mouse strain	*# experiments*	*# mice per expt*	*subtotal*
A	*6*	*10*	*60*
F1 (B x C)	*6*	*10*	*60*

F1 (D x E)	*6*	*10*	*60*

Estimated numbers of mice required as recipients or donors for stem cell transplantation

Mouse strain	*# experiments*	*# mice per expt*	*subtotal*
F1 (D x E)	*6*	*10*	*60*
F (recipient)	*6*	*10*	*60*

To obtain enough mice for the above experiments and for maintenance of mouse colonies, the following numbers are required:

Mouse strain	*# mating*	*# pups per mating*	*subtotal*	*cf # in Table, p. 2*
A and G (backcross)	*12*	*6*	*72*	*#1: 72*
B and G (backcross)	*6*	*6*	*36*	*#2: 36*
C and G (backcross)	*6*	*6*	*36*	
F1 (B and C)	*12*	*6*	*72*	*#3: 36 + 72 = 108*
D and G (backcross)	*6*	*6*	*36*	*#4: 36*
E	*12*	*6*	*72*	
F1 (D x E)	*18*	*6*	*108*	*#5: 72 + 108 = 180*
F	*12*	*6*	*72*	*#6: 72*

Total = 504