CALCULATING ANIMAL NUMBERS

Below are some examples to assist in calculating your animal numbers. You will find examples for: animals used for tissue harvest, animals used for experiments, animals used for breeding colonies:

A. ANIMAL FOR TISSUE HARVEST:

   a. Example I

   30 fetuses requires the use of 4 pregnant rats
   4 pregnant rats X 2 time points = 8 pregnant rats

   For Specific Aim 2, expression of 4 genes will be examined by the use of Northern blot. RNA extracted from pooled brain tissues from 60 fetuses each at 14 and 18 days of gestation will be examined to determine when the 4 genes are expressed. Expression of all 4 genes can be examined on one Northern blot, however we need to be able to repeat this experiment once to verify any unexpected results.

   60 fetuses requires the use of 8 pregnant rats
   8 pregnant rats X 2 time points = 16 pregnant rats - repeated one time = 32 pregnant rats

   Total for protocol = 40 pregnant rats per year

   b. Example II

   hsc70 cytosol: 3 rats/prep activity loss 1 of 10
   hsc70 lysosomal: 5 rats/prep activity loss 1 of 10
   LAMP-2A: 6 rats/prep activity loss 1 of 20
   LAMP-2B: 6 rats/prep activity loss 1 of 20
   LAMP-2C: 6 rats/prep activity loss 1 of 20
   cathepsin A: 6 rats/prep activity loss 1 of 20
   metalloprotease: 4 rats/prep activity loss 1 of 10

   Total per assay = 36 rats  Total loss in 10 preps = (3+5+3+3+3+3+4) = 24 per year
B. FOR EXPERIMENTS:

   a. Example I

Identification new components (proteomics):    16 proteins x 5 conditions x 6 experiments  =  480 rats
Isolation of new components:                   8 proteins x 3 conditions x 6 experiments  =  144 rats
Age-related studies:                          5 conditions (3 age/fed-restricted) x 8 exp  =  40 rats
                                              TOTAL = 663 rats
                                              Half-project* = 332 rats

* We have already carried out half of this project so we only include in this protocol half of the animals originally requested

   b. Example II

   6 experiments x 3 mice/experiment x 4 groups = 72
   8 experiments x 3 mice/experiment x 3 groups = 72

   camKII-Tet L2A:  6 experiments x 3 mice/experiment x 4 groups = 72
                    8 experiments x 3 mice/experiment x 3 groups = 72
                    8 experiments x 5 mice/experiment x 2 groups = 80

   rTA-Tet L2A:     6 experiments x 3 mice/experiment x 4 groups = 72
                    8 experiments x 3 mice/experiment x 3 groups = 72
                    8 experiments x 5 mice/experiment x 2 groups = 80

   TOTAL          592 mice
C. FOR TRANSGENIC COLONY: BREEDING COLONY MAINTENANCE

We need to make two different transgenics, with three to five founder lines from each construct (up to 10 mice). We will purchase 10 “mates” for the founder animals. Each founder will be bred for production of experimental mice. We need 6 mice of each transgenic line per experimental group to check the level of gene expression at each of 6 time points (3, 6, 9, 12, 15, 18 months). Since only 50% of the offspring will carry the transgene, 12 offspring must be produced per line for each time point to get 6 transgenic mice per line per time point. We will also purchase non-transgenic controls at matched ages from Jackson labs to avoid the need to raise these controls ourselves. Mice without the correct genotype will be humanely killed after genotyping results are complete. Therefore the total number of mice needed will be:

- 2 transgenic lines X 5 founders each = 10 founders
- 10 “mates” for founders purchased from Jackson labs
- 2 transgenic lines X 12 mice / group (6 transgenic, 6 non-transgenic) X 6 time points = 144 mice
- Controls for 2 transgenic lines X 6 mice / time point X 6 time points = 72 controls
- Total request = 10 + 10 + 144 + 72 = 236 mice

(In this example, the animals purchased for production of founders (donor females, recipient females, vasectomized males) have been approved in the core protocols for the transgenic and gene targeting core facilities.)