

Recommendations for Maintaining Transgenic Mouse Stocks

A. Cage Cards

Sample cage card and mating cage cards are shown on pages 7 and 9, respectively. If you wish to use our cage card system, we can provide the graphic file to make your own cage cards or a printout of the file so that Biocommunications can make cage cards for you using our graphics. The graphic file is a Macintosh Canvas document designed to be used with Avery Index cards that can be purchased from office stores. The spacing may need to be modified slightly to accommodate different printers. If you have a PC, we can provide this file in a Pict or Tiff format. This form is also available on our web site.

B. Mating Cages

1. A mating cage normally consists of 1 male and 1 female. It is possible to have 1 male and 2 females in a cage. The disadvantage of this type of mating is that pups often are not born at the same time; thus, it is possible to have old pups with young pups, with the young pups getting trampled by the older pups.
2. Food: We recommend a high fat diet (Purina 5021) for the breeding cages.
3. Light/dark cycle: We recommend that the mice be maintained on 12 hr on/12 hr off light cycle.
4. Mating cages should always contain nesting material.
5. When working in mouse rooms with matings, it is important to be as quiet as possible. Excess noise can have an adverse effect on breeding.
6. To maintain a transgenic line, 2-3 mating cages should be set up at any given time. If the mice are also needed for experiments, then more mating cages should be set up. If mice do not breed well or if the mice become sick at an early age, then more mating cages may be necessary.
7. When setting up matings, weaning mice from mating cages, or handling mice in different mating cages, you should change gloves in between cages (Reason: The scent on your gloves from different males/females can adversely affect breeding).
8. When a litter is born, the mating cage should not be opened for 2-3 days after birth (Reason: Disturbing the mating cage may result in the loss of the litter). The first litter of a breeding cage often may not survive because the mother may cannibalize the litter.
9. Caretakers of your room should be instructed: (1) to avoid changing mating cages with new litters for 2-3 days, (2) to change gloves in between cage changes or to use tongs to change cages (tongs are dipped in disinfectant between cages), and (3) to transfer some nesting material from the old cage to the new cage when changing cages.
10. The founder transgenic mice are on a C57BL/6 & C3H/He F2 genetic background (unless you requested transgenic mouse production on a different genetic background) and are called generation N0. Since genetic variation may affect your experimental results, you should backcross your founders to one of these inbred strains. The offspring of the first backcross are called N1, the 2nd N2, etc. Each successive backcross generation should result in less genetic variation. For the first several generations, we recommend

that you backcross offspring of each generation once they have achieved sexual maturity (5-6 wks of age) to minimize the possibility that different genetic backgrounds may affect your results. When setting up the next generation of mating, it is safer to wait until the new mating cages have produced litters before sacrificing the old mating cages.

11. Once the mouse line has been backcrossed for several generations, you may want to leave the mating cage set up for several months. You should monitor the mating cage to determine if it is productive. Reproductive performance declines in females between 6- 8 months of age and in males after 1 year of age (although males can successfully breed up to 1.5 years of age). When the mating cage becomes older or unproductive, you should set up new matings. As stated previously, it is safer to wait until the new matings produce transgenic offspring before sacrificing the old matings.

12. To maintain a line, you should keep 2 generations of offspring at any given time (3-4 cages) on the shelf. These mice will then be readily available whenever you need to set up new matings.

C. Setting up matings

1. If brother/sister matings are being set up, offspring should have been separated from each other for 2 weeks.
2. Mice should be at least 5 weeks of age before they are put into mating cages.
3. Always put the female into the cage of the male and never put the male in the cage of the female (Reason: If the male is put in the cage of the female, she becomes territorial).
4. Accurate records must be kept for all matings and offspring of matings (Samples records are shown on pages 5 and 8). A sample of a mating cage card is shown on page 9. Accurate record keeping is also an AALAC and NIH requirement. We recommend that you maintain a Mating Book and Pedigree book. It is important to maintain a “paper” trail should you ever need to check the history of a line (especially to check for mistakes). The mating should be recorded in the mating book. You should record on the cage card from which you take the mouse that it was transferred to a mating cage. You should also record in the Pedigree book that the mouse was transferred to a mating cage.
5. Designate that the cage is to be maintained on a high fat diet (we put small cards on the cage that says “5021 diet”).
6. When we have multiple mating cages for multiple lines, we arrange the cages by line, in the order in which the mating cages are set up (from oldest to youngest).

D. Weaning offspring

1. We wean mice at 21 days of age. Mice can become sexually mature (and active!) by 28 days of age.
2. Adult mice should not be housed more than 5 mice/cage.
3. Accurate records should be kept in the Pedigree book (see page 5). The number of offspring should be recorded on the mating cage card (see page 9).
3. We tag the mice by making ear punches as illustrated on page 4. Other types of ear punch schemes can be used to identify mice. The tails of the mice are clipped using a pair

of scissors and then cauterized. The tail is then placed in a microfuge tube (labeled with the animal #), and then the tube is placed on ice. Tail DNAs can be stored in a -20°C freezer for a few days, but should be stored at -80°C if you are not going to digest the tails with proteinase K within a few days.

4. We maintain our offspring on the diet used by the ARC (7021). We put a little of the 5021 diet inside of the cage to ease the transition.

E. Miscellaneous Information

1. If you anticipate that your transgenic mice may become sick, you should frequently monitor your mice for signs of sickness. You should also consider asking the Vet techs that examine your mice to inform you if any of your mice are sick. If you would like pathology on the mice, you should sacrifice the mice prior to their death.

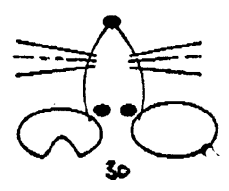
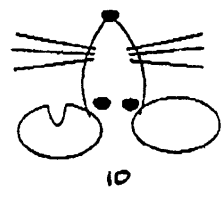
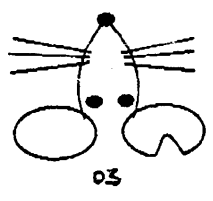
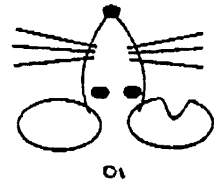
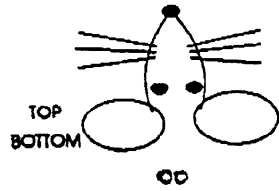
2. PIs are required by ARC to inform them about the number of mice that are being generated by breeding. Your ACUC protocol has been approved for a specific number of mice for a specific stress level. You are required to inform the ARC the number of mice that are used for each stress level.

F. Electronic Database for mating/pedigree records

We are using a program called Mendel's Lab for maintaining our mating and pedigree records electronically. If you are interested in obtaining more information about this program, please contact Jeffrey Ceci or Mendel's Software, 24 Andrews Lane, Princeton, NJ 08540 (FAX 609-924-4382). This program requires licensing from Mendel's Software.

G. Cryopreservation: The Transgenic Mouse Core Facility provides embryo and sperm cryopreservation services if (1) you would like to protect your transgenic mice against accidental loss, and/or (2) you would like to discontinue maintenance of your stocks.

Ear Clip Numbers



Sample pedigree records:

Animal	Sex	Genotype	Mating Cage /Litter	Gener-ation	Birth Date	Line/Parents	Mating cage #	Mating date	dissipation	dissipation date
T501	M	tg/+	8-4	N6	5/14/97	114 Lck-1A Tpl.trunc/			sick-sac	7/14/97
T502	M	tg/+	8-4	N6	5/14/97	1579 tg/+, B6 +/+			sac	7/14/97
T503	M	tg/+	8-4	N6	5/14/97		MC123	7/14/97		
T504	M	+/+	8-4	N6	5/14/97				sac	7/17/97
T505	F	tg/+	8-4	N6	5/14/97					
T506	F	+/+	8-4	N6	5/14/97					
T507	F	tg/+	8-4	N6	5/14/97					
T508	F	tg/+	8-4	N6	5/14/97					
T509	M	tg/+	10-2	N6	5/14/97	61 Lck-1A Tpl.trunc			sac-tissues taken	8/12/97
T510	M	tg/+	10-2	N6	5/14/97	B6 +/+, 1562 tg/+			sac-tissues taken	8/12/97
T511	M	tg/+	10-2	N6	5/14/97				sac-tissues taken	8/12/97
T512	M	tg/+	10-2	N6	5/14/97				sac-tissues taken	8/12/97
T513	M	tg/+	10-2	N6	5/14/97				found dead	6/5/97
T514	F	+/+	10-2	N6	5/14/97				found dead	6/10/97
T515	F	+/+	10-2	N6	5/14/97				sac	6/10/97
T516	F	+/+	10-2	N6	5/14/97				sac	6/10/97
T517	F	+/+	10-2	N6	5/14/97				sac	6/10/97
T518	F	+/+	10-2	N6	5/14/97				sac	6/10/97
T519	F	+/+	10-2	N6	5/14/97				sac	6/10/97
T520	M	tg/+	9-3	N6	5/14/97	61 Lck-1A Tpl.trunc	MC150	7/29/97		
T521	M	tg/+	9-3	N6	5/14/97	B6 +/+, 1561 tg/+			sac	7/2/97

We maintain a bound notebook devoted exclusively to maintaining pedigree records.

Place the animal #, sex, genotype, generation and birth date of the mouse in the appropriate columns.

Genotype: +/+ for wild-type, tg/+ for heterozygous for the transgene, tg/tg for homozygous for the transgene, KO/+ for heterozygous for the knockout allele, and KO/KO for homozygous for the knockout allele.

Generation: Backcrosses are indicated by N1 (1st backcross), N2 (2nd backcross), etc.

Intercrosses (brother/sister matings) are indicated by F1 (1st intercross), F2 (2nd intercross), etc.

MC/litter: MC refers to the mating cage that the offspring came from. Litter refers to the litter number, ie. the first litter born in the cage is litter 1, the 2nd litter 2, etc.

Line: refers to the transgenic line with the construct name. Parents (with genotype): By convention the female parent is listed first, the male parent listed second.

Mating cage # refers to the mating cage in which these offspring are later placed, and mating date refers the date that the mating is set up.

Dissipation and Dissipation date refer to the manner in which the mouse dies (i.e. sacrificed, dies due to sickness, etc.) and the date that the mouse dies.

The information listed above is the minimum amount of information that should be kept in the pedigree book. Additional information can be added to meet your individual needs.

Refer to sample cage cards (page 7) to see how we maintain the cage cards. We **NEVER** discard the cage cards in case we need to refer to them at a later date.

Sample Pedigree Cage Cards

CAGE/LITTER		GENER	BIRTH	♀	1579 tg/+
8 - 4		N6	5/14/97	♂	B6 +/-
114 Lck-1A Tpl.trunc ST.	N				
	1	M	T501 "00" tg/+ sick sac 7/14/97		
	2		T502 "01" tg/+ sac 7/14/97		
	3		T503 "03" tg/+ MC123 7/14/97		
	4	↓	T504 "10" +/- sac 7/17/97		
	5				
	6				
	7				
	8				
	9				
	10				
	11				
12					

"00" refers to ear punch #.

CAGE/LITTER		GENER	BIRTH	♀	1579 tg/+
8 - 4		N6	5/14/97	♂	B6 +/-
114 Lck-1A Tpl.trunc ST.	N				
	1	F	T505 "00" tg/+		
	2		T506 "01" +/-		
	3		T507 "03" tg/+		
	4	↓	T508 "10" tg/+		
	5				
	6				
	7				
	8				
	9				
	10				
	11				
12					

The construct name along with the founder line are placed in the St. (Strain) space.

Cage refers to the mating cage # and litter # from which the mice were born.

The generation of the mice and the birth date are recorded in the appropriate boxes.

The male and female parents are listed in the upper right-hand corner.

The sex of the mice, the animal #, and ear punch identification # are listed in the center of the cage card.

When the genotype of the mice has been determined, it is recorded on the cage card and in the pedigree book.

When animals are sacrificed, used in experiments, or placed in mating cages, this information is recorded on the cage card, along with the date (as shown with the male mice).

Sample Mating records (for parents of offspring shown in pedigree records).

Mating cage # Set up date	Transgenic Female parent, birth date Parents of female parent (include genotypes of mice)	Line Male parent, birth date Parents of male parent (include genotypes of mice)	Generation
MC8 2/13/97	1579 tg/+, 1/1/97 827 tg/+, B6 +/+	TG114 Lck-1A Tpl.trunc B6 +/+, 12/15/96 B6 +/+, B6 +/+	N6
MC9 3/13/97	B6 +/+, 12/20/96 B6 +/+, B6 +/+	TG61 Lck-1A Tpl.trunc 1561 tg/+, 12/9/96 845 tg/+, B6 +/+	N6
MC10 3/15/97	B6 +/+, 12/20/96 B6 +/+, B6 +/+	TG61 Lck-1A Tpl.trunc 1562 tg/+, 12/9/96 845 tg/+, B6 +/+	N6

Refer to sample mating cage cards on the next page.

Supplies needed for tail clipping

(1) cauterizer: Roboz, catalog # RS209

(2) ear punch: Fisher 01-337B

(3) scissors, Roboz, catalog # RS-5882 (This is what we use) or
Fisher: 08-951-20

(4) long forceps: Fisher 10-316C

(5) Cage cards can be printed by Biocommunications (we have the templates) or printed
using laser index cards (from Pict files)(these are more expensive).

Roboz Surgical Instrument Company, Inc.
9210 Corporate Bld., Suite 220
Rockville, MD 20850
1-800-424-2984