SOUTHWESTERN

THE UNIVERSITY OF TEXAS
SOUTHWESTERN MEDICAL CENTER
AT DALLAS

TRANSGENIC TECHNOLOGY CENTER

Obtaining Germ Line Transmission of Targeted Alleles from SM-1 (129Sv/Ev S6) ES Cells

10/25/10

1. Great news - Your chimeras have been sent to your animal room and they look lovely. You have received an email from the staff of the TTC that identifies the number of chimeras per clone and the approximate % chimerism of each of the animals that has been reassigned to your colony. This information is important and is an indication of the quality of the experiment.

2. Take a look at the distribution of male chimeras between the 2 to 3 clones of ES cells that were injected. The range of chimerism will be from ~50 to >90 % as evidenced by the extent of agouti coat color on the black background. The more agouti the mouse is, the more extensive the contribution of the injected ES cells to the formation of the animal.

3. While a high degree of chimerism is certainly reflective of a high contribution of ES cells to the body, it is not necessarily reflective of the degree of ES cell contribution to the germ line. This observation is important, and it will impact your strategy for propagating the targeted allele through the germ line.

4. It is difficult to predict which of the chimeric males will transfer the mutant allele through the germ line first so you will need to implement an effective, efficient breeding strategy to maximize your efforts.

5. In general, we set up chimeric males ranging in % chimerism from 60 to >90 % to breed with 2 young, ~4 to 5 week old females. The chimeric males will reach sexual maturity by approximately 8 weeks of age, so please be aware that the males you received from the Core are not likely to be sexually mature when they arrive. Be patient and don't set them up to breed before they are 8 weeks old.

6. If you received a large number of chimeric males from the Core, this is a good problem to have. No, you don't have to breed every male. The general guideline is to breed animals with a range of chimerism. Don't breed just the highest % chimeras. Some of these will be infertile and some will exhibit delayed sexual maturation. Again, be patient. We generally set up between 10 to 12 males to breed with WT B6 females. Be mindful of the colony! Visit it at least twice a week during the early stages of mating and once a day when the litters are due to observe near term females.

7. In general, the B6 females will not lactate effectively during their first pregnancy and as a consequence many will lose their first litters from lack of adequate milk. You can try to foster weak pups that remain from a partially lost litter but the process can be difficult without a sizeable foster colony. In general, we simply allow the multi-gravid females to mate during the post-partum estrous period and usually the second litters will survive.

8. If the chimeric males do not mate within the first few weeks after being set up there is no need to panic. The lack of mating does not necessarily mean the males are infertile. You can simply leave the females with the males for a few more weeks or check for copulatory plugs to verify that the males are breeding.

9. If the males have not mated within ~ 6 weeks, it is time to place 2 new females in the cage and document the change on the cage card. Monitor the status of the females and mark the cage if a pregnancy is noted. It is possible that she will lose her first litter but the good news is that you have verified that the male is fertile. Carry on!

10. If the first litter does not produce agouti offspring again, do not panic. In many chimeric males, the 129 sperm will mature more slowly that the B6 sperm and thus the first or second litters may not contain any or only have a few agouti offspring. Again, not to worry. It happens!

11. If a given chimeric male has transmitted the agouti locus through the germ line but not the mutant allele, verify your screening strategy and keep breeding! Unless the mutant allele has perturbed germ cell maturation, you should obtain germ line transmission.

12. It is certainly possible that the mutant allele will not be propagated through the germ line of a set of chimeric males. While this is an infrequent occurrence, it does happen. If none of the males from any of the clones transmits the mutant allele through the germ line, please contact the staff of the TTC to discuss alternative experimental strategies.

Frequently asked questions:

1. *What do I do with the chimeric females that the core sent me? Are they worth keeping for breeding?*

   Yes, you can keep some for breeding. There is always the remote possibility that a particular es cell clone will lose the Y chromosome and therefore females that arise from this clone will be XO. We generally either mate a small cadre of females or use a few for breeding.

2. *Should I keep the chimeric males once they have transmitted the mutant allele?*

   Keep them if you intend to cross them to the 129Sv/Ev- S6 strain to maintain them in an inbred 129 background. If you have no intention of doing this and the genotype of the heterozygous offspring is definitive, sacrifice the chimeras and save cage costs!

3. *If none of the chimeric males I set up to breed transmit the agouti allele, should I breed the other males?*

   Yes, you should mate the other males and you should continue to breed the males that have not transmitted the agouti locus. We generally stop breeding a male after he has had ~ 30 to 40 offspring.

4. *How many lines harboring the mutant allele do I need?*

   You only need one if all is “in order” with the mutant allele. Be sure that the targeting is correct in the line you intend to keep and recombination occurs before you sacrifice the other lines!

5. *What is the background of the WT blastocysts that were used to create the chimeras?*

   The targeted es cells are injected into blastocysts obtained from C57Bl/6N mice obtained from Charles River.

6. *If I want to establish an inbred 129 strain harboring this allele, what parental 129 strain should I purchase?*

   The strain is 129Sv/Ev:Tac or 129S6 and it can be obtained from Taconic Farms.