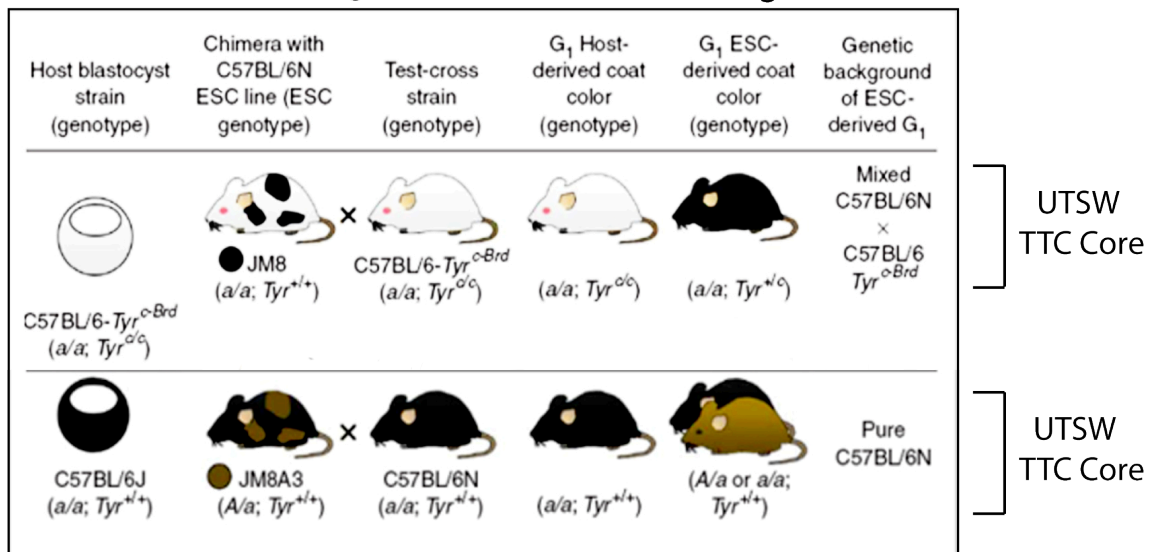


**TRANSGENIC TECHNOLOGY
CENTER**



10/22/10

Guide to microinjection, chimera breeding and germline transmission testing of KOMP JM8 and JM8A₃ ES cells



Coat color of chimeric mice and their offspring from injections of JM8 and JM8A₃ cells into blastocysts from several common mouse strains.

Agouti C₅₇BL/6N embryonic stem cells for mouse genetic resources.
S. J. Pettitt, Q. Liang, X. Y. Raidan, J. L. Moran, H. M. Prosser, D. R. Beier,
K. C. Lloyd, A. Bradley and W. C. Skarnes. Nature Methods **6**: 493-495 (2009)

1. The chimeric mice from the injection of the mouse embryonic ES cells you purchased from KOMP have been produced and are ready to be shipped to your colony.
2. You should have received an e-mail from the staff of the TTC that identifies the number of chimeric animals that were produced per clone and the

approximate % chimerism of each of the animals that was reassigned to your colony. This information is important and is an indication of the quality of the experiment.

3. Observe the distribution of the male chimeras between the clones that were injected. The range of chimerism will likely be from ~30 % to as high as 90% and will be evident by the extent of either agouti coat color (JM8A3 ES cells) on a black background or black coat color (JM8 ES cells) on an albino background.
4. While the degree of chimerism is a reflection of the contribution of the injected ES cells to the body, it is not necessarily reflective of the degree of ES cell contribution to the germ line. This is important and will impact your strategy for propagating the targeted allele through the germ line.
5. It is difficult to predict with certainty which of the chimeric males will transfer the mutant allele through the germ line first, so an efficient, effective breeding strategy to maximize your efforts will need to be implemented.
6. In general, with chimeras originating from KOMP clones we breed each of the chimeric males 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 going to be sexually mature when they arrive in your colony. Be patient!
7. If you received chimeras that have patches of black coat color on a white background, then the KOMP ES cells that were injected are from a B6 background and were injected into albino B6 blastocysts (1st example in the guide box). This means that you must mate the chimeric males to albino B6 females (available from the Jackson lab or Harlan). The progeny generated from such matings will be either albino or black in coat color. The progeny that are black in coat color are from germ cells that are ES cell derived and the albino progeny are from WT germ cells. Please keep the black progeny and genotype these. They will either harbor the mutant or wt allele from the ES cells. The female albino progeny can be discarded or used for future matings.
8. If you received chimeras that have patches of agouti coat color on a black background, then the KOMP ES cells that were injected are from a B6 parental strain of ES cells that have had the agouti locus corrected (heterozygous correction) by gene targeting and they were injected into B6 blastocysts (2nd example in the guide box). This means that you must mate the chimeric males to C57Bl/6 females. The progeny from such matings will be either agouti or black in coat color. The progeny that are agouti in coat color are from germ cells that are ES cell derived and the black progeny are from either ES cell

derived germ cells or WT germ cells. This means that you **must** genotype both the agouti and black coat color progeny for the mutant allele.

9. In general, the albino B6 and B6 females will not lactate effectively during their first pregnancies and as a consequence many will lose their first litter due to a lack of milk. You can try to foster weak pups that remain from a partially lost litter, but the effectiveness will be poor without a sizeable foster colony. In general, we simply allow the multi-gravid females to mate during the 1st post-partum estrous period and usually the second litters will survive.
10. If the chimeric males do not mate within the first few weeks after being bred, there is no need to panic. The lack of mating does not mean that the males are infertile. You can simply leave the females with the males for a couple of weeks or check for copulatory plugs to verify that the males are breeding.
11. If the males have not mated within 6 to 8 weeks, it is time to place two 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 they will lose their first litters but the good news is that you have verified that the male is fertile. Carry on!