

Germline testing of KOMP C57BL/6N male chimeric mice

October 4, 2007

ES cell genetic background:

KOMP-CSD: The JM8 (parental) ES cell line was isolated from the inbred C57BL/6N mouse strain. Injection of these cells into albino (Tyr^c/Tyr^c) C57BL/6 blastocysts will produce black and white chimeras.

KOMP-Regeneron: The VGB6 ES cell line was isolated from the inbred C57BL/6N mouse strain. Injection of these cells into albino (Tyr^c/Tyr^c) C57BL/6 blastocysts will produce black and white chimeras.

Chimera genetic background:

1. The chimera's donor blastocyst heritage is a/a (black) on the "Agouti gene" (e.g. AlbinoB6, FVB, CD1)
2. The chimera's ES cell heritage is a/a (black) on the "Agouti gene" and has no Tyr^c (non-albino) on the "Color gene" (e.g. AlbinoB6)
3. The female breeding with the chimera is a/a (black) on the "Agouti gene" and Tyr^c/Tyr^c (albino) on the "Color gene" (e.g. AlbinoB6)

Breeding chimeras with Albino B6 females:

1. Set highest-percentage (>70%) male chimeras to breed with 2 albino B6 females each at 6 weeks of age.
2. If no plugs are detected, and no pregnancies are observed for a period of 2 weeks, "rest" chimeras for 2-3 days before again breeding with new females. Repeat plug checking for a minimum of 2 weeks.
3. If still no plugs are detected and no pregnancies are observed, either select additional chimeras for breeding or consider alternatives to natural mating (e.g., IVF, ICSI).
4. If plugs and/or pregnancies are observed, do not disturb mice until birth.
5. If plugs are detected but no pregnancy ensues, repeat beginning at step 2. If still no pregnancy after plug, chimera will be noted as likely sterile.

Confirm germline transmission by genotyping:

1. When pup coat colors are evident, remove and sacrifice white pups to promote survival of black pups.
2. At ten days of age, harvest tissue (e.g., tail) samples from black pups for genotyping.
3. Remove and sacrifice non-germline pups to promote survival of germline positive black pups.
4. Wean germline-positve black pups at 3-4 weeks of age.
5. Any combination of breeding between germline chimerae and positive offspring can be used to achieve the necessary quantity, genotypes, and genders of animals for experiments.