Germline Testing Protocol for KOMP C57BL/6N (non-agouti) derived ES cells (JM8, JM8.F6, JM8.N4, VGB6)

Rev. 6/15/10

Strain Background:

1. The chimera's donor blastocyst is BALB/c and the heritage is Tyr^c/Tyr^c (albino) on the 'Color gene' and A/A (agouti) on the 'Agouti gene'
2. The chimera's ES cell is created from C57BL/6N and the heritage is a/a (black) on the "Agouti gene" and has no *Tyr*^{c} (non-albino) on the "Color gene"
3. The female (C57BL/6N) breeding with the chimera is *a/a* (black) on the "Agouti gene" and no *Tyr*^{c} on the "Color gene"
4. Chimeras derived from injection of black (a/a) ES cells into the BALB/c host embryo will have black, agouti, and white coat color. % chimerism is determined by the % of black and agouti hair on the mouse.[1]
5. These ES cells are XY and you will want to breed the male chimera. On rare occasion we encounter cells that are XØ, meaning they have lost the Y chromosome. We will notify you if this is the case for the clones or chimeras you receive. If cells are XØ you will breed the female chimera to C57BL/6N males.

Breeding chimeras with C57BL/6N females:

1. Set chimeras (6 wks of age), no more than on male per breeding cage, to breed with C57BL/6N females (6-8 wks of age), no more than 3 females per cage.
2. If there is no sign of plugs by 7 days, or pregnancy at 10 – 14 days if you do not perform plug checks, remove females and give males 2-3 days rest and then replace with new females. Repeat 3 times before retiring chimera, and/or consider an artificial reproductive technique (ART) for testing chimera.
3. If plugs or pregnancies are observed, leave one of the pregnant females in with male (or both if your vivarium allows it), await the birth, and allow for re-mating.
4. If plugs are detected but no pregnancy ensues, repeat step 1.

Germline testing:

1. When pup coat colors are evident, agouti pups should be removed as deemed necessary by the colony technician to ensure survival of black pups.
2. At ten days of age, number pups (toe clip, ear tag, etc) and take tissue samples (tail snips, ear punch, etc) from black pups and submit for genotyping analysis (PCR, Southern, etc).
3. Use germline-positive mice to establish breeding colony.

[1] *Pettitt et al.*: Agouti C57BL/6N embryonic stem cells for mouse genetic resources. Nature Methods, 2009. See Figure 2.