MMRRC @ UCDavis
Mutant Mouse Resource & Research Centers supported by NIH

Germline Testing Protocol for C57BL/6N (agouti) derived ES cells
JM8A3, JM8A3.N1, JM8A1.N3 (C57BL/6 host)
Revised 06/03/16

Strain Background:

1. The chimera's donor blastocyst C57BL/6 and the heritage is a/a (black) on the "Agouti gene" and has no *Tyr*^{c} (non-albino) on the "Color gene".
2. The chimera's ES cell is created from C57BL/6N with a repair at the non-agouti locus by gene targeting, and the heritage is A/a (agouti) on the 'Agouti gene', and has no *Tyr*^{c} (non-albino) on the "Color gene"[1]
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. Chimera derived from injection of the JM8 sub-line agouti (A/a) ES cells into a black host embryo (a/a) will be agouti and black. **% chimerism is based on the % of agouti coat color on the mouse.**
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 (6-8 wks of age), no more than one male per breeding cage, to breed with C57BL/6N females (6-8 wks of age), and no more than 3 females per cage.
2. If there is no sign of plug at 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 (or both if your vivarium allows it) of the pregnant females in with male, await the birth, and allow for re-mating.
4. If plugs are detected but no pregnancy ensues, repeat step 1.

Germline testing:

1. At ten days of age, number pups (toe clip, ear tag, etc) and **take tissue samples (tail snips, ear punch, etc) from *both* agouti and black pups and submit for genotyping analysis (PCR, Southern, etc).**
2. Use germline-positive* mice to establish breeding colony, and discard wild type mice. (Note: Wild type littermates should not be used for breeders as these may be host embryo derived rather than cell line derived. New C57BL/6N breeders should be requested for mating to the heterozygous positive mice for colony build, or sibling mating of heterozygous mice.)

***Warning/Recommendation**: All mutant mice (lacZ+ and/or short range PCR+) derived from CSD & EUCOMM ES cell created chimeras (F1 generation) should be confirmed for correct targeting using 5' long range PCR (LRPCR). Alternatively, targeting may be confirmed using quantitative (Taqman) Loss of Allele (LOA) for CSD targets. All mutant F1 mice from Knockout first (tm1a) alleles should also be confirmed for the presence of the distal loxP cassette. F1 mice passing LRPCR and LoxP testing should then be used for establishing your tm1a colony. Mice failing LRPCR may be incorrectly targeted, mice passing LRPCR but failing distal LoxP are considered tm1e, targeted, non-conditional. We have found around 5% of tm1a derived germline mice to be either non-targeted or missing the distal LoxP due to undetectable amounts of mixed populations of cells.

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