

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

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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 (6-8 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 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. **Only black pups are ES cell derived and expected to carry the ES cell mutation, however ES cells are heterozygous for the mutation, and screening is necessary. Not all black pups will carry the mutation.**
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.

***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](#). See Figure 2.