



Germline Transmission Testing Policy

Revised February 2019

NOTE: Germline Transmission is not a guaranteed service.

Assessment of chimerism: Pups are graded at day 10 of age as either 0%, 10%, 30%, 50%, 70%, 90%, or 100% “chimeric”, meaning the percent somatic cell (coat color) chimerism derived from the originating ES cell. All mice less than 50% chimerism or of the inappropriate sex for germline transmission testing will be euthanized prior to weaning (as early as 10 days of age).

Breeding: We will breed all appropriately sexed chimeras graded 50% or higher when they are 7 to 9 weeks of age; male chimeras are set with two females (6-7 weeks of age) of the appropriate genetic background, and female chimeras are set with one male of the appropriate genetic background. We mate the chimera until either they produce 25 wild-type pups each, the desired heterozygous pups, or are non-productive, whichever comes first.

Wildtype pups: If a chimera does produce at least 25 wildtype pups or only wildtype pups within 120 days, we feel Germline Transmission Testing will not be successful with the chimera, and the chimera will be euthanized.

Non-productive or unhealthy: If a male chimera is non-productive for 30-45 days, the male will be submitted for sperm collection, preservation, and genetic and morphological analysis for possible IVF services (additional fees apply). If a male chimera is unhealthy and requires euthanization, and has not yet produced at least 25 pups, he will be submitted for sperm cryo-preservation and analysis for possible IVF services (additional fees apply).

Germline: If germline transmission is successful and a heterozygous mouse is produced, all non-germline producing chimera will be euthanized. If a low number of heterozygous mice were produced, one or more will be bred to generate additional heterozygotes.

Once heterozygous pups are produced, we will then proceed with arranging shipment or any additional services requested, such as FLP or Cre recombination services.

For clones with a parental cell line of JM8A1.N3, JM8A3, or JM8A3.N1:

These cells were made from C57BL/6N (black) mice with the Agouti gene restored in the heterozygous form, thus the cells are A/a; Tyr^{+/+}. For more information please see [Pettitt et al. 2009](#). Because of this, tissue samples are taken from both agouti and black F1 pups for genotyping analysis. However, we have found that litters containing agouti pups or agouti *and* black pups are a good indication of germline transmission. Litters containing only black pups have a very small chance of producing a heterozygous mouse, but many samples may need to be taken. *Our policy is to discard any all-black litters once genotyping samples have been taken. Please note that we could be discarding a heterozygous mouse, and there is a small risk that the chimera will stop mating after producing the mouse. But this will give us an idea on what chimera to focus on if we do see a het and after that all pups from that chimera would be saved.

*Please note, as JM8A1.N3, JM8A3, or JM8A3.N1 require genotype testing of all pups produced, the fees will most likely be higher than estimated due to volume of samples.

General fees for germline transmission testing/breeding in our barrier facility:

Service	UC customer	Non-profit customer	For-profit customer
Strain/project management	\$134 per month	\$193.50 per month	\$206.90 per month
Per diem, barrier facility	\$1.59/cage/day	\$2.30/cage/day	\$2.46/cage/day
Colony management, breeder cages	\$0.53/cage/day	\$0.77/cage/day	\$0.82/cage/day
B6 or B6N mouse purchase	\$67.85 per mouse	\$98.25 per mouse	\$105.04 per mouse
Genotyping (qPCR, single rxn)	\$10 per sample	\$14.44 per sample	\$15.44 per sample
Genotyping (PCR)	\$13 per sample	\$18.77 per sample	\$20.07 per sample
Sperm cryo and analysis of chimera	\$182.04 per male	\$263.59 per male	\$281.80 per male