KOMP and EUCOMM Breeding Strategies and Recommendations

Targeting strategies: https://www.mousephenotype.org/understand/start-using-the-impc/allele-design/

If you are receiving (or produce from frozen germplasm) only a few heterozygous mice, we strongly recommend that you set your KOMP/EUCOMM targeted hets with purchased C57BL/6N wildtype mice for the first round of breeding, thus increasing the numbers of heterozygous mice you have to ensure a stronger breeding colony prior to setting up het x het matings. Additionally, you may want to continue a colony of het x wildtype B6N breeders in case there are viability or fertility issues in heterozygous or homozygous mutant mice.

Knock-out first (tm1a) allele - genetrap:

All the Knockout First alleles are technically genetrap firsts in which the "KO" is relying on the splicing of the LacZ into the upstream exons. Even in a homozygous tm1a mouse, it is possible for "leaky" expression for downstream exons, because the Beta Actin promoter of the Neomycin gene is very strong and can override the pA tail located immediately after that segment of the vector. If you do not require a conditional KO, we recommended converting to a tm1b (or tm1d if you already have the floxed tm1c allele) to make a constitutive KO that does not rely on splicing. See below for further details on converting to different alleles.

Alleles containing Neomycin selection cassette (tm1 or tm1a*):

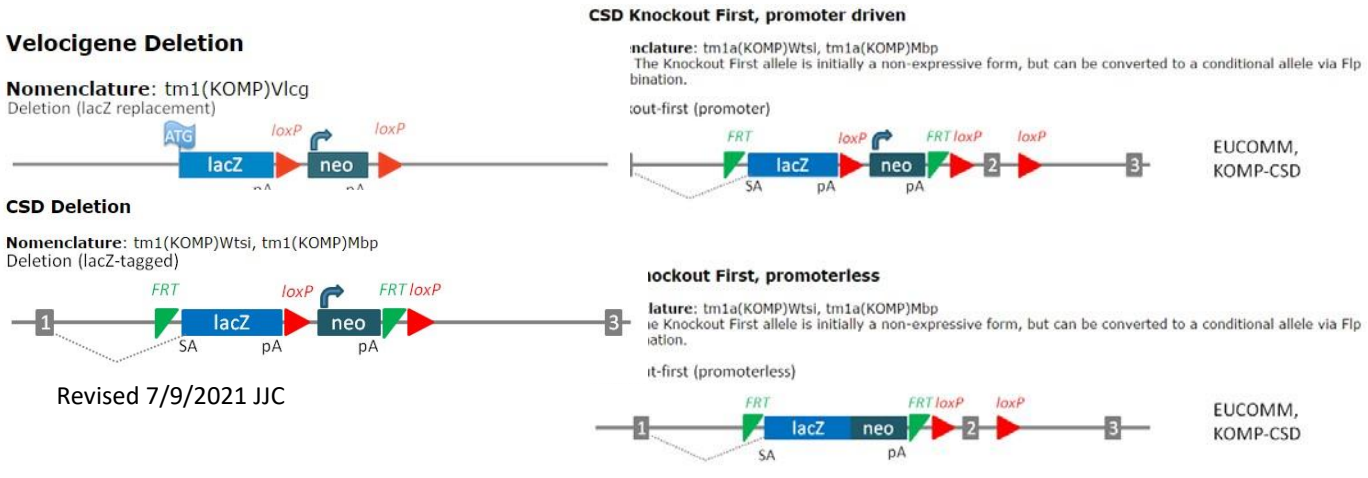
It is advisable to remove the positive selection cassette (necessary for ES cell selection); for KOMP alleles this is done via *in vivo* Cre recombination. This converts the alleles to tm1.1 or tm1b respectively; these lines remain a LacZ reporter and are non-conditional. There are numerous examples demonstrating that the PGKneo cassette includes a cryptic splice acceptor and donor that interfere with the expression of genes and neighboring genes. In addition, it has been demonstrated that the PGK promoter is bidirectional, which may also interfere with gene expression.

We recommend breeding post-Cre mutants with B6N wildtype mice after excision to remove the Cre transgene and ensure complete recombination, culling or further backcrossing mice which may be mosaic tm1/tm1.1 or tm1a/tm1b.

Here are a few examples:

- Fiering S, Epner E, Robinson K, Zhuang Y, Telling A, Hu M, Martin DIK, Enver T, Ley TJ, Groudine M. 1995. Targeted deletion of 5'HS2 of the murine beta-globin LCR reveals that it is not essential for proper regulation of the beta- globin locus. Genes and Development 9:2203-2213.
- Nagy A, Moens C, Ivanyi E, Pawling J, Gertsenstein M, Hadjantonakis AK, Pirity M, Rossant J. 1998. Dissecting the role of N-myc in development using a single targeting vector to generate a series of alleles. Curr Biol. 1998 May 21;8(11):661-4.
- Scacheri PC, Crabtree JS, Novotny EA, Garrett-Beal L, Chen A, Edgemon KA, Marx SJ, Spiegel AM, Chandrasekharappa SC, Collins FS. 2001. Bidirectional transcriptional activity of PGK-neomycin and unexpected embryonic lethality in heterozygote chimeric knockout mice. Genesis. 30:259-63.

**For the tm1a allele, excision of the selection cassette is recommended only if you will not be converting to the conditional model first (see next section for more details).*



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<u>Knock-out first (tm1a) allele - converting to the conditional (floxed) allele</u>:

Mice or sperm/embryos you receive from KOMP will be heterozygous for the knock-out first allele. **Further breeding will be necessary to produce fully conditional mice** as follows. Exposure of the construct to Flp recombinase (by *in vivo* Flp breeding) will remove the trapping cassette and render the mouse phenotype "wild type"; see representation of the tm1c allele below. A critical exon, or exons, would remain "floxed", but could later be removed conditionally after breeding to the appropriate Cre mouse model, resulting in allele tm1d.

We recommend breeding post-FLP mutants with B6N wildtype mice after excision to remove the FLP transgene and ensure complete recombination, culling or further backcrossing mice which may be mosaic tm1a/tm1c.