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Protocol 1: Controlling genetic background in crosses of mouse models of cancer

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Abstract

The design of a mouse cross can affect the distribution of variation in the control and experimental groups. This protocol assumes that one is interested in studying the effect of a gene of interest or intervention in different mouse groups without the confounding effects of strain background differences.

Materials:

The materials necessary depend on the method of genotyping being used. Publically available and commercial sources of genome-wide genotyping are now available.

Reagents:

1. Genetically engineered mice for breeding with information on strain background
 - a. e.g. Knockout models, conditional (floxed) models, tissue-specific Cre lines, transgenic models, etc
2. Kit for isolating genomic DNA from mouse tails or ear punches
 - a. e.g. Promega Wizard SV 96 Genomic DNA purification system (Promega cat# A2370, A2371, or A6780)
 - b. Standard DNA preparations using proteinaseK, phenol:chloroform , purification and ethanol precipitation, e.g. Jackson Laboratories DNA from Tail Biopsies: http://www.protocol-online.org/cgi-bin/prot/view_cache.cgi?ID=1171
3. Genotyping method for following the inheritance of the engineered genes
 - b. e.g. Southern blot or PCR assay
4. (optional) Genotyping method for assessing genome-wide variation
 - a. Illumina's Mouse Medium Density Linkage Panel available from the Center for Inherited Disease Research, Johns Hopkins University (http://www.cidr.jhmi.edu/supported/mouse_whole.html)

- b. MEGA Mouse Universal Genotyping Array available from Geneseek (<http://www.neogen.com/Agrigenomics/ResearchDevelop.html#top>)
- c. Mouse Diversity Genotyping Array available from Jackson Laboratory (<http://jaxservices.jax.org/mdarray/index.html>)
- d. Sequencing of SNPs. The genotypes of different strains in a particular region can be found on the Jackson Laboratory Phenome Database (<http://phenome.jax.org/db/q?rtn=snp/ret1>) and the surrounding genomic DNA is available in dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>). PCR primers can be designed to amplify the region of the SNP for sequencing by standard methods to follow specific strain contributions in crosses.

- 5. (optional) Genomic DNA from inbred strains found in the mixed strain background of your genetically engineered mice for use as controls. Samples of DNA from common inbred strains can be purchased from Jackson Laboratory (<http://www.jax.org/dnares/index.html>).

Equipment:

- 1. Computer with database to follow mouse breeding
 - a. Simple mouse colonies can be followed using a spreadsheet program such as Microsoft Office Excel
 - b. For more extensive breeding programs a relational database, such as Filemaker Pro or Microsoft Access, is highly recommended. Commercially available mouse colony maintenance programs are also available (but costly).
 - c. The database/spreadsheet should track the following information:
 - i. Mouse ID
 - ii. Mouse Genotype
 - iii. Mouse Sex
 - iv. Date of Birth
 - v. Date Died
 - vi. Strain Background (e.g. C57BL/6J, 129/Sv, mixed...)
 - vii. Cross (e.g. inbred, F1 if hybrid, N1, N2, N3, etc if backcrossed onto a pure background, or mixed if unknown)
 - viii. Mother's ID
 - ix. Mother's Genotype
 - x. Mother's Strain Background (e.g. C57BL/6J, 129/Sv, mixed...)

- xi.** Mother's Cross (e.g. inbred, F1 if hybrid, N1, N2, N3, etc if backcrossed onto a pure background, or mixed if unknown)
- xii.** Father's ID
- xiii.** Father's Genotype
- xiv.** Father's Strain Background (e.g. C57BL/6J, 129/Sv, mixed...)
- xv.** Father's Cross (e.g. inbred, F1 if hybrid, N1, N2, N3, etc if backcrossed onto a pure background, or mixed if unknown)
- xvi.** It is also helpful to record the coat color of each mouse, as this is often the first clue that strain contamination has occurred

- 2.** Vacuum manifold if using the Promega Wizard SV 96 Genomic DNA purification kit

Method:

Two methods are described as examples, but will depend on the details of the mouse model. The first example is for a simple model in which a single mutant allele is followed in crosses on an inbred strain background. The second example is a more complex cross in which the model is homozygous for a floxed allele of the gene of interest and carries a tissue-specific Cre transgene.

Example 1: Comparing a single mutant allele to wild-types on an inbred strain background

- 1.** If importing the model from another source, confirm the mutation in the gene of interest by isolating DNA from tail clips or ear punches using one of the methods listed under Reagents, and genotyping for the mutant allele.
- 2.** If the strain background is in question, use one of the SNP panels listed under Reagents to check the homozygosity of SNPs throughout the genome. Alternatively, select approximately 96 SNPs spread throughout the genome and polymorphic for common strains such as C57BL/6J and 129/Sv and test the tail DNA by PCR amplification of the region around the SNP and sequencing.
- 3.** If the mutant gene has been inbred onto the current strain background (e.g. C57BL/6J) from a different strain background (e.g. 129/Sv), check for presence of SNPs from the previous strain (129/Sv) particularly around the gene of interest (Figure 1A). Determine whether these polymorphisms may affect the expression or isoforms of other genes not related to your gene of interest. The role of this region in the phenotype can be tested with additional crosses (Bolivar et al. 2001).
- 4.** Cross the mutant mouse to wild-type mates of the same strain background to generate heterozygous offspring for study, keep wild-type siblings as controls (Figure 1A).

- Note: The inheritance of the mutation from the mother or the father can change the phenotype (Reilly et al. 2006). When collecting heterozygous mice from het X het crosses the parental origin of the mutation is unknown and cannot be controlled. By crossing het X wt, the parental origin of the mutation is controlled.
5. Generate equal numbers of mutant experimental mice and control wild-type mice, controlling for the sex of the mouse. To study strong effects, 20 mice per group (10 males and 10 females) is a good starting point. For more subtle phenotypes, 50 mice per group (25 males and 25 females) is recommended.
 6. Analyze the phenotype of interest for statistically significant differences between the mutant and wild-type mice. Analyze differences separately in males and females to look for sex biases. Analyze differences separately in mice inheriting the mutation from their mother and from their father to look for effects of parental inheritance.

Example 2: Comparing mice homozygous for a floxed allele of the gene of interest and carrying a tissue-specific Cre transgene to sibling controls on a mixed background.

1. Confirm the presence of the flox mutation and Cre transgene in parental lines by isolating DNA from tail clips or ear punches using one of the methods listed under Reagents, and genotyping for the mutant alleles.
2. Confirm that the Cre transgene is unlinked to the flox mutations (on a different chromosome) and will independently segregate in crosses. If the genomic location of the transgene is not already known it can be tested by fluorescent in situ hybridization (FISH) or by sequencing outward from the transgene into the adjacent genomic regions and using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the region of the mouse genome. If the transgene is on the same chromosome as the floxed allele it will be necessary to link the transgene to one of the floxed alleles using germline recombination.
3. Decide which parent (mother or father) will contribute the Cre transgene in the cross. In at least one example, Cre activity can vary depending on whether it is on the maternal or paternal allele (Dubois et al. 2006), so it is wise to control for the parental inheritance of the transgene.
4. Cross the floxed allele mouse to the Cre transgenic mouse in a fixed direction (e.g. floxed males to Cre female) and select for double mutant progeny (Figure 1B). All of these progeny should carry the same strain of mitochondria and Y chromosome (if male).
5. Take only one sex of the double mutant progeny and cross to the floxed allele mouse (e.g. flox+;Cre+ males X flox+ females). The progeny should be similarly randomized for autosomal variation and all carry the same strain of mitochondria and Y chromosome (if male).
6. Generate equal numbers of experimental and control groups, controlling for the sex of the mouse. Ideally one would keep all genotypes from the cross as

controls for the flox/flox;Cre+ experimental group, but at a minimum one should compare:

- a. flox/flox;Cre+ (experimental group)
- b. flox/flox; no Cre
- c. flox/wt;Cre+
- d. wt/wt; Cre+

These groups will allow one to determine whether any phenotypes observed require tissue-specific loss of the floxed gene, or are due to spurious effects of Cre, the strain background of the floxed allele or the strain background of the Cre transgene. To study strong effects, 20 mice per group (10 males and 10 females) is a good starting point. For more subtle phenotypes, 50 mice per group (25 males and 25 females) is recommended. Note in Figure 1B that flox/flox;no Cre and wt/wt;Cre progeny are on different strain backgrounds than the parental flox/flox and Cre lines.

7. Analyze the phenotype of interest for statistically significant differences between the flox/flox;Cre+ mice and other groups. Analyze differences separately in males and females to look for sex biases.
8. In order to use more of the offspring from the first generation cross (step 4) it is possible to breed reciprocal crosses in step 5 (e.g. flox+;Cre+ males X flox+ females and flox+ males X flox+;Cre+ females), so long as the number of offspring in each group is equally represented between the two types of crosses. In this scenario it is recommended that differences between the maternal and paternal Cre inheritance in the phenotype be checked.

Discussion:

Many factors can confound a mouse genetic experiment if the experimental groups and control groups are not treated equally and bred to have identical (or nearly identical) genetic backgrounds. Progeny often do not have the same genetic background as parents with the same mutation unless the line is well inbred. Differences between the sexes and differences due to parental inheritance effects also need to be taken into account. Given that mouse genetic experiments are costly it is recommended that these variables are at least tracked in experiments so that if biases are suggested experiments can be repeated with a larger cohort size to give sufficient statistical power to detect the true effects of the experimental variable.

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Figure 1:

Examples of cross designs for simple and complex mouse models of cancer. (A) On inbred strain backgrounds there can be a residual window of strain contamination (gold) around the gene mutation. This region will be uniquely inherited by the experimental group and should be taken into consideration in the interpretation of results. (B) On mixed strain backgrounds (indicated by different random colors for mix #1 and mix #2), the mitochondria (m) is inherited from the mother and the Y chromosome (Y) is inherited from the father. The rest of the genome undergoes germline recombination in every generation and evolves such that

the genetic background of progeny is different from the genetic background of parents and grandparents, even when the engineered mutation (e.g. Cre or flox) is selected. It is therefore important to use siblings as the control group, rather than parental lines, and to use multiple different control groups carrying different alleles to make sure that phenotypes track with the combination of all alleles in the experimental group and not any one genetic contribution.

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