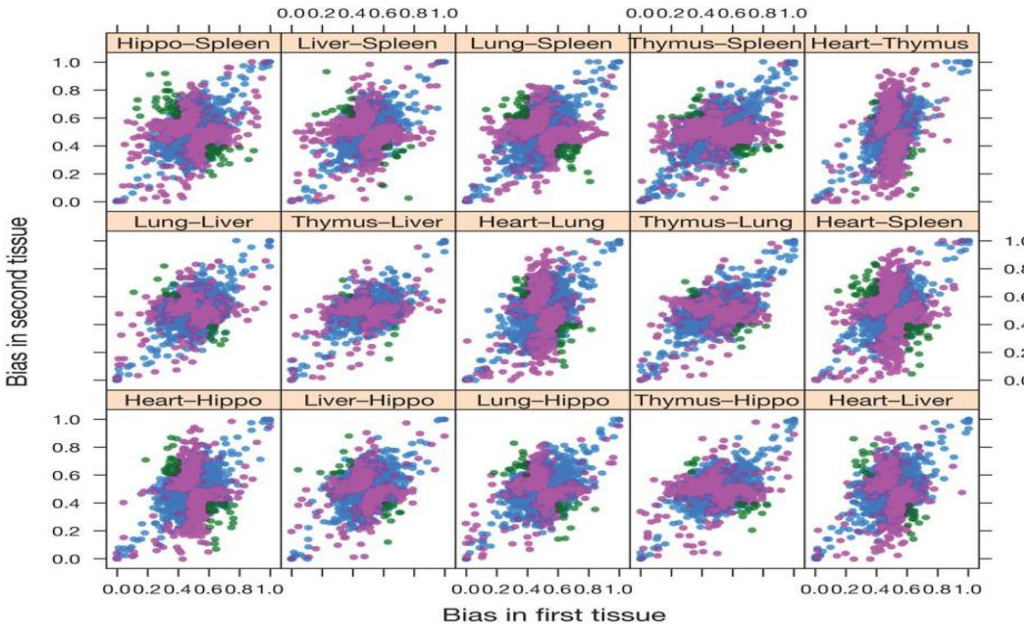

Allele-specific biases in RNA expression levels between tissues from C57BL/6J x DBA/2J F1 mice. Please read reference article for details. *Nature* (2011) 477:289–294.

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Genetic Background Management

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Genetic background

Genetic background refers to the genetic make-up of a genome, except the reference loci of interest or the donor allele (e.g. a transgene, a mutant allele or a knockout allele). It includes all alleles of all loci in the genome. For example, a spontaneous mutant arose in an inbred C57BL/6N colony has a genetic background of C57BL/6N. A transgenic strain generated by pronuclei DNA microinjection into an FVB zygote produces a mouse with the introduced transgene in an FVB background. If they are kept inbreeding or crossing with their respective parental strain, their original genetic background is maintained. However, if they are crossed with a second strain, the mutation or the transgene is said to be in a mixed genetic background.

Influence of Genetic Background on the phenotypic expression of genes of interests

The presence of modifier genes may explain physiological differences among different inbred strains. They modulate gene transcription, mRNA stability, and epigenetics directly or indirectly. It is not surprising that different sets of modifier genes in different genetic background can modulate phenotypic expression of a gene of interest differently.

The following are examples of different phenotypic expression of genes of interest in different genetic backgrounds.

Obesity model Severe diabetic condition is produced by the obesity model with *obese* mutation (*ob*) in the C57BL/KsJ genetic background but not in the C57BL/6J background. Ref: *Diabetologia* (1973) 9:287-93.

Alzheimer's disease model The amyloid precursor protein transgene, *Tg(APP695)*, merely induces amyloid plaques in the brain in an outbred genetic background. When crossing it to an inbred genetic background (inbred FVB/N or B6 mice), the amyloid precursor protein caused lethality. Ref: *Hum Mol Genet* (1997) 6:1951-9.

Dystonia model Life span of mouse with an in-frame deletion (GAG, "ΔE") in the *TOR1A* gene in 129/DBA/2J background was largely extended (> 18 days) when comparing with that in 129/C57BL/6J background (< 3 days postnatal). Ref: *PLoS* (2012) 7: e32245.

Lymphoma model *Pten* (Phosphatase and tensin homolog, a tumor suppressor gene) mutant develop lymphoma at an incident rate of about 90% in a C57/129 background but in 129/Balb/c background the incident dropped to less than 10%. Ref: *Cancer Res.* (2006) 66:6492-6.

Mammary Carcinoma model *Adenomatous polyposis coli* (*Apc*) gene mutation *multiple intestinal neoplasia* (*min*) in C57BL/6J background developed mammary tumor at an incident rate of 93% whereas in 129S6/SvEvTac/C57BL/6J mixed background, the incident rate was 41%, after a carcinogen ENU treatment. In 129X1/SvJ/C57BL/6J mixed background, the incident rate dropped to 9% only. Ref: *Cancer Research* (2001) 61:3480–3485.

Colitis Model *Interleukin-10-deficient* (*Il10*(-/-) mice in C3H/HeJBir background developed more severe colitis than those on a C57BL/6J background. Ref: *Genomics* (2002). 80:274-82.

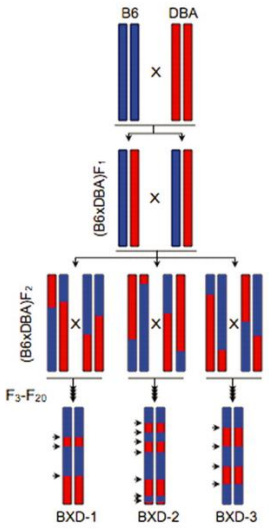
Breeding Scheme and Genetic Background Maintenance

Application of different breeding strategies lead to qualitative difference in genetic backgrounds. The following are two commonly used breeding schemes resulting in two extremes in genetic background qualities.

Congenic strain Continuously crossing a mutant strain originally in strain A to another inbred strain B (i.e. recipient inbred strain), will bring the donor allele to an increasing proportion of genetic background of the recipient strain B. Until the recipient strain contributes 99.9% to the genetic background, the donor allele is said to be in a congenic (Cg) background of the recipient strain B. It mathematically takes 10 backcross generations. In other words, except the genomic region flanking the donor allele, the

rest of the genome is generally considered being identical to the recipient strain B.

Are You Generating Recombinant inbred lines? Inbreeding a strain with a mixed genetic background (by sibling cross) continuously will eventually "fix" a unique combination of alleles segregated from their respective genetic background of origins, i.e. establishing a recombinant inbred line. The presence of different sets of modifier genes in different recombinant inbred lines may cause different, but consistent, phenotypic expression of the gene of interest, making data non-reproducible by another laboratory with the same mutant but in different recombinant inbred genetic background.



Generation of recombinant inbred strains. Different recombinant inbred lines inherited different sets of alleles segregated from the parental strains. Source: *Nucleic Acids Research* (2010) 38:2346-54.

On the contrary, inbreeding a mutant already in a congenic genetic background will not generate recombinant inbred strains. With the same set of modifier genes, congenic strains being established or maintained in different laboratories produce more reproducible experimental data.

Recommendation on breeding a transgenic/mutant strain and the choice of experimental control animals

Mixed genetic background A mutant strain may be maintained in a mixed genetic background by frequent back-crossing it to an appropriate F1 hybrid. This strategy maintains a mixed genetic background with consistent heterogeneity along different stages of a project and among different laboratories with the same breeding practice. The best control animals are the non-carrier or wildtype littermate of the mutant, followed by the F1 hybrid animals used for the backcrossing.

Congenic or inbred genetic background Maintaining a mutant in an inbred genetic background or establishing a congenic strain will make the phenotypic expression of the mutant allele constant. Both the non-carrier littermates and animals from the corresponding inbred strain are the choice of experimental control animals.

If both of the above are technically impossible (e.g. compound transgenic mice generation by crossing different transgenic strains), littermates are always the best control animals. However, one must consider the genetic background variation among littermates. Segregation of different modifier genes to different progenies may cause significant phenotypic variations. An appropriate sample size of both test and control animals should be recruited to ensure the conclusion is drawn from statistically sound data.

In all cases involving inbreeding, one should backcross the strain at least once every 10 generations to avoid fixing any accumulated spontaneous mutations and genetic drift.

Side Notes:

Reading a Generation Definition

N: Number of backcross generations

F: Number of filial/inbreeding (sibling cross) generations

p: History of being cryopreserved

Example:

N5F1pF2 is describing a line that has been backcrossed for five generations (N5), inbred for one generation (F1), being cryopreserved (p) and then two more inbreeding generations (F2) after recovery from the cryopreservation (JAX).

"Hemizygous" vs "heterozygous"

If the two alleles of a locus are different (e.g. one of the alleles is mutated), the individual is heterozygous at that locus (symbol: -/+). If a locus is missing one allele, or another allele never exist (e.g. an randomly integrated transgene with no matching pair of the transgene in the sister chromosome), the individual is hemizygous at that locus, i.e. the transgene (symbol: Tg/0).

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