Breeding of constitutive and conditional mouse mutants: a pragmatic approach

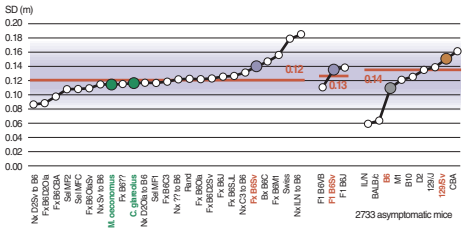
H.-P. Lipp, H. Welzl, R. Madani, D.P. Wolfer - University of Zurich, Institute of Anatomy, CH-8057 Zurich

NCCR Neural Plasticity and Repair, Swiss NF 31-54184.98 & 31.57139.99, Roche Research & Hartmann-Müller Foundations

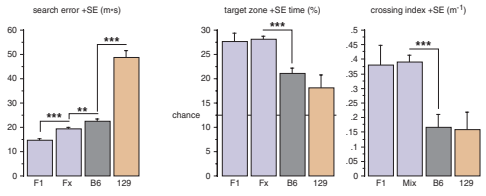
Summary

An uncontrolled bias or shift of genetic background can severely confound phenotypic characterization of genetically modified mice. It has therefore repeatedly been suggested that co-isogenic or at least congenic lines should be used exclusively. However, generation of such a homogeneous genetic background is often impossible because of limited resources and time. We provide evidence that studying samples with a mixed genetic background, typically a combination of C57BL/6 with a substrain of 129, is a valid alternative, provided that essential rules are respected. A set of breeding strategies is proposed which implement these rules with a minimum of resources and can be used with constitutive or conditional targeted mutations.

1 Reliable results and good base line performance from outbred samples



SD as measure of within sample variability varies over wide and similar ranges in outbred, F1 hybrid as well as inbred samples. Inbred mice are not more reliable.

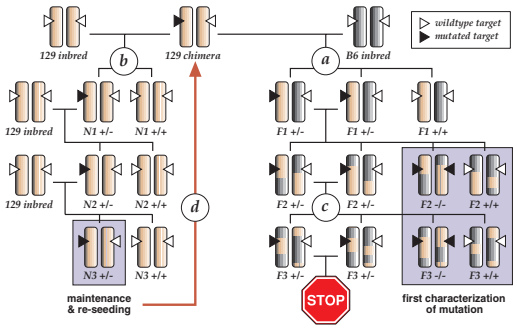


During swimming navigation, 129B6F1 mice outperform both parental strains. B6 beat 129 with respect to escape performance, but not on transfer tests. Outbred samples are only marginally inferior to F1 and beat both parental strains as well.

2 F2 to characterize a *constitutive* mutation, co-isogenic line for its later maintenance

Essential rules according to 1997 Banbury Conference:
- use littermates as controls
- background must be documented and reproducible
- use few and commonly available strains

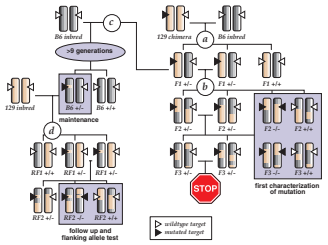
Basic scheme with maintenance of mutation in co-isogenic line (on ES-cell background):



(a) Rapid production of test samples for first phenotypic characterization: chimera crossed with B6 females, heterozygous F1 offspring interbred to obtain F2. (b) Co-isogenic line for mutation maintenance by backcrossing chimera to ES-cell donor strain. (c) Do not interbreed past F3! (d) Later, new F2 should be generated using co-isogenic maintenance line.

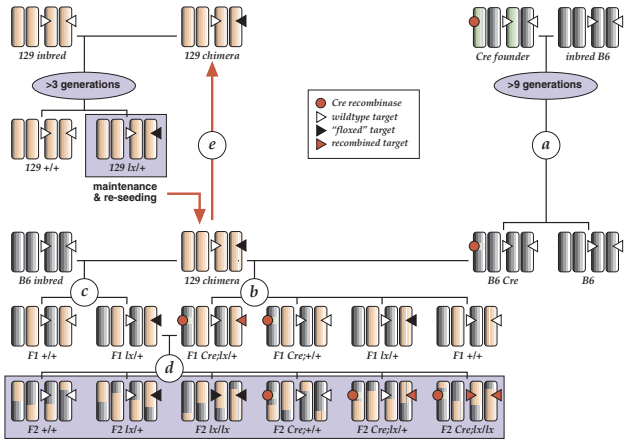
Alternative if mutation is in congenic (e.g. B6) line:

(a,b) As above. (c) Mutation maintained as congenic line after backcrossing to C57BL/6. (d) Creation of follow up samples similar to original F2 by outcrossing maintenance line again to the ES-cell donor strain (e.g. 129/SvEv). In resulting reverse F1, heterozygous animals are interbred to create a reverse F2. The latter provides test samples in which flanking allele effects cannot occur, because ES-cell donor alleles make equal contributions to the flanking region of the targeted locus in all genotypes.



3 Similar breeding strategy applied to *conditional* models, for example using the Cre/LoxP system

Basic scheme with floxed allele kept in co-isogenic line (ES-cell background) and Cre transgene on B6 background:



(a) Cre transgene maintained in congenic line by backcrossing to C57BL/6, over as many generations as time allows. (b) Chimera with floxed allele crossed with C57BL/6 congenic Cre transgenics and (c) with inbred C57BL/6. (d) F1 offspring of first cross carrying both floxed allele and Cre mated to heterozygous F1 offspring of second cross. F2 then contains all genotype combinations necessary for two-way analysis. (e) Floxed allele is maintained in co-isogenic line by backcrossing chimera to ES-cell donor strain. Heterozygous mice of maintenance line replace chimera.

Alternative if Cre and LoxP Line are both on B6 background:

(a) Cre recombinase transgene and the floxed allele only available in C57BL/6 congenic maintenance lines. (b) Re-introduce ES-cell donor background by outcrossing heterozygous mice of both congenic maintenance lines. (c) Cross of F1 animals carrying floxed allele and Cre recombinase transgene, respectively. (d) Double heterozygous F2 is backcrossed to F1 mice carrying floxed allele. Resulting F3 has genetic background close to the original F2, yet contains all genotype combinations necessary for a two-way analysis.