# KNOCK-OUT MICE: POSSIBLE SOLUTIONS TO THE GENETIC BACKGROUND AND FLANKING GENE PROBLEM

472.3

Swiss National Science Foundation Grant 31-46691.96

**W.E. Crusio[1], D.P. Wolfer[2] and H.-P. Lipp[2]**

[1]Institut de Transgénose, Génétique, Neurogénétique et Comportement, UPR 9074 CNRS, Orléans, France;
[2]Institute of Anatomy, University of Zürich, Switzerland.

SPONSOR:
International Behavioural and Neural Genetics Society (IBANGS)

## ? *Knockout effects in mice may be faked by incapacitating alleles flanking the targeted locus*

**It has been noted (R. Gerlai, TINS 19: 177-180, 1996) that mice carrying targeted null ("knock-out", KO) mutations differ from control animals not only by the invalidated gene, but also in the origin of any genes flanking that gene. Specifically, mutant mice derived from embryonic stem (ES) cells of the 129 strains will carry flanking alleles from that same strain. Since the 129 strains possess many alleles impairing performance in a variety of learning tasks, such flanking alleles may, with bad luck, induce spurious behavioral and neurophysiological impairments falsely attributed to the targeted mutation.**

**Based on the recommendations of the Banbury Conference (Neuron 19:755-759, 1997) we show here that this problem can be controlled using proper breeding schemes classically used in behavioral genetics. These breeding schemes are in any case necessary to maintain the mutation for further investigation.**

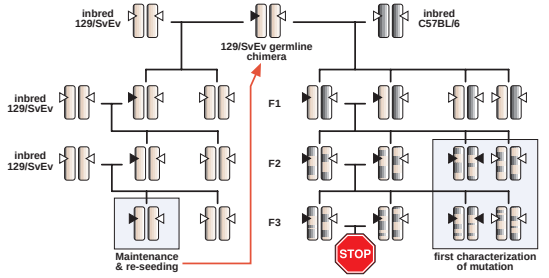
**As the large majority of KO mutants is based on 129 derived ES cells and phenotypes have been analyzed usually on a mixed 129 x C57BL/6 background, we use these two strains as an example. The principles remain the same for other strains.**

## ! *Recommendations*

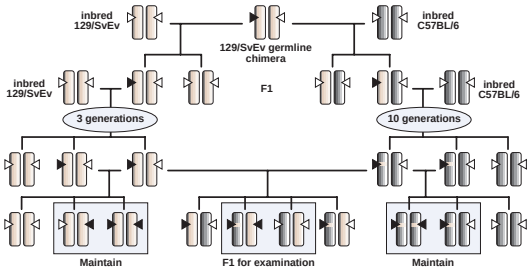
1. **Use proper breeding schedules, cull litter sizes, use littermates whenever possible**
2. **Measure your phenotype(s) as soon as possible in the parental strains!!!**
3. **Check your F1 generation (obtained by mating 129 germline chimeras with C57BL/6 mice) and compare it with the parental strains. This will show presence or absence of hybrid vigor. If present (F1 better than parental strains), the flanking gene problem is greatly diminished (we explain that). This comparison will also detect dominant flanking allele and mutation effects. If wildtypes show 129 features, there may be a problem!**
4. **Test initially for (typically) recessive KO-effects using an F2 on a mixed 129 x C57BL/6 background. It is not ideal but will work in most cases. Initiate backcrossing to the parental strains as soon as possible and continue backcrossing during the analysis of phenotypes.**
5. **Use then one of the testing methods indicated above. The most economical (but not fastest) solution is to obtain littermates which are different for the targeted locus but always homozygous for the flanking 129 derived region.**
6. **Now you may safely publish (or withdraw/correct) your data...**

## 1 *This needs to be done in any case*

*Short-term* control of genetic background during initial characterization of targeted mutations




Targeted mutations: generation of congenic lines for *long-term* control of genetic background

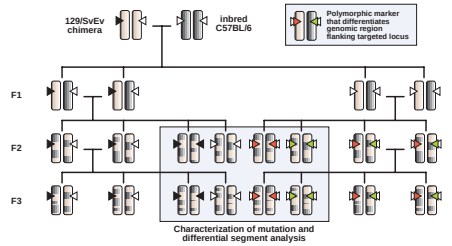



### *Some minor caveats for the interested reader*

1. **Theoretically, F1 hybrids are optimal for phenotypical analysis. Slight drawback: they often mask weak phenotypes.**
2. **In our experience (about 2000 mice, about 30 KO lines), a mixed background with enough 129 alleles is helpful in discovering KO phenotypes.**
3. **Our models apply only if there is no interaction between targeted locus and confounding loci!**
4. **Be wary that a KO dependent phenotype is not a function of the targeted locus but a function of the entire genome minus the targeted locus...**

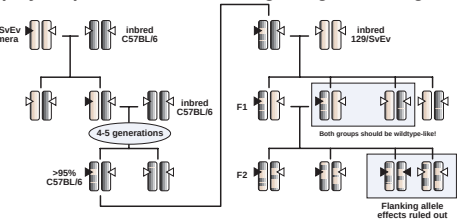
## 2 *Three techniques to check for flanking gene effects*

Polymorphic marker for targeted genomic region permits rapid exclusion of flanking allele effects



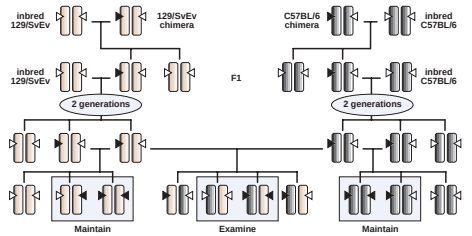

Molecular solution

Flanking allele effects can be ruled out without a polymorphic marker for the targeted genomic region




Poor man's choice

Double targeting in unrelated ES-cell lines: thorough but rarely applied solution to the flanking allele problem




Ideal but expensive