CROSSING PROCEDURE CHIMERA => HETEROZYGOTE => HOMOZYGOTE

Chimeras are generated by injection of 129/Ola ES cells (male) into C57Bl/6 blastocysts (male or female).

1. Test on germline chimerism => heterozygotes:

- the percentage of male chimeras is an indication for the potency of the male ES cells: although theoretically 50% of the blastocysts is female and 50% male, sexconversion by the potent male ES cells should lead to more than 50% of male chimeras.

- 6 male chimeras of reasonable chimerism (varying in coat color from black + agouti to agouti to beige) are used at the age of 7 weeks:
  in six cages : one male chimera + one female FVB (F1: grey and agouti)
  + one female 129/Ola (F1: beige and agouti)
  * FVB is a suitable strain for big litters, but the second generation will have a mixed background.
  * 129/Ola is the strain to obtain F1 pups with a pure 129/Ola background, but the litters are small.

- one or more grey or beige F1 pups indicate, that the ES cells are capable of coat color transmission: 20-30 pups is a reasonable amount to judge this capability.

- 50% of grey or beige pups are heterozygous for the knock-out allele: cross with FVB (big litters) to obtain ± 30 grey pups.

- at the time of weaning (± 3 weeks after birth) DNA of the tails of the grey pups can be analyzed to screen for heterozygotes.

2. Heterozygotes => homozygotes:

- theoretically 50% of the heterozygotes is male (7-8 pups), 50% female (8-7 pups): intercross the F1(FVB x chimera) heterozygotes to obtain homozygotes.

- Generate homozygotes derived from two independent knock-out ES cell clones.

NOTE: use F1 of FVB with mixed background to study embryonic lethality of homozygotes quickly; use F1 of 129/Ola with pure background once phenotypic effects in hematopoiesis in homozygotes have to be studied.

3. Establishing mouse lines

The F1 descendents of the chimeras obtained under **1** are used to establish mouse lines in 129OLA and FVB. The F1 mice are crossed back to fresh wild-type 129OLA or FVB to prevent inadvertant ES cell abnormalities to interfere with the viability of the descendant lines

- Set up lines for two independent knock-out ES cell clones:
  F1 (OLA x chimera) x w.t. OLA — two couples => Backcross 1
  F1 (FVB x chimera) x w.t. FVB — one couple => Backcross 1

- Determine genotype by tail analysis and use heterozygotes for the next generation:
  B1 (OLA x F1) x w.t. OLA — two couples => Backcross 2
  B1 (FVB x F1) x w.t. FVB — one couple => Backcross 2

- If nullizygosity for the knock-out mutation is lethal, continue with this scheme generating heterozygous offspring every four months.

- If homozygotes are viable and fertile, the strains can be maintained as homozygotes. However, start intercrossing after at least three backcrosses of the F1's to wild-type mice.

NOTE: If the lines derived from two independent ES cell knock-out clones show the same phenotype, one can be discarded.