INTRODUCTION OF SUBTLE MUTATIONS USING *CRE/LOX*

In this procedure a replacement DNA targeting vector is used carrying the desired mutation and the positive/negative selectable *Hprt* marker gene. The marker is flanked by *lox* sites and is located within an intron. In the first step both the mutation and the marker are introduced by double cross-over. In the second step, the *Cre* recombinase is expressed which catalyses site-specific recombination between *lox* sites thus removing the marker gene.

1. Introduction of the mutation by positive selection

- Prepare an isogenic replacement DNA targeting vector, carrying the mutation (usually within an exon) and the *Hprt* cassette flanked by *lox* sites located within an intron.
- Purify the targeting vector from an agarose gel.
- Introduce the targeting construct into HM-1 ES cells (*Hprt*$^-$) by electroporation and select colonies on HAT medium.
- Analyse colonies for correct integration of the mutation and the *Hprt* marker gene.

2. Removal of *Hprt* from targeted clones

- Grow individual clones till confluency on a 6-well plate (± $5x10^6$ cells).
- Introduce a PGK*Cre* expression vector by electroporation or Lipofectin.
- Reseed cells at a density of 4×10^5 / 10 cm dish in 60% BRL conditioned medium.
- Refresh medium next day with 60% BRL supplied with 10 μg/ml of 6-TG.
- Continue selection for 8-10 days.
- Pick colonies and expand for storage in N_2 and DNA analysis.