Supplementary Figures

Supplementary Figure S1: Donor plasmid.

The donor plasmid consists of the fluorescent marker gene (mEGFP in this case) which is flanked by 500-800 bp homology arms of the GOI. A linker is placed between the tag and the

gene to maintain functionality of the tagged protein. As a backbone vector pUC-based plasmids are used.

Supplementary Figure S2: Scheme of expected junction PCR result.

A forward primer binding at the 5' end outside of the left homology arm and a reverse primer binding to the fluorescent marker gene will result in one PCR product of the expected size. To test if all alleles are tagged with the fluorescent marker at the correct locus, a forward primer located 5' outside of the left homology arm and a reverse primer 3'outside of the right homology arm were used. Two PCR products using this primer set will indicate heterozygote clones and one PCR product which runs at the expected size of the tagged gene will indicate homozygosity.

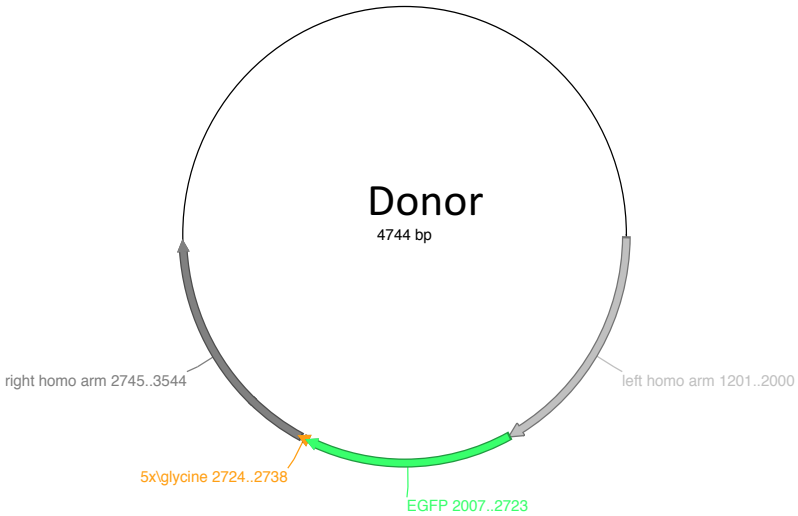
Supplementary Figure S3: Southern blot transfer of DNA.

This scheme depicts how to build up the southern blot transfer as described in step 48| Option B, step xii-xxiii). Two long filter papers are dipped into a tank filled with 10X SSC and used as wicks. Gel, nylon membrane and filter papers were sandwiched on a glass plate as depicted to transfer the DNA from the gel onto the membrane.

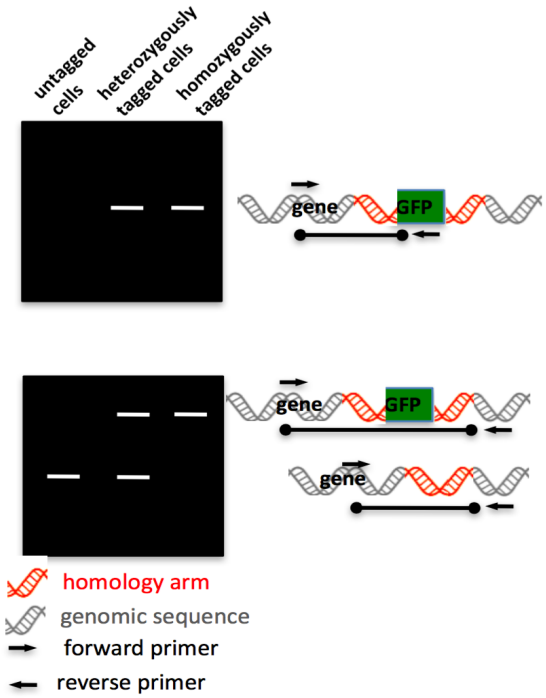
Supplementary Figure S4: Example of sequences containing point mutation.

Sanger sequencing was performed with a PCR fragment (lower sequence line #1-278) which was aligned to the expected sequence of the GOI (middle sequence line labeled with #3201-3500). The overlaying green and red lines indicate a point mutation at position 3411 of the expected sequence, i.e. one allele has the nucleotide T at this position (green line) whereas another allele has the nucleotide A (red line) at the same position. This indicates a point mutation (red A) within one of the alleles.

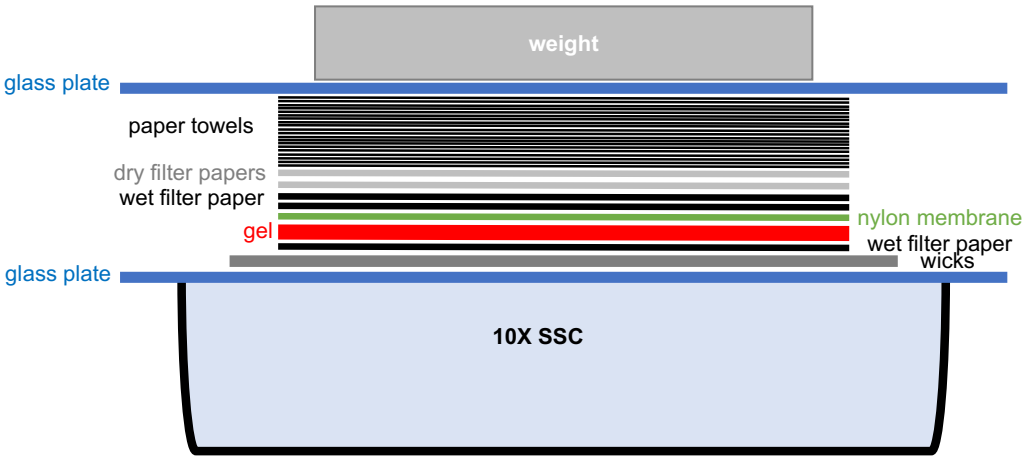
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4