

### **Ple50 (mEMS1645) ESC derivation**

pEMS1361 was electroporated into mEMS1204 (B6129F1-*Gt(ROSA)26Sor<sup>tm1Sor/+</sup>*, *Hprt1<sup>b-m3</sup>/Y*) embryonic stem cells (ESCs) generating positive constructs (identified by PCR – see Ple50 genotyping assay).

mEMS1642, mEMS1643, and mEMS1645 were identified as correctly targeted ESC lines.

These ESC lines were microinjected into ICR (CD-1 022 Charles River) and/or B6(Cg)-*Tyr<sup>c-2I</sup>/J* (JAX Stock#000058). Resulting chimeras were bred to B6(Cg)-*Tyr<sup>c-2I</sup>/J* females, and germline N1 progeny identified by the presence of the *Tyr<sup>+</sup>* allele in combination with the *A<sup>w</sup>* (agouti, white belly) and *a* (non-agouti/black) coat color alleles.

The Ple50 strain (B6129P2-*Hprt1<sup>tm6(Ple50-EGFP;mEMS1645)Ems</sup>*) was established from the mEMS1645 ESC line (see Ple50 (mEMS1645) Mouse description file).