

**Ple56 (mEMS2478) ESC derivation (DDC-A)**

pEMS1125 was electroporated into mEMS1204 (B6129F1-*Gi(ROSA)26Sor<sup>tm1Sor/+</sup>*, *Hprt1<sup>b-m3</sup>/Y*) embryonic stem cells (ESCs) generating positive constructs (identified by PCR – see Ple56 genotyping assay).

mEMS2475, mEMS2478, mEMS2479 and mEMS2482 was identified as the correctly targeted ESC lines.

The mEMS2478 ESC line were microinjected into ICRBAF1 (F1 hybrid of CD-1 (022 Charles River) and B6(Cg)-*Tyr<sup>c-2J</sup>/J* (JAX Stock#000058)). Resulting chimeras were bred to B6(Cg)-*Tyr<sup>c-2J</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 Ple56 strain (B6129P2-*Hprt1<sup>tm23(Ple56-EGFP/NLS)Ems</sup>*) was established from the mEMS2478 ESC line (see Ple56 (mEMS2478) Mouse description file).