

**Ple111 (mEMS1774) ESC derivation (HCRT-A)**

pEMS1417 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 Ple111 genotyping assay).

mEMS1767, mEMS1768, mEMS1774, and mEMS1775 were identified as correctly targeted ESC lines.

The ESC line mEMS1774 was microinjected into ICR (CD-1 022 Charles River) and/or ICRBAF1 (F1 hybrid of ICR 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 Ple111 strain (B6129P2-*Hprt1<sup>tm15(Ple111-EGFP/NLS)Ems</sup>*) was established from the mEMS1774 ESC line (see Ple111 (mEMS1774) Mouse description file).