

**Ple160 (mEMS955) ESC derivation (PITX3-B)**

pEMS1146 was electroporated into mEMS21 (E14TG2a) embryonic stem cells (ESCs) generating positive constructs (identified by PCR – see Ple160 genotyping assay).

mEMS955 was identified as the correctly targeted ESC line.

This ESC line was microinjected into C57BL/6J (JAX Stock#000664). Resulting chimeras were bred to C57BL/6J females, and germline N1 progeny identified by the presence of the  $A^w$  (agouti, white belly) coat color allele.

The Ple160 strain (B6129P2-*Hprt1*<sup>tm29(Ple160-EGFP/cre/NLS)Ems</sup>) was established from the mEMS955 ESC line (see Ple160 (mEMS955) Mouse description file).