Experimental Outline for Targeting ES Cells – Step by Step Protocol

General Considerations:
Plan out a flow chart from day 1 to day 16–19. Note, depending upon the growth of the ES cells, the days may have to be shifted. Electroporation, screening, picking and preparation of DNA will take 2–3 weeks, including weekends!

Day #	Application	Protocol ID No.
1	Prepare culture plates for plating	PC1796EN00
	Prepare PMEF Feeder cell plates for ES cell expansion & electroporation	PC1797EN00
2	Check PMEF Feeder cell plates, and thaw ES cells	PC1801EN00
3	Feed ES cells; Passage if required	PC1801EN00
4	Feed ES cells; Passage if required Prepare Targeting Vector: Linearize ~100 µg of targeting vector (each electroporation requires 15 - 30 µg of linearized vector), and precipitate with EtOH (no need to phenolize).	PC1801EN00
5	Electroporation of ES cells	PC1833EN00
6-10	Select for ES cell Transformants	PC1800EN00
9-11	Pick ES cells	PC1807EN00
12-13	Feed picked ES cells	PC1801EN00
14	Freeze ES cell clones & retain duplicate wells to grow ES cells for DNA isolation Preparation of Genomic DNA for Southern blot analysis	PC1808EN00 PC1799EN00
16-19	Recover clones following analysis using RESGRO Culture Medium	PC1804EN00