

APPENDIX

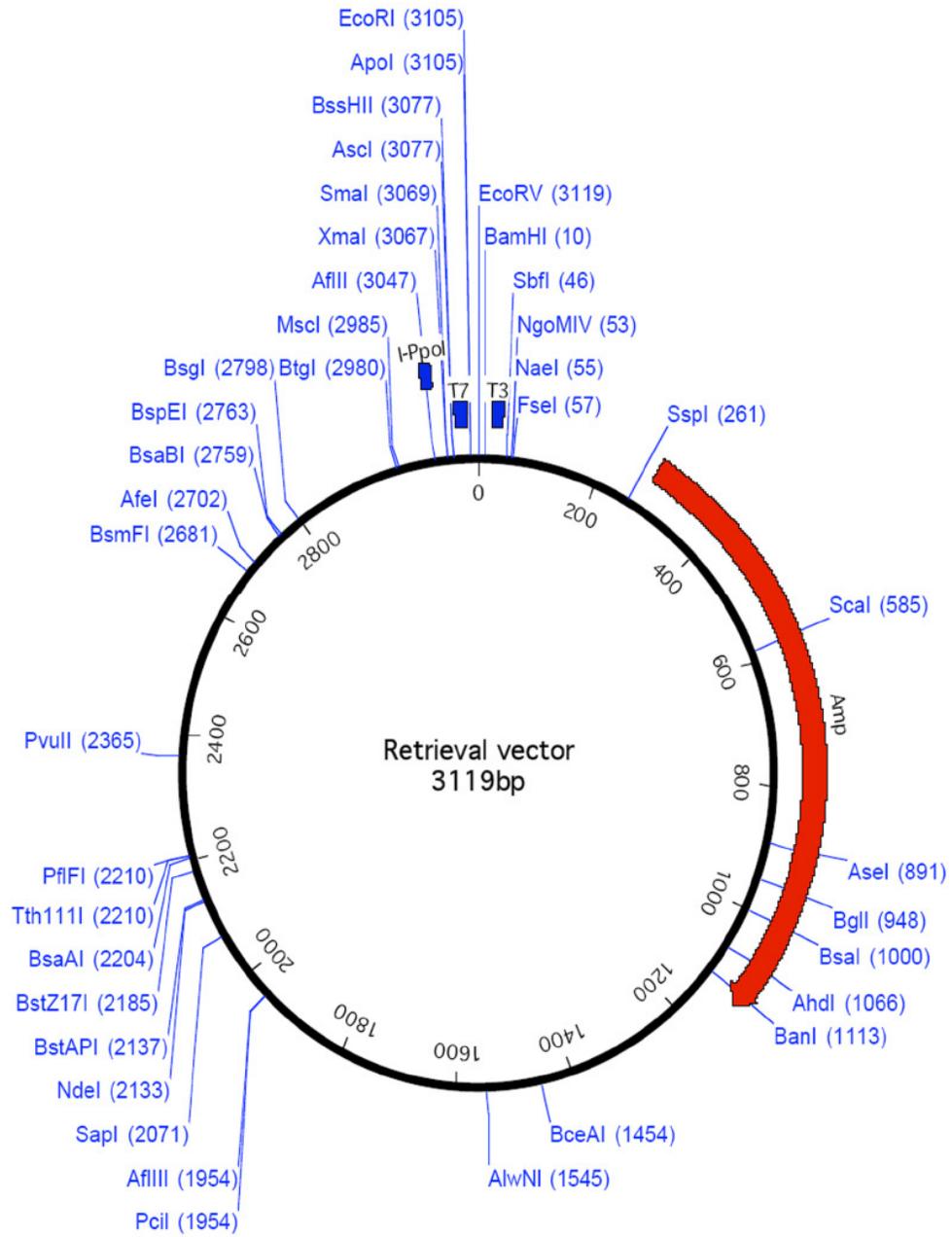


Figure A.1. Plasmid map of PL611 retrieval vector.

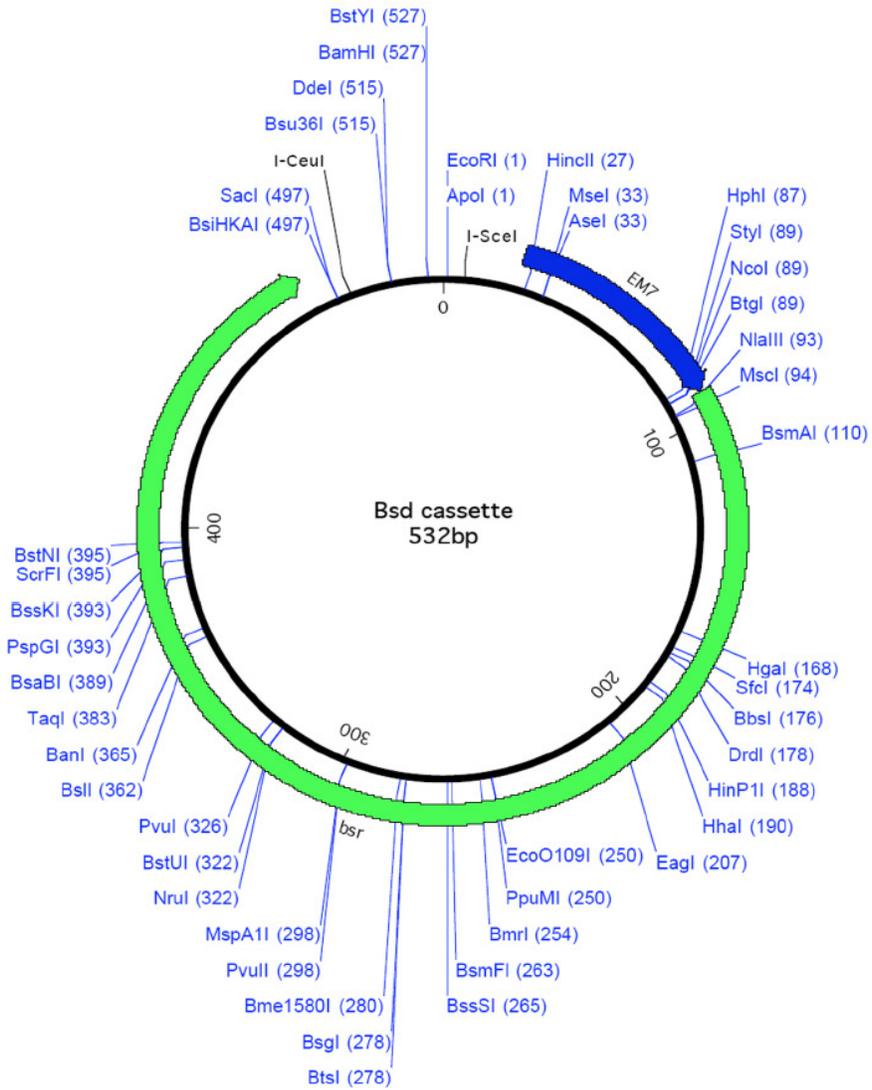


Figure A.2. Plasmid map of *Bsd* cassette.

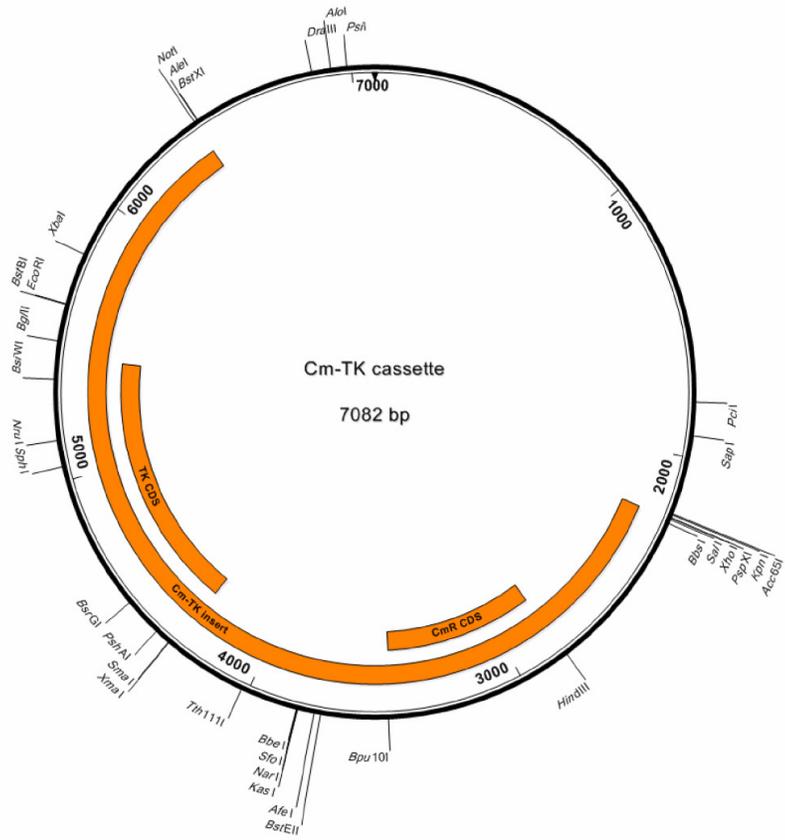


Figure A.5. Plasmid map of *Cm-TK* cassette.

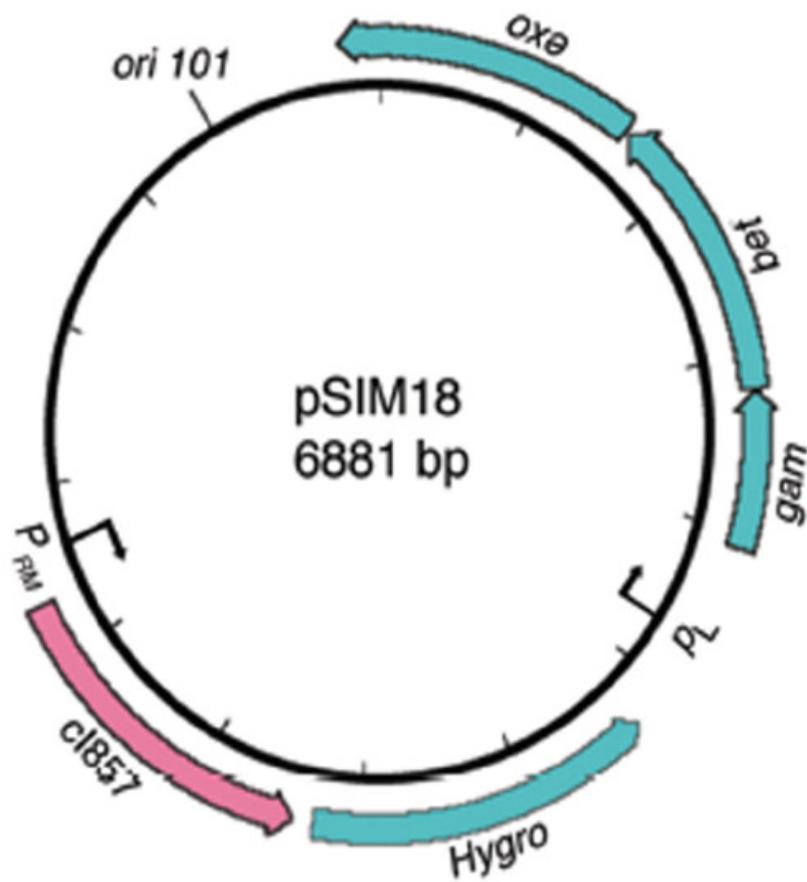


Figure A.6. Plasmid map of pSim18. Obtained from (Chan et al., 2007).

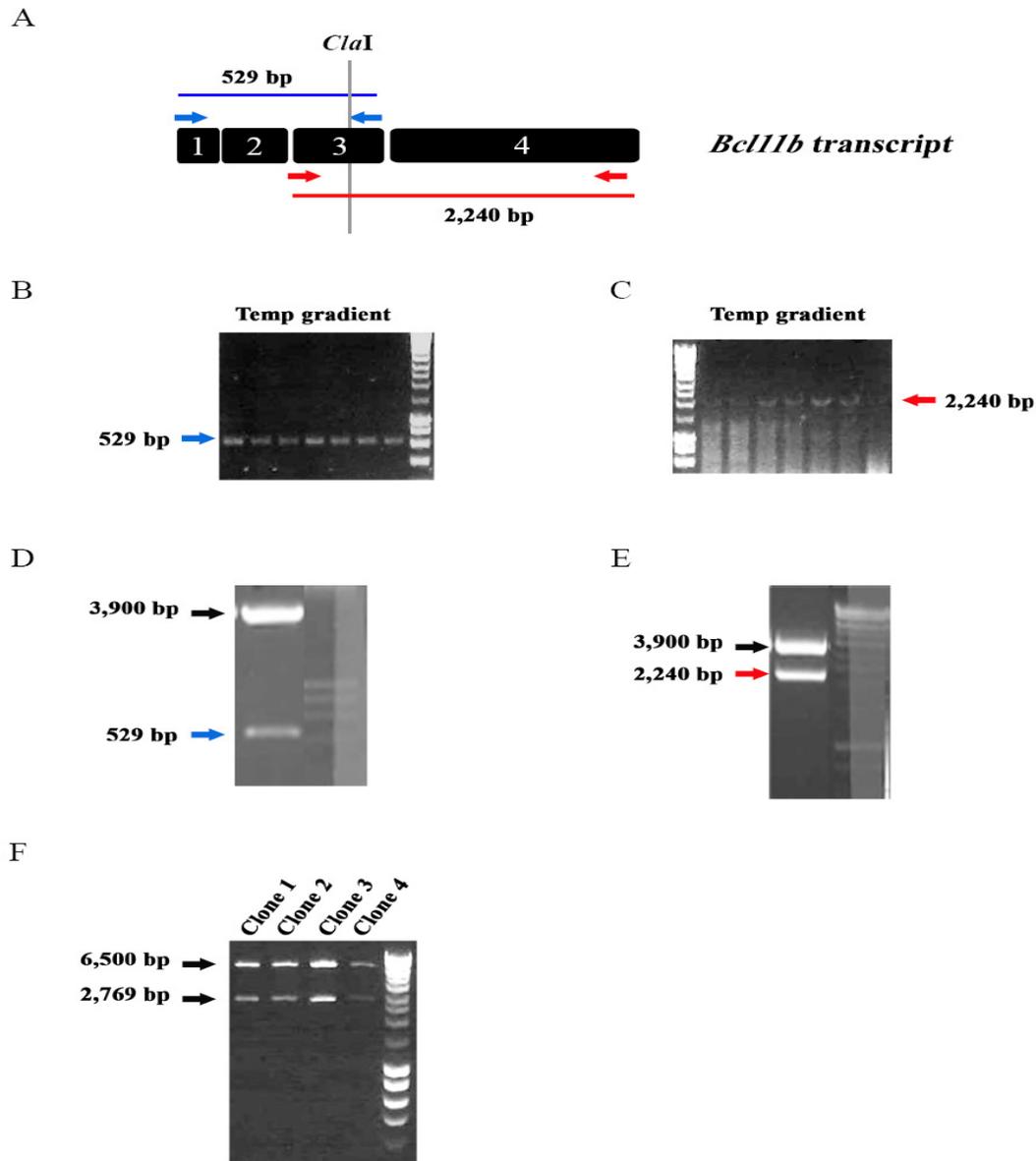


Figure A.7. Cloning of *Bcl11b* cDNA and construction of *Bcl11b* over-expression vector. (A) Positions of primer pairs used to amplify *Bcl11b* exons 1-2-3 (Blue arrows) and exons 3-4 (Red arrows). Gel images showing PCR products from the respective primer pairs. Fragments corresponding to (B) 529 bp and (C) 2,240 bp are purified and cloned into pCR-BluntII-TOPO vector (3,900 bp). Gel images showing restriction digestion products of (D) *Bcl11b* exons 1-2-3-pCR-BluntII-TOPO and (E) *Bcl11b* exons 3-4-pCR-BluntII-TOPO plasmids following *Bgl*III + *Cla*I and *Cla*I + *Eco*RI digestion respectively. Fragments corresponding to (D) 529 bp and (E) 2,240 bp are purified and cloned into *MSCV-IRES-eGFP* over-expression plasmid in a 3-way ligation reaction. (F) Gel images showing restriction digestion products of *MSCV-Bcl11b* cDNA-*IRES-eGFP* plasmid following *Bgl*III + *Eco*RI digestion. All four clones show the expected fragment sizes (*Bcl11b* cDNA insert – 2,769 bp and *MSCV-IRES-eGFP* backbone – 6,500 bp).

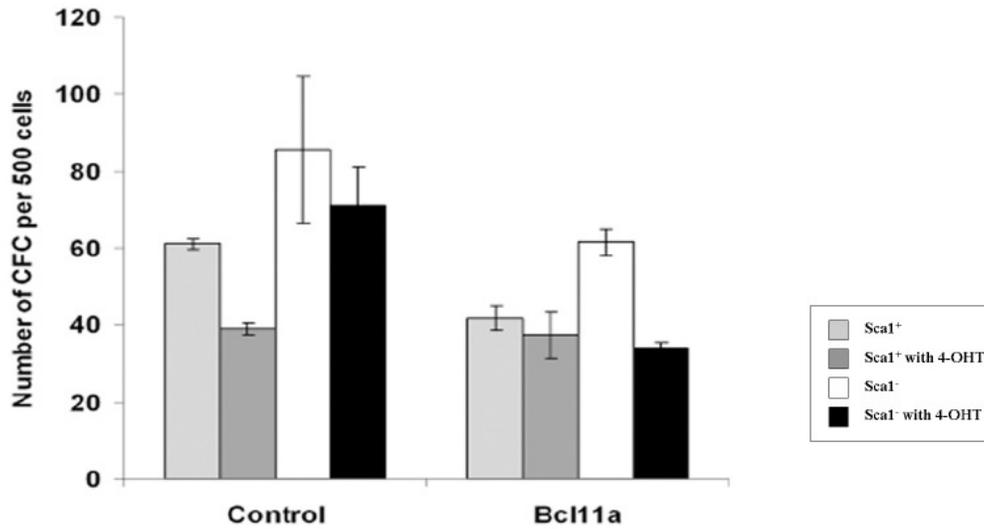


Figure A.8. *In vitro* mammary colony-forming cells (Ma-CFCs). Graphs showing number of Ma-CFCs per 500 cells after deletion of *Bcl11a* in sorted Lin⁻CD24^{hi}CD49b⁺Sca1^{+/-} luminal progenitors. CD24^{hi}CD49b⁺Sca1^{+/-} luminal progenitors from *Cre-ERT2*; *Bcl11a*^{flax/flax} mammary glands are sorted and plated with irradiated feeders in NSA media for 24 hours before 1 μ M of 4-hydroxytamoxifen (4-OHT) is added to induce deletion of *Bcl11a*. After 2 hours, fresh NSA media is replaced and cells are maintained at 37°C/5%CO₂ for another 6 days before the number of Ma-CFCs is enumerated.