

## **Canine Blastocyst cDNA Library**

A single canine blastocyst was harvested 14 days post LH surge. Due to the uncertainty in the timing of canine fertilization, this could correspond to a post-fertilization age of 6-10 days. The blastocyst structure was typical, with a thin expanded trophectoderm and a compact ICM constituting less than 10% of the blastocoel space.

Polyadenylated mRNA was purified with Oligo dT magnetic beads using a Miltenyi  $\mu$ MACS mRNA isolation kit (Cat. # 130-075-201). The entire mRNA sample was reverse transcribed with AffinityScript (Stratagene Cat. #200436). The cDNA was synthesized and amplified using reagents and primers from a Clontech "SMART" cDNA Library Construction Kit from Clontech (Cat. # 634901), which favors full-length cDNA. Amplified cDNA was cloned into the SfiI sites of  $\lambda$  TriplEX2 (Clontech) vector, using the SfiI sites embedded in the "SMART" primers, packaged as Lambda phage, and  $2.5 \times 10^6$  pfu were plated, grown and harvested as a pooled library. An aliquot was thawed and titered 4/11/08 and yielded  $4 \times 10^7$  pfu/ml, but this should be retested by the user.

The cDNA can be propagated as phage or excised with Cre recombinase and propagated as plasmid. Maps and instructions for propagation and manipulation of the vector can be found online in the Clontech Users Manual at <http://www.clontech.com/images/pt/PT3003-1.pdf>

As a test of the quality of the library, full length open reading frames for KLF1, SOX2, Oct4, cMyc, and Nanog were all amplified by PCR from the library, verified by sequencing, cloned into expression vectors, and expressed as full length proteins in transfected cells.