The CTV Vector
(CAG-STOP-EGFP-ROSA Targeting vector)
By Changchun Xiao, 12/6/07

1. pRosa26-1

This is the original vector from Dr. Philippe Soriano at Fred Hutchinson Cancer Research Center. It contains Rosa 26 5' fragment-XbaI-Rosa 26 3' fragment-PGK DTA bpA.

2. Rosa TV

Based on pRosa26-1, with a linker of SgfI-SnaBI added at the XhoI site of the plasmid backbone.

3. STOP-EGFP-ROSA TV

Generated and used successfully by Dr. Yoshiteru Sasaki/Rajewsky lab, based on Rosa TV. He introduced splicing acceptor (SA)-Neo-STOP-AscI-Ires-EGFP-pA at the XbaI site of Rosa TV.
Note that AscI site is not present in pRosa26-1 and Rosa TV.

4. CAG-STOP-EGFP-ROSA TV, also named CAG-SERTV-7, as well as **CTV** for convenience.

I introduced the CAG promoter from the pCXN2 vector to the PacI site of the STOP-EFGP-ROSA TV, between SA and the floxed Neo-STOP cassette.

5. R-A4

From the Stuart Orkin lab at the Children's Hospital, Boston.
This is not a targeting vector. Instead, it is a plasmid containing a 4kb genomic DNA from the Rosa 26 locus. Digest with EcoRI+PacI to obtain the Rosa probe A for Southern blot. Probe A runs at the position of 600bp on agarose gels, though it is 693bp in length.

Notes

1. All plasmids are Amp[r].
2. Restriction digestion:
   (1) AscI: We use it to insert the gene of interest. Gives a single band of 16kb.
   (2) SgfI (Promega): We use it to linearize the plasmid before ES cell transfection. Gives a single band of 16kb.
   (3) PacI: I used it to cloned the CAG promoter. Gives two bands: 1.7kb (the CAG promoter) and 14.4kb (the STOP-EGFP-ROSA TV).
   (4) XbaI: I used it to confirm the orientation of the CAG promoter. Gives four bands: 56bp (not visible), 1.4kb, 2.7kb, and 11.9kb.

Since this vector has passed through several labs and individuals, the sequence we have now may not be completely accurate. I only did the above-mentioned digestions. If they all give bands at expected patterns, you should feel confident that this is the CTV vector that has been used successfully in the Klaus Rajewsky lab.

CTV Annotation

By Changchun Xiao, 1/28/08

1. Rosa 5' homology region:	nt 3-1082
2. Splicing acceptor site (SA):	nt 1141-1158
3. CAG promoter	nt 1213-2930
3. loxp site (reverse orientation):	nt 2950-2983
4. Neo cassette (reverse orientation):	nt 2984-4578
5. STOP cassette:	nt 4579-5455
6. loxp site (reverse orientation):	nt 5456-5489
7. Frt site:	nt 5522-5569
8. IRES:	nt 5570-6170
9. EGFP;	nt 6171-6914
10. Frt site:	nt 6915-6962
11. Bovine growth factor poly A signal	nt 7010-7310
12. Rosa 3' homology region	nt 7334-11159
13. PGK DTA bpA	nt 11627-13082
14. Linker region for linearization	nt 13216-13245