TRANSDUCTION MURINE SPLENOCYTES (Schumacher Lab May 2013)

Materials/reagents needed:

- IMDM - 5% FCS – p/s
- IMDM serum free
- Phoenix Eco packaging cell line
- Maxiprep DNA of interest
- pCLECO DNA
- XtremeGene 9 transfection reagent (Roche)
- Mouse splenocytes
- Erylysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.4)
- Concanavalin A (Calbiochem)
- IL-7 (Santa Cruz)
- RPMI – 8% FCS – p/s
- RetroNectin™ (Takara)
- PBS with 2% BSA (BSA: Sigma)

Day 0 (Friday): Thaw & culture Phoenix Eco (P-ECO) Cells (3x10E6 cells in T175 Flask) in 35 ml IMDM + 5% FCS + P/S

Day 3 (Monday): Plate out P-ECO:
3 cm plates: 0.6x10E6 cells in 2.5 ml IMDM + 5% FCS + P/S
10 cm plates: 1.2x10E6 cells in 10ml IMDM + 5% FCS + P/S

Day 4 (Tuesday) **TRANSFECTION**

3 cm plates: 400 µl serum free medium (ISCOVE's) + 12.5 µl Fugene; leave at room temperature (RT) for 5 minutes. Then add 5 µg maxiprep DNA and 3 µg pCLECO DNA, leave at room temp 15 minutes.
10 cm plates: 400 µl serum free medium (IMDM) + 12 µl XtremeGene; leave at room temperature (RT) for 5 minutes. Add 4 µg maxiprep DNA and 3 µg pCLECO DNA, leave at room temp ~30 minutes.

Carefully add transfection mix to P-ECO cells, place back in incubator

ACTIVATION OF SPLENOCYTES

Transfer splenocytes through a nylon filter (70 um) to obtain single cell suspension
Spin, resuspend in sterile erylysis buffer. Add 3ml buffer per spleen, 3 minutes on ice. Add 1% IMDM (block erylysis) and wash cells
Count cells

Resuspend cells in RPMI + 8% FCS + P/S at concentration of 3x10E6 cells/ml.
Add Concanavalin A (2 μg/ml; 1:500 dilution of stock) and IL-7 (1 ng/ml; 1:10.000 dilution of stock).
Plate on 24 well tissue culture treated plates at 3x10E6 cells per well

Day 5 (Wednesday): Refresh medium on P-ECO with ISCOVE's w' 5% FCS

Day 6 (Thursday): **TRANSDUCTION**

Coat non-tissue culture treated 24 well plates with 0.5ml Retronectin (1:5 dilution of stock) for 2 hrs at RT (or O/N at 4^{o}C).
Incubate with 2% BSA, 30 mins RT. Wash 1x with PBS

Harvest splenocytes, count, wash.

Harvest virus supernatant, spin (2000 rpm 10 mins; remember to keep supernatant!!!)

Add virus supernatant to splenocytes; 0.5 ml virus per 1.5x10E6 cells
Plate on the Retronectin coated plates

Perform spin infection (90 mins/ 2000rpm/ no break)

Keep virus on cells and incubate O/N at 37 ^{o}C

Day 7 (Friday): Harvest splenocytes, ficoll to remove dead cells, measure transduction efficiency by FACS. Wash 3x with HBSS, adoptively transfer to recipient mice.