Retroviral Primary Fibroblast Immortalisation (hTERTing)

Reagents required

- Fugene (Roche)
- Polybrene (4mg/ml stock Sigma #H9268)
- DMEM (complete 10% FCS and serum free)
- MEM (complete 15% FCS)
- Trypsin
- Puromycin (1mg/ml stock)
- PT67 packaging cell line
- PBS
- pBABE-puro retroviral vector+hTERT

Safety considerations

Care must be taken when working with retroviruses
All waste must be autoclaved
Use independent vacuum pump
Use double gloves and disposable labcoat
Work in designated viral hood
Use Virkon to decontaminate equipment etc.

Transfection of hTERT plasmid into packaging cell line

Day 1:

- Seed PT67 cells into 1xT25cm^2 flask at 5x10^5 total.

Day 2:

- Add 4µg of hTERT plasmid DNA to 100µl of serum free DMEM.
- Add Fugene to 100µl serum free DMEM (3:1 Fugene to DNA).
- Mix together DNA and Fugene and incubate at room temperature for 30min.
- Aspirate media from the PT67 cells and replace 3ml serum free DMEM +DNA/Fugene mix.
- Incubate at 37^{o}C for 3-8h.
- Wash PT67 cells with PBS and change media to complete DMEM.

Day 3:

- Aspirate media, wash with PBS and replace with fresh DMEM.

Day 7:

- Wash PT67 cells with PBS and replace media with DMEM complete containing 5µg/ml of puromycin. Grow cells until confluent.

Day 12-13:

- When PT67 cells are confluent trypsinize and transfer to 2xT75cm^2 flasks. Maintain in complete DMEM + puromycin until confluent.

Infection of retrovirus into target cells

Day 20:

- Wash PT67 cells with PBS and change media to serum free DMEM.
- Seed target cells into 1xT25cm^2 flask at 5x10^5 total.

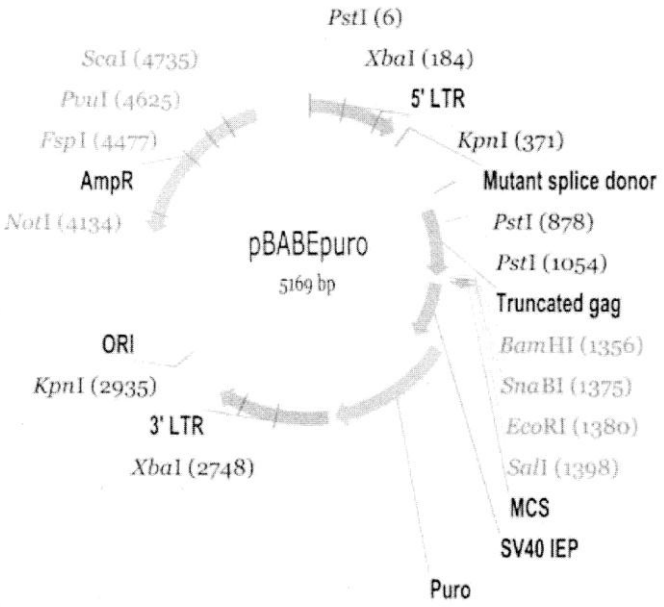
Day 21:

- Collect the virus containing media from the PT67 cells.
- Using a 0.45µM syringe filter, sterilise and add 4ml to each 1xT25cm^2 flask of target cells +4µl of polybrene (aliquot unused virus into 2ml cryovials and store at -80^{o}C for future use).
- Incubate at 37^{o}C for 18-24h.

Day 22:

- Aspirate virus containing media from target cells and wash with PBS.
- Add complete MEM +0.75µg/ml puromycin.
- Continue to culture the infected cells in selection replacing media every 3-4 days.

pBABE-Puro Retroviral Vector Map



For advice see: Sophie/Elena