STAT5 is required for long-term maintenance of normal and leukemic human stem/progenitor cells

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Day 1

- Coat 10 cm dishes for two hours with 0.1% gelatin at room temperature
- Plate 2.5 x 10^6 293T cells in 10ml DMEM plus 10% FCS into a gelatin coated 10 cm dish per group, incubate overnight at 37ºC, 5% CO_2

Day 2

- transfect with Fugene:

Tube 1:

- DMEM without FCS	100 µl
- packaging construct (pCMV Δ8.91)	3 µg
- glycoprotein envelop plasmid (pMD2.G = VSV-G)	0.7 µg
- vector construct (pTRIP)	3 µg

Tube 2:

- DMEM without FCS	400 µl
- Fugene6 (Roche)	21 µl

(in medium, not against the edges of the tube)

- Add tube 1 to tube 2, flick gentle and allow complex formation for 20 minutes at room temperature
- Add mixture dropwise to 293T cells, swirl real gentle, incubate overnight 37ºC, 5% CO_2

Day 3

- Change medium of 293T cells to 4.5 ml HPGM and incubate overnight 37ºC, 5% CO_2

Day 4

- Remove 4.5 ml medium from 293T cells and pass over two filters (low protein binding filters (Millex HV, Millipore) to remove residual 293T cells.
- Freeze virus containing supernatant in aliquots of 500 µl in cryotubes in -80ºC and use when necessary.