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## 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  $2.5 \times 10^6$  293T cells in 10ml DMEM plus 10% FCS into a gelatin coated 10 cm dish per group, incubate overnight at 37°C, 5% CO<sub>2</sub>

### 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 37°C, 5% CO<sub>2</sub>

### Day 3

- Change medium of 293T cells to 4.5 ml HPGM and incubate overnight 37°C, 5% CO<sub>2</sub>

### 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 -80°C and use when necessary.