**Stable cell line generation**

**Purpose:** To generate a cell line (or lines) that stably express a protein of interest.

**Reagents:**

Standard DMEM +1% P/S +10% FCS

Access to serum-free media

Ice cold PBS

Transfectant solution (DNA:PEI – see PEI mediated transfection protocol)

Selection agent (10mg/mL Zeocin™ stock)

Transfectees (HEK293T)

Reagents in brackets are those used in the procedure described below but, depending on your specific aims, others may be used.

**Outline:**

High Selection

Maintain

Seed cells

Transfect

Day 1

Day 2

Day 4

O/N

48hr

10-14 days

**Procedure:**

* Seed HEK293T at 250000 per well in 6-well dish in standard media overnight
* Three hours prior to transfection replace media with that containing 5% FCS (1:1 dilution of standard media with serum-free)
* After 3 hours add DNA:PEI solution (and mock transfectant if required)
* At 24 hours replace media with that containing 10% FCS (standard media)
* At 48 hours harvest in ice cold PBS and transfer to T25 flask containing 200ug/mL Zeocin™ (100µL 10mg/mL stock in 5mL media)\*
* Maintain 200µg/mL Zeocin™ by GENTLY replacing media until complete death of untransfected cells and the transfected colonies are approaching confluence (approx 10-14 days)
* Drop Zeocin™ to 40µg/mL (20μL stock in 5mL media) and maintain as such forever and ever\*

As soon as this process is complete, bulk up the cells to freeze down in liquid nitrogen

\*take sample for Western blot analysis