# Creating a Stable Cell Line

## Background

HeLa T-REx cells are stored in frozen aliquots, which were expanded and subsequently frozen from a vial given to us by Tarun Kapoor's laboratory. The cell line was originally purchased from Invitrogen, and more detailed information can be found in their manual (link listed below). To create the stable cell line, the HeLa T-REx cells are co-transfected with:

1) pcDNA5/FRT/TO - a vector that contains your gene of interest, under the control of a tetracyclin promoter, with Flp recombination sites. A list of multiple cloning sites for inserting your gene can be found in the manual for the vector (link below).

2) pOG44 – a Flp recombinase expression vector

Following co-transfection, the Flp recombinase will facilitate the insertion of your gene of interest into the genome of the cell. Following proper gene recombination, the cells will become hygromycin B resistant, as this marker is also incorporated into the genome during the recombination process. After maintaining co-transfected cells in media supplemented with 350 μg/mL hygromycin B for two weeks following the transfection, all remaining cells should have the gene of interest integrated into their genome. Addition of 1 μg/mL tetracycline to the growth medium should result in the expression of the target protein (if GFP-tagged, the expression can be checked via fluorescence 24 hrs post-tetracycline addition).

## Procedure

1. Thaw a vial of HeLa T-REx cells. The complete medium for these cells is:

EMEM
10% FBS
2 mM L-glutamine
1% Pen-Strep
5 μg/mL blasticidin

2. Passage the cells 3-4 times to ensure they are healthy enough for transfection.

3. In a 6-well plate, co-transfect HeLa cells that are 50-60% confluent with the pcDNA5/FRT/TO vector containing your gene of interest along with the pOG44 vector (different constructs can be used for each well, and be sure to include an empty vector control). Transfection is done with Lipofectamine 2000 following a standard transfection protocol, using a 9:1 ratio of pOG44:pcDN5/FRT/TO. The total DNA amount should total 1 μg per well (ie: use 0.9 μg pOG44 and 0.1 μg plasmid).

4. 24 hrs post-transfection, wash cells and replace with complete media (do NOT include hygromycin B at this point)

5. 48 hrs post-transfection, expand cells to a 10 cm dish (if they are becoming too confluent). Let cells attach overnight.

6. The following day, change the media. Hygromycin B (350 μg/mL) should now be included the media.

7. Continue to change the media every 2-3 days for the next 2 weeks. Significant cell death should be observed within 3-4 days after exposing the cells to hygromycin B.

8. Expand the selected cells and confirm that your gene of interest is present using media supplemented with 1 μg/mL tetracycline.

9. After confirmation, expand the cells normally (no longer need to select, so hygromycin B can now be omitted), and freeze cells for long-term storage according to the manufacturers instructions (see manual).

**Useful links**

T-REx Growth and Maintenance manual:
http://tools.thermofisher.com/content/sfs/manuals/trexcells_man.pdf

pcDNA5/FRT/TO vector manual:
http://tools.thermofisher.com/content/sfs/manuals/pcdna5frtto_man.pdf

pOG44 Flp-recombinase expression vector manual:
https://tools.thermofisher.com/content/sfs/manuals/flpin_pog44_man.pdf