Cryogenic Storage and Recovery of the HEK 293 cell lines

The materials needed for the following two procedures are as follows:

- Dulbecco's Modified Eagle Medium (DMEM; GibcoBRL, 11965-092) with 10% (v/v) Fetal Bovine Serum (FBS; GibcoBRL, 10439-024) and 1% (v/v) Penicillin/Streptomycin (P/S**;** GibcoBRL, 15070-063); (0.001% (v/v) Hygromycin B (GibcoBRL, 10687-010, 50mg/mL soln.) **required only** for the 293c7 cell line)
- Trypsin-EDTA (T/E; GibcoBRL, 25300-063)

The methods for freezing-down HEK 293 cell lines are as follows:

NOTE: The common practice up to this point has been to freeze-down one to two confluent 60-mm TC dishes (p60) of HEK 293 cells per cryovial. This protocol follows with that in mind.

1. Aspirate the medium from the confluent TC plate(s), keeping in mind that based on surface area, 1 x 100-mm dish = 2.8 x 60-mm dish and 1 x 150-mm dish = 6.25 x 60-mm dish.
2. Add Trypsin-EDTA solution: 1-2mL/p60, 3-6mL/p100, or 6-12mL/p150, and incubate for 3-5 minutes @ 37^{o}C and 5%CO_2.
3. Cells should be easily dislodged by gently tapping the side of the plate. Add DMEM w/ 10% FBS and 1%P/S (**NOTE:** supplemented with 0.001% Hygromycin in the case of the 293c7 cell line) to dilute the volume of Trypsin-EDTA added by four-fold, i.e. if you trypsinized 1 x p100 with 3mL of Trypsin-EDTA, add ~9mL of DMEM to neutralize the enzymatic activity.
4. Transfer the cellular solution into a polypropylene centrifugation tube (15 or 50mL BlueMax tube recommended) and spin down for 5 minutes @ 1000 rpm (Temp. setting = 23-25^{o}C (approximate room temperature)).
5. Aspirate off the supernatant, then replenish and resuspend the pellet in DMEM w/ 10%FBS and 1%P/S (supplemented w/ Hygromycin, if needed) as follows:
6. For the ATCC 293 cell line, add 0.9mL of DMEM per 2 x p60 trypsinized.
7. For the MI 293 or 293c7 cell lines, add 0.9mL of DMEM per 1 x p60 trypsinized.
8. Add in a drop wise fashion, DMSO, so that the final volume is 10% (v/v) DMSO, meaning, add 0.1mL of DMSO for every 0.9mL of medium added. Very gently invert the tube a few times to mix in the DMSO.
9. Into each pre-labeled cryovial, add 1mL of the cellular suspension. Make sure all the caps are tightly refastened.
10. Transfer the aliquoted vials to an empty 15mL Styrofoam BlueMax tube holder, and then align another empty holder on top to mirror the hole-pattern so it fits like a cap. With labeling-tape, securely fasten the top-holder to the bottom-holder sandwiching the vials within.
11. Store the "sandwich" container in the -80^{o}C freezer (in room 7731) for 16-24 hours for the initial freeze-down.
12. Quickly untape the "sandwich" and transfer (it is also a good idea at this point to check and make sure the vial caps are all still securely fastened, for they often loosen

during the initial freeze-down) to their predetermined storage location within the LN_2 tank (as of 02/07/2001, located in Ormond MacDougald's laboratory).

The methods for thawing and plating HEK 293 cell lines are as follows:

1. Warm DMEM w/ 10% FBS and 1%P/S to 37°C **before** thawing the cells. In the case of the 293c7 cell line, be sure to use DMEM w/10% FBS and 1%P/S, as well as 0.001% (v/v) Hygromycin solution.
2. Retrieve the vial of cells from the LN_2 tank storage, immediately starting the thawing process either by rubbing it between your hands, or incubating in a 37°C-water bath for approximately 2 minutes, or until thawed. **NOTE:** The next step is optional for the ATCC 293 cell line, but mandatory for either the MI or 293c7 cell lines for the removal of DMSO**. Draw up 9mL of DMEM w/10% FBS and 1%P/S (w/ or w/o 0.001% Hygromycin) into a 10mL-serological pipet, and then draw up the contents of the thawed cellular solution and transfer to a 15-mL BlueMax tube.
3. Spin down for 5 minutes @ 1000 rpm and ~23-25°C.
4. Aspirate the medium; resuspend the pellet in 10mL of DMEM w/10% FBS and 1%P/S (w/ or w/o 0.001% Hygromycin), then plate the cellular solution onto a 100-mm (p100) TC dish.
5. Incubate O/N @ 37°C and 5%CO_2.
6. The next day, aspirate off the existing medium and replenish with 10mL of DMEM w/10% FBS and 1%P/S (w/ or w/o 0.001% Hygromycin, as needed) to remove any cellular debris and/or residual DMSO.