HEK cell culturing:

Big petri dish holds: 10 million cells when 100% confluent
6-well dish holds: 1 million cells/well when 100% confluent

All cell work requires using aseptic technique. When transferring cells to and from incubator and culture hood, spray and wipe dishes with 70% ethanol. Never open dishes outside of the tissue culture hood.

To culture cells you will need:
DMEM (with 4.5g/L glucose, L-glutamine, and pyruvate), 10%FBS, 1mg/mL G418 & 5 ml Pen-Strep/500 ml DMEM bottle
0.05% Tryp/EDTA
1xPBS

1. First, put your Tryp/EDTA and DMEM in water bath to warm up. Then wash down the hood area and your gloves with 70% ethanol.
2. Aspirate out cell culture media.
3. Wash cells with 1x PBS (dispense PBS on side wall of dish, not directly onto cells). Aspirate PBS off.
4. Add 1 mL Tryp/EDTA directly to cells, rock plate gently to cover all cells, then put in incubator 5 min.
5. After 5 min, take cells out of incubator and check on microscope to see that cells have detached from plate. Add 4 ml DMEM to culture, triturate several times. Check to make sure majority of cells are single, then transfer into 15 ml conical tube.
6. Centrifuge on tissue culture setting.
7. When done, aspirate supernatant and resuspend pellet with media. Mix cells gently w/ media.
8. Take only a fraction of this (amount of cells you take out will depend on the confluency that you want to obtain), add to your new dish, and add more DMEM over top (big petri dishes should hold 10 ml solution total).
9. Now you are done and can store the cells in the incubator.

Notes:
Media has a shelf life. If your media is over 3-4 weeks old, your cells may not grow well.

Cells need to be split occasionally (1-2 times/week). Even when they are less than confluent. This is because, cells in the middle of clumps are starved for nutrients and will eventually send out cytotoxic factors into the medium. When splitting, you want to break the monolayer into single cells. After trypsinizing, the monolayer will lift off in sheets. This is fine. After quenching the trypsin by adding 4 mL media, you need to triturate w/ a P1000 10 times at least. Look at the cells under a microscope and see that the vast majority are single cells. If not, you may have to triturate more.

Don’t let your cells dry out. When washing cells or changing media, have the solution you will cover the cells with ready before aspirating the cells dry. Minimize the time your cells aren’t covered with solution.