Hi Mel!

Thank you again so so so so much for doing this, you are a gem. Did I mention you're welcome to my Anchor steam? You're welcome to my Anchor Steam, in the fridge and next to the fridge.

Here are dates w/ how I'd probably be doing them; please feel free to modify as you like/see fit, just let me know what you end up doing. t also turns out upon further examination that I fucked up the written lab notebook calendar, so…ignore it? Ish? This is way easier to read.

Relevant days in gray.

P19 day	**Date**	**action**
0	Sep 25	Plate new cells,
1	Sep 26 Friday	Switch 5 of 6 of the P19 dishes to 1% MEMalpha with 1 mM RA; the last dish stays on 5% to act as a stock
3	Sep 28 Sunday	Change media on all dishes Trizol lysing of one set of diff cells
6	Oct 1 Wednesday	Change media lyse one dish split stock P19s (maybe) note pass #, date, media type, cell type,
9	Oct 4 Saturday	Change media lyse one dish
	I get back October 5th! Yay!	
12	Oct 7 Tuesday	Change media lyse one dish split stock P19s

protocols for Baby Mel!

differentiating P19s

Why?

P19 stem cells differentiate into neuron-like cells when exposed to 1 mM trans-retinoic acid (RA). While RA technically only needs to be added for the first 3 days or so to set cell fate, we keep it on RA the whole time and on low levels of FBS to promote differentiation.

protocol

reagants

- retinoic acid (-20 C freezer, in the corner with trypsin; kept in tinfoil to prevent light exposure and degradation)
- MEMalpha with 1% FBS
- sparse-ish P19, preferably at a low pass number

do

1. Take off any current media (typically 5%) and wash the cells with PBS gently (if this is Day 0 of differentiation, otherwise no washing!). P19s throw off a lot of stuff.
2. Add 1.5 mls MEMalpha with 1% FBS
    - the less FBS, the better they differentiate, but also the faster they die. It's a balance.
3. Add 1.5 uls of the RA to the center of the dish; swirl!
    - a cool thing happens where the RA must be very hydrophobic, so a tiny clear spot will appear where you pipette it in
4. Do this again 3 days later!
    - P19s are delicate infants; please don't wash them if they're in the process of throwing out processes.

RNA extraction from P19s

Why?

Extracting, isolating, and quantiating RNA expression allows us to build a timeline of the "usual" P19 differentiation expression levels, and then compare it to what happens with the introduction of different transcription factors. Step 1: Kill cells, collect cell lysate to later do an RNA extraction en masse.

Do

1. Wash cells in 35mm dish 2x with PBS (wash= add ~1ml PBS and aspirate).
    - unless you're using delicate cells that don't need washing, like delicate differentiating P19s
2. Add 1 mL of TRIZOL Reagent to the dish and pipette up and down to make sure all the cells are really dead and really broken up.
3. Scrape TRIZOL/lysed cells into a 1.5 mL microcentrifuge; scrape! Get ALL of the stuff.
4. Incubate the homogenized samples for 5 minutes at room temperature
    - permits complete dissociation of nucleoprotein complexes.

Store the suspension @ -80 [in the Leeper box, green tape, top shelf next to the other mol. bio stuff] , label with date & any other pertinent information

Splitting P19s

why?

sometimes cells are too confluent and we need less of them! P19s grow fast, so even with a 1:9 split (no counting @ Hampshire), they need to be split every 5-6 days, and fed every three days.

do

reagants

- MEMalpha, with some percent FBS depending on your task. 5% for normal maintenance.
- trypsin

1. Aspirate media, rinse with PBS
2. Add .5 ml trypsin and wait for cells to shake loose
   - if you're not me, you'll actually let them hang out in the incubator and dissociate properly
3. Add 1.5 ml media to deactivate trypsin
4. add .2 mls cells and 1.8 mls media to each plate for a ~1:9 split