MIT Team Culturing Lab Notebook

Monday 7/1/19

Protocol: CULTURE OF HEK 293/293T CELLS

HEK 293 cell, the Human Embryonic Kidney 293 cells was originally derived from human embryonic kidney cells grown in tissue culture. HEK293 cells are available from ATCC (www.atcc.org), catalog# CRL-1573. It is easy to grow and transfect and have been widely used for cell biology research and also used by the biotechnology industry to produce therapeutic proteins and viruses for gene therapy.

Materials/Media Components:
- DMEM, high glucose (VWR Cat.# 10-013-CV)
- Heat Inactivated Fetal Bovine Serum (VWR, Cat.#35-010-CV)
- Dulbecco's Phosphate Buffered Saline (DPBS Invitrogen Cat.#14190-250)
- 0.05% Trypsin-EDTA 1X (Corning Cat.# 25-053-CI)
- Tissue Culture Dish (Corning, Cat.430599)
- Class II biological safety cabinet
- Hemocytometer
- Humidified 37 °C, 5% CO2 incubator
- Inverted microscope

Propagation of Cells
Culturing HEK293:

- Shiva taught us how to culture! Yay!
- The cells were super confluent.
- Detached cells and dispersed clumps.
    - Cells should be maintained between 10% and 90% confluency in a 37°C, 5% CO2 tissue culture incubator. This typically will require passaging the culture twice a week. The approximate cell number for 100% confluence for this cell line in a 150mm tissue culture dish is 2×10^7 cells. Volumes listed are for propagation in a 150mm tissue culture dish. When cells have reached the appropriate density, aspirate the medium from the flask.
        - Clean hood w/ ethanol; **All items (other than cells) that enter the hood must be sprayed with ethanol**
    - Wash 1 time with 10ml 1X DPBS.
    - Add 2ml of 37°C 0.05% Trypsin-EDTA. Evenly coat flask surface containing the cells. Trypsinize for 2 minutes.

        - Using a microscope, verify that the cells have detached and clumps have completely dispersed.
        - Stop trypsinization by adding 10 ml of growth medium.
            - Pipette up and down to prevent clumping
        - Transfer cell suspension to a conical tube. Determine cell number using a hemacytometer.
            - Use 10 ul for hemocytometer (mix of dye and cells, depending on confluence)
- Counted cells (30 uL dye + 30 uL cells, 10 uL per hemacytometer)- ~$50*10^5$ cells.
    - Count cells in 4 grids in hemocytometer, and then average them
        - cells/mL = avg cells * dilution factor * 10^4
- Prepared to replate cells
    - Pellet cells at 500 × g for 5 minutes at 25°C.
    - Aspirate the supernatant and resuspend cells in Growth Medium.
    - Seed new flasks at appropriate cell density depending on the size of flask. For example, use 1 × 10^6 cells for a 150mm TC dish.
- We plated 2 mL of cells and 8 mL of media for 1 million cells
    - Place dish in 5% CO2, 37°C incubator.
- IBIDI plating
    - Get IBIDI chambers and spray w/ ethanol; dry
    - Get 6-well-plate and stick chambers to middle of well plates with forceps
    - Make 10^5 cells/mL suspension of cells
    - Add 70 ul (~7 * 10^3 cells) of suspension to IBIDI chambers
    - Incubate at 37ºC

For Ibidi chamber and Glass ring, seed 10^5 cells/ml. Use 70 ul for Ibidi chamber and 100 ul for glass ring.

Lab

- Passaged HEK cells in two groups: G1 and G2 → Passage1
- Seeded cells in 2 Ibidi Chambers and 2 Glass Rings.
- For Ibidi Chambers, we used 100ul for one side and 50ul for the other.
- Group 2 - avg. # of cells: theoretically 6300/well plate in IBIDI for 70 ul (→ actually used 100ul and 50ul)

Tuesday 7/2/19

Lab:

- Transfect on Wednesday/Thursday
- Learn to make media
- Start culturing HL-60s next week
- Observed culture today

- Observed Ibidi and glass ring cell patterns: retained patterns well

	Initial Confluency	Seeded for passage/cells	Theoretical value: Seeded in Ibidi Chambers (70ul)	Confluency 7/2
Group 1 (G1)	>95%	2 x 10^6	7000 cells	40%
Group 2 (G2)	>90%	1 x 10^6	6300 cells	10%

IBIDI:

- Remove chambers with tweezers
- Add 2 mL of medium to each well plate
- View with microscope; borders should be clear and defined

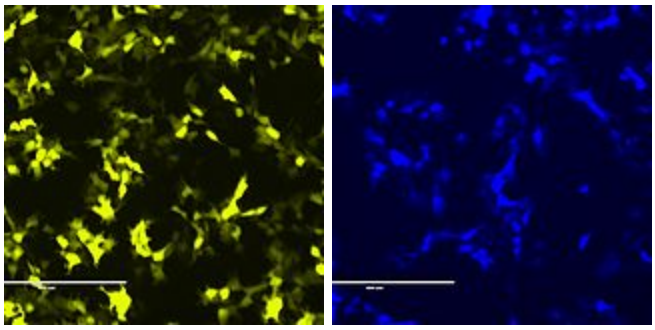
Wednesday 7/3/19

Protocol: HEK Transfection
Lab

- Learned how to do transfections

Fluorescent pL1 used	Stock DNA concentration (ng/ul)	Amount of DNA used (ul)	Amount of Viafect used (ul)
pIG_029 (Yellow)	55.5	5.4	16.2
pIG_030 (Red)	68	4.4	13.25
pIG_033 (Blue)	55.6	5.4	16.2

- Results: in transfection folder (Lab → Microscope Pictures → Transfections)



pIG_029 pIG_033

Friday 7/5/19

Protocol

- Passaging: Same protocol as Monday, minus cell counting

Lab

- Took pictures of transfections (pIG_029, pIG030, pIG033)
- Did passaging 1 for G2
- EYFP bled into green light channel
    - EYFP emits as green light as well
    - Need to consider spectral overlap in future experiments
    - Means iRFP transfection wasn't successful/it didn't work in the red light channel

Sunday 7/7/19

- G1 Hek cells→ 99% confluent
- G2 Hek cells→ 95% confluent
- Passaged G1 and G2 cultures, same protocol as Monday, minus cell counting

Monday 7/8/19

Lab:

- pIG_029 and pIG_033 were diluted to 100ng/ul and put in pL0 concentrated stocks (for group 2 transfection use) (Margaret)

Tuesday 7/9/19

Lab:

- Passaged group 1 and group 2's cells. Group 2's cells were plated with 1 mL of cells and 9 mL of pure media. Group 1's cells were plated with 2 mL of cells and 8 mL of media.

Wednesday 7/10/19

Plan for HL-60 culture:

- HL-60 Differentiation calendar
    - 5-6 day period
    - Tuesday-Saturday for Monday-Friday testing
    - 1.3% DMSO (2009 USCF and Aki Lab Protocol)

Differentiating Start Date	Tuesday	Wednesday	Thursday	Friday	Saturday
Testing Date	Monday	Tuesday	Wednesday	Thursday	Friday

Thursday 7/11/19

Protocol:
Lab:

- HEK cells confluency ~ 10% for both Group 1 and Group 2. Will check tomorrow if ready for transfection
- Mentors started the culture for HL-60 and Jurkat cells.
- HL-60s → 80% confluent.
- Jurkat cells → 5% confluent.
- We split the HL-60s into three flasks with different amount of cells:
    - Take cells + media out of flask and insert into 15 mL Falcon tubes
    - Spin cells down 100x for 10 minutes
    - Replace media with RPMI, 10% heat inactivated FBS
    - Add cells into flask and add appropriate amount of RPMI + HI FBS (5ml for each ml of HL60s)

Flask 1	3 ml of HL60s
Flask 2	2 ml
Flask 3	1 ml

- Froze rest of HL60s into stocks
    - Add 5% DMSO and freeze at -80C in ethanol bath

Friday 7/12/19

Protocol: Transfection of HEKs (see Shiva's protocol)

Lab:

- HEKs were transfected with TagBFP (pIG_029), EYFP (pIG_033) and mKate (from Shiva)
- Checked on HL60s— not very confluent

Saturday 7/13/19

Protocol:
Lab:

- Checked transfected HEK cells; transfections were successful
- HL-60s were not confluent enough for differentiation; the 2mL and 3mL were pooled together. Shiva will thaw another set and seed at higher concentration
- Jurkats still not very confluent
    - Didn't like heat-inactivated FBS
    - May get more cells from Jan

Monday 7/15/19

Lab:

- Passaged HEK cells → passage #6
- HL-60s flask 1 → media was pink → ~40% confluent
- HL-60s flask 2+3 → media was comparatively orange → ~70% confluent
- Attempted to count the cells with hemocytometer twice but only got an average of 1 cell per grid (corresponds to 2x10^4 cells/ml) but this doesn't match up with the confluency we saw.

Tuesday 7/16/19

Lab: Designed Media Compatibility experiment

Wednesday 7/17/19

Lab:

- Differentiated HL-60s.
    - Protocol called for RPMI-HEPES with 9% FBS. But we didn't have fresh RPMI media to make it so we used the RPMI- HEPES with 10% FBS that we already had.
- Ran our Media Compatibility Test for HEK cells. See Experiment Details.
    - scaled down to 10,000 cells and in 96 well plate (from original plan in 24 well
    - Used different RPMI with 10 mM HEPES and 10% FBS (instead of 25 mM HEPES, because we had to make sure there was no PenStrep)
    - Used pIG_029 (EYFP) for transfection

Thursday 7/18/19

Protocol:
Lab:
To Do:

- Check differentiation of HL-60s
- Check how our HEKs are doing
- Check on RPMI order
- Differentiate again tmr?

Friday 7/19/19

Protocol:
Lab:

- Passaged cells
- Took pictures of co-culture experiment - Ye Cheng
    - All wells were 100% confluent
    - Controls showed no fluorescence
    - Transfection efficiency of EYFP was similar for all ratios of DMEM/ RPMI
        - Place pictures here

Monday 7/20/19

To-do:

- ✓ Split HL-60s into multiple flasks
- ✓ Check HEK cells, differentiate if necessary
- ✓ Check differentiation
- ✓ Transfect HEKS with IL-8 constructs

Protocol:

Lab:

- Checked on cells:
    - HL-60s passaged on 7/17/19 were confluent
    - HL-60s differentiated on 7/17/19 were also confluent and some look differentiated? We're not sure.
    - Both have huge cell death populations

Tuesday 7/21/19

Protocol:

- See Transfection Protocol (from Shiva)
- https://docs.google.com/presentation/d/1Jz9OMOIheWHTib-NqcDaW6_Kf5v8jIyB2d5hFj0h9DA/edit#slide=id.g5e009bc5a6_0_0

Lab:

- Started IL8_NG secretion test:
    - Transfected HEK293 with IL8_NG and NG in 6-well-plates
- Split HEK cells → passage #9

Wednesday 7/22/19

To-do:

- ✓ Start growing HL-60s in greater amounts
- ✓ Differentiation

Protocol:

Lab:

- IL8_NG Secretion Test Check-up:
    - Transfection rate not as great as transfection with fluorescent proteins
    - Changed out media with serum-free media (DMEM with 1% NEAA, no FBS)

Thursday 7/23/19

Meeting Notes:

- Examined images → looks like IL8 is aggregating in the nuclei
- Message Shiva/Deepak about plate reader
- Talk to Shiva about what to do about HL-60s. We need to start culturing in large amounts
- Follow up with Cammie about T25 flasks
- Grow 15 T75 flasks at a time
- Read up about HL-60 cell density and how it affects viability
- Potentially use higher FBS conc. (source: Researchgate) ~ about 15-20% FBS
- Start writing up our part of the story.

- Start growing more HEK cells for next week

Protocol:

https://docs.google.com/presentation/d/1Jz9OMOIheWHTib-NqcDaW6_Kf5v8jIyB2d5hFj0h9DA/edit#slide=id.g5e009bc5a6_0_5

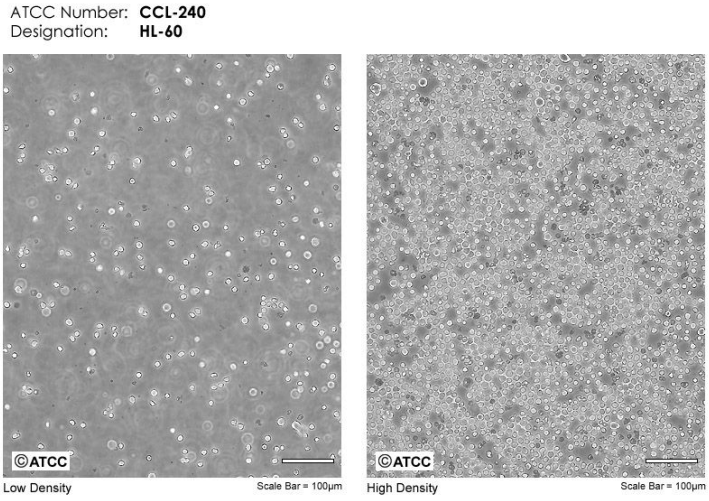
Lab:

- Filtered IL8-NG and NG control for test using AMICON Filters
- Completed the IL8_NG secretion test: Success! Found Neon Green in the supernatant of the transfected cells. But for some reason, there was no Neon Green in the supernatant of the control

Monday 7/29/19

Lab:

- Cultivating HL-60s has been difficult until now. We thawed a new vial last week and from now on, we will wait until it is more confluent
- Reference:



- (https://www.atcc.org/products/all/CCL-240.aspx#characteristics)

Tuesday 7/30/19

Lab:

To Do:

- Contact Cammie to get lysis buffer stuff by Friday
- Call Sigma. Ask about receiving protease inhibitors by Friday (and fibronectin)

Wednesday 7/31/19

Lab:

- Started IL8_NG secretion test #2
    - Added untransfected condition to our experiment
- Made a Calendar for culturing our different cell types

Thursday 8/1/19

Protocol:

- Second secretion test (with lysis buffer): https://docs.google.com/presentation/d/1gKggd6W_FGk-B0T_mJcZXoDLQsMMEnWiGMggcvAh6lk/edit#slide=id.p

Lab:

- Change out media with serum-free, phenol-red free media
- Split HL-60s
    - HL-60s should not be spun down
    - Can use media with serum
    - Need high density & large batches for Assay team
- Jurkats
    - Try to replace with serum-free media if possible
    - If Jurkats are ready
- Differentiate one batch
- Second secretion test

Plans for next week:

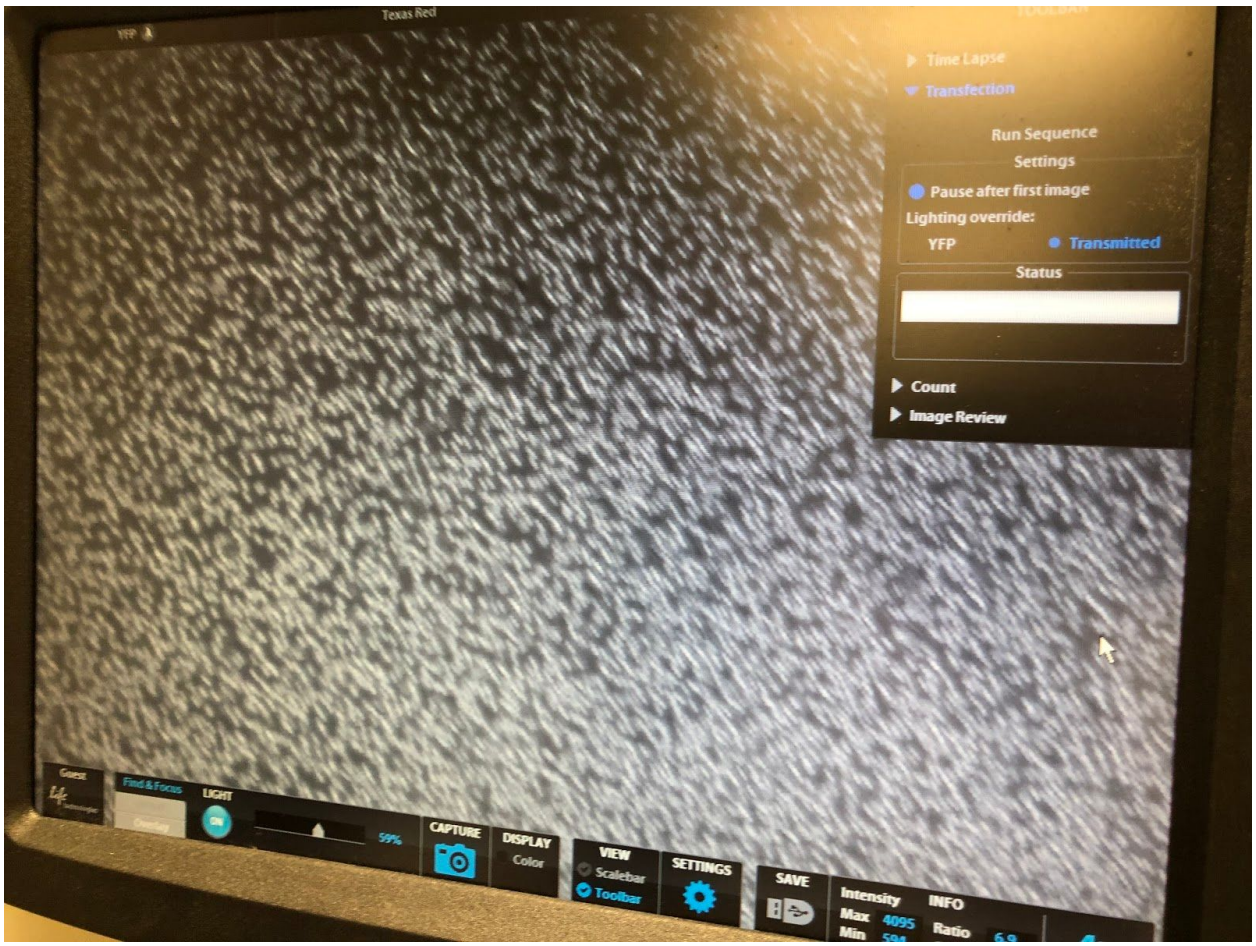
Thu Aug 1	Consult Shiva
Fri Aug 2	- HL60 differentiation - Split Jurkats
Sat/Sun	...
Mon Aug 5	- HEK transfection (IL8 and CCL5 only, no NG) - Jurkat FBS removal - HEK transfection into Ibidi chambers - Repeat HEK Media Compatibility Test
Tue Aug 6	- HEK transfection media change - Imaging Jurkats - Have Jan book the microscope

Wed Aug 7	- Imaging: FMLP - Boyden Chamber Assay -

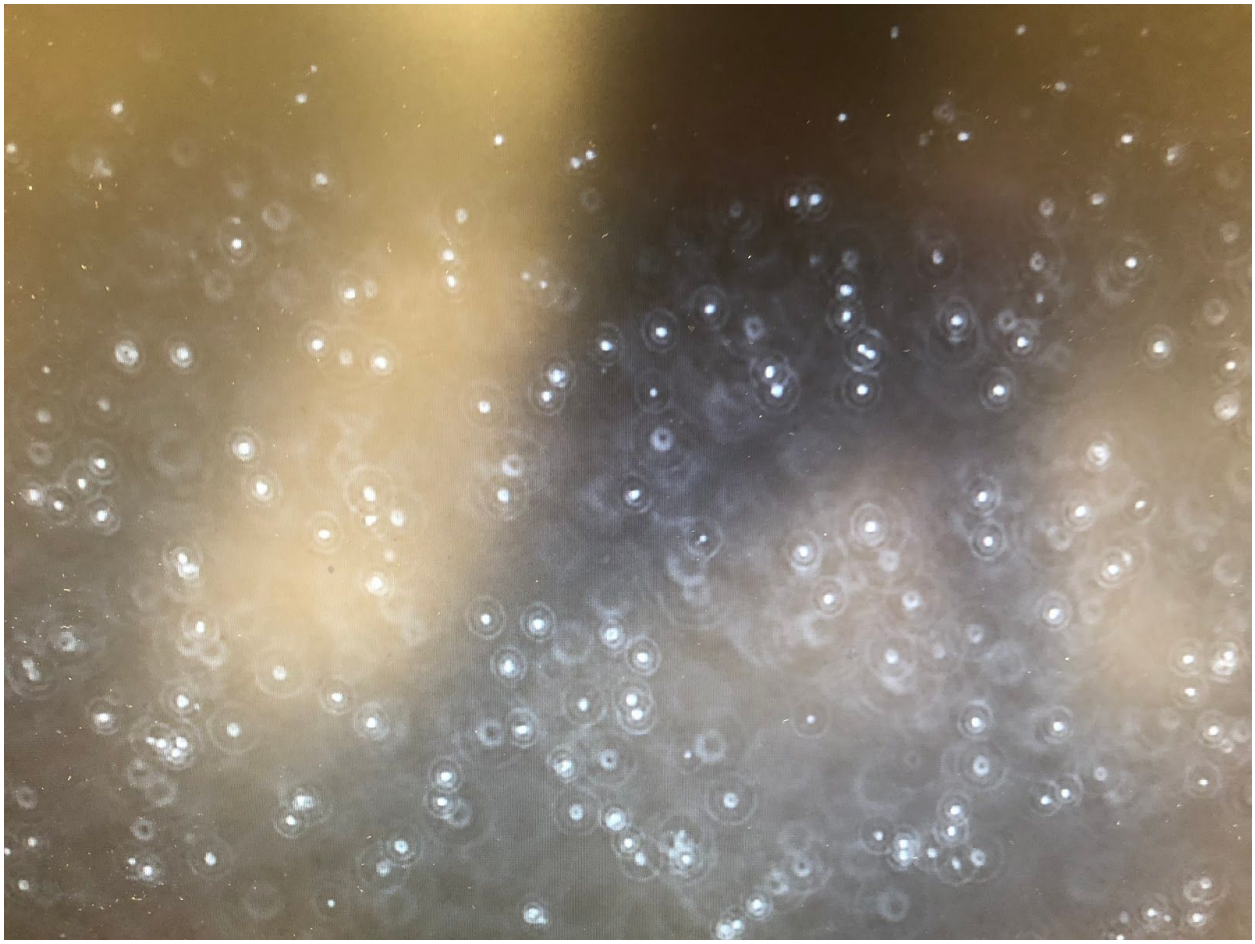
Friday 8/2/19

Protocol:

Lab:

- Differentiated 10 ml HL-60 at 2e+5 cells per mL
- HL60 sets (4 plates total; 2 diff. 2 not) set 1 was very confluent after changing media and ~8 hours
- 

- Cells from later passaging (set 2)

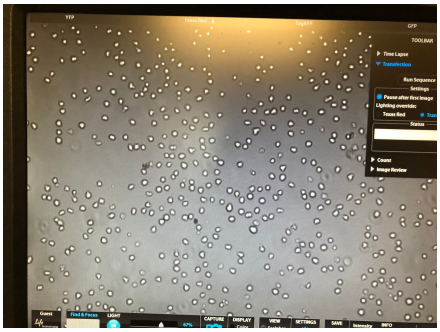
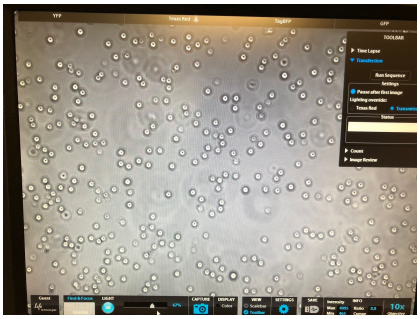
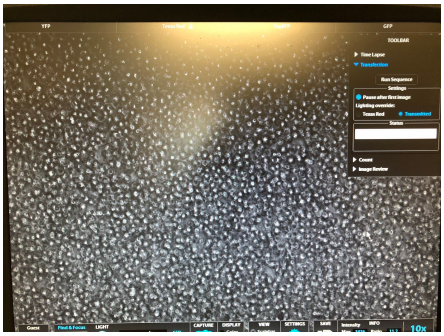
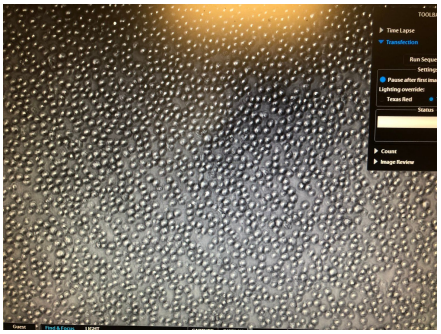


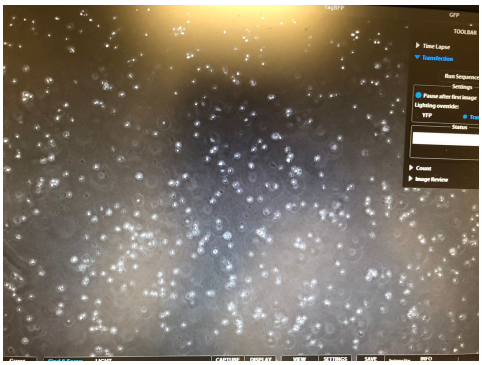
Monday 8/5/19

Protocol:
https://docs.google.com/presentation/d/1Npc7-dlzgqZd7b5wLq3_YMUnuL03sAYAaJUeeVPTffE/edit#slide=id.g5e75c79a31_0_185

Lab:

- Transfected HEKs (Ye Cheng)
    - Transfected in:
        - 6 well plates (3 wells each), seeded with 6.25e5 cells
        - Ibidi chambers (2 chambers each), seeded with 5e4 cells
    - Transfected with:
        - Both IL8 and NG
        - IL8_NG
        - Both CCL5 and NG
        - CCL5_NG
        - Included untransfected control
- Passaged HEKs (Ye Cheng)

- HEK transfection (cell count ~1.5*10^6 cells/mL)
- HL60 updates:
    - Saturday
        -  
        - 
        - 
            - For the two very confluent; split into two flasks (P4, 5) — not really passage because media was not changed
    - Sunday
        - Cells looked at relatively okay confluence
    - Today
        - HL60 undiff. iGEM (maybe ATCC? —need to ask Shiva)
            - Hemocytometer Counts: 10, 12, 13, 7
            - 21*10e4 cells
        - HL60 diff. iGEM
            - Counts: 2, 4, 3, 5
            - 7*10e4
    - Start differentiation on Friday if interested!
- Jurkats:
    - Low confluency according to Ye Cheng

- Split into two: starved (A) and unstarved (B) in T25s— should be moved to T75s if necessary today (ask Shiva)
- Hemocytometer count (done when in tubes)
  - A: 12, 17, 9, 6
  - A: 22*10e4 cells
  - B: 6, 6, 11, 3
  - B: 13*10e4 cells
- After putting into respective flasks ~3-4 hours later:
  - 

Tuesday 8/6/19

Protocol:

- Revised IBIDI transfection: slide 9 https://docs.google.com/presentation/d/1Npc7-dlzgqZd7b5wLq3_YMUnuL03sAYAaJUeeVPTffE/edit#slide=id.g5e75c79a31_0_66

Lab:

- Jurkats:
  - Unstarved T25: Look alright… but oddly not as confluent as starved T25
    - 2, 5, 2, 0, 1
    - 4*10e4 cells
  - Starved T25: lots of cells…
    - 3, 4, 3, 3
    - 7*10e4 cells
  - 50ul T75: Also not much growth/confluence
- HL60s:
  - Differentiated flasks looking super confluent
  - Undifferentiated flasks that were separated are starting to look super confluent
    - Wondering if it's a good idea to do one with 20% FBS and maybe one with 15% or 10% FBS to slow down growth?
  - Shiva froze down two cryovials of HL60s as undifferentiated flasks were very confluent —> **should follow up and ask about where she plans to store them!**

- Redid IBIDI transfections, this time mixing transfection mix with HEKs before adding to chamber (Ye Cheng)
- Replaced media in 6 well plate transfections
    - IL8 + NG, IL8_NG, untransfected - replaced DMEM with RPMI + 10% FBS
    - CCL5 + NG, CCL5_NG - replaced DMEM with RPMI

Wednesday 8/7/19

Protocol:

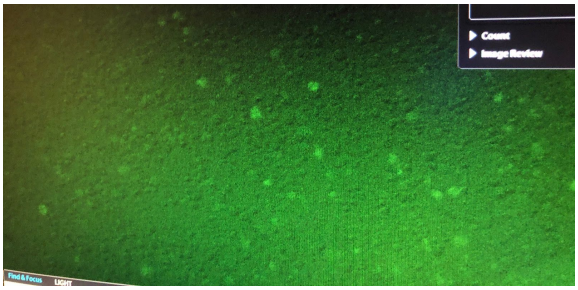
- Fibronectin coat/Electroporation

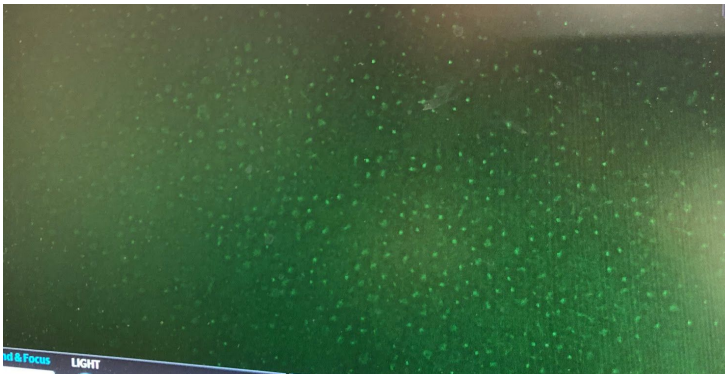
1. Coat 8 well chamber with 40 µg/mL Fibronectin and wait for 30 min at rt
2. Wash wells with DPBS and dry in tissue culture hood
3. Centrifuge 2.0 x 10^6 cells (100xg, 5–10 min, rt).
4. Resuspend cells with 100 µL Nucleofactor solution (Lonza Kit V)
5. Add 2 µg DNA and mix
6. Transfer cell mixture to cuvette
7. Electroporation (T-019)
8. Add 800 µL mHBSS (+0.2% BSA) immediately to cuvette (recipe of mHBSS is in the next page)
9. Transfer 405 µL cells for each well
10. Incubate cells in CO2 incubator (37˚C, 5%CO2) for 4–6 hours (Longer incubation doesn't enhance transfection efficienty file:///Users/hideakimatsubayashi_air/Downloads/21567.pdf)

8 well chamber: Nunc™ Lab-Tek™ 8-well Chambered Coverglass #155411

I tested a couple of media/buffer other than mHBSS. Cells didn't look healthy in DPBS (w/o BSA). RPMI (w/o FBS) and L-15 media with BSA (Orion's biorxiv protocol) looked good, but cells are motile even before fMLP stimulation (amino acids in the media could be stimulating the cells!?).

Lab:

- Differentiated flask iGEM P4 was used for boyden chamber assay (2x10^6 cells required per run) - Ye Cheng and Assay Team
- Transfection of HL60s by electroporation (see Shiva's HL60 protocols!): - Melody
    - In HBSS:
    - 
    - In RPMI + 20% FBS:

    - 
    - After running in flow cytometry, determined not much were actually transfected-- maybe 20%? (ask Nika)
- More HL60s were differentiated -- will be ready next Monday/Tuesday - Ye Cheng
    - 20 mL of 2.3e5 cells/ mL
- Starved a set of dHL60s - 4 mL of 2.6e5 cells/mL - Ye Cheng

Thursday 8/8/19

https://docs.google.com/spreadsheets/d/1Rad0Q88tSMvtBjQBRzKgg09WYDqTK4nz2CtXeAM0wAE/edit#gid=0
TO DO:

- HL60s Electroporation with synNotch parts: - Melody
    - 12, 16, 8, 10
    - 23*10^4 cells = 2.3*10^5 cells
    - Took 10mL 2x for electroporation
    - HL60s status: Still enough? may need to split/transfer to T75 today
- Jurkats
- HEKs

Friday 8/9/19

Update at some point…
https://docs.google.com/spreadsheets/d/1Rad0Q88tSMvtBjQBRzKgg09WYDqTK4nz2CtXeAM0wAE/edit#gid=0

- Results/Conclusion of HL60 Electroporation:
    - TRE EYFP high expression; not good
    - TagBFP low expression— curiously
- Results/Conclusion of HEK Transfection

    - Good; high transfection of HEKs with mKate, not sure about CD19 but assumption is they both worked
    - 

- Cell Statuses: - Melody
    - HL60s
        - Differentiated: 2 flasks
            - One flask looks slightly more confluent than the other (Day 2 now)
        - Undifferentiated: 3 flasks
            - All three are reaching full confluency, may need another day or two!
            - Weiss ATCC cells slowly recovering; needs another day or two but may need new media to be added
    - HEKs at 30% confluency
        - “We have stable landing pad (EYFP) HEK and CHO synNotch sender cells. Later tonight I will try to locate them and thaw them. In that case for HL60 you can use the TRE-mKate as the reporter and hef1-EBFP as your transfection marker - no need to transfect HEKs.” -Shiva
    - Jurkats
        - Some confluent, some not…
        - Small starved T25 surprisingly looks okay and getting very confluent - ~70% confluency
        - Other starved T75 also at 40% confluency…
        - Does starved affect Jurkat growth at all??
        - Unstarved at ~30% confluency; should come and check tomorrow or the day after!!

Saturday 8/10/19

- HEKs - Ye Cheng
    - Low confluency
    - Transfection was done with IL8 and CCL5
- HL60s - Melody
    - Two flasks confluent enough for differentiation for more experiments Thursday/Friday
    - One of the flasks was more confluent, split into 3 (~10-15mL) T25s— 2 of which were differentiated, 1 of which was not differentiated and passaged
    - Will need to check on all three T25s tomorrow!! Some may need to be moved to T75
    - Double check that number of differentiated cells is enough! If not, maybe differentiate the other one too?
- Jurkats - Melody
    - Unstarved at 80% confluency

Sunday 8/11/19

- HL60s: - Melody
    - T25s are confluent enough to be transferred (media is also orange looking now)
        - 
    - Differentiated T75 flasks are not too confluent so differentiation seems to be slowly happening; A looks more confluent than B though
        - Would it be possible to add more media if too confluent?
    - Note: seems like more media helps with growth

    - Differentiated ⅓ of another T75 flask
        - Split into 3 T25s, 2 of which were undifferentiated 1 of which was differentiated
    - One T75 still with undifferentiated HL60s; has not reached optimal confluency yet
    -

	differentiated	undifferentiated
T75	4	2
T25	1	2

    -
- Jurkats: - Melody
    - Look eh… maybe 60% confluency? not very confluent on either side although starved ones show more clumping???
- HEKs - Ye Cheng
    - Media for transfected HEKS (IL8 and CCL5) were replaced with FBS free RPMI

Monday 8/12/19

Protocol:
[Transfection protocol for HL60s]

Lab:
- HL60s
    - Concentrated HL60s differentiated on 8/9/19 T75 for Boyden chamber + under agarose assays - Ye Cheng
        - 2.5 mL of 1.0e5 cells/ 100ul
        - 1.5 mL of 2.34e5 cells/ 100ul
    - T75 MW - Melody
        - ~ 12.4*10^4 cells/ml (124,000 cells/mL); 500ul gives ~50,000 cells
    - Lipofectamine LTX with different color controls - Melody
        - https://docs.google.com/presentation/d/1OhNaKnmAFEQzGleau6HCiblHjg47vxBcHVeURmhZOMc
        -

	differentiated	undifferentiated
T75	4	4 (Inoue, one of which I added 10 mL too

		since it got too confluent)
T25	0	1 (Weiss)

- HEKs
    - Collected supernatant from HEKs transfected with IL8 and CCL5

- Jurkats
    -

	starved	unstarved
T75	1	1
T25	1	0

    -

Tuesday 8/13/19

Protocol:

- Neon Electroporation:
    - https://www.thermofisher.com/content/dam/LifeTech/migration/en/filelibrary/cell-culture/neon-protocols.par.55142.file.dat/hl-60-blood.pdf
    - https://tools.thermofisher.com/content/sfs/manuals/neon_device_man.pdf - pg 23

Lab:

- HL60s: - Ye Cheng
    - Neon Electroporation:
        - 2 x 10 ul of HL60s at 5 million cells/ mL
            - Transfected with 500 ng of NeonGreen
            - Control

	differentiated	undifferentiated
T75	4	4 (500ul of flask A)
T25	0	1 (Weiss)

- Jurkats: - Melody
    - None seem confluent enough still? Same amount of flasks as before. Don't know if we still plan to do anything with them as most assays are complete and focus is on IL8 and HL60s!
- HEKs: - Ye Cheng

Wednesday 8/14/19

Lab:

- HL60s:
  -

	differentiated	undifferentiated
T75	4	4
T25	0	1

  - Shiva split cells into multiple flasks for passaging as they were very confluent and differentiated some
- Jurkats:
  - N/A
- HEKs:
  - Transfect with IL8 + NG + NG control at low density (10^5)
    - Fibronectin coating
  - Transfect with IL8 only for Friday Boyden

Thursday 8/15/19

Lab:

- HL60s - Melody
  -

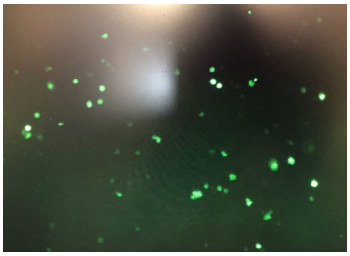
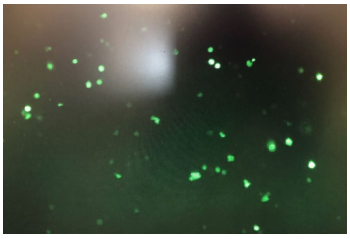
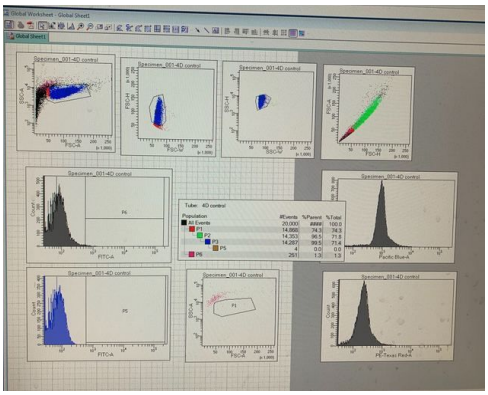
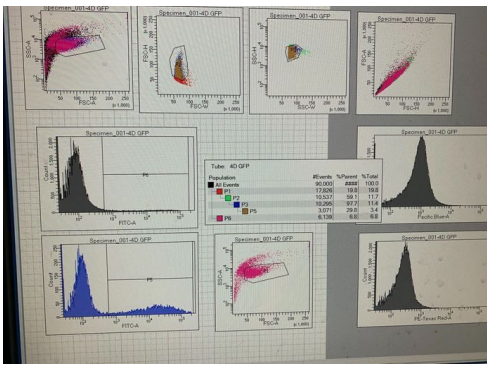
	differentiated	undifferentiated
T75	3	10
T25	0	0

  - transfected with Lonza protocol:
    - https://docs.google.com/presentation/d/1sFlqCtOMBKItNDmgDOJSosM4_MHXtjFHszZnMeFkbvM

Friday 8/16/19

Lab:

- Transfection relatively successful with pmaxGFP - Melody

- 
- 
- Checked by flow with Jan - Ye Cheng
  - 
  - 
  - See quantitative flow results pdf titled: iGEM-2019-08-16 flow batch export

Saturday 8/17/19

- Several flasks were aspirated (~3 undiff. And 3 diff.)

- 

- Blue = passaged today
- Green = not passaged; top undiff. 3 flasks were not passaged but 5mL media was added to help with growth
- Could potentially electroporate tomorrow/Monday with top undiff. 3 that were not passaged (since 8/15)
- one/two were trashed after getting too confluent on Sunday

Monday 8/19/19

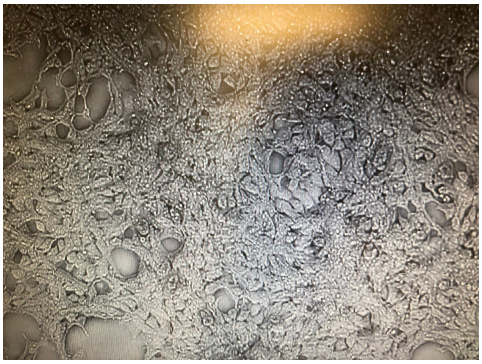
Protocol:
Lonza Transfection protocol:
https://docs.google.com/presentation/d/1sFlqCtOMBKItNDmgDOJSosM4_MHXtjFHszZnMeFkbvM

Lab:

- HL60s
    - Transfected ~ 2x10^6 * 6 cells with mKate, TagBFP, YFP, NG, pmaxGFP, and mKate + NG with Lonza electroporation protocol

	differentiated	undifferentiated
T75	2 (8/15), 3 (8/17)	7
T25	0	0

- HEKs
    - Probably confluent today and will need to be passaged!!

- 
    - 90% confluency!!
    - Transfect today too for imaging tomorrow!

- Jurkats
    - 30-40% confluency → Giving up?

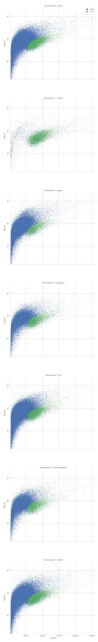
Tuesday 8/20/19

Lab:

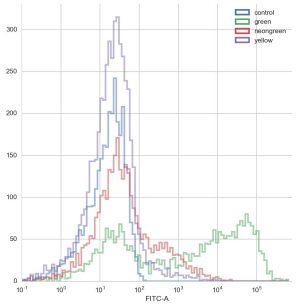
- HL60s:
    - Transfected cells were flowed with Jan's immense help
    - Current status:

	differentiated	undifferentiated
T75	2 (8/15), 3 (8/17)	7
T25	0	0

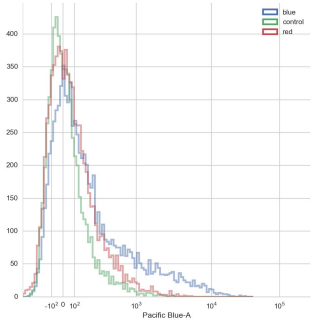
    - Most of these cells have already reached their passage limit, will need to thaw new batch (Wednesday morning/afternoon?)
    - Cell death (blue), live cells (green):



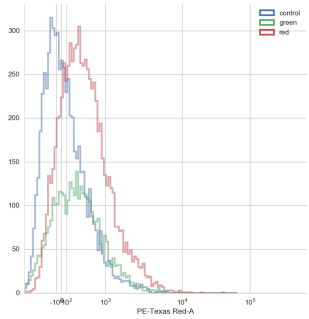
- Notes from Jan: I would conclude that control plasmid works quite well, mKate did not work, but the rest of our plasmids worked marginally. As you can see, the cells look quite unhappy after transfection, which is probably also related to the clumping that we see



- Log scale histogram for FITC, showing a nice peak transfected with GFP, and maybe also a peak in NeonGreen? - Jan



- Log scale histogram for mKate, GFP sample included as control

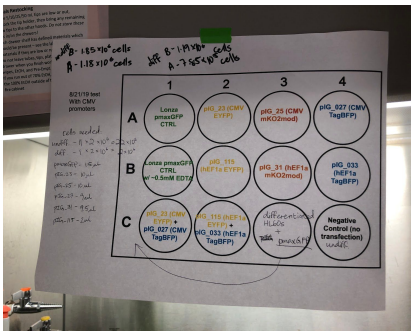
- 

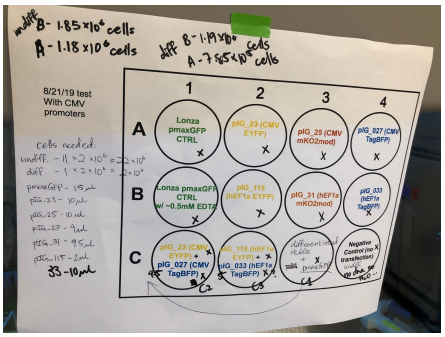
  Log scale histogram for mKate, GFP sample included as control

- ~~Jurkats:~~
- HEKs: passaged into two 10cm dishes; will need to be transfected for assays if assay team comes Friday

Wednesday 8/21/19

Lab:

- HL60s: - Melody
  - Did another transfection test (see 8/21 test layout)
    - Took 2 undifferentiated flasks for transfection (one of which was all used one of which I added
    - Took 1 differentiated flask (8/15) and took 300uL for transfection
    - 

    - 

  - Passaged rest of 3 flasks (P10 now)
  - Shiva thawed another set of HL60s
- Jurkats:
- HEKs:
  - Both plates are confluent and ready for transfection tomorrow

Thursday 8/22/19

Lab:

- HL60s
- HEKs: - Gabi
  - Passaged plates

Wednesday 9/4/19

Lab:

- Passaged HL60s - Melody (P6)

Thursday 9/5/19

Lab:

- Differentiated HL60s - Melody (P7)
- Differentiated count- 2 T25s
- Undifferentiated count- 2 T75s