MIT Team Assay Lab Notebook

Tuesday 7/23/19

Lab

- Boyden chamber was abandoned due to many unknown factors
- The inserts do not fit into regular 24 well plates
- Only have 0.25% Trypsin EDTA
- Played with insert, not as sensitive as imagined
- Sealed original chamber plate with parafilm for future use

Thursday 8/1/19

Lab

- ✓ Coated 8 wells A1-4 and B1-4 with 2ug/cm^2 of fibronectin
- ✓ Incubated in 37 degrees for one hour
- ✓ B4 does not have enough FN+PBS Coating and extra PBS during washing
- ✓ Washed with PBS and stored in PBS
- ✓ Sealed with parafilm in TC 4 degrees fridge

Monday (NEGEM) 8/5/19

Lab

- ✓ Coated 10 wells with fibronectin (A1-A5 and B1-B5) note B5 was last - Miles
    - 1 hour 37C
    - 4ug per well

Tuesday 8/6/19

Lab

- ✓ Coated 8 wells with fibronectin (A1-A4 and B1-B4) note B4 was last
    - ✓ 1 hour 37C
    - ✓ 4ug per well

Wednesday 8/7/19

Lab

- ❑ Boyden Assay: followed procedure as written, but incubated for 2 hours (same as UCSF)
    - ✓ Wells:

.1 nM IL8	1 nM IL8	10 nM IL8	100 nM IL8	100 nM fMLP	RPMI (20% FBS)
.1 nM IL8	1 nM IL8	10 nM IL8	100 nM IL8	100 nM fMLP	RPMI (20% FBS)
.1 nM IL8	1 nM IL8	10 nM IL8	100 nM IL8	100 nM fMLP	RPMI (20% FBS)

Thursday 8/8/19

Lab

Small Boyden Chamber, HL-60

	RPMI	10% FBS	10nM IL-8	100nM fmlp	RPMI No cell
Cells starved right before	RPMI	10% FBS	10nM IL-8	100nM fmlp	RPMI No Cell

- ❏ Chemoattractant dilutions
  - ✓ IL8 (highlighted made, others improved calculations)
    - ❏ 200 uL of 1000 nM into 1800 uL RPMI without FBS for 100 nM solution
    - ❏ 30 uL of 1000 nM into 2970 uL RPMI without FBS for 10 nM solution
    - ❏ 10 uL of 1000 nM in 9990 uL RPMI without for 1 nM solution
    - ❏ 200 ul of 1 nM into 1800 uL of RPMI without FBS for 0.1 nM solution
  - ✓ Fmlp
    - ❏ Work with 100 uM solution
    - ❏ Desired concentration: 100 nM
    - ❏ 1/1000 dilution factor
    - ❏ 10 ul of 100 uM solution in 9990 ul of RPMI in 20% FBS
  - ✓ Disposed of chemoattractants w/FBS
  - ✓ CCL5
    - ❏ Made 1000 nM stock in water
    - ❏ 100 uL into 900 uL RPMI w/out FBS for 100 nM CCL5 (made 3 mL)
    - ❏ 10 uL into 990 uL RPMI w/out FBS for 10 nM CCL5 (made 3 mL)
    - ❏ 1 uL into 999 uL RPMI w/out FBS for 1 nM CCL5 (made 3 mL)
    - ❏ 1 uL into 9999 uL RPMI w/out FBS for .1 nM CCL5 (made 10 mL, 10 last-little under volume)

Monday 8/12/19

Lab

- ✓ Made dilutions of iL-8, 3000 ul of each concentration

- ✓ Made 100 nM iL-8 working stock with water
- ✓ Boyden chamber with iL-8 concentration between 0.1 - 100 nM, RPMI, RPMI + 10% FBS, and supernatant
- ✓ Supernatant in A1 does not have enough cells
- ✓ IL-8 stock was not mixed well, very diluted
- ✓ Use fmlp as positive control instead of 10% FBS in the future
- ✓ Used unfiltered supernatant and had readings of over 7000, most likely read the dead hek cells
- ✓ In future, filter supernatant, remake iL8, use fmlp as positive control
- ✓ Use rest of iL8 for supernatant test at three dilutions

Tuesday 8/13/19

Lab

- ✓ Under agarose assay
    - ✓ 100nm fmlp - cells - media
    - ✓ Made 6 wells of agar plates
    - ✓ A1 cut out successfully

Wednesday 8/14/19

Lab

- ✓ Boyden Chamber
    - ✓ B1 and E1 did not have enough media when transferring to fluorescent reader
    - ✓ Did 0.1% and 0.01% dilution of iL8 stock as well as fmlp and rpmi

Thursday 8/15/19

Lab

- Imaging of hek and HL60s on fibronectin coating
- 10^5 hek cells per well
- Imaging for 2 hour, every 30 sec
- Replacing RPMI media right before seeding HL60s
- Let HL60s set for 15 minutes
- Test