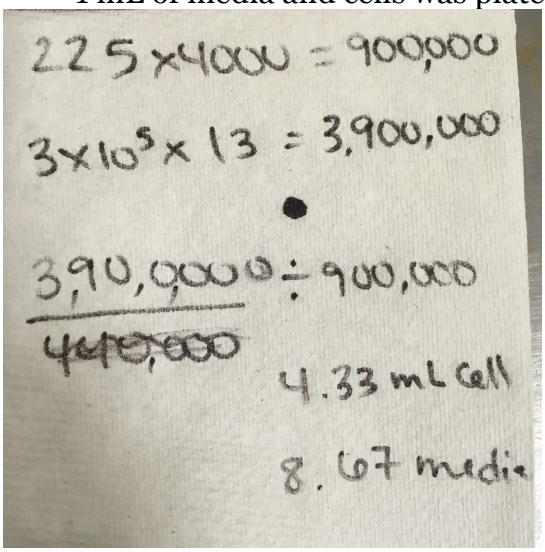
Week 2

June 13, 2016

What We Did Today:
- Made buffers
- Split big flask into big 1:5 and into 2 small 1:20
- Plated 12 well

Plating 12 Well:
- First counts were too high so rediluted with 10 mL of media
- New Counts:
  - 237
  - 322
  - Avg. 280
- 3X10^5 Calculations
  - 280 x 4000 = 1.12x10^6 cells/mL
  - 13 wells x 3x10^5 = 3.9x10^6 cells in 13 mL
  - 3.9/1.12 = 3.48 mL of cells
  - 13-3.48 = 9.52mL media added to cells
- 1 mL of media and cells was plated into each wells in the 12 wells plate



Buffer creation:

- 2xHebs (200ml)
  - Mixed 2.00g of HEPES with 3.39g of NaCl
  - Dissolved in 190mL of sterile water
  - Adjusted pH to ~7.1 by adding drops of 1N NaOH
  - Adjusted volume to 200mL by adding sterile water
  - Filtered with .22µm disposable filter

70mM Na_2HPO_4 (200mL)
Dissolved 5.01g $Na_2HPO_4.12H_2O$ with 200mL of sterile water
Filtered with .22µm disposable filter

2.5M $CaCl_2$ (200mL)
Dissolved 73.5g of $CaCl_2.12H_2O$ in 200mL of sterile water
Stored at -20°C

1/10 TE buffer (1mM Tris-HCL and .1mM EDTA)
100 mL of sterile water
100 µL of 1M Tris-HCL
40 µL of .25M EDTA

General Lab Work
Transformation of pmCherry C3 and pTagRFP C1 into DH5α *E.coli* cells on KAN plates
Maintenance of HEK293T cell cultures

MG, JW
Split cell 1:4
Cell count:
294, 232 Average: 263
Plated in 12 wells at $3*10^5$ cells/mL

- Confluence: >100%, discard
- Split cell culture (Matthew's culture, 1:4)
- Cell count
  Total count: count 1 = 294, count 2 = 232
  - 4 mL cell + 9 mL media
  - Plate in 12 wells at $3*10^5$ cells/mL

June 14, 2016

12 Well DNA Transfection Protocol

1.) CaPhos-DNA Mix
   a.) 50 µL 2.5 M CaCl2
   b.) 10 µg total DNA (5 µg each plasmid)
      I. I.) GFP: Use either pEGFPN3 -> 5 µg = 7.7 µL or pEGFPC1 -> 5 µg = 5.7 µL
      II. II.) RFP: Use PTagRFPC -> 5µg = 5.7 µL
   c.) q.s. to 500 µL with 0.1X TE
   d.) Pipet up and down to mix
2.) Add 1 equal volume (500 µL) of 2X HBS solution
3.) Pipet up and down to mix (volume of tube is now 1 mL)
4.) Incubate at room temp for 1 min
5.) Add carefully 100 µL each to 3 wells (this will be 1 µg total DNA per well)
6.) Add carefully 50 µL each to 3 wells (this will be 0.5 µg total DNA per well)

What We Did Today:

Transfected cells using GFP (pEGFPC1) and RFP
TouchTomorrow Data
Streaked InterLab colonies to new plates (Light Pink and Brown caps)
Sequenced rat APOBEC-XTEN-dCas9

Buffer Creation continued:

2xHBS (50mL) *all done in hood*

50mL of 2xHebs
1mL of Na_2HPO_4
Filtered with .22µm disposable filter

<u>General Lab Work</u>

Transfection of pTagRFP and eGFP into HEK293T cells
Maintenance of HEK293T cell cultures
Transformation of pmCherryC1 and pTagRFPC3 plasmids into 23716 *E.coli* cells

• Confluence: ~50%
• Transfection: pEGFP N3, pTag RFP
   • DNA 5 µg each (7.7 µL GFP, 5.7µL RFP)
   • Plated 100 µL of mixture in 3 wells, 50 µL in 3 wells
   • Change media after 4 hours

June 15, 2016

What We Did Today:

- Transformed pEGFPC1 and pEGFPN3
- Transformed InterLab Study brown and light pink cap (using 5 μL DNA for each)
- Prepared new transfection plates
- Took pictures of Transfection plate with florescent microscope
- Made more art
- Split cells 1:5
- Named our team (RICE CRISPRS)

Cell Count Math

- Count = 250 cells
- 250 x 4000 = 1,000,000 cells/mL
- 3x10^5 x 13 = 3,900,000
- 3,900,000/1,000,000 = 3.9 mL of cells
- 13 – 3.9 = 9.1 mL media

<u>GM, JW</u>
Liquid culture: pmCherry C3, pTag RFP C1 (2 each)
5mL LB in each tube
5uL antibiotic (Kan) in each tube
Pick colony and inoculate into conical tube
Shake for a day

Made 100 mL cell culture media (JW)
GM:
Wells: 80% confluent
1:20 flasks: 40%
1:5 flash: frozen for stock

Cell counts:
J: 228
G: 241
Avg: 234.5
Avg * 4000 = 938000
7*(3*10^5)=2.1*10^6 in 7mL
2.1/.938=2.238 mL of cells in 7mL
7-2.24= 4.76 media in 7mL
1mL per well 6 well

- Confluence: 90%
- Split culture
- Liquid culture: pmCherry C3, pTag RFP C1 (2 each)
- Cell count: $9.38*10^5$ cells/mL
  - Plated in 6 wells at $3*10^5$ cells/mL
- Made 100 mL new media

June 16, 2016

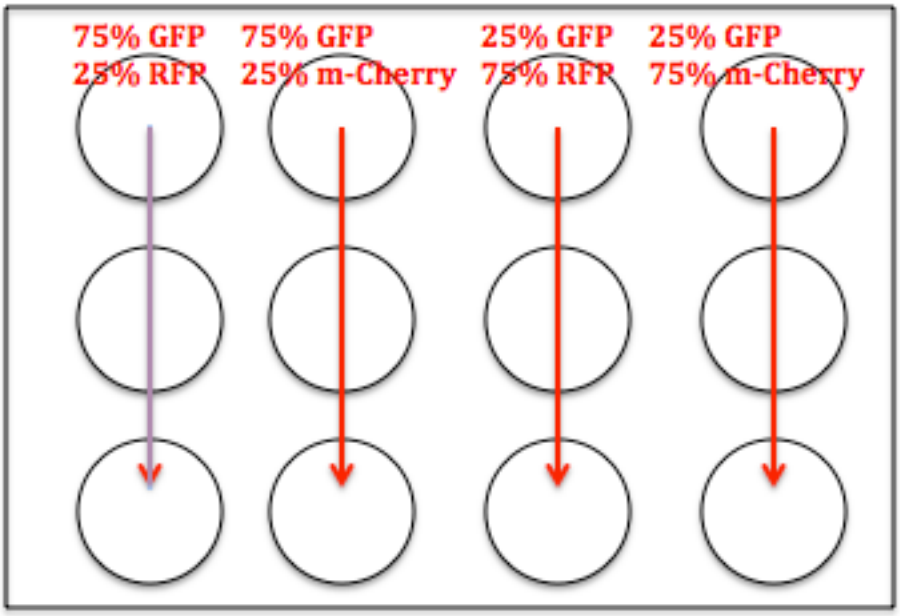
What We Did Today

- TouchTomorrow Report
- Poured Kan1 plates
- Transformed GFP plasmids
- Transfected cells with GFP and RFP
- Mini-Prep

Transfection for One Well

1.) 5 µL CaPhos
2.) 1 µg total DNA
3.) Dilute to 50 µL with 0.1x TE
4.) add 50 µL 2x HBS

Transfections for Today



GFP = 0.691 µg/µL
RFP = 0.87 µg/µL
M-Cherry = 0.54 µg/µL

Protocols Used:

- 75% GFP and 25% RFP
  1. 1.) 15 µL CaPhos
  2. 2.) 3.256 µL GFP

3. 3.) 0.862 µL RFP
4. 4.) Dilute to 150 µL with 0.1x TE
5. 5.) Add 150 µL 2x HBS

75% GFP and 25% m-Cherry
1.) 15 µL CaPhos
2.) 3.256 µL GFP
3.) 1.389 µL m-Cherry
4.) Dilute to 150 µL with 0.1x TE
5.) Add 150 µL 2x HBS

25% GFP and 75% RFP
1.) 15 µL CaPhos
2.) 1.085 VL GFP
3.) 2.586 µL RFP
4.) Dilute to 150 µL with 0.1x TE
5.) Add 150 µL 2x HBS

25% GFP and 75% m-Cherry
1.) 15 µL CaPhos
2.) 1.085 µL GFP
3.) 4.167 µL m-Cherry
4.) Dilute to 150 µL with 0.1x TE
5.) Add 150 µL HBS

GM, JW
Made glycerol stock of pTag C1 RFP and pmCherry
Mini-prep:
pmCherry: 17.6 ng/µL
pTag: 32.3 ng/µL (JW)
pmCherry 1: 54.0 ng/µL
pmCherry 2: 87.5 ng/µL

Transfection: 1 µg DNA/well
pmCherry only, 3 wells (GM)
pTag only, 3 wells (JW)
Liquid culture: pmCherry w/ kan added, 8 tubes

Transfections: (GM/JW)
Same protocol as before except:
Plated one row 100% pmCherry
one row 100% p-Tag

Transfection per one well:
5uL $CaPO_4$
1ug total DNA

Dilute to 50uL with .1xTE buffer
add 50uL 2xHBS

Use nanodrop to calculate total concentration and calculate off of that

pmCherry= .54ug/uL
1.85uL plasmid = 1ug DNA
pmCherry 3 wells:
15uL $CaPO_4$
5.56uL DNA
130uL .1xTE
150uL HBS

pTag = .87µg/µL
1.15µL plasmid = 1µg DNA
pTag 3 wells:
15µL $CaPO_4$
3.45µL DNA
131.55µL .1xTE
150uL HBS

- Glycerol stock of pTag C1 RFP
- Miniprep
  - pmCherry: 17.6 ng/µL
  - pTag: 32.3 ng/µL
  - (Discarded)
- Transfection: pTag RFP
  - 1 µg/well, plated in 3 wells
- Liquid culture:
  - pmCherry, 8 tubes with kan

June 17, 2016

What We Did Today
- Mini-Prep m-Cherry plasmids
- Split Cells
- Photographed Transfections
- Pet Dog

• Miniprep
  - pmCherry: 17.3 ng/μL
• Photo of 6/16 transfection: 50/50 w/ pmCherry, GFP, pTag, pmCherry
  - No significant bleed-over detected

GM:
Mini prepped pmCherry:
20ng/uL everyone got really low results (because of A4 buffer not containing ethanol)

Split 1:20 flasks to 1:10
forgot to bang one of the flasks after trypsin so some cells still stuck on flask

Took pictures of transfections
Checking for bleeding into other channels
Pictures on drive