
McDonald Lab

Lab Protocols and Daily Log

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Part I

Lab Protocols

1 Location of Common Materials

| Item | Location | Storage |
|-------------------------------|--|---|
| (In)complete Medium 199 | Incomplete Medium 199 is located in the cold room in the hallway immediately outside the lab. | Complete and serum-free Medium 199s are stored in the 4°C freezer in R2048. |
| Penicillin/Streptomycin Stock | P/S stock is located in the −20°C freezer in the hallway immediately outside the lab. | Leftover stock is stored in the 4°C freezer in R2048. |
| L-Glutamine Stock | Stock is located in the −20°C freezer in the hallway immediately outside the lab. | Leftover stock is stored in the 4°C freezer in R2048. |
| Amphotericin B stock | Stock is located in the −20°C freezer in the hallway immediately outside the lab. | Leftover stock is stored in the 4°C freezer in R2048. |
| 0.05% Trypsin-EDTA | Stock is located in the −20°C freezer in the hallway immediately outside the lab. | Leftover stock is stored in the 4°C freezer in R2048. |
| Trypsin (2mg/mL) | Stock is located in the −20°C freezer in the hallway immediately outside the lab. | Leftover stock should be refrozen in a box labelled with your name. |
| Fetal Bovine Serum | Stock is located in the −20°C freezer in the hallway immediately outside the lab. | Leftover stock is stored in the 4°C freezer in R2048. |
| (In)complete 2x EMEM | Stock is located in the cold room in the hallway immediately outside the lab. | Complete EMEM stock is stored in the 4°C freezer in R2048. |
| SeaPlaque Agarose | Stock is located in a jar above the benches immediately outside of the biosafety cabinet. | Leftover stock should be replaced where you found it. |
| 10x PBS | Stock is located in a plastic bottle above the benches immediately outside of the biosafety cabinet. | Leftover stock should be replaced where you found it. |
| SA11 Stock | Stock is located in the −20°C freezer in the hallway immediately outside the lab. | Leftover stock should be replaced where you found it. |
| Natural Red | Stock is located in the 4° freezer in R2048. | Leftover stock should be replaced where you found it. |
| Lentiviral stock | Stock is located at the −80°C freezer in the hallway immediately outside the lab. | Leftover stock should be replaced where you found it. |
| Polybreen | Stock is located in the −20°C freezer in the hallway immediately outside the lab. | Leftover stock should be returned where you found it. |

2 Recording Work and Labelling Materials

All solutions should be labeled with your name, the date of preparation, and what the solution contains. All cell flasks should be labeled with your name, the date of preparation, the type of cell contained, and the

passage of cell contained.

All work completed in the lab should be recorded in a laboratory notebook. Each page should contain entries from only a single day. The date of entry should be recorded at the top of each page. All writing must be easily readable and written in pen. Any errors should be struck through with a single solid line with the correction appearing next to it. Any space on a page not used at the end of a work day should be clearly crossed out in pen.

3 Procedures for Autoclaving

Ensure that all items have autoclave tape (if necessary). Place all items into the plastic bin found on the cart next to R2048. Take to the autoclave room. Add an autoclave quality indicator strip to the bin and insert into the autoclave. Close the door and select the appropriate options based on what is being sterilized. Start the cycle and fill out the autoclave use form on the bench next to the machine. Once the cycle is complete, retrieve the bin using the thick insulated gloves found in the lab (next to where the bin is stored). It is normal for there to be a small amount of water in the base of the bin.

4 Preparation of Medium 199 (Serum-Free)

Items Needed:¹

1. Incomplete Medium 199 (500mL)
2. Penicillin/streptomycin stock (5mL)
3. Amphotericin B stock (1mL; 250 μ g/mL)

In the biosafety cabinet, supplement 500mL incomplete Medium 199 with 5mL P/S stock and 1mL amphotericin B stock. Store at 4°C for up to 3 months.

5 Preparation of Medium 199 (Complete)

Items Needed:²

1. Incomplete Medium 199 (500mL)
2. Penicillin/streptomycin stock (5mL)
3. Amphotericin B stock (1mL; 250 μ g/mL)
4. Fetal bovine serum (55mL)

In the biosafety cabinet, supplement 500mL incomplete Medium 199 with 5mL P/S stock, 1mL amphotericin B stock, and 55mL fetal bovine serum. Store at 4°C for up to 3 months.

6 Preparation of 1.2% Agarose

Items Needed:

1. SeaPlaque agarose
2. Milli-Q filtered water

To a 500mL flask add 1.2g agarose for every 100mL water. Inadvisable to fill flask to more than 400mL. Cap and shake. Loosen lid. Apply autoclave tape to the lid. Autoclave approx. 20 min.

¹The paper *Culturing, Storage, and Quantification of Rotaviruses* advises using different quantities of some materials below. Nonetheless, the following are the recommended quantities for use in lab.

²See Note 1.

7 Preparation of 2x EMEM (Serum-Free)

Items Needed:

1. incomplete 2x EMEM (500mL)
2. 200mM L-glutamine (10mL)
3. P/S stock (10mL)
4. 250 μ g/ml amphotericin B stock (1mL)

In the biosafety cabinet, to 500mL incomplete 2x EMEM stock add 10mL L-glutamine, 10mL P/S stock, and 1mL amphotericin B stock. Store at 4°C for up to 3 months.

8 Preparation of PBS (Phosphate Buffered Saline)

Items Needed:

1. 10x PBS (80mL)
2. milli-Q water (720mL)

To a large graduated cylinder add 80mL 10x PBS solution (use a 25mL pipet). Fill the graduated cylinder to 800mL with milli-Q-filtered water. Apply autoclave tape to the lid. Autoclave for 30 minutes.

9 Procedure for Splitting MA104 Cells

Items Needed:

1. 1x PBS
2. 0.05% Trypsin-EDTA
3. Complete Medium 199
4. 150cm² flask

In a water bath, warm PBS, Trypsin, and complete Medium 199 to 37°C. Transfer all materials into the biosafety cabinet. Tilt the flask with your cells (having formed a confluent monolayer) such that the culture medium runs towards the neck of the flask. Vacuum out all culture medium using a small glass pipet connected to the rubber vacuum hose. Dispose of the pipet. To the cell culture, add 10mL 1x PBS. Tilt the flask to ensure that all cells are thoroughly covered by PBS. Aspirate using a small glass pipet. Dispose of the pipet.

To the cell culture, add 5mL Trypsin, tilting the flask forward and back to ensure that the cells are fully bathed. Vacuum out the Trypsin with a small glass pipet. Add a second 5mL portion of Trypsin to the cell culture. Incubate at 37°C until all cells have detached from the surface and are free-floating. Lightly tap the flask with your hand if any cells remain attached.

To the cell culture add 15mL complete Medium 199. (If you used less trypsin in the previous step, adjust the amount of Medium 199 applied such that there is 20mL solution in the flask.)

To each new flask that you wish to prepare, add complete Medium 199 such that the final flask volume after the cell mixture is added will be equal to 25mL. To determine the volume of cell mixture to add to each new flask, we use Table 1.1.

Cap the new flask(s) and tilt forward and back to evenly spread the cells. Loosen the lids and incubate at 37°C.

Table 1.1

| Cell dilution ratio | Cell mix volume to add |
|---------------------|------------------------|
| 1:2 | 10mL |
| 1:4 | 5mL |
| 1:8 | 2.5mL |

Note. For example, if we wished to prepare one 1:4 dilution and two 1:8 dilutions, to our first flask we would add 15mL complete Medium 199 and 10mL cell mixture; to each of our second two flasks we would add 20mL complete Medium 199 and 5mL cell mixture.

10 Procedure for Plating MA104 Cells

Items Needed:

1. 1x PBS
2. 0.05% Trypsin
3. Complete Medium 199
4. Trypan Blue
5. 6-well plates (x4)

Aspirate the cell culture medium from the flask. Wash the cell monolayer in 10mL 1x PBS and aspirate from the flask. Wash the cell monolayer in 5mL trypsin and aspirate from the flask. Bathe the cell monolayer in 5mL trypsin and incubate at 37°C until all cells have detached from the flask. (Check every 2 minutes.) Tap the flask with your hand to detach any remaining cells.

To the flask add 15mL complete Medium 199 and mix thoroughly with the pipet. On a piece of parafilm, combine 10 μ L cell mixture and 10 μ L trypan blue. Mix thoroughly with a pipet. To a slide, add 20 μ L such that the slide is completely filled with liquid. Insert into the automatic cell counter and record the result (total cells; live cells; percent alive). Use this to extrapolate the number of cells in the T150 flask and in each well as follow:

$$\begin{aligned}\frac{\text{cells}}{20\text{mL flask}} &= \frac{\text{cells}}{\text{mL}} \cdot \frac{20\text{mL}}{\text{flask}} \\ \frac{\text{cells}}{75\text{mL conical vial}} &= \left(\frac{1}{2} \cdot \frac{\text{cells}}{\text{flask}} \right) / \frac{75\text{mL}}{\text{conical vial}} \\ \frac{\text{cells}}{\text{well}} &= \frac{\text{cells}}{75\text{mL}} \cdot \frac{3\text{mL}}{\text{well}}\end{aligned}$$

To a 100mL conical vial add 65mL complete Medium 199. Supplement this with 10mL cell solution. (These values may be doubled if 8 plates are being prepared. A T75 flask can accommodate 4 6-well plates; a T150 flask 8 6-well plates.) Transfer the cell mix to each of the wells, adding 3mL cell solution per well. Spread the cells by tilting forward and back. Incubate the plates at 37°C for several days.

11 Procedure for activating RV SA11 and Infecting MA104 Cells

Items Needed:

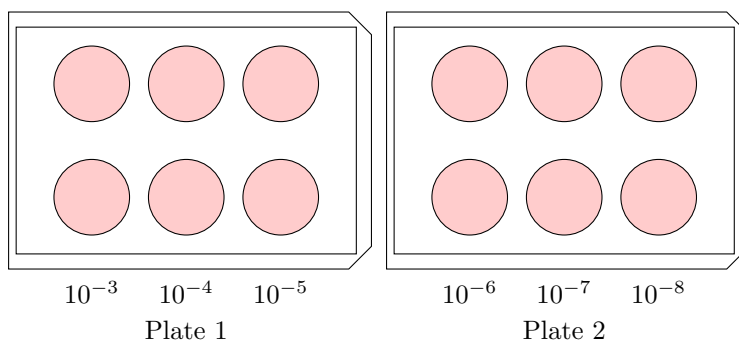
1. SA11 stock
2. Trypsin (2mg/mL)
3. Serum-free Medium 199
4. 2x EMEM

5. 1.2% agarose

In a large Eppie tube combine 400 μ L rotavirus SA11 stock and 2 μ L trypsin. Cap and incubate in a 37°C water bath for 1 hour. To each of 8 15mL plastic tubes add 2.7mL serum-free Medium 199.

Add 300 μ L virus mix to the first tube and label as 10^{-1} . Mix the solution by vertex to homogenize. Take 300 μ L of the 10^{-1} solution and add to the second tube. Label 10^{-2} , cap, and mix by vertex. Repeat this process across the remaining tubes, ending with a viral concentration of 10^{-8} . Store in the 4°C freezer if the cells are not going to be immediately infected. This is enough for 2 6-well plates. (Quantities may be increased proportionately if more than 2 plates are being infected at once.)

To 2 6-well plates, add the rotavirus titers. Each titer is to be performed in duplicate. To achieve this, add ~ 1 mL of the 10^{-8} titer to two wells, then 10^{-7} titer to two wells, and so on. You will end with two plates as follow:



Incubate the plates at 37°C for 1 hour.

Liquefy a jar of 1.2% agarose using a microwave. Warm 2x EMEM to 37°C in a water bath. For each plate infected, prepare a solution of 10mL agarose, 10mL 2x EMEM, and 5 μ L trypsin. (Add the EMEM first, followed by the trypsin, followed by the agarose. Do not add trypsin to hot agarose.)

One plate at a time, aspirate the viral inoculant. (You may use a new pipet for each well or may use one pipet per plate, aspirating from the lowest concentration well to highest.) Quickly add to each well ~ 3 mL of the agarose solution. Place the lid on the well and allow it to sit, undisturbed, for approximately 30 minutes (or until the agarose overlay has solidified). Incubate for several days at 37°C.

12 Procedure for Performing RV Plaque Assay

Items Needed:

1. Neutral red
2. 2x EMEM
3. 1.2% Agarose

Liquefy 1.2% agarose by microwave. For 4 6-well plates, mix 15mL 2x EMEM, 15mL 1.2% agarose, and 1.5mL neutral red. Add 1mL solution to each well and allow it to solidify. Incubate the plates at 37°C and count the plaques that have formed after 4 – 24 hours of incubation.

13 Procedure for Transfecting MA104 Cells with siRNA-Expressing Lentiviral Vectors

Items Needed:

1. 1000x Polybrene
2. Lentiviral vector

3. Non-silencing vector (control)
4. Complete M199
5. 0.05% Trypsin
6. 1x PBS
7. Trypan blue

This procedure should be performed when the plated MA104 cells are 70 – 80% confluent.

Select one well to use for a cell count. Aspirate the cell culture medium from this well. Add 1mL PBS, spread evenly, and aspirate. Add 500 μ L trypsin, spread evenly, and aspirate. Add 500 μ L trypsin, spread evenly, and incubate the plate at 37°C until all cells have detached from the well.

To the well add 1.5mL complete M199 and mix well. Take 10 μ L cell mix and combine with 10 μ L trypan blue. Apply this mixture to a slide and determine the number of cells per mL. Normalize this to the number of cells per well. (I.e., double the cell count per mL for the 2mL cell soln in the target well.)

Calculate the dilution factor for the lentiviral vector by:

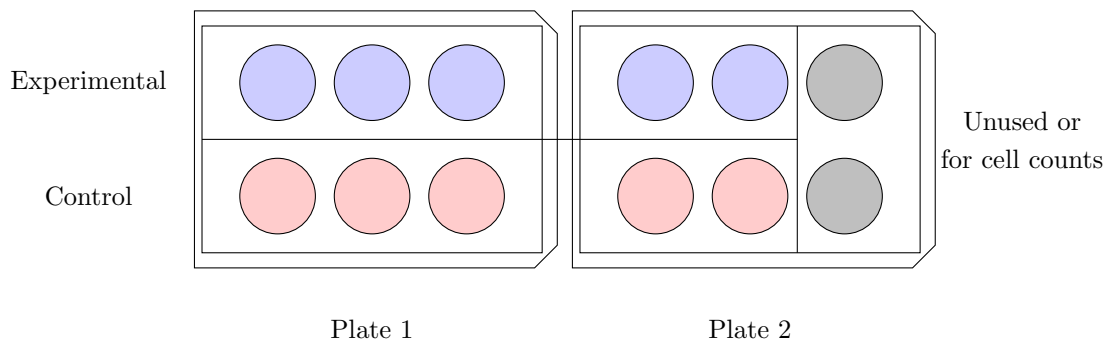
$$\left(\frac{\text{cells}}{\text{well}} \cdot \text{MOI} \right) / [\text{lentivirus}]$$

For example, if there are 1.86×10^5 cells per well, your original lentiviral concentration is 2.13×10^9 particles per mL, and you want to transfect your cells with a multiplicity of infection (MOI) of 10:

$$\begin{aligned} \text{dilution} &= \left(\frac{1.86 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}} \right) / \frac{2.13 \times 10^9 \text{ particles}}{\text{mL}} \\ &= \frac{1.86 \times 10^6 \text{ particles}}{\text{well}} / \frac{2.13 \times 10^9 \text{ particles}}{\text{mL}} \\ &= \frac{0.00087 \text{ mL}}{\text{well}} = \frac{0.87 \mu\text{L}}{\text{well}} \end{aligned}$$

Per well, prepare a solution of 1mL complete M199, 1 μ L polybrene (diluted by a factor of 1000), and the calculated volume of lentiviral vector. Repeat this procedure for the control NSV.

Aspirate the cell culture medium from each control and experimental well. To the experimental wells, add with one pipet 1mL lentiviral solution to each well. To the control wells, add with a second pipet 1mL control solution to each well. The final plates, using 5 control and 5 experimental wells, may look similar to the following:



Incubate these plates for 2 hours at 37°C. After 2 hours, add to each of the experimental wells 2mL complete M199. Using a second pipet, add to each of the control wells 2mL complete M199. The final volume of medium in each well should be 3mL. Incubate for 48 hours at 37°C.

14 Procedure for Infecting Cells Following Lentiviral Transfection

Items Needed:

1. Serum-free M199
2. Complete M199
3. SA11 stock
4. 0.05% Trypsin
5. Trypsin (2mg/mL)
6. Trypan blue
7. 1x PBS

Select one well to use for a cell count. Aspirate the cell culture medium from this well. Add 1mL PBS, spread evenly, and aspirate. Add 500 μ L 0.05% trypsin, spread evenly, and aspirate. Add 500 μ L 0.05% trypsin, spread evenly, and incubate the plate at 37°C until all cells have detached from the well.

To the well add 1.5mL complete M199 and mix well. Take 10 μ L cell mix and combine with 10 μ L trypan blue. Apply this mixture to a slide and determine the number of cells per mL. Normalize this to the number of cells per well. (I.e., double the cell count per mL for the 2mL cell solution in the target well.)

Calculate the dilution factor for the SA11 stock by:

$$\left(\frac{\text{cells}}{\text{well}} \cdot \text{MOI} \right) / [\text{SA11}]$$

For example, if there are 2.36×10^5 cells per well, your original SA11 stock concentration is 5×10^7 PFU per mL, and you want to infect your cells with a multiplicity of infection (MOI) of 5:

$$\begin{aligned} [\text{SA11}] &= \frac{5 \times 10^7 \text{ PFU}}{1\text{mL}} \\ \text{dilution} &= \left(\frac{2.36 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{5 \text{ particles}}{1 \text{ cell}} \right) / \frac{5 \times 10^7 \text{ PFU}}{1\text{mL}} \\ &= \frac{23.6\mu\text{L}}{\text{well}} \end{aligned}$$

Activate SA11 by combining 400 μ L SA11 stock and 2 μ L 2mg/mL trypsin. Incubate in a 37°C water bath for 1 hour. Prepare a vial of SA11 diluted in serum-free M199. 1mL of solution will be applied to each well. It is recommended to prepare a single solution containing a volume sufficient for inoculation of all transfected wells.

Wash all transfected wells twice using serum-free M199 by dumping. Add to each well 1mL viral inoculant. Spread evenly by shaking and incubate for 1 hour at 37°C.

Prepare a solution of 3mL serum-free M199 and 0.75 μ L 2mg/mL trypsin per well. Aspirate the viral inoculant from each well and replace with 3mL of the serum-free M199 solution just prepared. Spread evenly by shaking and incubate plates at 37°C overnight.

15 Procedure for Lysing Transfected Cells Following RV Infection

Freeze transfected cells completely at -80°C for approx. 30 minutes. Remove from freezer and allow to thaw. Refreeze cells overnight in -20°C freezer.

The following day, remove cells from the freezer and allow to thaw fully. Pipet the contents of each well into its own 15mL tube, cap tightly, and label. Use a new pipet for each solution transfer. Centrifuge the solutions at 500RPM for 10 minutes at 4°C.

Collect the supernatant from each centrifuged solution in its own fresh tube. Cap, label, and store at -20°C until you are ready to activate the viral content and infect plated cells.

Part II

Lab Notes

16 September 2014

02 September 2014

1. Prepared serum-free Medium 199
 - (a) Supplemented 500mL incomplete Medium 199 (incomplete M199) with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100 μg /mL streptomycin final concentration) and 1mL 250 μg /mL amphotericin B stock (0.25 μg /mL amphotericin B final concentration)
 - (b) Stored at 4°C
2. Prepared complete Medium 199
 - (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100 μg /mL streptomycin final concentration) and 1mL 250 μg /mL amphotericin B stock (0.25 μg /mL amphotericin B final concentration), 55mL fetal bovine serum
 - (b) Stored at 4°C
3. MA104 cell split — Passage 59
 - (a) A T75 flask was split by Shu from her maintained stock, labeled, and incubated at 37°C

03 September 2014

1. Prepared 2x EMEM, serum-free
 - (a) Supplemented 500mL 2x EMEM stock with 10mL 200mM L-glutamine stock (4mM L-glutamine final concentration), 10mL penicillin/streptomycin stock (200U/mL penicillin; 200 μg /mL streptomycin final concentration), and 1mL 250 μg /mL amphotericin B stock (0.5 μg /mL amphotericin B final concentration)
 - (b) Stored at 4°C
2. Prepared 400mL 1.2% agarose
 - (a) Combined 4.8020g SeaPlaque agar to $\sim 400\text{mL}$ milli-Q-filtered water
 - (b) Autoclaved for 20 minutes
 - (c) Stored at room temperature

09 September 2014

1. MA104 cell split — Passage 60, from Passage 59, Flask A
 - (a) Aspirated cell culture medium
 - (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
 - (c) Rinsed cells in 2mL 0.05% trypsin; aspirated trypsin
 - (d) Bathed cells in 2mL 0.05% trypsin
 - (e) Incubated cells at 37°C until all cells detached from flask

- (f) Added 18mL complete M199 to flask
- (g) Added to 3 T150 flasks 15mL complete M199/10mL cell mix; 20mL complete M199/5mL cell mix; and 20mL complete M199/5mL cell mix. Respectively, flasks A, B, and C
- (h) Gently shook flasks to distribute cells evenly
- (i) Incubated at 37°C

12 September 2014

1. Plated MA104 cells — Passage 60, Flask A

- (a) Aspirated cell culture medium
- (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
- (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
- (d) Bathed cells in 5mL 0.05% trypsin
- (e) Incubated cells at 37°C until all cells detached from flask
- (f) Added 15mL complete M199 to flask
- (g) Took cell count by combining 10μL cell mixture with 10μL trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{3.91 \times 10^5 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{\text{flask}} &= \frac{3.91 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} = \frac{7.62 \times 10^6 \text{ cells}}{20\text{mL}} \\
 \frac{\text{cells}}{10\text{mL cell mix}} &= \frac{7.62 \times 10^6 \text{ cells}}{20\text{mL}} \cdot \frac{1}{2} = \frac{3.81 \times 10^6 \text{ cells}}{10\text{mL}} \\
 \frac{\text{cells}}{75\text{mL vial}} &= \frac{3.81 \times 10^6 \text{ cells}}{75\text{mL}} = \frac{5.08 \times 10^4 \text{ cells}}{\text{mL}} \\
 \frac{\text{cells}}{3\text{mL well}} &= \frac{5.08 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} = \frac{1.52 \times 10^5 \text{ cells}}{\text{well}}
 \end{aligned}$$

- (h) Added 65mL complete M199 and 10mL cell mixture to 125mL conical vial for final volume of 75mL
- (i) Transferred 3mL solution to each well of 4 6-well plates
- (j) Spread cells evenly by shaking
- (k) Incubated at 37°C

15 September 2014

1. MA104 cell split — Passage 61, from P60, Flask B

- (a) Aspirated cell culture medium
- (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
- (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
- (d) Bathed cells in 5mL 0.05% trypsin
- (e) Incubated cells at 37°C until all cells detached from flask
- (f) Added 15mL complete M199 to flask
- (g) Added to 3 T150 flasks 20mL complete M199/5mL cell mix; 22.5mL complete M199/2.5mL cell mix; and 22.5mL complete M199/2.5mL cell mix. Respectively, flasks A, B, and C

- (h) Gently shook flasks to distribute cells evenly
 - (i) Incubated at 37°C
2. Prepared 1x PBS
- (a) Combined 80mL 10x PBS and 720mL milli-Q-filtered water in a 1L graduated cylinder
 - (b) Transferred to a 1L bottle
 - (c) Autoclaved for 30 minutes
 - (d) Stored at room temperature
3. Rotavirus activation and series dilution for P60A plated cells
- (a) Combined 400 μ L SA11 rotavirus stock and 2 μ L trypsin
 - (b) Incubated for 1 hour in a 37°C water bath
 - (c) Added 2.7mL serum-free M199 to each of 8 15mL tubes
 - (d) To the first tube, added 300 μ L rotavirus solution to a final concentration of 10^{-1}
 - (e) Mixed contents by vertex
 - (f) To the next tube, added 300 μ L rotavirus solution from the previous tube to a final concentration of 10^{-2}
 - (g) Mixed contents by vertex
 - (h) Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
 - (i) Stored at 4°C

16 September 2014

1. Plated MA104 cells — Passage 60, Flask C
- (a) Aspirated cell culture medium
 - (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
 - (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
 - (d) Bathed cells in 5mL 0.05% trypsin
 - (e) Incubated cells at 37°C until all cells detached from flask
 - (f) Added 15mL complete M199 to flask
 - (g) Took cell count by combining 10 μ L cell mixture with 10 μ L trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{2.16 \times 10^5 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{\text{flask}} &= \frac{2.16 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &= \frac{4.32 \times 10^6 \text{ cells}}{20\text{mL}} \\
 \frac{\text{cells}}{10\text{mL cell mix}} &= \frac{4.32 \times 10^6 \text{ cells}}{20\text{mL}} \cdot \frac{1}{2} &= \frac{2.16 \times 10^6 \text{ cells}}{10\text{mL}} \\
 \frac{\text{cells}}{75\text{mL vial}} &= \frac{2.16 \times 10^6 \text{ cells}}{75\text{mL}} &= \frac{2.88 \times 10^4 \text{ cells}}{\text{mL}} \\
 \frac{\text{cells}}{3\text{mL well}} &= \frac{2.88 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &= \frac{8.64 \times 10^4 \text{ cells}}{\text{well}}
 \end{aligned}$$

- (h) Added 65mL complete M199 and 10mL cell mixture to 125mL conical vial for final volume of 75mL

- (i) Transferred 3mL solution to each well of 4 6-well plates
 - (j) Spread cells evenly by shaking
 - (k) Incubated at 37°C
2. Rotavirus infection of P60A plated cells
- (a) Washed monolayer in each well twice with serum-free M199 by dumping
 - (b) Added $\sim 1\text{mL}$ rotaviral solution to wells in concentrations from 10^{-3} to 10^{-8} with 2 wells being infected at each concentration of rotavirus
 - (c) Spread cells evenly by shaking
 - (d) Incubated at 37°C for 1 hour
3. Agarose overlay of P60A plated cells
- (a) Liquefied 1.2% agarose by microwave and equilibrated to 55°C
 - (b) For each plate of cells, prepared 1 vial of 10mL agarose, 10mL 2x EMEM, 5 μL 2mg/mL trypsin
 - (c) Aspirated viral inoculant from each well
 - (d) To each well, added $\sim 3\text{mL}$ agarose solution
 - (e) Let agarose solidify
 - (f) Incubated at 37°C

19 September 2014

1. MA104 cell split — Passage 62, from Passage 61, Flask A
- (a) Aspirated cell culture medium
 - (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
 - (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
 - (d) Bathed cells in 5mL 0.05% trypsin
 - (e) Incubated cells at 37°C until all cells detached from flask
 - (f) Added 15mL complete M199 to flask
 - (g) Added to 3 T150 flasks 20mL complete M199/5mL cell mix; 22.5mL complete M199/2.5mL cell mix; and 22.5mL complete M199/2.5mL cell mix. Respectively, flasks A, B, and C
 - (h) Gently shook flasks to distribute cells evenly
 - (i) Incubated at 37°C
2. Neutral red overlay of P60A plated cells
- (a) Prepared 2 vials, each with 7.5mL 1.2% agarose, 7.5mL 2x EMEM, and 600 μL neutral red
 - (b) To each well of 2 6-well plates, added $\sim 1\text{mL}$ prepared agarose solution (using 1 vial per plate)
 - (c) Let agarose solution solidify
 - (d) Incubated at 37°C for 4 hours
 - (e) Observed $3 \times 10^6 \text{PFU/mL}$

21 September 2014

1. Rotavirus activation and series dilution for P60C plated cells
 - (a) Combined 400 μ L SA11 rotavirus stock and 2 μ L trypsin
 - (b) Incubated for 1 hour in a 37°C water bath
 - (c) Added 2.7mL serum-free M199 to each of 8 15mL tubes
 - (d) To the first tube, added 300 μ L rotavirus solution to a final concentration of 10^{-1}
 - (e) Mixed contents by vortex
 - (f) To the next tube, added 300 μ L rotavirus solution from the previous tube to a final concentration of 10^{-2}
 - (g) Mixed contents by vortex
 - (h) Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
 - (i) Stored at 4°C

22 September 2014

1. Rotavirus infection of P60C plated cells
 - (a) Washed monolayer in each well twice with serum-free M199 by dumping
 - (b) Added \sim 1mL rotaviral solution to wells in concentrations from 10^{-3} to 10^{-8} with 2 wells being infected at each concentration of rotavirus
 - (c) Spread cells evenly by shaking
 - (d) Incubated at 37°C for 1 hour
2. Agarose overlay of P60C plated cells
 - (a) Liquefied 1.2% agarose by microwave and equilibrated to 55°C
 - (b) For each plate of cells, prepared 1 vial of 10mL agarose, 10mL 2x EMEM, 5 μ L 2mg/mL trypsin
 - (c) Aspirated cell culture medium from each well
 - (d) To each well, added \sim 3mL agarose solution
 - (e) Let agarose solidify
 - (f) Incubated at 37°C

25 September 2014

1. Prepared complete Medium 199
 - (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100 μ g/mL streptomycin final concentration) and 1mL 250 μ g/mL amphotericin B stock (0.25 μ g/mL amphotericin B final concentration), 55mL fetal bovine serum
 - (b) Stored at 4°C
2. Plated MA104 cells — Passage 61, Flask B
 - (a) Aspirated cell culture medium
 - (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
 - (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
 - (d) Bathed cells in 5mL 0.05% trypsin
 - (e) Incubated cells at 37°C until all cells detached from flask

- (f) Added 15mL complete M199 to flask
- (g) Took cell count by combining 10 μ L cell mixture with 10 μ L trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{3.56 \times 10^5 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{\text{flask}} &= \frac{3.56 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &= \frac{7.12 \times 10^6 \text{ cells}}{20\text{mL}} \\
 \frac{\text{cells}}{10\text{mL cell mix}} &= \frac{7.12 \times 10^6 \text{ cells}}{20\text{mL}} \cdot \frac{1}{2} &= \frac{3.56 \times 10^6 \text{ cells}}{10\text{mL}} \\
 \frac{\text{cells}}{75\text{mL vial}} &= \frac{3.56 \times 10^6 \text{ cells}}{75\text{mL}} &= \frac{4.75 \times 10^4 \text{ cells}}{\text{mL}} \\
 \frac{\text{cells}}{3\text{mL well}} &= \frac{4.75 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &= \frac{1.42 \times 10^5 \text{ cells}}{\text{well}}
 \end{aligned}$$

- (h) Added 65mL complete M199 and 10mL cell mixture to 125mL conical vial for final volume of 75mL
- (i) Transferred 3mL solution to each well of 4 6-well plates
- (j) Spread cells evenly by shaking
- (k) Incubated at 37°C

3. Neutral red overlay of P60C plated cells

- (a) Prepared 2 vials, each with 7.5mL 1.2% agarose, 7.5mL 2x EMEM, and 600 μ L neutral red
- (b) To each well of 2 6-well plates, added \sim 1mL prepared agarose solution (using 1 vial per plate)
- (c) Let agarose solution solidify
- (d) Incubated at 37°C for 4 hours
- (e) Agarose was applied too hot and plaques observed were not sufficient

26 September 2014

1. Rotavirus activation and series dilution for P61B plated cells

- (a) Combined 400 μ L SA11 rotavirus stock and 2 μ L trypsin
- (b) Incubated for 1 hour in a 37°C water bath
- (c) Added 2.7mL serum-free M199 to each of 8 15mL tubes
- (d) To the first tube, added 300 μ L rotavirus solution to a final concentration of 10^{-1}
- (e) Mixed contents by vertex
- (f) To the next tube, added 300 μ L rotavirus solution from the previous tube to a final concentration of 10^{-2}
- (g) Mixed contents by vertex
- (h) Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
- (i) Stored at 4°C

2. MA104 cell split — Passage 63, from Passage 62, Flask B

- (a) Aspirated cell culture medium
- (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
- (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin

- (d) Bathed cells in 5mL 0.05% trypsin
- (e) Incubated cells at 37°C until all cells detached from flask
- (f) Added 15mL complete M199 to flask
- (g) Added to 3 T150 flasks 20mL complete M199/5mL cell mix; 22.5mL complete M199/2.5mL cell mix; and 22.5mL complete M199/2.5mL cell mix. Respectively, flasks A, B, and C
- (h) Gently shook flasks to distribute cells evenly
- (i) Incubated at 37°C

28 September 2014

1. Rotavirus infection of P61B plated cells
 - (a) Washed monolayer in each well twice with serum-free M199 by dumping
 - (b) Added $\sim 1\text{mL}$ rotaviral solution to wells in concentrations from 10^{-3} to 10^{-8} with 2 wells being infected at each concentration of rotavirus
 - (c) Spread cells evenly by shaking
 - (d) Incubated at 37°C for 1 hour
2. Agarose overlay of P61B plated cells
 - (a) Liquefied 1.2% agarose by microwave and equilibrated to 55°C
 - (b) For each plate of cells, prepared 1 vial of 10mL agarose, 10mL 2x EMEM, 5 μL 2mg/mL trypsin
 - (c) Aspirated viral inoculant from each well
 - (d) To each well, added $\sim 3\text{mL}$ agarose solution
 - (e) Let agarose solidify
 - (f) Incubated at 37°C
3. Plated MA104 cells — Passage 62, Flask C
 - (a) Aspirated cell culture medium
 - (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
 - (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
 - (d) Bathed cells in 5mL 0.05% trypsin
 - (e) Incubated cells at 37°C until all cells detached from flask
 - (f) Added 15mL complete M199 to flask
 - (g) Took cell count by combining 10 μL cell mixture with 10 μL trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{4.16 \times 10^5 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{\text{flask}} &= \frac{4.16 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &= \frac{8.32 \times 10^6 \text{ cells}}{20\text{mL}} \\
 \frac{\text{cells}}{10\text{mL cell mix}} &= \frac{8.32 \times 10^6 \text{ cells}}{20\text{mL}} \cdot \frac{1}{2} &= \frac{4.16 \times 10^6 \text{ cells}}{10\text{mL}} \\
 \frac{\text{cells}}{75\text{mL vial}} &= \frac{4.16 \times 10^6 \text{ cells}}{75\text{mL}} &= \frac{5.55 \times 10^4 \text{ cells}}{\text{mL}} \\
 \frac{\text{cells}}{3\text{mL well}} &= \frac{5.55 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &= \frac{1.67 \times 10^5 \text{ cells}}{\text{well}}
 \end{aligned}$$

- (h) Added 65mL complete M199 and 10mL cell mixture to 125mL conical vial for final volume of 75mL
- (i) Transferred 3mL solution to each well of 4 6-well plates
- (j) Spread cells evenly by shaking
- (k) Incubated at 37°C

29 September 2014

1. Lentiviral transfection of P62C plated cells

- (a) Counted cells per well
 - i. To one well, added 1mL 1x PBS; aspirated
 - ii. Rinsed in 500μL trypsin; aspirated
 - iii. Bathed in 500μL trypsin
 - iv. Incubated at 37°C until cells detached
 - v. Added 1.5mL complete M199
 - vi. Took cell count by combining 10μL cell mixture with 10μL trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{9.03 \times 10^4 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{2\text{mL well}} &= \frac{9.05 \times 10^4 \text{ cells}}{\text{mL}} \cdot 2\text{mL} = \frac{1.86 \times 10^5 \text{ cells}}{\text{well}}
 \end{aligned}$$

- (b) Calculated RIO3 dilution

$$\begin{aligned}
 [\text{RIO3}] &= \frac{2.13 \times 10^9 \text{ particles}}{1\text{mL}} \\
 \text{dilution} &= \left(\frac{1.86 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}} \right) / \frac{2.13 \times 10^9 \text{ particles}}{1\text{mL}} \\
 &= \frac{0.87\mu\text{L}}{\text{well}} \\
 &= \frac{4.4\mu\text{L}}{5 \text{ wells}}
 \end{aligned}$$

- (c) Calculated NSV dilution

$$\begin{aligned}
 [\text{NSV}] &= \frac{1.95 \times 10^8 \text{ particles}}{1\text{mL}} \\
 \text{dilution} &= \left(\frac{1.86 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}} \right) / \frac{1.95 \times 10^8 \text{ particles}}{1\text{mL}} \\
 &= \frac{9.54\mu\text{L}}{\text{well}} \\
 &= \frac{47.7\mu\text{L}}{5 \text{ wells}}
 \end{aligned}$$

- (d) Prepared RIO3 wells

- i. Combined 5mL complete M199, 5μL polybreen, and 4.4μL RIO3 and mixed by vortex
- ii. To each of the 5 experimental wells, added 1mL RIO3 solution
- iii. Spread evenly by gently shaking plates

- (e) Prepared NSV control wells

- i. Combined 5mL complete M199, 5 μ L polybrene, and 47.7 μ L NSV and mixed by vortex
- ii. To each of the 5 control wells, added 1mL NSV solution
- iii. Spread evenly by gently shaking plates
- (f) Incubated plates at 37°C for 2 hours
- (g) Supplemented each of the 5 experimental wells with an additional 2mL complete M199 (3mL final well volume)
- (h) With a separate pipet, supplemented each of the 5 control wells with an additional 2mL complete M199 (3mL final well volume)
- (i) Incubated plates at 37°C

17 October 2014

01 October 2014

1. Rotavirus infection of P62C plated cells (RIO3 and NSV transfected)

- (a) Counted cells per well
 - i. To one well, added 1mL 1x PBS; aspirated
 - ii. Rinsed in 500 μ L trypsin; aspirated
 - iii. Bathed in 500 μ L trypsin
 - iv. Incubated at 37°C until cells detached
 - v. Added 1.5mL complete M199
 - vi. Took cell count by combining 10 μ L cell mixture with 10 μ L trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{1.18 \times 10^5 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{2\text{mL well}} &= \frac{1.18 \times 10^5 \text{ cells}}{\text{mL}} \cdot 2\text{mL} = \frac{2.36 \times 10^5 \text{ cells}}{\text{well}}
 \end{aligned}$$

- (b) Calculated SA11 dilution

$$\begin{aligned}
 [\text{SA11}] &= \frac{5 \times 10^7 \text{ PFU}}{1\text{mL}} \\
 \text{dilution} &= \left(\frac{2.36 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{5 \text{ particles}}{1 \text{ cell}} \right) / \frac{5 \times 10^7 \text{ PFU}}{1\text{mL}} \\
 &= \frac{23.6\mu\text{L}}{\text{well}} \\
 &= \frac{118\mu\text{L}}{5 \text{ wells}}
 \end{aligned}$$

- (c) Prepared viral inoculant
 - i. To a vial, combined 400 μ L SA11 and 2 μ L trypsin
 - ii. Incubated in 37°C water bath for 1 hour
 - iii. Prepared vial of 10mL serum-free M199
 - iv. Added to vial 236mL viral solution (for infection of 10 wells)
- (d) Washed wells twice with serum-free M199 by dumping
- (e) Added to each well 1mL viral solution
- (f) Spread evenly by gently shaking plates

- (g) Incubated plates at 37°C for 1 hour
- (h) Aspirated viral inoculant from wells
- (i) Added to each well 3mL serum-free M199 and 0.75μL trypsin
- (j) Incubated plates at 37°C

2. Plated MA104 cells — Passage 63, Flask A

- (a) Aspirated cell culture medium
- (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
- (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
- (d) Bathed cells in 5mL 0.05% trypsin
- (e) Incubated cells at 37°C until all cells detached from flask
- (f) Added 15mL complete M199 to flask
- (g) Took cell count by combining 10μL cell mixture with 10μL trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{4.61 \times 10^5 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{\text{flask}} &= \frac{4.61 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} = \frac{9.22 \times 10^6 \text{ cells}}{20\text{mL}} \\
 \frac{\text{cells}}{10\text{mL cell mix}} &= \frac{9.22 \times 10^6 \text{ cells}}{20\text{mL}} \cdot \frac{1}{2} = \frac{4.61 \times 10^6 \text{ cells}}{10\text{mL}} \\
 \frac{\text{cells}}{75\text{mL vial}} &= \frac{4.61 \times 10^6 \text{ cells}}{75\text{mL}} = \frac{6.15 \times 10^4 \text{ cells}}{\text{mL}} \\
 \frac{\text{cells}}{3\text{mL well}} &= \frac{6.15 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} = \frac{1.84 \times 10^5 \text{ cells}}{\text{well}}
 \end{aligned}$$

- (h) Added 65mL complete M199 and 10mL cell mixture to 125mL conical vial for final volume of 75mL
- (i) Transferred 3mL solution to each well of 4 6-well plates
- (j) Spread cells evenly by shaking
- (k) Incubated at 37°C

3. Neutral red overlay of P62C plated cells

- (a) Prepared a vial with 7.5mL 1.2% agarose, 7.5mL 2x EMEM, and 750μL neutral red
- (b) To each well of 2 6-well plates, added ~ 1mL prepared agarose solution
- (c) Let agarose solution solidify
- (d) Incubated at 37°C overnight
- (e) Observed titer = 7×10^6 PFU/mL

02 October 2014

1. MA104 cell split — Passage 64, from Passage 63, Flask B

- (a) Aspirated cell culture medium
- (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
- (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
- (d) Bathed cells in 5mL 0.05% trypsin

- (e) Incubated cells at 37°C until all cells detached from flask
- (f) Added 15mL complete M199 to flask
- (g) Added to 3 T150 flasks 20mL complete M199/5mL cell mix; 22.5mL complete M199/2.5mL cell mix; and 22.5mL complete M199/2.5mL cell mix. Respectively, flasks A, B, and C
- (h) Gently shook flasks to distribute cells evenly
- (i) Incubated at 37°C

2. Lysed P62C transfected cells

- (a) Froze transfected cells in -80°C freezer for 30 minutes; thawed
- (b) Froze transfected cells in -20°C freezer overnight

03 October 2014

1. Collected viral load from lysed P62C transfected cells

- (a) Thawed P62C cells
- (b) Transferred content of each well to its own 15mL tube
- (c) Centrifuged tubes for 10 minutes at 500RPM and 4°C
- (d) Transferred supernatant from each centrifuged solution into fresh test tubes
- (e) Stored at -20°C

06 October 2014

1. RIO3 Rotavirus activation and series dilution for P63A plated cells

- (a) Combined 400 μL SA11 reclaimed from RIO3-transfected cells (vial 3 of 5) and 2 μL trypsin
- (b) Incubated for 1 hour in a 37°C water bath
- (c) Added 2.7mL serum-free M199 to each of 8 15mL tubes
- (d) To the first tube, added 300 μL rotavirus solution to a final concentration of 10^{-1}
- (e) Mixed contents by vertex
- (f) To the next tube, added 300 μL rotavirus solution from the previous tube to a final concentration of 10^{-2}
- (g) Mixed contents by vertex
- (h) Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
- (i) Stored at 4°C

2. RIO3 Rotavirus infection of P63A plated cells

- (a) Washed monolayer in each well of 2 6-well plates twice with serum-free M199 by dumping
- (b) Added $\sim 1\text{mL}$ rotaviral solution to wells in concentrations from 10^{-3} to 10^{-8} with 2 wells being infected at each concentration of rotavirus
- (c) Spread cells evenly by shaking
- (d) Incubated at 37°C for 1 hour

3. NSV Rotavirus activation and series dilution for P63A plated cells

- (a) Combined 400 μL SA11 reclaimed from NSV-transfected cells (vial 4 of 5) and 2 μL trypsin
- (b) Incubated for 1 hour in a 37°C water bath
- (c) Added 2.7mL serum-free M199 to each of 8 15mL tubes

- (d) To the first tube, added $300\mu\text{L}$ rotavirus solution to a final concentration of 10^{-1}
 - (e) Mixed contents by vertex
 - (f) To the next tube, added $300\mu\text{L}$ rotavirus solution from the previous tube to a final concentration of 10^{-2}
 - (g) Mixed contents by vertex
 - (h) Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
 - (i) Stored at 4°C
4. NSV Rotavirus infection of P63A plated cells
- (a) Washed monolayer in each well of 2 6-well plates twice with serum-free M199 by dumping
 - (b) Added $\sim 1\text{mL}$ rotaviral solution to wells in concentrations from 10^{-3} to 10^{-8} with 2 wells being infected at each concentration of rotavirus
 - (c) Spread cells evenly by shaking
 - (d) Incubated at 37°C for 1 hour
5. Agarose overlay of P63A plated cells
- (a) Liquefied 1.2% agarose by microwave and equilibrated to 55°C
 - (b) For each plate of cells (4 total), prepared 1 vial of 10mL agarose, 10mL 2x EMEM, $5\mu\text{L}$ 2mg/mL trypsin
 - (c) Aspirated viral inoculant from each well
 - (d) To each well, added $\sim 3\text{mL}$ agarose solution
 - (e) Let agarose solidify
 - (f) Incubated at 37°C
6. Plated MA104 cells — Passage 64, Flask A
- (a) Aspirated cell culture medium
 - (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
 - (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
 - (d) Bathed cells in 5mL 0.05% trypsin
 - (e) Incubated cells at 37°C until all cells detached from flask
 - (f) Added 20mL complete M199 to flask³
 - (g) Took cell count by combining $10\mu\text{L}$ cell mixture with $10\mu\text{L}$ trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{1.10 \times 10^5 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{\text{flask}} &= \frac{1.10 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 25\text{mL} &= \frac{2.75 \times 10^6 \text{ cells}}{25\text{mL}} \\
 \frac{\text{cells}}{20\text{mL cell mix}} &= \frac{1.10 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &= \frac{2.20 \times 10^6 \text{ cells}}{20\text{mL}} \\
 \frac{\text{cells}}{150\text{mL vial}} &= \frac{2.20 \times 10^6 \text{ cells}}{150\text{mL}} &= \frac{1.47 \times 10^4 \text{ cells}}{\text{mL}} \\
 \frac{\text{cells}}{3\text{mL well}} &= \frac{1.47 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &= \frac{4.40 \times 10^4 \text{ cells}}{\text{well}}
 \end{aligned}$$

³I goofed and added 5mL too much complete M199. Small goof, but FYI.

- (h) Added 130mL complete M199 and 20mL cell mixture to 250mL conical vial for final volume of 150mL
- (i) Transferred 3mL solution to each well of 8 6-well plates
- (j) Spread cells evenly by shaking
- (k) Incubated at 37°C

09 October 2014

1. Disposal of P64A Plated Cells

An unknown error occurred when plating cells on 06 Oct. causing none to survive. The culture medium was aspirated from each well and the plates disposed of appropriately.

2. Neutral red overlay of P63A plated cells

- (a) Prepared a vial with 7.5mL 1.2% agarose, 7.5mL 2x EMEM, and 750 μ L neutral red
- (b) To each well of 2 6-well plates, added \sim 1mL prepared agarose solution
- (c) Let agarose solution solidify
- (d) Incubated at 37°C for 4 hours
- (e) Observed titer = $x \times 10^6$ PFU/mL

3. Plated MA104 cells — Passage 64, Flask B

- (a) Aspirated cell culture medium
- (b) Rinsed cells in 10mL 1x PBS; aspirated PBS
- (c) Rinsed cells in 5mL 0.05% trypsin; aspirated trypsin
- (d) Bathed cells in 5mL 0.05% trypsin
- (e) Incubated cells at 37°C until all cells detached from flask
- (f) Added 15mL complete M199 to flask
- (g) Took cell count by combining 10 μ L cell mixture with 10 μ L trypan blue:

$$\begin{aligned}
 [\text{cells}] &= \frac{3.46 \times 10^5 \text{ cells}}{1\text{mL}} \\
 \frac{\text{cells}}{\text{flask}} &= \frac{3.46 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &= \frac{6.92 \times 10^6 \text{ cells}}{25\text{mL}} \\
 \frac{\text{cells}}{150\text{mL vial}} &= \frac{6.92 \times 10^6 \text{ cells}}{150\text{mL}} &= \frac{4.61 \times 10^4 \text{ cells}}{\text{mL}} \\
 \frac{\text{cells}}{3\text{mL well}} &= \frac{4.61 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &= \frac{1.38 \times 10^5 \text{ cells}}{\text{well}}
 \end{aligned}$$

- (h) Added 130mL complete M199 and 20mL cell mixture to 250mL conical vial for final volume of 150mL
- (i) Transferred 3mL solution to each well of 8 6-well plates
- (j) Spread cells evenly by shaking
- (k) Incubated at 37°C