McDonald Lab
Lab Protocols and Daily Log

Christopher Wetherill
Virginia Polytechnic Institute and State University

Audit log available at https://github.com/faulconbridge/TBMH/tree/master/Rotations

Part I
Standard Operating Procedures

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° freezer in the hallway immediately outside the lab.	Leftover stock should be refrozen in a box labeled 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 2× 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.
10× 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° freezer in the hallway immediately outside the lab.	Leftover stock should be replaced where you found it.
Neutral 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.
Polybrene	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 Labeling Materials

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

cell contained, the passage of cell contained, and, if applicable, any agents with which the cell has been transduced or infected.

All work completed in the lab should be recorded in a laboratory notebook. Each page should contain entries from only a single day if handwritten. 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.

If an electronic lab notebook is used, the same general guidelines apply; however, multiple entries may appear on a single printed page. There must additionally be maintained an audit log of all changes and alterations made to the notebook to which the author has read-only access (i.e., the author must be unable to tamper with the audit log).

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; 250μg/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; 250μg/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.

[1] The paper *Culturing, Storage, and Quantification of Rotaviruses* advises using different quantities of some materials listed. Nonetheless, those listed here are the recommended quantities for use in lab.

[2] See Note 1.

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. (It is inadvisable to fill flask to more than 400mL.) Cap tightly and shake. Loosen lid. Apply autoclave tape to lid. Autoclave for 20 minutes.

7 Preparation of 2× EMEM (Serum-Free)

Items Needed:

1. Incomplete 2× EMEM (500mL)
2. 200mM L-glutamine (10mL)
3. Penicillin/streptomycin stock (10mL)
4. 250μg/ml amphotericin B stock (1mL)

In the biosafety cabinet, to 500mL incomplete 2× 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. 1× PBS
2. 0.05% Trypsin-EDTA
3. Complete Medium 199
4. 150cm^2 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 (Pasteur) pipet connected to the rubber vacuum hose. Dispose of the pipet. To the cell culture, add 10mL 1× 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. (The solution

in the flask will turn cloudy as cells detach. Detachment may be verified through the microscope.) 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.

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 20mL complete Medium 199 and 5mL cell mixture; to each of our second two flasks we would add 22.5mL complete Medium 199 and 2.5mL cell mixture.

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

10 Procedure for Plating MA104 Cells

Items Needed:

1. 1× PBS
2. 0.05% Trypsin
3. Complete Medium 199
4. Trypan Blue
5. 6-well plates (x8)

In a water bath, warm PBS, Trypsin, and complete Medium 199 to 37°C. Aspirate the cell culture medium from the flask. Wash the cell monolayer in 10mL 1× 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 10μL cell mixture and 10μL trypan blue. Mix thoroughly with a pipet. To a cell counter slide, add the 20μL solution 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:

$$\begin{aligned}
\frac{\text{cells}}{\text{20mL flask}} &= \frac{\text{cells}}{\text{mL}} \cdot \frac{\text{20mL}}{\text{flask}} \\
\frac{\text{cells}}{\text{150mL conical vial}} &= \left(\frac{1}{2} \cdot \frac{\text{cells}}{\text{flask}}\right) / \frac{\text{150mL}}{\text{conical vial}} \\
\frac{\text{cells}}{\text{well}} &= \frac{\text{cells}}{\text{150mL}} \cdot \frac{\text{3mL}}{\text{well}}
\end{aligned}$$

To a 250mL conical vial add 130mL complete Medium 199. Supplement this with 20mL cell solution. (These values may be halved if 4 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

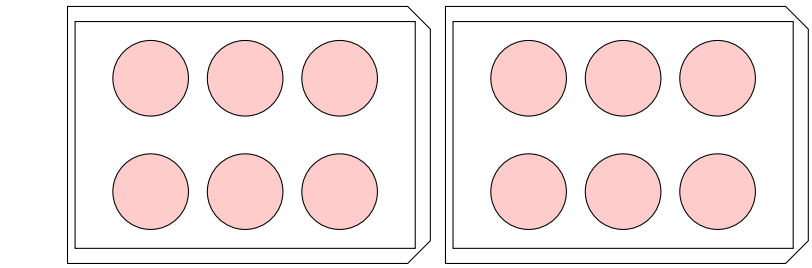
Items Needed:

1. SA11 stock
2. Trypsin (2mg/mL)
3. Serum-free Medium 199
4. 2× EMEM
5. 1.2% agarose

In a large Eppie tube combine 400μL rotavirus SA11 stock and 2μL trypsin. Cap and incubate in a 37°C water bath for 1 hour. To each of 8 14mL plastic tubes add 2.7mL serum-free Medium 199.

Add 300μL virus mix to the first tube and label as 10^{-1}. Mix the solution by vertex to homogenize. Take 300μL 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.)

Wash each plate twice with serum-free Medium 199 by dumping. (See detailed procedure below.) To each plate add the rotavirus titers. Each titer is to be performed in duplicate. To achieve this, add ∼ 1mL 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:



NOTE: Detailed Procedure to Avoid Cell Dessication

During the washing and infection steps, cells may dry out and die. To avoid this, we typically:

1. Prepare in a glass Pyrex bowl a small amount (0.5cm deep) of bleach
2. Prepare one 50mL tube of serum-free M199
3. For one plate, flip upside down to dump cell culture medium into the bleach solution
4. Quickly pour into each well ∼ 3mL of serum-free M199 from the prepared vial (pour along the bottom edge of each well to make as little direct contact with the monolayer as possible while pouring)
5. Tilt the plate to gently wash the cells with the solution
6. Dump the culture medium into the bleach solution
7. Again pour into each well ∼ 3mL of serum-free M199 from the prepared vial and spread evenly
8. Uncap the 3 vials of viral titer that are to be used for the plate
9. Dump the culture medium into the bleach solution
10. Quickly pour ∼ 1mL of viral solution into each corresponding well

11. Label the plate cover with the titers used in each well and set the plate aside
12. Repeat the above steps sequentially for all remaining plates needing infection (a new tube of serum-free M199 should be used for each plate; the bleach solution may be reused across all plates)

Here, it is important to both work quickly to ensure that cells do not dry out, but also carefully so that you do not pour M199 or viral inoculant into the wells too heavily, causing cells to wash away. If your plaque assays have sickle-shaped holes, this is likely due to cells having been washed away by heavy pouring. Likewise, large, irregular, blotchy holes are likely caused by cell drying. Generally, if you work too quickly, the halo of washed-away cells will appear; too slowly and the cells will dry out. If you're having trouble finding a happy medium, err towards the sickles. These do not *necessarily* ruin a plaque assay whereas cell desiccation will generally render the entire assay unusable.

Incubate the plates at 37°C for 1 hour.

Liquify a jar of 1.2% agarose using a microwave. Equilibrate the agarose to no more than 55°C (hotter and the cells will be killed) and no cooler than 37°C (the agarose will resolidify). Warm 2× EMEM to 37°C in a water bath. For each plate infected, prepare a solution of 10mL agarose, 10mL 2× EMEM, and 5μL 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. Tilt the plates downward towards you and keep the pipet along the lower edge of the well. Do not make contact between the pipet and the monolayer.) Quickly add to each well $\sim$ 3mL of the agarose solution. Place the lid on the well and allow it to sit, undisturbed, for approximately 30 to 45 minutes (until the agarose overlay has solidified). Incubate approximately 3 days at 37°C.

12 Procedure for Performing RV Plaque Assay

Items Needed:

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

Liquefy 1.2% agarose by microwave. Equilibrate agarose to no more than 55°C. For 4 6-well plates, mix 15mL 2× 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 Transducing 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. 1× 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 500μL trypsin, spread evenly, and aspirate. Add 500μL 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 10μL cell mix and combine with 10μL 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 lentiviral vector by:

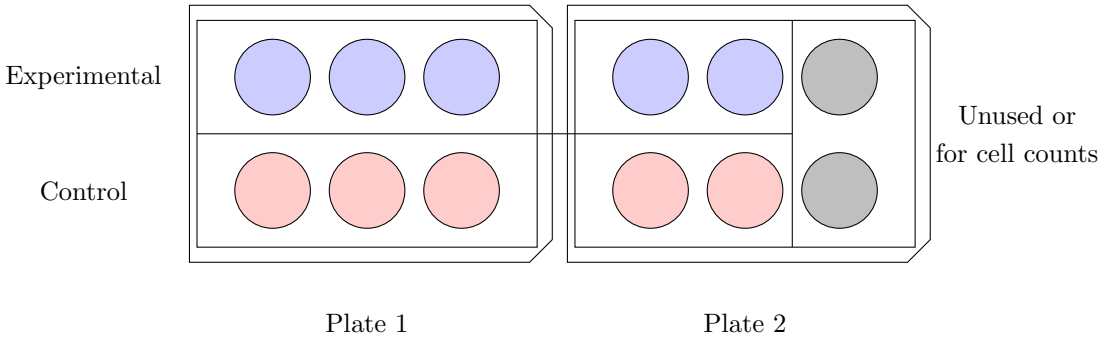
$$\left(\frac{\text{cells}}{\text{well}} \cdot \text{MOI}\right) / \text{[lentivirus]}$$

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 transduce 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, 1μL 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 for example 5 control and 5 experimental wells, may look similar to the following:



Typically, only biological triplicate is needed (the minimum number of data points to calculate standard deviation/error). More may be prepared, however, if desired. *If preparing only 3 experimental and control wells, respectively, be sure to make up a second plate of cells to be used exclusively for cell counts. Otherwise you'll be destroying your experimental and control wells. That's bad times.*

Incubate these plates for 2 hours at 37°C. After 2 hours, supplement each of the experimental wells with 2mL complete M199. Using a second pipet, supplement each of the control wells with 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 Transduction

Items Needed:

1. Serum-free M199
2. Complete M199
3. SA11 stock
4. 0.05% Trypsin
5. Trypsin (2mg/mL)
6. Trypan blue
7. 1× 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 500μL 0.05% trypsin, spread evenly, and aspirate. Add 500μL 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 10μL cell mix and combine with 10μL 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 400μL SA11 stock and 2μL 2mg/mL trypsin. Incubate in a 37°C water bath for 1 hour. Prepare a vial of SA11 diluted according to the equation above 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 transduced wells.

Wash all transduced 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 0.75μL 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 transduced Cells Following RV Infection

Freeze transduced cells completely at −80°C for approximately 30 minutes. (Depending on the number of plates, the exact time will vary. What is important is that all wells freeze completely.) 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 500×g 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. The solutions may be stored at −80°C if no cells will be infected immediately.

Part II
Lab Notes & Daily Log

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; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/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; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/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; 200μg/mL streptomycin final concentration), and 1mL 250μg/mL amphotericin B stock (0.5μg/mL amphotericin B final concentration)
   (b) Stored at 4°C
2. Prepared 400mL 1.2% agarose
   (a) Combined 4.8020g SeaPlaque agar to $\sim$ 400mL 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 400μL SA11 rotavirus stock and 2μL 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 300μL rotavirus solution to a final concentration of 10^{-1}

(e) Mixed contents by vertex

(f) To the next tube, added 300μL 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 10μL cell mixture with 10μL 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 $\sim$ 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

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, 5μL 2mg/mL trypsin
   (c) Aspirated viral inoculant from each well
   (d) To each well, added $\sim$ 3mL 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 600μL 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) Observed 3×10^6PFU/mL

21 September 2014

1. Rotavirus activation and series dilution for P60C plated cells
   (a) Combined 400μL SA11 rotavirus stock and 2μL 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 300μL rotavirus solution to a final concentration of 10^{-1}
   (e) Mixed contents by vertex
   (f) To the next tube, added 300μL 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

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, 5μL 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; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/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 10μL cell mixture with 10μL trypan blue:

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

(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 600μL 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 400μL SA11 rotavirus stock and 2μL 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 300μL rotavirus solution to a final concentration of 10^{-1}

(e) Mixed contents by vertex

(f) To the next tube, added 300μL 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 $\sim$ 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 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, 5μL 2mg/mL trypsin
   (c) Aspirated viral inoculant from each well
   (d) To each well, added $\sim$ 3mL 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 10μL cell mixture with 10μL 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 transduction 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:

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

   (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 polybrene, and 4.4μL RIO3 and mixed by vertex
      ii. Aspirated cell culture medium from experimental wells
      iii. To each of the 5 experimental wells, added 1mL RIO3 solution
      iv. Spread evenly by gently shaking plates
   (e) Prepared NSV control wells
      i. Combined 5mL complete M199, 5μL polybrene, and 47.7μL NSV and mixed by vertex
      ii. Aspirated cell culture medium from control wells
      iii. To each of the 5 control wells, added 1mL NSV solution
      iv. 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. Prepared serum-free Medium 199

   (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration)

   (b) Stored at 4°C

2. 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 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:

$$[\text{cells}] = \frac{1.18 \times 10^5 \text{ cells}}{1\text{mL}}$$

$$\frac{\text{cells}}{2\text{mL well}} = \frac{1.18 \times 10^5 \text{ cells}}{\text{mL}} \cdot 2\text{mL} = \frac{2.36 \times 10^5 \text{ cells}}{\text{well}}$$

   (b) Calculated SA11 dilution

$$[\text{SA11}] = \frac{5 \times 10^7 \text{ PFU}}{1\text{mL}}$$

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

   (c) Prepared viral inoculant

      i. To a vial, combined 400μL SA11 and 2μL trypsin
      ii. Incubated in 37°C water bath for 1 hour
      iii. Prepared vial of 10mL serum-free M199
      iv. Added to vial 236μL 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

3. 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:

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

(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

4. 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 $\sim$ 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 400μL SA11 reclaimed from RIO3-transfected cells (vial 3 of 5) and 2μL 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 300μL rotavirus solution to a final concentration of 10^{-1}

(e) Mixed contents by vertex

(f) To the next tube, added 300μL 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 $\sim$ 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

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

(a) Combined 400μL SA11 reclaimed from NSV-transfected cells (vial 4 of 5) and 2μL 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 300μL rotavirus solution to a final concentration of 10^{-1}

(e) Mixed contents by vertex

(f) To the next tube, added 300μL 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 $\sim$ 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

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, 5μL 2mg/mL trypsin

(c) Aspirated viral inoculant from each well

(d) To each well, added $\sim$ 3mL 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 flask[3]

(g) Took cell count by combining 10μL cell mixture with 10μL trypan blue:

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

[3] I 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 (NSV)

   (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 $\sim$ 1mL prepared agarose solution
   (c) Let agarose solution solidify
   (d) Incubated at 37°C for 4 hours
   (e) Observed titer = 2×10^8 PFU/mL

3. Neutral red overlay of P63A plated cells (RIO3)

   (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 $\sim$ 1mL prepared agarose solution
   (c) Let agarose solution solidify
   (d) Incubated at 37°C for 4 hours
   (e) Observed titer = 5.5×10^8 PFU/mL

4. 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 10μL cell mixture with 10μL trypan blue:

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

   (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

10 October 2014

1. MA104 cell split — Passage 65, from Passage 64, 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) Added to 4 T150 flasks 20mL complete M199/5mL cell mix; 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, C, and D
   (h) Gently shook flasks to distribute cells evenly
   (i) Incubated at 37°C
2. Prepared complete Medium 199
   (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration), 55mL fetal bovine serum
   (b) Stored at 4°C
3. Prepared serum-free Medium 199
   (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration)
   (b) Stored at 4°C

12 October 2014

1. RIO3 and NSV rotavirus activation and series dilution
   (a) For each of four samples (NSV vial 3; NSV vial 5; RIO3, vial 4; RIO3, vial 5):
      i. Combined 400μL SA11 reclaimed from transfected cells and 2μL trypsin
      ii. Incubated for 1 hour in a 37°C water bath
      iii. Added 2.7mL serum-free M199 to each of 8 15mL tubes
      iv. To the first tube, added 300μL rotavirus solution to a final concentration of 10^{-1}
      v. Mixed contents by vertex
      vi. To the next tube, added 300μL rotavirus solution from the previous tube to a final concentration of 10^{-2}
      vii. Mixed contents by vertex
      viii. Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
      ix. Stored at 4°C
   (b) Ended with 24 total vials, 8 of each of the SA11 samples identified previously

13 October 2014

1. Rotavirus infection of P64B plated cells
   (a) For each of four samples (NSV vial 3; NSV vial 5; RIO3, vial 4; RIO3, vial 5):
      i. Washed monolayer in each well of 2 6-well plates twice with serum-free M199 by dumping
      ii. 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
      iii. Spread cells evenly by shaking
      iv. Incubated at 37°C for 1 hour

2. Agarose overlay of P64B plated cells
   (a) For each of four samples (NSV vial 3; NSV vial 5; RIO3, vial 4; RIO3, vial 5):
      i. Liquefied 1.2% agarose by microwave and equilibrated to 55°C
      ii. For each plate of cells (8 total), prepared 1 vial of 10mL agarose, 10mL 2x EMEM, 5μL 2mg/mL trypsin
      iii. Aspirated viral inoculant from each well
      iv. To each well, added $\sim$ 3mL agarose solution
      v. Let agarose solidify
      vi. Incubated at 37°C
   (b) Ended with 8 infected 6-well plates with 2 infected plates (viral titers from $10^{-3} - 10^{-8}$ in duplicate) for each of the 4 viral samples

14 October 2014

1. Plated MA104 cells — Passage 65, 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:

$$
\begin{aligned}
[\text{cells}] &= \frac{2.31 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{\text{flask}} &= \frac{2.31 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &&= \frac{4.62 \times 10^6 \text{ cells}}{20\text{mL}} \\
\frac{\text{cells}}{150\text{mL vial}} &= \frac{4.62 \times 10^6 \text{ cells}}{150\text{mL}} &&= \frac{3.08 \times 10^4 \text{ cells}}{\text{mL}} \\
\frac{\text{cells}}{3\text{mL well}} &= \frac{3.08 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &&= \frac{9.24 \times 10^4 \text{ cells}}{\text{well}}
\end{aligned}
$$

   (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

15 October 2014

1. RIO3 and NSV rotavirus activation and series dilution
   (a) For each of four samples (NSV vial 1; NSV vial 2; RIO3, vial 1; RIO3, vial 2):
      i. Combined 400μL SA11 reclaimed from transfected cells and 2μL trypsin
      ii. Incubated for 1 hour in a 37°C water bath
      iii. Added 2.7mL serum-free M199 to each of 8 15mL tubes
      iv. To the first tube, added 300μL rotavirus solution to a final concentration of 10^{-1}
      v. Mixed contents by vertex
      vi. To the next tube, added 300μL rotavirus solution from the previous tube to a final concentration of 10^{-2}
      vii. Mixed contents by vertex
      viii. Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
      ix. Stored at 4°C
   (b) Ended with 24 total vials, 8 of each of the SA11 samples identified previously

16 October 2014

1. Plated MA104 cells — Passage 65, 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 10μL cell mixture with 10μL trypan blue:

$$
\begin{aligned}
[\text{cells}] &= \frac{2.46 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{\text{flask}} &= \frac{2.46 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &&= \frac{4.92 \times 10^6 \text{ cells}}{20\text{mL}} \\
\frac{\text{cells}}{\text{5mL sample}} &= \frac{4.92 \times 10^6 \text{ cells}}{20\text{mL}} \cdot \frac{1}{4} &&= \frac{1.23 \times 10^6 \text{ cells}}{5\text{mL}} \\
\frac{\text{cells}}{\text{37.5mL vial}} &= \frac{1.23 \times 10^6 \text{ cells}}{37.5\text{mL}} &&= \frac{3.28 \times 10^4 \text{ cells}}{\text{mL}} \\
\frac{\text{cells}}{\text{3mL well}} &= \frac{3.28 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &&= \frac{9.84 \times 10^4 \text{ cells}}{\text{well}}
\end{aligned}
$$

   (h) Added 32.5mL complete M199 and 5mL cell mixture to 125mL conical vial for final volume of 37.5mL
   (i) Transferred 3mL solution to each well of 2 6-well plates
   (j) Spread cells evenly by shaking
   (k) Incubated at 37°C for 1 hour and then transferred to 31°C incubator
2. MA104 cell split — Passage 66, from Passage 65, Flask D

   (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 4 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

3. Neutral red overlay of P64B plated cells (RIO3, vial 4)
   (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 $\sim$ 1mL prepared agarose solution
   (c) Let agarose solution solidify
   (d) Incubated at 37°C overnight
   (e) Observed titer = 2×10^8 PFU/mL
4. Neutral red overlay of P64B plated cells (RIO3, vial 5)
   (a) Procedure was identical to above
   (b) Observed titer = 3×10^8 PFU/mL
5. Neutral red overlay of P64B plated cells (NSV, vial 3)
   (a) Procedure was identical to above
   (b) Observed titer = 2×10^8 PFU/mL
6. Neutral red overlay of P64B plated cells (NSV, vial 5)
   (a) Procedure was identical to above
   (b) Observed titer = 1×10^8 PFU/mL

18 October 2014

1. Rotavirus infection of P65A plated cells
   (a) For each of four samples (NSV vial 1; NSV vial 2; RIO3, vial 1; RIO3, vial 2):
      i. Washed monolayer in each well of 2 6-well plates twice with serum-free M199 by dumping
      ii. 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
      iii. Spread cells evenly by shaking
      iv. Incubated at 37°C for 1 hour
2. Agarose overlay of P65A plated cells
   (a) For each of four samples (NSV vial 1; NSV vial 2; RIO3, vial 1; RIO3, vial 2):
      i. Liquefied 1.2% agarose by microwave and equilibrated to 55°C
      ii. For each plate of cells (8 total), prepared 1 vial of 10mL agarose, 10mL 2x EMEM, 5μL 2mg/mL trypsin
      iii. Aspirated viral inoculant from each well

iv. To each well, added $\sim$ 3mL agarose solution
v. Let agarose solidify
vi. Incubated at 37°C

(b) Ended with 8 infected 6-well plates with 2 infected plates (viral titers from $10^{-3} - 10^{-8}$ in duplicate) for each of the 4 viral samples

20 October 2014

1. Disposed of P65C plated cells.

2. Prepared complete Medium 199

   (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration), 55mL fetal bovine serum

   (b) Stored at 4°C

3. Prepared serum-free Medium 199

   (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration)

   (b) Stored at 4°C

4. MA104 cell split — Passage 67, from Passage 66, 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; 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

5. Plated MA104 cells — Passage 66, 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 10μL cell mixture with 10μL trypan blue:

$$
\begin{aligned}
[\text{cells}] &= \frac{2.41 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{\text{flask}} &= \frac{2.41 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &&= \frac{4.82 \times 10^6 \text{ cells}}{20\text{mL}} \\
\frac{\text{cells}}{150\text{mL vial}} &= \frac{4.82 \times 10^6 \text{ cells}}{150\text{mL}} &&= \frac{3.28 \times 10^4 \text{ cells}}{\text{mL}} \\
\frac{\text{cells}}{3\text{mL well}} &= \frac{3.21 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &&= \frac{9.63 \times 10^4 \text{ cells}}{\text{well}}
\end{aligned}
$$

(h) Added 120mL 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 for 1 hour and then transferred to 31°C incubator

21 October 2014

1. Neutral red overlay of P64B plated cells (RIO3, vial 1)

   (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 $\sim$ 1mL prepared agarose solution

   (c) Let agarose solution solidify

   (d) Incubated at 37°C overnight

   (e) Observed titer = 1.5×10^8 PFU/mL

2. Neutral red overlay of P64B plated cells (RIO3, vial 2)

   (a) Procedure was identical to above

   (b) Observed titer = 1×10^8 PFU/mL

3. Neutral red overlay of P64B plated cells (NSV, vial 1)

   (a) Procedure was identical to above

   (b) Observed titer = 2.5×10^8 PFU/mL

4. Neutral red overlay of P64B plated cells (NSV, vial 2)

   (a) Procedure was identical to above

   (b) Observed titer = 2×10^8 PFU/mL

5. Prepared 400mL 1.2% agarose

   (a) Combined 4.8106g SeaPlaque agar to $\sim$ 400mL milli-Q-filtered water

   (b) Autoclaved for 20 minutes

   (c) Stored at room temperature

22 October 2014

1. Lentiviral transduction of P66B 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:

$$[\text{cells}] = \frac{1.00 \times 10^5 \text{ cells}}{1\text{mL}}$$
$$\frac{\text{cells}}{2\text{mL well}} = \frac{1.00 \times 10^5 \text{ cells}}{\text{mL}} \cdot 2\text{mL} = \frac{2.00 \times 10^5 \text{ cells}}{\text{well}}$$

   (b) Calculation of kinase dilutions

      i. Calculated NSV dilution

$$[\text{NSV}] = \frac{1.95 \times 10^8 \text{ particles}}{1\text{mL}}$$
$$\begin{aligned}\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{1.95 \times 10^8 \text{ particles}}{1\text{mL}} \\ &= \frac{10.3\mu\text{L}}{\text{well}} \\ &= \frac{30.8\mu\text{L}}{3 \text{ wells}}\end{aligned}$$

      ii. Calculated RIO3 dilution

$$[\text{RIO3}] = \frac{2.13 \times 10^9 \text{ particles}}{1\text{mL}}$$
$$\begin{aligned}\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{2.13 \times 10^9 \text{ particles}}{1\text{mL}} \\ &= \frac{0.939\mu\text{L}}{\text{well}} \\ &= \frac{2.82\mu\text{L}}{5 \text{ wells}}\end{aligned}$$

      iii. Calculated GUCY2D dilution

$$[\text{GUCY2D}] = \frac{2.43 \times 10^9 \text{ particles}}{1\text{mL}}$$
$$\begin{aligned}\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{2.43 \times 10^9 \text{ particles}}{1\text{mL}} \\ &= \frac{0.823\mu\text{L}}{\text{well}} \\ &= \frac{2.47\mu\text{L}}{3 \text{ wells}}\end{aligned}$$

iv. Calculated CSNK2B dilution

$$\begin{aligned}
[\text{CSNK2B}] &= \frac{1.77 \times 10^9 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{1.77 \times 10^9 \text{ particles}}{1\text{mL}} \\
&= \frac{1.13\mu\text{L}}{\text{well}} \\
&= \frac{3.39\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

v. Calculated EPHA1 dilution

$$\begin{aligned}
[\text{EPHA1}] &= \frac{3.59 \times 10^9 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{3.59 \times 10^9 \text{ particles}}{1\text{mL}} \\
&= \frac{0.557\mu\text{L}}{\text{well}} \\
&= \frac{1.67\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

vi. Calculated ZMYND8 dilution

$$\begin{aligned}
[\text{ZMYND8}] &= \frac{3.42 \times 10^9 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{3.42 \times 10^9 \text{ particles}}{1\text{mL}} \\
&= \frac{0.585\mu\text{L}}{\text{well}} \\
&= \frac{1.75\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

vii. Calculated CKM dilution

$$\begin{aligned}
[\text{CKM}] &= \frac{2.38 \times 10^9 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{2.38 \times 10^9 \text{ particles}}{1\text{mL}} \\
&= \frac{0.840\mu\text{L}}{\text{well}} \\
&= \frac{2.52\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

viii. Calculated PIK3CA dilution

$$\begin{aligned}
[\text{PIK3CA}] &= \frac{1.04 \times 10^9 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{1.04 \times 10^9 \text{ particles}}{1\text{mL}} \\
&= \frac{1.92\mu\text{L}}{\text{well}} \\
&= \frac{5.77\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

ix. Calculated STK11IP dilution

$$\begin{aligned}
[\text{STK11IP}] &= \frac{3.66 \times 10^9 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{3.66 \times 10^9 \text{ particles}}{1\text{mL}} \\
&= \frac{0.546\mu\text{L}}{\text{well}} \\
&= \frac{1.64\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

x. Calculated SKAP1 dilution

$$\begin{aligned}
[\text{SKAP1}] &= \frac{2.79 \times 10^9 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{2.79 \times 10^9 \text{ particles}}{1\text{mL}} \\
&= \frac{0.717\mu\text{L}}{\text{well}} \\
&= \frac{2.15\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

xi. Calculated UCK2 dilution

$$\begin{aligned}
[\text{UCK2}] &= \frac{2.35 \times 10^9 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{2.35 \times 10^9 \text{ particles}}{1\text{mL}} \\
&= \frac{0.851\mu\text{L}}{\text{well}} \\
&= \frac{2.55\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

xii. Calculated TGF-α dilution

$$\begin{aligned}
[\text{TGF-}\alpha] &= \frac{1.50 \times 10^8 \text{ particles}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{2.00 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{10 \text{ particles}}{1 \text{ cell}}\right) / \frac{1.50 \times 10^8 \text{ particles}}{1\text{mL}} \\
&= \frac{13.3\mu\text{L}}{\text{well}} \\
&= \frac{40.0\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

(c) Lentiviral transduction of wells

i. Prepared NSV control wells
   A. Combined 3mL complete M199, 3μL polybrene, and 30.8μL NSV and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL NSV solution
   D. Spread evenly by gently shaking plates

ii. Prepared RIO3 wells
   A. Combined 3mL complete M199, 3μL polybrene, and 2.82μL RIO3 and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL RIO3 solution

   D. Spread evenly by gently shaking plates
iii. Prepared GUCY2D wells
   A. Combined 3mL complete M199, 3μL polybrene, and 2.47μL GUCY2D and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL RIO3 solution
   D. Spread evenly by gently shaking plates
iv. Prepared CSNK2B wells
   A. Combined 3mL complete M199, 3μL polybrene, and 3.39μL CSNK2B and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL NSV solution
   D. Spread evenly by gently shaking plates
v. Prepared EPHA1 wells
   A. Combined 3mL complete M199, 3μL polybrene, and 1.67μL EPHA1 and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL RIO3 solution
   D. Spread evenly by gently shaking plates
vi. Prepared ZMYND8 wells
   A. Combined 3mL complete M199, 3μL polybrene, and 1.75μL ZMYND8 and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL NSV solution
   D. Spread evenly by gently shaking plates
vii. Prepared CKM wells
   A. Combined 3mL complete M199, 3μL polybrene, and 2.52μL CKM and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL RIO3 solution
   D. Spread evenly by gently shaking plates
viii. Prepared PIK3CA wells
   A. Combined 3mL complete M199, 3μL polybrene, and 5.77μL PIK3CA and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL NSV solution
   D. Spread evenly by gently shaking plates
ix. Prepared STK11IP wells
   A. Combined 3mL complete M199, 3μL polybrene, and 1.64μL STK11IP and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL RIO3 solution
   D. Spread evenly by gently shaking plates
x. Prepared SKAP1 wells
   A. Combined 3mL complete M199, 3μL polybrene, and 2.15μL SKAP1 and mixed by vertex
   B. Aspirated cell culture medium from wells
   C. To each of the 3 wells, added 1mL NSV solution
   D. Spread evenly by gently shaking plates
xi. Prepared UCK2 wells
   A. Combined 3mL complete M199, 3μL polybrene, and 2.55μL UCK2 and mixed by vertex
   B. Aspirated cell culture medium from wells

C. To each of the 3 wells, added 1mL RIO3 solution
D. Spread evenly by gently shaking plates

xii. Prepared TGF-α wells
A. Combined 3mL complete M199, 3μL polybrene, and 40.0μL TGF-α and mixed by vertex
B. Aspirated cell culture medium from wells
C. To each of the 3 wells, added 1mL NSV solution
D. Spread evenly by gently shaking plates

(d) Incubated plates at 37°C for 2 hours
(e) Supplemented each of the wells with an additional 2mL complete M199 (3mL final well volume)
(f) Incubated plates at 37°C for 48 hours

2. MA104 cell split — Passage 67-2, from Passage 66, 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) Added to 3 T150 flasks 20mL complete M199/5mL cell mix; 20mL complete M199/5mL cell mix; and 20mL complete M199/5mL cell mix. Respectively, flasks A2, B2, and C2
(h) Gently shook flasks to distribute cells evenly
(i) Incubated at 37°C

23 October 2014

1. Prepared complete Medium 199
(a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration), 55mL fetal bovine serum
(b) Stored at 4°C

2. Prepared serum-free Medium 199
(a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration)
(b) Stored at 4°C

3. Plated MA104 cells — Passage 67, 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:

$$\begin{aligned}
[\text{cells}] &= \frac{3.31 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{\text{flask}} &= \frac{3.31 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &&= \frac{6.62 \times 10^6 \text{ cells}}{20\text{mL}} \\
\frac{\text{cells}}{150\text{mL vial}} &= \frac{6.62 \times 10^6 \text{ cells}}{150\text{mL}} &&= \frac{4.41 \times 10^4 \text{ cells}}{\text{mL}} \\
\frac{\text{cells}}{3\text{mL well}} &= \frac{4.41 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &&= \frac{1.32 \times 10^5 \text{ cells}}{\text{well}}
\end{aligned}$$

(h) Added 120mL 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) Labeled plates A1–A8

(l) Incubated at 37°C for 1 hour and then transferred to 31°C incubator

4. Plated MA104 cells — Passage 67, 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 10μL cell mixture with 10μL trypan blue:

$$\begin{aligned}
[\text{cells}] &= \frac{4.72 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{\text{flask}} &= \frac{4.72 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &&= \frac{9.44 \times 10^6 \text{ cells}}{20\text{mL}} \\
\frac{\text{cells}}{150\text{mL vial}} &= \frac{9.44 \times 10^6 \text{ cells}}{150\text{mL}} &&= \frac{6.29 \times 10^4 \text{ cells}}{\text{mL}} \\
\frac{\text{cells}}{3\text{mL well}} &= \frac{3.21 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &&= \frac{1.89 \times 10^5 \text{ cells}}{\text{well}}
\end{aligned}$$

(h) Added 120mL 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) Labeled plates B1–B8

(l) Incubated at 37°C for 1 hour and then transferred to 31°C incubator

5. Plated MA104 cells — Passage 67, 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 10μL cell mixture with 10μL trypan blue:

$$\begin{aligned}
[\text{cells}] &= \frac{1.76 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{\text{flask}} &= \frac{1.76 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &&= \frac{3.52 \times 10^6 \text{ cells}}{20\text{mL}} \\
\frac{\text{cells}}{150\text{mL vial}} &= \frac{3.52 \times 10^6 \text{ cells}}{150\text{mL}} &&= \frac{2.35 \times 10^4 \text{ cells}}{\text{mL}} \\
\frac{\text{cells}}{3\text{mL well}} &= \frac{2.35 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &&= \frac{7.04 \times 10^4 \text{ cells}}{\text{well}}
\end{aligned}$$

(h) Added 120mL 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) Labeled plates C1–C8
(l) Incubated at 37°C for 1 hour and then transferred to 31°C incubator

24 October 2014

1. Rotavirus infection of P66B plated cells (lentivirus-transduced)
   (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:

$$\begin{aligned}
[\text{cells}] &= \frac{3.46 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{2\text{mL well}} &= \frac{3.46 \times 10^5 \text{ cells}}{\text{mL}} \cdot 2\text{mL} &&= \frac{6.92 \times 10^5 \text{ cells}}{\text{well}}
\end{aligned}$$

   (b) Calculated SA11 dilution

$$\begin{aligned}
[\text{SA11}] &= \frac{5 \times 10^7 \text{ PFU}}{1\text{mL}} \\
\text{dilution} &= \left(\frac{6.92 \times 10^5 \text{ cells}}{\text{well}} \cdot \frac{5 \text{ particles}}{1 \text{ cell}} \right) \Big/ \frac{5 \times 10^7 \text{ PFU}}{1\text{mL}} \\
&= \frac{69.2\mu\text{L}}{\text{well}} \\
&= \frac{207.6\mu\text{L}}{3 \text{ wells}}
\end{aligned}$$

(c) Prepared viral inoculant
   i. To 12 vials, added 400μL SA11 and 2μL trypsin
   ii. Incubated in 37°C water bath for 1 hour
   iii. For each of 12 lentivirus transfections, prepared a vial of 3mL serum-free M199
   iv. Added to each vial 207.6μL viral solution (for infection of 3 wells transduced by a single lentivirus)

(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

25 October 2014

1. Lysed P66B transfected cells

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

   (b) Froze transfected cells in -20°C freezer overnight

26 October 2014

1. Collected viral load from lysed P66B transfected cells

   (a) Thawed P66B 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

2. Rotavirus activation and series dilution for P66B plated cells

   (a) For each of the 3 samples reclaimed from NSV, RIO3, TGF-α, and SKAP1-transduced cells (12 samples total):
      i. Combined 400μL SA11 reclaimed from LV-transfected cells and 2μL trypsin
      ii. Incubated for 1 hour in a 37°C water bath
      iii. Added 2.7mL serum-free M199 to each of 8 15mL tubes
      iv. To the first tube, added 300μL rotavirus solution to a final concentration of 10^{-1}
      v. Mixed contents by vertex
      vi. To the next tube, added 300μL rotavirus solution from the previous tube to a final concentration of 10^{-2}
      vii. Mixed contents by vertex
      viii. Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
      ix. Stored at 4°C

27 October 2014

1. Prepared serum-free Medium 199
   (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration)
   (b) Stored at 4°C
   (c) Repeated to prepare a second bottle
2. Rotavirus infection of P66B plated cells
   (a) For each of the 3 samples reclaimed from NSV, RIO3, and TGF-α-transduced cells (12 samples total):
      i. Washed monolayer in each well of 2 6-well plates twice with serum-free M199 by dumping
      ii. 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
      iii. Spread cells evenly by shaking
      iv. Incubated at 37°C for 1 hour
3. Agarose overlay of P66B plated cells
   (a) Liquefied 1.2% agarose by microwave and equilibrated to 55°C
   (b) For each plate of cells (24 total), prepared 1 vial of 10mL agarose, 10mL 2x EMEM, 5μL 2mg/mL trypsin
   (c) Aspirated viral inoculant from each well
   (d) To each well, added $\sim$ 3mL agarose solution
   (e) Let agarose solidify
   (f) Incubated at 37°C
4. MA104 cell split — Passage 68, from Passage 67-2, 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) Added to 2 T150 flasks 22.5mL complete M199/2.5mL cell mix and 22.5mL complete M199/2.5mL cell mix. Respectively, flasks A and B
   (h) Gently shook flasks to distribute cells evenly
   (i) Incubated at 37°C
5. 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; 200μg/mL streptomycin final concentration), and 1mL 250μg/mL amphotericin B stock (0.5μg/mL amphotericin B final concentration)
   (b) Stored at 4°C

28 October 2014

1. Plated MA104 cells — Passage 67-2, Flask B and C
   (a) Aspirated cell culture medium from each flask
   (b) Rinsed cells in each flask with 10mL 1x PBS; aspirated PBS
   (c) Rinsed cells in each flask with 5mL 0.05% trypsin; aspirated trypsin
   (d) Bathed cells in each flask with 5mL 0.05% trypsin
   (e) Incubated cells in each flask at 37°C until all cells detached from flask
   (f) Added 15mL complete M199 to each flask
   (g) Took cell count (per flask) by combining 10μL cell mixture with 10μL trypan blue:

$$
\begin{aligned}
[\text{B cells}] &= \frac{2.72 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{\text{flask B}} &= \frac{2.72 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &&= \frac{5.44 \times 10^6 \text{ cells}}{20\text{mL}} \\
[\text{C cells}] &= \frac{5.02 \times 10^5 \text{ cells}}{1\text{mL}} \\
\frac{\text{cells}}{\text{flask B}} &= \frac{5.02 \times 10^5 \text{ cells}}{1\text{mL}} \cdot 20\text{mL} &&= \frac{1.01 \times 10^7 \text{ cells}}{20\text{mL}} \\
\frac{\text{cells}}{40\text{mL}} &= \frac{5.44 \times 10^6 \text{ cells}}{20\text{mL}} + \frac{1.01 \times 10^7 \text{ cells}}{20\text{mL}} &&= \frac{1.55 \times 10^7 \text{ cells}}{40\text{mL}} \\
\frac{\text{cells}}{337.5\text{mL vial}} &= \frac{1.55 \times 10^7 \text{ cells}}{337.5\text{mL}} &&= \frac{4.60 \times 10^4 \text{ cells}}{\text{mL}} \\
\frac{\text{cells}}{3\text{mL well}} &= \frac{4.60 \times 10^4 \text{ cells}}{\text{mL}} \cdot 3\text{mL} &&= \frac{1.38 \times 10^5 \text{ cells}}{\text{well}}
\end{aligned}
$$

   (h) Added 297.5mL complete M199 and 20mL cell mixture from each flask (40mL cell mixture total) to a 500mL conical vial for final volume of 337.5mL
   (i) Transferred 3mL solution to each well of 18 6-well plates
   (j) Spread cells evenly by shaking
   (k) Labeled plates
   (l) Incubated at 37°C

2. Rotavirus infection of P66B plated cells
   (a) For each of the 3 samples reclaimed from SKAP1-transduced cells (12 samples total):
      i. Washed monolayer in each well of 2 6-well plates twice with serum-free M199 by dumping
      ii. 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
      iii. Spread cells evenly by shaking
      iv. Incubated at 37°C for 1 hour

3. Agarose overlay of P66B plated cells
   (a) Liquefied 1.2% agarose by microwave and equilibrated to 55°C
   (b) For each plate of cells (24 total), prepared 1 vial of 10mL agarose, 10mL 2x EMEM, 5μL 2mg/mL trypsin
   (c) Aspirated viral inoculant from each well
   (d) To each well, added $\sim$ 3mL agarose solution
   (e) Let agarose solidify
   (f) Incubated at 37°C

30 October 2014

1. Neutral red overlay of P66B plated cells (RIO3, vial 1)
   (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 $\sim$ 1mL prepared agarose solution
   (c) Let agarose solution solidify
   (d) Incubated at 37°C for 4 hours
   (e) Observed titer = 1×10^8 PFU/mL
2. Neutral red overlay of P66B plated cells (RIO3, vial 2)
   (a) Procedure was identical to above
   (b) Observed titer = 1×10^8 PFU/mL
3. Neutral red overlay of P66B plated cells (RIO3, vial 3)
   (a) Procedure was identical to above
   (b) Observed titer = 1.5×10^8 PFU/mL
4. Neutral red overlay of P66B plated cells (NSV, vial 1)
   (a) Procedure was identical to above
   (b) Observed titer = 4×10^8 PFU/mL
5. Neutral red overlay of P66B plated cells (NSV, vial 2)
   (a) Procedure was identical to above
   (b) Observed titer = 1×10^8 PFU/mL
6. Neutral red overlay of P66B plated cells (NSV, vial 3)
   (a) Procedure was identical to above
   (b) Observed titer = 2×10^8 PFU/mL
7. Neutral red overlay of P66B plated cells (TGF-α, vial 1)
   (a) Procedure was identical to above
   (b) Observed titer = 3×10^8 PFU/mL
8. Neutral red overlay of P66B plated cells (TGF-α, vial 2)
   (a) Procedure was identical to above
   (b) Observed titer = 1.75×10^8 PFU/mL
9. Neutral red overlay of P66B plated cells (TGF-α, vial 3)
   (a) Procedure was identical to above
   (b) Observed titer = 3×10^8 PFU/mL
10. Prepared serum-free Medium 199
    (a) Supplemented 500mL incomplete M199 with 5mL penicillin/streptomycin stock (100U/mL penicillin; 100μg/mL streptomycin final concentration) and 1mL 250μg/mL amphotericin B stock (0.25μg/mL amphotericin B final concentration)
    (b) Stored at 4°C
11. Prepared 400mL 1.2% agarose
    (a) Combined 4.8043g SeaPlaque agar to $\sim$ 400mL milli-Q-filtered water
    (b) Autoclaved for 20 minutes
    (c) Stored at room temperature

31 October 2014

1. Neutral red overlay of P66B plated cells (SKAP1, vial 1)
   (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 $\sim$ 1mL prepared agarose solution
   (c) Let agarose solution solidify
   (d) Incubated at 37°C for 4 hours
   (e) Observed titer = 1×10^8 PFU/mL
2. Neutral red overlay of P66B plated cells (SKAP1, vial 2)
   (a) Procedure was identical to above
   (b) Observed titer = 1×10^8 PFU/mL
3. Neutral red overlay of P66B plated cells (SKAP1, vial 3)
   (a) Procedure was identical to above
   (b) Observed titer = 1.5×10^8 PFU/mL
4. Rotavirus activation and series dilution for P67-2 A/B plated cells
   (a) For each of the 3 samples reclaimed from GUCY2D, CSNK2B, and EPHA1-transduced cells (9 samples total):
      i. Combined 400μL SA11 reclaimed from LV-transfected cells and 2μL trypsin
      ii. Incubated for 1 hour in a 37°C water bath
      iii. Added 2.7mL serum-free M199 to each of 8 15mL tubes
      iv. To the first tube, added 300μL rotavirus solution to a final concentration of 10^{-1}
      v. Mixed contents by vertex
      vi. To the next tube, added 300μL rotavirus solution from the previous tube to a final concentration of 10^{-2}
      vii. Mixed contents by vertex
      viii. Repeated serially for the remaining tubes ending with a concentration of 10^{-8} in the final tube
      ix. Stored at 4°C

01 November 2014

1. Rotavirus infection of P67-2 A/B plated cells
   (a) For each of the 3 samples reclaimed from GUCY2D, CSNK2B, and EPHA1-transduced cells (9 samples total):
      i. Washed monolayer in each well of 2 6-well plates twice with serum-free M199 by dumping
      ii. 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
      iii. Spread cells evenly by shaking
      iv. Incubated at 37°C for 1 hour
2. Agarose overlay of P67-2 A/B plated cells
   (a) Liquefied 1.2% agarose by microwave and equilibrated to 55°C
   (b) For each plate of cells (18 total), prepared 1 vial of 10mL agarose, 10mL 2x EMEM, 5μL 2mg/mL trypsin

(c) Aspirated viral inoculant from each well
(d) To each well, added $\sim$ 3mL agarose solution
(e) Let agarose solidify
(f) Incubated at 37°C

03 November 2014

1. MA104 cell split — Passage 69, from Passage 68, Flask A
   (a) Aspirated cell culture medium
   (b) Rinsed cells in 10mL 1× 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 2 T75 flasks 15mL complete M199/2.5mL cell mix and 15mL complete M199/2.5mL cell mix. Respectively, flasks A and B
   (h) Gently shook flasks to distribute cells evenly
   (i) Incubated at 37°C
2. Disposed of P68B plated cells

04 November 2014

1. Neutral red overlay of P68A/B plated cells (GUCY2D, vial 1)
   (a) Prepared a vial with 7.5mL 1.2% agarose, 7.5mL 2× EMEM, and 750μL 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 = 2.5×10^8 PFU/mL
2. Neutral red overlay of P68A/B plated cells (GUCY2D, vial 2)
   (a) Procedure was identical to above
   (b) Observed titer = 5×10^8 PFU/mL
3. Neutral red overlay of P68A/B plated cells (GUCY2D, vial 3)
   (a) Procedure was identical to above
   (b) Observed titer = 2×10^8 PFU/mL
4. Neutral red overlay of P68A/B plated cells (CSNK2B, vial 1)
   (a) Procedure was identical to above
   (b) Observed titer = 1×10^8 PFU/mL
5. Neutral red overlay of P68A/B plated cells (CSNK2B, vial 2)
   (a) Procedure was identical to above
   (b) Observed titer = 2×10^8 PFU/mL
6. Neutral red overlay of P68A/B plated cells (CSNK2B, vial 3)

   (a) Procedure was identical to above
   (b) Observed titer = 8×10^7 PFU/mL

7. Neutral red overlay of P68A/B plated cells (EPHA1, vial 1)
   (a) Procedure was identical to above
   (b) Observed titer = 1×10^8 PFU/mL
8. Neutral red overlay of P68A/B plated cells (EPHA1, vial 2)
   (a) Procedure was identical to above
   (b) Observed titer = 1×10^8 PFU/mL
9. Neutral red overlay of P68A/B plated cells (EPHA1, vial 3)
   (a) Procedure was identical to above
   (b) Observed titer = 4×10^8 PFU/mL