BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

Last modified: June 2016
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Protocol: No-Spin Infection Protocol for adherent cell lines

While the Spinfection protocol (ie includes a centrifugation step after the addition of virus) uses lower virus volumes per number of cells infected, this alternative protocol reduces handling time, necessity of centrifugation and over-confluence issues present in the Spinfection protocol.

The GFP-expressing, puromycin/blasticidin-resistant pRosetta virus is available from GPP or the plasmid can be obtained from Addgene (plasmid # 59700).

Procedure

1. **For a New Cell Line:**

   Step 1. Determine Number of Cells to Seed

# of cells/well *	Total Vol in each well (mL)
2.00E+04	1.1
5.00E+04	
1.00E+05	
3.00E+05	
6.00E+05	
1.50E+06	

*This range of values is for cell lines without especially large or small size and doubling times of ~1 day

Day 0, pm: Seed cells in 6 wells of a 6-well plate

Day 1, am (17hr post-seed)

Aspirate viral media and add 2.75 mL media per well

Day 2, pm (48hr post-seed)

Check confluence of all wells.

Use condition that is 90-95% confluent.

# of cells seeded per well on Day 0	% confluence 48hr post-seed
1.00E+05	
2.50E+05	
5.00E+05	
7.50E+05	
1.00E+06	
2.00E+06	

Examples of appropriate cell numbers at time of seeding:

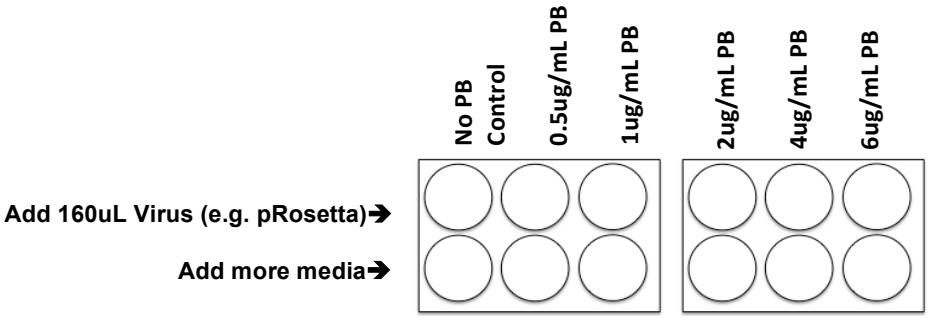
- A375: ~ 2.5e5 cells
- HT29: ~ 1.0e6 cells
- 293T: ~ 2.0e5 cells

Step 2. Determine optimal Polybrene (PB) concentration

Day 0, pm: Seed cells

1) Create 1mL of 0.05 ug/uL PB stock
2) Create a cell + media master mix:
   - Master mix is made in 20% excess: 1.2*12 ml =14.4ml
   - Total volume: 14.4*0.800µL = 11.5 ml
   - Cells: 14.4 * (# of cells from Part 1 Step1)
3) Seed 12 wells in two 6-well plates with 800uL of cell + media master mix.

PB Condition	Media + Cells (uL)	0.05 ug/uL PB Stock (uL)	Additional Media (uL)	Virus (uL)
0	800	0	140	160
			300	----------
0.5	800	11	129	160
			289	----------
1	800	22	118	160
			278	----------
2	800	44	96	160
			256	----------
4	800	88	52	160
			212	----------
6	800	132	8	160
			168	----------



4) Add appropriate volume of PB stock, additional media and virus

Day 1, am (17hr post-seed)

Aspirate media from all wells and add 2.75mL new media to each well

Day 2, pm (48hr post-seed)

1) Determine PB toxicity & infection efficiency
   -Count cells to determine PB toxicity
   -Flow for % GFP+ (or other easily measured marker) to determine efficiency
2) Looking at the infection efficiencies and cell counts, determine the PB concentration for your cell line that best balances increased infection efficiency with a toxicity level that you are willing to accept.
3) If no PB concentration works, repeat using higher virus volume. Reduce the amount of media used so that the total volume is 1.1mL per well.

2) <u>For a new virus - Determine virus volume to use</u>

<u>Step 1. Infect cells with different virus vol.</u>

Day 0, pm: Seed cells

1) Create 2mL of PB Stock at 10X of PB concentration selected in **Part 1 Step 2.**
2) Create a cell + media master mix using:
   -Master mix is made in 20% excess: 1.2*12 ml =14.4ml
   -Total volume: 14.4*0.6ml = 8.65 ml
   -Cells: 14.4 * (# of cells from Part 1 Step1)
   -PB: 1.6 ml of 10X stock
3) Seed 12 wells of 2 x 6 well-plates with 600uL cells-PB-media master mix
4) Add additional media so that the final volume after virus addition will be 1.1mL
5) Add Virus: 0 (NIC), 100, 200, 300, 400 and 500uL

	Cells-PB-Media Master Mix per well (uL)	**Additional Media (uL)**	**Virus (uL)**
No Infection Control (NIC)	**600**	**500**	**0**
100uL Virus		**400**	**100**
200uL Virus		**300**	**200**
300uL Virus		**200**	**300**
400uL Virus		**100**	**400**
500uL Virus		**0**	**500**

Day 1, am (17hr post-seed)

Aspirate media from all wells and add 2.75mL new media.

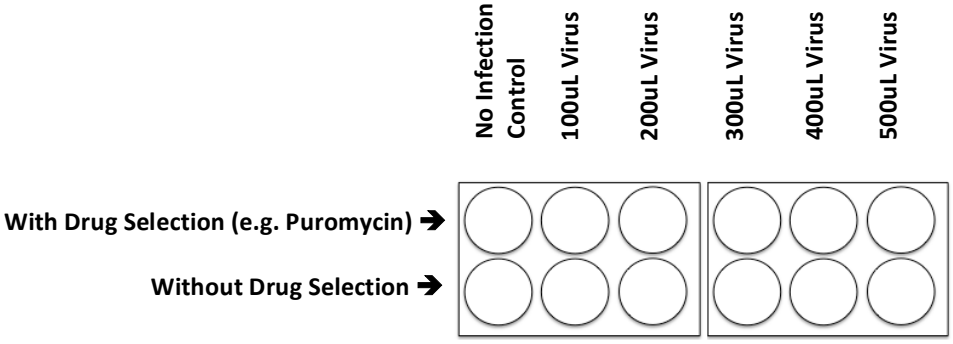
Day 2, pm (48hr post-seed)

- ***If virus expresses GFP or other easily visualizable marker:*** flow for %GFP+ or marker
- ***If virus confers drug (e.g. puromycin) resistance:*** make a titer plate and drug-select infected cells (Explained in **Part 2 Step 2**)

Step 2. Making a titer plate to assess infection efficiency by drug selection (e.g. Puromycin)

Day 2, pm (48hr post-seed/end of infection)

1) Passage all 6-well plate conditions.
2) Seed ½ number of cells determined in **Part 1 Step 1** in 5mL total vol. in 2 x 6-well plates using 2 wells per virus volume
   - For the 1st well of each 2-well set, select with drug (e.g. Puromycin).
   - For the 2nd well of each set, don't add drug selection.



Day 4-6

1) Check confluence of wells in the 6-well plates:
If No Infection Control well *with* Drug Selection is not dead and some wells are approaching confluence, passage cells.
2) Titer is ready to read when No Infection Control *with* Drug Selection is dead (*ensure selection is complete*).
3) When ready to read, count cells in each well.
4) For each 2-well set, determine infection efficiency:

$$\frac{(\#cells\ in\ well\ \boldsymbol{with}\ selection)}{(\#cells\ in\ well\ \boldsymbol{without}\ selection)} X\ 100\% = Infection\ Efficiency$$

5) Pick a virus volume that is the closest to the efficiency desired.

3) Repeating and scaling up using titered virus and previously used cell line

Step 1. Infection and Titer Guide

Day 0, pm: Seed Cells

In T175s, seed 18.191 * (# cells from **Part 1 Step 1**)

- Scale up screen arms

		Tissue Culture Format	6-well	T175
		Surface Area mm^2	962	17500
Day 0, pm:	Seeding & Infection Start	Time (hr)	0	
		Seed	**Part 1 Step 1** value	**Part 1 Step 1** value * 18.191
		PB conc. (ug/mL)	**Part 1 Step 2** value	
		Virus vol. (mL)	**Part 2 Step 1** value	**Part 2 Step 1** value * 18.191
		Total infection/Seeding vol. (mL)	1.1	20

18.191 scaling factor based on the surface area of a T175 vs. 6 well: $\left(\frac{17500mm^2}{962mm^2} = 18.191\right)$

Day 1, am (17hr post-seed)

Aspirate viral media and add 2.75mL media to the 6-well and 50mL to the T175s

		Tissue Culture Format	6 Well	T175
		Surface Area mm^2	962	17500
Day 1, am	Change Media	Time (hr)	17	
		Media (mL)	2.75	50

Day 2, pm (48hr post-seed)

1) Determine in-line titer:
   Read out visualizable titer (e.g. GFP), if applicable

OR

   Pool infected cells from T175, determine cell concentration, seed ½ number of cells determined in **Part 1 Step 1** in 6 wells and start drug selection for drug-based titer (see **Part 2 Step 2)**

2) Seed the remaining pooled infected cells into flasks for screening and start drug selection.