The RNAi Core

Version 2 (12/11/2014)

Puromycin kill curve

Optimization of puromycin selection condition

To generate a fully transduced population of cells it is important to determine the minimum amount of puromycin required to eliminate non-transduced cells. This is accomplished by performing a kill curve to determine the optimal puromycin concentration needed to eliminate non-transduced cells. A kill curve is a dose-response experiment where the cells are subjected to increasing amounts of antibiotic to determine the minimum antibiotic concentration needed to kill all the cells over the course of 2 to 7 days. For puromycin, the optimal concentration is the lowest concentration that kills 100% of non-transduced cells and shows maximal survival of transduced cells in 48-72 hours. The optimal concentration is cell type dependent. Performing a kill curve is recommended with each new cell type or when a new selection antibiotic or different lot of selection antibiotic is used.

Puromycin Kill Curve

1. Plate cells at appropriate density in 100ul complete growth medium per well in a 96-well clear bottom tissue culture plate. The recommended cell density for most cell types is 30-50% confluence at the day of transduction. Incubate at 37℃ overnight.

2. Replace media with 45ul media containing 4ug-8ug/ml polybrene (depend on cell type).

3. Infect the cells with shRNA control virus (C6-4) at MOI 2 and MOI 6:

Virus MOI												
A												
B												
C												
D												
E		**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	
F		**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	
G		**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	
H		**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	

4. Spin the plates at 1100g at 30℃ for 15-30min. Incubate at 37℃ overnight.

5. Replace the media from the infected cell plates and add 100ul media containing various puromycin concentrations as shown below. Incubate at 37℃ for 48-72 hours.

Puromycin concentration, ug/mL												
A		**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**	
B		**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**	
C		**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**	
D		**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**	
E		**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**	
F		**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**	
G		**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**	
H		**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**	

6. Remove the puromycin containing media from the cell plates.

7. Add 100ul of phenol red free DMEM medium containing 10% MTS. Incubate at 37℃ for 0.5-3hrs.

8. Record the absorbance at 490 nm using a 96-well plate reader.

9. Determine the optimal puromycin concentration by choosing the minimum concentration of puromycin that causes complete cell death in non-transduced cells and maximal viability in transduced cells.