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Selection kill curve

Since cell lines differ in their sensitivity to antibiotics, the optimal concentration (pre-transduction) should be determined. Once transfection/transduction has occurred, the cells can be treated to select for cells expressing antibiotic resistance. In the following protocol the lowest concentration that provides adequate selection is determined for the experimental cell line. If using an inducible vector, a doxycycline optimization curve also needs to be performed.

Puromycin and blasticidin have a similar range of concentration that is toxic to most cell lines. The same kill curve can be used for both as shown in the example in **Table 2**. For Hygromycin, use a concentration range of 50-500 µg/ml. For Neomycin (G418), use a concentration range of 100-800 µg/ml as shown in the example in **Table 3**.

Materials

- Complete media for experimental cell line
- Appropriate antibiotic for selection
  - Blasticidin S HCl antibiotic (Life Technologies, Catalog# A11139-03)
  - Puromycin Dihydrochloride (Life Technologies, Catalog# A11138-03)
  - Hygromycin B (50 mg/ml) (ThermoFisher, Catalog# 10687010)
  - G418 Sulfate (50 mg/ml)(ThermoFisher, Catalog# 10131035)
- 24-well tissue culture plate

Equipment

- Automatic pipette/Pipette-aid
- Disposable or autoclaved tissue culture pipettes
- CO_2 cell culture incubator at 37°C

Protocol

1. Make the appropriate stock solution of antibiotic based on the recommended concentration range in **Table 1** below:

Table 1. Stock solutions for antibiotics

Antibiotic	Concentration range for kill curve
Puromycin	1-10 µg/ml (use a 1.25 µg/µl stock solution)
Blasticidin	2-10 µg/ml (use a 1.25 µg/µl stock solution)
Hygromycin	100-800 µg/ml (use a 50 µg/µl stock solution)
Neomycin (G418)	50-500 µg/ml (use a 50 µg/µl stock solution)

2. Plate 5 x 10^4 cells per well in 11 wells of a 24-well tissue culture plate using media without antibiotics.
3. Prepare antibiotic dilutions in culture media for titration as shown in Tables 2 and 3 below:
   a Prepare puromycin or blasticidin dilutions in culture media for titration as shown in the example in **Table 2**.

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Table 2. Example dilutions and volumes required for establishing optimal antibiotic concentration for puromycin and blasticidin

Volume of Puromycin or Blasticidin Stock Solution Added (µl)	Total Volume of Media plus Antibiotic per 24 Well (µl)	Final Concentration (µg/ml)
0	500	0
0.2	500	0.5
0.4	500	1
0.6	500	1.5
0.8	500	2
1	500	2.5
1.2	500	3
1.6	500	4
2	500	5
3	500	7.5
4	500	10

b Prepare hygromycin or neomycin (G418) dilutions in culture media for titration such as shown in the example **Table 3**.

Table 3. Example dilutions and volumes required for establishing optimal antibiotic concentration for hygromycin and G418

Volume of Hygromycin or neomycin (G418) Stock Solution Added (µl)	Total Volume of Media plus Antibiotic per 24 Well (µl)	Final Concentration (µg/ml)
0	500	0
0.5	500	50
1	500	100
2	500	200
3	500	300
4	500	400
5	500	500
6	500	600
7	500	700
8	500	800

4. Begin antibiotic selection the following day by replacing antibiotic free media with media containing the appropriate concentrations of antibiotic.
5. Incubate cells with 5% CO_2 at 37°C, or use conditions normal for the target cells.
6. Check cells daily to estimate rate of cell death.
7. Replenish the media containing the appropriate concentrations of antibiotic every 2 days for 6 days.

Note: The optimal antibiotic concentration will kill the cells rapidly (2 - 4 days). This is particularly important for screens involving essential genes that may be selected against prior to the experiment.