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RNAi, Gene Expression & Gene Editing

Lentiviral titering by crystal violet staining

The following protocol provides an example for titering lentiviral particles using crystal violet staining in a 6-well format to obtain transducing units per mL (TU/mL). Once lentiviral particles are packaged and collected, small aliquots should be frozen at -80 °C. One aliquot should be thawed on ice and used for titering the lentiviral particles.

Note: Lentiviral particles must be stored at -80 °C. Repeated freeze-thaw cycles should be avoided, as this is expected to negatively affect titer. Once thawed, unused lentiviral particles should be kept on ice, divided into smaller aliquots (if necessary) and immediately returned to -80 °C.

Materials Required:

- Lentiviral particles
- Multi-well tissue culture plates or tissue culture dishes
- Blasticidin S (Fisher Scientific, Cat #BP2647-25; InvivoGen, Cat #ant-bl-1) or Puromycin (Fisher Scientific, Cat #BP2956-100; InvivoGen, Cat #ant-pr-1)
- Crystal Violet (Fisher Scientific, Cat C581-25) or similar assay for assessing cell viability
- Growth Medium: antibiotic-free cell culture medium (with serum and/or supplements) recommended for maintenance of the cells of interest
- Transduction Medium: the base cell culture medium for transduction of lentiviral particles (with transduction additives and serum, if necessary)

Day 1

1. Plate an appropriate number of cells per well in a 6-well plate, using standard Growth Medium for the cells of interest.
2. Incubate cells at 37 °C in a humidified CO_2 incubator overnight.

Day 2

1. Prepare the Transduction Medium (with or without serum and necessary supplements) and equilibrate the medium to 37 °C.
2. Thaw lentiviral particles on ice.
3. Perform 10-fold serial dilutions of the lentiviral particles in 1.5 mL Transduction Medium starting at 1:100 to obtain $10^{-2} \rightarrow 10^{-3} \rightarrow 10^{-4} \rightarrow 10^{-5} \rightarrow 10^{-6}$ dilutions.
4. Remove Growth Medium from cells and add 1.5 mL Transduction Medium containing dilutions of lentiviral particles. To the 6th well, add 1.5 mL Transduction Medium without lentiviral particles (untransduced control). Return plate to incubator overnight.

Day 3

1. Change medium containing predetermined concentration of antibiotic, such as puromycin or blasticidin.

Note: Prior to transduction for titering, determine the minimum concentration of antibiotic required to kill non-transduced cells between three and 10 days by generating an antibiotic kill curve.

Day 5-14

1. Change medium containing antibiotic every 2-3 days until all of the untransduced cells are killed, and transduced colonies are growing.

Day 12-16

1. Remove medium and gently wash cells with PBS.
2. Add 1 mL crystal violet solution (1% crystal violet in 10% EtOH).
3. Incubate at room temperature for 10 minutes.
4. Wash gently with PBS 2-3 times.
5. Count colonies where reasonable (Figure 1) and calculate titer using: # colonies/mL × dilution = TU/mL

Note: Too high of a multiplicity of infection (MOI; i.e., too many particles per cell) or lentiviral particles directly from unpurified supernatant can result in cell death and no colony formation. In this case, go to further dilutions to count colonies for titer calculation.

Example calculation:
57 colonies/1.5 mL × 10^5 = 3.8 × 10^6 TU/mL

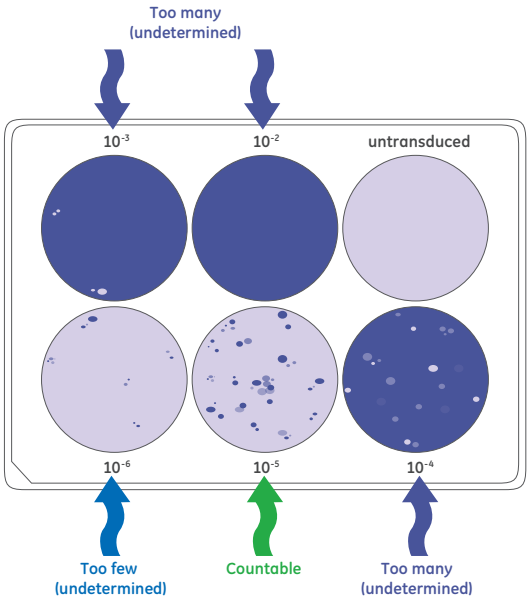

Figure 1. Crystal violet stained cell colonies for titering lentiviral particles. All cells in the untransduced well are dead. In wells transduced with 10^{-2}, 10^{-3} and 10^{-4} dilutions there are too many colonies to distinguish or count. The well with 10^{-5} dilution has a reasonable number of colonies to count, while there are too few colonies in the 10^{-6} dilution.

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