Spin Infections

Reference: Kotani H; Newton PB 3rd; Zhang S; Chiang YL; Otto E; Weaver L; Blaese RM; Anderson WF; McGarrity GJ
Improved methods of retroviral vector transduction and production for gene therapy.
Genetic Therapy, Inc., Gaithersburg, MD 20878.
Language: Eng
Source: Hum Gene Ther 1994 Jan;5(1):19-28
Unique Identifier: 94207070

1. plate 1-2 x 106 cells/well in 24-well dish. Density should be adjusted so that surface of plate is covered after spinning. Wash cells x 1 before adding virus.

2. Add 1 ml of retroviral supe or medium plus supe PLUS polybrene (Sigma) such that final polybrene concentration is 8 μg/ml.

3. Spin @ 2000 rpm in Sorvall RT6000B or equivalent centrifuge for 1.5-2 hours. I use room temp 22-26 degrees.

4. Return plate to incubator for 2 hour to overnight. I usually change the media after 2 hours. For some cells 8 μg/ml polybrene may be toxic. The polybrene concentration does not appear to make a difference (as long as it is there!!!) and I have had good success with concentrations ranging from 2-8 μg/ml.

5. It is usually necessary to change the media after 24 hour. In fact, I usually return the cells to a flask at this point.

6. Stain for x-gal 24 hours after infection.