

24-well Plate Spin Infection

Infect Raji B cells using lentivirus by spinning in a centrifuge.

Materials:

- * Polybrene
- * Virus
- * 24-well plate
- * Complete Media (RPMI + 2% FBS + P/S/B-mercaptoethanol)
- * Raji B Cells

Methods:

1. Count cells.
2. Pre-heat the centrifuge to 33°C by spinning.
3. For each well, plate 500,000 cells in 1 ml of complete media (RPMI, 10% FBS, p/s/ β -mercaptoethanol) supplemented with 8 ug/ml polybrene. Use as many wells as necessary to infect the amount of cells you want, hence 4 wells for 2 million cells...etc.
4. Add virus to the medium. Mix by rocking plate back and forth a couple of times. For Raji cells: MOI of 0.3 generally gives about 20% infected cells.
5. Spin the plates at 33°C for 2 hours @ 2250 rpm.
6. Resuspend the cells in each well by pipetting up and down. (Note: Cells tend to stick to the edges of the plate)
7. Transfer the cells of each well to a 6-well plate. Add 4 mL of complete media to each well. Incubate overnight at 37°C. If cells need to grow over the weekend, transfer cells to a 10-cm plate and resuspend in 12 mL of complete media. If more than 1 million cells are infected AND the cells need to incubate over the weekend, transfer cells to a 15cm plate and resuspend in 30 mL of complete media.

NOTE: Virus infections need to be performed in the viral hood. Wear a lab coat and gloves. All materials that come in contact with virus need to be treated with 10% bleach for approximately 1 hour before being discarded!! After contact with virus, gloves need to be removed and discarded before touching anything else!!