

### Retroviral Infection Protocol

Virus used:

Producer cells:      Bosc                      Bing                      PhoenixAmpho                      PhoenixEco  
 Date made:              Exp#                      from plasmid(s)

Cells infected:              A7r5                      RASM                      other: \_\_\_\_\_  
 plated:                      passage#

Day/Time	notes	procedure
		<b>1. Dilute polybrene (0.8mg/ml stock solution) 1:100 into thawed or fresh virus supernatant.</b>
		<b>2. Aspirate media from cells, replace with virus. Use 2 ml per 35 mm well.</b>
		<b>3. 32° in 5%CO2 for 15 min</b>
		<b>4. Spin 2500 rpm 30 min 32° in tabletop centrifuge (make sure centrifuge is pre-run to warm it up, set temp gauge to 40C)</b>
		<b>5. After spin, aspirate viral supernatant and replace with fresh media.</b>
		<b>6. Return cells to 37°</b>
		<b>Infect total of 1-3 times over 24 hour period, so every 8-12 hours. You can infect with even more rounds if you care to achieve greater efficiency.</b>
		<b>After last infection, remove virus and add fresh media to cells</b>
		<b>Add selection drugs (if necessary) 24 h after the last spin/infection.</b>