pBabe Retrovirus Production

293 Transfection

1. Plate 12 x 10^6 293.T in 20 ml on a 15 cm^2 plate 24 hours before transfection. In general, two 15cm plates per virus. It is essential that the cells be well-maintained and of relatively low passage number.
2. Mix the following DNAs (made w/ Endo-free Qiagen Kits) in a FACS tube. The DNAs should be in Endo-free TE at a concentration of 0.5µg/µl.

   For 3 plasmid system (reference: Cheng HL,et al: PNAS 100:10794-10799, 2003)
   - 20 µg pBabe vector,
   - 10 µg VSVG
   - 10 µg pMDL g/p RRE
3. Add 400 µl 1.25 M $CaCl_2$ and 1.5 ml H_20 and mix by tapping gently.

The following steps are done 1 plate at a time.

4. Add 2 ml of 2X HBS dropwise to DNA mixture while bubbling with a Pasteur pipette. When finished, continue to bubble for 12-15 seconds.
5. Take plate of 293T out of the incubator (plate remains in incubator for long as possible), and add transfection mixture dropwise all over the plate. Gently swirl plate from front to back, and return immediately to incubator.
6. 3.5 to 4 hours later, remove media, wash 2x with 10ml warm PBS, and add 20 ml warm D10 onto plate and place in incubator.
7. 36-48 hours after transfection, harvest viral supernatant and spin @ 2000 rpm, 7 min at 4°C in a 50ml tube.
8. Filter viral SN through .45 um filter. Add 35ml of filtered supernatant to an ultracentrifuge tube. Balance tubes with additional media. Cover tubes with small piece of parafilm. (It is useful to titer some of the leftover supernatant to determine if there is loss of virus during concentration.)
9. Spin tubes using a SW-28 rotor @ 25,000 rpm, 90 min, 4°C. Decant liquid and leave tube upside down on kimwipe for 10 min. Aspirate remaining media being careful not to touch bottom of tube.
10. Add 15µl cold PBS (for embryo infections, or any volume you wish) and leave tube at 4°C O/N with no shaking.
11. To resuspend, hold tube at angle and pipet fluid over pellet 20 times, being careful not to touch pellet with tip. It is expected that the pellet not be resuspended after this is complete. This pellet does not contain virus and can be discarded.
12. Aliquot or use virus. Virus should be aliquoted, flash-frozen in liquid nitrogen and stored at -80. There should be no change in titer with freezing concentrated virus. Avoid multiple freeze-thaws.
13. **Stable cell line**: Transfection with virus in 2 ug/ml polybrene, and selection with 2.5 ug/ml puromycin.