12/17/12

Bullet Protocol

Preparation of Gene Gun Bullets and Biolistic Transfection of Neurons in Slice Culture (http://www.jove.com/video/675/preparation-of-gene-gun-bullets-and-biolistic-transfection-of-neurons-in-slice-culture)

Au solution (200mg/mL in 50% glycerol, size 1.6 µm Gold Microcarriers - 165-2264)
Spermidine (50mM stock in deep freezer)
1M CaCl2 (help to stick DNA to Au)
EtOH, anhydrous (Sigma 459836). Use 10mL syringe.
20mg/mL PVP in dw (make fresh, sonication will help to dissolve). PVP will help the Au to stick to the tube. polyvinylpyrrolidone

10 µg.

1. Prepare DNA solution. (about 3ug / construct). Total DNA volume should be less than 6uL. DNA should be maxi prep. If using two different construct, mix well
2. Combine 15uL of Au solution with 17uL of 50mM spermidine. Au solution should be well vortexed (20) and (24) sonicated. After combined, (22) (26) Sonicate 5sec, 3times. cut end of tip -
3. Combine DNA and Au/spermidine solution.
4. Drop wise 17uL of 1M CaCl2 while vortexing.
5. Incubate for (20) 3min, RT
6. Cfg 6,000rpm, 30sec and remove sup → absol. - use syringe
7. Wash and spin (full speed for 30sec) three times with 1mL of 100% EtOH to remove all traces of water. Sonicate for 1sec (don't over Sonicate) before each centrifugation making sure there is no aggregated Au prior to spinning. - brief
8. In a separate tube prepare 0.05mg/mL PVP EtOH by adding 3.75uL of 20mg/mL PVP (dessicated) in 1.5mL EtOH. → make better stick on tubing 1.5
9. Combine 1.5mL 0.05mg/mL PVP with Au pellet. (use less than 1.5mL may concentrate the Au particle per tube)
10. Vortex immediately prior to loading into tube.
11. Use syringe to slowly suck Au into two foot piece of tubing (1.5mL will fill about half of the tube; mark the Au boundary on the tube. Leave a few inches dry from the end). Careful to avoid bubbles.
12. Let Au settle to bottom of tube for 10min