Biolistics Bullet Preparation

Materials/Equipment:

DNA stocks
Water (HPLC Grade)
100% anhydrous ethanol (Either fresh or dried with molecular sieves)
PVP (polyvinylpyrollidone, MW 360,000)
Dry N_2 Tank and bullet board
Tefzel tubing
Centrifuge for 15 ml conical tubes
Bullet vials and labels
Tubing chopper
0.5 M spermidine free-base
15 ml centrifuge tubes (VWR orange or purple cap, polypropylene)
1 M $CaCl_2$
1.6 micron gold powder (Bio-Rad)
Syringes, with tubing adaptor
Scissors
Desiccator tablets

Procedure:

1. Prepare appropriate number of tubing (cutting 30" stretches for a full bullet).
2. Attach the tubing to the syringe. Set aside.
3. Label vials appropriately and insert a desiccator tablet. Set aside.
4. Prepare PVP-Ethanol by measuring out 100% EtOH into a beaker, cover and stir (you will need 3.5 ml for each 30" bullet):

   0.07 mg PVP per 1 ml 100% EtOH
5. Thaw the 0.5 M spermidine and DNA stocks at room temperature.
6. Weigh out gold. Our current protocol calls for **6 mg total for a full 30" bullet**.
7. Once spermidine is thawed, dilute down to 0.05 M with HPLC water (i.e., 100 ul 0.5 M spermidine into 900 ul of water). Set aside.
8. In each centrifuge tube, measure out appropriate amount of DNA, making sure that all stock is mixed in properly at the bottom of the tube:

   Measure so that **6 ug of total DNA is applied to each 1 mg Au**. For example:

	Per mg Au	**Per 6mg bullet**
YFP	2 ug	12 ug
Construct A	2 ug	12 ug
Construct B/Stuffer	2 ug	12 ug
Total DNA	**6 ug**	**36 ug**

9. Add the diluted spermidine to the gold, measuring out 200 ul for each bullet prep.

10. Sonicate the resulting gold slurry for 30 seconds.
11. Add 200 ul of the gold slurry to each tube containing DNA, vortexing the slurry between applications.
12. While vortexing the DNA-gold slurry mixture, add 200 ul $CaCl_2$ slowly, drop-wise. This type of application prevents localized DNA precipitation.
13. Incubate the slurry at room temperature for 10 minutes.
14. Place the tubes in a tabletop centrifuge and spin for 1 minute at 3000rpm.
15. Remove supernatant.
16. Add 1 ml 100% EtOH and vortex.
17. Centrifuge for 1 minute at 3000 rpm.
18. Remove supernatant and add 1 ml of 100% EtOH and vortex.
19. Repeat the rinse step twice. After pouring off the supernatant from the last rinse, add 3.5 ml of PVP-EtOH to each (30" length bullet) and vortex.
20. Place tubing end of the syringe+tubing into the tube, vortex the gold/EtOH mixture while pulling the liquid into the tubing with the syringe with a slow, steady movement.
21. Slide the filled tubing into place on the bullet board. Try to position it so that the portions of the tube that hold liquid are horizontal so that gold does not gather on a hanging end.
22. Let the gold settle onto the inside of the tubing (about 10 minutes or longer).
23. With a slow, steady motion, pull the ethanol off the inside of the tubing using the syringe. Discard the ethanol.
24. Attach the end of the tubing to the flowmeter's connective tubing.
25. Open the N_2 tank. Increase the flow of gas to the flowmeters slowly and steadily. If needed, adjust the valve on the flowmeter so that it reads 0.3-0.5 lpm.
26. Allow the bullet to dry for 15-20 minutes. The flowmeter is not perfect, so check the progress of the bullets often.
27. When bullets are sufficiently dry, remove the bullet tubing from the flowmeter tubing, bleed the line to the flowmeter and turn off the N_2 tank
28. Feed the bullet tubing into the chopper, fitting the bullet vial underneath.
29. Seal and store at 4 degrees C.

Loading Bullet Cartridges Protocol

Materials:

Bullets
Cartridges
Cartridge Extractor Tool

Loading procedure:

1. Place white cartridge holder in one hand
2. The cartridge should be handled such that the small black numbers located on the top of the cartridge, beginning with the number 1 continue around clockwise through number 12
3. Carefully insert one DNA/gold coated, pre-cut tubing (gold line facing you outside the cartridge) into each numbered hole with a firm downward motion
4. Use the number on the top/face of the cartridge as your guide

   Note: **DO NOT use the numbers on the outside rim of the cartridge!**
5. Lightly shake the cartridge once loading is complete to assure that the tubing (bullet) is secured

Unloading procedure:

1. To remove tubing (bullets), line up the cartridge extractor tool to the back of the cartridge and push downward
2. Discard used bullets

Brain slice culture medium recipe

Materials:

Material	Volume
Neurobasal A medium	750 ml
Heat-inactivated horse serum	150 ml
1 M KCl	10 ml
1 M Hepes	10 ml
Penicillin/Streptomycin	10 ml
100 mM MEM Sodium Pyruvate	10 ml
200 mM L-Glutamine	5 ml

Filter Sterilize with 0.22 micron filter in the hood

Coronal Slicing (Hemi-sections)

Materials:

Vibratome & parts (i.e. tray, screws, mounting stage)	Rat pups (age P10)
Fine sable paint brushes	Spatulas
Small petri dish	Razor blades
Valet or Personna razor blades	Dissecting scissors
Forceps	Pipet bulbs
Pasteur pipets (broken & unbroken)	Crazy glue (cyanoacrylate)
6-well culture plates	Ice buckets
Transwell (Millicell) inserts	
Ice trays	Diaper
Paper towels	Disposable/Biohazard bags
Buffers and media	Beaker

Procedure:

1. Get ice.
2. Spray all instruments with 70% ethanol and allow to dry.
3. Using forceps take Valet or Personna razor blade and place in 50 ml tube containing acetone, gently swirl, then using forceps place blade in 95-100% ethanol, gently swirl, then attach blade to apparatus and spray with 70% ethanol and allow to dry.
4. Remove culture plates and buffer from the refrigerator and place on ice.
5. Keep plates on ice (cold) at all times.
6. Fill vibratome ice bath with ice.
7. Place buffer tray on vibratome for the Leica machine.
8. Set up vibratome:
   a. Thickness of slices: 250 µm
   b. Blade settings

	Leica	Pelco
Frequency:	7-8	7.5
Speed:	3-4	4

9. Remember on the Pelco you have to wind for thickness after each slice.
10. Set mounting stage on ice.
11. Loosen blade and change blade between each round.

Surgery/Dissection:

1. Keep Petri dish with media on ice.
2. Decapitate 2-3 rat pups at a time, dispose of bodies in biohazard bags.
3. Dissection:
   a. Using scissors cut along midline (sagittally), and also lateral cuts rostrally and caudally.
   b. Pull back scalp, pull back skull.
   c. For cortico-striatal slice, for example, use to blade cut laterally across the "lambda" at the back of the brain.
   d. Using spatula lift entire brain on flat cut edge and place in Petri dish containing chilled medium.

Mounting and Slicing:

1. Place epoxy/crazy glue on mounting stage or tray (Pelco).
2. Using spatula lift brain on flat cut edge, and dab on paper towel to remove excess moisture.
3. Place brain on epoxy/crazy glue on mounting stage or tray.
4. Wait for glue to dry (~1 minute).
5. Cut brain in half along the midline.
6. Mount stage/tray on to vibratome.
7. Tighten screws.
8. Secure blade.
9. Position and wet the blade.

   Note: Never attempt to cut with a dry blade!
10. Set and program the vibratome for desired slicing position.
11. Start slicing, use brush as guide, clean up little pieces.

   Note: Discard the first couple of slices.

12. Use back end of Pasteur pipet to pick up slice(s).
13. Place slice on transwell insert.
14. Remove all excess liquid.
15. Place insert into well of 6-well plate and add 1 ml of medium ***below*** the insert only.
16. Place plates in 37 degree Celsius incubator for ~1 hour.
17. Shoot slices at 95 psi.
18. Return plates to incubator after shooting.

Cleaning

1. Scrape brain remnants off mounting stage using a razor blade.
2. Remove blades and dispose of blades in sharps container.
3. Tie up rat bags and place in -20 degree Celsius freezer.
4. Clean and wash all items used.
5. Spray all items(instruments, vibratome & parts) with 70% ethanol.

   Note: Autoclave all instruments once a month.