

Protocol

Coating Particles with Dextran-Conjugated Fluorescent Dyes or Other Hydrophilic Compounds

Josh L. Morgan and Daniel Kerschensteiner

Imaging and reconstruction of developing neurons require cells that are labeled in a way that distinguishes them from their neighbors. This can be achieved with ballistic labeling, which refers to the delivery of a cell label by means of carrier particles (tungsten or gold) propelled from a pressurized gun. Ballistic delivery can reach many dispersed cells in one shot and can deploy a wide variety of cell markers to neurons in diverse preparations. The three most commonly used types of ballistic labels are carbocyanine dyes, dextran-conjugated fluorescent markers, and DNA plasmids. The primary advantage of ballistic labeling is that multiple dispersed cells can be labeled quickly in live or fixed tissue. This article describes a protocol for coating tungsten particles with dextran-conjugated fluorescent dyes or ion indicators. Such hydrophilic compounds conjugated to dextran are water soluble, and therefore they are excellent indicators for functional studies within living cells. This protocol was developed for labeling ganglion cells in retinal flat mounts.



MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

Reagents

Dextran-conjugated dyes and ion indicators (Invitrogen)

Labels used in this protocol are fluorescent dextrans (e.g., 10,000 MW; Oregon Green 488, Fluoro-Ruby dextran) and calcium indicators conjugated to dextrans (e.g., Fura-2 dextran, Oregon Green 488 BAPTA-1, Calcium Green dextran).

Tungsten particles (1.3 μm or 1.7 μm) (Bio-Rad Laboratories)

Equipment

Desiccant pellet

Glass slides

Guillotine (Bio-Rad Laboratories)

Microcentrifuge tubes (1.5 mL)

Nitrogen gas (ultrapure)

Parafilm

Adapted from *Imaging in Developmental Biology* (ed. Sharpe and Wong). CSHL Press, Cold Spring Harbor, NY, USA, 2011.

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Cite this article as *Cold Spring Harbor Protoc*; 2012; doi:10.1101/pdb.prot067058

- Pipette tip (1 mL; cut off at the end; see Step 7)
- Razor blades
- Tefzel tubing
- Tubing preparation station (Bio-Rad Laboratories) (optional; see Step 1)
- Vial (e.g., screw-cap scintillation vial)

METHOD

1. Cut a 20-cm-long piece of Tefzel tubing, and dry the interior with a stream of ultrapure nitrogen gas.
A tubing preparation station designed as part of the Helios Gene Gun system (Bio-Rad Laboratories) is convenient for drying the tubing.
2. Place 1.5 mg of fluorescent dextran in a 1.5-mL microcentrifuge tube, and dissolve it in 15 μ L of distilled H₂O.
3. Place 20–25 mg of tungsten particles (1.3 μ m or 1.7 μ m diameter) onto a clean glass slide.
4. Add the dissolved dextran to the tungsten particles, and use the tip of the pipette to mix the dye with the tungsten particles while spreading the slurry into a thin film across the surface of the slide (see Fig. 1A,B).
5. Allow the particles to dry for several minutes.
6. Use a clean razor blade to gently scrape the coated particles from the slide, and chop up any clumps that might remain. The result should be a fine powder (see Fig. 1C).

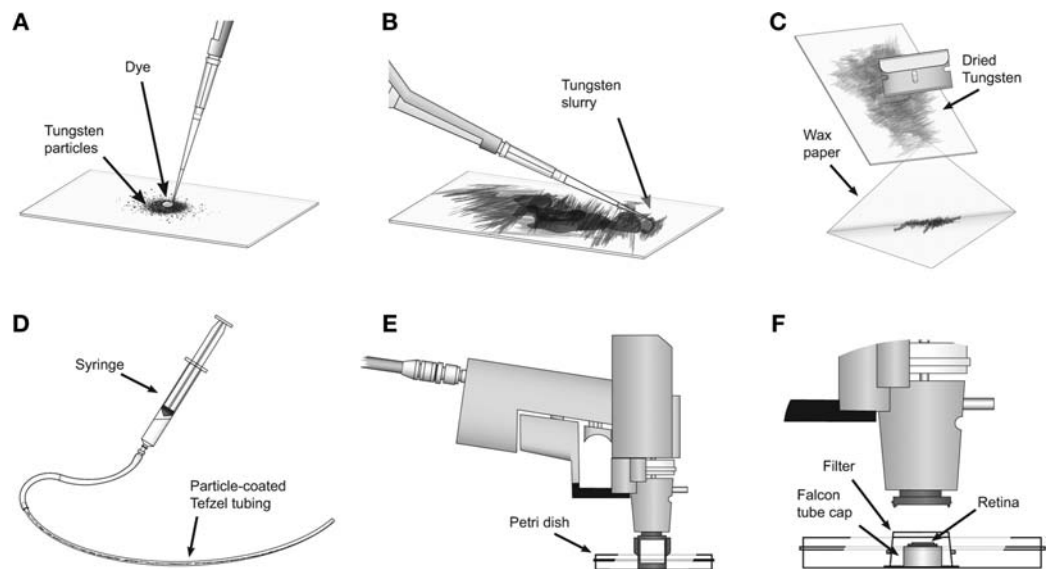


FIGURE 1. Particle coating and shooting tissue with a gene gun. (A) Add dye to tungsten particles on a clean glass slide. (B) Mix dye with tungsten, and spread across the slide to create a thin smooth film. (C) Gently scrape dried particles onto wax paper. (D) Use a 5-mL syringe to load the Tefzel tube with suspended carrier particles and to draw off the fluid once the particles have settled. (E) When shooting DNA-coated particles at low pressure, the standard Helios Gene Gun barrel can be used without a filter between the gun and the tissue. (F) Shooting particles coated in dye or shooting DNA-coated particles at high pressures requires that a filter be placed between the gun and the tissue. The front spacer of the Helios Gene Gun barrel can be removed to achieve the proper distance between the gun and the filter (~1 cm). The protocol for shooting coated particles into tissue slices is described in **Shooting DNA, Dyes, or Indicators into Tissue Slices Using the Gene Gun** (Morgan and Kerschensteiner 2011a).

7. Seal one end of the dry Tefzel tubing with Parafilm, and pour the dried particles into the other end using a 1-mL pipette tip cut off at the end to act as a funnel.
8. Seal the second end of the tubing with Parafilm, and flick the tube until particles have been distributed evenly across the inner surface of the tubing.
See Troubleshooting.
9. Cut the dried tubing using the guillotine, and collect the cartridges on a large Kimwipe.
10. Gently shake the cartridges in the Kimwipe (holding the ends of the Kimwipe together).
11. Store the cartridges in a capped vial with desiccant at 4°C.

*The cartridges containing the "biobullets" can be stored in the refrigerator for several months, provided they are kept dry. Proceed to the protocol described in **Shooting DNA, Dyes, or Indicators into Tissue Slices Using the Gene Gun** (Morgan and Kerschensteiner 2011a) for details on shooting fluorescent dextran indicators into tissue slices using the gene gun.*

TROUBLESHOOTING

Problem (Step 8): Carrier particles fail to stick to Tefzel tubing.

Solution: PVP can be used to increase adhesion. For dextran-coated particles, the tubing can be pre-coated with PVP as described for carbocyanine dyes (see **Coating Particles with Carbocyanine Dyes** [Morgan and Kerschensteiner 2011b]). Higher concentrations of PVP can be used to

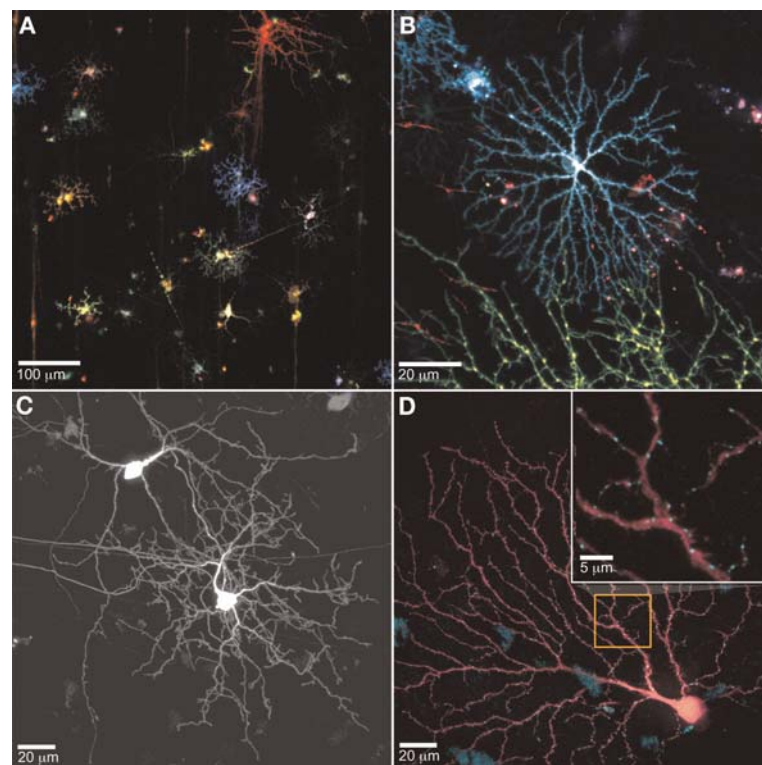


FIGURE 2. Examples of retinal neurons ballistically labeled with different types of molecules. (A) Multicolor labeling of cells in the neonatal mouse retinal ganglion cell layer using particles coated with different combinations of carbocyanine dyes (DiI, DiO, DiD) (see Gan et al. 2000). (B) Spectrally distinct membrane labeling of cells with multicolored carbocyanine dye. (C) Cytosolic labeling of two neonatal mouse retinal cells with dextran-conjugated Fluoro-Ruby. (D) A neonatal mouse retinal ganglion cell expressing cytosolic td-Tomato (magenta) and yellow fluorescent protein-tagged (YFP-tagged) postsynaptic density 95 (PSD95, cyan puncta). PSD95 is a scaffolding protein found at glutamatergic postsynaptic sites. Expression was driven by coating particles with two plasmids, CMV: td-Tomato and CMV: PSD95-YFP (Morgan et al. 2008). *Inset* shows the region within the orange box at higher magnification. (A,B, Reprinted with permission from Lohmann et al. 2005.)

increase particle adhesion during coating but may require higher helium pressures to purge the cartridge.

DISCUSSION

The procedure described here was optimized for labeling ganglion cells in retinal flat mounts using a Helios Gene Gun (Bio-Rad Laboratories). The large surface area ($\sim 35 \text{ mm}^2$) of this preparation and superficial location of ganglion cell somata ($< 20 \mu\text{m}$ deep) make them an ideal target for ballistic labeling.

Carbocyanine dyes are bright lipophilic dyes that rapidly label cell membranes (Fig. 2A,B; see **Coating Particles with Carbocyanine Dyes** [Morgan and Kerschensteiner 2011b]). In contrast, dextran-conjugated dyes such as Fluoro-Ruby (Fig. 2C) and Oregon Green rapidly fill the cytosol of a cell and are generally less toxic than carbocyanine dyes (Stacy and Wong 2003). In addition, many dextran-conjugated calcium indicators can be successfully delivered using the gene gun (Kettunen et al. 2002). However, labeling cells with DNA-coated particles provides greater flexibility because genetic constructs can be made from fluorescent proteins that have been fused to other proteins to specifically label specific subcellular structures (Fig. 2D) (Lo et al. 1994; Wong et al. 2000; Morgan et al. 2008). For a procedure describing the preparation of DNA-coated gold particles, see **Coating Gold Particles with DNA (Biolistics)** (Morgan and Kerschensteiner 2012).

Tissue that has been labeled by ballistic delivery can be imaged using standard imaging methods. The multicolor labeling of superficially located cells lends itself to confocal imaging, although most fluorescent labels can also be efficiently (two-photon) excited with an infrared laser. A sufficiently dense layer of carrier particles will reduce image quality, but the particle density at which image quality is significantly reduced is generally greater than the density at which tissue health is compromised.

ACKNOWLEDGMENTS

The above techniques were developed in the laboratory of Rachel O. Wong under the support of the National Institutes of Health (EY10699 to R.O.W.; T32 EY07031 to J.L.M.), the McDonnell Foundation at Washington University (to R.O.W.), and the Deutsche Forschungsgemeinschaft (KE 1466/1-1 to D.K.).

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