Protocol

Coating Gold Particles with DNA (Biolistics)

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 gold particles with plasmid DNA, which can be used to label developing 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

$CaCl_2$ (1 M)
Ethanol (100%)
Gold particles (1.0–1.6 µm) (Bio-Rad Laboratories)
Plasmid DNA (1–5 µg/µL)
Spermidine

Equipment

Centrifuge
Desiccant
Guillotine (Bio-Rad Laboratories)
Microcentrifuge tubes (1.5 mL)
Nitrogen gas (ultrapure)
Parafilm
Sonicator
Syringe (5 mL fitted with flexible connector to fit Tefzel tube)
Tefzel tubing (Bio-Rad Laboratories)

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

Cite this article as *Cold Spring Harbor Protoc*; 2012; doi:10.1101/pdb.prot067066

Tubing preparation station (Bio-Rad Laboratories) (optional; see Step 6)

Vial (e.g., screw-cap scintillation vial)

METHOD

1. Place 12 mg of gold particles (1.0–1.6-µm diameter) in a 1.5-mL microcentrifuge tube.
2. Add 50 µL of 0.05 M spermidine to the gold particles. Sonicate, and vortex the mixture to produce a uniform suspension.
3. Add 25 µL of plasmid DNA (1 µg/µL), and vortex for 5 sec.

   The amount of DNA used may need to be titrated according to the DNA construct and the tissue used. See Discussion.
4. Add 50 µL of 1 M $CaCl_2$ in H_2O dropwise while vortexing.
5. Allow the DNA to precipitate onto the gold particles for 10 min.
6. While the DNA is precipitating, dry 40 cm of Tefzel tubing for 15 min using ultrapure nitrogen at ~0.4 L/min.

   A tubing preparation station designed as part of the Helios Gene Gun system (Bio-Rad Laboratories) is convenient for drying the tubing.
7. Centrifuge the DNA-coated gold particles at low speed until the supernatant is clear (~10 sec at 1000*g*).
8. Remove the supernatant, and replace it with 1.5 mL of ethanol. Vortex to resuspend the particles.
9. Centrifuge, remove the supernatant, and resuspend the particles in ethanol three more times to wash the gold particles.
10. Use a 5-mL syringe to draw the resuspended gold particles into the dried length of Tefzel tubing.
11. Lay the tubing on the bench to allow the particles to settle for ~1 min. Slowly draw off the supernatant with the 5-mL syringe.
12. Using the syringe, remove any excess ethanol, and load the tubing into the tube coating station. (Be sure that the nitrogen flow is off.) Rotate the tubing until gold particles coat the inner surface of the tubing.
13. Dry the gold particles in a flow of nitrogen (~0.4 L/min) for ~5 min.

    See Troubleshooting.
14. Remove the tubing from the drying station, and cap the ends with Parafilm. Flick the tubing until the gold particles are evenly distributed across the inner surface of the tube.
15. Cut the tubing into sections using the guillotine. Store the cartridges at 4°C in a screw-cap vial with desiccant.

    Cartridges can be cut into halves or thirds to reduce the density of cell labeling. This protocol produces enough cartridges to shoot tissue samples approximately 20–60 times. Because the quality of biolistic cartridges decreases with time, it is best to produce an amount that will be used within 3 wk. 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 transfecting tissue slices with DNA-coated particles using the gene gun.*

TROUBLESHOOTING

Problem (Step 13): Carrier particles fail to stick to Tefzel tubing.

Solution: PVP can be used to increase adhesion. In the case of DNA-coated particles, the final suspension of particles can be performed with PVP added to the ethanol.

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 (~35 mm^2) of this preparation and superficial location of ganglion cell somata (<20 μm deep) make them an ideal target for ballistic labeling. Cell membranes can be labeled with lipophilic carbocyanine dyes (see **Coating Particles with Carbocyanine Dyes** [Morgan and Kerschensteiner 2011b]), whereas the cytosol can be labeled with water-soluble dextran-conjugated dyes and calcium indicators (see **Coating Particles with Dextran-Conjugated Fluorescent Dyes or Other Hydrophilic Compounds** [Morgan and Kerschensteiner 2012]). However, labeling cells with DNA-coated particles provides far greater flexibility because genetic constructs can be made from the many spectral variants of jellyfish and coral fluorescent proteins that are available (Tsien 1998; Shaner et al. 2004; Verkhusha and Lukyanov 2004) and that have been fused to other proteins to specifically label specific subcellular structures (Lo et al. 1994; Wong et al. 2000; Morgan et al. 2008). The range of applications for ballistic DNA transfection of cells (biolistics) is limited primarily by the relatively long incubation time (6–24 h in retinal ganglion cells) required for sufficient protein expression.

Labeling intensities and toxicity effects depend on the plasmid used for ballistic labeling. Exceptionally bright cytosolic labeling with jellyfish and coral fluorescent proteins can be achieved with no apparent ill effects, whereas very high expression of membrane-targeted versions of these proteins, in our experience, seems to be toxic. Particular care must be taken when fusing fluorescent molecules to other proteins to verify correct targeting and control for interference with normal cell function. For example, when expressing synaptic fusion proteins, we generally use lower levels of DNA (20%–50%) compared with what is used for cytosolic labeling. Expression can usually be assayed by the brightness of the labeling and the distribution of the transfected protein. Severe overexpression often results in accumulation of fluorescently tagged proteins within the Golgi apparatus or endosomes. The amount of DNA used in the protocol described in **Shooting DNA, Dyes, or Indicators into Tissue Slices Using the Gene Gun** (Morgan and Kerschensteiner 2011a) produces appropriate levels of cytosolic fluorescent proteins in early postnatal mouse retina using the CMV promoter and 12 h of incubation at physiological temperatures. Different species, cell types, ages, and promoters will likely require different levels of DNA and incubation times. For instance, mature retinas typically require 50% longer incubation times compared with early postnatal retinas to achieve the same intensity of fluorescence.

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 of the fluorescent labels described can also be efficiently (two-photon) excited with an infrared laser. Although a sufficiently dense layer of carrier particles will reduce image quality, the particle density at which image quality is significantly reduced is generally greater than the density at which tissue health is compromised. However, gold particles, even at low densities, can cause other problems for two-β photon imaging. Reflection of infrared light off of gold particles can cause some photomultiplier tubes to saturate and shut off, and infrared light focused on gold particles can heat up the labeled cells. These problems can usually be overcome by limiting laser power in regions where the gold is present.

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 ROW), and the Deutsche Forschungsgemeinschaft (KE 1466/1-1 to D.K.).

REFERENCES

Lo DC, McAllister AK, Katz LC. 1994. Neuronal transfection in brain slices using particle-mediated gene transfer. *Neuron* **13:** 1263–1268.

Morgan JL, Kerschensteiner D. 2011a. Shooting DNA, dyes, or indicators into tissue slices using the gene gun. *Cold Spring Harbor Protoc* doi: 10.1101/pdb.prot067074.

Morgan JL, Kerschensteiner D. 2011b. Coating particles with carbocyanine dyes. *Cold Spring Harbor Protoc* doi: 10.1101/pdb.prot067041.

Morgan JL, Kerschensteiner D. 2012. Coating particles with dextran-conjugated fluorescent dyes or other hydrophilic compounds. *Cold Spring Harbor Protoc* doi: 10.1101/pdb.prot067058.

Morgan JL, Schubert T, Wong RO. 2008. Developmental patterning of glutamatergic synapses onto retinal ganglion cells. *Neural Dev* **3:** 8 doi: 10.1186/1749-8104-3-8.

Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. 2004. Improved monomeric red, orange, and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nat Biotechnol* **22:** 1567–1572.

Tsien RY. 1998. The green fluorescent protein. *Annu Rev Biochem* **67:** 509–544.

Verkhusha VV, Lukyanov KA. 2004. The molecular properties and applications of Anthozoa fluorescent proteins and chromoproteins. *Nat Biotechnol* **22:** 289–296.

Wong WT, Faulkner-Jones BE, Sanes JR, Wong RO. 2000. Rapid dendritic remodeling in the developing retina: Dependence on neurotransmission and reciprocal regulation by Rac and Rho. *J Neurosci* **20:** 5024–36.

Coating Gold Particles with DNA (Biolistics)

Josh L. Morgan and Daniel Kerschensteiner

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot067066