Gold Particle Preparation for Bombardment

Materials

- 0.6 µ gold particles (Bio-Rad, Hercules, CA)
- Macro-carrier holders (Bio-Rad, Hercules, CA)
- Macro carriers (Bio-Rad, Hercules, CA)
- 100% ethanol
- $CaCl_2$ (2.5M, filter-sterilized)
- Spermidine (0.1M, free-base, filter-sterilized, aliquoted and frozen at –20ºC)
- Sterile ddH_2O

Methods

- **Washing Gold**

1. Weigh 15 mg gold particles and transfer to sterile, 1.5 ml microcentrifuge tubes. These tubes are considered 10X gold quantities. Note that at least two tubes of gold are always washed at one time so that they balance each other in the centrifuge steps.

2. In the laminar flow hood, add 500 µl 100% ethanol, straight from the freezer, to each tube of 15 mg gold and sonicate in an ultrasonic water bath for 15 sec. Tap the closed tube on the bench to gather all droplets to the tube bottom and let tube sit until all the particles have settled out (about 30 minutes).

3. Spin in a tabletop centrifuge for 60 sec. at 3000 rpm, and remove the ethanol supernatant (keep tear-drop shaped pellet facing down or it will fall into the pipet). To rinse, add 1 ml ice cold, sterile ddH_20 by dribbling the water down the side of the microcentrifuge tube. Slightly disturb the pellet by finger vortexing, then let the gold settle out again. Spin at 3000 rpm for 60 sec. Repeat the rinse-step two more times, the third time centrifuging at 5000 rpm for 15 sec. After removing the final wash, suspend the pellet in 500 µl sterile water. Ultra-sonicate this suspension for 15 sec, then immediately place the tube on a vortex to keep it shaking as rapidly as possible (vortex setting of 3). Leave the tube shaking at this setting while you open it and aliquot the washed gold for storage.

⌘ Aliquoting Gold to 1X tubes

1. For each 10X tube of washed gold, set out ten, 2.0 ml microcentrifuge tubes in a microcentrifuge tube rack. While the 10X tube is shaking, aliquot 25 µl of the gold suspension to each of the 10 tubes. Then, beginning with the last tube, start backwards, aliquoting another 25 µl of the gold suspension to each tube. When finished, each "1X" tube of gold should contain 1.5mg gold in 50 µl water at a 1X concentration. Label the top of each tube as 1X, close and freeze (-20ºC) until use.

⌘ Coating the gold with DNA

1. On bombardment day, leave the gold in the freezer until just before you begin the gold coating procedure. BRIEFLY, thaw one, 1X tube of gold for 8-10 plates to be bombarded. Ultra-sonicate the tube for 15 sec. In the flow bench, add, in sequence, the appropriate amount of selection construct and GOI construct. We currently use 0.03 µg of selectable marker per 1X gold tube + 3-fold more GOI, adjusted for molar equivalents depending on relative sizes of the plasmids. Finger vortex very well, tapping the tube on the bench top to gather all the droplets to the bottom of the tube. Add 50 µl $CaCl_2$. Using the same pipet tip, gently suck the suspension up and down once, and place on the vortex at low speed (around 2-3 shaking on a Fisher brand Vortex Genie 2). Add 20 µl spermidine while the tube is still shaking on the vortex. Wait 30 sec, close the tube and finger vortex well. Return the tube to the vortex and let shake for 10 min.

2. Next, remove the tube from the vortex and let the gold settle out for several minutes. Centrifuge for 15 seconds at 5000 rpm (just enough to pellet the gold), then pipet off the supernatant. Take the 100% ethanol from the freezer (do not take it out sooner because it must be cold) and add 250 µl ethanol to the 1X pellet. Finger vortex to dislodge the pellet, then rock the tube back and forth until the gold achieves a very "silty" smooth consistency dispersed on the base of the tube. Let the tubes sit until the gold settles out (3-5 min). Centrifuge for 15 seconds at 5000 rpm. Remove the supernatant and add 120-140 µl 100% ethanol. Finger vortex well to ensure complete suspension of the gold pellet and place on the vortex (setting 2-3) for loading the macro-carriers.

⌘ Loading the macro-carriers

1. Fit pre-sterilized macro-carriers (10 min soak in 70% ethanol then air-dry over night) into their stainless steel holders in a sterile dish surrounded by indicating drierite. While the 1X tube of DNA-coated gold particles is still shaking, open the tube and aliquot 10 µl of the suspension onto the center of each macro-carrier. While aliquoting the suspension, draw a half-spiral with the pipet tip from the center and outward of each macro-carrier to ensure the even distribution of the suspension over the inner, target circle. IMPORTANT! Work quickly to avoid evaporation of the remaining suspension. After loading the macros, let them sit for 5-10 minutes to be sure they are dry before bombardment.

References

Sanford, J.C., Smith, F. D., Russell, J.A. 1993. Optimizing the biolistic process. Methods in Enzymology 217: 483-509.