

Gene-Gun Gold Bullet Preparation

Supplies:

100% ethanol
spermidine (S2501-1g; Sigma)
PVP (BioRad)
gold beads (1 micron particles)
sterile H₂O
sterile 1 M CaCl₂
sterile 15-ml conical tubes
Tubing
Sterile 5 ml syringe
Silica crystals (Dri-Rite)

Equipment:

Analytical balance
Bath sonicator
N₂ gas cylinder
Tubing Prep Station
microfuge
peristaltic pump

Gold preparation:

1. Weigh 10 mg PVP to a sterile microfuge tube.
2. Add 500 μ l dry/fresh 100% ethanol.
3. Add 35 μ l of the above PVP solution to 7 ml dry/fresh 100% ethanol in sterile 15 ml tube (giving 0.02 mg/ml final concentration).
4. Weigh 25 mg of gold beads (1 micron particles) into a sterile 1.5 ml microfuge tube.
5. Make a fresh spermidine hydrochloride stock: in a sterile microfuge tube add 31.75 mg spermidine to 250 μ l sterile H₂O (= 0.5M). Then 5 μ l of this 0.5M solution to 45 μ l sterile H₂O for 0.05M solution.

Binding DNA to gold particles:

6. Add 50 μ l of 0.05M spermidine hydrochloride to the 25 mg of gold.
7. Vortex for 10sec, sonicate 10sec, vortex 10sec, sonicate 10sec.
8. Add required volume of DNA to reach 50 μ g of DNA (generally DNA stocks at 1 μ g/ μ l, hence volume will tend to be 50 μ l).
9. Vortex for 5 seconds.
10. Immediately add an equal volume of autoclaved 1 M CaCl₂ (typically this is 50 μ l if your DNA was at 1 μ g/ μ l).
11. Vortex immediately for 5 seconds.
12. Stand for 10 minutes at room temperature.
13. Spin in microfuge for 15 seconds, remove & discard supernatant.
14. Vortex for 5 seconds.
15. Add 1 ml dry/fresh 100% ethanol, 15 second spin, remove & discard supernatant.

16. Gently pipette up and down, 15 second spin, remove & discard supernatant.
17. Repeat for a total of 3 ethanol washes.
18. Resuspend pellet in 200 μ l of initially prepared PVP solution (from step 3).
19. Transfer to sterile 15 ml tube.
20. Use more PVP solution to remove all gold from Eppendorf to 15 ml tube if necessary. Sonicate **only** if needed.

Bullet-tube coating:

21. Turn on N₂ cylinder.
22. Turn pressure gauge to approximately 1 bar.
23. Push through bullet tubing from the RHS of bullet coater till tubing reaches the end.
24. Turn on Pa gauge on bullet maker and leave for 10 min.
25. Turn off N₂.
26. Uncouple black bullet tube holder from left hand side and keep approximately horizontal.
27. Push bullet tube through a few more inches from the right hand side.
28. Cut tube approximately 5 inches from coater.
29. Vortex & invert gold solution (from step 20).
30. Attach the tube to a 10 ml syringe and suck up a little air.
31. Suck up gold solution into the syringe.
32. Attach syringe tube to bullet tube and depress syringe.
33. Remove tube attached syringe and connect the bullet tubing to peristaltic pump tube.
34. Leave for 5 minutes.
35. Pull bullet tube back from right hand side until left hand side is flush with o-ring of black bullet tube holder.
36. Check to make sure the O-ring is securely in position.
37. Set peristaltic pump to give pump rate of 0.5 -10 inches/sec.
38. Turn on peristaltic pump.
39. Cut bullet tube when all solution has been pumped away from the right hand side.
40. Rotate the black bullet tube holder 180° by switching to setting 2 on white box.
41. Leave for 4 seconds.
42. Switch to setting 1 for continuous rotation.
43. After 30 seconds Turn N₂ on to 0.35-0.4 on coater gauge
44. Leave for 5 minutes.

45. Stop rotation.
46. Turn off N₂.
47. Pull out tube and cut into 0.5" long bullets.
48. Add silica crystals to 15-ml conical tube up to the 2mL mark.
49. Scrunch up small piece of foil and push down the tube on top of the silica crystals to prevent bullets and silica contact.
50. Place bullets on top of the foil, seal tube tightly and store at 4°C
51. Before using the bullets, allow tube to reach room room temperature before opening to prevent development of condensation in tube.

Notes: Tubing Prep Station (Bio-Rad part # 165-2418 is a part of the amazingly expensive Helios Gene Gun System, Bio-Rad part # 165-2431)