PARTICLE BOMBARDMENT OF *PHYTOPHTHORA INFESTANS*

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(For additional details look in the PDS-1000/He Biorad instruction manual)

A. Overview:

Germinate spores overnight on membranes.
Transfer membranes to rye-mannitol.
Shoot using the Biorad PDS-1000/He.
Transfer to selection plates.

B. Set up cultures of *Phytophthora infestans* for spore preparation

Inoculate plates with *P. infestans* for spore preparation (grow 7-10 days). Use rye media plates (1.5 % agar with 50 µg/ml ampicillin and 4 ml/liter 10000 U/ml Nystatin)

C. Spore harvesting and germination

After 7-10 days, isolate spores as follows (this is normally done around 5-6 pm in the evening:

1. Add 5-10 ml sterile water to each plate and resuspend the spores with a bent glass rod.
2. Filter through sterile 50 µ nylon mesh to separate the spores from mycelial fragments.
3. Concentrate the spores by centrifugation at low speed (500 x g), followed by resuspension in the desired amount of sterile water for plating. Plate approx. 6 x 10^5 spores per plate, on top of rye media plates, without selection, covered with sterile polycarbonate membranes (Osmonics, bought through Fisher).

Per transformation shot, you will need two plates of germinating spores (rye agar,100 mm plates with 50 µg/ml ampicillin and 4 ml/liter 10000 U/ml Nystatin).

Per shot, you will also need one rye-mannitol plates (100 mm plates containing 0.8 M mannitol, 4 % agar, 50 µg/ml ampicillin and 4 ml/liter 10000 U/ml Nystatin), two selection plates (150 mm rye agar plates containing 5-10 µg/ml G418 1.5 % agar, 50 µg/ml ampicillin and 4 ml/liter 10000 U/ml Nystatin.

D. Preparing materials for transformation

(this is normally done the morning of the transformation)

<u>microcarriers</u>

For 10 transformations, scale up as necessary:

a. Weigh 6 mg 0.6 µ gold into a 1.5 ml screw cap tube (preferably silanized).
b. Add 100-200 µl 100 % EtOH and vortex for 3 min at max. speed.
c. Centrifuge 10 sec at 10000 rpm
d. Remove supernatant
e. Repeat the ethanol washes twice (total 3 washes).
f. Add 100 µl sterile dd water
g. Vortex 2 min max. speed
h. Centrifuge 30 sec,10000 rpm
i. Discard supernatant
j. Resuspend in 100 µl sterile dd water
k. Add to 50 µl of the gold particles in water, while vortexing, 5 µl 1 µg/µl DNA plus 20 µl 0.1 M spermidine plus 50 µl 2.5 M $CaCl_2$
l. Let the particles settle for 10 min at room temp (or just do a short spin).
m. Remove supernatant
n. Add 50 µl 100 % EtOH, vortex at slow speed to resuspend.
o. Distribute samples (5-10 µl) onto the center of the sterile macrocarriers and let them dry. While pipetting the suspension it is important to maintain the pipette in a vertical position to ensure to ensure that the suspension spreads evenly on the surface of the macrocarriers.

other supplies

a. You will need forceps, autoclaved stopping screens, gold+DNA mixture, 70 % and 95 % EtOH, 70 % isopropanol, and the target tissue. Helium tank should have at least 200 psi in excess of the desired rupture disk (we will use 1750 psi). Sterilize area and the gun with 70 % EtOH.
b. You will also need sterile macrocarriers, rupture disks, and macrocarrier holders (160 mm). Prepare these as follows:

   This is normally done at least 30 min before bombardment, to ensure that everything dries in time.

   The macrocarriers and macrocarriers holders are sterilized in 95-100 % EtOH and dried. Once dry, insert the macrocarriers in the holders. They are then ready for DNA loading.

   The rupture disks are sterilized in 70 % isopropanol for a few seconds (NOT LONGER!!) and dried. Handle with forceps.

E. Transformation

1. Transfer germinating spores (on the membranes) to the rye-mannitol plates, incubate at 18 degrees C until shooting.
2. After four hours, shoot.

(it is during this point that the above supplies are prepared)

3. Shoot!

General conditions for shooting are 1100-1550 psi, shelf NR. 5 (9cm), 27 inches of Hg vacuum. We advise making a test shoot without cells or microcarrier to ensure that the system works properly.

a. Turn on power.
b. Load sterile rupture disk into retaining cap and secure it to end of gas acceleration tube, tighten with torque wrench.
c. Load macrocarrier and **stopping screen** into microcarrier launch assembly.
d. Place microcarrier launch assembly and target cells in the chamber and close door.
e. Apply vacuum, when 25-27 inches of Hg are reached (it takes about 30 s), press quickly the vacuum control switch through the middle VENT position to the HOLD position.
f. Bombardment: press FIRE button until rupture disk bursts and helium pressure gauge drops to zero, then release button.
g. Release vacuum by setting the switch in VENT position.
h. Remove target cells, then the macrocarrier and stopping screen, then the rupture disk (sometimes I reuse the stopping screen more than once for transformation with same DNA).
i. When all the day's experiments are completed: remove helium pressure as described in PDS-1000/He Biorad protocol, page 30.

F. Selection

1. After about 24 hours, slice the membranes into 3-mm wide strips and transfer to rye-agar plates containing 5-10 µg/ml G418, ampicillin and nystatin.
2. Periodically check the plates for colonies. They will become visible after 2-5 days. They will need to be transferred within 1 day, otherwise they will grow into each other.
3. Transfer growing colonies to 60 mm rye agar plates containing 10 µg/ml G418.