Obtaining progeny of *P. infestans*

1. Setting up the cross

a. Prepare approximately 5 plates per cross of rye (preferred) or V8 media containing 50 µg/ml Bsitosterol (add before autoclaving from a 95% ethanol stock solution).

b. Cut out approximately 2 mm x 90 mm strips of the A1 and A2 strains. Lay them parallel to each other on a fresh 100 mm plate, about 1.5 to 2 cm away from each other.

c. Incubate the plates at 18°C for 10-14 days. Leave the plates right-side up for 2-3 days and then turn upside down.

2. Germinating oospores

a. Oospores are formed within the agar where the A1 and A2 strains contact. Check for oospore production by examining the plug from this zone under the microscope.

b. Cut out the region containing the oospores. Typically this will be about 4 x 90 mm. Avoid including any parts of the culture with aerial hyphae. Place the strips in a 50 ml plastic conical tube. Add 7.5 ml of sterile water.

c. Sterilize the probe of the polytron by immersing the probe in 70% ethanol. Wait a few seconds to let most of the ethanol drip off and then ignite using a flame. BE CAREFUL! It will take 5-10 seconds for the flame to subside.

d. Liquify the agar-water mixture. Blend at setting 4 for 10-20 seconds or until all the chunks are broken up, and then blend for 20 seconds at setting 7. (Wash the polytron probe by blending in sterile water at low speed, and then flaming, before using on a new sample).

e. The volume should now be about 15 ml. Add an equal volume of 10 mg/ml Novozyme234 in water (filter sterilized). Incubate at 18C, in the dark, shaking at 40 rpm, overnight.

f. Pour the mixture through sterile 100 µm mesh. Use sterile forceps to press the oozy mixture through the mesh. Wash twice with sterile water (about 15 mls each time). Count the flowthrough with a haemocytometer to determine the yield (you will need to sum up several fields since the concentration will be low).

g. Pellet in the swinging bucket rotor, 5 min, 4000 rpm. Pour off the supernatant. Resuspend the mushy pellet in 40 mls of water. Respin and pour off the supernatant. Repeat twice more (totalling three water washes).

h. Resuspend the final pellet with water to about 10 ml. Spread 1 ml on 0.8% water agar plates. Incubate 17-18°C under blue-white light. Germination will occur over 2-6 days.

3. Recovering progeny

a. After about 5-7 days, dissect out germinated oospores. Use the inverted microscope in the hood and a thin scalpel probe. Be careful not to transfer any persistent vegetative mycelia or asexual sporangia! Transfer to fresh rye media (plus amp and PCNB) and incubate at 18°C. Place about 5-6 oospores per plate. Colonies should become visible at 7 days or so. Transfer as needed to individual plates.

b. Confirm outcrossing using RAPDs or other markers.