Non-protoplast Method for Generating Karyotypes of Zymoseptoria tritici

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Abstract

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Protocol

Prepare fungal biomass

Step 1.

Inoculate YMA plates or YSB solution with *Zt.* Incubate at 18°C (4 to 6 days). Do not try to grow the culture for very long. We have more success with very young (log) cultures than with old cultures.

Harvest and wash the spores

Step 2.

Harvest spores by adding 3- 5 mL of sterile TE Buffer/plate (or sterile water) if on the plate, and transfer spores to a 50 mL Falcon tube. Add TE Buffer (or water) to 45 mL mark, gently shake the tube and filter through a nylon mesh (Miracloth) or 1-2 layers of cheesecloth into a new 50 mL Falcon tube (if the spores are very fine you can skip this step). Centrifuge at 3500 rpm for 5-15 min at room temperature. Pour off supernatant (you should have around 500 to 750 ul of spores as a pellet, but it depends on the number of plates you use). Optionally resuspend to 45ml of TE Buffer or water and repear centrifuge.

Add 1-2mL of TE Buffer in the tube (or more; no water here) and dissolve the pellet by vortexing. Count the spores with a Thoma chamber. Dilute the spores before counting (1:100 or more). Normally you should have 10^8 to 10^9 spores/mL.

Make plugs

Step 3.

Suspend the pellet with the desired conc. in an equal volume of molten (55°C) 2% low melting agarose (LMA) and cast plugs in a plug-casting apparatus (previously cooled on ice). You can mix 1.5 mL of diluted spores with 1.5 mL of LMA (keep both volumes in a pre-warmed water bath before mixing). This is enough for making 10 plugs. The final conc. can vary between 1 to 5 x 10^8 spores (but it depends on the isolates).

Prepare plugs for use

Step 4.

Incubate plugs in 5 mL 0.45 M EDTA (pH 8) buffer containing 1% SDS and 1mg/mL protease (type XIV, Sigma P5147). Incubate at 60°C for 48h. Change the incubation buffer and enzyme after 24 h.

Rinse the plugs 2 to 3 times with 6 mL (or more) of 0.5 M EDTA (pH 8.0) and store them in 0.5 M EDTA (pH 8.0) at 4°C.

Note: it work also well with 0.25 M EDTA (instead of 0.45 and 0.5 M EDTA).