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Non-protoplast Method for Generating Karyotypes of Zymoseptoria tritici

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Abstract

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Prepare fungal biomass

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

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

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 × 10^8 spores (but it depends on the isolates).

Prepare plugs for use

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).