Lysis Aspergillus niger, extracting and purifying DNA

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Grow the Biomass

1 -4 days before the lysis, inoculate on MMA (Minimal Media Agar - see Transformation protocol for MMA recipe) at 30^{O}C the strain that you want to analyze.

Harvest the spores

2 -Once enough biomass grew on the slants, you are ready to do the lysis

-Make sure that before you use the entire slant for the lysis, back up your strain by harvesting some spore into a new slant (30^{O}C for 4 days).

-Add 1mL of 0.2% tween-20 into the slant and vortex to have the spores into the solution. You don't need super concentrated spores to make the lysis worked.

Extracting the DNA

3 -Add 20 uL of mycelia, into 480 uL of the 1st lysis buffer (400 mM of Tris-HCl pH 8.0, 60 mM of ethylene diaminetetraacetic acid (EDTA) pH 8.0, 150 mM NaCl and 1% (v/v) sodium dodecyl sulfate (SDS)). The total volume is 500 uL, mix and incubate for 10 minutes at room temperature.

-After incubation add 100 uL of the 2nd buffer (2 M potassium acetate, and 7.6% glacial acetic at pH 4.8), the solution turns white opaque. Centrifuge at 10,000 rpm for 2 minutes.

-Right after the centrifuge, 500uL of the supernatant is transferred to a new 1.5-mL Eppendorf tube and centrifuged again for 2 minutes at 10,000 rpm.

Purifying the DNA

4 -Right after centrifuge, 400 uL of the supernatant is transferred to a new 1.5-mL Eppendorf tube and washed with 400 uL of isopropyl alcohol (>99%). Invert the tube couple of times before centrifuge for another 2 min at 10,000 rpm.

- Right after centrifuge discard the supernatant and rinse the rest of the DNA (contain at the bottom of the tube) with 300 uL 70% EtOH. Invert the tube couple of times before centrifuge for another 2 min at 10,000 rpm.

-Right after centrifuge discard the supernatant and evaporate the rest of the EtOH using a rotavapor (30C, Alcohol mode, 1h15min).

-After all the alcohol has been evaporated, the DNA is dilute in 50 uL of dH_2O, and ready for PCR (LongAmp Taq Master Mix- 1uL of DNA for 25 uL of reaction).