Isolation of Neurospora crassa genomic DNA

kdcastillo[1]

[1]Texas A&M University

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kdcastillo

ABSTRACT

Here, we describe the isolation of fungal genomic DNA from *Neurospora crassa* mycelial mats.

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ABSTRACT

Here, we describe the isolation of fungal genomic DNA from *Neurospora crassa* mycelial mats.

FUNGAL GENOMIC DNA ISOLATION (MINIPREP) FROM N. CRASSA MYCELIA MINI-MATS

Materials:

DNA extraction buffer:

To make 50 ml in a 50 mL conical tube:

5 ml 1M Tris-HCl, pH 8.0, 2 ml 0.5M EDTA, 5 ml 5M NaCl, 5 ml 10% SDS, 33 ml H_2O

Prepare prior to collecting mycelia for DNA extraction (# will depend on the # of your samples):

- Labeled 1.5-ml tubes for collecting mini-mats
- Labeled 1.5 ml microfuge tubes with 500 µl chloroform.
- Labeled 1.5 ml microfuge tubes with 600 µl isopropanol.

Part 1: COLLECTING THE MYCELIA

1. If your mats are coming from a tube, vortex the tube quickly.
2. Using a sterile wooden stick (RNA-free cabinet), collect the mycelia from each tube and lay on a stack of paper towel (they will look like mycelia lollipops).Usually, 7 samples can fit on the length of a regular paper towel.Press with another layer of paper towel to get excess media off.
3. Transfer each mycelia mat into the pre-labeled 1.5-ml tubes (set 1).
4. You can freeze them in -20^{O}C or proceed to the extraction process.

Part 2: DNA EXTRACTION

1. Measure out the total amount of extraction buffer you need, and add Proteinase K (Freezer #4, tubes labeled PK).Each sample gets 500 µl extraction buffer with 3 µl Proteinase K.Just do the math for making a “master mix” depending on the # of samples you have.
2. Add ~500-503 µl of the buffer/Proteinase K mix to each sample.Vortex for 5 mins (using the vortex mixer with the round holder for multiple samples or the one which holds racks).Incubate the samples at 65^{O}C for 1 hr (or more, not that critical).
3. Place tubes on ice, then add 200 µl of 7.5M ammonium acetate. Vortex for 1 min.Place on ice or 4^{C}C fridge for 5 min.
4. Centrifuge at max speed for 5 min.
5. Transfer supernatant to TUBE 2 (set with 500 µl chloroform).Vortex for 5 mins. Centrifuge at max speed for 5 min. * Tubes with chloroform go into the phenol/chloroform orange waste bucket next to chemical hood.
6. While avoiding the interface, transfer ~500 µl of the supernatant into TUBE 3 (set with 600 µl isopropanol).Mix by inversion, then let sit for 10 min. Alternatively, can let sit at room temp. overnight for higher yield.
7. Centrifuge at max speed for 10 min.
8. Discard supernatant.Wash pellet with 70% ethanol. Centrifuge at max speed for 5 mins.
9. Take out the ethanol. Air dry or speed vac.
10. Suspend in 100 µl 1X TE with 1µl RNAse A (Freezer #4).Once again, you can make a “master mix” of TE buffer and RNase A depending on the number of your samples.Put in the 37^{O}C water bath for 1 hr.
11. Centrifuge at max speed for 1 min. Quantitate DNA concentration with NanoDrop with 1X TE buffer as blank.

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