

***Aspergillus fumigatus* Genomic DNA Prep**

1. Inoculate broth (usually GMM) with AF spores by gently scraping colony until tip is green and dunking it in the media. Twice should be enough. Grow overnight. Usually 10 ml of broth in a 50 ml centrifuge is adequate but 50 ml in a 250 ml flask may be necessary for large scale extractions.
2. There are two ways to collect the tissue. The preferred way is to pour the media over a buchner funnel and scrape the relatively dry tissue into a 2 ml centrifuge tube. The other, if tissue is scarce, is to spin the liquid down in a 50 ml tube and pour off supernatant, then spoon the wet pellet at the bottom into a 2 ml tube. When dealing with large amounts of tissue use the 50 ml rather than the 2 ml tube and proceed.
3. Lyophilize tissue...first place the tubes at -80 C for 20 minutes or flash freeze tissue with liquid nitrogen. Lyophilize samples overnight. Alternatively, liquid nitrogen and a mortar and pestal may be used.
4. The lyophilized tissue is transferred to a 2 ml microcentrifuge tube. The tissue should take up about $\frac{1}{2}$ of the 2 ml tube. Add $\frac{1}{2}$ of a 0.2 ml scoop of 0.1mm glass beads to the tube. Place the tubes in the bead mill and beat until tissue becomes a powder.
5. Add 0.6 ml AF extraction TENS buffer (pre-heated to 65 C in the hyb oven) and vortex vigorously.
6. Incubate tubes at 65 C for 30 minutes. Depending on time constraints this can be compressed to 15 minutes if the buffer was pre-heated.
7. Add 0.6 ml of 25:24:1 Phenol:Chloroform:Isoamyl alcohol and vortex vigorously.
8. Centrifuge at max speed (13,000 rpm) for 10 minutes.
9. Transfer supernatant (top layer) to a new 1.7 ml tube. Avoid the interface of the bottom layer and debris. This should yield around 0.5 to 0.55 ml.
10. Add equal volume of Chloroform:Isoamyl alcohol 24:1 and mix by inversion.
11. Centrifuge at max speed for 10 minutes and transfer supernatant in the same manner as step 9.
12. Add 1/10 volume of NaOAC (pH 5.3) and 0.7 volumes Isopropanol to supernatant. DNA should precipitate out (but you don't have to visually see it). Mix by inversion.

13. Centrifuge at full speed for 30 minute at 4 C.
14. You should have a respectable white pellet in the bottom corner of the tube. Carefully remove the supernatant and wash the pellet with 500 ul of chilled 70% ethanol. You want the pellet to break free of the wall and break apart, but the debris doesn't have to completely disappear.
15. Centrifuge for 5 minutes at 4 C. After removing supernatant, sit the tube upside down on a paper towel on the bench top for 2 minutes. This lets some residual alcohol dry up and run off.
16. Re-suspend pellet in 200 ul of EB. You want to gently pipet up and down with a large bore tip until the pellet and debris have completely disappeared visually. Usually this requires upwards of 30-60 seconds of continuous motion per sample. Your sample is now ready to spec. Ideal concentrations are between 1000-2500 ng/ul. For PCR, create a working solution in a separate tube by diluting to 100 ng/ul and using 2 ul of template in a 25 ul reaction.

Optional: If you need higher quality DNA

17. Add RNase A to final concentration of about 20 ug/ml, about 0.5 ul if a 100 mg/ml stock and incubate for 60 minutes at 37 C.
18. Repeat steps 7-16.

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