

CTAB Method for Fungal DNA Isolation of *A. flavus*

1. Turn on water bath to 65°C
2. Add 500µl of CTAB buffer to 1.5ml tubes
3. Add one scoop of glass beads (425-600µ; Sigma G9772) to the 1.5ml tubes
4. Collect spores from colony by pipetting 50µl of Triton-X100 up and down several times over the same spot on the plate
5. Add spore/Triton-X100 mix to 1.5ml tubes
6. Vortex using Disruptor Genie for 2 minutes
7. Incubate at 65°C for 15 minutes
8. Add 233µl of isopropanol and 32µl of 7.5M NH₄OAc into a new 1.5ml tube and set aside
9. Vortex using Disruptor Genie for 1 minute
10. Incubate at 65°C for 15 minutes
11. Add 500µl of 24:1 Chloroform:isoamyl alcohol
12. Mix well by shaking
13. Centrifuge for 5 minutes at maximum speed
14. Transfer 400µl of aqueous phase into tube containing isopropanol and NH₄OAc
15. Mix well by shaking
16. Centrifuge for 5 minutes at maximum speed
17. Pour off liquid
18. Add 500µl of cold 70% Ethanol
19. Centrifuge for 5 minutes at maximum speed
20. Pour off liquid being careful not to lose pellet (barely visible)
21. Dry pellet in speed vac
22. Resuspend samples with 20µl of water
23. Use 5µl for PCR

CTAB stock (1L)

- 100ml 1M Tris pH 8
- 280ml 5M NaCl
- 40ml 0.5M EDTA
- 20g CTAB (cetyltrimethyl ammonium bromide)
- Autoclave

CTAB Buffer (use within 1 week, dissolve right before starting extractions)

CTAB stock	polyvinylpyrrolidone	b-mercaptoethanol
5ml	0.2g	25ul
20ml	0.8g	100ul