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CTAB DNA extraction protocol for fungi

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Black Yeast Fungal Prot...

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Protocol status: Working
We use this protocol and it's working

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Abstract

CTAB Fungal DNA Extraction Protocol
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*Adapted from Cubero et al., 1999 and Lagonigro et al., 2004
February 2020; Updated August 15th, 2022

Attachments

DOCX

2022-05-20_CTAB_Fung...

21KB

Materials

Reagents Needed:
Liquid Nitrogen
CTAB Extraction Buffer + PVP
CTAB Precipitation Buffer (pH=7.2-7.6)
24:1 Chloroform: Isoamyl alcohol
100% Isopropanol (ice cold)
70% Ethanol (ice cold)
100% Ethanol (ice cold)
3M Sodium Acetate (pH=5.2)
5M NaCl
UltraPure water or TE buffer
Proteinase K 20mg/mL
RNase A 10 mg/mL

Instruments/Materials Needed:
Bead beating tubes
0.5 mm Glass beads
Dewar for Liquid Nitrogen
Water bath or heat block set to 70° C
1.5mL Microcentrifuge tubes
2.0mL Microcentrifuge tubes
15mL Centrifuge tubes
Fridge and Freezer (4° C and -20° C)
Pipettes
Refrigerated Microcentrifuge
Fungal samples, **0.03g** minimum

Recipes:
CTAB Extraction Buffer + PVP
1% w/v CTAB
1M NaCl
100mM Tris
20mM EDTA
*RIGHT BEFORE USE ADD: 1% w/v Polyvinyl pyrrolidone (PVP)
*RIGHT BEFORE USE ADD: 1% of 20mg/mL Proteinase K

CTAB Precipitation buffer:
1% w/v CTAB
50mM Tris-HCl
10mM EDTA
40mM NaCl

*Adjust pH to 7.2-7.6

Preparing Yeast from liquid culture for DNA extraction

1 Grow fungi in preferred media type till fungi are in mid-exponential phase

2 Centrifuge fungal samples at 10,000x g for 1 minute and wash with water 3 times, centrifuging in between, then obtain as tight of a fungal pellet as possible and remove all liquid from the tube.

3 Place tube of cell pellet in liquid nitrogen to freeze cells, then keep tube in -80 freezer until you are ready to perform the DNA extraction.

Preparing Yeast from solid culture for DNA extraction

4 Grow fungi on preferred media type till fungi grows to its maximum on the plate

5 Place 800µL of water in a 1.5mL tube and weigh recording the weight

6 Scrape 0.03g of fungus off the plate and place into the tube, avoiding agar

7 Centrifuge at 10,000x for 1 minute to obtain a fungal pellet and remove all water

8 Place tube in liquid nitrogen to flash freeze, and place frozen tube in -80 freezer until you are ready to do the DNA extraction

Lysing Filamentous fungi

9 Grab as many mortar and pestles as necessary for the number of samples you have (or wash one out thoroughly with 70% ethanol between samples)

10 Obtain liquid nitrogen placed in Dewar

11 Place tubes with processed fungi in a small container and pour liquid nitrogen over the tubes until the fungi pellets are frozen completely

12 With tube lids closed, tap the lids against the table to dislodge the pellet from the tube

13 Put the frozen fungal pellet into the mortar and pour additional sterile Liquid nitrogen over the pellet

14 Carefully grind the fungus being sure not to launch it out of the mortar, grind till the fungus is a powder, adding more liquid nitrogen if the fungus gets too liquidy

15 Scrape ground up fungus out of the mortar and place in a fresh 1.5mL tube, place sample in -80° C freezer until ready for DNA extraction.

DNA extraction Protocol (from cell growth to final DNA product):

16 Grow fungi in preferred media type till fungi are in mid-exponential phase (3-7 days)

17 Pipette 1 mL of fungus into a 1.5 mL tube

18 Centrifuge fungal samples at 10,000x g for 1 minute

19 Wash with 1 mL of ddH2O 3 times, centrifuging in between, then obtain as tight of a fungal pellet as possible and remove all liquid from the tube

20 Take your 1.5 mL tube of washed cell pellet and place in liquid nitrogen to flash freeze and place tubes in -80 freezer till you're ready to extract DNA

21 START OF DNA EXTRACTION

22 Turn on water bath/heat block to 70° C

23 Determine the number of samples you will be processing, multiply that number by 750 µL to determine the amount of CTAB Extraction buffer you will need (700 µL per sample)

and aliquot that out into a 15 mL centrifuge tube

24 Add 1% w/v PVP (.01g/mL) and 1% v/v 20mg/mL Proteinase K (5µL/500µL) to your aliquoted CTAB Extraction buffer

25 Grab as many bead beating tubes as you have samples

26 Pour ~300uL of glass beads into tubes and label tubes accordingly

27 Remove tubes of frozen fungus from the freezer

28 Dislodge pellet from bottom of tube by pipetting 700 µL of the complete CTAB Extraction buffer into each frozen sample tube

29 Transfer sample in CTAB Extraction buffer to Bead beating tube

30 Put tubes in 70 °C water bath or heat block for 5 minutes, then bead beat for 5 minutes; repeat 2 more times (30 mins total) (change temperature of heat block/water bath to 37° C after this step, unless you have a 37° C incubator)

31 Confirm cells have lysed via microscopy, 5 µL of sample onto a microscope slide. If cells have not lysed, bead beat for additional 2-5 minutes.

32 Remove tubes from the water bath/heat block and transfer as much liquid as possible into a new 2 mL microcentrifuge tube, avoiding the glass beads

33 Add 1x the volume of 24:1 Chloroform: Isoamyl to the tubes, invert tubes to mix and then centrifuge tubes at 10,000x g for 5 minutes

34 Carefully remove tubes from centrifuge, collect upper layer only, and transfer the upper layer into a new 2 mL microcentrifuge tube

35 Add 2 volumes of CTAB Precipitation buffer to the tubes and mix by inverting for 2 minutes

36 Centrifuge tubes at 13,000x g for 15 minutes

37 Remove supernatant, being sure to leave the pellet behind in the tube

38 Re-suspend pellet in 350 µL of 5M NaCl

39 Add 2µL of 10 mg/mL RNase A to tubes and place tubes at 37° C for 30 minutes

40 Remove tubes from 37° C and add one volume of 24:1 Chloroform: Isoamyl; mix thoroughly by inverting

41 Centrifuge tubes at 10,000x g for 5 minutes

42 Remove upper phase and transfer to a new 1.5 mL tube

43 31. Add 6 volumes of ice cold isopropanol and mix by inverting

44 (PAUSE POINT) Place tubes in -20 °C freezer for 15 minutes OR OVERNIGHT/FOR MULTIPLE DAYS

45 Centrifuge tubes in 4 °C at 13,000x g for 20 mins

46 Remove all liquid and let air dry for 30 mins to over night, until it's dry to remove excess alcohol

47 Resuspend pellet in 30-50 µL of UltraPure water or TE buffer

DNA clean-up

48 Add 0.1 volumes of 3M Sodium Acetate and 3 volumes of ice cold 100% Ethanol to your DNA samples, mix by inverting.

49 Freeze samples at -80° C for 20mins or at -20° C overnight.

50 Centrifuge tubes at 4° C at max speed for 30 minutes

51 Remove supernatant and resuspend pellet in 1mL of ice cold 70% Ethanol, being sure the DNA pellet becomes loose from the tube and floats freely in the liquid.

52 Centrifuge at 4° C at max speed for 10 minutes

53 Remove Ethanol and let tubes dry completely with no ethanol left in the tubes. Overnight with running air over the open tubes works best.

54 Pre-heat elution buffer (TE or water) at 65° C, then re-suspend DNA in 30-50µL of warmed elution buffer