Extraction of ribosomal DNA according to Möller (modified)

1. Subject

By mechanical lysis of yeast cells or mycelium ribosomal DNA is extracted for PCR (polymerase chain reaction) purposes. The method contains several purification steps with acetate, chloroform etc. but no phenol.

2. Principal

Lysis of biological material gives ribosomal DNA in solution. Proteins, enzymes and non-lysed cells will be precipitated by adding acetate and chloroform. The water phase (CTAB-buffer) contains DNA. The increased concentration of sodium chloride and sodium dodecylsulphate precipitates polysaccharides. The DNA can be precipitated by adding ice cold ethanol 96% and centrifuged. After washing the pellet with ice cold ethanol 70%, the DNA is resuspended in TE-buffer (Tris/EDTA) with addition of RNAse.

3. Material and reagents

3.1 roundbottom Eppendorf cups 2 ml
3.2 silica-gel (Merck 7736)
3.3 Celite 545 (Macherey, Nagel & Co)
3.4 Eppendorfcups 1.5 ml (Sarstedt 72.690)
3.5 micropestles (Eppendorf 0030 120.973)
3.6 vortex
3.7 centrifuge 14.000 rpm (Sigma 112, Eppendorf 5417R)
3.8 pipets 5-50 µl, 50-200 µl, 200-1000 µl
3.9 pipettips
3.10 waterbaths 37°C, 65°C
3.11 Tris(hydroxymethyl)-aminomethane (Merck 8382)
3.12 Na-EDTA (Titriplex III, Bio Rad 161-0729)
3.13 CTAB (hexadecyltrimethylammoniumbromide, Sigma H-5882)
3.14 1 N HCl (Ferak 11448)
3.15 pancreatic RNAse 20U/mg (ribonuclease, Merck 24570)
3.16 NH4-acetate (Fluka 09688)
3.17 NaCl (Merck 6404)
3.18 latex gloves
3.19 Proteinase K (Merck 124568)
3.20 sodiumdodecylsulphate, SDS (Merck 13760)
3.21 GFX columns, DNA Purification Kit (Amersham Pharmacia 27-9602-01)

4. Solutions

4.1 chloroform
4.2 ethanol 96% (-20°C) [6.1]
4.3 ethanol 70% (-20°C)
Mix 700 ml ethanol 96% with ultrapure water to a volume of 960 ml.
4.4 iso-amylalcohol
4.5 iso-propanol (Sigma I-0398)
4.6 RNAse-solution:
Dissolve 10 mg pancreatic RNAse [3.15] in 1 ml 0.01 M Na-acetate, pH 5.2 [4.15]. Heat the solution till 100°C during 15 min. Cool slowly to roomtemperature. Adjust pH by adding 100 µl 1 M Tris, pH 7.4 [4.16]. Make aliquot of 100 µl and store at -20°C.

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4.7 10% CTAB:
Dissolve 10 g CTAB [3.13] in 100 ml ultrapure water. Keep at roomtemperature. Do not autoclave.

4.8 TES-buffer:
Add 1.2 g Tris [3.11], 0.38 g Na-EDTA [3.12] and 2 g sodiumdodecylsulphate (SDS) [3.20] to 80 ml ultrapure water. Adjust pH to 8.0 with 1 N HCl [3.14]. Heat the solution if EDTA doesn't dissolve very well. Adjust volume to 100 ml with ultrapure water. Aliquot the buffer in 50 ml tubes. Filter sterilize the solution if need be. Do not autoclave. Store at room temperature.

4.9 Silical-mix:
Mix 30 g silica-gel [3.2] with 15 g Celite 545 [3.3]. Fill roundbottomcups with 80-100 mg silica-mix. When handling the silica work in a chemical safety cabinet. Autoclave the cups 15 min. at 121°C. Store at roomtemperature.

4.10 Proteinase K 10 mg/ml:
Dissolve 50 mg proteinase K [3.19] in 5 ml ultrapure water. Make aliquots of 500 µl and store at -20°C.

4.11 5 M NaCl:
Dissolve 29 g NaCl [3.17] in 100 ml ultrapure water.

4.12 SEVAG
Mix 240 ml chloroform [4.1] with 10 ml isoamylalcohol [4.4].

4.13 5 M NH_4-acetate
Dissolve 38.54 g CH_3COONH^4 [3.16] in 100 ml ultrapure water.

4.14 TE-buffer:
Add 0.12 g Tris [3.11] and 0.04 g Na-EDTA[3.12] to 80 ml ultrapure water. Adjust pH to 8.0 with 1 N HCl [3.14]. Heat the solution if EDTA doesn't dissolve very well. Adjust volume to 100 ml with ultrapure water. Aliquot the buffer in 50 ml tubes. Autoclave the solution during 15 min. at 121°C. Store at roomtemperature.

4.15 0.01 M sodium acetate, pH 5.2:
Fill a Falcon tube with 45 ml ultrapure water. Discard 150 µl and add 150 µl sodium acetate 3M [4.17].

4.16 1 M Tris, pH 7.4:
Dissolve 121.1 g Tris in a total volume of 800 ml ultrapure water. Adjust pH to 7.4 with 5N HCl and later with 1N HCl. Add ultrapure water to a volume of 1000 ml.

4.17 3 M sodium acetate, pH 5.2:
Dissolve 20.4 g $CH_3COONa3H_2O$ [3.16] in 50 ml ultrapure water. Adjust pH to 5.2. Make aliquots of 1 ml in eppendorfcups. Store at -20°C.

5. Protocol

5.1 Switch on the waterbaths to 37°C and 65°C (6.2).
5.2 Pipet 300 µl TES buffer (4.8) into an eppendorfcup with silica-mix (4.9).
5.3 Transfer ± 1 cm² of fungal material from pure culture into the buffer (6.3).
5.4 Grind the material with a micropestle during 1-2 min. (6.4).
5.5 Add an extra 200 µl TES-buffer and vortex.
5.6 Pipet 10 µl Proteinase K (4.10) to the grinded material.
5.7 Incubate the mixture during 10 min. in a waterbath of 65°C (swirl occasionally).
5.8 Increase the saltconcentration by adding 140 µl 5M NaCl (4.11).
5.9 Add 1/10 volume CTAB 10% (4.7) (≈ 65 µl).
5.10 Incubate again for 30 min. at 65°C.
5.11 Add 1 volume SEVAG (4.12) (≈ 700 µl) and mix carefully by hand, e.g. invert 50 times.
5.12 Incubate during 30 min. at 0°C (on icewater).
5.13 Spin 10 min. at 4°C at 14.000 rpm (20.400 rcf).
5.14 Pipet ONLY the toplayer (water phase) in a clean, sterile eppendorfcup.
5.15 Add 225 µl 5M NH_4-acetate (4.13) and mix carefully.
5.16 Incubate for at least 30 min. on icewater; the longer, the better.
5.17 Spin 10 min. at 4°C at 14.000 rpm (20.400 rcf).
5.18 Transfer the supernatant to a clean, sterile eppendorfcup [3.4].
5.19 Add 0.55 volume isopropanol (4.5) to the supernatant (≈ 510 µl) and mix carefully.

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5.20 Spin directly during 5 min. at 14.000 rpm (20.400 rcf).
5.21 Decant supernatant and wash the pellet 2x with icecold ethanol 70% (4.3).
5.22 Air dry the pellet and resuspend in ±50 µl TE-buffer with RNAse (4.6) added (48.5 µl TE-buffer + 1.5 µl RNAse).
5.23 Incubate samples for 15-30 min. at 37°C.
5.24 Perform a extra purification with GFX-columns (Protocol 12, p. 41).

6. Remarks

6.1 Ethanol 96% (4.2) should be non-denatured ethanol.
6.2 Wear latex gloves when starting the 'Protocol' (5).
6.3 When liquid media was used, the mycelium should be washed first with sterile water.
6.4 Micropestles are kept in concentrated HCl (5 N); before use they should be washed in sterile, ultrapure water. Used pestles should be cleaned, autoclaved and stored in concentrated HCl again.

7. Literature

7.1 Möller EM, Bahnweg G, Sandermann H, Geiger HH. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. NAR 20 (22): 6115-6116 (1992).

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