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DNA extraction protocol

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We use this protocol and it's working

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Disclaimer

Abstract

This DNA extraction protocol has been developed at the CIRM-Levures (INRAE, SPO, FRANCE) to extract yeast chromosomes. It is based on a phenol/chloroform DNA extraction procedure.

Troubleshooting

CELL LYSIS AND NUCLEIC ACID EXTRACTION

1 - Perform a 3 ml YPD (yeast extract 10g/l, bacto peptone 10g/l, glucose 10g/l) culture for 60h in order to harvest cells at stationary phase.

2 - Centrifuge 5 min at 5000 rpm and remove the supernatant.

3 - Wash the pellet with 5 ml of 50mM EDTA, transfer to a 2 ml screw cap tube and centrifuge for 5 min at 12000 rpm. Remove the supernatant.

4 - Add 0.2ml lysis buffer (50mM Tris pH 8, 50mM EDTA, 100mM NaCl, 2% Triton, 1% SDS), 0.2ml TE 1X (10mM Tris pH 8, 1mM EDTA), 0.2ml phenol/chloroform and 0.3g glass beads.

5 - Grind with the Digital Disruptor Genie (Scientific Industries, Bohemia, NY 11716 U.S.A) for 2 minutes at 2850 rpm.

6 - Centrifuge 5 min at 12000 rpm.

7 - Transfer the aqueous phase to a new tube, add 0.5 ml of chloroform and mix.

8 - Centrifuge 5 min at 12000 rpm.

9 - Transfer the aqueous phase to a new tube, add 0.5 ml of isopropanol and mix.

10 - Centrifuge for 5 min at 12000 rpm.

11 - Wash the pellet with 0.4 ml of 70% ethanol and let the tube dry.

12 - Re-suspend the DNA pellet in 490 µL of TE 1X and let overnight at 4 °C.

13 - Add 10 µl of RNase (100 mg/ml, Qiagen ref: 19101). Incubate for 60 min at 37 °C.

DNA PURIFICATION, Magnetic Beads BINDING

14 - To each sample, add 50 µl of 5 M NaCl, 15 µl of Chemagic CMG-252-A magnetic bead (PerkinElmer, Waltham, Massachusetts, U.S.A) suspension, 250 µl of 7.8M Guanidium chloride and 800 µl of isopropanol.

15 - Mix by inversion for 5 minutes.

16 - Set up the tubes on the magnetic rack (DynaMag™-2 Magnet type, Invitrogen ThermoFisher Scientific).

17 - Wait for 2 min. Remove the liquid.

18 - Perform a first wash with 1 ml of AMMLAV/E buffer (10 mM Tris pH 8.0, 0.1 mM EDTA, 60 mM potassium acetate, 65% ethanol) and mix gently to disintegrate the beads.

19 - Place the tubes on the magnetic rack. Wait 2 min. Remove the liquid.

20 - Repeat a second wash with 1 ml of AMMLAV/E buffer.

21 - Perform two more washes as above with 1 ml of 75% ethanol

22 - Air dry the beads at room temperature for 5 min.

23 - Add 0.1 ml of 1X TE. Mix well to re-suspend the beads in TE 1X. Incubate 10 min at room temperature.

24 - Place the tubes on the magnetic rack. Wait 5 min. Transfer the DNA solution to new storage tubes.

25 - The DNAs are then stored at -20°C.