# DNA extraction protocol

Cecile Grondin[1], Jean-Luc Legras[1], hugo.devillers[2]

[1]CIRM-Levures, SPO, INRAE, France; [2]ADEL, SPO, INRAE, France

Sep 06, 2022

1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.5qpvorx47v4o/v1

hugo.devillers

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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.

DOI

dx.doi.org/10.17504/protocols.io.5qpvorx47v4o/v1

PROTOCOL CITATION

Cecile Grondin, Jean-Luc Legras, hugo.devillers 2022. DNA extraction protocol . **protocols.io** https://protocols.io/view/dna-extraction-protocol-cf64trgw

CREATED

Sep 05, 2022

LAST MODIFIED

Sep 06, 2022

PROTOCOL INTEGER ID

69564

DISCLAIMER:



## 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.



**Citation:** Cecile Grondin, Jean-Luc Legras, hugo.devillers DNA extraction protocolÃÂ
https://dx.doi.org/10.17504/protocols.io.5qpvorx47v4o/v1

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.



**Citation:** Cecile Grondin, Jean-Luc Legras, hugo.devillers DNA extraction protocolÃÂ
https://dx.doi.org/10.17504/protocols.io.5qpvorx47v4o/v1

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.



**Citation:** Cecile Grondin, Jean-Luc Legras, hugo.devillers DNA extraction protocolÃÂ
https://dx.doi.org/10.17504/protocols.io.5qpvorx47v4o/v1