



## Mechanical disruption of frozen yeast cells and RNA isolation method

### 1. Subject

Total RNA can be extracted from balls of fungal cells frozen in liquid nitrogen. The RNA is of sufficient quality for use in microarray experiments and other methods for testing gene expression.

### 2. Principle

Fungal cells are harvested as quickly as possible and droplets frozen in liquid nitrogen to maintain RNA integrity. The frozen cell balls can then be stored at -80 °C. Total RNA is then extracted from the frozen droplets of cells after mechanical disruption. The cells balls are transferred to liquid nitrogen cooled Teflon vessel, a tungsten bead is added and the vessel closed and placed into a Braun Micro-Dismembrator. The vessel is rapidly shaken smashing the cell balls into a fine powder. Trizol is then added to the frozen cell powder and RNA extracted.

### 3. Materials and Reagents

RNase away® (Molecular Bioproducts **MB-ME-7005**)

Sterile 50 ml Falcon® tubes

Centrifuge with rotor that takes 50 ml tubes

Liquid nitrogen

Sartorius Micro-Dismembrator

5 ml Teflon vessel and 7 mm tungsten carbide bead

Trizol® (Invitrogen 15596-026)

Pipettes 0.5-20, 20-100, 50-200, 200-1000

Sterile RNase-free pipette tips white, yellow and blue

Sterile RNase-free microcentrifuge tubes 1.5 ml

Microcentrifuge

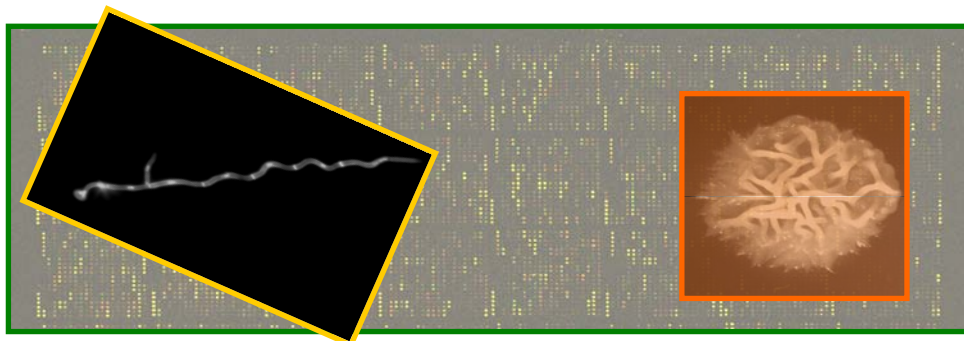
Chloroform

Isopropanol

DEPC-treated H<sub>2</sub>O

LiCl buffer

Ice-cold 70% (v/v) ethanol





## 4. Solutions

Ensure weighing is done without spatulas, mark all solutions RNase free and don't touch without gloves.

### 4.1 DEPC Treated H<sub>2</sub>O

|                             | 100 ml | 200 ml | 500 ml  | 1000 ml |
|-----------------------------|--------|--------|---------|---------|
| dH <sub>2</sub> O           | 200 ml | 500 ml | 1000 ml |         |
| Diethyl polycarbonate(0.1%) | 0.2 ml | 0.5 ml | 1 ml    |         |

Leave overnight at room temperature. Autoclave.

### 4.2 70 % Ethanol

|                               | 100 ml | 200 ml | 500 ml |
|-------------------------------|--------|--------|--------|
| RNase free ethanol            | 70 ml  | 140 ml | 350 ml |
| DEPC treated H <sub>2</sub> O | 30 ml  | 60 ml  | 150 ml |

### 4.3 4 M Lithium Chloride

|                       | 100 ml  | 200 ml  | 500 ml |
|-----------------------|---------|---------|--------|
| DEPC H <sub>2</sub> O | 100 ml  | 200 ml  | 500 ml |
| LiCl (MW = 42.4)      | 16.96 g | 33.92 g | 84.8 g |

### 4.4 Lithium Chloride Buffer

|                             | 20 ml | 50 ml | 100 ml |
|-----------------------------|-------|-------|--------|
| 4 M LiCl (4 M)              | 20 ml | 50 ml | 100 ml |
| 1 M Tris.HCl pH 7.4 (20 mM) | 4 µl  | 10 µl | 20 µl  |
| 0.5 M EDTA (10 mM)          | 4 µl  | 10 µl | 20 µl  |

## 5. Protocol

### *Preparation of frozen yeast cell drops*

- 5.1 Grow 5 ml overnight cultures @ 30°C @ 200 rpm.
- 5.2 Grow cells to an OD of at least 0.5 in a volume of ~50-100 ml.
- 5.3 Split cultures into 2 Falcon tubes.
- 5.4 Centrifuge for 3 min @ 4,000 rpm.
- 5.5 Pour off as much supernatant as possible
- 5.6 Resuspend pellets in the remaining supernatant (~200 µl in total).
- 5.7 Fill a 50ml Falcon tube with liquid N<sub>2</sub>.
- 5.8 Wearing safety goggles, carefully, slowly release small drops of cell suspension into the liquid N<sub>2</sub> to form discrete frozen balls.
- 5.9 Frozen cell balls can be stored at -80°C or continue with RNA isolation straight away.



## **Isolation of Total RNA**

- 5.10 Soak a 5 ml Teflon vessel and 7 mm tungsten carbide bead in RNase Away®, then pre-cool in liquid N<sub>2</sub>
- 5.11 Wearing disposable gloves add the frozen cell drops and the bead to the Teflon vessel and screw shut.
- 5.12 Insert into the Micro-Dismembrator. Shake for 2.5 min @ 2000 rpm.
- 5.13 Ensure that there are no un-powdered lumps of cells left and resuspend immediately in 2 ml Trizol® (Toxic, dispose of tips and tubes in cytotoxic bins).
- 5.14 Transfer to 2 microcentrifuge tubes. Vortex for 1 min.
- 5.15 Leave for 5 min @ room temperature to allow for dissociation of the nucleoprotein complexes.
- 5.16 Centrifuge for 10 min @ 12,000 g.
- 5.17 Tip supernatant into a new microcentrifuge tube.
- 5.18 Add 0.4 vol chloroform. Shake samples vigorously by hand for 15 s.
- 5.19 Leave for 3-10 min @ room temperature.
- 5.20 Centrifuge for 5 min @ 12,000 g.
- 5.21 Transfer the top (colourless) layer into a new microcentrifuge avoiding the interphase.
- 5.22 Add 0.5 vol isopropanol. Precipitate RNA for 5-15 min @ room temperature.
- 5.23 Centrifuge for 10 min @ 12,000 g. Carefully remove the supernatant.
- 5.24 Wash the pellet with 1 ml ice-cold 70% ethanol. Vortex.
- 5.26 Centrifuge for 10 min @ 12,000 g. Carefully remove the supernatant.
- 5.27 Briefly air dry the RNA pellet
- 5.28 Resuspend the pellet in 500 µl DEPC-treated H<sub>2</sub>O. Vortex.
- 5.29 Add 500 µl LiCl buffer. Vortex.
- 5.30 Leave to precipitate @ -20°C for at least 1 h or overnight.
- 5.31 Centrifuge for 30 min @ max @ 4°C.
- 5.32 Wash the pellet with 1 ml ice-cold 70% ethanol. Centrifuge for 10 min @ max @ 4°C.
- 5.33 Wash the pellet with 500 µl ice-cold 70% ethanol. Centrifuge for 10 min @ max @ 4°C.
- 5.34 Remove ethanol with a pipette.
- 5.35 Air dry pellet for ~15 min
- 5.36 Resuspend in ~50-100 µl DEPC H<sub>2</sub>O.
- 5.37 Run 1 µl on a 1% TBE-agarose or formaldehyde gel to visualise and check for RNA integrity.
- 5.38 Take the OD<sub>260</sub> of 1/1000 dilution to quantify the RNA.  
OD<sub>260</sub> of 1.0 = 40 µg µl<sup>-1</sup>  
therefore [RNA] = OD<sub>260</sub> x 40 x 1000 / 1 x 1000 = OD<sub>260</sub> x 40 µg µl<sup>-1</sup>



## 6. Literature

- 6.1 Hauser, N.C., Vingron, M., Scheideler, M., Krems, B., Hellmuth, K., Entian, K-D, and Hoheisel, J.D. (1998) Transcriptional profiling on all open reading frames of *Saccharomyces cerevisiae*. *Yeast* **14**: 1209–1221.
- 6.2 Enjalbert, B., Smith, D.A., Cornell, M.J., Alam, I., Nicholls, S., Brown, A.J., Quinn, J. (2006) Role of the Hog1 stress-activated protein kinase in the global transcriptional response to stress in the fungal pathogen *Candida albicans*. *Mol Biol Cell* **17**: 1018-1032.

