Upload image

Version 1

Jul 21, 2020

RNA Extraction from Cryptococcal Cells. V.1

Liz Hughes[1], Edward Wallace[1], Elizabeth Ballou[2]

[1]University of Edinburgh; [2]University of Birmingham

1 Works for me This protocol is published without a DOI.

Wallace lab for Fungal RNA
Tech. support email: Edward.Wallace@ed.ac.uk

Liz Hughes
University of Edinburgh

ABSTRACT

This protocol is for extracting RNA from Cryptococcal cell cultures.
Cell pellets are fixed in methanol, lyophilised overnight, the cell wall mechanically disrupted and RNA extracted using the Zymogen DNA/RNA miniprep kit.

The Zymogen Kit is designed to purify RNA from small amounts of starting material.
They provide a fast and simple method for preparing up to 100 µg total RNA per sample. The purified RNA is ready for use in downstream applications such as:

- Next-Gen sequencing
- RTPCR
- Microarray
- Hybridization

The*Quick*-DNA/RNA Miniprep Kit provides a quick method for the isolation of high quality genomic DNA and total RNA from small amounts of cells and tissue. The kit isolates both genomic DNA and a broad range of RNA species without the use of phenol. Small RNAs (e.g., tRNAs, microRNAs) can be recovered following a simple adjustment within the RNA isolation protocol – no extra steps are required! Both DNA and RNA from up to 5x10^{6}cells can be eluted into volumes as little as 25 µl in less than 15 minutes.

The procedure represents a well-established technology for RNA purification. This technology combines the selective binding properties of a silica-based membrane with the speed of micro-spin technology. A specialised high-salt buffer system allows up to 50 µg of RNA to be extracted.

Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine-chlodride containing buffer, which immediately inactivates RNases to ensure purification of intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to a spin column, where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30–100 µl water.

Size range: Genomic DNA ≥40 kb and Total RNA ≥17 nt
Yield: 25 µg DNA and RNA (binding capacity), ≥25 µl (elution volume)
A260/A280, A260/A230: >1.8.

https://files.zymoresearch.com/protocols/_d7001_quick-dna-rna_miniprep_kit.pdf
https://files.zymoresearch.com/datasheets/ds2503-02_quick-dna_rna_kit_datasheet.pdf

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://files.zymoresearch.com/protocols/_d7001_quick-dna-rna_miniprep_kit.pdf
https://files.zymoresearch.com/datasheets/ds2503-02_quick-dna_rna_kit_datasheet.pdf

PROTOCOL CITATION

Liz Hughes, Edward Wallace, Elizabeth Ballou 2020. RNA Extraction from Cryptococcal Cells.. **protocols.io** https://protocols.io/view/rna-extraction-from-cryptococcal-cells-73dhqi6

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

https://files.zymoresearch.com/protocols/_d7001_quick-dna-rna_miniprep_kit.pdf

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

https://files.zymoresearch.com/datasheets/ds2503-02_quick-dna_rna_kit_datasheet.pdf

KEYWORDS

RNA extraction, Cryptococcus, Lyophilize, RNeasy plant mini kit, RNA

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Oct 09, 2019

LAST MODIFIED

Jul 21, 2020

PROTOCOL INTEGER ID

28485

GUIDELINES

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
An RNase free environment is essential when working with RNA samples.
RNases are very hardy and removing them can be very difficult.
RNAses are a ubiquitous component of skin.
Always wear gloves when handling RNA and any associated materials/equipment.
This protocol is for small volumes of RNA using a 1.5ml microcentrifuge tube.
Always use filtered tips.

NB: Usually 5 x 10^6 yeast cells can be processed. Depending on the strain and growth conditions, 30–50 µg RNA can be expected.

MATERIALS

NAME	CATALOG #	VENDOR
Methanol	PA-33900HPLCCS4L	P212121
Beta-mercaptoethanol		
Ethanol	100983	Merck Millipore
0.5mm zirconia/sililca beads	11079105z	BioSpec Products
Zymogen DNA/RNA Miniprep Kit	D7001	

SAFETY WARNINGS

Guanidine salts can form highly reactive compounds when combined with bleach.
If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

https://files.zymoresearch.com/sds/_d7010-2_dna_rna_prep_buffer.pdf (safety data sheet for DNA/RNA Prep buffer)
https://files.zymoresearch.com/sds/_d7010-3-6_d7010-3-12_d7010-3-24_dna_rna_wash_buffer.pdf (safety data sheet for DNA/RNA Wash buffer)

1 Prepare Cryptococcal cells for RNA extraction.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

1.1 Streak out appropriate glycerol stock on YPD plate. Incubate @ 30ºC for 2 days.

1.2 Pick a single colony from the freshly streaked plate and put in required volume of appropriate media. Incubate in appropriate conditions depending on expected experimental conditions/outcome.

1.3 ⚠

Following growth of cells they **MUST** be fixed with methanol prior to downstream processing.
Cool 5 ml of Methanol per culture sample on dry ice prior to collecting cultures.
Mix 10 ml of culture with 5 ml 100% Methanol on dry ice, invert a few times to mix and leave on dry ice for 3-5 mins. Leaving any longer will result in the culture freezing.

1.4 After fixing the cells on dry ice move to normal ice for a few mins and then spin cells down at 4°C, 3000 g for 2 mins.
Wash cells with 15 ml sterile ice cold water.
Spin cells down at 4°C, 3000 g for 2 mins, pour off supernatant.
Repeat wash step.
Re-suspend pellets in 1-2 ml sterile ice cold water and transfer to a 2 ml **screw cap** tube.
Spin cells at 4°C, 3000 g for 2 mins.
Remove all the water and store at -80°C for **AT LEAST** 2 hours before freeze drying on the lyophiliser.

2 Lyophilise cell pellets overnight.

2.1 Cool lyophiliser at least an hour before using to bring the temperature down to -60°C.
Switch lyophiliser on and follow instructions on machine to cool it down.

NB: Cell pellets **must** be at -80°C for at least 2 hours prior to lyophilisation to allow sublimation to take place.

2.2 When the lyophiliser is at -60°C, switch off and release vacuum by removing silver plug from orange tube.
Place samples on the mesh.
Replace the lid and silver plug.
Switch on the pump.

2.3 Dry cell pellets overnight under vaccum @ -60°C.

Before leaving apparatus check the pellets are still in the tube and have not started to rise or pop out. If the pellets are cold enough this should not happen.

NB: Freeze drying, also known as lyophilisation or cryodesiccation, is a low temperature dehydration process that involves freezing the product, lowering pressure, then removing the ice by sublimation (a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapour without passing through a liquid phase). This is in contrast to dehydration by most conventional methods that evaporate water using heat, and allows removal of water without excessive heating.

3 Mechanical disruption of Cryptococcal cells.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

3.1 Take dry lyophilised pellets and add 200µl of dry 0.5 mm zirconium beads.
Add 700 µl of RLT Buffer + β_2M
(10 µl β_2M + 1 ml RLT buffer = lysis buffer).

3.2 'Bead-beat' Using PreCellys machine.
Carry out 10 cycles of the EW Yeast Program. (1 cycle = 3 x 10 sec with 20 sec pause)
Place tubes on ice for 1 min between each cycle.
You should now have a fine powder.

NB:' Bead-beating' machines warm the sample about 10 degrees/minute. This is due to frictional collisions of the beads during homogenization. Sample warming can be controlled by 'bead-beating' for short time intervals with cooling on ice between each interval.

4 RNA Extraction of mechanically disrupted Cryptococcal cells.

4.1 Use the Zymogen kit.
After mechanical disruption, perform all steps of the procedure at room temperature.
Work quickly.

Spin cells @ 1000 x g for 3 mins to pellet the beads.

4.2 ⚠

KEEP the supernatant, **DO NOT** throw it away.
Add the supernatant to the Zymo spin IIIC column.
Place the column into a collection tube and spin @ 12,000g for 1 min.

This step binds the RNA to the IIIC column.
Optimisation has been carried out on 5 x 10^7 cells.

4.3 ⚠

KEEP the flow through.
Transfer the flow through to micro-centrifuge tube leaving any pellet behind.
Add 400µl of 100% Ethanol to the flow through, mix immediately by pipetting and transfer to the Zymo Spin IIC Column (RNA binding capacity of this column is 50µg).
Spin @ 12,000 x g for 1 min.
Discard flow through.

4.4 Add 400µl of DNA/RNA Prep buffer to the column.
Spin @ 12,000 x g for 1 min.
Discard the flow through.

NB: DNA/RNA Prep buffer contains guanidine chloride and ethanol. This is a stringent washing buffer that removes carbohydrates, proteins, fatty acids etc that are non-specifically bound to the silica membrane.

4.5 Add 700µl of DNA/RNA Wash buffer to column.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Tip column up and down to ensure all previous buffers are caught from the lid etc.
Spin @ 12,000 x g for 30 sec.
Discard the flow through.

NB: DNA/RNA Wash buffer is a mild washing buffer. It's main function is to remove traces of salts that remain on the column due to earlier buffers.

4.6 Add 400µl of DNA/RNA Wash buffer to column.
Tip column up and down to ensure all previous buffers are caught from the lid etc.
Spin @ 12,000 x g for 1 min.
Discard the flow through.

NB: DNA/RNA Wash buffer is a mild washing buffer. It's main function is to remove traces of salts that remain on the column due to earlier buffers.

4.7 Centrifuge the column in a clean and dry collection tube @ full speed for 1 minute to dry the membrane.

4.8 Place the column in a fresh RNase free micro-centrifuge tube.
Add 30µl of RNase free water.
Close the lid gently and leave at RT for 3-5 mins.
Spin @ 10,000 x g for 1 min to elute the RNA.
Discard the column.

4.9 Check the RNA purity and integrity using the NanoDrop and Fragment Analyzer.
Store the RNA at -80ºC.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited