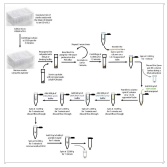

Aug 09, 2021

RNA extraction with spin columns from yeast cells grown on 12-column deep well plates

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ABSTRACT

This protocol is for yeast cell growth, lysis, and RNA extraction using silica spin columns. We use this to prepare RNA for RT-qPCR analysis from many strains at once. We have particularly used it on S288C-background *Saccharomyces cerevisiae*, containing a plasmid encoding a fluorescent reporter and a URA3 selection cassette, grown in SC-URA media. Then we use RT-qPCR to measure reporter gene expression compared to reference genes. In principle the protocol should work for similar strains/species and growth media, as long as the cell shape and cell walls are similar enough that the lysis method works.

Yeast strains are grown in parallel, in a 12 column deep well plate, on a shaking incubator allowing for easier strain handling. Yeast cells then are lysed by bead bashing with zirconia beads using the Precellys Evolution homogeniser. RNA is subsequently purified using a silica column. The RNA extraction method was a modification of the Quick-DNA/RNA miniprep kit from Zymo Research (D7001, https://www.zymoresearch.com/collections/quick-dna-rna-kits/products/quick-dna-rna-miniprep). The key difference is that we find RNA binding buffer (R1013) is far more effective as a lysis buffer for our yeast samples than the buffer supplied with that kit. The protocol makes extensive use of Zymo Research columns and buffers, that can be purchased individually. Other manufacturers make similar columns and buffers or you can make your own, although we have not tested those (Yaffe et al. 2012; Jue et al., 2020).

References:
Jue, E., Witters, D. & Ismagilov, R.F. Two-phase wash to solve the ubiquitous contaminant-carryover problem in commercial nucleic-acid extraction kits. Sci Rep 10, 1940 (2020). https://doi.org/10.1038/s41598-020-58586-3
Yaffe, H., Buxdorf, K., Shapira, I. *et al.* LogSpin: a simple, economical and fast method for RNA isolation from infected or healthy plants and other eukaryotic tissues. *BMC Res Notes* **5,** 45 (2012). https://doi.org/10.1186/1756-0500-5-45

DOI

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PROTOCOL CITATION

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RNA extraction, S. cerevisiae

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34995

MATERIALS TEXT

Labware:

Gas Permeable Adhesive Seals **Thermo Scientific Catalog #AB-0718**

RNA binding buffer (Optional) **Zymo Research Catalog #R1013-2-100**

DNA/RNA Prep buuffer **Zymo Research Catalog #D7010-2**

DNA/RNA Wash buffer **Zymo Research Catalog #D7010-3**

Zymo Spin IIICG column **Zymo Research Catalog #C1006**

Zymo Spin IIC columns **Zymo Research Catalog #C1011**

10 ml 24 Deepwell Plate Square Wells with V-Shape Bottoms **StarLab Catalog #10 ml 24 Deepwell Plate, Square**

12 Column Deep Well Polypropylene Storage Reservoir Plate **4titude Catalog #PCR0244**

0.5 mm Zirconia/Silica Beads **Bio Spec Products Inc. Catalog #11079105z**

2 ml screw cap micro tubes **Sarstedt Catalog #72.694.005**

Optional alternative tubes pre-loaded with beads:

ZR BashingBead Lysis Tubes (2mm) **Zymo Research Catalog #S6003-50**

Growth media. Prepare a growth media appropriate for your experiment, e.g. SC, SC-URA, YPD.

Grow single colonies of yeast 2d

1 Streak out yeast from glycerol stocks to single colonies on petri dishes poured with solid media that is appropriate for[2d] your experiment, and grow as desired. We usually grow for 2 days (**48:00:00**) at **30 °C** . To minimize experimental variability, it is best to use fresh plates, i.e. that have never been stored in a fridge.

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Plate plan 10m

2 **Plan your 24 well culture plate layout** - 5m

Subdivide strains into groups of 6 then fill out the strain names on the following pre-culture (24 well) plate plan to log the locations of each strain on the 24 well plate

Template 24 well plate plan - **24well_preculture_plateplan_template.xlsx**

Example 24 well plate plan - **24well_preculture_plateplan_example1.xlsx**

24 well pre-culture plate plan

	1	2	3	4	5	6
A						
B	strain1_biorep1	strain1_biorep2	strain1_biorep3	strain2_biorep1	strain2_biorep2	strain2_biorep3
C	strain3_biorep1	strain3_biorep2	strain3_biorep3	strain4_biorep1	strain4_biorep2	strain4_biorep3
D				Media	Media	Media

Make sure you have media only wells to serve as a negative control!

By biological replicate (biorep), we usually mean single colonies of the same genetically identical stock, grown on the same agar plate.

3 **Plan your 12 column culture plate layout** - 5m

Subdivide the strains into groups of 12 then fill out the strain names on the following 12 well plate plan to log the locations of each strain on plate.

Template 12 column deep well plate plan - **12column_culture_plateplan_template.xlsx**

Example 12 column deep well plate plan - **12column_culture_plateplan_example1.xlsx**

Plate 1 - 12 column deep well plate plan

	1	2	3	4	5	6	7	8	9	10	11	12
A	strain1_biorep1	strain1_biorep2	strain1_biorep3	strain2_biorep1	strain2_biorep2	strain2_biorep3	Media	Media	Media			

Plate 2 - 12 column deep well plate plan

	1	2	3	4	5	6	7	8	9	10	11	12
A				Media	Media	Media	strain3_biorep1	strain3_biorep2	strain3_biorep3	strain4_biorep1	strain4_biorep2	strain4_biorep3

Strains have been divided into 2 groups of 6 so that we can have media-only wells.

Again, make sure you have media-only wells!

Pre-culture growth 1d 0h 0m 17s

4 Take a sterile 24 (deep) well plate (Star lab cat no. E2824-0000) and using a pipette controller, transfer **1 mL** sterile media to each well of the 24 well plate according to the plate plan above (24 well plate plan in Step 2). 5m

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5 Using sterile P20 tips, inoculate the media in each well with a single colony of the appropriate strain according to the plate plan above (24 well plate plan in Step 2). 5m

Use a new sterile tip for each strain - do not re-use tips for multiple wells or multiple strains.

6 Seal the deep well plate with a gas permeable seal (Thermo cat #AB0718) and grow overnight in a shaking incubator set at **30 °C** and 200 rpm. 2m

7 After 16-20 hours, for each strain transfer **100 µl** of the overnight culture to a cuvette then add **900 µl** of media and mix well (1:10 dilution of the overnight culture) and measure the OD600 using a spectrophotometer. 1d

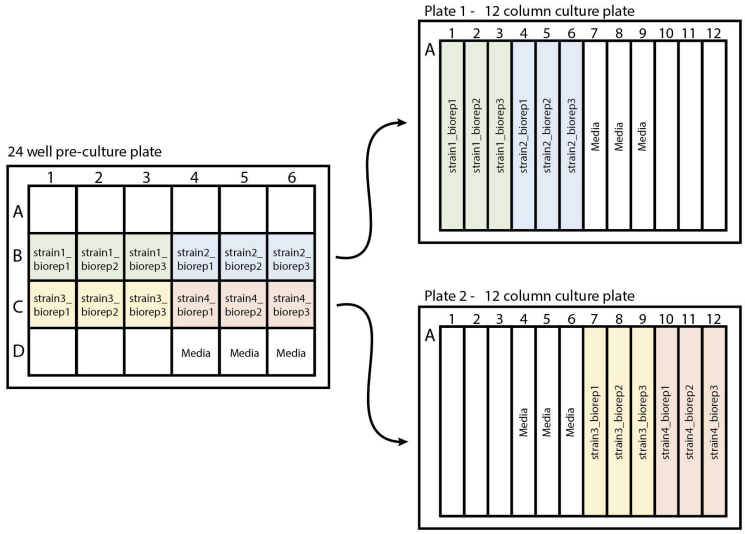
16-20 hours is enough time for the culture to reach saturation.

8 Calculate the amount of the overnight culture to add to 8 ml of sterile media to reach a starting OD600 of 0.2. 5m

Culture growth from an OD600 of 0.2 to 0.5-0.7 3h 57m

9 Take sterile 12 column deep well plates (4titude cat #PCR0244) (based on the plate plans above, we will need 2 for this example). Using a pipette controller (Eppendorf cat #4430000018), dispense **7 mL** of media into each well of the 12 column deep well plate. 5m

10 Transfer the calculated amount of the overnight culture into their respective wells on the 12 column deep well plate, based on the amount calculated in step 8. 10m



Change the pipette tip for each strain/biorep

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11 Take **1 mL** of the diluted culture and measure their OD600 using a spectrophotometer (to check that their starting ODs are betwee 0.15 - 0.2). 10m

12 Seal with a gas permeable seal and grow in a shaking incubator set at **30 °C** and 90 rpm to an OD600 of 0.5 - 0.7. 2m

This should take ~3.5 - 4 hours.

13 After ~3.5 - 4 hours, measure the OD of each culture by transferring **1 mL** of the culture using a spectrophotometer. 3h 30m

Harvesting and lysis of cells 1h 7m

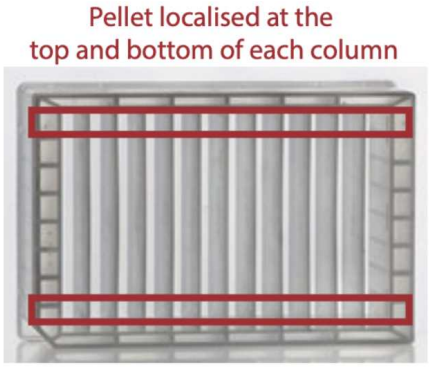
14 Once the OD600 of the culture has reached between OD600 of 0.5-0.7, pellet the cells on the 12 column deep well plate by centrifugation at **3000 x g, 00:03:00** . 3m

15 Using an aspirator, remove the media in each well. Avoid touching the pellet. 10m

DO NOT decant! The pellet gets loosened and increases the chance of cross contamination between wells.

When using the aspirator, change the tip for each well to avoid cross contamination.

Try to remove as much media as possible. Leaving a bit of media in the well can affect the RNA extraction efficiency.



Localisation of pellet after centrifugation in a swinging bucket centrifuge. The pellets localise at two areas in the 12 column deep well plate, making it easy to resuspend the pellet with a small volume (400 ul).

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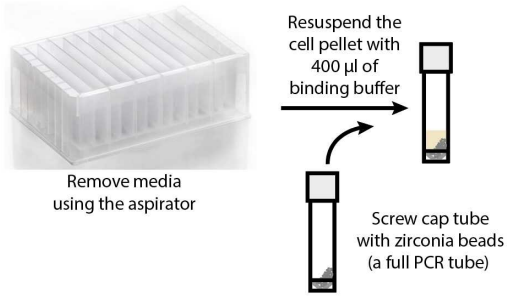
This is a possible pause step - The pellets in the deep well plate can be frozen at **-80 °C . It is better to freeze it this way than in RNA binding buffer with the zirconia beads.

16 Take a screw cap tube (Sarstedt cat #12705493) and use a PCR tube to measure zirconia beads (BioSpec #11079105z) (note - fill the entire PCR tube with beads) . Add the zirconia beads into the screw cap tubes. Label each tube. 10m

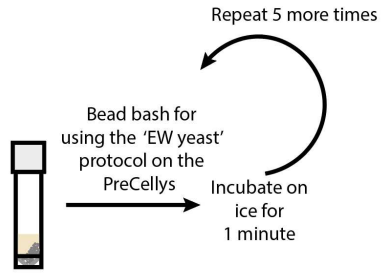
17 Resuspend the pellet in the 12 column deep well plate with **400 µl** of **RNA binding buffer** (Zymo cat no. R1013-2) and transfer them to the screw cap tubes containing the zirconia beads (keep on ice) 15m



18 Transfer the tubes to the PreCellys (Bertin Instruments PreCellys Evolution) and run the **EW Yeast protocol** (this is a **00:00:50** method). Chill tubes on ice for **00:01:00** . Repeat this step 5 more times (Total of 6 cycles) 1m 50s

The Precellys Evolution EWYeast protocol is set to 3 cycles shaking at a speed of 6000 RPM, each with a duration of 10s and with a pause between each cycle of 10s.

Cell mixture tends to freeze when placed too long on ice. Thaw before putting it on the PreCellys. A frozen pellet can cause problem in the RNA extraction protocol and ultimately low yields.



19 Transfer the tubes to a tabletop centrifuge and spin at **12000 x g, Room temperature , 00:01:30** . 1m 30s

The tubes tend to freeze and solidify when kept on ice for too long. Make sure that it is thawed before you transfer it to the centrifuge. A frozen pellet can cause problem in the RNA extraction protocol

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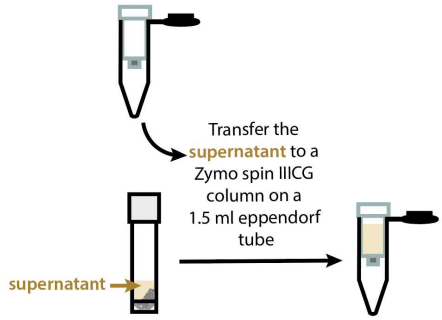
DO NOT cool the centrifuge in this and all following steps. This causes problems in the RNA extraction process. Keep the centrifuge at room temperature.

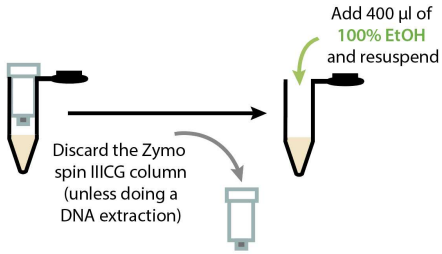
If you have a refrigerated centrifuge, set it to 22°C.

20 Transfer supernatant to a **Zymo Spin IIICG** column (Zymo cat no. C1006) on a collection tube (Label tubes). Spin the Zymo Spin IIICG containing the supernatant at **12000 x g, Room temperature , 00:01:00** . (This step binds DNA to IIICG column). 1m



RNA extraction 1h 10m

21 Discard the Zymo IIICG and **keep the flow through**! Add **400 µl** of **100% EtOH** to the flow through on the collection tube and mix well by pipetting up and down at least 4x. 10m



Mix well in this step!

22 Transfer the flow-through and ethanol mixture to a **Zymo Spin IIC** column (Zymo cat no. C1011) on a collection tube and spin at **12000 x g, Room temperature , 00:01:00** . 10m

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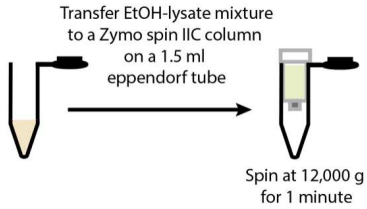
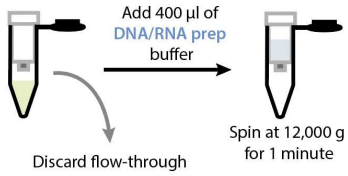


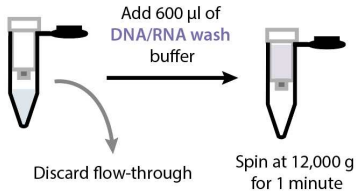
23 10m

Discard the flow-through and keep the column. Add **400 µl** of **DNA/RNA Prep Buffer** (Zymo cat no. D7010-2) to the column. Spin at **12000 x g, Room temperature , 00:01:00** .



24 10m

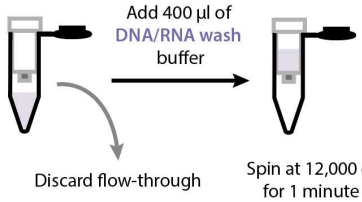
Discard the flow-through and add **600 µl** of **DNA/RNA Wash Buffer** (Zymo cat no. D7010-3) to the column. Spin at **12000 x g, Room temperature , 00:01:00** .



Make sure that you have added Ethanol to the concentrated DNA/RNA wash buffer, following the manufacturer's instructions. We recommend that this dluted wash buffer is not too old, as Ethanol and water evaporate at different rates, which disturbs the concentrations.

25 10m

Discard the flow-through and add **400 µl** of **DNA/RNA Wash Buffer** to the column. Spin at **12000 x g, Room temperature , 00:01:00** .



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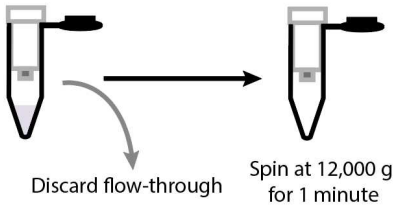
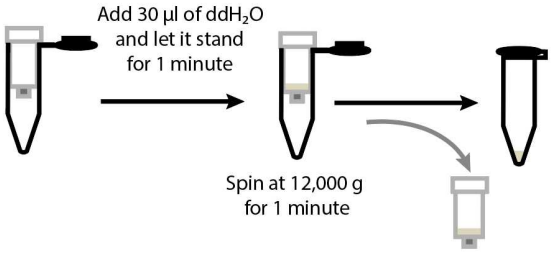
26 1m

Discard the flow-through spin again at **12000 x g, Room temperature , 00:01:00** .



27 10m

Transfer the Zymo IIC column to a clean 1.5 ml tube, labelled with your sample name as this will be used to store the RNA. Add **30 µl** of **nuclease free water** to the column (add this on the filter without touching the filter with your tip) and incubate at room temperature for **00:01:00** . Spin at **12000 x g, Room temperature , 00:01:00** and discard the column. The RNA is now in the eluted flow-through.



28 5m

Measure the concentration of the RNA using a microvolume spectrometer (e.g.nanodrop). Verify the quality of the RNA by running samples on the fragment analyzer.

Expected result - The RNA concentration should be between 1000-2000 ng/ul.

A gel or capillary electrophoresis trace should show 2 clear ribosomal RNA bands. See *RNA: a laboratory manual* (Rio, Ares, Hannon, Nilssen) for more on RNA quality.

Store the RNA in **-80 °C** .

For long-term storage or to send to a sequencing facility, it is often recommended to store in low-binding tubes and in low TE buffer.

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