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A TRIzol-Beadbeater RNA extraction method for yeast V.1

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Abstract

Saccharomyces cerevisiae yeast cells were harvested by centrifugation and preserved in TRIzol reagent at −80 °C. Cell lysis was achieved via bead-beating in the presence of zirconium oxide beads using a high-speed homogenizer (Omni Bead Ruptor 12), resulting in >85% lysis after a single 30-second cycle, as verified by phase-contrast microscopy. RNA was extracted via chloroform-mediated phase separation and purified using the Ambion RiboPure™ Yeast RNA Purification Kit. This protocol provides an efficient method for isolating purified RNA suitable for downstream applications such as RT-qPCR and RNA-seq.

Guidelines

Materials and Methods

Fifty-eight 50 ml samples of yeast cells (with an average of 4.2×10^8 cells) were harvested by centrifugation at 3,000 rpm for 5 minutes at 4 °C using a Sorvall Super T21 centrifuge. The supernatant was removed by vacuum aspiration, and the cell pellet was resuspended in 1 mL TRIzol reagent (an acidic solution containing guanidinium thiocyanate, phenol, and chloroform) and aliquoted in two tubes (each containing 0.5 ml sample). Samples were stored at −80 °C until further processing. One set of frozen yeast in TRIzol was sent to Novogene and the second set was processed at Stony Brook, see below.

Local (Stony Brook) Methods

Yeast Cell Lysis

For lysis, one set of frozen yeast in TRIzol was thawed on ice. Samples were transferred into 2 ml screw-cap tubes with an O-ring. Zirconium oxide beads (0.5 mm diameter) were added from a plastic weigh boat until the bead level reached approximately 1 mm below the liquid meniscus. Tubes were pre-chilled on ice to 0 °C, then loaded in a balanced configuration into an Omni International Bead Ruptor 12. Homogenization was performed at 3.1 m/s for 30 seconds.

After homogenization, tubes were placed on ice. A 1 µL sample (avoiding beads) was mixed with 6 µL of water on a microscope slide and inspected using phase-contrast microscopy to assess cell disruption. Typically, >85% of cells were lysed after a single cycle. If lysis is insufficient, a second 30-second cycle can be performed (Prolonged agitation is not recommended, as it may cause overheating.) No second cycle was needed for any of the samples considered here.

RNA Recovery and Processing

The cell lysate was transferred to a 1.5 mL Eppendorf tube using a pipette tip, leaving the zirconium oxide beads behind. Chloroform was added (20% of the TRIzol volume, i.e., 200 µL per 1 mL TRIzol), and the mixture was gently rocked for 3 minutes at room temperature. Samples were then centrifuged at 14,000 rpm for 15 minutes at room temperature in a tabletop centrifuge.

Phase separation resulted in three layers: a clear upper aqueous phase (containing RNA), a white interphase (containing DNA), and a red lower organic phase (containing proteins). The aqueous phase was carefully transferred to a 15 mL centrifuge tube, avoiding disruption of the interphase.

To the aqueous phase, 350 µL of Binding Buffer (from the Ambion RiboPure™ Yeast RNA Purification Kit, Cat. #AM1926) was added per 100 µL of solution. Ethanol (200-proof) was added to reach a final concentration of 70%, and the sample was then processed using the kit's filter cartridges, following the manufacturer's protocol. RNA was eluted in 2 × 25 µL of 10 mM Tris-HCl (pH 8.0).

RNA concentration was determined using a NanoDrop spectrophotometer.

Cell Lysis and RNA Recovery (Novogene)

The second set of frozen yeast in TRIzol was processed at Novogene. Per customer service at Novogene, their team used mechanical disruption using type C bead tubes from MN and a TissueLyser II by Qiagen operated at 30 Htz for 5 minutes per side and RNA may have been recovered using the RNeasy Mini kit-TRIzol. There was no mention of an assay for cell breakage.

Expected results

Table 1 shows the number of cells in each sample, the theoretical amount of RNA in each sample, the amount of RNA recovered by Stony Brook or Novogene for each sample, the ratio of the amount of Novogene RNA to theoretical RNA; and the ratio of the amount of Novogene RNA to Stony Brook RNA.

Summary of Table 1 (per-sample data shown in the table): mean values across the 58 samples are reported as follows — Mean theoretical RNA: 143 µg; Mean Stony Brook (SB) RNA recovered: 49.4 µg; Mean Novogene RNA recovered: 0.71 µg; Mean Novogene/Theoretical ratio: 0.007; Mean Novogene/SB ratio: 0.0193.

"Cells (x 10^7)" is the number of cells in each sample.

"Theoret. RNA" is the theoretical amount of RNA in each sample, based on each cell containing 7.1×10^{-13} g RNA per cell [7]. However, this is likely a slight to moderate over-estimate in most of these cases, since cells in most of these samples were smaller than usual, due to the nature of the experiment that produced them.

"SB RNA" is the number of micrograms of RNA produced locally (i.e., at Stony Brook) from these samples. For SH8, SH30, and SH55, the sample was the residual cell suspension, much less than a full 0.5 ml sample.

"Novogene RNA" is the number of micrograms of RNA produced by Novogene from these samples.

"Novo/Theoret." is the ratio of the amount of RNA produced by Novogene to the theoretical amount of RNA.

"Novo/SB" is the ratio of the amount of RNA produced by Novogene to the amount of RNA produced at Stony Brook. For SH8, SH30, and SH55, this is "NA" because a full sample was not available at Stony Brook.

Since a yeast cell contains about 7.1×10^{-13} g RNA per cell [7], one expects an average of nearly 300 micrograms of total RNA per 50 ml sample. Since 0.5 ml was half the total sample, the theoretical expectation for the average amount of total RNA was about 150 micrograms (or somewhat less, given the small average cell size of elutriated cells).

Novogene reported an average recovery of only 0.71 micrograms of total RNA per sample, ranging from 0.03 micrograms to 2.63 micrograms (Table 1). This is only a small fraction of the ~150 micrograms of total RNA

expected.

Our method yielded an average of 49.4 micrograms of total RNA per sample, compared to 0.71 micrograms of total RNA per sample at Novogene. When the yields in micrograms were compared between each sample by a paired t-test, the difference was significant with a p-value of 2×10^{-16}.

Compared to our protocol, which achieved approximately 40% RNA extraction efficiency from S. cerevisiae, the method used by Novogene resulted in a substantially lower yield of around 1%.

This low efficiency could be because only about 1% of cells in the sample were lysed. Although there is no proof that inefficient lysis is the cause, there are two supporting arguments. First, the method possibly used by Novogene to lyse the cells would be expected to be inefficient (see Methods). Second, the yeast samples where Novogene was more successful (~ 3% efficiency) were samples that contained a relatively high proportion of large, aberrant cells, and the yeast samples where Novogene was less successful (~ 0.05% efficiency) were samples containing mainly small, round cells. Alternatively, the low efficiency could have been due to inefficient RNA recovery (see Methods).

Since only a few nanogram of RNA is enough to prepare library for RNA-Seq, so even if cell breakage was inefficient but completely random, then the ensuing RNA-Seq results could still be useful. However, if cell breakage occurred preferentially for large, aberrant cells, then the results from RNA-Seq would represent gene expression in these aberrant cells, and so would be misleading with regard to gene expression in the entire sample population. Thus it could be potentially misleading to sequence RNA samples when extraction efficiency is very low.

In the apparent absence of any quality control for the extraction step, it seems prudent for investigators to check the RNA yield for any kind of sample. This is a parameter that should be reported in publications.

Materials

List of materials and equipment explicitly mentioned on these pages:
- TRIzol reagent (described as an acidic solution containing guanidinium thiocyanate, phenol, and chloroform)
- Ambion RiboPure™ Yeast RNA Purification Kit
- Binding Buffer (from the Ambion RiboPure™ Yeast RNA Purification Kit, Cat. #AM1926)
- Kit filter cartridges (from Ambion RiboPure™ Yeast RNA Purification Kit)
- Ethanol (200-proof)
- Zirconium oxide beads (0.5 mm diameter)
- Omni International Bead Ruptor 12 (Omni Bead Ruptor 12) high-speed homogenizer
- Sorvall Super T21 centrifuge
- Tabletop centrifuge capable of 14,000 rpm
- Vacuum aspiration apparatus (for supernatant removal)
- 50 mL sample tubes (for initial yeast cultures)
- 15 mL centrifuge tubes (for collection of aqueous phase)
- 2 mL screw-cap tubes with O-ring (for bead-beating; pre-chilled on ice to 0 °C)
- Plastic weigh boat (to hold beads until bead level reached ~1 mm below liquid meniscus)
- 1.5 mL Eppendorf tubes (for lysate transfer)
- Pipettes and pipette tips
- Phase-contrast microscope (to inspect lysis)
- Chloroform (for phase separation)
- Nuclease-free water
- −80 °C freezer (for sample storage)
- Ice (for thawing and pre-chilling tubes)
- Microcentrifuge tubes and reagents as required by downstream purification kit
- NanoDrop spectrophotometer (for RNA quantitation)
- Type C bead tubes from MN (used by Novogene customer service)
- TissueLyser II by Qiagen (used by Novogene customer service)
- RNeasy Mini kit-TRIzol (possible kit used by Novogene)

Troubleshooting

Safety warnings

Explicit cautionary statements present on these pages:
- Prolonged agitation is not recommended, as it may cause overheating.
- When inspecting lysate under the microscope, avoid transferring beads (sample should be taken avoiding beads).
- A second 30-second bead-beating cycle can be performed if lysis is insufficient, but prolonged agitation/overheating should be avoided.
- When transferring the aqueous phase after centrifugation, avoid disruption of the interphase to prevent DNA/protein contamination.
- Handle 200-proof ethanol and chloroform with appropriate laboratory safety precautions (flammability and toxicity) and in compatible containers (not explicitly detailed on these pages).
- Table 1 contains some entries marked NA for certain ratio calculations where values were not available.

Before start

Preparatory notes and sample setup described on these pages:
- Harvest yeast culture by centrifugation (example used: centrifugation at 3,000 rpm for 5 minutes at 4 °C).
- Remove supernatant by vacuum aspiration and resuspend pellet in 1 mL TRIzol reagent.
- Aliquot resuspended samples into two tubes (each containing 0.5 mL sample) and store at −80 °C until processing.
- Pre-chill 2 mL screw-cap tubes with O-ring on ice to 0 °C before adding samples and beads.
- Add zirconium oxide beads (0.5 mm) to tubes until bead level is ~1 mm below liquid meniscus.
- Balance tubes when loading into the Omni Bead Ruptor 12.
- If using chloroform for phase separation, prepare 20% (v/v) chloroform relative to TRIzol (example: 200 µL chloroform per 1 mL TRIzol).
- Prepare Binding Buffer (Ambion RiboPure™ kit) and 200-proof ethanol to reach 70% final ethanol for binding step.
- Prepare appropriate centrifuge tubes (e.g., 15 mL) to receive aqueous phase after phase separation.

Materials and Methods

1 Harvest 50 mL yeast culture samples by centrifugation at 3,000 rpm for 5 minutes at 4 °C using a Sorvall Super T21 centrifuge.

2 Remove the supernatant by vacuum aspiration and resuspend each cell pellet in 1 mL TRIzol reagent. Aliquot each resuspended sample into two tubes (each containing 0.5 mL) and store at −80 °C until further processing.

Local (Stony Brook) Methods

3 For lysis, thaw one set of frozen yeast in TRIzol on ice.

Yeast Cell Lysis

4 Transfer thawed samples into 2 mL screw-cap tubes with an O-ring.

5 Add zirconium oxide beads (0.5 mm diameter) from a plastic weigh boat until the bead level reaches approximately 1 mm below the liquid meniscus.

6 Pre-chill tubes on ice to ~0 °C, then load tubes in a balanced configuration into an Omni International Bead Ruptor 12.

7 Homogenize at 3.1 m/s for 30 seconds using the Omni Bead Ruptor 12.

8 Immediately place tubes on ice after homogenization. To assess lysis, take a 1 µL aliquot (avoiding beads), mix with 6 µL of water on a microscope slide, and inspect under phase-contrast microscopy to assess cell disruption (typically >85% of cells are lysed after a single cycle).

8.1 If lysis is insufficient, perform a second 30-second homogenization cycle; avoid prolonged agitation to prevent overheating.

RNA Recovery and Processing

9 Transfer the cell lysate to a 1.5 mL Eppendorf tube using a pipette tip, leaving the zirconium oxide beads behind.

10 Add chloroform equal to 20% of the TRIzol volume (e.g., 200 µL chloroform per 1 mL TRIzol). Gently rock the mixture for 3 minutes at room temperature.

11 Centrifuge the samples at 14,000 rpm for 15 minutes at room temperature in a tabletop centrifuge to effect phase separation.

12 Identify the three phases after centrifugation: a clear upper aqueous phase (containing RNA), a white interphase (containing DNA), and a red lower organic phase (containing proteins). Carefully transfer the aqueous phase to a 15 mL centrifuge tube, avoiding disruption of the interphase.

13 To the recovered aqueous phase, add 350 µL of Binding Buffer (Ambion RiboPure™ Yeast RNA Purification Kit) per 100 µL of aqueous solution.

14 Add ethanol (200-proof) to the sample to reach a final ethanol concentration of 70% and mix according to the kit instructions.

15 Process the sample using the Ambion RiboPure™ Yeast RNA Purification Kit filter cartridges following the manufacturer's protocol.

16 Elute RNA in 2 × 25 µL of 10 mM Tris-HCl (pH 8.0).

17 Determine RNA concentration using a NanoDrop spectrophotometer.

RNA Recovery and Processing

18 As a quality-control recommendation, measure and record the RNA yield for every extracted sample (e.g., using NanoDrop) and report the RNA yield when publishing results.

Protocol references

1. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 2009;10(1), 57-63. doi:10.1038/nrg2484

2. Collart MA, Oliviero S. Preparation of yeast RNA. Curr Protoc Mol Biol. 2001; Chapter 13, Unit13 12. doi:10.1002/0471142727.mb1312s23

3. DeCaprio J, Kohl TO. Lysing Yeast Cells with Glass Beads for Immunoprecipitation. Cold Spring Harb Protoc. 2020; (11). doi:10.1101/pdb.prot098590

4. Green MR, Sambrook J. Total RNA Extraction from Saccharomyces cerevisiae Using Hot Acid Phenol. Cold Spring Harb Protoc. 2021; (12). doi:10.1101/pdb.prot101691

5. Sasidharan K, Amariei C, Tomita M, Murray DB. Rapid DNA, RNA and protein extraction protocols optimized for slow continuously growing yeast cultures. Yeast. 2012; 29(8), 311-322. doi:10.1002/yea.2911

6. Schmitt ME, Brown TA, Trumpower BL. A rapid and simple method for preparation of RNA from Saccharomyces cerevisiae. Nucleic Acids Res. 1990; 18(10), 3091-3092. doi:10.1093/nar/18.10.3091

7. von der Haar TA. quantitative estimation of the global translational activity in logarithmically growing yeast cells. BMC Syst Biol. 2008; 2, 87. doi:10.1186/1752-0509-2-87

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