RNA Preparation for Microarray Experiments

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[Abstract] This protocol describes a simple and relatively general method to extract total RNA from a yeast culture using the Qiagen RNeasy kit. In it, total RNA is treated with DNase I and cleaned up to be suitable for microarray experiments. Therefore, this protocol can be used to generate a large yield of high quality total RNA from yeast cells (*S. cerevisiae* and *S. pombe*).

Materials and Reagents

1. *S. pombe* cells
2. Qiagen RNeasy Mini Kit (QIAGEN, catalog number: 74104)
3. DNase I (Roche Diagnostics, catalog number: 04716728001)
4. RNA STAT based on "*S. Pombe* RNA Prep"
5. DEPC water
6. EDTA
7. RLT buffer

Equipment

1. Standard bench-top centrifuge with 1.5 ml Eppendorf tube capacity
2. Calibrated standard laboratory pipettes

Procedure

A. Total RNA extraction

Grow 5-10 OD log phase *S. pombe* cells, extract total RNA using RNA STAT based on "*S. Pombe* RNA Prep".

Note: Using DEPC water to dissolve RNA. Usually RNA concentration is > 1.2 μg/μl and A_{260}/A_{280}=1.95-2.15.

B. DNase I treatment

Prepare the following mixture:

Total RNA	50 µg
10x incubation buffer	5 µl
DNase I	1 µl (10 units)
Water (RNase free)	up to 50 µl
Total	50 µl

Incubate at room temperature (RT) for 20 min, then stop the reaction by adding 2 µl of 0.2 M EDTA (pH 8.0) to a final concentration of 8 mM and heating to 75 °C for 10 min.

Notes:

1. *If RNA samples are not prepared right before the DNase I treatment, it is better to re-measure RNA concentrations.*
2. *Digestion time should not be more than 20 min. Higher temperatures and longer time could lead to Mg^{2+}-dependent hydrolysis of RNA. Additionally, it is vital that EDTA be added to at least 2 mM prior to heat-inactivation to avoid this problem.*
3. *RNA cleanup is strongly recommended right after DNase I digestion. If you could finish step 1 of RNA cleanup (add RLT and mix well) within 1 min, DNase I inactivation step is not necessary.*

C. RNA cleanup (Qiagen RNeasy Mini Kit)

1. Before RNA cleanup, make sure:
   a. There is no precipitate in RLT buffer.
   b. Buffer RPE is ready to use (EtOH added).
   c. All reagents and the centrifuge are at RT 20-25 °C.
   d. Enough columns, collection tubes are available.
2. Adjust the sample to 100 µl with RNase-free water. Add 350 µl Buffer RLT and mix well.
3. Add 250 µl EtOH, mix well by pipetting.
4. Transfer the sample to a RNeasy Mini spin column placed in a 2 ml collection tube. Close the lid gently and centrifuge for 15 sec at 10,000 *x g*. Discard the flow-through.
5. Add 500 µl buffer RPE to the column. Close the lid gently, and centrifuge for 15 sec at 10,000 *x g*. Discard the flow-through.
6. Add 500 µl buffer RPE to the column. Close the lid gently, and centrifuge for 2 min at 10,000 *x g*. Carefully remove the column from the collection tube so that the column does not contact the flow-through.
7. Place the column to a new collection tube (2 ml), close the lid gently and centrifuge for 1 min at 10,000 *x g*.
8. Place the column in a new collection tube (1.5 ml). Add 30 µl RNase-free water directly to

the spin column membrane. Close the lid gently and centrifuge for 1 min at 10,000 *x g* to elute the RNA.

9. Add the eluate from STEP 7 back to the spin column membrane. Close the lid gently and centrifuge for 1 min at 10,000 *x g* to elute the RNA.
   Note: Work quickly!

D. Sample preparation for microarray
   1. Measure RNA concentrations.
   2. Check whether RNA samples are degraded (Northern blot, check rRNA).
   3. Optional: Analyse one negative control (tube 1 etc) and one positive control using northern blot or real-time RT-PCR.
   4. Adjust RNA concentration to 1.0 µg/µl with RNase-free water. Prepare 10 µg total RNA for microarray analysis.

Notes

1. All steps need to be conducted rapidly to minimize chances of RNA degradation. All materials and laboratory space used in the experiment needs to be kept clean to ensure maximum likelihood of undegraded RNA.

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References

1. Manual for DNase I recombinant, RNase-free (Roche, Catalog number: 04716728001)
2. RNeasy Mini Handbook (p. 56-57)

