

Tips and tricks of the trade

Soft-Boiled Yeasts

Cell walls of the yeast *Saccharomyces cerevisiae* are rather hard and thick, whereas the RNA inside the cell is extremely sensitive. Thus, isolating RNA from yeast cells is like cracking open a walnut shell without destroying the nut.

Lab Hint

Most yeast labs rely on two different methods for RNA isolation: vortexing the cell suspension with glassbeads or extracting the RNA with acid-equilibrated phenol. Glass beads effectively disrupt the cells and high yields of RNA are obtained. Usually, the yeast suspension is pipetted into a small reaction vessel, some glass beads are added and the mixture is vortexed by hand. This protocol works fine with small sample numbers. If, however, you are dealing with hundreds of probes, e.g. in high-throughput applications, you get completely lost and after hours of extensive vortexing your arm is numb.

Extracting RNA with acid-equilibrated phenol is quite simple and delivers large amounts of RNA, however, working with the toxic phenol is not what most people in a lab dream about.

That's why Jin Li *et al.* from the Shandong University School of Medicine in Shandong, China sought an alternative method, devoid of glass beads and acid phenol. The group finally came up with a



A hot waterbath may be useful for canning but also for extraction of yeast RNA.

protocol, published in a recent 'Notes & Tips' paper in *Analytical Biochemistry* (Vol. 384 (2009) 189-190), which is so simple that it's staggering as to why nobody has tried it before. The yeast cells are simply incubated at 65 °C in RNA isolation buffer containing 10 mM EDTA, 50 mM Tris-HCl (pH

6.0) and 5% SDS. Accordingly, Li *et al.* call their new RNA isolation technique the "waterbath method".

To isolate RNA corresponding to Li *et al.*, log phase yeast cells (OD_{600} : 2.5, approx. 7.5×10^7 cells) are harvested by centrifugation and washed with 400 μ l of DEPC treated water. After washing, the cell suspension is centrifuged at 12,000 rpm for 2 minutes and the resulting cell pellet is re-suspended in 400 μ l isolation buffer and incubated at 65 °C for 5 minutes. The sample is rapidly cooled in ice/water and 200 μ l of 0.3 M KCl (pH 6.0) are added. The solution is mixed thoroughly and centrifuged at 12,000 rpm for 10 minutes at 4 °C. The supernatant is recovered and extracted with the same volume of phenol/chloroform/isoamylalcohol (25:24:1) and the mixture is centrifuged at 12,000 rpm for 5 minutes at 4 °C. In the next step, the supernatant is again recovered and precipitated by adding 0.1 volume of sodium acetate (pH 5.2) and 2.5 volumes of ethanol followed by incubation at -20 °C for 10 minutes. Finally, the extracted RNA is pelleted by centrifugation at 13,000 rpm for

10 minutes at 4 °C. Washing, drying and storing the RNA pellet is done as usual.

According to Li *et al.*, the whole procedure takes about one hour and yields 50 to 80 micrograms of total RNA from a 2.5 OD_{600} log phase culture. The isolated RNA may be used in downstream applications such as northern blots or RT-PCR. Though the Chinese group doesn't mention whether the quality of the RNA extracted with the waterbath method is also suitable for microarray experiments, it may be worth a try.

HARALD ZÄHRINGER

Do you have any useful tips?

Contact us at: editors@lab-times.org