Supplementary Material For:

Extraction of nucleic acids from yeast cells and plant tissues using ethanol as medium for sample preservation and cell disruption

Bettina Linke[1], Kersten Schröder[1], Juliane Arter[1], Tatiana Gasperazzo[1], Holger Woehlecke[2], and Rudolf Ehwald[1]
[1]Department of Biology, Cell Biology, Humboldt University, Berlin, Germany and [2]Dr. Lerche KG, Berlin, Germany

BioTechniques 49:655-657 (September 2010) doi 10.2144/000113476
Keywords: cell wall disruption; cell wall dehydration; ethanol; zeolite; in situ preservation of RNA; small RNA

Materials and methods

Biological materials

Compressed baker's yeast (*Saccharomyces cerevisiae*) was washed twice with a large excess of deionized water and recovered by centrifugation. The pellet was diluted with water to obtain a suspension containing ~8 mg dry weight and 2.7 × 10^8 cells in 100 µL. Plant material was obtained from *Arabidopsis thaliana* (rosette leaves, flowers, siliques), *Daucus carota* (leaves, flowers), *Perlagonium zonale* (leaves), and *Larix decidua* (young needles).

Light microscopy

Digital brightfield images were taken with a Leitz DMRB microscope (Leica Microsystems, Wetzlar, Germany) combined with the digital camera (SPOT Insight Firewire Color Mosaic; Diagnostic Instruments, Sterling Heights, MI, USA). Staining of nondisrupted cells was carried out by dilution of 10 µL cell suspension and/or cell homogenates in 50 µL solubilizing buffer (50 mM EDTA, 1.4% SDS) containing 0.2% methylene blue. Cells were counted on at least 40 fields of a Thoma chamber to determine the efficiency of disruption.

Fixation and dehydration of cells and tissues

One milliliter of 96% ethanol was added to 50–100 mg plant material or up to 100 µL yeast cell suspension in a 2-mL tube. After 30 min, the liquid medium was replaced by 1 mL absolute ethanol. An amount of 400 mg zeolite beads (MolSIV 3A AGS, diameter of 1.5 or 3 mm, water binding capacity 220 mg/g; UOP LLC, Des Plaines, IL, USA) was enclosed with the sample, and the tube was gently agitated for ~16 h. The zeolite beads (400 mg) were applied either unfixed or attached to strips that were provided by Dr. Lerche KG (Berlin, Germany).

Tissue and cell disruption

The ethanolic yeast suspension or the ethanol with the suspended plant material was aspirated or decanted from the zeolite beads. For subsequent disruption of yeast cells, a mixture of 200 mg glass beads with a diameter of 0.25–0.5 mm and 10 glass beads with a diameter of 2.5 mm (Carl-Roth GmbH, Karlsruhe, Germany) were added. For disruption of plant tissues, 200 mg cerium-stabilized zirconium oxide beads with a diameter of 0.4–0.6 mm and 10 cerium-stabilized zirconium oxide beads with a diameter of 2–2.4 mm (Mühlmeier GmbH & Co, Bärnau, Germany) were enclosed. The tubes were shaken in a horizontal position on a vortex mixer. A Vortex L46 (Labinco BV, Breda, The Netherlands) or a VortexGenie 2 (Scientific Industries, Bohemia, NY, USA) were used with suitable adapters at maximum speed. Disruption of yeast cells was almost complete after 15 min. For plant materials, a disruption time of 1 h was applied. The homogenate could be easily separated from the large grinding beads by aspiration.

Extraction

An aliquot (usually 100 or 200 µL) of the ethanolic homogenate was centrifuged, absolute ethanol was decanted, and the pellet was completely dried at room temperature. Extraction of the dry cells or cell debris with a defined volume (usually 100 µL) of the solubilizing buffer (50 mM Na-EDTA, pH 7.5, 1.4% SDS) was carried out on the vortex shaker at room temperature for 15 min (standard treatment) or by using longer periods if indicated. The volume ratio between the ethanolic homogenate and the solubilizing buffer could be increased up to 10-fold, if the dry weight concentration in the homogenate was low. A ratio of 1 was suitable when the homogenate contained debris of yeast cells with a dry weight concentration of approximately 5 mg/mL or of plant tissues with a dry weight concentration of approximately 15 mg/mL. To extract nucleic acids for purification of RNA by means of the guanidinium thiocyanate-phenol method (1), the homogenate pellet was solubilized in the sarcosyl buffer recommended for this method.

Quantitative analysis of DNA and total nucleic acids

The DNA quantitation kit of Sigma-Aldrich (St. Louis, MO, USA) was used according to the original procedure of the bisbenzimide method (2) with a Hoefer DQ 300 fluorimeter (Hoefer, San Francisco, CA, USA). Total nucleic acids in the yeast crude extract were estimated roughly by measuring the optical density at 260.

RNA quality

RNA isolated from *S. cerevisiae* was analyzed with an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and graded by RNA integrity number (RIN) (3).

Gel electrophoresis

Separation of DNA and RNA on denaturing and nondenaturing agarose gels was performed as previously described (4).

Purification of nucleic acids for PCR and RT-PCR analysis

DNA was purified from proteins, SDS, and enzymatically hydrolized RNA on size exclusion columns provided by Dr. Lerche KG according to the supplier's protocols and applied to PCR. As a control, DNA isolated according to the protocols of Qiagen (Hilden, Germany) was included. Purification of RNA from crude extracts containing SDS was performed by NucleoSpin-RNA-cleanup columns (Machery Nagel, Düren, Germany). The RNA obtained was immediately subjected to RT-PCR by using primers that cover intron regions. Alternatively, RNA was purified by means of the guanidinium thiocyanate-phenol method (1) with subsequent precipitation in isopropanol.

Restriction enzyme analysis and Southern hybridization

Between 3 and 10 µg purified DNA were treated with restriction enzymes (*Eco*RI, *Eco*RV, *Pvu*II) according to the supplier's

Supplementary Table S1. Influence of SDS concentration on the yield of nucleic acids from *S. cerevisiae*.

SDS concentration in the buffer (%)	Nucleic acids extracted within 15 min	
	DNA (mg/g dry weight)	Total nucleic acids (mg/g dry weight)
0.7	4.51 ± 1.28	75.6 ± 5.3 (1.69)
1.4	9.3 ± 0.64	96.9 ± 2.55 (1.56)
3	9.2 ± 0.15	104.4 ± 3.2 (1.54)

Cell disruption was carried out with a yeast dry weight of 7.7 mg in samples. Ethanolic homogenates were stored for 30 days at room temperature before extraction. Figures represent mean values ± SD of three parallel extractions. Complete disruption was proven by microscopy. Figures represent mean values of three independent extractions ± SD. Total nucleic acid were determined photometrically. The ratio E_{260}/E_{280} is given in brackets.

Supplementary Table S2. The influence of extraction time and storage time on the yield of DNA extracted from *S. cerevisiae*.

Extraction time at room temperature (h)	Extracted DNA (µg/mg dry weight)	
	Ethanolic homogenates stored at room temperature: 1 day (n = 1)	Ethanolic homogenates stored at room temperature: 9 days (n = 3)
0.25	8.07	6.79 ± 2.9
1.0	8.97	9.13 ± 0.25
5.0	8.86	8.64 ± 0.09
18	9.53	8.04 ± 0.07

Extracts were separated from solids after the given extraction time and set on ice before the fluorimetric DNA assay was carried out. Further details are in Supplementary Table S1.

recommendation (Fermentas, St. Leon-Rot, Germany). For Southern hybridization, 6 µg yeast DNA were digested with *Eco*RV and *Pvu*II, fractionated on a 0.8% agarose gel in 1× TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.3), and transferred onto positively charged nylon membrane Hybond N+ (GE Healthcare, Buckinghamshire, UK) by capillary transfer in 10× SSC (1.5 M NaCl, 0.15 M sodium citrate, pH 7.0). Probe labeling of the mitochondrial gene cytochrome c oxidase subunit II (*COXII*) with digoxigenin (DIG) was performed according to the manufacturer's instructions (DIG-PCR labeling kit; Roche Diagnostics, Penzberg, Germany). Hybridization was done with a probe concentration of 100 ng/mL at 42°C overnight, followed by stringent washing procedures with 0.1× SSC/0.1% SDS twice for 15 min at 42°C (Roche Diagnostics). Blocking procedures, incubation with anti-DIG antibodies, and detection by chemiluminescence using CDP-Star was done as described (Roche Diagnostics) and followed by exposure to Hyperfilm ECL (GE Healthcare) for 3–10 min.

PCR and RT-PCR analyses

The cycle number and temperature of the PCR were adjusted according to primer T_m and expected lengths of amplification products. A 127-bp fragment of a genomic sequence of *S. cerevisiae* (FM177899, 5.8S rRNA gene, *its1* and *its2*) covering the 3′ part of the gene encoding 5.8S rRNA and the transcribed *its2* spacer was amplified by using the primers 5.8S/ITS2-fw, 5′-GCCTGTTTGAGCGTCATTTC-3′ and 5.8S/ITS2-rev, 5′-CGCAGAGAAACCTCTCTTTGGA-3′. Genomic DNA fragments of 700 bp of *S. cerevisiae* actin (*act1*, V01288) were amplified using the primers ACT1-fw, 5′-CAATGGATTCTGGTATGTTCTAG-3′ and ACT1-rev, 5′-GATGGAAACGTAGAAGGCTGG-3′.

For Southern hybridization, a 320-bp fragment of the 5′ coding region of the mitochondrial cytochrome c oxidase subunit II gene (*coxII*, NC_001224) of *S. cerevisiae* was amplified using the primers CII-fw, 5′-GAATGATGTACCAACACCTTATGCATG-3′ and CII-rev, 5′-CCAATAGCTTTAATAGTTATAGCTGGTG-3′. Synthesis of cDNA was performed with Superscript II (Invitrogen, Karlsruhe, Germany) using oligo (dT) primers (3′-RACE primer; Invitrogen) according to the manufacturer's protocols. Genes encoding for *S. cerevisiae* actin (*act1*, V01288), for *D. carota* α-tubulin (*tba*, AAG02564), and for *A. thaliana* tubulin α-3/α-5 chain (*tua3*, NM_121982; *tua5*, NM_121983) were analyzed. RT-PCR was done with a template dilution of 1:10 for 26–28 cycles. The following primer pairs were used: ACT1-fw, 5′-CAATGGATTCTGAGGTTGCTGC-3′ and ACT1-rev, 5′-GATGGAAACGTAGAAGGCTGG-3′ to generate a 400-bp fragment of actin; TBA-fw, 5′-GTGCATTTGAGCCCTCTTCTATGATG-3′ and TBA-rev, 5′-CATAGAGAACAGCATAAAGAGTTGTTG-3′ for amplification of a 560-bp fragment of α-tubulin; TUA-fw 5′-TGGTTCTGGATTGGGTTCTC-3′ and TUA-rev 5′-ACAGCATGAAATGGATACGG-3′ for amplification of a 370-bp of *tua3/tua5*C. Control samples without reverse transcriptase were routinely conducted.

References

1. **Chomczynski, P. and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. *162*:156-159.
2. **Labarca, C. and K. Paigen.** 1980. A simple, rapid, and sensitive DNA assay procedure. Anal. Biochem. *102*:344-352.
3. **Schroeder, A., O. Mueller, S. Stocker, R. Salowsky, M. Leiber, M. Gassmann, S. Lightfoot, W. Menzel, et al.** 2006. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol. Biol. *7*:3.
4. **Sambrook, J., E.F. Fritsch, and T. Maniatis.** 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. CSH Laboratory Press, Cold Spring Harbor, New York.