

Gene expression analysis in yeast cells

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Method Article

Keywords: gene expression yeast

Posted Date: January 29th, 2019

DOI: <https://doi.org/10.1038/protex.2019.009>

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Abstract

Protocol for gene expression analysis using *Saccharomyces cerevisiae* cells

Reagents

-LETS buffer: 0.1 M LiCl, 0.01 M EDTA, 0.01 M Tris-HCl pH 7.4, 0.2% SDS - Phenol:Chloroform (5:1) - Chloroform:Isoamyl Alcohol (24:1) - Lithium Chloride 5M - Ethanol 70% - NaAc 0.3M - TURBO DNase (AM2238, Ambion) - EDTA 150 mM - OligodT - Improm-II® Reverse Transcriptase (M314A, PROMEGA) - RNasin® Ribonuclease Inhibitor (N2118, PROMEGA) - SYBR® Premix Ex Taq™ Tli RNase H Plus Green with ROX (RR420A, Takara).

Equipment

- FastPrep Precellys24 (Bertin technologies) - NanoDrop 2000 Spectrophotometer® (Thermo scientific). - DNA Engine Peltier Thermal Cycler (Bio Rad)

Procedure

Sample collection 1. Collect approximately 2×10^8 cells from exponential growing yeast cultures 2. Pellet cells by centrifugation at 4000xg at 4°C and wash once with cold RNase-free water 3. Cells can be stored at -20°C 4. Lysis and RNA extraction 4. Resuspend cells with 500 µL of LETS buffer 5. Add one volume of saturated phenol (pH 4.5) and 200 µL of glass beads 6. Broke cells in a FastPrep Precellys24 (Bertin technologies) 7. Centrifuge cells at 13400xg for 5 min at 4°C 8. Transfer the aqueous phase to a new eppendorf 9. Add one volume of phenol:chloroform (5:1) 10. Centrifuge cells at 13400g for 10 min at 4°C 11. Repeat steps 8 to 10 12. Transfer the aqueous phase to a new eppendorf 13. Add one volume of chloroform:isoamyl alcohol (24:1) 14. Precipitate the RNA overnight with one volume of 5 M lithium chloride at -80°C. 15. Centrifuge cells at 13400xg for 15 min at 4°C 16. Wash the precipitate was with 70% ethanol 17. Centrifuge cells at 13400xg for 15 min at 4°C to collect the RNA 18. Resuspend RNA pellet with 200 µL of RNase-free water 19. Re-precipitate RNA at -80°C for 3h adding Na Acetate 0.3M and two volumes of ethanol 20. Centrifuge cells at 13400g for 15 min at 4°C 21. Wash the precipitate was with 70% ethanol 22. Centrifuge cells at 13400xg for 15 min at 4°C to collect the RNA 23. Resuspend RNA pellet in 30-50 µL of RNase-free water 24. RNA was quantified by measuring the absorbance at 260 nm with NanoDrop 2000 Spectrophotometer® (Thermo scientific) 25. Load 1 µg of RNA in a 1% TAE agarose gel and run the electrophoresis 15 minutes at 120V to check the purified RNA and the sample integrity 26. cDNA synthesis 26. Incubate 5 µg of RNA with Turbo DNase (Ambion) for 1 hour at 37°C 27. Inactivate DNase by incubating the sample 10 minutes at 75°C in 15 mM EDTA 28. Incubate 1 µg of this RNA with 100 pmoles of oligo dT 5 minutes at 70°C 29. Transfer the sample to ice for 10 minutes 30. Incubated 5 min at 25°C, 60 min at 42°C and 5 min at 72°C with Improm-II® Reverse Transcriptase and Recombinant RNasin® (Promega) following the manufacturer instructions 31. Transfer the sample to ice 32. The

cDNA was analyzed by semiquantitative RT-PCR or by quantitative RT-PCR in a DNA Engine Peltier Thermal Cycler (Bio Rad) using the SYBR® Premix Ex Taq™ Tli RNase H Plus Green with ROX (Takara)