Yeast colony Northern: a fast method for detection of transcripts by colony hybridization

Piotr P. Stepień and Ronald A. Butow*
Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235 – 9038, USA

Submitted November 28, 1989

We have developed a rapid and simple method for screening yeast colonies in order to detect changes in the level of a specific transcript. Total RNA from yeast colonies is immobilized on Nylon filters and hybridized to a radiolabeled DNA probe. Colonies differing about five-fold in the abundance of a specific RNA can be easily recognized. We are using this method to clone a nuclear gene influencing the abundance of the excised 1.1 kb 28w55 intron of the mitochondrial 21S rRNA gene (1). We believe this method can be applied to the cloning of any gene affecting specific RNA abundance.

Colonies of yeast Saccharomyces cerevisiae are grown on Hybond-N nylon membranes or are deposited on the membrane by use of a replicator. All subsequent steps are performed at the room temperature unless otherwise indicated. Filters are layered on 500 μl of 1M sorbitol, 0.1M sodium citrate, pH 5.8, 0.01 EDTA, 50 mM mercaptoethanol and incubated for 10 min, blotted on 3MM filter paper and layered on 500 μl of 10 mg/ml solution of mureinase in the above buffer and incubated at 30°C for 1 hr. After incubation, filters are blotted and layered on 500 μl of 2% SDS, 7.3% formaldehyde, 50 mM Tris-HCl, pH 7.5 and 10 mM EDTA (lysis buffer). After 5 min filters are blotted and layered on the lysis buffer for an additional 5 min. The colony lysis can be performed at any temperature up to 60°C. Finally, filters are blotted, layered on 6 × SSC, 0.1% SDS for one min, blotted again and UV-irradiated for 3 min. Filters are prehybridized for 1 hr in 50% formamide, 5 × SSPE, 5 × Denhardts, 1% SDS, 100 μg/ml ssDNA and a DNA probe (40 × 10⁶ cpm), which is a 0.5 kb HindIII fragment of the ω intron (2), labeled by oligonucleotide priming. Hybridization is performed overnight at 43°C. The filters are then washed with 5 × SSPE, 0.1% SDS and then with 1 × SSPE, 0.1% SDS at 43°C for a total of 2 hrs and exposed to X-ray film for 4 hrs.

The experiment shown in Fig. 1 presents the gene dosage effect of the yeast nuclear mutation SUV3−1 (1) on accumulation of the excised intron. The SUV3−1 allele affects a number of posttranscriptional events in yeast mitochondria (1). Homozygous wild-type diploids, which accumulate relatively small amounts of the excised 28w55 RNA, show only a faint hybridization signal (Fig. 1a). However, diploids heterozygous for the SUV3−1 allele show a 5-fold increase in the signal (Fig. 1, b and c), while SUV3−1 homozygous diploids show about 90-fold stronger signal than the wild-type (Fig. 1, d and e). This technique allows screening of a large number of yeast colonies for differences in abundance of specific RNA transcripts in a very short time.

ACKNOWLEDGEMENTS

Supported by grants from the NIH and the Robert A. Welch Foundation.

REFERENCES


Figure 1. The autoradiogram presents five rows of colonies, grown on 1% yeast extract, 1% bactopeptone and 2% glucose, with identical mitochondrial genomes that contain the ω intron: a) homozygous wild-type diploids; b) heterozygous SUV3−1 diploids; c) homozygous SUV3−1 diploids. Colonies lacking the ω intron do not show any hybridization signal (data not shown).

* To whom correspondence should be addressed