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A rapid assay for quantitative detection of specific RNAs

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The detection of specific RNAs in a complex mixture of RNAs is typically accomplished by Northern blot (1). Here we report an alternative method for rapid and quantitative detection of RNA species, in which a radioactively-labeled oligonucleotide complementary to the RNA of interest is hybridized with the RNA in solution, and the resulting hybrid is resolved on a nondenaturing gel and detected by autoradiography. Because transfer to a membrane is not required and hybridization occurs in solution, our method is quicker and more quantitative than a Northern blot. Our application of this solution hybridization method to the small nuclear RNAs of the yeast *Saccharomyces cerevisiae* demonstrates its specificity, sensitivity and ability to resolve similar RNA species.

Figure 1A shows that ^{32}P-labeled *S.cerevisiae* U6 snRNA (2) is quantitatively bound by an oligonucleotide (U6D) complementary to its 3′ end after slowly cooling from 70°C. The protocol had no effect on the mobility of *Xenopus laevis* U1 snRNA, which was included in the hybridization mixture as a nonspecific RNA control. When ^{32}P-labeled U6D oligo was annealed to unlabeled yeast total cellular RNA and applied to a nondenaturing gel, a single major hybrid species was detected (Figure 1B). A band of identical mobility was seen when *in vitro* synthesized U6 snRNA was annealed to oligo U6D (data not shown), indicating that the U6D oligo is specific for U6 snRNA. The melting temperature (T_m) of the oligo U6D/U6 snRNA hybrid in hybridization buffer is 45°C. Incubation of the hybridization mixture at either 42°C or 37°C for 15 minutes instead of slowly cooling from 70°C gave efficient and specific hybrid formation, and was used in subsequent experiments. We have also used the solution hybridization method to detect U4 and U5 snRNAs (Figure 1C). Two U4 oligos shift the mobility of U4 snRNA (160 nt.) differently and bind with different efficiencies (Figure 1C, lanes 1 and 2). The two bands seen in lane 3 presumably correspond to the long (L; 214 nt.) and short (S; 179 nt.) forms of U5 snRNA (3).

Formation of hybrids at 37° to 42°C permits detection of the U4/U6 snRNA complex, which has a T_m of 53°C (4). In Figure 1D, deproteinized yeast splicing extract was incubated with oligo U6D at 37°C for 15 minutes and applied to a nondenaturing gel. Both free U6 snRNA and U4/U6 snRNA complex were detected. As expected, the U4/U6 snRNA complex could also be detected with an oligo complementary to U4 RNA (data not shown).

Our solution hybridization assay is sensitive and responds linearly to increasing amounts of RNA. One fmol of U6 snRNA (synthesized *in vitro* with T7 RNA polymerase) was easily detected (Figure 2A) and a plot of radioactivity in the hybrid band with 1 to 8 fmol of input U6 snRNA gave a straight line (correlation coefficient 0.994; Figure 2B). Therefore, quantitation of nonabundant RNAs can be accomplished with this method. From preliminary experiments we estimate that the average *S.cerevisiae* cell contains one to two thousand molecules of U6 snRNA.

To test if subtly different forms of RNA can be resolved by this method, we took advantage of a mutant yeast U6 snRNA gene, TATAbox-sub, which produces 5′-truncated forms of the 112-nucleotide U6 snRNA via initiation of RNA polymerase III at positions +1, +5, +7 and +12 (2). In the experiment shown in Figure 3, a glycerol gradient (8) was used to fractionate splicing extract made from a TATAbox-sub mutant strain, but similar results were obtained with total cellular RNA. All but the +5 and +7 species of U6 snRNA were clearly resolved from one another.

We conclude that solution hybridization combined with visualization of hybrids on a nondenaturing gel provides a rapid and sensitive method of detection of snRNAs, and we anticipate this method will be applicable to other RNAs as well.

Oligodeoxynucleotides used in this experiment:
U6D: 5′-AAAACGAAATAAATCTCTTTG-3′ (complementary to positions 92 to 112)
U4B: 5′-AGGTATTCCAAAAATTCCC-3′ (complementary to positions 140 to 158)
U4C: 5′-ACAATCTCGGACGAATCCTC-3′ (complementary to positions 32 to 51)
U4D: 5′-AGACGGTCTGGTTTA-3′ (complementary to positions 77 to 91)
U5B: 5′-AAGTTCCAAAAAATATGGCAAGC-3′ (complementary to positions 158 to 180)

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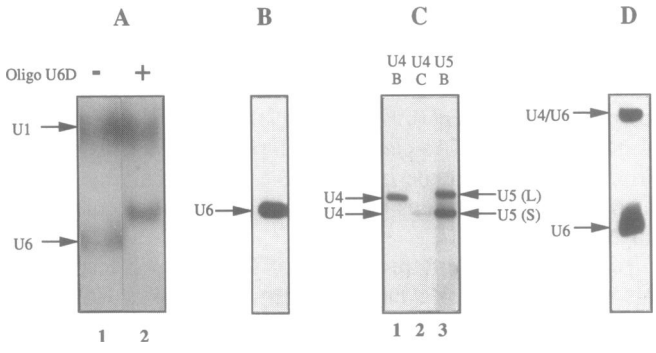

Figure 1. **(A)** Gel mobility shift of U6 snRNA bound to a complementary oligonucleotide. ^{32}P-labeled *S.cerevisiae* U6 snRNA (2) and *Xenopus laevis* U1 snRNA were combined with (lane 2) or without (lane 1) 100 pmol oligo U6D in 10 μl of hybridization buffer (150 mM NaCl, 50 mM Tris–HCl pH 7.4 and 1 mM EDTA) and heated at 70°C for 5 min, then slowly cooled to 9°C over a period of about 2 hrs. Four μl of loading buffer (15% glycerol, 0.15% Bromophenol blue, 0.15% xylene cyanol) was added prior to loading on a 40 cm tall, 0.4 mm thick, 9% polyacrylamide nondenaturing gel with 30:1 acrylamide to bisacrylamide (BDH Electran) in 50 mM Tris–borate pH 8.3 and 1 mM EDTA. The gel was run at 1000 volts for 5.5 hrs at 4°C. **(B)** Detection of U6 snRNA in yeast total cellular RNA with oligo U6D. Oligo U6D was end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (US Biochemicals) and was gel-purified (1). One-half μg of yeast total cellular RNA (2) was combined with 100 fmol U6D in 4 μl hybridization buffer, heated at 70°C for 1 min and slowly cooled down to 37°C over a period of about 1 hr. Two μl of loading buffer was added before loading a 10 cm tall, 1 mm thick, 9% polyacrylamide nondenaturing gel formulated as in (A). The gel was run at 300 volts for 1 hr at 4°C. Free oligo ran out of the gel and the U6 snRNA/oligo U6D hybrid migrated more slowly than the xylene cyanol tracking dye. **(C)** Detection of U4 and U5 snRNAs in yeast total cellular RNA with oligos complementary to U4 snRNA (U4B, U4C) or U5 snRNA (U5B). Five hundred fmol of each ^{32}P-labeled oligo was incubated with 2 μg RNA in 4 μl of hybridization buffer at 42°C for 15 min and 37°C for another 15 min. Hybrids were electrophoresed as in (B). **(D)** Detection of U6 snRNA and U4/U6 snRNA complex in yeast splicing extract with oligo U6D. Two μl of yeast splicing extract (5) was deproteinized (4) and the naked RNA was hybridized with 2 pmol ^{32}P-labeled oligo U6D at 42°C for 15 min. Hybrids were resolved as in (B).

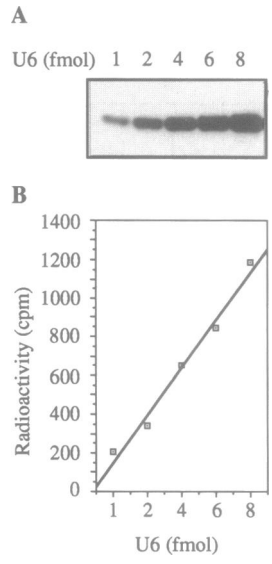

Figure 2. Sensitivity and linearity of the solution hybridization assay. **(A)** *S.cerevisiae* U6 snRNA was synthesized by transcription of the EcoRI/DraI fragment of pT7U6 (6) with T7 RNA polymerase in a large-scale reaction (7), to which a trace amount (1μCi/μmol) of [α-^{32}P]GTP was added to allow accurate quantitation of the transcript. After decay of the incorporated ^{32}P, the indicated amounts of gel-purified U6 snRNA were incubated in 4 μl of hybridization buffer with 200 fmol ^{32}P-labeled oligo U6D at 70°C for 3 min and at 42°C for 15 min. Electrophoresis was as in Figure 1B and the gel was exposed to Kodak XAR-5 film with a DuPont Lightning Plus intensifying screen for 24 hrs at −70°C. **(B)** The bands in panel A were excised from the gel and their radioactivity measured in a scintillation counter. A background value was subtracted from each before plotting.

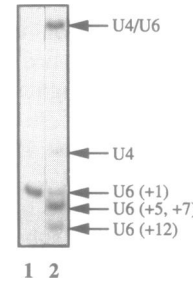

Figure 3. Resolution of variant forms of U6 snRNA in splicing extract from the TATAbox-sub mutant. Splicing extract was fractionated on a glycerol gradient and RNA extracted from a fraction in the U4/U5/U6 snRNP region (8) was hybridized with oligos U6D and U4D at 42°C for 30 minutes (lane 2). Wild type yeast total cellular RNA hybridized to U6D is in lane 1. Electrophoresis was as in Figure 1B. The assignment of positions of the 5′-truncated U6 snRNAs (2) was confirmed by primer extension (data not shown).