A simple method for isolation of intact RNA from dried polyacrylamide gels

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We have developed a simple, rapid method to isolate intact radioactively labelled RNA (either with $^{32}$P or $^{35}$S) from dried polyacrylamide gels on Whatman paper, thus allowing its further investigation even after several months (in the case of a $^{35}$S-label) of handling the gels without any protection, e.g. from RNases. The isolated RNA molecules are still biologically active, since we could demonstrate the catalytic activity of an isolated selfsplicing group II intron RNA from yeast mitochondria.

A $^{32}$P-radioactively labelled RNA of 1031 nt harbouring a selfsplicing intron was electrophoresed on two lanes on a denaturing 5% polyacrylamide gel. One of the lanes was dried on Whatman paper at 80°C under vacuum after electrophoresis, the second lane was used for the usual isolation from a wet gel as a control (Peattie, 1979). The position of the RNA in the gel was determined by autoradiography.

RNA isolation from dried gel

The piece containing the RNA was cut out of the dried gel and incubated for 5 minutes at 80°C in a reaction tube containing 400 μl elution buffer (0.5 M NH$_4$OAc, 0.1% SDS, 0.1 mM EDTA). The Whatman paper was carefully removed from the rehydrated gel slice with sterile forceps. In order to destroy any possible RNases on the gel slice, 40 μl phenol were added to the elution buffer and the solution mixed by vortexing for 1 minute. The elution assay was incubated at 4°C overnight, so that the RNA could diffuse out of the gel slice. The buffer containing the RNA was transferred into a new reaction tube. After extraction with 1 volume chloroform/isoamyl alcohol (24:1 vol.) to remove the phenol, the eluted RNA was concentrated by ethanol precipitation and redissolved in 20 μl water.

Control: isolation of RNA from wet gel

The gel slice containing the RNA was incubated overnight in 400 μl elution buffer at 4°C (Peattie, 1979). After the elution of the RNA was completed the gel slice was removed and the RNA precipitated and redissolved in 20 μl water.

To quantitate the efficiency of the isolation assay, we measured the radioactivity of the isolated RNA and compared it with the radioactivity remaining in the gel prior to elution. The table shows that the elution efficiency for an RNA of 1031 nt is about 70% compared with isolation from wet gels and even higher for shorter RNA molecules (data not shown). As can be seen in the figure, RNA isolated from dried gel is only somewhat more degraded than RNA isolated from wet gel. To test the biological activity, the isolated catalytic intron RNA was incubated under selfsplicing conditions (Schmelzer and Schweyen, 1986) and subsequently electrophoresed. The autoradiography shows that the RNA isolated from the dried gel is still able to catalyze the splicing reaction. Experiments with a $^{35}$S labelled version of the catalytic RNA, which was left in the gel for several months, yielded the same results with similar rates of recovery.

This clearly demonstrates that the RNA maintained its structural and functional integrity despite drying the gel at 80°C and long-term storage.

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REFERENCES


Table 1. Measured radioactivity

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<thead>
<tr>
<th>RNA isolated from:</th>
<th>wet gel</th>
<th>dried gel</th>
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<tbody>
<tr>
<td>gel slice (prior to elution)</td>
<td>128,790 cpm (100%)</td>
<td>134,825 cpm (100%)</td>
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<tr>
<td>isolated RNA</td>
<td>87,793 cpm (68%)</td>
<td>64,225 cpm (46.7%)</td>
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