Isolating *Haloferax volcanii* Mature tRNATrp-ProM From Total RNA Using the Whole Gel Eluter

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Introduction

This report describes the use of the whole gel eluter to effectively electroelute tRNA from a polyacrylamide gel. The tRNATrpΔ13115'-ProM hybrid RNA was expressed in vivo in *Haloferax volcanii* strain WFD11, and the mature RNA isolated from pre-tRNA and other cleavage components using the whole gel eluter. The mature tRNA, produced in vivo by both cleavage and ligation, was 182 nt in size. This isolated tRNA product was subsequently used in primer extension experiments in order to determine if nucleoside modifications can occur independently of mature tRNA structure.

Methods

Sample Preparation

Haloferax volcanii strain WFD11 was grown aerobically at 37°C in complex medium to an A_{560} of 1.0. Cells were pelleted by centrifugation. Total RNA was isolated from cells via Trizol reagent per manufacturer's directions (GIBCO BRL, Gaithersburg, MD). The resulting RNA pellet was resuspended in ~1 ml of RNA loading buffer (7 M urea, 10% glycerol, 0.05% Bromophenol Blue, 0.05% xylene cyanol).

Electrophoresis Conditions

Approximately 1 ml of sample was loaded on a single-well (15 x 1.5 cm), 6% polyacrylamide, 8 M urea, 1.5 mm thick preparative gel. Upper and lower buffer chambers contained 1x TBE, ~pH 8.3 (89 mM Tris-borate, 2 mM EDTA). The gel was run overnight at 30 V until the Bromophenol Blue marker was ~1 cm from the bottom of the gel (shorter (2 hr) runs can be used without sacrificing any resolution). The electrophoresis apparatus was disassembled and the gel placed between two pieces of Saran wrap to protect the gel from RNase contamination. The sandwiched gel was trimmed with a razor blade to the appropriate dimensions (14 x 16 cm) using the whole gel eluter template as a guide.

The whole gel eluter was cleaned by soaking the base plate, bottom electrode, elution chamber core, and upper plate/electrode in a 2% solution of Micro cleaning detergent. These parts were then rinsed with distilled water, wiped with 70% ethanol, and dried thoroughly before use.

The preparative RNA gel was placed in the elution chamber, and the whole gel eluter was assembled as described per manufacturer's instructions with the following exceptions: (1) TBE (0.5x) was used as the elution buffer, (2) the eluter was operated at 12 V for an elution time of 30 min, and (3) at the end of the elution period, the power was turned off and the electrical leads reversed. Power was applied for 30 sec to dislodge any RNA that was bound to the cellophane membrane.

Harvesting Fractions

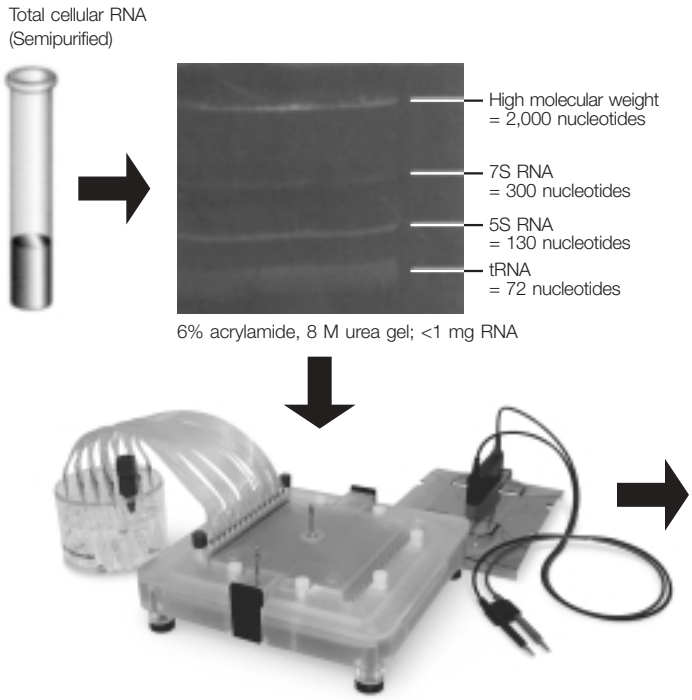
Plastic tubes (12 x 75 mm) were used in lieu of glass tubes for harvesting to minimize the potential loss of RNA due to binding of nucleic acids to the inner tube surfaces. Immediately after harvesting, the RNA fractions were transferred separately to 50 ml screwcap tubes containing 2.5 vol 95% ethanol, 1/10 volume 3 M NaOAc, pH 5.5 (9 ml 95% EtOH, 350 ml NaOAc). Samples were stored at –70ºC until needed.

Analysis

RNAs from each fraction were pelleted, dried, and resuspended individually in 30 µl of RNA loading buffer. The fractions and a size control of total RNA were analyzed as follows: (1) A 10-well, 8% polyacrylamide, 8 M urea gel (18 cm x 16 cm x 1.5 mm) was run at 200 V until the Bromophenol Blue marker was 1 cm from the bottom of the gel, (2) the gel was stained with a solution of 1x TBE containing 1 µg/ml of ethidium bromide, then visualized under ultraviolet light (Figure 1), (3) RNAs were transferred to Zeta-Probe® membranes for northern analysis, and probed with a radiolabeled primer complementary to the sequences in exon 2 (Figure 2).

Results

Northern blot analysis shows that the mature tRNATrpΔ 13115'-ProM was the only hybridizing product in fraction 14, although a small amount is detectable in fraction 13. Absolute single-species RNA was not required for this study, and Figure 1 shows very small amounts of other RNAs in fraction 14. This study, however, clearly demonstrated the separation of different species of the same RNA.



Discussion

The purification of intact RNA has been a challenge. Separation methods that require several steps subject RNA to the potential hazards of RNase degradation, and often result in poor yields. Other methods used to recover RNA (for example, the crush and soak method) can be time-consuming and labor-intensive. Approximately 50–60% of the RNA was recovered from the preparative gel. The whole gel eluter combines the advantage of the high resolution of polyacrylamide gel electrophoresis with a single-step purification method and provides more clean TAM in a shorter amount of time (30 min) than other available methods.

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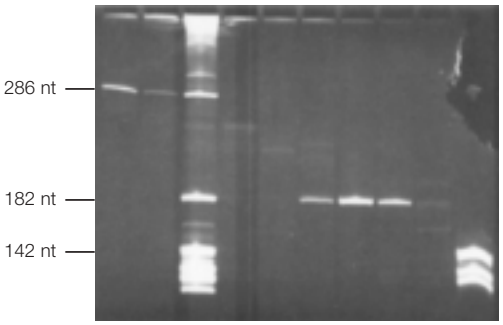

Fig. 1. Ethidium bromide-stained 8% acrylamide gel of whole gel eluter fractions. T = total RNA (starting material).

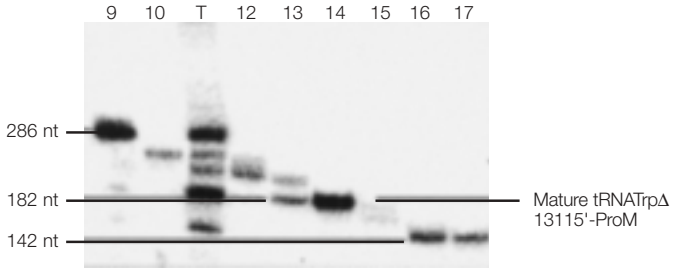

Fig. 2. Radiolabled northern blot of whole gel eluter fractions. T = total RNA prior to fractionation.

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