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# Denaturing agarose gels for large RNAs with glyoxal

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

PAGE gels work great for separating small RNAs under 500 nt or so, but cannot separate larger RNAs. This protocol uses glyoxal to denature RNAs, which can then be run on an agarose gel and will migrate according to their length rather than secondary structure etc. These gels work great and are an easier alternative to making formaldehyde gels when trying to separate large RNAs. Glyoxal reversibly reacts with guanine, adenine and cytidine bases in RNA preventing secondary structure formation. It is critical to maintain acidic pH (< 7.0), as the glyoxylation reaction is reversible in basic conditions.

The glyoxal reagent is NorthernMax loading dye from Ambion.
http://www.lifetechnologies.com/order/catalog/product/AM8551

There are two steps: pouring the agarose gel and glyoxylating the RNA to be loaded onto the gel. Note that the buffer for this is not TAE!  Further note that the loading dye already has EtBR in it so staining is unnecessary, and **do not add stain to the gel**.

References:
http://link.springer.com/protocol/10.1385%2F0-89603-127-6%3A1
http://cshprotocols.cshlp.org/content/2006/1/pdb.rec328
RNA: A Laboratory Manual by Rio, Ares, Hannon and Nilsen (CSHP)

## Materials

MATERIALS

ssRNA Ladder - 25 gel lanes **New England Biolabs Catalog #**N0362S

Agarose

Ambion NorthernMAX glyoxal loading dye **Thermo Fisher Scientific Catalog #**AM8551

10X BPTE buffer

- 100 mM PIPES
- 300 mM Bis-Tris
- 10 mM EDTA
- pH 6.5 (RT)

## Glyoxylate RNA

1 Mix 500ng to 1ug of RNA sample 1:1 with glyoxal loading dye in ~10 ul

2 Mix the required amount of ladder 1:1 with glyoxal loading dye

3 Incubate at 50 degrees for 30 minutes

Note

Note: proceed to pouring gel while glyoxylation reaction is occuring

4 Snap cool reaction by placing it on ice immediately from 50 degrees to minimize formation of secondary structure

## Pouring gel

5 Make 1 liter of 1X BPTE buffer (won't use this much but can save the rest).

6 Make a 1% agarose solution in BPTE and dissolve by microwave

Note

do not add stain to the gel! stain is in the glyoxal loading dye

7 Pour gel and let cool to RT

## Running gel

8 Load samples after 1-2 minutes on ice

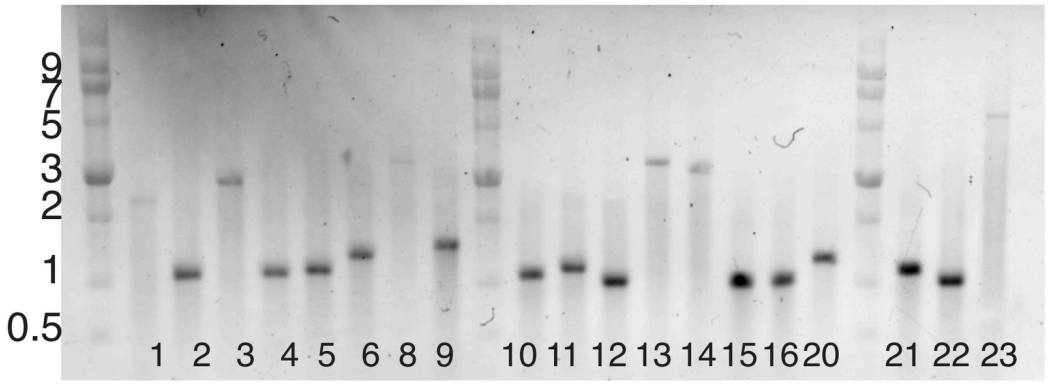
9 Run the gel at around 5V per cm between electrodes (typically ~80V). This usually takes 1-2 hours.

Note

[**CRITICAL**] Excessive heating may reverse glyoxylation and/or degrade RNA

10 Image gel using the EtBR channel

Expected result




Numbers on the left are the RNA ladder in kb. Numbers 1-23 on the bottom are different in vitro transcribed RNA samples. Three ladders are loaded. The background smear is normal and not a concern unless there is not a well-defined band at the correct size. Actual concentration of the product can be estimated using densitometry of the full-length band to normalize between samples.