Sep 25, 2020

Denaturing PAGE for resolving RNA

Forked from Denaturing RNA Urea-PAGE

Diep R Ganguly[1], Anna Behle[2]

[1]The Australian National University; [2]Institute for Synthetic Microbiology

1 Works for me dx.doi.org/10.17504/protocols.io.bj8rkrv6

Pogson Genomics Group

Diep Ganguly
The Australian National University

ABSTRACT

Protocol denaturing PAGE separation and identification of total RNA extracts using Urea-PAGE with the BioRad Mini-PROTEAN II / Tetra Cell electrophoresis system. Modified from dx.doi.org/10.17504/protocols.io.gszbwf6.

DOI

dx.doi.org/10.17504/protocols.io.bj8rkrv6

PROTOCOL CITATION

Diep R Ganguly, Anna Behle 2020. Denaturing PAGE for resolving RNA. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bj8rkrv6

FORK FROM

Forked from Denaturing RNA Urea-PAGE, Anna Behle

KEYWORDS

RNA, Electrophoresis, Denaturing-PAGE, PAGE

CREATED

Aug 24, 2020

LAST MODIFIED

Sep 25, 2020

PROTOCOL INTEGER ID

40945

GUIDELINES

Use filter tips and ensure you are using RNase free components and surfaces.

This is a fantastic resource to help planning and troubleshooting: RNA: A Laboratory Manual (https://www.cshlpress.com/default.tpl?cart=15988567819019717 30&fromlink=T&linkaction=full&linksortby=oop_title&--eqSKUdatarq=725).

Also see 10.3791/1485.

MATERIALS

NAME	CATALOG #	VENDOR
ssRNA Ladder - 25 gel lanes	N0362S	New England Biolabs
dsRNA Ladder - 25 gel lanes	N0363S	New England Biolabs
Low Range ssRNA Ladder - 25 gel lanes	N0364S	New England Biolabs
SYBR Gold	S-11494	Thermo Scientific
TEMED	1610801	Bio-rad Laboratories
Mini Protean tetra cell		Bio-rad Laboratories
SYBR Green		Thermo Fisher Scientific
Ethidium bromide [EB, EtBr]	EB0195.SIZE.1g	Bio Basic Inc.
DEPC Water	DD1005(D0121).SIZE.4x500ML	Bio Basic Inc.
Axygen 0.5 mL PCR tubes, 0.5 mL, thin wall, clear, flat caps	PCR-05-C	Corning
Thermal cycler		
RNaseZap® RNAse Decontamination Solution	AM9780	Life Technologies
Filter tips		
Depc (Diethyl Pyrocarbonate)	80730-888	VWR international Ltd
40% Acrylamide-bisacrylamide (19:1)		
30% Acrylamide / Bis. solution	161-0158	Bio-rad Laboratories
0.1% SDS solution		
10% Ammonium persulfate (APS)		Sigma Aldrich
Gel Loading Buffer II (Denaturing PAGE)	AM8546G	Thermo Fisher
TBE (10X), RNase-free	AM9865	Thermo Fisher
Mini-PROTEAN® Spacer Plates with 0.75 mm Integrated Spacers	1653310	BioRad Sciences
Mini-PROTEAN® Short Plates	1653308	BioRad Sciences

SAFETY WARNINGS

Wear vinyl or nitrile gloves when handling PAA (neurotoxic).
Make sure to thoroughly wash wells before loading samples.
2x RNA loading buffer contains formamide.

BEFORE STARTING

Thoroughly clean all components with 70% ethanol and RNase away (or 0.05 - 0.1 % SDS). Ensure RNA samples of interest have been normalized to a known concentration with nuclease-free/DEPC-treated water.

Gel preparation

1 Before preparing the gel, clean all components, gel tank, and surfances with 70 % ethanol (can use 0.1% SDS or RNase Zap to be extra thorough, we do this periodically).

2 Assemble gel plates in the green brackets and place on casting unit. Ensure glass plates are not cracked and are sitting flush against the separator plate and on the rubber mats (to prevent gel leakage).

3 Use the following table to prepare the required gel recipe using either 30 % *or* 40 % PAA (~4 mL=1x0.75 mm gel).

PAA percentage	8 %	10 %	12 %	15 %
30 % / 40 % PAA	2 / 2.67 mL	2.5 / 3.33 mL	3 / 4 mL	3.75 / 5 mL
8 M Urea	5 g	5 g	5 g	5 g
10x TBE	1 mL	1 mL	1 mL	1 mL
10 % APS	80 µL	80 µL	80 µL	80 µL
TEMED	10 µL	10 µL	10 µL	10 µL

Citation: Diep R Ganguly, Anna Behle (09/25/2020). Denaturing PAGE for resolving RNA. https://dx.doi.org/10.17504/protocols.io.bj8rkrv6

DEPC-treated H_2O	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL

Urea-PAGE recipe

4 Dissolve urea in 10x TBE, PAA, and 1/2 volume DEPC-treated H_2O.

Warming solution (e.g. 37˚C water bath) with gentle agitation will help to dissolve urea.

Wear vinyl or nitrile gloves and work in fumehood when handling PAA (neurotoxic!)

5 Once dissolved, allow to cool to room temperature and add DEPC-treated H_2O up to 10 mL.

6 Add 80 µL APS (10 %) and 10 µL TEMED to the gel solution and gently swirl once. Immediately pipette solution into gel apparatus (~4.5 mL, fill until just before it overflows to prevent bubbles when inserting comb). Retain some gel mixture to check for complete polymerisation.

7 Immediately insert comb tilted at an angle (to minimise trapping air bubbles). Make sure you are wearing appropriate gloves. Lower completely and allow gel to polymerise for >15 mins. Gels can be stored at 4˚C wrapped in plastic (use with 3-5 days).

RNA preparation

8 Determine desired loading amount of RNA per well (e.g. 0.5 - 2 µg RNA). Normalize RNA concentrations accordingly (e.g. 100 - 200 ng/µL).

9 Add required RNA to equal volume 2x gel loading buffer II (Ambion). Up to to 50% can be RNA sample although less is better. Final volumes for loading may vary, however, less than 5 µL results in poor banding when using the Mini protean system (10 µL / gel works well). Keep prepared samples @ 4 °C until ready for denaturation and loading.

RNA loading dye contains formamide. Wear goggles/lab coat/ gloves!

RNA loading

10 Once remaining gel solution has polymerised, remove from casting bracket and insert into running bracket (keep combs). Fill interior with 1x TBE and ensure there are no leaks (~5 mins). If leaking, re-adjust and check again. Place bracket into gel tank and fill with 1x TBE.

11 Remove combs and rinse individual wells thoroughly.

12 Connect electrodes, and pre-run gel @ 10 mA / gel for ~20-30 minutes to remove residual urea.

13 During the pre-run, denature RNA samples and ladder, in gel loading buffer II, at 72˚C for 5 min. Once finished, immediately keep at 4˚C until ready to load (minimise time kept at 4˚C).

Citation: Diep R Ganguly, Anna Behle (09/25/2020). Denaturing PAGE for resolving RNA. https://dx.doi.org/10.17504/protocols.io.bj8rkrv6

14 Turn power supply off and rinse the well a second time.

15 Pipette denatured samples into wells slowly while ensuring there are no air bubbles trapped in the pipette tip (Ambion recommends loading while samples are still hot).

16 Close the lid and plug electrodes into power supply. Separate RNA @ 10 mA / gel for ~1-2 hours (make sure you have a reliable power supply that holds current, particularly as urea has poor conductivity). Allowing the upper marker, from the Ambion loading buffer II, to run off the gel is a reasonable indictor for running time.

Visualization

17 After sufficient running time switch off power supply, pour out buffer, disassemble gel and clean components. Transfer gel to clean container and rinse once with deionized water.

18 Incubate gel in 0.5 µg ethidium bromide / mL 1x TBE for ~15 minutes (or use SYBR gold/green safe stains). Rinse 3x with water and allow to destain for ~2 minutes. Visualize.

Citation: Diep R Ganguly, Anna Behle (09/25/2020). Denaturing PAGE for resolving RNA. https://dx.doi.org/10.17504/protocols.io.bj8rkrv6