# Radioactive Northern Blot

**Anna Behle**

## Abstract

Protocol for Northern Blot using radioactive RNA probes.

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## Before start

Generate a PCR product with a T7 promoter for generating riboprobes. Make sure the riboprobe is antisense to the RNA of interest.

## Protocol

### Step 1.

Separate RNA by PAGE:

PROTOCOL

**. Denaturing RNA Urea-PAGE**

CONTACT: Anna Behle
Gel preparation
**Step 1.1.**

Before preparing the gel, clean all components with 70 % ethanol and RNase away.

Recipe for one small Hoefer gel (10 mL):

| PAA percentage | 5 % | 6 % | 8 % | 10 % | 12 % | 15 % |
|---|---|---|---|---|---|---|
| **40 % PAA (19:1)** | 1.25 mL | 1.5 mL | 2 mL | 2.5 mL | 3 mL | 3.74 mL |
| **Urea** | 5 g | 5 g | 5 g | 5 g | 5 g | 5 g |
| **10x TBE** | 1 mL | 1 mL | 1 mL | 1 mL | 1 mL | 1 mL |

| | | | | | | |
|---|---|---|---|---|---|---|
| **10 % APS** | 80 µL | 80 µL | 80 µL | 80 µL | 80 µL | 80 µL |
| **TEMED** | 10 µL | 10 µL | 10 µL | 10 µL | 10 µL | 10 µL |
| **ddH$_2$O** | ad 10 mL | ad 10 mL | ad 10 mL | ad 10 mL | ad 10 mL | ad 10 mL |

Gel preparation

**Step 1.2.**

Dissolve 5 g urea in PAA, 10x TBE and $H_2O$. This can be done at RT or at 40˚C. Agitate/shake occasionally.

SAFETY INFORMATION

**Wear vinyl gloves when handling PAA (neurotoxic!)**

Gel preparation

**Step 1.3.**

Let the liquid cool down before pouring gel.

Assemble gel caster. Take a 1 mL aliquot of gel mixture, add 20 µL APS and 1 µL TEMED and quickly pour to prevent gel from running out.

Gel preparation

**Step 1.4.**

Add 80 µL APS and 12 µL TEMED to the rest of the gel and quickly pour. Insert comb immediately and clasp tight to prevent leakage. Let polymerize for an hour or over night.

Gels can be stored for a week by wrapping with wet paper towels and Saran wrap.

RNA preparation

**Step 1.5.**

For Northern Blot Analysis, at least 2-3 µg of RNA should be loaded per well.

Adjust concentration and volume of all RNA samples by adding RNase-free ddH2O. Add desired amount of 2x or 5x RNA loading dye.

Denature RNA at 95˚C for 5 min. Snap cool on ice.

DURATION

00:05:00

SAFETY INFORMATION

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

RNA loading

**Step 1.6.**

Add running buffer (1x TBE). Remove comb. Before loading samples, wash all wells and each well individually just before loading with 1xTBE and a syringe or a pipette tip. Carefully pipet samples into wells.

**Step 1.7.**

Close the lid and plug electrodes into power supply.

Separate RNA at 20 mA/gel, 1 hour.

Visualization

**Step 1.8.**

Pour out buffer, then disassemble gel.

Incubate gel in 0.5x TBE + GelRed for 10 minutes, then visualize.

DURATION
00:10:00

Buffers needed

**Step 2.**

- Blotting Buffer: 0.5x TBE (50 mM Tris-HCl, 50 mM boric acid, 1 mM EDTA
- 5x Ribomax buffer: 400 mM Hepes-KOH pH 7.5, 60 mM $MgCl_2$, 10 mM spermidine, 200 mM DTT
- 20x Roti-Stock SSC buffer (Roth): 3 M NaCl, 300 mM sodium citrate, pH=7.0
- Hybridization buffer: 50% (v/v) formamide, deionized, 7 % (w/v) SDS, 120 mM Na-Phosphate buffer, pH=7.2 (Na2HPO4, NaH2PO4, 250 mM NaCl)
- Washing buffer I: 2x SSC, 1 % (w/v) SDS
- Washing buffer II: 1x SSC, 0.5 % (w/v) SDS
- Washing buffer III: 0.5x SSC, 0.1 % (w/v) SDS

Blot

**Step 3.**

Measure the size of the gel.

Cut a piece of nylon membrane, as well as 4-6 Whatman papers to the corresponding size.

Incubate in 0.5xTBE.

Blot

**Step 4.**

On the BioRad TransBlot semi-dry blotting system, assemble blotting sandwhich:

(-)Cathode

3 layers of Whatman paper

Nylon membrane

Gel

3 layers of Whatman paper

(+)Anode

Make sure to remove any air bubbles.

Blot

**Step 5.**

Blot at 2 mA/cm$^2$ for 45 minutes.

DURATION
00:45:00

Crosslinking

**Step 6.**

Crosslink transferred RNA with the membrane using UV-light. This can be done while documenting transfer.

In vitro transcription

**Step 7.**

PROTOCOL

**. Radioactive in vitro transcription**

CONTACT: Anna Behle

Buffers and reagents required

**Step 7.1.**

- 5x Ribomax buffer: 400 mM Hepes-KOH, pH 7.5; 60 mM $MgCl_2$; 10 mM spermidine; 200 mM DTT

Pipetting scheme

**Step 7.2.**

PCR Product containing the T7-Promoter (100 ng - 1 µg)

| | |
|---|---|
| Ribomax buffer | 6 µL |
| Pyrophosphatase | 1 µL |
| RNase inhibitor | 0.5 µL |
| DTT (1 M) | 2 µL |
| ATP 10 mM | 1.8 µL |
| CTP 10 mM | 1.8 µL |
| GTP 10 mM | 1.8 µL |

α-[$^{32}$P]-UTP 3 µL

T7 RNA-Polymerase 1 µL

$H_2O$ *ad* 30 µL

Incubate reaction for at least 2 hours at 37˚C.

DURATION
02:00:00

Clean-up

**Step 7.3.**

Remove excessive radioactive nucleotides by cleaning the probe using a G-50 column (GE-Healthcare).

Before start:

**Step 7.4.**

Generate a PCR product using primers containing the T7-promoter. Gel-extract or clean up using column purification.

**Step 8.**

While the probe is transcribed:

Prehybridize membrane at 42 ˚C for 1 hour in hybridization buffer.

DURATION
01:00:00

**Step 9.**

Add radioactive probe directly to the hybridization buffer. Make sure not to pipet onto membrane.

Hybridize for 6 hours or over night. Use calculated annealing temperature or 62˚C.

Screen prepara

**Step 10.**

Prepare phosphor screen by illuminating for at least 30 minutes. This erases previously obtained signals.

Stringency washes:

**Step 11.**

Wash membrane:

- 10 min in Washing buffer I (42 °C)
- 5 min in Washing buffer II (42 °C)
- A few seconds in Washing buffer III (RT)

Detection

**Step 12.**

Wrap membrane in transparent plastic foil to prevent leakage of radioactive material.

Place a phosphor plate/screen on top.

Incubate depending on signal strength.

5S rRNA: 30 minutes

Other RNA: An hour to over night

**Step 13.**

Detect signals using a phosphoimager (Fujifilm).

## Warnings

Always wear a lab coat, goggles and gloves when working with radioactive materials.

Limit exposure to radioactive materials.

Measure all materials with a Geiger counter.