Capillary Blotting of total RNA from agarose gels

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Abstract

This protocol can be used to transfer total RNA from thick formaldehyde/agarose gels to Nylon membranes for downstream Northern Blot Analysis. This is useful for analyzing larger RNA molecules such as mRNA.

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Guidelines

Always wear gloves to prevent degradation of RNA.

Protocol

Required buffers and components

Step 1.

- 20x Roti-Stock SSC buffer (Roth): 3 M NaCl, 300 mM sodium citrate, pH=7.0
- 2 layers of 1 mm Whatman paper (2x length of gel)
- 4 layers of 1 mm Whatman paper (1x length of gel)
- N+ Nylon membrane, size of gel
- Paper towels, cut into pieces roughly the size of the gel

Separation of total RNA

Step 2.

PROTOCOL

. Denaturing formaldehyde agarose gel-electrophoresis

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Buffers and reagents needed:

Step 2.1.

- 10x MEN buffer: 200 mM MOPS, 50 mM NaOAc, 10 mM EDTA, pH = 7.0
- Formaldehyde
- Ultra pure water
- Agarose

Gel preparation

Step 2.2.

Recipe for one 100 mL gel (e.g. PeqLab Mini L electrophoresis chamber):

Assemble gel chamber with suitable comb.

Heat 1.3 g in 85 mL ultra pure water. Let cool.

Under the hood, quickly add 10 mL 10x MEN buffer and 5 mL formaldehyde and pour gel.

RNA preparation

Step 2.3.

For Northern Blot Analysis, 1-3 µg of RNA should be loaded per well.

Adjust concentration and volume of all RNA samples by adding RNase-free ddH_2O. Add equal amounts of 2x RNA Loading Dye.

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

RNA loading and electrophoresis

Step 2.4.

Add 1x MEN to the chamber (running buffer). Make sure wells are covered in buffer.

Load RNA.

Separate RNA at 5-6 V/cm for at least 30 minutes or longer, if desi

Visualization

Step 2.5.

Document the gel using the BioRad ChemiDoc MP Imaging System.

Use either the GelRed or the Ethidium bromide protocol.

Equilibration

Step 3.

Equilibrate Whatman papers, membrane and gel in 10x SSC buffer.

Assembly of Blot

Step 4.

Use a horizontal gel chamber with a bridge (e.g. Peqlab). Fill with 10x SSC buffer, but don't cover the bridge.

On top of bridge, assemble in ascending order:

- 2 layers of Whatman paper, 2x length of gel, connecting the 10x SSC buffer bridge
- 2 layers of Whatman paper, 1x length of gel
- Agarose gel (wells down)
- Nylon membrane
- 2 layers of Whatman paper, 1x length of gel

Assembly of Blot

Step 5.

Stack as many paper towels as possible on top and press down with a glas weight (800 g).

RNA transfer

Step 6.

RNA transfer can be carried out over night or even over the weekend. Make sure to supply fresh 10x SSC buffer when required.

Crosslinking

Step 7.

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

Optional: Mark size standard with a pencil.

Standard Northern Blot analysis can be performed with membrane.

Warnings

Formaldehyde agarose gel should be kept under the fume hood. Always wear gloves.