

Northern blotting

RNA-Preparation

use your favorite method

Elektrophoresis

Gel (for 100ml):

1. mix together 1g Agarose and 84ml H₂O
2. heat in the microwave until boiling
3. let cool down to approx. 60°C
4. add 10 ml 10xMOPS and 6 ml 37% HCHO (hood !)
5. pour into tray and let cool (approx. 30-60')

Running buffer for elektrophoresistank:

1xMOPS

Samplepreparation (for total volume of 15µl):

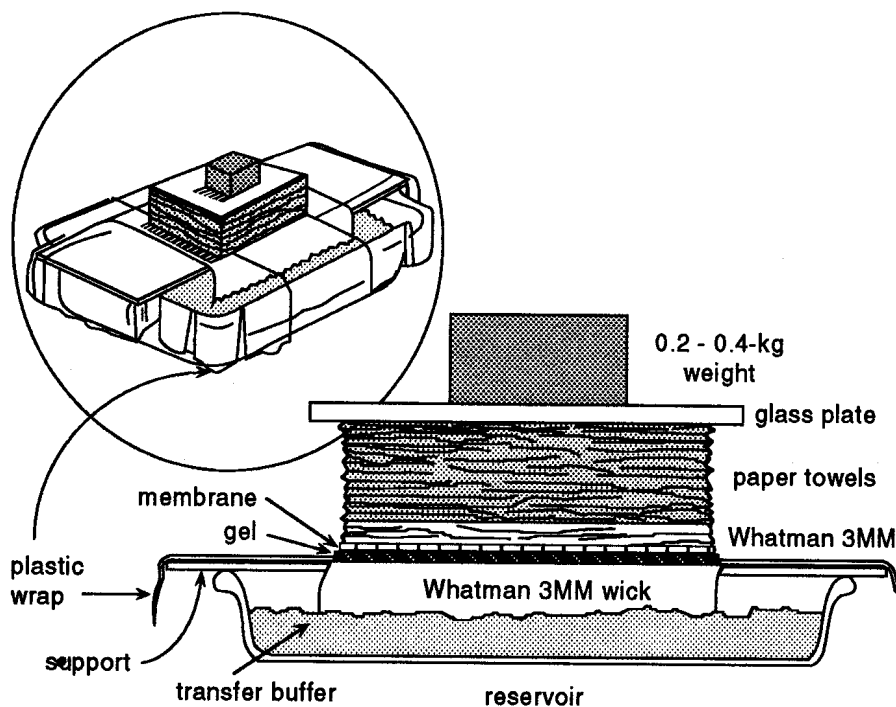
1. mix together 1,25µl 10xMOPS, 0,75µl 37% HCHO and 10,5 µl Formamide containing RNA
2. heat for 10' at 60°C
3. chill on ice
4. add 2,5µl Loading-Buffer
5. load on gel

Elektrophoresis:

approx. 3 to 4 hours at 60-70 V (not longer, otherwise buffer will change pH) or over night at 10-20 V

Blotting

Blotting in 20xSSC over night to nylonmembrane (Duralon-UV), see figure



Cross-linking

1. take membrane out of the blott
2. put membrane into the UV-crosslinker with the "gelside" (=RNA-side) up and crosslink ("autocrosslink")
3. pack membrane into plastic-foil and freeze at -80°C or
4. continue with prehybridisation (see protocol "Radioactive Labelling")

Buffers

H₂O:

always DEPC-H₂O (dest. H₂O + 0,1% DEPC, 37°C over night, autoclave)

10xMOPS:

0,4 M MOPS
0,5 M NaCH₃COO
0,01 M EDTA
pH 7,0

1xMOPS:

10xMOPS 1:10 diluted with H₂O

Loading-Buffer:

10 mM NaPO₄
0,25 % Bromphenolblue
0,25 % Xylencyanol
50 % Glycerol

20xSSC:

3 M NaCl
0,3 M Na-Citrat.2H₂O
pH 7,0