Northern Blot

Electrophoresis

- clean gel box with NaOH and/or SDS, 2 hours to overnight, rinse with water
- prepare **Agarose gel solution** [1 % Agarose, 1 x MOPS, H_2O to 95 % of endvolume]
- microwave until completely dissolved
- cool down to **60-70 °C**, add **Formaldehyde (37 %) to 0.6 M** endconcentration, pour immediately
- allow gel to harden at least 30 min
- prepare **running buffer** [1 x MOPS, 0.2 M Formaldehyde]

Sample preparation

- use **10-20 µg** total RNA per lane (up to 30 µg)
- bring RNA with H_2O_{DEPC} to equal volume (5-10 µl), add same vol. loading buffer
- add 0.5 µl EtBr (0.5 µg/µl)
- heat for **5 min @ 90 °C**, cool on ice

Northern transfer of RNA

Wash the formaldehyde gel 2X in sterile water 30min
Wash the gel in **10X SSC** 1h30min

Cut the nylon membrane as the same size of the gel

Cut 10-15 pieces of 3mm paper as the same size of the gel

Cut a 3mm paper “bridge”

set up capillary blot with **10 x SSC** transfer buffer:

2 wet Whatman - gel - membrane - 2 wet Whatman - 2 dry Whatman - papertowel - glasplate - weight transfer **16-24 h** with changes of the papertowel

mark lanes, remove membrane, wash briefly in **2 x SSC**

place membrane on wet Whatman paper and **UV-crosslink** damp (auto crosslink setting, 254 nm, Stratagene, Stratalinker)