

buffer (50mM Tris pH 6.8, 4% SDS, 0.2% bromophenol blue), boiled at 95°C for 5 minutes, and loaded onto the gel. Gels were fixed and immersed in running buffer (25 mM Tris-glycine/0.1% SDS) at a constant 200 V electric field, for which an electrophoresis constant power supply ECPS 3000/15 (Pharmacia) was used (here and for the other electrophoretic techniques). To visualize the migration pattern, the gel system was de-assembled and the SDS-PAGE rinsed with water before staining. A coomassie-based staining solution (0.5mg/ml coomassie brilliant blue R-250, 25% v/v isopropanol, 10% v/v acetic acid) was used to visualize proteins and/or 0.1% v/v toluidine-based staining solution to visualize RNA. Optimal staining took around 20 minutes. Stained gels were rinsed with water and immersed in a de-staining solution (60% v/v H₂O, 10% v/v ethanol, 30% v/v acetic acid) for about 3 hours. To keep record of the experiments, de-stained gels were rinsed with water and dried on Whatman paper. The drying system consisted of a gel- dryer (Zabona AG, Basel) connected to a cooling trap (UNICRYO MC 4L -80°C) and a vacuum pump (Vaccubrand GMBH).

3.1.2) Denaturing 8M urea polyacrilamide gel electrophoresis (urea-PAGE)

Preparative (20x40 cm) 8% urea-PAGE gels were used for the purification of large-scale *in-vitro* transcription reaction products. Gels were cast with a commercially available, ready-to-use ultrapure 8 % gel casting solution, SequaGel-8 (National Diagnostics). Glass plates, spacers and electrophoretic gel systems of appropriate dimensions, were built and provided in-house by the EMBL-Mechanical Workshop. Assembled urea-PAGE gels were fixed on a gel system, immersed in 0.5X TBE (0.45 M Tris pH 8.0, 0.45 M borate acid, 10 mM EDTA) buffer and pre-ran during 30 minutes. Electrophoretic migration was triggered with a constant 70 mA current, achieved with an electrophoresis constant power supply ECPS 3000/15 (Pharmacia). The temperature was kept between 50-60°C with a metallic cover leaf embracing most of the front glass plate. The RNA sample was mixed with a 1/10 volume of loading buffer (20% v/v glycerol, 10 mM EDTA, 1X TBE and trace amounts of xylen cyanol FF and bromophenol blue), heated for 15 minutes at 70°C to assure complete unfolding of all RNA moieties in the mixture, and slowly loaded onto the urea-PAGE. xylen cyanol FF and bromophenol blue are organic dyes that migrate on a urea-PAGE

as if they were an RNA moiety with a specific nucleotide length (table 7) (Fritsch *et al.*, 1989). This allowed a controlled monitoring of the sample migration.

TABLE 7

% Polyacrilamide/urea gel	Xylene cyanol FF (nucleotides)	Bromophenol blue (nucleotides)
4	155	30
6	110	25
8	75	20
10	55	10

Table taken from Fritsch *et al.*, 1989.

3.1.3) Native polyacrylamide gel electrophoresis (native-PAGE)

Native-PAGE gels were used to estimate the binding activity and integrity of RNA and protein aliquots. A 20x20 cm 5% native-PAGE gel was cast at 4°C, as follows:

31 ml distilled H₂O

5 ml 40% Acrylamide: N, N'- Methylenebisacrylamide 19:1 solution

4 ml 10x RNA running buffer (500 mM Tris-acetate, pH 7.5; 100 mM Mg-Acetate)

50 μ L N, N, N, N', N'- Tetramethylethylenediamine (TEMED)

250 μ L Amonium persulfate (APS) 10% w/v

Total volume 40 ml

As a first step, the distilled H₂O, the acrylamide solution and the 10X running buffer were mixed gently and degassed. Immediately before casting the gel, APS and TEMED were added. Assembled gels were pre-ran during 30 minutes at 4°C. The samples were loaded onto the gel and ran overnight or during 8-12 hours, at 4°C and 80 volts. Finally, the gel was de-assembled, gently rinsed with water and stained with 0.1% v/v toluidine. In keeping record of the experiments, native-PAGE gels were dried as described in section 3.1.1.

