Denaturing Urea Poly-Acrylamid Gel Electrophoresis (PAGE)

Aim of the Experiment

This experiment can be used to perform the analysis of RNA samples based on their size. The denaturating conditions help to disrupt the natural structure of the RNA, causing it to unfold into a linear chain.

Materials

- *dd* H_2O
- nuclease-free H_2O (nf H_2O, Sigma Aldrich, Germany)
- Urea (Carl Roth, Germany)
- 10x TBE buffer (Carl Roth, Germany)
- 40 % Acrylamide (Rotiphorese Gel 40 (29:1), Carl Roth, Germany)
- Tetramethylethylenediamine (TEMED, Carl Roth, Germany)
- 10 % Ammonium persulfate (APS, Thermo Fisher Scientific, Germany)
- 10 % Sodium dodecyl sulfate (Carl Roth, Germany)
- Gel chamber (Mini Protean System, Bio-Rad, USA)
- Electrophoresis power source (VWR, Germany)
- Novex gel cassette (Thermo Fisher Scientific, Germany)
- SybR Green II (Thermo Fisher Scientific, Germany)
- 2x RNA loading dye (NEB, Germany)
- ssRNA ladder (Thermo Fisher Scientific, Germany)
- UV detector (Peqlab, Germany)

Procedure

1. Mix 6.25 g urea (will account for 4,73mL once dissolved), 2 ml dd H_2O and 1.3 ml TBE buffer (10x) in a glass beaker.
2. Heat at 70 $^{\circ}C$ with magnetic agitation for about 10 min until the urea is fully dissolved.
3. Take out the gel cassette and 15 well comb.
4. Add 4.875 ml Acrylamide (for 15 % gel), 13 µl TEMED and 130 µl APS.
5. The resulting gel mixture of 13 ml contains 8M urea and 15% polyacrylamide. Adjust the polyacrylamide concentration accoring to the length of the analyzed RNA.
6. Pour immediately into the cassette and place the comb since the polymerization is very quick.
7. Leave it to polymerize for 30 min and then fix the cassette in the chamber.
8. Fill the chamber with 1 x TBE buffer,heat the chamber to 45 $^{\circ}C$ using a water bath and let the gel run for 30 min at 100 V to bring it to denaturing temperature before loading.
9. Dilute the RNA 1:10 (only in case of *in vitro* transcribed samples).
10. Mix each sample and the ssRNA ladder with loading dye and heat the samples at 95 $^{\circ}C$ for 10 min.
11. Put them directly on ice and load the samples into the gel.
12. Run the gel at 100 V for 1 hour 15 min .
13. Stain the gel with 10 µl SybR Green II for 10-20 min and take the image of the gel.