General remarks

Wear laboratory gloves to prevent RNase contamination and to protect hands from ethidiumbromide (potential carcinogen!) used for agarose gel electrophoresis.

Material

- Agarose, DNA grade
- TBE or TAE electrophoresis buffer: RNase-free, autoclaved; agarose gel and buffer contain 30 ng ethidiumbromide/ml
    - TBE: 90mM Tris, 90mM boric acid, 2mM EDTA, pH8.3
    - TAE: 40mM Tris, 1mM EDTA, pH8.0 adjusted with glacial acetic acid
- RNase-free gel loading buffer, e.g. 25% Ficoll 400 in TE (10mM Tris, 1mM EDTA, pH 7.5)
- Standard total RNA of known concentration and amount ratio of 28S/18S rRNA ~2
- UV photometer with quartz cuvette
- Electrophoresis apparatus: buffer chamber and tray should be cleaned thoroughly with detergent and subsequently rinsed with aqua dest. to get rid of RNase contamination.
- Nanodrop Spectrometer (Company, cat. no. ?)
- Agilent Bioanalyzer 2100 and electrophoresis set (Agilent, cat. no. G2938C and G2947CA)

Methods

A. Gel electrophoresis:

Analyse 1-3 µl (depending on the RNA concentration and size of the biopsy) total RNA of biopsy sample together with standard total RNA in a 1.2% agarose gel.

The amount ratio of 28S/18S rRNA in the sample RNA may be estimated by comparing the fluorescence signals of the 28S and 18S rRNA bands. The ratio should range between 1.5 and 2. Lower ratios indicate RNA degradation.

The amount of RNA in the sample can be estimated by comparing the fluorescence signals of the 28S and 18S rRNA bands from the standard RNA with those from the sample RNA.

The comparisons may be performed visually by an experienced person (though somewhat subjective) or by a fluorescence quantitation software tool provided with a gel documentation system.

B. UV photometer:

Prepare a series of dilutions of the RNA sample and measure the optical density (OD) at wavelengths of 260 nm and 280 nm using a quartz cuvette. Use RNase-free H_2O or, if TE buffer was used for dissolving and/or diluting the RNA, TE as reference. For exact measurement, the OD_{260} value of the RNA sample dilution should lie between 0.05 and 0.5.

An OD_{260nm} = 1 corresponds to an RNA concentration of ~40 µg/ml. Consider the dilution factor when calculating the concentration from the OD_{260nm} value.

The OD260/OD280 ratio should be between 1.8 and 2. A lower ratio indicates contamination with proteins.

Be aware that contamination with DNA or degradation of the RNA cannot be recognized with the photometer.

C. Nanodrop Spectrometer:

Allows quantitation of nucleic acids and proteins in very small volumes, e.g., 1 µl.

For optimal measurements, the RNA concentration should be between 50 ng/µl – 500 ng/µl.

The measurement is started with the settings for nucleic acid quantitation and RNA.

Use 1 µl H_2O or, if TE buffer was used for dissolving and/or diluting the RNA, TE as a reference.

Consider the dilution factor when calculating the concentration from the OD_{260nm} value.

Be aware that contamination with DNA and RNA degradation cannot be recognized with the Nanodrop Spectrometer.

D. Agilent Bioanalyzer 2100:

Aliquot RNA 6000 ladder and store at –80°C. Place aliquot on ice when in use. Store the kit reagents at 4°C. The gel matrix with the dye concentrate is good for 8 weeks and has to be protected from light in a dark Eppi.

Kit reagents: gel mtrix (red tube)
RNA dye concentrate (blue tube)
sample buffer

Gel preparation: 400 µl gel matrix
4 µl RNA dye concentrate

Vortex the substances and apply to the column, centrifuge 10 min at 3,000 rpm.

Chip preparation:

Apply 9 µl of the gel-dye mix at the bottom of the well marked G. Place the chip on the chip priming station and set the plunger at 1 ml. Press the plunger until it is held by the syringe clip. Wait 30 sec, lose the plunger and remove the chip. Apply 9 µl each to the two G-wells left.

Chip loading:

Denature RNA 6000 ladder and samples for 3 min at 70°C, then place on ice.

Apply 5 µl RNA sample buffer to wells 1-12 and ladder (rightmost position below).

In case, a well remains free, apply 6 µl RNA sample buffer.

Apply 1 µl RNA ladder to the ladder well. Apply 1 µl sample (e.g., 200 ng RNA) to the other wells.

Vortex chip for 1 min.

Electrophoresis run:

Start program Agilent Bioanalyzer 2100.

Apply 350 µl RNase Away to the electrode cleaner.

Open the lid and place the electrode cleaner in the Agilent 2100 bioanalyzer for 1 min.

Repeat with second electrode cleaner filled with 350 µl RNase-free H_2O.

Wait a few minutes, remove the electrode cleaner and briefly let dry the electrodes.

Chose assay “Eucaryotic total RNA (or mRNA) Nano (or Pico)”, insert the chip and start the program. Fill the table, confirm and save data.

After the run, remove the chip and clean the electrodes with the two electrode cleaners.

The amount ratio of 28S/18S rRNA in the sample RNA should range between 1.5 and 2. Lower ratios indicate RNA degradation.

Version	Tracking of changes	Name	Date