# RNA quality control

Outline

Any extracted RNA must be devoid of contaminants such as salt, protein, solvents and genomic DNA. Poor quality RNA will lead to problems when performing the reverse transcription and labelling and might affect data quality. We quality control our extracted RNA using gel electrophoresis and optical density measurements. Gel electrophoresis is used to check for Genomic DNA contamination and RNA decay. Optical density is used to assay the RNA yield and to check for contamination by salt, solvent, protein, *etc*.

Gel electrophoresis to check for genomic DNA:

Genomic DNA from *D. melanogaster* will be visible as a tight DNA band of high molecular weight. Whereas rRNA will be visible as two sharp bands half way down the gel the mRNA is the smear in the background. Presence or absence of genomic DNA is easy to detect. mRNA decay is inferred from fuzzy rRNA bands and the presence of low molecular weight smearing (from the rRNA band and below). The following is an example of good quality *D. melanogaster* RNA.

Gel electrophoresis of *D. melanogaster* RNA
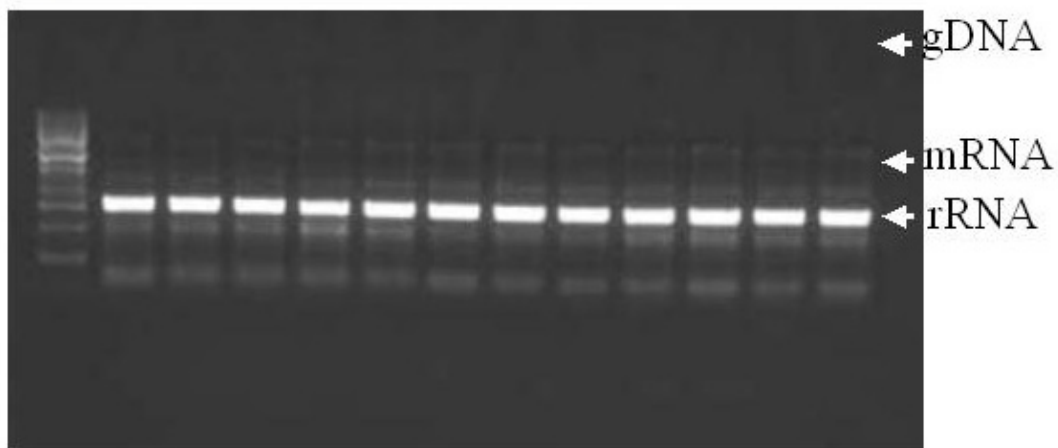
Protocol − ethidium bromide (Medium to large−scale RNA extractions)

1. Make a 1% agarose gel and add 5 µl ethidium bromide (10 mg / ml) per 100 ml of gel
2. Load 0.5 µl RNA extract with 4.5 µl water and 1 µl 6 x loading buffer
3. Run the gel at 80 V until the fastest dye has moved 2/3 of the gel length
4. Visualise the gel using a UV transilluminator and then take a digital photograph

Protocol − SYBR Gold (Small−scale RNA extractions)

When following this protocol please note that the size ladder should be diluted 1:20 − otherwise it will be too bright.

1. Make a 0.8% agarose gel, then load 0.5 µl RNA extract with 4.5 µl water and 1 µl 6 x loading buffer
2. Run the gel at 80 V until the fastest dye has moved 2/3 of the gel length
3. Add 30 µl SYBR Gold (Molecular Probes; Cat. No. S−11494) to 300 ml 1 x TAE (1:10,000 dilution)
4. Remove gel from the gel tank, immerse in SYBR Gold and leave to stain for 30 minutes (with an orbital shaker, in the dark)

5. Destain the gel by washing in 1 x TAE for 10 minutes (with an orbital shaker, in the dark)
6. Visualise the gel using a UV transilluminator and then take a digital photograph

Optical density measurements:

Optical density measurements are made using a Nanodrop ND−1000 spectrophotometer (http://www.nanodrop.com) and have been calibrated using dilutions of RNA type III from *S. cerevisiae* (Sigma; Cat. No. R−7125). This calibration showed that our linear range is within the reading range of 10 to 1000 ng/μl. Therefore, our samples are first diluted to fall within this range. Any measurement taken from outside of this range will be inaccurate.

Good quality RNA will have an OD 260/280 ratio of 1.8 to 2 and an OD 260/230 of 1.8 or greater. This is because nucleic acid is detected at 260 nm, whereas protein, salt and solvents are detected at 230 and 280 nm. A high OD 260/280 and OD 260/230 ratio therefore indicates that you have extracted RNA devoid of any of these contaminants. This is shown in the following graph that includes examples of good samples and samples with a poor OD 260/230 ratio.

Optical density measurements of *D. melanogaster* RNA
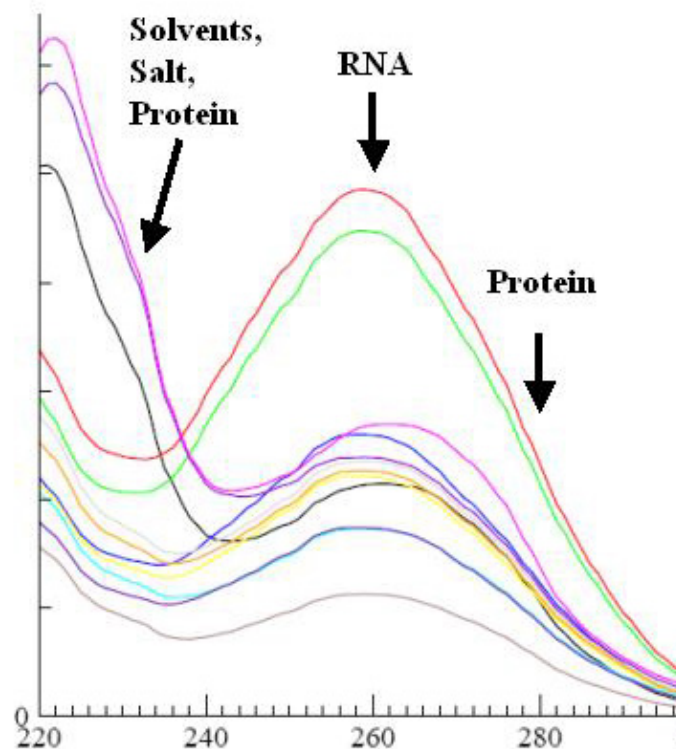
Protocol

1. Open the Nanodrop icon and select 'Nucleic Acid Measurements'.
2. Add 2 μl of the solvent the RNA has been dissolved in ("the solvent"); the instrument will then initialise.
3. After each and all subsequent measurements clean the pedestal by wiping with a dry lint−free tissue.
4. Add 2 μl of solvent and press 'Blank'
5. Repeat the blanking until there is a stable baseline, close to zero
6. Confirm that the baseline is correct by measuring 2 μl of solvent, as if it were your first sample by pressing 'Measure'
7. Add 2 μl of the first sample making sure to add the sample ID (or name) to the 'Sample ID' field and then press 'Measure'

8. Repeat step 3 and then 7 for all samples
9. Confirm that the baseline is correct after taking all measurements by measuring 2 μl of solvent, as if it were your last sample by pressing 'Measure'
10. Each of the measurements is automatically saved by the instrument and these can then be calibrated using the in house script