Quantifying RNA by spectral absorption

RNA can be quantified using absorption of light at 260 and 280 nm (A260/280). Ideally, this ratio should be close to 2 for high quality nucleic acid. However, for retinal RNA, we typically get values more like 1.8 and values as low as 1.6 still give useful real time RT PCR results (consistent with other samples).

Warm up the spectrometer with the UV light on for at least 15 min.

Add 0.6 ml of deI water to one of the matched quartz cuvettes (the ones which have a 4 mm width but a 1 cm pathlength). Put the cuvette in and go to 280 nm. Zero the instrument. Move to 260 nm and record the blank reading. Add 5 µl of RNA to the cuvette and mix briefly (this is a 1:125 dilution).

Read the absorption at 260nm and then at 280 nm. The A260/280 ratio is then given by

[A260 - A260blank] / A280

A crude way to calculate the concentration from the absorption at 260 nm is to use Beer's law : A = e C l

where :

A is the measured absorption at 260 nm
e is the RNA extinction coefficient (25 µl / µg / cm)
C the RNA concentration
l is the pathlength (1 cm)

From this C(meas) = A / (e l) = A * 40 µg/ml. However, this is the concentration of the diluted solution in the quartz cuvette. The actual RNA concentration C(sample)=C(meas) * dilution factor. In the above case the dilution factor is 125.

For example, if we measured A=0.08, then C(meas)=0.08 * 40 µg/ml = 3.2 µg/ml
and C(sample)=125*3.2 µg/ml = 400 µg/ml = 0.4 µg/ul.

In order to add 1 µg to an RT reaction requires 2.5 µl of this RNA solution.

A more precise way to get RNA concentration (and take account of protein absorption which is important when A260/A280 is much less than 2) is to use the method described in Glasel, Biotechniques 18: 62-63 (1995). I have an excel spreadsheet which does this calculation if anyone is interested.