Small RNA/miRNA samples for preparation of Illumina libraries

The CGR cannot guarantee good results from samples that do not meet the requirements set out in this document.

Maximising sample quality

Please be aware that many RNA extraction kits will only retain RNA fragments above a certain size, meaning that samples extracted using these products will contain no small RNA species. Some manufacturers do sell kits specifically designed to retain small RNA species, so please ensure that your extraction kit of choice is suitable for this application before submitting samples for small RNA library preparation. We do not recommend enriching your samples for small RNA species prior to library preparation.

To maximise quality, it is essential that your RNA samples:

- have been stored at -20/-80C and have not undergone multiple freeze-thaw cycles, which can affect RNA quality.
- have not been exposed to high temperatures or extremes of pH.
- have a 260:280 ratio and a 260:230 ratio of ≥1.8.
- do not contain insoluble material.
- are free from DNA contamination. On-column DNase treatment tends to be less effective than in-solution DNase treatment.
- have been eluted and stored in nuclease-free water or low TE buffer. Please do not use DEPC treated water, as this may interfere with enzymatic steps in the workflow.
- do not contain contaminating salts, metal ions, ethanol, phenol, polysaccharides or pigments.

Before extracting or treating your samples in any way, we recommend cleaning benches and equipment with an RNase decontamination solution and using fresh buffers/solutions that are free from RNase.

ERCC spike in RNAs should not be used when constructing small RNA libraries. The ERCC RNAs will not be properly represented in the small RNA libraries because they are outside of the size range of RNAs selected for these libraries.

Assessing the quality and quantity of samples prior to submission

As part of the sample submission process, we will ask you to provide quantification data for your samples. It is important that the RNA is quantified accurately – we would recommend a dye-based, RNA-specific method, such as Qubit or a gel-based method such as the Agilent Bioanalyzer.

NanoDrop readings alone are not sufficient for accurate quantification but can help with assessing the quality of the sample. Submitted samples should have 260:280 and 260:230 ratios ≥1.8. If your samples require clean-up, the cost of this will be added to your invoice.

Please provide a gel image of all samples to confirm RNA integrity. We recommend working with intact total RNA with RIN values ≥ 7 as starting material for depletion or enrichment. This value is calculated by the Agilent Bioanalyzer software, or similar instrument, for most types of RNA. However, for some species the software cannot compute the RIN value. In those cases, RNA integrity can be estimated by the sharpness of the rRNA bands and a value close to zero in the 200-1200 bp range.

Sample submission requirements

Please supply **500-1000 ng total RNA** (including the miRNA fraction) for each sample in **8 µl** of nuclease-free water or TE buffer. Although our library prep protocol states that it is sufficient to use as little as 200-1000 ng total RNA, in our experience the library yield is often too little for sequencing if we use less than 500 ng input RNA.

Please note that we cannot QC samples with concentrations below 0.5 ng/ul or a total quantity of RNA below 5 ng. Therefore, we will not be able to guarantee successful library preparation, but we will charge for any work carried out on such samples.

We request that samples submitted in tubes (only for projects of <24 samples) are clearly labelled in numerical order for ease of sample identification. Please underline any numbers that could be misread upside-down (e.g. 6/9, 16/91).

For projects that involve ≥24 samples, please submit the samples in a 96-well plate, arranged down the plate in a column-wise fashion (sample 1 = well A1, sample 2 = B1, sample 3 = C1, etc.). For projects with over 24 samples submitted in tubes, a fee will be charged to transfer them into plates and the costs will depend on the number of samples. Please pay careful attention to the sealing of 96-well plates prior to shipping: unfortunately, we do occasionally receive poorly sealed plates in which samples have leaked from their wells, leading to cross contamination.

If you are unable to meet the stated requirements for your library type, please contact us at CGR.Enquiries@liverpool.ac.uk and we will be happy to offer further advice.