Centre for Genomic Research

UNIVERSITY OF LIVERPOOL

Samples for preparation of PacBio Iso-Seq libraries

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

Maximising sample quality

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.
- have been eluted and stored in nuclease-free water. 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.

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 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

The concentrations required will depend on the type of RNA being submitted.

Sample type	Concentration	Volume	Optimal quantity
Total RNA	≥ 10 ng/µl	≤ 10 µl	400 ng
PolyA selected RNA	≥ 5 ng/µl	≤ 6 µl	≥ 50 ng
SMARTer amplified cDNA, which has been size selected and further amplified and is ready for PacBio library preparation	Fraction 1-2 Kb: ≥ 15 ng/µl Fraction 2-3 Kb: ≥ 30 ng/µl Fraction 3-6 Kb: ≥ 45 ng/µl Fraction > 6 Kb: ≥ 45 ng/µl	≤ 40 µl	Fraction 1-2 Kb: 500 ng Fraction 2-3 Kb: 1000 ng Fraction 3-6 Kb: 1500 ng Fraction > 6 Kb: 1500 ng

We request that 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).

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.