Submission of samples for preparation of PacBio IsoSeq 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

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).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41	Sample 49	Sample 57	Sample 65	Sample 73	Sample 81	Sample 89
B	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34	Sample 42	Sample 50	Sample 58	Sample 66	Sample 74	Sample 82	Sample 90
C	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35	Sample 43	Sample 51	Sample 59	Sample 67	Sample 75	Sample 83	Sample 91
D	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	Sample 44	Sample 52	Sample 60	Sample 68	Sample 76	Sample 84	Sample 92
E	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37	Sample 45	Sample 53	Sample 61	Sample 69	Sample 77	Sample 85	Sample 93
F	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38	Sample 46	Sample 54	Sample 62	Sample 70	Sample 78	Sample 86	Sample 94
G	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87	Empty
H	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80	Sample 88	Empty

Guidance on Sample Randomisation

To ensure reliable sequencing data, it is important to address batch effects as part of your experimental design. Batch effects are caused by processing samples in multiple batches and can introduce biases that can confound biological effects. Randomising sample layouts by distributing replicates and experimental groups across plates and positions within plates helps to mitigate batch effects. We do not provide a sample layout randomisation service, but we strongly recommend considering this when designing your sample layout, prior to submission. Effective randomisation approaches include:

- **Interleaving replicates**: Spread replicates across plates.
- **Balancing groups**: Represent all conditions on each plate.
- **Avoiding positional bias**: Randomly assign wells.

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