Submission of samples for preparation of DNA fragment 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 DNA samples:

- are double-stranded. Single-stranded DNA is not compatible with any of our library prep protocols.
- are submitted in nuclease-free water, TE buffer or low-TE buffer. Other sample buffers have not been tested and may affect the final library size or yield. If buffer exchange is required, this will incur additional costs.
- have been stored at 4°C (short term) or -20/-80°C (long term) and have not undergone multiple freeze-thaw cycles, which can affect DNA quality.
- have not been exposed to high temperatures or extremes of pH.
- have a 260:280 and a 260:230 ratio of ≥ 1.8.
- do not contain insoluble material.
- are free from RNA contamination.
- have not been exposed to intercalating fluorescent dyes or ultraviolet radiation.
- do not contain denaturants (such as guanidinium salts or phenol), divalent metal cations (such as Mg2+) or detergents (such as SDS or Triton-X100).
- do not contain ethanol.
- do not contain contamination from the original organism/tissue (haeme, humic acid, polyphenols, etc.).

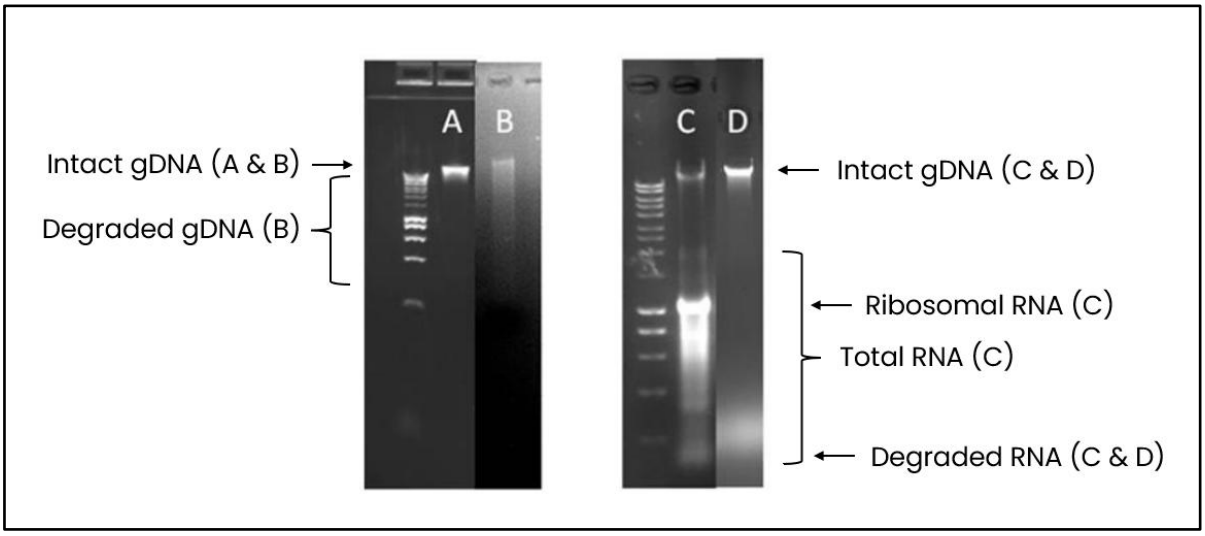
Assessing the quality and quantity of samples prior to submission

As part of the sample submission process, we will ask you to provide quantification data for your samples. It is important that the DNA is quantified accurately – we recommend a dye-based, dsDNA-specific method, such as Qubit. NanoDrop readings alone are not sufficient for accurate quantification but help with assessing the quality of the sample. For chromatin immunoprecipitation (ChIP) DNA samples, we recommend validating the success of the ChIP by qPCR prior to submission, to confirm increased levels of expected regions.

Concentration measurements by Qubit and NanoDrop should not differ significantly. A significant difference in those values may indicate that the sample contains single-stranded gDNA, RNA and/or other contaminating compounds (which may not be reflected in reduced NanoDrop 260:280 and 260:230 ratios). Unfortunately, we do not have an exact acceptable difference between the readings.

Please provide a gel image or trace of all submitted samples to confirm sample integrity, including the type of ladder and/or indication of fragment size(s). If there is more than one band or a smear, the sample may contain degraded gDNA, be contaminated with RNA, or contain a contaminant that could affect the library preparation.

The gel image below illustrates intact, degraded and RNA-contaminated genomic DNA, run alongside Hyperladder I (Bioline). If a smear exists in the range of 1-2000 bp, RNase treatment can be used to reveal whether it is RNA or degraded gDNA.



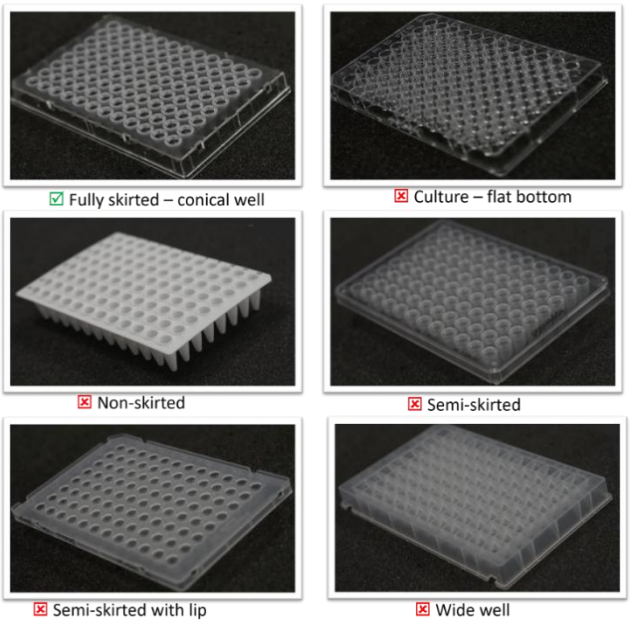
Sample submission requirements

The concentrations required will depend on the type of libraries being generated. The table below outlines the minimum input for each type of library.

Library type	DNA per sample	Volume per sample
Tru-Seq PCR-free DNA kit	≥1 µg	10-50 µl
Illumina DNA PCR-free kit (Tagmentation)	≥100-500 ng	10-30 µl
NEBNext Ultra II DNA kit	≥10-1500 ng	10-50 µl
NEBNext Ultra II FS DNA kit	≥10-500 ng	10-30 µl
NEBNext Ultra II FS DNA kit (1/2 volume protocol)	≥10-200 ng	10-30 µl
NEBNext Ultra II FS DNA kit (1/10 volume automated protocol)	≥100 ng	~10 µl
NEBNext Enzymatic Methyl-Seq kit	~300 ng	10-50 µl
SRSLY PicoPlus Uracil+	≥10-50ng	~10ul

Please note that we cannot QC samples with concentrations below 0.5 ng/µl or a total quantity of DNA below 5 ng. 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.

For samples that do not meet these requirements, we will not be able to guarantee successful library preparation, and we will have to charge for any work undertaken, regardless of the quality of the results.



For projects that involve ≥24 samples, we require samples to be submitted in a 96-well, fully-skirted plate. Please arrange your samples down the plate in a column-wise fashion, leaving 2 empty wells per plate so that we can add internal controls, as shown in the diagram below.

Sample position is very important for our workflows. If the submitted samples are not arranged as in the diagram below, you will be charged an additional £50 per plate to cover the cost of re-ordering the samples. It may also take longer for us to complete your project.

	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

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.

For projects involving <24 samples, submission in a 96-well plate is still recommended but we will also accept tubes. We require that samples submitted in tubes 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).

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 have any further questions, please contact us at CGR_Lab@liverpool.ac.uk .