Submission of externally prepared libraries for Illumina sequencing

The CGR cannot guarantee the performance of externally prepared libraries. We will carry out projects as described on the quote uploaded during the project submission process, and will charge for any work undertaken, regardless of the quality of results.

Maximising library quality

- Libraries should be free from contaminants. We recommend purifying libraries using columns or Agencourt AMPure XP beads and eluting in nuclease-free water.
- After purification, please run each library on an agarose gel or automated electrophoresis instrument (such as Bioanalyzer, TapeStation or Fragment Analyzer) to evaluate quality. Please attach a copy of the gel image or trace on the order form, including information about the ladder or size markers used. If available, please provide Bioanalyzer traces of the (pooled) libraries and calculate the average fragment size for the entire range of fragments using the Bioanalyzer software.
- Library quantification should be performed using a dye-based method such as Qubit, rather than a spectrophotometric method such as NanoDrop.
- Libraries should be provided free from traces of primer and/or adapter dimers.

Library submission requirements

The concentrations and volumes required will depend on the type of platform and run configuration to be used for your project.

Sequencing platform and chemistry	Total number of lanes/runs required per pool of libraries	Required amount of library *
NovaSeq X Plus 1.5B	1	≥ 2.5 nM in a volume of ≥ 20 µl
	2 (full flow cell)	≥ 5 nM in a volume of ≥ 20 µl
NovaSeq X Plus 10B	1	≥ 2.5 nM in a volume of ≥ 20 µl
	2	≥ 5 nM in a volume of ≥ 20 µl
	3	≥ 7.5 nM in a volume of ≥ 20 µl
	4	≥ 10 nM in a volume of ≥ 20 µl
	5	≥ 10 nM in a volume of ≥ 30 µl
	6	≥ 10 nM in a volume of ≥ 30 µl
	7	≥ 10 nM in a volume of ≥ 40 µl
	8 (full flow cell)	≥ 10 nM in a volume of ≥ 40 µl
NovaSeq X Plus 25B	1	≥ 3 nM in a volume of ≥ 30 µl
	2	≥ 6 nM in a volume of ≥ 30 µl

	3	≥ 9 nM in a volume of ≥ 30 µl
	4	≥ 12 nM in a volume of ≥ 30 µl
	5	≥ 15 nM in a volume of ≥ 30 µl
	6	≥ 15 nM in a volume of ≥ 30 µl
	7	≥ 15 nM in a volume of ≥ 40 µl
	8 (full flow cell)	≥ 15 nM in a volume of ≥ 40 µl
NovaSeq 6000 SP or S1	1	≥ 2.5 nM in a volume of ≥ 20 µl
	2 (full flow cell)	≥ 5 nM in a volume of ≥ 60 µl
NovaSeq 6000 S2	1	≥ 2.5 nM in a volume of ≥ 20 µl
	2 (full flow cell)	≥ 5 nM in a volume of ≥ 80 µl
NovaSeq 6000 S4	1	≥ 3 nM in a volume of ≥ 20 µl
	2	≥ 3 nM in a volume of ≥ 40 µl
	3	≥ 3 nM in a volume of ≥ 60 µl
	4 (full flow cell)	≥ 10 nM in a volume of ≥ 100 µl
MiSeq	1	≥ 2.5 nM in a volume of ≥ 20 µl

* These quantities are expected to include sufficient material for library QC and qPCR. In order to convert concentrations from ng/µl to nM, use the following equation: nM = (Concentration in ng/µl x 1,000,000) / (Average size of library in base pairs x 660).

Please supply each sample in a tube labelled with the sample number and/or name exactly as given on the online order form. If more than one tube is provided, please label them 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_Lab@liverpool.ac.uk and we will be happy to offer further advice.