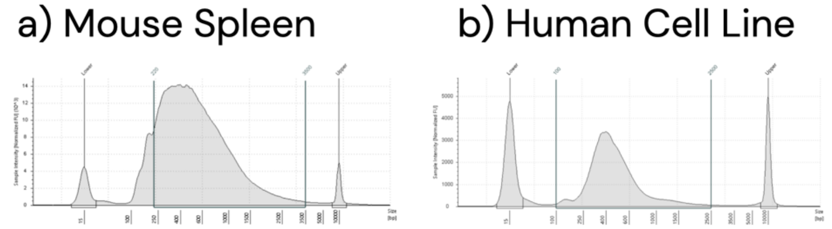
QC Information for the single cell Xpress-seq Service

We here provide QC information for your submitted Xpress-seq plates. QC is performed at the final sequencing ready library stage (ie. no intermediary QC of full-length cDNA on the single cell level).

Every submitted 384-well plate of Xpress-seq generates a single QC trace using the Agilent TapeStation platform.

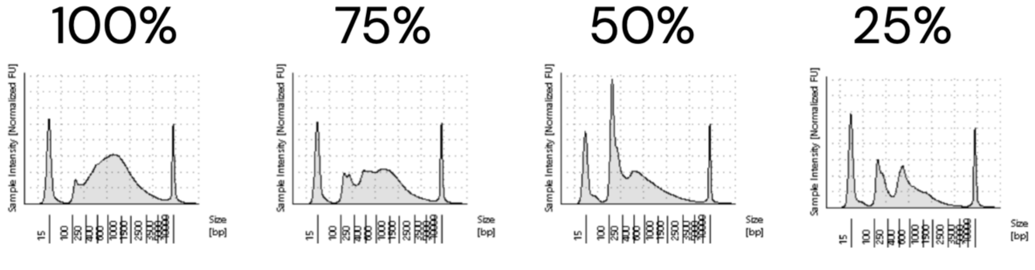
The expected results are a uniform library distribution with DNA fragments sized between approx. 200-1,500 base-pairs centered on an average of around 500-800 base-pairs (see Fig. 1a,b).


a) Mouse Spleen

b) Human Cell Line

Figure 1: QC profiles of successful libraries

If significant deviation from the expected peak and size distribution occur it may indicate low quality libraries that could lead to decreased data performance. Lowered performance may include (a) decreased sequencing yield (less reads/cell), (b) decreased read mapping ratios, (c) fewer wells with QC-passing single cells or (d) degraded RNA. For examples of suboptimal library profiles, please refer to the following examples where we intentionally filled only 25%, 50%, 75% or 100% of wells of 384-well plates and performed Xpress-seq (Fig. 2).


100%

75%

50%

25%

Figure 2: Influence of sorting success rate on library QC.

If you receive a notification about your ongoing Xpress-eq library QC, it will be because it has been deemed to deviate too much from expectation, indicating potential performance limitation, as described above. As the customer, you will have the following three options to proceed with your Xpress-seq service order:

1. Proceed with submitted plates as planned. Having considered our potential concerns, you chose to sequence as specified at the time of ordering.
2. Perform a low-throughput QC sequencing run according to the specification listed below on all or specified plates. This QC sequencing can be useful in determining the number of high-quality cells per plate and to inform which plates to retain for deeper sequencing in case the TapeStation-based QC analysis is inconclusive. The list price cost for whole flow cell sequencing on DNBSEQ G99RS (PE150) is 8,000 SEK.
3. Exclude specific or all plates from sequencing and terminate their processing their further processing after library QC.

   For non-sequenced plates, a cost of 4,000 SEK / 400 EUR / 400 USD depending on the currency of your quote is charged for handling instead of the quoted per-plate price incl. sequencing.

Sequencing Type	Sequencer Type	Read Length	Approximate Yield
Whole flow cell sequencing	DNBSEQ G99RS	PE150	80M reads