UNIVERSITY OF LIVERPOOL | CENTRE FOR GENOMIC RESEARCH

DNA sample requirements for preparation of Chromium 10X DNA libraries

The Chromium Genome Protocols generate long-range information across the length of individual DNA molecules. Starting the process with High Molecular Weight (HMW) genomic DNA (gDNA) will typically result in better application performance, such as increased haplotype phase block length and ability to call structural variants. Optimal performance has been characterized on input gDNA with a mean length >50 Kb.

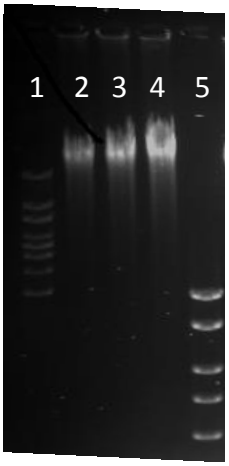
DNA extraction methods can be found here:
10x DNA extraction from whole blood
10x DNA extraction from fresh frozen tissue
10x DNA extraction from single insects

In our experience, size selection by use of the Blue Pippin instrument is required for most samples. Therefore, please submit a **minimum of 400 ng gDNA at a concentration of ≥15 ng/µl**.

The size of the gDNA can be assessed by one of the methods described in the document, which can be downloaded from this link 10x HMW DNA QC

Alternatively, to assess the true size of gDNA, run an aliquot of the sample on a 0.5% agarose gel overnight at 30-35 V for 17-18 hours. The ladder on the gel needs to have a marker of >40 Kb (as examples, please use the GeneRuler High Range DNA Ladder [10,171 to 48,502 bp] from Thermo or the 1 Kb DNA Extension ladder from Life Technologies).

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



0.5% agarose gel run at 30V for 15 hours

1. GeneRuler High Range DNA Ladder (10,171 to 48,502 bp)
2. Sample (488 ng)
3. Sample (632 ng)
4. Sample (976 ng)
5. HyperLadder I (200 to 10,000 bp)

Sample labelling: If possible, please label the samples and tubes 1, 2, 3 etc. in order to ease our sample identification. Please remember to underscore numbers that can be read upside-down such as 6, 9, 16, 91, 69, 96 etc.