# DNA extraction and quality check

The length of the enriched target DNA will depend on the length of the input DNA. Low DNA integrity may negatively affect the enrichment in two ways as the DNA fragment length directly correlates to the length of the resulting area of sequencing coverage and reversely correlates with the number of off-target fragments per droplet. More off-target DNA fragments in the droplets would result in a lower enrichment as more off-target DNA would be sorted with each correctly sorted on-target fragment. Therefore, we recommend that the Xdrop™ system is run with **high molecular weight DNA as input**, possibly above 60 kb (see Fig. 1)

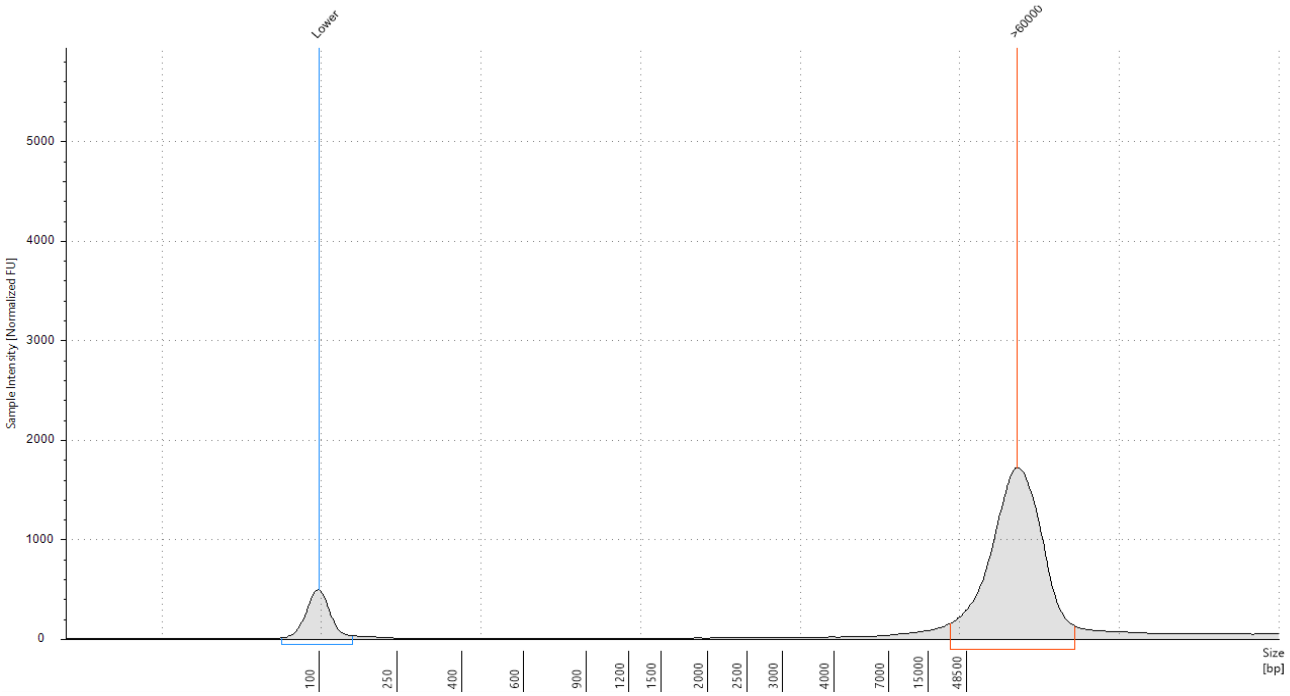


**Figure 1.** Example of TapeStation measurement of HMW DNA > 60 kb.

Note: when the input DNA amount available is above 200 ng we recommend a **bead-purification** before starting with the Xdrop workflow. Any bead-based purification kit is suitable, e.g. MAGBIO HighPrep™ PCR cleanup or AMPure XP cleanup kits. Make sure to follow manufacturer's recommendations. We recommend performing the final elution at 55 ºC for 3 minutes to ensure that HMW DNA is released from the beads.

The Xdrop™ enrichment technology can be affected by contamination of the DNA sample by RNA, proteins, carbohydrates, salt and phenol among others. To ensure high Xdrop™ performance we recommend a high purity HMW DNA with the following absorbance ratios: **A260/280 ~ 1.8 -2.0** and **A260/230 ~ 2.0-2.2.**

**Note: We recommend eluting extracted DNA into Molecular Grade Water, free of DNase, Protease and RNase.** e.g. Invitrogen™ UltraPure™ DNase/RNase-Free Distilled (Catalog number: 10977035)

In the following table we show some of the extraction methods successfully implemented for the Xdrop™ workflow.

| Organism | Tissue Type | Extraction method |
|---|---|---|
| **Human** | Blood | Circulomics Nanobind |
| **Human** | Blood | Salt extraction |
| **Plant** | Leaves | DNeasy Plant kit |
| **Plant** | Leaves | Circulomics Nanobind |

However, as a general recommendation, we suggest purifying the DNA to the same quality as required for long read sequencing. In the following table you find the links to the different long-reads sequencing platforms for HMW DNA extraction recommendations.

| Platform | Guidelines | Link |
|---|---|---|
| **PacBio** | Comprehensive guidelines for most sample types | Technical note |
| **Oxford Nanopore** | Comparative Guidelines for: Animal, Plant, Bacteria, Yeast | Nanopore Community* |
| **10x Genomics** | Specific Guidelines for: Blood, Cells, Fresh Frozen Tissue | Links to Recommendations |

*to access Oxford Nanopore recommendations, you need to log in into their community (free of charge). Recommended extraction methods are found under Knowledge > Extraction Methods.

Apart from the recommendations provided in the technical note, **PacBio** facilitates a more extensive collection of publications describing extraction protocols for high-molecular weight DNA followed by PacBio sequencing at this website, including non-model organisms.

## Plant DNA extraction recommendations

Plant HMW DNA extraction might be particularly challenging. Here you can find a comprehensive list of protocols suggested for long read sequencing by PacBio, 10x Genomics and Oxford Nanopore Technologies (ONT) specifically focusing on HMW plant DNA extraction methods.

**PacBio** generally recommends the following plant HMW DNA purification:

- Qiagen Genomic Tip 20/100/500/G kit
- Circulomics Nanobind Plant Kit
- CTAB/Phenol extraction

Other protocols have been successfully combined with PacBio sequencing for the following species

- Sunflower leaf
- Melon leaf
- Switchgrass
- Forage, crop, horticultural and common model species
- Leaves or cambium from 8 genera of plants and mycelium

**10X Genomics** provides a list of protocols for HMW DNA isolation here.

**ONT** Community also links to tested protocols for plant leaves extraction methods (Log in into Nanopore Community, then select Knowledge > Extraction Methods > Plant Leaves). For example, this is an article recommending a combination of CTAB, Qiagen Genomic Tip protocol, including an Amicon buffer exchange and using the Short Read Eliminator kit from Circulomics.