

Best Practices: Preparation of High Molecular Weight DNA

High-quality high molecular weight (HMW; ≥ 50 kb) genomic DNA is critical to achieving long read lengths on platforms such as 10x Genomics® Chromium™ and PacBio® Sequel®. Since HMW DNA is extremely susceptible to shearing, which may adversely affect data quality, please consider the following guidelines when extracting and handling for submission to GENEWIZ.

Handling HMW DNA

- Whenever pipetting HMW DNA, use wide-bore tips. Be sure to pipet slowly and minimally to avoid shearing.
- Never vortex genomic DNA since this can cause shearing. Instead, mix samples by inversion or gentle flicking of the tube.
- Minimize freeze-thaw cycles. DNA can be stored short-term at 4°C or aliquoted for long-term storage at -20°C/-80°C.
- Avoid heating or over drying. If using a vacuum concentrator, do not heat the sample.
- DNA should be eluted in a neutral, buffered solution (e.g., 10 mM Tris-HCl, pH 8). Do not elute or submit DNA in nuclease-free water or unbuffered solution.
- Do not expose DNA to intercalating fluorescent dyes (e.g., ethidium bromide) or ultraviolet radiation.

Extracting HMW DNA

- In general, we recommend extraction of HMW DNA using the QIAGEN® Genomic-tip 100/G kit (cat. # 10243) to obtain genomic DNA in the size range of 100-200 kb. If using this kit for the first time, a pilot extraction is strongly suggested.
- For yeast/fungal HMW DNA extraction, we recommend using the Lucigen® MasterPure™ Yeast DNA Purification Kit (cat. # MPY80200), which can yield ~40-50 kb DNA. Performing a pilot extraction is strongly recommended before working with limited or precious samples.
- Alternative extraction approaches may be suitable for your sample type assuming they yield DNA ≥ 50 kb, use a neutral, buffered solution for elution, and avoid carryover of organic solvents (e.g., phenol or guanidinium salts) or inhibitory species (e.g., high levels of polysaccharides, phenolics, and secondary metabolites) in the final product.

Submitting Samples

All HMW DNA samples should be shipped to GENEWIZ overnight on dry ice.