

DNA Sample requirements for PacBio® Sequel sequencing

(adapted from PacBio® official guidelines)

Genomic DNA: Recommended DNA Quantity for submission of gDNA is **10-15µg** needed for one SMRTbell library prep and includes extra quantity needed for any additional QC (with conservative excess).

Amplicon DNA: Input material for library preparation is **500ng**, please provide some additional material for upfront QC.

The Pacific Biosciences® library preparation process for genomic DNA does not utilize amplification techniques and resulting library molecules are directly used as templates for the sequencing process. High-quality, high-molecular-weight genomic DNA is imperative for obtaining long read lengths and optimal sequencing performance. Data yields may vary depending on sample DNA quality.

Important measures impacting DNA quality

For optimal sequencing performance, it is **essential** that your DNA sample:

- Is double-stranded; single-stranded DNA is not compatible with the library preparation process.
- Has not undergone multiple freeze-thaw cycles as they can lead to DNA damage.
- Has not been exposed to high temperatures (e.g.: > 65°C for 1 hour can cause a detectable decrease in sequence quality), pH extremes (< 6 or > 9).
- Has an OD260/OD280 ratio between 1.8 and 2.0.
- Has an OD260/OD230 ratio between 2.0 and 2.2
- Does not contain insoluble material.
- Does not contain RNA contamination.
- Has not been exposed to intercalating fluorescent dyes or ultraviolet radiation. SYBR dyes are not DNA damaging, but do avoid ethidium bromide.
- Does not contain denaturants (e.g., guanidinium salts or phenol) or detergents (e.g., SDS or Triton-X100).
- Does not contain carryover contamination from the original organism/tissue (e.g., heme, humic acid, polyphenols, etc.)
- eluted in 1xTE

Guidelines for DNA extraction to obtain high molecular weight and clean genomic DNA

These are general recommendations to help obtain clean, high molecular weight genomic

DNA.

- 1) Before DNA extraction:
 - a) Avoid incubation in complex or rich media
 - b) Harvesting from several cultures rather than a single, high-density culture during early- to mid-logarithmic growth phase is preferred.
 - c) Extraction of small volumes is preferred over large volumes to avoid accumulating high concentrations of potentially inhibiting secondary components.
- 2) Options for DNA Extraction: (not an official endorsement from PacBio)
 - a) Qiagen Genomic-tip kit (50-100 kb)
 - b) Phenol-chloroform extraction
 - Ensure phenol is fresh and not oxidized; use within three months of opening the reagent bottle.

Quality control of HMW DNA at the VBCF NGS facility

- Spectrophotometric measurement using Nanodrop to determine the purity - of the samples
- Fluorespectrometric Nanodrop Picogreen assay for concentration measurement
- Large Fragment Analysis on Fragment Analyzer

High molecular weight DNA is visible on gels or Fragment/Bio- Analyzer as one clear band or peak. A smear or several bands indicate degradation of the DNA.

SMRT bell library preparation including several DNA damage repair steps on degraded DNA will lead to incalculable loss of material and results in insufficient amount of library for sequencing.

A discrepancy of concentrations between Spectrophotometric and Fluorespectrometric analysis might indicate contamination.