

PACBIO® PROJECT SUBMISSION RECOMMENDATIONS

I. Estimating library yield on the PacBio RS II (Table 1)

The [PacBio Binding Calculator](#) should be used to estimate yield for all samples. The table below provides estimates of expected yields for various DNA libraries. Please note the assumptions used to generate the table.

Target Library Insert Size	Recommended DNA Quantity for Submission*	Min Input DNA Amount Required (Post-Shearing)	Purified DNA SMRTbell™ Library Concentration (ng/μl)	Est. Total Data Yield (Gb)	
				MIN	MAX
250 bp	600 ng	250 ng	5	95	190
500 bp	600 ng	250 ng	5	18	36
1 kb	1.2 μg	500 ng	10	340	680
2 kb	1.2 μg	500 ng	10	170	340
5 kb	2.4 μg	1 μg	25	170	340
10 kb	2.4 μg	1 μg	25	85	170
10 - 20 kb (AMPure)	15 μg	5 ug	125	35	70
20 kb (BluePippin)	15 μg	5 μg	50	10	20

* DNA input amounts recommended for submission represent quantities needed for one (1) SMRTbell library prep and includes extra quantity needed for any additional QC (with conservative excess). Estimated Min and Max total sequencing data yields (Gb) are based on the assumption of a starting SMRTbell DNA template volume of 10 uL, a purified SMRTbell DNA template concentration of 5 - 125 ng/ μl and a throughput of 500 - 1000 MB per SMRT Cell using P6-C4 chemistry. For insert sizes ≥ 1-kb, a Magnetic Bead (one cell per well) loading protocol is used in the SMRT Cell yield calculations. Preparation of 20-kb large-insert libraries can be achieved using either AMPure or BluePippin size selection strategies. Actual data yields may vary depending on sample DNA quality.

II. Sample requirements for PacBio sequencing

The Pacific Biosciences® library preparation process does not utilize amplification techniques and resulting library molecules are directly used as templates for the sequencing process. As such, the quality of the DNA starting material will be directly reflected in the sequencing results. Any irreversible DNA damage present in the input material (e.g., interstrand crosslinks, etc.) will result in impaired performance in the system. High-quality, high-molecular-weight genomic DNA is imperative for obtaining long read lengths and optimal sequencing performance.

III. 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.)

IV. 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)
 - i. [Product Information](#)
 - b. Qiagen Gentra® Puregene® kit (100–200 kb)
 - i. [Product Information](#)
 - c. Phenol-chloroform extraction
 - i. [Product Information](#)
 - ii. Ensure phenol is fresh and not oxidized; use within three months of opening the reagent bottle.

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