PACBIO® Project Submission Instructions

I. DNA Quantity

The table below provides the recommended quantity of double stranded DNA necessary for submission. Please submit samples in dH2O or EB **(**10 mM Tris-Cl, pH 8.5), ***not* *TE***. Please contact HTSF with any questions about submission requirements.

|  |  |
| --- | --- |
| Library Insert Size\* | Recommended Quantity for Submission |
| 500bp-1000bp | 1ug |
| 2kb-5kb | 4ug |
| 10kb | 10ug |
| 20kb (AMPure kit) | 15ug |
| 20kb (Blue Pippin) | 20ug |

\* Amounts recommended for submission represent quantities needed for one SMRTbell library prep and includes extra quantity needed for any additional QC and conservative excess. In most cases one library prep produces enough template for multiple SMRT cells. For insert sizes >1 KB, a magnetic bead loading protocol is used in the SMRT Cell calculation. Two size-selection protocols for large-insert libraries are available using either AMPure or BluePippin strategies.

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

To maximize read length and quality, 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.: > 65ºC for 1 hour can cause a detectable decrease in sequence quality), pH extremes (< 6 or > 9).
* Has an A260/A280 ratio of 1.8 to 2.0.
* 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. General guidelines for DNA extraction to maintain high molecular weight and clean DNA

Before DNA extraction:

* Avoid incubation in complex or rich media
* Harvesting from several cultures rather than a single, high-density culture during early- to mid-logarithmic growth phase is preferred.
* Extraction of small volumes is preferred over large volumes to avoid accumulating high concentrations of potentially inhibiting secondary components.

Options for DNA Extraction: (not an official endorsement from PacBio)

* Qiagen Genomic‐tip kit (50-100 kb)
* Qiagen Gentra® Puregene® kit (100-200 kb)
* Phenol‐chloroform extraction
* Ensure phenol is fresh and not oxidized; use within three months of opening the reagent bottle.

V. Removal of inhibitors. Some of the genomic DNA extraction methods (see II and IV) – especially fungi and plant ones – can leave substances in the solution that can inhibit the PacBio sequencing reaction. PacBio recommends using MoBio PowerClean DNA Clean-Up Kit to remove them. We can do this for you (please mark the *Additional genomic DNA clean-up* box) but it results in the loss of some of the DNA. Choosing this option means we may request additional DNA if there is not enough after clean-up.