PacBio Submission Workflow

The PacBio system utilizes Single Molecule, Real-Time (SMRT) technology, eliminating the need for amplification. The DNA submitted serves as a direct template for the sequencing reaction via the ligation of hairpin adapters that create a circular template. Real-time sequencing occurs continuously across this circular template, producing extra-long read lengths and high consensus accuracy. These advantages make the PacBio a powerful tool in accurately calling SNPs and sequencing through CG-rich areas. For more information see the PacBio RSII Sequencing System brochure (PDF).

The PacBio RSII system can be used for de novo assembly, targeted sequencing, and base modification detection. PacBio sequence data provides:

- Extraordinarily long read lengths - up to 40kb with high consensus accuracy.
- Extremely high accuracy - few apparent miscalls (99.999% consensus accuracy)
- Direct measurement of base modification (epigenetics)

We can also prepare libraries from amplicons, without shearing. The RSII upgrade allows us to maximize read length up to 40kb with a 1x240 minute movie while capturing all 150,000 ZMWs (reaction wells) on a SMRTcell (where sequencing takes place). Typically, 35,000-75,000 reads on a good run. Larger DNA fragments tend to produce fewer reads.

Before starting a project, we strongly recommend discussing your project with the HTSF team. Contact Tara Skelly or Tristan De Buysscher to discuss project needs.

Submission Requirements

- All samples submitted must be measured by a fluorometer (e.g. Qubit) or qPCR. Please also include a copy of your QC gel or tapestation with your submission so we can accurately determine the quality of sample submitted. If samples have not been measured by fluorometer or qPCR and/or no gel image is provided, your sample must be QA/QC by HTSF (at an additional cost) after receipt of your submission.

- No amplification is required for the PacBio library preparation. Prepared libraries are used directly as the sequencing template.
- Submitting high quality, high molecular weight, genomic DNA is imperative for obtaining long read lengths and optimal sequencing performance.
- DNA must be double-stranded. Single-stranded material is incompatible with PacBio library preparation.

  The amount of DNA necessary for library preparation varies based on the insert size requested. The table below lists the minimum amount of double-stranded DNA accepted.
### Desired Library Insert Size vs. Minimum Quantity Required

<table>
<thead>
<tr>
<th>Desired Library Insert Size</th>
<th>Minimum Quantity Required (optimal conc. 200-300 ng/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 - 1000 bp</td>
<td>1 μg</td>
</tr>
<tr>
<td>2000 - 5000 bp</td>
<td>4 μg</td>
</tr>
<tr>
<td>10 kb</td>
<td>10 μg</td>
</tr>
<tr>
<td>20+ kb</td>
<td>20 μg</td>
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</tbody>
</table>

**Output per SMRTcell**

The number of SMRTcells required for your project depends on the application. For more information please discuss your project with an HTSF PacBio Research Specialist and the center's bioinformatician familiar with PacBio data analysis (*bottom of page*).

**Informatics Options for Improving Large Genome Assemblies**

Contact Tristan de Buysscher or Hemant Kelkar to discuss data needs.

**Submitting Samples**

Fill out a PacBio Submission Form, making certain to include information on the size of the material being submitted and the insert size desired.

Samples can be physically dropped off at 1153 Genome Sciences Building from Mon-Fri between 10am and 4pm only please (unless otherwise arranged). Due to UNC security considerations, our facilities may not be accessible outside of these hours.

**PacBio Contacts**

- For information on pricing, turnaround times, or submitting PacBio samples contact Piotr Mieczkowski or Corbin Jones.
- For PacBio library preparation questions contact Tara Skelly.
- For questions regarding PacBio bioinformatics support contact Tristan De Buysscher.
- For questions regarding PacBio systems and technology please contact George Yuan at Pacific Biosciences.