

## PACBIO® RS II ISO-SEQ™ PROJECT SUBMISSION FORM

DISCLAIMER: Failure to fill out this form accurately and in its entirety may result in additional costs and/or sample failure in downstream sequencing preparation applications.

General Information	
Project ID	
P.I.	
P.O. #	
Contact Name	
Contact Phone	
Contact Email	
Contact Address	
Number of submitted samples	
Date Submitted	
Submitted By	
Application Service Requested	
<p>Transcriptome:</p> <p><input type="checkbox"/> Targeted, gene-specific isoform characterization for one or more gene families</p> <p><input type="checkbox"/> <i>De novo</i> whole transcriptome isoform characterization</p> <p><input type="checkbox"/> Other (Please specify):</p>	<p>Size-Selection Protocol (Select one of the following):</p> <p><input type="checkbox"/> No size selection</p> <p><input type="checkbox"/> With size selection:</p> <p style="margin-left: 20px;"><input type="checkbox"/> Gel Cut   <input type="checkbox"/> BluePippin™ size selection   <input type="checkbox"/> SageELF™ Size Selection</p> <p>Transcript Size-Selection Library Bins:</p> <p><input type="checkbox"/> 1-2 kb   <input type="checkbox"/> 2-3 kb   <input type="checkbox"/> 3-6 kb   <input type="checkbox"/> 6-10 kb*   <input type="checkbox"/> Other Size Fractions:</p> <p><i>*BluePippin™ Size Selection Required</i></p> <p>Perform double BluePippin size selection for insert size fractions &gt;3 kb:</p> <p><input type="checkbox"/> Yes   <input type="checkbox"/> No</p> <p><i>Note: Double BluePippin size selection improves sequencing performance of longer transcripts.</i></p>
	<p>Sequencing Chemistry</p> <p><input type="checkbox"/> P6-C4   <input type="checkbox"/> Other:</p>
	<p>Loading Method</p> <p><input type="checkbox"/> Diffusion   <input type="checkbox"/> Mag Bead (Recommended)</p>
	<p>Other Notes:</p>
Type of Input Sample Submitted	
<p><input type="checkbox"/> Total RNA (Recommended: 2 µg)      <input type="checkbox"/> PolyA+ RNA (Recommended: 40 ng)</p> <p><input type="checkbox"/> cDNA (Minimum for no-size selection: 500 ng / Minimum for BluePippin size selection: 2 – 2.5 µg)</p> <p>Was double-stranded cDNA library generated following the PacBio Iso-Seq Protocol? <input type="checkbox"/> Yes   <input type="checkbox"/> No   <input type="checkbox"/> N/A</p> <p>If No, please describe:</p> <p>Source: <input type="checkbox"/> Bacteria   <input type="checkbox"/> Fungus   <input type="checkbox"/> Plant   <input type="checkbox"/> Animal   <input type="checkbox"/> Human   <input type="checkbox"/> Cell Culture/Tissue   <input type="checkbox"/> Other :</p> <p>Other Notes:</p>	
<p>Submitted Sample (ng/µl):</p> <p>Total (ng) :</p>	<p>Quantitation Method:</p> <p><input type="checkbox"/> Bioanalyzer®   <input type="checkbox"/> Nanodrop®   <input type="checkbox"/> Qubit® Fluorometer</p> <p><input type="checkbox"/> Other (Specify):</p>

RNA Extraction Method
<input type="checkbox"/> Automated <input type="checkbox"/> Manual <input type="checkbox"/> Information not provided  Description < Please provide kit information > :  
RNA Quality Check
<input type="checkbox"/> Absorbance (e.g., Nanodrop®) [OD <sub>260</sub> /OD <sub>280</sub> ratio:                      OD <sub>260</sub> /OD <sub>230</sub> ratio:                      ] <input type="checkbox"/> Agarose Gel* ( <input type="checkbox"/> SYBR® Gold <input type="checkbox"/> SYBRi Green II) <input type="checkbox"/> Bioanalyzer* [RIN:              28S/18S Ratio:                      ] <input type="checkbox"/> Other (Please Specify): <input type="checkbox"/> Information not provided  *Please provide agarose gel images and Bioanalyzer trace files for direct data visualization as secondary evaluation of RIN and OD ratios.

cDNA Synthesis Method [If Submitted Starting Input is cDNA]
Starting RNA material: <input type="checkbox"/> Total RNA <input type="checkbox"/> PolyA+ RNA  Method of conversion: <input type="checkbox"/> Clontech® SMARTer® PCR cDNA Synthesis Kit <input type="checkbox"/> Other (Please specify):  Were PCR optimization performed to obtain adequate dsDNA synthesis for SMRTbell™ Library? <input type="checkbox"/> Yes <input type="checkbox"/> No PCR amplification kit used: Optimal number of PCR amplification cycles performed:  Other Notes:
cDNA Quality Check
<input type="checkbox"/> Absorbance [OD <sub>260</sub> /OD <sub>280</sub> ratio:              OD <sub>260</sub> /OD <sub>230</sub> ratio:              ] <input type="checkbox"/> Agarose Gel <input type="checkbox"/> Bioanalyzer <input type="checkbox"/> Other (Please specify): <input type="checkbox"/> Information not provided

Experimental Design	* Please see PacBio Project Submission Recommendations *
List Number of SMRT® Cells Requested per Size Fractionation	<input type="checkbox"/> 1-2 kb : <input type="checkbox"/> 2-3 kb: <input type="checkbox"/> 3 -6 kb: <input type="checkbox"/> 6 -10 kb: <input type="checkbox"/> Other Size Fraction: <input type="checkbox"/> No Size Selection:
Bioinformatics and Data Delivery	
<input type="checkbox"/> Basic Data Delivery (bas.h5, bax.h5, metadata.xml files) <input type="checkbox"/> Basic Analysis (Filtered subreads, Iso-Seq Analysis via SMRT Portal) <input type="checkbox"/> Custom Solution (Description): <input type="checkbox"/> Consulting	

**PACBIO® ISO-SEQ™ PROJECT SUBMISSION RECOMMENDATIONS**
**I. Estimating no. of SMRT Cells for Iso-Seq Application (Table 1)**

The table below provides general guidelines for the number of SMRT Cells needed for experimental design goals. Number of SMRT Cells for each project is defined per size-selected library and depends on the research goals and transcriptome complexity. For insert sizes >3 kb, the estimated number of SMRT Cells recommended is based on implementing the double BluePippin size-selection protocol, which improves sequencing performance for these longer transcript lengths. The number of SMRT Cells required generally increases for libraries that are not cleanly size-selected (due to the presence of shorter transcripts that were not removed from each size bin). Approximately 25,000 full-length transcripts can be sequenced with each SMRT Cell under optimized sample loading conditions.

<b>Number of SMRT Cells (per sample)</b>	<b>Experimental Goals</b>
1 SMRT Cell	Targeted, gene-specific isoform characterization. (e.g., using PCR products as input). Multiple genes may be analyzed on a single SMRT Cell by employing an appropriate <a href="#">PacBio Barcoding</a> strategy.
1-4 SMRT Cells	General survey of full-length isoforms in a transcriptome using a no-size-selection protocol. Resulting transcript sizes may be skewed towards representation in favor of shorter isoforms (i.e., 1-3 kb).
4-8 SMRT Cells	A focused study on one or two size fractions of full-length transcripts with 4 SMRT Cells per fraction (e.g. 2-3 kb and 3-6 kb).
16 SMRT Cells	A more comprehensive survey of full-length isoforms in the whole transcriptome ranging from 1 kb to 10 kb. General guidance is to run 4 SMRT Cells per size-selected library: e.g., (a) 1-2 kb; (b) 2-3 kb; (c) 3-6 kb; and (d) 6-10 kb library size bins are recommended for enabling coverage of highly expressed isoforms.
>16 SMRT Cells	Add additional sequencing of specific size-selected libraries post-analysis to saturate isoform discovery, or increase the dynamic range for very low abundance transcript discovery and detection.

**II. RNA sample requirements for PacBio sequencing**

The Iso-Seq Application from Pacific Biosciences provides researchers the ability to sequence intact, full-length transcripts with the demonstrated detection of 5'/3' ends and a polyA+ tail. Project success is highly dependent on the quality of the starting RNA / cDNA material since any damages to transcripts will be directly reflected in the sequencing results. High-quality RNA extractions and clean size-selection of samples are imperative for obtaining long read lengths and optimal sequencing performance.

**III. Important measures impacting RNA input sample quality**

For optimal sequencing performance, it is **essential** that the RNA sample:

- Has not undergone multiple freeze-thaw cycles as they can lead to additional RNA damage.
- Has not been exposed to high temperatures (e.g.: > 65°C for 1 hour can cause a detectable decrease in sequence quality) or pH extremes (< 6 or > 9).
- Has an OD<sub>260</sub>/OD<sub>280</sub> ratio between 2.0 and 2.2.

- Has an OD<sub>260</sub>/OD<sub>230</sub> ratio between 1.8 and 2.1.
- Has a RIN number ≥ 9 (Recommended).
- Does not contain insoluble material.
- Does not contain DNA contamination.
- Has not been exposed to intercalating fluorescent dyes or ultraviolet radiation. SYBR dyes are not RNA 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.).
- Note: RNA samples should only be shipped with dry ice.

#### IV. Important measures impacting cDNA input sample quality

The most important factor impacting the quality of cDNA libraries is the quality of the starting RNA (see Section III above).

Please take all precautions against additional damages and ensure that the cDNA sample:

- Has not been exposed to the same types of damaging agents, conditions, and contaminants listed above for RNA input samples (see Section III above).
- Has an OD<sub>260</sub>/OD<sub>280</sub> ratio of 1.8 to 2.0.

#### V. Recommendations for RNA extraction/purification (not an official endorsement from PacBio)

The minimum starting amount is 2 ng of total RNA or 1 ng of polyA+ RNA. The following kits are recommended for polyA+ extraction/purification:

- Ambion® Poly(A) Purist™ MAG Kit (<http://products.invitrogen.com/ivgn/product/AM1922>)
- Qiagen® RNeasy Plus Kits (<http://www.qiagen.com/qdm/rna/rneasy-plus-kits?cmpid=Qven10GARneasy>)

#### VI. Recommendations for cDNA library construction

- Clontech® SMARTer® PCR cDNA Synthesis Kits ([http://www.clontech.com/US/Products/cDNA\\_Synthesis\\_and\\_Library\\_Construction/cDNA\\_Synthesis\\_Kits/SMA RTer\\_Kits](http://www.clontech.com/US/Products/cDNA_Synthesis_and_Library_Construction/cDNA_Synthesis_Kits/SMA RTer_Kits))
- [PacBio User Bulletin – Guidelines for Preparing cDNA Libraries for Isoform Sequencing \(Iso-Seq™\)](#)

---

For Research Use Only. Not for use in diagnostic procedures. © Copyright 2015, Pacific Biosciences of California, Inc. All rights reserved. Information in this document is subject to change without notice. Pacific Biosciences assumes no responsibility for any errors or omissions in this document. Certain notices, terms, conditions and/or use restrictions may pertain to your use of Pacific Biosciences products and/or third party products. Please refer to the applicable Pacific Biosciences Terms and Conditions of Sale and to the applicable license terms at <http://www.pacificbiosciences.com/licenses.html>.

Pacific Biosciences, the Pacific Biosciences logo, PacBio, SMRT, SMRTbell, and Iso-Seq are trademarks of Pacific Biosciences in the United States and/or certain other countries. All other trademarks are the sole property of their respective owners.