

## RNA sample requirements for PacBio® Sequel sequencing

(adapted from PacBio® official guidelines)

The Iso-Seq Application from Pacific Biosciences provides 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 of samples are imperative for obtaining long read lengths and optimal sequencing performance.

### RNA input:

Please provide 1µg total RNA in maximal 3.5µl. This doesn't include extra quantity needed for QC, please provide excess volume.

For standard Iso-Seq protocol the Clontech® SMARTer® PCR cDNA Synthesis Kit is used followed by SMRTbell library preparation.

The aim of the standard protocol is an even distribution of length throughout the transcripts.

For non standard input material e.g. cDNA or preparation e.g. enrichment for a specific size-fraction, please contact us.

### Important measures impacting RNA quality

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

- Has not undergone multiple freeze-thaw cycles as they can lead to RNA degradation.
- 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 2.0 and 2.2.
- Has an OD260/OD230 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.)
  - Store RNA in DEPC treated or nuclease free water at -80°C.
  - Best to freshly prepare total RNA before starting library preparation/bringing the material to the facility.
  - Note: RNA samples should only be shipped on dry ice.
- 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
  - Has an OD260/OD280 ratio of 1.8 to 2.0.
- Recommendations for RNA extraction/purification:
- Ambion® Poly(A) Purist™ MAG Kit
  - Qiagen® RNeasy Plus Kits



## HOW MANY SMRT CELLS?

RS // SMRT Cells (per sample)	Sequel SMRT Cells (per sample)	Experimental Goals
1	<1	Targeted, gene-specific isoform characterization
1-8	1	General survey of full-length isoforms in a transcriptome (moderate to high expression levels) with or without size selection
12-16	1-2	A comprehensive survey of full-length isoforms in the transcriptome across 3-4 size fractions
>16	2+	Deep sequencing for comprehensive isoform discovery and identification of low abundance transcripts across 3-4 size fractions