THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Pacbio Sequel SMRT Sequencing

The PacBio Sequel Sequencing is a proven third generation Single Molecule, Real-Time (SMRT) technology that provides ultra-long sequencing reads (8-12Kb) with high consensus accuracy (99.9999%), uniform coverage, and simultaneous epigenetic characterization at single-molecule resolution. Sequel SMRT sequencing directly measures individual molecules, using long reads to fully characterize genetic complexity, including rare SNPs, indels, structural variants, haplotypes and phasing. Single molecule resolution allows comprehensive characterization of heterogeneous samples and identification of variation invisible to multi-molecule sequencing technologies. The PacBio Sequel SMRT sequencing system provides extra-long read lengths for *de novel* that simplify and improve genome assembly without reference. Iso-Seq RNA sequencing that provide long read full length transcripts and isoforms cross the poly(A) tail to the 5' end without the need of assembly and is ideal for improving annotations in reference genomes, characterizing gene isoforms, alternative splicing and gene fusion events. The PacBio Sequel SMRT sequencing detects single nucleotide modifications in real time, directly measuring the kinetic properties of base additions during the sequencing process. These kinetic measurements present characteristic patterns in response to a variety of base modifications such as 5-methylcytosine, 5-hydroxymethylcytosine, 6-methyladenine, 8-oxoguanine, and more. Sequel SMRT sequencing expects that researchers will use this capability to study a broad range of base modifications at single base resolution.

Pacbio Sequel Services: "Sequencing and ncRNA program core" offers 1) Sequel SMRT bell DNA and RNA library preparation and SMRT sequencing services. The SMRT bell library preparation can be started from either high molecular weight genomic DNA for whole genome assembly and multiplexed PCR amplicon(s) for targeted genome sequencing. Or cDNA SMRT bell library converted from high quality intact total RNA for whole transcriptome sequencing (Iso-Seq method).

Sequel System Applications:

- Create high-quality whole genome*de novo* assemblies of eukaryotic organisms
- Survey large population cohort studies for structural variants
- Sequence full-length transcriptomes or targeted transcripts
- Target regions not currently accessible by other technologies
- Detect genomic variation in complex populations on all size scales
- Detect epigenetic modifications

Sample Requirements

High-quality, high-molecular-weight genomic DNA is crucial for obtaining long read lengths and optimal sequencing performance The SMART library preparation process does not utilize amplification techniques and resulting library molecules are directly used as templates for the sequencing process. The quality of the DNA and RNA starting material will be directly reflected in the extent of sequencing success or failure. Any unrepaired or irreversible DNA damage present in the input material (e.g., interstrand crosslinks, nicks, etc.) will result in impaired performance in the system.

Pacific Biosciences recommends resuspending your DNA samples in either water or 10 mM Tris-HCl. RNA samples in RNase –free Water. Please see the table and list below for additional DNA quantity and quality requirements and recommendations.

Sequencing Application	Minimum Quantity Needed (per Library)
250 bp to 3 Kb insert library preparation	250 ng - 2 µg of DNA
3 - 10 Kb insert library preparation	2 µg - 5 µg of DNA
10 - 20 Kb insert library preparation	10 µg - 20 µg of DNA
20 - 30 Kb insert library preparation	20 µg - 50 µg of DNA
Total RNA Iso-Seq (no-cut)	2 µg - 5 µg of total RNA

Recommended Characteristics of DNA Suitable for Single-Molecule Sequencing

- Minimal DNA quality: OD $_{260/280}$ and OD $_{260/230}$ should be 1.8-2.0
- Must be double-stranded. Single-stranded DNA will not be made into a SMRTcell template in the template preparation process and can interfere with quantitation and polymerase binding.
- Has undergone a minimum of freeze-thaw cycles.
- Has not been exposed to high temps (> 65°C for more than one hour can cause a detectable decrease in sequence quality).
- Has not been exposed to pH extremes (< 6 or > 9).
- Does not contain insoluble material.
- Does not contain RNA.
- Has not been exposed to intercalating fluorescent dyes or ultraviolet radiation

References

PacBio Guidelines for Successful SMRTbell Libraries
Q&A for "DNA Quality Requirements for Single Molecule, Real-Time (SMRT) Sequencing"

Pricing and quotation

Please request service through iLab
https://mdanderson.ilabsolutions.com/service_center/show_external/3659/sequencing_and_nc_rna_program

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Data Retrieval

1. Primary analysis files will be transferred to customers or destinated bioinformatic support team. The following files are an example of the primary analysis output files: • *.adapters.fasta • *.scraps.bam.pbi • *.subreads.bam • *.subreadset.xml • *.txt • *.scraps.bam • *.sts.xml • *.subreads.bam.pbi • *.transferdone.

2. The primary analysis files will be permanently deleted after 6 months from the data transfer date.