cDNA Sequencing

Oxford Nanopore Technologies Services

Summary

Renew Biotechnologies (Renew), a certified Oxford Nanopore Technologies (ONT) sequencing service provider, offers comprehensive cDNA sequencing to analyze full-length cDNA transcripts. Leveraging ONT's long-read sequencing technology, this service enables the detection of complete gene isoforms, splice variants, and fusion genes. Designed for robust and accurate transcriptomic analysis, Renew's cDNA sequencing is ideal for researchers seeking detailed insights into the transcriptome without epigenetic data.

Key Features & Benefits

- **Full-Length Transcript Characterization:** Captures complete cDNA transcript sequence data for comprehensive analysis of structural variants.
- **Maximizes Data Output:** Enables multiplexing of up to 24 samples per flow cell, optimizing throughput and cost-efficiency.
- **Flexible Input Options:** cDNA derived from total or enriched RNA (e.g., poly(A)+ or ribodepleted).
- **Reduced Amplification Bias:** Incorporating molecular identifiers (UMI) during the strand-switching step of the protocol reduces PCR amplification biases.

Ideal Applications

- Gene expression quantification
- Transcriptome Profiling
- Isoform detection and alternative splicing analysis
- Fusion gene and rare transcript detection

Protocol Overview

ONT Kit: cDNA-PCR Sequencing Kit V14 (SQK-PCS114)

Platform: PromethION

Workflow

1. **RNA Extraction:** Clients prepare RNA from their samples following their project's specific extraction protocol.
2. **Sample Receipt:** Extracted RNA is received at Renew and quality checked to ensure that sample requirements are met (minimum 10 ng of enriched RNA (poly(A)+ or ribodepleted RNA) or 500 ng of total RNA per sample.
3. **Library Preparation (Figure 1):** Reverse transcription and strand-switching are performed to synthesize full-length, double-stranded cDNA from the RNA sample. A unique molecular identifier (UMI) is incorporated during the strand-switching process, followed by PCR amplification of the double-stranded cDNA using primers containing 5' tags. Sequencing adapters are ligated to the PCR products.
4. **Loading onto Flow Cell:** The prepared cDNA library is loaded onto the PromethION flow cell for sequencing.
5. **Real-Time Sequencing & Analysis:** Long-read, real-time sequencing occurs with direct data streaming and analysis.
6. **Results Generation:** Raw nanopore reads are basecalled and uploaded as BAM files in Renew's client portal. Clients receive notifications when sequencing is complete and when results are accessible in the portal.

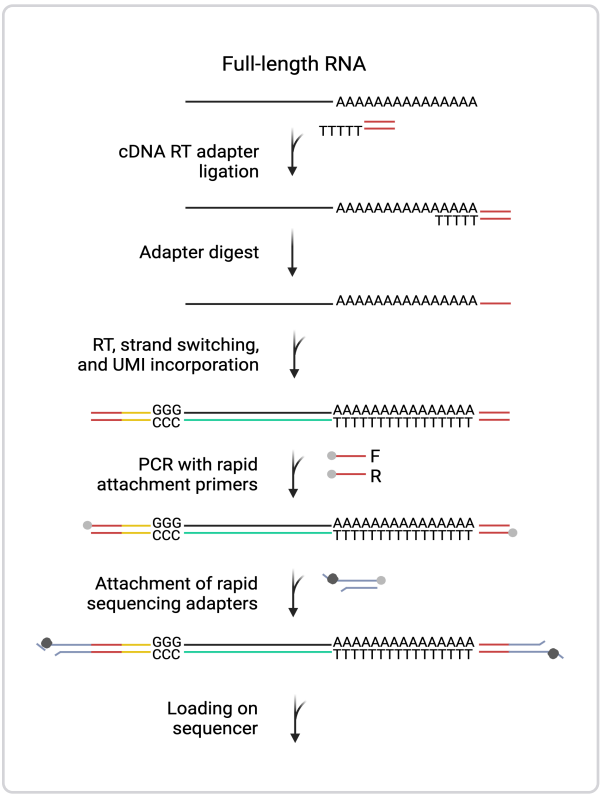

Figure 1. ONT DNA PCR Sequencing Library Preparation Workflow. This schematic illustrates the key steps involved in preparing cDNA samples for sequencing using Oxford Nanopore Technologies' (SQK-PCS114) kit. The process begins with reverse transcription of full-length RNA, followed by a strand-switching mechanism to generate double-stranded cDNA. During this step, a Unique Molecular Identifier (UMI) is incorporated to reduce PCR amplification bias. The cDNA is then amplified by PCR, and sequencing adapters are ligated to the PCR products, preparing the sample for loading onto the flow cell for real-time, long-read sequencing (PromethION). This method is optimized for full-length transcript analysis and isoform detection. The figure was adapted from ONT and created with BioRender.com.

Sample Requirements

Parameter	Requirement
Sample Type	Total RNA, mRNA, or other purified RNA (e.g., lncRNA)
Minimum Input RNA	4 ng enriched RNA (Poly(A)+ or ribodepleted RNA) or 200 ng total RNA
Recommended Input RNA	10 ng enriched RNA (Poly(A)+ or ribodepleted RNA) or 500 ng total RNA
Quality Control (QC)	RIN/RQN: > 7.0 (>8.0 preferred) 260/280 ratio: 1.8 - 2.0 260/230 ratio: 1.9 - 2.5

Throughput & Capacity

Parameter	Requirement
Read Lengths	Read lengths vary depending on the sample type, quality, and transcription. Full-length transcripts typically range from 1 to 2 kb, with longer reads observed in higher-quality RNA samples.
Samples per Flow Cell	Up to 24
Sequencing Yield per Sample	Varies based on samples per flow cell (e.g., 4-6 Gb per sample for a 24-sample run).

Data Output

File Format

Data is delivered via the Renew client portal, including sequencing files, methylation calls, variant outputs, and quality control reports. Optional services (available for an additional fee) include POD5 file delivery, alignment to client-supplied reference genomes, and custom bioinformatics analyses.

File	Description
POD5	Raw signal data
BAM	Sequencing reads + base modifications
QC Report	Sequencing metrics and run statistics

QC Reports

For each batch of samples, we provide Quality Control Reports that include read length distribution, base quality, and yield, along with summary statistics such as total number of reads, average read length, and error rates.

Sequencing Coverage

Typical Coverage: Variable based on sample quality and RNA quantity. Ideal for projects requiring full transcriptome coverage or isoform resolution.

Service Inclusions

Library Preparation: Full library prep services are included, utilizing the specified ONT kit.

Data Delivery: All sequencing data is provided as unaligned BAM files through Renew's secure cloud-based Laboratory Information Management System (LIMS), offering clients a clear and transparent view of the entire process.

Choosing the Right Approach

cDNA vs. Direct RNA Sequencing: Key Differences

For researchers deciding between cDNA and Direct RNA sequencing, it is essential to consider the specific needs of your project. Each approach offers distinct advantages depending on your focus, whether you are analyzing the transcriptome at the epigenetic level or seeking complete transcriptomic insights without epigenetic data. Below, we outline the key differences to help you choose the most suitable service.

Feature	cDNA	Direct RNA
Full Length Transcripts	Sequences full-length cDNA molecules, capturing complete gene isoforms and splice variants.	Directly sequences full-length native RNA molecules, including rare transcripts and isoforms, without reverse transcription or PCR.
Epigenetic Modifications	Cannot detect epigenetic modifications, as RNA must be reverse transcribed into cDNA, which removes these modifications.	Enables epigenetic modification analysis (e.g., m6A, m5C, inosine) by sequencing native RNA, preserving modifications in their natural state

PCR Amplification Bias	PCR amplification is required in this protocol, but the use of unique molecular identifiers (UMIs) helps reduce over-representation of certain sequences, improving the accuracy of transcript quantification.	No amplification required, reducing bias and preserving native transcript integrity.
Quantification Accuracy	Can achieve quantitative accuracy through PCR amplification with the incorporation of unique molecular identifiers, though reverse transcription is required	Preserves quantitative accuracy by sequencing RNA directly, avoiding artifacts from reverse transcription or amplification.
Processing Complexity	Requires additional processing steps, such as reverse transcription to convert RNA into cDNA.	Minimal processing required, as RNA is sequenced directly from the sample.
Sample Integrity Requirement	cDNA synthesis is more tolerant of lower-quality RNA samples, making it useful when RNA integrity may be compromised.	RNA needs to be high quality to maintain transcript integrity.
Ideal For Researchers	Researchers focused on transcriptomic analysis-isoform detection, splice variant analysis, and gene expression quantification-without the need for epigenetic modifications..	Researchers interested in studying RNA modifications, capturing the transcriptome at the epigenetic level, or those seeking a reverse transcription-free workflow for more accurate, native RNA analysis.

Common Challenges and Solutions

Concern	Renew's Solution
Low RNA Input or Quality	Renew's cDNA Sequencing Service is optimized for high sensitivity, handling inputs as low as 10 ng of RNA with reliable results. For projects with lower input or quality, we offer custom sample preparation solutions.
Sequencing Efficiency	Our rigorous quality control protocols are designed to optimize sequencing yield. We employ refined QC processes to enhance performance and efficiency for every run, providing reliable results.
Sample Contaminants	To provide reliable and accurate data, stringent purification protocols are used to remove contaminants and prepare samples to high-quality standards.

Contact and Pricing

Contact us for more information or request a quote

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