Direct RNA Sequencing

Oxford Nanopore Technologies Services

Summary

Renew Biotechnologies (Renew), a certified Oxford Nanopore Technologies (ONT) sequencing service provider, offers Direct RNA Sequencing that captures native, full-length polyadenylated RNA transcripts without reverse transcription or CDNA synthesis. This approach preserves the integrity of RNA molecules, including epigenetic modifications, providing accurate long-read sequencing and real-time analysis that reflects real biology. This robust service is ideal for discovery research and clinical diagnostics, offering the following key benefits for various use cases:

Key Features & Benefits

- **Full-Length Transcript Characterization:** Captures complete polyadenylated RNA transcripts.
- **Wide Range of Read Lengths:** From short (20 bp) to ultra-long (25 kb) reads.
- **Flexible Input Options:** Total or enriched RNA (e.g., poly(A)+ or ribodepleted).
- **No Reverse Transcription or PCR Bias:** Eliminates steps that could introduce errors.
- **Native RNA Sequencing:** Preserves key epigenetic modifications for comprehensive analysis.

Epigenetic Modifications

Abbreviation	Modification	Description
m5C	5-methylcytosine	Regulates gene expression and RNA stability
m6A	N6-methyladenosine	Influences RNA stability and translation
inosine	A-to-I RNA editing	Alters codons and affects protein translation
pseU	pseudouridine	Plays roles in splicing, translation, and stability

Ideal Applications

- Viral RNA sequencing
- Transcriptome profiling
- Single-cell RNA analysis
- RNA modification analysis
- Isoform and alternative splicing detection
- Sequencing transcripts that are difficult to reverse transcribe

Protocol Overview

ONT Kit: Direct RNA Sequencing Kit (SQK-RNA004)

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 300 ng of poly(A) tailed RNA or 1 ug of total RNA in 8 ul per sample).
3. **Library Preparation (Figure 1):** A single strand of complementary RNA is synthesized and sequencing adapters are ligated to the ends of the RNA-cDNA hybrid molecule (Figure 1).
4. **Loading onto Flow Cell:** The prepared RNA 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.

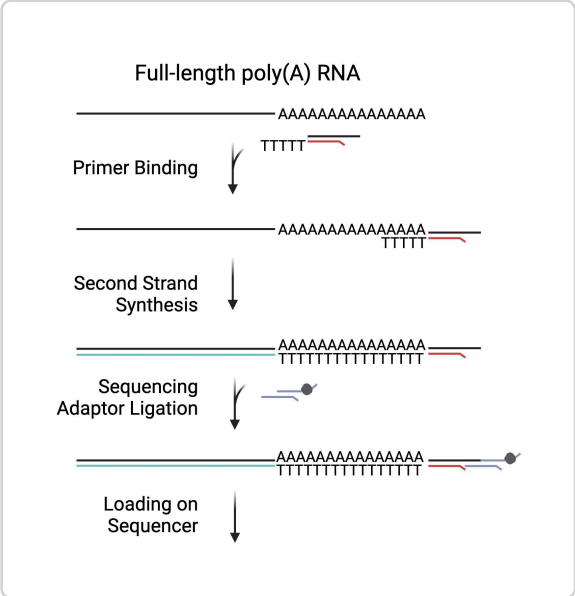

Figure 1. ONT Direct RNA Sequencing Library Preparation Workflow. This schematic illustrates the key steps involved in preparing RNA samples for direct sequencing using Oxford Nanopore Technologies' (SQK-RNA004) kit. The process includes second strand synthesis, direct adapter ligation to the RNA molecule, and loading onto the flow cell for real-time long-read sequencing (PromethION). This method avoids the need for reverse transcription or amplification, preserving native RNA features for comprehensive sequencing and epigenetic analysis. This 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	300 ng of poly(A) tailed RNA or 1 µg of total RNA in 8 uL
Recommended Input RNA	500 ng of poly(A) tailed RNA or 1.5 µg of total RNA in 8 uL
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 RNA reads typically range from 1 to 2 kb, with longer reads observed in higher-quality RNA samples.
Sequencing Yield per Sample	10-20 Gb
Samples per Flow Cell	1

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 sequencing data
BAM	Sequencing reads + base modifications

File	Description
CH3	Base modification output format
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

Direct RNA vs. cDNA Sequencing: Key Differences

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

PCR Amplification Bias	No amplification required, reducing bias and preserving native transcript integrity.	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.
Quantification Accuracy	Preserves quantitative accuracy by sequencing RNA directly, avoiding artifacts from reverse transcription or amplification.	Can achieve quantitative accuracy through PCR amplification with the incorporation of unique molecular identifiers, though reverse transcription is required
Processing Complexity	Minimal processing required, as RNA is sequenced directly from the sample.	Requires additional processing steps, such as reverse transcription to convert RNA into cDNA.
Sample Integrity Requirement	RNA needs to be high quality to maintain transcript integrity.	cDNA synthesis is more tolerant of lower-quality RNA samples, making it useful when RNA integrity may be compromised.
Ideal For Researchers	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.	Researchers focused on transcriptomic analysis-isoform detection, splice variant analysis, and gene expression quantification-without the need for epigenetic modifications..

Common Challenges and Solutions

Concern	Renew's Solution
Low RNA Input or Quality	Renew's Direct RNA Sequencing service is optimized for high sensitivity, handling inputs as was 300 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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