Revelo™ RNA-Seq

PRODUCT SHEET

High sensitivity RNA-seq library preparation kit.

One-day sample to sequencer solution for pathogen detection.

RNA-Seq library preparation from degraded or mixed samples, where detection of low abundance sequences is critical, poses a serious challenge to many researchers. Most commercially available RNA-Seq kits lack the sensitivity to detect and characterize low-titer viral or other pathogenic agents in samples with high background levels of human RNA, such as nasal swabs from patients infected with SARS-CoV-2. These samples may have a wide range of background contamination based on the sample collection method, impacting the ability to detect low viral copy numbers with confidence.

Revelo RNA-Seq is a whole transcriptome solution, integrated with proven SPIA® (Single Primer Isothermal Amplification) and optimized for the detection and characterization of rare and low abundance transcripts from degraded samples. This library preparation kit is integrated with an array of novel technologies for end-to-end processing of degraded samples with inputs as low as 250 pg.

- New SPIAboost™ technology offers improved depletion of human rRNA to maximize informative sequencing reads
- Enzymatic fragmentation for efficient sample preparation
- Unique Dual Indexed Adaptors for increased multiplexing and detection of index hopping
- DimerFree® technology eliminates adaptor dimers without the need for adaptor titration, regardless of sample input
- NuQuant® library quantification eliminates the need for expensive, time-consuming quantification methods, such as qPCR. Libraries can be quantified in minutes by a simple fluorescent measurement with a Qubit fluorometer or standard plate reader
- Ready-to-go automation scripts available with the DreamPrep™ NGS workstation

The Revelo RNA-Seq kit offers end-to-end processing of human samples, enabling complete RNA library preparation – including library quantification – in ~6.5 hours.

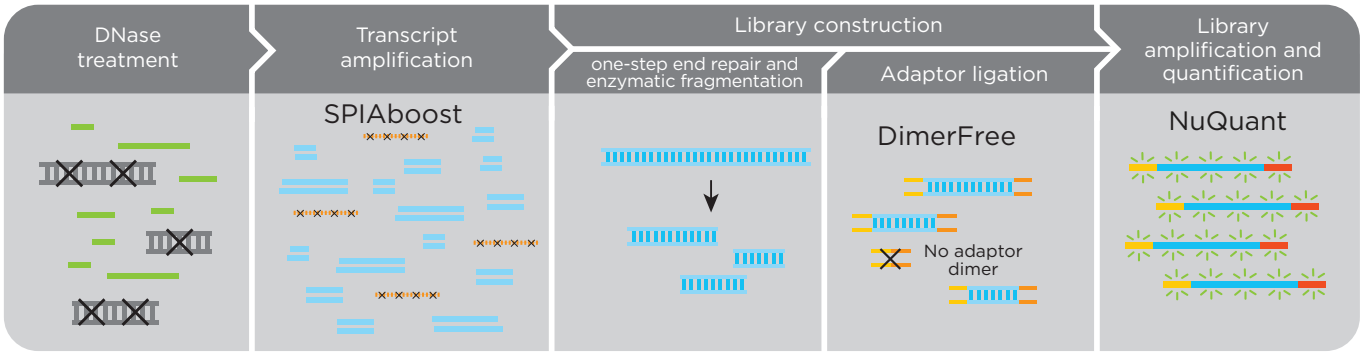

Figure 1: Save time and resources with Revelo RNA-Seq, an one-day sample-to-sequencer workflow optimized for degraded sample types.

Revelo RNA-Seq demonstrates increased viral coverage at low viral titers

To compare viral detection sensitivity at low titers, RNA-Seq libraries were prepared using 500 pg K562 human RNA spiked with synthetic SARS-CoV-2 genome at 100 to 100,000 viral copies. Revelo RNA-Seq libraries show significantly better viral coverage and human rRNA depletion compared to competitor kit. (Illumina NovaSeq® 6000, 20 million (M) reads/sample) (Figures 2 and 3).

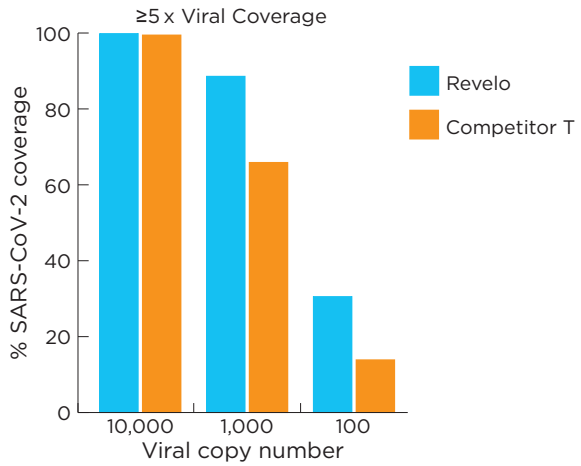

Figure 2: **Revelo RNA-Seq demonstrates increased viral genome coverage at ≥5 x compared to a competitor kit at 100 and 1,000 copies.** The increased detection sensitivity achieved with Revelo RNA-Seq enables access to high-quality data from as little as 100 viral copies and uncovers previously inaccessible information from as little as 500 pg total RNA input. ≥1 x and ≥10 x showed a similar trend of increased viral genome coverage.

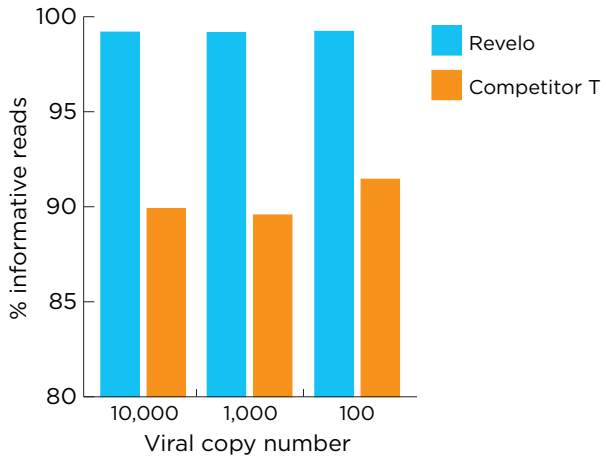

Figure 3: **Revelo RNA-Seq uses proprietary SPIAboost technology for an improved depletion, effectively eliminating unwanted human ribosomal reads (<1% rRNA reads).** Libraries generated using competitor kit show 8-10 % rRNA reads, compared to <1% in Revelo RNA-Seq. This leads to increased informative reads, reduced sequencing costs and simplified data analysis.

Sensitive detection of SARS-CoV-2 and Influenza A from highly degraded nasal swab samples

Revelo RNA-Seq was used to successfully generate high quality libraries from total RNA extracted from nasal swab samples of varying quality and viral copies. RNA samples with RIN values as low as 2.20 and Ct values for SARS-CoV-2 detection ranging from 19 - 32 were processed using Revelo RNA-Seq. Table 1 and Figure 4 show superior sensitivity of Revelo RNA-Seq compared to the competitor kit in detecting SARS-CoV-2 without the need for deeper sequencing. (Illumina MiniSeq®, 1 M reads/sample)

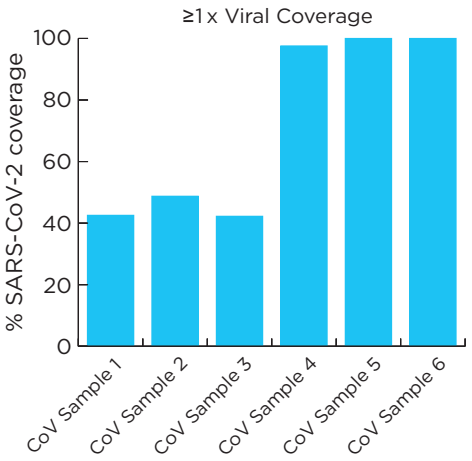

Figure 4: **Revelo RNA-Seq features high-sensitivity detection of viral sequences from as low as ~500 copies per sample** at low read depth of 1 M reads/sample. This exceptional sensitivity leads to a reduction in sequencing costs while still maintaining reliable high quality results from samples of varying quality.

Revelo RNA-Seq is a robust discovery tool for other pathogens such as the Influenza A/H1-2009 strain. The libraries generated from this kit can successfully detect the virus at nearly 100 % viral coverage (≥5 x) at 1 M sequencing read depth.

CoV Sample ID	Viral copies per sample	Avg Ct value	RIN values	Revelo				Competitor T			
				Viral coverage			Viral reads	Viral coverage			Viral reads
				≥1x	≥5x	≥10x		≥1x	≥5x	≥10x	
Sample 1	537	32	2.6	42.55%	24.72%	14.59%	1293	1.33%	0.0%	0.0%	13
Sample 2	647	32	*	48.75%	31.54%	23.21%	2797	0.0%	0.0%	0.0%	5
Sample 3	1052	31	2.5	42.24%	19.37%	8.80%	639	0.48%	0.0%	0.0%	14
Sample 4	17638	30	2.5	97.57%	73.13%	40.07%	1995	4.37%	0.0%	0.0%	37
Sample 5	216827	23	5.9	100.00%	99.99%	99.99%	688987	13.90%	0.0%	0.0%	33
Sample 6	7026072	19	2.4	100.00%	100.00%	99.99%	843342	99.87%	38.57%	5.81%	717

*too low to measure

Table 1: **Revelo RNA-Seq achieves greater detection sensitivity across a range of viral load (500–7 M) compared to the competitor kit.** Competitor kit only detects the virus at higher copy numbers, a noteworthy contrast to Revelo's sensitivity at low copy numbers. Revelo RNA-Seq showed consistent virus detection from samples with varying integrity (RIN values <3.0) and viral load at shallow sequencing depth (1M reads/sample).

Benefits of the Revelo RNA-Seq library preparation kit:

- **Very high sensitivity** – works across a broad dynamic range down to 250 pg to uncover previously inaccessible information at low copy number (as low as ~100 copies at 1M reads)
- **Rapid protocol** – six-and-a-half hour workflow, including library quantification to enables a complete NGS workflow, from RNA sample to data, in under 24 hours
- **Reliable** – consistent results every time, even with low quality samples
- **Co-infection detection** – identify co-infective agents in the same sample without *a priori* knowledge
- **Built-in depletion** – only sequence the transcripts you want and eliminate unwanted human ribosomal RNA to <1% rRNA reads with enhanced SPIAboost technology
- **Built-in quantification** of the NGS library in less than six minutes using any plate reader or a Qubit fluorometer
- **Automation ready** – for high throughput applications, on the DreamPrep NGS workstation

Description	Specs
Sample input	250 pg-10 ng total RNA
rRNA depletion	Human rRNA
Total workflow time	~ 6.5 hours
Multiplexing	Up to 96 single index and 96 UDI
Automation	DreamPrep NGS (Fluent® 780 workstation). Automation on other platforms available
Applications	• RNA-Seq from degraded and mixed samples • RNA virus sequencing • Gene expression • Rare transcript detection • Whole transcriptome profiling
Compatible platforms	Illumina HiSeq®, MiSeq®, NextSeq®, MiniSeq, NovaSeq

Table 2: Revelo RNA-Seq product specifications.

Ordering information

Product Name	Part number	No. of reactions
Revelo RNA-Seq, Human rRNA	30184147	08
Revelo RNA-Seq, Human rRNA	30184149	32
Revelo RNA-Seq, Human rRNA	30184151	96 (Automation fill)
Revelo RNA-Seq, UDI Human rRNA	30184204	96 (Automation fill)

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