LEXOGEN

The RNA Experts

Whole Transcriptome Library Prep Kits • RNA Enrichment and Depletion • RNA Extraction and cDNA Amplification • Single-Cell RNA-Seq • NGS Data Analysis • Resea

Expression Profiling Library Prep Kits • Spike-In RNA Variant Controls • Metabolic RNA Labeling • Service • Small RNA •

Product Catalog

WE ARE THE RNA EXPERTS

COMPANY

Established in 2007, Lexogen is a global leading company in transcriptomics, next-generation sequencing, RNA analysis, and bioinformatics. Lexogen is the leader in 3' mRNA sequencing, a technology proven for its efficiency, its robustness, and its sensitivity.

Lexogen is a global leading company in transcriptomics, next-generation sequencing, RNA analysis, and bioinformatics. Lexogen's portfolio includes innovative kits developed and produced in Vienna, Austria, for true single-cell as well as bulk RNA sequencing, RNA extraction, ribosomal RNA depletion, spike-in RNA variant controls, and nascent RNA labeling for transcriptome-wide analysis of RNA kinetics. Lexogen also provides first-class, fully integrated, customizable NGS services, from experimental design consulting to sample processing and tailored bioinformatics analysis and reporting.

Lexogen is a privately held company headquartered in Vienna, Austria, with a subsidiary in New Hampshire, USA. Lexogen is ISO 9001:2015 and EN ISO 13485:2016 certified for medical device manufacturing.

MISSION

Empower our customers with innovative top quality RNA analysis solutions & support, in order to improve health and well-being for everyone and our planet.

Table of Contents

Product Overview

Sampling

SLAMseq for High-Throughput Kinetic RNA Sequencing

» Explorer Modules
» Kinetics Modules

- Analyze RNA synthesis and degradation (RNA kinetics) in a time-resolved manner.
- Combined with QuantSeq to gain novel insights into the control of gene expression.

RNA/DNA Defender Solution NEW

- Stabilize RNA and DNA in fresh tissue and cell culture samples.
- Postpone RNA or DNA extraction to when more convenient.
- Various storage options: one day at 37 °C, one week at room temperature, one month at 4 °C, or up to one year at -20 °C.

RNA Preparation

TraPR Small RNA Isolation

- Isolation of pure, functional sRNA.
- Fast and easy column-based purification.
- Species independent.

SPLIT RNA Extraction

- Extraction of high-quality, high-purity RNA from a wide range of biological samples, including cell culture, animal and plant tissue, and fluid samples.

RiboCop rRNA Depletion

- rRNA depletion (>99 %) at lowest cost, without off-target effects.
- Suitable for demanding downstream applications (e.g., NGS).
- Available for human, mouse, rat, bacteria and yeast. NEW
- Simultaneous rRNA and globin mRNA depletion for human, mouse, rat.

Poly(A) RNA Selection

- Highly specific poly(A) RNA enrichment.
- Rapid turnaround, automation-friendly, easy scaling.

NGS Library Prep

Expression Profiling

» QuantSeq 3′ mRNA-Seq V2 with UDI NEW

- New and improved PCR system.

» QuantSeq 3′ mRNA-Seq FWD and REV
» QuantSeq-Pool Sample-Barcoded 3′ mRNA-Seq
» QuantSeq-Flex Targeted RNA-Seq

- Cost-efficient gene expression profiling - including low-input, low-quality, and blood samples.
- Unique Molecular Identifiers (UMIs).
- Up to 384 Unique Dual Indices (UDI).
- Easily scalable to 36,864 samples for large screening projects.

» LUTHOR 3′ mRNA-Seq

- True single-cell expression profiling.
- High resolution for ultra-low input.

Whole Transcriptome

» CORALL RNA-Seq V2 NEW

- Fragmentation-free library size adjustment. NEW
- Uniform transcript coverage.
- UMIs included.
- UDIs included.
- Fast (4.5 h) and easy protocol.

» CORALL mRNA-Seq V2 NEW
» CORALL Total RNA-Seq V2 NEW

Small RNA-Seq

- User-friendly workflow, high level of multiplexing (up to 96 unique i7 indices included).
- Optimized for low RNA content samples such as plasma, serum, and urine.

NGS Data Analysis

Demultiplexing and Index Error Correction Tool

QuantSeq Data Analysis

- Automated, in-house data analysis pipeline, including SLAMdunk for SLAMseq data analysis.
- User-friendly, streamlined data analysis.

CORALL Data Analysis

Automated, in-house data analysis pipeline.

- Uses Mix² for accurate transcript abundance estimation.
- User-friendly, streamlined data analysis.

Mix² RNA-Seq Data Analysis Software

- Accurate estimation of isoform concentration with best quantity, quality, and accuracy measures,
- Fast and small memory footprint.
- Identification of positional biases in RNA-Seq data.

cDNA Preparation

TeloPrime Full-Length cDNA Amplification

- Best 5′ cap specificity for full-length cDNA generation.
- Preservation of short and long RNA molecules, suitable for long-read sequencing (e.g. PacBio™ and Oxford Nanopore™).

NGS Controls

Spike-In RNA Controls

» SIRV-Set 1 (Iso Mix E0, E1, E2)
» SIRV-Set 2 (Iso Mix E0)
» SIRV-Set 3 (Iso Mix E0 / ERCC)
» SIRV-Set 4 (Iso Mix E0 / ERCC / long SIRVs)

- Comprehensive design - isoform and abundance complexity.
- Validate, monitor, and compare RNA-Seq experiments and pipelines.

SIRV Suite Spike-In Analysis Software

- Design and evaluate your SIRV experiment, derive RNA-Seq quality measures and compare experiments.

Lexogen NGS Services

Library Preparation

- Flexible options from nucleic acid extraction to data analysis.
- Library preparation solutions tailored to your needs.
- Focus on highest quality data and reproducibility using internal standard controls.
- Excel with low-input and low-quality samples.

Automation and Sequencing

- Automated liquid handling for high-throughput projects.
- From tens to thousands of samples.
- Flexible sequencing platforms, read mode and depth options.

Data Analysis

- Fully integrated data analysis pipelines.
- Data analysis report including standard metrics, and (differential) gene expression.
- Custom design data analyses available.

Library Prep Kits Overview

Kit Selection Criteria	CORALL RNA-Seq			QuantSeq				LUTHOR 3′ mRNA-Seq	Small RNA-Seq	Te-lo-Prime Full-Length cDNA
		+ Poly(A) (CORALL mRNA)	+ RiboCop	QuantSeq 3′ FWD	QuantSeq-Pool 3′	QuantSeq 3′ REV	QuantSeq -Flex			
Application										
Gene Expression Profiling	✓	✓	✓	✓*	✓*	✓	✓	✓	✓	✗
Whole Transcriptome (full-transcript coverage)	✓	✓	✓	✗	✗	✗	✗	✗	✗	✓
Alternative Polyadenylation	✗	✓	✗	✓	✗	✓*	✓	✓	✗	✓
Isoform discovery & quantification	✓	✓*	✓*	✗	✗	✗	✓	✗	✗	✓
Targeted Sequencing	✗	✗	✗	✗	✗	✗	✓*	✗	✗	✓
RiboSeq (ribosomal profiling)	✗	✗	✗	✗	✗	✗	✗	✗	✓*	✗
Transcript (re)annotation	✓	✓	✓	✗	✗	✗	✉	✗	✓ sRNA only	✓
De novo assembly	✓	✓	✓	✗	✗	✗	✗	✗	✗	✓
SLAMseq (kinetic RNA-Seq)	✓	✓	✓	✓	✓	✓	✓	✉	✓	✓
Small RNA (e.g., miRNA) analysis	✗	✗	✗	✗	✗	✗	✗	✗	✓	✗
Long non-coding RNA analysis	✓	✓ poly(A) only	✓*	✗	✗	✗	✓	✗	✗	✓pA
RNA Input Type										
Human or animal cells, protoplasts and lysates	✗	✗	✗	✗	✗	✗	✗	✓	✗	✗
Total RNA	✓	✓*	✓*	✓*	✓*	✓	✓	✓	✓	✓
Poly(A)+ RNA (≙ mRNA)	✓*	✗	✓	✓	✓	✉	✉	✉	✗	✓
rRNA depleted RNA	✓*	✉	✗	✉	✉	✉	✉	✉	✗	✉
Small RNA	✗	✗	✗	✗	✗	✗	✗	✗	✓	✗
High quality (RIN ≥8)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Moderate quality (RIN 5 - 8)	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗
Highly degraded (RIN <5; DV_{200} <50 %)	✓	✗	✓	✓M	✉	✓M	✓M	✗	✓	✗
Low Input <100 pg	✗	✗	✗	✗	✗	✗	✗	✓	✗	✗
Organism										
Human, mouse, rat	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Other animals	✓	✓	✉	✓	✓	✓	✓	✓	✓	✓
Plant	✓	✓	✗	✓	✓	✓	✓	✓	✉	✓
Yeast	✓	✓	✓	✓	✓	✓	✓	✓	✉	✓
Bacteria	✓	✗	✓	✗	✗	✗	✓	✗	✓	✉
Virus	✓	✓pA	✉	✓pA	✓pA	✓pA	✓	✓pA	✉	✓pA
Sample Type (other sample types will also be compatible for most kits, please contact support@lexogen.com for further information)										
Tissues	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cells (cell lines, primary, sorted cells, etc.)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Blood	✓	✓	✓	✓	✓	✓	✓	✓	✓	✉
Liquid biopsy (CSF, serum, plasma, urine)	✉	✗	✉	✉	✉	✉	✉	✓	✓*	✗
FFPE / Biobank samples	✓	✗	✓	✓M	✉	✓M	✓M	✗	✓	✗
Exosomes	✉	✉	✉	✉	✉	✉	✉	✓	✓*	✗
Recommended / compatible sequencing platforms / formats										
Illumina*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Single-read	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓
Paired-end	✓	✓	✓	✗	✓	✓	✓	✗	✗	✓
Additional Options										
Unique Dual Indexing (UDI) available	included	included	included	✓	✓	✓	✓	✓	✗	✗
Automation-compatible	✓	✓	✓	✓	✉	✓	✓	✉	✗	✗
UMIs available	included	included	included	✓	included	✗	✓	✗	✗	✉
Data Analysis pipeline available	✓	✓	✓	included	✓	included	✉	✓	✓	✓
Service available	✓	✓	✓	✓	✓	✓	✉	✓	✓	✗
Spike-In control recommendation	SIRV-Set 1/2/3			SIRV-Set 3			✗	SIRV-Set 3	✗	SIRV-Set 1/2/3/4 ✉

✓* Optimal Choice
✓ Yes
✉ For more information please contact support@lexogen.com.
* Contact us at support@lexogen.com for information on compatibility with sequencing platforms

✓pA Possible only if a poly(A) tail (and a 5′ cap for TeloPrime) is present or with prior poly-adenylation
✓M Protocol modifications apply
✗ Not possible or not useful

Customer testimonials

“We can support our researchers very well using Lexogen’s **CORALL** RNA-Seq Library preparation due to its excellent performance on low starting RNA input, combined with unique molecular identifiers and 12 nt unique dual indexes. We find the protocol to be straight-forward and can generate very consistent libraries perfect for standard RNA-Seq projects. In addition, CORALL delivers great results for degraded RNA or FFPE samples with minimal adaptations in very few protocol steps. **CORALL RNA-Seq V2**, the latest version of the kit, is even better because we now have the choice to generate libraries with longer insert sizes compatible with sequencing at 2 x 150 bp. This is very useful, for example, for RNA-Seq for non-model organisms, for improved mapping for demanding projects, and for projects characterizing isoforms.”

Dr. Pamela Nicholson
Manager Next Generation Sequencing Platform
University of Bern, Switzerland

“We have extensively tested Lexogen’s **RiboCop META** rRNA depletion kit on bacteria for transcriptomics. Due to a research focus in infection biology at our university, we are working on RNA sequencing projects with various types of bacteria. The RiboCop META is a versatile tool with very good depletion results for all types of bacteria tested so far. Therefore, we would like to continue to use it for future transcriptome profiling projects.”

Tobias Heckel
Core Unit Systems Medicine (NGS & Bioinformatics),
University of Würzburg, Germany

“Throughout my career, leading genomic projects and departments, I have always been a fan of Lexogen chemistry. When I finally decided to co-found a genomics company, I was delighted to hear that Lexogen had a services arm, and signed up immediately. We work with extremely challenging samples, from biobanked FFPEs to micro-organ samples. At every step Lexogen has worked closely with us, even helping to optimise our protocols, to ensure the best possible results.”

Dr. Quin Wills
Co-founder and CSO, Ochre Bio

“We have established **QuantSeq-Pool** on lysates in our facility and are very happy with its performance. In our hands, QuantSeq-Pool convinced with very low technical variability which boosts the performance for Differential Gene Expression analysis. QuantSeq-Pool now offers us a robust and time-saving procedure that we can scale up to 1,000s of samples for our customer projects.”

Pieter Mestdagh
Biogazelle, Belgium

“Using the **QuantSeq 3’ mRNA-Seq library prep kits,** we were able to multiplex >40 samples per sequencing lane and obtain between 2 to 5 million reads per sample. This enabled us to analyze numerous different strains with various exosome and roadblocking factors inactivated, showing that inactivating roadblocks shifted the window of NNS termination downstream.”

Kevin Roy
Lars Steinmetz Lab, Department of Genetics,
Stanford University School of Medicine, USA

“Transcriptional profiling is one of our key approaches to study and understand the molecular mechanism of action of cancer drugs. We have made excellent experiences with the **CORALL** kit both in terms of experimental feasibility but also data quality. Through integration with synthetic spike-ins SIRVs, It was particularly helpful when testing drugs that affect overall transcriptional output and thus cause a global decrease in mRNA levels.”

Georg Winter, PhD, Principal Investigator
CeMM, the Research Center for Molecular Medicine of the
Austrian Academy of Sciences, Austria

“The **TraPR** column greatly improve my preparation of sRNA libraries, as it speeds up the otherwise more laborious sRNA preparation. Moreover, by using TraPR I could increase the number of samples loaded per lane of the sequencing flow cell, without decreasing the amount of reads per sample.”

Heinrich Bente
Mittelsten Scheid Group, GMI - Gregor Mendel Institute of
Molecular Plant Biology, Austria

QuantSeq Expression Profiling Library Prep Kits

The QuantSeq 3′ mRNA-Seq Kits generate Illumina compatible NGS libraries of sequences close to the 3′ ends of poly(A) RNA. Only one fragment per transcript is generated, thereby enabling accurate gene expression quantification while saving sequencing depth and allowing for a high level of multiplexing.

Advantages

- **NEW!** QuantSeq 3′ mRNA-Seq V2 with UDI, with new and improved PCR system
- Cost-efficient and automation-friendly genome-wide analysis of gene expression
- Saving sequencing depth by generating only one fragment per transcript
- Fast and easy all-in-one protocol: from total RNA to ready-to-sequence libraries in less than 4.5 hours
- Suitable for low-input (1 ng total RNA) and low-quality RNA (including FFPE samples)
- Complimentary user-friendly data analysis pipeline for non-bioinformaticians
- UMIs for detection of PCR duplicates available
- Efficient analysis of blood samples with optional Globin Block
- Bundles with up to 384 Unique Dual Indices (UDIs) available

Workflow

QuantSeq generates only one fragment per transcript. Oligo(dT) priming in the reverse transcription step ensures poly(A) specificity (mRNA). Second strand synthesis is initiated by random priming. As the insert size is determined by the distance between the second strand synthesis primer and the poly(A) tail, no additional RNA fragmentation is required. In the subsequent PCR amplification up to 384 UDIs can be introduced, enabling a high degree of multiplexing.

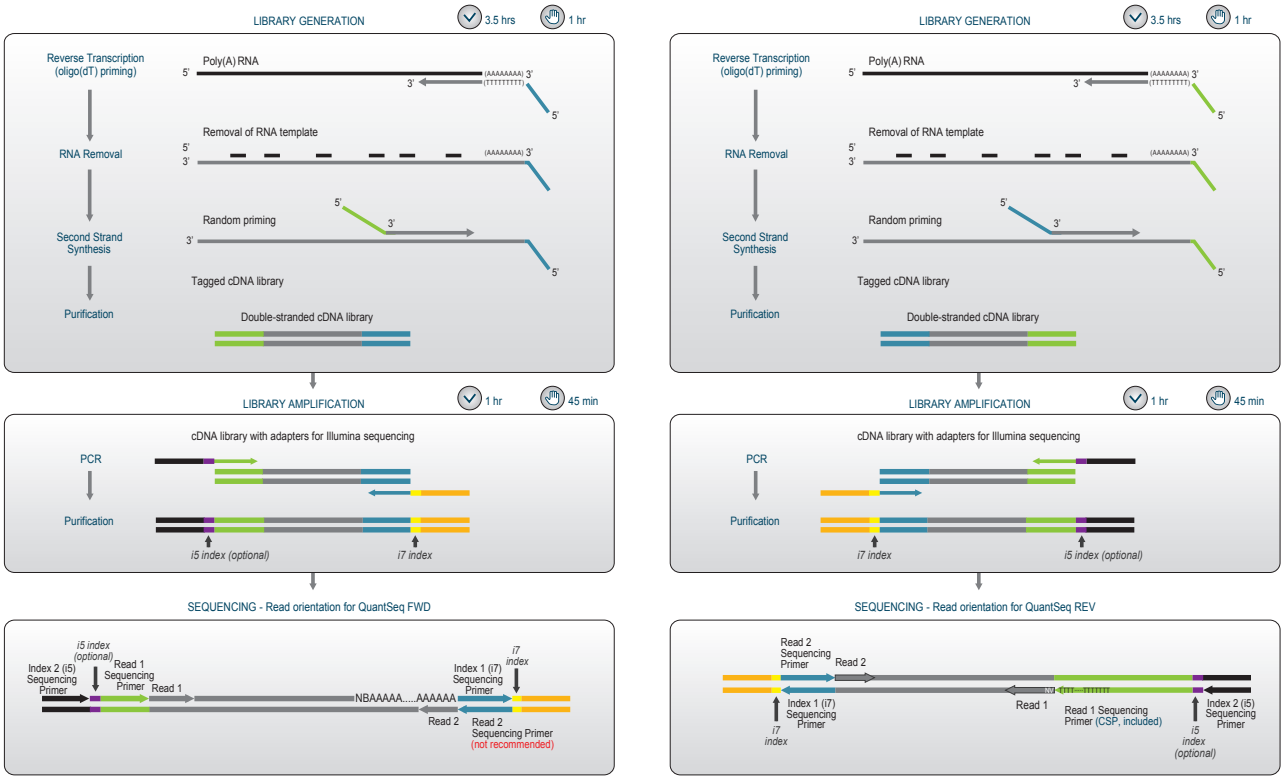

Figure 1 | Schematic overview of QuantSeq 3′ mRNA-Seq FWD (left panel) and QuantSeq 3′ mRNA-Seq REV (right panel) Library Preparation workflow. Left Panel) Read 1 (sequencing starts from the green P5 adapter part) of the QuantSeq FWD Kit corresponds to the mRNA sequence close to the 3′ end, allowing for an economical NGS run with Illumina standard sequencing primers. **Right Panel)** In the QuantSeq REV kit the position of adapters for Read 1 and Read 2 are switched, enabling the exact determination of the transcription end-site in Read 1. A Custom Sequencing Primer (CSP, included in the kit) is required for QuantSeq REV Read 1 sequencing.

Two versions of QuantSeq 3′ mRNA-Seq for Illumina are available, differing in the final positioning of Read 1. In QuantSeq Forward (FWD) NGS reads are generated towards the poly(A) tail (Fig. 1, left). This version is the recommended format for performing gene expression analysis. If the research focus is on the exact 3′ end of transcripts or if paired-end sequencing is intended, QuantSeq Reverse (REV) is recommended. Here, Read 1 starts directly at the poly(A) tail (Fig. 1, right). QuantSeq kits are ideally combined with Lexogen UDI 12 nt Unique Dual Indexing System (p.36) for maximized preservation of reads. QuantSeq FWD with UDI is now available in "V2" format with an improved library amplification PCR system.

Performance

QuantSeq Saves Sequencing Depth

One major benefit of QuantSeq can be visualized by plotting the relative coverage across the normalized transcript length, as shown for the FDA Sequencing Quality Control (SEQC) standard samples A and B reference RNAs spiked-in with the ERCC external RNA control ExFold Spike-In Mixes 1 and 2 [1, 2] (Fig. 2). Standard mRNA-Seq distributes reads across the entire length of transcripts, whereas QuantSeq (by design a 3′ mRNA-Seq) covers the very 3′ end of transcripts. Therefore, when the RNA-Seq experiment focuses on studying differential gene expression only, QuantSeq is a perfect choice. In this example, QuantSeq saves more than 90 % of sequencing depth while still determining gene expression accurately (Fig. 2). The additional sequencing space gained by focusing on the 3′ end can be used for a higher degree of multiplexing (more libraries in the same flow cell).

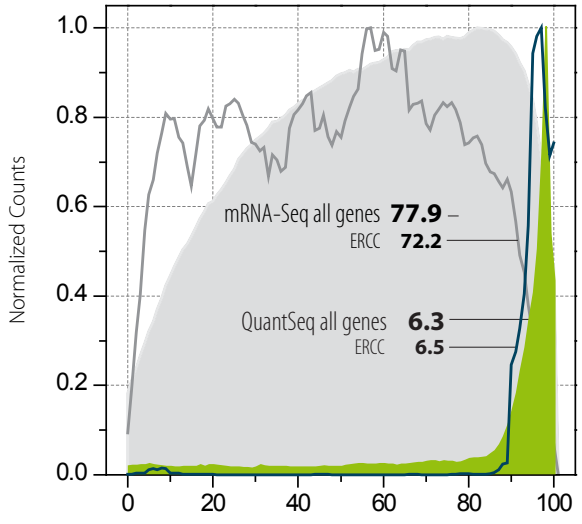

Figure 2 | Coverage versus normalized transcript length in QuantSeq and standard mRNA-Seq. QuantSeq libraries were prepared from reference RNA samples and compared with an mRNA-Seq data set available in the Association of Biomolecular Resource Facilities (ABRF) NGS study [3]. RSeQC-derived coverage is plotted for all transcripts (areas) and the ERCC mix only (lines), for QuantSeq (colored) and mRNA-Seq (gray). Numbers give the Area Under the Curve (AUC) values as a measure for sequence coverage.

Strand-Specific Mapping of 3′ Ends

Strand-specificity can be assessed independent of any genomic annotation using the ERCC external RNA control ExFold Spike-In Mixes since the spike-in transcripts exist only in sense direction. QuantSeq shows >99.9 % strandedness in all cases, while the two assessed mRNA-Seq SEQC data sets are stranded to only 93.4 % and 97.8 %, respectively [3]. This reduced experimental noise enables accurate detection and quantification of antisense transcripts.

QuantSeq Quantifies Proportionally Across Six Orders of Magnitude

QuantSeq shows very high input-output correlation and accuracy in gene expression determination, as assessed in a linear model and by Spearman correlation (Fig. 3).

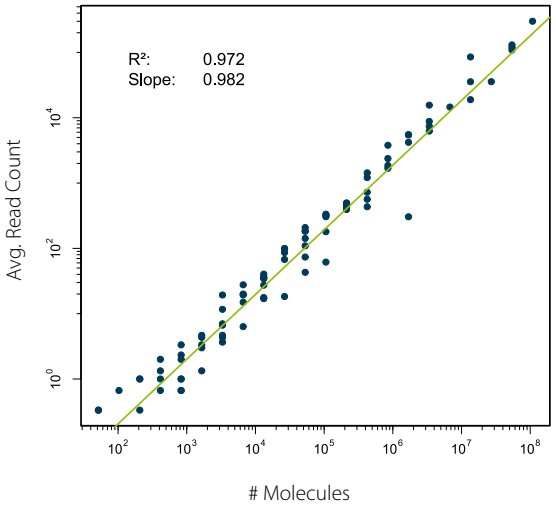

Figure 3 | QuantSeq-derived ERCC read counts correlate exceedingly well with the documented input. ERCC spike-in transcripts' average read count plotted against input molecules.

Performance with FFPE Samples

QuantSeq enables accurate gene expression quantification independently of the RNA quality. Since it only generates one fragment at the transcript's 3′ end, QuantSeq successfully produces libraries even from degraded RNA, such as RNA derived from FFPE samples. Standard mRNA-Seq protocols aim to cover the whole transcript, but will result in a heavy 3′ bias when used on degraded RNA input. Therefore, QuantSeq 3′ mRNA-Seq is an efficient tool to generate NGS libraries from low-quality samples compared to other mRNA-Seq protocols using poly(A) selection.

Lexogen provides a dedicated protocol for low RNA amounts (<10 ng), low RNA quality, and FFPE-derived RNA input. QuantSeq libraries can be successfully generated from FFPE-derived RNA with a DV_{200} from as low as 9 % (Fig. 5). Resulting libraries show a smooth size distribution with no visible linker-linker by-products and a shift towards shorter fragments (Fig. 4).

References
1. The External RNA Controls Consortium. (2005) The External RNA Controls Consortium: a progress report. Nature Methods 2:731-734 2. DOI:10.1038/nmeth1005-731.
2. Ambion. ERCC RNA Spike-In Control Mixes. Cat. No. 4456740, 4456739.
3. Li, S., et al. (2014) Multi-platform assessment of transcriptome pro ling using RNA-seq in the ABRF next-generation sequencing study. Nature Biotechnology. 32, 915–925. DOI: 10.1038/nbt.2972.
4. Munro, S.A., et al. (2014) Assessing technical performance in differential gene expression experiments with external spike-in RNA control ratio mixtures. Nature Communications 5, 5125. DOI:10.1038/ncomms6125

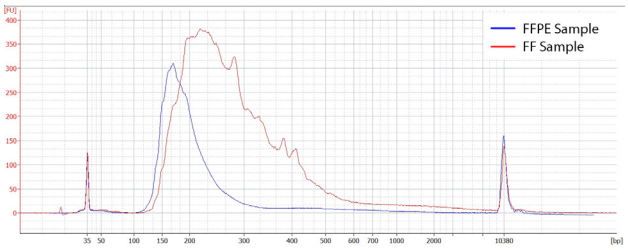

Figure 4 | Bioanalyzer traces of QuantSeq 3′ mRNA-Seq FWD libraries from FFPE (blue) or fresh frozen (FF) (red) samples. A xenograft of the MOLP-8 human tumor cell line was split, and processed either as fresh frozen cryo-block or embedded FFPE material, leading to different RNA qualities (FF sample: RNA Integrity Number (RIN) of 8.3, FFPE sample: distribution value of RNA fragments >200 nucleotides (DV_{200}) of 87 %, RIN of 2.8). Libraries were generated with QuantSeq 3′ mRNA-Seq FWD using 50 ng total RNA input. For the FFPE sample the protocol recommendations for low-quality RNA input were followed, for the FF sample the standard protocol was applied. The libraries were analyzed on a Bioanalyzer 2100 HS DNA chip. The average library size is 204 bp (FFPE) and 286 bp (FF sample), respectively.

When QuantSeq 3′ mRNA-Seq V2 with UDI kit is used for library preparation on samples from differently stored and prepared mouse spleen tissue, i.e., FFPE samples or fresh frozen (FF), consistent gene counts and correlations are obtained, as seen when comparing sequencing data (Fig. 5).

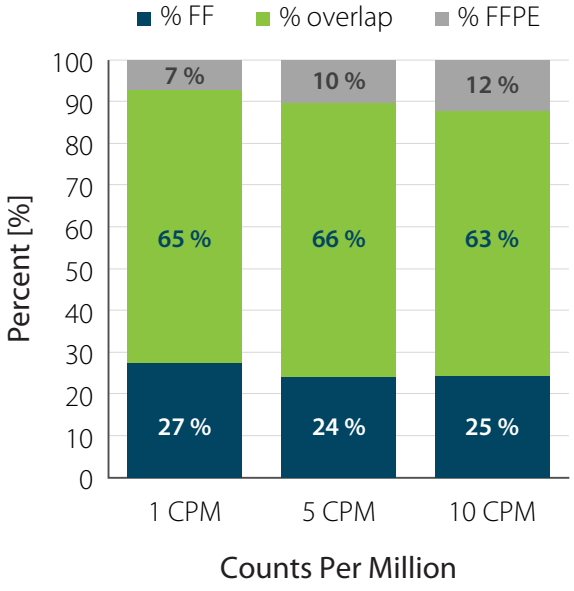

Figure 5 | Fresh Frozen (FF) vs. FFPE correlation with QuantSeq. Sequencing data obtained with 10 ng input from two different mouse spleen samples, 1 M reads, data shown for 1 CPM, 5 CPM, and 10 CPM (from left to right). RIN values: 3.7 (FF) and 2.9 (FFPE); DV_{200} values: 90 % (FF) and 9 % (FFPE). CPM: Counts Per Million.

In addition, gene expression correlation between libraries derived from FFPE and FF RNA is high (R^2 0.75) and indicates that QuantSeq performs consistently well on samples of different RNA quality (Fig. 6).

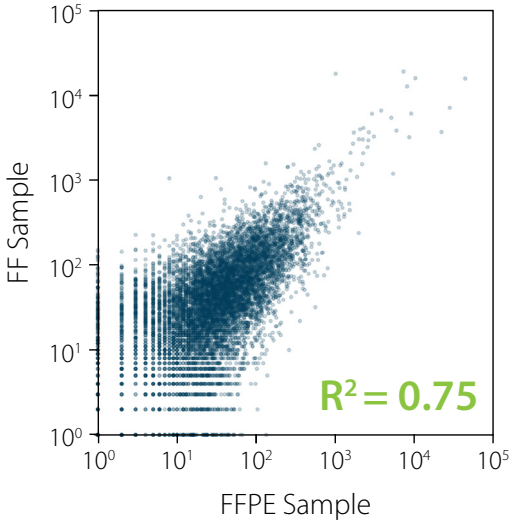

Figure 6 | Correlation of gene counts of FFPE and FF samples.

QuantSeq Automation

Automation saves hands-on time, maximizes throughput, and avoids pipetting and sample tracking errors. Lexogen supports implementation of automated QuantSeq protocols for 3′ mRNA-Seq Library Preparation.

QuantSeq has been successfully implemented on the following platforms / liquid handlers: Perkin Elmer: Sciclone®/ Zephyr®; Hamilton: Microlab STAR / STARlet; Agilent: NGS Workstation (NGS Bravo Option B); Beckman Coulter: Biomek FXP, Biomek i5, Biomek i7; Eppendorf: EpMotion® 5075; Opentrons® OT-2. QuantSeq automation on other platforms may also be possible.

The two most important parameters when automating a protocol are volume optimization and script compatibility. If it comes to the implementation of Lexogen-manufactured kits on liquid handlers, Lexogen will help you find the best solution, tailored to your needs. We recommend contacting the respective vendor's support team for hardware or software issues linked to the original liquid handling instrument.

If you are interested in automation or need more information on data analysis, please get in touch with us at support@lexogen.com.

For high-throughput applications without automation on a liquid handler, we recommend QuantSeq-Pool Expression Profiling Kit (p.11).

Performance with Blood RNA - Globin Block Modules

Seamless Globin Block during QuantSeq Library Prep

Blood is a highly informative, accessible tissue for biological and disease-related discovery. Globin mRNAs (HBA1, HBA2, HBB) account for 50 - 80 % of total RNA in blood and thus sequester the majority of sequencing reads, severely limiting gene detection and quantification sensitivity.

Existing globin depletion methods require RNA pre-processing, high amounts of RNA, and incur additional costs. Lexogen's Globin Block Modules for QuantSeq enable globin depletion during the library prep itself. Lower input amounts starting from 50 ng of total RNA from blood can be used and no additional pre-processing or protocol steps are required.

The Globin Block Modules are specifically designed for use with the QuantSeq 3' mRNA-Seq Library Prep Kits for Illumina. Globin Blockers are introduced during the QuantSeq protocol through a simple solution exchange. The Globin Blockers bind to globin first strand cDNA and prevent the generation of double-stranded cDNA from globin mRNAs during second strand synthesis (see also Fig. 1). Globin Block is compatible with automation systems and is available for human and pig samples. For use with other species contact us at support@lexogen.com.

Globin Block Reduces Globin Mapped Reads to 0.7 %

Libraries prepared with Globin Block Modules show significant reduction of total globin mapped read percentages, compared to libraries prepared with standard QuantSeq (Fig. 9). Total globin mapped read percentages drop down to 0.7 % for leukocyte-enriched blood, and 9.7 % for whole blood in +Globin Block libraries.

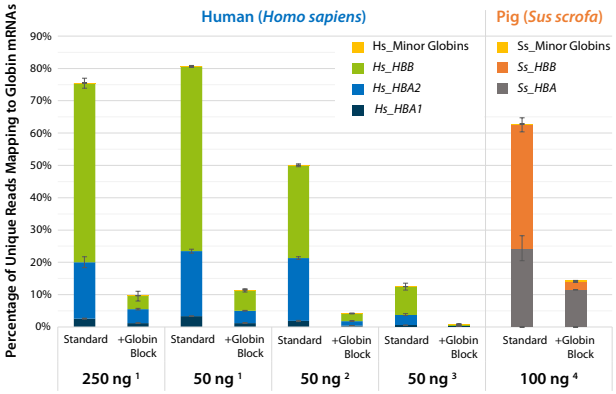

Figure 9 | Percentage of reads uniquely mapping to human and pig globin mRNAs. Libraries were prepared from whole blood RNA with the Standard QuantSeq FWD protocol, versus QuantSeq +Globin Block. [1] SPLIT RNA Extraction Kit without red blood cell lysis (Lexogen), [2] PAXgene® Blood RNA Kit (Qiagen, includes red blood cell lysis). [3] SPLIT RNA Extraction Kit with red blood cell lysis, [4] Preserved Blood RNA Purification Kit I (Norgen Biotek, without red blood cell lysis).

Enhanced Gene Detection in Globin Block Libraries

Globin Block increases gene detection rate compared to standard QuantSeq library prep (Fig. 10). The majority of genes are similarly detected in Standard and +Globin Block libraries, while up to 3,690 more genes are uniquely detected in +Globin Block libraries. In contrast, only very few transcripts are uniquely detected in Standard QuantSeq libraries.

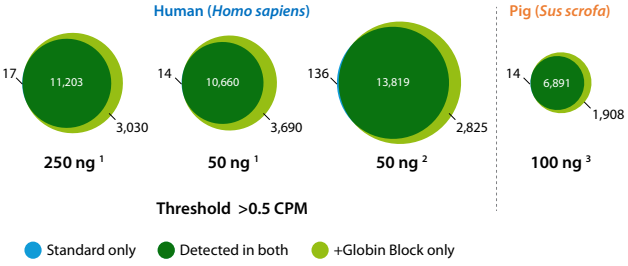

Figure 10 | Increased gene detection in human and pig blood QuantSeq libraries using Globin Block. Libraries were prepared from whole blood RNA with the Standard QuantSeq FWD protocol or QuantSeq +Globin Block. Number of detected genes was calculated from CPM-normalized read counts (threshold >0.5). Gene lists were compared to determine the overlap (dark green), versus genes uniquely detected in Standard (blue) or +Globin Block (light green) libraries. [1] SPLIT RNA Extraction Kit without red blood cell lysis (Lexogen), [2] PAXgene® Blood RNA Kit (Qiagen, includes red blood cell lysis). [3] Preserved Blood RNA Purification Kit I (Norgen Biotek, without red blood cell lysis). CPM: Counts Per Million.

Automated QuantSeq Data Analysis

Users of all QuantSeq 3' mRNA-Seq Library Prep Kits receive a code for free data analysis including differential expression (DE) analysis on Lexogen's web-based data analysis platform.
For other QuantSeq products' data analysis solutions contact us at support@lexogen.com.

Find out more about the complementary QuantSeq data analysis pipeline - for any user even without bioinformatics background at:
www.lexogen.com/lexogen-data-analysis-solutions

Analysis of QuantSeq data is also implemented on several third-party data analysis platforms, such as the ROSALIND™ platform, Partek Flow software and open-source data analysis tool Chipster.

Associated Products

- Lexogen UDI 12 nt Unique Dual Indexing System (p.36)
- Globin Block Modules for QuantSeq (p.38)
- UMI Second Strand Synthesis Module for QuantSeq FWD (p.38)
- SPLIT RNA Extraction Kits (p.31)
- SIRVs - Spike-In RNA Variant Controls (p.32)
- PCR Add-on Kit for Illumina (p.38)
- Purification Module with Magnetic Beads (p.39)
- SLAMseq Kits for High-Throughput Kinetic RNA Sequencing (p.22)

This product is available as part of the Lexogen NGS Services.
For details, contact our Services department:
services@lexogen.com.

QuantSeq-Pool Expression Profiling Kit

The QuantSeq-Pool Sample-Barcoded 3′ mRNA-Seq Library Prep Kit is the optimal solution for gene expression profiling for large screening projects using sample barcoding, early pooling, and batch processing of up to 96 samples in one reaction providing a workflow that is easily scalable for multiplexing up to 36,864 samples.

Advantages

- Cost-efficient gene expression analysis method for screening projects
- Early pooling and batch processing of up to 96 samples save time and consumables, and increase robustness and reproducibility
- Easily scalable from a few to 36,864 samples thanks to triple indexing (addition of 12 nt UDI)
- UMIs included to eliminate PCR duplicates
- Simple data analysis by counting mapped reads to calculate gene expression

Workflow

QuantSeq-Pool is based on the QuantSeq FWD method and generates one fragment per transcript from the 3′ end. In the first step, the Read 1 linker (blue), Unique Molecular Identifiers (UMIs, red), and an i1 sample-barcode (light blue) are introduced (Fig. 1). After reverse transcription, up to 96 individually tagged samples can be combined by pooling. All further reactions are carried out in batch on the combined samples saving time, effort, and consumables. Optionally, up to 384 UDIs can be introduced in the final PCR to increase multiplexing capacity, enabling multiplexing of up to 36,864 samples.

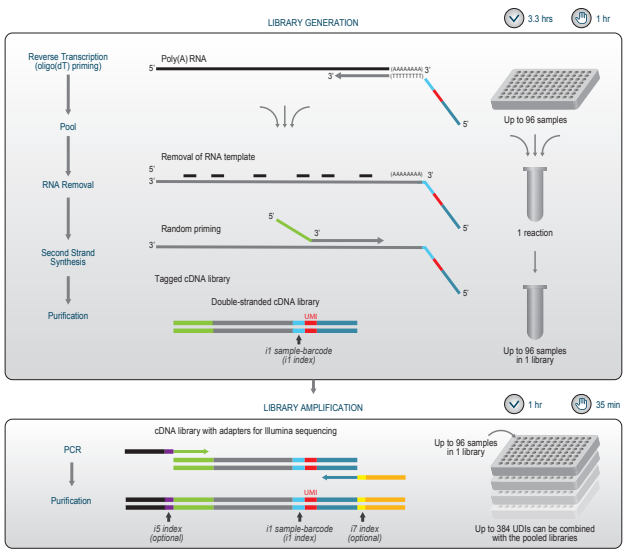

Figure 1 | Schematic overview of the QuantSeq-Pool library preparation workflow.

Performance

High-throughput screening projects require robust and reliable gene expression profiling also at low read depth. QuantSeq-Pool reliably detects 7,500 to 9,000 highly expressed genes at very shallow read depths of 100 K to 1 M reads per sample.

QuantSeq-Pool Saves Time and Consumables

QuantSeq-Pool enables completion of the RNA-to-sequencing workflow for 96 samples in ~5.5 hours. Sample barcoding in the first step followed by pooling of up to 96 samples and batch processing saves handling time and consumables and eliminates the need for quality control (QC), quantification, and equimolar pooling of individual libraries to generate the final lane mix for sequencing.

QuantSeq-Pool vs QuantSeq Decision

QuantSeq-Pool combines the benefits of the well-established QuantSeq methodology with sample-barcoding and early pooling. Figure 2 gives an overview over the factors that contribute to the decision, whether QuantSeq or QuantSeq-Pool is the most suited tool for your application.

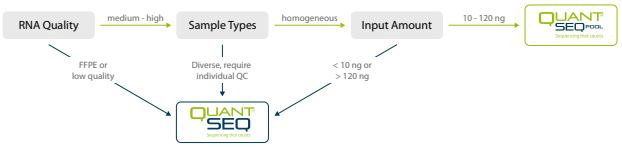

Figure 2 | Overview over QuantSeq vs QuantSeq-Pool decision parameters.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- SPLIT RNA Extraction Kits (p.31)
- Lexogen UDI 12 nt Unique Dual Indexing System (p.36)
- SIRVs - Spike-In RNA Variant Controls (p.32)

This product is available as part of the Lexogen NGS Services.
For details, contact our Services department: services@lexogen.com.

Find more information about this product at its web page:

QuantSeq-Flex Targeted RNA-Seq Library Prep Kits

The QuantSeq-Flex RNA-Seq Kit enables tailored RNA-Seq library preparations by employing custom-designed primers for first and/or second strand synthesis. Maintaining the QuantSeq principle of generating one read per transcript, the kit empowers fully flexible targeted sequencing and the generation of custom RNA-Seq panels.

Advantages

- Flexible kit for generating RNA-Seq panels and targeted sequencing
- Multiplex >100 custom primers per reaction
- Accurate gene expression quantification with minimal number of reads
- Identification of known and unknown fusion transcripts
- 4.5 hours from total RNA to ready-to-sequence libraries
- 9,216 dual indexing and 384 unique dual indexing combinations available

Workflow

With QuantSeq-Flex Targeted RNA-Seq, four different library types can be generated depending on the combination of primers used:

1) Oligo(dT) priming for reverse transcription, random priming for second strand synthesis (QuantSeq 3′ mRNA-Seq FWD).

2) Oligo(dT) priming for reverse transcription, target-specific priming for second strand synthesis (targeted 3′ mRNA-Seq).

3) Target-specific priming for reverse transcription, random priming for second strand synthesis (targeted RNA-Seq, allows for identification of novel fusions).

4) Target-specific priming for both first and second strand synthesis (targeted RNA-Seq, only known targets detected).

Figure 1 | Schematic overview of the QuantSeq-Flex Targeted RNA-Seq Library Preparation workflow. The reverse transcription reaction can be primed either using an oligo(dT) primer (included in the kit) or a target-specific primer (not included, custom designed). Second strand synthesis can be initiated by either random priming (included in the kit) or by using a target-specific primer (not included, custom designed). The RNA removal step is only required for random primed second strand synthesis and is omitted when using target-specific primers. Depending on the combination of different priming options four different libraries can be generated. Sequencing starts from the P5 adapter (green) with Read 1 corresponding to the RNA sequence.

Performance

Examples for target-specific priming are depicted in Figure 2. Primers were designed against *BRAF*, *ERBB2*, and *KRAS* genes and used with QuantSeq-Flex on total RNA derived from K562 cells. Figure 3 shows an RNA input amount series (500 ng, 100 ng, and 10 ng) for the detection of *BCR-ABL* fusion transcripts.

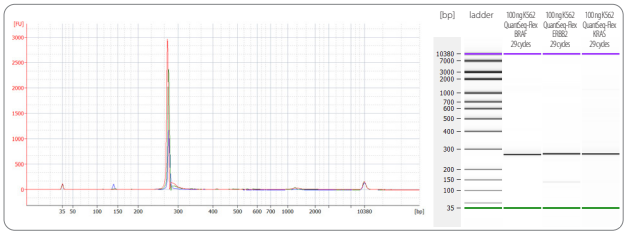

Figure 2 | Bioanalyzer traces of QuantSeq-Flex libraries with targeting in first and second strand synthesis. Amplicons were amplified from *BRAF* (serine / threonine-protein kinase B-raf, red trace), *ERBB2* (receptor tyrosine-protein kinase erbB-2, blue trace), and *KRAS* (proto-oncogene, GTPase, green trace) transcripts.

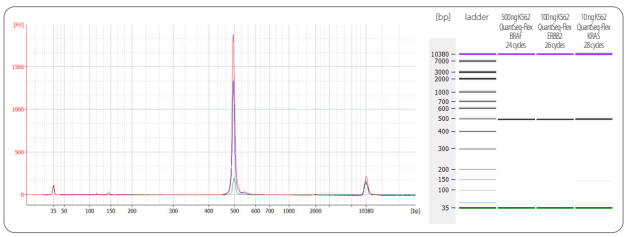

Figure 3 | Bioanalyzer traces of QuantSeq-Flex libraries with target-specific primers for BCR-ABL fusion transcripts. Input series with 10 ng, 100 ng, and 500 ng total RNA.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- Lexogen UDI 12 nt Unique Dual Indexing System (p.36)

Find more information about this product at its web page:

This product is available as part of the Lexogen NGS Services. For details, contact our Services department: services@lexogen.com.

LUTHOR 3′ mRNA-Seq Library Prep Kit

LUTHOR combines a direct RNA amplification technology with a highly efficient, one-step 3′ library preparation for RNA-Seq from purified ultra-low input RNA and single cells with unprecedented sensitivity and reproducibility.

Advantages

- Unlock the true variability of singularized cells with the first comprehensive high-resolution single-cell 3′ mRNA-Seq library prep
- Optimized for individual cells, cell suspensions (1 – 100 cells), and ultra-low input RNA (~10 pg to 1 ng)
- Proprietary THOR Technology for RNA amplification directly from the original mRNA molecule
- Unparalleled sensitivity and reproducibility
- High-quality results even from challenging or degradation-prone sample types
- Compatible with Lexogen UDI 12 nt Unique Dual Indexing Sets for maximized sequencing output
- Time-efficient and straight-forward data analysis for precise gene expression quantification

Workflow

LUTHOR 3′ mRNA-Seq library generation is initiated by RNA amplification using THOR (T7 High-resolution Original RNA) Amplification Technology. THOR generates RNA copies directly from the endogenous mRNA template in a linear manner (Fig. 1). In a highly efficient process, the original mRNA is fused to a T7 promoter which is required for amplification. Then, *in vitro* transcription templated by the mRNA - Promoter fusion molecule generates antisense RNA copies. These copies are void of the promoter sequence. Therefore, only the original mRNA molecule serves as template and is amplified repeatedly. Following RNA amplification, 3′ libraries are generated using a highly-efficient one-step library conversion which results in a single-stranded cDNA library with partial Illumina-compatible adapters.

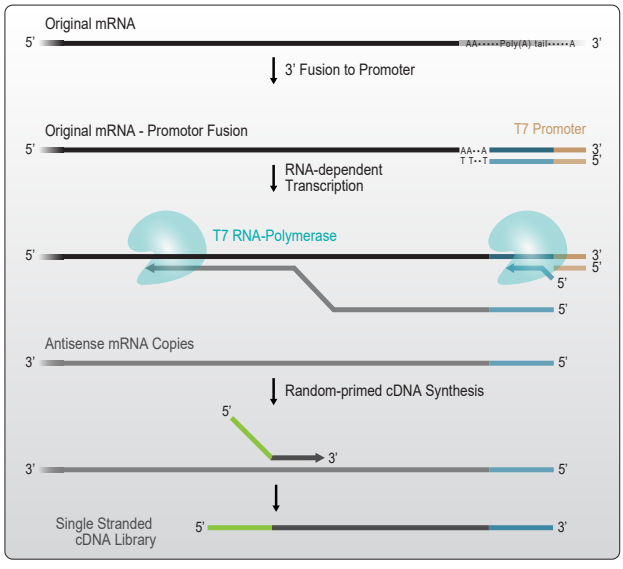

Figure 1 | THOR Amplification and RNA-Seq library template generation.

During the subsequent PCR step, the second strand is generated, and the cDNA is amplified. Furthermore, i7 and i5 indices are introduced and complete adapter sequences for cluster generation on Illumina instruments are added. LUTHOR is designed for use with Lexogen's UDI 12 nt Unique Dual Indexing Sets. These sets are available with up to 384 pre-mixed i5 / i7 Unique Dual Indices (UDIs) and offer superior index error correction capacity for maximized sequencing output (p.36).

Performance

LUTHOR Generates High Quality Results Even from Challenging Sample Types

LUTHOR 3′ mRNA-Seq reliably represents endogenous mRNA composition and efficiently excludes ribosomal rRNAs focusing sequencing reads on coding sequences (Fig. 2).

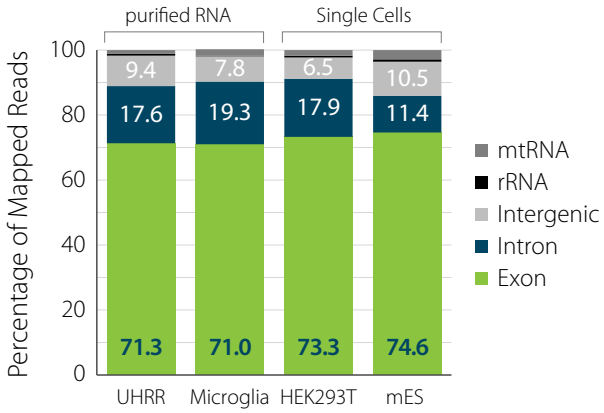

Figure 2 | Feature distribution of uniquely mapped reads for LUTHOR 3′ mRNA-Seq. The majority of LUTHOR reads generated from 100 pg UHRR and murine microglia RNA, or single HEK293T and mES cells map to exonic sequences.

Unparalleled Sensitivity

LUTHOR 3' mRNA-Seq offers unprecedented sensitivity for ultra-low input RNA and single cells. LUTHOR reliably detects ~13,000 to 16,000 genes from ultra-low input RNA (10 - 100 pg Universal Human Reference RNA, UHRR) at 1 M reads / sample (Fig. 3 A). For one single HEK293T cell, ~11,000 - 12,500 genes are detected. A much smaller single mouse embryonic stem (mES) cell yields ~9,500 - 10,500 detected genes, and ~6,000 - 7,000 genes are detected per single *Drosophila* S2 cell (Fig. 3 B).

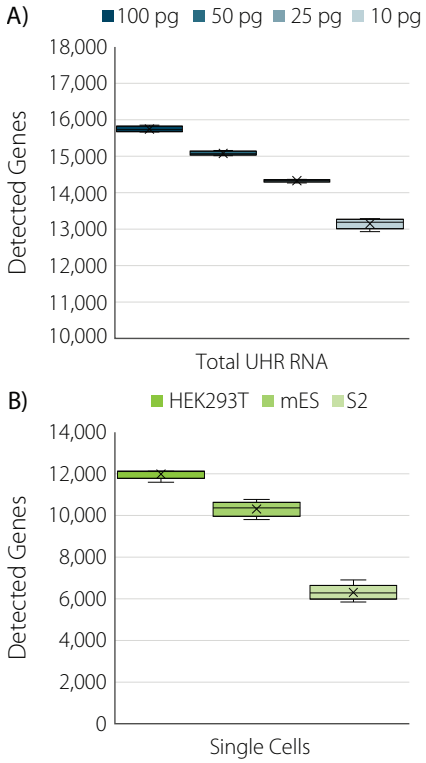

Figure 3 | LUTHOR 3' mRNA-Seq enables highly sensitive and consistent gene detection already at low sequencing depth. Gene detection was assessed at 1 M reads / sample at a threshold of >1 Counts Per Million (CPM) for LUTHOR libraries generated from **A)** ultra-low input UHRR (4 replicates) or **B)** frozen single cells: HEK293T cells, mES cells, and S2 cells (8 replicates per cell type).

Applications

LUTHOR's exceptional sensitivity and robust performance renders this kit highly suitable for gene expression profiling for ultra-low input RNA and single cells. LUTHOR 3' mRNA-Seq is especially useful to study small perturbations in cellular transcriptomes, e.g., analyses of cell heterogeneity, responses to treatments on the cellular level, studies of disease and immunity, single-cell CRISPR-screens, and biomarker discovery even from difficult samples types or limited input material.

Associated Products

- SPLIT RNA Extraction Kits (p.31)
- PCR Add-on Kit for Illumina (p.38)
- Purification Module with Magnetic Beads (p.39)
- Lexogen UDI 12 nt Unique Dual Indexing System (p.36)

Robust performance and excellent reproducibility

The combination of innovative THOR Amplification Technology and robust library generation not only allows for the most sensitive gene detection but also delivers excellent reproducibility between technical replicates from ultra-low input UHRR (Fig. 4A). LUTHOR also delivers excellent cell-to-cell correlation as exemplified for two frozen mES cells (Fig. 4B).

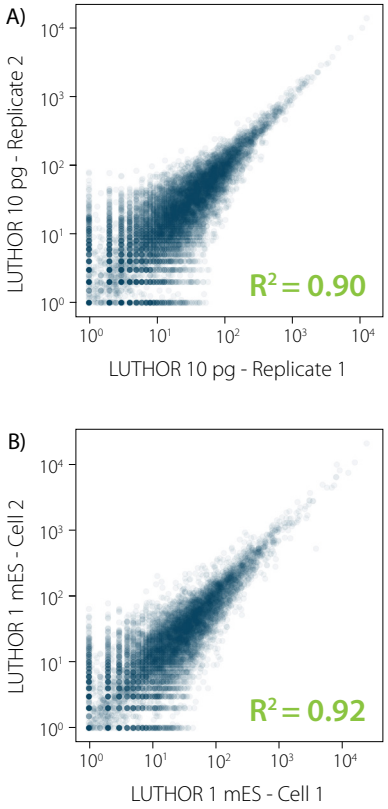

Figure 4 | Excellent reproducibility for LUTHOR 3' mRNA-Seq. A) Correlation plot of gene counts for replicates from 10 pg UHRR and **B)** from two individual FACS-sorted mES cells at 1 M reads / sample. Cells were frozen after sorting and stored at -80 °C prior to processing with LUTHOR 3' mRNA-Seq.

LUTHOR 3' mRNA-Seq uses Lexogen's proprietary THOR Amplification Technology to enable high-resolution sequencing of single cells with unprecedented sensitivity. RNA is amplified directly from the original mRNA eliminating the need for amplification of cDNA intermediates. The protocol enables 3' mRNA-Seq even from challenging ultra-low input samples, or individual cells that are prone to degradation. LUTHOR allows in-depth analysis of the transcriptome profile of individual cells. Thereby, LUTHOR outperforms all conventional single-cell RNA-Seq methods which detect only highly abundant genes and require extremely large numbers of cells.

Find more information about this product at its web page:

This product is available as part of the Lexogen NGS Services.
For details, contact our Services department: services@lexogen.com.

CORALL RNA-Seq V2 Library Prep Kits

CORALL RNA-Seq V2 enables streamlined generation of Illumina-compatible libraries within 4.5 hours, with seamless integration of Unique Molecular Identifiers (UMIs) and exceptional protocol-inherent strand specificity (>99 %). The fragmentation-free protocol enables complete transcript coverage including start sites.

Advantages

- Wide range of total RNA input (1 ng to 1 µg)
- Excellent performance on low-input / lowly concentrated samples
- Ideal for challenging and/or degraded samples (e.g., FFPE)
- 6-step protocol delivers ready-to-sequence libraries within 4.5 hours
- Includes Lexogen's 12 nt Unique Dual Indices (UDIs) for superior index error correction
- Unique Molecular Identifiers (UMIs) seamlessly integrated
- Excellent protocol-inherent strandedness (>99 %)
- **NEW!** Fragmentation-free library size adjustments

Workflow

CORALL library generation is initiated by random hybridization of Displacement Stop Primers (DSPs) with partial Illumina-compatible P7 sequences to the RNA template (Fig. 1). The complete workflow is fragmentation-free as the insert size is determined by the distance between hybridized DSPs. Reverse transcription extends each DSP to the next, where transcription is effectively stopped.

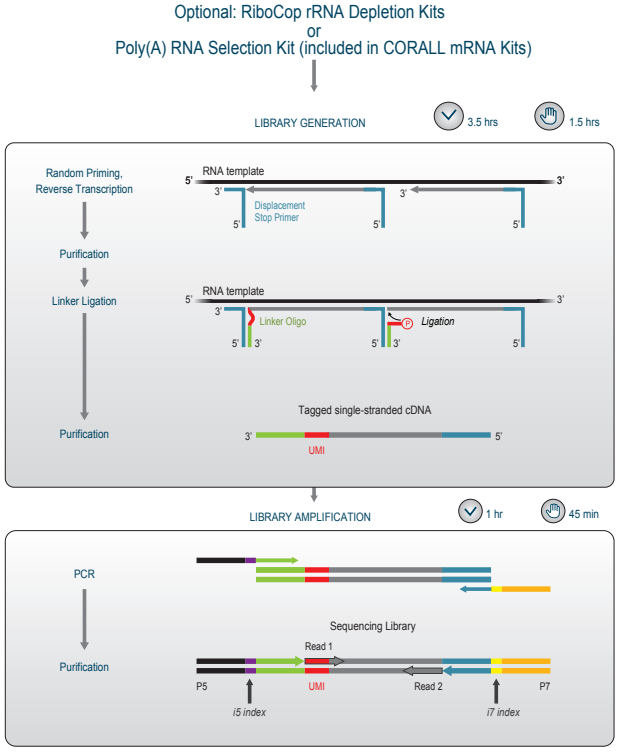

Figure 1 | Schematic overview of the CORALL Library Preparation workflow.

This stop prevents spurious second strand synthesis and maintains excellent strand specificity. Highly efficient ligation of Linker Oligos to the 3' ends of first strand cDNA fragments then introduces partial Illumina-compatible P5 sequences and UMIs. Lexogen's 12 nt UDIs are introduced during the PCR amplification step, together with complete adapter sequences required for cluster generation on Illumina instruments. UDIs are designed to provide superior error correction, accuracy, and highest quality sequencing data.

All purification steps are based on magnetic beads, rendering the protocol highly suitable for automation.

CORALL RNA-Seq V2 libraries are compatible with single-read and paired-end sequencing on Illumina instruments.

CORALL RNA-Seq V2 Kits come with two reverse transcription chemistries: RTM and RTL, allowing library size adjustments. RTL Chemistry can be used to increase the average library size to approximately 550 bp compared to RTM Chemistry which generates libraries with an average size of approximately 350 bp.

Input RNA can be either depleted of ribosomal RNA (rRNA), or poly(A) enriched. Alternatively, total RNA without prior depletion or enrichment can be used for CORALL library preparation.

CORALL RNA-Seq V2 Kits are available in form of various convenient bundles, e.g., including RiboCop for rRNA depletion in CORALL Total RNA-Seq V2 (p.18), or including poly(A) selection in CORALL mRNA-Seq V2 bundles (p.18).

CORALL Total RNA-Seq V2 Library Prep Kits

CORALL Total RNA-Seq V2 with UDIs enables streamlined generation of Illumina-compatible libraries with exceptional protocol-inherent strand specificity (>99 %). Libraries are generated within 6 hours starting with rRNA depletion. Built-in Unique Molecular Identifiers (UMIs) allow identification and removal of PCR duplicates for accurate transcript quantification. The fragmentation-free protocol enables complete transcript coverage with excellent start site representation.

Advantages

- Wide range of total RNA input (1 ng to 1 µg)
- Ready-to-sequence libraries within 6 hours
- Excellent performance on low-input / lowly concentrated samples
- Ideal for challenging and/or degraded samples (e.g. FFPE)
- Robust performance and a complete workflow for whole blood RNA-Seq, combined with RiboCop H/M/R + Globin
- Including Lexogen's 12nt Unique Dual Indices (UDIs) for superior index error correction
- Unique Molecular Identifiers (UMIs) seamlessly included
- Excellent protocol-inherent strandedness (>99 %)
- **NEW!** Fragmentation-free library size adjustments

Workflow

In contrast to conventional Whole Transcriptome RNA-Seq, CORALL Total RNA-Seq V2 library preparation is free from fragmentation, requires only 6 steps and can be completed in 6 hours (Fig. 1).

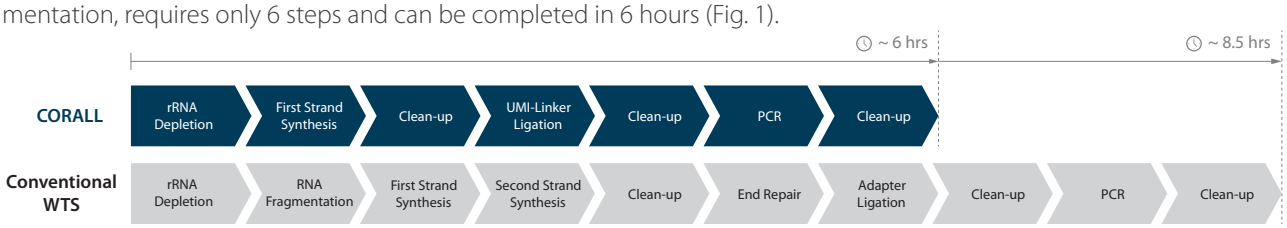

Figure 1 | Comparison of CORALL and conventional WTS RNA-Seq library preparation workflows.

Performance

Transcript Coverage

CORALL Total RNA-Seq V2 generates transcriptome-wide, smooth and uniform read coverage, including exceptional 5' coverage (Fig. 2), with gene body coverage comparable to competitor protocols.

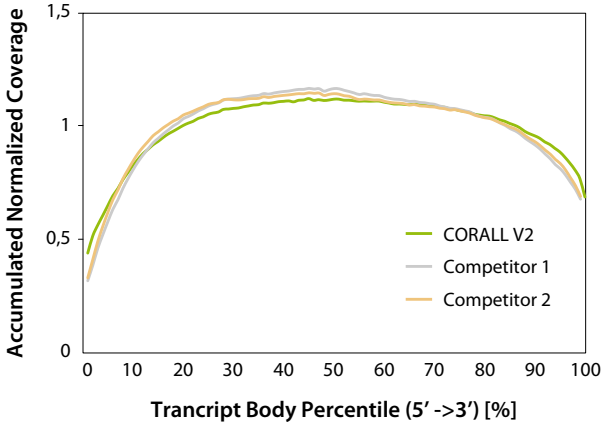

Figure 2 | Accumulated transcript body coverage (whole transcriptome). Coverage across all transcripts was generated using the geneBody_coverage.py tool provided by RSeQC (transcripts length normalized to 100 %).

Gene detection

CORALL Total RNA-Seq V2 delivers excellent gene discovery rates matching renown competitor kits (Fig. 3). This high level of library complexity ensures faithful representation of the transcriptome, enabling sensitive expression profiling.

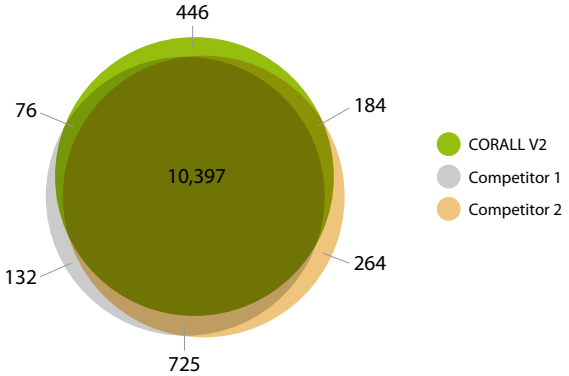

Figure 3 | Gene detection overlap. The Venn diagram illustrates the overlap of detected genes between CORALL Total RNA-Seq V2 and two competitor kits at normalized expression levels of > 10 CPM.

Superior End-to-End Coverage

CORALL's comprehensive coverage delivers improved transcription start site representation. Read coverage was analyzed using the ERCC spike-in controls, which feature precise, known transcription start sites (TSS). CORALL reads map more accurately to the exact ERCC TSS (Fig. 4) than reads from competitor library preps.

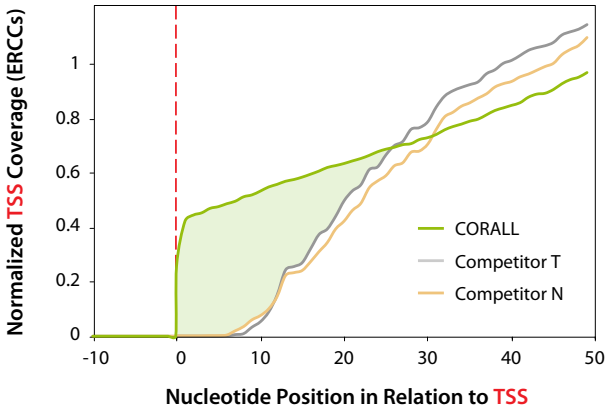

Figure 4 | Normalized ERCC coverage of transcription start sites. Normalized coverage of accumulated mapped reads for all detected ERCCs. The absolute nucleotide positions relative to the TSS (red dotted line) are shown.

Performance on FFPE Samples

CORALL Total RNA-Seq V2 is a perfect solution for processing degraded and compromised samples, such as FFPE material. Gene discovery rates are highly comparable between fresh frozen and FFPE matched tissue samples from human liver. Qualitative analysis shows a large overlap of detected genes at normalized expression levels >5 CPM between FFPE and fresh frozen samples demonstrating consistent and robust performance of CORALL Total RNA-Seq even for low-quality / FFPE RNA samples (Fig. 5).

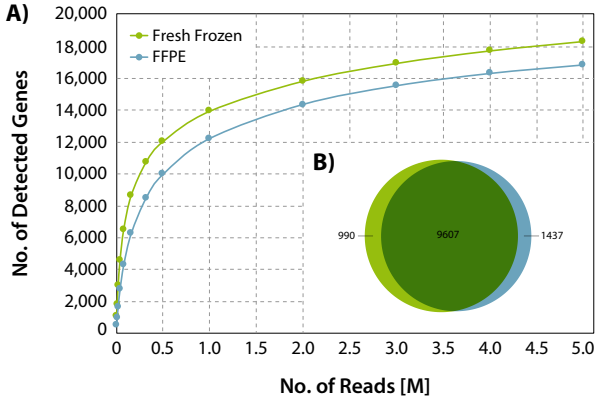

Figure 5 | Gene discovery rates for fresh frozen and FFPE-derived RNA. Matched human liver RNA samples were treated with DNase I, rRNA was depleted with RiboCop HMR V2 and libraries were prepared with CORALL RNA-Seq V2. A) Gene detection rates for fresh frozen or FFPE fixed human liver. The number of detected genes is plotted against the total number of reads mapping uniquely to exons. B) Overlap of detected genes from fresh frozen and FFPE input RNA at normalized expression levels >5 CPM (for uniquely mapping reads).

Performance on Whole Blood Samples

Whole blood RNA is comprised of highly abundant undesired RNA species, such as ribosomal RNA (rRNA), accounting for ~80 – 90 % of total RNA, and globin mRNA, representing 30 – 80 % of all mRNAs.

CORALL Total RNA-Seq V2 combined with RiboCop for Human/Mouse/Rat plus Globin for simultaneous removal of rRNA and globin mRNA provides a highly convenient workflow for processing whole blood samples. Depletion of rRNA and globin mRNA frees up sequencing space for RNAs of interest (Fig. 6). This workflow allows to study coding and non-coding RNAs of the blood, including mRNA, lncRNAs, snoRNAs, and snRNAs. For convenience, bundles of CORALL Total RNA-Seq V2 with RiboCop for Human/Mouse/Rat plus Globin are available (Cat. No. 185 and 186).

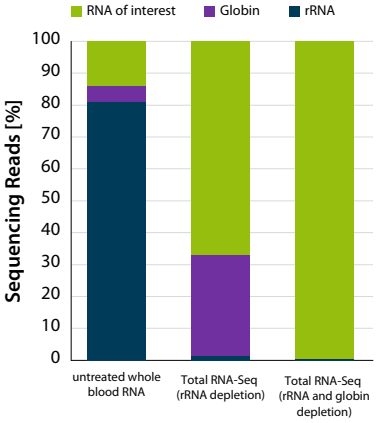

Figure 6 | CORALL Total RNA-Seq V2 with RiboCop rRNA Depletion for Human/Mouse/ Rat plus Globin efficiently removes rRNA and globin mRNA from human whole blood RNA. RNA was extracted from human donor whole blood. 5 ng whole blood RNA were depleted with RiboCop HMR V2 or HMR+Globin and libraries were prepared with the CORALL Total RNA-Seq V2 Library Prep (Cat. No. 183, and 185). Reads mapping to rRNA (blue), RNA of interest (green) and globin (purple) are shown.

Applications

CORALL Total RNA-Seq V2 is suitable for all whole transcriptome RNA-Seq applications, including: gene expression profiling, isoform discovery and quantification, alternative splicing studies, transcript (re)annotation, and *de novo* assembly. CORALL can also be used for SLAMseq Kits for High-Throughput Kinetic RNA Sequencing (p.17)

Data Analysis

Lexogen offers an optimized CORALL Data Analysis Pipeline, which performs read quality control, mapping, Unique Molecular Identifier (UMI) deduplication, and transcript quantification. The pipeline is available for download from our Lexogen Tools Github page (https://github.com/Lexogen-Tools/corall_analysis).
Additional web-based, automated data analysis solutions are available via the Lexogen Data Analysis Solutions webpage (www.lexogen.com/lexogen-data-analysis-solutions).
For further inquiries, please contact support@lexogen.com.

Associated Products

Find more information about this product at its web page:

- SPLIT RNA Extraction Kits (p.31)
- PCR Add-on Kit for Illumina (p.38)
- SIRVs - Spike-In RNA Variant Controls (p.32)
- RiboCop rRNA Depletion Kits (p.24)
- Poly(A) RNA Selection Kit V1.5 (p.27)
- Purification Module with Magnetic Beads (p.39)
- SLAMseq Kits for High-Throughput Kinetic RNA Sequencing (p.22)

This product is available as part of the Lexogen NGS Services.
For details, contact our Services department: services@lexogen.com.

CORALL mRNA-Seq V2 Library Prep Kits

The CORALL mRNA-Seq V2 Library Prep Kit enables fast and cost-efficient generation of stranded, UMI-labelled, and unique dual indexed libraries for whole transcriptome poly(A) RNA analyses using Illumina NGS platforms. The fragmentation-free workflow uses Lexogen's proprietary Strand Displacement Stop and Ligation technologies and is suited for input amounts down to 1 ng total RNA.

Advantages

- Complete workflow solution for mRNA-Seq including poly(A) selection
- Wide range of total RNA input (1 ng to 1 µg)
- Ready-to-sequence libraries in 5.5 hours total
- Unique Molecular Identifiers (UMIs) seamlessly included
- Maximized sequencing output with Unique Dual Indices (UDIs) for superior index error correction
- Excellent protocol-inherent strandedness (>99 %)
- High sensitivity and excellent reproducibility also at low-input amounts
- Convenient workflow for mRNA-Seq from whole blood in combination with RiboCop H/MR + Globin
- **NEW!** Fragmentation-free library size adjustments

Workflow

CORALL mRNA-Seq V2 Kits provide a complete solution for mRNA sequencing. RNA is poly(A) enriched in 1 hour and can be directly transferred into CORALL library preparation, generating ready-to-sequence libraries in total of 5.5 hours (Fig. 1). CORALL mRNA-Seq includes Unique Molecular Identifiers (UMIs) and is suitable for input amounts down to 1 ng total RNA prior to poly(A) selection. Thus, CORALL is perfectly suited for expression analysis delivering gene and transcript level quantification even for low-input samples. The Poly(A) RNA Selection Kit V1.5 (p.27) is included in CORALL mRNA-Seq bundles. Poly(A) selection and all purification steps are based on magnetic beads rendering the complete workflow suitable for full automation.

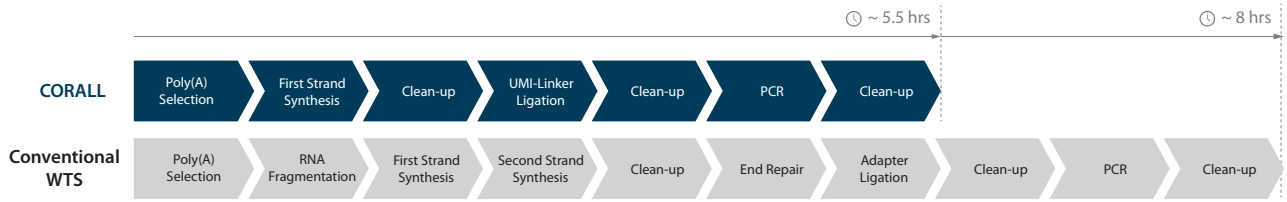

Figure 1 | CORALL mRNA-Seq V2 workflow.

Performance

Excellent Reproducibility and Sensitivity

Correlation analysis of CORALL mRNA-Seq V2 libraries from 1 ng total RNA input prior to poly(A) selection reveals excellent reproducibility for libraries of both medium (Fig. 2A) and long length sizes (Fig. 2B).

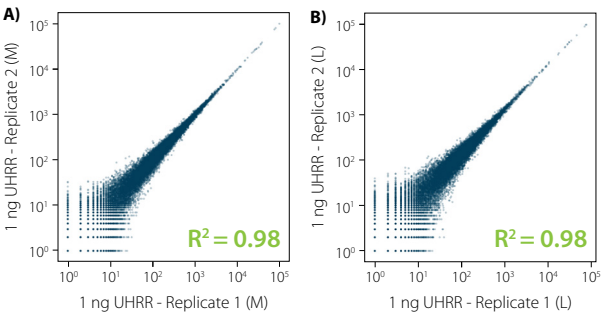

Figure 2 | Excellent reproducibility between replicates for low input RNA. Correlation analysis between replicates for CORALL mRNA-Seq with 1 ng total RNA input for libraries with A) medium length (average size ~350 bp) and B) long length (average size ~550 bp).

Gene Detection

CORALL mRNA-Seq V2 delivers excellent gene discovery rates across a wide range of RNA input amounts and is highly sensitive even at low input levels (Fig. 3).

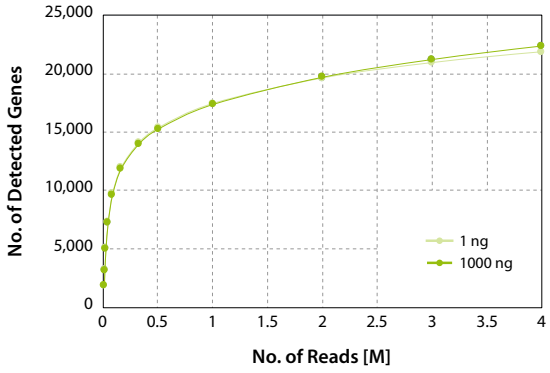

Figure 3 | Gene discovery rates for libraries with length of (average size) 550 bp. 1 ng, and 1000 ng RNA were used as input for poly(A) selection and library preparation using CORALL mRNA-Seq V2. The number of detected genes is plotted against the number of reads mapping uniquely to exons (calculated with featureCounts).

Performance on Blood

CORALL mRNA-Seq V2 is a perfect solution for mRNA-Seq analysis of the whole blood samples, combined in a best practice workflow with RiboCop H/M/R + Globin for removal of ribosomal RNAs (rRNAs) and globin mRNAs, enabling focus on mRNAs of interest (Fig. 4).

Figure 4 | Workflow for mRNA-Seq analysis of whole blood combines CORALL mRNA-Seq V2 with an additional rRNA and globin depletion step.

This workflow proved to have an exceptional performance on blood samples, and leads to a significant increase in gene detection (Fig 5. A and B).

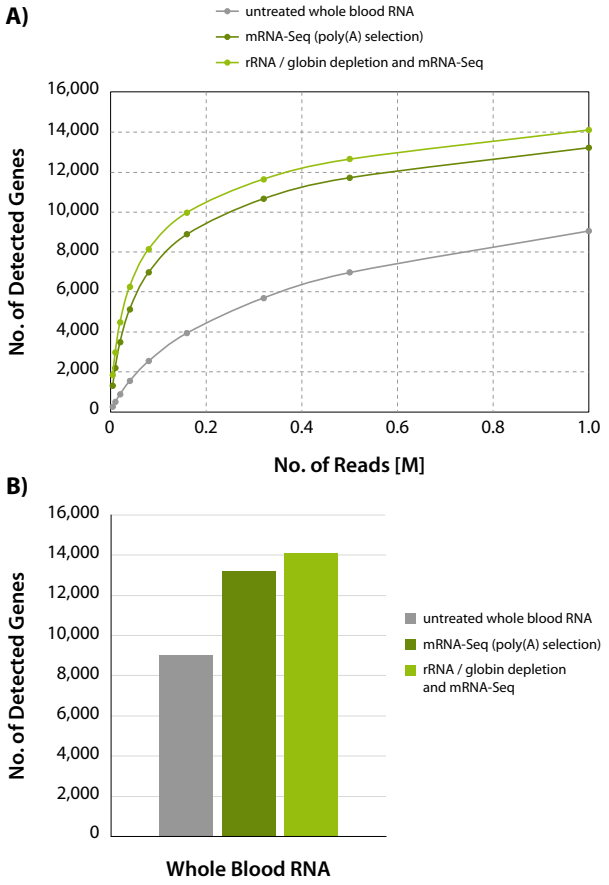

Figure 5 | Increased gene detection for CORALL mRNA-Seq V2 with additional rRNA and globin depletion. A) The number of detected genes per number of reads uniquely mapping to exons (FeatureCounts) B) Bar plot illustrating the number of detected genes at 1 M reads /sample, normalized to Counts Per Million (CPM) at a threshold of CPM > 1.

This workflow allows to study predominantly coding RNAs and polyadenylated long non-coding RNAs of the blood. For this workflow we recommend purchasing the following products together:

- CORALL Total RNA-Seq V2 with RiboCop for Human/Mouse/Rat plus Globin (Cat. No. 185 and 186).
- Poly(A) RNA Selection Kit V1.5 (p.27).

Data Analysis

Lexogen offers an optimized CORALL Data Analysis Pipeline, which performs read quality control, mapping, Unique Molecular Identifier (UMI) deduplication, and transcript quantification. The pipeline is available for download from our Lexogen Tools Github page (https://github.com/Lexogen-Tools/corall_analysis). Additional web-based, automated data analysis solutions are available via the Lexogen Data Analysis Solutions webpage (https://www.lexogen.com/lexogen-data-analysis-solutions/). For further inquiries, please contact support@lexogen.com.

Featured Products

- CORALL RNA-Seq V2 Kits with UDIs (Cat. No. 171 - 176)
- CORALL mRNA-Seq V2 Kits with UDIs (Cat. No. 177 - 182)
- RiboCop (HMR) and CORALL Total RNA-Seq V2 Kits with UDIs (Cat. No. 183 and 184)
- RiboCop (HMR+Globin) and CORALL Total RNA-Seq V2 Kits with UDIs (Cat. No. 185 and 186)

Associated Products

- SPLIT RNA Extraction Kits (p.31)
- PCR Add-on Kit for Illumina (p.38)
- Purification Module with Magnetic Beads (p.39)
- SIRVs - Spike-In RNA Variant Controls (p.32)
- RiboCop rRNA Depletion Kits (p.24)
- SLAMseq Kits for High-Throughput Kinetic RNA Sequencing (p.22)

This product is available as part of the Lexogen NGS Services. For details, contact our Services department: services@lexogen.com.

Find more information about this product at its web page:

Small RNA-Seq Library Prep Kit

The Small RNA-Seq Library Prep Kit offers a streamlined procedure for generating Illumina ready-to-sequence libraries from total or enriched small RNA in less than 5 hours. The protocol enables the discovery and profiling of small RNAs such as microRNA or small interfering RNA, which play key roles in the regulation of gene expression.

Advantages

- Ready-to-sequence libraries in less than 5 hours
- Wide input range from 50 pg to 1 µg of enriched or total RNA
- Optimized for low RNA content samples such as plasma, serum, and urine
- Compatible with TraPR Small RNA Isolation for efficient sRNA screening
- Indices for multiplexing of up to 96 samples included

Workflow

Library generation is based on adapter ligation to the 3′ and 5′ ends of total or enriched small RNA. The input RNA, flanked by 5′ and 3′ adapters, is then converted into cDNA and up to 96 external i7 indices are added during a PCR step, enabling a high degree of multiplexing. The workflow is fully compatible with upstream enrichment of functional sRNAs using TraPR Small RNA Isolation and for low RNA content samples. A bundled version with Lexogen's Purification Module with Magnetic Beads is available to prevent potential linker-linker artifacts.

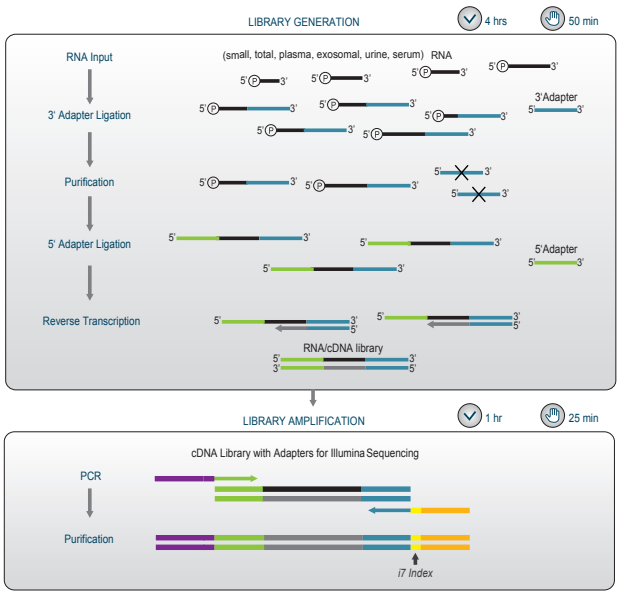

Figure 1 | Schematic overview of the Small RNA-Seq Library Prep workflow.

Performance

The Small RNA-Seq Kit allows for exceptional miRNA discovery and outperforms other workflows especially at lower RNA inputs (Fig. 2). The high sensitivity makes it suited very well for challenging, low content RNA sources, such as liquid biopsies (plasma, serum, and urine), including exosomes.

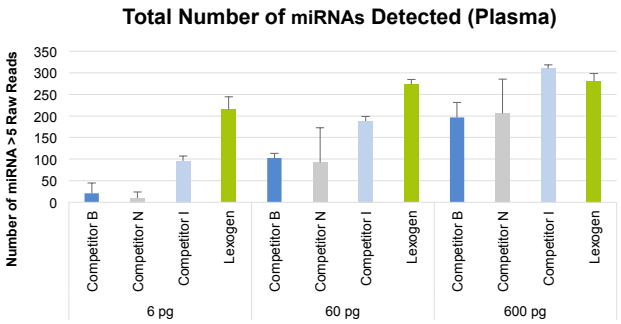

Figure 2 | Lexogen's Small RNA-Seq Kit allows for detection of a higher number of microRNAs, especially for lower RNA inputs. Total number of miRNAs detected across 4 different protocols. Dilution series (6 pg, 60 pg, and 600 pg) of purified plasma RNA was used with library prep kits from different vendors. The obtained libraries were sequenced at equal molarity at ~1.5 – 2 M total raw reads per sample. Lexogen's Small RNA-Seq Library Prep Kit showed much higher numbers of miRNAs detected at ≥5 raw reads, in particular for lower input amounts.

Associated Products

- TraPR Small RNA Isolation Kit (p.28)
- SPLIT RNA Extraction Kits (p.31)
- Gel Extraction Module (p.39)

This product is available as part of the Lexogen NGS Services.

For details, contact our Services department: services@lexogen.com.

Find more information about this product at its web page:

TeloPrime Full-Length cDNA Amplification Kit

The TeloPrime Full-Length cDNA Amplification Kit is based on Lexogen's unique Cap-Dependent Linker Ligation (CDLL) and long Reverse Transcription (long RT) technologies, making it highly selective for full-length RNA molecules that are both capped and polyadenylated. TeloPrime provides for a faithful representation of the mRNA transcriptome, thus also empowering long-read NGS.

Advantages

- Full-length cDNA generation with exceptional 5′ cap and poly(A) tail specificity
- Optimized second strand synthesis and PCR for high yield of long cDNAs (>2 kb)
- Ideal for long-read NGS library generation (PacBio™ and Oxford Nanopore™)
- All-in-one protocol
- 1 ng - 2 µg total RNA input
- Flexible protocol for targeted, cap-specific cDNA synthesis

Workflow

First, cDNA synthesis is initiated by oligo(dT) primed long reverse transcription from total RNA (Fig. 1). Then, an adapter is ligated to the 3′ end of cDNAs, but only if they were extended up to the RNA cap structures. Thus, in the subsequent second strand synthesis exclusively 5′- and 3′-tagged, full-length cDNA is converted into double-stranded cDNA, which is then amplified using Lexogen's optimized TeloPrime PCR protocol.

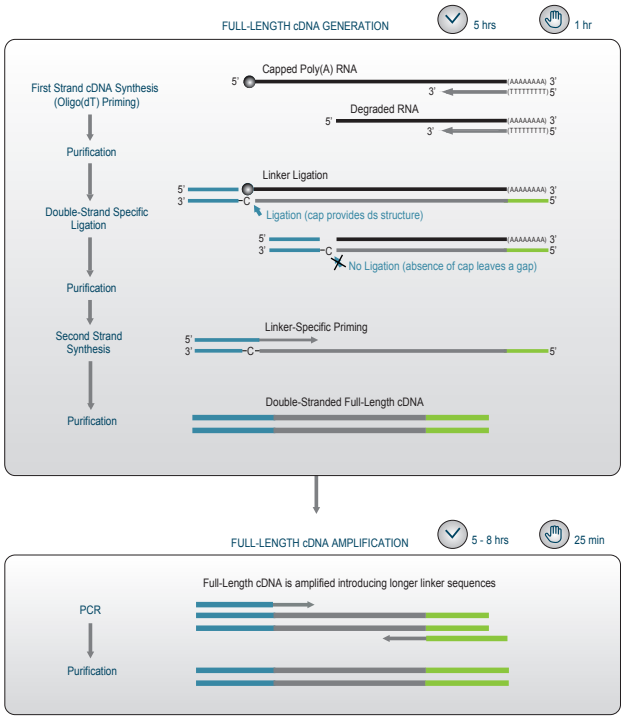

Figure 1 | Schematic overview of the TeloPrime workflow. After full-length cDNA synthesis, the inverted G of the cap structure of the RNA/cDNA hybrid takes part in an atypical base- pairing with the 5′C overhang of a double-stranded adapter. By using a double-strand specific ligase, ligation to the cDNA's 3′ end only takes place if the cap is present (e.g., the RNA is intact) and if the reverse transcription has extended fully to the 5′ end of the mRNA.

Performance

TeloPrime delivers superior 5′ cap specificity compared to other full-length cDNA preparation methods, such as Template Switch and Oligo Capping, enabling the highest precision of transcription start site (TSS) mapping (Fig. 2).

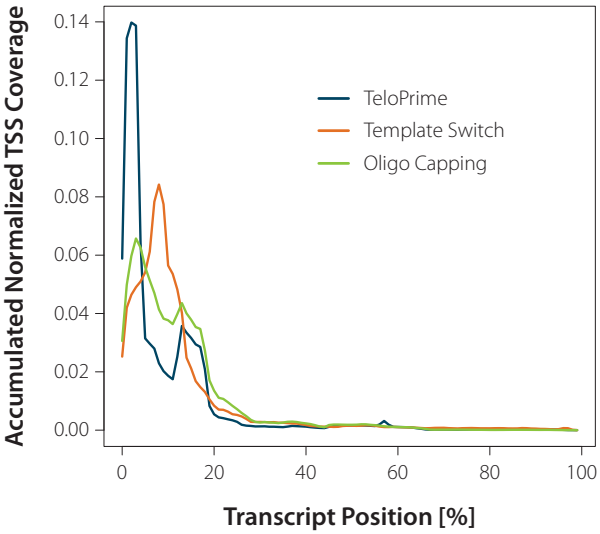

Figure 2 | Lexogen's TeloPrime Full-Length cDNA Amplification Kit enables high precision of transcription start sites (TSS) mapping. In an mRNA-Seq experiment, mRNAs were tagged at their 5′ end using Template Switch, Oligo Capping, or TeloPrime's CDLL technology. Illumina- compatible libraries were then generated from the full-length cDNA and sequenced. The accumulated read coverage of TSS mapped to the human genome is plotted versus the normalized annotated transcript length to show relative TSS mapping for the top 500 expressed genes.

Associated Products

- SIRVs - Spike-In RNA Variant Controls (p.32)
- SPLIT RNA Extraction Kits (p.31)

Find more information about this product at its web page:

SLAMseq Kits for High-Throughput Kinetic RNA Sequencing

Standard RNA-Seq methods measure total RNA abundances but cannot resolve the underlying kinetics of RNA transcription and degradation that determine overall steady-state levels. The SLAMseq product family adds time as a new dimension to RNA-Seq and enables quantitative analysis of newly synthesized and existing RNA from a single total RNA sample in parallel, without the need for biochemical isolation.

Advantages

- Analyze transcriptome-wide kinetics of RNA synthesis and turnover
- Gain novel insights into the control of gene expression
- Establish stimulus-triggered sequence of changes in transcription and RNA decay
- Significantly enhance the resolution of differential gene expression by comparing nascent transcript levels
- Just two steps are added to an RNA-Seq workflow, no need for pull-down or biochemical isolation
- Fully integrated pipeline for time-resolved RNA-Seq provided by Lexogen's SLAMseq labeling kit, QuantSeq 3' mRNA-Seq library preparation kit, and SLAMdunk analysis software

Workflow

The SLAMseq kit family is based on thiol (SH)- Linked Alkylation for the Metabolic Sequencing of RNA, a transcriptome-wide, quantitative, fast, and reliable labeling method [1]. For the metabolic labeling, cells are incubated with the nucleotide analogue 4-Thiouridine (S4U), which is taken up and incorporated into newly transcribed, nascent RNA. S4U labeling conditions can be optimized using the Cell Viability Titration Module (059.24) and the S4U Incorporation Module (060.24) of Lexogen's SLAMseq Explorer Kit. The key feature of the workflow is an alkylation step, which uses iodoacetamide (IAA) to modify the S4U nucleotides, leading to nucleotide conversion during reverse transcription. This results in thymine-to-cytosine (T>C) mutations in sequencing reads derived from S4U-labeled transcripts. In addition to the total RNA level bioinformatics analysis of T>C-containing reads can therefore quantify the fraction of nascent RNA in the same sample (Fig. 1).

For cost-efficient, time-resolved gene expression studies, SLAMseq is best combined with QuantSeq Expression Profiling Library Prep Kits (p.7), followed by SLAMdunk data analysis.

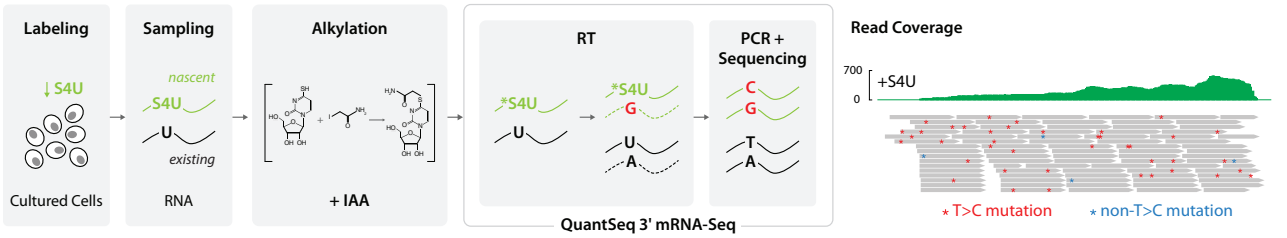

Figure 1 | Schematic overview of the SLAMseq workflow. Cultured cells are treated with 4-Thiouridine (S4U) for labeling of nascent RNA (green). Total RNA is purified, and alkylation of the 4-thiol group is induced by the addition of iodoacetamide (IAA). During library preparation, shown here using the QuantSeq 3' mRNA-Seq Library Prep Kit, the presence of the resulting carboxyamidomethyl-group causes reverse transcriptase to incorporate guanine (G, in red) instead of adenine (A, in black) at any position where a *S4U-modified nucleotide is encountered. In this way, nascent RNA can be distinguished from existing RNA by the presence of T>C mutations (red asterisks) during subsequent data analysis.

Lexogen offers access to SLAMdunk [1], a customized pipeline for analyzing SLAMseq RNA-Seq data from libraries prepared with QuantSeq 3' mRNA-Seq Library Prep Kits for Illumina. The SLAMdunk software allows easy analysis of sequencing data from a SLAMseq experiment. The user uploads compressed fastq files and selects the appropriate species. After processing the data, SLAMdunk yields statistics about T>C conversion rates and alignments falling in unique 3' UTR regions. The results can then be downloaded to derive RNA turnover kinetics, transcriptional cascades, tissue-specific gene expression, etc. Together, SLAMseq, QuantSeq, and SLAMdunk provide a complete and user-friendly solution for high-throughput time-resolved RNA-Seq experiments. Please contact us at support@lexogen.com for all the information on how to access the SLAMdunk pipeline.

Performance

Measuring RNA Synthesis and Decay Rates

Mouse embryonic stem cells (mESCs) were subjected to S4U labeling, followed by total RNA extraction, alkylation, QuantSeq 3' mRNA library preparation and sequencing (SLAMseq Kinetics Kit Anabolic Module, 061.24). In a second setup, mESCs were first subjected to S4U metabolic RNA labeling for 24 hours, followed by a washout and Uridine-stop (U Stop) using unlabeled uridine (SLAMseq Kinetics Kit Catabolic Module, 062.24). SLAMseq data was analyzed using the SLAMdunk pipeline. RNA turnover rates were determined for the whole transcriptome, with Figure 2 showing exemplary synthesis and decay kinetics for two genes.

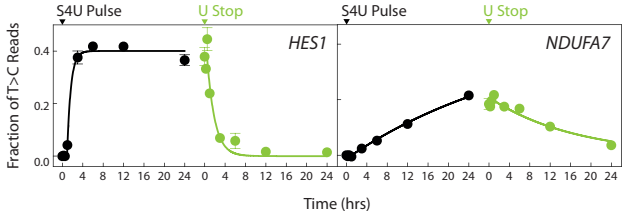

Figure 2 | S4U labeling kinetics experiments reveal individual RNA synthesis and degradation rates. To measure RNA synthesis, cells were treated with S4U (100 µM) for 24 hours, whereas for RNA decay measurements, cells were first saturated with S4U, and rates were measured after removal of S4U and addition of unlabeled uridine (U Stop).

The Anabolic and Catabolic Modules of Lexogen's SLAMseq Kinetics Kit enable these comprehensive transcriptome dynamics analyses.

Differential Expression Profiling of Nascent and Total RNA in Parallel

SLAMseq enables expression profiling of total RNA and nascent RNA from the same sample. To illustrate the difference between standard, steady-state and a SLAMseq-based, metabolic RNA-Seq analysis, differential gene expression was analyzed in a human cell line [2]. SLAMseq significantly enhanced the sensitivity of differential gene expression detection and quantification (Fig. 3A). Analyzing nascent mRNA levels using SLAMseq revealed transcriptional responses to inhibitor treatment that cannot be resolved by standard RNA-Seq (Fig. 3B). By enabling the discovery of direct transcriptional targets and the underlying mechanisms of cellular responses, SLAMseq is therefore perfectly suited to empower drug discovery studies.

SLAM-ITseq: Determining Tissue- and Cell Type-Specific Gene Expression *in vivo*

For this application, 4-Thiouracil is injected into a transgenic organism expressing uracil phosphoribosyltransferase (UPRT) in a given cell type. The nascent RNA becomes labeled exclusively in this cell type, and tissue- and cell type-specific *in vivo* gene expression can be quantified due to the telling base conversions in RNA-Seq data sets [3]. This approach can also be used to monitor trafficking of RNA between tissues [4].

Differentiation of Cell Infection from Carryover of Viral Nucleic Acids

Using SLAMseq, RNA from viruses and bacteria replicating in cell cultures can be unambiguously distinguished from nucleic acid carryover. While detection of contaminations by standard RNA-Seq is highly sensitive, it can lead to false positive results. In contrast, S4U labeled RNA is an unequivocal sign of the presence of a transcriptionally active virus or bacterium [5, 6].

Service

While keeping absolute control of the SLAMseq experiment itself, you can opt to leave the part downstream of RNA alkylation to the experts from Lexogen NGS Services. They will perform library preparations, NGS run and data evaluation, so you can confidently derive your conclusions.

Associated Products

Find more information about this product at its web page:

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- TraPR Small RNA Isolation Kit (p.28)
- Small RNA-Seq Library Prep Kit (p.20)
- SPLIT RNA Extraction Kits (p.31)

This product is available as part of the Lexogen NGS Services. For details, contact our Services department: services@lexogen.com.

References

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5. Cheval, J., et al. (2019) mSphere, DOI: 10.1128/mSphere.00298-19
6. Desbrousses, C., et al. (2020), Biologicals DOI: 10.1016/j.biologicals.2020.03.002

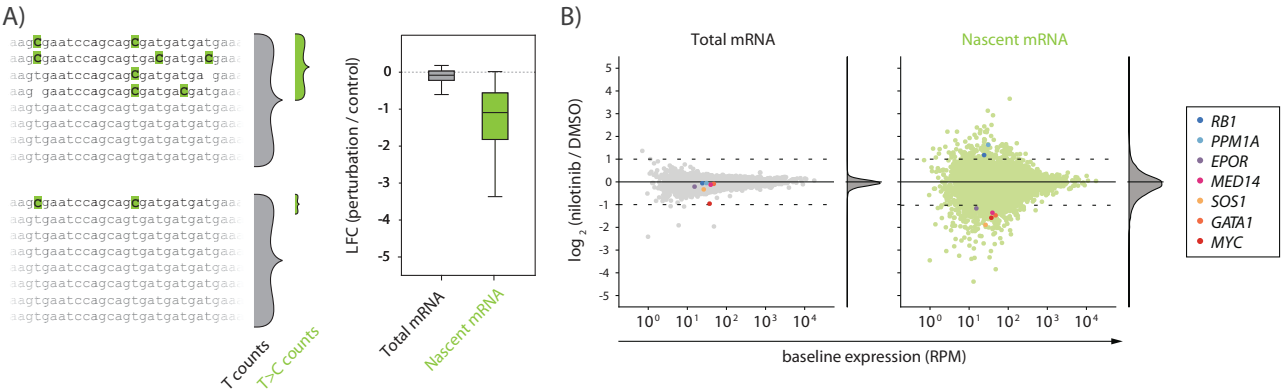


Figure 3 | SLAMseq enhances differential expression detection. A) Nascent RNA levels represented by T>C-containing reads (green) show a higher log fold change (LFC) compared to total mRNA (grey) levels when analyzing control (DMSO) and BCR/ABL-inhibitor-treated (nilotinib) K562 cells. **B)** More differentially expressed genes are detected when analyzing nascent mRNA (green) versus total mRNA levels (grey). Figure modified from Muhar et al, 2018 [2].

RiboCop rRNA Depletion Kits

Total RNA is comprised of large amounts of undesired transcripts, such as ribosomal RNA (rRNA), which account for ~80 - 98 % of the total RNA sample, and globin mRNA, accounting for ~35 - 80 % of mRNA in blood samples. Lexogen's RiboCop rRNA Depletion Kits efficiently remove rRNA and globin mRNA from intact as well as degraded total RNA samples. The resulting depleted RNA can be directly used for Next Generation Sequencing (NGS) as well as other demanding RNA applications.

Advantages

- **NEW!** Depletion of human/mouse/rat, bacterial, or yeast rRNA
- Suitable for intact and degraded RNA including FFPE samples
- Flexible RNA input amounts (1 ng - 1 µg of total RNA input)
- Automation-friendly protocol – magnetic bead-based purification
- No enzymatic reactions or mechanical shearing steps required
- Seamless integration with any RNA-Seq library prep
- Fragmentation-free complete workflow solution: RiboCop bundles with CORALL RNA-Seq V2
- Innovative probe design minimizing off-target effects
- Combined rRNA and globin mRNA depletion for whole transcriptome analysis of blood samples

Workflow

RiboCop's rRNA depletion is based on the removal of rRNA via hybridization to specifically designed oligos and subsequent affinity purification of the rRNA-oligo hybrids with magnetic beads. The entire protocol is automation-friendly by utilizing magnetic beads for depletion and purification and can be completed in only 1.5 hours (Fig. 1).

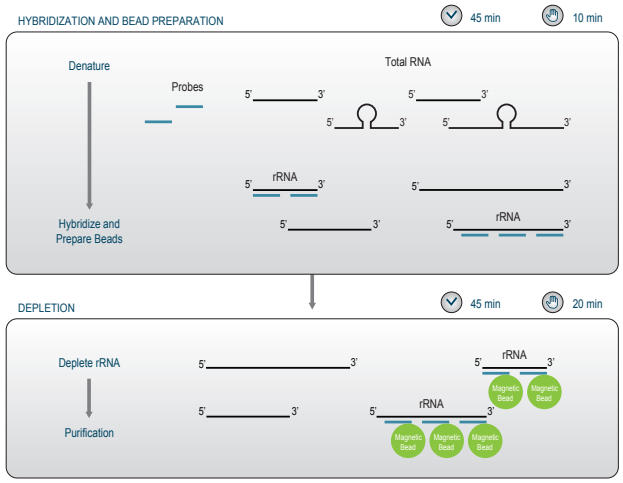


Figure 1 | Schematic overview of the RiboCop workflow.

RiboCop Kits for Human/Mouse/Rat and for Bacteria or Yeast can conveniently be combined for simultaneous depletion of host and bacterial rRNA, or host and yeast rRNA. RiboCop Kits are compatible with all random primed RNA-Seq library preparation kits.
For a complete workflow solution, RiboCop Kits for Human/Mouse/Rat are also available as convenient bundles with CORALL RNA-Seq V2 library prep kits (Cat. No. 183 – 186).

Performance

RiboCop for Human/Mouse/Rat V2

Lexogen's RiboCop rRNA Depletion Kit for Human/Mouse/Rat V2 removes undesired cytoplasmic (28S, 18S, 5.8S, 5S, and 45S) and mitochondrial (mt16S, mt12S) rRNA from intact as well as degraded material, including formalin-fixed, paraffin-embedded (FFPE) RNA. Standard recovery ranges between 1 – 3 % of input RNA and is dependent on the input material.
RiboCop for Human/Mouse/Rat V2 (Cat. No. 144) performs robustly across a wide range of input amounts and efficiently removes rRNA from all three species while maintaining constant transcript expression regardless of the input amount (Fig. 2 and Fig. 3).

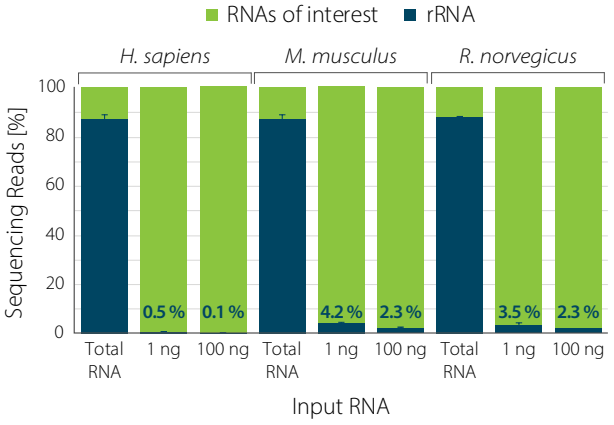


Figure 2 | RiboCop rRNA Depletion for Human/Mouse/Rat efficiently removes rRNA across a wide range of input amounts. Two RNA amounts of the indicated species were rRNA depleted using the HMR V2 Probe Mix. CORALL Total RNA-Seq libraries were prepared and sequenced. The percentage of reads mapping to rRNA is plotted in blue for all species.

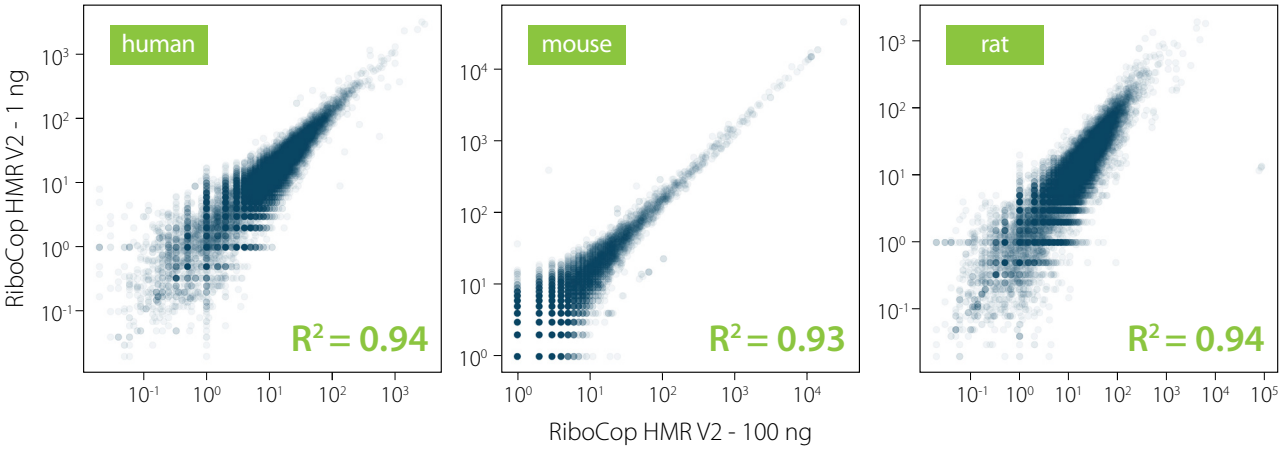


Figure 3 | RiboCop HMR V2 maintains constant transcript abundance across various input amounts. Correlation of transcript abundance in samples depleted with RiboCop HMR V2 for 100 ng RNA vs. 1 ng RNA input for indicated species.

RiboCop for Human/Mouse/Rat Plus Globin

Globin mRNA constitutes ~35 - 80 % of all blood mRNA. RiboCop for Human/Mouse/Rat Plus Globin (Cat. No. 145) simultaneously removes rRNA and globin mRNA from human, mouse, or rat whole blood, thus providing a convenient workflow for whole transcriptome analysis of blood samples while freeing up sequencing space for RNAs of interest (Fig. 4).

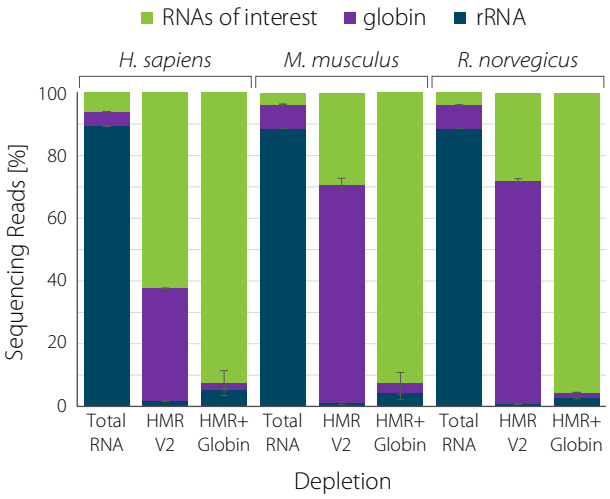


Figure 4 | RiboCop rRNA Depletion for Human/Mouse/ Rat plus Globin (HMR+Globin) enriches for RNAs of interest in blood sample across species. RNA was extracted from fresh human donor blood using SPLIT RNA Extraction and depleted with either RiboCop HMR V2 or RiboCop HMR+Globin. Libraries were prepared with CORALL RNA-Seq. Reads mapping to rRNA are shown in blue and in purple for globin.

RiboCop for Bacteria

RNA extracted from bacterial species comprises up to 98 % of the ribosomal RNAs, presenting a challenge especially when analyzing the transcriptome capacity from complex bacterial communities.

Lexogen's RiboCop rRNA Depletion Kits for Bacteria remove 23S, 16S, and 5S rRNA from mixed bacterial samples and monocultures. Intact as well as degraded material may be processed using RiboCop Bacteria depletion kits with dedicated probe mixes for Gram negative (G-), Gram positive bacteria (G+), and mixed bacterial species (META).

RiboCop performs robustly across wide range of input amounts, from 1 ng to 1 µg, efficiently reducing rRNA reads from 98 % to 1-3 % as shown by using the RiboCop for Gram Negative Bacteria on RNA isolated from *E. coli* (Fig. 5).

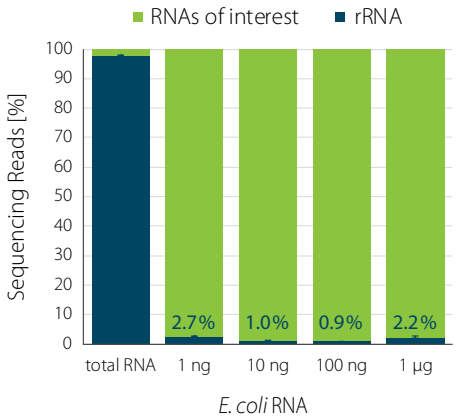


Figure 5 | RiboCop rRNA Depletion for Bacteria efficiently removes rRNA across a wide range of input amounts. Libraries were prepared using CORALL RNA-Seq and depletion was monitored by sequencing and subsequent analysis of remaining rRNA reads from untreated (total RNA) and depleted *E. coli* RNA of different input amounts.

The RiboCop rRNA Depletion Kit for Mixed Bacterial Samples (META) is specifically designed for depletion of complex, mixed populations such as environmental communities or microbiome samples. META Kit performs robustly across a wide range of input amounts across species, as shown by rRNA depletion from two Gram negative and one Gram positive species (Fig. 6).

RiboCop for Bacteria is offered in three different versions for:

- Mixed bacterial samples (META, Cat. No. 125)
- Gram negative bacteria (G-, Cat. No. 126)
- Gram positive bacteria (G+, Cat. No. 127)

If you are uncertain which kit to choose, then the RiboCop for Bacteria Kit Selection Tool can help you find the best-suited kit for your species of interest.

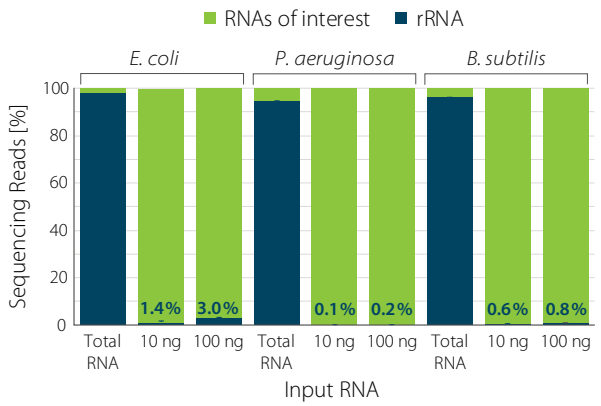


Figure 6 | RiboCop rRNA Depletion for Mixed Bacterial Samples (META) efficiently removes rRNA from various bacterial species. Two RNA amounts from monocultures of the indicated species were subjected to rRNA depletion using the META Probe Mix. CORALL Total RNA-Seq libraries were prepared and sequenced. Reads were mapped against the respective genomes of *E. coli* MG1655, *P. aeruginosa* PAO1, and *B. subtilis* 168. The percentage of reads mapping to rRNA is plotted in blue.

RiboCop for Yeast

RiboCop rRNA Depletion Kits for Yeast remove cytoplasmic (25S, 18S, 5.8S, 5S, and 35S) and mitochondrial (21S, 15S, ACI60_gr01 and ACI60_gr02) rRNA from intact as well as degraded input RNA.

Ribodepletion enables an unbiased view of the transcriptome, including non-coding and non-polyadenylated RNA species. RiboCop for Yeast (Cat. No. 190) is ideally suited for NGS in combination with CORALL RNA-Seq V2 Library Preparation Kits and is also compatible with other random primed total RNA library prep kits.

RiboCop for Yeast efficiently reduces rRNA from *Saccharomyces cerevisiae* total RNA to ~1 % across broad range of inputs, from 10 ng to 1 µg (Fig. 7).

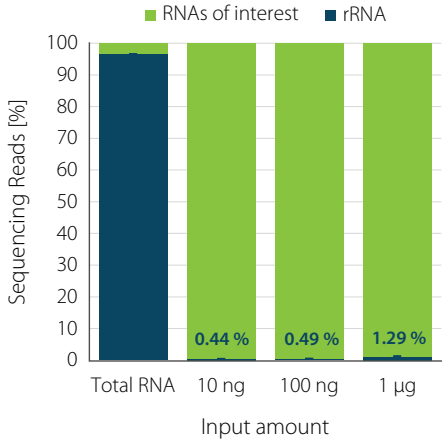


Figure 7 | RiboCop rRNA Depletion for Yeast efficiently removes rRNA across a wide range of input amounts. NGS libraries were prepared using CORALL RNA-Seq V2 Library Prep Kit. Successful depletion was monitored by analysis of remaining rRNA reads from untreated and depleted *S. cerevisiae RNA*. The percentage of reads mapping to rRNA is plotted in blue.

Besides saving valuable sequencing space, removal of rRNAs provides an unobstructed view of the yeast transcriptome and thereby increases gene detection (Fig. 8).

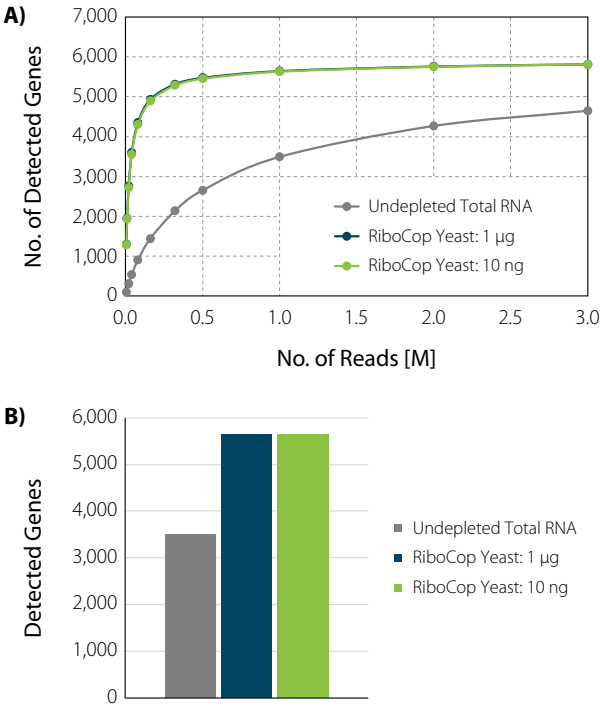


Figure 8 | Increased gene detection upon rRNA depletion. A) The number of detected genes per number of reads uniquely mapping to exons (FeatureCounts) was plotted for undepleted total RNA as well as depleted *S. cerevisiae* RNA across different input amounts. **B)** Bar plot illustrating the number of detected genes at 1 M reads /sample. *S. cerevisiae* samples depleted with RiboCop for Yeast using 1 µg or 10 ng input RNA. Libraries were prepared using CORALL RNA-Seq V2. Reads were mapped against the *S. cerevisiae* reference genome using STAR aligner and counted with FeatureCounts.

Featured Products

- RiboCop for Human/Mouse/Rat V2 (Cat. No. 144)
- RiboCop for Human/Mouse/Rat Plus Globin (Cat. No. 145)
- RiboCop for Mixed Bacterial Samples (META, Cat. No. 125)
- RiboCop for Gram Negative Bacteria (G-, Cat. No. 126)
- RiboCop for Gram Positive Bacteria (G-, Cat. No. 127)
- RiboCop for Yeast (Cat. No. 190)
- RiboCop HMR V2 and CORALL RNA-Seq V2 bundles (Cat. No. 183, 184)
- RiboCop HMR +Globin and CORALL RNA-Seq V2 bundles (Cat. No. 185, 186)
- RiboCop (HMR) and CORALL RNA-Seq V2 with UDIs bundles (Cat. No. 183, 184)
- RiboCop (HMR +Globin) and CORALL RNA-Seq V2 with UDIs bundles (Cat. No. 185, 186)

Do you need other RiboCop versions bundled with CORALL RNA-Seq? Contact sales@lexogen.com for other kit combinations.

Associated Products

- SPLIT RNA Extraction Kits (p.31)
- SIRVs - Spike-In RNA Variant Controls (p.32)
- SLAMseq Kits for High-Throughput Kinetic RNA Sequencing (p.22)

Find more information about this product at its web page:

This product is available as part of the Lexogen NGS Services.

For details, contact our Services department: services@lexogen.com.

Poly(A) RNA Selection Kit V1.5

Lexogen's Poly(A) RNA Selection Kit V1.5 enables the rapid and highly specific enrichment of polyadenylated RNAs from total RNA samples and thereby efficiently eliminates highly abundant cytoplasmic ribosomal RNA (rRNA) which is often of little interest. Samples are suited for various demanding RNA analysis applications such as RNA-Seq.

Advantages

- Highly specific poly(A) enrichment from total RNA (>95 % protein coding RNA)
- Flexible RNA input amounts (1 ng - 5 µg of total RNA)
- Included in CORALL mRNA Kits with Unique Dual Indices (UDIs) for use with 1 ng - 1 µg total RNA input
- Poly(A) RNA purifications of up to 480 µg (96x 5 µg)
- Automation-compatible protocol – magnetic bead-based purification
- Rapid turnaround – only 20 minutes hands-on time
- Poly(A) RNA elution or beads-bound seamless integration in various downstream applications

Workflow

Total RNA is briefly denatured and the polyadenylated 3' ends present in most mRNAs are hybridized to oligo(dT) beads. Any RNA without poly(A) stretches (e.g., rRNA, tRNA) will not be captured and hence be removed during subsequent washing. Polyadenylated RNA can either be eluted from the oligo(dT) beads and used in e.g., cDNA library construction, RT-PCRs, as well as RNA-Seq, or directly inserted into downstream applications while still being bound to the oligo(dT) beads (e.g., priming first strand cDNA synthesis). This Poly(A) RNA Selection Kit is included in Lexogen's CORALL mRNA-Seq V2 Library Prep Kits (p.18).

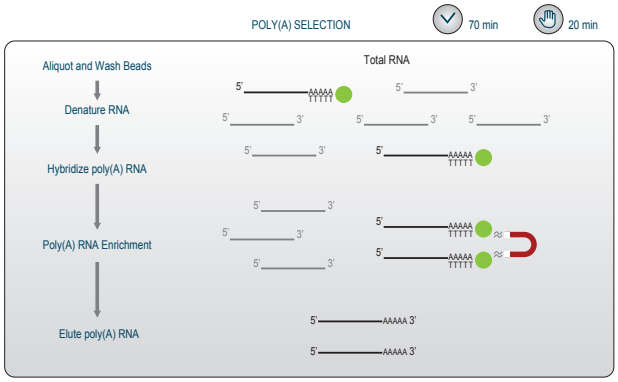


Figure 1 | Schematic overview of the Poly(A) RNA Selection workflow.

Performance

The Poly(A) RNA Selection Kit V1.5 enables highly specific poly(A) RNA enrichment and efficient removal of rRNA without a poly(A) tail (Fig. 2). Protein coding genes account for 95 % of mapped reads in poly(A) RNA isolated using Lexogen's Poly(A) RNA Selection Kit V1.5 (Fig. 3). Standard recovery ranges from 1 - 3 % of input RNA and is highly dependent on the input material.

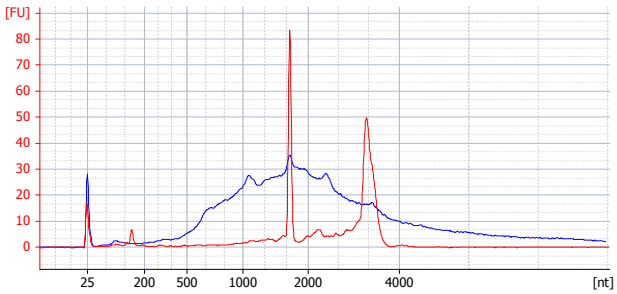


Figure 2 | Lexogen's Poly(A) RNA Selection Kit V1.5 enables highly specific poly(A) RNA enrichment and efficient removal of rRNA. Bioanalyzer traces before (red) and after poly(A) RNA selection (blue).

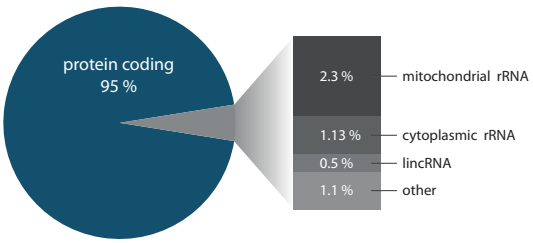


Figure 3 | Highly efficient removal of rRNA using Lexogen's Poly(A) RNA Selection Kit. RNA-Seq biotype analysis shows that the Poly(A) RNA Selection Kit enriches RNAs containing a poly(A) tail such as mRNAs and mitochondrial rRNAs.

Featured Products

- Poly(A) Selection Kit (Cat. No. 157)
- CORALL mRNA-Seq V2 Library Prep Kits (p.18)

Find more information about this product at its web page:

Associated Products

- SPLIT RNA Extraction Kits (p.31)
- SIRVs - Spike-In RNA Variant Controls (p.32)

This product is available as part of the Lexogen NGS Services.
For details, contact our Services department: services@lexogen.com.

TraPR Small RNA Isolation

TraPR (Trans-kingdom, rapid, affordable Purification of RISCs) presents a gel- and bias-free, column-based method for isolation of functional small RNAs from RNA-induced silencing complexes (RISCs) of all organisms. Within 15 minutes, TraPR enables purification of RISC fractions even from challenging or inconsistent samples, cell types, tissues, and bio-fluids.

Advantages

- Fast and easy isolation of physiologically relevant silencing small RNAs
- Unique, gel-free, single column workflow capturing even low abundant sRNAs
- Universally applicable to all eukaryotic organisms, even unknown species
- Consistent and reproducible results even from challenging and variable sample types
- Saving sequencing cost by focusing the reads on functional sRNAs only

Workflow

TraPR is an easy and robust column purification: the sample is lysed, and the clarified lysate is loaded onto the TraPR column. TraPR exploits the conserved properties of RISCs to elute them while bulk RNA and DNA are retained on the column (Fig. 1). After RISC elution, sRNAs can be isolated by phenol / chloroform extraction, and the resulting pure sRNAs are suitable for all molecular biology and NGS applications.

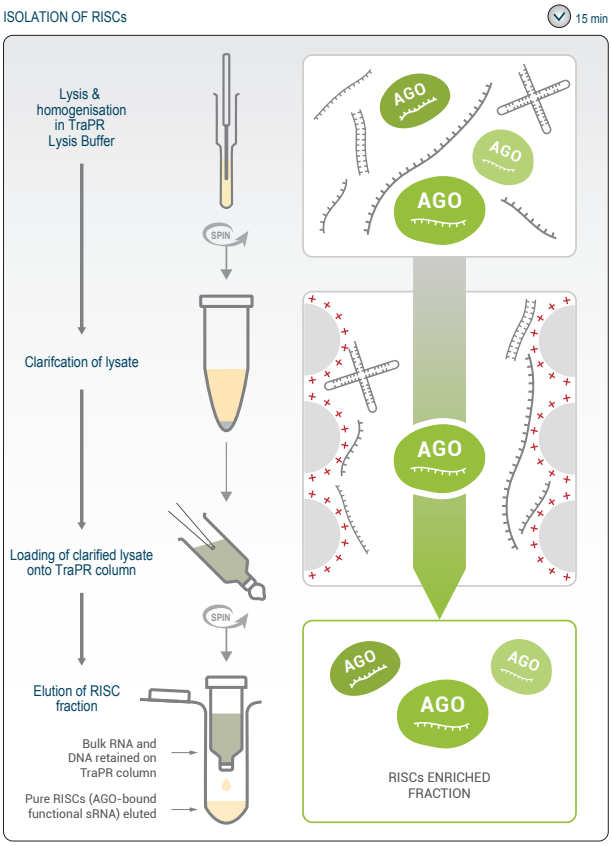


Figure 1 | Schematic depiction of the TraPR workflow and the principle of RISC isolation.

sRNAs can be isolated even from degradation-prone material or from samples that are notoriously hard to work with (e.g., plasma).

Performance

Lexogen's TraPR Small RNA Isolation Kit specifically isolates RISC-associated sRNAs, including piRNAs, siRNAs, miRNAs (Fig. 2) *via* a single purification column, and subsequent RNA extraction channels only functional sRNAs into the library preparation. Thus, TraPR decreases sequencing costs by re-directing reads from by-products towards functional sRNAs, dramatically lowering the overall cost per sample for sRNA-Seq (Fig. 3).

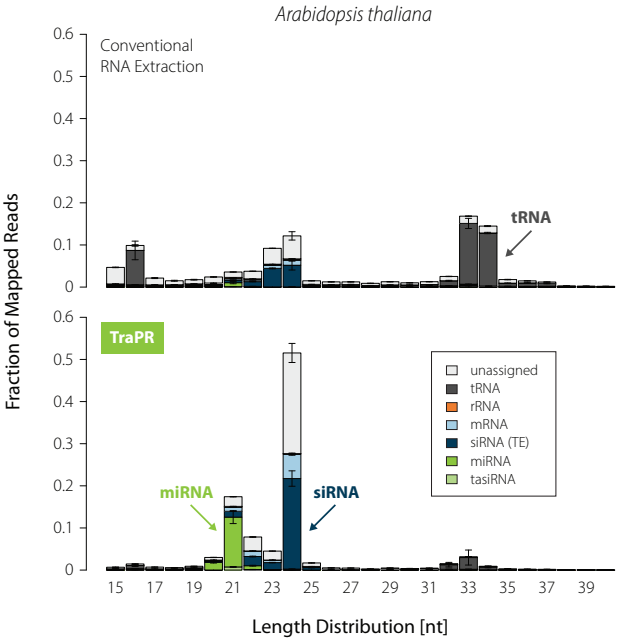


Figure 2 | TraPR enriches functional sRNA. Size distribution and biotypes of mapped reads from NGS libraries prepared from total RNA (conventional RNA extraction) or TraPR-isolated sRNA from *Arabidopsis thaliana* [1].

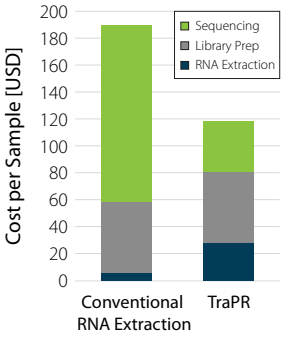


Figure 3 | TraPR significantly reduces overall sRNA analysis cost. Almost all reads obtained from TraPR-isolated RNA map to functional sRNAs (see also Fig. 2) whereas using conventional RNA extraction, only ~40 % of reads map to functional sRNAs. Hence, TraPR allows pooling of more libraries per lane resulting in decreased sequencing and overall cost per sample.

TraPR Overcomes Current Obstacles in sRNA Isolation

Current methods for specific isolation of functional sRNAs involve co-immuno-precipitation (Co-IP) via their respective AGO proteins. Co-IP is a very specific and sensitive method to isolate functional sRNAs but it is limited to one organism of interest and the availability of suitable, expensive antibodies. On the other hand, isolation with commercial sRNA extraction kits (using spin columns) is fast, cheap, and easy, but it does not confer any specificity since all RNAs smaller than a common threshold of ~200 nucleotides are purified. Thus, the majority of RNA-Seq reads will be wasted on non-functional RNA fragments derived from degradation.

Subsequent approaches to increase specificity use tedious gel extraction steps. But since selection is still purely based on the size, a distinction between functional sRNAs and degraded RNA fragments is not possible.

So far, there has been no method that combines the specificity of AGO Co-IP with the ease of commercial sRNA extraction kits (Tab. 1).

Table 1 | TraPR combines the favorable features of sRNA-specific AGO Co-IP with fast and easy sRNA extraction kits.

	TraPR	Other sRNA Isolation Methods		
		AGO Co-IP	sRNA Extraction Kits	Gel Extraction
Specific	✔	✔	✖	✔*
Sensitive	✔	✔	✖	✖
Robust	✔	✔	✔	✖
Universal	✔	✖	✔	✔
Fast	✔	✖	✔	✖
Easy	✔	✖	✔	✖

* Specific for sRNA length, not functionality.

TraPR Combines Fast and Easy Handling with High Specificity for Functional sRNAs

TraPR enables the specific isolation of functional sRNAs from RISCs using an easy 15 minutes column purification followed by standard RNA extraction of 1 hour (Fig. 4). No long lasting and tedious gel extraction or prior knowledge of the species' AGO composition for specific Co-IP is needed. This is the fastest possible method that also confers high specificity for functional, physiological relevant sRNAs, even low abundant ones. The TraPR Small RNA Isolation Kit generates high-quality sRNA preparations suitable for NGS applications and thus provides a highly reproducible, time-saving method to enrich pure sRNAs from challenging or inconsistent samples, cell types, tissues, and biofluids that outperforms all current gold-standard procedures for sRNA profiling.

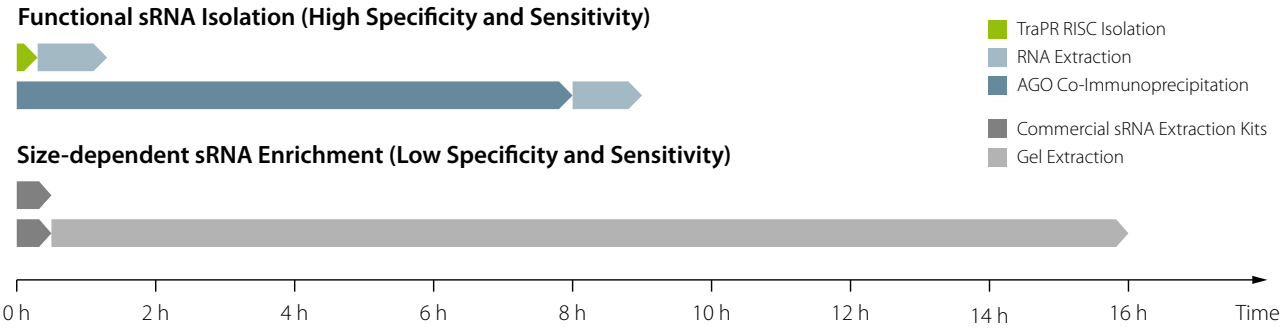


Figure 4 | TraPR is the fastest method for functional sRNA isolation. TraPR isolates functional sRNA in only 1 hour and 15 minutes. Common sRNA extraction kits are very fast, but not selective for functional sRNAs. AGO Co-Immunoprecipitation or gel extraction increase specificity but take one to two full working days.

Associated Products

- Small RNA-Seq Library Prep Kit (p.20)
- Gel Extraction Module (p.39)

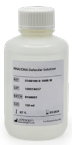
This product is available as part of the Lexogen NGS Services.

For details, contact our Services department: services@lexogen.com.

Find more information about this product at its web page:

References
1. Grentzinger T., Oberlin, S., Schott, G., et. al. (2020) A universal method for the rapid isolation of all known classes of functional small RNAs. Nucleic Acids Res, DOI: 10.1093/nar/gkaa472.

RNA/DNA Defender Solution



Lexogen's RNA/DNA Defender Solution is a non-toxic storage solution that stabilizes and protects fragile RNA molecules in fresh tissue and cell culture samples, as well as DNA molecules. It eliminates the need to process samples immediately or freeze them in liquid nitrogen for later processing.

Advantages

- Preserve the genetic integrity and expression profile of the sample
- Freedom from needing to immediately process samples or freeze them in liquid nitrogen
- Safely postpone RNA or DNA extraction, especially when the experiment calls for numerous samples
- No need to have access to liquid nitrogen or a -80 °C freezer at the place of sampling
- Store one day at 37 °C, one week at room temperature, one month at 4 °C, or up to one year at -20 °C
- Suitable for most animal and plant tissues as well as cultured cells

Workflow

RNA/DNA Defender Solution rapidly permeates the tissues and cells. It stabilizes cellular RNA (and DNA) molecules, allowing temporary storage of the sample at temperatures where RNA degradation would typically occur. Immediately after collection, fresh tissue should be completely submerged in the RNA/DNA Defender Solution. Cultured cells are pelleted upon harvesting and resuspended in the RNA/DNA Defender Solution. Tubes containing submerged tissue or resuspended cells can be stored for up to one year at -20 °C, one month at +4 °C, at room temperature for one week, or one day at 37 °C (Fig. 1).

Storage of tissue and cells in RNA/DNA Defender Solution is compatible with most RNA extraction methods, such as acid phenol extractions or column-based one-step kits. To extract high-quality RNA from samples stored in RNA/DNA Defender Solution, we recommend Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008). Samples stored in RNA/DNA Defender Solution can also be used for extraction of DNA using any method of choice.

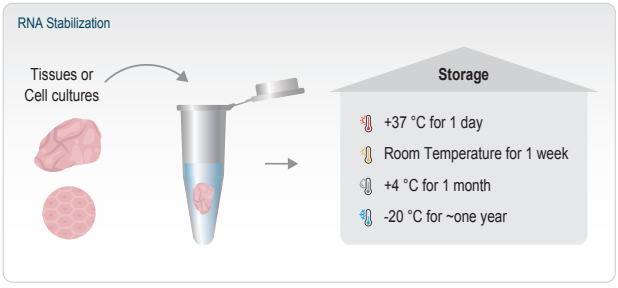


Figure 1 | Workflow for storage of fresh tissue or cells in RNA/DNA Defender Solution for downstream RNA or DNA extraction.

Performance

Storage of tissue pieces in RNA/DNA Defender Solution stabilizes contained RNA even at temperature of 37 °C or over longer periods of time at -20 °C (Fig. 2).

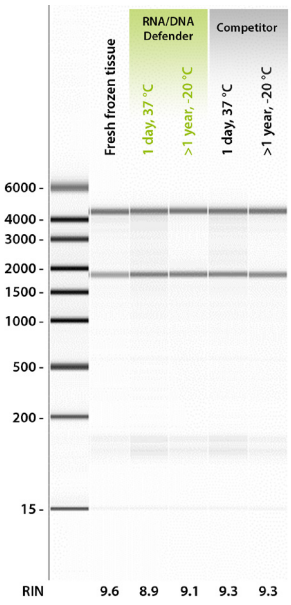


Figure 2 | RNA/DNA Defender Solution stabilizes RNA in tissue samples. Mouse liver tissue was either fresh frozen or stored in RNA/DNA Defender Solution or a competitor RNA stabilization solution for one day at 37 °C or for over one year at -20 °C. After storage RNA was extracted using SPLIT RNA Extraction kit and analyzed on Fragment Analyzer.

Associated Products

– SPLIT RNA Extraction Kits (p.31)

This product is available as part of the Lexogen NGS Services.

For details, contact our Services department: services@lexogen.com.

Find more information about this product at its web page:

SPLIT RNA Extraction Kits

Lexogen's SPLIT RNA Extraction Kit is specifically designed to purify RNA for demanding downstream applications such as RNA-Seq. It enables fast, clean, and highly efficient RNA extraction from a broad range of biological samples. SPLIT recovers the complete RNA size ranges, including small RNA.

Advantages

- Total RNA from <17 nt to >10,000 nt
- Universal, species-independent RNA extraction
- Convenient and fast protocol requiring only 30 minutes (Phase Lock Gel tubes included)
- High RNA integrity and purity (RIN >8 for tissue, up to RIN 10 for cell culture)
- Protocol for extraction of large and small RNA-enriched fractions
- Supplementary protocol for FFPE samples available

Workflow

The SPLIT workflow enables easy, fast, and reliable RNA extraction from a variety of materials (plasma, tissue, cell lines) and from various organisms (mammals, plants, insects, fungi, bacteria, virus, etc.). The sample is homogenized in a highly chaotropic isolation buffer for RNase deactivation and easy solubilization (Fig. 1). This is followed by acidic phenol / chloroform extraction aided by Phase Lock Gel tubes for a clean separation of the aqueous phase (containing the RNA) from the organic and interphase (comprising DNA and proteins). The aqueous phase is mixed with isopropanol and the RNA is precipitated onto a silica column.

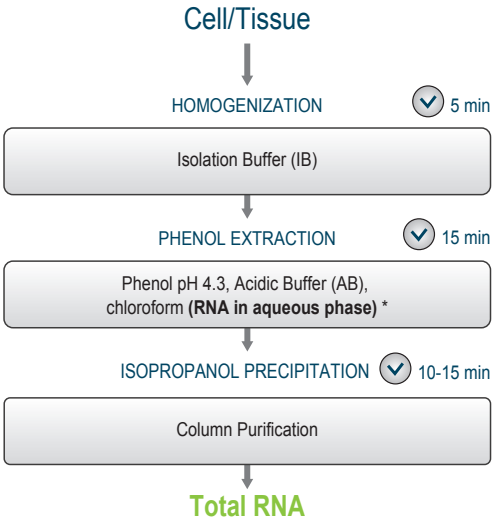


Figure 1 | Schematic overview of the SPLIT workflow for cells/tissues.

Performance

Samples extracted with the SPLIT kit deliver the entire RNA size range for RNA-Seq, from miRNAs (17 nt) to mRNAs of over 10,000 nucleotides length, which can be isolated as total RNA or as large and small RNA-enriched fractions by following a supplemental protocol (Fig. 2).

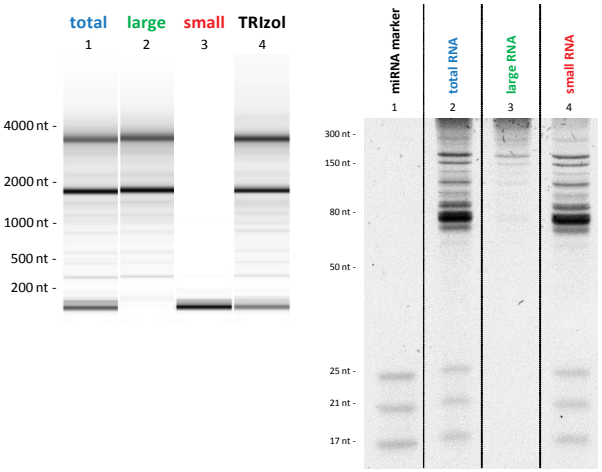


Figure 2 | The SPLIT protocol enables the extraction of high-quality RNA and efficient recovery of small RNA. A) RNA from mouse liver stored in RNAlater was extracted either as total RNA (lane 1) or as large and small RNA-enriched fractions (lanes 2 and 3). A control sample was extracted following a TRIzol protocol (lane 4). All samples have a RIN of 8.2 – 8.3 (not applicable for small RNA-enriched fraction). **B)** Separation of SPLIT RNA samples on a polyacrylamide gel, showing the large and small RNA-enriched fractions at a threshold of ~150 nt. The total RNA sample comprising small and large RNA is shown as a comparison. The homogenate was spiked with a miRNA marker to assess efficient miRNA recovery. Small RNAs down to at least 17 nt are efficiently recovered in the total RNA sample and in the small RNA-enriched fraction.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- CORALL Total RNA-Seq V2 Library Prep Kits (p.16) and CORALL mRNA-Seq V2 Library Prep Kits (p.18)
- Small RNA-Seq Library Prep Kit (p.20)
- LUTHOR 3' mRNA-Seq Library Prep Kit (p.13)

This product is available as part of the Lexogen NGS Services.

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Find more information about this product at its web page:

SIRVs - Spike-In RNA Variant Controls

Each step in RNA sequencing workflows imposes biases that the final data processing algorithms aim to compensate afterwards. Markers and controls are the only way to unambiguously determine the reliability of your RNA-Seq experiment, and Lexogen's Spike-In RNA Variants (SIRVs) provide the means to control for both, RNA-Seq experiment quality and data set comparability.

Advantages

- Comparison and monitoring of RNA-Seq experiments by using external RNA controls
- Identification of error sources and biases to validate and improve RNA-Seq workflows
- Compatible with any transcriptomics platform and with RNA from any organism, down to single-cell level
- Representation of transcriptomic isoform, concentration, and length complexity
- Optimized SIRV sets for straightforward sample-to-sample comparisons, full-length assessments, and complex isoform expression measurements

Workflow and Modular Design

Minimal Read Share for Maximum Control

The Spike-In RNA Variant (SIRV) controls are sets of artificial RNAs for transcriptome analyses targeting long coding and non-coding RNAs. The SIRVs comprehensively mirror transcriptome complexity and represent a ground truth by providing *a priori* knowledge of RNA sequences and concentrations. Due to their artificial sequence, they can be spiked into the RNA of any organism. In RNA-Seq workflows the spike-in RNAs are subjected to the same reactions, restrictions, and biases as the endogenous RNA (Fig. 1). In the final step (NGS data analysis), the spike-in information is separated, and only 1-2 % of the final sequencing reads have to be dedicated to the controls to evaluate an RNA-Seq workflow. Biases and "blind spots" are revealed unambiguously, and qualitative measures such as precision, accuracy, and coefficient of deviation can be calculated. Based on these measures, small SIRV data subsets can be compared between samples to determine the degree of their concordance and suitability for meaningful differential expression analyses.

Data Analysis with SIRVsuite Spike-In Analysis Software

Lexogen developed a publicly-available command line tool, SIRVsuite, that can be used to quality control RNA-Seq workflows using Lexogen's SIRVs. To get started with SIRVsuite, please visit Lexogen's GitHub page: https://github.com/Lexogen-Tools/SIRVsuite.

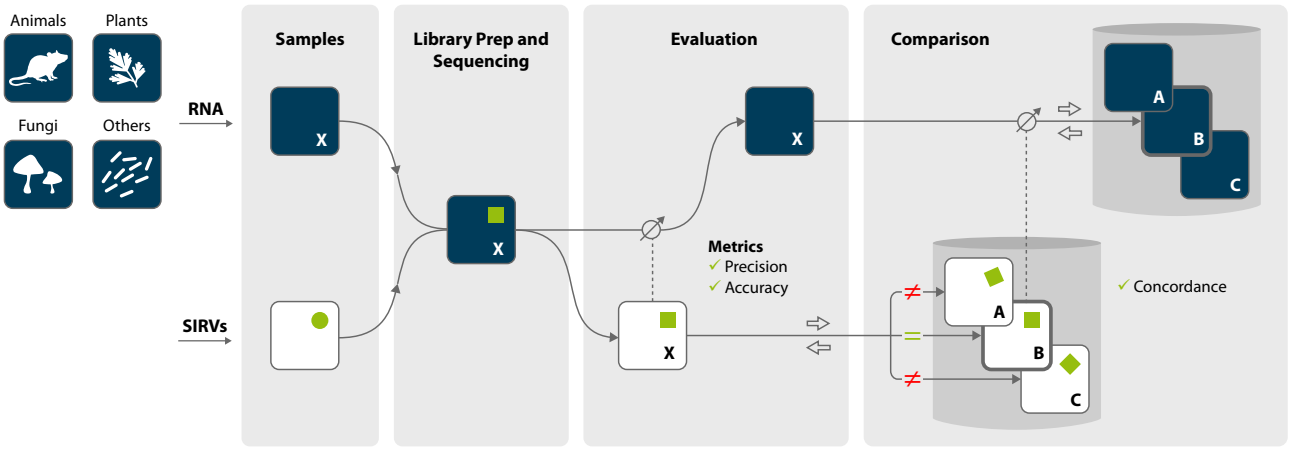

Figure 1 | Using SIRV controls in RNA-Seq. SIRVs are defined artificial RNA molecules that mimic the main aspects of transcriptome complexity. They are added in minuscule amounts to samples before the library preparation and are processed alongside endogenous RNA. After mapping the reads to the combined genome and SIRVome, the SIRV control data is used to analyze the quality metrics and to categorize the experiments.

Modular Concept

The RNA spike-in controls are available in three modules, the SIRV isoform, the ERCC, and the long SIRV module, each probing a specific component of the transcriptome (Fig. 2)

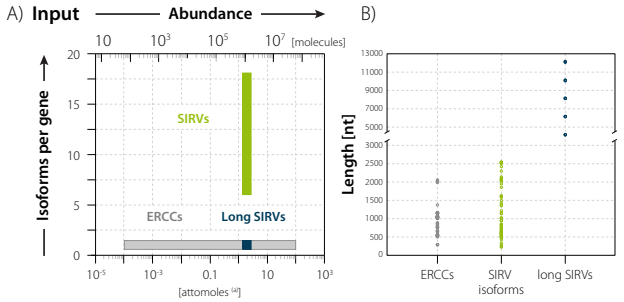

Figure 2 | The representation of isoform complexity, transcript abundance, and length by the three SIRV modules. SIRV Isoform Mix E0 represents isoform complexity at equimolar concentrations, whereas ERCCs and long SIRVs both follow the one gene, one isoform rule covering abundance and length, respectively. Note that in SIRV-Set 1 the SIRV isoforms are also available in concentration ranges of 8-fold (Mix E1) and 128-fold (Mix E2).

Isoform Module

The SIRV isoform module mimics transcriptome complexity in a condensed manner, with 69 artificial transcript variants addressing alternative splicing, alternative transcription start- and end-sites, overlapping genes, and antisense transcripts (Fig. 3). Between 6 and 18 transcript variants map to seven SIRV loci, resulting in high isoform complexity for in-depth probing of RNA-Seq workflows [1]. Correct,as well as (exemplary) insufficient and over-annotations are provided for the testing of workflow robustness towards different transcript annotations [2]. The SIRV isoform module is available in three mixes, with equimolar concentrations of all transcripts in Isoform Mix E0, and molar ratios of up to 8-fold (Mix E1) and up to 128-fold (Mix E2), respectively.

ERCC Module

The External RNA Controls Consortium (ERCC) has developed 92 artificial transcripts with non-overlapping sequences (Fig. 3). Due to their unique sequence identities, the ERCC controls are well suited for measuring technical parameters irrespective of isoforms. By covering a 2^{20} (10^6) dynamic range, ERCC Mix 1 addresses the entire spectrum of transcript concentration complexity [3, 4]. Comparison of the assigned and evaluated reads with known concentrations allows for the assessment of dynamic range, dose response, lower limit of detection, and workflow efficiency.

Long SIRV Module

The introduction of long read sequencing platforms has significantly increased the available read length, now easily exceeding the average transcript length. Lexogen has therefore developed the "long SIRVs", a module that contains three different transcripts for each of the five length categories 4 kb, 6 kb, 8 kb, 10 kb, and 12 kb (Fig. 3). The sequence of each of these 15 RNAs is unique and does not overlap with any other spike-in or endogenous transcripts, making them optimal tools to evaluate the transcript length aspect in RNA-Seq workflows.

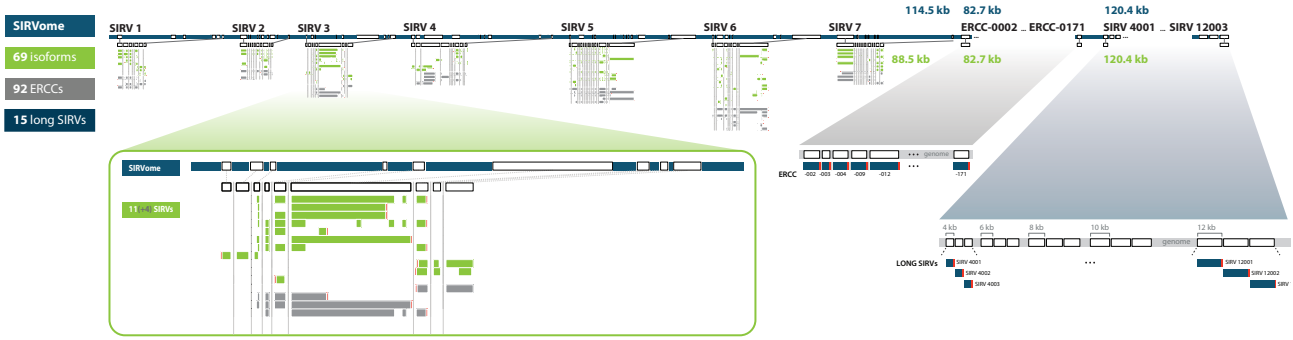

Figure 3 | SIRVs design overview. SIRV isoform genomic loci, ERCC genes, and long SIRV genes are lined up on a SIRVome. SIRV1 to SIRV7 of the SIRV Isoform module mimic human model genes to comprehensively represent all main aspects of alternative splicing and transcription in numerous repeats and variations. Pullout, compact coverage visualization of the SIRV3 locus which provides 11 transcript variants (shown in green); transcript variants shown in grey are additional annotations for alternative evaluation procedures. The ERCC module follows the one gene, one transcript concept, covering a concentration range of 6 orders of magnitude. The long SIRV module contains RNAs 4, 6, 8, 10, or 12 kb long with 3 transcripts in each length category.

Performance

Figure 4 shows for one example, locus SIRV6, the expected read coverage derived from summing up the equimolar contributions of the 18 individual transcripts of Isoform Mix E0. The difference between expected and measured read coverage can be condensed into a single value, the Coefficient of Deviation (CoD), which is a measure for performance and bias of RNA-Seq experiments [1].

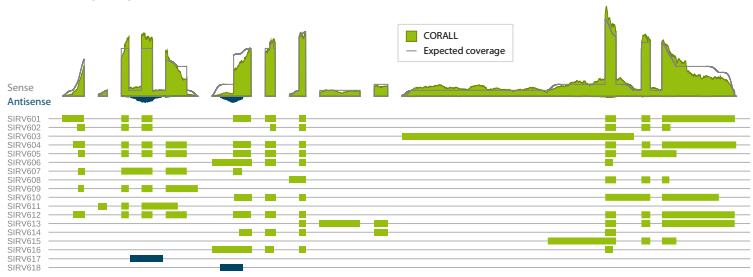

Figure 4 | Coverage of SIRV6. The exon-intron structure of all 18 transcripts of the SIRV6 locus is shown in the lower panel (with antisense transcripts in blue). The equimolar individual SIRV transcripts contribute in equal parts to the expected coverage (fine line, with modeling of transcript ends). Reads generated with the CORALL RNA-Seq Library Preparation Kit were mapped to the SIRVome and visualized on locus level.

The ERCC module is ideal for unambiguous input-output concentration correlation measurements and to derive lower limit of detection (LLoD) values (Fig. 5A). Input-output correlations for more complex settings with multiple isoforms of a given gene also distributed across a wide concentration range can be evaluated using Isoform Mixes E0, E1, and E2. Further, RNA-Seq pipelines can be assessed for their performance in determining differential expression on the transcript level (Fig. 5B).

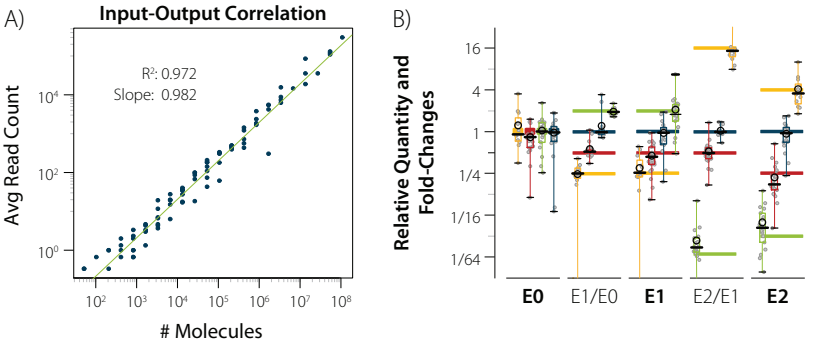

Figure 5 | Measuring input-output correlation using ERCC and SIRV Isoform modules. A) The one gene, one transcript spike-ins of ERCC Mix 1 in SIRV-Set 3 were assessed in a QuantSeq 3′ mRNA-Seq experiment. Correlation of the theoretical and the measured concentrations was high with an R^2 of 0.972. **B)** Box plot overview of calculated concentration values for transcripts in SIRV mixes E0, E1, and E2 and mix ratios. Results are shown in reference to the known inputs, the colored bars correspond to SIRV SubMixes with isoforms in 1:1 (E0), 1:8 (E1) and 1:128 (E2) concentration ranges. The black circles mark the mean and the bold dashes the medians of the data points which are shown in grey, the boxes span the 25 to 75 % region of the data points, and the black whiskers with connecting lines reach up to the min and max values and indicate also outliers outside the scale of the graph.

Applications

The SIRV modules are available in different sets to provide the perfect mix for different applications (Table 1). They cater for in-depth isoform workflow analysis (SIRV-Set 1), cost-efficient data set comparison (SIRV-Set 2), concentration-probing (SIRV-Set 3), and comprehensive assessments including long spike-in RNAs (SIRV-Set 4).

Table 1 | SIRV set selection guide. SIRV-Set 1 contains the isoform mixes E0, E1 and E2 of the isoform module, SIRV-Set 2 provides the isoform Mix E0 only, SIRV-Set 3 has the SIRV Isoform Mix E0 in a mixture with the ERCCs, and SIRV-Set 4 is a mixture of the long SIRVs with SIRV Isoform Mix E0 and the ERCCs. *Refers to number of vials, 1 or 3. The ERCC Module includes ERCC Mix 1 [5] ✔: applicable, ✖: not applicable, and partly applicable (or parts of the set applicable).

		SIRV-Set 1	SIRV-Set 2	SIRV-Set 3	SIRV-Set 4
Cat. No		**025.03**	**050.0***	**051.0***	**141.0***
Module(s)	Isoforms	Isoform Mixes E0, E1, E2	Isoform Mix E0	Isoform Mix E0	Isoform Mix E0
	ERCC	✖	✖	ERCC Mix 1	ERCC Mix 1
	long SIRVs	✖	✖	✖	long SIRVs
Property	Isoform detection and quantification	✔	✔	✔	✔
	Dynamic range	partially	✖	✔	✔
	Length >2.5 kb	✖	✖	✖	✔
Applications	Pipeline Validation	✔	partially	partially	partially
	Sample Control	✖	✔	✔	✔
Number of spike-in transcripts in each mix		69 (69 isoforms in each mix)	69 (69 isoforms)	161 (69 isoforms, 92 ERCCs)	176 (69 isoforms, 92 ERCCs, 15 long SIRVs)

References
1. Paul, L., et al. (2016) SIRVs: Spike-In RNA Variants as external isoform controls in RNA-sequencing. bioRxiv. DOI:10.1101/080747
2. Weirather, J. L., et al. (2017) Comprehensive comparison of Pacific Biosciences and Oxford Nanopore Technologies and their applications to transcriptome analysis. F1000Res 6, 100. DOI: 10.12688/f1000research.10571.1
3. Baker, S. C., et al. (2005) The External RNA Controls Consortium: a progress report. Nature Methods 2, 731–734. DOI:10.1038/nmeth1005-731
4. The External RNA Controls Consortium. (2005) Proposed methods for testing and selecting the ERCC external RNA controls. BMC Genomics 6, 150. DOI:10.1186/1471-2164-6-150
5. Munro, S. A., et al. (2014) Assessing technical performance in differential gene expression experiments with external spike-in RNA control ratio mixtures. Nature Communications 5, 5125. DOI:10.1038/ncomms6125
6. SEQC/MAQC-III Consortium. (2014) A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. Nature Biotechnology 32, 903–914. DOI:10.1038/nbt.2957

This product is available as part of the Lexogen NGS Services. For details, contact our Services department: services@lexogen.com.

Associated Products

Find more information about this product at its web page:

- CORALL Total RNA-Seq V2 Library Prep Kits (p.16) and CORALL mRNA-Seq V2 Library Prep Kits (p.18)
- TeloPrime Full-Length cDNA Amplification Kit (p.21)
- LUTHOR 3′ mRNA-Seq Library Prep Kit (p.13)
- QuantSeq Expression Profiling Library Prep Kits (p.7)
- SPLIT RNA Extraction Kits (p.31)
- Mix² RNA-Seq Data Analysis Software (p.35)

Mix² RNA-Seq Data Analysis Software

Mix² (Mix-Square) computes highly accurate concentration estimates for gene isoforms by adapting to the positional coverage bias in RNA-Seq data. Isoform quantification with Mix² is repeatable across variable conditions and leads to accurate detection of differential expression.

Advantages

- Precise and repeatable transcript concentration estimates across different sequencing facilities, library preparations, and RNA integrity states
- Accurate detection of differential expression
- Detection and classification of bias types in RNA-Seq data
- Extremely fast run-times and small memory footprint

Working Principle

Accuracy and repeatability of isoform quantification in RNA-Seq is adversely affected by fragment bias in RNA-Seq data. Mix² makes no assumptions about coverage bias but fits a mixture model to the data for each gene isoform (Fig. 1). Mix² can therefore, for instance, accurately represent the 5′ bias, as shown in Fig. 1A and B, whereas Cufflinks is restricted to the uniform distribution (Fig. 1C).

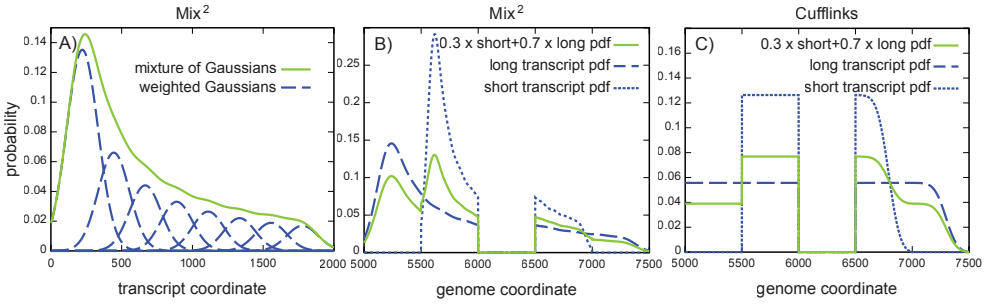

Figure 1 | Working principle of Mix². **A)** Exemplary representation for 5′ positional fragment bias over a 2,000 nt transcript modelled by Mix² with a mixture of 8 normal distributions. **B)** Gene with 2 isoforms with 5′ bias modelled by Mix². **C)** Uniform distribution of Cufflinks in gene B) irrespective of fragment bias.

Performance

Mix² was tested on publicly available RNA-Seq data sets from multiple sequencing facilities, library preparations, and from RNA with different types of degradation. In comparison to other bias correcting methods, quantification estimates of Mix² are better correlated with the qPCR assessed ground truth and lead to more accurate detection of differential expression (Fig. 2) [1]. Further, Mix² enables the investigation of biases in RNA-Seq data by visualizing clusters of estimated fragment distributions (not shown).

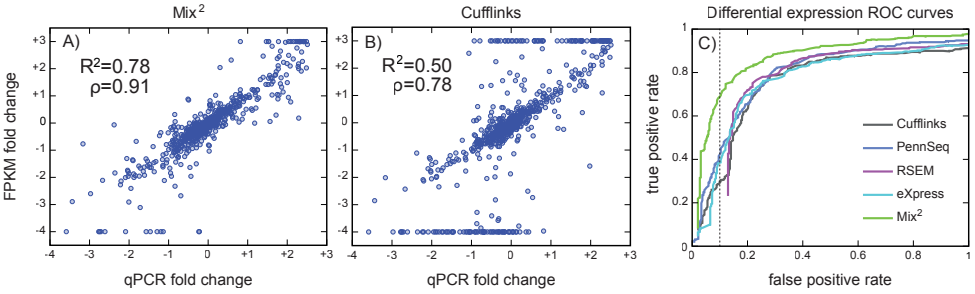

Figure 2 | Correlation between qPCR expression changes and expression changes estimated by **A)** Mix² and **B)** Cufflinks. **C)** Receiver Operating Characteristic (ROC) curve of detection of differential expression based on isoform quantification of different methods. True positive rates are significantly higher for Mix².

Associated Products

– CORALL Total RNA-Seq V2 Library Prep Kits (p.16) and CORALL mRNA-Seq V2 Library Prep Kits (p.18)

This product is available as part of the Lexogen NGS Services. For details, contact our Services department: services@lexogen.com.

Find more information about this product at its web page:

References

1. Tuerk, A., et al. (2017) Mixture models reveal multiple positional bias types in RNA-Seq data and lead to accurate transcript concentration estimates. PLOS Computational Biology 13(5): e1005515. DOI:10.1371/journal.pcbi.1005515

Lexogen UDI 12 nt Unique Dual Indexing System

The Lexogen UDI 12 nt Unique Dual Indexing Sets contain up to 384 pre-mixed UDIs with patented advanced index sequence design. All indices bear optimal distance to each other which allows superior error correction for maximal sequencing data output.

Advantages

- 384 Unique Dual Indices (UDIs) cover all barcoding needs in RNA-Seq
- Rescue of reads maximizes data output and saves sequencing costs
- Adjustable index read-out of 8, 10, or 12 nt length accommodates each experiment scale
- Optimized distances translate into superior index error correction

Workflow

Lexogen's UDI 12 nt Unique Dual Indexing Sets feature superior error correction for maximal sequencing data output and are introduced at the PCR step of Lexogen's library kits. In this step the previously introduced partial Illumina adapters are completed and the libraries finalized for sequencing. Lexogen 12 nt UDIs can thus be used for all RNA-Seq library preps that use this principle which is the case for most commercially available methods (for details and questions, please contact support@lexogen.com).

A critical consideration for any multiplexed RNA-Seq workflow is to avoid errors in the index read-out, as these can result in the mis-assignment of sequencing reads to the wrong samples. While the majority of the raw reads will have the expected index combinations (Fig. 1A), read mis-assignment can occur, due to two main events: Index Hopping and random Index Sequence Errors. Unique Dual Indexing (UDI), where each library is barcoded with unique i7 and unique i5 index sequences, unambiguously identifies reads with hopped indices which can be removed from downstream analysis (Fig. 1C). Read mis-assignment due to random Index Sequence Errors (an error in one index sequence transforms the index into another one present within the same multiplexed sample pool) can be resolved with UDIs and discarded (Fig. 1C). If the index sequence is different enough from the other index sequences in this pool, then error correction can be applied to recover a significant share of these reads (up to 7 % of the initial reads, Fig. 1B).

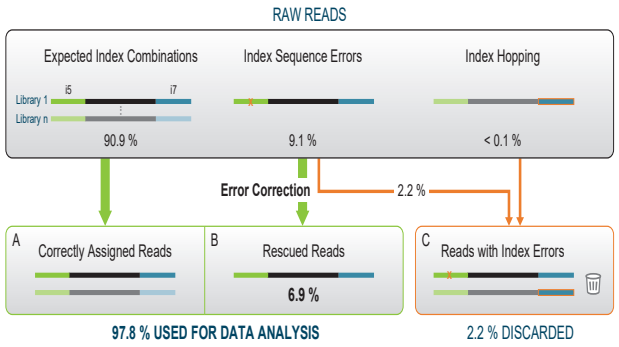

Figure 1 | The effects of Index Hopping and Index Sequence Errors in a pool of libraries with Unique Dual Indexing. Correctly assigned reads (A) Reads with hopped indices are irreversibly discarded (C). Reads with random Index Sequence Errors in the pool are classified undetermined, and can be rescued (B). The percentages were derived from an RNA-Seq experiment pooling 96 libraries with Lexogen's 12 nt UDIs and full 12 nucleotide index read-out on an Illumina NextSeq500.

Performance

Lexogen's 12 nt UDIs are 12 nucleotides (nt) long and designed to maximize inter-index distance for different sample numbers and index read-out lengths. In a typical experiment around 9.1 % of the initial raw reads contain a random Index Sequence Error (Fig. 2A, orange). This renders them undetermined, hence removing these reads from downstream analysis. Lexogen's advanced index design enables the rescue of 76 % of these undetermined reads (6.9 % of the initial reads), even if multiple nucleotides of the index contain errors. The useful output thereby increases to 97.8 % of the initial reads, an unprecedented performance due to the cutting-edge index design (Fig. 2B).

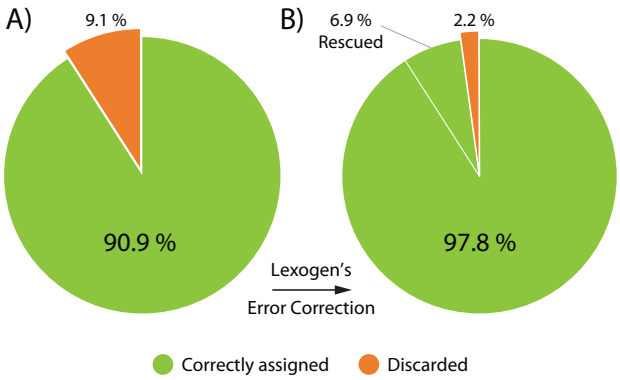

Figure 2 | Maximizing read output with Lexogen's 12 nt UDIs and error correction. 96 multiplexed libraries were sequenced on an Illumina NextSeq 500 with 12 nt UDI read-out. **A)** 9.1 % of reads were undetermined (orange) due to random Index Sequence Errors. **B)** Lexogen's Error Correction Tool confidently rescued almost 76 % of originally undetermined reads and correctly assigned them to the respective library.

Lexogen's 12 nt UDIs can be read out in 8, 10, or 12 nt index read lengths, suiting every customer's need. For higher level of multiplexing longer index read-out is recommended to gain full advantage of the error correction. Further, the different Sets A (Forward Strand Workflow) and B (Reverse Complement Workflow) are designed for perfect fit with the different workflows on Illumina instruments. The 12 nt UDI Sets are conveniently available in bundles with QuantSeq and CORALL V2 library prep kits and as Add-on kits for other vendors' library preps.

Demultiplexing and Error Correction Tool – iDemux

Lexogen has generated an alternative tool, iDemux, which is freely available on Github (https://github.com/Lexogen-Tools/idemuxcpp). iDemux can demultiplex indices in the i7 and i5 position as well as i1 inline indices that are part of the read. The program was originally designed to demultiplex Quantseq-Pool libraries which can be triple-indexed and thus contain all three index types. By allowing for simultaneous demultiplexing and error correction of all indices, the tool saves valuable processing time and can maximize data output by rescuing reads with index errors. iDemux is a highly flexible tool and can be used for demultiplexing of any set of barcodes, however without error correction.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- CORALL Total RNA-Seq V2 Library Prep Kits (p.16) and CORALL mRNA-Seq V2 Library Prep Kits (p.18)
- LUTHOR 3' mRNA-Seq Library Prep Kit (p.13)

This product is available as part of the Lexogen NGS Services.
For details, contact our Services department: services@lexogen.com.

Find more information about this product at its web page:

Modules and Add-ons

PCR Add-on Kit for Illumina

The PCR Add-on Kit for Illumina provides PCR Mix, Enzyme Mix, and compatible primers for qPCR assays to determine endpoint PCR cycle numbers for various Library Prep Kits and library reamplification. Please consult our webpage and FAQs for compatibility information and contact support@lexogen.com for inquiries.

UMI Second Strand Synthesis Module for QuantSeq FWD

The UMI module allows unique tagging of individual transcripts with 6 nucleotides long Unique Molecular Identifiers (UMIs), which are read out at the beginning of Read 1. The module contains the UMI Second Strand Synthesis Mix (USS), which simply replaces the Second Strand Synthesis Mix 1 (SS1) from the standard QuantSeq FWD Kit (NOTE: This module is not compatible with QuantSeq REV).

Globin Block Modules for QuantSeq

The Globin Block (RS-Globin Block) Modules for QuantSeq prevent the generation of library fragments from globin mRNAs, by blocking their extension during second strand synthesis. The module is compatible only with QuantSeq 3′ mRNA-Seq Library Prep Kits for Illumina (FWD, Cat. No., 191-196, and REV), and is intended for the preparation of libraries from human (*Homo sapiens*) or pig (*Sus scrofa*) blood samples.

QuantSeq-Flex First Strand Synthesis Module

The QuantSeq-Flex First Strand Synthesis Module enables the exchange of the reverse transcription primer with a target-specific primer in the QuantSeq protocol. It can furthermore be used to generate longer libraries with the standard QuantSeq 3′ mRNA-Seq kit if needed. Compatible only with QuantSeq FWD Kits for Illumina (Cat. No., 191-196).

QuantSeq-Flex Second Strand Synthesis Module

With the QuantSeq-Flex Second Strand Synthesis Module the random second strand synthesis primer can be substituted with a target-specific primer in the QuantSeq protocol. Compatible only with QuantSeq FWD Kits for Illumina (Cat. No., 191-196).

Lexogen UDI 12 nt Unique Dual Indexing Add-on Kits

The Lexogen UDI 12 nt Unique Dual Indexing Add-on Kits contain up to 384 premixed UDIs with superior error correction for maximal sequencing data output. Two 96-well plate format index sets are available: Sets A1 – A4 for Workflow A and Set B1 for Workflow B. UDI Add-on Kits are provided with a dedicated amplification enzyme and buffer.

Purification Module with Magnetic Beads

The Purification Module with Magnetic Beads contains all the necessary reagents to carry out additional purifications, e.g., after PCR or to concentrate the libraries if needed. Compatible with QuantSeq, CORALL, and Small RNA-Seq Library Prep Kits.

Gel Extraction Module

The Gel Extraction Module enables the size selection of Small RNA-Seq libraries in a convenient spin column format. It prevents unwanted sequences (e.g., linker-linker artifacts and longer library fragments) dominating the sequencing results and helps focus the sequencing depth on the desired microRNA fraction.

TeloPrime PCR Add-on Kit

The TeloPrime PCR Add-on Kit contains 16 reactions of the optimized PCR Mix, polymerase, and Forward and Reverse Primers. These primers can be easily exchanged with gene-specific primers. The kit can furthermore be used to run multiple PCR amplifications of your sample to generate sufficient template for any downstream application.

Lexogen NGS Services

Advantages

- Fully customized services, from initial consultations and experimental planning to delivering publication-ready data
- Expertise in handling challenging material (e.g., FFPE curls), low-input and low-quality samples
- Standard as well as custom data analysis options

First-class NGS solutions with Lexogen NGS Services

We provide first-class, fully customizable workflows, where you can choose from nucleic acid extraction, library preparation, sequencing to data analyses. By developing our own products, we have gained invaluable NGS expertise that we use to adapt our pipelines to your specific experimental objectives, depending on your needs.

Consultation

Every project with Lexogen NGS Services begins with a consultation to ensure the best experimental design for your experiment to be successful. Together with you, we determine a tailored setup to ensure you get the data needed for your project.

Shipment Support

We can offer our customers various types of shipment support, such as providing help with customs and export documents. In addition, under the fulfillment of certain conditions, we can also organize the shipment for you.

Sample Extraction

We have extensive expertise in nucleic acid extraction from tissues, cells, and biofluids, both manual and automated. We have great experience and success with challenging samples such as FFPE-derived, microdissections, and degraded RNA - we identify the best approach to maximize the data quality for your given sample type.

Library Preparation

We offer various library preparation solutions tailored to your needs. By using, but not being limited to Lexogen products, we take advantage of our proprietary technologies and with the support of our R&D team, can adapt our pipelines to your specific experimental objectives.

Our portfolio includes:

- Gene expression sequencing
- Whole transcriptome RNA-Seq
- Small RNA-Seq
- Single-cell RNA-Seq
- Ultra-low input RNA-Seq
- DNA-Seq

Additional highlights:

- High-throughput library preparation solutions.
- Deplete and block globin mRNA in blood and BC1 mRNA in mouse brain samples for significantly improved sensitivity.
- Analysis of RNA kinetics: we prepare RNA-Seq libraries from SLAMseq samples.
- Unique Molecular Identifiers (UMIs) enabling the most accurate quantification of RNA transcripts.
- Lexogen's 12 nt multiplexing solutions (UDIs) provide superior error correction for maximal data output.
- Routine use of internal standard controls for workflow validation and data set concordance.

Sequencing

Samples are sequenced with the read mode and read depth most suitable for your application.

Data Analysis and Report

Sequencing data are analyzed in-house and standardly include: demultiplexing, read quality control, trimming and filtering procedures, mapping, read counting, and reporting (differential) gene expression. We also offer on-demand, custom design data analyses and custom pipeline development.

Contact us to discuss you project and/or to obtain an example report at www.lexogen.com/services/#inquire and services@lexogen.com.

Find more information about Lexogen NGS Services at its web page www.lexogen.com/services.

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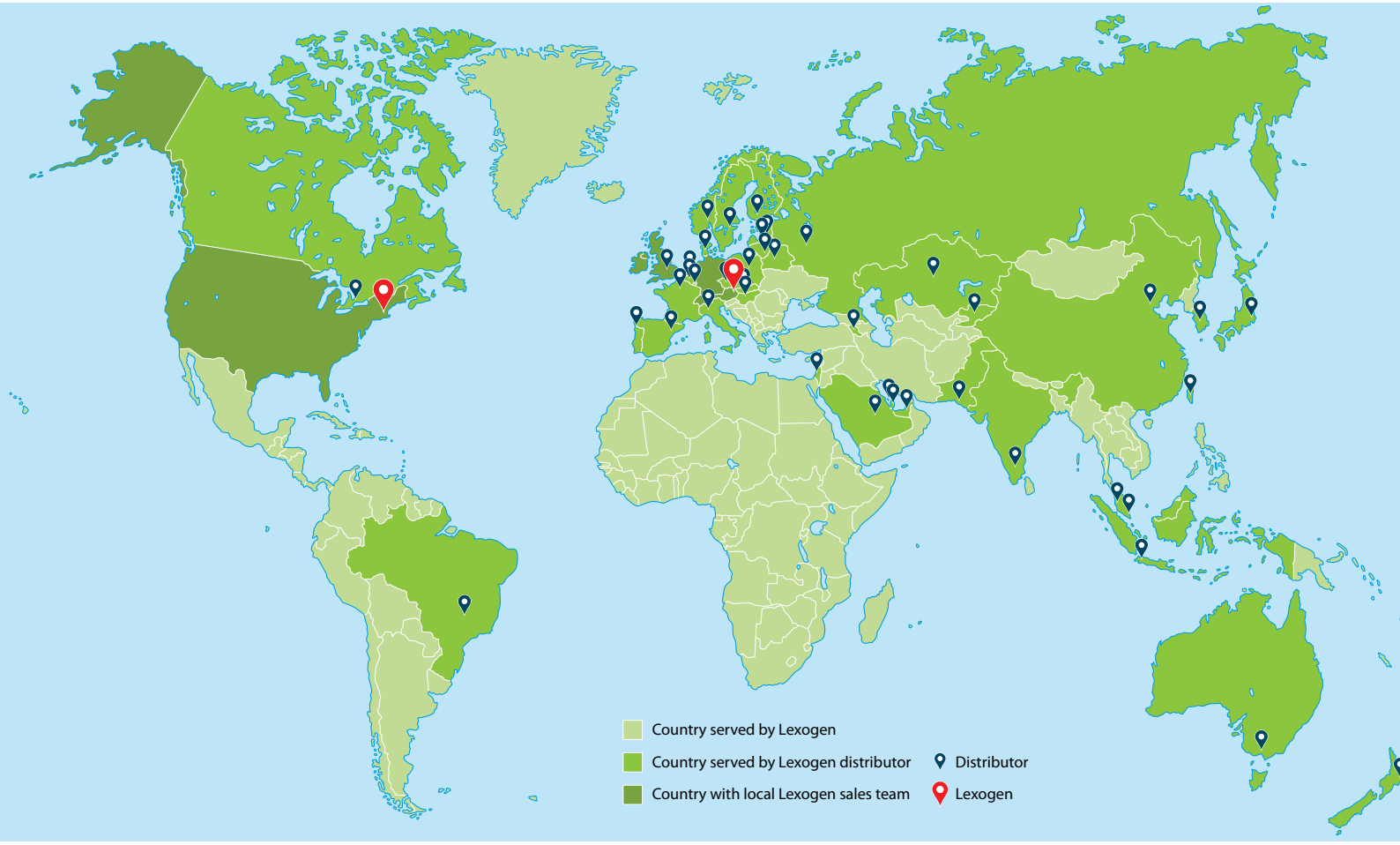

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  - Name of the product.
  - Catalog number (see Product List (p.46)).
- Contact Information:
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  - E-invoice email, if applicable.
  - Purchase Order Number (PO #), if applicable.
  - TAX/VAT ID Number for orders purchased and shipped within Europe. For US customers sales tax may be added unless a sales tax exemption certificate is provided.
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Technical support:

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- +43 (0) 660 744 0536
  Working hours: 9:00 – 18:00 (CET)

Product List

Product №	Product Description	Kit Size
Expression Profiling Library Prep Kits		
NEW! Version 2: 3′ mRNA-Seq Library Prep Kits with UDI		
	Illumina™ compatible (including Lexogen 12 nt UDIs)	
191.24	QuantSeq 3′ mRNA-Seq V2 Library Prep Kit FWD with UDI 12 nt Set A1, (UDI12A_0001-0024), 1 rxn/UDI	24
191.96	QuantSeq 3′ mRNA-Seq V2 Library Prep Kit FWD with UDI 12 nt Set A1, (UDI12A_0001-0096), 1 rxn/UDI	96
192.24	QuantSeq 3′ mRNA-Seq V2 Library Prep Kit FWD with UDI 12 nt Set B1, (UDI12B_0001-0024), 1 rxn/UDI	24
192.96	QuantSeq 3′ mRNA-Seq V2 Library Prep Kit FWD with UDI 12 nt Set B1, (UDI12B_0001-0096), 1 rxn/UDI	96
193.384	QuantSeq 3′ mRNA-Seq V2 Library Prep Kit FWD with UDI 12 nt Sets A1-A4, (UDI12A_0001-0384), 1 rxn/UDI	384
194.96	QuantSeq 3′ mRNA-Seq V2 Library Prep Kit FWD with UDI 12 nt Set A2, (UDI12A_0097-0192), 1 rxn/UDI	96
195.96	QuantSeq 3′ mRNA-Seq V2 Library Prep Kit FWD with UDI 12 nt Set A3, (UDI12A_0193-0288), 1 rxn/UDI	96
196.96	QuantSeq 3′ mRNA-Seq V2 Library Prep Kit FWD with UDI 12 nt Set A4, (UDI12A_0289-0384), 1 rxn/UDI	96
Version 1: 3′ mRNA-Seq Library Prep Kits		
	Illumina™ compatible (including 6 nt barcodes 5001-5004, 7001-7024 and/or 7001-7096)	
015.24	QuantSeq 3′ mRNA-Seq Library Prep Kit (FWD), 24 preps	24
015.96	QuantSeq 3′ mRNA-Seq Library Prep Kit (FWD), 96 preps	96
015.2x96	QuantSeq 3′ mRNA-Seq Library Prep Kit (FWD), 2x96 preps	192
015.384	QuantSeq 3′ mRNA-Seq Library Prep Kit (FWD) HT with i5 Dual Indexing Add-on Kit (5001-5004), 384 preps	384
	QuantSeq REV kits (including custom sequencing primer and 6 nt barcodes 7001-7024 or 7001-7096)	
016.24	QuantSeq 3′ mRNA-Seq Library Prep Kit (REV) with Custom Sequencing Primer, 24 preps	24
016.96	QuantSeq 3′ mRNA-Seq Library Prep Kit (REV) with Custom Sequencing Primer, 96 preps	96
	Illumina™ compatible (including Lexogen 12 nt UDIs)	
113.96	QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set A1, (UDI12A_0001-0096), 1 rxn/UDI	96
129.96	QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set A2, (UDI12A_0097-0192), 1 rxn/UDI	96
130.96	QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set A3, (UDI12A_0193-0288), 1 rxn/UDI	96
131.96	QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set A4, (UDI12A_0289-0384), 1 rxn/UDI	96
115.384	QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Sets A1-A4, (UDI12A_0001-0384), 1 rxn/UDI	384
114.96	QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set B1, (UDI12B_0001-0096), 1 rxn/UDI	96
Sample-Barcoded 3′ mRNA-Seq Library Prep Kit		
139.96	QuantSeq-Pool Sample-Barcoded 3′ mRNA-Seq Library Prep Kit for Illumina, 96 preps *For multiplexing of more than 96 samples additional indexing is required. We recommend the Lexogen UDI 12 nt Sets (Cat. No. 101 - 105).*	96
Whole Transcriptome Library Prep Kits		
NEW! Version 2: CORALL RNA-Seq V2 Library Prep Kits		
	Stand-alone Kits without rRNA Depletion, including Lexogen 12 nt UDI Sets	
171.24	CORALL RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0024), 24 preps	24
171.96	CORALL RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0096), 96 preps	96
172.96	CORALL RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A2, (UDI12A_0097-0192), 96 preps	96
173.96	CORALL RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A3, (UDI12A_0193-0288), 96 preps	96
174.96	CORALL RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A4, (UDI12A_0289-0384), 96 preps	96
175.24	CORALL RNA-Seq V2 Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0024), 24 preps	24
175.96	CORALL RNA-Seq V2 Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0096), 96 preps	96
176.384	CORALL RNA-Seq V2 Library Prep Kit with UDI 12 nt Sets A1-A4 (UDI12A_0001-0384), 384 preps	384

Product №	Product Description	Kit Size
Version 1: CORALL RNA-Seq Library Prep Kits		
	Stand-alone Kits without rRNA Depletion, including barcodes	
095.24	CORALL Total RNA-Seq Library Prep Kit, 24 preps	24
095.96	CORALL Total RNA-Seq Library Prep Kit, 96 preps	96
117.96	CORALL Total RNA-Seq Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0096), 1 rxn/UDI	96
132.96	CORALL Total RNA-Seq Library Prep Kit with UDI 12 nt Set A2, (UDI12A_0097-0192), 1 rxn/UDI	96
133.96	CORALL Total RNA-Seq Library Prep Kit with UDI 12 nt Set A3, (UDI12A_0193-0288), 1 rxn/UDI	96
134.96	CORALL Total RNA-Seq Library Prep Kit with UDI 12 nt Set A4, (UDI12A_0289-0384), 1 rxn/UDI	96
119.384	CORALL Total RNA-Seq Library Prep Kit with UDI 12 nt Sets A1-A4, (UDI12A_0001-0384), 1 rxn/UDI	384
118.96	CORALL Total RNA-Seq Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0096), 1 rxn/UDI	96
NEW! Version 2: CORALL Total RNA-Seq V2 Library Prep Bundles with RiboCop rRNA Depletion		
	With rRNA Depletion, including Lexogen 12 nt UDI Sets	
183.24	RiboCop (HMR) and CORALL Total RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0024), 24 preps	24
183.96	RiboCop (HMR) and CORALL Total RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0096), 96 preps	96
184.24	RiboCop (HMR) and CORALL Total RNA-Seq V2 Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0024), 24 preps	24
184.96	RiboCop (HMR) and CORALL Total RNA-Seq V2 Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0096), 96 preps	96
185.24	RiboCop (HMR+Globin) and CORALL Total RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0024), 24 preps	24
185.96	RiboCop (HMR+Globin) and CORALL Total RNA-Seq V2 Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0096), 96 preps	96
186.24	RiboCop (HMR+Globin) and CORALL Total RNA-Seq V2 Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0024), 24 preps	24
186.96	RiboCop (HMR+Globin) and CORALL Total RNA-Seq V2 Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0096), 96 preps	96
Version 1: CORALL Total RNA-Seq Library Prep Bundles with RiboCop rRNA Depletion		
	With rRNA Depletion, including barcodes 7001-7096	
146.24	CORALL Total RNA-Seq Library Prep Kit with RiboCop (HMR), 24 preps *This product contains RiboCop rRNA Depletion Kit for Human/Mouse/Rat (HMR) V2.*	24
146.96	CORALL Total RNA-Seq Library Prep Kit with RiboCop (HMR), 96 preps *This product contains RiboCop rRNA Depletion Kit for Human/Mouse/Rat (HMR) V2.*	96
147.24	CORALL Total RNA-Seq Library Prep Kit with RiboCop (HMR+Globin), 24 preps	24
147.96	CORALL Total RNA-Seq Library Prep Kit with RiboCop (HMR+Globin), 96 preps	96
NEW! Version 2: CORALL mRNA-Seq V2 Library Prep Kits		
	With Poly(A) Selection, including Lexogen 12 nt UDI Sets	
177.96	CORALL mRNA-Seq V2 Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0096), 96 preps	96
178.96	CORALL mRNA-Seq V2 Library Prep Kit with UDI 12 nt Set A2, (UDI12A_0097-0192), 96 preps	96
179.96	CORALL mRNA-Seq V2 Library Prep Kit with UDI 12 nt Set A3, (UDI12A_0193-0288), 96 preps	96
180.96	CORALL mRNA-Seq V2 Library Prep Kit with UDI 12 nt Set A4, (UDI12A_0289-0384), 96 preps	96
181.96	CORALL mRNA-Seq V2 Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0096), 96 preps	96
182.384	CORALL mRNA-Seq V2 Library Prep Kit with UDI 12 nt Sets A1-A4 (UDI12A_0001-0384), 384 preps	384
Version 1: CORALL mRNA-Seq Library Prep Kits		
	With Poly(A) Selection, including Lexogen 12 nt UDI Sets	
158.96	CORALL mRNA-Seq Library Prep Kit with UDI 12 nt Set A1, (UDI12A_0001-0096), 96 preps	96
159.96	CORALL mRNA-Seq Library Prep Kit with UDI 12 nt Set A2, (UDI12A_0097-0192), 96 preps	96
160.96	CORALL mRNA-Seq Library Prep Kit with UDI 12 nt Set A3, (UDI12A_0193-0288), 96 preps	96

Product №	Product Description	Kit Size
161.96	CORALL mRNA-Seq Library Prep Kit with UDI 12 nt Set A4, (UDI12A_0289-0384), 96 preps	96
162.96	CORALL mRNA-Seq Library Prep Kit with UDI 12 nt Set B1, (UDI12B_0001-0096), 96 preps	96
163.384	CORALL mRNA-Seq Library Prep Kit with UDI 12 nt Sets A1-A4 (UDI12A_0001-0384), 384 preps	384
Single-cell and Low-input RNA-Seq Library Prep Kits		
LUTHOR 3' mRNA-Seq Library Prep Kits		
143.24	LUTHOR 3' mRNA-Seq Library Prep Kit for Illumina, 24 preps *For full flexibility, the LUTHOR kits are delivered without the required indices. We recommend the Lexogen UDI 12 nt Sets (Cat. No. 101 - 105)*	24
143.96	LUTHOR 3' mRNA-Seq Library Prep Kit for Illumina, 96 preps *For full flexibility, the LUTHOR kits are delivered without the required indices. We recommend the Lexogen UDI 12 nt Sets (Cat. No. 101 - 105)*	96
Metabolic RNA Labeling		
059.24	SLAMseq Explorer Kit - Cell Viability Titration Module, 24 preps	24
060.24	SLAMseq Explorer Kit - S4U Incorporation Module, 24 preps	24
061.24	SLAMseq Kinetics Kit - Anabolic Kinetics Module, 24 preps	24
062.24	SLAMseq Kinetics Kit - Catabolic Kinetics Module, 24 preps	24
Small RNA Profiling & Discovery (including barcodes 7001-7096)		
052.08	Small RNA-Seq Library Prep Kit for Illumina, 8 preps	8
052.24	Small RNA-Seq Library Prep Kit for Illumina, 24 preps	24
052.96	Small RNA-Seq Library Prep Kit for Illumina, 96 preps	96
058.08	Small RNA-Seq Library Prep Kit for Illumina including Purification Module with Magnetic Beads, 8 preps	8
058.24	Small RNA-Seq Library Prep Kit for Illumina including Purification Module with Magnetic Beads, 24 preps	24
058.96	Small RNA-Seq Library Prep Kit for Illumina including Purification Module with Magnetic Beads, 96 preps	96
135.08	Small RNA-Seq Library Prep Kit for Illumina with TraPR, 8 preps	8
135.24	Small RNA-Seq Library Prep Kit for Illumina with TraPR, 24 preps	24
RNA Depletion		
Human/Mouse/Rat (HMR)		
144.24	RiboCop rRNA Depletion Kit for Human/Mouse/Rat (HMR) V2, 24 preps	24
144.96	RiboCop rRNA Depletion Kit for Human/Mouse/Rat (HMR) V2, 96 preps	96
145.24	RiboCop rRNA Depletion Kit for Human/Mouse/Rat plus Globin (HMR+Globin), 24 preps	24
145.96	RiboCop rRNA Depletion Kit for Human/Mouse/Rat plus Globin (HMR+Globin), 96 preps	96
Yeast		
190.24	RiboCop rRNA Depletion Kit for Yeast, 24 preps	24
190.96	RiboCop rRNA Depletion Kit for Yeast, 96 preps	96
Bacteria		
125.24	RiboCop rRNA Depletion Kit for Mixed Bacterial Samples (META), 24 preps	24
125.96	RiboCop rRNA Depletion Kit for Mixed Bacterial Samples (META), 96 preps	96
126.24	RiboCop rRNA Depletion Kit for Gram Negative Bacteria (G-), 24 preps	24
126.96	RiboCop rRNA Depletion Kit for Gram Negative Bacteria (G-), 96 preps	96
127.24	RiboCop rRNA Depletion Kit for Gram Positive Bacteria (G+), 24 preps	24
127.96	RiboCop rRNA Depletion Kit for Gram Positive Bacteria (G+), 96 preps	96
NGS Controls		
025.03	SIRV-Set 1 (Iso Mix E0, E1, E2)	-
050.01	SIRV-Set 2 (Iso Mix E0), 1 vial	1 vial
050.03	SIRV-Set 2 (Iso Mix E0), 3 vials	3 vials
051.01	SIRV-Set 3 (Iso Mix E0 / ERCC), 1 vial	1 vial

Product №	Product Description	Kit Size
051.03	SIRV-Set 3 (Iso Mix E0 / ERCC), 3 vials	3 vials
141.01	SIRV-Set 4 (Iso Mix E0 / ERCC / long SIRVs), 1 vial	1 vial
141.03	SIRV-Set 4 (Iso Mix E0 / ERCC / long SIRVs), 3 vials	3 vials
RNA Stabilization / Extraction / Isolation		
008.48	SPLIT RNA Extraction Kit, 48 extractions	48
168.100	RNA/DNA Defender Solution, 100 ml	100 ml
128.08	TraPR Small RNA Isolation Kit, 8 preps	8
128.24	TraPR Small RNA Isolation Kit, 24 preps	24
RNA Enrichment		
157.96	Poly(A) RNA Selection Kit V1.5, 96 preps	96
Full-Length cDNA Amplification Kit		
013.08	TeloPrime Full-Length cDNA Amplification Kit, 8 preps	8
013.24	TeloPrime Full-Length cDNA Amplification Kit, 24 preps	24
Lexogen Indexing Solutions		
	UDI 12 nt Sets (index plates only)	
101.96	Lexogen UDI 12 nt Set A1 (UDI12A_0001-0096), 1 rxn/UDI	96
102.96	Lexogen UDI 12 nt Set A2 (UDI12A_0097-0192), 1 rxn/UDI	96
103.96	Lexogen UDI 12 nt Set A3 (UDI12A_0193-0288), 1 rxn/UDI	96
104.96	Lexogen UDI 12 nt Set A4 (UDI12A_0289-0384), 1 rxn/UDI	96
105.96	Lexogen UDI 12 nt Set B1 (UDI12B_0001-0096), 1 rxn/UDI	96
156.384	Lexogen UDI 12 nt Sets A1-A4 (UDI12A_0001-0384), 1 rxn/UDI	384
	NEW! Version 2: UDI 12 nt Unique Dual Indexing V2 Add-on Kits contain primer plates, PCR Enzyme and PCR Buffer	
198.96	Lexogen UDI 12 nt Unique Dual Indexing V2 Add-on Kit, Set A1 (UDI12A_0001-0096), 1 rxn/UDI	96
199.96	Lexogen UDI 12 nt Unique Dual Indexing V2 Add-on Kit, Set A2 (UDI12A_0097-0192), 1 rxn/UDI	96
200.96	Lexogen UDI 12 nt Unique Dual Indexing V2 Add-on Kit, Set A3 (UDI12A_0193-0288), 1 rxn/UDI	96
201.96	Lexogen UDI 12 nt Unique Dual Indexing V2 Add-on Kit, Set A4 (UDI12A_0289-0384), 1 rxn/UDI	96
202.96	Lexogen UDI 12 nt Unique Dual Indexing V2 Add-on Kit, Set B1 (UDI12B_0001-0096), 1 rxn/UDI	96
203.384	Lexogen UDI 12 nt Unique Dual Indexing V2 Add-on Kit, Sets A1-A4 (UDI12A_0001-0384), 1 rxn/UDI	384
	Version 1: UDI 12 nt Unique Dual Indexing Add-on Kits contain primer plates, PCR Enzyme and PCR Buffer	
107.96	Lexogen UDI 12 nt Unique Dual Indexing Add-on Kit, Set A1 (UDI12A_0001-0096), 1 rxn/UDI	96
108.96	Lexogen UDI 12 nt Unique Dual Indexing Add-on Kit, Set A2 (UDI12A_0097-0192), 1 rxn/UDI	96
109.96	Lexogen UDI 12 nt Unique Dual Indexing Add-on Kit, Set A3 (UDI12A_0193-0288), 1 rxn/UDI	96
110.96	Lexogen UDI 12 nt Unique Dual Indexing Add-on Kit, Set A4 (UDI12A_0289-0384), 1 rxn/UDI	96
111.96	Lexogen UDI 12 nt Unique Dual Indexing Add-on Kit, Set B1 (UDI12B_0001-0096), 1 rxn/UDI	96
120.384	Lexogen UDI 12 nt Unique Dual Indexing Add-on Kit, Sets A1-A4 (UDI12A_0001-0384), 1 rxn/UDI	384
	Illumina compatible 6 nt i7 barcodes (index plate only)	
044.96	Lexogen i7 6 nt Index Set (7001-7096), 1 rxn/Index	96
	Illumina compatible 6 nt i5 barcodes (index plate and PCR buffer)	
047.4x96	Lexogen i5 6 nt Dual Indexing Add-on Kit (5001-5004), 96 rxn/Index	4x96
047.96	Lexogen i5 6 nt Unique Dual Indexing Add-on Kit (5001-5096), 1rxn/Index	96
Add-ons and Modules		
018.16	TeloPrime PCR Add-on Kit, 16 rxn	16
020.96	PCR Add-on Kit for Illumina, 96 rxn	96
022.96	Purification Module with Magnetic Beads, 200 rxn	200

Product №	Product Description	Kit Size
054.24	Gel Extraction Module, 24 extractions	24
QuantSeq Modules		
166.96	QuantSeq-Flex First Strand Synthesis Module for Illumina V2, 96 preps	96
028.96	QuantSeq-Flex Second Strand Synthesis Module for Illumina, 96 preps	96
070.96	RS-Globin Block, *Homo sapiens*, 96 rxn	96
071.96	RS-Globin Block, *Sus scrofa*, 96 rxn	96
167.96	RS-BC1 Block, *Mus musculus*, 96 rxn	96
081.96	UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1), 96 rxn	96
NGS Data Analysis Software		
165.01	ROSALIND Analysis Unit (AU)	1 AU
023	Mix^2 RNA-Seq Data Analysis Software	

All Lexogen sample preparation kits are also offered as a service at the Lexogen NGS Services department. For any service inquiries, complete the form at www.lexogen.com/services/#inquire or e-mail to services@lexogen.com.

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Our RNA experts have prepared RNA Lexicon, a practical guide that will equip you with the necessary information to plan and execute your RNA-Seq experiments successfully. We cover all the basics of working with RNA, performing RNA-Seq, and analyzing data. Moreover, RNA Lexicon contains many invaluable, advanced tips and tricks on further excelling your RNA-Seq projects.

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- ✓ Types of RNA pretreatment, including enrichment and depletion.
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- ✓ RNA-Seq primary, secondary and tertiary data analysis.

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