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

December 2021

WE ARE THE RNA EXPERTS

COMPANY

Lexogen is a biotech company that provides innovative solutions for one of the fastest developing technologies in the last decade – Next Generation Sequencing (NGS). The company's main focus is the development of RNA sequencing technologies to resolve the complexity of the transcriptome.

Lexogen was founded in 2007. The headquarters of the company are located in Vienna, Austria, and the North American office is based in New Hampshire, in the USA.

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 Metabolic RNA Labeling

» Explorer Modules
» Kinetics Modules

- Analyze RNA synthesis and degradation by distinguishing between newly synthesized and existing RNA.
- Gain novel insights into the control of gene expression, best combined with QuantSeq.

RNA Preparation

TraPR Small RNA Isolation NEW

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

SPLIT RNA Extraction

- No need for DNase, high yield, efficient recovery, applicable for a wide range of species.
- Splitting option - purify total RNA or small and/or large RNA fractions from one sample.
- Also available for RNA extraction from blood, and for viral RNA/DNA extraction. NEW

RiboCop rRNA Depletion

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

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
» 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 (UMI).
- Up to 384 Unique Dual Indices (UDI). NEW
- Easily scalable to 36,864 samples for large screening projects.

» LUTHOR 3′ mRNA-Seq NEW

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

Whole Transcriptome

» CORALL mRNA-Seq
» CORALL Total RNA-Seq

- Uniform transcript coverage.
- UMIs included.
- UDIs available.
- mRNA-Seq with poly(A) selection.
- Fast (4.5 h) and easy protocol.

Small RNA-Seq

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

NGS Data Analysis

i1 Demultiplexing and Index Error Correction Tool NEW

QuantSeq Data Analysis

» QuantSeq Pipeline on BlueBee®
» SLAMdunk Pipeline for QuantSeq-SLAMseq data on BlueBee®
» QuantSeq Pipeline in Partek® Flow®
» QuantSeq Pipeline on ROSALIND™

- User-friendly, streamlined data analysis.
- No bioinformatics knowledge required.

CORALL Data Analysis

» CORALL Pipeline on BlueBee®

- Uses Mix² for accurate transcript abundance estimation.
- User-friendly, streamlined data analysis.
- No bioinformatics knowledge required.

Mix² RNA-Seq Data Analysis Software

- Accurate estimation of isoform concentration with best quantity, quality, and accuracy measures.
- Fast and small memory footprint.

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, abundance, and length complexity.
- Validate, monitor, and compare RNA-Seq experiments and pipelines.

Services

Library Preparation

» Service for all Lexogen products
» Sample prep by the RNA-Seq experts

- RNA extraction available.
- SLAMseq projects.
- Spike-in RNA controls (SIRVs).
- UMIs, UDIs, Globin Block.
- Custom Solutions and Custom Design.

Automation and Sequencing

- Project size flexibility.
- Automated liquid handling for high-throughput projects.
- Sequencing on in-house NextSeq 500 and other Illumina® platforms (incl. NovaSeq).

Data Analysis and Reporting

- Fully integrated data analysis pipeline for all Lexogen library preps, including SLAMdunk.
- Standard analysis including reporting of (differential) gene expression.
- Advanced and custom analyses available.

Library Prep Kits Overview

Kit Selection Criteria	CORALL RNA-Seq			QuantSeq				LUTHOR 3′ mRNA-Seq	Small RNA-Seq	Telo-Prime Full-Length cDNA
		+ Poly(A) (CORALL mRNA)	+ Ribo-Cop	Quant-Seq 3′ FWD	Quant-Seq-Pool 3′	Quant-Seq 3′ REV	Quant-Seq -Flex			
Application										
Expression Profiling	✓	✓	✓	✓*	✓*	✓	✓	✓	✓	✗
Whole Transcriptome (full-transcript coverage)	✓	✓	✓	✗	✗	✗	✗	✗	✗	✓
Alternative Polyadenylation / 3′UTR isoforms	✗	✗	✗	✓	✗	✓*	✓	✓	✗	✓
Isoform discovery & quantification	✓	✓*	✓*	✗	✗	✗	✓	✗	✗	✓
Target-Specific	✗	✗	✗	✗	✗	✗	✓*	✗	✗	✓
RiboSeq (Ribosomal profiling)	✗	✗	✗	✗	✗	✗	✗	✗	✓*	✗
Polysome profiling	✗	✉	✉	✓*	✉	✉	✉	✗	✗	✓
Transcript (re)annotation	✓	✓	✓	✓ 3′UTR only	✓ 3′UTR only	✓ 3′UTR only	✉	✓ 3′UTR only	✓ sRNA only	✓*
De novo assembly	✓	✓	✓	✗	✗	✗	✗	✗	✗	✓*
SLAMseq (Nascent RNA-Seq)	✓	✓	✓	✓*	✓*	✓	✓	✉	✓	✓
Small RNA (e.g., miRNA) analysis	✗	✗	✗	✗	✗	✗	✗	✗	✓*	✗
Long non-coding RNA analysis	✓	✓	✓	✗	✗	✗	✓	✗	✗	✓
rRNA Sequencing	✓*	✗	✗	✗	✗	✗	✓	✗	✗	✗
RNA Input Type										
Human or animal cells and protoplasts	✗	✗	✗	✗	✗	✗	✗	✓*	✗	✗
Total RNA	✓	✓*	✓*	✓*	✓*	✓	✓	✓	✓	✓
Poly(A)+ RNA (≙ mRNA)	✓*	✗	✉	✓	✓	✉	✉	✉	✗	✓
rRNA-depleted RNA	✓*	✉	✗	✉	✉	✉	✉	✉	✗	✉
Small RNA	✗	✗	✗	✗	✗	✗	✗	✗	✓*	✗
High quality (RIN ≥8)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Moderate quality (RIN 5 - 8)	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗
FFPE / highly degraded (RIN <5; DV200 <50%)	✓M	✗	✓M	✓M	✉	✓M	✓M	✗	✓	✗
Low Input <100 pg	✗	✗	✗	✗	✗	✗	✗	✓	✗	✗
Organism										
Human, mouse, rat	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Other animals	✓	✓	✉	✓	✓	✓	✓	✓	✓	✓
Plant	✓	✓	✗	✓	✓	✓	✓	✓	✉	✓
Yeast	✓	✓	✗	✓	✓	✓	✓	✓	✉	✓
Bacteria	✓	✗	✓	✓pA	✗	✓pA	✓	✗	✓	✉
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, cell lines, primary or sorted cells etc.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Blood	✓	✓	✓	✓*	✓	✓*	✓	✓	✓	✉
Liquid biopsy (CSF, serum, plasma, urine)	✉	✗	✉	✓M	✓	✉	✓	✓	✓*	✗
Exosomes	✉	✉	✉	✉	✉	✉	✉	✓	✓*	✗
Recommended / compatible sequencing platforms / formats										
Illumina	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ion Torrent	✗	✗	✗	✓	✗	✗	✗	✗	✗	✉
Oxford Nanopore	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓M
Pacific Biosciences	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓M
Single-read	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓
Paired-end	✓	✓	✓	✗	✓	✓	✓	✗	✗	✓
Additional Options										
Dual indexing compatible	✓	✓	✓	✓	✓	✓	✓	✓	✉	✗
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
✉ Possible, but not evaluated. For more information please contact support@lexogen.com.
✓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 have been using **QuantSeq** for over 5 years to profile samples that we have found previously difficult to target, such as formalin fixed, paraffin embedded (FFPE) samples. We have found that QuantSeq allows good reproducibility, low sample input requirements and rapid turnaround, allowing us to make the most of these precious samples."

Andrew Beggs
Institute of Cancer and Genomic Sciences, University of Birmingham, UK

"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

"**QuantSeq** from Lexogen is everything we dreamed of and more. All we had to do was a quick Trizol RNA prep, send our samples off for analysis, and wait shortly to hear back from Lexogen. As an added bonus everyone at Lexogen was extremely knowledgeable, kind, and more than eager to answer any questions we had. We highly recommend using **QuantSeq service** of Lexogen."

Gary Cantor
University of North Carolina at Chapel Hill, USA

"We used the new **CORALL kit** for performing transcriptome-analysis of CRISPR-modified cells in order to understand the consequences of deregulated epigenetic modifiers. In our hands the kit performance was highly satisfying in terms of data-quality and reproducibility across biological replicates. It furthermore convinced us with the ease of use, clarity of instructions, details in the manual and handling of reagents."

Max Koeppel
Functional Tumor Genomics Group, Leibniz-Institute DSMZ, Germany

"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

"With Lexogen **Poly(A) Selection Kit** I was able to isolate reasonable amounts of polyadenylated RNA with no trace of rRNA contamination. I see great advantages of this kit in clear-cut protocol and quite low starting concentration of total RNA that you can easily scale-up based on chosen downstream application. The other thing that should be appreciated is Lexogen customer service that provides you with very high level of support."

Květoslava Brožinová
Research Group: ERA Chair – RNA and Immunity, CEITEC, Brno, Czech Republic

"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

- 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
- **NEW!** 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.

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 paired-end sequencing is intended, QuantSeq Reverse (REV) is recommended. Here, Read 1 starts directly at the poly(A) tail (Fig. 1, right).

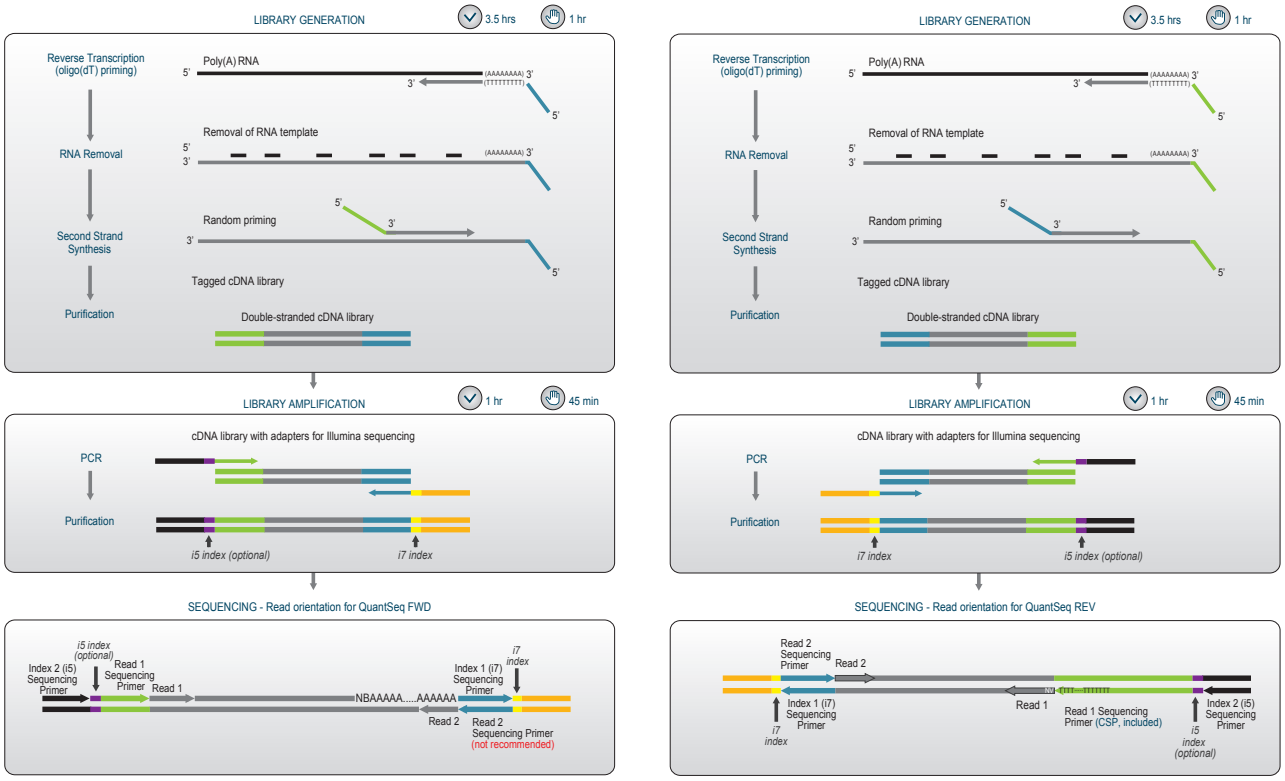

Figure 1 | Schematic overview of QuantSeq 3′ mRNA-Seq FWD (left panel) and QuantSeq 3′ mRNA-Seq REV (right panel) Library Preparation workflow. Left Panel) Read 1 (sequencing starts from the green P5 adapter part) of the QuantSeq FWD Kit corresponds to the mRNA sequence close to the 3′ end, allowing 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.

Performance

QuantSeq Saves Sequencing Depth

QuantSeq libraries cover the very 3′ ends of transcripts, whereas standard mRNA-Seq distributes reads across the entire length of transcripts, 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). In this example, QuantSeq saves more than 90 % of sequencing depth while still determining gene expression accurately.

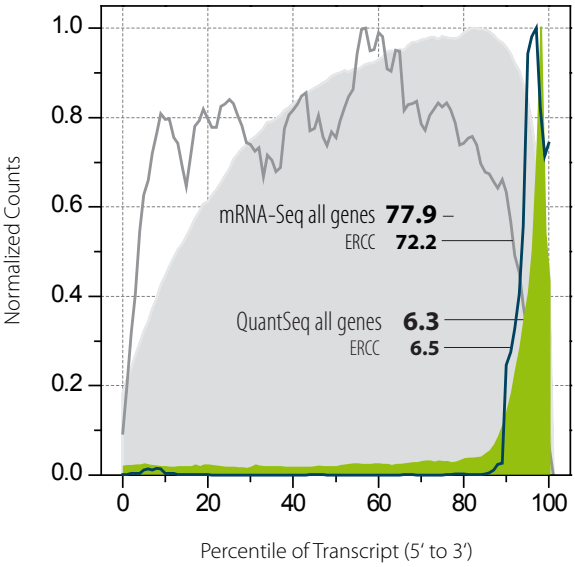

Figure 2 | Coverage versus normalized transcript length in QuantSeq and standard mRNA-Seq. QuantSeq libraries were prepared from reference RNA samples and compared with an mRNA-Seq data set available in the Association of Biomolecular Resource Facilities (ABRF) NGS study [3]. RSeQC-derived coverage is 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).

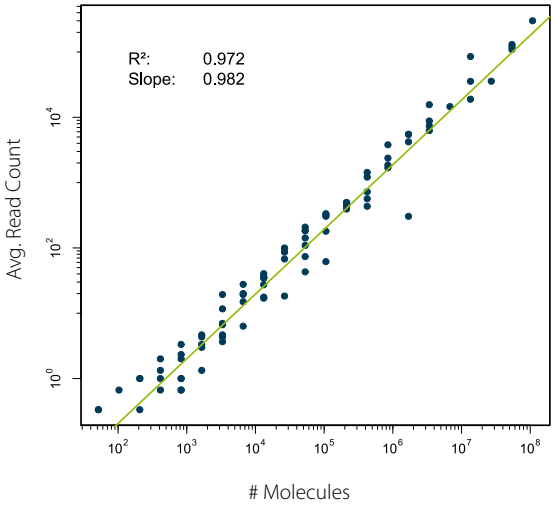

Figure 3 | QuantSeq-derived ERCC read counts correlate exceedingly well with the documented input. ERCC spike-in transcripts' average read count plotted against input molecules.

Accurate Detection of Differential Gene Expression

QuantSeq is superior to mRNA-Seq in detecting differential gene expression, even for small changes and low read depth, as shown in an experiment with external controls and predetermined fold changes (Fig. 4).

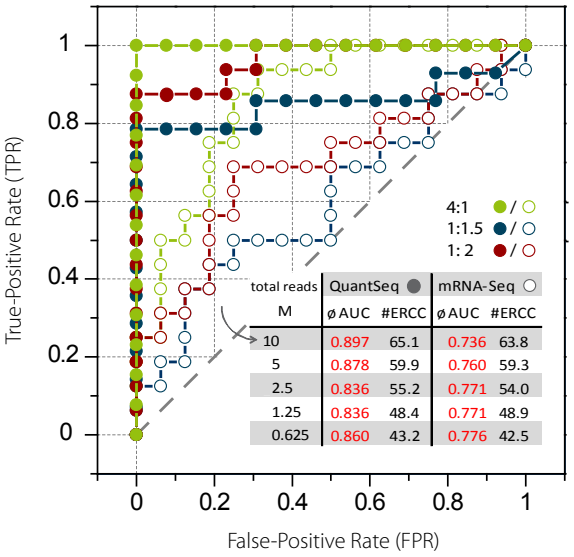

Figure 4 | Differential gene expression performance of QuantSeq and mRNA-Seq. The predetermined fold-changes (4:1 ●/○, 1:1.5 ●/○, 1:2 ●/○) between ERCC ExFold Spike-In Mix 1 and 2 were used to assess true and false positive rates (TPRs and FPRs). Optimal detection of differential gene expression using the "erccdashboard" software [4] is indicated by a maximum AUC (Area Under the Curve) value of 1. AUC values were assessed together with the number of ERCC RNAs detected (#ERCC) for reads down-sampled from 10 M to 0.625 M.

Find more information about this product at its web page:

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. Ambion. ERCC RNA Spike-In Control Mixes. Cat. No. 4456740, 4456739.
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

Performance with FFPE Samples

QuantSeq enables accurate gene expression quantification independent of the RNA quality (including FFPE samples) since it only generates one fragment at the transcript's 3' end. 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 23 % (data not shown). Resulting libraryies show a smooth size distributing with no visible linker-linker by-products and a shift towards shorter fragments (Fig. 5).

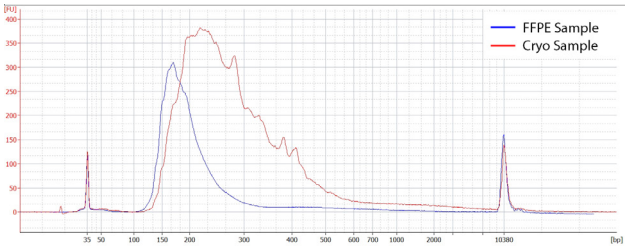

Figure 5 | Bioanalyzer traces of QuantSeq 3' mRNA-Seq FWD libraries from FFPE (blue) or Cryo (red) samples. A xenograft of the MOLP-8 human tumor cell line was split, and processed either as fresh frozen cryoblock or embedded FFPE material, leading to different RNA qualities (Cryo sample: RNA Integrity 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 Cryo 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 (Cryo sample), respectively.

QuantSeq Yields Good Correlation Between High and Low Quality (FFPE) Samples

Relative coverage across the normalized transcript length shows that coverage is focused on the transcripts' 3' ends, independent of the input RNA quality (Fig. 6). Since FFPE sample libraries are significantly shorter than the Cryo sample's, the ends of the transcripts are reached more frequently, which is reflected in the coverage plots.

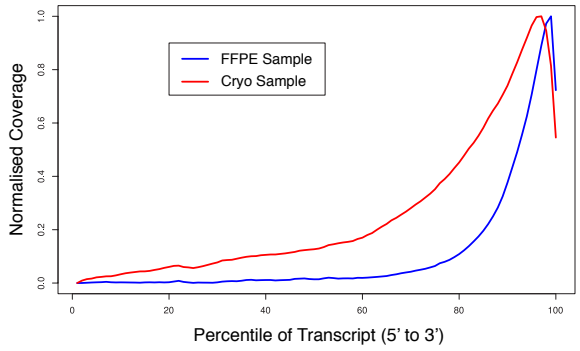

Figure 6 | QuantSeq read coverage versus normalized transcript length of NGS libraries derived from FFPE-RNA (blue) and cryo-preserved RNA (red). Libraries were prepared as detailed in Fig. 5 and sequenced on a HiSeq 2500 instrument at 1x 50 bp read length.

Gene expression correlation between libraries derived from FFPE and cryo-preserved RNA is high (R^2 0.86) and indicates that QuantSeq performs consistently well on samples of different RNA quality (Fig. 7).

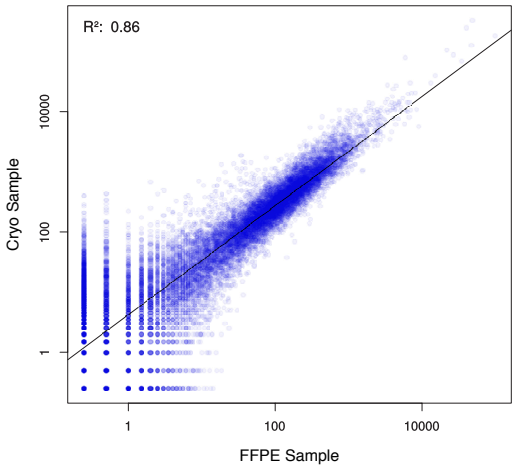

Figure 7 | Correlation of gene counts of FFPE and Cryo samples.

QuantSeq reliably detects gene expression in both cryo-preserved and FFPE samples. With a uniform sampling of 2.5 M reads, 20,081 genes are detected in the Cryo sample with at least 1 read, compared to 15,190 genes in the FFPE samples, which represents a 24 % difference (Fig. 8). However, increasing the detection level to 5 or 10 reads / gene reduces the difference to 3 % and 1 % respectively.

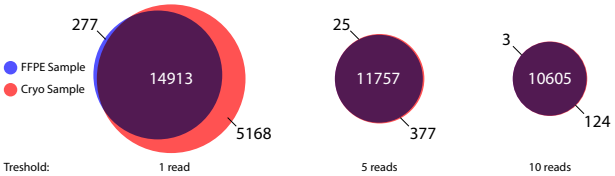

Figure 8 | Venn diagrams of genes detected by QuantSeq at a uniform read depth of 2.5 M reads in FFPE and Cryo samples with 1, 5, and 10 reads / gene thresholds. The difference in detection of lowly expressed genes (1 read) is due to the higher susceptibility of their low copy transcripts to degradation during FFPE treatment, storage, and recovery.

QuantSeq Automation

Lexogen offers autoQuantSeq, the automated version of the QuantSeq 3' mRNA-Seq Library Prep protocol. Generate Illumina-compatible 3' mRNA-Seq libraries on an automated platform for high throughput, scalable gene expression profiling with up to 384 UDIs or 9,216 i5 / i7 index combinations. Automation saves hands-on time, maximizes throughput, and avoids pipetting and sample tracking errors.

AutoQuantSeq is currently available for the following platforms: Perkin Elmer Sciclone® NGS and Zephyr® liquid handlers, Hamilton Microlab STAR / STARlet / NGS STAR Workstations, the Agilent NGS Workstation (NGS Bravo Option B), Eppendorf EpMotion® 5075, and Beckman Coulter Biomek FX^P, and Biomek i7. Please contact us at support@lexogen.com if you are interested in automation or need more information on data analysis.

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.

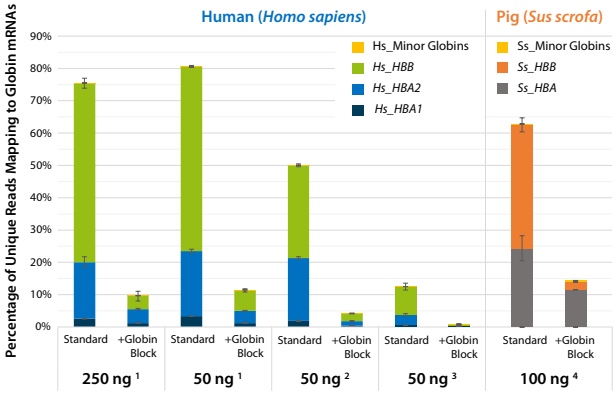

Figure 9 | Percentage of reads uniquely mapping to human and pig globin mRNAs. Libraries were prepared from whole blood RNA with the Standard QuantSeq FWD protocol, versus QuantSeq +Globin Block. [1] SPLIT RNA Extraction Kit without red blood cell lysis (Lexogen), [2] PAXgene® Blood RNA Kit (Qiagen,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

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

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.

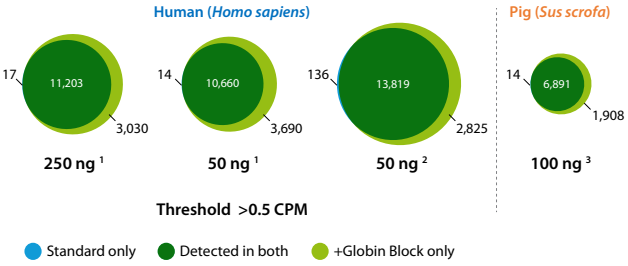

Figure 10 | Increased gene detection in human and pig blood QuantSeq libraries using Globin Block. Libraries were prepared from whole blood RNA with the Standard QuantSeq FWD protocol or QuantSeq +Globin Block. Number of detected genes was calculated from CPM-normalized read counts (threshold >0.5). 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

Free access to the QuantSeq data analysis pipeline hosted by the BlueBee® Genomics Platform is included with all QuantSeq 3' mRNA-Seq Library Prep Kits (FWD and REV) for Illumina. Codes are supplied with each kit, enabling the user to explore their data with complimentary access to our customized pipeline.
QuantSeq data analysis is also available on Partek® Flow® (license required). Users can import raw sequencing files directly into Partek Flow and automate their analysis. Customizable data analysis options are also available.
Analysis of QuantSeq data is implemented on the ROSALIND™ platform as well, which enables experiment design, quality control, differential expression, and pathway exploration in a real-time collaborative environment.

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

Associated Products

- Lexogen UDI 12 nt Unique Dual Indexing System (p.34)
- Globin Block Modules for QuantSeq (p.35)
- UMI Second Strand Synthesis Module for QuantSeq FWD (p.35)
- SPLIT RNA Extraction Kits (p.27)
- SIRVs - Spike-In RNA Variant Controls (p.29)
- PCR Add-on Kit for Illumina (p.35)
- Purification Module with Magnetic Beads (p.36)

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
- The automation-friendly protocol is easily scalable from a few to 36,864 samples
- 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.

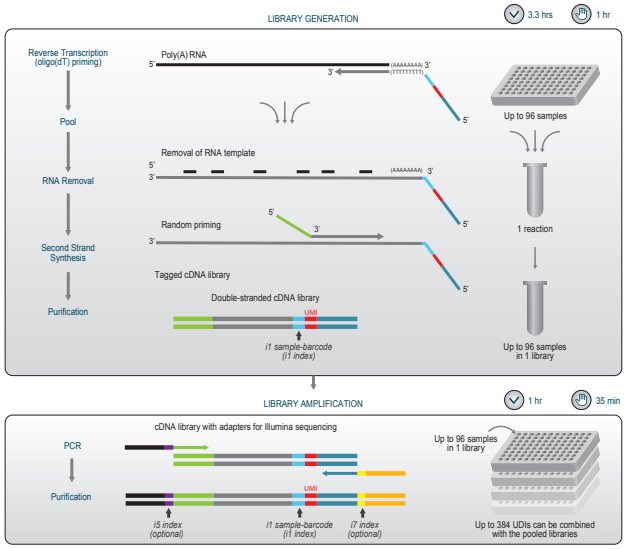

Figure 1 | Schematic overview of the QuantSeq-Pool library preparation workflow.

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.

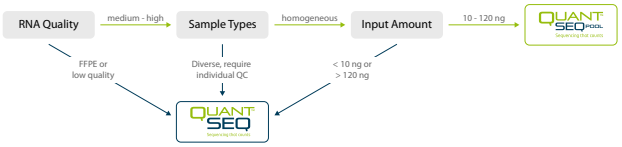

Figure 2 | Overview over QuantSeq vs QuantSeq-Pool decision parameters.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- SPLIT RNA Extraction Kits (p.27)
- Lexogen UDI 12 nt Unique Dual Indexing System (p.34)
- SIRVs - Spike-In RNA Variant Controls (p.29)

Find more information about this product at its web page:

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

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.

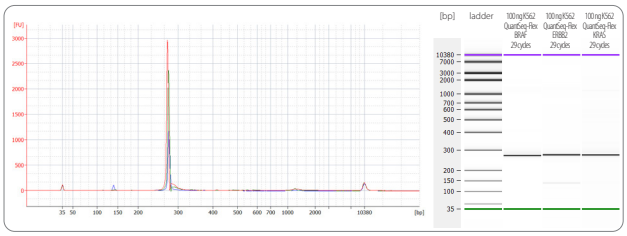

Figure 2 | Bioanalyzer traces of QuantSeq-Flex libraries with targeting in first and second strand synthesis. Amplicons were amplified from *BRAF* (serine / threonine-protein kinase B-raf, red trace), *ERBB2* (receptor tyrosine-protein kinase erbB-2, blue trace), and *KRAS* (proto-oncogene, GTPase, green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

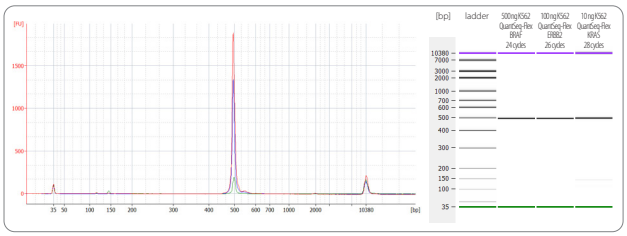

Figure 3 | Bioanalyzer traces of QuantSeq-Flex libraries with target-specific primers for BCR-ABL fusion transcripts. Input series with 10 ng, 100 ng, and 500 ng total RNA.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- Lexogen UDI 12 nt Unique Dual Indexing System (p.34)

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

Find more information about this product at its web page:

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.

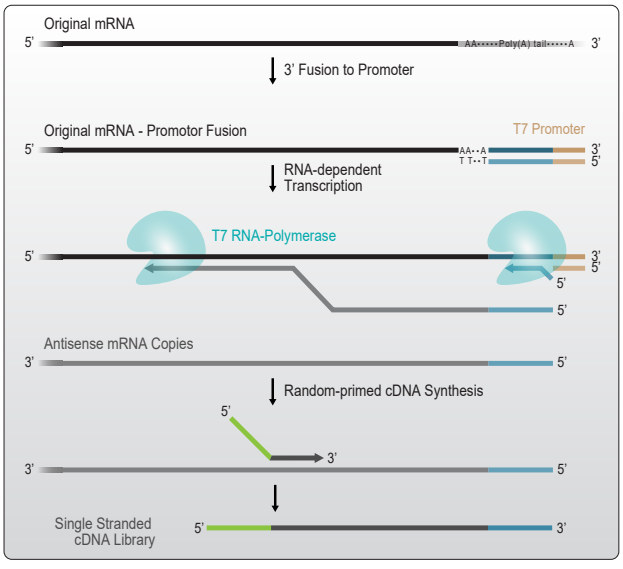

Figure 1 | THOR Amplification and RNA-Seq library template generation.

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.34).

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).

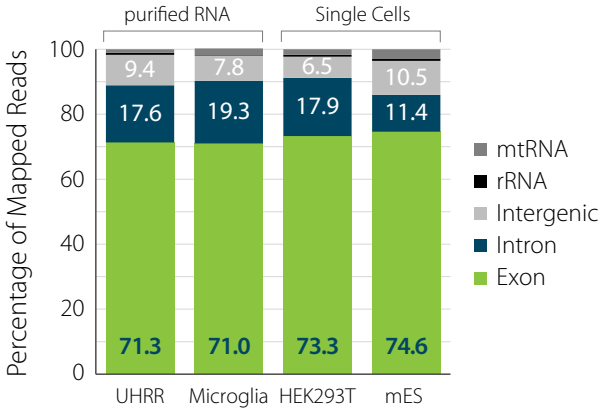

Figure 2 | Feature distribution of uniquely mapped reads for LUTHOR 3' mRNA-Seq. The majority of LUTHOR reads generated from 100 pg UHRR and murine microglia RNA, or single HEK293T and mES cells map to exonic sequences.

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).

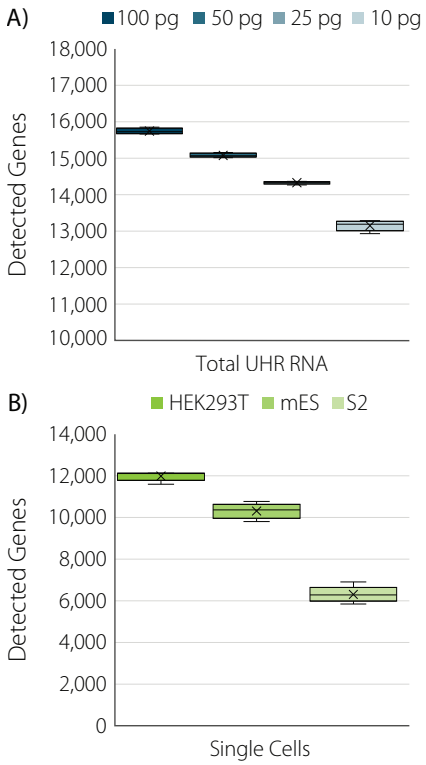

Figure 3 | LUTHOR 3' mRNA-Seq enables highly sensitive and consistent gene detection already at low sequencing depth. Gene detection was assessed at 1 M reads / sample at a threshold of >1 Counts Per Million (CPM) for LUTHOR libraries generated from **A)** ultra-low input UHRR (4 replicates) or **B)** 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.27)
- PCR Add-on Kit for Illumina (p.35)
- Purification Module with Magnetic Beads (p.36)
- Lexogen UDI 12 nt Unique Dual Indexing System (p.34)

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).

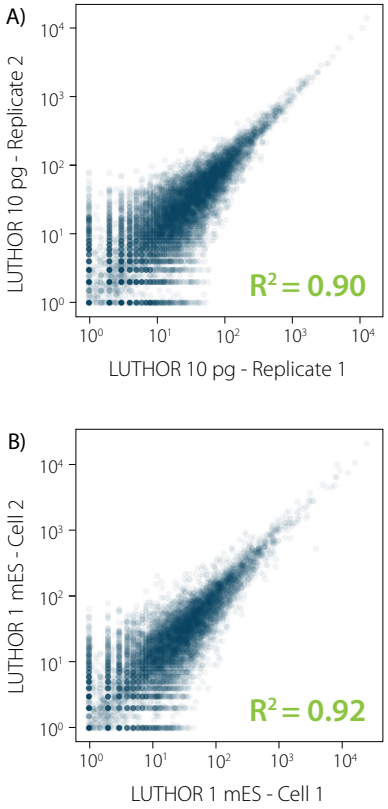

Figure 4 | Excellent reproducibility for LUTHOR 3' mRNA-Seq. A) Correlation plot of gene counts for replicates from 10 pg UHRR and **B)** from two individual FACS-sorted mES cells at 1 M reads / sample. Cells were frozen after sorting and stored at -80 °C prior to processing with LUTHOR 3' mRNA-Seq.

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

CORALL Total RNA-Seq Library Prep Kits

CORALL enables streamlined generation of Illumina-compatible libraries within 4.5 hours, featuring seamless integration of Unique Molecular Identifiers (UMIs) and exceptional protocol-inherent strand specificity (>99 %). The fragmentation-free protocol uses Lexogen's proprietary Strand Displacement Stop and Ligation technologies to deliver complete transcript representation, including genuine transcription start sites.

Advantages

- Wide range of total RNA input (1 ng to 1 µg)
- Compatible with poly(A) selection for mRNA-Seq or rRNA depletion for total RNA-Seq
- Ready-to-sequence libraries within 4.5 hours
- Unique Molecular Identifiers (UMIs) seamlessly included
- Excellent protocol-inherent strandedness (>99 %)
- Robust performance at low input and with FFPE samples
- **NEW!** CORALL Kits with Unique Dual Indices (UDIs) with superior index error correction
- **NEW!** Convenient complete workflow solution: CORALL Total RNA-Seq and RiboCop rRNA Depletion

Workflow

CORALL library generation is initiated by random hybridization of Displacement Stop Primers (DSPs) with partial Illumina-compatible P7 sequences to the RNA template. 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.

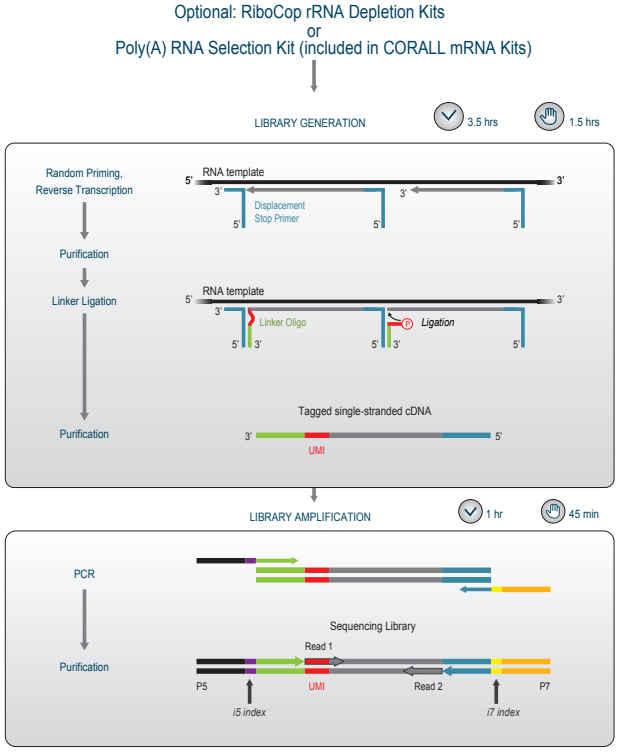

Figure 1 | Schematic overview of the CORALL Library Preparation workflow.

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. During PCR, the cDNA is amplified, i7 and (optional) i5 indices are introduced and complete adapter sequences for cluster generation on Illumina instruments are added. All purification steps use magnetic beads, rendering the protocol compatible for automation.

CORALL is available with up to 384 pre-mixed i5 / i7 Unique Dual Indices (UDIs) with superior index error correction capacity (p.34). Optionally, libraries can be multiplexed using single indexing with up to 96 i7 indices. The UMI information is contained in Read 1 and therefore directly accessible in cost-efficient single-read mode.

CORALL Total RNA-Seq is available in form of convenient bundles with RiboCop Kits for Human/Mouse/Rat or as CORALL mRNA-Seq including poly(A)-selection and UDIs (p.17).

Performance

CORALL Total RNA-Seq

CORALL Total RNA-Seq demonstrates excellent coverage with true representation of genuine transcription start sites while matching gene detection rates of conventional RNA-Seq kits (Figs. 2 – 4). CORALL has been tested with RNA from various species (including human, mouse, rat, plant, and bacteria), different tissue types and body fluids, as well as with degraded and FFPE RNA samples (Fig. 5).

Transcript Coverage

CORALL generates transcriptome-wide smooth, uniform read coverage, comparable to that of competitor kits (Fig. 2).

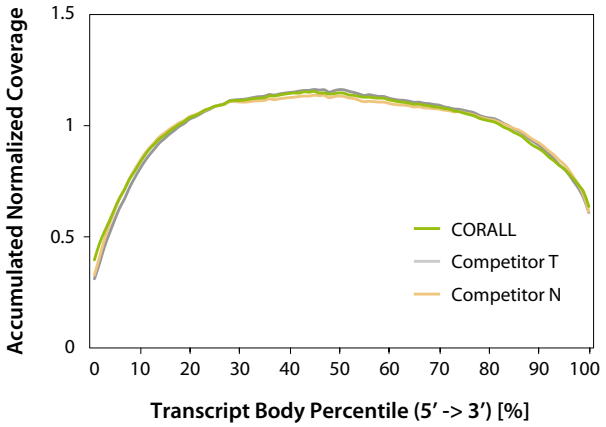

Figure 2 | Accumulated transcript body coverage (whole transcriptome). Coverage across all transcripts was generated using geneBody_coverage.py provided by RSeQC (transcripts length normalized to 100 %).

Gene Detection

CORALL delivers excellent gene discovery rates matching conventional competitor kits (Fig. 3A). 95 % of genes are commonly detected between CORALL and each of the two competitors (at >10 Counts Per Million (CPM), Fig. 3B). This high level of library complexity ensures faithful representation of the transcriptome, enabling sensitive expression profiling.

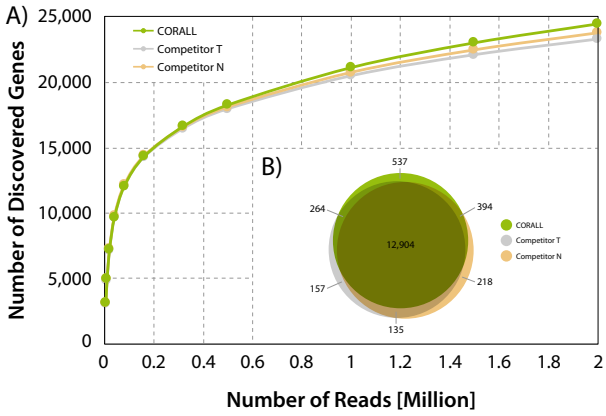

Figure 3 | Gene detection. A) Gene discovery rates. The number of detected genes is plotted against the total number of reads mapping uniquely to exons. **B)** Overlap of detected genes. The Venn diagram illustrates overlaps between CORALL and competitor kits for genes detected with normalized expression levels >10 CPM.

Associated Products

Find more information about this product at its web page:

- SPLIT RNA Extraction Kits (p.27)
- PCR Add-on Kit for Illumina (p.35)
- SIRVs - Spike-In RNA Variant Controls (p.29)
- RiboCop rRNA Depletion Kits (p.22)
- Poly(A) RNA Selection Kit V1.5 (p.24)
- Purification Module with Magnetic Beads (p.36)
- Lexogen UDI 12 nt Unique Dual Indexing System (p.34)

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

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 competitor libraries.

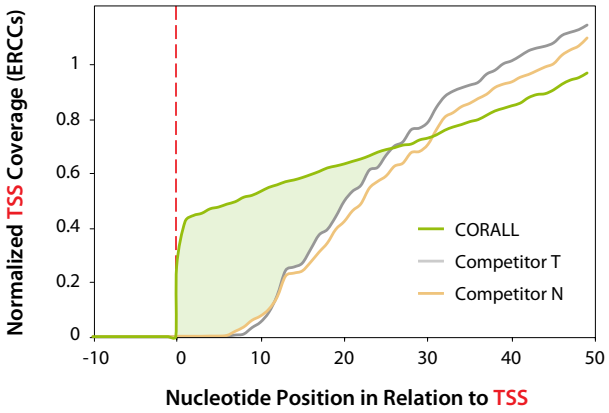

Figure 4 | Normalized ERCC coverage of transcription start sites. Normalized coverage of accumulated mapped reads for all detected ERCCs. The absolute nucleotide positions relative to the TSS (red dotted line) are shown.

Performance on FFPE Samples

CORALL is suitable for processing degraded and compromised samples, including FFPE material. Gene discovery rates are highly comparable between fresh frozen and FFPE mouse spleen samples (DV_{200} = 6 %). Qualitative analysis shows a large overlap of detected genes at normalized expression levels >10 CPM between FFPE and fresh frozen samples demonstrating consistent and robust performance of CORALL even for low quality and FFPE RNA samples (Fig. 5).

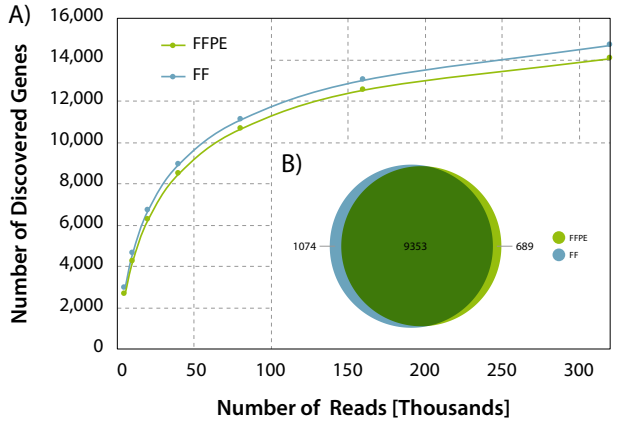

Figure 5 | Gene discovery rates for fresh frozen and FFPE derived RNA. A) Gene detection rates for fresh frozen (FF) or FFPE fixed mouse spleen RNA samples. The number of detected genes is plotted against the total number of reads mapping uniquely to exons. **B)** Overlap of detected genes from FF and FFPE input material at normalized expression levels >10 CPM (for uniquely mapping reads).

Applications

CORALL 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 with the SLAMseq Metabolic Labeling Kits (p.20).

CORALL mRNA-Seq Library Prep Kits

The CORALL mRNA-Seq 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
- 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

Workflow

CORALL mRNA-Seq Kits provide a complete solution for mRNA sequencing. RNA is poly(A) enriched and can be directly transferred into CORALL library generation. The streamlined RNA fragmentation-free protocol generates ready-to-sequence libraries in 4.5 hours using the CORALL technology (p.15). CORALL mRNA-Seq includes Unique Molecular Identifiers (UMIs) and is suitable for input amounts down to 1 ng total RNA inserted into poly(A) selection. Additionally, CORALL mRNA-Seq Kits are available with up to 384 pre-mixed Unique Dual Indices (UDIs) with superior error correction capability (p.34) and thus provide the optimal choice for robust and sensitive whole transcriptome mRNA sequencing. Poly(A) RNA Selection Kit V1.5 (p.24) and all purification steps are based on magnetic beads rendering the complete workflow suitable for full automation.

Performance

Gene Detection

CORALL mRNA-Seq delivers excellent gene discovery rates across a wide range of RNA input amounts and is highly sensitive even at low input levels (Fig. 1).

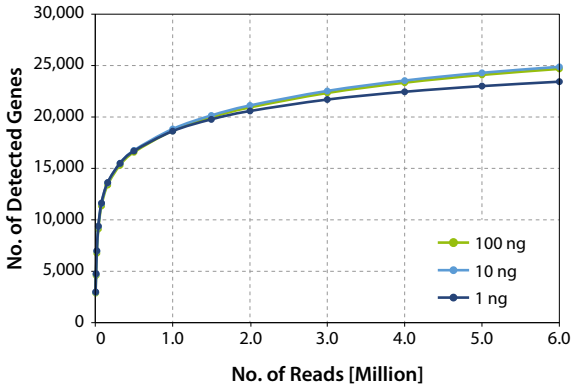

Figure 1 | Gene discovery rates. The number of detected genes for CORALL mRNA-Seq from 100, 10, and 1 ng input RNA is plotted against the total number of reads mapping uniquely to exons.

Excellent Reproducibility and Sensitivity

Correlation analysis comparing replicates for CORALL mRNA-Seq from 10 ng Universal Human Reference RNA (UHRR) reveals excellent reproducibility for the complete workflow (Fig. 2 A). Additionally, correlation plots show high consistency across input amounts emphasizing consistent transcript expression levels even at low input RNA amounts (Fig. 2 B).

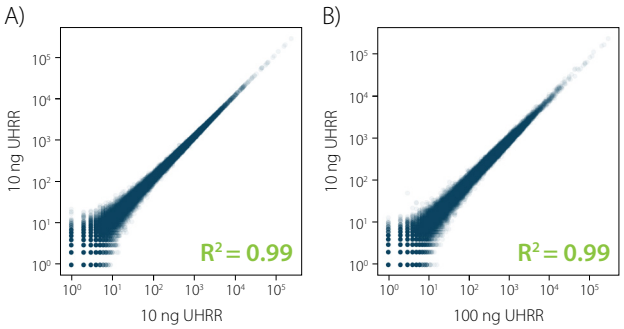

Figure 2 | Excellent reproducibility and consistent transcript abundance across input amounts. Correlations between **A)** replicates for CORALL mRNA-Seq from 10 ng RNA and **B)** transcript abundance from CORALL mRNA samples using 100 ng vs. 10 ng RNA as input.

Associated Products

- SPLIT RNA Extraction Kits (p.27)
- PCR Add-on Kit for Illumina (p.35)
- Purification Module with Magnetic Beads (p.36)
- SIRVs - Spike-In RNA Variant Controls (p.29)

Find more information about this product at its web page:

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

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.

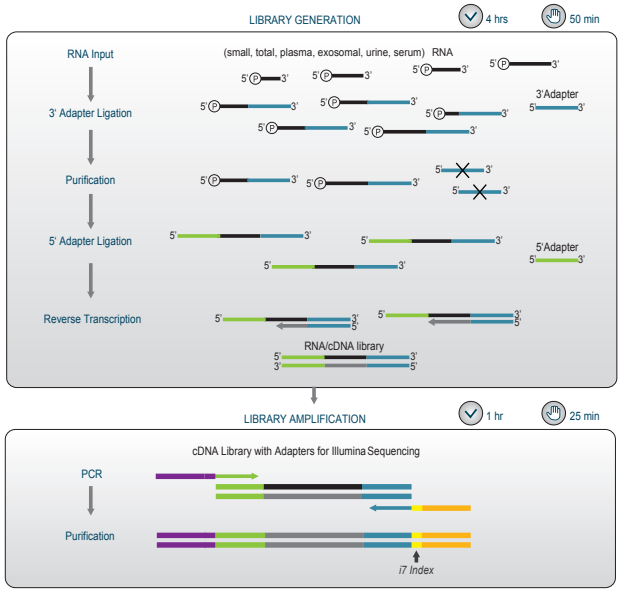

Figure 1 | Schematic overview of the Small RNA-Seq Library Prep workflow.

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.

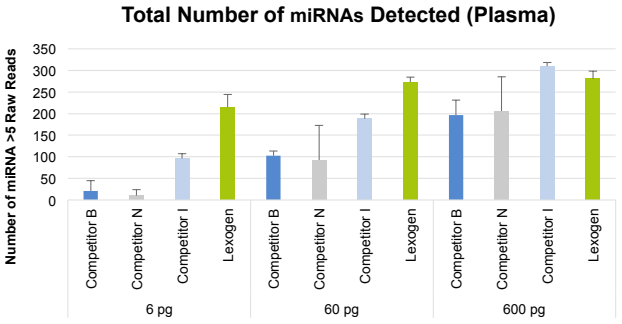

Figure 2 | Lexogen's Small RNA-Seq Kit allows for detection of a higher number of microRNAs, especially for lower RNA inputs. Total number of miRNAs detected across 4 different protocols. Dilution series (6 pg, 60 pg, and 600 pg) of purified plasma RNA was used with library prep kits from different vendors. 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.25)
- SPLIT RNA Extraction Kits (p.27)
- Gel Extraction Module (p.36)

This product is available as part of the Lexogen 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.

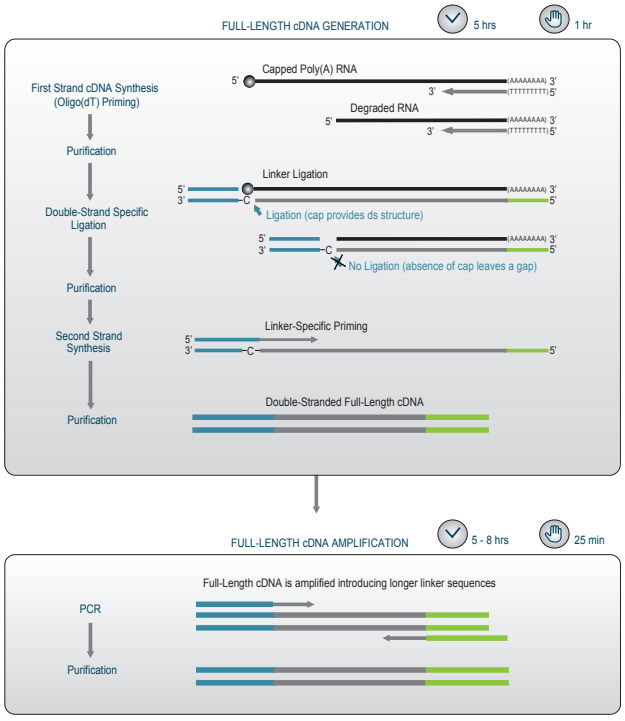

Figure 1 | Schematic overview of the TeloPrime workflow. After full-length cDNA synthesis, the inverted G of the cap structure of the RNA/cDNA hybrid takes part in an atypical base- pairing with the 5′C overhang of a double-stranded adapter. By using a double-strand specific ligase, ligation to the cDNA's 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).

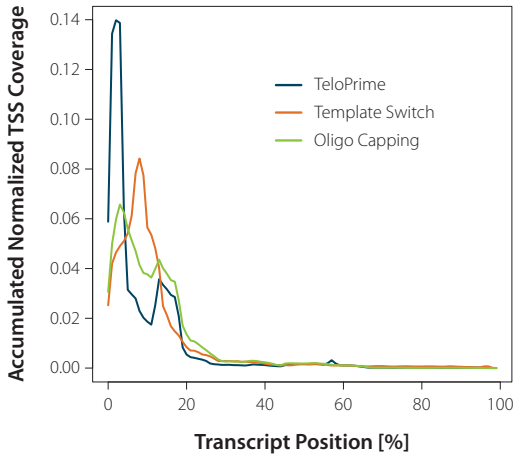

Figure 2 | Lexogen's TeloPrime Full-Length cDNA Amplification Kit enables high precision of transcription start sites (TSS) mapping. In an mRNA-Seq experiment, mRNAs were tagged at their 5′ end using Template Switch, Oligo Capping, or TeloPrime's CDLL technology. Illumina-compatible libraries were then 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.29)
- SPLIT RNA Extraction Kits (p.27)

Find more information about this product at its web page:

SLAMseq Metabolic Labeling Kits

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 new 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.

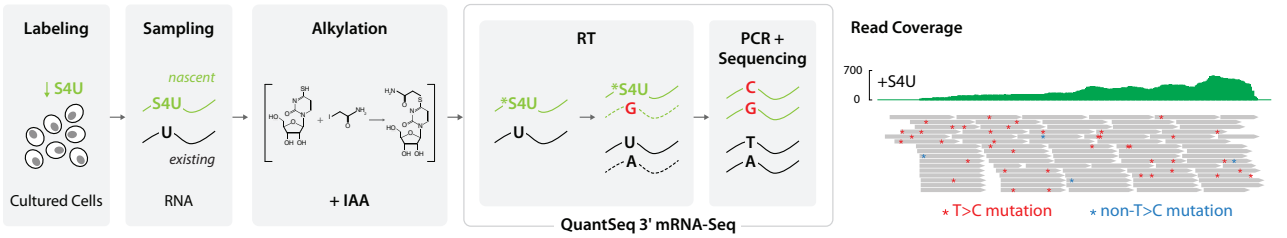

Figure 1 | Schematic overview of the SLAMseq workflow. Cultured cells are treated with 4-Thiouridine (S4U) for labeling of nascent RNA (green). Total RNA is purified, and alkylation of the 4-thiol group is induced by the addition of iodoacetamide (IAA). During library preparation, shown here using the 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 is available on the BlueBee® Genomics platform and 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 from the BlueBee® platform 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.

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.

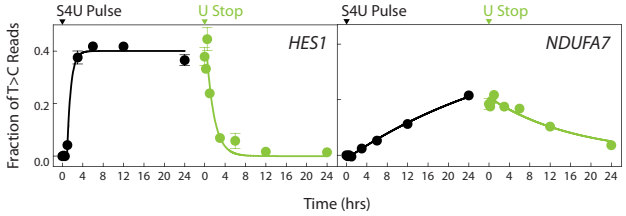

Figure 2 | S4U labeling kinetics experiments reveal individual RNA synthesis and degradation rates. To measure RNA synthesis, cells were treated with S4U (100 µM) for 24 hours, whereas for RNA decay measurements, cells were first saturated with S4U, and rates were measured after removal of S4U and addition 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 this 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 Services. They will perform library preparations, NGS run and data evaluation, so you can confidently derive your conclusions.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- TraPR Small RNA Isolation Kit (p.25)
- Small RNA-Seq Library Prep Kit (p.18)
- SPLIT RNA Extraction Kits (p.27)

Find more information about this product at its web page:

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

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4. Sharma, U., et al. (2018) Developmental Cell, DOI: 10.1016/j.devcel.2018.06.023
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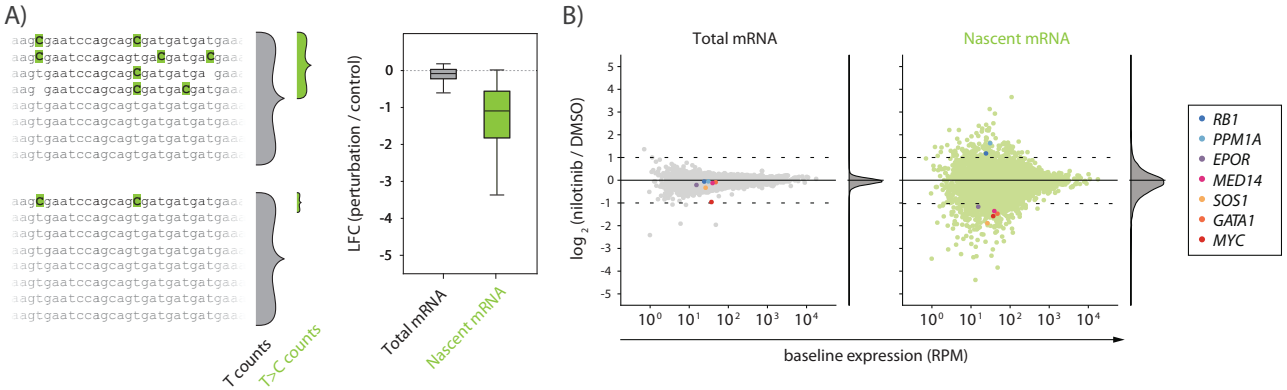


Figure 3 | SLAMseq enhances differential expression detection. A) Nascent RNA levels represented by T>C-containing reads (green) show a higher log fold-change (LFC) compared to total mRNA (grey) levels when analyzing control (DMSO) and BCR/ABL-inhibitor-treated (nilotinib) K562 cells. **B)** More differentially 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

- Depletion of human/mouse/rat, or bacterial rRNA
- Suitable for intact and degraded RNA (e.g., 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 Total RNA-Seq
- **NEW!** Innovative probe design minimizing off-target effects
- **NEW!** Combined rRNA and globin mRNA depletion

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).
RiboCop Kits for Human/Mouse/Rat and for Bacteria can conveniently be combined for simultaneous depletion of host and bacterial 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 Total RNA-Seq library prep kits.

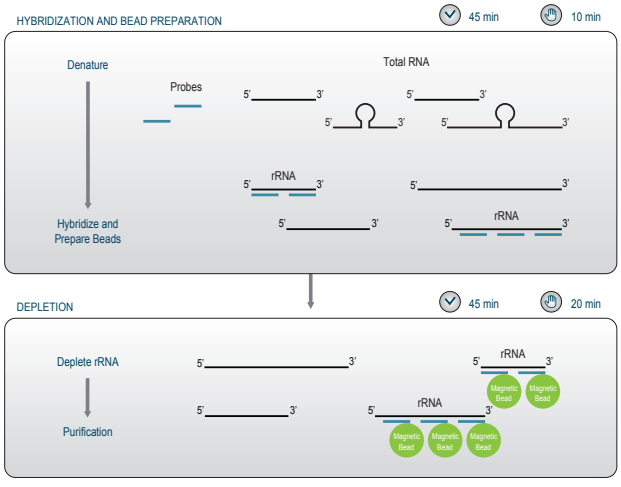


Figure 1 | Schematic overview of the RiboCop workflow.

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 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).

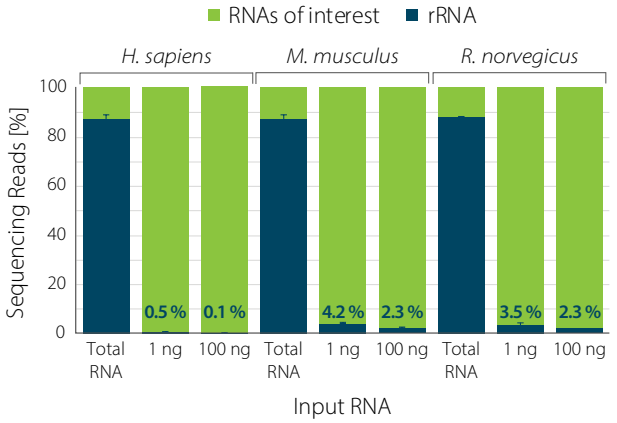


Figure 2 | RiboCop rRNA Depletion for Human/Mouse/Rat efficiently removes rRNA across a wide range of input amounts. Two RNA amounts of the indicated species were rRNA-depleted using the HMR V2 Probe Mix. CORALL Total RNA-Seq libraries were prepared and sequenced. The percentage of reads mapping to rRNA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

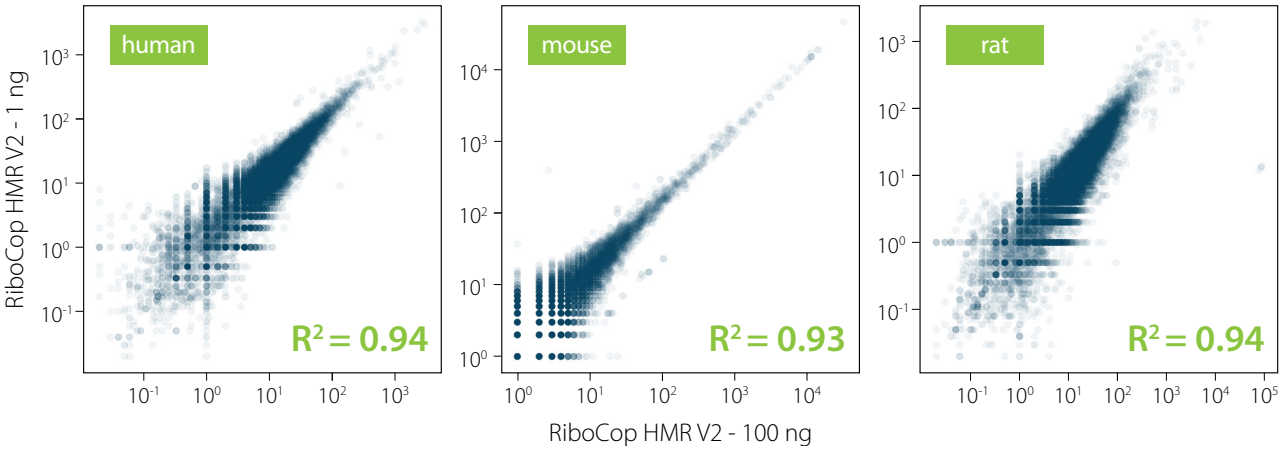


Figure 3 | RiboCop HMR V2 maintains constant transcript abundance across various input amounts. Correlation of transcript abundance in samples depleted with RiboCop HMR V2 for 100 ng RNA vs. 1 ng RNA input for indicated species.

RiboCop for Human/Mouse/Rat Plus Globin

Globin mRNA constitutes ~35 - 80 % of all blood mRNA. RiboCop for Human/Mouse/Rat Plus Globin simultaneously removes rRNA and globin mRNA from whole blood RNA, thus providing a convenient workflow for whole transcriptome analysis of blood samples while freeing up sequencing space for RNAs of interest (Fig. 4).

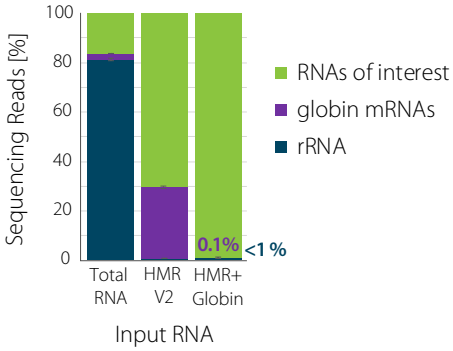


Figure 4 | RiboCop rRNA Depletion for Human/Mouse/Rat plus Globin (HMR+Globin) efficiently removes rRNA and globin mRNAs from human blood RNA. 10 ng human whole blood RNA were used as input for RiboCop using either the HMR V2 or the HMR+Globin Probe Mix. CORALL Total RNA-Seq libraries were prepared and sequenced. The percentage of reads mapping to rRNA is plotted in blue, globin mRNA reads in purple.

RiboCop for Bacteria

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 for Bacteria performs robustly across a wide range of input amounts and across species as exemplified by using the META kit for rRNA depletion from two Gram negative and one Gram positive species (Fig. 5).

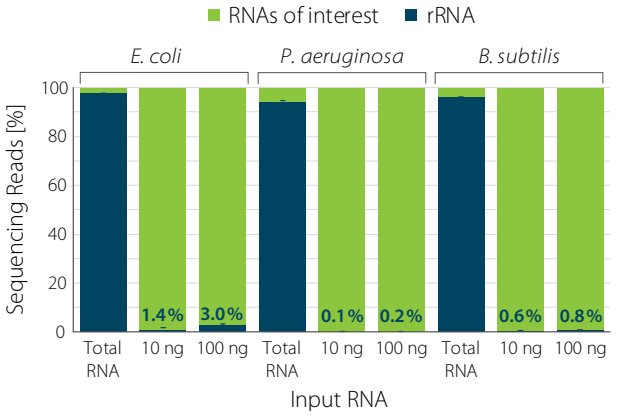


Figure 5 | RiboCop rRNA Depletion for Mixed Bacterial Samples (META) efficiently removes rRNA from various bacterial species. Two RNA amounts from monocultures of the indicated species were subjected to rRNA depletion using the META Probe Mix. CORALL Total RNA-Seq libraries were prepared and sequenced. 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.

Associated Products

- SPLIT RNA Extraction Kits (p.27)
- SIRVs - Spike-In RNA Variant Controls (p.29)
- CORALL Total RNA-Seq Library Prep Kits (p.15)

Find more information about this product at its web page:

This product is available as part of the Lexogen 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 Library Prep Kits (p.17).

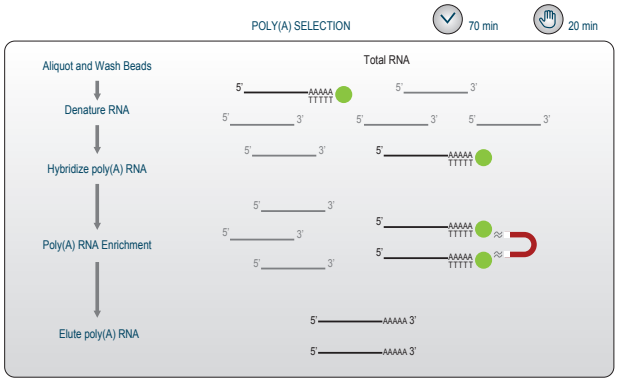


Figure 1 | Schematic overview of the Poly(A) RNA Selection workflow.

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.

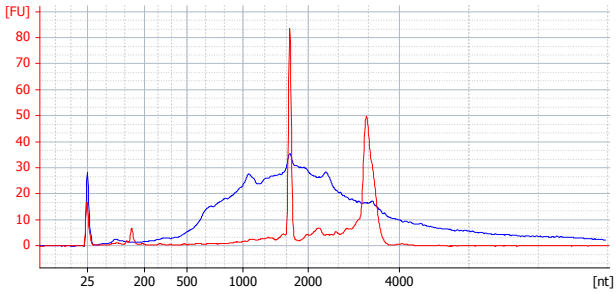


Figure 2 | Lexogen's Poly(A) RNA Selection Kit V1.5 enables highly specific poly(A) RNA enrichment and efficient removal of rRNA. Bioanalyzer traces before (red) and after poly(A) RNA selection (blue).

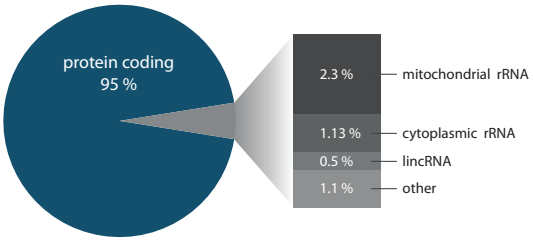


Figure 3 | Highly efficient removal of rRNA using Lexogen's Poly(A) RNA Selection Kit. RNA-Seq biotype analysis shows that the Poly(A) RNA Selection Kit enriches RNAs containing a poly(A) tail such as mRNAs and mitochondrial rRNAs.

Associated Products

- SPLIT RNA Extraction Kits (p.27)
- SIRVs - Spike-In RNA Variant Controls (p.29)
- CORALL mRNA-Seq Library Prep Kits (p.17)

Find more information about this product at its web page:

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

TraPR Small RNA Isolation Kit

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.

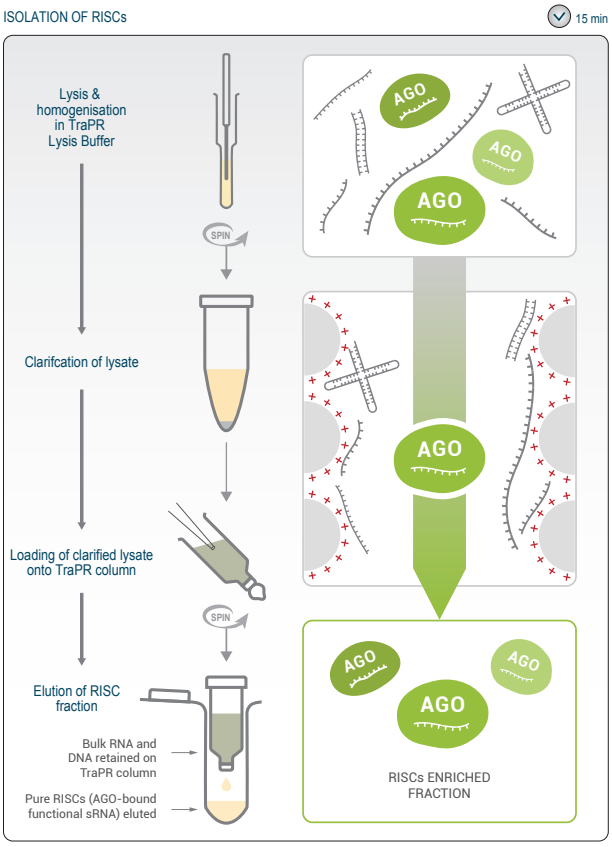


Figure 1 | Schematic depiction of the TraPR workflow and the principle of RISC isolation.

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).

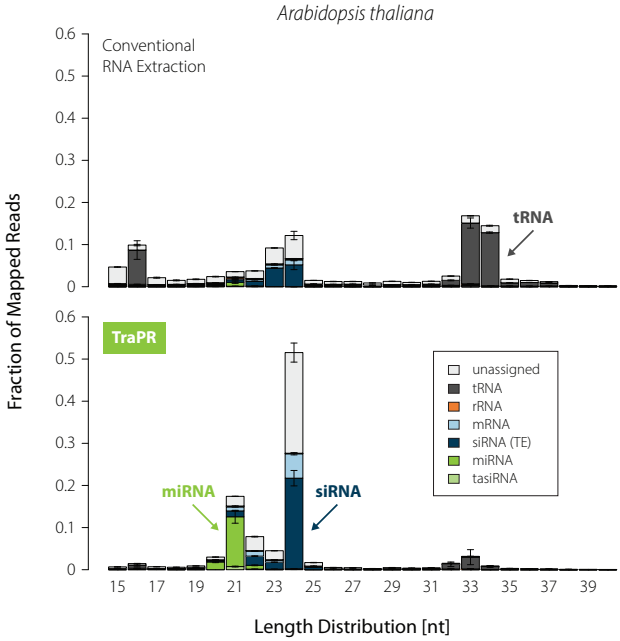


Figure 2 | TraPR enriches functional sRNA. Size distribution and biotypes of mapped reads from NGS libraries prepared from total RNA (conventional RNA extraction) or TraPR-isolated sRNA from *Arabidopsis thaliana* [1].

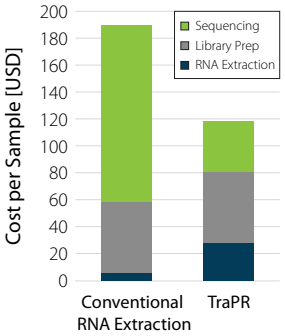


Figure 3 | TraPR significantly reduces overall sRNA analysis cost. Almost all reads obtained from TraPR-isolated RNA map to functional sRNAs (see also Fig. 2) whereas using conventional RNA extraction, only ~40 % of reads map to functional sRNAs. Hence, TraPR allows pooling of more libraries per lane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

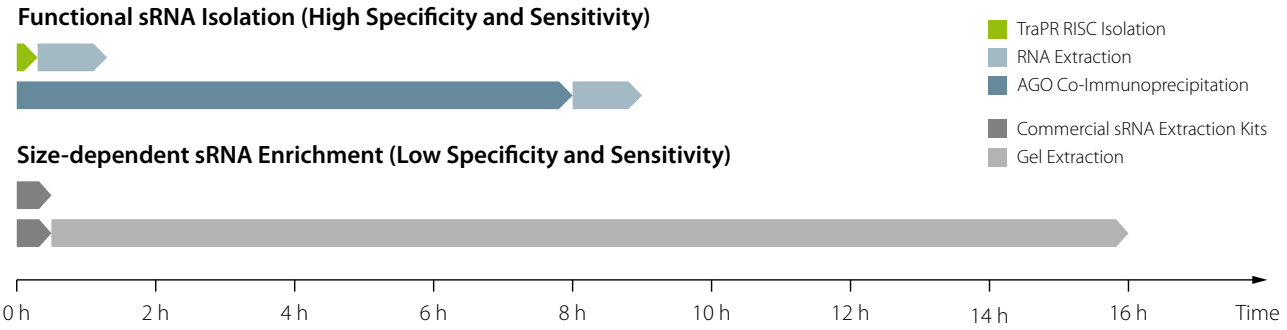


Figure 4 | TraPR is the fastest method for functional sRNA isolation. TraPR isolates functional sRNA in only 1 hour and 15 minutes. Common sRNA extraction kits are very fast, but not selective for functional sRNAs. AGO Co-Immunoprecipitation or gel extraction increase specificity but take one to two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

Associated Products

- Small RNA-Seq Library Prep Kit (p.18)
- Gel Extraction Module (p.36)

This product is available as part of the Lexogen 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.

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 extraction of RNA. Additionally, SPLIT allows recovering total RNA or splitting into small and large RNA fractions.

Advantages

- Total RNA from <17 nt to >10,000 nt
- Option to split into large RNA and small RNA fractions (cutoff ~150 nt)
- Convenient and universal protocol requiring only 30 minutes
- High RNA integrity and purity (RIN >8 for tissue, up to RIN 10 for cell culture)
- Supplementary protocol for FFPE samples available
- SPLIT for Blood enables concomitant depletion of globin mRNAs from human blood samples
- **NEW!** SPLIT Rapid Viral RNA/DNA Extraction Kit: phenol-free, 15-minute workflow

Workflow

The SPLIT workflow enables easy, fast, and reliable RNA extraction from a variety of materials (plasma, tissue, cell lines) from different 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). RNA fractions are purified via silica column purifications which enable the recovery of either total RNA or large and/or small RNA fractions. For extraction of viral nucleic acids, SPLIT Rapid Viral RNA/DNA Extraction provides a fast workflow without phenol / chloroform.

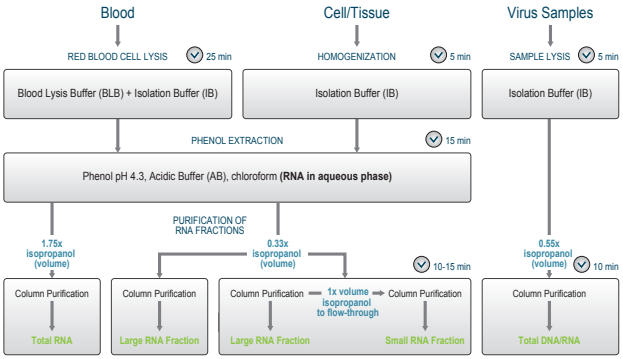


Figure 1 | Schematic overview of the SPLIT, SPLIT for Blood, and SPLIT Rapid Viral workflows.

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 purified in one or in separate fractions (total, or large, and/or small). Extracted RNA has negligible residual gDNA content without inducing RNA degradation or size biases frequently caused by DNase treatment, heat inactivation, or gDNA removal columns (Fig. 2).

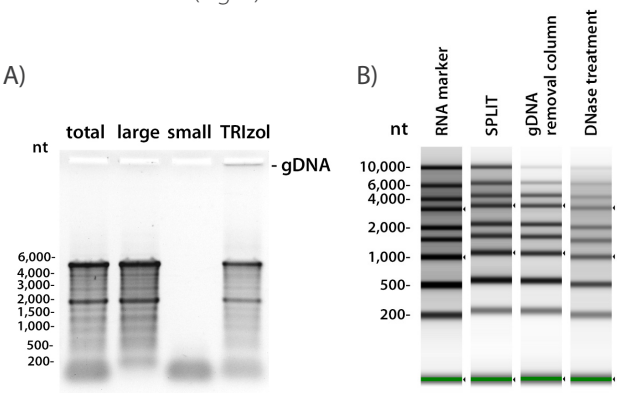


Figure 2 | The SPLIT protocol extracts RNA and reduces the level of genomic DNA contamination. **A)** RNA fractions (total, or large, and/or small) extracted from mouse liver are shown on a denaturating formaldehyde agarose gel. A TRIzol-extracted control sample shows a significant amount of genomic DNA whereas SPLIT extracted RNAs are free from gDNA. **B)** RNA integrity of an RNA marker (200 - 10,000 nt) was assessed on Agilent's TapeStation after employing different gDNA removal methods – SPLIT outperforms other methods in terms of RNA integrity and unbiased size distribution.

SPLIT RNA Extraction Kit for Blood

The SPLIT RNA Extraction Kit for Blood includes a red blood cell lysis step prior to sample homogenization of the conventional SPLIT RNA Extraction Kit. The protocol yields high-integrity RNA from fresh human blood (Fig. 3). After red blood cell lysis, samples can be resuspended in Isolation Buffer and stored for up to three months at -80 °C for later RNA extraction.

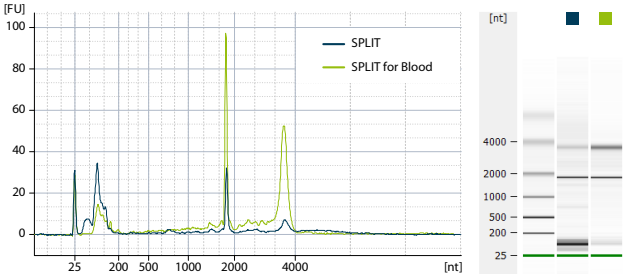


Figure 3 | The SPLIT for Blood protocol improves the RNA quality from whole blood extractions. Bioanalyzer traces of RNA extracted from fresh human blood using the SPLIT RNA Extraction Kit for Blood (green, RIN 8.7) or the conventional SPLIT protocol without prior red blood cell lysis (blue, RIN 7.0), respectively.

Efficient Depletion of Globin mRNA from Human Blood

The additional red blood cell lysis step enables efficient depletion of globin mRNA species from blood samples (Fig. 4).

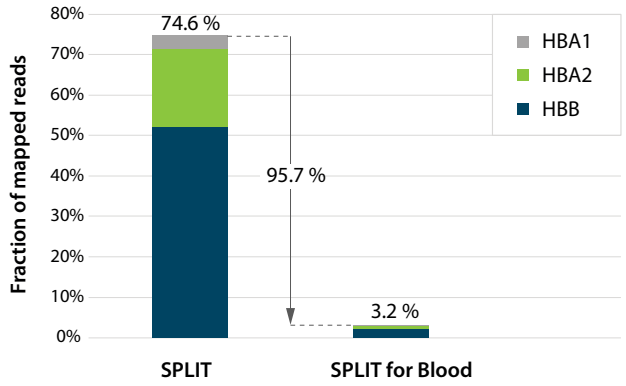


Figure 4 | The SPLIT for Blood protocol depletes >95 % of globin mRNA species from the extracted RNA samples. RNA was extracted from fresh human blood using the SPLIT and SPLIT for Blood protocol, respectively. RNA-Seq libraries were prepared with Lexogen's QuantSeq 3' mRNA-Seq (FWD) kit, sequenced reads were mapped to the human reference genome, and the percentage of reads mapping to globin mRNAs was calculated.

Increased Gene Detection after Globin mRNA Depletion

The efficient depletion of the predominant globin mRNA species from the blood RNA samples frees up sequencing space and thereby results in increased gene discovery rates (Fig. 5), saving sequencing costs.

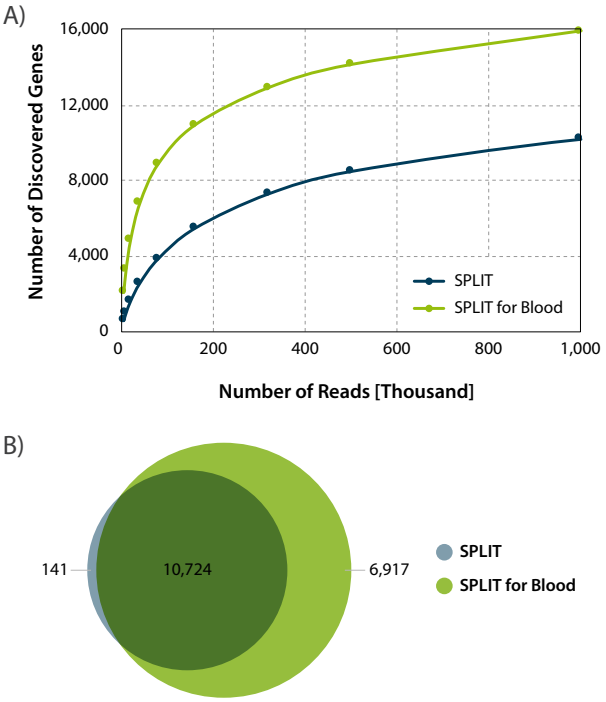


Figure 5 | Increased gene detection in human blood QuantSeq libraries using SPLIT RNA Extraction Kit for Blood. Libraries were prepared with Lexogen's QuantSeq 3' mRNA-Seq (FWD) protocol with RNA extracted from fresh human blood using the SPLIT RNA Extraction Kit (blue) or the SPLIT RNA Extraction Kit for Blood (green). **A)** Gene Discovery Plot. The number of discovered genes was calculated from CPM (Counts Per Million reads) normalized read counts (threshold >0.5 CPM). **B)** Venn Diagram depicting the number of genes detected in libraries from RNA extracted using the SPLIT and SPLIT for Blood protocols and the overlap between the two.

SPLIT Rapid Viral RNA/DNA Extraction

The SPLIT Rapid Viral RNA/DNA Extraction Kit specifically enables isolation of high-quality RNA and DNA from liquid samples, such as nasopharyngeal and buccal swabs within 15 minutes. Since the workflow is based on sample lysis and column purification, only a standard centrifuge is required. The kit yields high-quality RNA suitable for reverse transcription, RT-qPCR, and targeted RNA-Seq. Furthermore, the protocol is transferable to magnetic beads for plate-based, automatable high-throughput RNA extraction.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- CORALL Total RNA-Seq Library Prep Kits (p.15) and CORALL mRNA-Seq Library Prep Kits (p.17)
- Small RNA-Seq Library Prep Kit (p.18)
- LUTHOR 3' mRNA-Seq Library Prep Kit (p.13)

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

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, and in RNA-Seq workflows the spike-in transcripts 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.

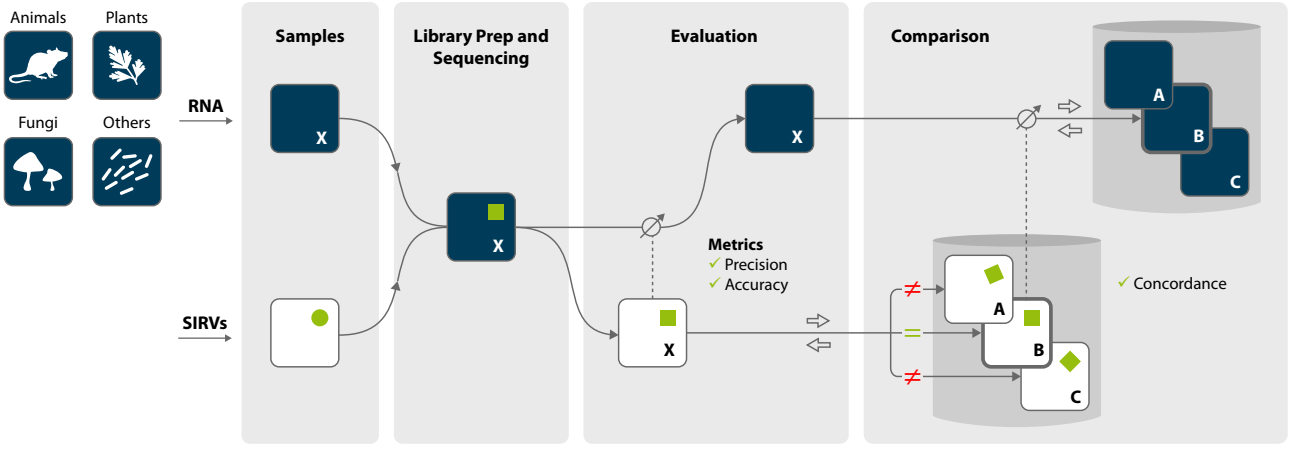


Figure 1 | Using SIRV controls in RNA-Seq. SIRVs are defined artificial RNA molecules that mimic the main aspects of transcriptome complexity. They are added in minuscule amounts to samples before the library preparation and are processed alongside endogenous RNA. After mapping the reads to the combined 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)

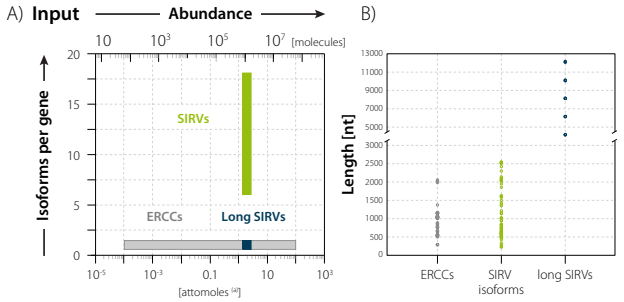


Figure 2 | The representation of isoform complexity, transcript abundance, and length by the three SIRV modules. SIRV Isoform Mix E0 represents isoform complexity at equimolar concentrations, whereas ERCCs and long SIRVs both follow the one gene, one isoform rule covering abundance and length, respectively. 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 genes, 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.

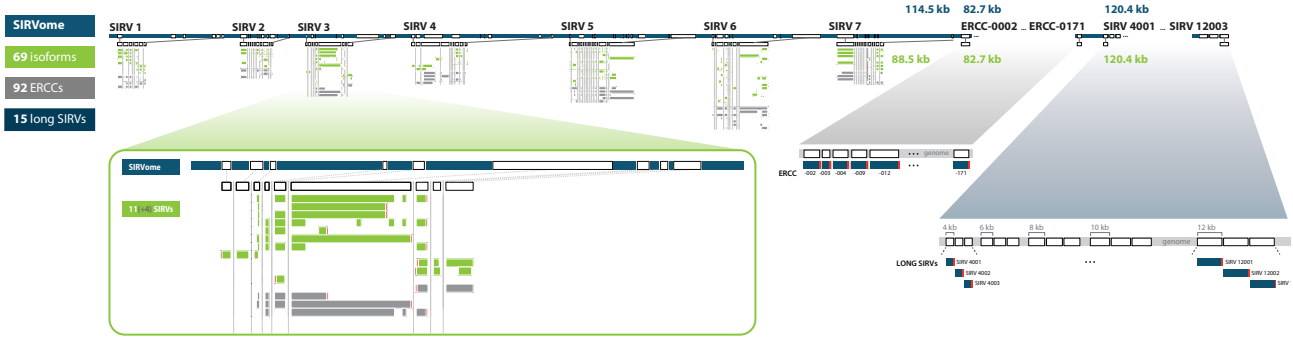


Figure 3 | SIRVs design overview. SIRV isoform genomic loci, ERCC genes, and long SIRV genes are lined up on a SIRVome. SIRV1 to SIRV7 of the SIRV Isoform module mimic human model genes to comprehensively represent all main aspects of alternative splicing and transcription in numerous repeats and variations. 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

Fig. 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].

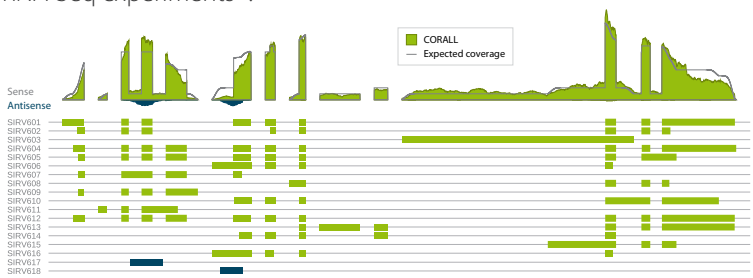


Figure 4 | Coverage of SIRV6. The exon-intron structure of all 18 transcripts of the SIRV6 locus is shown in the lower panel (with antisense transcripts in blue). The equimolar individual SIRV transcripts contribute in equal parts to the expected coverage (fine line, with modeling of transcript ends). 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).

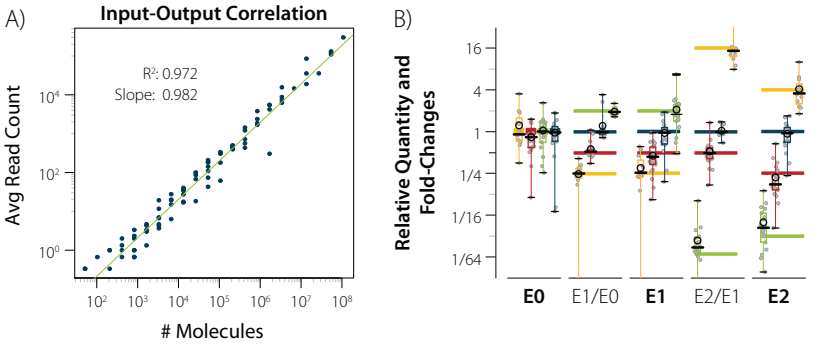


Figure 5 | Measuring input-output correlation using ERCC and SIRV Isoform modules. A) The one gene, one transcript spike-ins of ERCC Mix 1 in SIRV-Set 3 were assessed in a QuantSeq 3′ mRNA-Seq experiment. Correlation of the theoretical and the measured concentrations was high with an R^2 of 0.972. **B)** 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 services. For details, contact our Services department: services@lexogen.com.

Associated Products

- CORALL Total RNA-Seq Library Prep Kits (p.15) and CORALL mRNA-Seq Library Prep Kits (p.17)
- TeloPrime Full-Length cDNA Amplification Kit (p.19)
- LUTHOR 3′ mRNA-Seq Library Prep Kit (p.13)
- QuantSeq Expression Profiling Library Prep Kits (p.7)
- SPLIT RNA Extraction Kits (p.27)
- Mix² RNA-Seq Data Analysis Software (p.33)

Find more information about this product at its web page:

QuantSeq-Pool Targeted SARS-CoV-2 Panel

The QuantSeq-Pool Targeted SARS-CoV-2 Panel leverages the ultra-high throughput capacity of Next Generation Sequencing (NGS) to allow mass screening at population scale. It combines convenient gargle sampling with high specificity and scalability.

Advantages

- The most economical solution for SARS-CoV-2 mass screening
- Scalable to population size, analysis of up to 36,864 samples per NGS run
- Individual barcoding of samples for tracing positive results
- Batch processing for high-quality results in 20 hours and for reduction of plasticware requirements

Workflow

Lexogen's QuantSeq-Pool Targeted SARS-CoV-2 Panel works with RNA extracted from convenient gargle samples, allowing for painless self-sampling. Samples are individually barcoded during cDNA synthesis, and all samples of a 96-well plate are then pooled in a single well in another plate for second strand synthesis. In the subsequent PCR amplification, each pool obtains one of 384 Unique Dual Indices (UDIs), enabling multiplexing of up to 36,864 samples for analysis in a single NGS run (Fig. 1). The streamlined workflow can be completed within 20 hours, and automation provides for highest throughput scalability.

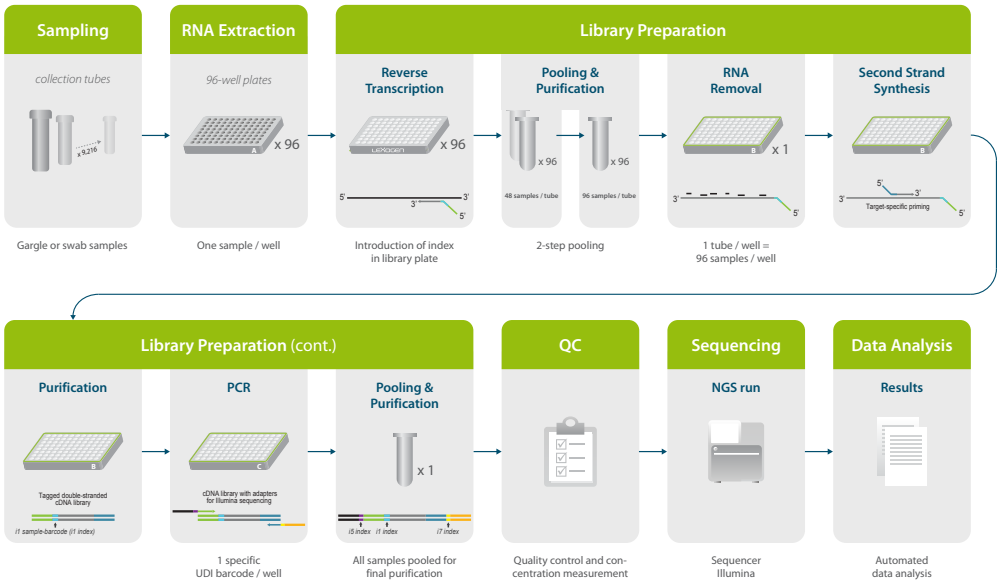


Figure 1 | Schematic overview of the QuantSeq-Pool SARS-CoV-2 Panel workflow. RNA is extracted from gargle (or other) samples and added to wells of a plate containing dried reverse transcription primers and controls. During cDNA synthesis, each sample is individually labeled with an i1 barcode. All 96 cDNA samples of a plate are then pooled and transferred into a single well of another plate. In the PCR step, each sample pool (well) obtains one (of 96) UDI barcode combinations, allowing for pooling of all 9,216 samples in a single NGS run. By using all 384 available UDIs, 36,864 samples can be barcoded.

Performance

Lexogen's SARS panel reliably amplifies four regions of the SARS-CoV-2 genome (Fig. 2). The SARS test showed a sensitivity of 99.03 % at a PCR Ct ≤30 with a specificity of >99 % when tested on 5,000 PCR-validated samples with a 3.32 % positive rate.

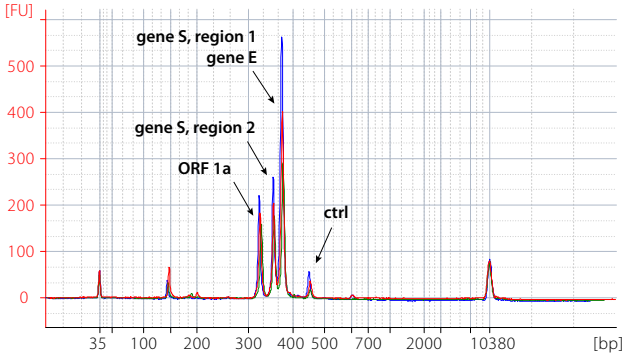


Figure 2 | Bioanalyzer trace of a positive sample. Lexogen's SARS panel amplifies four regions of the SARS-CoV-2 genome in genes ORF 1a, Gene E, Gene S (region 1), Gene S (region 2), and a spike-in control (ctrl).

Associated Products

- SARS-CoV-2 Rapid RNA Extraction Kit (Cat. No. 142.8x96)
- Lexogen UDI 12 nt Unique Dual Indexing System (p.34)

Find more information about this product at its web page:

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).

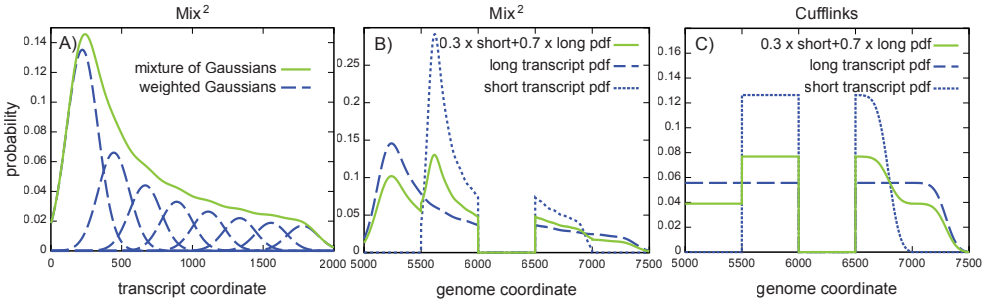


Figure 1 | Working principle of Mix². **A)** Exemplary representation for 5′ positional fragment bias over a 2,000 nt transcript modelled by Mix² with a mixture of 8 normal distributions. **B)** Gene with 2 isoforms with 5′ bias modelled by Mix². **C)** Uniform distribution of Cufflinks in gene B) irrespective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

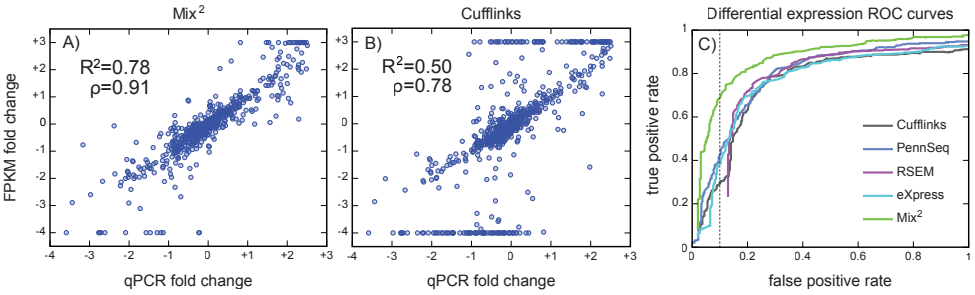


Figure 2 | Correlation between qPCR expression changes and expression changes estimated by **A)** Mix² and **B)** Cufflinks. **C)** Receiver Operating Characteristic (ROC) curve of detection of differential expression based on isoform quantification of different methods. True positive rates are significantly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

Associated Products

– CORALL Total RNA-Seq Library Prep Kits (p.15) and CORALL mRNA-Seq Library Prep Kits (p.17)

This product is available as part of the Lexogen 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 advanced index sequence design. All indices have maximum 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 12 nt UDIs are added to the prepared libraries during the final PCR amplification. In this step the previously introduced partial Illumina adapters (Fig. 1, green and blue) 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).

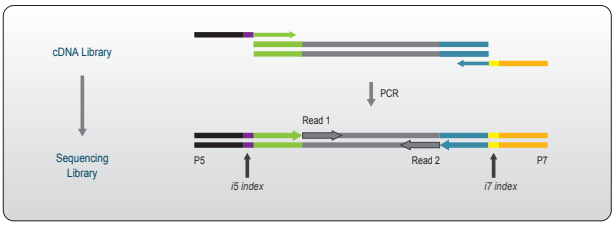


Figure 1 | Schematic overview of the introduction of Lexogen's 12 nt UDIs to cDNA libraries with partial Illumina adapters (green and blue).

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).

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

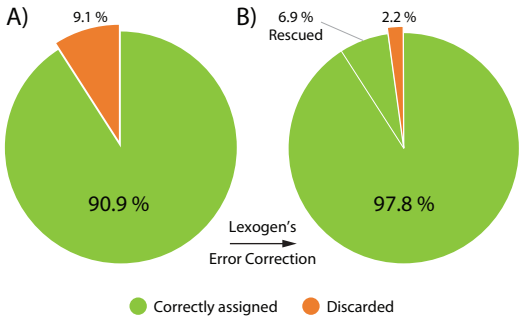


Figure 2 | Maximizing read output with Lexogen's 12 nt UDIs and error correction. 96 multiplexed libraries were sequenced on an Illumina NextSeq 500 with 12 nt UDI read-out. **A)** 9.1 % of reads were undetermined (orange) due to random Index Sequence Errors. **B)** Lexogen's Error Correction Tool confidently 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 library prep kits and as Add-on kits for other vendor's library preps.

Associated Products

- QuantSeq Expression Profiling Library Prep Kits (p.7)
- CORALL Total RNA-Seq Library Prep Kits (p.15) and CORALL mRNA-Seq Library Prep Kits (p.17)
- LUTHOR 3' mRNA-Seq Library Prep Kit (p.13)

Find more information about this product at its web page:

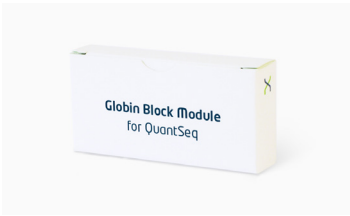
Modules and Add-ons

PCR Add-on Kit for Illumina

The PCR Add-on Kit for Illumina provides PCR Mix, Enzyme Mix, and a compatible P7 primer that are required for performing qPCR assays to determine endpoint PCR cycle numbers for all QuantSeq, CORALL, and LUTHOR Library Prep Kits. A reamplification primer is also included that can be used for reamplification of single-indexed (i7) libraries of QuantSeq (Cat. No. 015, 016) and CORALL (Cat. No. 095).

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). QuantSeq UMI data analysis is also available on the BlueBee Genomics Platform.



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. 015, 113 – 115, 129 - 131, and REV, Cat. No. 016), 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. 015, 113 – 115, 129 - 131).

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. 015, 113 – 115, 129 - 131).

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.

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 Services

Advantages

- Fully integrated RNA-Seq Services from the RNA Experts
- Automated setups for highly multiplexed gene expression profiling
- Renowned expertise for difficult samples such as FFPE-derived, degraded, and low-input RNA
- Standard data reporting included, with advanced analysis services available

Your samples & Lexogen's expertise

Lexogen develops world-class RNA-Seq technologies, and Services applies this knowledge to your samples.

Consultation

Profit from our expertise even before we begin working with your samples. In a consultation with you, we determine a tailored setup to ensure you get the data you need for your project.

Sample Extraction

We start from purified RNA or by extracting RNA from submitted tissue, cells, biofluids, and single cells. Benefit from our expertise with difficult samples such as FFPE-derived and degraded RNA - we identify the best approach to maximize the data quality for your given sample type.

Library Preparation

Depending on your application, the appropriate Lexogen library preparation kit is selected:

- Gene expression profiling: QuantSeq
- Gene expression high-throughput screening: QuantSeq-Pool
- mRNA-Seq and total RNA-Seq: CORALL
- sRNA-Seq: Small RNA-Seq Library Prep
- Single-cell and ultra low-input RNA-Seq: LUTHOR

Further, we can

- prepare libraries from SLAMseq samples (metabolic labeling).
- deplete and block globin mRNA in blood samples for significantly improved sensitivity.
- include Unique Molecular Identifiers (UMIs) to take care of PCR duplication in low-complexity samples.
- use Lexogen's 12 nt UDI barcodes to provide superior error correction for maximal data output.
- include SIRVs spike-in control transcripts for workflow validation and data set concordance.
- handle large-scale projects on automated liquid handling platforms.
- flexibly adjust to your requirements for advanced setups and workflows.

Sequencing

Your samples are sequenced on Illumina platforms with the read mode and read depth most suitable for your application. We routinely exceed output specifications.

Data Analysis and Reporting

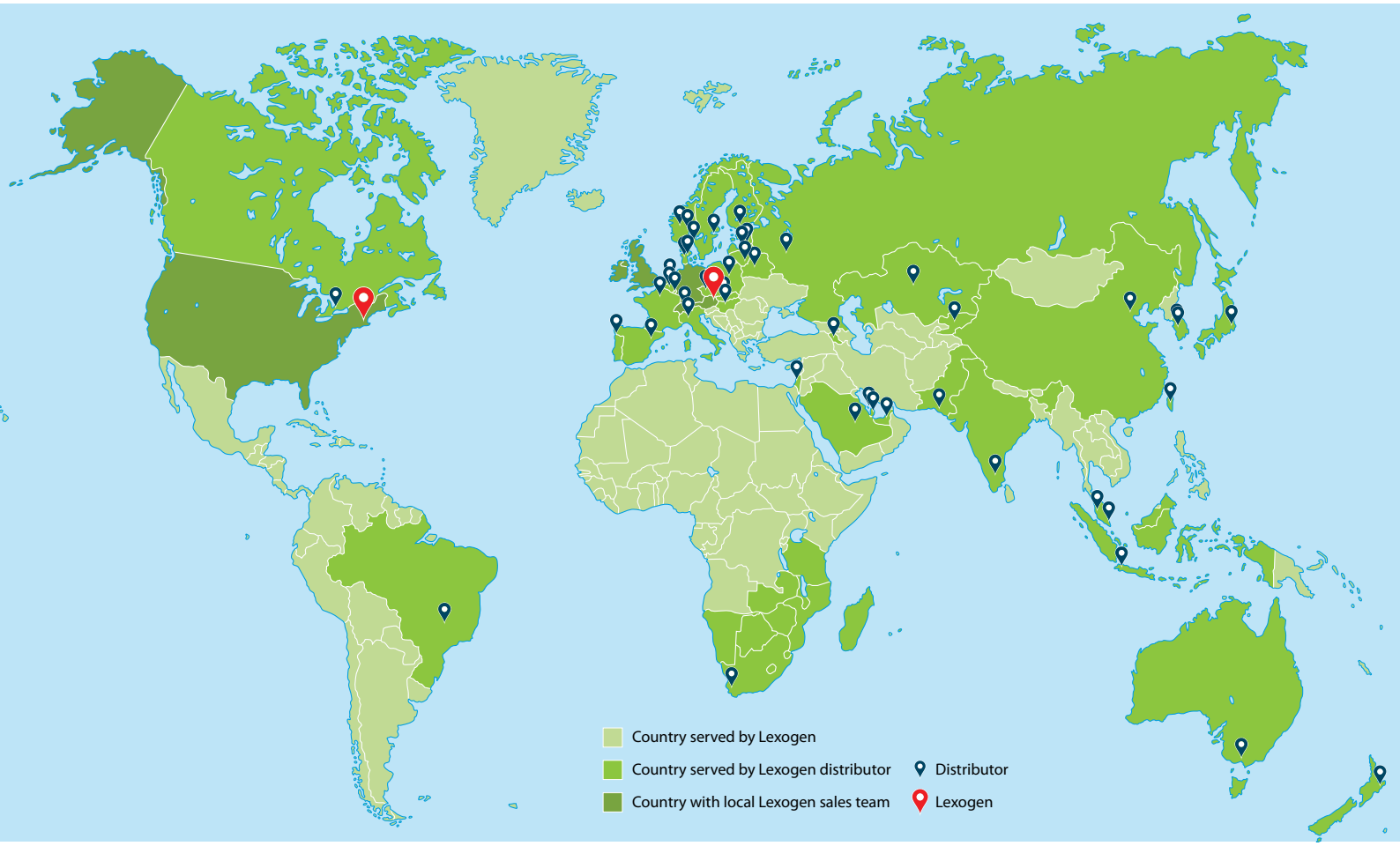
RNA-Seq data is analyzed in-house, and our standard analysis includes: demultiplexing, read quality control, trimming and filtering procedures, mapping, read counting, and reporting (differential) gene expression. Advanced services are available e.g., for: mapping of polyadenylation sites, evaluation of SLAMseq experiments, and handling non-standard genomes and annotations.

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 Services at its web page www.lexogen.com/services.

Worldwide Distribution and Partner Network

At Lexogen, we are committed to offering our RNA-Seq solutions on a global scale. To provide personal attention to every customer, we work with distribution partners worldwide. Our network of trained local distributors is available to deliver you proficient sales and technical support.
Check the list of countries below to locate a distributor in your area. If no distributor is listed for your region or if you need assistance, please contact us at sales@lexogen.com. The United States, the United Kingdom, Ireland, Germany, Austria, and Switzerland are served by Lexogen directly.



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Ordering Information for Kit Products

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Order via Distributor:

We are happy to have trained local distributors in more than 30 different countries. If your country has a local representative, orders have to be placed via the distributor. The complete list of our distributors can be found in the section Worldwide Distribution and Partner Network (p.38).

Information Needed:

When placing an order, please provide us with the following information:

- **Product Information:**
  - Name of the product.
  - Catalog number (see Product List (p.43)).
  - Amount.
- **Contact Information:**
  - Your name, phone number, and email address.
  - Shipping and billing address.
  - 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 in CA, MA, and NJ, please submit a Sales Tax Exemption certificate (if applicable) or state sales tax will be applied to the sale.

Ordering Information for Software

QuantSeq, CORALL, and SLAMdunk data analyses on the BlueBee® Platform can be purchased online via our webstore at www.lexogen.com/store. You will receive an email from Lexogen including an activation code to use at the BlueBee® platform lexogen.bluebee.com.

Please be aware that the SLAMdunk/BlueBee codes are currently not automatically generated. It might therefore take up to one working day for you to receive your valid code.

To purchase analysis units (AUs) for data analysis on the ROSALIND™ platform, please contact sales@lexogen.com.

Mix² RNA-Seq Data Analysis Software can be purchased online through our webstore. You will be redirected to an external partner website. After completing the payment, you will receive an email with a download link and licensing key for activating the software.

Ordering Information for Services

The RNA experts are ready to provide you with a fully integrated service for your RNA-Seq project. To contact us, please use the form at www.lexogen.com/services/#inquire or send an e-mail to services@lexogen.com, providing basic information about your project such as sample number, sample source, and requested service scope (RNA preparation, library prep, sequencing, data analysis).

Shipping Information

Depending on the product storage conditions, shipment is performed at -20 °C with cool packs, - 80 °C on dry ice, or at room temperature (storage at + 4 °C). Deliveries are made through UPS or FEDEX, unless required otherwise. You will receive a notification e-mail including the delivery date provided by the courier service company. An electronic invoice from Lexogen is sent to you, once your order leaves our warehouse. For US customers invoices are sent electronically if specified in a PO, otherwise they are mailed.

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- Northeast: +1-609-605-3102
- Southeast: +1-609-233-0426

Technical support:

- 877-539-6436 extension 700
- 603-498-1666 (Working hours: 9:00 – 18:00 (EST))
- 949-767-1631 (Working hours: 8:00 – 17:00 (PST))

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Technical support:

- +43 (0) 1 345 1212-41
- +43 (0) 660 946 4554

Working hours: 9:00 – 18:00 (CET)

Product List

Catalog №	Product Description	Kit Size
Expression Profiling Library Prep Kits		
3′ mRNA-Seq Library Prep Kits		
	Illumina™ compatible (including barcodes)	
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
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
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
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
Targeted RNA-Seq Library Prep Kits		
	Illumina™ compatible (including barcodes 7001-7096)	
033.24	QuantSeq-Flex Targeted RNA-Seq Library Prep Kit V2 with First Strand Synthesis Module for Illumina, 24 preps	24
033.96	QuantSeq-Flex Targeted RNA-Seq Library Prep Kit V2 with First Strand Synthesis Module for Illumina, 96 preps	96
034.24	QuantSeq-Flex Targeted RNA-Seq Library Prep Kit V2 with Second Strand Synthesis Module for Illumina, 24 preps	24
034.96	QuantSeq-Flex Targeted RNA-Seq Library Prep Kit V2 with Second Strand Synthesis Module for Illumina, 96 preps	96
035.24	QuantSeq-Flex Targeted RNA-Seq Library Prep Kit V2 with First and Second Strand Synthesis Modules for Illumina, 24 preps	24
035.96	QuantSeq-Flex Targeted RNA-Seq Library Prep Kit V2 with First and Second Strand Synthesis Modules for Illumina, 96 preps	96
Whole Transcriptome Library Prep Kits		
Total RNA-Seq Library Prep Kits		
	Illumina™ compatible (without rRNA Depletion Kit, 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
	Illumina™ compatible (with rRNA Depletion Kit, including barcodes 7001-7096)	
146.24	CORALL Total RNA-Seq Library Prep Kit with RiboCop (HMR), 24 preps	24
146.96	CORALL Total RNA-Seq Library Prep Kit with RiboCop (HMR), 96 preps	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

Catalog №	Product Description	Kit Size
mRNA-Seq Library Prep Kits		
	Illumina™ compatible (including UDIs)	
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
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	-
060.24	SLAMseq Explorer Kit - S4U Incorporation Module, 24 preps	-
061.24	SLAMseq Kinetics Kit - Anabolic Kinetics Module, 24 preps	-
062.24	SLAMseq Kinetics Kit - Catabolic Kinetics Module, 24 preps	-
Small RNA Profiling & Discovery		
	Illumina™ compatible (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
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

Catalog №	Product Description	Kit Size
051.01	SIRV-Set 3 (Iso Mix E0 / ERCC), 1 vial	1 vial
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 Extraction Kits		
008.48	SPLIT RNA Extraction Kit, 48 extractions	48
099.48	SPLIT RNA Extraction Kit for Blood, 48 extractions	48
138.400	SPLIT Rapid Viral RNA/DNA Extraction Kit, 400 extractions	400
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 V2, 8 preps	8
013.24	TeloPrime Full-Length cDNA Amplification Kit V2, 24 preps	24
Lexogen Indexing Solutions		
	UDI 12 nt Sets contain primer plates and are intended for use with QuantSeq-Pool and LUTHOR Library Prep Kits 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
	UDI 12 nt Unique Dual Indexing Add-on Kits contain primer plates and 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
i7 / i5 6 nt Indexing Add-on Kits		
044.96	Lexogen i7 6 nt Index Set (7001-7096), 1 rxn/Index	96
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), 1rx/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
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

Catalog №	Product Description	Kit Size
NGS Data Analysis Software		
063.02	SLAMdunk Data Analysis for SLAMseq Integrated on BlueBee® Platform, 0 - 2 GB	2 GB
063.06	SLAMdunk Data Analysis for SLAMseq Integrated on BlueBee® Platform, 0 - 6 GB	6 GB
063.12	SLAMdunk Data Analysis for SLAMseq Integrated on BlueBee® Platform, 0 - 12 GB	12 GB
063.25	SLAMdunk Data Analysis for SLAMseq Integrated on BlueBee® Platform, 0 - 25 GB	25 GB
090.24	QuantSeq Data Analysis (FWD/FWD-UMI) on BlueBee® Platform, 24 runs, 0 – 1.5 GB each	24
091.24	QuantSeq Data Analysis (REV) on BlueBee® Platform, 24 runs, 0 – 1.5 GB each	24
093.03	QuantSeq Data Analysis (FWD/FWD-UMI) on BlueBee® Platform, 1 run, 0 - 3 GB	3 GB
093.06	QuantSeq Data Analysis (FWD/FWD-UMI) on BlueBee® Platform, 1 run, 0 - 6 GB	6 GB
094.03	QuantSeq Data Analysis (REV) on BlueBee® Platform, 1 run, 0 - 3 GB	3 GB
094.06	QuantSeq Data Analysis (REV) on BlueBee® Platform, 1 run, 0 - 6 GB	6 GB
106.24	CORALL Data Analysis on BlueBee® Platform, 24 runs	24
023	Mix^2 RNA-Seq Data Analysis Software	
165.01	ROSALIND™ Analysis Unit	1 AU

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

Please find more information about our products at **www.lexogen.com** or contact us at info@lexogen.com.

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