LEXOGEN

Enabling complete transcriptome sequencing

SLAMseq: High-Throughput Metabolic Sequencing of RNA

- Analyze transcriptome-wide kinetics of RNA synthesis and turnover
- Measure nascent RNA expression and transcript stability
- Enhance the temporal resolution of differential expression
- No pull-down or biochemical isolation required
- Use in combination with QuantSeq 3′ mRNA-Seq for cost-effective, high-throughput metabolic sequencing

Introduction - Metabolic Sequencing of RNA

Metabolic RNA sequencing combines labeling of newly synthesized RNA transcripts with RNA-Seq readout. Existing approaches utilize nucleotide derivatives such as 4-Thiouridine and biochemical pull-down to separate nascent and existing RNA for library preparation[1]. These protocols are typically cost-, time-, and labor-intensive, require high amounts of RNA input, and often produce low signal quality[1].

SLAMseq Technology

Lexogen now offers a family of kits based on a new transcriptome-wide, quantitative, fast, and reliable method: SLAMseq (thiol (SH)-Linked Alkylation for the Metabolic Sequencing of RNA)[2]. SLAMseq uses 4-Thiouridine (S4U) to label nascent RNA in cultured cells (Fig. 1). The key feature of the workflow is an alkylation step, which uses iodoacetamide (IAA) to modify S4U nucleotides, leading to nucleotide conversion during reverse transcription. This results in thymine-to-cytosine (T>C) mutations in sequencing reads from S4U-labeled transcripts. Bioinformatic analysis of T>C-containing read counts can then be used to analyze nascent RNA levels.

SLAMseq Kits

Lexogen's SLAMseq kits provide a complete solution for metabolic RNA-Seq experiments, right through from optimizing S4U labeling conditions, to performing S4U labeling kinetics experiments, and subsequent RNA isolation and alkylation (Table 1).

Table 1 | Lexogen's SLAMseq kits.

Kit Type	Module	Application
SLAMseq Explorer Kit	Cell Viability Titration Module (Cat. No. 059.24)	• Assess S4U toxicity in target cell lines • Optimize S4U labeling concentrations
	S4U Incorporation Module (Cat. No. 060.24)	• Measure S4U uptake and incorporation rates using HPLC analysis
SLAMseq Kinetics Kit	Anabolic Kinetics Module (Cat. No. 061.24)	• Label newly transcribed RNA with S4U • Measure RNA synthesis kinetics • Analyze nascent RNA differential expression
	Catabolic Kinetics Module (Cat. No. 062.24)	• Label existing RNA with S4U • Assess transcript stability • Analyze RNA degradation kinetics

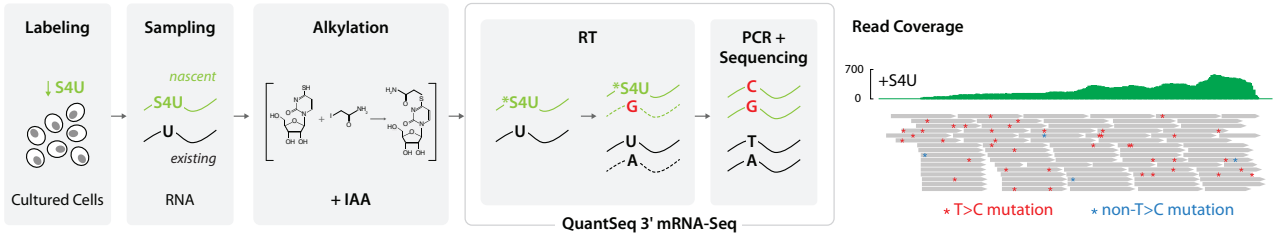

Figure 1 | The SLAMseq workflow. Cultured cells are treated with 4-Thiouridine (S4U) for labeling of nascent RNA (green). Total RNA is purified, and alkylation of the 4-thiol group is induced by the addition of iodoacetamide (IAA). During library preparation, shown here using the QuantSeq 3′ mRNA-Seq 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 reduced *S4U-modified nucleotide is encountered. In this way, nascent RNA can be distinguished from existing RNA by the presence of T>C mutations (red stars) during subsequent data analysis.

Ordering Information

Catalog Numbers:
059 (SLAMseq Explorer Kit - Cell Viability Titration Module)
060 (SLAMseq Explorer Kit - S4U Incorporation Module)
061 (SLAMseq Kinetics Kit - Anabolic Kinetics Module)
062 (SLAMseq Kinetics Kit - Catabolic Kinetics Module)
063 (SLAMdunk Data Analysis for SLAMseq Integrated on Bluebee Platform)

Find more about SLAMseq at www.lexogen.com
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SLAMseq Applications

Differential Expression Profiling of Nascent and Total RNA in Parallel

By defining nascent and total RNA levels for each sample, SLAMseq extends the depth of information provided by differential expression experiments. To demonstrate this, an anabolic kinetics experiment was conducted, using a 2-hour (120 minute) S4U labeling duration, comparing two treatment conditions to untreated controls at each timepoint (Table 2).

To assess the maximal effect of treatment, normalized log fold changes (LFC) were calculated for the highest treatment level (T2) versus control, and compared at distinct timepoints (Table 2, Fig. 2). At the start of the experiment (t0), nascent and total RNA LFC are similar. However, nascent RNA LFC were increased at t120 compared to total RNA. The general downregulation of nascent RNA levels in T2 versus control indicates an inhibitory effect on transcriptional output in response to the treatment. Normalized LFC for T2 versus control comparisons for total RNA however, showed only a modest increase over the same duration (Fig. 2, black).

Time (min)	Control	T1	T2
0	1	2	3
30	4	5	6
60	7	8	9
120	10	11	12

Table 2 | SLAMseq kinetics experiment for differential expression. Increasing strength treatment series (T1, T2) and control (no treatment) with S4U labeling (12 sample groups, numbered 1-12).

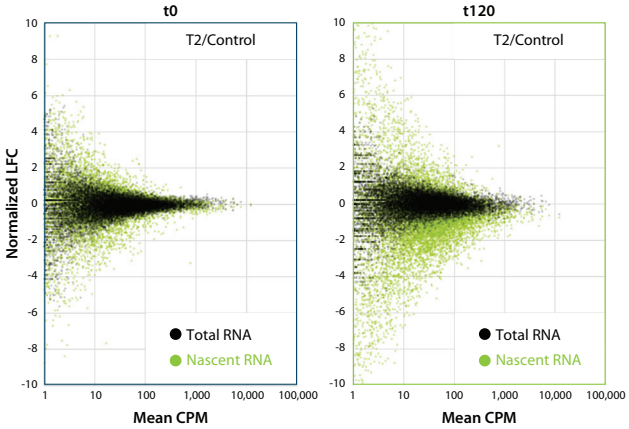

Figure 2 | Normalized LFC plots comparing treatment and control samples from a SLAMseq anabolic kinetics experiment. HeLa cells were grown in S4U-containing medium and exposed to a treatment series (T1 and T2) for 120 minutes. RNA was sampled according to Table 2. Following alkylation, QuantSeq FWD libraries were prepared from SLAMseq total RNA and sequenced, yielding 5-10 M reads per sample. Data was analyzed using the SLAMdunk Data Analysis Pipeline. Read counts were normalized using the formula: (CPM+0.1) x (CR_{gene} - $CR_{global\text{-}t0}$). This method corrects for zero values (CPM+0.1) and accounts for T>C conversion rates (CR = No. T>C conversions / total No. T nucleotides), by subtracting the average CR for all genes at t0 ($CR_{global\text{-}t0}$) from the per-gene CR (CR_{gene}).

Analyze the Kinetics of mRNA Synthesis and Turnover Rates

SLAMseq can specifically measure the RNA synthesis and degradation kinetics of individual transcripts (Fig. 3). Similarly, transcript synthesis, stability, and decay can be globally assessed, providing novel insights into the control of gene expression at transcriptional and post-transcriptional levels.

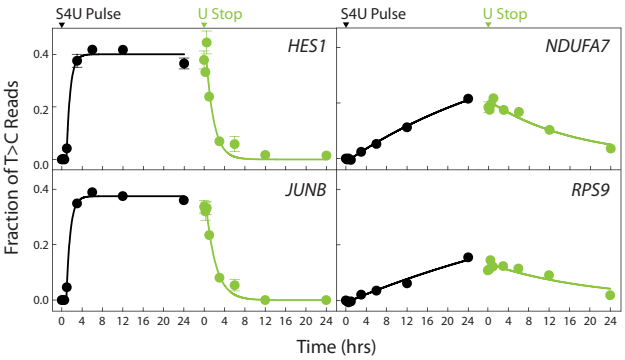

Figure 3 | S4U labeling kinetics experiments reveal individual RNA synthesis and degradation rates. Cells were first treated with S4U for 24 hours to measure RNA synthesis. RNA degradation rates were then measured over the next 24 hours after replacing S4U with unlabeled uridine (U Stop). mRNAs that encode for regulatory proteins such as the transcription factors *HES1* and *JUNB* usually have high synthesis and turnover rates, as shown. In comparison, housekeeping genes such as *NDUFA7* and *RSP9* have slower transcription and degradation rates. Reproduced from *Herzog et al.*, (2017) [2].

Combine SLAMseq with QuantSeq for Complete High-Throughput Metabolic RNA-Seq

Total RNA from SLAMseq experiments can be used as direct input for library preparation with Lexogen's QuantSeq 3' mRNA-Seq Library Prep Kits (Cat. Nos. 012, 015, and 016). QuantSeq generates stranded libraries that require 10x less reads than standard RNA-Seq. This enables samples from more complex experiments to be multiplexed together in a single sequencing lane or run. Therefore, both technical and biological replicates for treatment versus control can be included in kinetics experiments.

SLAMdunk - The SLAMseq Data Analysis Pipeline

Lexogen offers access to SLAMdunk [2], a customised pipeline for analyzing SLAMseq RNA-Seq data from libraries prepared with QuantSeq 3' mRNA-Seq Library Prep Kits (Fig. 4). SLAMdunk is now available on the Bluebee genomics platform. Together, SLAMseq, QuantSeq, and SLAMdunk provide a complete, user-friendly solution for high-throughput metabolic RNA-Seq experiments.

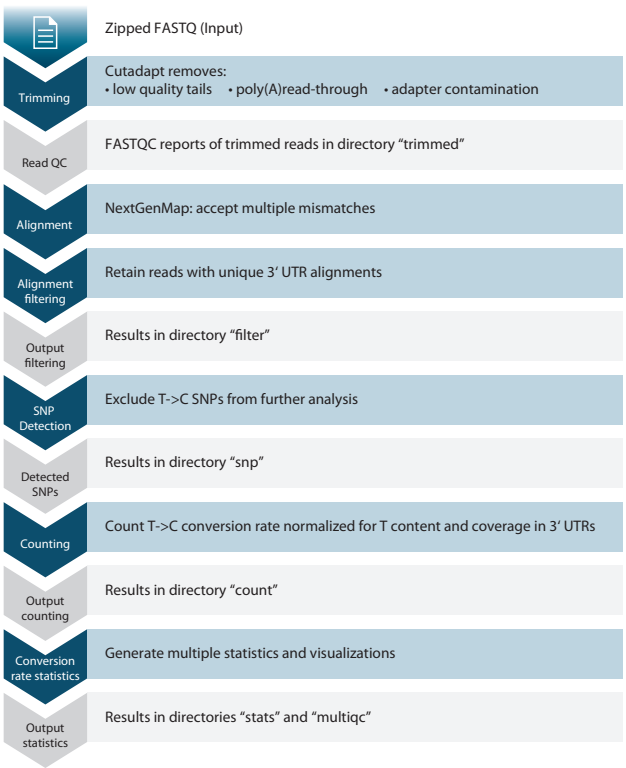

Figure 4 | The SLAMdunk Data Analysis Pipeline.

References:

[1] Neymotin B., et al. (2014). Determination of *in vivo* RNA kinetics using RATE-seq. *RNA* 20: 1645-1652.

[2] Herzog V., et al. (2017).Thiol-linked alkylation of RNA to assess expression dynamics. *Nature Methods*, doi: 10.1038/nmeth.4435.