

SLAMseq: THE Method for High-throughput Kinetic RNA Sequencing

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Introduction

Lexogen's SLAMseq Kits for high-throughput kinetic RNA sequencing enable transcriptome-wide analysis of RNA synthesis and decay by measuring nascent RNA expression and turnover. Newly synthesized transcripts are labeled with the ribonucleoside analog 4-thiouridine (S4U) to measure and monitor anabolic or catabolic processes. SLAMseq is compatible with whole transcriptome sequencing, small RNA sequencing, single-cell RNA-Seq, and also 3' mRNA-Seq for cost-efficient high-throughput screening setups, including drug discovery workflows.

Workflow

Metabolic RNA labeling with SLAMseq is based on accurate nucleotide conversion induced during library preparation. To capture anabolic processes, cells are treated with a drug prior to labeling; for catabolic processes, cells are first labeled until saturation, then treated with a drug, before a uridine chase is performed.

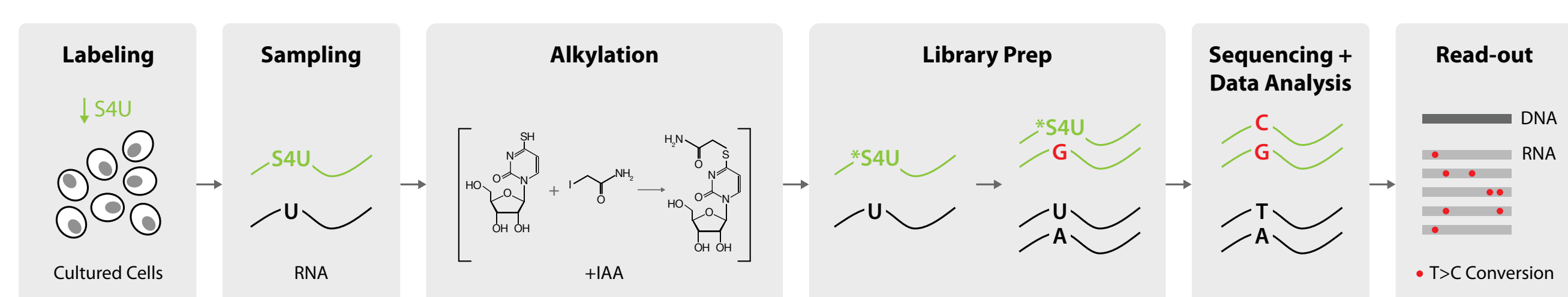


Figure 1 | The SLAMseq workflow. Cultured cells are treated with 4-thiouridine (S4U) for labeling of nascent RNA (green). Total RNA is purified, and alkylation of the 4-thiol group is induced by the addition of iodoacetamide (IAA). During the library preparation, the presence of the resulting carboxyamidomethyl-group 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) during subsequent data analysis.

Performance

Enhanced Sensitivity of Differential Expression Analysis

SLAMseq enables expression profiling of total RNA and nascent RNA from the same sample. To illustrate the difference between standard, steady-state RNA-Seq and a SLAMseq-based, metabolic RNA-Seq analysis, differential gene expression was analyzed in a human cell line. SLAMseq significantly enhanced the sensitivity of differential gene expression detection and quantification (Muhar *et al.*, 2018; Fig. 2).

Analyzing nascent mRNA levels using SLAMseq revealed transcriptional responses to inhibitor treatment that cannot be resolved by standard RNA-Seq. Thus, SLAMseq can reveal the underlying mechanisms of cellular responses making it ideally suited for drug discovery studies.

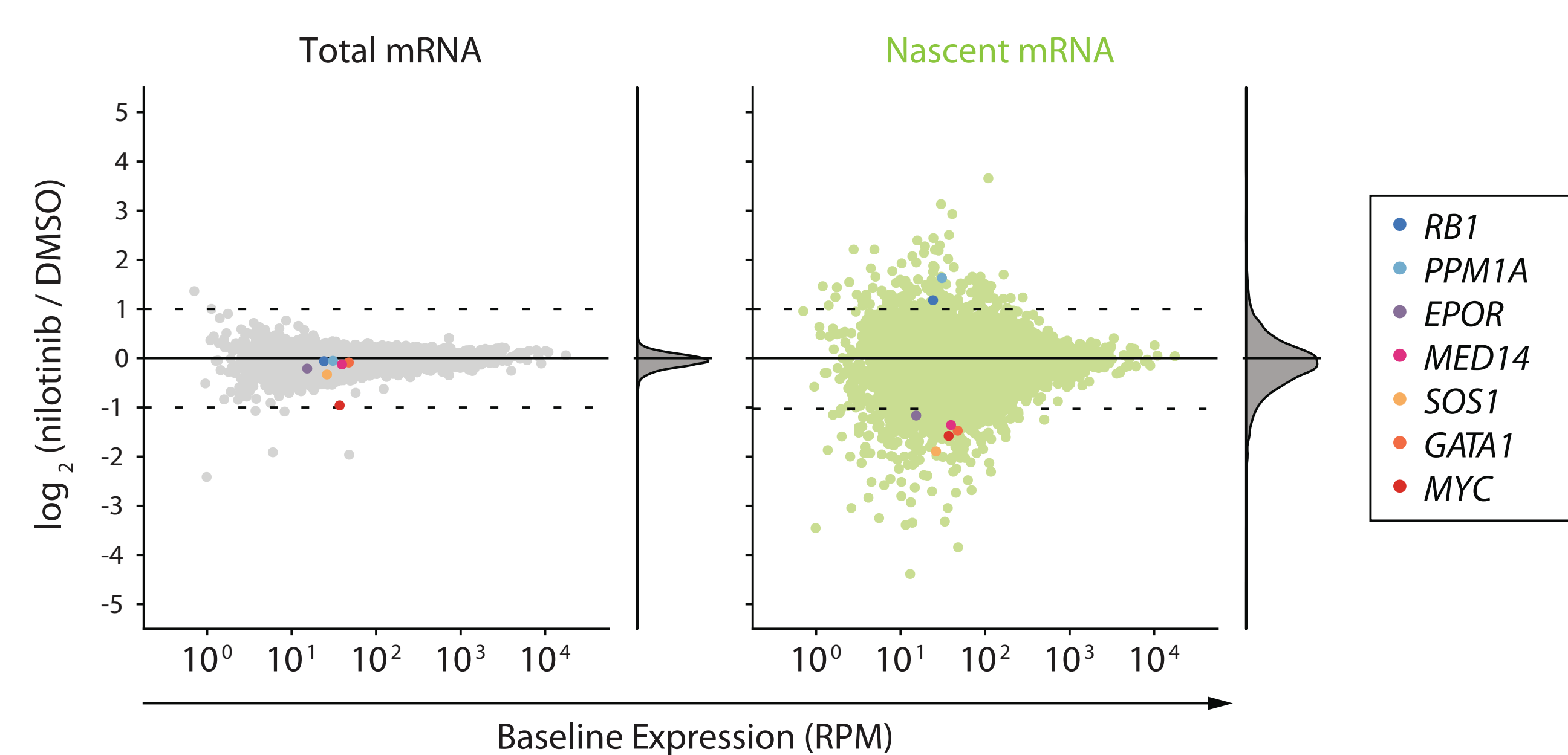


Figure 2 | SLAMseq enhances differential expression detection. More differentially-expressed genes are detected when analyzing nascent mRNA (green) vs. total mRNA levels (grey). Figure modified from Muhar *et al.*, 2018.

Identify Direct Transcriptional Targets of Any Gene

The catabolic kinetics SLAMseq experiment uses a longer initial S4U labeling duration to enable RNA metabolism to reach an approximate steady-state level. The exchange of S4U for unlabeled uridine in cell media stops the labeling at t0 and samples are collected over time. Existing RNA is therefore labeled with S4U, while nascent RNA synthesized after the uridine chase is unlabeled.

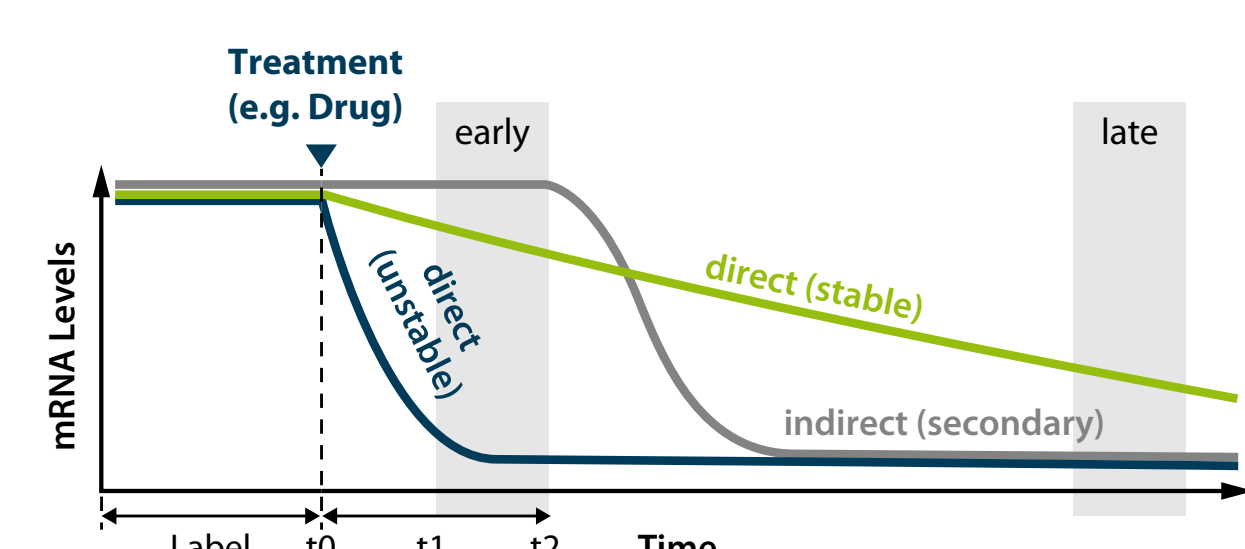


Figure 3 | SLAMseq identifies direct targets of genes. S4U labeling followed by drug treatment and early-stage sampling (time points: t1 – t2) measures mRNA stability changes to map sequential drug responses.

Combining SLAMseq with protein modulators, or drug treatments distinguishes direct (primary) and indirect (secondary) target responses (Fig. 3). SLAMseq enables dissecting signaling pathways underlying biological processes and characterize drug-target responses on the transcriptional level (Muhar, M *et al.*, 2018).

Analyze the Kinetics of mRNA Synthesis and Turnover Rates

SLAMseq can specifically measure the RNA synthesis and degradation kinetics of individual transcripts (Fig. 4). The nature of a transcript and its molecular regulation dictate the speed of synthesis and degradation. Housekeeping genes are naturally slowly synthesized and better protected against degradation (see *NDUFA7* and *RPS9*), compared to transcription factors (*HES1* and *JUNB*) which show high synthesis and turnover rates.

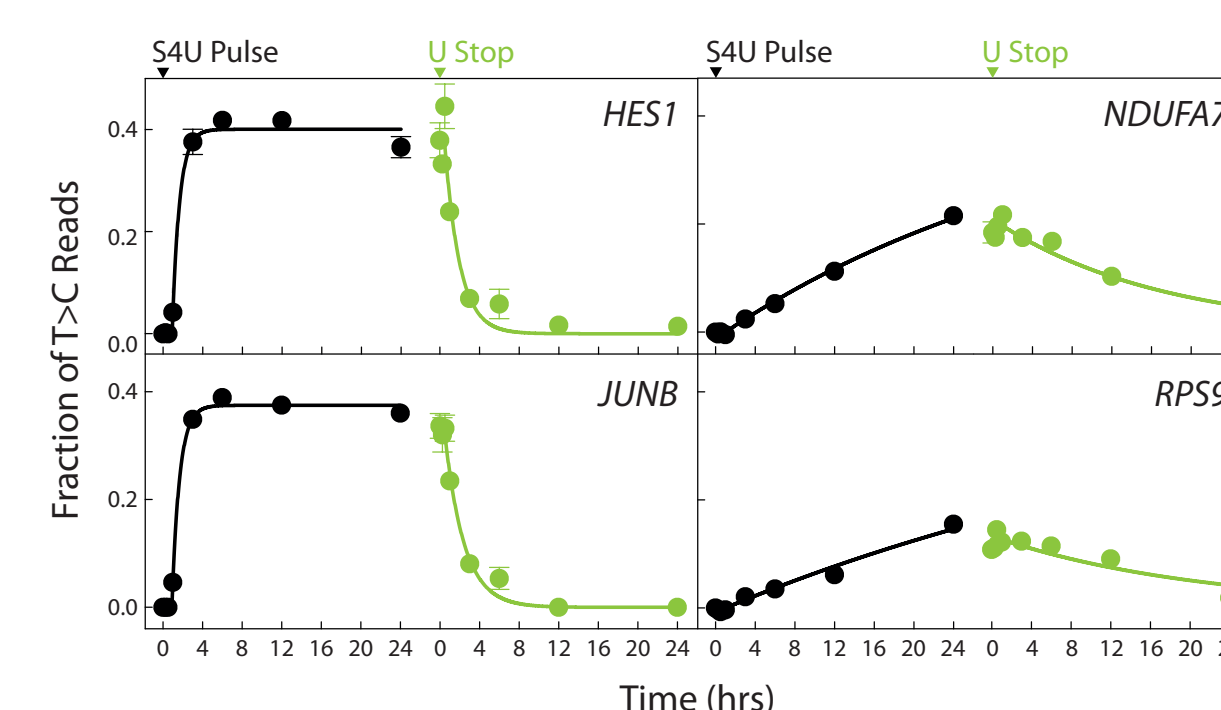


Figure 4 | S4U labeling kinetics experiments reveal individual RNA synthesis and degradation rates. Cells were first treated with S4U for 24 hours to measure RNA synthesis. RNA degradation rates were then measured over the next 24 hours after replacing S4U with unlabeled uridine. Reproduced from Herzog *et al.*, (2017).

Applications

Gene regulation Thiol-linked alkylation of RNA to assess expression dynamics, Herzog, 2017	Cell-signaling pathways Global SLAMseq for accurate mRNA decay determination and identification of NMD targets, Alalam 2022	Drug effects and synergies SLAM-seq defines direct gene-regulatory functions of the BRD4-MYC axis, Muhar, 2018	Small RNA silencing processes Small RNAs are trafficked from the epididymis to developing mammalian sperm, Sharma, 2018
RNA kinetics in transgenic animals SLAM-ITseq: sequencing cell type-specific transcriptomes without cell sorting, Matsushima, 2018	Microbe & mycoplasma contamination High-Throughput Sequencing (HTS) of newly synthesized RNAs enables one shot detection and identification of live mycoplasmas and differentiation from inert nucleic acids, Desbrousses, 2020	Adventitious virus testing Adventitious virus detection in cells by high-throughput sequencing of newly synthesized RNAs: unambiguous differentiation of cell infection from carryover, Cheval, 2019	Monitoring of RNA transfer SLAMseq reveals transfer of RNA from liver to kidney in the mouse, Hunter, 2024

Working with Lexogen SLAMseq kits and solutions

End-to-end Solution for Your High-throughput Metabolic Sequencing

Lexogen covers the entire SLAMseq kinetic RNA-Seq workflow, from optimization of metabolic RNA labeling to data analysis, providing comprehensive solutions for each experimental phase. Individual SLAMseq modules are available and can be combined with QuantSeq 3'mRNA-Seq for cost-effective, high-throughput metabolic sequencing, or other RNA-Seq library preps. Additionally, you can also have your SLAMseq samples processed at our Services facility.

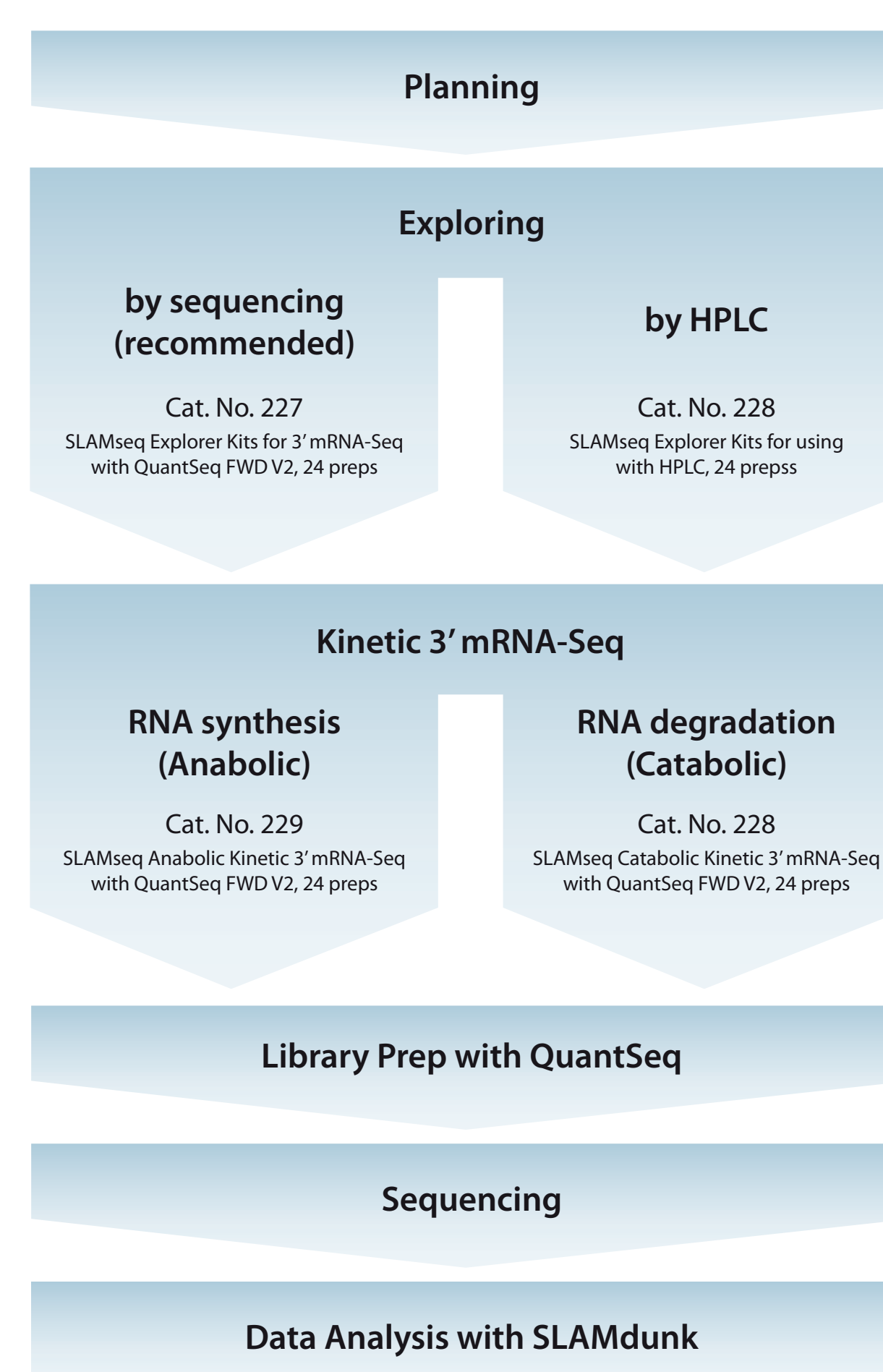


Figure 5 | Lexogen SLAMseq workflow for cost-effective, high-throughput metabolic sequencing with QuantSeq 3' mRNA-Seq.

Licensed Technology

Be fully compliant when working with our SLAMseq kits and profit from production-grade quality for reliable, robust, and reproducible results.

Technical Support

Benefit from our experienced technical support. Our team is here to guide you through experimental design, assist with data analysis, and resolve any troubleshooting challenges you may face.

References:
Herzog V., *et al.*, (2017). Thiol-linked alkylation of RNA to assess expression dynamics. *Nature Methods*, doi: 10.1038/nmeth.4435.
Muhar, M., *et al.*, (2018). SLAM-seq defines direct gene-regulatory functions of the BRD4-MYC axis. *Science*, DOI: 10.1126/science.aaa2793.

