LEXOGEN

Enabling complete transcriptome sequencing

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NEW

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LUTHOR 3' mRNA-Seq Library Prep Kit

- First comprehensive single-cell 3' mRNA-Seq library prep
- Proprietary THOR Technology directly amplifies original RNA molecules
- Unprecedented sensitivity for ultra-low input RNA and single cells
- Empower analysis of challenging samples

Introduction

Cell-to-cell variability is universal among multi-cellular organisms. Sequencing of individual cells allows assessing heterogeneity in gene expression even between cells of similar type or origin, and provides a basis for identification of cell populations, classes, and subtypes. Conventional library preps convert only ~10 - 20 % of all transcripts. Thus, current single-cell approaches rely on sequencing of thousands of cells at shallow read depth to compensate for the low conversion efficiencies. Cells are then clustered using only highly abundant marker transcripts.

Lexogen's LUTHOR 3' mRNA-Seq Library Prep Kit enables RNA-Seq from individual cells or ultra-low input RNA with unprecedented sensitivity using the proprietary THOR (T7 High-resolution Original RNA) Amplification Technology.

THOR Amplification and LUTHOR 3' mRNA-Seq

The robust THOR Amplification Technology generates RNA copies directly from the endogenous mRNA template in a linear process (Fig. 1). The original mRNA is fused to a T7 promoter required for amplification. Then, RNA-templated *in vitro* transcription generates antisense RNA copies void of the promoter sequence. Only the original mRNA molecule serves as template and is amplified repeatedly.

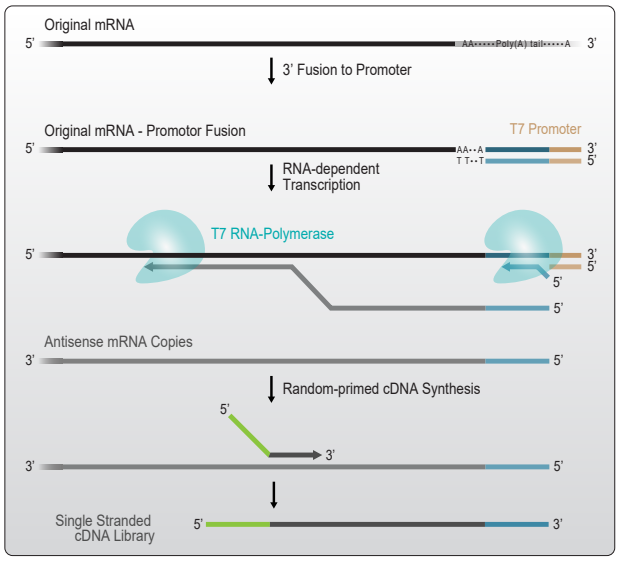

Figure 1 | THOR Amplification and RNA-Seq library template generation.

Thereby, the RNA concentration is increased for library preparation. Libraries are generated in a highly strand-specific conversion step (Fig. 1). The complete LUTHOR workflow is free from ligation, template switch, and fragmentation and thus also supports challenging samples.

The resulting single-stranded cDNA is amplified by PCR to introduce Illumina®-compatible full-length adapters for cluster generation and Unique Dual Indices (UDIs) at the i5 and i7 position. Lexogen offers pre-mixed 12 nt Unique Dual Indexing Sets (Cat. No. 101 - 105, 156).

Unparalleled Sensitivity

LUTHOR 3' mRNA-Seq offers unprecedented sensitivity for ultra-low input Universal Human Reference RNA (UHRR) and single cells (Tab. 1).

Table 1 | LUTHOR provides unprecedented sensitivity.

Method	Input	Detected Genes*
LUTHOR 3' mRNA-Seq	1 cell (HEK293T)	~11,000 - 15,000
SMART-Seq v4 3' DE	1 cell (K562)	~ 6,000 - 8,000[1]
SMART-Seq Single Cell (WTS)	1 cell (GM22601)	~9,000 - 10,500[1]
CEL-Seq 2 (C1 HT) (WTS)**	1 cell (mES)	~7,000[2]
SMART-Seq 2 (WTS)**	1 cell (mES)	~8,000 - 9,000[2]
SMART-Seq 3 (WTS)**	1 cell (HEK293FT)	~11,000 - 12,000[3]

*Obtained from manufacturers specifications[1] and from published benchmarking studies[2, 3] at 1 M reads / sample, threshold of >1 Counts Per Million (CPM). For more details and references[1–3], visit www.lexogen.com. Methods are shown for 3'-Seq and whole transcriptome sequencing (WTS). **Academic protocol.

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. 2 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. 2 B).

LUTHOR

Explore further

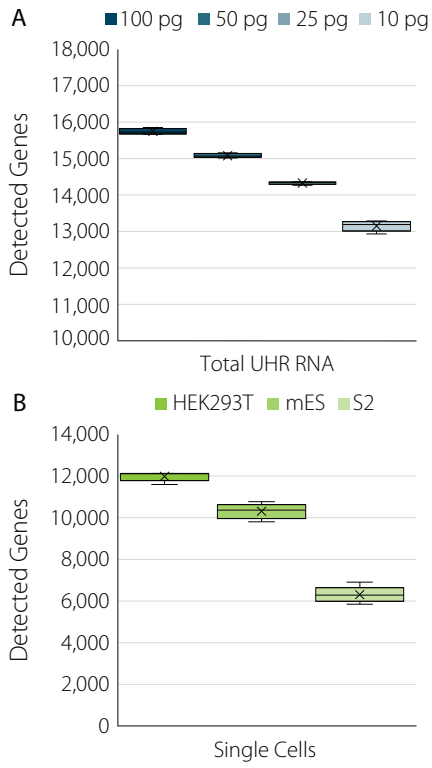

Figure 2 | LUTHOR 3' mRNA-Seq enables highly sensitive and consistent gene detection already at low sequencing depth. A) LUTHOR libraries were generated from ultra-low input UHRR (4 replicates) or **B)** from frozen single HEK293T cells, single mES cells, and single S2 cells (8 replicates per cell type). Libraries were sequenced, and detected genes were analyzed for 1 M reads / sample. The number of detected genes was counted at a threshold of >1 Counts Per Million (CPM).

High Quality Performance for Ultra-low Input and Single Cells

LUTHOR 3' mRNA-Seq reliably represents endogenous mRNA composition and efficiently excludes ribosomal rRNAs focusing sequencing reads on coding sequences (Fig. 3). Therefore, LUTHOR is the optimal choice for gene expression analysis for ultra-low input samples down to single-cell level.

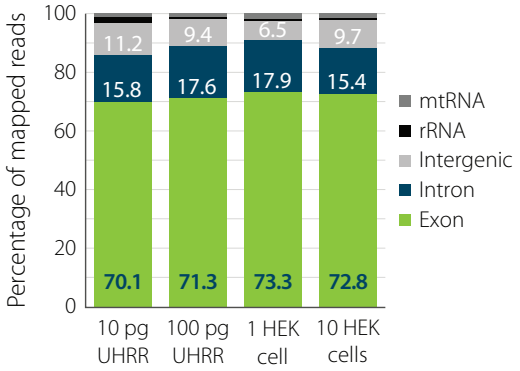

Figure 3 | Feature distribution of uniquely mapped reads for LUTHOR 3' mRNA-Seq. The majority of LUTHOR reads generated from 10 and 100 pg UHRR, or 1 and 10 single HEK293T cells map to exonic sequences.

Excellent Cell-to-Cell 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 even for individual mES cells after freezing (Fig. 4).

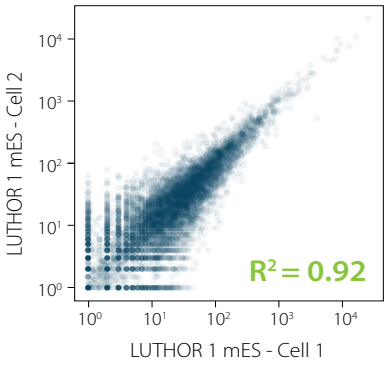

Figure 4 | Excellent cell-to-cell reproducibility for LUTHOR 3' mRNA-Seq. Correlation plot of gene counts for two individual mES cells at 1 M reads / sample. Cells were frozen prior to processing with LUTHOR 3' mRNA-Seq.

Summary

LUTHOR 3' mRNA-Seq uses Lexogen's proprietary THOR Amplification Technology to enable true 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. This cannot be achieved by conventional single-cell RNA-Seq which requires extremely large numbers of cells and only offers resolution enough for detection of the highest abundant genes.

Benefits

- **Accurate:** The novel THOR Amplification Technology directly amplifies only the original RNA molecules.
- **Sensitive:** Efficient RNA amplification and library preparation offer the most comprehensive gene expression profiles from single cells. Detect ~13,000 genes (per 1 M reads) from ~10 pg purified RNA input or up to ~15,000 genes (per 1 M reads) from one singularized cell.
- **Cost-efficient:** No need to analyze thousands of single cells to obtain reliable data. Use individual FACS-sorted cells as direct input.
- **Empower challenging samples:** The template switch-, ligation-, and fragmentation-free protocol supports ultra-low input, degradation-prone cells, and even degraded samples.
- **Simple data analysis:** Save time and computing capacity by simply counting mapped reads to calculate gene expression.

Ordering Information

Catalog Number:
143 (LUTHOR 3' mRNA-Seq Library Prep Kit for Illumina)
Associated Products:
101 - 104, 156, or 105 (Lexogen UDI 12 nt Sets A1 - A4, or B1)

For more information and additional resources on LUTHOR visit our website.

Find more about LUTHOR at www.lexogen.com.
Contact us at info@lexogen.com, +43 1 345 1212-41, or find your local contact at www.lexogen.com/distributors.