LEXOGEN

Enabling complete transcriptome sequencing

139PF280V0100

NEW

QuantSeq-Pool Sample-Barcoded 3′ mRNA-Seq Library Prep

- Cost-efficient gene expression analysis method for screening projects
- Early pooling and batch processing save time effortlessly
- Easily scalable from a few to 36,864 samples

Introduction

To date, unbiased gene expression profiling is the most commonly used application for RNA-Seq in biomedical research and diagnostics. Conventional gene expression projects use mRNA sequencing technologies that offer comprehensive single-nucleotide resolution across the full-length transcript but are limited in the number of samples that can be processed and multiplexed cost-efficiently and economically. Although price per base is continuously decreasing, sample preparation, sequencing, and data processing are still major cost factors that limit high-throughput screenings.

3′ mRNA-Seq is a Powerful Tool for Gene Expression Profiling

3′ mRNA-Seq methods generate Next-Generation Sequencing (NGS) libraries close to the 3′ end of polyadenylated RNAs without the need of prior poly(A) enrichment (Tab. 1).

Table 1 | Comparison of conventional mRNA-Seq and QuantSeq 3′ mRNA-Seq solutions for gene expression profiling.

	Conventional mRNA-Seq	3′ mRNA-Seq
Samples	High quality required	Low quality tolerated
Time*	7 - 11 hours	< 5 hours
Coverage	Multiple fragments, complete transcript coverage	One fragment per transcript close to the poly(A)-tail
Multiplexing	Low to medium	Low to massive
Data Analysis	Complex, multi-step analysis	Simple and accurate analysis using counting
Cost	++++	+

*overall library preparation time, excluding QC

Since 3′ mRNA-Seq generates only one fragment per transcript, the number of reads mapped to a given gene is directly proportional to its expression. 3′ mRNA-Seq does not rely on correct isoform annotation and identification for determining unambiguous gene expression values. Furthermore, it is less sensitive to variations in RNA quality. Conventional mRNA-Seq on the other hand requires high-quality RNA for mRNA enrichment and is often biased towards longer transcripts as these generate more fragments. Thus, 3′ mRNA-Seq offers a reliable and affordable solution for gene expression profiling regardless of transcript length[1].

QuantSeq-Pool Accelerates Digital Gene Expression Projects

Lexogen's QuantSeq-Pool combines early sample barcoding with all other benefits of 3′ mRNA-Seq (Fig. 1). Thereby, QuantSeq-Pool tremendously increases multiplexing capacities, and the highly streamlined protocol reduces hands on time as well as consumables and sequencing expenses.

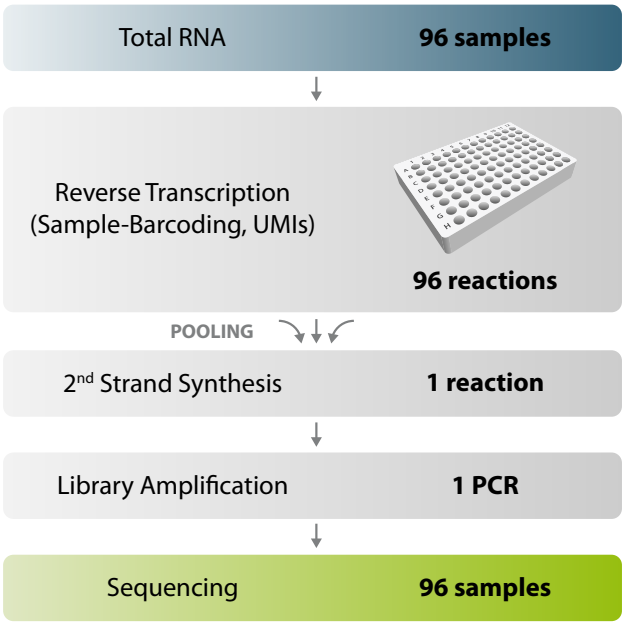

Figure 1 | Early pooling of sample-barcoded libraries allows batch processing of 96 samples in one reaction.

During the initial library generation step, sample barcodes are introduced enabling subsequent pooling of the individually labeled samples. In addition, Unique Molecular Identifiers (UMIs) are incorporated at this step and enable elimination of PCR duplicates. Library generation is completed within 4.5 hours by combining up to 96 samples into one reaction. Batch processing significantly shortens the complete RNA-to-Sequencing workflow by reducing handling time and eliminating the need for quantification, quality control, and equimolar pooling of individual libraries (Fig. 2).

Scalable Multiplexing for Massive-Throughput Projects

During the library amplification step additional Unique Dual Indices (UDIs) can be introduced at the i5 and i7 positions of QuantSeq-Pool libraries for higher multiplexing capacity.

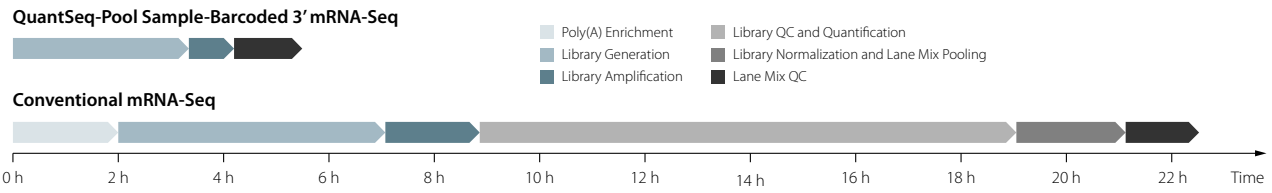

Figure 2 | QuantSeq-Pool enables completion of the RNA-to-sequencing workflow for 96 samples in ~5.5 hours. Sample barcoding in the initial step of the QuantSeq-Pool protocol enables convenient pooling of up to 96 samples and batch processing for subsequent library generation and amplification steps in a single reaction. Following PCR, the library pool containing all 96 samples is purified thereby eliminating the need for quality control (QC), quantification, and equimolar pooling of individual libraries to generate the final lane mix for sequencing.

Multiplexing of QuantSeq-Pool is highly scalable and reaches 36,864 individually barcoded samples, if all 96 i1 sample-barcodes are combined with the largest available set of 384 UDIs (Tab. 2). Thus, triple-indexed QuantSeq-Pool libraries (containing i1 sample-barcodes and 12 nt UDIs) are highly suitable for large-scale screening projects and massive multiplexing on Illumina® NovaSeq™ instruments. The efficiency of the early-pooling workflow enables manual handling of high-throughput projects without the need to invest in specialized automated handlers. If desired, the automation-friendly protocol can be adapted for processing of thousands of samples.

Table 2 | QuantSeq-Pool is scalable up to 36,864 samples.

Number of pools	Samples per pool	Number of samples per sequencing lane
1	96	96
96 *	96	9,216
384 *	96	36,864

*the same number of external indices, e.g. UDIs is required for multiplexing in one sequencing run

Robust Gene Detection Even for Shallow Sequencing

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 (Fig. 3).

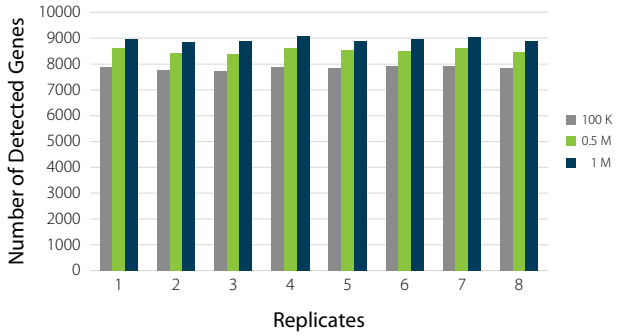

Figure 3 | QuantSeq-Pool enables robust and consistent gene detection already at low sequencing depth. QuantSeq-Pool libraries were generated from 8 x 10 ng UHRR, sequenced, and gene detection was analyzed for 100 K, 0.5 M and 1 M reads / sample. Number of detected genes was counted at a threshold of > 10 Counts Per Million (CPM).

Reference

[1] Ma, F., Fuqua, B.K., Hasin, Y. et al. A comparison between whole transcript and 3′ RNA sequencing methods using Kapa and Lexogen library preparation methods BMC Genomics, DOI: 10.1186/s12864-018-5393-3.

This high sensitivity is consistent across eight replicates, owed to early pooling and reduction of technical variation through batch processing.

Thus, more replicates can be included easily to increase confidence in differential gene expression while maintaining cost-efficiency.

Summary

QuantSeq-Pool combines sample barcoding and early pooling with the benefits of 3′ mRNA-Seq and is the gene expression profiling method of choice saving time, sequencing requirements, and data analysis efforts. This translates into up to 10 times lower overall cost compared to conventional mRNA-Seq projects. The input can routinely be as low as 10 ng total RNA, and built-in UMIs allow elimination of PCR duplicates for high confidence, robust gene expression analysis. The convenient-protocol enables manual high-throughput processing and is also automation-friendly for projects requiring massive multiplexing.

Benefits

- **Fast:** Accelerate your workflow by early pooling and batch processing of up to 96 samples in one reaction.
- **Scalable throughput:** Process large-scale projects without the need for expensive automation equipment. Massive multiplexing of up to 36,864 samples is supported by an automation-friendly protocol.
- **Cost efficient:** Save up to 10 times sequencing and consumable cost.
- **Reproducible:** Reduce technical variability and consistently process large numbers of samples in parallel.
- **Accurate:** Eliminate PCR duplicates with built-in UMIs for high-precision expression profiling.
- **Simple data analysis:** Save time and computing capacity by simply counting mapped reads to calculate gene expression.

Ordering Information

Catalog Number:
139 (QuantSeq-Pool Sample-Barcoded 3′ mRNA-Seq Library Prep)
Associated Product:
105 (Lexogen UDI 12 nt Set B1 (UDI12B_0001-0096))

For more information and additional resources on QuantSeq-Pool visit our website.

Find more about QuantSeq-Pool 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.