LEXOGEN

Enabling complete transcriptome sequencing

QuantSeq 3′mRNA-Seq Library Prep Kit for Illumina

- ideal for gene expression analysis and eQTL studies
- exact 3′UTR tagging
- 4.5 hours from total RNA to ready-to-sequence libraries
- cost-effective sequencing of up to 96 samples per lane
- strand-specificity of >99.9 %

Introduction

QuantSeq 3′mRNA-Seq Library Prep Kit generates Illumina-compatible libraries of the sequences close to the 3′end of the polyadenylated RNA. Only one fragment per transcript is generated and therefore no length normalization of reads is needed. This results in extremely accurate gene expression values.

QuantSeq is available in two versions: using the first kit (Cat. No.: 015.96) NGS reads are generated towards the oligodT, and to pinpoint the exact 3′ end paired-end sequencing may be required. This version of QuantSeq results in reads directly reflecting the mRNA sequence. With the second version of the kit (Cat.No.:016.96) it is possible to exactly pinpoint the 3′ end during Read 1. The reads generated here during Read 1 reflect the cDNA sequence. In order to achieve cluster calling on the Illumina platform a T-fill reaction is required first for this version of the kit.

QuantSeq maintains exceptional strand-specificity of more than 99.9 % and allows to map reads to their corresponding strand on the genome, enabling the discovery and quantification of antisense transcripts and overlapping genes.

Workflow

QuantSeq has a short and simple workflow and can be completed within 4.5 hours (Figure). The required hands-on time is less than 2 hours. The kit can be used for down to 50 ng of input total RNA. No prior poly(A) enrichment or rRNA depletion is required.

Library generation is initiated by oligodT priming. The primer already contains Illumina-compatible linker sequences. After the first strand synthesis the RNA is removed and second strand synthesis is initiated by random priming and a DNA polymerase. The random primer also contains Illumina-compatible linker sequences. No purification is required between first and second strand synthesis. The insert size is optimized for shorter reads (SR50, PE50, SR100, PE100). Second strand synthesis is followed by a magnetic bead-based purification step rendering the protocol compatible with automation. The library is then amplified, introducing the sequences required for cluster generation.

Optional multiplexing of libraries can be carried out using up to 96 external barcodes. Libraries are compatible with both single-end and paired-end sequencing reagents.

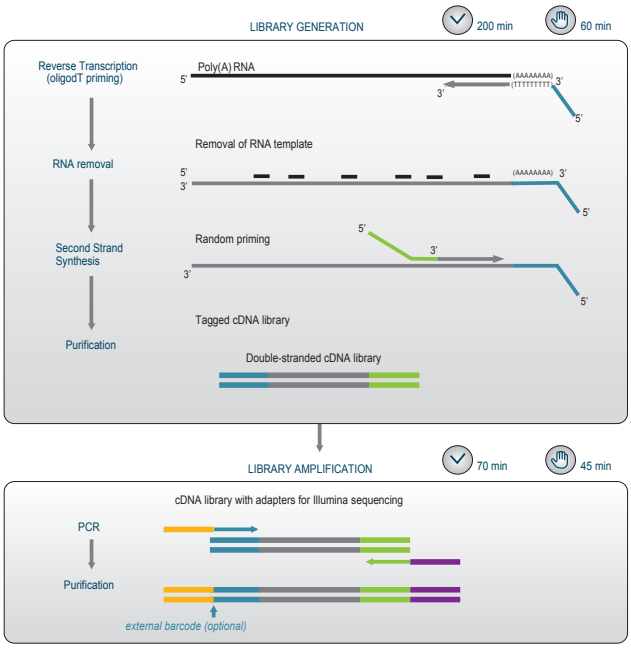

Figure. Schematic overview of the QuantSeq library preparation workflow.

Find more about QuantSeq at www.lexogen.com.
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QUANT SEQ™

Simply counting 3′ends