LEXOGEN

Enabling complete transcriptome sequencing

QuantSeq-Flex Targeted RNA-Seq Library Prep Kit

- Flexible kit suited for targeted sequencing and molecular barcoding
- Enables identification of known and unknown fusion transcripts
- 4.5 hours from total RNA to ready-to-sequence libraries
- Cost-effective sequencing of up to 96 samples per lane

Introduction

The QuantSeq-Flex Targeted RNA-Seq Library Prep Kit uses the QuantSeq Forward (FWD, Cat. No. 015) reagents where Read 1 linker sequence is introduced by the second strand synthesis primer. Flexible modules for the First Strand and/or Second Strand Synthesis (SSS) allow the use of custom primers and render the kit suitable for targeted sequencing. The kit provides a library preparation protocol designed to generate Illumina compatible libraries from total RNA. With this highly flexible QuantSeq kit the following types of libraries can be generated:

1) OligodT primed during RT (with and without molecular barcoding), random primed during SSS (QuantSeq 3′ mRNA-Seq).

2) OligodT primed during RT, target-specifically primed during SSS (targeted 3′ mRNA-Seq).

3) Target-specifically primed during RT, random primed during SSS (targeted RNA-Seq, allows for identification of novel fusions).

4) Target-specifically primed during RT, target-specifically primed during SSS (targeted RNA-Seq, known targets detectable only).

Figure 1 | Schematic overview of the QuantSeq-Flex workflow.

Workflow

Reverse transcription can be primed with an oligodT primer (included in the kit) or target-specific primers including an Illumina P7 sequence at their 5′ end (to be provided by the user). After removing the RNA template, second strand synthesis can either be initiated by random priming simply by using SS1 from QuantSeq 015 or target-specific primers (to be provided by the user). Targeted primers for the second strand synthesis should include the Illumina P5 sequence at the 5′ end. 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. Molecular barcoding options are also available with the QuantSeq-Flex modules.

Ordering Information

Catalog Numbers:
015 (QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina (FWD))
026 (QuantSeq Flex First Strand Synthesis Module compatible with QuantSeq 015)
028 (QuantSeq Flex Second Strand Synthesis Module compatible with QuantSeq 015)
033 (QuantSeq-Flex Targeted RNA-Seq Library Prep Kit with First Strand Synthesis Module)
034 (QuantSeq-Flex Targeted RNA-Seq Library Prep Kit with Second Strand Synthesis Module)
035 (QuantSeq-Flex Targeted RNA-Seq Library Prep Kit with First and Second Strand Synthesis Modules)
020 (PCR Add-on Kit for Illumina)
022 (Purification Module with Magnetic Beads)

Results

A known fusion transcript BCR-ABL was successfully amplified from 500 ng K562 total RNA using QuantSeq-Flex targeted primers.

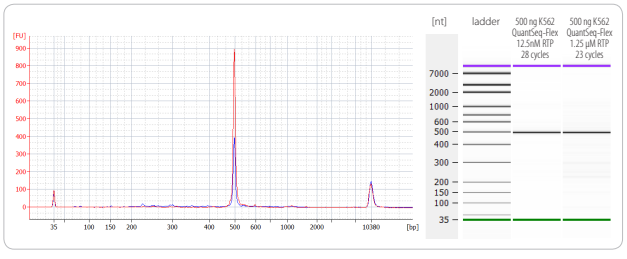

Figure 2 | Bioanalyzer traces of QuantSeq-Flex libraries targeting BCR-ABL fusion transcripts. 500 ng K562 total RNA was used as input material. 12.5 nM final concentration of a targeted first strand synthesis primer (5′ CGTGTGCTCTTC-CGATCTTGTTGACTGGCGTGATGTAGTTGCTTGG 3′) (red trace) was amplified for 28 cycles. Increasing the first strand synthesis primer concentration by a factor of 100 (1.25 µM final concentration) reduced the required cycle number to 23 cycles (blue trace).100 nM final concentration of the second strand synthesis primer (5′ CACGACGCTCTTCCGATCTACAGAATTCCGCTGACCATCAATAAG 3′) was used for all three libraries. First and second strand syntheses were performed at 50 °C.

Find more about QuantSeq-Flex at www.lexogen.com
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QUANT SEQ™
Sequencing that counts