



The Future of RNA-Seq

Improved quantitation • Streamline protocols • Improved results



NEXTflex™ qRNA-Seq™ Kit

NEXTflex™ Rapid Directional qRNA-Seq™ Kit

NEXTflex™ Rapid RNA-Seq Kit

NEXTflex™ Rapid Directional RNA-Seq Kit

THE NGS EXPERTS™

NEXTflex™ qRNA-Seq™ Kit

- Enables high precision measurement of RNA concentration
- Enhanced Adapter Ligation Technology offers a larger number of unique sequencing reads
- Input 10 - 100 ng of mRNA or rRNA-depleted total RNA
- Embedded sample index barcodes for multiplexing of up to 96 samples
- Automation-friendly workflow is compatible with liquid handlers
- Functionally validated with Illumina® sequencing platforms



Bioo Scientific's NEXTflex™ qRNA-Seq™ Kit (patent pending), enables high precision gene expression analysis by RNA-Seq. Developed in conjunction with Cellular Research Inc., this new kit efficiently generates libraries equivalent to conventional RNA-Seq libraries, but with the added feature of molecular indexing. Similar to conventional RNA-Seq, sample RNA is converted to cDNA fragments. Prior to PCR amplification, all cDNA fragment ends are ligated to a pair of adaptors chosen at random from a total set of 9,216 molecular indices. Individual DNA molecules of identical sequence become distinct through indexing (figure 1), allowing for differentiation between re-sampling of the same molecule and sampling of a different molecule of identical sequence. Analysis using molecular indexing information provides an absolute, digital measurement of gene expression levels, irrespective of common amplification distortions observed in many RNA-Seq experiments. This kit is also ideal for improved quantitation of ChIP-Seq.

Stochastic Labeling of Individual DNA Molecules

The NEXTflex qRNA-Seq Kit contain two sets of 96 distinct molecular labels on the sequencing adaptors. Each label or index consists of an 8 nucleotide barcode tag. In the ligation reaction, these 96 adaptors are present in vast molar excess over the concentration of the cDNA fragments, and therefore serve as a non-depleting reservoir of molecular labels. Each end of a cDNA fragment independently and randomly chooses and ligates to a single label from this pool of 96 adaptors to result in a total of $96 \times 96 = 9,216$ possible combinations across both ends. For every clone sequenced, paired-end reads reveal the chosen label on each end along with adjoining cDNA sequence. In addition to encoding DNA fragments at the molecular level, the kit also allows for the application of sample-specific barcodes during the library preparation PCR step. A more detailed description of the use of molecular indexing for RNA-Seq is available in our product application note.

Multiplexing

NEXTflex qRNA-Seq Kits contain barcodes so additional barcodes do not need to be purchased. The 8 rxn kit includes 4 barcodes for multiplexing. Four different versions of the 48 reaction kit are available, each contain 24 unique barcodes. Up to 96 samples can be multiplexed together.

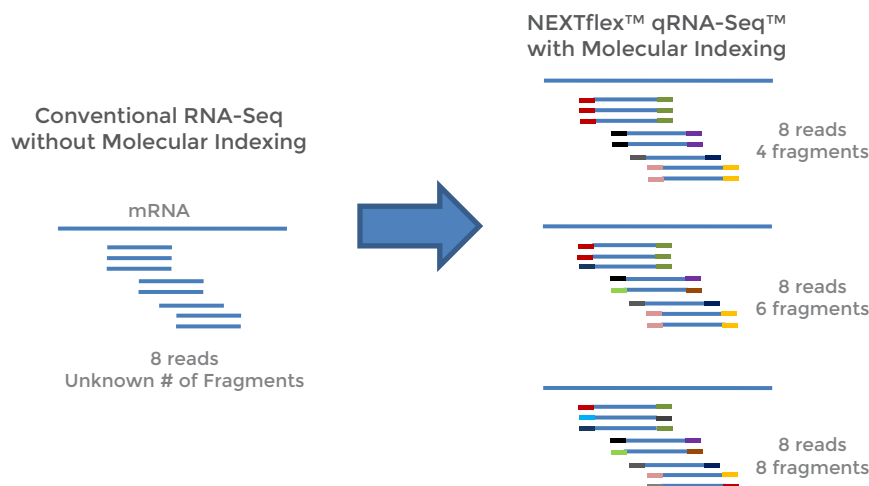


Figure 1. An illustration with 8 mapped reads to an mRNA transcript either with or without molecular indexing. Individual molecular indices at fragment ends are represented by different colors. The number of cDNA fragments represented by the reads is unknown without molecular indexing, but can be determined with molecular indexing.

NEXTflex™ Rapid Directional qRNA-Seq™ Kit

- Generates stranded, molecular indexed libraries
- Enables high precision, quantitative measurement of RNA expression
- Enhanced Adapter Ligation Technology offers a larger number of unique sequencing reads
- 2 hours faster than traditional RNA-seq protocols



- Input 10 - 100 ng of mRNA or rRNA-depleted total RNA
- Embedded sample index barcodes for multiplexing of up to 96 samples
- Automation-friendly workflow is compatible with liquid handlers
- Functionally validated with Illumina sequencing platforms

The patent pending NEXTflex™ Rapid Directional qRNA-Seq™ Kit enables high precision directional gene expression analysis by RNA-seq. Developed in conjunction with Cellular Research Inc., this kit generates directional libraries equivalent to conventional RNA-Seq libraries, but with the added feature of molecular indexing. The NEXTflex Rapid Directional qRNA-Seq Kit can be used to obtain “stranded” information which identifies the specific DNA strands a given RNA transcript was derived from.

Protocol

Using the NEXTflex Rapid Directional qRNA-Seq Kit, mRNA or rRNA-depleted RNA is fragmented using a cationic buffer. Fragmented RNA undergoes first and second strand synthesis, followed by adenylation, ligation and PCR. This kit incorporates the NEXTflex™ Rapid Reverse Transcriptase, a robust, thermostable RT that outperforms other RTs on the market for NGS applications.

NEXTflex™ Rapid Directional RNA-Seq™ Kit



- 2 hours faster than traditional RNA-seq protocols
- Minimal hands-on time required
- Streamlined protocol reduces sample loss
- Utilizes dUTP-based methodology
- Offers measurement of mRNA strand orientation
- Robust, reliable performance
- Input 10 - 100 ng of mRNA or rRNA depleted total RNA
- Up to 96 barcodes available for multiplexing
- Automation-friendly workflow is compatible with liquid handlers
- Functionally validated with Illumina sequencing platforms
- Allows for single read or paired end transcriptome sequencing

The NEXTflex™ Rapid Directional RNA-Seq Kit provides an easy and flexible solution for generating single read, paired-end and multiplexed libraries from mRNA or rRNA depleted total RNA. This kit can be used to obtain “stranded” information which identifies the specific DNA strands a given RNA transcript was derived from. This stranded information improves transcript annotation and improves read alignment, reducing per sample sequencing costs. dUTP-based strand orientation also enables the detection of anti-sense expression regulatory relationships.

New - NEXTflex™ Rapid Directional mRNA-Seq Kit Bundles

To simplify your mRNA sequencing, Bioo Scientific now offers affordable bundles containing the NEXTflex Rapid Directional RNA-Seq library prep reagents, NEXTflex RNA-Seq Barcodes and NEXTflex Poly(A) Beads. There are two different versions of this kit. They each contain the 48 rxn NEXTflex Rapid Directional RNA-Seq Kit, NEXTflex Poly(A) Beads (48 rxns) and 24 unique NEXTflex RNA-Seq Barcodes (in aliquots of 2 reactions each).

Multiplexing

The NEXTflex Rapid Directional RNA-Seq Kit was designed to be used in conjunction with the NEXTflex™ RNA-Seq Barcodes or NEXTflex-96™ RNA-Seq Barcodes for multiplexing. The availability of up to 96 unique adapter barcodes makes this the most high-throughput kit available for Illumina compatible RNA-seq.

NEXTflex™ Rapid RNA-Seq™ Kit



- Faster than traditional RNA-seq protocols
- Enhanced Adapter Ligation Technology offers a larger number of unique sequencing reads
- Input 10 - 100 ng of mRNA or rRNA depleted total RNA
- Up to 96 barcodes available for multiplexing
- Automation-friendly workflow is compatible with liquid handlers
- Functionally validated with Illumina sequencing platforms

The patent pending NEXTflex™ Rapid RNA-Seq Kits provide an easy and flexible solution for generating single, paired-end and multiplexed libraries from mRNA or rRNA depleted total RNA. Using the NEXTflex™ RNA-Seq library prep protocol, mRNA or rRNA depleted RNA is fragmented using a cationic buffer. Fragmented RNA undergoes first and second strand synthesis, followed by end-repair, adenylation, ligation and PCR. This kit incorporates the NEXTflex™ Rapid Reverse Transcriptase, a robust, thermostable RT that outperforms any other RT on the market for NGS applications.

Multiplexing

This kit was designed to be used in conjunction with the NEXTflex™ RNA-Seq or NEXTflex-96™ RNA-Seq Barcodes for multiplexing. The availability of up to 96 unique adapter barcodes makes this the most high-throughput kit available for RNA-seq.

Barcodes for Multiplexing

For multiplexing purposes, Bio Scientific offers barcodes separately from the sequencing kit to allow researchers the flexibility to order the number of barcodes they need for each experiment. They are available in sets of 6, 12, 24, 48 and 96 unique barcodes. Using the NEXTflex™ RNA-Seq Barcodes and the NEXTflex-96™ RNA-Seq Barcodes, the index is added at the adapter ligation step (not during PCR). These Y-shaped barcodes are ideal for Illumina-compatible RNA-Seq library prep applications including use with the NEXTflex Rapid Directional RNA-Seq Kit and the NEXTflex Rapid RNA-Seq Kit.

Citations

Denzler, R., Agarwal V., Stefano J., Bartel DP. and Stoffel, M. (2014) Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. *Mol. Cell.* 54:4 pp 766-76.

Nam, J., Rissland, O.S., Koppstein, D. et al. (2014) Global Analyses of the Effect of Different Cellular Contexts on MicroRNA Targeting. *Molecular Cell.* <http://dx.doi.org/10.1016/j.molcel.2014.02.013>.

NEXTflex™ qRNA-Seq™ Kit		
Cat #	Product	Quantity
5130-01	NEXTflex™ qRNA-Seq™ Kit - 4	8 rxns
5130-02	NEXTflex™ qRNA-Seq™ Kit - 24 set A	48 rxns
5130-03	NEXTflex™ qRNA-Seq™ Kit - 24 set B	48 rxns
5130-04	NEXTflex™ qRNA-Seq™ Kit - 24 set C	48 rxns
5130-05	NEXTflex™ qRNA-Seq™ Kit - 24 set D	48 rxns
NEXTflex™ Rapid Directional qRNA-Seq™ Kit		
Cat #	Product	Quantity
5130-01D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit - 4	8 rxns
5130-02D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit - 24 set A	48 rxns
5130-03D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit - 24 set B	48 rxns
5130-04D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit - 24 set C	48 rxns
5130-05D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit - 24 set D	48 rxns
NEXTflex™ Rapid Directional RNA-Seq™ Kit		
Cat #	Product	Quantity
5138-07	NEXTflex™ Rapid Directional RNA-Seq™ Kit	8 rxns
5138-08	NEXTflex™ Rapid Directional RNA-Seq™ Kit	48 rxns
5138-10	NEXTflex™ Rapid Directional mRNA-Seq Kit Bundle with RNA-Seq Barcodes 1 - 24 and beads	48 rxns
5138-11	NEXTflex™ Rapid Directional mRNA-Seq Kit Bundle with RNA-Seq Barcodes 25- 48 and beads	48 rxns
NEXTflex™ Rapid RNA-Seq™ Kit		
Cat #	Product	Quantity
5138-01	NEXTflex™ Rapid RNA-Seq™ Kit	8 rxns
5138-02	NEXTflex™ Rapid RNA-Seq™ Kit	48 rxns
NEXTflex™ RNA-Seq Barcodes		
Cat #	Product	Quantity
512911	NEXTflex™ RNA-Seq Barcodes - 6	48 rxns
512912	NEXTflex™ RNA-Seq Barcodes - 12	96 rxns
512913	NEXTflex™ RNA-Seq Barcodes - 24	192 rxns
512914	NEXTflex™ RNA-Seq Barcodes - 48	384 rxns
512915	NEXTflex-96™ RNA-Seq Barcodes	768 rxns

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