NEXTflex™ qRNA-Seq™ Kit

产品特点：

- 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

产品描述：

The patent pending, NEXTflex™ qRNA-Seq™ Kit, 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. But prior to any PCR amplification steps, all DNA fragment ends are ligated to a pair of adapters chosen at random from a total set of 9,216 molecular indices. Individual DNA molecules of identical sequence become distinct through indexing, 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.

Stochastic Labeling of Individual DNA Molecules

The NEXTflex qRNA-Seq Kit contains two sets of 96 distinct molecular labels on the sequencing adapters. Each label or index consists of an 8 nucleotide barcode tag. In the ligation reaction, these 96 adapters 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 adapters to result in a total of 96 x 96 = 9,216 possible combinations across both ends. For every clone sequenced, paired-end reads reveals 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.

Protocol

Using the NEXTflex qRNA-Seq Kit, 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.

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.

Magnetic Beads for mRNA Purification

The NEXTflex™ Poly(A) Beads now provide a convenient method for batch purification of pure, intact mRNA upstream NEXTflex qRNA-Seq library preparation. NEXTflex Poly(A) Beads beads use oligo(dT) primer to isolate polyadenylated messenger RNAs from 0.1 µg – 10 µg of previously isolated total RNA.

For more information read our recent white paper written in collaboration with Cellular Research, which demonstrates the capabilities of the NEXTflex qRNA-Seq Kit for quantitative transcriptome analysis.