MGIEasy RNA Directional Library Prep Set V2.1

Features

Low RNA input	Use as low as 10 ng total RNA per sample
Compatible with various sample types	Including low-quality RNA, FFPE RNA
Excellent coverage quality	Accurate and comprehensive mapping of transcripts
High uniformity	Enables high uniform from transcripts 5 'to 3' ends
Accurate detection of strand orientation	Identify transcript origin, detect antisense transcripts, enhances transcript annotation
Easy to Use	Simple protocol and short operation time, suitable for automated library preparation instrument
Flexible Workflow	Various RNA enrichment methods and sequencing read lengths to meet the needs of different sample types

Introduction

RNA sequencing is a powerful method for comprehensive and rapid analysis of gene expression changes, examining rare and novel transcripts, discovery of alternate splicing events, gene fusions, SNPs and allele-specific expression in tissues or cells.

As the complexities of gene regulation become better understood, stranded information specifically identifies from which of the two DNA strands a given RNA transcript originates. Identifying strand origin increases the percentage of reads that align to the transcriptome and provides more accurate information for studies in regulation of gene expression and gene functional analysis. Maintaining strand orientation also allows identification of antisense expression and novel genes.

MGIEasy RNA Directional Library Prep Set provides an efficient workflow for generating libraries suitable for MGI high-throughput sequencing platforms from 10 ng - 1 μg of total RNA.

Product Specification

Assay Time	~7 hours
Hands-On Time	~30 min
Input Quantity	10 ng - 1 μg of total RNA
Sample types	tissues and FFPE sample
Species Compatibility	Human, animals, plants, fungi and bacteria, such as mouse, rice, Arabidopsis, yeast and E.coli.
Applications	RNA-Seq, Transcriptome Sequencing, total RNA sequencing, lncRNA Sequencing
Platform Compatibility	BGISEQ-500*, MGISEQ-2000*, DNBSEQ-G400*
Recommended Read Length	SE50/PE100/PE150
Recommended sequencing data per sample	25 M raw reads（SE50）/8 Gb raw data（PE100/PE150）

Data Performance

Wide range of total RNA input

MGIEasy RNA Directional Library Prep Set is compatible with a range of total RNA input (10 ng -1 μg) and exhibits excellent quality sequencing data and transcript annotation.

With just 8 Gb of sequencing data from Universal Human Reference RNA (UHRR) [1][2], the genome mapping and gene mapping rate were >90% and >70% respectively and the numbers of genes or transcripts detected were similar and in high proportions across all input amounts. These uniform results demonstrate performance stability for all the input amounts tested (Fig. 1).

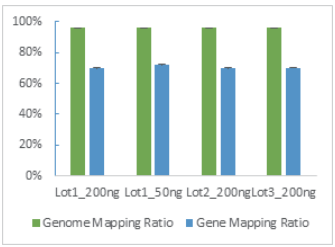

Fig. 1a The ratios of alignment in different input amounts of total RNA and different kit batches

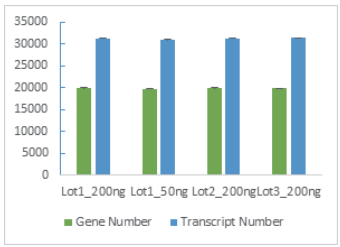

Fig. 1b The number of genes and transcripts detected in different input amounts of total RNA

Fig. 1 Libraries were prepared from an input of UHRR ranging from 10 ng -1 μg using the MGIEasy RNA Directional Library Prep Set and sequenced on MGISEQ-2000 at PE100 read-length. After data filtering, approximately 8 Gb of data was collected per library for analysis. The data were mapped with genome database (hg19 Human Genome) and gene database (refMrna. fa).

High concordance of gene expression

The concordance of gene expression on libraries constructed with the MGIEasy RNA Directional Library Prep Set was tested using either different (Fig. 2a) or identical (Fig. 2b) input amounts of total RNA. Using Pearson and Spearman correlation tests, stable and accurate results were demonstrated with r values >0.993 for all data analyzed.

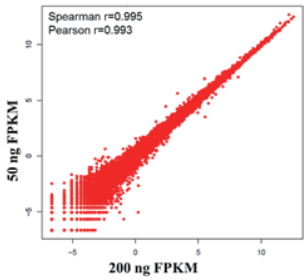

Fig.2a Gene expression level reproducibility and concordance in different input amounts of total RNA

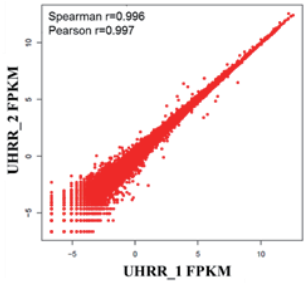

Fig.2b Gene expression level reproducibility and concordance in identical input amounts of total RNA

Fig. 2 Libraries were prepared from UHRR using the MGIEasy RNA Directional Library Prep Set and sequenced on MGISEQ-2000 at PE100 read-length. After data filtering, approximately 8 Gb were collected per library for analysis.

High uniformity

Uniformity of coverage across the entire transcript is important for analysis of gene structure[3]. A comparison was performed between the MGIEasy RNA Directional Library Prep Set sequenced on an MGI sequencer and comparable RNA kits from Company-I sequenced on another platform. The results in Figure 3 demonstrate that the libraries constructed using MGIEasy RNA Directional Library Prep Set have superior 3'end coverage than Company-I kits (Fig. 3).

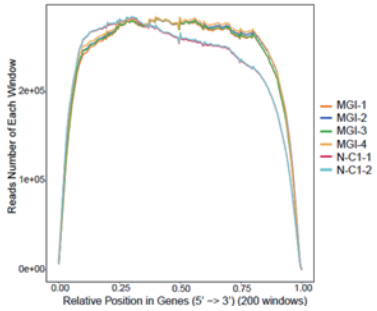

Fig.3 The reads randomness of different platforms and kits

The libraries MGI -1/2/3/4 were generated from UHRR using the MGIEasy RNA Library Prep Set and sequenced on MGISEQ-2000 at PE150 read-length. The libraries N-CI-1/2 were prepared using Company-I kits and sequenced on "N" sequencing platform at PE150 read-length. After data filtering, approximately 10 Gb were collected per library for analysis. The analysis of sequencing data was based on the same instruction.

High-Quality Stranded Information, High accuracy of gene abundance

Based on the libraries from an enriched poly(A) mRNA sample of UHRR, the percentage of unique mapped reads that present accurate strand origin information was determined to be above 99% (Fig. 4a), which confirmed accurately preserved direction of transcripts. RNA abundance is also accurately reflected by high consistency between libraries constructed using MGIEasy RNA Directional Library Prep Set and MGIEasy RNA Library Prep Set (Fig. 4b). Therefore, MGIEasy RNA Directional Library Preparation Set can be applied to detect transcript strand information and analysis of gene expression.

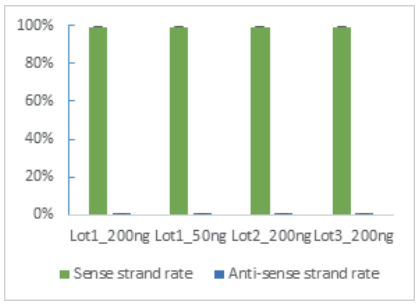

Fig.4a Residual ratio in different FFPE RNA Samples

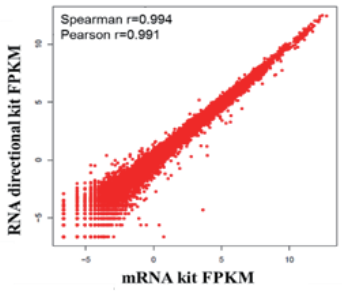

Fig.4b Concordance of MGIEasy RNA Directional Library Prep Set and MGIEasy RNA Library Prep Set RNA

Fig.4 Libraries were prepared with from an enriched poly(A) mRNA of UHRR using the MGIEasy RNA Directional Library Prep Set and sequenced on MGISEQ-2000 at PE100 read-length. After data filtering, approximately 8 Gb were collected per library for analysis.

Easy to use and suitable for automatic library preparation instrument

Optimization and simplification of complicated and time-consuming steps in previous methods has allowed the MGIEasy RNA Library Prep Set easy to use with simple protocol and short operation time, and to become suitable for automated sample preparation. Utilizing the automated sample preparation system MGISP-100,sample preparation of libraries for directional RNA sequencing analyses has become less labor intensive and time-consuming for users.

Flexible Packages

MGIEasy RNA Directional Library Prep Set provides flexible packages with various RNA enrichment methods (poly(A) enrichment or rRNA depletion) and sequencing read lengths to meet the needs of different species and sample types.

Table 1 Corresponding use options for different sample types poly(A) enrichment or rRNA depletion

Sample type	RNA enrichment method	Read length	Applications
Eukaryotic total RNA with high integrity	Poly(A) mRNA enriched by oligo(dT) beads	SE50/PE100/PE150	mRNA quantification and transcriptome analysis
	rRNA depleted with rRNA depletion kit	PE100/PE150	RNA quantification and transcriptome analysis
Prokaryotic total RNA	rRNA depleted with rRNA depletion kit	PE100/PE150	RNA quantification and transcriptome analysis
Degraded RNA from FFPE samples or plasma cell-free RNA etc.	rRNA depleted with rRNA depletion kit	SE50/PE100	RNA quantification and transcriptome analysis

Summary

The MGIEasy RNA Directional library prep Set provides an efficient workflow for library construction, enabling excellent coverage quality, high uniformity, and stability using different input amounts and qualities of total RNA, from a wide range of samples, including nonhuman and FFPE. Importantly, it can be used to accurately identify DNA origins of the transcripts and enable greater detection of antisense transcripts. The MGIEasy RNA Directional Library Prep Set can be widely used in human, animal, plant and microbe RNA sequencing research to help users achieve their research goals faster and more easily.

Ordering information

Product	Configuration	Catalog No.
MGIEasy RNA Directional Library Prep Set	16 RXN	1000006385
	96 RXN	1000006386

Reference

【1】Zhenqiang Su, *et al*. A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. Nature Biotechnology, 2014, 32: 903-914.
【2】Charles Wang, *et al*. The concordance between RNA-seq and microarray data depends on chemical treatment and transcript abundance. Nature Biotechnology, 2014, 32: 926–932.
【3】Sheng Li, *et al*. Detecting and correcting systematic variation in large-scale RNANA sequencing data. Nature Biotechnology, 2014, 32: 888–895.

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