

MGIEasy RNA Library Prep Set V3.1

■ 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	Accurate and comprehensive mapping of transcripts
High uniformity	Enables high uniform from transcripts 5' to 3' ends
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. Researchers aim to understand known or novel factors that alter gene expression during particular biological processes or development of diseases. Therefore, RNA-Seq has been widely applied to many fields such as biological research, disease research, drug development, agriculture and environmental research.

MGIEasy RNA Library Prep Set provides an efficient workflow for generating libraries from a wide range (10 ng - 1 µg) of total RNA that is suitable for MGI high-throughput sequencing platforms. MGIEasy RNA Library Prep Set is easy to use and can be widely applied to human, animal, plant and microbe RNA Sequencing research.

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
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

As low as 10 ng total RNA input

MGIEasy RNA 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).

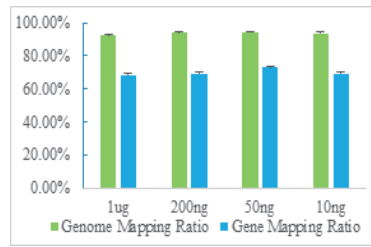


Fig. 1a Ratios of alignment in different input amounts of total RNA and different kit batches

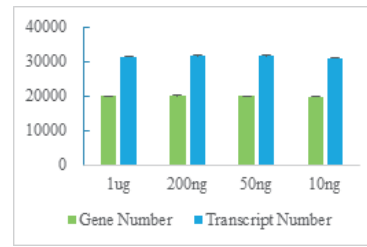


Fig. 1b Number of genes and transcripts detected in different input amounts of total RNA

Fig. 1 Libraries were prepared from an input of UHRR ranging from 10 ng -1 µg using the MGIEasy RNA 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 (reflMna. fa).

High concordance of gene expression

The concordance of gene expression on libraries constructed with the MGIEasy RNA Library Prep Set was tested using different input amounts of total RNA. Using Pearson and Spearman correlation tests, stable and accurate results were demonstrated with r values >0.995 for all data analyzed (Fig. 2).

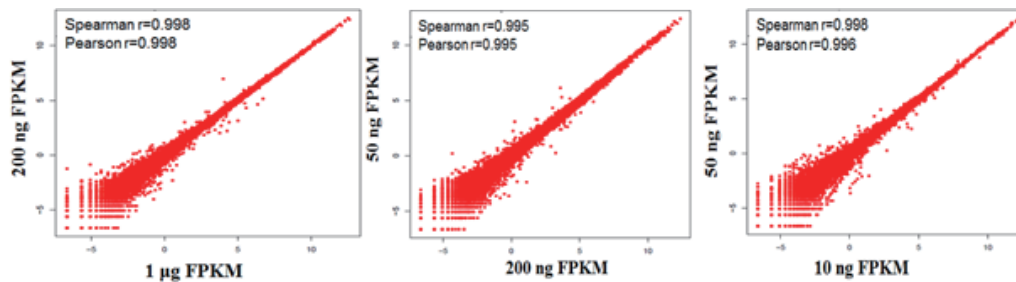


Fig. 2 Gene expression level reproducibility and concordance in different input amounts of total RNA

The libraries were prepared from an input of UHRR ranging from 10 ng -1 µg using the MGIEasy RNA 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.

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 Library Prep Set sequenced on MGISEQ-2000 sequencer and comparable RNA kits from Company-I and Company-N sequenced on another platform. The results in Figure 3 demonstrate that the libraries constructed using MGIEasy RNA Library Prep Set have superior 3'end coverage than Company-I kits (Fig. 3).

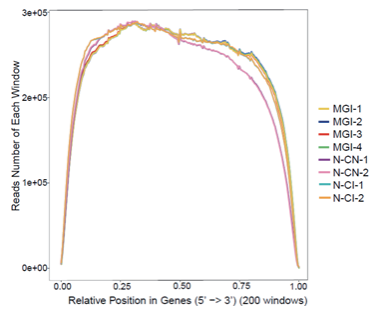


Fig.3 Reads randomness of different platforms and kits

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-CN-1/2 and N-CI-1/2 were prepared using Company-N and Company-I kits respectively and sequenced on "N" platform at PE150 read-length. After data filtering, about 10 Gb were collected per library for analysis. The analysis of sequencing data was based on the same instruction.

Compatible with FFPE sample

Lung Cancer FFPE RNA samples with low RNA Integrity Numbers (RIN) ranges (FFPE-1= 5 to 7, FFPE-2= 3 to 5 and FFPE-3 <3) were used to prepare libraries with the MGIEasy RNA Library Prep Set. The sequencing results of these libraries show that the numbers of genes detected are highly abundant and map accurately to the transcriptome (Fig. 4).

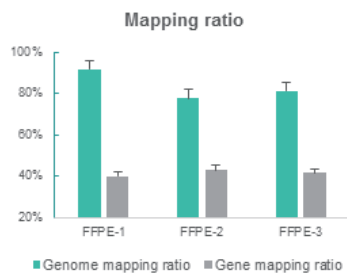


Fig.4a Residual ratio in different FFPE RNA Samples

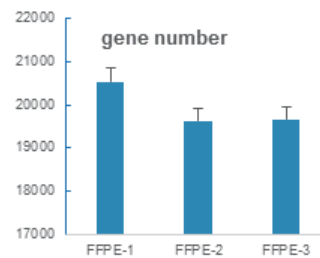


Fig.4b Numbers of genes detected in different FFPE RNA Samples

Fig.4 Libraries were prepared from Lung Cancer FFPE RNA samples using the MGIEasy RNA Library Prep Set and sequenced on BGISEQ-500 at PE100 read-length. After data filtering, approximately 1 Gb of data was 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 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 varied species and sample types.

Table 1 Applications for different sample types with 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
Prokaryotic total RNA	rRNA depleted with rRNA depletion kit	SE50/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 Library Prep Set provides an efficient workflow for library construction, enabling excellent coverage quality, high uniformity, and stability using different inputs amounts and qualities of total RNA, from a wide range of samples, including nonhuman and FFPE. MGIEasy RNA Library Prep Set is ideal for transcriptome sequencing, helping users achieve their research goals faster and more easily.

Ordering information

Product	Configuration	Catalog No.
MGIEasy RNA Library Prep Set	16 RXN	1000006383
	96 RXN	1000006384

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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