MGIEasy Fast RNA Library Prep Set

Introduction

The MGIEasy Fast RNA Library Prep Set is a specifically designed RNA rapid library prep product for DNBSEQ™ sequencers. The library prep set is optimized to convert 10 ng to 1 μg of total RNA into a DNA library, providing high-quality sequencing data under different total RNA input amounts, with uniform coverage of transcript regions, precise quantification of gene expression, and stable performance. The library prep set is applicable for transcriptome sequencing and abundance analysis, discovery of novel transcripts and analysis of transcript structural variation. It is suitable for various species such as humans, animals, plants, fungi, bacteria, and viruses.

The MGIEasy Fast RNA Library Prep Set combines cDNA second-strand synthesis and end repair steps, which significantly shortening the library preparation time. The second-strand synthesis is equipped with two types of buffers, allowing for preparing common or directional libraries according to needs. Up to 288 unique dual barcodes enable flexible throughput for multiplexing 4 to 288 samples. The library prep set is automation-friendly and compatible with MGISP-100, MGISP-960, and MGISP-Smart 8 platforms.

Highlights

Fast library prep workflow

Combine cDNA second strand synthesis, end repair and A-tailing into one step, significantly simplifying the experimental process, shortening library preparation time, and minimizing hands-on time.

Flexible library prep with two library types

Provide two types of cDNA second-strand synthesis buffer, supporting the preparation of either a common RNA library or a directional RNA library.

Wide range of total RNA input and various sample types

Compatible with a range of total RNA input (10 ng - 1 μg) and various sample types, including low-quality RNA, FFPE RNA.

Higher and flexible throughput

Up to 288 selected Unique Dual Barcodes enable flexible throughput for 4 - 288 samples multiplexing.

Excellent performance

High uniformity coverage across transcripts, high concordance of gene expression, and stable performance.

Flexible solutions

Adapt to various RNA enrichment methods, sequencing read lengths, and two DNB method (universal circularization or OneStep DNB) to meet the needs of different sample types.

Efficient automatable workflow

Adapt to MGISP-100, MGISP-960, and MGISP-Smart 8, providing efficient automated solutions.

Specification

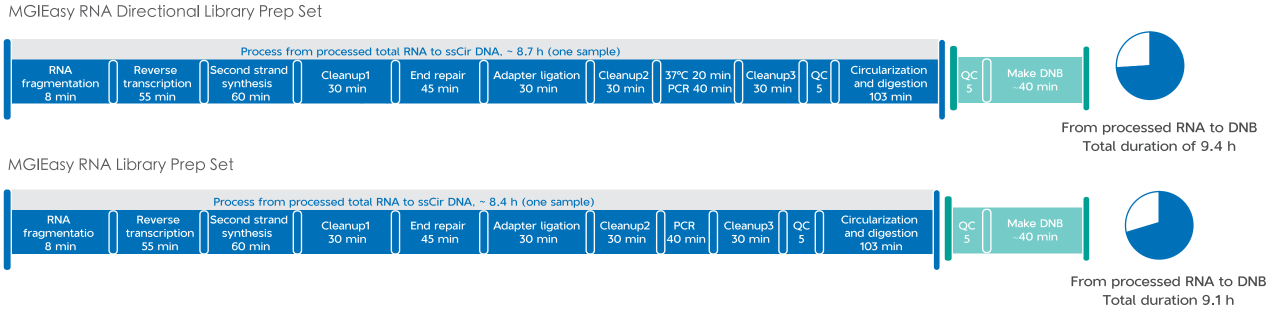
Assay Time	~ 4 h (from processed RNA to PCR library)
Hands-On Time	~ 1 h
Input Quantity	10 ng - 1 μg total RNA
Barcode	~ 288 barcodes
Sample Types	Total RNA from blood, saliva, fresh tissue, FFPE RNA, Plant total RNA, Animal total RNA, Microbial total RNA, Meta RNA,
Compatible Species	Human, Animal, Plant, Microbe
Applications	Whole-Transcriptome Sequencing， mRNA Sequencing, Long non-coding RNA Sequencing, RNA-Seq, Metatranscriptome Sequencing
Sequencers	DNBSEQ-G400, DNBSEQ-G50, DNBSEQ-G99, DNBSEQ-T7, DNBSEQ-T10×4
Sequencing Types	SE50 / PE100 / PE150
Automation Platform	MGISP-100 (16 RXN), MGISP-960 (96 RXN, 192 RXN), MGISP-Smart 8

Product performance

Fast and flexible workflow

The MGIEasy Fast RNA Library Prep Set combines cDNA second strand synthesis, end repair and A-tailing into one step, while comprehensively optimizing multiple steps including cDNA first strand synthesis, adapter ligation and PCR. These optimizations greatly simplify the experimental process, shorten the library preparation time to within 4 h (from processed RNA sample to PCR library).

The MGIEasy Fast RNA Library Prep Set provides two kinds of cDNA second strand synthesis buffer, supporting either common (non-strand-specific libraries) or directional RNA library preparation (strand-specific libraries). It also offers up to 288 unique dual barcodes, enabling flexible throughput for 4 to 288 samples multiplexing. The MGIEasy Fast RNA Library Prep Set adapts to two circularization methods, including MGI universal circularization or MGI OneStep DNB. By using Fast RNA combined with the MGI OneStep DNB, the whole process from RNA to DNB can be complete in 4.7 h (Fig.1).



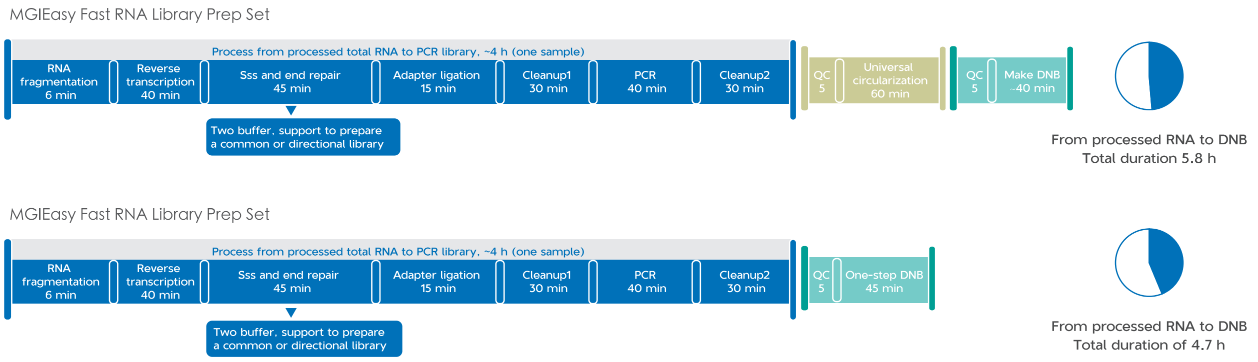


Fig. 1 The whole process from enriched RNA to DNB under different library preparation methods and circularization methods

Calculate the process time by manually preparing one library once. The MGIEasy Fast RNA Library Prep Set is compatible with two circularization methods:

1) MGI universal circularization method. The prepared PCR library is circularized using the MGI circularization kit (MGI Cat. No. 1000020570) to obtain single-stranded circular DNA (ssCir DNA), followed by conventional DNB making by using the DNB making reagent in the MGI sequencing reagent set. The process from enriched RNA to DNB takes 5.8 h.

2) MGI OneStep DNB method. Use the MGI OneStep DNB kit (MGI Cat. No. 940-000036-00) to complete the library circularization and DNB making in one step, directly obtaining DNB. This simplifies the process from the PCR library to DNB and reduces the DNB preparation time. The entire process from enriched RNA to DNB can be shortened to less than 4.7 h.

*Sss: second strand synthesis.

Excellent performance with high uniformity coverage across transcripts

Using the Universal Human Reference RNA (UHRR) as the test samples, a comparison was performed between the MGIEasy Fast RNA Library Prep Set and competitive RNA kits by sequencing on MGI sequencers and another sequencing platforms.

The results showed the libraries prepared from MGIEasy Fast RNA Library Prep Set exhibit excellent quality on sequencing data, genome mapping rate, gene mapping rate and gene detection, and have higher correlation with qPCR quantification, better reads randomness from the 5’ to the 3’ ends of transcripts and higher uniformity of transcript region coverage (Fig. 2).

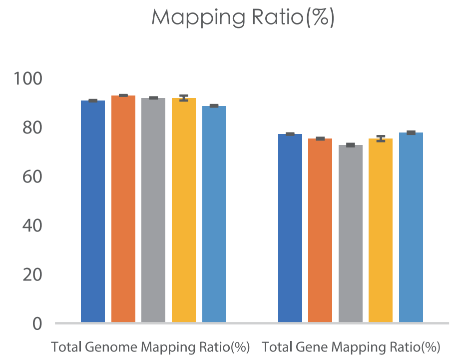


Fig. 2a Mapping Ratios of different RNA kits and platforms

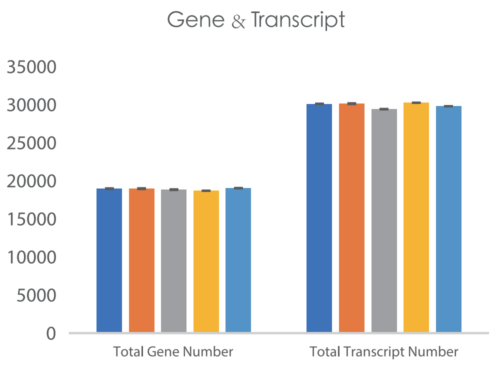
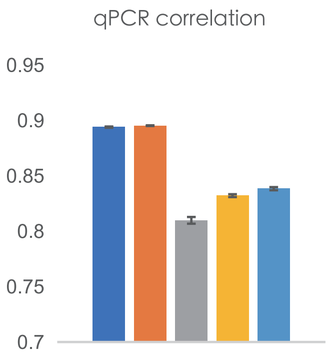


Fig. 2b Detected genes of different RNA kits and platforms

🟦 DNBSEQ-T7 Fast 🟧 DNBSEQ-G400 Fast ⬜ N seq-K lib 🟨 N seq-N lib 🟦 DNBSEQ-G400-V lib



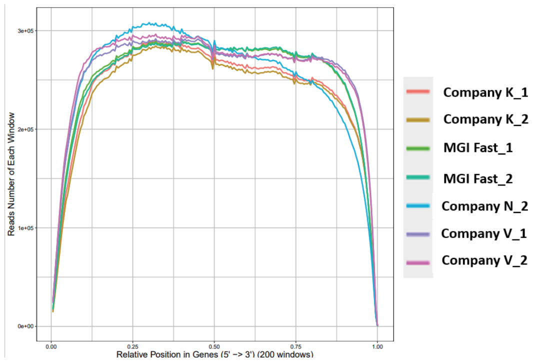


Fig. 2c The qPCR correlation qPCR of different RNA kits and platforms

Fig. 2d Reads randomness of different RNA kits and platforms

■ DNBSEQ-T7 Fast ■ DNBSEQ-G400 Fast ■ N seq-K lib ■ N seq-N lib ■ DNBSEQ-G400-V lib

Fig. 2 Sequencing data of different RNA kits and platforms

Wide range of total RNA input amounts

The MGIEasy Fast RNA Library Prep Set is compatible with a wide range of total RNA input (10 ng -1 μg) and exhibits excellent quality sequencing data and stable performance under different input amounts. Libraries were prepared from an input of UHRR ranging from 10 ng - 1 μg using the MGIEasy Fast RNA Library Prep Set and sequenced on DNBSEQ-G400 PE100. The results demonstrate performance stability for all the input amounts tested under the same library type, and even at low input amounts, the MGIEasy Fast RNA Library Prep Set can detect expressed genes with high sensitivity and have a high degree of uniform coverage, which aids in gene structure analysis.

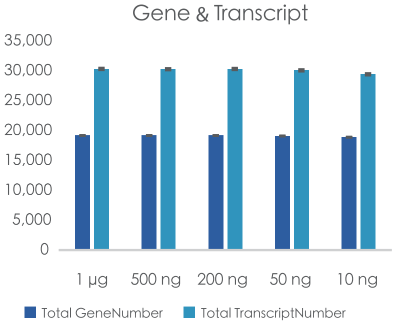


Fig. 3a Detected genes under different input amounts for common RNA libraries (non-strand-specific libraries)

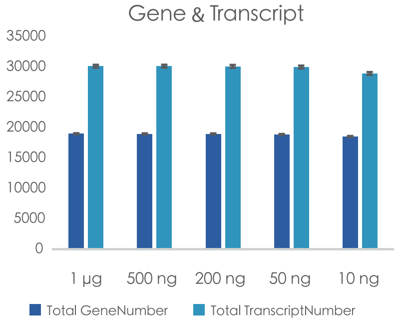


Fig. 3b Detected genes under different input amounts for directional RNA libraries (strand-specific libraries)

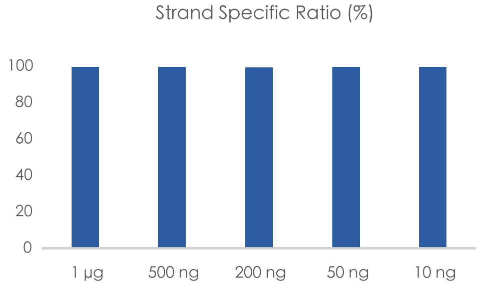


Fig. 3c Strand specific ratio under different input amounts for directional RNA libraries (strand-specific libraries)

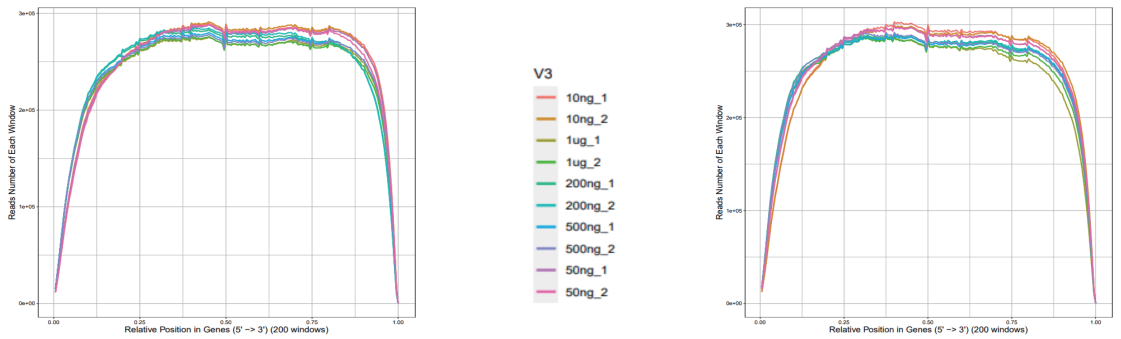


Fig. 3d Reads randomness from the 5’ to the 3’ ends of transcripts under different input amounts for common RNA libraries (non-strand-specific libraries)

Fig. 3e Reads randomness from the 5’ to the 3’ ends of transcripts under different input amounts for directional RNA libraries (strand-specific libraries)

Fig. 3 Performance under different input amounts

After mRNA enrichment, UHRR samples with different input amounts were used for common RNA library or directional RNA library preparation. Libraries were sequenced on DNBSEQ-G400 at PE100 read-length. After data filtering, approximately 10 Gb of data was collected per library for analysis.

Effective detection of low-concentration pathogen Meta samples

Fresh normal saliva samples, fresh normal throat swabs samples and simulated Meta samples were used to prepare RNA libraries with the MGIEasy Fast RNA Library Prep Set for Meta-transcriptome Sequencing. These libraries were sequenced on DNBSEQ-G400 PE100 and the sequencing data were analyzed using MGI PFI software. High library yields were obtained for each sample type under the corresponding library preparation conditions. The analysis results showed that the majority of microbial species identified in saliva and swab samples were oral/respiratory tract colonizers, which is consistent with normal conditions. The known simulated pathogen species can be accurately detected in simulated Meta samples even at low concentrations.

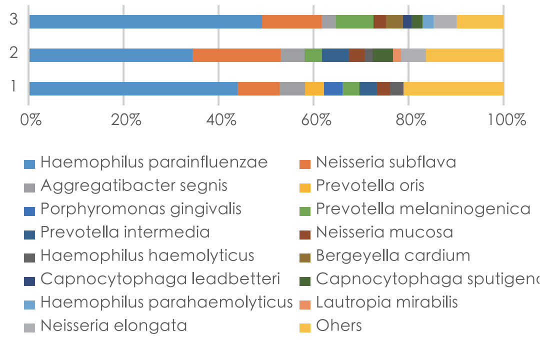


Fig. 4a Bacterial species identification and distribution in saliva samples

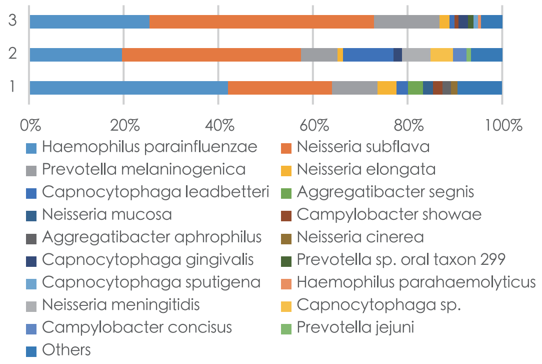


Fig. 4b Bacterial species identification and distribution in throat swabs samples

Type	Scientific Name	Chinese Name	Real Read	Estimate Read	Abundance
Bacteria	*Escherichia coli*	大肠埃希氏杆菌	122	17,032	61.92%
Viruses	*Alphapapillomavirus 7*	乳头瘤病毒7	2,536	2,536	9.22%
Bacteria	*Pseudomonas aeruginosa*	铜绿假单胞菌	115	1,747	6.35%
Viruses	*Severe acute respiratory syndrome-related coronavirus*	严重急性呼吸道综合征相关冠状病毒	1,329	1,329	4.83%
Bacteria	*Comamonas aquatica*	水丛毛单胞菌	184	1,188	4.32%
Bacteria	*Stenotrophomonas acidaminiphila*	嗜酸寡养单胞菌	154	722	2.62%
Bacteria	*Cutibacterium acnes*	痤疮丙酸杆菌	648	710	2.58%
Bacteria	*Acinetobacter junii*	琼氏不动杆菌	296	637	2.32%
Bacteria	*Cloacibacterium normanense*	Cloacibacterium normanense	304	460	1.67%
Bacteria	*Pseudomonas putida*	恶臭假单胞菌	81	329	1.20%

Fig. 4c Accurately detected the severe acute respiratory syndrome-related coronavirus sequence

Fig. 4 Performance under different sample types for Meta-transcriptome Sequencing

Saliva and swab samples: About 200 ng Meta RNA from fresh normal saliva samples, about 200 ng Meta RNA from fresh swab samples. Simulated Meta samples: 10^5 (CN5) and 10^6 (CN6) copies of the COVID-19 coronavirus standards are spiked into 5 ng of UHRR, respectively. Library preparation: After rRNA depletion, perform common RNA library preparation with a 200 bp insert size. Libraries were sequenced on DNBSEQ-G400 at PE100 read-length. After data filtering, approximately 8 Gb of data was collected per library for analysis.

Compatible with FFPE sample

FFPE RNA samples with different quality (degradation) were used to prepare libraries with the MGIEasy rRNA Depletion Kit V1.3 and the MGIEasy Fast RNA Library Prep Set. The results showed that FFPE samples with different degrees of degradation can all be successfully used to construct high-quality libraries with low rRNA residue rate and abundant detected genes. Better quality samples have better rRNA depletion ratio and gene detection.

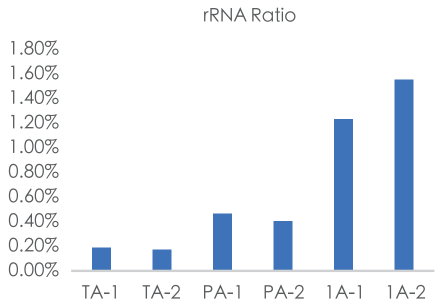


Fig. 5a rRNA residual ratio in different FFPE RNA Samples

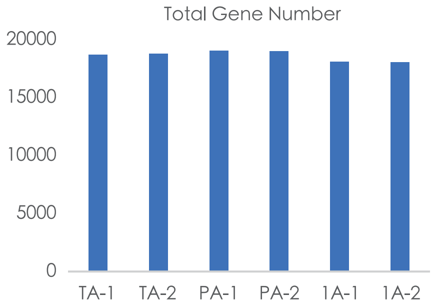


Fig. 5b Detected genes in different FFPE RNA Samples

Fig. 5 Sequencing data from FFPE samples with different degrees of degradation

FFPE samples with different quality (degradation) were used for common RNA library preparation, including 200 ng TA (RIN 7.1, DV_{200} approximately 66%), 200 ng PA (RIN 7.6, DV_{200} approximately 63%), and 500 ng 1A (RIN 2.3, DV_{200} approximately 48%). The libraries were sequenced on DNBSEQ-G400 at PE100 read-length. After data filtering, approximately 8 Gb of data was collected per library for analysis.

Stable performance on Automated Sample Preparation System

The MGIEasy Fast RNA Library Prep Set is adapted to MGISP-100, MGISP-960, and MGISP-Smart 8 automated platforms, providing efficient automation solutions and simplifying your library preparation. Directional libraries were prepared from an input of 50 ng UHRR (200 bp insert size) on different automated platforms. The results indicate that the MGIEasy Fast RNA Library Prep Set has stable library preparation performance on different automated platforms, with high library yield, high reproducibility, low deviation, and consistent library size.

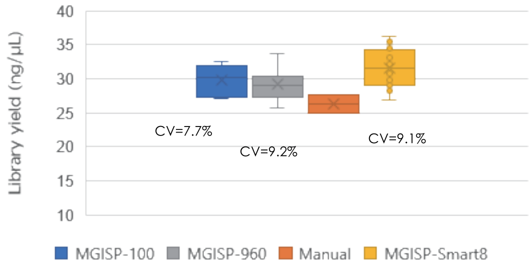


Fig. 6a Library yield on different automated platforms

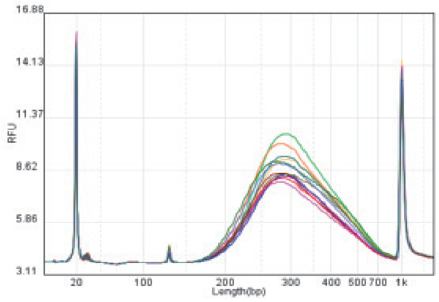


Fig. 6b Library size distribution on MGISP-100 platform

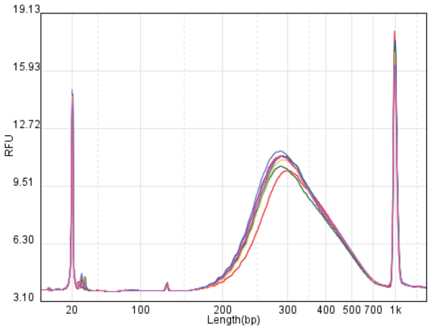


Fig. 6c Library size distribution on MGISP-Smart 8 platform

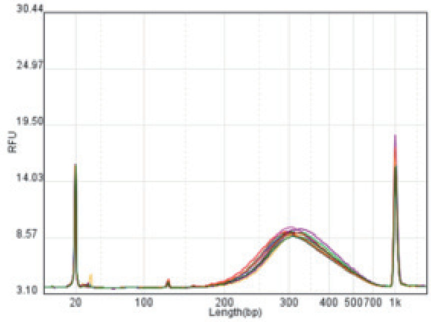


Fig. 6d Library size distribution on MGISP-960 platform

Fig. 6 Stable performance on Automated Sample Preparation System

Summary

The MGIEasy Fast RNA Library Prep Set provides an efficient solution for rapid RNA library preparation. The libraries obtained perform excellent coverage quality, high uniformity coverage across transcripts, precise gene expression quantification, and stable performance across different sample input amounts. MGIEasy Fast RNA Library Prep Set supports a wide range of applications and is suitable for a wide range of species and sample types, compatible with multiple Automated Sample Preparation Systems. MGIEasy Fast RNA Library Prep Set is ideal for transcriptome sequencing, helping you achieve your research goals more quickly and easily.

Ordering Information

Product	Configuration	Cat. No.	Note
MGIEasy Fast RNA Library Prep Set	16 RXN	940-000890-00	
	96 RXN	940-000889-00	
	192 RXN	940-000961-00	
MGIEasy Fast RNA Library Prep Set C	96 RXN	940-001835-00	
MGIEasy rRNA Depletion Kit V1.3	16 RXN	940-001751-00	Suitable for total RNA samples of humans, mice, and rats
	96 RXN	940-001752-00	
MGIEasy rRNA & Globin Depletion Kit V1.3	16 RXN	940-001761-00	Suitable for human blood total RNA samples
	96 RXN	940-001760-00	
MGIEasy Dual Barcode Circularization Kit	16 RXN	1000020570	
DNBSEQ OneStep DNB Make Reagent Kit V2.0 (OS-DB)	4R XN	940-000036-00	

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