LeXPrep RNA & DNA Library DualPrep Module

Background

Infectious diseases caused by pathogenic microorganisms are among the significant causes leading high mortality and morbidity in humans. The types of pathogenic infections are diverse, including parasites, fungi, bacteria, mycoplasma/chlamydia/spirochetes/Rickettsia, viruses, etc. Specific diagnostic and therapeutic measures are often required for different pathogenic infe- ctions. Therefore, rapidly and accurately detecting the causative pathogen is crucial for the diagnosis, treatment, and management of infectious diseases. DNA detection process is suitable for suspected DNA pathogen infections, while RNA detection process is applicable for suspected RNA virus infections. When viral infection are challenging to exclude, it is recommended to simultaneously perform RNA and DNA co-detection. However, the dual-process detection of RNA and DNA is costly , and there is an urgent need for an RNA & DNA single-tube library dual preparation scheme to reduce detection costs and shorten detection time. Combining targeted next-generation sequencing (tNGS) of pathogenic microorganisms further aids in rapid and accurate clinical diagnosis and treatment of infections. To expedite the entire process of pathogenic microorganisms detection in tNGS, LexigenBio has launched LeXPrep RNA & DNA Library DualPrep Module, enabling a single process for RNA & DNA codetection.

Introduction

LeXPrep RNA & DNA Library DualPrep Module is developed for high-throughput sequencing platforms, enabling a single process for RNA & DNA dual preparation from mixed pathogenic microbial samples. This module consist of 1st Strand Synthesis, 2nd Strand Synthesis, Fragmentation and End Repair & A-Tailing, Adapter Ligation and Amplification. It can be used in conjunction with the LeXPrep Universal Stubby Adapter (UDI) Module and LeXPrep Universal Adapter (MDI) Module (for MGI) for different sequencing platforms.

Feature

Flexible Compatibility

- Supports mixed samples with varying ratios of 10-100 ng RNA and 1-100 ng DNA
- Compatible with both MGI and Illumina sequencing platforms

Simple and Rapid

- Single-tube operation, quick and convenient
- RNA & DNA library preparation in a single process in 3-3.5 hours

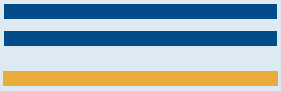
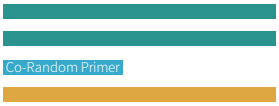
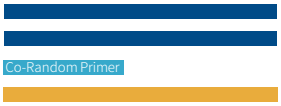
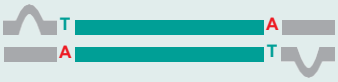
Efficiency and Uniformity

- High efficiency and stability of library yield
- Uniform size distribution of fragment
- Low GC bias

Background Pathogenic Microorganism Control

- Comprehensive control of background contamination of pathogenic microorganisms throughout the entire process, including environmental control, consumables control, raw material control, process control, manufacturing process control, quality control, etc.

Workflow

MGI	RNA & DNA	Duration	Illumina
	RNA & DNA Mixed Sample		
	RNA Degeneration & Random Primer Hybridization	5 min	
	1st Strand Synthesis	25 min	
	2nd Strand Synthesis	30 min	
	Fragmentation / End Repair & A-tailing	45-60 min	
	Adapter Ligation	15 min	
	Post-ligation Cleanup	30 min	
	PCR Amplification	20 min	
	Post-amplification Cleanup	30 min	

LeXPrep M-Adapter (DI) LeXPrep Universal Stubby Adapter

Performance

Flexible Compatibility

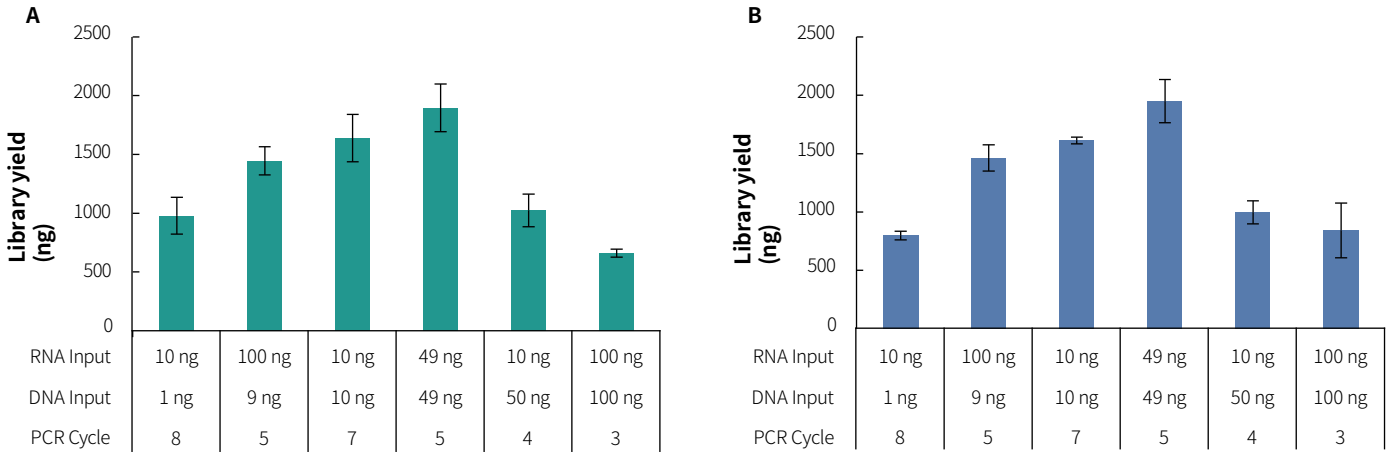


Figure 1. LeXPrep RNA & DNA Library DualPrep Module for library preparation with mixed RNA & DNA samples in varying initial ratios. A. Library yield (MDI); **B.** Library yield (UDI). Utilize LeXPrep RNA & DNA Library DualPrep Module in conjunction with LeXPrep Universal Adapter (MDI) Module (for MGI) and LeXPrep Universal Stubby Adapter (UDI) Module for library preparation.

Note: The RNA samples are from K562 cell lines; the DNA samples are Human Genomic DNA Standards (Promega, G1521).

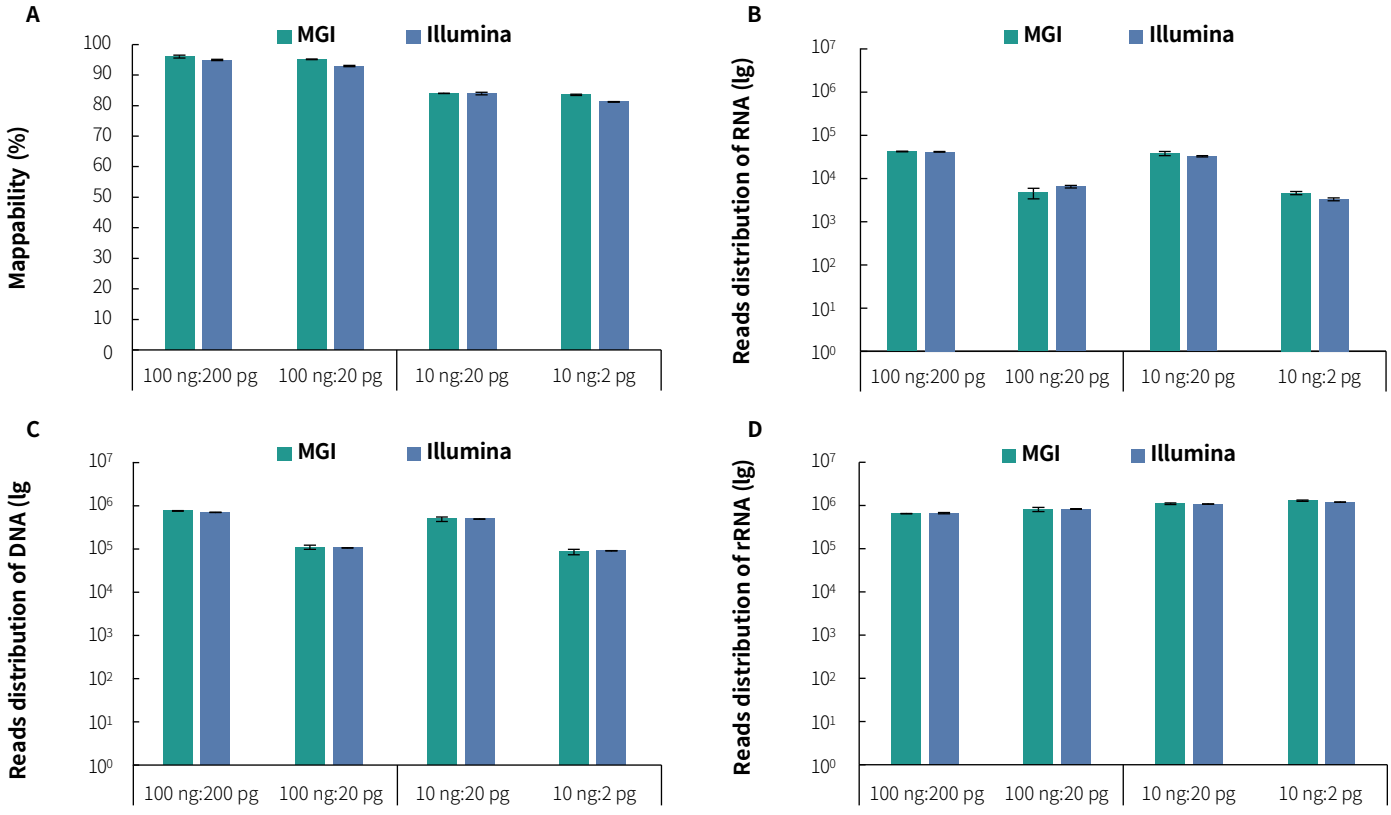


Figure 2. Detection of tNGS Total Solution with LeXPrep RNA & DNA dualprep on sequencing platforms. A. Mappability; **B.** Reads distribution of Coronavirus; **C.** Reads distribution of *Candida albicans*; **D.** Reads distribution of rRNA. Pre-libraries preparation were performed using LeXPrep RNA & DNA Library DualPrep Module. Hybridization capture were completed with LeXPrep ES Hybrid Capture Reagents and NEX-t Panel v1.0, followed by sequencing on DNBSEQ-T7, PE150 and Illumina NovaSeq 6000, PE150. For each sample, 0.5 Gb was used for data analysis.

Note: The scale on the X-axis represents the proportional initial input amounts of the host-mimicking mixed nucleic acid samples to the input amounts of the pathogenic mixed nucleic acid samples. The host-mimicking mixed nucleic acid samples consist of Human Brain Total RNA Standard (Clontech, 636530) and Human Genomic DNA Standard (Promega, G1521) mixed at a 1:1 ratio. The pathogenic mixed nucleic acid samples consist of the Reference material for SARS-CoV-2 Omicron Variant Genomic RNA (National Institute of Metrology, NIM-RM 5225; 1 pg equals 50 copies) and *Candida albicans* standard (ATCC, 10231; 1 pg equals 6000 copies) mixed at a 1:1 ratio.

Higher Sensitivity

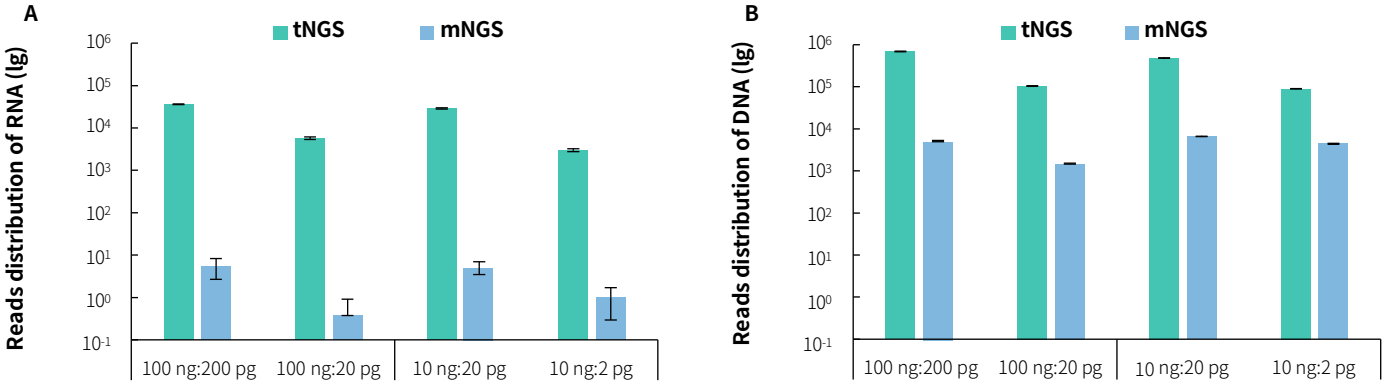


Figure 3. Distribution of detected reads in simulated pathogenic microbial samples using tNGS and mNGS based on the LeXPrep RNA & DNA Library DualPrep Module. A. Reads distribution of Coronavirus; **B.** Reads distribution of *Candida albicans*.

Note: Sequencing platform: Illumina NovaSeq 6000, PE150.

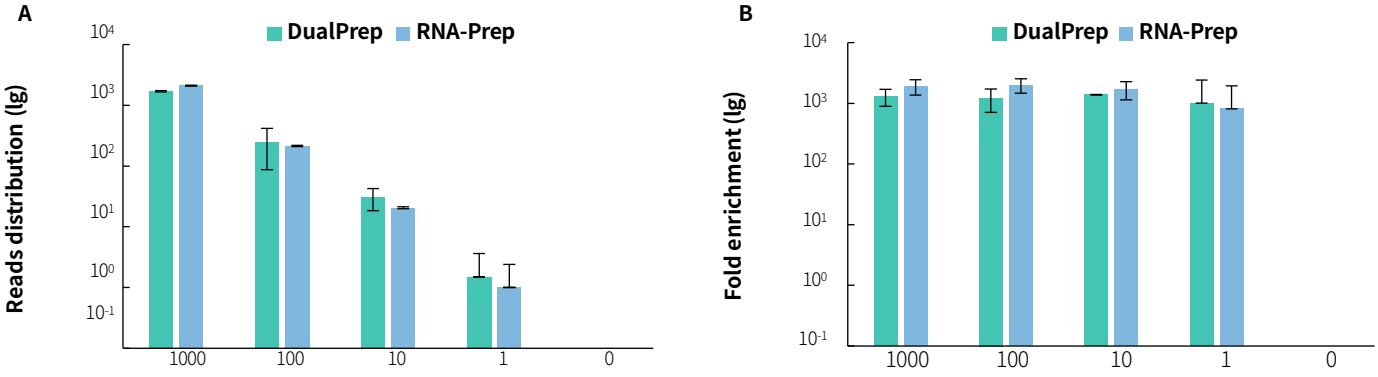


Figure 4.Capture performance of mixed RNA samples in varying copy numbers with dual preparation and RNA-preparation.

A. Reads distribution; **B.** Enrichment fold.

Note: The X-axis represents the copy number of the Reference material for SARS-CoV-2 Omicron Variant Genomic RNA. The samples are derived from a mixture of Human Brain Total RNA Standard (Clontech, 636530) and Omicron virus samples in varying copy numbers.

	DualPrep Actual coverage depth after removing duplication					DNA-Prep Actual coverage depth after removing duplication					Theoretical copy number				
	1%	0.10%	0.01%	0.001%	0%	1%	0.10%	0.01%	0.001%	0%	1%	0.10%	0.01%	0.001%	0%
Bifidobacterium_adolescentis	5	0	0	0	0	6	1	0	0	0	15	2	0	0	0
Deinococcus_radiodurans	6	0	0	0	0	6	0	0	0	0	15	2	0	0	0
Enterococcus_faecalis	1	0	0	0	0	2	0	0	0	0	15	2	0	0	0
Phocaeicola_vulgatus	6	0	0	0	0	5	0	0	0	0	15	2	0	0	0
Schaalia_odontolytica	4	1	0	0	0	2	0	0	0	0	15	2	0	0	0
Acinetobacter_baumannii	18	2	0	0	0	14	2	0	0	0	135	14	2	0	0
Cutibacterium_acnes	58	9	2	4	3	44	4	2	2	1	135	14	2	0	0
Helicobacter_pylori	41	6	0	0	0	42	3	0	0	0	135	14	2	0	0
Lactobacillus_gasseri	41	3	0	0	0	35	3	0	0	0	135	14	2	0	0
Neisseria_meningitidis	39	4	1	0	0	32	3	0	0	0	135	14	2	0	0
Bacillus_pacificus	473	47	5	0	0	469	37	6	0	0	1347	135	14	2	0
Clostridium_beijerinckii	497	52	5	0	0	592	52	6	1	0	1347	135	14	2	0
Pseudomonas_aeruginosa	455	24	4	0	0	272	22	4	0	0	1347	135	14	2	0
Staphylococcus_aureus	438	51	5	1	0	465	56	8	1	0	1347	135	14	2	0
Streptococcus_agalactiae	135	16	1	0	0	140	9	2	0	0	1347	135	14	2	0
Cereibacter_sphaeroides	5792	263	78	5	0	4809	492	67	3	0	13463	1347	135	14	0
Escherichia_coli	4510	413	57	5	0	3475	357	50	4	0	13463	1347	135	14	0
Porphyromonas_gingivalis	4372	556	52	5	0	2798	289	54	2	0	13463	1347	135	14	0
Staphylococcus_epidermidis	2455	289	29	4	0	2384	282	41	4	0	13463	1347	135	14	0
Streptococcus_mutans	2713	354	59	3	0	2307	222	31	2	0	13463	1347	135	14	0

Figure 5. Captured coverage depth after duplication removal for samples with different microbial contents with DualPrep and DNA-preparation.

The simulated microbial community samples were prepared using the LeXPrep RNA & DNA Library DualPrep Module (DualPrep) and LeXPrep EZ DNA Library Preparation Module v2 (DNA-Prep), in conjunction with LeXPrep Universal Stubby Adapter (UDI) Adapter for library preparation, followed by hybrid capture using LeXPrep ES Hybrid Capture Reagents and the NEX-t Panel v1.0.

Note: Simulated microbial community samples of 0.001% - 1% MSA-1003 were created by diluting a mixture of 20 strains of genomic material (ATCC, MSA-1003) using the Human Genomic DNA Standard (Promega, G1471) at various ratios. Sequencing platform: Illumina NovaSeq 6000, PE150.

Low GC Bias

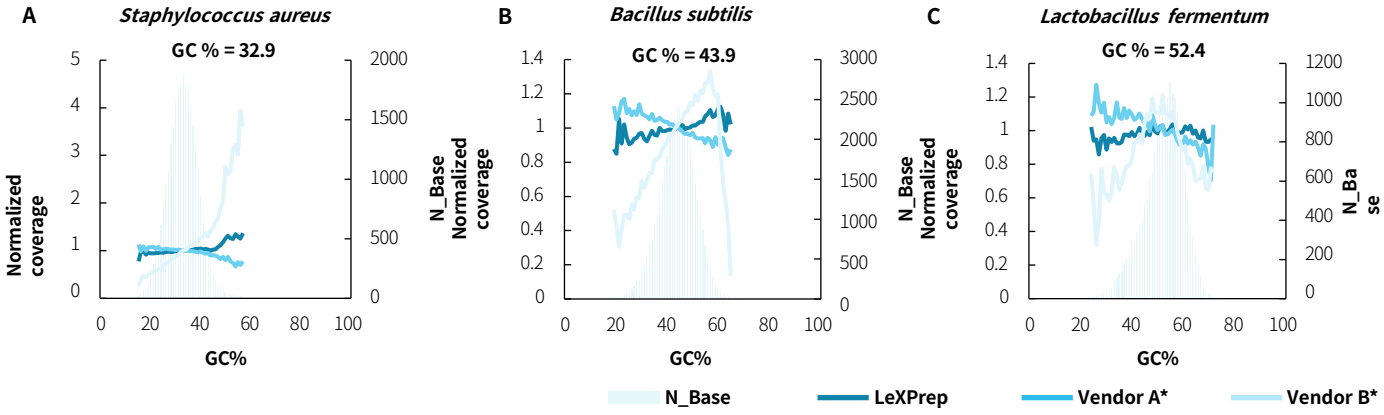


Figure 6. Uniform coverage of regions with various GC content using LeXPrep RNA & DNA Library DualPrep Module. A. *Staphylococcus aureus,* **B.** *Bacillus subtilis,* and **C.** *Lactobacillus fermentum* with different GC content.

Application Example in Clinical Sample

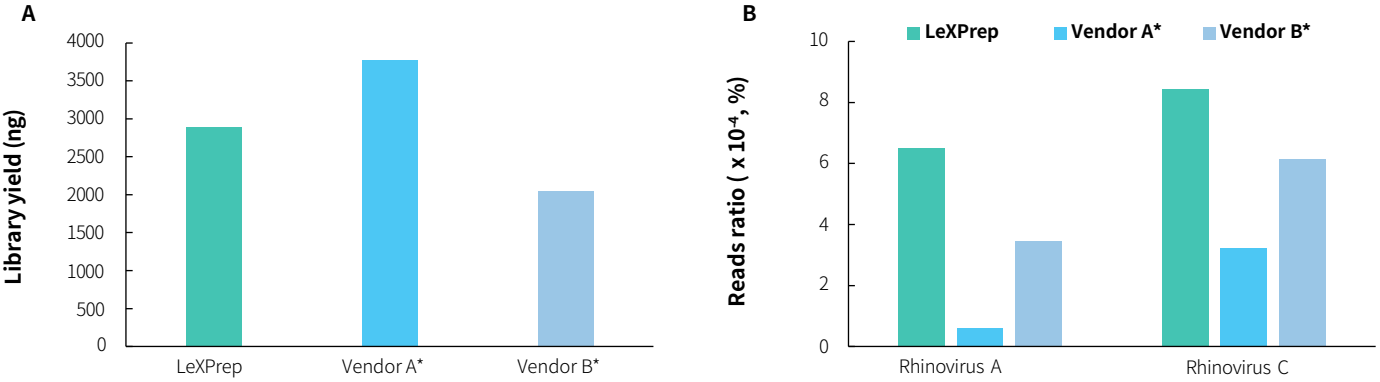


Figure 7.Detection performance of tNGS Total Solution for clinical samples with LeXPrep RNA & DNA Library DualPrep Module.
A. Library yield; **B**.Reads ratio of Rhinovirus A and Rhinovirus C.

Note: The samples were derived from 50 ng of clinical rhinovirus nucleic acid samples. Sequencing platform: Illumina NovaSeq 6000, PE150.

Ordering Information

Product	Catalog#
LeXPrep RNA & DNA Library DualPrep Module, 24 rxn	LX02411
LeXPrep RNA & DNA Library DualPrep Module, 96 rxn	LX02412

Statement

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