

### Pathogenic Microorganism Detection

mNGS

TransGen, To Achieve Life Science Dreams

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### **Library Preparation**

TransNGS® Microbiome DNA Library Prep Kit for Illumina®(Cat. No. KP141) TransNGS® Microbiome RNA Library Prep Kit for Illumina® TransNGS® Microbiome DNA/RNA Library Prep Kit for Illumina® Pathogenic microorganisms can invade the human body and cause infectious diseases or epidemic, which have been attracting clinical attention. The global pandemic of COVID-19 has brought severe challenges to the world economy and human health, demanding for accurate and rapid detection of pathogens an urgent social need.

Metagenomics next-generation sequencing (mNGS) is a laboratory diagnostic technology based on highthroughput sequencing technology to sequence all biological genomes in various clinical samples. Compared with other common microbial identification methods, it has the advantage of high throughput and can detect tens of thousands of microorganisms at the same time. In recent years, it has been gradually applied to the diagnosis of various infectious diseases and has played an important role in the etiological diagnosis of unexplained critical reinfection and pathogen discovery of emerging infectious diseases.

Based on the high-throughput advantage of underlying sequencing technology, metagenomic NGS (mNGS) can simultaneously obtain sequencing data from all the species of pathogenic microorganisms and compare with special pathogen databases to identify the species of suspected pathogens., providing rapid and accurate diagnosis basis for difficult and critical infections. For the detection of pathogenic microorganisms, the whole solution of upstream and downstream, metagenomic & metagenomic transcriptome library preparation, was developed by TransGen.

#### Identification Methods of Clinical Pathogenic Microorganism

Identification Method	Time	Features
Smear microscopy	1 hour	Low throughput, suitable for a small number of microorganism detection
Isolation culture	2-3 days	Low throughput, and the positive rate is about 20%
Serum immunology	2-5 days	Low throughput, suitable for individual microorganisms, each detection of 1 microorganism
Mass spectrometry identification	2-8 days	Unable to detect unknown pathogens
PCR detection	1 day	One organism at a time and no unknown pathogens can be detected
mNGS detection	2-3 days	High throughput, and tens of thousands of microorganisms are detected simultaneously

#### **TransGen mNGS Integrated Solution**



Preserve viral nucleic acids of nasopharyngeal and oropharyngeal swab samples DNA/RNA extraction from pathogenic microorganisms

Metagenomic & Macrotranscriptome library Suitable for Illumina, MGI and other sequencing platforms Metagenomic & Macrotranscriptome analysis

#### **Our Advantages**



# TRANSGEN

# **Host DNA Depletion**



## TransNGS<sup>®</sup> Host DNA Depletion Kit (Cat. No. EH301)

- Simple and time-saving: simple operation, and the nucleic acid removal of the human host is completed in 50 minutes.
- High-efficiency removal: special lysate can efficiently remove human host nucleic acid and improve the detection rate of microorganisms.
- Wide range of samples: suitable for buccal/throat swabs, sputum, pleural fluid, ascites, cerebrospinal fluid, amniotic fluid and other biological fluid samples.
- · Compatible with a variety of extraction platforms: compatible with column extraction and magnetic bead method and other downstream extraction methods.



#### Effective Depletion of Host Nucleic Acid

TransGen, Company V and Company Q were used to process different samples, and the corresponding the column extraction products of pathogenic microorganism nucleic acid were used to extract nucleic acid, and the host conserved genes were quantitatively detected by aPCR. The results showed that TransGen could effectively remove host nucleic acid, and the removal efficiency was significantly better than that of Company V and Company Q products.

Sputum







Without host DNA depletion Host DNA depletion

Magnetic Bead Column Extraction Method Method

Cq value

value

ð





Different samples were processed with TransGen products, and nucleic acids were extracted with column extraction method and magnetic bead method, and the extracted nucleic acids were quantitatively detected by qPCR for host conserved genes. The results showed that the TransGen product was compatible with downstream extraction methods and could effectively remove host nucleic acid.

Applicable to the analysis of library construction, improving the level of microbial detection



The products extracted from the throat swab, sputum, ascites and pleural fluid after the removal of host nucleic acid were used as samples for library construction and PE150 sequencing analysis. The results showed that TransGen products could significantly reduce the proportion of host sequence.



The products extracted from throat swabs before and after removal of host nucleic acid were used as samples for library construction and PE150 sequencing analysis with TransGen, Company V and Company Q products. The results showed that TransGen products could significantly improve microbial detection rate.

# TRANSGEN

# **Nucleic Acid Extraction**



#### **Extraction Flow Chart**



# 01

## EasyPure<sup>®</sup> Microbiome DNA Isolation Kit (Cat. No. EE401)

- The extracted nucleic acid is of high quality and meets a variety of downstream testing needs.
- Strictly control the contaminations of background bacteria in reagents to reduce the risk of false positives.
- Suitable for microbial nucleic acid extraction from the throat swab, sputum, ascites and pleural fluid samples.

#### Efficient Extraction of Multiple Samples





TransGen - High input
 Company V - High input
 Company Q - High input
 TransGen - Low input
 Company V- Low input
 Company Q - Low input



TransGen - High input
 Company V - High input
 Company Q - High input
 TransGen - Low input
 Company V- Low input
 Company Q - Low input



Sputum

Ascites



TransGen - High input
 Company V - High input
 Company Q - High input
 TransGen - Low input
 Company V- Low input
 Company Q - Low input

High and low initiation volume of different bacteria diluents were added to throat swabs, sputum, pleural effusion and ascites samples, and nucleic acid was extracted using TransGen, Company V and Company Q products, respectively. qPCR was used to detect the extraction effect. The results showed that the extraction efficiency of TransGen products for different microorganisms in different samples was better than competing products.

# 02

## MagicPure<sup>®</sup> Microbiome DNA Isolation Kit (Cat. No. EC107)

- The extracted nucleic acid is of high quality and meets a variety of downstream detection requirements.
- Strictly control the contaminations of background bacteria in reagents to reduce the risk of false positives.
- Suitable for microbial nucleic acid extraction from the throat swab, sputum, ascites and pleural fluid samples.
- Suitable for high-throughput magnetic-rod nucleic acid extractor.

#### Efficient Extraction of Multiple Samples

#### Throat Swab





- Magnetic bead method: Low input
- Column extraction method:
- Column extraction method:



- Magnetic bead method:
  High input
  Magnetic bead method:
- Low input
- Column extraction method High input
- Column extraction method: Low input



Sputum



- Magnetic bead method: High input Magnetic bead method:
- Low input
  Column extraction method:
- High input Column extraction method: Low input





- Magnetic bead method: Hiah input
- High input Magnetic bead method: Low input
- Column extraction method: High input
- Column extraction method: Low input

The high and low initiation volume of different bacteria diluents were added to the throat swab, sputum, ascites and pleural fluid samples. The nucleic acid was extracted using the TransGen column-based method and magnetic bead method, and the extraction effect was detected by qPCR. The results showed that both TransGen magnetic bead method and column based method can efficiently extract samples with high and low initiation volume.

## EasyPure<sup>®</sup> Viral DNA/RNA Kit (Cat. No. ER201)

- Unique lysis buffer , specifically binding to a silica-based spin column, efficient purification of viral DNA/RNA.
- High yield, high purity.
- Suitable for viral DNA/RNA extraction from a variety of samples such as whole blood, swabs and cell supernatants.

#### Efficient extraction





The RNA of the SARS-CoV-2 pseudovirus was extracted using TransGen and Company TH products, and the ORF1ab and N genes were detected by qRT-PCR. The results showed

that the TransGen product had high efficiency in nucleic acid extraction.



## MagicPure<sup>®</sup> Fly 96 Viral DNA/RNA Kit (Cat. No. EC331-96-11)

- Easy to operate, only one rinse.
- High sensitivity, high virus detection rate.
- Fast, 14 minutes to complete.
- Suitable for viral DNA/RNA extraction from serum, swabs and cell supernatant samples.
- Specific adsorption and purification of viral DNA/RNA using silicon-based magnetic beads, suitable for a variety of 96-channel automated nucleic acid extractor.

#### **Efficient Extraction**



SARS-CoV-2 pseudovirus diluted by 10<sup>4</sup> and 10<sup>6</sup> times were used as templates. The nucleic acid was extracted using TransGen EC331-96 and EC311-96 products, and the extraction

effect was detected by qPCR (TransGen, AQ322). The results showed that the yield amount of EC331-96 was higher.

# TRANSGEN

## **Library Preparation**



## TransNGS<sup>®</sup> Microbiome DNA Library Prep Kit for Illumina® (Cat. No. KP141)

- Fast: no more than 90 minutes.
- Easy to operate: single-tube enzymatic reaction, Simultaneous DNA fragmentation and adapter ligation.
- Wide compatibility: suitable for a variety of DNA template library construction requirements.
- Low input: as low as 1 ng.

#### Schematic Diagram of the Metagenome Library Preparation



#### Library Preparation Profiles from Various Samples



TransGen and Company V products were used for library construction using 1 ng of genome samples from oropharyngeal swabs, sputum, and blood, respectively.

#### Library Preparation of Artificial Clinical Specimens



1:100 pg Bacillus DNA+100 pg Staphylococcus DNA+1 ng Homo DNA; 2:10 pg Bacillus DNA+10 pg Staphylococcus DNA+1 ng Homo DNA; 3:10 pg Bacillus DNA+10 pg Staphylococcus DNA+1 ng Homo DNA; 4:100 pg Bacillus DNA+10 pg Staphylococcus DNA+1 ng Homo DNA;

TransGen and Company V products were used to construct the library with artificial clinical pathogenic microorganism specimens vitro, and the results showed that the library

detection rate of TransGen products was closer to the ratio of artificial specimens.

02

## TransNGS<sup>®</sup> Microbiome RNA Library Prep Kit for Illumina<sup>®</sup>



#### Multi-sample Library Preparation Peak Type

- Simple process: library , No need of cDNA twostrand synthesis.
- Low sample volume: as low as 1 ng.
- Good compatibility rRNA removal step.







TransGen products were used to construct the library using 1 ng, 10 ng, and 100 ng initial volumn of Hela cell RNA, respectively.

The results showed that the TransGen product could efficiently construct the library for the initial samples of 1-100 ng.



## TransNGS® Microbiome DNA/RNA Library Prep Kit for Illumina®

- Cost-effective: complete DNA & RNA library construction simultaniously, reducing sequencing costs.
- Display the overall condition of microbiota: the results of metagenomic and macrotranscriptome sequencing are obtained simultaniously, showing the overall condition of the microbiota and the interaction with the body.
- Simple process: one-step DNA and RNA library preparation in one step to save time.

#### Flow Chart of DNA and RNA Library Preparation

#### Strategy 1



#### Strategy 2



#### Library Preparation with Different Initiation Volume



Sample: Arabidopsis DNA(100 ng) + E.Coli DNA(1 ng) + Hela RNA(100 ng) + Xanthomonas RNA(100 ng) + PRRSV RNA (100 ng) + BVDV RNA (100 ng)



Sample . Arabidopsis DNA(100 ng) + *E.Coli* DNA(1 ng) + Hela RNA(100 ng) + Xanthomonas RNA(10 ng) + PRRSV RNA (10 ng) + BVDV RNA (10 ng)



Sample : Arabidopsis DNA(100 ng) + *E.Coli* DNA(1 ng) + Hela RNA(100 ng) + Xanthomonas RNA(1ng) + PRRSV RNA (1 ng) + BVDV RNA (1 ng)

DNA and RNA mixtures from different sources were used as samples for library preparation using TransGen and Company V products, respectively, and PE150 sequencing was performed on the Illumina HiSeq X10 platform. The results showed that the sensitivity of the TransGen product was higher with different initiation volume of DNA&RNA.

Туре	Product Name	Cat. No.
Host DNA Depletion	TransNGS® Host DNA Depletion Kit	EH301
Nucleic Acid Extraction	EasyPure® Microbiome DNA Isolation Kit	EE401
	MagicPure® Microbiome DNA Isolation Kit	EC107
	EasyPure® Viral DNA/RNA Kit	ER201
	MagicPure® Fly 96 Viral DNA/RNA Kit	EC331-96-11
Library Preparation	TransNGS® Microbiome DNA Library Prep Kit for Illumina®	KP141
	TransNGS® Microbiome RNA Library Prep Kit for Illumina®	-
	TransNGS® Microbiome DNA/RNA Library Prep Kit for Illumina®	-



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