Health Gene Tech (HGT) tNGS Solution Compatible with MGI's DNBSEQ Platform Enables Detection of Pathogens and Drug Resistance Genes

Performance Evaluation for HGT AccuGen Pathogen Multiplex-PCR Library Prep Kit on DNBSEQ Platform

A tNGS solution composed of HGT AccuGen Pathogen Multiplex-PCR Library Prep Kit,DNBSEQ sequencing platforms (DNBSEQ-E25/DNBSEQ-G50), and AccuGen Rapid Identification of Pathogen Microorganisms Software was evaluated to achieve accurate identification of the composition and abundance of pathogens, drug resistance genes in both standard and real samples. The results exhibited high repeatability and consistency across different sequencers, validating the precision and effectiveness of the combined solution.

Recommended application: Pathogens & drug resistance genes detection

Recommended models: DNBSEQ-E25, DNBSEQ-G50, DNBSEQ-G99, DNBSEQ-G400

- ### Detect multi-pathogen & resistance gene and offer customized panel

  AccuGen Pathogen Multiplex-PCR Library Prep Kit can identify clinically common 330 pathogens and 150 drug resistance genes, and it supports panel customization(can reach ≥ 5000 ×) to precisely identify low-abundance and similar bacterial strains.

- ### AccuGen Rapid Identification of Pathogen Microorganisms Software can be be installed in DNBSEQ platform

  HGT AccuGen Rapid Identification of Pathogen Microorganisms Software offer seamless integration for analysis and report output, and can be installed in DNBSEQ-E25RS.

- ### The combined solution show high detection efficiency

  This combined solution can facilitate the rapid identification of pathogens, in which the process from sample extraction to report generation within about 11 hours.

- ### Automatic operation compatible

  MGI provides automatic solutions for experiment process, which can significantly save labor cost and improve efficiency.

Background

In recent years, infectious diseases, caused by a range of microorganisms including bacteria, viruses, fungi, and parasites, have emerged as a significant threat to human health, contributing to a substantial global disease burden[1]. Therefore, it's very urgent to invent a cost-effective and efficient detection method that can quickly identify pathogens and detect drug resistance genes.

Nucleic acid detection technology offers a more efficient and sensitive alternative to traditional microbial culture methods for pathogen identification. While 16S rRNA PCR is effective for detecting most types of bacteria, it is uncapable of identifying fungi or microbial co-infection and can't quantitatively identify infectious bacteria[2]. Metagenomics next-generation sequencing (mNGS) surpasses the limitations of 16S rRNA PCR and could detect both known and unknown pathogens, offering comprehensive quantitative results for the identification of multiple microorganisms[3]. However, mNGS faces challenges due to unbiased sampling, which includes human genome and thus increase costs and reduce sensitivity for pathogen or drug resistance gene detection[4]. Moreover, it can't conduct DNA and RNA dual process detection simultaneously[5].

The targeted next-generation sequencing (tNGS) based on multiplex PCR technology, uses the primer panel designed for specific sequences of certain pathogens to amplify targeted genes and then obtains nucleic acid information through high-throughput sequencing, identifying pathogens and providing quantitative results via bioinformatic analysis. This method eliminates human genome interference and allows for simultaneous DNA and RNA detection. It is more cost-effective and sensitive than mNGS and offers customization. At present, this technology has been used in many settings for diagnosing various infections including lung and brain, or in detecting drug resistance genes, showcasing its clinical potential[6,7,8,9].

This study used HGT AccuGen Pathogen Multiplex-PCR Library Prep Kit and MGI DNBSEQ-E25 sequencing platform for library preparation and sequencing, respectively. Afterwards, AccuGen Rapid Identification of Pathogen Microorganisms Software was used to map the sequencing data with the pathogen database for analysis. The results suggested that this combined solution could effectively identify pathogens and drug resistance genes, offering quick and accurate diagnosis along with medication guidance for infected patients.

Materials and methods

Sample preparation

The RNA of SARS-CoV-2 and respiratory syncytial virus (RSV) extracted from positive samples were mixed with the ZymoBIOMICS™ Microbial Community DNA Standard (PN: D6306) approximately at the ratio of 5:1 (4 replications, hereinafter referred to as standard samples). Additionally, pathogen nucleic acid was extracted from bronchial alveolar lavage (BAL) fluid of 3 positive samples (hereinafter referred to as real samples) for further verification. All extractions were performed using the AccuGen Pathogen DNA/RNA Kit.

Library preparation and sequencing

The AccuGen Pathogen Multiplex-PCR Library Prep Kit was used for library preparation, detailed operations could be referred to in the related instructional manuals. Sequencing was conducted on DNBSEQ-E25/DNBSEQ-G50 with single-end 50bp (SE50) sequencing strategy.

Bioinformatic analysis

AccuGen pathogenic microorganism rapid detection software (can be installed in DNBSEQ-E25ARS) was used for bioinformatic analysis. The analysis process was as follows: quality control of raw data, sequence alignment via BWA-mem, identification of pathogens and detection of drug resistance genes. This software can perform qualitative and quantitative analysis of microorganisms, and the analysis results are presented in a clear and comprehensible format. A representative example of the report is shown as Figure 1.

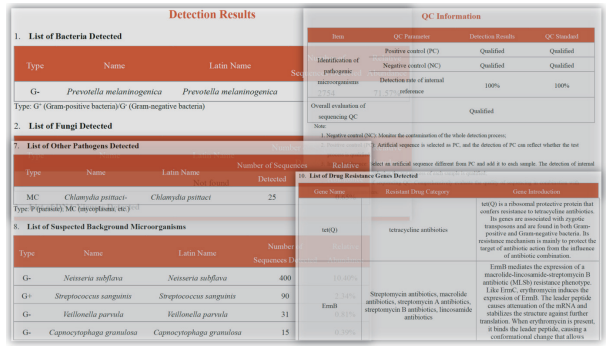

Figure 1. A representative example of the tNGS report.

Sample preparation (~1.5 h)	Library preparation (~6 h)	Sequencing (~3 h)	Data analysis (~0.5 h)
Nucleic acid extraction from blood, bone marrow, alveolar lavage fluid, sputum, urine, cerebrospinal fluid, etc.	AccuGen Pathogen Multiplex-PCR Library Prep kit	DNBSEQ-E25	AccuGen Rapid Identification of Pathogen Microorganisms Software (Can be installed in DNBSEQ-E25)

Results

DNBSEQ-E25 provides high-quality sequencing data for HGT tNGS solution

For standard samples, the libraries prepared from HGT tNGS solution generated 27.75 M reads on DNBSEQ-E25, with high-quality Q30(97.85%) and split rate(95.55%). For real samples, 24.66 M reads were generated, with Q30 at 93.65%, and split rate at 95.86% (Table 1).The sequencing data's quality all meet the requirements of subsequent bioinformatics analysis.

HGT tNGS solution enables co-detection of DNA and RNA

Four standard samples were analyzed and the pathogenic microorganisms' abundance were calculated. The results showed that all pathogens were detected consistently (low SD values) among four samples, with RSV has the highest relative abundance as expected and no drug resistance genes detected (Table 2, Figure 3).

Conclusively, this combined solution is of good repeatability and is capable of co-detecting both DNA and RNA.

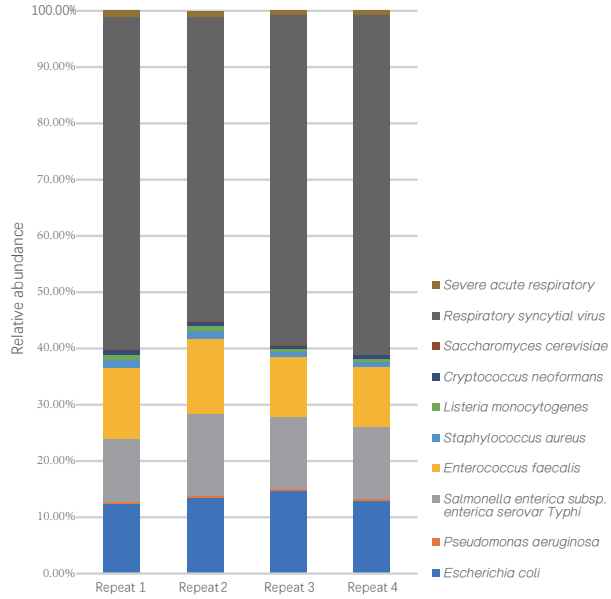

Figure 2. Relative abundance of the pathogenic microorganisms detected in the four repeated standard samples.

Sample name	Total Reads (M)	Q30(%)	Split Rate(%)
Standard (DNA+RNA)	27.75	97.85	95.55
Bronchial-alveolar lavage fluid	24.66	93.65	95.86

Table 1. Basic sequencing metrics generated by DNBSEQ-E25 with libraries constructed from HGT tNGS solution.

Type	Name	Relative abundance					
		Repeat 1	Repeat 2	Repeat 3	Repeat 4	Average	SD
G-	*Escherichia coli*	12.36%	13.44%	14.73%	12.89%	13.36%	0.010
G-	*Pseudomonas aeruginosa*	0.45%	0.50%	0.34%	0.35%	0.41%	0.001
G-	*Salmonella enterica subsp. enterica serovar Typhi*	11.22%	14.41%	12.79%	12.91%	12.83%	0.013
G+	*Lactobacillus fermentum**	--	--	--	--	--	--
G+	*Enterococcus faecalis*	12.50%	13.27%	10.76%	10.56%	11.77%	0.013
G+	*Staphylococcus aureus*	1.43%	1.56%	0.76%	0.88%	1.16%	0.004
G+	*Listeria monocytogenes*	0.94%	0.86%	0.58%	0.60%	0.75%	0.002
G+	*Bacillus subtilis**	--	--	--	--	--	--
F	*Cryptococcus neoformans*	0.90%	0.78%	0.55%	0.62%	0.71%	0.002
F	*Saccharomyces cerevisiae*	0.17%	0.12%	0.10%	0.10%	0.12%	0.000
V	*Respiratory syncytial virus***	58.92%	54.00%	58.66%	60.35%	57.98%	0.028
V	*Severe acute respiratory***	1.11%	1.05%	0.72%	0.74%	0.91%	0.002

Table 2. Abundance of microorganisms detected in each repeated sample. * : the species is not covered by the primer design, ** : the viruses are added to the standard, G+: gram-positive bacteria, G-: gram-negative bacteria, F: fungi, V: virus, MC: mycoplasma, etc.

MGI DNBSEQ sequencing platform can be perfectly compatible with HGT tNGS solution

Afterwards, this study tested 3 positive samples(tNGS-5-d, tNGS-11-d, and S0312-2), further verified that HGT tNGS solution combined with DNBSEQ-E25 or DNBSEQ-G50 could effectively detect the fungi, bacteria, viruses and mycoplasmas in the real samples (Table 3, 4 and Figure 3A, B, C). The pathogen abundances identified by DNBSEQ-G50 were highly consistent with DNBSEQ-E25 and Vendor X (R2 >0.99, Figures 3D, E,F), further validating the accuracy of pathogen detection across different DNBSEQ platforms with the tNGS solution. Meanwhile, no drug resistance genes were detected in these samples.

Sample	Type	Name	Relative abundance	
			Vendor X	MGISEQ-200
S0312-2	G-	*Veillonella parvula*	0.48%	0.30%
	G-	*Prevotella melaninogenica*	1.32%	2.77%
	G-	*Prevotella intermedia*	0.76%	1.74%
	G-	*Prevotella denticola*	5.78%	1.86%
	G-	*Prevotella buccae*	0.49%	0.15%
	G-	*Porphyromonas gingivalis*	0.64%	2.23%
	G-	*Neisseria subflava*	0.58%	1.55%
	G-	*Neisseria*	0.14%	0.91%
	G-	*Haemophilus parainfluenzae*	3.02%	5.25%
	G-	*Haemophilus influenzae*	79.61%	80.91%
	G+	*Parvimonas micra*	2.07%	1.15%
	G+	*Streptococcus mitis*	0.44%	0.07%
	G+	*Streptococcus anginosus*	1.22%	0.23%
	G+	*Streptococcus pneumoniae*	3.14%	0.74%
	G+	*Streptococcus sanguinis*	0.30%	0.14%

Table 3. Relative abundance of the pathogens detected in S0312-2.

Sample	Type	Name	Relative abundance	
			DNBSEQ-E25	MGISEQ-200
tNGS-5-d	G-	*Prevotella melaninogenica*	71.57%	73.57%
	G-	*Neisseria subflava*	10.40%	9.25%
	G-	*Veillonella parvula*	0.81%	1.03%
	G-	*Capnocytophaga granulosa*	0.39%	0.52%
	G-	*Veillonella dispar*	10.68%	9.40%
	G-	*Campylobacter concisus*	0.57%	0.58%
	G-	*Neisseria flavescens*	0.47%	0.58%
	G-	*Capnocytophaga gingivalis*	0.13%	-
	G+	*Streptococcus sanguinis*	2.34%	1.54%
	G+	*Cutibacterium acnes*	1.40%	1.64%
	G+	*Schaalia odontolytica*	0.21%	0.64%
	G+	*Streptococcus gordonii*	0.21%	0.17%
	G+	*Staphylococcus capitis*	0.18%	0.25%
	MC	*Chlamydia psittaci*	0.65%	0.83%
tNGS-11-d	G-	*Pseudomonas aeruginosa*	42.67%	39.75%
	G-	*Klebsiella pneumoniae*	6.25%	6.36%
	G-	*Haemophilus influenzae*	2.45%	3.24%
	G-	*Prevotella melaninogenica*	5.53%	7.53%
	G-	*Veillonella parvula*	0.32%	0.27%
	G-	*Prevotella denticola*	0.24%	0.46%
	G-	*Porphyromonas gingivalis*	0.14%	0.08%
	G-	*Prevotella intermedia*	0.07%	0.11%
	G-	*Capnocytophaga gingivalis*	0.03%	0.03%
	G-	*Morganella morganii*	0.55%	0.64%
	G-	*Stenotrophomonas maltophilia*	0.52%	0.37%
	G-	*Prevotella buccae*	0.20%	0.23%
	G-	*Neisseria subflava*	0.04%	0.06%
	G-	*Fusobacterium nucleatum*	0.02%	0.03%
	G-	*Campylobacter concisus*	0.02%	0.02%
	G-	*Prevotella loescheii*	0.01%	0.02%
	G-	*Fusobacterium periodonticum*	0.03%	0.02%
	G+	*Streptococcus pneumoniae*	29.46%	25.37%
	G+	*Corynebacterium diphtheriae*	2.54%	3.71%
	G+	*Streptococcus mitis*	2.44%	2.74%
	G+	*Streptococcus anginosus*	0.41%	0.73%
	G+	*Enterococcus faecalis*	0.08%	0.10%
	G+	*Streptococcus sanguinis*	0.01%	-
	G+	*Schaalia odontolytica*	0.01%	0.01%
	G+	*Parvimonas micra*	5.15%	6.90%
	G+	*Streptococcus salivarius*	0.05%	0.10%
	G+	*Streptococcus intermedius*	0.62%	1.00%
	G+	*Streptococcus constellatus*	0.11%	0.10%
	G+	*Streptococcus milleri*	0.01%	0.01%
	V	*Human betaherpesvirus 7*	0.01%	0.01%

Table 4. Relative abundance of the pathogens detected in tNGS-5-d and tNGS-11-d.

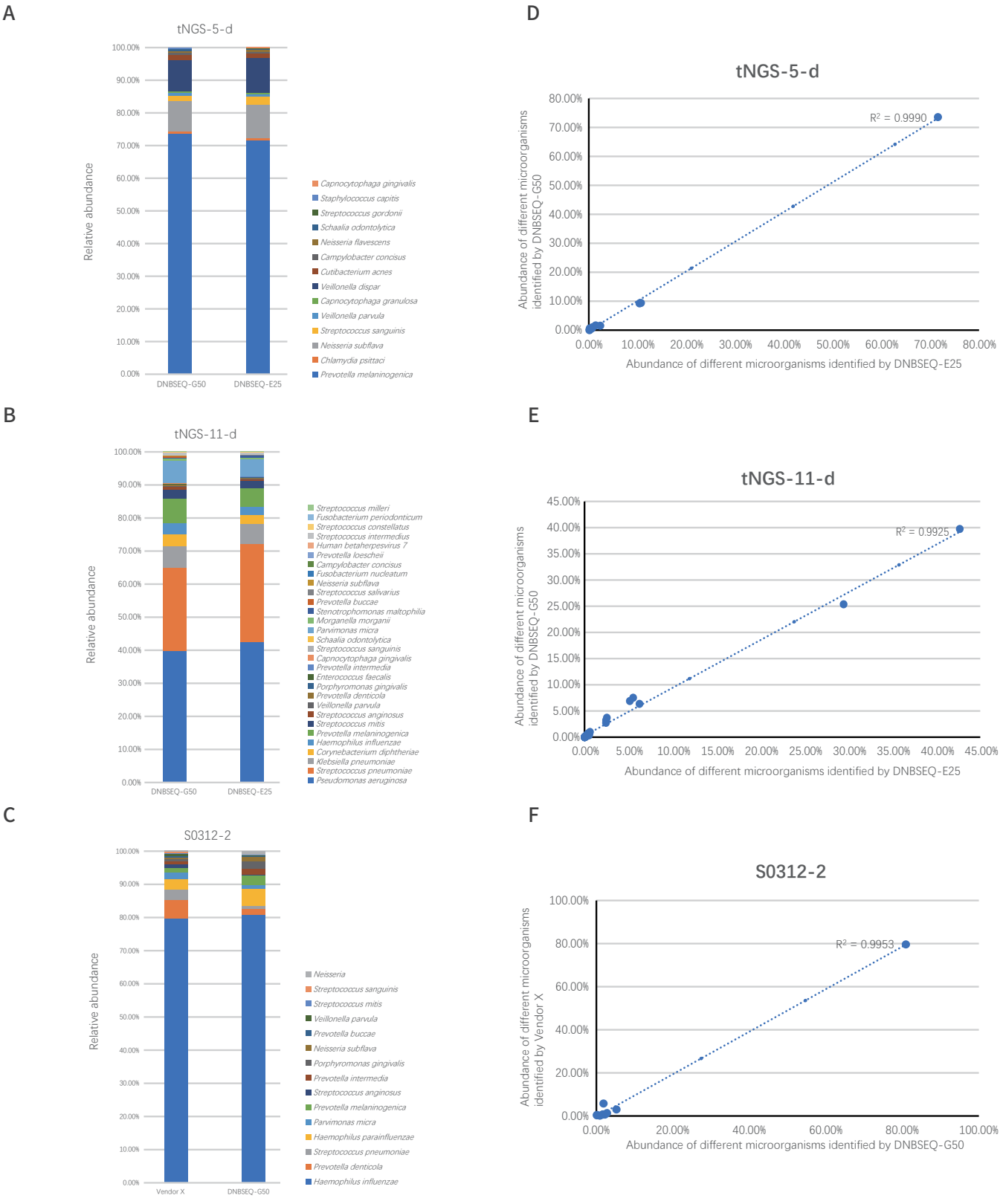

Figure 3. Comparative analysis of microbial composition across different sequencing platforms.

Summary

This study validated the compatibility of the HGT tNGS solution with the DNBSEQ platform using both standard and real positive samples for dual verification. It was found that the combination of HGT tNGS and DNBSEQ-E25 produced high-quality, accurate, and reproducible sequencing data for pathogen identification, with consistent results across different repeats and sequencers. Meanwhile, this combined solution is also applicable to various sample types like blood, bone marrow, sputum, and urine.

DNBSEQ-E25, a compact and lightweight sequencer, is easy to install and maintain, lowering the sequencing barrier and it's well-suited for pathogen detection and small genome sequencing. DNBSEQ-G50 is a flexible desktop sequencer with different hardware configuration options for diverse applications and budgets. It offers different specifications of flow cells and reagent sets for data outputs from 10 Gb to 150 Gb, supporting a wide range of researches and clinical applications such as low-pass whole genome sequencing, targeted sequencing, small whole genome sequencing, RNA sequencing and whole exome sequencing, etc.

Both DNBSEQ-E25 and DNBSEQ-G50 combined with HGT tNGS solution can achieve rapid and precise pathogen detection, which can greatly promote infectious disease research and reduce disease burdens.

Genetic Sequencer DNBSEQ-E25

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Recommended Ordering Information

Category	Product	Cat. NO.
Genetic Sequencer	Genetic Sequencer DNBSEQ-E25RS	900-000537-00
	Genetic Sequencer DNBSEQ-G50RS	900-000353-00
Automated instruments	MGISP-100RS Automated Sample Preparation System	900-000206-00
Software	AccuGen Rapid Identification of Pathogen Microorganisms Software*	/
Extraction reagent	AccuGen Pathogen DNA/RNA extraction Kit（50 RXN)	961-000013-00
Librany Prep reagent	AccuGen Pathogen Multiplex-PCR Librany Prep Kit (96 RXN)	961-000014-00
Sequencing reagent	DNBSEQ-E25RS High-throughput Sequencing Set (FCL SE100)	940-000573-00
	MGISEQ-200RS High-throughput Rapid Sequencing Set(FCS PE100)	1000019846

* The relevant products are available and can be ordered on the Health Gene Tech (HGT) official website (https://www.genebook.com.tw/)

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