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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 burden¹. 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 bacteria². 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 microorganisms³. 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 detection⁴. Moreover, it can't conduct DNA and RNA dual process detection simultaneously⁵.

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.

Detection Results				QC Information				
. List	of Bacteria Detected			Item	QC Parameter	Detection Results	QC Standard	
					Positive control (PC)	Qualified	Qualified	
			e	Identification of mathematic	Negative control (NC)	Qualified	Qualified	
G-	Prevotella melaninoge	votella melaninogenica Prevotella melaninogenica 2754		microorganisms	Detection rate of internal 71.579 reference	100%	100%	
	(Gram-positive bacteria)/G [.] (Gran of Fungi Detected	n-negative bacteria)		Overall evaluation of sequencing QC Note:		Qualified		
List	of Other Pathogens Detected	Latin Name	Number	2. Positive costrol	(NC): Monitor the contamination of the whole d		effect whether the test	
			mber of Sequence Detected	Relative	elect as artificial sequence different from 1 ince Genes Detected	PC and add it to each sam	ple. The detection of inter	
Type MC	Name Chlamydia psittaci-	Nu Latin Name Not found Chlamadia psittaci				Gene	Introduction	
MC		Latin Name Not found	Detected	. List of Drug Resists	nce Genes Detected	Gene tet(Q) is a riboson confers resistance	Introduction ad protective protein f	
MC ype: P (p	Chlamydia psittaci-	Latin Name Not found Chlamydia psittaci	Detected	. List of Drug Resists	nce Genes Detected	Gene tet(Q) is a riboson confers resistance Its genes are a transposons and	Introduction nal protective protein f to terracycline artibiot noceinted with rypotie are found in both Gran	
MC ype: P (p	Chlamydia psittaei- atsiale); MC (mycoplasnia; etc.)1	Latin Name Not found Chlamydia psittaci	Detected 25 25 Number of	List of Drug Resists Gene Name	nee Grines Detected Resistant Drug Category	Gene tet(Q) is a riboson confers resistance Its genes are a transposons and positive and Gra resistance mechanic target of arthbotic	Introduction nal protective protein fi to tetracycline antibion sociated with zygotic	
MC ype:P(pi	Chlamydia psittaci- atsiale) MC (nycoplasini, etc.) ed of Suspected Background Mice	Latin Name Not found Chlamydla psittaci roorganisms	Detected 20 25	List of Drug Resists Gene Name	nee Grines Detected Resistant Drug Category	Gene bet(Q) is a riboson confers resistance Its genes are a positive and Gra resistance mechani taget of antibiotic antibiotic ErmB mediati macrolide-linco antibiotic (MLS)	Introduction all protective protein it to terrosychine embios sociated with ergotie arr found in both Gran menegative bacteris. It arrite combination is mainly to protect action from the influe is combination. Is the expression of a samide-streptomychin beresistance otherotyce	
MC ype: Pipi List Type	Chlomydia prittact- arskie), MC (riscoptachi, itc) ^{eed} of Suspected Background Mict Name	Latin Name Not found Chlawydia psttaet roorganisms Latin Name	Detected 25 25 Number of Sequences De	 List of Drug Resists Gene Name tel(Q) rel(P) 10.40% 2.34% 	Resident Drug Cargory Resident Drug Cargory Intracycline antibiotics Streptomycia antibiotics, macroide	Gene tet(Q) is a ribotom confere resistance Its spees are a transposons and positive and Gra resistance mechanis target of antibiet of antibiet EmmB mediatu macoidde-linco antibiotis (MLS) Likk Ernot, eng expression of Er	Introduction and protective protein fit to intracyclina antibios are found in both Gean menegative basteria. In sociated with protect aution from the influe discontinuitors. In the expression of a similal-extreptorycini thromycin induces the fit of the state period	
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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.

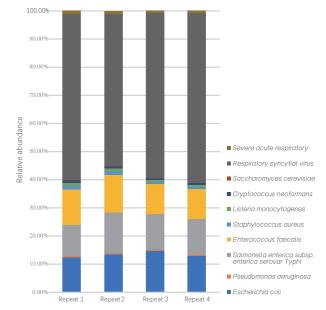


Figure 2. Relative abundance of the pathogenic microorganisms detected in the four repeated standard samples.

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.

Tunc	Nama	Relative abundance					
Туре	Name	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.

Constant	T	Marria	Relative abundance		
Sample	Туре	Name	Vendor X	MGISEQ-200	
	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%	
S0312-2	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%	

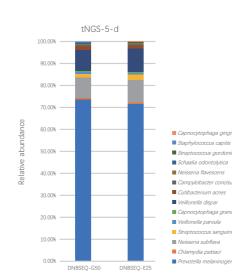
Table 3. Relative abundance of the pathogens detected in S0312-2.

Sample Type Name DNBSEQ-E25 MGISEQ-200 G- Prevotella melaninogenica 71.57% 73.57% G- Neisseria subflava 10.40% 9.25% G- Veillonella parvula 0.81% 10.03% G- Capnocytophaga granulosa 0.39% 0.52% G- Capnocytophaga granulosa 0.39% 0.52% G- Capnocytophaga granulosa 0.47% 0.58% G- Campylobacter concisus 0.47% 0.58% G- Capnocytophaga granulosa 0.47% 0.58% G- Capnocytophaga granulosa 0.21% 0.44% G+ Streptococcus gardonii 0.21% 0.44% G+ Schaalia odontolytica 0.21% 0.44% G+ Schaphylococcus apritis 0.18% 0.25% MC Chlamydia psittaci 0.45% 0.83% G- Prevotella melaninogenica 5.53% 7.53% G- Prevotella previnoa 5.53% 7.53% G-				Relative al	oundance
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		G+	Streptococcus constellatus	0.11%	0.10%
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		V	Human betaherpesvirus 7	0.01%	0.01%

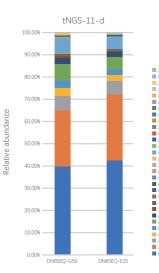
Table 4. Relative abundance of the pathogens detected in tNGS-5-d and tNGS-11-d.

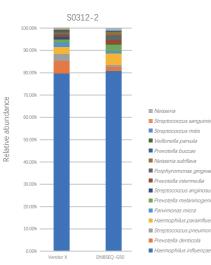
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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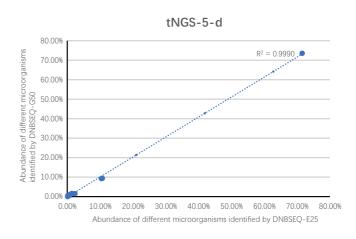


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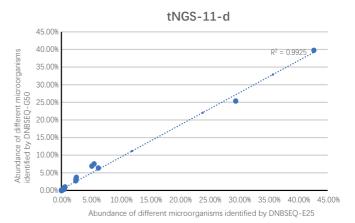






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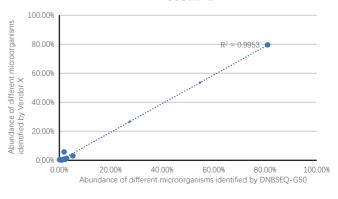


Figure 3. Comparative analysis of microbial composition across different sequencing platforms.

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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.
Constin Serverser	Genetic Sequencer DNBSEQ-E25RS	900-000537-00
Genetic Sequencer	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 reacent	DNBSEQ-E25RS High-throughput Sequencing Set (FCL SE100)	940-000573-00
Sequencing reagent	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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