Health Gene Tech (HGT) Analysis Software Combined with DNBSEQ Sequencing Platform Enables the Accurate Detection of *BRCA1/2* Variants

This application note shows the perfect compatibility of the HGT bioinformatics platform with the MGI DNBSEQ sequencing platform, ensuring accurate identification of *BRCA1/2* variants and their pathogenicity.

Recommended application: Cancer Genomics (*BRCA1/2* detection)
Recommended models: DNBSEQ-E25, DNBSEQ-G99, DNBSEQ-G400

- **Data output is efficient and high-quality**

  DNBSEQ sequencing technology has excellent features such as high accuracy, low duplication rate and low index hopping rate.

- **Perfect compatibility with DNBSEQ sequencing platform**

  The HGT analysis software can swiftly analyze sequencing data from the DNBSEQ platform in ~1 hour.

- **Automatic operation compatible**

  MGI offers automated solutions that significantly reduce labor costs and enhance efficiency.

Background

The proteins encoded by *BRCA1/2* play crucial roles in damaged DNA repair, cell cycle control, and chromosome stability maintenance[1], closely related to the pathogenesis of breast and ovarian cancers[2]. *BRCA1/2* mutation is also related to the increased risk of various cancers such as ovarian, prostate and pancreatic cancer[3,4]. Cancer cells harboring deleterious *BRCA1/2* mutations are highly sensitive to DNA-damaging agents, such as DNA interstrand crosslinking agents (e.g.: platinum or alkylating agents), topo-isomerase II inhibitors (e.g.: anthracyclines) or PARP inhibitors[5,6,7]. Those agents might be utilized as targeted therapeutic agents for cancer treatment[8]. The detection of *BRCA1/2* mutations is of paramount clinical importance for cancer risk assessment, early diagnosis, and personalized medicine.

Health Gene Tech (HGT) has developed a bioinformatics analysis solution for *BRCA1/2* mutation detection. By mapping sequencing data to clinical information databases (ClinVar and InterVar)[9], this solution is able to analyze and annotate different variants, and grade the variants according to ACMG (American College of Medical Genetics and Genomics) classification standard, to provide a scientific basis for clinical decision-making. The DNBSEQ sequencing platform developed by MGI has the advantages of high accuracy and sensitivity, ultra-low duplication rate and low index hopping rate. The mainstream sequencers include DNBSEQ-G99, DNBSEQ-G400, DNBSEQ-T7, etc., which can meet the research needs of medical science, scientific research, public health, food safety and other related fields.

To test the compatibility of this bioinformatics analysis solution to DNBSEQ sequencing platform, an anonymous *BRCA1/2* library preparation kit and DNBSEQ-E25/DNBSEQ-G99/DNBSEQ-G400 were used in this test, followed by analysis with the HGT solution. The results showed that the high-quality sequencing data yielded on DNBSEQ sequencing platform can be perfectly analyzed with the HGT solution, generating precise identification of the *BRCA1/2* variants and pathogenicity. This combined solution offers robust support for cancer research, particularly breast and ovarian cancer.

Materials and methods

Sample preparation

This study utilized HD793 and HD795 DNA standards purchased from Horizon Discovery, covering 13 verified germline and somatic mutations in *BRCA1* and *BRCA2* (Tables 2 and 3).

Library preparation and sequencing

An anonymous *BRCA1/2* library preparation kit was used for library preparation. Libraries were sequenced on DNBSEQ-E25 / DNBSEQ-G99 / DNBSEQ-G400 with paired-end 150bp (PE150) sequencing strategy.

Bioinformatics analysis

In this study, HGT software was used for bioinformatics analysis, enabling direct generation of clinical reports from sequencing data (Figure 1). The analysis process is as follows: uploading FASTQ files to HGT platform; secondary analysis (alignment and variant calling) and tertiary analysis (variant annotation and interpretation). Finally, variations are classified as pathogenic variants (P), likely pathogenic variants (LP), benign variants (B), likely benign variants (LB) and variants of unknown significance (VUS) according to ACMG guidelines, and associated clinical drugs were also listed.

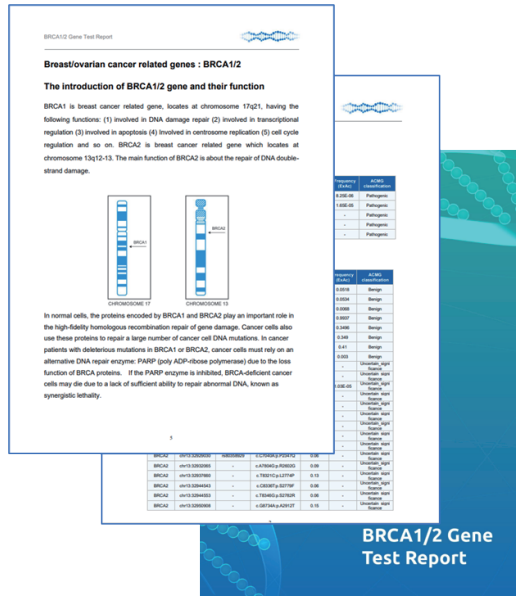
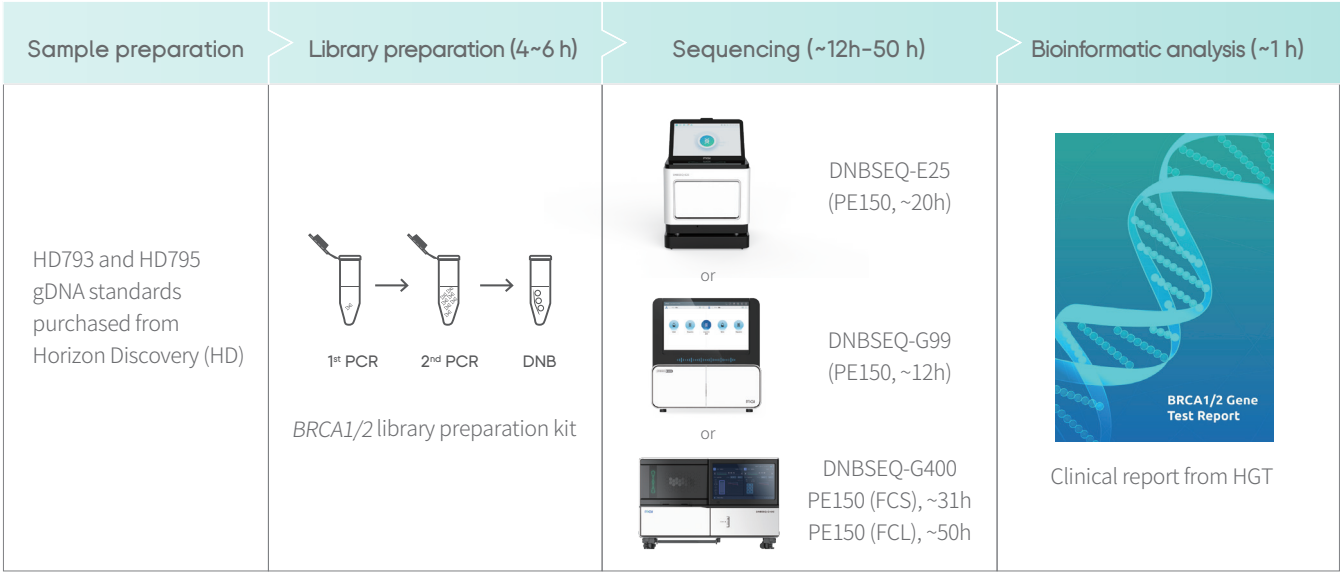


Figure 1. A representative example of the HGT *BRCA1/2* gene test report.



Results

DNBSEQ platform generates high-quality data

High sequencing quality is crucial for the accurate detection of the *BRCA1/2* genes. In this test, DNBSEQ-E25/DNBSEQ-G99/DNBSEQ-G400 generated high quality data with Q30 >90%, 'mapping rates' >99% and 'target rates'>96%, all of which meet the subsequent bioinformatics requirements.

The HGT solution can accurately detect *BRCA1/2* variants

The obtained sequencing data was analyzed by the HGT software. 13 known *BRCA1/2* mutations were all successfully identified by DNBSEQ-E25, DNBSEQ-G99 and DNBSEQ-G400 (Table 2, 3). The detected Variant Allele Frequencies (VAF) were all highly consistent with the theoretical values, with a correlation coefficient (R^2) exceeding 0.95 (Figure 2).

Genetic Sequencer	Sample	Clean Q30	Mapping rate	Target rate
DNBSEQ-E25	HD793	96.73%	99.70%	96.10%
	HD795	96.69%	99.63%	97.00%
DNBSEQ-G99	HD793	92.66%	99.86%	96.50%
	HD795	93.04%	99.92%	96.80%
DNBSEQ-G400	HD793	96.86%	99.00%	98.90%
	HD795	97.04%	99.92%	96.80%

Table 1. Basic sequencing metrics of DNBSEQ-E25/DNBSEQ-G99/DNBSEQ-G400.

Gene	GRCh37 coordinates	Coding	Protein	Expected VAF	DNBSEQ-E25 VAF	DNBSEQ-G99 VAF	DNBSEQ-G400 VAF
BRCA1	17:41246245	c.1303G>T	p.Asp435Tyr	50.00%	50.57%	50.41%	33.00%
BRCA1	17:41244000	c.3548A>G	p.Lys1183Arg	50.00%	46.35%	49.14%	53.00%
BRCA1	17:41245090	c.2458A>G	p.Lys820Glu	50.00%	48.49%	49.51%	48.00%
BRCA1	17:41244936	c.2612C>T	p.Pro871Leu	100.00%	99.84%	99.81%	100.00%
BRCA1	17:41234451	c.4327C>T	p.Arg1443Ter	0.00%	0.00%	0.00%	0.00%
BRCA1	17:41223094	c.4837A>G	p.Ser1613Gly	50.00%	58.05%	53.85%	67.00%
BRCA2	13:32912750	c.4258G>T	p.Asp1420Tyr	0.00%	0.00%	0.00%	0.00%
BRCA2	13:32937355	c.8021dup	p.Ile2675AspfsTer6	0.00%	0.00%	0.00%	0.00%
BRCA2	13:32913559	c.5073del	p.Lys1691AsnfsTer15	0.00%	0.00%	0.00%	0.00%
BRCA2	13:32913837	c.5351del	p.Asn1784ThrfsTer7	50.00%	52.78%	48.64%	50.00%
BRCA2	13:32906480	c.865A>C	p.Asn289His	50.00%	56.04%	51.14%	54.00%
BRCA2	13:32911463	c.2971A>G	p.Asn991Asp	50.00%	50.93%	51.93%	50.00%
BRCA2	13:32929387	c.7397T>C	p.Val2466Ala	100.00%	99.80%	99.86%	100.00%

Table 2. Variation detection results of HD793.

Gene	GRCh37 coordinates	Coding	Protein	Expected VAF	DNBSEQ-E25 VAF	DNBSEQ-G99 VAF	DNBSEQ-G400 VAF
BRCA1	17:41246245	c.1303G>T	p.Asp435Tyr	7.50%	8.95%	7.68%	6.50%
BRCA1	17:41244000	c.3548A>G	p.Lys1183Arg	7.50%	7.70%	8.09%	10.00%
BRCA1	17:41245090	c.2458A>G	p.Lys820Glu	7.50%	7.96%	8.25%	6.50%
BRCA1	17:41244936	c.2612C>T	p.Pro871Leu	15.00%	18.03%	18.66%	15.00%
BRCA1	17:41234451	c.4327C>T	p.Arg1443Ter	32.50%	25.28%	19.49%	14.00%
BRCA1	17:41223094	c.4837A>G	p.Ser1613Gly	7.50%	10.02%	9.43%	10.00%
BRCA2	13:32912750	c.4258G>T	p.Asp1420Tyr	32.50%	23.58%	30.09%	27.00%
BRCA2	13:32937355	c.8021dup	p.Ile2675AspfsTer6	10.00%	7.93%	10.10%	10.00%
BRCA2	13:32913559	c.5073del	p.Lys1691AsnfsTer15	32.50%	33.54%	32.76%	33.00%
BRCA2	13:32913837	c.5351del	p.Asn1784ThrfsTer7	40.00%	40.75%	37.85%	42.00%
BRCA2	13:32906480	c.865A>C	p.Asn289His	7.50%	8.43%	7.87%	7.00%
BRCA2	13:32911463	c.2971A>G	p.Asn991Asp	7.50%	8.21%	8.01%	8.00%
BRCA2	13:32929387	c.7397T>C	p.Val2466Ala	100.00%	99.80%	99.84%	100.00%

Table 3. Variation detection results of HD795.

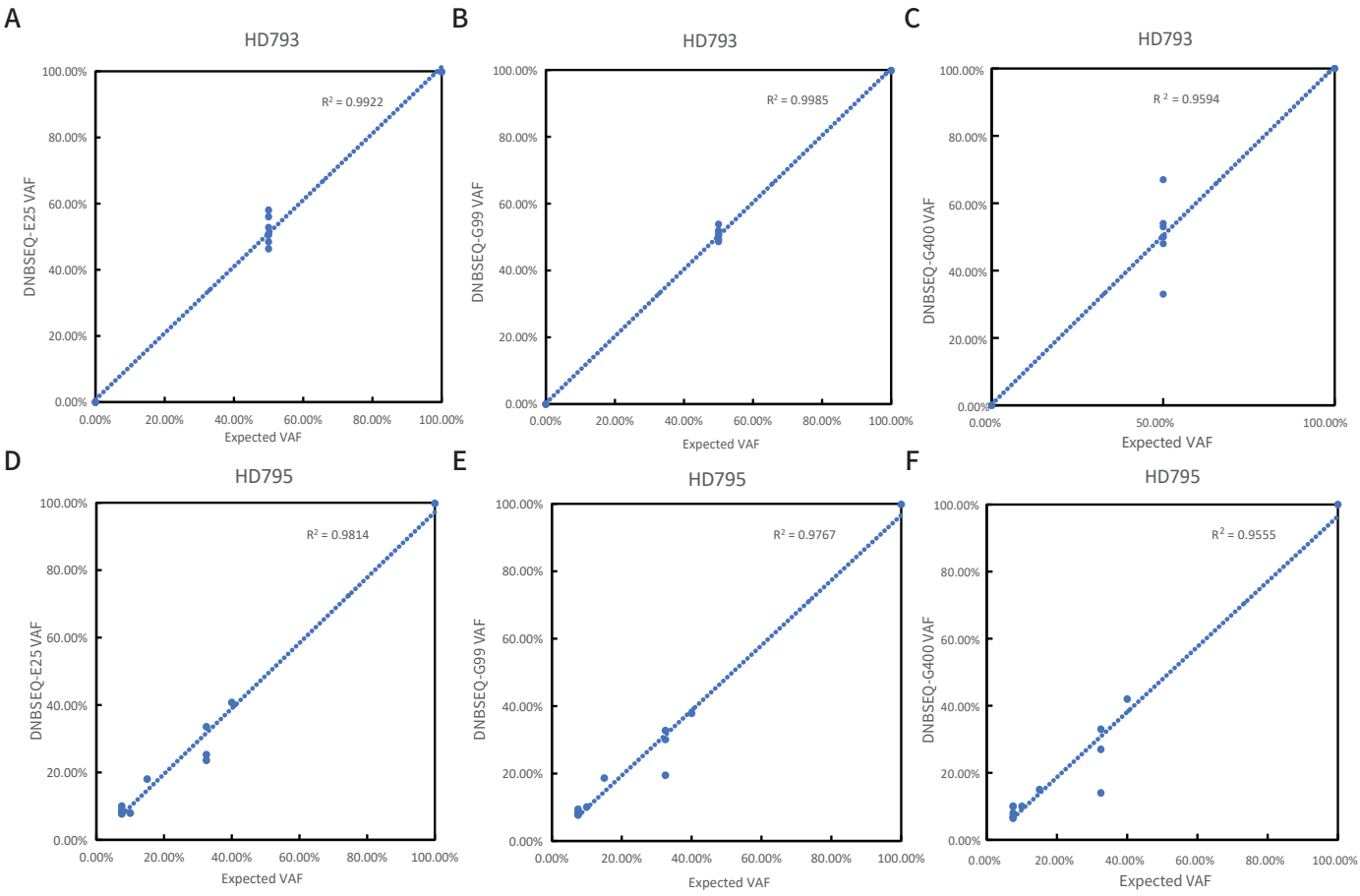


Figure 2. Correlation analysis of variation detection in HD793 and HD795 with DNBSEQ-E25/DNBSEQ-G99/DNBSEQ-G400.

HGT analysis platform combined with DNBSEQ platform can accurately identify *BRCA1/2* pathogenic sites

The HGT solution classifies *BRCA1/2* mutations into categories including pathogenic (P), likely pathogenic (LP), benign (B), likely benign (LB), and variants of unknown significance (VUS), facilitating rapid diagnosis. In this study, both HD793 and HD795 standards were found to have pathogenic variants, and the results between DNBSEQ-E25, DNBSEQ-G99, and DNBSEQ-G400 were consistent (Table 4).

Sample	Gene	GRCh37 coordinates	Protein	DNBSEQ-E25 VAF	DNBSEQ-G99 VAF	DNBSEQ-G400 VAF	ACMG classification
HD793	*BRCA2*	13:32913837	p.Asn1784ThrfsTer7	52.78%	48.64%	50.00%	Pathogenic
HD795	*BRCA1*	17:41234451	p.Arg1443Ter	25.28%	19.49%	14.00%	Pathogenic
HD795	*BRCA2*	13:32937355	p.Ile2675AspfsTer6	7.93%	10.10%	10.00%	Pathogenic
HD795	*BRCA2*	13:32913559	p.Lys1691AsnfsTer15	33.54%	32.76%	33.00%	Pathogenic
HD795	*BRCA2*	13:32913837	p.Asn1784ThrfsTer7	40.75%	37.85%	42.00%	Pathogenic

Table 4. Pathogenic variants in HD793 and HD795 standards identified by DNBSEQ-E25/DNBSEQ-G99/DNBSEQ-G400.

Conclusion

HGT bioinformatics analysis platform combined with DNBSEQ sequencing platform offers an accurate solution for the efficient detection of *BRCA1/2* mutations and robustly supports the in-depth research into these genes. This solution significantly advances our understanding of breast and ovarian cancers related gene mutations, providing a solid scientific basis for clinical diagnosis and personalized medicine.

DNBSEQ-E25 is a compact, portable and fast genetic sequencer, requiring only 0.1 m^2 of space and featuring a unique self-luminous biochemical system. It offers high-speed sequencing with minimal laboratory requirements and no optical path adjustments needed, making it ideal for pathogen detection and small genome sequencing.

DNBSEQ-G99 is among the world's fastest mid-low throughput sequencers, integrating sequencing and bioinformatics analysis with an in-built computing module. Capable of completing PE150 sequencing in just 12 hours, it is suitable for various applications including targeted sequencing, small whole genome sequencing, WGS, individual identification, and 16S metagenome sequencing, with efficient and high-quality data output.

The DNBSEQ-G400 offers flexible throughput, supporting 1~2 flow cells per run simultaneously. It supports different flow cell specifications (FCS and FCL) for independent operation and supports a variety of sequencing read lengths, which can comprehensively meet the wide sequencing requirements, making it a good choice for global laboratories.

Acknowledgements

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DNBSEQ-E25
genetic sequencer

DNBSEQ-G99
genetic sequencer

DNBSEQ-G400
genetic sequencer

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Recommended Ordering Information

Category	Product	Cat. NO.
Genetic Sequencers	Genetic Sequencer DNBSEQ-E25RS	900-000537-00
	Genetic Sequencer DNBSEQ-G99RS	900-000607-00
	Genetic Sequencer DNBSEQ-G400RS	900-000170-00
Automation Systems	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000147-00
Software	Health Gene Tech (HGT) bioinformatics analysis system*	/
Sequencing Reagents	DNBSEQ-E25RS High-throughput Sequencing Set (FCL PE150)	940-000567-00
	DNBSEQ-G99RS High-throughput Sequencing Set (G99 FCL PE150)	940-001269-00
	DNBSEQ-G99 Cleaning Reagent Kit	940-000624-00
	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)	940-000810-00

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

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