Amoy Diagnostics Co., Ltd.
Add: No.39 Dingshan Road, Xiamen 361027, China
Tel: 86-592-6806835 Fax: 86-592-6806839

Comparative Analysis of AmoyDx® NGS panels on Element AVITI System and Illumina NextSeq 550 platforms

Abstract

This white paper presents comparative analysis of the representative AmoyDx® NGS panels (Table 1) across the major sequencing platforms: Element AVITI System and Illumina NextSeq 550. Results demonstrate that Element AVITI System deliver comparable sequencing data quality, supporting their suitability for reliable NGS testing in tumor mutation detection and research.

Table 1. AmoyDx® NGS panels used for performance assessment on Element AVITI System

Product	Technology	Panel Coverage	Sample Type	Variant Type	Sample Quantity
BRCA Pro	HANDLE	*BRCA1* and *BRCA2* genes	FFPE	SNV, InDel	27
			Whole Blood	SNV, InDel, LR	48
HRD Complete	HANDLE	20 HRR genes plus HRD	FFPE	SNV, InDel, HD, and HRD status (GSS)	88
Classic	HANDLE	40 genes plus MSI	FFPE	SNV, InDel, fusion, CNV, MSI	75
HRR Liquid	ddCAP	24 genes	Liquid biopsy	SNV, InDel, PTEN Loss	38

Note:

BRCA Pro: AmoyDx® BRCA Pro Panel

HRD Complete: AmoyDx® HRD Complete Panel

Classic: AmoyDx® HANDLE Classic NGS Panel

HRR Liquid: AmoyDx® HRR Liquid NGS Panel

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Introduction

The AmoyDx® NGS Panels have been optimized for use on the Illumina NextSeq 550 platform. With the growing demand for more flexible and efficient sequencing options, this study aims to validate the performance of AmoyDx® NGS Panels on the Element AVITI System. By comparing key performance metrics between the Element AVITI System and Illumina platform, including sequencing quality and mutation detection consistency, this white paper explores the potential for expanding the use of AmoyDx® NGS Panels beyond the Illumina platform to the Element AVITI System.

Methodology

1. Sample Collection

To validate the sequencing performance on Element AVITI System, specimens with diverse sample types were collected to evaluate the agreement of the variant detection in accordance with the detection scope of each NGS panel.

1) BRCA Pro Panel:

A total of 48 whole blood samples and 27 FFPE samples were tested for performance evaluation of the Element AVITI System and NextSeq 550. The sequencing quality and the concordance across different variant types, including single nucleotide variants (SNVs), insertions and deletions (InDels), and large rearrangements (LRs) were evaluated on both sequencing platforms.

2) HRD Complete Panel:

A total of 88 FFPE samples from ovarian cancer and prostate cancer were tested for performance evaluation of the Element AVITI System and NextSeq 550. The sequencing quality and the concordance across different variant types, including SNVs, InDels, homozygous deletions (HDs), and HRD status were evaluated on both sequencing platforms.

3) Classic Panel:

A total of 75 FFPE samples from multiple cancer types were tested for performance evaluation of the Element AVITI System and NextSeq 550. The sequencing quality and the concordance across different variant types, including SNVs, InDels, gene fusions, copy number amplifications (CNAs) and microsatellite instability (MSI) were evaluated on both sequencing platforms.

4) HRR Liquid Panel:

A total of 20 plasma cfDNA samples and 18 cell line-derived reference samples were tested for performance evaluation of the Element AVITI System and NextSeq 550. The sequencing quality and the concordance across different variant types, including SNVs, InDels, and PTEN loss were evaluated on both sequencing platforms.

2. DNA/RNA Extraction, Library Preparation and Sequencing

The sample DNA (or RNA) was extracted using the AmoyDx extraction kits, following the manufacturer's instructions for the respective sample type. Library preparation was carried out in accordance with the manufacturer's instructions for each AmoyDx NGS panel. Sequencing was carried out for each library on both the Element AVITI System and NextSeq 550 platforms for comparative analysis.

3. Data Processing and Analysis

When the sequencing is finished, adopt AmoyDx ANDAS Data Analyzer to analyze the sequencing data. Select the appropriate analysis module according to the manufacturer's instructions for each AmoyDx NGS panel. Key metrics including sequencing depth, quality control parameters, and variant detection concordance were evaluated to assess the sequencing performance. The concordance of variant detection between different platforms, were investigated using overall/positive/negative percentage agreement (OPA/PPA/NPA).

Results

Sequencing and Data QC Performance Comparison

Table 2. Sequencing and data QC assessment for each product

Product	Metrics	Element AVITI System	Illumina NextSeq 550
BRCA Pro	Sequencing Quality (Q30)	≥75%	≥75%
	Depth	FFPE ≥400×; Blood ≥50×	FFPE ≥400×; Blood ≥50×
HRD Complete	Sequencing Quality (Q30)	≥75%	≥75%
	Coverage (180×) BRCA	≥ 95%	≥ 95%
	Coverage (180×) CDS	≥ 95%	≥ 95%
	DepthNoise for GSS	≤0.35	≤0.35
	BRAFNoise for GSS	≤0.05	≤0.05
	CDSDepthNoise for HD	≤0.40	≤0.40

	CDSBRAFNoise for HD	≤0.05	≤0.05
Classic Panel	Sequencing Quality (Q30)	≥75%	≥75%
	Depth	≥400×	≥400×
	RNA-Control	≥20	≥20
HRR Liquid	Sequencing Quality (Q30)	≥75%	≥75%
	CoverageRatioUNIQ1000	≥90%	≥90%
	CNVNoise	≤0.20	≤0.20

BRCA Pro Panel Testing Results

Table 3. Testing result summary of BRCA Pro panel

Platform	**Sample Type and Variant Type**	**Status**	**Illumina NextSeq 550**		**Concordance**
			Positive	**Negative**	
Element AVITI System	**Blood - Germline SNV/InDel**	**Positive**	21	0	PPA = 100.00% NPA = 100.00% OPA = 100.00%
		Negative	0	27	
	Blood - Germline LR	**Positive**	19	0	PPA = 100.00% NPA = 100.00% OPA = 100.00%
		Negative	0	29	
	Tissue - SNV/InDel	**Positive**	10	0	PPA = 90.91% NPA = 100.00% OPA = 96.30%
		Negative	1	16	

HRD Complete Panel Testing Results

Table 4. Testing result summary of HRD Complete panel

Platform	**Variant Type**	**Status**	**Illumina NextSeq 550**		**Concordance**
			Positive	**Negative**	
Element AVITI System	**SNV/InDel**	**Positive**	44	0	PPA = 91.67% NPA = 100.00% OPA = 95.45%
		Negative	4	40	
	HD	**Positive**	6	0	PPA = 100.00% NPA = 100.00% OPA = 100.00%
		Negative	0	82	
	HRD Status	**Positive**	52	0	PPA = 91.23% NPA = 100.00% OPA = 94.32%
		Negative	5	31	

Note: *A positive HRD status result is defined by either the presence of a pathogenic/likely pathogenic variant in BRCA1 and/or BRCA2 genes or a positive Genomic Scar Score (GSS).*

Classic Panel Testing Results

Table 5. Testing result summary of Classic panel

Platform	Variant Type	Status	Illumina NextSeq 550		Concordance
			Positive	Negative	
Element AVITI System	SNV/InDel	Positive	52	0	PPA = 98.11% NPA = 100.00% OPA = 98.67%
		Negative	1	22	
	Fusion	Positive	19	0	PPA = 100.00% NPA = 100.00% OPA = 100.00%
		Negative	0	56	
	CNV	Positive	15	0	PPA = 88.24% NPA = 100.00% OPA = 97.33%
		Negative	2	58	
	MSI	Positive	8	0	PPA = 100.00% NPA = 100.00% OPA = 100.00%
		Negative	0	67	

HRR Liquid Panel Testing Results

Table 6. Testing result summary of HRR Liquid panel

Platform	Variant Type	Status	Illumina NextSeq 550		Concordance
			Positive	Negative	
Element AVITI System	SNV/InDel	Positive	7	0	PPA = 100.00% NPA = 100.00% OPA = 100.00%
		Negative	0	31	
	PTEN Loss	Positive	14	0	PPA = 100.00% NPA = 100.00% OPA = 100.00%
		Negative	0	24	

Discussion

The comparative analysis highlights several key findings:

Sequencing Quality and Data QC Performance:
The Element AVITI System have been evaluated and exhibit high Q30 value and data quality comparable to the Illumina NextSeq 550 platform, indicating high sequencing accuracy and data quality, which supports their compatibility for downstream biomarker assessment and data analysis.

Detection Performance:
Both platforms demonstrated high accuracy and specificity across multiple variant types, including SNVs/InDels, CNVs, homozygous deletions, HRD status, and MSI status.

For the BRCA Pro panel, the germline SNV/InDel/LR evaluation from blood samples showed a 100% overall concordance on Element AVITI System, and the SNV/InDel evaluation from tissue samples showed a 96.3% overall concordance on Element AVITI System. The one discrepancy observed was caused by a potential oxidative damage-leading variant with low frequency, which was filtered out and is not attributable to differences between sequencing platforms.

For the HRD Complete panel, the BRCA SNV/InDel, HD, and HRD evaluation showed an overall concordance of 95%, 100%, 94%, respectively, when assessed on the Element AVITI System. The discrepancies observed in BRCA variants or HRD status were caused by the variant frequency or the GSS value being near the limit of detection (LoD) of the assay.

For the Classic panel, all the biomarkers investigated demonstrated over 95% consistency on the Element AVITI System. The minor discrepancies observed were caused by variants being filtered out due to the frequency/copy number below the the cut-off of the assay.

For the HRR Liquid panel, all the biomarkers investigated demonstrated 100% consistency on the Element AVITI System.

In summary, these data confirm the high accuracy and consistency of the Element AVITI System in variant detection and biomarker analysis performance at both DNA and RNA levels, positioning them as reliable alternatives to the NextSeq 550 system.

Conclusion
The study confirms that the AmoyDx® NGS Panels demonstrate exceptional performance on Element AVITI System. These platforms have demonstrated superior performance in sequencing quality and variant detection, comparable to the capabilities of Illumina platforms. These findings validate Element AVITI System as a robust and reliable platform for reliable NGS testing in tumor mutation detection and research.

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Disclaimer:

The results presented in this document are for informational purposes only and should not be considered as definitive medical conclusions. These results are based on the analysis of genetic data and are intended to support, not replace, professional medical evaluation, diagnosis, or treatment.

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