Validation of the AmoyDx® HANDLE Breast Cancer NGS Panel in FFPE and Liquid Biopsy Samples

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Introduction

Tissue and liquid biopsy are complementary for breast cancer profiling. Tissue defines baseline alterations, while liquid biopsy enables non-invasive monitoring of tumor evolution and resistance. Endocrine therapy (ET) remains the main treatment for HR+ disease (~70–80%). Acquired ESR1 mutations—rare at baseline—occur in 20–40% of advanced cases after ET and drive resistance. Based on the EMERALD trial, cfDNA ESR1 testing is recommended at progression. Guidelines also recommend screening for AKT1, PIK3CA, or PTEN mutations to identify candidates for targeted therapies. Moreover, ERBB2 mutations recently emerged as another potential target, as investigated in the SUMMIT basket trial. Integrating tissue and liquid biopsy provides a more complete view of tumor biology and resistance mechanisms. [1-6]

AmoyDx® HANDLE Breast Cancer NGS Panel

The AmoyDx® HANDLE Breast Cancer NGS Panel is a next-generation sequencing (NGS)-based assay designed for the qualitative detection of single nucleotide variants (SNVs) and insertions and deletions (InDels) in the targeted regions of five genes (see Table S1): *AKT1, ERBB2, ESR1, PIK3CA*, and *PTEN*. The assay is compatible with DNA extracted from formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue samples, as well as circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood samples from individuals with breast cancer.

The kit is intended to be used by trained professionals in a laboratory environment. The test results are for research use only, not for use in diagnostic procedures.

Figure 1: Principle of library construction (HANDLE system)

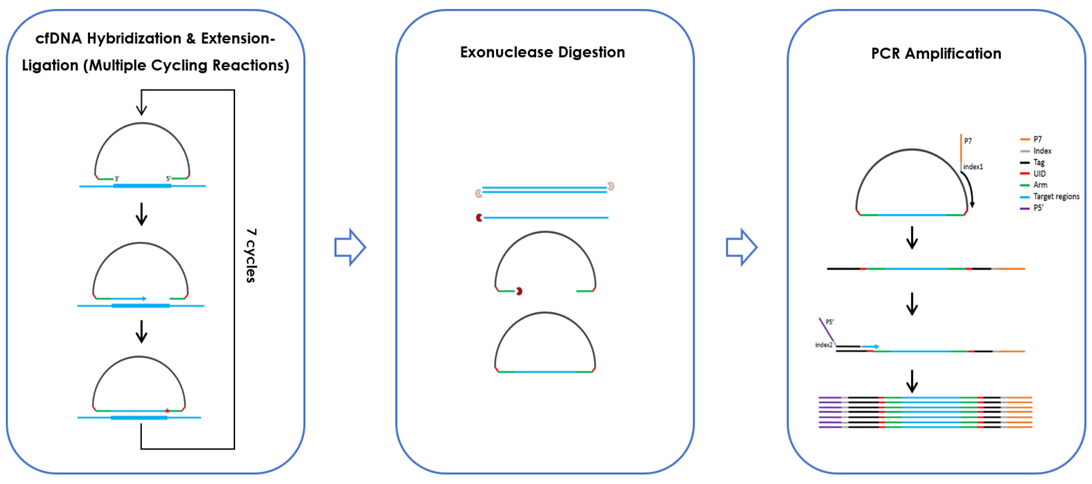
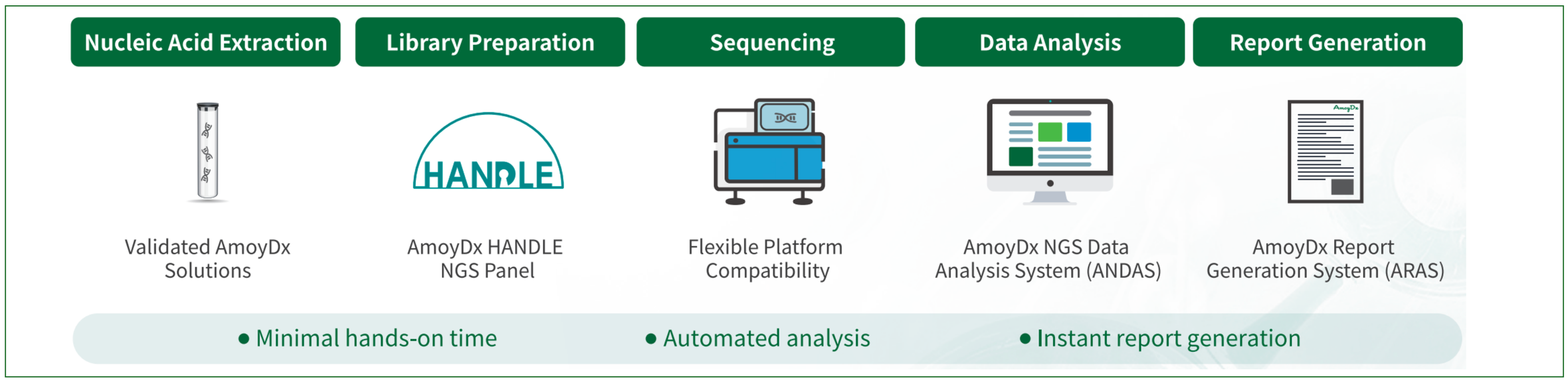


Table 1: Target Region

No.	Gene	Transcript	Target Regions	Mutation
1	*AKT1*	NM_001382430	Exon4 chr14:105246406-105246584	SNV, InDel
2	*ERBB2*	NM_004448	Exon18 chr17:37879856-37879940 Exon19 chr17:37880188-37880285 Exon20 chr17:37880968-37881048 Exon21 chr17:37881285-37881442	SNV, InDel
3	*ESR1*	NM_000125	Exon4 chr6:152265538-152265643 Exon5 chr6:152332777-152332857 Exon6 chr6:152382087-152382247 Exon7 chr6:152415508-152415577 Exon8 chr6:152419882-152419978	SNV, InDel
4	*PIK3CA*	NM_006218	Exon2 chr3:178916849-178916912 Exon5 chr3:178921503-178921610 Exon8 chr3:178927951-178928017 Exon10 chr3:178936051-178936115 Exon21 chr3:178952055-178952114	SNV, InDel
5	*PTEN*	NM_000314	Whole coding regions and intron/exon boundaries	SNV, InDel

Streamlined Workflow from Sample to Report within 4 Days



Materials and Methods

Accuracy study

A total of 297 plasma and 49 FFPE clinical samples (pre-characterized by reference methods) were analyzed, including 109 plasma / 29 FFPE positives and 188 plasma / 20 FFPE negatives. Performance was evaluated using PPA, NPA, OPA, with 95% CIs.

Acceptance criterion: PPA, NPA, and OPA ≥95% for all qualified samples.

Sensitivity (limit of detection, LOD) study

For plasma, sensitivity was evaluated for VIP and Hotspot variants using 5 ng and 30 ng cfDNA inputs.

- VIP variants: 6 reference samples (pre-verified SNV/InDel); 240 reactions (6 samples × 2 conditions × 20 replicates).
- Hotspot variants: 4 reference samples; 160 reactions (4 samples × 2 conditions × 20 replicates).

For FFPE, 3 reference samples containing VIP and Hotspot variants (pre-verified SNV/InDel) were tested with 10 replicates each (30 reactions).

Acceptance criterion: detection rate ≥95% at each LOD.

Precision study

For plasma, three pre-verified reference samples (two positive for SNV/InDel and one negative) were tested across three operators. One operator performed three batches, while the other two each performed one batch, with five replicates per batch (75 total reactions).

For FFPE, 4 reference samples with known SNV/InDel (pre-verified) were tested with 5 replicates each (20 reactions).

Acceptance criterion: ≥95% agreement among replicates.

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Evaluation with the Seraseq® ctDNA *ESR1* Mix

Analytical performance was further assessed using a fragmented ctDNA reference (150–220 bp) containing 22 predefined *ESR1* and *PIK3CA* variants (VAF 1%). VAFs and copy numbers were verified by digital PCR and cross-validated by NGS. The panel was tested at 0.5%, 0.2%, 0.1%, 0.05%, and 0% wild-type with 30 ng and 10 ng inputs. Non-zero levels were run in triplicate; wild-type controls once per input (26 total reactions). Variant detection rates at each VAF/input combination were evaluated.

Table 2: Variants in the Seraseq® ctDNA *ESR1* Mutation Mix (AF 1%)

#	Gene	Nucleic Acid Change	Amino Acid Change	Variant Type	#	Gene	Nucleic Acid Change	Amino Acid Change	Variant Type
1	*ESR1*	c.1138G>C	p.E380Q	SNV	12	*ESR1*	c.1609T>A	p.Y537N	SNV
2		c.1387T>C	p.S463P	SNV	13		c.1608_1609delinsTA	Y537N	INDEL
3		c.1603C>A	P535T	SNV	14		c.1610A>G	p.Y537C	SNV
4		c.1607_1608delinsAT	L536H	INDEL	15		c.1609T>G	p.Y537D	SNV
5		c.1607T>A	p.L536H	SNV	16		c.1613A>G	p.D538G	SNV
6		c.1607T>C	p.L536P	SNV	17		c.1610_1615dupATGACC	D538_L539insHD	INDEL
7		c.1607T>G	p.L536R	SNV	18		c.1625A>G	E542G	SNV
8		c.1607_1608delinsAG	p.L536Q	INDEL	19	*PIK3CA*	c.1624G>A	E542K	SNV
9		c.1610_1611delinsCA	Y537S	INDEL	20		c.1633G>A	E545K	SNV
10		c.1609_1610delinsAG	Y537S	INDEL	21		c.3140A>G	H1047R	SNV
11		c.1610A>C	p.Y537S	SNV	22		~~c.3203dupA~~	~~p.N1068Kfs*5~~	~~INDEL~~

Note: *PIK3CA* c.3203dupA (Chr3:178952148) falls outside the panel's coverage range.

Results

Accuracy study

All metrics met the predefined acceptance criteria, confirming the high analytical accuracy of the assay. Detailed agreement values are shown in Table 3:

Table 3: Summary of Accuracy Study Results

Accuracy Study, Plasma Samples					**Accuracy Study, FFPE Samples**				
Plasma (N=297)		Reference Assay		Total	**FFPE (N=49)**		Reference Assay		Total
		Positive	Negative				Positive	Negative	
HANDLE BC NGS Panel	Positive	107	0	107	HANDLE BC NGS Panel	Positive	29	0	29
	Negative	2	188	190		Negative	0	20	20
Total		109	188	297	Total		29	20	49
PPA [95% CI]		98.17% [93.56%, 99.50%]			PPA [95% CI]		100.00% [88.30%, 100.00%]		
NPA [95% CI]		100.00% [98.00%, 100.00%]			NPA [95% CI]		100.00% [83.89%, 100.00%]		
OPA [95% CI]		99.33% [97.58%, 99.82%]			OPA [95% CI]		100.00% [92.73%, 100.00%]		

Sensitivity (LOD) study

Plasma:

- VIP variants: At 0.2% VAF (30 ng DNA input), all 120 libraries passed QC, with 100% variant detection (140/140). At 1% VAF (5 ng), all 120 libraries passed QC; with 98.57% variant detection (138/140).
- Hotspot variants: At 0.5% VAF (30 ng), all 80 libraries passed QC, with 98.08% variant detection (255/260). At 3% VAF (5 ng), all 80 libraries passed QC, with100% variant detection (260/260).
- Established plasma LODs: 0.2% VAF for VIP variants and 0.5% VAF for hotspot variants in plasma at 30 ng cfDNA input; 1% (VIP) and 3% (hotspot) in plasma at 5 ng cfDNA input.

FFPE:

- VIP and Hotspot variants: At 1% VAF (10 ng DNA input), all 30 libraries passed QC, with 96% variant detection (96/100).
- Established FFPE LOD: 1% VAF for both VIP and hotpsot variants in FFPE at 10 ng DNA input.

All metrics met the predefined acceptance criteria, confirming sensitive variant detection at established LODs.

Precision study

For both plasma and FFPE assessments, all positive and negative results were correctly identified across replicates, operators, and batches, achieving 100% agreement. All metrics met the predefined acceptance criteria, demonstrating high precision.

Evaluation with the Seraseq® ctDNA *ESR1* Mix

Specificity: Using wild-type controls, no false positives were observed at 10 ng and 30 ng inputs (100% specificity).

Sensitivity: Tested at 0.05%, 0.10%, 0.20%, and 0.50% VAF for VIP (n=13), Hotspot (n=3), and Other (n=5) variants (see Table 4).

- VIP variants: 100% detection at 0.50% (10 ng) and 0.20–0.50% (30 ng)
- Hotspot variants: 100% detection at 0.50% (10 ng or 30 ng).
- These findings are consistent with the LODs established in the aforementioned broader sensitivity study and provide additional confirmation of the panel's high analytical sensitivity.

Figure 2. Variant Detection Rates of Seraseq® ctDNA *ESR1* Mix for VIP and Hotspot Variants

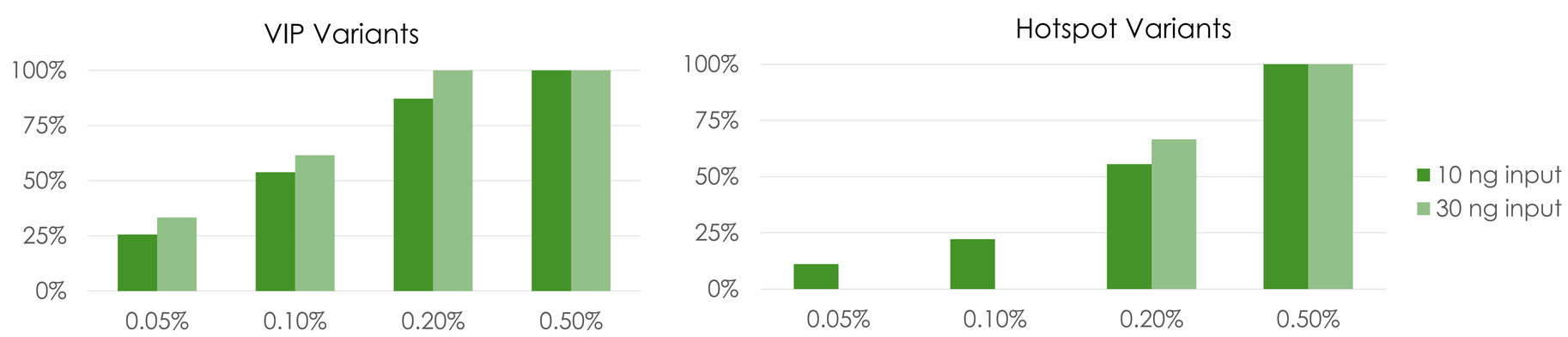

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Table 4: Summary of Seraseq® ctDNA *ESR1* Mix Variant Detection Results

ADx Tags	Nucleic acid change	Verified VAF	Variant Detection Rate	
			10 ng Input	30 ng Input
VIP Variants	*ESR1* c.1138G>C	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	3/3	2/3
		0.05%	1/3	1/3
	ESR1 c.1387T>C	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	2/3	2/3
		0.05%	1/3	0/3
	ESR1 c.1607T>A	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	2/3	3/3
		0.05%	0/3	1/3
	ESR1 c.1607T>C	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	1/3	2/3
		0.05%	1/3	2/3
	ESR1 c.1607T>G	0.50%	3/3	3/3
		0.20%	2/3	3/3
		0.10%	1/3	0/3
		0.05%	2/3	1/3
	ESR1 c.1607_1608delinsAG	0.50%	3/3	3/3
		0.20%	2/3	3/3
		0.10%	1/3	0/3
		0.05%	2/3	1/3
	ESR1 c.1610A>C	0.50%	3/3	3/3
		0.20%	2/3	3/3
		0.10%	0/3	2/3
		0.05%	0/3	0/3
	ESR1 c.1609T>A	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	2/3	1/3
		0.05%	0/3	2/3
	ESR1 c.1610A>G	0.50%	3/3	3/3
		0.20%	2/3	3/3
		0.10%	2/3	3/3
		0.05%	1/3	2/3
	ESR1 c.1609T>G	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	1/3	1/3
		0.05%	0/3	1/3
	ESR1 c.1613A>G	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	1/3	3/3
		0.05%	0/3	1/3
VIP Variants	*PIK3CA* c.1633G>A	0.50%	3/3	3/3
		0.20%	2/3	3/3
		0.10%	2/3	2/3
		0.05%	1/3	1/3
	PIK3CA c.3140A>G	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	3/3	3/3
		0.05%	1/3	0/3
Hotspot Variants	*ESR1* c.1610_1611delinsCA	0.50%	3/3	3/3
		0.20%	2/3	2/3
		0.10%	2/3	0/3
		0.05%	0/3	0/3
	ESR1 c.1609_1610delinsAG	0.50%	3/3	3/3
		0.20%	3/3	3/3
		0.10%	0/3	0/3
		0.05%	1/3	0/3
	PIK3CA c.1624G>A	0.50%	3/3	3/3
		0.20%	0/3	1/3
		0.10%	0/3	0/3
		0.05%	0/3	0/3
Other Variants	*ESR1* c.1603C>A	0.50%	1/3	3/3
		0.20%	0/3	0/3
		0.10%	0/3	0/3
		0.05%	0/3	0/3
	ESR1 c.1607_1608delinsAT	0.50%	2/3	3/3
		0.20%	1/3	0/3
		0.10%	0/3	0/3
		0.05%	0/3	0/3
	ESR1 c.1608_1609delinsTA	0.50%	3/3	3/3
		0.20%	0/3	0/3
		0.10%	0/3	0/3
		0.05%	0/3	0/3
	ESR1 c.1610_1615dupATGACC	0.50%	2/3	2/3
		0.20%	0/3	0/3
		0.10%	0/3	0/3
		0.05%	0/3	0/3
	ESR1 c.1625A>G	0.50%	3/3	2/3
		0.20%	0/3	0/3
		0.10%	0/3	0/3
		0.05%	0/3	0/3

Conclusion

The AmoyDx® HANDLE Breast Cancer NGS Panel enables efficient testing of both plasma and FFPE samples. Its proprietary HANDLE technology supports a simple, streamlined workflow with a turnaround time of 4 working days.

Analytical validation demonstrated high accuracy, sensitivity, and precision, with consistent performance across operators and batches. The Seraseq® *ESR1* reference series further confirmed analytical sensitivity and specificity.

Overall, the panel provides a rapid, sensitive, and reproducible solution for breast cancer testing across both tissue and liquid biopsy. By integrating key actionable targets in a single assay, it supports timely, evidence-based molecular findings.

References

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