AmoyDx® HANDLE Breast Cancer Liquid NGS Panel Performance Evaluation



Introduction

Liquid biopsy is an emerging non-invasive method for detecting and monitoring key biomarkers in breast cancer. Endocrine therapy (ET) remains the primary treatment for hormone receptor-positive (HR+) disease, comprising 70–80% of cases. Acquired ESR1 mutations, typically rare in primary tumors, occur in 20–40% of advanced cases following ET and represent a major mechanism of resistance. Based on EMERALD trial findings, guidelines now endorse ESR1 mutation testing in cfDNA at recurrence or progression. NCCN guidelines also recommend screening for AKT1, PIK3CA, or PTEN mutations to identify candidates for targeted therapies such as alpelisib or capivasertib, with category 1 evidence. Moreover, ERBB2 mutations recently emerged as another potential target, as investigated in the SUMMIT basket trial.

AmoyDx[®] HANDLE Breast Cancer Liquid NGS Panel

The AmoyDx® HANDLE Breast Cancer Liquid NGS Panel is a next-generation sequencing (NGS)-based assay intended for the qualitative detection of single nucleotide variants (SNVs) and insertions and deletions (InDels) in the targeted regions of 5 genes (see Table 1), including *AKT1*, *ERBB2*, *ESR1*, *PIK3CA*, and *PTEN*, using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood specimens.

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

Reference Material for Evaluation (Seraseq[®] ctDNA ESR1 Mix)

The Seraseq[®] ctDNA ESR1 Mix is intended for use with molecular testing assays that identify variants present in circulating tumor DNA (ctDNA) present in the blood. The Seraseq ctDNA ESR1 Mutation Mix AF1% contains 22 variants relevant to ctDNA monitoring, most of which are actionable mutations (see Table 2). The product is designed to simulate ctDNA fragment sizes, with a peak distribution between 150-220 bp. Variant allele frequency (VAF) and copy number gain are confirmed using digital PCR. VAF is additionally assessed by NGS and reported in the batch-specific TPR.

Table 2: Variants covered

#	Gene	Nucleic Acid Change	Amino Acid Change	Variant Type	#	Gene	Nucleic Acid Change	Amino Acid Change	Variant Type
1		c.1138G>C	p.E380Q	SNV	12		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	ESR1	c.1609T>G	p.Y537D	SNV
5		c.1607T>A	p.L536H	SNV	16		c.1613A>G	p.D538G	SNV
6	ESR1	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		c.1624G>A	E542K	SNV
9		c.1610_1611delinsCA	Y537S	INDEL	20		c.1633G>A	E545K	SNV
10		c.1609_1610delinsAG	Y537S	INDEL	21	PIKJCA	c.3140A>G	H1047R	SNV
11		c.1610A>C	p.Y537S	SNV	22		c.3203dupA	p.N1068Kfs*5	INDEL

Note: The mutation "c.3203dupA" with strikethrough on PIK3CA is not covered, with genomic position in Chr3:178952148 out of detection range.

AmoyDx® HANDLE Breast Cancer Liquid NGS Panel Performance Evaluation

Method

The sensitivity of the kit was evaluated using commercially available Seraseq ctDNA ESR1 reference materials with variant allele frequencies (VAFs) of 0.5%, 0.2%, 0.1%, 0.05%, and 0%. Testing was conducted at total DNA inputs of 30 ng and 10 ng. Each VAF and input combination was executed in triplicate on the Illumina NextSeq Sequencer(see Table 3).

VAF (%)	Input 1 (ng)	Input 2 (ng)	Instrument	Replicate
0.5	30	10		3
0.2	30	10		3
0.1	30	10	Illumina NextSeq 550Dx	3
0.05	30	10		3
0	30	10		1

Table 3: Experiment Design and Evaluation Parameters

The Cut-Off value and LoD specified in the IFU are as follows.

Cut-Off Metrics

The LoD of SNV/InDel

Devenuetor	Cut-off								
rarameter	Freq	AltDepth							
VIP mutations	$\geq 0.1\%$	≥ 5							
HotSpot mutations	$\geq 0.22\%$	≥ 10							
	When Depth < $100 \times$, Freq $\ge 20\%$								
Other mutations	When Depth $\geq 100 \times$ and $< 600 \times$, Freq $\geq 5\%$	≥ 10							
	When Depth \geq 600×, Freq \geq 0.5%								

Parameter	30 ng cfDNA input	5 ng cfDNA input
VIP mutations	0.2% MAF	1% MAF
HotSpot mutations	0.5% MAF	3% MAF

Result

Analytical Specificity Verification

• Analytical specificity was assessed using Seraseq ctDNA ESR1 Mix WT. The results demonstrated a 100% specificity at both 30 ng and 10 ng total DNA inputs (see appendix Table 5 WT testing results for details).

Analytical Sensitivity Verification

The Limit of Detection (LoD) was determined to be 0.2% Variant Aelle Frequency (VAF) at a DNA input of 30 ng for "VIP" mutations, and 0.5% VAF at a DNA input of 30 ng for "Hotspot" mutations (see Table 4). Detailed NGS detection information for each variants is provided In appendix.

Gene	#	Nucleic acid change	ADx Tags	Verified LoD	Claimed LoD	10 ng	30 ng	Gene	# Nucleic acid change	ADx Tags	Verified LoD Claimed LoD	10 ng	30 ng
				0.500/		2/2	2/2				0.500/	2/2	2/2

Table 4: Summary of Variant Detection Rates

				0.0070	010	010				0.000		ere	0,0	
	1	c 1138G>C	VIP	0.20% 5 ng 1%	3/3	3/3				VID	0.20%	5 ng 1% 30 ng 0.2%	2/3	3/3
		C.1130U>C		0.10% 30 ng 0.2%	6 3/3	2/3		20	c.1633G>A	VIP	0.10%		2/3	2/3
				0.05%	1/3	1/3				-	0.05%		1/3	1/3
				0.50%	3/3	3/3	PIK3CA				0.50%		3/3	3/3
	2	c.1387T>C	VIP	0.20% 5 ng 1%	3/3	3/3	_		c.3140A>G	-	0.20%		3/3	3/3
			-	0.10% 30 ng 0.2%	2/3	2/3		21		VIP	0.10%	30 ng 0.2%	3/3	3/3
				0.05%	1/3	0/3				-	0.05%		1/3	0/3
			-	0.50% 5 mg 10/	$\frac{3/3}{2/2}$	3/3	-				0.50%		3/3	3/3
	5	c.1607T>A	VIP	$\frac{0.20\%}{0.10\%} = \frac{5 \text{ ng } 1\%}{30 \text{ ng } 0.2\%}$	$\frac{3/3}{2/3}$		_			-	0.30%	-5 ng $3%$	2/3	2/3
			-	0.10% 50 lig 0.2%	$\frac{2/3}{0/3}$	1/3	-	9	c.1610_1611delinsCA	HotSpot	0.2070	-30 ng 0.5%	$\frac{2/3}{2/3}$	0/3
				0.03%	3/3	3/3	-			-	0.10%		$\frac{2/3}{0/3}$	0/3
			-	0.20% 5 ng 1%	3/3	3/3	ESR1				0.03%		$\frac{0/3}{2/2}$	2/2
	6	c.1607T>C	VIP	0.10% 30 ng 0.2%	$6 \frac{1/3}{1/3}$	2/3	_				0.30%		3/3	$\frac{3}{3}$
7		-	0.05%	1/3	2/3	-	10	c.1609_1610delinsAG	HotSpot	0.20%	-5 ng 3%	3/3	3/3 0/2	
		c.1607T>G	VIP	0.50%	3/3	3/3				-	0.10%	5 ng 3%	0/3	0/3
				0.20% 5 ng 1%	2/3	3/3	-				0.05%		1/3	0/3
				0.10% 30 ng 0.2%	ó <u>1/3</u>	0/3				-	0.50%		3/3	3/3
				0.05%	2/3	1/3	PIK3CA	19	c.1624G>A	HotSpot	0.20%		0/3	1/3
			-	0.50%	3/3	3/3	_			1	0.10%	30 ng 0.5%	0/3	0/3
ESR1 8	8	c 1607 1608delinsAG	VIP	0.20% 5 ng 1%	2/3	3/3	_				0.05%		0/3	0/3
			• •	0.10% 30 ng 0.2%	6 1/3	0/3	_			-	0.50%	_	1/3	3/3
				0.05%	2/3	1/3	_	3	c 1603C>A	Other	0.20%	/	0/3	0/3
			VIP	0.50%	3/3	3/3	_		C.1003C/11	Other	0.10%		0/3	0/3
	11	c.1610A>C		0.20% 5 ng 1%	$\frac{2/3}{0/2}$	3/3	_				0.05%		0/3	0/3
				0.10% 50 ng 0.2%	$\begin{array}{c} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 3 \\ 0 \\ 3 \\ 0 \\ 3 \\ 0 \\ 3 \\ 0 \\ 3 \\ 0 \\ 3 \\ 0 \\ 3 \\ 0 \\ 0$	$\frac{2/3}{0/3}$					0.50%		2/3	3/3
				0.03%	3/3	3/3	_		a 1607 1608 daling AT		0.20%		1/3	0/3
			-	0.30% 5 ng 1%	3/3	3/3	_	4	c.1007_1008defffisA1	Other	0.10%		0/3	0/3
	12	c.1609T>A	VIP	0.10% 30 ng 0.2%	$6 \frac{3/3}{2/3}$	1/3	-			-	0.05%		0/3	0/3
			-	0.05%	0/3	2/3	-				0.50%		3/3	3/3
				0.50%	3/3	3/3		10			0.20%		0/3	0/3
	11		VID	0.20% 5 ng 1%	2/3	3/3		13	c.1608_1609delins1A	Other	0.10%	_ /	0/3	0/3
		C.1010A>G	VIP	0.10% 30 ng 0.2%	ó 2/3	3/3				_	0.05%		0/3	0/3
				0.05%	1/3	2/3					0.50%		2/3	2/3
			-	0.50%	3/3	3/3	_		c 1610 1615dupATGA	-	0.20%		0/3	0/3
	15	c 1609T>G	VIP	0.20% 5 ng 1%	3/3	3/3	_	17	CC	Other	0.10%	— /	0/3	0/3
			• •	0.10% 30 ng 0.2%	6 1/3	1/3	_			-	0.10%		0/3	0/3
				0.05%	0/3	1/3	_				0.09%		3/3	2/3
			-	0.50%	3/3	3/3	_			-	0.30%		0/3	$\frac{2}{0}$
	16	c.1613A>G	VIP	0.20% 5 ng 1%	3/3	3/3	_	18	c.1625A>G	Other	0.2070	/	$\frac{0/3}{0/2}$	0/3
				0.10% 30 ng 0.2%	$\begin{array}{c c} 0 & 1/3 \\ \hline 0/2 \end{array}$	<u> </u>	_			-	0.10%		0/3	0/3
				0.03%	0/3	1/3					0.05%		0/3	0/3

Conclusion

The AmoyDx[®] HANDLE Breast Cancer Liquid NGS Panel demonstrates high sensitivity and specificity in detecting variants within the SeraSeq ESR1 and *PI3KCA* reference materials. Leveraging ultrasensitive HANDLE Liquid technology, the assay delivers superior detection performance while offering a streamlined NGS workflow as easy as PCR.

AmoyDx[®] HANDLE Breast Cancer Liquid NGS Panel Performance Evaluation

Appendix

Detailed NGS detection information for each variants including allele frequency (Freq) and allele depth(ADP) is provided below. Better sensitivity could be achieved by further optimizing the assay cut-off.

Table 5: "VIP", "HotSpot" and "other" mutations detection Results at 10 ng and 30 ng Input

								10 ng										30 ng						
Gene	#	Nucleic acid change	ADx Tag	s Repeat	W		0.0	5%	0.10	0%	0.20)%	0.50)%	W	T	0.0	5%	0.1	0%	0.2	0%	0.5	0%
					Freq	ADP	Freq	ADP	Freq	ADP	Freq	ADP	Freq	ADP	Freq	ADP	Freq	ADP	Freq	ADP	Freq	ADP	Freq	ADP
				Repeat1			0.19%	20	0.31%	24	0.11%	11	0.47%	59			0.17%	91	0.13%	64	0.15%	82	0.52%	322
	1	c.1138G>C	VIP	Repeat2			0.05%	6	0.17%	16	0.16%	23	0.52%	69			0.05%	25	0.07%	35	0.36%	193	0.66%	423
				Repeat3			0.06%	8	0.12%	11	0.17%	19	0.97%	123			0.07%	35	0.14%	76	0.18%	103	0.51%	302
				Repeat1			0.30%	31	0.25%	18	0.10%	9	0.42%	54	0.01%	2	0.05%	20	0.13%	47	0.17%	69	0.67%	303
	2	c.1387T>C	VIP	Repeat2					0.18%	17	0.13%	14	0.87%	107			0.08%	32	0.10%	40	0.22%	84	0.52%	235
				Repeat3			0.05%	6	0.14%	14	0.38%	37	1.12%	131			0.09%	35	0.09%	33	0.25%	102	0.73%	312
				Repeat1			0.01%	1	0.17%	9	0.44%	32	1.25%	132	0.01%	3	0.07%	21	0.11%	33	0.29%	97	0.57%	231
	5	c.1607T>A	VIP	Repeat2					0.23%	18	0.59%	60	0.78%	87			0.13%	41	0.10%	35	0.53%	180	0.60%	262
	C C			Repeat3			0.02%	2	0.11%	9	0.21%	18	0.74%	79			0.06%	17	0.12%	39	0.34%	121	0.63%	247
-				Repeat1			0.20%	16	0.04%	2	0.44%	32	0.31%	33			0.12%	35	0.06%	18	0.23%	75	0 59%	238
	6	c 1607T>C	VIP	Repeat?			0.2070		0.29%	23	0.12%	12	0.31%	54			0.12%	16	0.20%	67	0.12%	40	0.67%	295
	0	C.100712C	V II	Repeat3			0.01%	1	0.22%	25	0.12%	14	0.45%	101			0.0370	35	0.2070	31	0.1270 0.34%	122	0.59%	273
				Repeat1			0.03%	2	0.02%	2	0.1770	6	0.55%	71			0.12%	3	0.1076	6	0.27%	74	0.5570	186
	7	c 1607T\G	VID	Repeat?			0.05%	15	0.0070	<u> </u>	0.0070	24	0.0770	03			0.01%	32	0.0270	10	0.2270	83	0.4070	296
	1	C.10071>C	V II	Repeat2 Dopost3			0.1070	15	0.1070	14	0.2470	<u></u> 17	0.0470	<u> </u>				<u> </u>		<u> </u>	0.2370 0.240/	<u>83</u> <u>84</u>	0.0770	$\begin{array}{c} 290 \\ \hline 214 \end{array}$
				Repeats Depeat			0.10%	13	0.14%	11	0.20%		0.22%	<u> </u>			0.03%	20	0.09%	<u> </u>	0.24%	04 07	0.34%	$\begin{array}{c} 214 \\ \hline 240 \end{array}$
	0	~ 1.007 1.000 dollars A.C.		Repeat					0.21%	<u> </u>	0.37%	<u> </u>	0.18%	19				52	0.05%	$\frac{10}{20}$	0.25%	$\frac{62}{101}$	0.00%	$\begin{array}{c} 240 \\ \hline 216 \end{array}$
ESRI	8	c.1607_1608definsAG	VIP	Repeat2			0.28%	20	0.00%	5	0.48%	49	0.08%	/5			0.05%	15	0.09%	30	0.30%	121	0.49%	210
_				Repeat3			0.14%	13	0.07%	0	0.09%	8 07	0.83%	88			0.04%	13	0.000/	<u> </u>	0.24%	85	0.82%	322
	1 1	1 (10) 0	LUD	Repeat			0.13%	10			0.35%	25	0.80%	84			0.08%	23	0.22%	68	0.24%	/8	0.63%	256
		c.1610A>C	VIP	Repeat2			0.09%	8	0.03%	2	0.09%	9	0.56%	63			0.05%	15	0.10%	33	0.30%	101	0.60%	267
				Repeat3							0.13%		0.94%	100			0.08%	24	0.04%	13	0.13%	46	0.56%	222
				Repeat1			0.14%	11	0.32%	17	0.35%	25	0.58%	61			0.14%	40	0.08%	23	0.28%	92	0.92%	371
	12	c.1609T>A	VIP	Repeat2			0.04%	4	0.29%	23	0.13%	13	0.66%	74			0.01%	2	0.14%	48	0.20%	69	0.63%	278
				Repeat3					0.05%	4	0.38%	32	0.72%	77			0.10%	28	0.09%	30	0.31%	112	0.75%	296
				Repeat1			0.08%	6	0.47%	25	0.40%	29	0.45%	48			0.10%	29	0.16%	50	0.14%	47	0.41%	166
	14	c.1610A>G	VIP	Repeat2			0.05%	5			0.24%	25	0.62%	69			0.09%	29	0.17%	56	0.17%	59	0.50%	221
				Repeat3			0.12%	11	0.26%	21	0.06%	5	0.42%	45			0.14%	40	0.16%	53	0.24%	86	0.29%	116
				Repeat1			0.19%	15			0.60%	43	0.38%	40			0.02%	7	0.08%	24	0.28%	92	0.54%	220
	15	c.1609T>G	VIP	Repeat2					0.06%	5	0.27%	28	0.47%	52			0.10%	30	0.18%	61	0.19%	63	0.74%	327
				Repeat3					0.28%	23	0.14%	12	0.81%	86			0.07%	20	0.05%	16	0.30%	109	0.44%	175
				Repeat1	0.08%	3	0.11%	9	0.23%	12	0.24%	17	0.53%	56	0.02%	4	0.04%	12	0.10%	30	0.36%	118	0.68%	273
	16	c.1613A>G	VIP	Repeat2			0.11%	10	0.01%	1	0.30%	30	0.29%	32			0.06%	18	0.17%	55	0.20%	69	0.34%	149
				Repeat3			0.07%	7			0.39%	33	0.52%	55			0.10%	28	0.11%	36	0.29%	102	0.55%	217
				Repeat1	0.05%	2					0.11%	9	0.41%	43	0.01%	3	0.03%	8	0.17%	43	0.31%	84	0.32%	100
	20	c.1633G>A	VIP	Repeat2			0.12%	12	0.58%	53	0.01%	1	0.45%	51			0.13%	34	0.03%	8	0.19%	51	0.45%	131
				Repeat3			0.01%	1	0.23%	22	0.57%	53	0.70%	72			0.04%	10	0.00%	53	0.20%	56	0.51%	151
PIK3CA				Repeat1	0.04%	3	0.01%	2	0.13%	12	0.29%	39	0.73%	114	0.01%	4	0.05%	28	0.10%	50	0.19%	102	0.52%	344
	21	c.3140A>G	VIP	Repeat?			0.01%	1	0.13%	26	0.22%	77	0.75%	70	0.0170	•	0.09%	<u> </u>	0.10%	65	0.12%	66	0.32%	284
		0.51101120		Repeat3			0.01%	22	0.10%	39	0.1370	28	0.30%	59			0.03%	15	0.12%	92	0.12%	129	$\frac{0.1270}{0.41\%}$	250
							0.1570		10	nσ	0.1770	20	0.5270	57			0.0570	10	30	ησ	0.2270	127	0.1170	230
Gene	#	Nucleic acid change	ADy Tag	s Reneat	W	/T	0.0	5%	0.10	<u>በይ</u> ገ%	0.20)%	0.50)%	W	т	0.0	5%	0.1	<u>118</u> 0%	0.20	ገ%	0.5	0%
Oche	11	i delete dela enange	TIDA Tugi	s Repeat	Freq		Freq		Freq		Freq		Freq		Freq		Freq		Freq		Freq		Frea	
				Reneat1			0.05%	4	0.32%	17	$\frac{1109}{0.44\%}$	32	0.61%	64	Incq				0.16%	48	0.16%	52	0.40%	163
	Q	c 1610 1611delinsCA	HotSpot	Repeat?			0.0370	11	0.32%	21	0.11%	19	0.33%	37					0.13%	45	0.10%	102	0.10%	239
			Interpor	Repeat3			0.1270		$\begin{array}{c} 0.20\% \\ 0.09\% \end{array}$	<u></u> 7	0.15%	21	0.3370	$\frac{37}{43}$			0.08%	23	0.12%		0.30%	78	0.5470	259
ESR1				Repeat1					0.07%	/ 	0.2370	35	0.70%				0.0070	23	0.1270	<u> </u>	0.2270	105	0.0070	$\begin{array}{c} 237 \\ \hline 238 \end{array}$
	10	c 1600 1610 deline AG	HotSpot	Repeat?			0.05%	5	0.1770		0.4070	36	0.2270	<u> </u>					0.10%		0.3270	<u> </u>	0.57%	$\begin{array}{c} 230 \\ \hline 221 \end{array}$
	10			Repeat?			0.00/	2 28			0.3370	22	0.50%	56			0 1104	21	0.10/0	<u> </u>	0.20/0	2 1 81	0.5070	100
				Repeats Depent1			0.3070	20	0 110/	6	0.3770	15	0.3270	38			0.1170	18	0.1070	<u>J1</u>	0.2370 0.210/2	57	0.3070	177
DIKSCV	10	$c 1624G > \Lambda$	Hotenot	Repeat?			0.1070	U U	0.1170	16	0.1070	1J 7	0.3070	20 20			0.0070	10	0.000/	71	0.2170	<u> </u>	0.7470	07
IINJCA	17	C.10240/A	Ποιωροι	Dopost2			0.070/	Q	0.1770	10	0.0770	6	0.7070	26					0.0970	1.0	0.2970 0.170/	19	0.5470	192
				Кереаіз			0.0770	0	10	na	0.00%	0	0.33%	30					20	10	0.1770	4/	0.0270	105
Gana	#	Nucleic acid change		c Ronant	11	/ T	0.0	5%		<u>п</u> <u>у</u> 70%	0.20)%	0.50)0⁄~	TX 7	Ϋ́Τ	0.0	5%		Π <u>β</u> Π%	0.20	ጋ0⁄ራ	0.50	0%
UEIIE	#	Inucleic actu change	ADX Tag	s Repeat	Eroa		Eroa		Eroa		Eroa		Eroa		Frog		Erog		Eroa		Eroa		Eroa	
				Dopost1	Tieq	ADF	rieq	ADF	Tieq	ADF			0.65%	ADF	rieq	ADF	rieq	ADF	0.27%		0.210/	ADF 102	1 1eq	$\frac{ADF}{226}$
	2		Other	Repeat 1							0.22%	10	0.03%	<u> 00</u> <u> </u>					0.27%	<u> </u>	0.31%	102	$\frac{0.81\%}{0.76\%}$	$\begin{array}{c} 520 \\ \hline 224 \end{array}$
	3	c.1603C>A	Other	Repeat2			0.210/	20	0.220/	26	0.30%	<u> </u>	0.49%	<u> </u>			0.120/	25	0.15%	49	0.14%	40	0.70%	$\begin{array}{c} 334 \\ \hline 246 \end{array}$
				Repeat3			0.31%	29	0.110	20	0.31%	<u>31</u>	0.2001	4/			0.12%	50 50	0.23%	15	0.170	ð/	0.03%	240 250
				Repeat			0.30%	24	0.11%	6	0.31%	22	0.39%	41			0.18%	52	0.23%	69	0.15%	49	0.64%	258
	4	c.1607/_1608delinsAT	Other	Kepeat2			0.12%	11	0.10%	8	0.39%	40	0.84%	93							0.34%	114	0.56%	247
				Repeat3							0.54%	46	0.57%	61					0.12%	40	0.24%	86	0.56%	
				Repeat1			0.21%	17	0.23%	12	0.21%	15	1.01%	107					0.14%	42	0.24%	78	0.73%	297
ESR1	13	c.1608_1609delinsTA	Other	Repeat2			0.19%	18					0.72%	80					0.14%	47	0.32%	108	0.62%	273
				Repeat3					0.12%	10	0.38%	32	0.53%	57					0.12%	37	0.36%	130	0.55%	216
		c 1610 1615 due ATCAC		Repeat1			0.16%	13	0.04%	2	0.19%	14	0.40%	42			0.10%	30	0.06%	17	0.13%	43	0.55%	222
	17		Other	Repeat2			0.04%	4	0.21%	17	0.06%	6	0.70%	78			0.06%	18	0.14%	46	0.18%	60	0.48%	211
				Repeat3			0.01%	1	0.28%	23	0.09%	8	1.16%	123			0.01%	4	0.14%	46	0.13%	48	0.51%	199
				Repeat1			0.18%	14			0.22%	16	0.61%	64					0.15%	46	0.21%	68	0.48%	193
	18	c.1625A>G	Other	Repeat2			0.12%	11			0.28%	29	0.81%	90			0.23%	72	0.12%	41	0.15%	51	0.62%	274
				Repeat3					0.12%	10	0.12%	10	0.58%	62			0.24%	70	0.28%	90	0.25%	90	0.54%	214

Note: The numbers in RED indicate variant detection results below the established Cutoff, included for the purpose of exploring the product's limit of detection.