Enhanced detection of ESR1 mutations in breast cancer: validation of the GALEAS uPCR:ESR1 kit on multiple commercially available reference standards.

Charlotte Morgan, Agata Stodolna, Michael Parks (Nonacus Ltd, Birmingham, United Kingdom)

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Introduction

Mutations in the ESR1 gene are a significant cause of resistance to hormone therapy in estrogen receptor (ER)-positive breast cancer patients and arise in 20-40% of metastatic endocrine-therapy resistant cancers. Detecting them through liquid biopsy testing can guide treatment towards more effective targeted therapies (1).

Clinical guidelines issued by the European Society of Medical Oncology (ESMO) (2) and American Society of Clinical Oncology (ASCO) (3) recommend the use of liquid biopsy for ESR1 mutation testing in metastatic breast cancer during administration of endocrine therapy to guide treatment decisions in the event of relapse. However, current options for ESR1 variant detection methods can be complex and highly technical, require expensive equipment and intricate analysis, and often have long turnaround times.

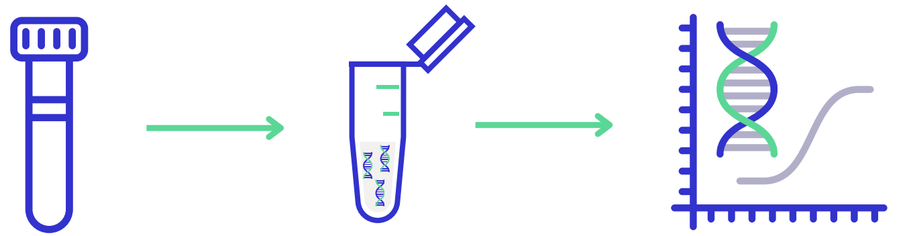
To help overcome these challenges, the GALEAS uPCR:ESR1 kit uses proprietary ultrasensitive qPCR technology developed at Nonacus to offer laboratories a simple, cost-effective alternative that is designed to detect eleven of the most prevalent ESR1 variants (Table 1.) known to be associated with endocrine therapy resistance. Analytical sensitivity exceeds 97% across targets and specificity is ≥98%. When combined with our cfDNA Blood collection tubes and extraction kits, Nonacus provides a complete workflow for ESR-1 mutation detection (Figure 1).

Here we describe the validation of this assay using three commercially available reference standards and demonstrate that ESR1 resistance mutations can be detected at allele frequencies as low as 0.1%.

Table 1. Variants detected by the GALEAS uPCR:ESR1 assay.

MULTIPLEX	ESR1 VARIANT (PROTEIN CHANGE)	VARIANT PREVALENCE	MULTIPLEX	ESR1 VARIANT (PROTEIN CHANGE)	VARIANT PREVALENCE
1	p.E380Q	HIGH	2	p.L536H	LOW
1	p.D538G	HIGH	3	p.L536R	LOW
1	p.Y537S	HIGH	3	p.P535H	LOW
1	p.Y537N	HIGH	3	p.L536Q	LOW
2	p.S463P	LOW	3	p.Y537D	LOW
2	p.Y537C	LOW	3	Endogenous control	N/A

Figure 1. Complete workflow from sample collection to mutation detection.



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Materials and methods

REFERENCE MATERIAL VALIDATION

To evaluate the performance and analytical sensitivity of the assay, three commercially available ESR1 cfDNA reference materials were used:

SensID ESR1 Reference Set 1% AF cfDNA – includes 5 vials, containing wild-type and a subset of 9 ESR1 mutations (Table 2.), of which 8 are relevant to the GALEAS uPCR:ESR1 kit.

Zeptometrix ESR1 control kit – includes 5 vials, containing wild-type and a subset of 16 ESR1 mutations (Table 2.), of which 10 are relevant to the GALEAS uPCR:ESR1 kit.

Seraseq® ctDNA ESR1 Mutation Mix AF1 – includes 2 vials, containing wild-type and a subset of 18 ESR1 mutations (Table 2.), 10 of which are relevant to the GALEAS uPCR:ESR1 kit.

A total of 15 ng of each reference material was tested at nominal mutant allele frequencies (MAF) of 1%, 0.3%, and 0.1%, diluted in a background of wild-type cfDNA. Each dilution of the reference material was tested across all primer–probe mixes included in the assay in duplicate. The Ct value cut off for a positive result is 32 for distinguishing mutant from wild-type. Each of the 3 runs also included positive controls (FAM and HEX), negative controls, and no-template controls (NTCs) that are included with the kit to ensure assay validity. Ct cut-offs for controls are defined within the kit protocol.

Table 2: Mutation coverage of GALEAS uPCR:ESR-1 by each reference standard tested.

ESR-1 VARIANT	SensID	ZeptoMetrix	seracare
E380Q	✓	✓	✓
D538G	✓	✓	✓
Y537S	✓	✓	✓
Y537N	✓	✓	✓
S463P	✓	✓	✓
L536H	✓	✓	✓
Y537C	✓	✓	✓
P535H	✓	✓	✓
L536R	✓	✓	✓
L536Q	✓	✓	✓
Y537D	✓	✓	✓

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Results

1. All reference standards were able to discriminate between the wild-type and variants, see an example of amplification plots.
2. All variants were detected in all reference standards down to 0.1%, with the exception of the S463P target in the Zeptometrix reference standard (Table 3).
3. With both SensID and Zeptometrix it is possible to discriminate between mutations due to the targets being split in different multiplexes.

Table 5: Lowest mutation detection for SensID, Zeptometrix and Seraseq ESR1 reference material using 15ng input.

PRIMER/ PROBE MIX	ESR1 MUTATION	SensID	ZeptoMetrix	seracare
1	p.E380Q	0.1%	0.1%	0.1%
1	p.D538G	0.1%	0.1%	0.1%
1	p.Y537S	0.1%		
1	p.Y537N	0.1%	0.1%	
2	p.S463P	0.1%	0.3%	0.1%
2	p.Y537C	0.1%	0.1%	0.1%
2	p.L536H		0.1%	
3	p.L536R	0.1%	0.1%	0.1%
3	p.L535H	-	0.1%	-
3	p.L536Q	-	0.1%	0.1%
3	p.Y537D	-	-	

Figure 2: SensID - Amplification plot of Y537N target showing no WT bleed through (multiplex 1 of GALEAS uPCR:ESR1 kit).

PC1: Positive control, FAM channel; SensID reference set at 0.3% and 0.1% duplicate repeats, FAM channel; Wild-type, negative control and NTC

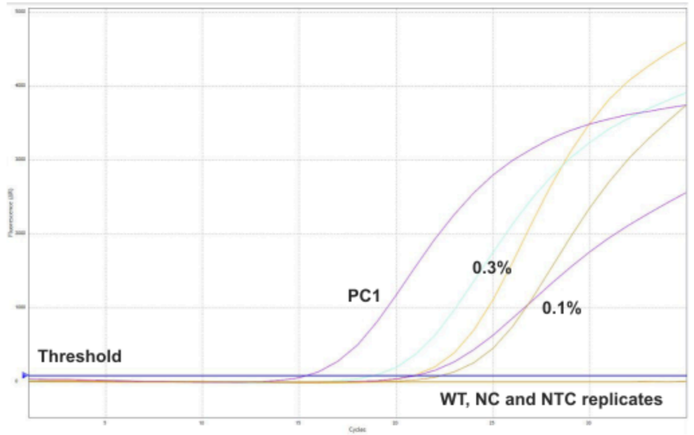


Figure 3: Zeptometrix - Amplification plot of E380Q target showing no WT bleed through (multiplex 1 of GALEAS uPCR:ESR1 kit).

PC1: Positive control, HEX channel; Zeptometrix reference set at 1%, 0.3% and 0.1% duplicate repeats; Wild-type, negative control and NTC

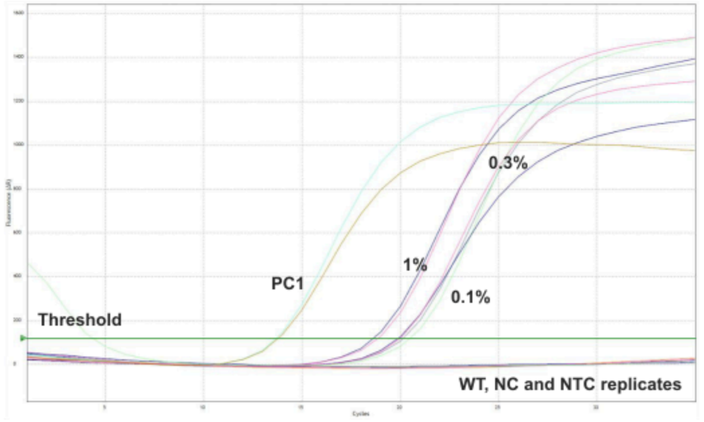
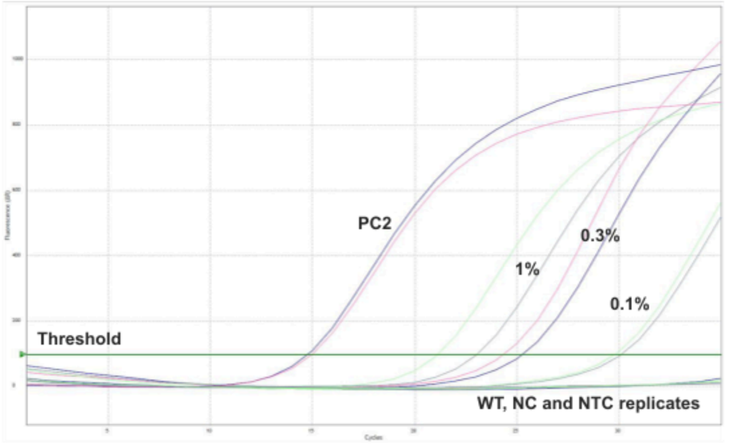


Figure 4: Amplification plot of S463P target showing no WT bleed through (multiplex 2 of GALEAS uPCR:ESR1 kit).

PC2: Positive control, HEX channel; Seraseq reference set at 1%, 0.3% and 0.1% duplicate repeats; Wild-type, negative control and NTC



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Conclusion

The validation with SensID, ZeptoMetrix, and SeraCare reference standards further supports the robustness and reliability of the GALEAS™ uPCR: ESR1 kit to detect variants down to 0.1%. Each reference standard could serve as independent control material for all 3 primer probe mixes and can be used as an additional control for internal instrument / method validation. SensID and Zeptometrix controls are able to discriminate between targets.

In summary, The GALEAS uPCR:ESR1 qPCR kit presents a simple, highly sensitive solution for the detection of ESR1 mutations linked with endocrine therapy resistance in breast cancer. This new technology offers a faster, accurate, and cost-effective alternative to existing sequencing methods, potentially improving treatment outcomes for breast cancer patients.

Acknowledgements

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References

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