GALEAS™ uPCR: ESR1

An ultrasensitive qPCR assay for detecting *ESR1* mutations in cell-free DNA

Highlights

High accuracy and sensitivity
Confidently detect mutations in the estrogen receptor gene, *ESR1*, with high sensitivity and specificity.

Easy to use
Deliver results in under 3 hours with a simple qPCR protocol that requires no specialist equipment and can be deployed in most clinical laboratories.

Clinically relevant variants
Detect eleven of the most important variants in the *ESR1* gene from cell-free DNA in plasma.

Introduction

Mutations in the estrogen receptor gene, *ESR1*, are known drivers of resistance to standard endocrine therapy and are present in up to 40% of ER+, HER2- metastatic breast cancers[1]. Liquid biopsy studies have led to a greater understanding of the role these mutations play in resistance and clinical guidelines increasingly support the use of *ESR1* testing in metastatic breast cancer[2].

Current methods for detecting *ESR1* mutations tend to deploy either next generation sequencing (which although comprehensive, can be expensive to set up and run) or digital PCR (dPCR) (which although sensitive, also requires specialist and often expensive equipment).

GALEAS™ uPCR: ESR1 offers laboratories a simple, cost effective alternative; a qPCR assay designed to detect eleven of the most prevalent *ESR1* variants with high sensitivity and specificity using standard equipment that can be found in almost every clinical laboratory.

GALEAS™ uPCR: ESR1

GALEAS uPCR: ESR1 detects eleven of the most prevalent *ESR1* variants known to be associated with endocrine therapy resistance (Table 1). The assay uses ultrasensitive PCR (uPCR), a Nonacus proprietary technology, to deliver improved sensitivity and specificity over standard qPCR, making it comparable to digital PCR and significantly faster and less expensive than next generation sequencing.

Designed for use with cell-free DNA (cfDNA) from plasma, where input material can often be limited, the assay has been developed to minimise the number of multiplexes required and reduce the amount of input material needed.

Table 1: Variants detected by GALEAS uPCR: ESR1

Multiplex	ESR1 Variant (Protein Change)	Variant prevalence
1	p.E380Q	High
	p.D538G	High
	p.Y537S	High
	p.Y537N	High
2	p.S463P	Low
	p.Y537C	Low
	p.L536H	Low
3	p.L536R	Low
	p.P535H	Low
	p.L536Q	Low
	p.Y537D	Low
	Endogenous control	N/A

Eleven *ESR1* mutations and endogenous control are covered just with three multiplexes. The kit contains sufficient qPCR reagents for 32 reactions per multiplex.. Positive and negative controls should be included with every run such that one kit can accommodate a maximum of 29 samples. To maximise full kit capacity, a minimum of 5 samples are required per run. An endogenous control has been included to ensure confidence in results for each run. A full list of specifications can be found in Table 2.

Table 2: GALEAS uPCR: ESR1 specifications

Parameter	Specification
Method	Qualitative qPCR test
Number of Targets	11 ESR1 mutations
Sample type	cell-free DNA (cfDNA)
Input amount	1-25 ng
Kit format	32 reactions per multiplex, accommodates 29 samples when running x3 controls
Controls	3 positive controls and 1 negative control
Protocol length	Under 3 hours

GALEAS™ uPCR: ESR1 Performance

GALEAS uPCR: ESR1 was tested using synthetic controls and commercially available reference standards containing multiple known *ESR1* variants.

Analytical Sensitivity

The sensitivity of the kit was determined using synthetic controls at 60 replicates across three independent qPCR runs. The Limit of Detection (LoD) was determined to be between 0.04% and 0.33% Mutant Alle Frequency (MAF) at an input of 25 ng, depending on the target. The analytical sensitivity at LoD averaged across all targets was >97% (range of 93.3% to 100%) (Table 3).

Table 3. Analytical Sensitivity of the GALEAS uPCR: ESR1 assay

ESR1 Variant (Protein Change)	LoD (% MAF)	Mutant copies at LoD	Percentage detection overall
E380Q	0.13%	10	100%
D538G	0.33%	25	96.66%
Y537S	0. 20%	15	100%
Y537N	0.07%	5	100%
S463P	0.33%	25	96.66%
Y537C	0.33%	25	100%
L536H	0.13%	10	100%
L536R	0.26%	20	100%
P535H	0.07%	5	100%
L536Q	0.04%	3	93.33%
Y537D	0.13%	10	90%

Analytical Specificity in cfDNA

The analytical specificity was determined for extracted cfDNA using 25 ng of commercially sourced cfDNA wild type reference standard per reaction and testing 75 technical replicates for each multiplex. To demonstrate that genomic DNA (gDNA) contamination would not impact specificity, the same testing was conducted on 25 ng of gDNA wild type reference standard per reaction with 90 replicates for each multiplex. Overall **≥98% specificity** was obtained for all multiplexes across both cfDNA and gDNA(Table 4).

Analytical Specificity in FFPE samples

In addition, analytical specificity for gDNA extracted from 25 individual FFPE human tissue specimens were tested at 25 ng input per reaction for each multiplex to confirm kit compatibility for FFPE material.
Overall **100% specificity** was obtained for all multiplexes across all FFPE samples.

Table 4. Analytical Specificity of GALEAS uPCR: ESR1 Robust specificity testing for different types of sample material.

ESR1 Variant	Detection channel	SensID cfDNA WT standard (25ng)	SensID gDNA WT standard (25ng)
E380Q	HEX	0/75 (100%)	0/90 (100%)
D538G	FAM	1/75 (>98%)	0/90 (100%)
Y537S	FAM		
Y537N	FAM		
S463P	HEX	1/75 (>98%)	0/90 (100%)
Y537C	FAM	0/75 (100%)	0/90 (100%)
L536H	FAM		
L536R	FAM	1/75 (>98%)	1/90 (>98%)
P535H	FAM		
L536Q	FAM		
Y537D	FAM		
Internal Control	HEX	Ct 16.15 - 17.86	Ct 14.16 - 15.65

Validation of GALEAS™ uPCR: ESR1 using SensID Reference standard

GALEAS™ uPCR: *ESR1* kit performance was validated using commercially available *ESR1* reference standards from SensID, GmbH. 15ng of the reference standard at 1%, 0.3% and 0.1% MAF was tested in a background of SensID wild-type cfDNA. 0.1% MAF was detected, and 100% specificity was achieved (Table 5). No wild type bleed through was observed in any of the three MAFs tested for any of the *ESR1* variants targeted, an example for one of the variants, D538G, is shown in Figure 1.

Table 5. Assay performance validation with reference material.
P535H, L536Q and Y537D have not been tested as these targets are not included in the reference standard set.

ESR1 Variant	Lowest MAF	Detected?
E380Q	0.1% in 15ng total	Yes
D538G	0.1% in 15ng total	Yes
Y537S	0.1% in 15ng total	Yes
Y537N	0.1% in 15ng total	Yes
S463P	0.1% in 15ng total	Yes
Y537C	0.1% in 15ng total	Yes
L536H	0.1% in 15ng total	Yes
L536R	0.1% in 15ng total	Yes

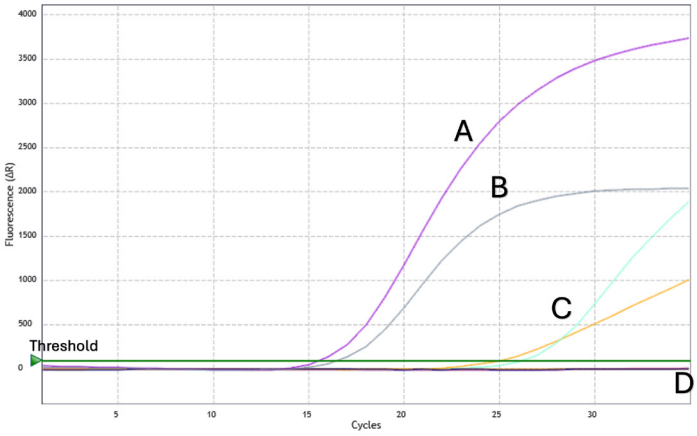
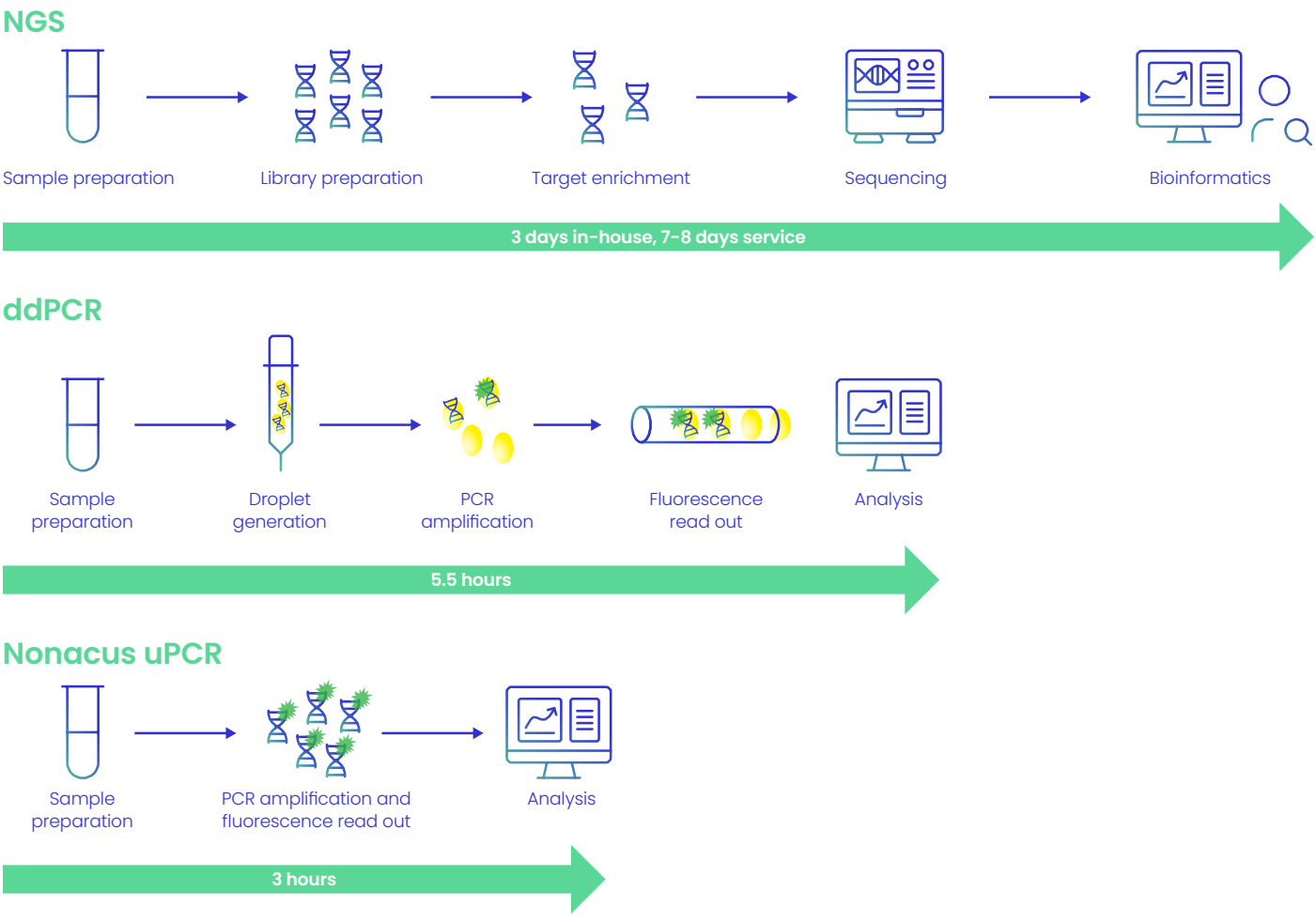


Figure 1. Amplification plot of D538G target showing no WT bleed through.
A) Positive control PC1, FAM channel; B) Positive Control PC1, HEX channel; C) SensID Tube 3, 0.3% duplicate repeats, FAM channel (D538G); D) wild-type control.

Streamlined workflow - quick and easy protocol

GALEAS™ uPCR: ESR1 achieves the sensitivity of digital PCR with the simplicity of qPCR. As it uses standard laboratory qPCR cyclers, there is no need for expensive specialist equipment and it can be deployed in almost any clinical laboratory worldwide. Easily scalable and with less steps and a less complex workflow than digital PCR or next generation sequencing, GALEAS uPCR: ESR1 supports a fast turnaround time from sample to result.

Figure 2. Workflow comparisons for uPCR, digital PCR and Next Generation Sequencing (NGS).



Summary

GALEAS uPCR: ESR1 detects 11 of the most prevalent mutations in the ESR1 gene with high sensitivity and specificity in cfDNA samples. The assay uses ultrasensitive PCR (uPCR), a proprietary qPCR-based technology, to deliver improved sensitivity over standard qPCR offering laboratories a simple, fast, inexpensive assay for *ESR1* mutation detection using standard molecular laboratory equipment.

Learn more

To learn more about GALEAS™ uPCR: ESR1 and to download the protocol, MSDS and other documents please visit: **www.nonacus.com**

References:

1. Will, M., Liang, J., Metcalfe, C., & Sarat Chandarlapaty. (2023). Therapeutic resistance to anti-oestrogen therapy in breast cancer. *Nature Reviews Cancer, 23*(10), 673–685. https://doi.org/10.1038/s41568-023-00604-3

2. Loibl, S., Varga, A., Bachelot, T., Barrios, C. H., Bergh, J., Burstein, H. J., Cardoso, L., Carey, L. A., Dawood, S., Lucia Del Mastro, Carsten Denkert, Eva Maria Fallenberg, Francis, P. A., H. Gamal El Din, Gelmon, K. A., Geyer, C. E., Gnant, M., Guarneri, V., Gupta, S., & Kim, S. (2023). Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up†. Annals of Oncology, 35(2). https://doi.org/10.1016/j.annonc.2023.11.016

Ordering information

Product	Pack size	Catalog No.
GALEAS™ uPCR: ESR1	96 reactions (32 reactions per multiplex)	PCR_GAL_ESR1_96

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