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SafeSEQ

Breast Cancer Liquid Biopsy NGS Panel

Ultra-sensitive mutation detection

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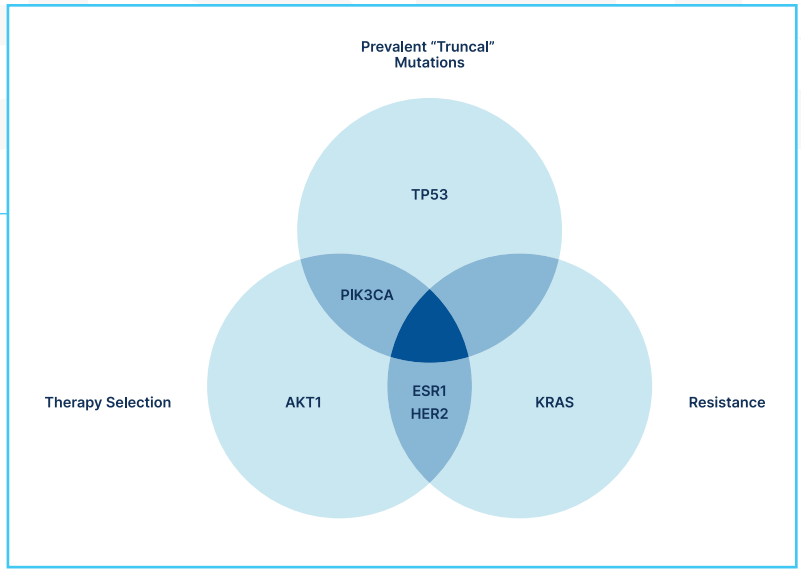


PIK3CA, ESR1, AKT1, ERBB2, TP53, and KRAS

Reliable detection of low-frequency plasma mutations

Sysmex's SafeSEQ Breast Cancer Panel is a clinical grade, ultra-sensitive liquid biopsy solution for the identification of gene mutations in PIK3CA, ESR1, AKT1, ERBB2, TP53, and KRAS to detect established and emerging therapeutic indications, resistance mutations, and frequently occurring somatic alterations in breast cancer.

The SafeSEQ Breast Cancer Panel can detect clinically relevant mutations in circulating tumor DNA (ctDNA) from patients with breast cancer across a broader range of genomic regions with a sensitivity equivalent to Sysmex Inostics' OncoBEAM digital PCR liquid biopsy breast cancer test.

The SafeSEQ Breast Cancer Panel is available as a CLIA testing service.

Panel Features

Ultra-high sensitivity coverage of the most relevant targets for HR+ breast cancer yields more information, with greater efficiency.

SAFESEQ BREAST CANCER PANEL COVERAGE

GENE	TRANSCRIPT	AMINO ACIDS COVERED	EXAMPLE VARIANTS WITH KNOWN CLINICAL RELEVANCE
AKTI	ENST00000554581	17-23	c.49G>A (p.E17K)
ERBB2	ENST00000269571	303-315, 754-769, 770-786	c.929C>T (p.S310F), c.929C>A (p.S310Y), c.2301C>G (p.I767M), c.2313_2324dup (p.V772_A775dup), c.2314_2325dup (p.V772_A775dup)
ESR1	ENST00000440973	370-381, 460-473, 529-538	c.1138G>C (p.E380Q), c.1387T>C (p.S463P), c.1607T>G (p.L536R), c.1607T>C (p.L536P), c.1607T>A (p.L536H), c.1607_1608delinsAG (p.L536Q), c.1607_1608delinsAT (p.L536H)
KRAS	ENST00000256078	4-14	c.34G>A (p.G12S), c.34G>C (p.G12R), c.34G>T (p.G12C), c.35G>A (p.G12D), c.35G>C (p.G12A), c.35G>T (p.G12V)
PIK3CA	ENST00000263967	86-92, 111-117, 119-122, 345-352, 363-371, 418-421, 450-462, 538-553, 714-728, 1040-1056	c.263G>A (p.R88Q), c.353G>A (p.G118D), c.1633G>A (p.E545K), c.1633G>C (p.E545Q), c.1634A>C (p.E545A), c.1634A>G (p.E545G), c.3127A>G (p.M1043V), c.3129G>A (p.M1043I), c.3129G>C (p.M1043I)
TP53	ENST00000269305	49-77, 99-125, 126-141, 151-179, 192-219, 233-260, 262-285, 297-306, 308-331, 332-360	c.488A>G (p.Y163C), c.524G>A (p.R175H), c.817C>T (p.R273C), c.818G>A (p.R273H)

Performance Specification

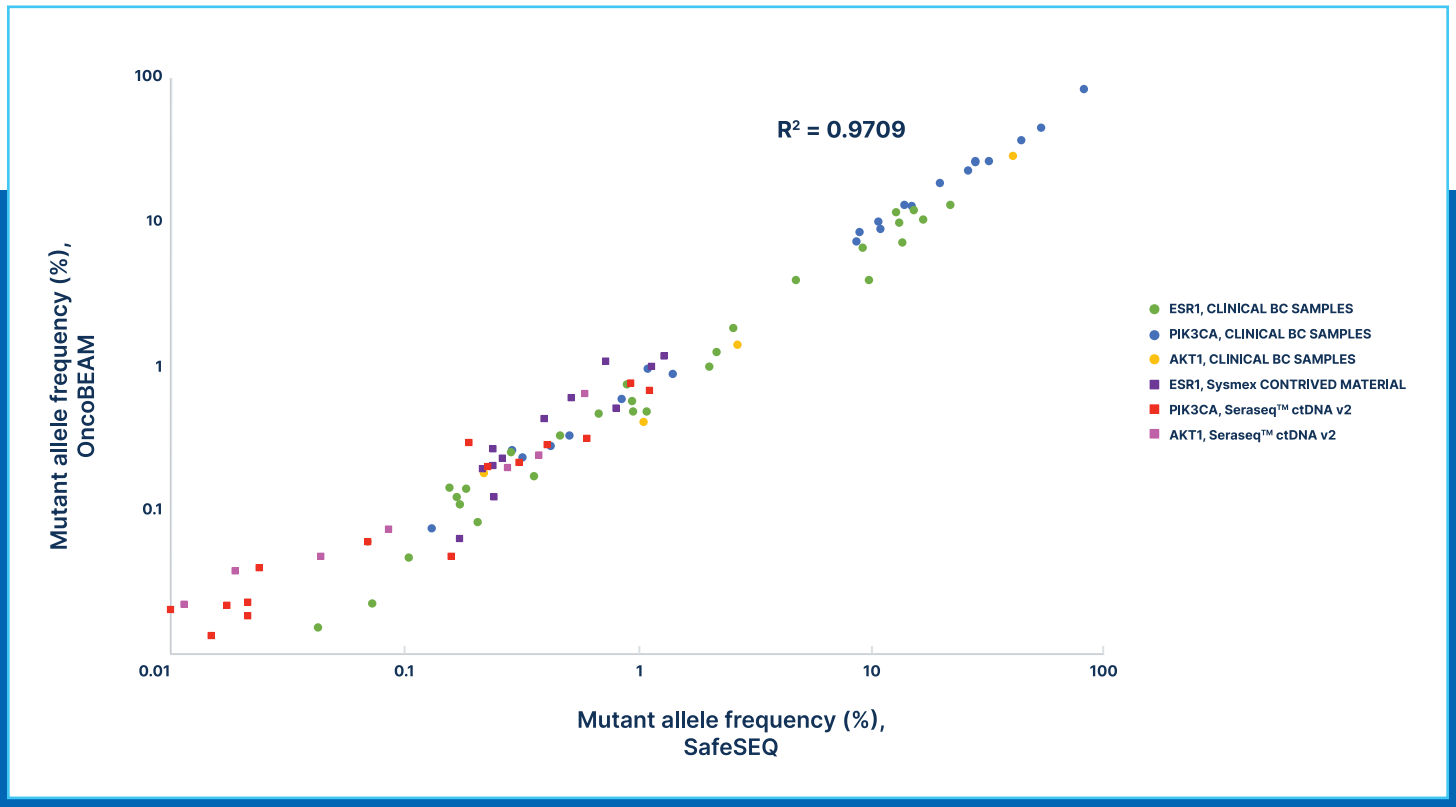
The SafeSEQ Breast Cancer Panel achieves an order of magnitude higher sensitivity compared to pan-cancer NGS tests, while maintaining robust specificity.

ANALYTICAL SENSITIVITY AND SPECIFICITY OF THE SAFESEQ BREAST CANCER PANEL

# OF ctDNA MOLECULES	MUTANT ALLELE FREQUENCY (MAF)*	ANALYTICAL SENSITIVITY	ANALYTICAL SPECIFICITY	REPORTING THRESHOLD*
20	0.10%	100%	100%	6 ctDNA molecules (0.030% MAF)
10	0.050%	98%		
5	0.025%	94%		
3	0.015%	78%		

*Based on cell-free DNA input of 66ng. Analytical sensitivity and specificity cited above is for targeted, clinically important regions. Reporting Threshold is set above LoD95.

SAFESEQ NGS SENSITIVITY SIMILAR TO ONCOBEAM™



Thirty-five clinical samples (2 ml plasma) and replicate testing (3x/method) of contrived material using SafeSEQ and OncoBEAM Breast Cancer Tests.[2]

SafeSEQ comparison to the gold-standard OncoBEAM technology

OncoBEAM set the gold standard for ctDNA analysis being the most sensitive digital PCR approach available. It has been used to detect subclonal resistance mutations, such as those in ESR1 for breast cancer patients on adjuvant aromatase inhibitor therapy who demonstrate endocrine resistance.[1] With robust detection as low as 0.02% mutant allele frequency (MAF), OncoBEAM ensures reliable molecular information for real-time therapy selection as well as monitoring of tumor response.

The figure to the left shows the robust accuracy at low allelic frequencies observed for SafeSEQ and demonstrates reliable detection of ctDNA across a broad dynamic range, which can provide new insights into tumor response and resistance as well as expedite validation of biomarker hypotheses.[2]

Clinical Relevance

Therapeutic selection: Enables maximum identification of biomarker-positive patients eligible for therapy. Less sensitive technologies can miss a significant subset of patients who may benefit from targeted therapy.

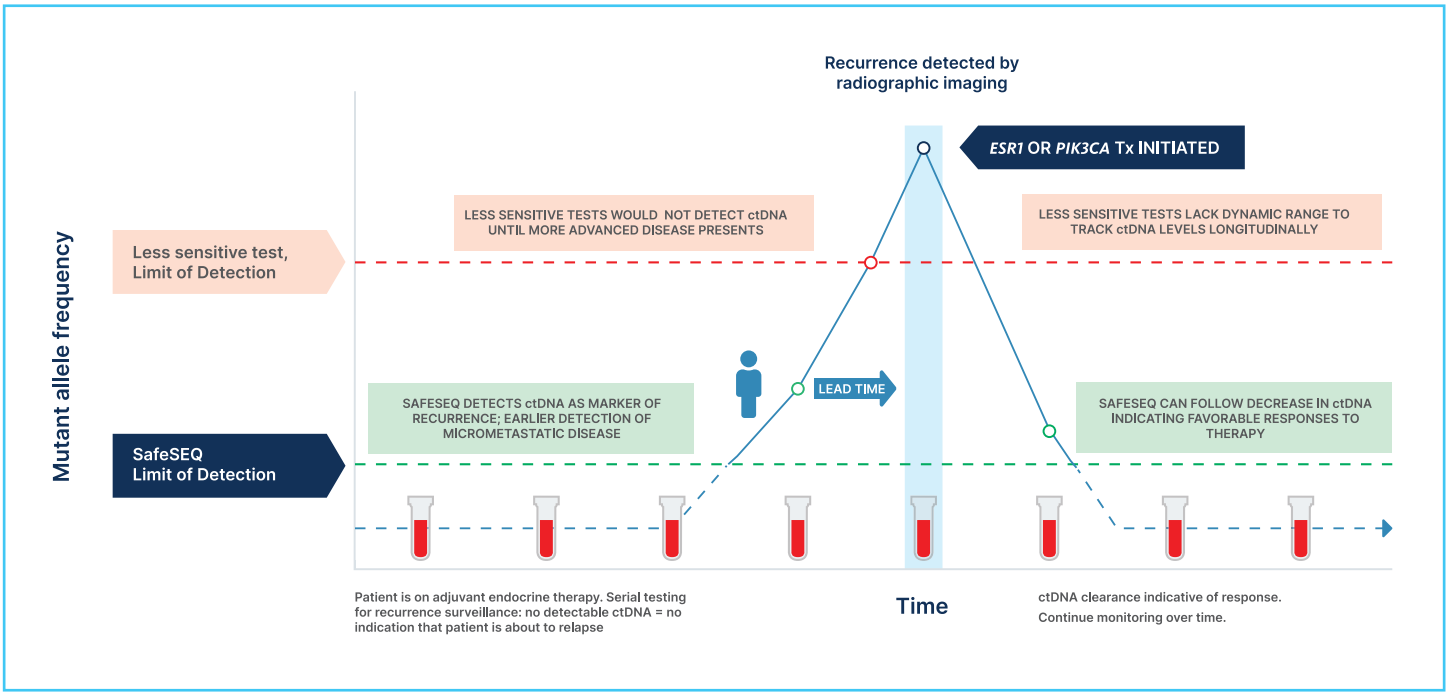
- **PIK3CA**—Identifying patients with PIK3CA mutations who derive benefit from PI3K-targeted therapy could help to guide treatment decisions. The BELLE-2 trial showed that a non-invasive ctDNA technique, such as SafeSEQ, can be used for detection of PIK3CA mutations in plasma and may provide a more accurate measure of mutational status over time and treatment, compared with archival tumor tissue.[3]

- **ESR1**—mediated resistance to endocrine therapy—SafeSEQ overcomes the challenges of detecting subclonal ESR1 mutations, a primary mechanism of resistance to aromatase inhibitors. These mutations are particularly difficult to detect since local biopsy of heterogeneous tissue may fail to capture ESR1-mutant tumor cells; additionally, ctDNA carrying ESR1 mutations is present at very low levels in circulation, making detection using less-sensitive methods very difficult.[1]

- **AKT1**—Ultra-sensitive detection of the activating mutation E17K as well as other rare mutations ensures robust and efficient investigation into the efficacy of novel therapies targeting this PI3K signaling pathway member.[4]

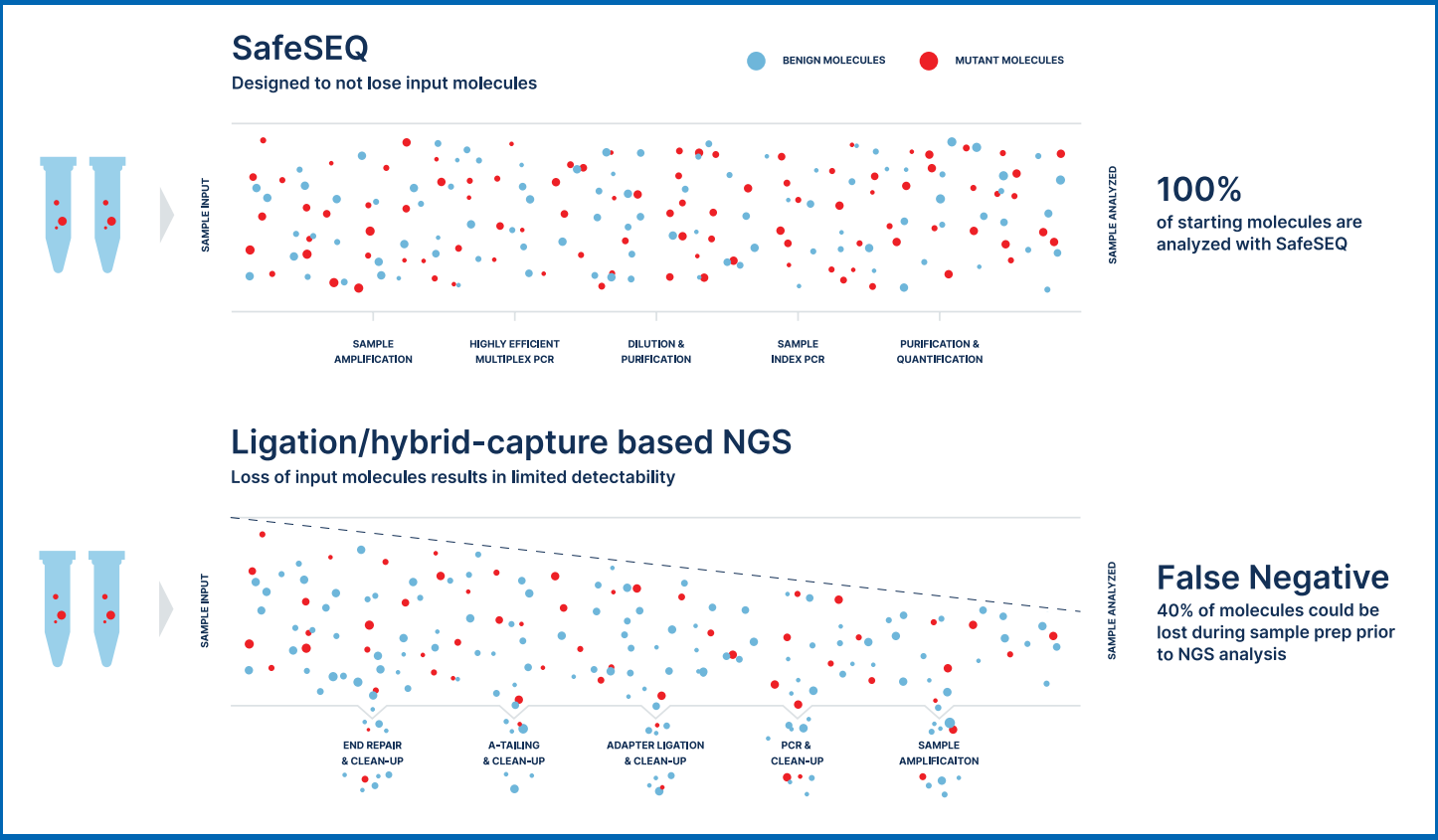
Monitor treatment response: Quantitative detection of somatic mutations enables meaningful comparison of ctDNA levels across timepoints for longitudinal monitoring. Correlating SafeSEQ results with clinical observations may reveal promising opportunities for novel therapeutic strategies.[5]

Minimal residual disease (MRD) detection and recurrence surveillance: Ultra-high sensitivity is essential for reliable detection of extremely small quantities of ctDNA that may be present post-intervention, as well as for early identification of increasing ctDNA levels which may herald relapse.[6]



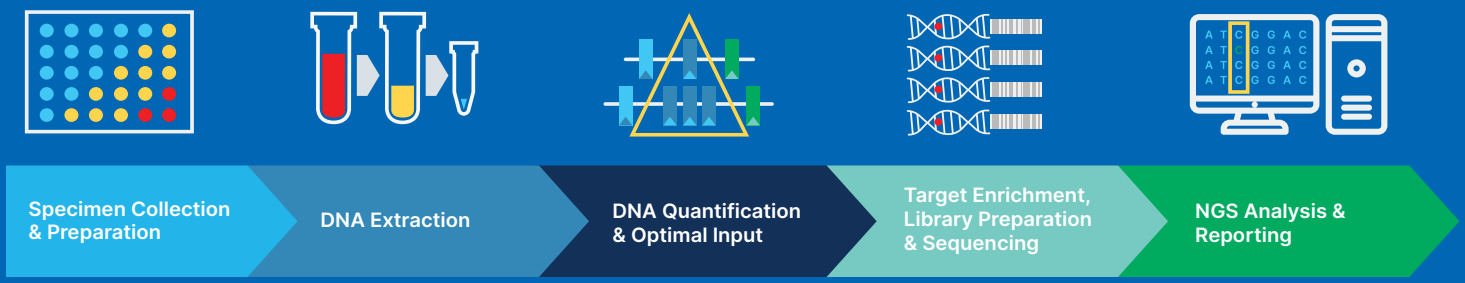
No molecule left behind

SafeSEQ technology was designed not to lose mutant molecules throughout the workflow. Other assays, particularly broad, hybrid-capture-based pan-cancer panels, are known to lose up to 40% of all Input DNA during sample prep, which decreases reliable detection of ctDNA.



SafeSEQ workflow

The SafeSEQ workflow delivers exquisite performance for ctDNA analysis through optimization of all steps, from pre-analytics through data analysis and reporting.



Overview of the SafeSEQ Workflow in Sysmex Inostics' CLIA-certified Laboratory.

Sample Requirements and Processing Time

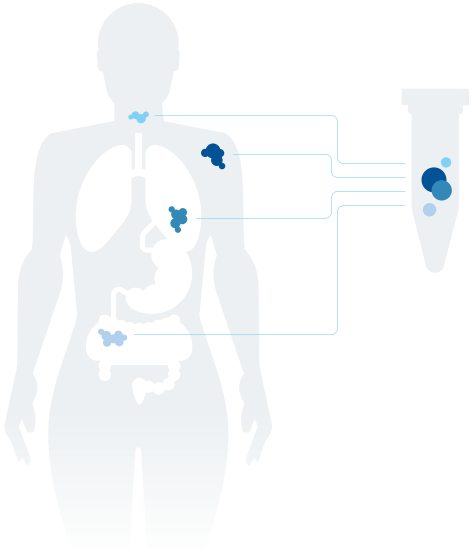
Sample Specification
2×10mL tubes of whole blood

Sample Storage & Shipping
Overnight at room temperature

Result Turnaround Time
7 – 10 days

SAFESEQ TECHNOLOGY

OncoBEAM-level sensitivity across a larger genomic area



Sysmex Inostics' SafeSEQ offers highly sensitive mutation detection across the most clinically relevant gene targets. SafeSEQ technology was designed specifically for measurement of ctDNA and panels are developed for specific clinical intended uses where high sensitivity detection may provide unique insights, and improve outcomes.

SafeSEQ is an order of magnitude more sensitive than other liquid biopsy NGS methods to accelerate trial enrollment and evaluate biomarker hypotheses with greater power.

Coverage of a larger genomic area, with sensitivity and specificity equivalent to our digital PCR platform OncoBEAM, yields unique information that can accelerate clinical studies.

Have a question about SafeSEQ Breast Cancer Panel, please visit **www.sysmex-inostics.com/contact-us.**

REFERENCES

1 Spoerke, J. M. et al. (2016) Heterogeneity and clinical significance of ESR1 mutations in ER-positive metastatic breast cancer patients receiving fulvestrant. Nat Commun. 7, 11579.
2 Rugo, H.S. et al. (2019, April). Palbociclib in combination with fulvestrant or tamoxifen as treatment for hormone receptor positive metastatic breast cancer with prior chemotherapy for advanced disease (TBCRC 035): A Phase II study with pharmacodynamic markers. Presented at the American Association of Cancer Research annual conference, Atlanta, Georgia.
3 Baselga, J. et al. (2017) Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 18, 904–916.
4 Rudolph, M. et al. (2016) AKT1 E17K mutation profiling in breast cancer: prevalence, concurrent oncogenic alterations, and blood-based detection. BMC Cancer. 16:622. doi:10.1186/s12885-016-2626-1.
5 Dawson, S.J. et al. (2013) Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med. 368:1199-209. doi:10.1056/NEJMoa1213261.
6 Rodriguez, et al. (2019) Detection of TP53 and PIK3CA mutations in circulating tumor DNA using next-generation sequencing in the screening process for early breast cancer diagnosis. J Clin Med. 8(8), 1188.doi: 10.3390/jcm8081183.

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