Oncology
Service Specification Sheet

GenomeScan® Onco-*ct*Trace

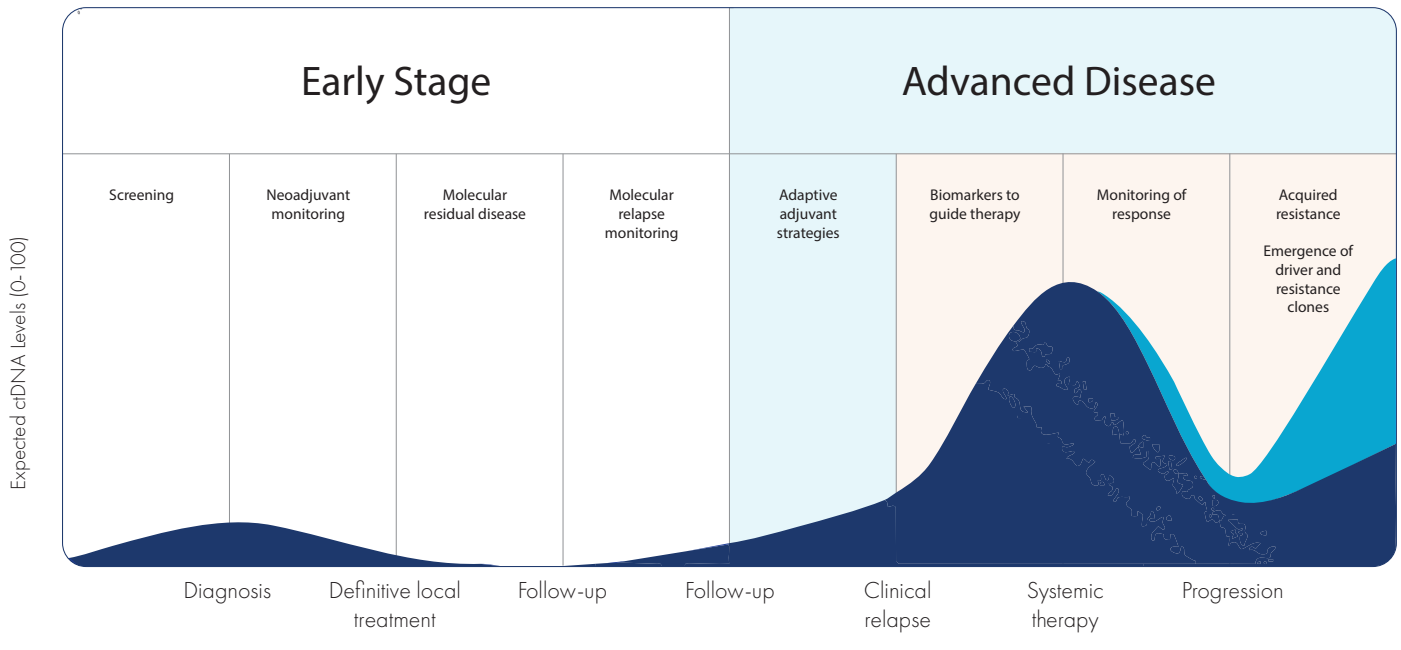
Empower your cancer research with cutting-edge ctDNA insights.

One in three people will likely be diagnosed with cancer in their lifetime. With the rapid evolution of precision oncology and personalized treatments, it's crucial to swiftly analyze cancer on a molecular level and match it with the best therapy options to improve patient outcomes. In precision oncology, understanding cancer genes and genome-wide alterations in cell-free DNA (cfDNA) provides valuable insights. These insights help identify actionable variants, assess potential therapeutic strategies, and stratify patients for clinical trials, ultimately enhancing patient outcomes.

Circulating tumor DNA (ctDNA) has multiple clinical applications, including screening, early disease characterization, detection of molecular residual disease (MRD) after definitive local treatment, prediction of relapses, genotyping advanced cancer, early assessment of treatment efficacy, monitoring response, and identifying mechanisms of resistance to therapy.

The Hedera Profiling 2 ctDNA test panel (HP2) (RUO) by HederaDx is a 32-gene assay optimized for detecting various genomic somatic alterations, including Single Nucleotide Variants (SNVs), Insertions and Deletions (Indels), Copy Number Variations (CNVs), splice variants, Gene Fusions, and Microsatellite Instability (MSI). Utilizing a streamlined DNA-only workflow enhanced with Unique Molecular Identifiers (UMIs) for molecular barcode error detection, this assay ensures sensitive identification of genomic alterations by generating duplex consensus reads from duplicates and removing sequencing artifacts. The HP2 panel covers clinically relevant biomarkers and promising molecular alterations included in major guidelines for common solid tumors. The HP2 assay is intended for *in vitro* examination of blood plasma and other samples with sufficient cfDNA, such as urine and saliva, using a targeted hybrid-capture NGS workflow, focusing on ESCAT (ESMO Scale of Clinical Actionability for molecular Targets) Evidence tier I - II genomic alterations. This solution is currently

Table adapted from HederaDx & Pascual et al. Ann. Oncol. 2022



Clinical applications for ctDNA testing

labeled for Performance Studies Only (PSO), for evaluating actionable tumor biomarkers supporting clinical decision-making strategies in non-small cell lung cancer (NSCLC), colorectal cancer (CRC), thyroid cancer, breast cancer (BC), melanoma, cholangiocarcinoma (CHOL), gastrointestinal stromal tumor (GIST), and other solid tumors .

This non-invasive and innovative approach greatly facilitates dynamic monitoring of tumor genetic evolution, precise identification of somatic alterations, and real-time assessment of therapeutic response, all while minimizing patient morbidity. Liquid biopsies (LBx) enhance traditional tissue biopsies in several significant ways: they are less burdensome for patients, facilitate frequent sampling, are representative of tumoral heterogeneity, and inherently reduce the sampling bias associated with tissue biopsies.

SNVs/Indels
AKT1, ALK, BRAF, EGFR, ERBB2, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, KEAP1, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, PTEN, RET, ROS1, STK11, TP53
CNVs
ALK, BRAF, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, HRAS, KRAS, MET, NRAS, PIK3CA, RET, TP53
Fusions
ALK, EGFR, FGFR1, FGFR2, FGFR3, MET, NTRK1, RET, ROS1
MSI
36 markers

The panel encompasses SNVs, Indels, CNVs, Fusions, and MSI

These technologies enable the identification of actionable somatic genomic alterations at very low variant allele frequencies with enhanced analytical sensitivity and specificity. The increasing recognition of cancer-derived somatic mutation detection in advanced solid tumors aligns with guidelines from the European Society for Medical Oncology (ESMO), the American Society of Clinical Oncology (ASCO), and the National Comprehensive Cancer Network (NCCN). These guidelines emphasize the importance of plasma-based cell-free and circulating tumor nucleic acids testing, particularly in the domain of evidence-based context of ctDNA analysis.

Key benefits

- Comprehensive: 32 gene-panel for the detection of SNVs, Indels, CNVs, Fusions and MSI across >10 most common tumor types in a single, DNA-only liquid biopsy assay.
- Accurate: The sensitivity and specificity for SNV/InDel detection are > 96% and > 99%, respectively, and for gene fusions, the sensitivity and specificity are 100%. Microsatellite instability (MSI) with a VAF of 0.5% and CNVs with a VAF of ≥ 2% are detected with 100% sensitivity[1].
- Fast TAT: Efficient workflow ensures quick results.
- Actionable: > 80% of the ESCAT Level I genes are included in the panel including ESR1, METex14 and MSI status[2].
- Advanced bioinformatic workflow: Dedicated software for accurate variant interpretation while ensuring accurate and reliable results.

1. Pascual, J et al. "ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group." Annals of oncology : official journal of the European Society for Medical Oncology vol. 33,8 (2022): 750-768. doi:10.1016/j.annonc.2022.05.520.

2. Lescuyer, G et al. "Analytical Validation of a Pan-Cancer NGS Assay for In-House Liquid Biopsy Testing: An International Multicenter Study." (2024) Manuscript in preparation. Preprint available at: https://www.medrxiv.org/content/10.1101/2024.10.17.24313324v2.

Table adapted from HederaDx & Pascual et al. Ann. Oncol. 2022

NSCLC	Colorectal	Chol-angio-carcinoma	Gastric	Thyroid	Breast	Prostate	Pancreatic	Hepato-cellular	Ovarian	Endo-metrial	Urothelial	Soft tissue sarcoma
EGFR ALK MET KRAS BRAF RET ROS1 NTRK1	BRAF KRAS** NRAS** NTRK 1 EGFR-ECD ERBB2 MSI-H	IDH1 FGFR2 NTRK1 MSI-H	ERBB2 NTRK1 MSI-H	BRAF RET NTRK1	PIK3CA ERBB2 ESR1 NTRK1 MSI-H	MSI-H	NTRK1 MSI-H	NTRK1 MSI-H	MSI-H	MSI-H	FGFR3-TACC3 NTRK1	NTRK1
MET ERBB2 BRAF						PTEN						

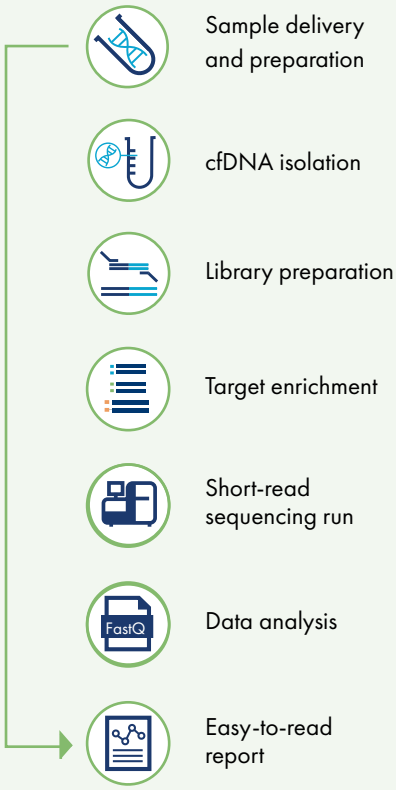
**Mechanism of resistance, non applicable for ESCAT

ESCAT Evidence tier I genes present in HP2

ESCAT Evidence tier II genes present in HP2

A 32-gene NGS panel designed for optimal performance across various solid tumor types

GenomeScan Onco-*ct*Trace Workflow



Input material

Samples

- Plasma samples (minimum of 5 mL required)

Isolated cell-free DNA requirements

- Optimal total cfDNA input: > 30 ng/sample
- Minimal volume: Sample should be in 35 µL Molecular Biology Grade Water, 10 mM Tris-HCl pH8.0, or Buffer EN.
- Minimal quality: free from gDNA contamination

Raw data output

Sequencing on Illumina NovaSeq (PE 150)

- Standard sequencing read depth 25 M paired-end reads/sample

Deliverables

- FastQ files
- Quality Score Q30 > 80%
- Optional data analysis with comprehensive report (Hedera Prime)

GenomeScan Onco-*ct*Trace Workflow

Our workflow incorporates library preparation, quality control steps, and short-read sequencing using Illumina sequencing platforms. This workflow has been validated using cfDNA from plasma samples, ensuring accuracy and reliability in our results.

The data generated by GenomeScan Onco-*ct*Trace can provide valuable insights for a wide range of clinical applications, such as cancer diagnostics, monitoring disease progression and treatment efficacy, identification of novel therapeutic targets and biomarkers, and facilitation of precision medicine and personalized treatment planning. For research use only. Not for use in diagnostic procedures.

Biological Insights

Single Nucleotide Variations (SNVs)

SNVs are the most common type of genetic variation, involving a change of a single nucleotide in the DNA sequence. These variants can have significant implications for cancer biology, including:

- Oncogene activation: Mutations in genes like KRAS or EGFR can lead to uncontrolled cell growth.
- Tumor suppressor gene inactivation: Mutations in genes such as TP53 can prevent the normal regulation of cell division and apoptosis.

Insertions and Deletions (Indels)

Indels are small insertions or deletions of bases in the genome. They can disrupt gene function by:

- Frameshift mutations: These can alter the reading frame of a gene, leading to dysfunctional proteins.
- Gene disruption: Indels can lead to loss of function in critical genes involved in cell cycle regulation.

Copy Number Variations (CNVs)

CNVs involve changes in the number of copies of a particular gene. These variations can contribute to cancer progression by:

- Gene amplification: Increased copies of oncogenes like HER2 can drive aggressive tumor growth.
- Gene deletion: Loss of tumor suppressor genes can lead to reduced control over cell proliferation.

Gene Fusions

Gene fusions occur when parts of two different genes combine to form a new hybrid gene. These can result in:

- Aberrant signaling: Fusion genes like BCR-ABL can lead to continuous activation of growth pathways.
- Novel protein functions: Fusion proteins can acquire new functions that promote cancer cell survival.

Microsatellite Instability (MSI)

MSI is a condition of genetic hypermutability resulting from impaired DNA mismatch repair. It is associated with:

- Increased mutation rates: This can lead to the accumulation of mutations in critical genes.
- Cancer predisposition: MSI is commonly seen in colorectal and endometrial cancers, indicating a higher risk for these malignancies.

Data analysis

We use HederaPrime, a CE-IVD certified medical device software designed for the interpretation of somatic NGS data. This platform streamlines the transition from raw sequencing data to clinically actionable reports by integrating variant calling, and pathogenicity assessment. HederaPrime incorporates curated databases such as ClinVar and OncoKB™, enabling evidence-based classification of genomic alterations and alignment with EMA- and FDA-approved therapies. The software supports a scalable, quality-controlled workflow that ensures accurate, reproducible results, facilitating informed clinical decision-making in precision oncology.

Trustworthy results

Data quality guarantee

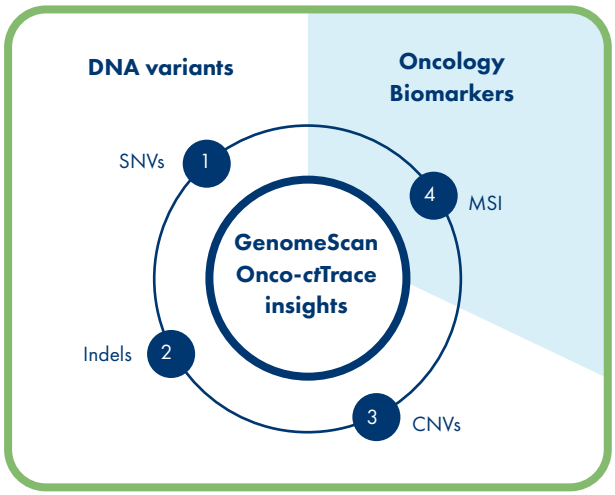
Depth of coverage, base quality and data quality are essential metrics to evaluate the quality of your NGS data.

ISO 17025 accreditation

GenomeScan strives for excellence and is committed to driving up standards in the field of NGS services. For our customers, this means consistency in our level of quality, service, and support.

Custom solutions

Custom bioinformatics can be performed allowing more in-depth mining of your data set. Functional gene information mining, gene enrichment set, gene ontologies may be additionally provided when required.



About GenomeScan

As an ISO-accredited leading Dutch Next Generation Sequencing service provider, GenomeScan develops customizable NGS solutions for pharmaceutical and biotech companies, healthcare providers and academic institutions. By providing state-of-the-art tools to analyze genetic disorders fast, affordably, and effectively, GenomeScan fosters innovation through partnership with medical centers and research laboratories.

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