DISCOVERY LIFE SCIENCES™

Science at your Service™

TECHNICAL DATA SHEET

Matched Solid Tumor & ctDNA TruSight™ Oncology 500 (TSO500) NGS Services

A fast path to reliable liquid biopsy cancer biomarkers

Service Overview

Liquid biopsies hold the promise of providing non-invasive, cost-effective and rapid evaluation of circulating tumor DNA (ctDNA) cancer biomarkers. However, biomarker signatures identified in ctDNA are not always concordant with those found in the primary tumor. Thus, discovery and development of robust, reliable ctDNA biomarkers require a detailed study of matched plasma and tumor samples.

Discovery Life Sciences' sequencing and bioinformatics laboratory, HudsonAlpha Discovery, accelerates cancer biomarker programs by identifying and validating ctDNA biomarkers using high coverage next generation sequencing (NGS) of matched resected tumor tissue and plasma ctDNA. Our research use only (RUO) service leverages highly sensitive TSO500 sequencing assays from Illumina and includes comprehensive bioinformatics analysis to simplify decision-making. The TSO500 EtDNA assays assesses 523 genes for single nucleotide variants (SNVs), insertions/deletions (InDels), copy number variants (CNVs), gene rearrangements, Tumor Mutation Burden (TMB), and Microsatellite Instability (MSI) to provide pan-cancer genomic biomarker evaluation.

Discovery also offers stand-alone TSO500 analysis of resected solid tumor tissue, which adds analysis of 55 RNA gene fusions to the 523 genes reported on with the matched gDNA and ctDNA service.

Visit dls.com/TSO500 to learn more.

Services Highlights

- Accelerate reliable analysis of solid tumor tissues and plasma using Discovery's biospecimen procurement, optimized dual DNA/RNA extractions and robust sequencing workflows
- Obtain highly sensitive genomic data with variant allele fractions (AFs) as low as 1% starting with as little as 30 ng of ctDNA from liquid biopsy
- Gain insights into the concordance between liquid biopsy and solid tumor cancer matched patient samples
- Determine TMB and MSI biomarker signatures that facilitate evaluation of potential immunotherapy response
- Streamline interpretation of clinically relevant genomic variants with an annotated TSO500 PDF report from PierianDx

ACCURACY: >99%

LIMIT OF DETECTION: >1% ALLELE FRACTIONS

REPRODUCIBILITY: >99%

ctDNA CONCORDANCE WITH SOLID TUMOR: 99%

Performance Data: *Using commercially available reference standards in our optimized and automated nucleic acid extraction, library construction, and sequencing workflows, we demonstrate the accuracy, sensitivity, reproducibility, and concordance of our matched liquid biopsy ctDNA – solid tumor gDNA TSO500 NGS assay service.*

Accuracy

With the OncoSpan cfDNA reference standard from Horizon Discovery and Seraseq® ctDNA Complete™ Mutation Mixes from SeraCare, our TSO500 ctDNA assay detected 22 of 22 (Table 1) and 24 of 24 (Table 2) expected variants at various allelic fractions (AF).

Reproducibility

The robust reproducibility of both the TSO500 Solid Tumor and ctDNA assays is demonstrated with two replicates, both within and across batches, with R^2 values of 0.99.

Limit of Detection

Multiple replicates of both the TSO500 ctDNA (Tables 1 and 2) and TSO500 Solid Tumor (Table 2) assays demonstrate highly sensitive detection of SNVs, InDels, and CNVs at the expected AFs, in some cases as low as 1%.

Table 1. TSO500 ctDNA Variant AF Detection in Reference Standard Samples.

Variant Type	Gene	Amino Acid Variant	5% AF	2.5% AF	1% AF
SNV	*AKT1*	p.E17K	3.9	2.5	0.7
	ALK	p.G1202R	4.4	2.4	1.0
	ALK	p.F1174L	4.5	2.3	0.8
	BRAF	p.V600E	4.4	2.9	0.7
	EGFR	p.T790M	5.7	2.3	1.3
	EGFR	p.L858R	5.1	3.5	1.1
	KIT	p.D816V	5.7	1.6	1.0
	KRAS	p.G12C	3.6	5.0	0.7
	KRAS	p.G12D	4.1	2.0	1.0
	KRAS	p.Q61H	4.1	6.1	1.3
	NRAS	p.Q61R	4.2	3.8	1.2
	PIK3CA	p.H1047R	4.4	2.8	1.0
Del	*BRCA1*	p.K654fs*47	4.1	3.1	1.0
	EGFR	p.L747_P753>S	6.6	3.0	0.8
	BRCA2	p.R2645fs*3	4.2	3.5	1.3
	EGFR	p.S752_I759 del SPKANKEI	4.7	1.6	1.1
	EGFR	p.E746_A750 del ELREA	6.7	2.6	0.8
Ins	*PIK3CA*	p.N1068fs*4	4.0	3.1	1.0
	ERBB2	p.A775_G776 ins YVMA	3.4	2.0	1.0
CNV	*ERBB2*	Amplification	Yes	Yes	Yes
	MET	Amplification	Yes	Yes	Yes
	MYC	Amplification	Yes	Yes	Yes

Concordance Between TSO500 Solid Tumor and ctDNA Genomic Variant Detection

Evaluation of both TSO500 ctDNA from Horizon OncoSpan cfDNA and TSO500 Solid Tumor Horizon OncoSpan gDNA reference standard samples with equivalent expected variant allele fractions shows our ability to achieve highly concordant results (R^2 = 0.99) between the two assays (Table 2).

We identified 22 of 22 (Table 1) and 23 of 23 (Table 2) variants at expected AFs from the TSO500 ctDNA assay and 23 of 23 variants at 2% or higher expected AFs from the TSO500 Solid Tumor assay.

Table 2. Variant AF Concordance Between TSO500 ctDNA and TSO500 Solid Tumor Assays.

Gene	Amino Acid Variant	Expected Allelic Frequency %	ctDNA	gDNA
APC	p.T1493T	35.0%	32.0%	34.7%
BRAF	p.V600E	10.5%	9.3%	8.2%
BRCA2	p.K1691fs*15	32.5%	31.5%	31.6%
CTNNB1	p.S33Y	32.5%	30.9%	35.5%
CTNNB1	p.S45del	10.0%	8.6%	9.3%
EGFR	p.G719S	24.5%	24.0%	23.7%
EGFR	p.L858R	3.0%	2.3%	1.9%
EGFR	p.E746_A750 delELREA	2.0%	1.4%	2.0%
EGFR	p.Q787Q	15.0%	14.8%	16.2%
FBXW7	p.S668fs*39	32.5%	31.3%	28.0%
FLT3	p.P986fs*>8	10.0%	9.7%	10.9%
KIT	p.D816V	10.0%	9.1%	10.1%
KIT	p.L862L	7.5%	5.3%	4.7%
KRAS	p.G13D	15.0%	14.5%	15.8%
KRAS	p.G12D	6.0%	6.0%	6.1%
MET	p.A1357A	7.0%	6.8%	6.2%
MET	p.L238fs*25	7.0%	6.5%	6.5%
NOTCH1	p.P668S	30.0%	30.1%	33.9%
NRAS	p.Q61K	12.5%	11.9%	12.9%
PIK3CA	p.E545K	9.0%	8.0%	7.7%
PIK3CA	p.H1047R	17.5%	16.8%	17.5%
RET	p.L769L	60.0%	62.2%	65.6%
TP53	p.P72R	92.5%	92.8%	93.3%

Conclusions

We have optimized both TSO500 Solid Tumor and ctDNA assays and the bundled applications are now offered as high-quality, robust services on a RUO fee-for-service basis with delivery of FASTQ, BAM, VCF, and other output files as well as an optional annotated PierianDx PDF report.

Get Started

Learn more about how Discovery Life Sciences can advance your liquid biopsy biomarker discovery and development programs by contacting us at **info@dls.com or calling (866) 838-2798.**

HUDSONALPHA DISCOVERY

SEQUENCING + BIOINFORMATICS™

A WORLD-CLASS GENOMIC SERVICES LAB WITH INDUSTRIAL-SCALE CAPABILITIES

HudsonAlpha Discovery brings together advanced technologies, workflows optimized for quality and reproducibility, and an innovative, service-oriented scientific team that delivers reliable scientific insight rapidly and at scale.