

Scaling Liquid Biopsy Biomarker Discovery: multi-center, Analytical Validation of high resolution ctDNA Profiling Using TSO500 ctDNA v2 on NovaSeq X Plus

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INTRODUCTION

- CtDNA profiling using Illumina’s TruSight™ Oncology 500 (TSO500) ctDNA v2 assay¹:
- Enables comprehensive genomic profiling from plasma, allowing non-invasive, repeatable monitoring of tumor evolution during clinical studies.
 - Covers a broad panel of 523 clinically relevant cancer genes.
 - Provides sensitive detection of key biomarkers such as SNVs, indels, CNVs, gene fusions, MSI, and TMB, all from a simple blood draw.
 - Return results within less than 4 days.
 - Works across multiple solid tumor types.
 - Well-suited for exploratory biomarker discovery, response monitoring, and mechanistic insights during immunotherapy and targeted therapy development without the need for fresh tissue biopsies.
 - Analytical performance validated in two CellCarta sites (Antwerpen and Naperville).

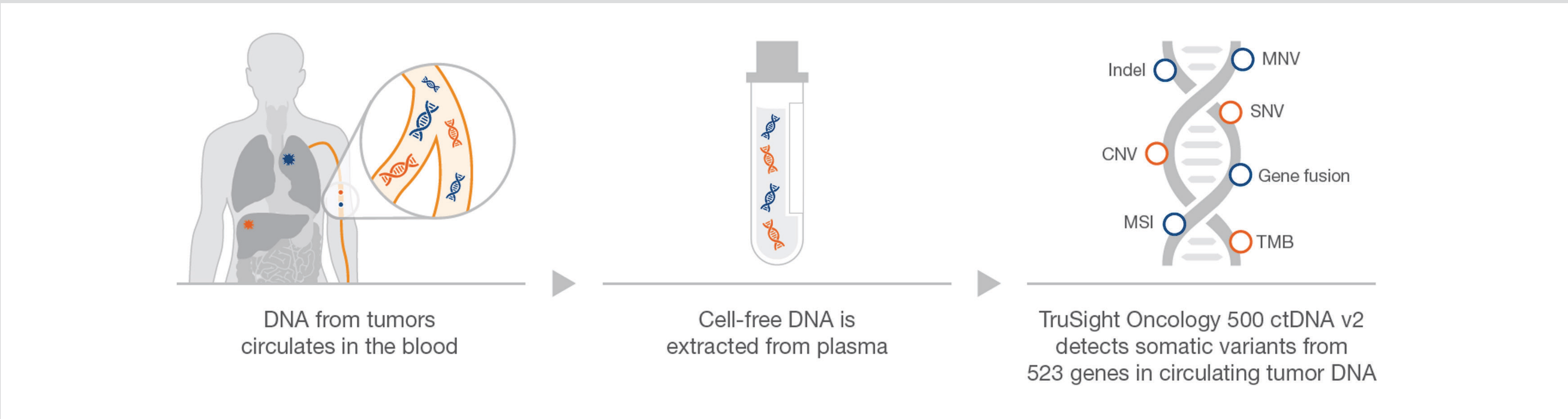


Figure 1: TruSight™ Oncology 500 (TSO500) schematic summary.

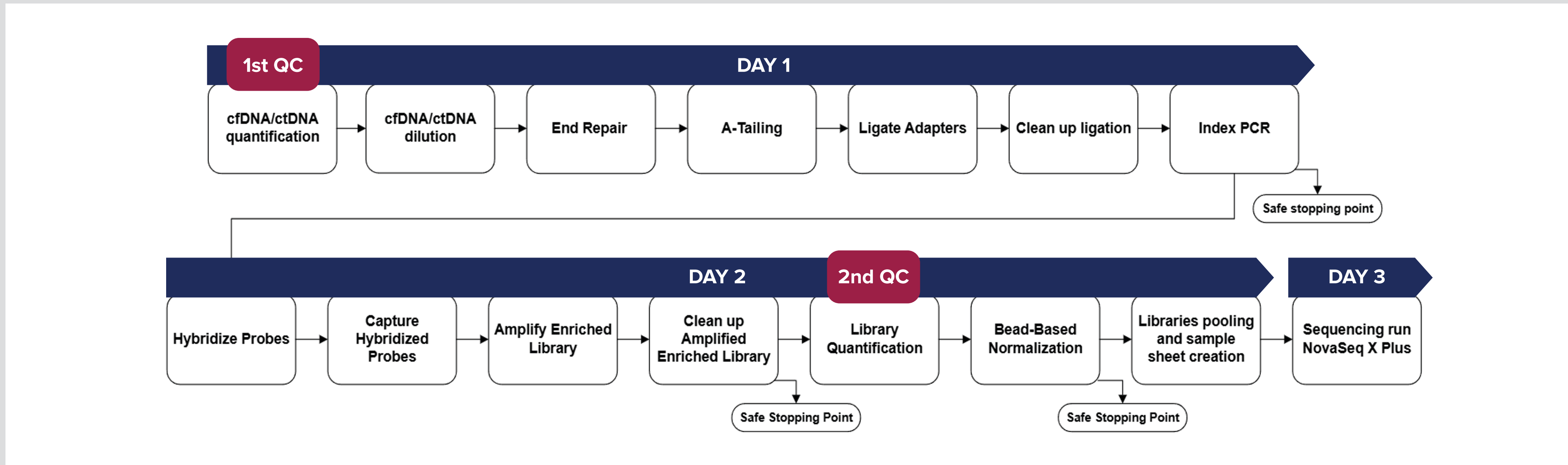


Figure 2: TruSight™ Oncology 500 (TSO500) summarized workflow.

METHODS

- Input: 20 ng of ctDNA from a blood-based liquid biopsy.
- 8-24 samples in the library preparation (manual): 8.5h.
- 24 samples per 10B flow cell.
- Reads: 2 X 151.
- Sequenced in NovaSeq™ X Plus platform.
- Validated in two sites: CellCarta Antwerp (CC-ANT) and CellCarta Naperville (CC-NPV).
- Finalized in less than 4 days.

RESULTS

- All performance characteristics for accuracy, precision and LOD are described in table 1 and met acceptance criteria for SNVs, indels, CNV, MSI and TMB when using an input of 20 ng.
- Input can be a limitation in clinical trial samples. Therefore, lower inputs were tested to assess assay performance (Table 2). Although input QC failed as expected, concordance was met for SNV (98.82% for 10 ng, and 98.63% for 5 ng) and for indels (95.59% for 10 ng, and 95.59% for 5 ng).
- Number of ctDNA samples used for validation:
- Accuracy: 20 samples.
 - Intra-run precision: 12 samples.
 - Inter-run precision: 12 samples.
 - Input: 4 samples.

Table 1: Assay performance and acceptance criteria for accuracy, precision and LOD in ANT (left) and NPV (right).

Assay performance		SNVs	Indels	CNV	MSI	TMB
Accuracy	OPA	98.80%	95.20%	97.44%	100%	100%
	PPA	98.53%	93.55%	100%	100%	100%
	NPA	100%	100%	96.77%	NA*	NA*
Acceptance criteria		≥ 95%	≥ 90%	≥ 95%	≥ 95%	≥ 95%
Precision	Intra-run	100%	98.63%	98.73%	100%	100%
	Inter-run	100%	98.50%	98.70%	100%	100%
Acceptance criteria		≥ 99%	≥ 95%	≥ 95%	≥ 95%	≥ 95%
LOD	Observed	100% (0.25% VAF)	100% (0.5% VAF)	100%	NA	NA
	Acceptance criteria	≥ 90% (0.25% VAF)	≥ 90% (0.5% VAF)	≥ 90%	NA	NA

Table 2: Assay performance and acceptance criteria for input in ANT (left) and NPV (right).

Assay performance		Sample QC	SNVs	Indels
Accuracy	5 ng	FAIL	98.63%	95.59%
	10 ng	FAIL	98.82%	95.59%
	20 ng	PASS	100%	100%
Acceptance criteria		Median Exon Coverage, PTC Exon Coverage and Median Bin Count CNV Target	≥ 95%	≥ 95%

CONCLUSION

- TSO500 ctDNA v2 assay delivers reproducible, high-resolution genomic profiling in both Antwerpen and Naperville sites, supporting its applicability for exploratory biomarker analyses and longitudinal response monitoring in clinical studies. All acceptance criteria and QC checks are met for VAF accuracy, precision and LOD when using 20ng as input.
- Acceptance criteria for variant accuracy is met for samples with lower inputs (5 and 10 ng) although input QC checks is not met, which allows accurate and reliable performance of clinical samples with limited inputs.

REFERENCES

¹ <https://www.illumina.com/products/by-type/clinical-research-products/trusight-oncology-500-ctdna.html>

Send inquiries to isales@cellcarta.com with AACR 2026 in email subject.