Rapid Automated Circulating Tumor DNA (ctDNA) Enrichment Directly from Plasma

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UPDATED ABSTRACT

INTRODUCTION: Blood liquid biopsy contains circulating cell-free DNA (cfDNA), a portion of which may be shed from tumors. This circulating tumor-derived DNA (ctDNA) can be tested to identify cancerous mutations. DNAe is developing the first rapid, sample-to-result, NGS-based solution, the LiDia-SEQ™ platform, enabling clinically relevant sequencing results to be reported within a single work shift for use in routine care. As part of its automated NGS workflow development, DNAe has developed a proprietary method to rapidly capture and enrich mutations of cancer ctDNA directly from plasma, named Specific Variant Capture (SVC).

METHODS: cfDNA multiplex reference material containing 5%, 1%, or 0.1% variant allele frequency (VAF) mutations of KRAS G12D, EGFR L858R, and PIK3CA E545K was spiked into 1- or 3-mL DNA-free synthetic plasma to mimic low VAF ctDNA in the plasma fraction of whole blood. Samples were processed using the SVC method in an automated sample preparation subsystem instrument prototype for enriching and isolating ctDNA, and the resulting template was evaluated with ddPCR for absolute allele-specific probe-based quantitation.

RESULTS: DNAe achieved a 19-fold, 73-fold, and 419-fold enrichment at 5%, 1%, or 0.1% VAF inputs respectively for the KRAS target in automated SVC singleplex. DNAe accomplished similar fold enrichment for all three targets at 5% and 1% VAF inputs and variable but high fold enrichment at 0.1% VAF input in SVC multiplex.

CONCLUSIONS: DNAe has developed SVC, a rapid, automated method for enriching mutant ctDNA from a background of wild-type cfDNA directly from plasma. The automated SVC method performs better than the manual (bench) protocol at the very low, but clinically relevant VAF levels, and enables a rapid, easy-to-use NGS workflow to detect and monitor cancer from a simple blood sample.

MATERIALS & METHODS

cfDNA multiplex reference material (Horizon Discovery, Cambridge, UK) containing 5%, 1%, or 0.1% variant allele frequency (VAF) mutations of KRAS G12D, EGFR L858R, and PIK3CA E545K was spiked into 1- or 3- mL DNA-free synthetic plasma to mimic low VAF ctDNA in the plasma fraction of whole blood. Targets were selected as a representative test panel to challenge the SVC enrichment method with GC-rich (EGFR), GC-normal (KRAS) and GC-poor (PIK3CA) targets. Samples were processed using the manual SVC bench method (1 mL plasma input) or the automated Lidia-SEQ™ prototype instrument (3 mL plasma input) with single-use cartridges for enriching and isolating ctDNA. The manual bench and automated instrument outputs were then evaluated with target-specific ddPCR assays, for absolute allele-specific probe-based quantitation.

SINGLEPLEX SVC TESTING

Sample input spikes of 100 ng for 5% and 1% VAF and 1000 ng for 0.1% VAF were chosen to be reliably detected with allele-specific ddPCR for the KRAS target. Yield was calculated from ddPCR positive controls for each target spiked at the same input amounts and VAF. 100% wild-type inputs and no template controls were run through the same processes on bench and instrument with no false positive mutants detected.

MULTIPLEX SVC TESTING

The same input spikes as used for singleplex SVC testing were used with multiplex SVC testing, with outputs being evaluated using singleplex allele-specific ddPCR assays for the KRAS, PIK3CA and EGFR targets.

BACKGROUND

Blood liquid biopsy contains circulating cell-free DNA (cfDNA), a portion of which may be shed from tumors. This circulating tumor-derived DNA (ctDNA) can be tested to detect cancers and identify their mutation profiles. Early cancer detection before metastasis is important as this is when treatment is most effective. Sensitive and specific detection of the cancer and its mutation profile is critical to guide selection of the correct treatment regimens and lead to improved patient outcomes[1].

VAF is a measure of the variant fraction at a specific gene locus, and in the context of blood cfDNA, represents the total variant fraction present in the plasma, determined by NGS or ddPCR assays. Emerging data shows this metric to have both prognostic and diagnostic clinical value, however, high confidence detection of ultra-low VAF in cfDNA is complex[2]. Enriching the variant fraction of a sample may increase assay sensitivities and improve confidence in results, potentially prompting earlier clinical intervention.

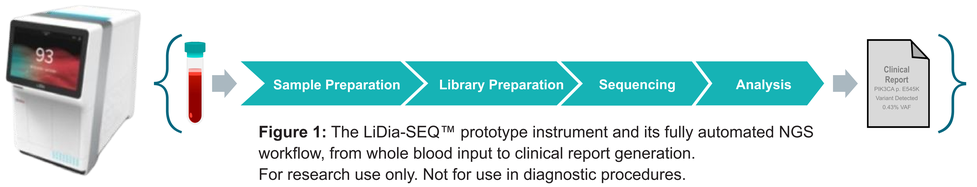

Figure 1: The LiDia-SEQ™ prototype instrument and its fully automated NGS workflow, from whole blood input to clinical report generation.
For research use only. Not for use in diagnostic procedures.

DNAe is developing the first rapid, sample-to-result, NGS solution, the LiDia-SEQ™ platform, enabling clinically relevant sequencing results to be reported within a work shift for use in routine care. As part of its automated NGS workflow development, DNAe has developed a proprietary pre-amplification sample preparation method to rapidly capture and enrich mutations of cancer ctDNA directly from plasma, named Specific Variant Capture (SVC). The SVC enrichment method can be used on a single target or multiple targets simultaneously.

RESULTS

SINGLEPLEX TESTING: DNAe achieved a 19-fold, 73-fold, and 419-fold enrichment for 5%, 1%, or 0.1% VAF input respectively for the KRAS G12D target in singleplex with SVC performed on the Lidia-SEQ™ prototype instrument. Results were similar with the manual SVC bench protocol at 5% and 1% VAF inputs but were notably improved at 0.1% VAF input on instrument.

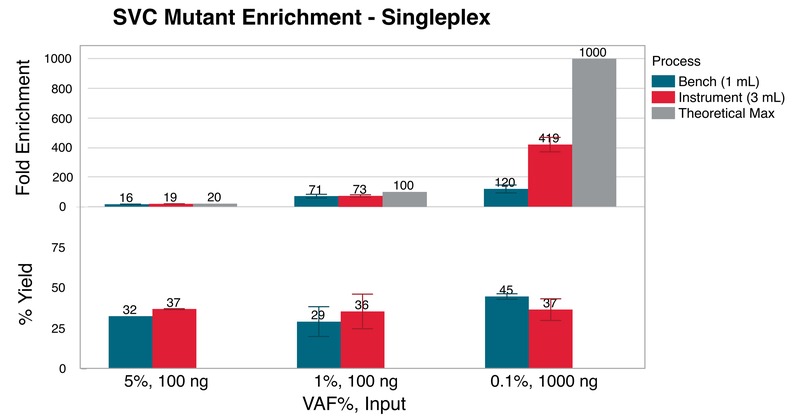

Figure 2: SVC Mutant Enrichment - Singleplex. Spiked cfDNA reference material into synthetic plasma (DNA-free) evaluated with an allele-specific KRAS ddPCR assay

MULTIPLEX TESTING: DNAe achieved similar results in the simultaneous SVC enrichment of the three targets at 5% and 1% VAF input, and varying high levels of enrichment at 0.1% VAF input for the KRAS G12D, PIK3CA E545K and EGFR L858R.

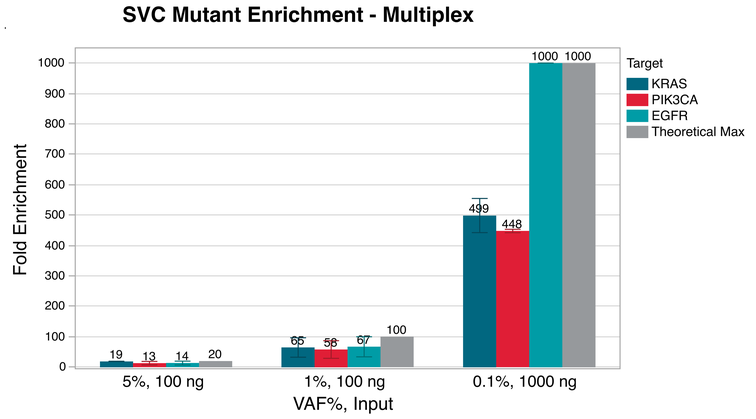

Figure 3: SVC Mutant Enrichment - Multiplex. Spiked cfDNA reference material into synthetic plasma (DNA-free) evaluated with allele-specific KRAS, PIK3CA, and EGFR ddPCR assays

CONCLUSION & DISCUSSION

High confidence detection of ultra-low frequency mutations in cfDNA is difficult but clinically important. SVC is a mutant enrichment method that can capture and enrich ctDNA directly from plasma with ultra-low VAF, potentially increasing assay sensitivities and improving confidence in assay results. SVC is fully automatable and can be integrated into a complete NGS workflow, like on the LiDia-SEQ™ platform, allowing for rapid and highly sensitive mutation detection from a liquid biopsy and potentially other sample types. Integration of SVC into platforms like LiDia-SEQ™ will enable the timely, sensitive and specific detection of cancers and help provide clinicians with the relevant information to make informed decisions regarding selection of treatment regimens.

REFERENCES
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