Complete Workflow for Automated cell-free DNA Extraction and Somatic Mutation Detection

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INTRODUCTION

Circulating cell-free DNA (cfDNA) has emerged as an important biomarker in cancer research and for non-invasive monitoring of various other clinical conditions. In oncology detection of cfDNA has also been considered as a potential prognostic marker for outcome in various cancers [1]. One of the main challenges encountered in these developments relate to the efficient extraction of cfDNA from "liquid biopsies", often yielding suboptimal quantities of highly fragmented DNA. As assays for cfDNA are typically intended to identify genetic variants present at very low allelic frequencies, many of the established detection technologies are driven to the edge of their performance. The purpose of this study was to compare the performance of two cfDNA extraction systems: a column-based kit (QIAamp Circulating Nucleic Acid Kit) and a novel automated magnetic beads-based system (*Sentosa*® SX cfDNA Kit (4x8)).

MATERIAL & METHODS

We developed a magnetic bead-based cfDNA extraction kit, the *Sentosa*® SX cfDNA Kit (4x8) and optimized it for use on the Vela *Sentosa*® SX101 platform. *Sentosa*® SX101 is a CE-IVD certified robotic liquid handling system for nucleic acid extraction, PCR set-up and Next-Generation Sequencing (NGS) library preparation **(Fig. 1**). We compared performance of the *Sentosa*® SX cfDNA Kit (4x8) with a column-based cfDNA extraction kit. Integrity of cfDNA extracted by both methods was assessed using ALU repeats qPCR assay. Quality of the extracted cfDNA was tested using the NGS-based *Sentosa*® SQ CRC Panel (4x8) (**Table 1**).

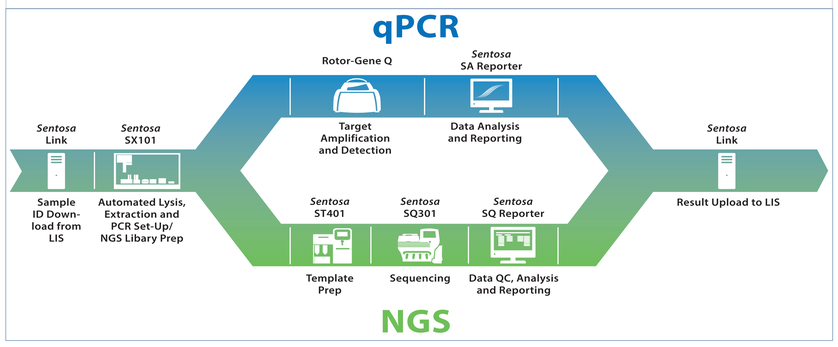


Figure 1. Vela's combined PCR and NGS *Sentosa*® Workflows.

The *Sentosa*® SX cfDNA Kit (4x8) utilizes 4 mL of human plasma as sample input and can process up to eight samples per run. The turnaround time is about 3.5 hours (with 15 minutes operator hands-on time).

RESULTS

In this study DNA was extracted from plasma samples spiked with 3 concentrations of fragmented HCT116 gDNA (*KRAS* G13D positive): 109, 54 and 27 mutant genome equivalents (GE) per 1mL of plasma using the *Sentosa*® SX cfDNA kit and a column-based cfDNA extraction kit, respectively. Fragment size of the cfDNA extracted by both methods was ~170 bp (confirmed by Bioanalyzer). The cfDNA portion in DNA samples extracted by the *Sentosa*® SX cfDNA kit was ~80% and for the column-based extraction method ~25% **(Fig. 2**).

Table 1. Target genes for the *Sentosa*® SQ CRC Panel.

Target Genes	Number of Amplicons	Number of Target Mutations	Amplicon Locations in Exon(s)
NRAS	3	19	2, 3, 4
CTNNB1	1	5	2
PIK3CA	2	14	11, 22
FGFR3	3	9	7, 9, 14
KIT	3	8	11, 13, 17
EGFR	4	9	18, 19, 20, 21
BRAF	2	15	11, 15
RET	1	1	16
PTEN	3	3	5, 7
KRAS	3	22	2, 3, 4
TP53	3	7	4, 6, 7
Total	**28**	**112**	

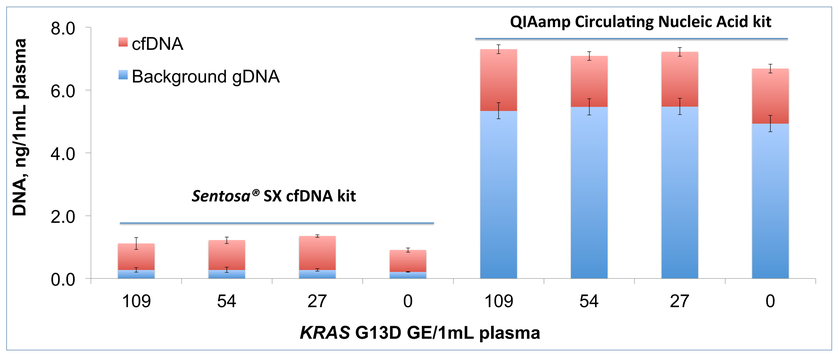
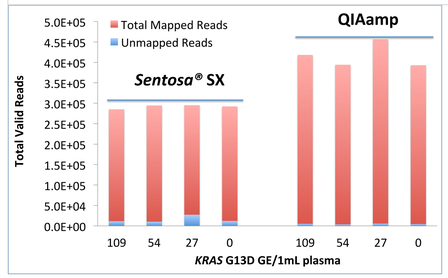
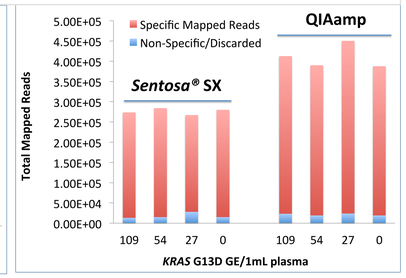
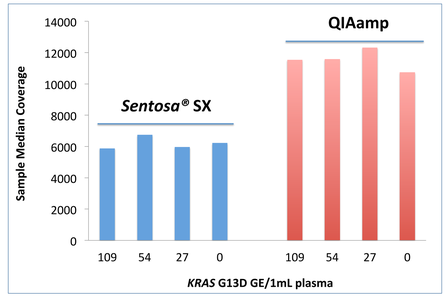


Figure 2. Assessment of cfDNA yield using the *Sentosa*® SX cfDNA Kit compared to the QIAamp Circulating Nucleic Acid Kit.

Amount and quality of DNA for all samples extracted by both methods was sufficient to prepare NGS libraries using the *Sentosa*® SQ CRC Panel (4x8) (**Fig. 3**). The *KRAS* G13D mutation was detected in all samples extracted by the *Sentosa*® SX cfDNA kit, but surprisingly no *KRAS* G13D mutation was detected in any of the column-based extracted samples.







	KRAS G13D GE/1mL plasma	KRAS G13D, %
Sentosa® SX cfDNA kit	109	8.32
	54	5.07
	27	3.55
	0	0
QIAamp Circulating Nucleic Acid Kit	109	0
	54	0
	27	0
	0	0

Figure 3. Assessment of cfDNA quality and quantity extracted by *Sentosa*® SX cfDNA Kit and QIAamp Circulating Nucleic Acid Kit using the NGS-based *Sentosa*® SQ CRC Panel (4x8).

CONCLUSIONS

The *Sentosa*® SX cfDNA Kit (4x8) selectively extracts cfDNA in the presence of high molecular weight gDNA. The *Sentosa*® SX cfDNA Kit (4x8) thus appears as an efficient and reliable solution for cfDNA extraction from human plasma samples. Integration into the *Sentosa*® qPCR- and NGS-based workflows makes the *Sentosa*® SX cfDNA Kit (4x8) a comprehensive *in vitro* tool, which can be used in combination with various assays for detection of tumour derived cfDNA.

REFERENCES

1) *De Mattos-Arruda L. et al.* Future Oncol. 2011 7:1385–97.