A Novel Cell-Free DNA Extraction System for Cancer Diagnostics

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BACKGROUND

Circulating cell-free DNA (cfDNA) in human plasma has shown potential as biomarker in various cancers and becoming an important source for mutation detection in cancer diagnostics and non-invasive progression monitoring of various clinical conditions. cfDNA has also been considered a potential prognostic marker for outcome in various cancers [1]. This has been resulted in the development of new *in vitro* diagnostics (IVD) cfDNA extraction solutions. Challenges encountered in these developments related to the efficient extraction of cfDNA from liquid biopsies, often yielding low quantities of highly fragmented DNA in the presence of high level of background gDNA. Since 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. Objective 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 used a newly developed magnetic beads-based cfDNA extraction kit. This kit was optimized for use on a robotic liquid handling platform (*Sentosa* SX101). *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**). Integrity of cfDNA extracted by both methods was assessed using ALU repeats qPCR assay. Quality of the extracted cfDNA was tested using an NGS-based *Sentosa* SQ CRC Panel (4x8) (**Table 1**).

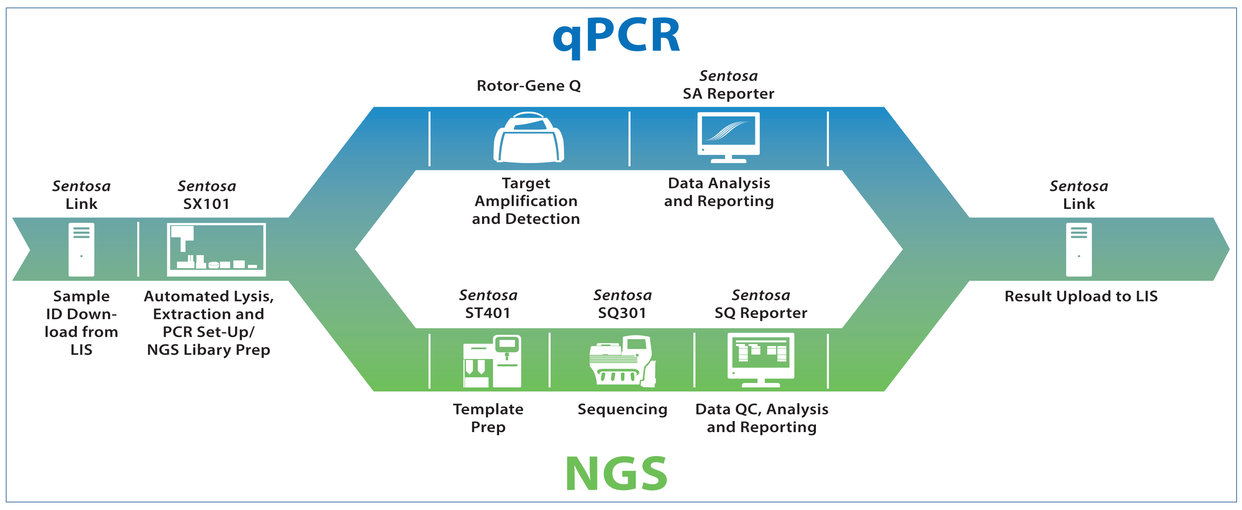


Figure 1. Vela's combined PCR and NGS *Sentosa*® Workflows.

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 only 15 minutes operator hands-on time)

Table 1. *Sentosa*® SQ CRC Panel Target Genes

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**	

RESULTS

In this pilot study DNA was extracted from plasma samples with 3 concentrations of spiked-in fragmented HCT116 gDNA (*KRAS* G13D positive) at 109, 54 and 27 mutant genome equivalents (GE) per 1 ml of plasma using *Sentosa* SX cfDNA and column-based cfDNA extraction kits, respectively. Fragment size of extracted DNA was ~170 bp (confirmed by Bioanalyzer). The ALU247/115 ratio for DNA extracted by the *Sentosa* SX cfDNA kit was 0.19-0.28 and for the column-based extraction method >0.7 (expected ratio for cfDNA is less than 0.5 and for gDNA is 1.0) (**Fig. 2**). Amount and quality of DNA for all samples extracted by both methods was sufficient to prepare NGS libraries using *Sentosa* SQ CRC Panel (4x8) (**Fig. 3**). *KRAS* G13D mutation was detected in all samples extracted by the *Sentosa* SX cfDNA kit and no *KRAS* G13D mutation was detected in any of column-based extracted samples.

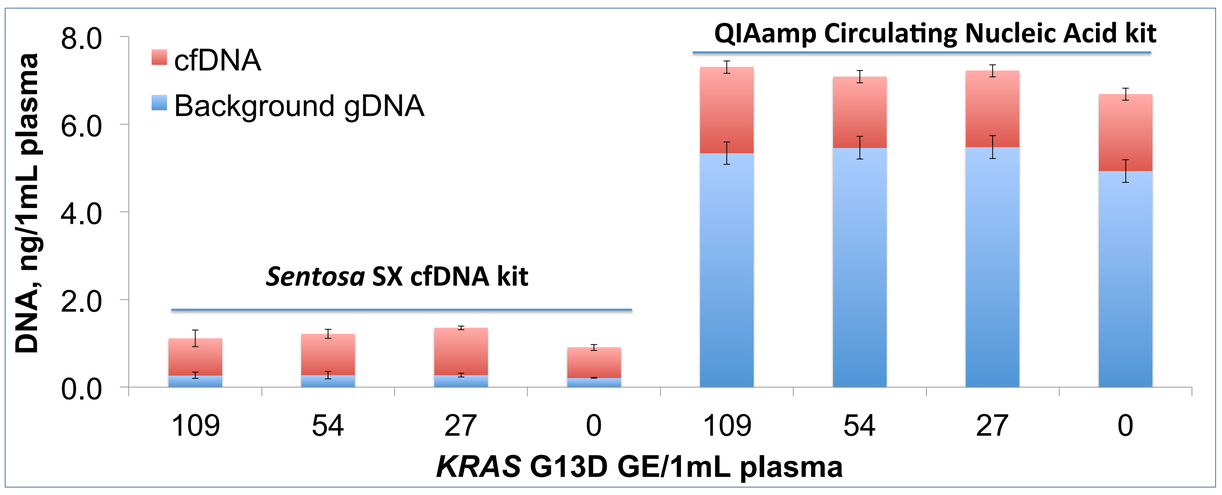
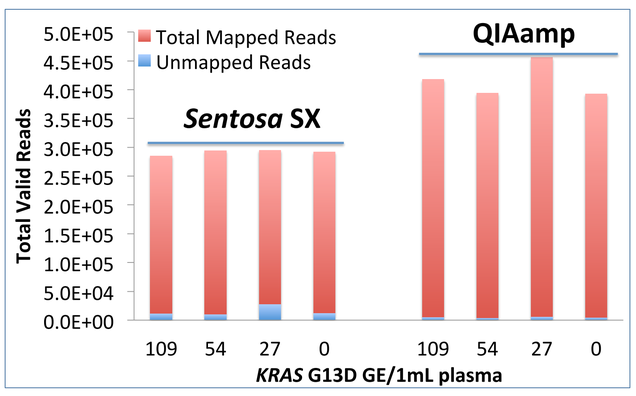
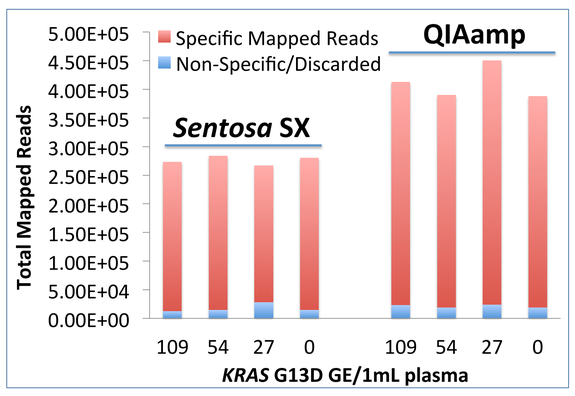
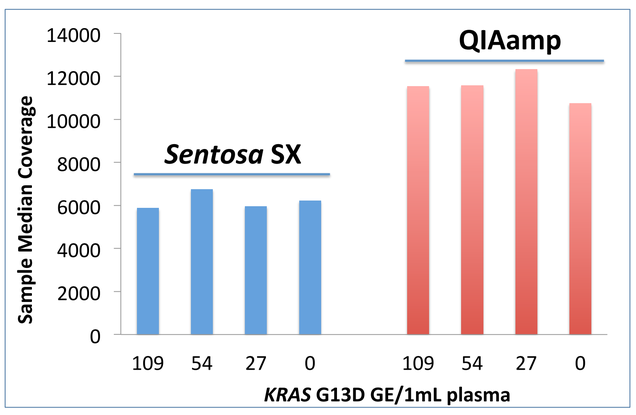


Figure 2. Assessment of cfDNA yield using *Sentosa* SX cfDNA Kit and QIAamp Circulating Nucleic Acid Kit.







	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 an NGS-based *Sentosa* SQ CRC Panel (4x8).

CONCLUSIONS

The *Sentosa* SX cfDNA Kit (4x8) selectively extracts cfDNA over high molecular weight gDNA. The *Sentosa* SX cfDNA Kit (4x8) 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 universal *in vitro* diagnostics tool, which can be used in combination with various IVD assays.

REFERENCES

1) De Mattos-Arruda L. et al. Future Oncol. 2011 7:1385–97
2) Misale S. et al. Nature. 2012 486:532–6

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