Integrated Next-Generation Sequencing and qPCR Workflow in Clinical Diagnostics

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INTRODUCTION

Sanger sequencing and polymerase chain reaction (PCR) methods have been the standard molecular methods in clinical diagnostics for decades. Next-Generation Sequencing (NGS) technology revolutionized the field of genomics, transcriptomics and metagenomics and is now swiftly becoming a routine method in different areas of clinical diagnostics [1,2].

RESULTS

Vela Diagnostics developed an integrated automated multi-purpose *Sentosa* workflow, which consists of:

1) a customized version of the epMotion 5075 (Eppendorf) robotic liquid handling system for nucleic acid extraction, PCR set-up and/or NGS library preparation (*Sentosa*® SX101);
2) instruments for real-time PCR (Rotor-Gene Q or ABI 7500) or template preparation and deep sequencing (PGM, Ion Torrent) [3];
3) kits for nucleic acid extraction, real-time PCR-based tests, NGS library preparation assays and reagents for deep sequencing;
4) flexible and easily customizable assay specific applications;
5) data analysis and reporting software.

Different diagnostic applications employ the same robotic platform for qPCR set-up and preparation of NGS libraries (**Fig. 1**).

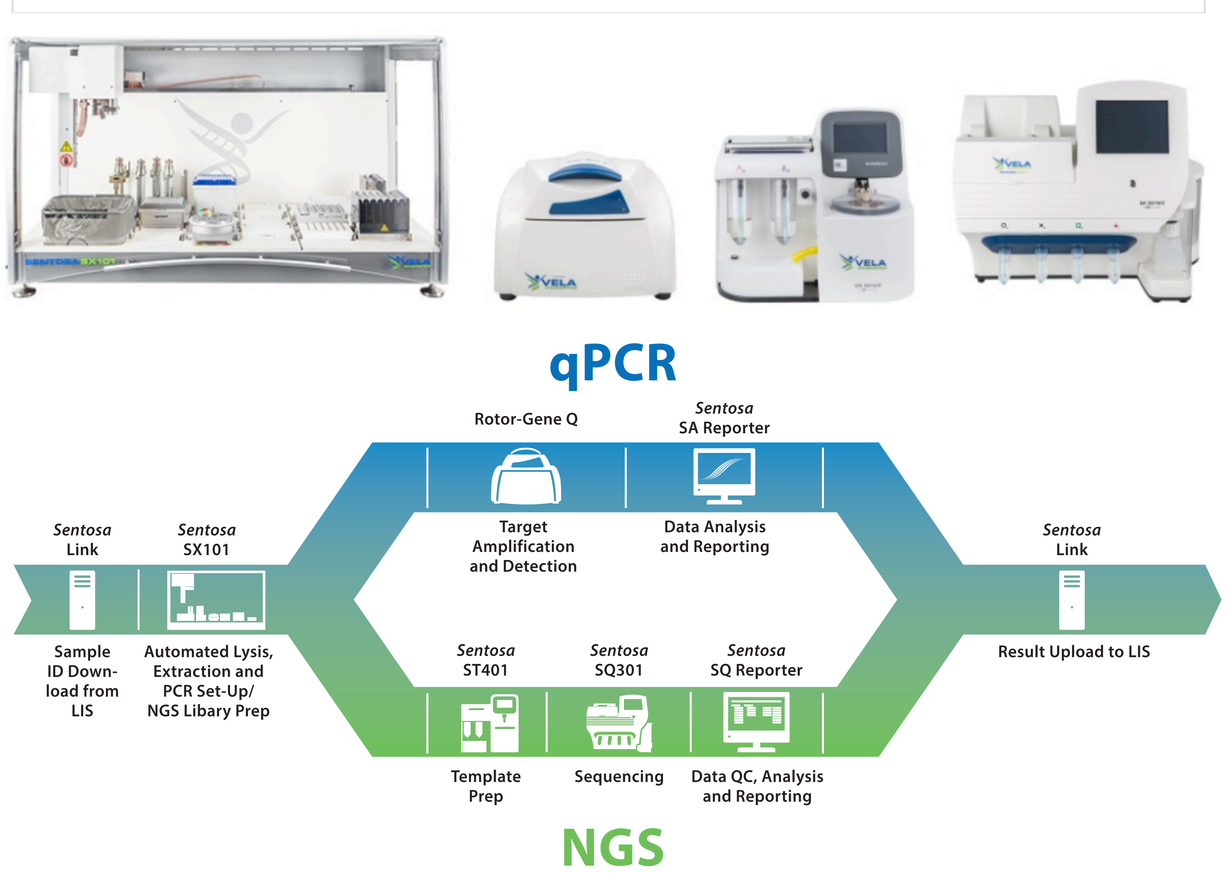

Figure 1. Combined PCR and NGS *Sentosa*® Workflows.

Vela Diagnostics offers 7 NGS (**Fig. 2**) and more than 30 PCR verified and validated viral, microbial and oncology CE-IVD tests (**Fig. 3**), which can be run on the same system.

Oncology

Sentosa® SQ Melanoma Panel (4x8)
Sentosa® SQ CRC Panel (4x8)
Sentosa® SQ NSCLC Panel (4x8)
Sentosa® SQ Thyroid Panel (4x8)
Sentosa® SQ Leukaemia Panel (4x8)*

Virology

Sentosa® SQ HCV Genotyping Assay (4x16)
Sentosa® SQ HIV Genotyping Assay (4x16)*

*Products in development

Figure 2. NGS-based CE-IVD Tests.

Respiratory Infections

Influenza A/B & RSV
MTC
H7N9
MERS
Strep A

Gastroenteritis

Norovirus (GI & GII)
Salmonella
C. diff (combination)
Rotavirus

ISP

HSV 1/2 Quant
CMV Quant
VZV Quant
BKV Quant
EBV Quant
HHV6 Quant

Bloodborne Viruses

HBV Quant
HCV Quant

Tropical Infections

Dengue (I-IV)
Chikungunya
Zika*

Microbiology

MRSA/SA (extraction)
MRSA/SA direct
VRE (vanA/vanB)
C. Diff
Strep A

Oncology

NRAS
KRAS
BRAF (V600)

Leukemia

BCR-ABL (M)
BCR-ABL (m)

*Products in development

Figure 3. PCR and qPCR-based CE-IVD tests.

The SX101 extraction kits were developed by Vela Diagnostics are able to isolate nucleic acids from various types of clinical samples, including FFPE, whole blood, plasma/serum, swabs, sputum, stool and urine (**Table below**).

Extraction Kit	Sample Type
Sentosa SX Bacterial gDNA Kit	Throat, Wound, Perianal, Rectal and Nasal swabs, Stool, Sputum
Sentosa SX FFPE gDNA Kit	FFPE
Sentosa SX Total RNA Kit	Peripheral blood
Sentosa SX Virus Total Nucleic Acid Kit	Serum, Plasma, Swab in UTM, Stool, Cerebrospinal fluid (CSF), Urine
Sentosa SX Whole Blood Kit	Whole Blood
Sentosa SX Lysis Beads	Nasal swabs

Integration with *Sentosa* middleware connects the system to the laboratory network and ensures sample traceability. Highly automated extraction, PCR set-up and NGS libraries preparation in conjunction with fully automated data analysis and reporting system reduce hands-on time up to 0.5 hrs. for the PCR and 3.5 hrs. for the NGS tests. The flexibility of the PCR platform allows for consolidated testing with more than 30 PCR tests and 14 clinically relevant and validated human sample types.

CONCLUSION

Combined automated qPCR and NGS *Sentosa* workflow is a reliable and efficient *in vitro* diagnostics tool for the detection and/or quantitation of a wide range of bacterial and viral pathogens as well as gene mutations. Unique abilities of the *Sentosa* workflow provide complete and relevant information to aid clinical decision-making and patient management.

REFERENCES

1) *Lefterova M. et al.* The J. of Molecular Diagnostics. 2015 Nov, 17(6): 623-34.
2) *Barson L. et al*. J. of Clinical Virology. 2013, 58: 346-350.
3) *Loman N. et al.* Nat. Biotechnol. 2012 May, 30(5):434-9.