Automated Integrated NGS and qPCR Workflow for *In Vitro* Diagnostics

AACC 2016
ANNUAL MEETING &
CLINICAL LAB EXPO
July 31 – August 4
Philadelphia, PA

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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 workflow, which consists of:

1) Middleware from Data Innovations compatible with more LIS providers and Vela Diagnostics instruments
2) 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);
3) Workflows with instruments for real-time PCR (Rotor-Gene Q or ABI 7500) or template preparation and deep sequencing (PGM, Ion Torrent) [3];
4) kits for nucleic acid extraction, real-time PCR-based tests, NGS library preparation assays and reagents for deep sequencing;
5) Data analysis and reporting software for both qPCR and NGS workflows.

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

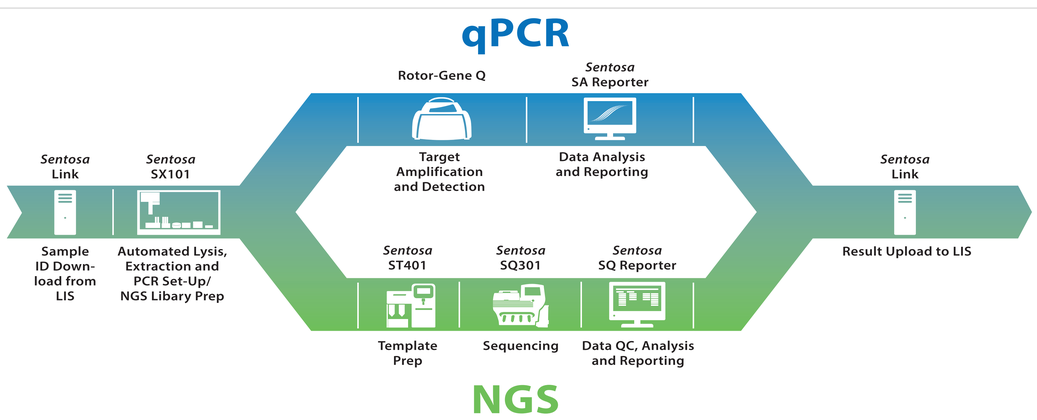

Figure 1. Combined PCR and NGS *Sentosa*® Workflows.

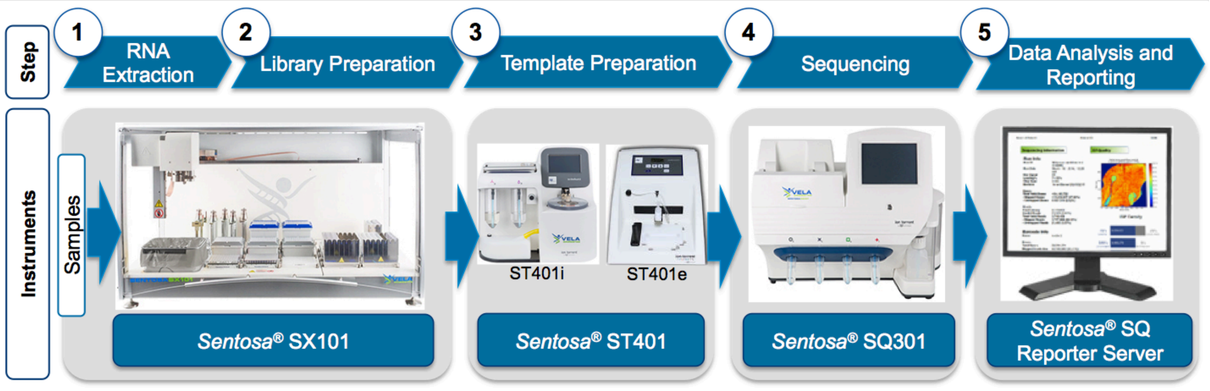

Figure 2. Vela Diagnostics *Sentosa*® NGS Workflow.

Vela Diagnostics NGS workflow **(Fig. 2)** provides solution for both Oncology and Virology samples using the customized instruments from Eppendorf (*Sentosa*® SX101) and Ion Torrent (*Sentosa*® SQ301). The flexibility in pipetting programs of the *Sentosa*® SX allows for 8 (Oncology) or 16 (Virology) barcoded libraries to be processed on deck. Dedicated reagents for nucleic acid extraction and library preparation are supplied for simplicity to the user.
The *Sentosa*® ST401 and SQ301 by Ion Torrent performs clonal emulsion amplification in 6 hours and sequencing in 3.5 hours respectively. The use of semiconductor sequencing technology allows for targeted sequencing workflow to be performed in a relatively short amount of time.
Sample traceability and instruments connectivity is seamlessly maintained through the network with Vela Diagnostics customized robotics and instrument software. Automatic data analysis and reporting of genotypes and mutations is available with the SQ Reporter software, providing integrated solution for sample to results within 2 days.

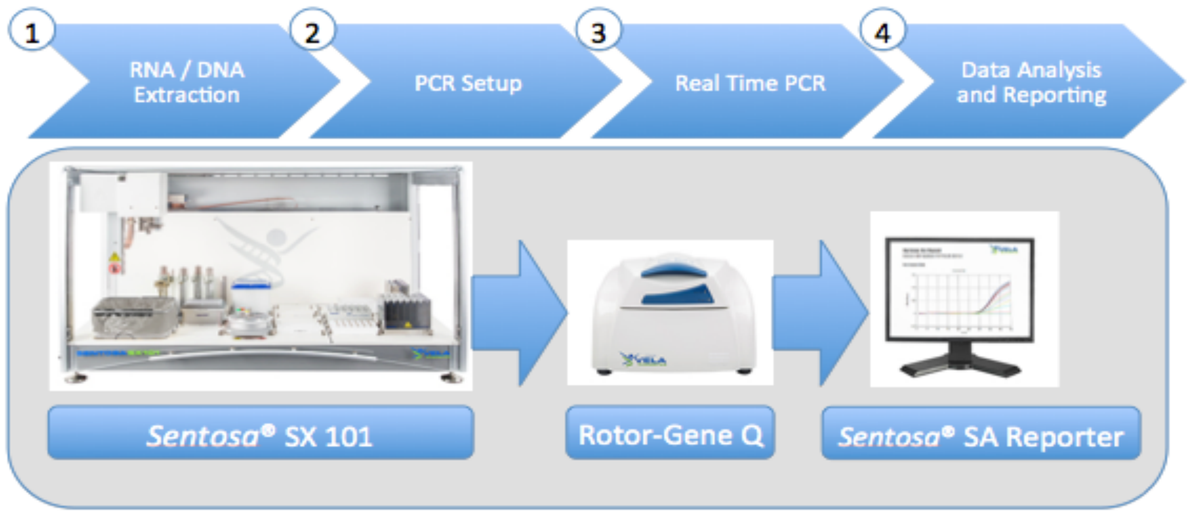

Figure 3. Vela Diagnostics *Sentosa*® qPCR Workflow.

Vela Diagnostics qPCR workflow **(Fig. 3)** allows for nucleic acid extraction and PCR setup on various sample types (Table 1) with run throughput ranging from 8 – 96 samples. With more than 30 available tests covering applications for Immunosuppression, Virology, Oncology and Gastroenteritis, the flexibility of the platform allows for a wide selection of menu.
Using the same robotic system (*Sentosa*® SX101) as the NGS workflow, it enables both NGS and qPCR workflows to be performed on a relatively small laboratory footprint.
The PCR tests are compatible with the Rotor-Gene Q (QIAGEN) or the *Sentosa*® SA201 (Applied Biosystems). Customized color coded reagent tube holders allows for easy placement of PCR reagents on the robotic deck
Sample traceability and instruments connectivity is seamlessly maintained through the network with Vela Diagnostics customized robotics and instrument software. Automatic analysis and reporting of qPCR results is available with the SA Reporter software, providing integrated solution for sample to results within 3 hours.

The SX101 extraction kits were developed by Vela Diagnostics are able to isolate nucleic acids from various types of clinical samples (**Table 1. below**).

Sample Type	Extraction Throughput
Throat, Wound, Perianal, Rectal and Nasal swabs, Stool	8 – 96 Samples
Whole Blood, Serum, Plasma, Sputum, Swab in UTM, Cerebrospinal fluid (CSF), Urine	8 – 24 Samples
Formalin-fixed, paraffin-embedded (FFPE)	8 – 16 Samples

Integration with *Sentosa*® Link 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.* J. Mol. Diagn. 2015, 17: 623-634
2) *Barson L. et al.* J. Clin. Virol. 2013, 58: 346-350
3) *Loman N. et al.* Nat. Biotechnol. 2012, 30: 434-439

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