✉ **CONTACT:** Elian Rakhmanaliev elian.rakhmanaliev@veladx.com

Highly Automated "Sample-to-Result" NGS and PCR Workflow for Molecular Diagnostics

Elian Rakhmanaliev, Tatiana Ivanova, Yin Kum Ng, Alex Yeo, Harry Suhardi, Caroline Villy, Mei Qi Yee, Kevin Tan, Amanda Fan, Andy Soh, Pramila Ariyaratne, Si Kee Tan, Jocelyn Wong, Jiang Xia, Grace Tan, Jasmin Ang, John Ong, Gerd Michel, Charlie Lee, Wen Huang.

Vela Research Singapore Pte. Ltd., Singapore.

MOLECULAR DIAGNOSTICS
PREMIER ONLINE-ONLY CONFERENCE
APRIL 12-13, 2017

INTRODUCTION

PCR and Sanger sequencing methods have been the standard molecular methods in clinical diagnostics for decades. Deep sequencing or Next Generation Sequencing (NGS) technology revolutionized the field of genomics, transcriptomics and metagenomics and is now rapidly becoming a routine method in different areas of clinical diagnostics such virology, oncology, drug-resistance monitoring, non-invasive prenatal tests, microbiology, precision medicine, etc. [1,2].

RESULTS

We developed a complete ("Sample-to-Result") highly automated multi-purpose *Sentosa* workflow, which consists of:

① A robotic liquid handling system for nucleic acid extraction, PCR set-up and/or NGS library preparation (*Sentosa* SX101);
② Instruments for real-time PCR or template preparation and deep sequencing (Ion Torrent's PGM system);
③ Kits for nucleic acid extraction, target specific real-time PCR-based tests, NGS library preparation assays and deep sequencing;
④ 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. In less than 5 years Vela Diagnostics developed 7 NGS-based viral and oncology assays and more than 20 qPCR-based viral, microbial and oncology CE-IVD tests, which can be run on the same system. In addition, several extraction kits were developed to isolate nucleic acids from various types of clinical samples, including FFPE, whole blood, plasma/serum, swabs, stool, etc. **(Table**).

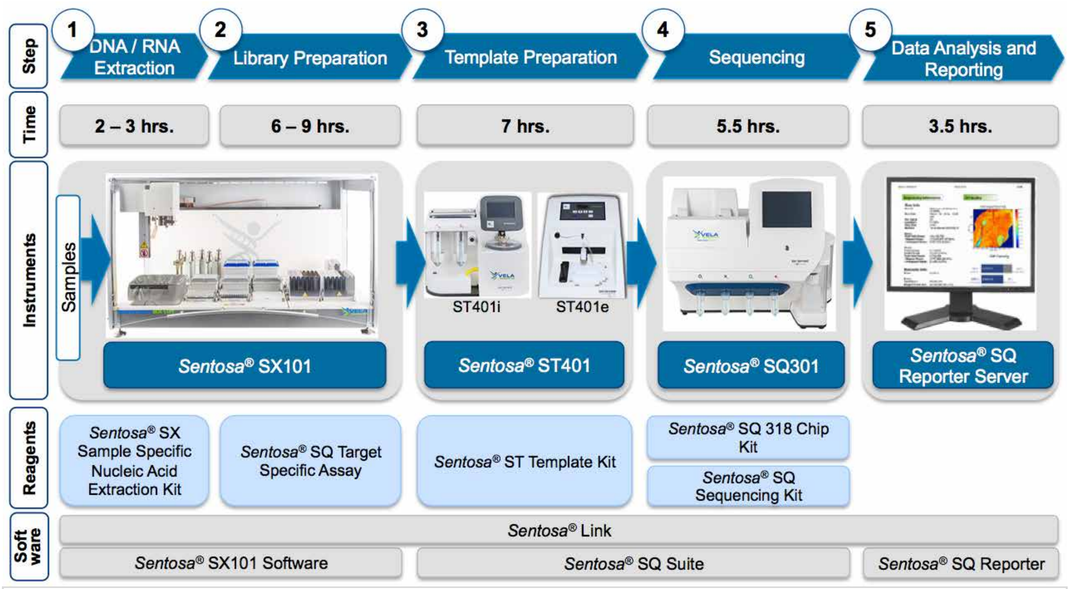

Figure 1. Vela Diagnostics *Sentosa*® NGS Workflow.

Vela Diagnostics' NGS workflow **(Fig. 1)** 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 and sequencing 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 maintained through the network with Vela Diagnostics customized 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.

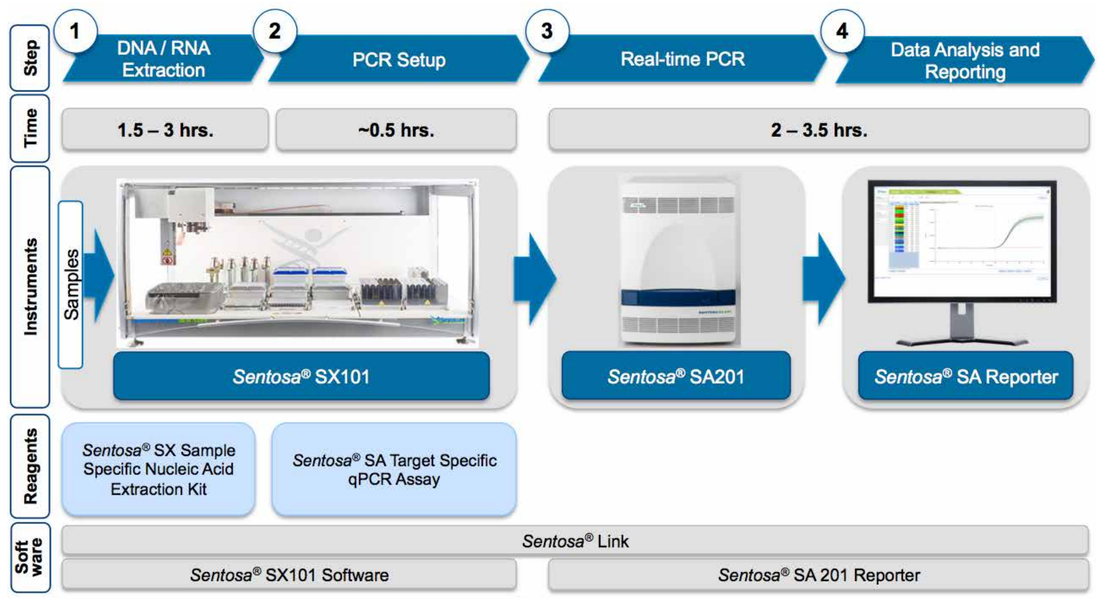

Figure 2. Vela Diagnostics *Sentosa*® qPCR Workflow.

Vela Diagnostics qPCR workflow **(Fig. 2)** allows for nucleic acid extraction and PCR setup on various sample types **(Table)** with run throughput ranging from 8 to 96 samples. With more than 20 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 *Sentosa*® SA201 (Applied Biosystems) or the Rotor-Gene Q (QIAGEN). 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 3-4 hrs.

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
Plasma cell-free DNA	Up to 8 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 20 PCR tests and 14 clinically relevant and validated human sample types.

CONCLUSION

Sentosa PCR and NGS workflow appears as a robust and efficient *in vitro* diagnostics (IVD) tool for the detection and/or quantitation of a wide range of bacterial and viral pathogens as well as mutations in human genes. Such comprehensive combined NGS and qPCR workflow that can help fill some existing gaps in the quality of molecular diagnostics will take the necessary steps towards improving patient outcomes.

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

www.veladx.com

T +1.714.463.4673 18340 Yorba Linda Blvd. Ste 107
F +1.714.970.2173 PMB 427, Yorba Linda, CA 92886
info@labroots.com • labroots.com