

# Highly Accurate HCV Genotyping by Targeted Next Generation Sequencing



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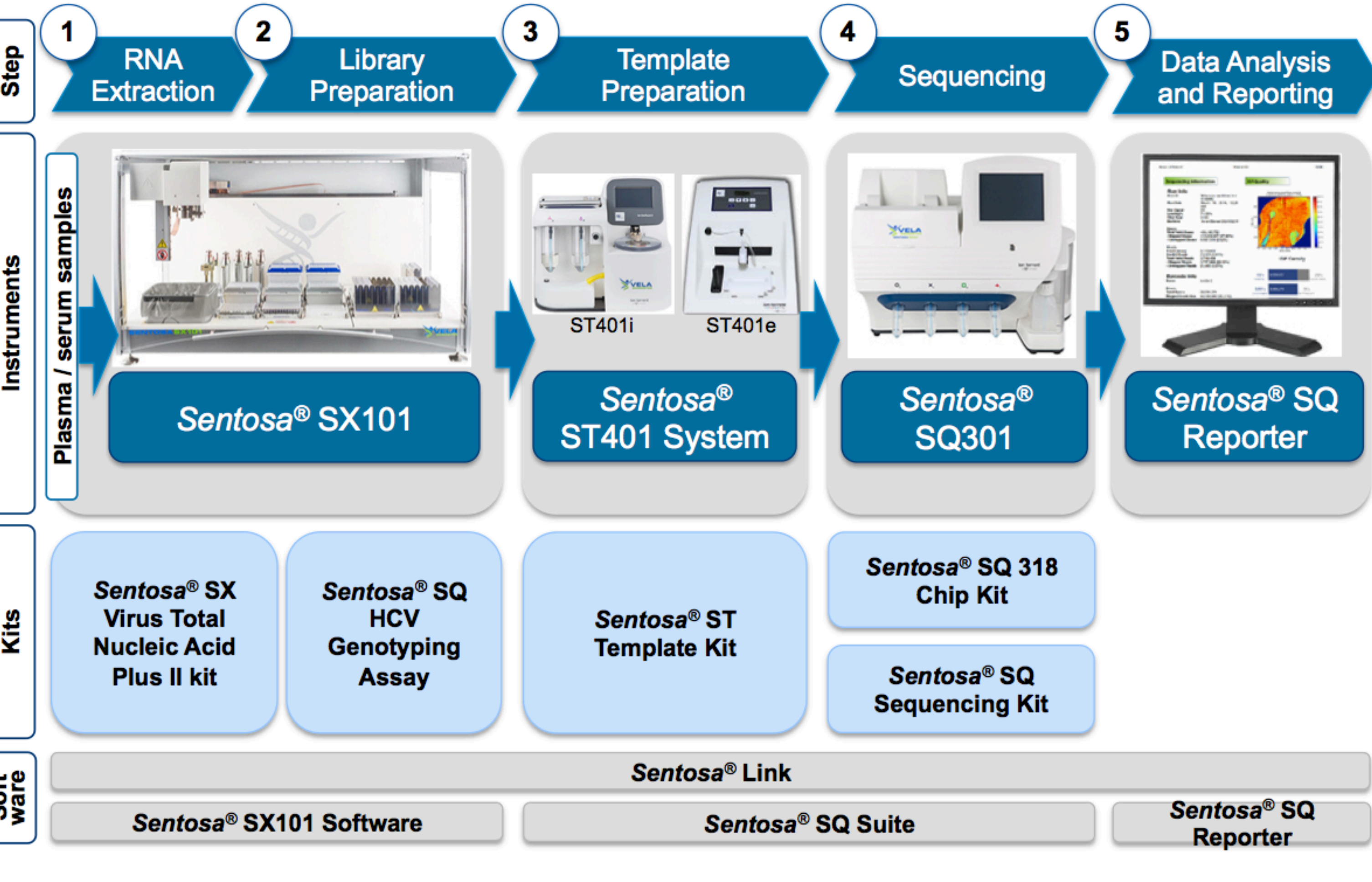
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## INTRODUCTION

Determination of HCV genotype (GT) provides essential information for decision making on treatment regimens in HCV infection [1]. DNA hybridization employing a line probe test format (VERSANT HCV Genotype 2.0 LiPA), first introduced about 20 years ago, still represents the most widely used laboratory method [2]. The recent fast advancement of next generation sequencing (NGS) technologies allowing for unprecedented speed and accuracy in analyzing viral genomes are opening new ways to further improve diagnostic genotyping of HCV [3].

## MATERIAL & METHODS

Figure 1. Sentosa® SQ HCV Genotyping Assay Workflow.



We have used NGS in combination with workflow automation, from sample preparation to result reporting (Fig. 1), to demonstrate high accuracy of GT assessment in clinical samples. This newly developed platform based on a customized version of the epMotion 5075 robotic system (Eppendorf, Germany) consists of a continuous robotic process starting with plasma or serum extraction and RT-PCR followed by automated library preparation, Ion Torrent deep sequencing [4] and direct online data analysis to determine HCV GTs. In contrast to the commonly used 5'UTR region, the limitations of which are well recognized, we have employed target sequences from the HCV NS3, NS5A and NS5B regions (Fig. 2).

## RESULTS

Figure 2. Regions targeted by Sentosa® SQ HCV Genotyping Assay.

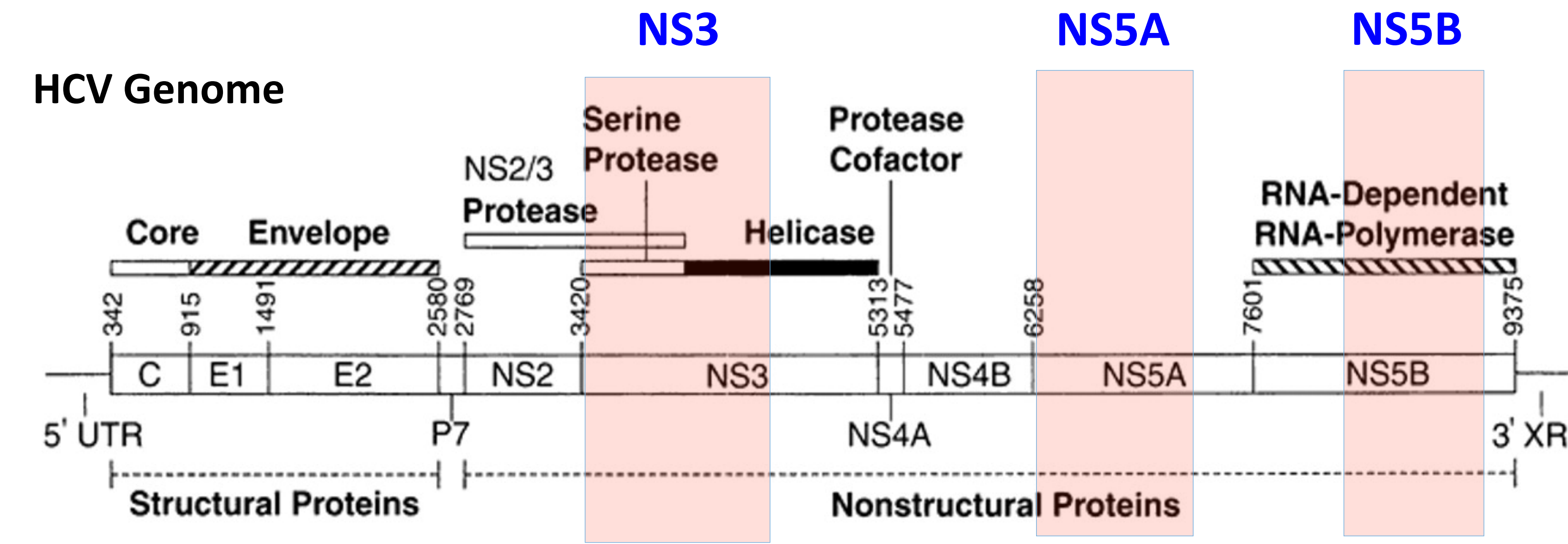
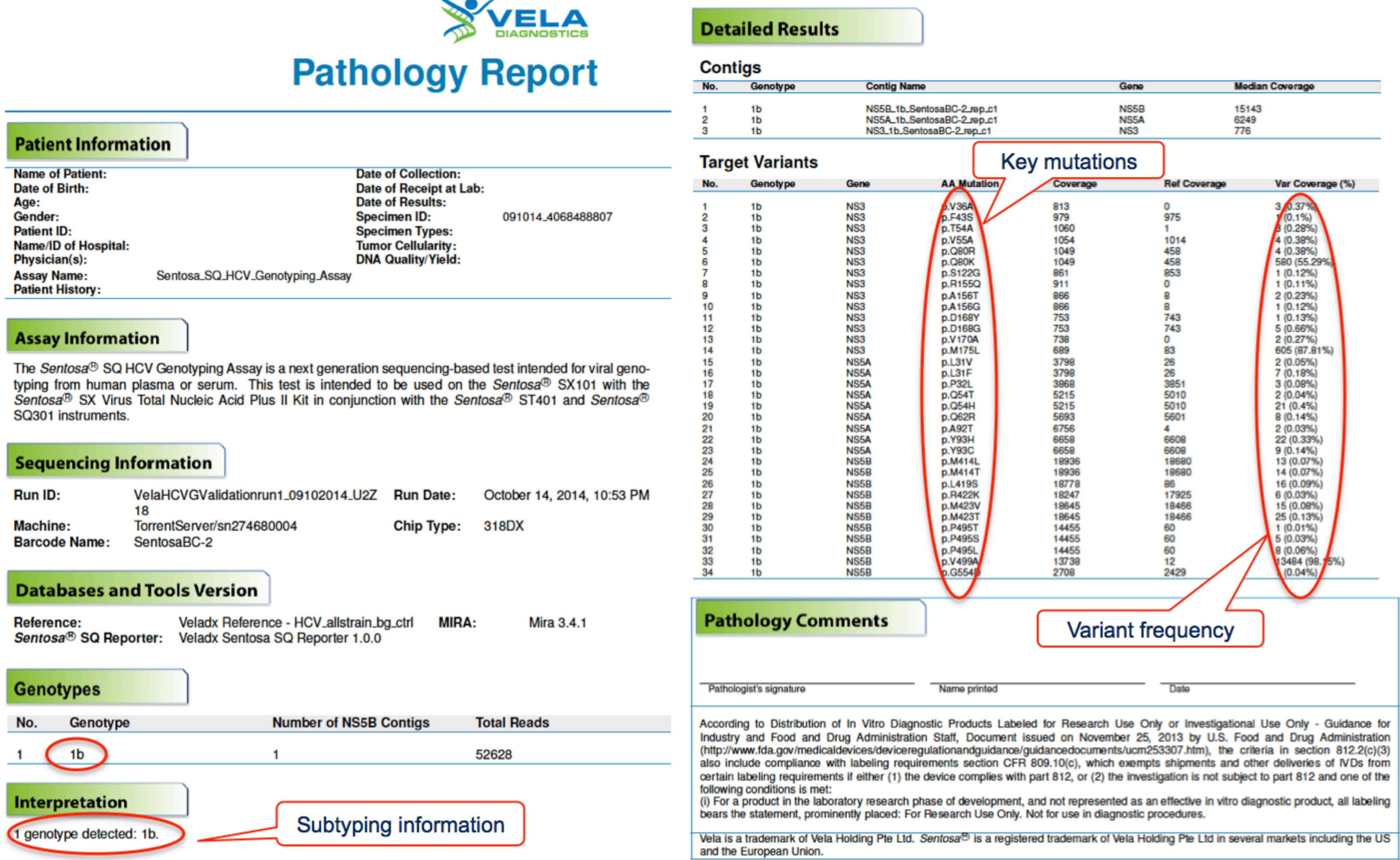


Figure 3. An example of a Pathology Report generated by Sentosa® SQ Reporter.



Results were compared to line probe assay data obtained from the same samples. Randomly selected archived serum or plasma samples from 143 Asian and 7 African patients with chronic HCV infection, viral loads ranging from 5.50x10<sup>2</sup> to 1.04x10<sup>8</sup> IU/mL (median 6.10x10<sup>6</sup>), were included in the study. GT distribution was as follows: 11 GT1a, 14 GT1b, 12 GT2, 58 GT3, 9 GT4, 7 GT5, and 39 GT6. In 16/150 (11%) of the samples, discordant results between the two methods were obtained. Confirmation testing by Sanger sequencing indicated that the ability to discriminate at the major GT level was 89.3% (95%CI: 83.4 – 93.3) for VERSANT and 100% (95%CI: 97.5-100) for NGS. Correct GT subtype calls were found to be 89% for VERSANT and 100% for NGS. Among the 16 discordant samples, 8 GT6 were erroneously classified as GT1b by line probing, 6 GT3 as GT4, and another 2 GT3 as GT6 (Table 1).

Table 1. Genotypes identified by different methods for discordant results.

No.	Viral Load (IU/mL)	Sample Type	Genotype by VERSANT HCV Genotype 2.0 LiPA	Genotype by Sentosa SQ HCV Genotyping Assay	Genotype by Sanger Sequencing*
1	364100	Serum	1b	6	6
2	397650	Serum	1b	6	6
3	3130050	Serum	1b	6	6
4	1978350	Serum	1b	6	6
5	1997600	Serum	1b	6	6
6	73150	Serum	1b	6	6
7	96800	Serum	1b	6	6
8	821150	Serum	1b	6	6
9	44000	Serum	4	3	3
10	2253900	Serum	4	3	3
11	78650	Serum	4	3	3
12	111100	Serum	4	3	3
13	5529700	Serum	4	3	3
14	155650	Serum	4	3	3
15	550	Serum	6	3	3
16	1042800	Serum	6	3	3

\*NS5B region has been used as a target region for Sanger Sequencing.

## CONCLUSION

In conclusion, considering the crucial role of correct genotyping in HCV treatment management, workflow automated HCV NGS appears as a highly reliable tool for differentiating HCV GTs, which can help to prevent diagnostic errors potentially leading to suboptimal treatment.

Not least, the library generated DNA contigs are fully user accessible for further sequence analysis thereafter, e.g., enabling assessment of additional mutations specific to the case under investigation (Fig. 3). This additional feature may prove useful for monitoring resistance to novel drug treatments.

## REFERENCES

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