Performance of the newly designed Sentosa SQ HCV Genotyping v2.0 assay in HCV genotyping and resistance testing within the PEPSI study

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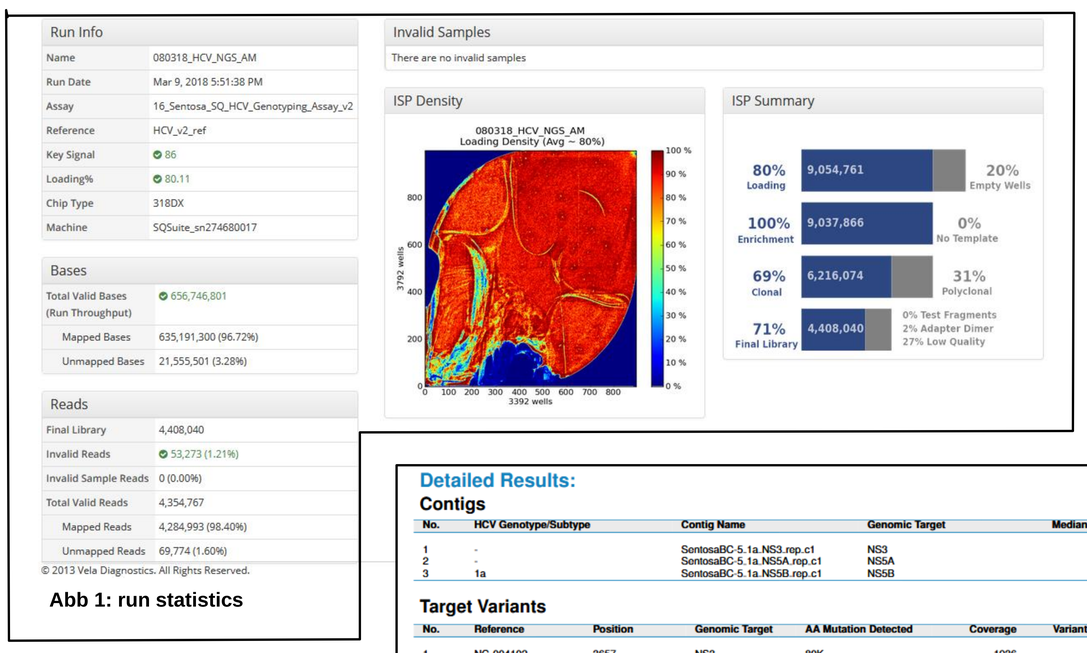
BACKGROUND

The Sentosa® SQ HCV Genotyping Assay v2.0 (Vela diagnostics) is a NGS-based test for qualitative identification of Hepatitis C Virus (HCV) genotypes 1 to 6 and the detection of resistance-associated substitutions (RAS) in HCV subtypes 1a/1b and 3. We used the research use only assay for genotyping and resistance detection in the NS3, NS5A and NS5B genes for samples within the PEPSI study(1). The further enhancement of version 2 is the extended length of sequence for NS5B starting at aminoacid (aa) 1 (instead of aa 339 in version 1) up to aa 565, and the inclusion of genotype 3 in resistance analysis.

METHODS

75 samples (so far) were analysed. Beside the included analysis in the Vela system we interpreted the raw data using the torrent suite (5.6) to map the sequences with a minimum of 100/20/4 reads for each base with minority cut-offs at 30/20/15/10/5 and 2%. Sequence-interpretation was performed with the geno2pheno [hcv] 0.92 online tool (http://hcv.geno2pheno.org). Predicted genotype and resistance mutation for NS3, NS5A and NS5B were analysed.

RESULTS



Run Info

Name	080318_HCV_NGS_AM
Run Date	Mar 9, 2018 5:51:38 PM
Assay	16_Sentosa_SQ_HCV_Genotyping_Assay_v2
Reference	HCV_v2_ref
Key Signal	86
Loading%	80.11
Chip Type	318DX
Machine	SQSuite_sn274680017

Bases

Total Valid Bases (Run Throughput)	656,746,801
Mapped Bases	635,191,300 (96.72%)
Unmapped Bases	21,555,501 (3.28%)

Reads

Final Library	4,408,040
Invalid Reads	53,273 (1.21%)
Invalid Sample Reads	0 (0.00%)
Total Valid Reads	4,354,767
Mapped Reads	4,284,993 (98.40%)
Unmapped Reads	69,774 (1.60%)

Invalid Samples

There are no invalid samples

ISP Density

ISP Summary

80% Loading	9,054,761	20% Empty Wells
100% Enrichment	9,037,866	0% No Template
69% Clonal	6,216,074	31% Polyclonal
71% Final Library	4,408,040	0% Test Fragments, 2% Adapter Dimer, 27% Low Quality

Abb 1: run statistics

Detailed Results:

Contigs

No.	HCV Genotype/Subtype	Contig Name	Genomic Target	Median Coverage
1	-	SentosaBC-5.1a.NS3.rep.c1	NS3	488
2	-	SentosaBC-5.1a.NS5A.rep.c1	NS5A	480
3	1a	SentosaBC-5.1a.NS5B.rep.c1	NS5B	460

Target Variants

No.	Reference	Position	Genomic Target	AA Mutation Detected	Coverage	Variant Freq. (%)
1	NC.004102	3657	NS3	80K	1936	97.52%

* NS5A region is sequenced successfully. No drug resistance mutations were detected

* NS5B region is sequenced successfully. No drug resistance mutations were detected

Abb 2: analysis report, details

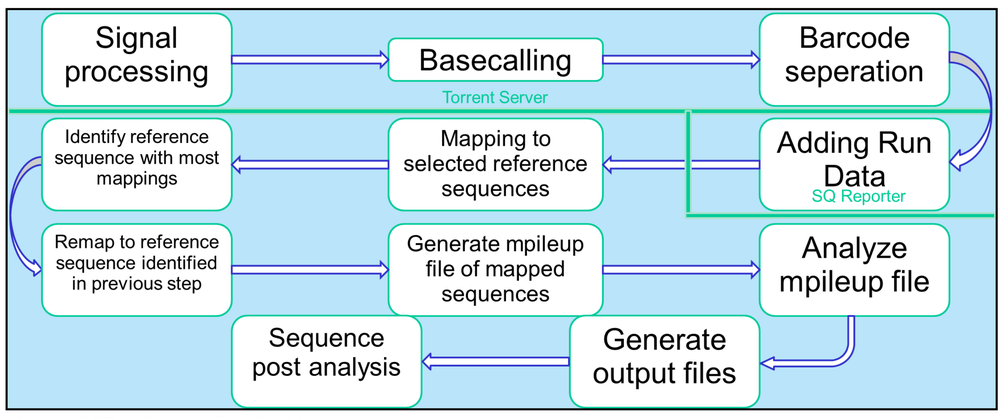


Abb 3: MIB NGS pipeline for Vela

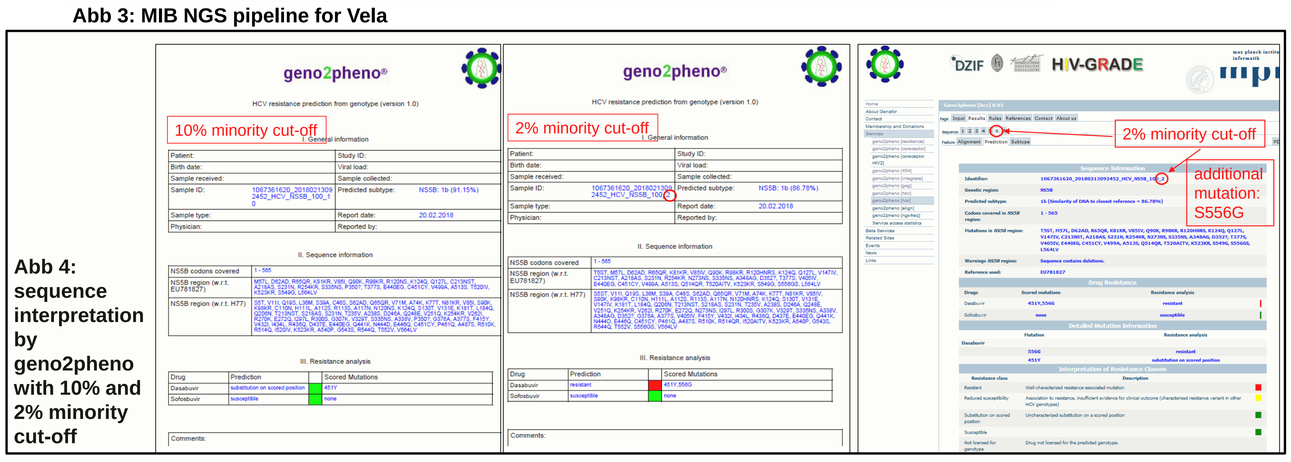


Abb 4: sequence interpretation by geno2pheno with 10% and 2% minority cut-off

RESULTS

Sequences for all samples could be generated with declining quality below 1000 IU/mL (three samples; one 1a with 450 IU/mL ok for all three genes; one 1a and one 3a, 250 and 460 IU/mL, only with NS5A sequence). There were drop-outs for single genes (NS3 12/75, NS5A 4/75 and NS5B 2/75) mainly in non-1/3 genotypes. Genotype-distribution was 1a (25), 3a (23), 4d (9), 1b (8), 2b (4), 4a (3) and each of 1g, 2c and 6a. No RAS were detected in genotypes 2b/c, 4a/d and 6a. Seven of eight 1b samples showed resistance, one sample to three classes. 14/25 1a samples had resistance mutations, NS3 Q80K (11x), NS5A (3x 28VT, one 58S) and NS5B (one 553V and 556R). 6/23 3a samples had NS5A mutations (4x 30K, one 30V, two 93H), none had NS3/NS5B mutations. Overall 32 RAS were detected (15/12/5 for NS3/NS5A/NS5B), additionally 7 RAS in minorities between 10% and 2% of population.

CONCLUSIONS

The Sentosa® SQ HCV Genotyping Assay v2.0 performed excellent, even in samples with low viral load. Designed to genotype all HCV strains and RAS in genotype 1a/1b and 3 we got success rates per gene of 96.4% (168 sequences/6 failures). In non-1a/b or 3 genotypes the success rate was 78.9% (57/12). With regard to the high genetic variability of HCV and the diversity of its genotypes and subtypes this is an exceptionally good success rate, which is reached otherwise only by stepwise approach using multiple in-house protocols. While for many sequencing protocols to determine the occurrence of RASs the HCV genotype has to be known on advance, this assay allows to gather this information in one step. Launch of HCV version 2 assay is planned for 2018.

(1) Kalaghatgi P, Sikorski AM, Knops E, et al. Geno2pheno[HCV] – A Web-based Interpretation System to Support Hepatitis C Treatment Decisions in the Era of Direct-Acting Antiviral Agents. Menéndez-Arias L, ed. PLoS ONE. 2016;11(5):e0155869. doi:10.1371/journal.pone.0155869.