

GGAGGGAATATG
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RUO

HCV

Solution

Rev. 01/2023

HCV SOLUTION

Background

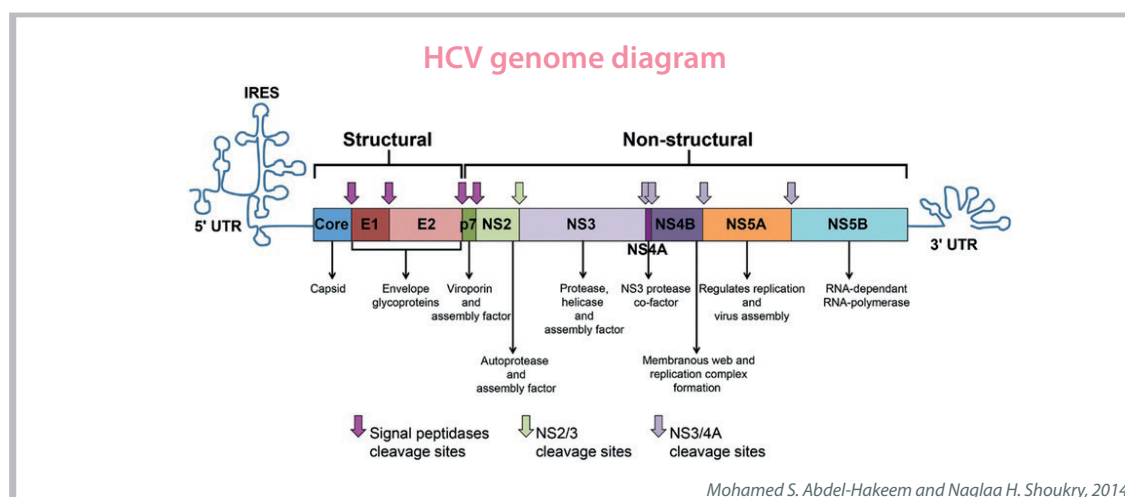
Hepatitis C virus (HCV) belongs to the Flaviviridae family. Six major genotypes and more than 60 subtypes have been described. HCV virus is endemic worldwide and represents a serious health problem. About 130 million people become infected with hepatitis C virus (*Fonseca-Coronado et al;* 2012). Since 2011, the treatment of chronic hepatitis C has been significantly improved by the introduction of direct-acting pangenotypic antiretroviral drugs (DAA). Despite this, therapeutic failures can occur in about 3-5% of treated patients. Many factors can contribute, including patient' therapy compliance and acquired drug-resistance (*Di Stefano et al.,* 2021).

Particularly, RAS (*Resistance Associated Substitution*) is often cause of the DAA regimes failure (*Sorbo et al.,* 2018; *Di Maio et al.,* 2018). During replication, HCV virus develops many mutations, which may include variants with reduced DAA susceptibility. For this reason, it is critical to define the viral genotype and identify the presence of associated drug-resistances.

Intended use

The AD4SEQ HCV Solution kit is an *in vitro* standardized device for HCV genotype detection and drug-resistance determination based on the RT-PCR technique and Next Generation Sequencing (NGS) of the complete viral genome (Whole Genome Sequencing - WGS), starting from plasma/serum samples positive for HCV.

A dedicated software allows visualization and computer analysis of sequencing data.



Sample Type

- ✓ Plasma
- ✓ Serum
- ...Positive for HCV virus

Performance features

- ✓ Sequencing library preparation in ~ 16 hrs
- ✓ Input: ≥ 1000 IU/ml
- ✓ Drug-resistance identification
- ✓ HCV genotyping
- Data analysis by dedicated software (developed by SmartSeq S.r.l)

RNA Extraction – Validated Methods

- ✓ QIAamp® Ultrasens Virus kit (Qiagen)
- ✓ Any other extraction method available in the laboratory may be used upon users' validation

NGS Instruments

- ✓ Illumina® MiSeq™
- ✓ Illumina® iSeq™ 100
- ✓ Illumina® MiniSeq™

Workflow Overview

15
MIN

"setup"

- ✓ HCV virus genomic RNA extraction

35
MIN

RT-PCR

- ✓ Whole genome RT-PCR

330
MIN

Target
PCR

- ✓ Whole genome amplification

35
MIN

"setup"

- ✓ Target PCR quality control
(e.g agarose gel electrophoresis or digital alternatives)
- ✓ Target PCR products purification via AMPure® XP magnetic beads

40
MIN

TAG
PCR

- ✓ Target PCR purified products digestion
- ✓ TAG PCR products purification via AMPure® XP magnetic beads

70
MIN

Index
PCR

- ✓ Samples indexing with unique indexes
(up to 96 indexes)

35
MIN

"setup"

- ✓ Index PCR quality control
(e.g. agarose gel electrophoresis or digital alternatives)

60
MIN

Purification
& Pool
Normalization

- ✓ Index PCR products purification via AMPure® XP magnetic beads
- ✓ Index PCR products quantification
(e.g. Qubit™ fluorometer or alternative methods)
- ✓ Library normalization and sequencing setup

up to
24
hours

NGS
Sequencing

- ✓ Illumina® MiSeq™
- ✓ Illumina® iSeq™ 100
- ✓ Illumina® MiniSeq™



Data
Analysis

- ✓ Data analysis by dedicated software (developed by SmartSeq s.r.l.)
(Recommended software for data analysis)

Performance features

- ✓ Whole genome sequencing
- ✓ Sequencing library preparation in ~ 16 hrs
- ✓ Input: ≥1000 IU/ml
- ✓ Drug-resistance identification
- ✓ HCV genotyping
- ✓ Data analysis by dedicated software (developed by SmartSeq S.r.l)

Sequencing Information

Instrument	Illumina® Kit	Number of Samples	Sequencing Time <small>(Illumina® Official Data)</small>
Illumina MiSeq™	MiSeq™ Reagent Nano Kit v2 (300 cycles)	up to 10	~ 20 h
Illumina MiSeq™	MiSeq™ Reagent Nano Kit v2 (500 cycles)	up to 10	~ 24 h
Illumina MiSeq™	MiSeq™ Reagent Micro Kit v2 (300 cycles)	up to 40	~ 39 h
Illumina MiniSeq™	MiniSeq™ Mid Output (300 cycles)	up to 75	~ 28 h
Illumina iSeq™ 100	iSeq™ 100 i1 Reagent (300 cycles)	up to 72	~ 20 h

Ordering Information

Cat. No.

AD4SEQ

HCV Solution

30 rxns

AD-006.030