

# DeepChek® Assay & DeepChek® Software

## Question & Answers

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**Advanced Biological Laboratories (ABL SA)**

5, boulevard de Trèves

57070 Metz France

Phone: +33 (0)3 51 56 60 77

Fax: +33(0)3 55 94 70 55

<http://diag.ablsa.com>

Support: support@ablsa.com

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## I. DeepChek® Assay – Extraction, PCR and Purification

### Q1) what genotypes were used to validate the HCV kit

Our kit for NS3 (K-16-NS3DR) is pan-genotypic. It has been validated for subtypes 1 to 6.

### Q2) Could you please confirm that the assay we use is just for GT1a and 1B?

For HCV Drug resistance, we now have pan-genotypic kits for NS3 (K-16-NS3DR), NS5A (K-16-NS5ADR) and NS5B (K-16-NS5BDR). Our former version of NS5A (K-15-027-C1-NS5ADR) has been validated for subtypes 1a and 1b.

### Q3) There is mentioned in the DeepChek® SingleRound RT-PCR and Sequencing NS5B / 5'UTR Assay v2, that MagNA Pure Compact Nucleic Acid Isolation Kit I is recommended to use for extraction. Would there be any trouble if RNA is purified with the kit of Anatolia kit?

We never tested this kit. It should probably work. Maybe we could think about starting a pilot in one of your sites (through demo package/kits) to test this approach?  
To be more sensitive, we anyway recommend to use 1ML of plasma.

### Q4) Is it fine to have different signals?

Yes it is fine. Different signals can be due to the fact that HCV NS5A is an hypervariable region. The signal will depend on the HCV genotypes. It can also depend on the viral load but it looks all good for genotyping.

### Q5) There are three variants of PCR purification: STEP 3 - Sephadex purification / STEP 3 - PCR Cleanup reagent / STEP 3 - Ethanol purification. Which is better to choose? If I choose the second one – PCR cleanup reagent, which product is the best to use?

The PCR Cleanup reagents work very well like Exosap and BDX purification.

## II. DeepChek® Assay – Sequencing

### Q1) What is the difference between Big Dyes Terminator kit v1.1 and Big Dyes Terminator kit v3.1 kits?

V3.1 is an evolution of the v1.1 which comes with better performances.

### Q2) Sanger Sequencer settings?

The DeepChek assays have been validated by our R&D department with the ABI 3130XL (System Upgrade: 3100 to 3130XL).

The Run Module for the ABI 3130xl uses these parameters:

- Oven Temp: 60 °C
- Poly Fill Volume: 6500 steps

- Current Stability: 5.0 Amps
- PreRun Voltage: 15.0 kVolts
- PreRun Time: 180 sec
- Injection Voltage: 1.2 kVolts
- Injection Time: 9 sec
- Voltage Number of Steps: 30 nk
- Voltage Step Interval: 15 sec
- Data Delay Time: 120 sec
- Run Voltage: 8.5 kVolts
- Run Time: 2780 sec

ABI uses POP7 capillaries and the procedure uses Z-Big Dye Version 3 as the dye for analysis.

**Q3) The Nextera XT protocol requires 1ng of dsDNA. In our case this 1ng will be from the pool of the 3 pcr products or we will perform 3 individual reactions per sample?**

For example to perform HIV with three regions RT, Protease and Integrase for the same patient before to start the Nextera XT protocol : pooled the 3 amplicons according to the relative concentration to be equimolar and used this pool as an unique sample.

**Q4) Can we combine HIV and HCV samples with the same index? I mean that one sample from HIV and one sample from HCV linked to the same index to use less reagent.**

Yes, that is possible. Keep in mind that the total amount reads you would normally get for a sample is now divided over the HIV and HCV sample. So for example, if you would (theoretically) get 1.000.000 reads when you sequence HIV alone with 1 index, you would now (when you use one index for HIV and HCV) get 500.000 for HIV and 500.000 for HCV.

This is of course very theoretical. The number of reads you will get for each virus will also depend on the genome or amplicon size. The Nextera kit has a bias for smaller DNA fragments. This means that an amplicon of 300bp will get sequenced more than a amplicon of 1500bp.

**Q5) How much PhiX do we need?**

For samples with low diversity, use a high-concentration spike-in (5% or higher) of PhiX to create a more diverse set of clusters.

**Q6) Which coverage do we need for the amplicons?**

For targeting a 1% interpretation, a 500x coverage is required at least. Depending on the type of chip that will be used you could, theoretically include the following number of samples:

For example:

PGM:

- Ion 314™ Chip v2 (400bp): 40 patients ( NS5ADR/NS5BDR)
- Ion 316™ Chip v2 (400bp): 400 patients ( NS5ADR/NS5BDR)

S5:

- Ion 520™ Chip (400bp): 800 patients (NS5ADR/NS5BDR)

### III. DeepChek® Software

#### Q1) What is the High performance subtyping

Based on *Struck et al Nucleic Acids Research, 2014*. This work introduces COMET (Context-based Modeling for Expedient Typing), COMET is a powerful genotyping tool specialized in HIV-1 and HCV.

#### Q2) Covered positions?

*Chen et al. Nature 2016* reported the frequencies for HCV (GT1-6) variants resistant to direct acting antiviral agents for NS3 protease inhibitors, NS5A inhibitors and NS5B polymerase inhibitors.

**The covered positions for our HCV kits are :**

NS3 :1-206

NS5A : 1-222

NS5B : 1- 579 ( in 2 fragments)

**The covered positions for our HIV kits are :**

RT:1-312

PR: 1-99

INT v3: 49-282

INT v2: 51-276

#### Q3) The reference sequences, are different for different suptypes, are from Geno2Pheno?

The reference sequences were chosen from the COMET test set which in turn came from ICTV HCV Reference Sequence Alignment (2013-09-11).

#### Q4) What kind of an input file goes into the DeepChek software

Using DeepChek, you can use several formats:

- NGS Raw sequence: FastaQ, FastaQ paired, Fasta
- NGS alignment: Fasta-AVA, BAM+ reference
- Sanger: Fasta, AB1

For MiSeq data management, we recommend you to use the Paired-end FASTQ format, DeepChek will handle the entire bioinformatics process.

#### Q5) How to make drug resistance analysis

1/ be sure that Genotyping analysis is set to « Variant population analysis » in the « Input Configuration » step

2/ Activate at least one Algorithm + be sure that « Drug Resistance Determination » section is checked in « Setup report » step

#### **Q6) How to gets all mutations, even filtered ones?**

During the analysis process, DeepChek will determined all variant. You can find them :

- For NGS, in the quality information CSV (AA and NT)
- For SANGER, all AA mutation is reported in the pdf

#### **Q7) Why my analysis is still in "queue" ?**

DeepChek portal server is a "cloud" solution based, means that you shared ressources with other customer and the server processing pipe is unfortunately limited. The system pickup one by one analysis for running by follow a FIFO queue. The "Queued" status means that the system waiting for an empty slot to process your analysis.

#### **Q8) How long could take an analysis ?**

It really depends on DATA size it selves and the type of analysis (Variant calling and/or subtyping).

- SANGER analysis or 454 AVA consensus should take approximatively 5 to 10 minutes to process.
- NGS analysis are more variant, could take from 10 minutes to hours...

#### **Q9) Does DeepChek works with multiplexed files?**

Deepchek analysis only works on single sample file.

#### **Q10) How to works directly on chromatogram files?**

AB1 entry is currently only available in ViroScore (soon in DeepChek). To access, you have to select « Chromatogram (AB1) Format » when you launch a new ViroScore analysis.

Please note that a "Premium" license is needed to activate the Chromatogram entry.

#### **Q11) Why Comet Subtyping prevalence differs a bit if I reanalyse the same files?**

It 's due of Comet way of working, is based on predictive algorithm. Difference should not difers more than 10%

#### **Q12) In Quality CSV information file, what does passed expert system show?**

The expert System is our system that filter irrelevant mutations. There are at least 4 major filters

- Noisy mutations filtering
- Coverage filtering
- Forward/Reverse unbalanced frequency
- Forward/Reverse unbalanced coverage

Only mutations that pass the expert system are displayed in the PDF report and are used for drug interpretation.

#### **Q13) What is mutation prevalence?**

Prevalance shows the frequency of mutation, right? It's the mutation frequency (mutation count / coverage at position)

**Q15) How to analyse V3 loop region (tropism) in DeepChek?**

To get tropism results you need:

- Upload file in GP120 region, V3 loop can be uploaded also as GP120 entry
- select Phylogenic algorithm (Geno2pheno)