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DeepChek® HIV v1.0., a reliable tool for the bioinformatics analysis and resistance interpretation of Massive Ultra Deep Sequencing of HIV genomes

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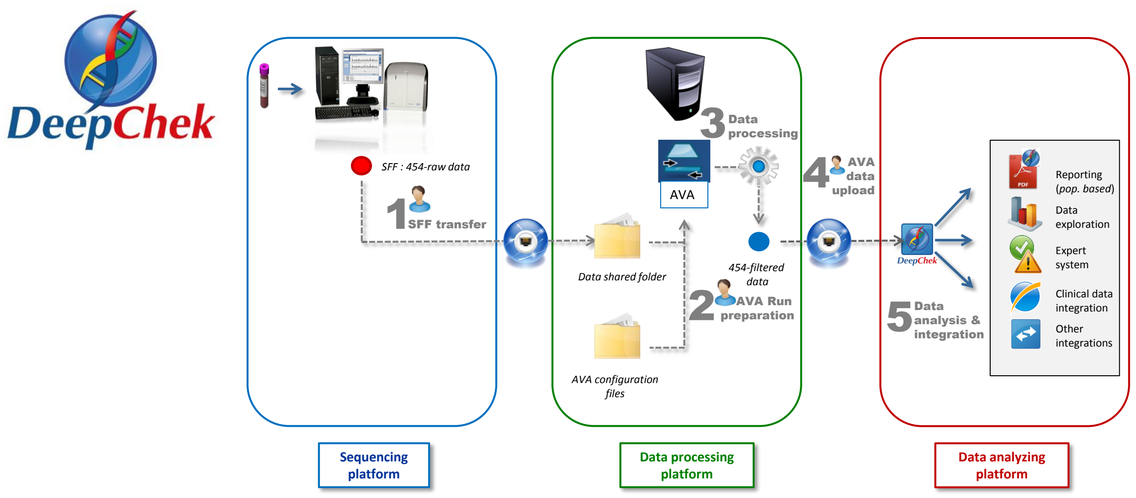
Background and Aim

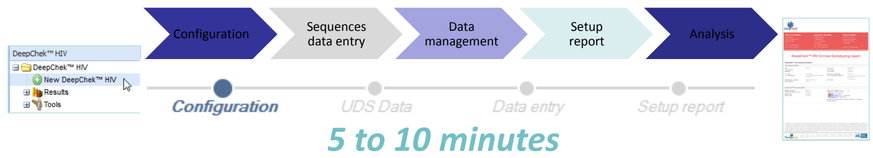
The detection of minor variants of NNRTI resistance mutations has been related with a higher risk of virological failure for patients on an NNRTI containing first line regimen. Ultra Deep Parallel Sequencing (UDPS) using 454 platforms allow for an accurate estimation of the presence of minor variants. As this technology comes into the diagnostic arena, there is a need for rapid and reliable bioinformatics tools for sequences analysis, data management and interpretations, for routine and research uses. In this report we present our results using the DeepChek®-HIV system, a new released IT solution for HIV UDPS sequence analysis.

Patients and Methods

50 HIV positive patients (viral load >10,000 copies/mL) have been studied. After RT & Pro sequencing, the information from AVA® was analyzed in DeepChek®-HIV software (part of TherapyEdge™, a global HIV database system), and results were compared to confirm its usefulness. The prevalence of low abundance drug resistant variants was compared using AVA® and the Next Generation Sequencing new data management and analysis system, DeepChek®-HIV v1.0 and DeepChek®-HIV v1.1.

DATA WORKFLOW – DEEPCHEK®-HIV (V1.0 AND 1.1)





- Type of analysis : DeepChek-HIV
- Include Sanger-based analysis
- Include AVA variants file
- File upload of direct integration (not available)

- 454 format selection
- 454 data upload (PROT, RT, INT, GP41, GP120)
- Sanger data upload (PROT, RT, INT, GP41, GP120) - *optional*

- Sample data entry
- Clinical data entry (GSS drugs…)
- Healthcare providers data entry
- Population based data management :
  - **Thresholds definition**
  - 454 data parsing
  - **Consensus sequences creation**

- Reminder sample/454 captured data
- **Algorithms selection** + version
- Report configuration
  - Language
  - Display resistance data
  - Display mutation comments
  - Display quality
  - GSS cutoffs definition

- 454/Sanger sequences localization
- 454/Sanger sequences quality check
- 454/Sanger sequences translation
- Nº of seqs/cutoff validation
- Resistance testing
- Subtyping determination
- Classification mutations of interest
- Overtime variants analysis
- Contamination check
- Cumulative population analysis

Results

The full workflow of database processing, analysis and reporting using DeepChek®-HIV was 5 minutes/sample (3,000-5,000 sequences per sample) comparable to the TruGene® HIV system. Overall, a high concordance between DeepChek®-HIV (considered as reference) and AVA®, was observed. Discordant results were observed with DeepChek®-HIV v1.0, mainly at the 1% threshold (mainly associated to the "population based" analysis) and did not lead to different drug resistance interpretations. Main discordances are highlighted in the table below. With DeepChek®-HIV v1.1, offering a per variant analysis in addition to all the others functionalities of v1.0, no discordance was observed. Furthermore, DeepChek®-HIV presented additional information not presented in the AVA® output files such as, any HIV possible mutation in the sequenced regions, all possible drug resistance interpretations (with the options to use several algorithms and personalize the definitive clinical genotyping report), which are up to date and updated in real time, HIV subtype determination and possibilities to integrate with global HIV molecular and patient databases. In addition, the optional DeepChek®-HIV Expert System includes several types of filters to optimize reliability and precision of drug resistance determination.

Sample	UDS seqs	AVA (%)		DeepChek HIV 1 v1.0 detected at 1-5 (%)*		DeepChek HIV v1.1 (%) **	
		RT	PROT	RT	PROT	RT	PROT
1	7896	-	L63P (0.19)	-	L63P	-	L63P (0.20)
2	5192	V179I (88.10), G190E (3.42)	K20M (0.19)	V179I,G190E	K20M	V179I (88.22), G190E (3.43)	-
3	3414	-	L33I (0.91), M46I (0.32), L63P (0.60)	-	L33I ,M46I , L63P	-	L33I (0.91), M46I (0.33), L63P (0.60)
4	645	T69A (0.38)	L10F (0.07), K20M (0.07), K20V (0.15), M36V (0.29)	T69A	L10F (>20), K20M (>20), K20V , M36V	T69A (0.38)	L10F (0.15), K20M (0.07), K20V (0.15), M36V (0.29)
5	2266	T69I (0.79), T215I -T215S (nd), K219Q (0.52)	-	T69I, T215I , T215S , K219Q	-	T69I (0.79), T215S (0.06), K219Q (0.52)	-
6	2877	-	L10F, K20M (nd), L33V (0.83)	-	L10F , K20M ,L33V	-	L10F (0.19), K20M (0.03), L33V (0.83)
7	1607	K103E (0.67)	-	K103E	-	K103E (0.67)	-
8	1303	F77L (0.76)	L33V (0.61), L63P (0.60)	F77L	L33V, L63P	F77L (0.65)	L33V (0.61), L63P (0.59)
9	5017	T69N (0.98)	L10F (nd), V11I (0.79), G16E (0.95), K20M (0.11)	T69N	L10F , V11I , G16E, K20M	T69N (0.89)	L10F (0.16), V11I (0.80), G16E (0.95), K20M (0.16)
10	8335	L210M (nd)	M36I (0.92)	L210M (5-10)	M36I	-	M36I (0.93)
11	9281	L210M (nd), L210W (0.05)	K20M (0.09)	L210M , L210W (5-10)	K20M	L210W (0.05)	K20M (0.09)
12	5287		M36I (nd)		M36I	-	-
13	1169	K70E (0.26), K103R (nd)	-	K70E , K103R	-	K70E (0.26)	-
14	1908	V90I (1)		V90I (<1)		V90I (1)	-
15	1447	-	L10F (nd), K20M (nd)	-	L10F , K20M	-	-
16	1951	-	M36I (0.26)	-	M36I	-	M36I (0.26)
17	1829	-	V82I (1), L10F (0.24), K20M (0.24), L89I (0.14)	-	V82I (<1), L10F (1-20), K20M (1-20)	-	V82I (1), L10F (0.24), K20M (0.24), L89I (0.14)
18	2296	-	L10F (nd), K20M (0.42)	-	L10F , K20M	-	K20M (0.42)
19	1956	-	K20M (nd)	-	K20M	-	-
20	1768	D67E, D67H, T215I (nd)	L10F , K20M (nd)	D67E , D67H , T215I (1-10)	L10F, K20M (1-10)	-	L10F (0.51)
21	2019	D67N (0.98)	-	D67N	-	D67N (0.99)	-
22	6190	V179E , L210M (nd)	L10F (0.12)	V179E , L210M (1-10)	L10F	-	L10F (0.12)

* Unless specified; ** raw data from v1.1 (DeepChek®-HIV Expert System disabled); nd: Not Detected

DeepChek™ HIV drug resistance Mutation analysis

HIV Reverse Transcriptase drug resistance mutations

Position	Sanger based sequencing	Threshold 20%	Threshold 10%	Threshold 5%	Threshold 1%
K20	R	R	R	R	R
M41			L	L	L
K43			N	N	N
V60	I	I	I	I	I
D67	N	N	N	N	N
T69	D	D	D	D	D
K103	N	N	N	N	N
E122	K	K	K	K	K
I135	T	T	T	T	T
M184	V	V	V	V	V
Q197	R				
L214	F	F	F	F	F
K220					N
H221					N
P225	H	H	H	H	H
L228					H
Y271					X
P272	A	A	A	A	A/C/F/G/L/R/S/V/W/X
G273					R/W/X

DeepChek™ HIV drug resistance Susceptibility analysis

HIV Nucleoside Reverse Transcriptase Inhibitors

	Algorithm	Sanger based sequencing	Threshold 20%	Threshold 10%	Threshold 5%	Threshold 1%
Zidovudine	ANRS	S	S	S	S	S
	Rega institute	S	S	I	I	I
	Stanford	S	S	I	I	I
Didanosine	ANRS	S	S	S	S	S
	Rega institute	R	R	R	R	R
	Stanford	I	I	I	I	I
Stavudine	ANRS	S	S	S	S	S
	Rega institute	I	I	I	I	I
	Stanford	I	I	I	I	I
Lamivudine	ANRS	R	R	R	R	R
	Rega institute	R	R	R	R	R
	Stanford	R	R	R	R	R
Emtricitabine	ANRS	R	R	R	R	R
	Rega institute	R	R	R	R	R
	Stanford	R	R	R	R	R
Abacavir	ANRS	S	S	I	I	I
	Rega institute	S	S	I	I	I
	Stanford	I	I	I	I	I
Tenofovir	ANRS	S	S	I	I	I
	Rega institute	S	S	S	S	S
	Stanford	S	S	S	S	S

DeepChek™ VIH Coverage

Reverse Transcriptase

Number of sequences	Umbral 50%	Umbral 20%	Umbral 10%	Umbral 5%	Umbral 1%
1169	ok (10)	ok (25)	ok (50)	ok (100)	ok (500)

Protéase

Number of sequences	Umbral 50%	Umbral 20%	Umbral 10%	Umbral 5%	Umbral 1%
1169	ok (10)	ok (25)	ok (50)	ok (100)	ok (500)

DeepChek™ VIH Subtyping

Reverse Transcriptase

	Threshold 50%	Threshold 20%	Threshold 10%	Threshold 5%	Threshold 1%
Subtyping	B	B	B	B	B
Similarity	97.9	97.9	96.72	96.07	93.32

Protéase

	Threshold 50%	Threshold 20%	Threshold 10%	Threshold 5%	Threshold 1%
Subtyping	B	B	B	B	B
Similarity	99.29	98.57	98.57	98.57	97.5

Conclusion

DeepChek®-HIV v1.1 seems to provide improved reliability compared to v1.0, especially for minor viral population analysis. DeepChek®-HIV offers an efficient and simplified global and reliable database system wherein UDS-454® data may be fully integrated to analyze its usefulness on clinical and biological outcomes with statistical input; this next generation IT solution for the data management and interpretation of UDPS data may be used for clinical and diagnostic routine and research applications with ease of use.