Comparison of short-read and long-read next-generation sequencing technologies for determining HIV-1 drug resistance

AQO0605

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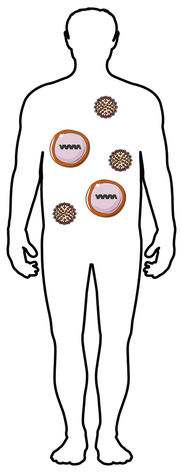
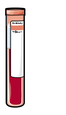
BACKGROUND

Accurate HIV-1 genome sequencing is necessary to identify drug resistance mutations in PWH. NGS allows the detection of minor variants and is now accessible in many laboratories.

METHODS



PWH (N=14)



Plasmas (n=11 + 5 dilutions) → RNA
Buffy coats (n=3) → DNA

« Short reads »

DeepChek® Whole Genome HIV-1 Assay

iSeq100

« Long reads »

pol and *env* SMRT NGS

PacBio
(CSS mode)

RESULTS

Table 1. Comparison of detection of HIV-1 drug-resistance mutations at cut-off 3%

Sample ID	Subtype	Viral load[a]	PR[b]		RT[c]		INT[d]		ENV[e]	
			DeepChek® WG	PacBio SMRT	DeepChek® WG	PacBio SMRT	DeepChek® WG	PacBio SMRT	DeepChek® WG	PacBio SMRT
1	B	5.9	33V (99%), 62V (99%)	33V (100%), 62V (100%)	*none*	*none*	*none*	*none*	**43K (23%)**	**43K (68%)**
2	F1	6.6	10I (99%), 20M (98%), 36I (99%), 89M (99%)	10I (100%), 20M (97%), 36I (100%), 89M (100%)	*none*	*none*	*none*	*none*	**375M (99%)**	**375M (99%)**
3	CRF02	5.8	20I (99%), 36I (99%), 63P (99%), 69K (99%), 89M (99%)	20I (99%), 36I (100%), 63P (100%), 69K (100%), 89M (100%)	103R (95%), 106I (99%)	103R (100%), 106I (100%)	*none*	**138K (13%)**	*none*	*none*
4	CRF06	6.4	16E (98%), 20I (99%), 36I (98%), 62V (3%), 69K (99%), 77I (6%), 82A (4%), 89M (99%)	16E (100%), 20I (100%), 36I (100%), 62V (4%), 69K (100%), 77I (5%), 82A (3%), 89M (100%)	*none*	*none*	*none*	*none*	*none*	*none*
5	CRF02	5.6	20I (99%), 36I (70%), 64L (99%), 69K (99%), 82A (5%), 89M (99%)	20I (100%), 36I (65%), 64L (100%), 69K (100%), 89M (100%)	*none*	*none*	*none*	*none*	*none*	*none*
6	CRF02	5.0	**10V (99%), 16E (99%),** 20R (70%), 20T (29%), 36I (97%), 69K (99%), 89I (99%)	**10V (100%) + 16E (100%),** 20R (100%), 36I (100%), 69K (100%), 89I (100%)	*none*	**65E (5%),** 101E (5%)	*none*	*none*	*none*	*none*
7	B	5.5	60E (99%), 63P (99%), 71T (12%), 77I (25%)	60E (73%), 63P (99%), 71T (24%), 77I (23%)	*none*	*none*	*none*	*none*	*none*	*none*
8	CRF06	5.0	16E (99%), 20I (18%), 36I (87%), 69K (99%), 89I (100%)	16E (100%), 36I (100%), 69K (100%), 89I (100%)	*none*	*none*	*none*	*none*	*none*	*none*
9	CRF02	5.8	20I (99%), 36I (100%), 46I (3%), 69K (99%), 89M (99%)	20I (98%), 36I (100%), 69K (100%), 89M (100%)	*none*	*none*	*none*	*none*	*none*	*none*
10	CRF02	4.5	10V (8%), 20I (100%), 36I (99%), 69K (99%), 77I (11%), 89M (99%)	20I (99%), 36I (100%), 69K (100%), 89M (100%)	108I (100%)	108I (100%)	*none*	*none*	*none*	*none*
11	CRF02	5.5	20I (100%), 36I (100%), 63P (99%), 69K (99%), 89M (99%)	20I (99%), 36I (100%), 63P (100%), 69K (100%), 89M (100%)	*none*	*none*	**148K (3%)**	*none*	*none*	*none*
12: 1 D1/100	B	3.8	33V (99%), 62V (100%)	33V (100%), 62V (100%)	*none*	*none*	*none*	*none*	**43K (17%)**	**43K (100%)**
13: 1 D1/1000	B	2.8	33V (100%), 62V (100%)	x	*none*	x	*none*	x	**43K (31%)**	x
14: 2 D1/1000	F1	3.6	10I (99%), 20M (98%), 36I (100%), 89M (100%)	10I (100%), 20M (100%), 36I (100%), 89M (100%)	**138Q (9%)**	*none*	*none*	*none*	x	x
15: 2 D1/10000	F1	2.6	10I (99%), 20M (99%), 36I (100%), 89M (100%)	10I (100%), 20M (100%), 36I (100%), 89M (100%)	*none*	*none*	*none*	*none*	**375M (99%)**	x
16: 3 1/500	CRF02	3.1	20I (100%), 36I (100%), 63P (99%), 69K (99%), 89M (100%)	x	106I (100%)	x	*none*	x	x	*none*
DNA 1	B	<1.4	16E (15%), 36I (100%)	36I (99%)	179I (99%), **184I (17%)[f], 230I (19%)[f]**	179I (100%)	*none*	*none*	x	x
DNA 2	B	1.5	63P (53%), 77I (19%)	63P (99%)	**184I (27%)[f], 230I (27%)[f]**	*none*	*none*	*none*	*none*	*none*
DNA 3	B	1.4	x	62V (100%), 77I (100%) 63P (99%)	x	*none*	x		*none*	x

[a] log_{10} copies/mL for RNA or log_{10} copies/10^6 PBMCs for DNA, [b] protease, [c] reverse transcriptase, [d] integrase, [e] envelope, [f] the following drug-resistance mutations could reflect APOBEC activity, [x] genotyping failure, bold: mutation with drug-resistance, red: difference between the two approaches.

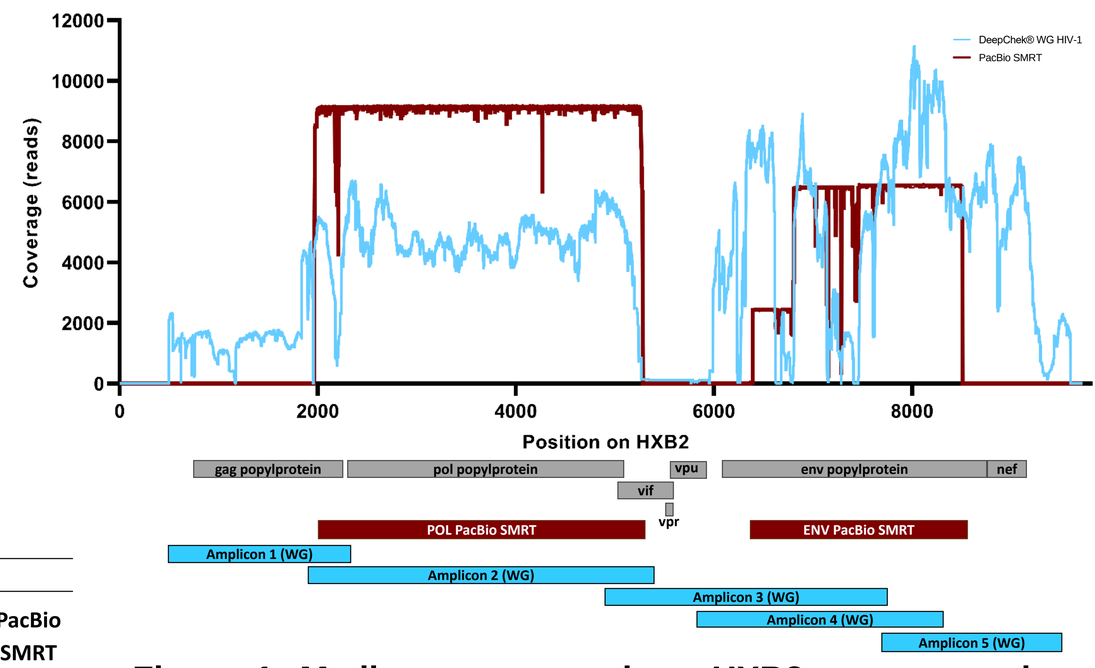


Figure 1. Median coverage along HXB2 genome and localization of the DeepChek® WG and PacBio SMRT amplicons. Grey, HIV-1 proteins; blue, DeepChek® Whole Genome HIV-1 assay and red, PacBio SMRT.

Table 2. HIV-1 *pol* and *env* sequencing success and failure using DeepChek® WG and PacBio SMRT. Green, sequencing success; orange, partially covered; red, sequencing failure.

Sample ID Plasma HIV-1 RNA	Subtype	Viral load (log_{10} copies/mL)	DeepChek® WG HIV-1 *POL*	PacBio SMRT *POL*	DeepChek® WG HIV-1 *ENV*	PacBio SMRT *ENV*
1	B	5.9				
2	F1	6.6				
3	CRF02	5.8				
4	CRF06	6.4				
5	CRF02	5.6				
6	CRF02	5.0				
7	B	5.5				
8	CRF06	5.0				
9	CRF02	5.8				
10	CRF02	4.5				
11	CRF02	5.5				
12: 1 D1/100	B	3.8				
13: 1 D1/1000	B	2.8				
14: 2 D1/1000	F1	3.6				
15: 2 D1/10000	F1	2.6				
16: 3 1/500	CRF02	3.1				

Sample ID Cell HIV-1 DNA	Subtype	Viral load (log_{10} copies/10^6 PBMCs)	DeepChek® WG HIV-1 *POL*	PacBio SMRT *POL*	DeepChek® WG HIV-1 *ENV*	PacBio SMRT *ENV*
1	B	<1.4				
2	B	1.5				
3	B	1.4				

CONCLUSION

DeepChek® WG HIV-1 Assay on Illumina and SMRT sequencing on Sequel IIe exhibit overall good performance, allowing the detection of drug resistance mutations, accurate quantification of variants and identification of the HIV-1 subtype.

ADDITIONAL KEY INFORMATION

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