EUROPEAN MEETING ON HIV & HEPATITIS 2024
TREATMENT STRATEGIES & ANTIVIRAL DRUG RESISTANCE
BARCELONA, SPAIN | 22 - 24 MAY 2024

ABL Advanced Biological Laboratories Diagnostics

Complete GENOMICS™

DNBSEQ™ Sequencing to Identify HIV-1 Drug Resistance Mutations

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Introduction

Subtype and drug-resistance mutations were mostly assessed routinely using Capillary Electrophoresis (CE) sequencing which does not detect co-infection or minor variants (frequency below 15-20%). Next Generation Sequencing (NGS) has become the new standard for genotypic drug resistance testing on the full HIV-1 polymerase gene. The objective of this study was to evaluate the performances of ABL DeepChek® DNBSEQ Native kit on Complete Genomics' DNBSEQ-G99 sequencing platform using HIV-1 Quality Control for Molecular Diagnostics (QCMD) samples.

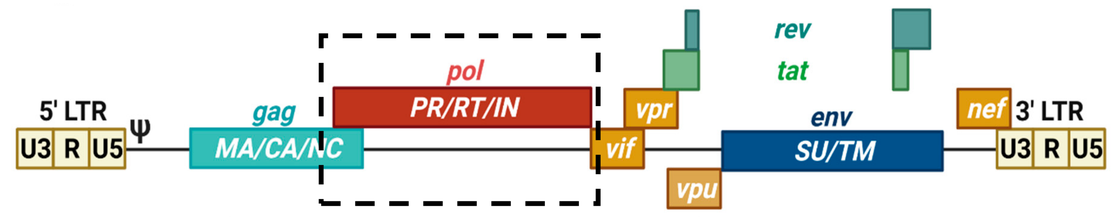
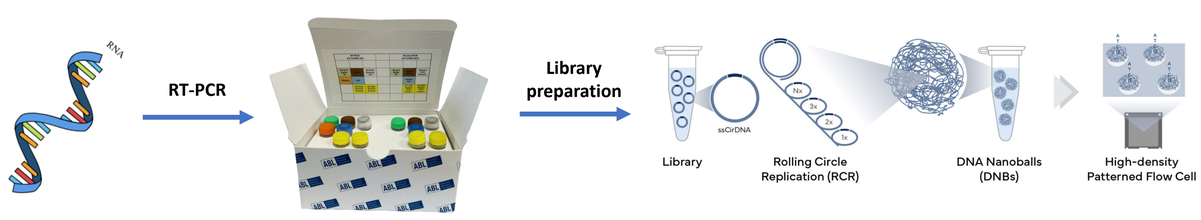
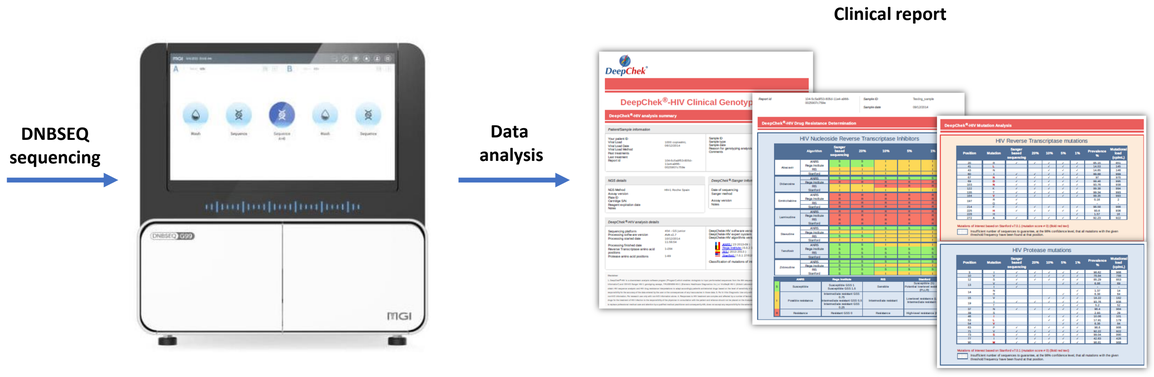


Figure 1: A diagram of the 9.8 kb HIV-1 genome. The pol portion of the genome is transcribed into the protease, reverse transcriptase and integrase.

Methods

A total of 5 HIV RNA positive plasmas (QCMD) at a range of 4.20 to 5.31 Log10 copies/mL were amplified and sequenced using Complete Genomics DNBSEQ sequencing technology. The protease, reverse transcriptase and integrase genes were amplified, libraries were prepared and sequenced using DeepChek® native kit for DNBSEQ (ABL) on the DNBSEQ-G99 sequencing platform. DeepChek® HIV software (ABL) was used for the interpretation of subtype and drug resistance according to the French ANRS v33 (National Agency for AIDS Research), Grade 9-2021 and HIVdb 9.4 12-2022.





Complete Genomicss DNA Nanoball Sequencing:
- ☑Linear amplification Low
- ☑ amplification bias
- ☑ No amplification error accumulation
- Lower ☑ index hopping

DeepChek® (ABL, Luxembourg) is a CE marked:
The pipelines consist of 10 major steps which are :
1) Data cleaning, 2) Subtyping, 3) Tropism analysis, 4) Alignment, 5) Post-alignment cleaning, 6) Consensus creation, 7) Variant calling, 8) Expert system filtering, 9) Drug resistance calculation, 10) Reporting

- ☑ DeepChek® HIV version 2.0 / expert system 2.3 /algorithms 13.1
- ☑ ANRS 33 10-2022 / Grade 2021 9-2021 / HIVdb 9.4 12-2022
- ☑ Classification of mutations of interest ANRS 33

Figure 2: End-to end Solution for HIV-1 Genotyping and Drug Resistance for Routine Diagnostic Sequencing.

Results

All samples were accurately genotyped, two subtypes B, two subtypes C and one recombinant from 02_AG were identified. The median coverage per sample was 9.000.000 of reads and the Q30 was 94% on DNBSEQ-G99. 100 % of concordance was found for the detection of drug resistant mutations (>5%) for the protease, reverse transcriptase, and integrase regions. On protease, for one sample the minority mutation V32I at 3 % was found.

Table 1 : QCMD results using DNBSEQ-G99 and DeepChek ® software.

Sample	Viral load	Subtype	Protease mutation	Reverse transcriptase	Integrase mutation
QCMD 1	5.31	B	L10I (92%) L10V (7%) K20R (40%) L33I (17%) M46I (97%) I54V (99%) L63P (97%) A71T (99%) V82A (98%) L90M (96%)	No	No
QCMD 2	4.20	A/G	No	V179I (99%)	No
QCMD 3	4.97	C	K20R (98%) L63P (98%)	M41L (99%) E44D (34%) D67N (99%) T69D (99%) A98G (99%) M184I (99%) Y188L (99%) G190A (99%) L210W (99%) T215Y (97%)	No
QCMD 4	4.40	B	No	M41L (98%) M184V (97%) L210W (99%) T215Y (98%)	No
QCMD 5	4.69	C	G16E (98%) K20R (95%) M46I (99%) I54V (98%) V82A (96%) V32I (3%)	M184V (99%)	No

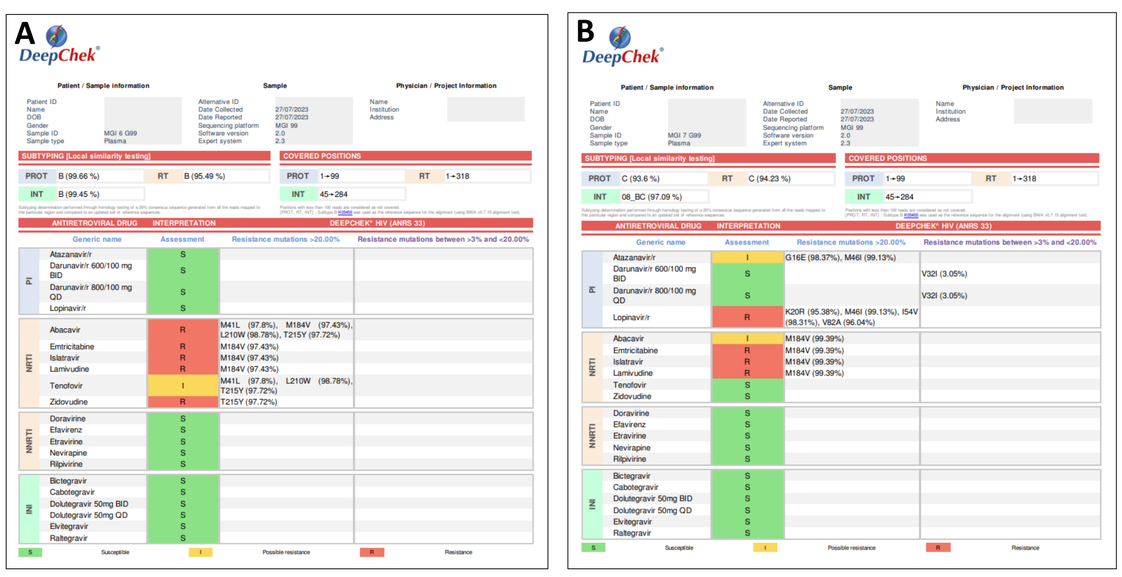


Figure 3 : Example of DeepChek ® reports with covered positions, subtyping, mutation of interest and antiretrovural drug interprétations. **(A)** QCMD4; **(B)** QCMD5

Conclusions

This study is the first evaluation of HIV-1 QCMD samples using the DeepChek® assays on Complete Genomics DNBSEQ Sequencing platform. DNBSEQ Sequencing platforms are suitable for evaluation of HIV-1 QCMD samples. The DNBSEQ-G99 can identify HIV-1 minor variants (3-20%) conferring drug resistance. The NGS should occupy a major place in HIV, HCV and HBV applications testing for subtyping, mutation determination and analysis, and drug resistance surveillance.