ID037

Comparative Performance of iSeq100 and MiSeq i100 for Pooled Sequencing of HIV, HCV, TB, and 16S rRNA

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Background

Next-generation sequencing (NGS) is a powerful tool for analyzing complex pathogens such as HIV, HCV, tuberculosis (TB), and microbial communities like 16S rRNA[1,2]. Pooling samples in a single sequencing run reduces costs and increases throughput[2,3]; however, its impact on sequencing quality and sensitivity is not fully understood. This study compares the Illumina iSeq100 and MiSeqi100 platforms for pooled sequencing of HIV, HCV, TB, and 16S rRNA.

Materials & Methods

A total of 27 pooled samples were included in this study, targeting HIV, HCV, Mycobacterium tuberculosis (TB), and bacterial 16S rRNA. RNA extraction was performed using the EZ1 DSP Virus Kit (Qiagen), while DNA was extracted using the EZ1 DSP DNA Blood Kit (Qiagen). Amplification of the respective targets and library preparation were carried out using the DeepChek® assay kits (ABL). Sequencing was performed on both the MiSeqi100 and iSeq100 platforms. Lyophilized reagents were used for the MiSeqi100, allowing for ambient temperature storage and shipment, thereby enhancing ecological sustainability by reducing reliance on cold-chain logistics.

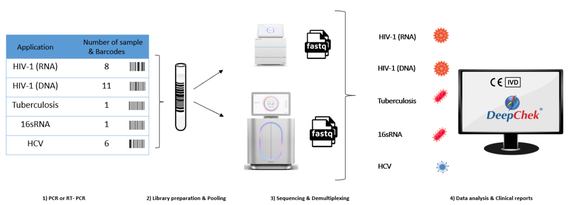

Figure 1. Integrated Workflow for HIV-1 and Tuberculosis Genomic Analysis: From Clinical Samples to NGS-Based Interpretation

Standard reagents were used for the iSeq100. Sequencing data were analyzed using the MicrobioChek® software (ABL) for subtype identification and resistance mutation analysis. Performance parameters including read quality, depth of coverage, sensitivity to low-frequency variants, and overall sequencing throughput were compared between the two platforms. Resistance-associated mutations were identified using the most up-to-date reference databases: ANRS for HIV, geno2pheno for HCV, and WHO guidelines for TB.

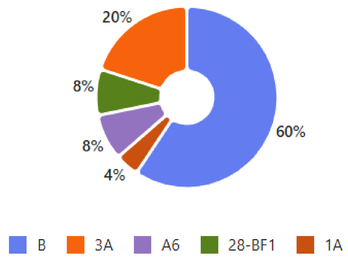

Figure 2. Subtype Distribution in the Study (HIV = B, 28-BF1, A6 ; HCV = 3A, 1A)

Results

Both platforms provided high-quality data across all pooled sample types. MiSeqi100 had faster turnaround and deeper coverage. For HIV, both platforms detected similar mutations in PR/RT/IN/capsid/env regions, with consistent subtyping, except for one blood sample where iSeq100 failed. Both sequenced HIV capsid and envelope, enabling detection of mutations related to new treatments (e.g., enfuvirtide, fostemsavir, lenacapavir). HCV samples had no resistance mutations, all subtyped as 3a. The 16S rRNA sample identified Aggregatibacter, and the TB sample showed no mutations in the 13Plex region. Results were consistent across platforms, supporting reliability.

Table1. Genotyping Results: Viral Load, Amplified Genes, Mutations, and Subtypes

					iSeq100		MiSeq100	
Application	Sample	Viral Load	Sample Type	Amplified Gene	Mutation of Interest	Subtype	Mutation of Interest	Subtype
HIV	2502-270515	104 copies /10^6PBMCs	Blood	PR/RT/IN/CAPSID	RT:M41L(68,29),M184V(56,03%),L210W(58,27%),T215Y(56,31%),E44D(69,02%) INT:T97A(62,49%),Y143R(48,96%)	28-BF1	RT:M41L(62,91),M184V(57,67%),L210W(57,73%),T215Y(51,78%),E44D(66%) INT:T97A(61,01%),Y143R(51,816%)	28-BF1
HIV	2502-260441	125 copies /10^6PBMCs	Blood	PR/RT/IN/CAPSID	IN:L71I(99,26%),E138K(20,94%)	A6	IN:L71I(99,85%),E138K(19,82%)	A6
HIV	2502-250220	932 copies/ml	Plasma	PR/RT/IN/CAPSID/ENV	PR:L10I(99,27%),M46I(6,68%),A71V(96,93%) RT:M14I(6,61%) ENV:M434I(97,97%)	B	PR:L10I(99,92%),M46I(7,31%),A71V(99,88%) RT:M14I(6,49%) ENV:M434I(97,9%)	B
HIV	2502-200945	567343 copies/ml	Plasma	PR/RT/IN/CAPSID/ENV	RT:L33V(84,55%),D60E(99,41%),L63P(97,82%) ENV:S375N(69,45%)	B	RT:L33V(84,83%),D60E(99,9%),L63P(96,21%) ENV:S375N(71,6%)	B
HIV	2502-200411	<20 copies /10^6PBMCs	Blood	PR/RT/IN/CAPSID/ENV	Failed	B	RT:M184I(99,52%),E138K(99,24%),M230I(99,23%) INT:G140S(99,44%) ENV:M434I(51,24%)	B
HIV	2502-130434	1798 copies/ml	Plasma	PR/RT/IN/CAPSID/ENV	RT:M41L(98,75%),M184V(99,21%),L210W(66,2%),T215Y(56,27%),E44D(69,51%) INT:L74I(59,76%)T97A(65,76%),E138K(64,38%)N155H(67,18%)Y143R(98,73%)	28-BF1	RT:M41L(99,97%),M184V(99,97%),L210W(65,61%),T215Y(57,58%),E44D(69,4%) INT:L74I(64,37%)T97A(66,77%),E138K(65,84%)N155H(69,18%)Y143R(99,85%)	28-BF1
HIV	2502-040540	102 copie /10^6PBMCs	Blood	PR/RT/IN/CAPSID	INT:L74I(99,03%)S153F(44,47%)	A6	INT:L74I(99,85%),S153F(46,35%)	A6
HIV	2504-010281	616 copies /10^6PBMCs	Blood	PR/RT/INT	RT:T215Y(97,8)	B	RT:(T215Y(99,8)	B
HIV	2503-260687	88485 copies/ml	Plasma	PR/RT/IN/CAPSID/ENV	PR:D60E(95,4%),L63P(94,3%) RT:K103N(99,8%),M230I(5,18%) ENV:S375T(99,052%,M475I(5,2%)	B	PR:D60E(95,85%),L63P(95,85%) RT:K103N(99,8%),M230I(5,25%) ENV:S375T(99,5%,M475I(5%)	B
HIV	2503-100648	Not done	Blood	PR/RT/IN/CAPSID	PR:G16E(13,4%)D60E(99,86%),L63P(99,88%) RT:M184I(17,34%),M230I(16,45%)	B	PR:G16E(13,6%)D60E(99,86%),L63P(99,88%) RT:M184I(17,36%),M230I(17,89%)	B
HIV	2503-130159	45 copies /10^6PBMCs	Blood	PR/RT/INT	PR:K20R(99,9)L63P(99,9)	B	PR:K20R(99,9)L63P(99,9)	B
HIV	2504-030766	Not done	Blood	PR/RT/INT	PR:A71T(99,9)L63P(99,9)	B	PR:A71T(99,9)L63P(99,9)	B
HIV	2503-260381	225 copies /10^6PBMCs	Blood	PR/RT/IN/CAPSID/ENV	PR:L10I(99,9%)A71V(99,69%) RT:V106I(14,5%) ENV:M434I(50,2%)	B	PR:L10I(99,9%),A71V(99,69%) RT:V106I(15,64%) ENV(51,23%)	B
HIV	2503-110272	Not done	Blood	PR/RT/IN/CAPSID/ENV	PR:L10I(99,9%)L63P(99,56%) RT:T215D(99,47%),V90I(99,82%) ENV:M434I	B	PR:L10I(99,9%)L63P(99,84%) RT:T215D(99,84%),V90I(99,83%) ENV:M434I	B
HIV	2503-120812	493 copies/ml	Plasma	PR/RT/IN/CAPSID/ENV	RT:K103N(97,5%) ENV:M426L(99,5)	B	RT:K103N(98,57%) ENV:M426L(98,8)	B
HIV	2503-110453	155530 copies/ml	Plasma	PR/RT/IN/CAPSID/ENV	PR:L33V(98,7%,A71T(22,5%),L63P(99,4%) RT:D67N(38,2%),T215V(33,5%),K70R(45,12%),K219Q(38,4%),Y181C(31,92%) ENV:S375T(99,25%)	B	PR:L33V(98,89%,A71T(24,55%),L63P(99,59%) RT:D67N(38,31%),T215V(32,81%),K70R(47,15%),K219Q(38,56%),Y181C(31,92%) ENV:S375T(99,25%)	B
HIV	2503-270862	5309 copies/ml	Plasma	PR/RT/IN/CAPSID/ENV	ENV:S375N(38,28%),S375T(31,5%)I(23,5%),S375	B	ENV:S375N(24,09%),S375T(38,28%)IS375N(31,74)	B
HIV	2503-310595	Not done	Blood	PR/RT/IN/CAPSID/ENV	RT:K70R(99,9%)	B	RT:K70R(99,9%)	B
HIV	2503-280534	217579 copies/ml	Plasma	PR/RT/IN/CAPSID/ENV	PR:L10I(99,9%)G16E(5,15%)A71T(99,4%) RT:M184V(92,4%) INT:E157Q(7,1%)	B	PR:L10I(99,71%)G16E(6,5%)A71T(99,5%) RT:M184V(92,7%) INT:E157Q(7,1%)	B
HCV	2502-120650	Not done	plasma	NS5B/NS5A/NS3	No mutation	3a	No mutation	3a
HCV	2502-100597	977347 UI/ml	plasma	NS5B/NS5A/NS3	No mutation	3a	No mutation	3a
HCV	2502-060977	1678 UI/ml	plasma	NS5B/NS5A/NS3	No mutation	3a	No mutation	3a
HCV	2502-280535	2776 UI/ml	plasma	NS5B/NS5A/NS3	NS3:S122G	1a	NS3:S122G	1a
HCV	2503-030606	33393503 UI/ml	plasma	NS5B/NS5A/NS3	No mutation	3a	No mutation	3a
HCV	2504-050300	553809 UI/ml	plasma	NS5B/NS5A/NS3	No mutation	3a	No mutation	3a
16SRNA	5	Not done	Souche	V1-V9	aggretibacter	Not applicable	aggretibacter	Not applicable
TB	6	Not done	MGIT	13Plex	No mutation	Not applicable	No mutation	Not applicable

Figure 3. Example of Whole Genome HIV Clinical Genotyping Report: Summary of Antiretroviral Drug Resistance and Viral Subtyping.

DeepChek® report summarizes subtype, genomic coverage, and key resistance mutations in PR/RT/INT regions. Major mutations include M184V (NRTI) and K103N/Y181C (NNRTI), reducing susceptibility to several reverse transcriptase inhibitors. The report highlights how genotypic analysis guides precise HIV treatment decisions.

Conclusion

Both platforms are suitable for high-throughput sequencing of pooled HIV, HCV, TB, and 16S rRNA samples. MiSeqi100 offers superior sensitivity, depth, and speed, making it well-suited for research and diagnostics. iSeq100 remains a cost-effective tool for routine diagnostics. Pooling multiple microbiological targets in a single NGS run improves efficiency, lowers costs, and enhances data output using a single, continuously updated software platform.

References

1. Vellas, et al. Comparison of short-read and long-read next-generation sequencing technologies for determining HIV-1 drug resistance. J Med Virol. 2024.
2. Martinez, et al. Advancing Drug Resistance Detection: Comparative Analysis Using Short-Read and Long-Read NGS Technologies. LabMed 2025.
3. Mohamed, et al. From Capillary Electrophoresis to Deep Sequencing: An Improved HIV-1 Drug Resistance Assessment Solution Using In Vitro Diagnostic (IVD) Assays and Software. Viruses. 2023.