HIV-1 Drug Resistance Sequencing Products Package

——Powerful,rapid and easy to use,empowering HIV drug resistance monitoring and epidemiological research.

Features

- **Covering the main drug-resistant regions**

  Only one panel covered three main drug resistance regions of HIV-1, protease (PR), reverse transcriptase (RT), and integrase (IN).

- **Short TAT**

  Combined with the DNBSEQ-G99 sequencer, from RNA to report, can be completed within 20.5 hours for 16 samples.

- **Work for low frequency drug resistance**

  Based on deep sequencing, capable of detecting drug resistance frequency less than 20%, which cannot be detected by the Sanger.

- **Automation friendly**

  Combined with the MGISP-100, MGISP-960 and HIV-GenomePro platform, can achieve automated library prep and data analysis, save labor.

- **Various analysis functions**

  Features genotyping, genome assembly, mutation detection, and evolutionary tracing. Based on the Stanford HIVdb, it can precisely analyze and annotate the drug resistance. Additionally, by merging multiple samples sequences, HIV-trace analysis can be conducted to obtain a visual molecular network.

Introduction

Drug resistance due to HIV mutations is the most common cause of antiviral treatment failure in HIV patients. Timely drug resistance testing and monitoring to understand the resistance status of individuals and populations play an important role in the prevention and treatment of AIDS. MGI HIV-1 drug resistance sequencing products package, based on self-developed library prep reagents, automated sample preparation systems, sequencer, and software, covering from library prep to reporting, enabling rapid and accurate detection of HIV-1 .

Table 1. Parameters

Intended use	HIV-1 genotyping and drug resistance detection
Target region	PR, RT, IN genes
Sample type	Viral RNA from plasma, serum and cultured strains
Sequencer	DNBSEQ-G99/E25/G50
Recommended read length	PE150
Recommended data volume	1 M PE150 Reads
Recommended sample input	⩾100 copies per rxn
Recommended throughput	16 samples/FC for DNBSEQ-E25
	60 samples/FC for DNBSEQ-G99
	60 samples/FC for DNBSEQ-G50 FCS
	300 samples/FC for DNBSEQ-G50 FCL
TAT for library prep	<8.5h for 16 samples
TAT for analysis	<2h for 16 samples by DNBSEQ-G99ARS
	≈1h for 16 samples by HIV-GenomePro platform

Figure 1. Workflow

Performance

To test the consistency with the gold standard Sanger platform, RNA extracted from plasma and cultured strains were used for library prep. Sequencing and analysis were performed on the DNBSEQ-G99ARS. As the result shown in Table 2, MGI has a sequence consistency with Sanger of >98%, and the genotyping results are totally consistent.

Table 2. Result comparison with Sanger

Sample ID	Sequence consistency	Genotyping result by Sanger	Genotyping result by MGI
S1	99.06%	B	B
S2	98.36%	CRF01_AE	CRF01_AE
S3	99.62%	CRF07_BC	CRF07_BC
S4	98.94%	CRF08_BC	CRF08_BC
S5	100%	CRF01_AE	CRF01_AE

To test the performance of detecting low-frequency drug resistance, RNA extracted from a plasma sample was used for library preparation. Sequencing and analysis were conducted on the DNBSEQ-G99ARS, and compared with Sanger's results. the results shown in Table 3, the MGI's products solution capable of detecting mutations frequency as low as 2.75% (marked in red) which cannot be detected by Sanger. the mutation frequency indicated in the parentheses.

Table 3. Drug resistance detection result

Drug type	NRTI	NNRTI
MGI	K219N(97.27%), M41L(9.65%)	K103N(28.31%), Y181C(99.88%), H221Y(2.75%)
Sanger	K219N	K103N, Y181C

To test the LoD, RNA extracted from a cultured strain sample (reference drug resistance mutations are Y115F, M184V, K219E, K103N, E138G, V179E) was serially diluted. Sequenced and analyzed on the DNBSEQ-G99ARS. As the results shown in Table 4, all reference drug resistance- can be accurately detected when the viral copy number in the reaction is 7.67E+02. While the viral copy number as low as 7.67E+01, the K103N drug resistance cannot be detected, the other five reference drug resistance and the mutation frequencies still accurately detected. Therefore, the LoD of this products solution is approximately 10^2 cps/rxn*. For detection of low-frequency drug resistance mutations, viral input of $\geqslant 10^3$ cps/rxn is recommended. Additionally, the mutation frequencies of each resistance are very close among different concentration, indicated excellent repeatability.

*The conversion formula from cps/rxn to plasma viral load cps/mL is as follows:
The HIV copies per reaction (cps/rxn) = [Plasma viral load (cps/mL) * Volume of plasma used for extraction (mL) * Volume of viral for library prep (μL)] / Eluted volume of viral RNA extraction (μL). For example, if a plasma sample's HIV viral load is measured by QPCR to be 10.000 cps/mL. the volume of plasma used for extraction is 0.2 mL. the eluted volume of viral RNA extraction is 30 μL. and the volume of viral RNA for library prep is 20 μL. so the HIV copies per reaction is calculated as: ((10.000 cps/mL* 0.2 mL * 20 μL) / 30 μL) ≈ 1333 cps/rxn

Table 4. Drug resistance result for copies gradient

cps / rxn	100× Coverage(%)	NRTI	NNRTI
7.67E+06	93.8	Y115F(99.67),M184V(99.67), K219E(99.72)	K103N(99.82),E138G(99.87), V179E(99.72)
7.67E+05	93.8	Y115F(99.74),M184V(99.81), K219E(99.69)	K103N(99.79),E138G(99.9), V179E(99.74)
7.67E+04	90.33	Y115F(99.63),M184V(99.69), K219E(99.79)	K103N(99.77),E138G(99.85), V179E(99.77)
7.67E+03	90.83	Y115F(99.72),M184V(99.86), K219E(99.67)	K103N(99.86),E138G(99.81), V179E(99.71)
7.67E+02	86.32	Y115F(99.59),M184V(99.79), K219E(99.75)	K103N(99.74),E138G(99.62), V179E(99.44)
7.67E+01	83.94	Y115F(99.62),M184V(98.94), K219E(100.0)	E138G(99.67),V179D(99.66)

To study the consistency of results among different MGI sequencers, RNA extracted from four cultured strain samples was used for library preparation. Sequencing was performed on the DNBSEQ-G99ARS, DNBSEQ-E25RS, and DNBSEQ-G50 platforms respectively. The consistency of the output data evaluated by indicators such as the effective sequences proportion, HIV sequences proportion, 1x coverage, 100x coverage, and the sequence rate without N. As the result shown in Figure 2, the output data from the three platforms for the same sample have high consistency. Furthermore, as shown in Table 5, the drug resistance detection results also completely consistent among different platforms for the same sample.

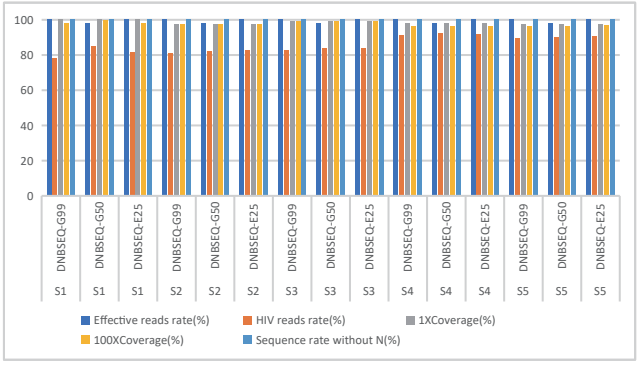

Figure 2. Bar plot for QC data for different MGI sequencers

Table 5. Drug resistance result for different MGI sequencers

Sample ID	Sequencer	NRTI	NNRTI
S1	DNBSEQ-G99	M41L(10.85),K219N(96.99)	K103N(27.93),Y181C(99.81), H221Y(2.78)
S1	DNBSEQ-G50	M41L(10.98),K219N(97.19)	K103N(27.75),Y181C(99.76), H221Y(2.38)
S1	DNBSEQ-E25	M41L(10.08),K219N(96.66)	K103N(27.14),Y181C(99.56), H221Y(3.16)
S2	DNBSEQ-G99	--	V106I(3.0)
S2	DNBSEQ-G50	--	V106I(2.7)
S2	DNBSEQ-E25	--	V106I(2.56)
S3	DNBSEQ-G99	--	--
S3	DNBSEQ-G50	--	--
S3	DNBSEQ-E25	--	--
S4	DNBSEQ-G99	--	--
S4	DNBSEQ-G50	--	--
S4	DNBSEQ-E25	--	--
S5	DNBSEQ-G99	Y115F(99.86),M184V(99.63), K219E(99.62)	K103N(99.96),E138G(99.97), V179E(99.79)
S5	DNBSEQ-G50	Y115F(99.8),M184V(99.64),K 219E(99.72)	K103N(99.83),E138G(99.42), V179E(99.54)
S5	DNBSEQ-E25	Y115F(99.77),M184V(99.68), K219E(99.53)	K103N(99.86),E138G(99.55), V179E(99.75)

Take sample S1 as an example to present analysis report, the Table 6 showed the basic analysis results which indicate that the subtype of the sample is HIV-1 subtype B, with Q30 of 95.1%, effective sequence ratio of 99.99%, 1x coverage of 100%, 100x coverage of 98.18%, and 100% sequence without N. showed excellent sequencing quality, meeting the requirements for downstream analysis. Additionally, the system generates genome coverage maps for the PR, RT, and IN gene as the Figure 3 shown. The genome coverage maps show that the sequencing reads evenly distributed and cover the target regions well.

Table 6. Testing and data QC result

Sample ID	Sex	Age	Type	Subtype	Mutation	Surveillance
S1	-	-	HIV-1	B	5	4

Raw reads	Q30 rate(%)	GC content(%)	Clean rate(%)	Mapping rate(%)
2000000	95.1	44.23	99.99	100.0

Clean reads	HIV reads	HIV reads pct(%)	1X cov(%)	100X cov(%)	Assembly	Absolute consentration(cp/ml)	Relative consentration(cp/ml)
1999860	1557994	77.91	100.0	98.18	yes	-	-

Region	Ref_Length	Assembly Length	Non-N Length	Non-N pct(%)	Download
2253-3680;4043-5242	2628	2628	2628	100.0	HIVjt_1_2_MGI_G99.genome.fa

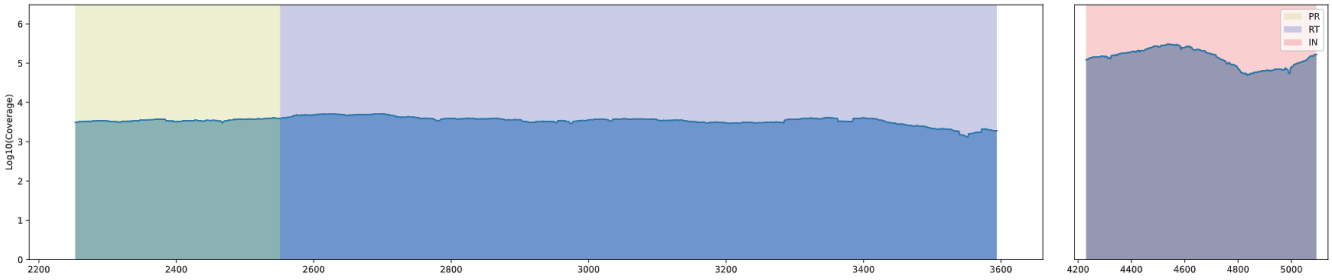

Figure 3. genome coverage graph

Subsequently, by mapping to the reference sequence, the system analyzes the mutations, as shown in Table 7. The system also determined whether the mutations belong to monitored sites and showed the information such as wild-type base, mutant base, mutation frequency, and sequencing depth. The system generated the gene mutation graph as the Figure 4 shown, which allows for visual of the positions and frequency of amino acid mutations on the reference genome.

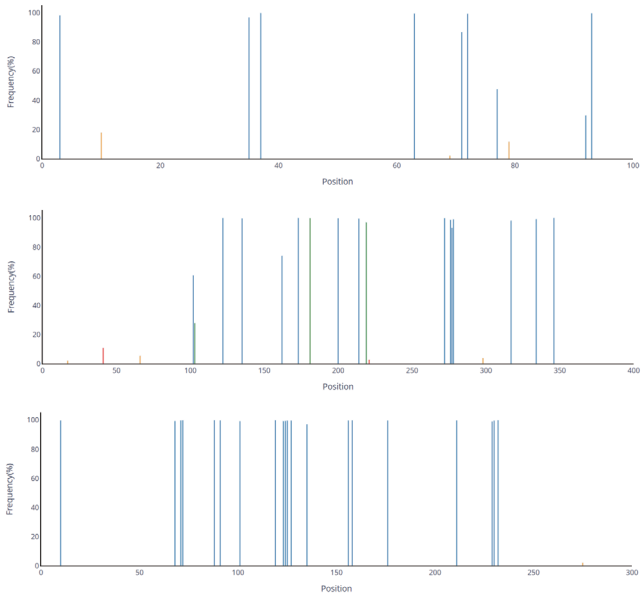

Figure 4. Mutation graph for RT, PR, and IN genes

Table 7. Mutations result

Gene	Classification	Monitored site	Wildtype	Position	Mutation	Wild-type base	Genome location	Vutant base	Frequency (%)	Sequencing depth
PR	Other	no	V	3	I	G	2259	A	98.09	262
PR	Other	no	L	10	I	C	2280	A	17.99	2974
PR	Other	no	E	35	D	A	2357	C	96.65	3586
PR	Other	no	S	37	N	G	2362	A	99.72	3623
PR	Other	no	L	63	P	T	2440	C	99.36	3455
PR	Other	no	H	69	Y	C	2457	T	2.17	3370
PR	Other	no	A	71	T	G	2463	A	86.72	2907
PR	Other	no	A	71	V	C	2464	T	12.76	2907

Furthermore, based on the Stanford HIVdb, the system predicted, annotated, and scored the drug resistance, as shown in Table 8. The drug resistance for S1 sample located in the RT region only, included the drug classes NRTI and NNRTI. There is high level of resistance to efavirenz, nevirapine, and lopinavir.

Table 8. Drug resistance analysis result

Drug Type	Drug Name	Drug Abbreviation	Gene	Mutation	Resistance Score	Resistance Degree
NRTI	Epivir	3TC	RT	M41L(10.85),K219N(96.99)	0	Susceptible
	Emtricitabine	FTC	RT	M41L(10.85),K219N(96.99)	0	Susceptible
	Abacavir	ABC	RT	M41L(10.85),K219N(96.99)	10	Potential low-level resistance
	Di-deoxyinosine	DDI	RT	M41L(10.85),K219N(96.99)	15	Low-level resistance
	Tenofovir	TDF	RT	M41L(10.85),K219N(96.99)	10	Potential low-level resistance
	Stavudine	D4T	RT	M41L(10.85),K219N(96.99)	25	Low-level resistance
	Azidothymidine	AZT	RT	M41L(10.85),K219N(96.99)	25	Low-level resistance
NNRTI	Doravirine	DOR	RT	K103N(27.93),Y181C(99.81),H221Y(2.78)	25	Low-level resistance
	Efavirenz	EFV	RT	K103N(27.93),Y181C(99.81),H221Y(2.78)	100	High-level resistance
	Etravirine	ETR	RT	K103N(27.93),Y181C(99.81),H221Y(2.78)	40	Intermediate resistance
	Nevirapine	NVP	RT	K103N(27.93),Y181C(99.81),H221Y(2.78)	135	High-level resistance
	Rilpivirine	RPV	RT	K103N(27.93),Y181C(99.81),H221Y(2.78)	60	High-level resistance

Finally, the system aligned the assembled genome with published representative HIV subtype reference genomes. to generate evolutionary tree, as shown in Figure 5. The results indicate that the S1 is highly homologous to the B-KP178435 strain reported in China in 2010, pointed by red arrow.

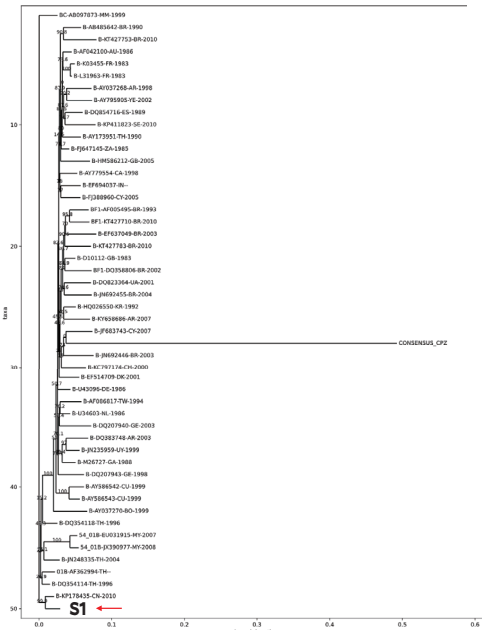

Figure 5. Evolutionary tree

Conclusion

In summary, the MGI HIV-1 Drug Resistance Sequencing Products package is characterized by excellent performance, various functions, time and labor-saving features, making it an ideal tool for HIV drug resistance monitoring and epidemiological research.

Ordering information

Type	Name	Specification	PN
Instrument	DNBSEQ-G99ARS sequencer	Include server	900-000609-00
	DNBSEQ-E25RS sequencer	Standard	900-000537-00
	DNBSEQ-G50 sequencer	Config 2	900-000354-00
	MGISP-100RS Automated Sample Prep System	Standard	900-000206-00
	MGISP-960RS Automated Sample Prep System	Config 2	900-000147-00
	MGISP-960RS Automated Sample Prep System	Config 7	900-000152-00
Reagents	ATOPlex HIV-1 Amplification Kit	16 RXN	940-000722-00
	MGIEasy Fast PCR-FREE FS DNA Library Prep Set	16 RXN	940-000019-00
	MGIEasy Fast PCR-FREE FS DNA Library Prep Set	96 RXN	940-000021-00
	MGIEasy DNA Clean Beads	8 mL/tube	1000005278
	DNBSEQ OneStep DNB Make Reagent Kit	4 RXN	1000026466
	CPAS barcode primer 3	3.5 mL	1000020834
	High-throughput Sequencing Set	G99 SM FCL PE150	940-000410-00
	High-throughput Sequencing Set	G99 FCL PE150	940-001269-00
	DNBSEQ-G50RS High-throughput Rapid Sequencing Set	FCS PE150	1000019862
	DNBSEQ-G50RS High-throughput Sequencing Set	FCL PE150	1000019858
	DNBSEQ-E25RS High-throughput Sequencing Set	FCL PE150	940-000567-00
Analysis	HIV-GenomePro Software	-	970-000281-00
	HIV-GenomePro Platform	-	900-000818-00

MGI Tech Co., Ltd.
Building 11, Beishan Industrial Zone, Yantian District, Shenzhen, CHINA
MGI-service@mgi-tech.com en.mgi-tech.com +86-4000-688-114