USE OF NEXT-GENERATION SEQUENCING TECHNOLOGIES IN THE CLINICAL HIV LABORATORY

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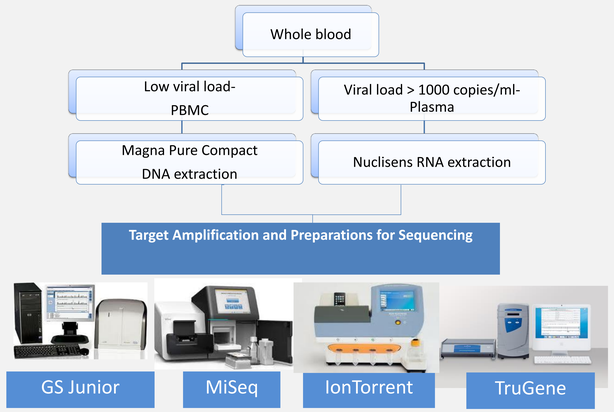
BACKGROUND

Sanger-based HIV sequencing is currently the method of choice for the identification and follow-up of HIV antiretroviral drug resistance. It has limited sensitivity and detects the more dominant viral species.

We have analyzed the results obtained by Roche GS Junior (GSJ) and Illumina MiSeq and compared the mutation load and the prediction of HIV resistance to Trugene (TG). In three cases IonTorrent resistance esults were also compared. Plasma samples from nine HIV carriers, representing the major HIV subtypes in Israel, were utilized. The protease and reverse transcriptase sequence and mutation load detected was compared between the systems. DeepChek®-HIV software was used for data analysis and resistance interpretation of all sequencing results.

A1. Experimental Design



A2. Library Preparation, Sequencing & Analysis

	GSJ	MiSeq	Ion Torrent	Trugene
Target amplification	Roche Kit for RT_PR .	Home made RT-_PR		Trugene HIV kit for RT_PR
Library preparation		Nextera	Ion Xpress Plus Fragment Library Kit	NONE
Sequencing	Reads/sample: ~10,000 Tot # samples:10	Reads/sample: 500,000-million Tot # samples:10	Reads/sample: ~150,000. Tot # samples: 4	Open Gene system for each sample
Output	Reads alignments (FASTA, AVA)	Reads alignments (BAM)	Reads alignments (BAM)	Open Gene Software,17.01 guidelines
	Mutation Threshold 3%			
Post NGS analysis	DeepChek®-HIV			

A3. Demographics, clinical information and NGS coverage

Patient Number	Sex	Current Age (years)	Risk Group	Treatment	HIV Sub-type	VL (copies/ml)	CD4, (counts)	GSJ		MiSeq	
								Total # Reads	Coverage per PR_RT (average)	Total # Reads	Coverage per PR_RT (average)
2333	M	28	MSM	-	B	120,000	483	8,885	1,646	156,000	21,750
2334	F	NA	OGE-F	-	C	830,000	NA	8,300	1,548	114,000	11,414
1188	M	30	MSM	-	B	24,000	437	7,133	1,282	142,000	20,310
1939	F	39	OGE-IL	-	C	6,500	436	4,201	922	102,000	21,389
2352	M	37	MSM	-	FB	500,000	429	10,137	1,875	115,000	19,883
2498	M	52	MSM	-	B	53,000	NA	4,775	867	88,000	22,705
2261	M	23	MSM	Truvada, Atazanavir	B	620	596	5,715	1,130	107,000	25,945
2054	M	31	MSM	Truvada, Atazanavir	B	510	494	5,706	1,015	162,000	18,876
2275	F	40	NA	Truvada, Atazanavir	A	980	NA	7,088	1,362	90,000	17,959

A4. Number of amino acid mutations detected by GSJ, MiSeq (MIS), at above a threshold of 3% , and by Trugene (TG) (n=9)

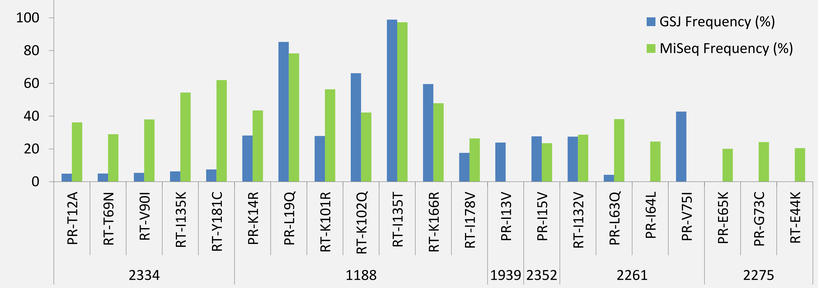
	PR			RT			PR and RT		
	GSJ	MIS	TG	GSJ	MIS	TG	GSJ	MIS	TG
Total number of mutations [a]	103	105	79	129	138	102	232	243	181
Mutations identified by MIS & GSJ & TG	77	77	77	93	93	93	170	170	170
Mutations identified by MIS & GSJ only	13	13	0	17	17	0	30	30	0
Mutations identified by TG& GSJ only	0	0	0	1	0	1	1	0	1
Mutations identified by TG & MIS only	0	1	1	0	6	6	0	7	7
Mutations identified by GSJ only	13	0	0	19	0	0	32	0	0
Mutations identified by MIS only	0	14	0	0	23	0	0	37	0
Mutations identified by TG only	0	0	1	0	0	2	0	0	3

The total number of mutations >3% frequency was significantly different between MiSeq and Trugene ($p<0.005$, 95% CI: 2.91-10.87) and between GSJ and Trugene ($p<0.009$, 95% CI: 1.92- 9.41).

A5. Mutations detected by Trugene but undetected or detected at a frequency below 20% by GSJ and/or MiSeq

Patient Number	Amino Acid	GSJ Frequency (%)	MiSeq Frequency (%)
2333	PR-L63S	NI	75.0
	RT-T215LF [a]	NI	NI
2334	PR-L63T [a]	NI	NI
	RT-I135K	6.3	54.4
	RT-E138A	33.8	14.9
	RT-K238R	23.2	5.9
1939	RT-E40D	NI	38.3
	RT-I50V	18.8	8.3
	RT-K101R	NI	51.5
	RT-D123S	NI	98.9
	RT-I135V	NI	98.0
	RT-I142V	NI	83.1
	RT-E169G	8.4	99.0
	RT-Q174R	90.8	NI
2352	RT-R211K	NI	NI
2261	RT-K173S	61.5	17.1
2054	RT-I94L	NI	59.7
2275	RT-K104R	15.8	28.4

A6. Mutations detected by GSJ and/or Miseq at frequencies above 20% and not detected by Trugene



A7. DRMs identified by MiSeq, GSJ, PGM and TruGene

	MiSeq			GSJ			PGM			TruGene		
	PI	NRTI	NNRTI	PI	NRTI	NNRTI	PI	NRTI	NNRTI	PI	NRTI	NNRTI
2333	L90M (97%)	T215L (98.7%)	A98G(97.9%), K103N(96.5%) E138A(98.3%)	L90M (99%)	D67G(9.7%), T215L(98.2%)	A98G(99.6%), K103N(99.5%), E138A(99.4%)	L90M (98.2%)	T215L (97.5%)	A98G(96.5%), K103N(96.8%), E138A(98.9%),	L90M	T215LF	A98G, K103N, E138A
2334	NONE	K65R(4.1%), T69AN(29.0%)	V90I(38.0%), E138A(14.9%), Y181C(62.0%)	NONE	T69AN(5.0%)	V90I(5.4%), E138A(33.8%), Y181C(7.5%)	ND	ND	ND	NONE	NONE	E138A
1188	NONE	NONE	NONE	NONE	NONE	NONE	ND	ND	ND	NONE	NONE	NONE
1939	NONE	NONE	NONE	NONE	NONE	NONE	ND	ND	ND	NONE	NONE	NONE
2352	NONE	NONE	NONE	NONE	NONE	NONE	ND	ND	ND	NONE	NONE	NONE
2498	NONE	NONE	NONE	NONE	NONE	NONE	ND	ND	ND	NONE	NONE	NONE
2261	NONE	D67N(6.5%)	NONE	NONE	NONE	NONE	NONE	NONE	NONE	NONE	NONE	NONE
2054	NONE	NONE	NONE	NONE	NONE	NONE	ND	ND	ND	NONE	NONE	NONE
2275	G73C (24.4%)	T215I (17.6%)	NONE	NONE	NONE	L100F (9.4%)	G73C (26.4%)	T215I(16.6%) K219E(3.3%)	NONE	NONE	NONE	NONE

CONCLUSIONS

- **More drug resistance mutations were identified by the NGS platforms, primarily, but not only, of low abundance**
- **Though platform-specific mutations were identified in all outputs and require corroboration and further assessment of their clinical significance, our study shows that the tested NGS systems are more sensitive and can replace population sequencing for HIV resistance analysis**