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Next-Generation Sequencing Technology in the Clinical HIV Laboratory: A More Sensitive Alternative to Sanger Sequencing

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BACKGROUND

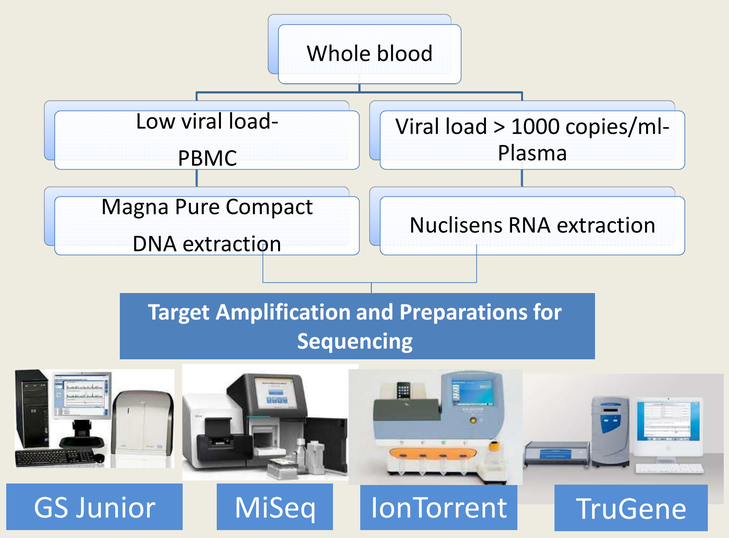
Sanger based HIV sequencing is currently the method of choice for the identification and follow-up of HIV drug resistance. To assess the utility of bench-top next generation sequencing (NGS) platforms for the clinical HIV diagnostic laboratory, we have analyzed results obtained by Roche GS Junior (GSJ), Illumina MiSeq and ABI's IonTorrent (ION), and compared the mutations load and the prediction of HIV resistance derived from GS Junior and Trugene (TG) in all samples and between the four platforms in representative samples.

MATERIALS & METHODS

A1. Patients' Characteristics

Patient Number	TG Sample	NGS Sample	Sub-type	Risk Group	VL (cop./ml)	CD4 (counts)	T/N	ART
2333	64061 (DNA & RNA)		B	MSM	120,000	483	N	-
2334	64064 (DNA & RNA)		C	OGE-F	8,300,000	NA	N	-
2352	64006	64301	FB	MSM	500,000	429	N	-
2261	63308	64340	B	MSM	620	596	N	-
2275	63716		A	UK	980	NA	T	FTC,TDF, RTV,ATZ
2355	64288		AE	MSM	4,800,000	NA	N	-
1101	62026		C	OGE-IL	74,700	NA	T	FTC,TDF RTV,DRV
187	61486		C	OGE-IL	47,900	170	T	FTC,TDF, RTV,RAL

A2. Experimental Design



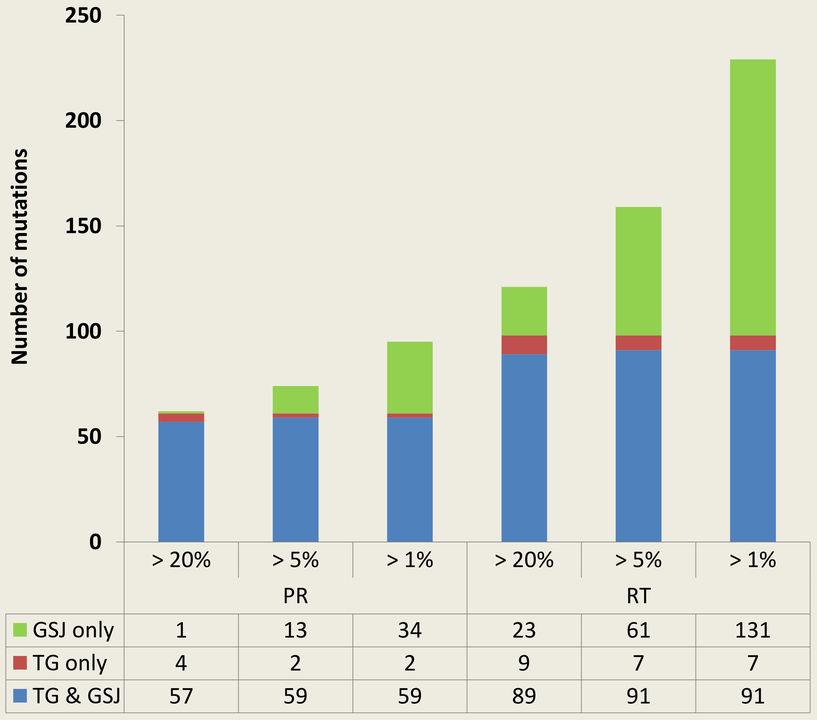
A3. Library Preparation, Sequencing & Analysis

	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) ; mutation scores (>0%) & resistance	Reads alignments (BAM)	Reads alignments (BAM); mutation scores (>%5)	Open Gene Software,17.01 guidelines
Post NGS analysis	DeepChek®-HIV on original AVA files	Realignment GATK, Trimming, mapping BWA	None	Stanford HIV resistance DB

RESULTS

A. COMPARISON BETWEEN GSJ & TRUGENE

A 1. Total number and percentage of amino acid mutations identified in 6 samples assessed by GSJ & TG



	PR			RT		
	> 20%	> 5%	> 1%	> 20%	> 5%	> 1%
GSJ only	1	13	34	23	61	131
TG only	4	2	2	9	7	7
TG & GSJ	57	59	59	89	91	91

Mutations (>1% in GSJ)	RT	PR
% TG only	7.1%	3.3%
% GSJ only	57.2%	35.8%

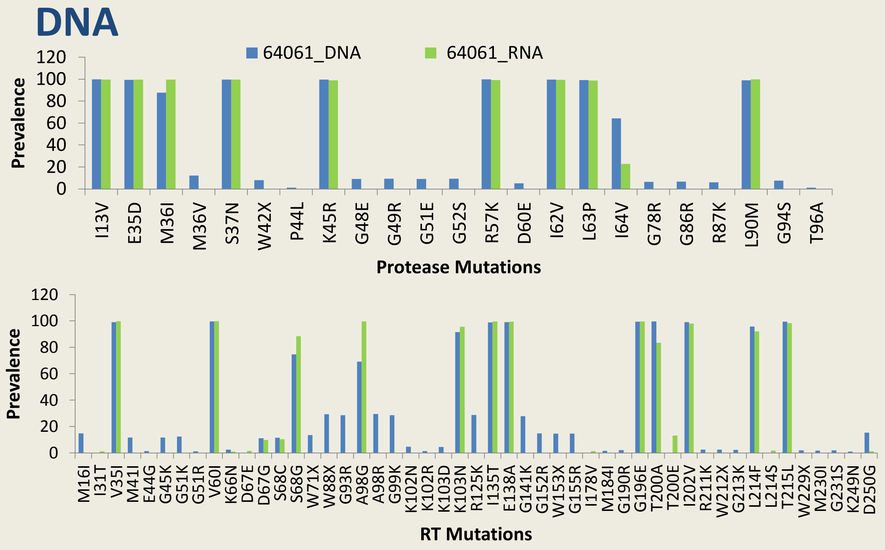
✓ 94% of all substitutions detected by TG were also detected by GSJ

✓ Only 48% of the amino acids substitutions detected by GSJ were also found by TG

A2. Resistance Mutations identified by GSJ & TG (results of 8 samples)

	RT DRMs			PR DRMs		
Sample	Common	TG Only	GSJ only	Common	TG Only	GSJ only
64061	T215L(98%), A98G(99%), K103N(95%), E138A(99%)	T215F(0.05%)	D67E(1.98%), D67G(9.75%)	L90M(99%)	None	None
64064	None	None	T69N(5%), K65N(6.6%), Y181C(7.5%)	None	None	None
64301	None	None	None	None	None	None
64340	None	None	None	A71T(99%)	None	None
63716	None	None	None	None	None	None
64288	None	None	L100F(35%), K103E(3%)	None	None	None
62026	None	None	K65N(1.03%)	None	None	L10MI(3.2%,11%)
61486	None	None	K65N(9.7%), D67N(3%), K101R(15%), V118I(20%), M184V(9%)	None	None	None

B . Mutations identified at >1% frequency in plasma viral RNA versus PBMCs proviral DNA



C. Spearman correlation between frequency of 688 sequence variants identified in MiSeq and/or GSJ

	Illumina_ N701	IonTorrent N701	Illumina_ N702	Illumina_ N703	Illumina_ N704	Illumina_ N705	Illumina_ N707	Illumina_ N708	Illumina_ N709
454_1 % if both strands >0	0.62	0.53	0.11	0.20	0.04	0.15	0.18	0.21	0.14
454_2 % if both strands >0	0.12	0.11	0.77	0.16	0.34	0.17	0.07	0.11	0.25
454_3 % if both strands >0	0.18	0.16	0.18	0.72	0.11	0.18	0.06	0.13	0.14
454_4 % if both strands >0	0.09	0.05	0.43	0.13	0.61	0.19	0.03	0.09	0.30
454_5 % if both strands >0	0.17	0.12	0.15	0.16	0.09	0.64	0.14	0.11	0.12
454_7 % if both strands >0	0.16	0.12	0.13	0.14	0.10	0.16	0.61	0.47	0.06
454_8 % if both strands >0	0.20	0.18	0.11	0.15	0.10	0.09	0.45	0.66	0.06
454_9 % if both strands >0	0.09	0.08	0.25	0.16	0.17	0.13	0.00	0.03	0.69

Average correlation 0.67, considered as rather good correlation

D. Comparison between specific & total number of mutations identified by 4 platforms

Samples	ION				MiSeq		GSJ		TG	
	>1%		>3%		>1%		>1%			
	PR	RT	PR	RT	PR	RT	PR	RT	PR	RT
61486	22	57	17	29	ND	ND	14	51	16	19
63716	19	49	13	24	13	28	12	32	8	15
64061	14	24	13	11	17	27	11	22	11	13
64340	30	36	16	16	14	13	11	17	8	10

Conclusions:

- ✓ Deep sequencing may be more sensitive for DRM detection, though the clinical significance of our observations requires long term follow-up.
- ✓ PBMCs derived HIV DNA can be utilized as an alternative to plasma RNA samples, for more sensitive prediction of HIV mutations.
- ✓ Correlation between the sequences obtained by the 3 NGS platforms is rather strong, most TG mutations were detected by NGS at frequency higher than 20%.
- ✓ DeepChek®-HIV (CE-IVD marked) is compatible for routine clinical genotyping of 454, GSJ, data.
- ✓ A complete bioinformatics solution such as the DeepChek®-HIV to analyze IonTorrent and MiSeq data is, to enable efficient use in the clinical HIV laboratory.