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QUALITY CONTROL for MOLECULAR DIAGNOSTICS

Final Report

QCMD 2009 ENVA9 HIV Drug Resistance Typing EQA Programme

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on behalf of QCMD and its Scientific Advisory Board
January 2010

The QCMD programme is organised in collaboration with the European Society for Clinical Virology and the European Society for Clinical Microbiology & Infectious Diseases.

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1. Programme Aims

The primary aims of this external quality assessment programme were to assess the performance of laboratories in the detection of drug resistance mutations in the HIV-1 Protease and Reverse transcriptase genes.

2. Programme details

Table 1: Programme Details

QCMD ENVA9	
Date programme distributed	21/09/2009
Number of participants	119
Number of countries	42
Number of respondents	105 (88%)
Number of non-respondents	14 (12%)
Number of datasets submitted	108

All participants who had not submitted results prior to the closing date for submission were contacted by e-mail. Five participants withdrew from the programme citing "Technical issues" (n=2), "Test not performed" (n=1), "Unable to amplify from material provided" (n=1) & "Purchased for validation purposes" (n=1) while eight did not respond or submit results and one participant did not submit sequence data. In the previous HIV Drug Resistance Typing EQA Programme (ENVA8) 8 % of participants (9 laboratories) were non-respondents.

3. Panel composition

The ENVA9 panel consisted of five well-characterised samples derived from cultured clinical isolates or recombinant viruses. The sample materials were heat inactivated (30 minutes at 56 degrees centigrade) and dispensed into 1ml aliquots. Viral loads of the materials were determined before and after lyophilisation (Table 2).

Table 2: Panel composition & viral load determination

Sample	Matrix[1]	Subtype	Release testing[2] Lyophilised samples Viral load (copies/ml)
ENVA09-02	Citrate plasma	G	1.13×10^4
ENVA09-03	Citrate plasma	B	1.06×10^4
ENVA09-05	Citrate plasma	C	2.79×10^4
ENVA09-06	Citrate plasma	C	1.21×10^4
ENVA09-07	Citrate plasma	A	1.13×10^4

1. All samples were diluted in HIV, HBV and HCV negative citrate plasma and lyophilised prior to distribution.
2. Release testing was performed using the Roche COBAS TaqMan HIV-1 Test.

4. Programme results

Analysis of the Sequence Data

Table 3(a): Summary of submitted results by individual dataset

		ENVA9-02		ENVA9-03		ENVA9-05		ENVA9-06		ENVA9-07	
Lab. Code[2]	Method	PR2[3]	RT2[3]	PR3[3]	RT3[3]	PR5[3]	RT5[3]	PR6[3]	RT6[3]	PR7[3]	RT7[3]
AU008.SP	IH										
BE007.CD	IH										
CA008.CD	IH										
CH020.SP	IH										
CY002.CD	IH										
DE006.CD	IH										
DE012.CD	IH										
DE017.SP	IH										
DE026.CD	IH										
DE084.CD	IH										
DE093.CD	IH										
DE112.CD	IH										
ES036.CD	IH										
FR002.SP	IH										
FR003.CD	IH										
FR039.SP	IH										
FR084.SP	IH										
FR102.SP	IH										
GB003.CD	IH										
GB006.CD	IH										
GB007.CD	IH										
GB010.CD	IH										
GB011.1.CD	IH										
GB016.CD	IH										
GB019.CD	IH										
GB064.CD	IH										
GB098.CD	IH										
HK001.CD	IH										
HK004.CD	IH										
HK020.CD	IH										
HU008.SP	IH										
IT043.CD	IH										
NO010.CD	IH										
NZ001.CD	IH										
SE001.CD	IH										
SE002.SP	IH										
SK005.SP	IH										
UG002.CD	IH										
US048.CD	IH										
YU002.CD	IH										
ZA018.1.CD	IH										
ZA018.2.CD	IH										
AT002.CD	TG										
AU012.1.SP	TG										
BE001.SP	TG										
BE002.CD	TG										
BE005.CD	TG										
BE010.CD	TG										
BE020.CD	TG										
BE026.CD	TG										
BG002.SP	TG										
DE003.CD	TG										
DE027.SP	TG										
DE081.CD	TG										
ES014.CD	TG										
ES018.CD	TG										
ES028.CD	TG										
ES031.SP	TG										
ES044.CD	TG										
FR053.CD	TG										
GR013.CD	TG										
HR004.SP	TG										
IE002.CD	TG										
IL003.SP	TG										
IT066.SP	TG										
IT092.CD	TG										
LV005.CD	TG										
PL010.CD	TG										
TH001.SP	TG										
US051.CD	TG										
ZA006.CD	TG										
ZA024.CD	TG										
AT012.CD	VS										
AU012.2.CD	VS										
BE049.CD	VS										
CZ001.CD	VS										
DE004.CD	VS										
DE007.CD	VS										
DE014.CD	VS										
DE020.CD	VS										
FI001.CD	VS										
GB001.CD	VS										
GB009.CD	VS										
GB011.2.CD	VS										
GB020.CD	VS										
IT011.CD	VS										
IT012.CD	VS										
IT023.CD	VS										
IT037.CD	VS										
IT051.CD	VS										
LT001.CD	VS										
LU001.CD	VS										
NL003.CD	VS										
NL008.CD	VS										
NL009.CD	VS										
NL022.CD	VS										
NL032.CD	VS										
NL037.CD	VS										
NO006.CD	VS										
PL047.CD	VS										
PT008.CD	VS										
PT020.CD	VS										
SE003.CD	VS										
SI006.CD	VS										
US042.CD	VS										
ZA003.CD	VS										
ZA009.CD	VS										
ZA030.CD	VS										

Please see legend on page 4.

Table 3(a): This table summarises the datasets submitted by participants, depending on whether combined or separate analysis of the Protease and Reverse transcriptase genes was performed. Details of the alignments are provided in Figures 1 to 5.

1. Shaded cell = sequence submitted and non-shaded cell = sequence NOT submitted.
2. CD = combined amplification for Protease and Reverse transcriptase and SP = separate amplification for Protease and Reverse transcriptase.
3. PR = Protease and RT = Reverse transcriptase.

These data are presented by panel sample and method of analysis. IH = In-house techniques, TG = Siemens TRUGENE HIV-1 Genotyping Test and VS = Celera Diagnostics ViroSeq HIV-1 Genotyping System.

Table 3(b): Summary of the number of reported datasets per sample and technology

Sample		Number of full datasets (PR and RT) [1, 2]		
	Cumulative (%)	In-house (%)	TruGene (%)	ViroSeq (%)
ENVA09-02	103/108 (95.37 %)	39/42 (92.85 %)	30/30 (100 %)	34/36 (94.44 %)
ENVA09-03	104/108 (96.29 %)	40/42 (95.24 %)	30/30 (100 %)	34/36 (94.44 %)
ENVA09-05	105/108 (97.22 %)	39/42 (92.85 %)	30/30 (100 %)	36/36 (100 %)
ENVA09-06	95/108 (87.96 %)	37/42 (88.09 %)	29/30 (96.77 %)	29/36 (80.56 %)
ENVA09-07	91/108 (84.26 %)	39/42 (92.85 %)	16/30 (51.61 %)	36/36 (100 %)
ALL SAMPLES	78/108 (72.22 %)	35/42 (83.33 %)	15/30 (50 %)	28/36 (77.78 %)

1. PR = Protease and RT = Reverse transcriptase.
2. Datasets were counted only if data were submitted for both the Protease and Reverse transcriptase genes.

Calculation of the consensus sequences

The ENVA9 consensus sequences were prepared by aligning the sequences submitted by all participants in the programme. The sequences returned for each codon were then analysed. The majority result (observed in >60% of the sequences) was recorded as the consensus sequence for each codon. The consensus sequences calculated from all the sequences submitted by the participants were in substantial agreement with the consensus sequences calculated from the independent testing results.

The ENVA Scoring system

In order to compare the results of participants, a scoring system was applied in which the correct detection of a codon genotype (i.e. identical to the codon genotype of the consensus sequence) was given 1 point and an incorrect codon (not matching the consensus sequence) was given 0 points. For codons containing a mixture of 2 or 3 nucleotides at a certain position, 1 point was given if the mixture was reported or if the correct mutation was reported.

Table 3(c): Scoring Table

Expected Result / Participants Result	Wild Type	Mutant Type	Mixed Type
Wild Type	1	0	0
Mutant Type	0	1	1
Mixed Type	0	1	1

The number of codons involved was 38 for Protease and 30 for Reverse transcriptase. Therefore the maximum performance score that could be achieved was 68 points for all panel members. This resulted in an overall maximum score achievable of 340 points.

Table 4: Genotypic composition of the ENVA9 samples at each of the IAS defined resistance codons

Amino Acid position[1]	WT[2]	ENVA9				
		-02	-03	-05	-06	-07
PR-10	CTC	CTA	ATC	TTT	CTT	TTA
PR-11	GTC					
PR-13	ATA	GTA	GTA			GTA
PR-16	GGG			GAG		
PR-20	AAG	ATA			AGG	ATA
PR-24	TTA				CTA	
PR-30	GAT			AAC		
PR-32	GTA					
PR-33	TTA	TTG		CTA		
PR-34	GAA					
PR-35	GAA	GAC	GAC	GAC	GAT	GAC
PR-36	ATG	ATA	ATA	GTA	ATA	ATA
PR-43	AAA					
PR-46	ATG		ATA			
PR-47	ATA					
PR-48	GGG			GGA	GGA	
PR-50	ATT					
PR-53	TTT					
PR-54	ATC		GTC			
PR-58	CAG				CAA	CAA
PR-60	GAT					
PR-62	ATA		GTA	GTA		
PR-63	CTC	CTT	CCC	CTT	CTT	CCT
PR-64	ATA					
PR-69	CAT	AAA		AAA	AAG	AAA
PR-71	GCT		ACT			GCC
PR-73	GGT	GGG		GGC		
PR-74	ACA			TCA		
PR-76	TTA					
PR-77	GTA	RTA			GTG	GTG
PR-82	GTC	ATC	GCC			
PR-83	AAC					
PR-84	ATA					
PR-85	ATT					
PR-88	AAT			GAT	AAC	
PR-89	CTG	ATG		ATG	ATG	ATG
PR-90	TTG		ATG			
PR-93	ATT		CTT	CTT	CTT	

Amino Acid position[1]	WT[2]	ENVA9				
		-02	-03	-05	-06	-07
RT-41	ATG			TTG		
RT-62	GCC				GYC	
RT-65	AAA			AAG	ARG	
RT-67	GAC			AAC	RAC	GAT
RT-69	ACT			GAT	AYT	ACC
RT-70	AAA			AAG	AAG	
RT-74	TTA					
RT-75	GTA				RYA	
RT-77	TTC	TTT		TTT		
RT-90	GTT	GTC				GTC
RT-98	GCA	GCG		GGA		GCG
RT-100	TTA					
RT-101	AAA				CAA	
RT-103	AAA				AAC	
RT-106	GTA			GTG	ATG	
RT-108	GTA					
RT-115	TAT					
RT-116	TTT					
RT-138	GAG				GCA	
RT-151	CAG					CAA
RT-179	GTC	GTG	GTT	GTT	GTT	GTG
RT-181	TAC		TAT	TAT	TAT	
RT-184	ATG			GTA	GTR	
RT-188	TAT					
RT-190	GGA					
RT-210	TTG			TGG		CTG
RT-215	ACC			TAC		
RT-219	AAA			AAG	AAR	
RT-225	CCT			CCC	CCA	CCC
RT-230	ATG					

Key: Genotypic mixture using the IUB nucleotide ambiguity system

IUB Code	K	M	R	S	W	Y	B	D	H	V	N
Mixture of	G or T	A or C	A or G	G or C	A or T	C or T	C, G or T	A, G or T	A, C or T	A, C or G	Any Base

Cornish-Bowden A. IUPAC-IUB Symbols for Nucleotide Nomenclature. Nucleic Acids Research. 1985; 13: 3021-3030.

The Protease and Reverse transcriptase gene codons presented are those associated with clinical drug resistance, according to Johnson VA *et al* .(2008). Update of the Drug Resistance Mutations in HIV-1: December 2008. Topics in HIV Medicine, 16(5):138-145.

1. PR = Protease and RT = Reverse transcriptase.
2. WT = Wild type sequence pNL4-3.

An empty box signifies that the consensus sequence was in agreement with the wild type sequence pNL4-3.

Additional legend information for Figures 1 to 5

The consensus sequences are presented at the top of figures 1, 2, 3, 4 & 5 and were determined by analysing all sequences submitted by participants for the Protease (PR) and Reverse transcriptase (RT) genes.

A period (.) indicates agreement with the consensus sequence.

A nucleotide letter or IUB code indicates a difference in sequence composition compared with the consensus sequence.

Figure 1: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA9-02

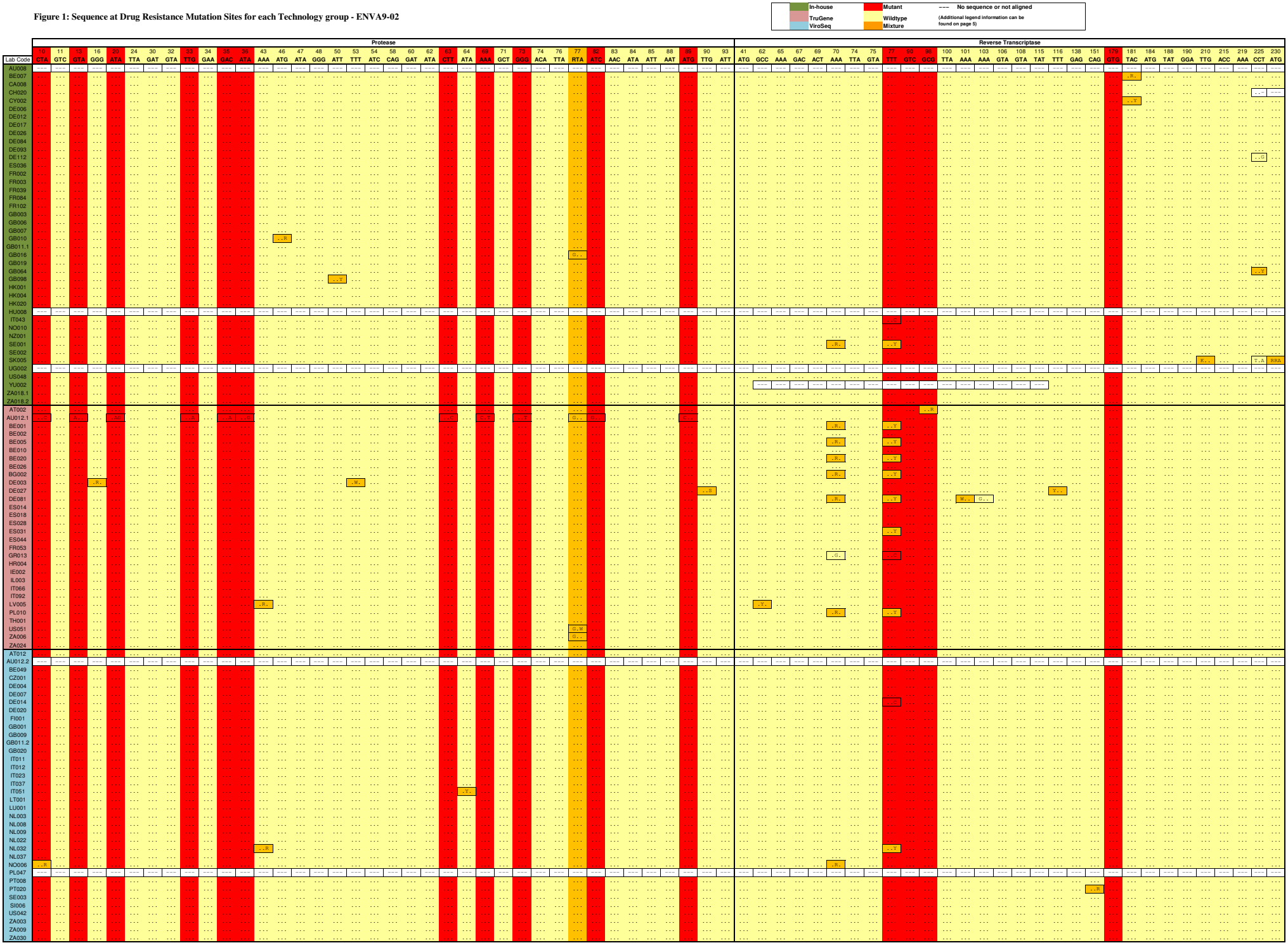
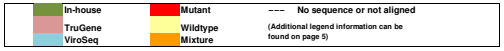


Figure 2: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA9-03



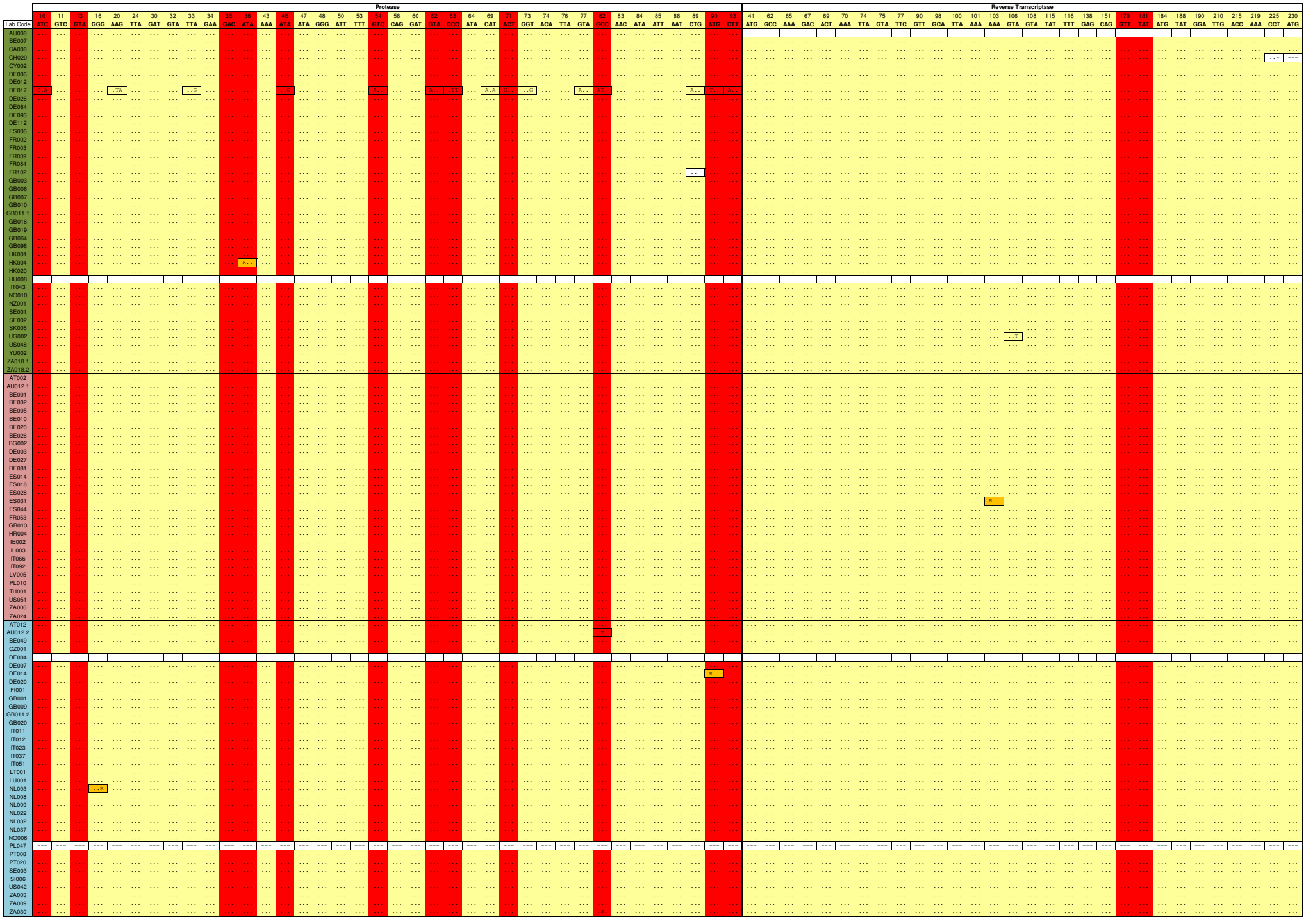


Figure 3: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA9-05

Figure 4: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA9-06

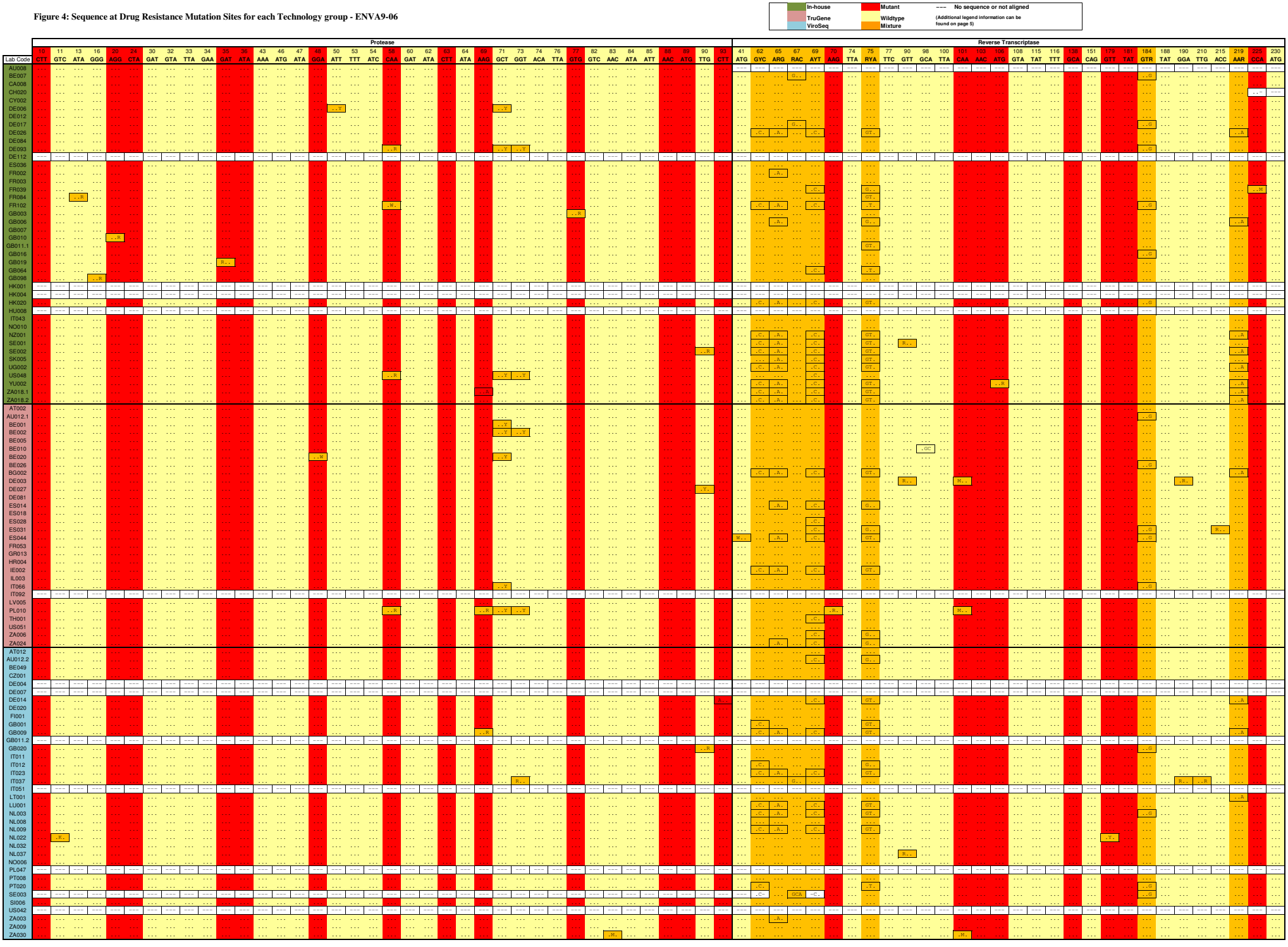


Figure 5: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA9-07

In-house | TruGene | ViroSeq | Mutant | Wildtype | Mixture | --- No sequence or not aligned | (Additional legend information can be found on page 5)

Protease

Lab Code	10 TTA	11 GTC	13 GTA	16 GGG	20 ATA	24 TTA	30 GAT	32 GTA	33 TTA	34 GAA	35 GAC	36 ATA	43 AAA	46 ATG	47 ATA	48 GGG	50 ATT	53 TTT	54 ATC	58 CAA	60 GAT	62 ATA	63 CCT	64 ATA	69 AAA	71 GCC	73 GGT	74 ACA	76 TTA	77 GTG	82 GTC	83 AAC	84 ATA	85 ATT	88 AAT	89 ATG	90 TTG	93 ATT
AU008	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
BE007	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CA008	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CH020	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CY002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE006	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE012	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE017	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	C..	..T	...	...	...	G..	...
DE026	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE084	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE093	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE112	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ES036	...	..Y	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
FR002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
FR003	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
FR039	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.Y.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
FR084	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
FR102	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB003	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB006	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB007	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB010	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB011.1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB016	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB019	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB064	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB098	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
HK001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
HK004	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
HK020	AGC	CC.	.GC	CTT	GG.	C.C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
HU008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IT043	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
NO010	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
NZ001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SE001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SE002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SK005	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
UG002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
US048	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
YU002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
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ZA018.2	...	..Y	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
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BE005	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
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DE081	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
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ES031	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ES044	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
FR053	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
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IT066	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
IT092	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
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TH001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..Y	...	...	...	...	...	...	...
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ZA024	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
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Reverse Transcriptase

Lab Code	41 ATG	62 GCC	65 AAA	67 GAT	69 ACC	70 AAA	74 TTA	75 GTA	77 TTC	90 GTC	98 GCG	100 TTA	101 AAA	103 AAA	106 GTA	108 GTA	115 TAT	116 TTT	138 GAG	151 CAA	179 GTG	181 TAC	184 ATG	188 TAT	190 GGA	210 CTG	215 ACC	219 AAA	225 CCC	230 ATG
AU008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
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ES036	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
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GB064	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB098	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
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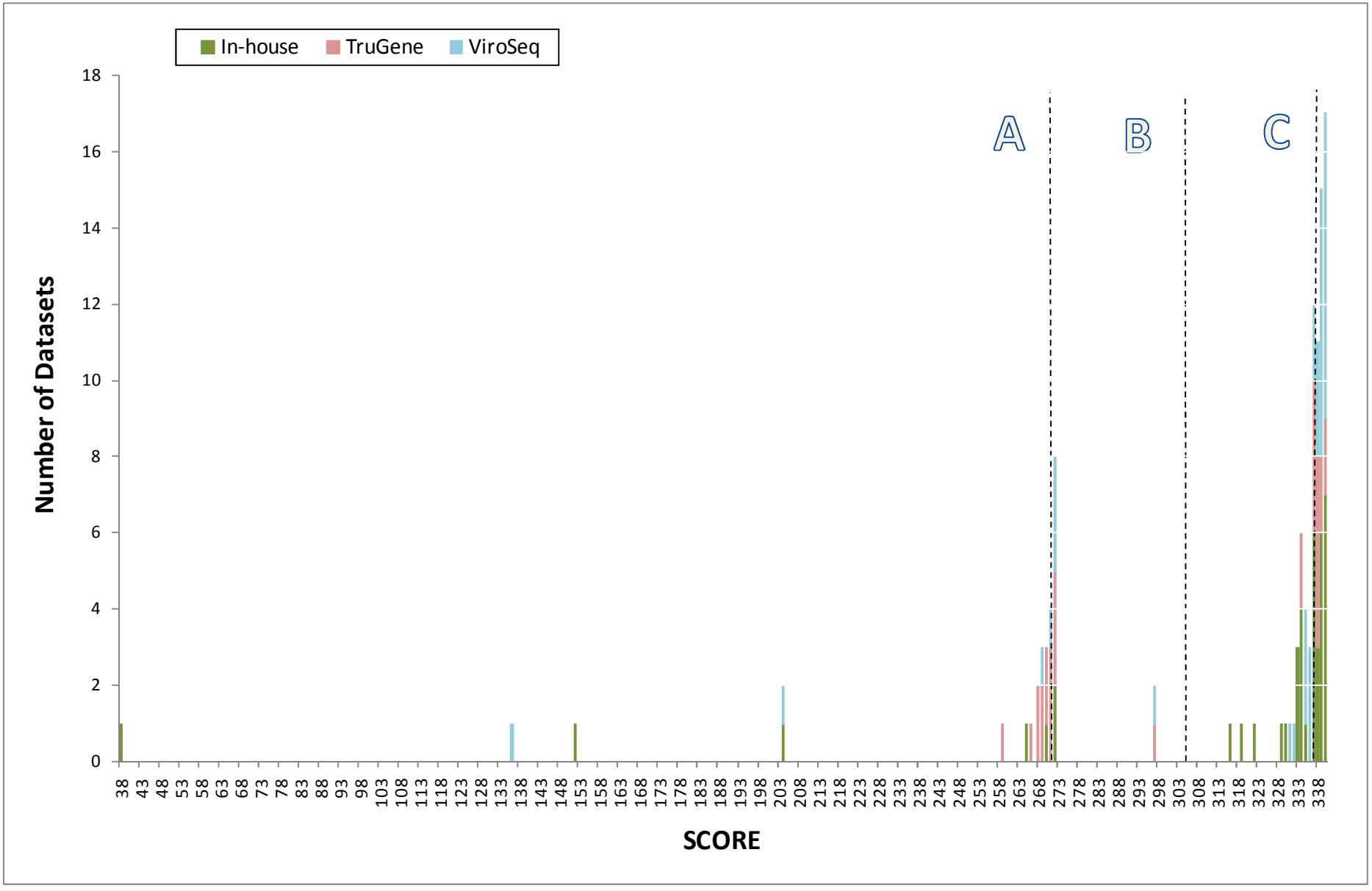
SE002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SK005	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	T.W
UG002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
US048	...	...	...	...	...	...	...	...	...	...	..R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
YU002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZA018.1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZA018.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
AT002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AU012.1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
BE002	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
BE005	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
BE010	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE020	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
BE026	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
BG002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE003	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE027	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE081	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ES014	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ES018	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ES028	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ES031	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...
ES044	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
FR053	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GR013	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
HR004	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IE002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IL003	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
IT066	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
IT092	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
LV005	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
PL010	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
TH001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
US051	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ZA006	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ZA024	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AT012	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
AU012.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
BE049	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
CZ001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE004	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE007	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE014	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
DE020	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
FI001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB009	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB011.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
GB020	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
IT011	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
IT012	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
IT023	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
IT037	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
IT051	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
LT001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
LU001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
NL003	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
NL008	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
NL009	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
NL022	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	T..	...	...	...	...
NL032	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
NL037	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
NO006	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
PL047	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
PT008	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
PT020	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SE003	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
SI006	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
US042	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZA003	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZA009	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ZA030	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.K.	...	...	...	...	...	...	...	...	...

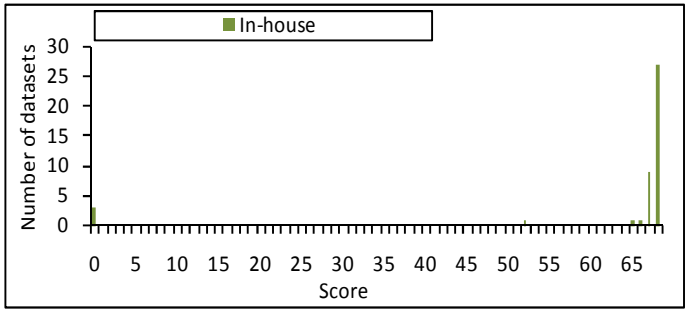
Figure 6: Summary of overall performance scores by technology

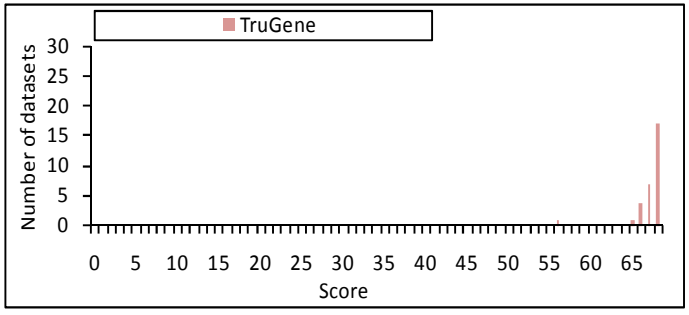


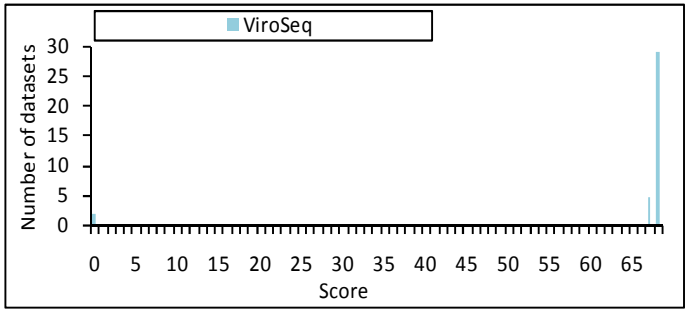
A: 80% (272) of maximum achievable score (340), B: 90% (306) of maximum achievable score (340), C: 99% (336) of maximum achievable score (340).

Figure 7(a): Summary of performance scores by panel sample

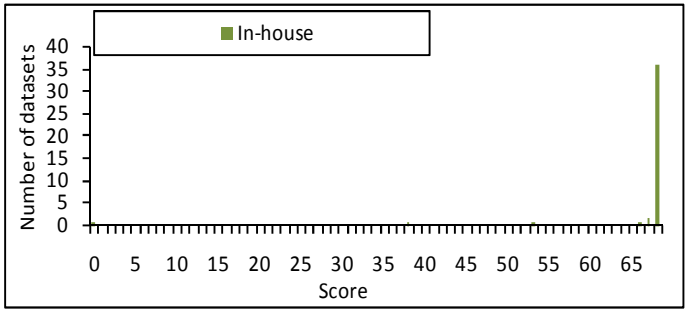
ENVA9-02

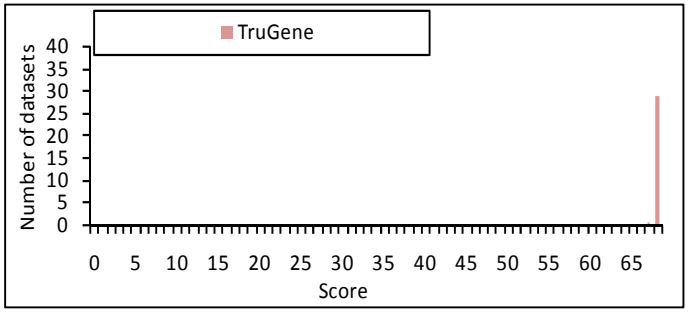


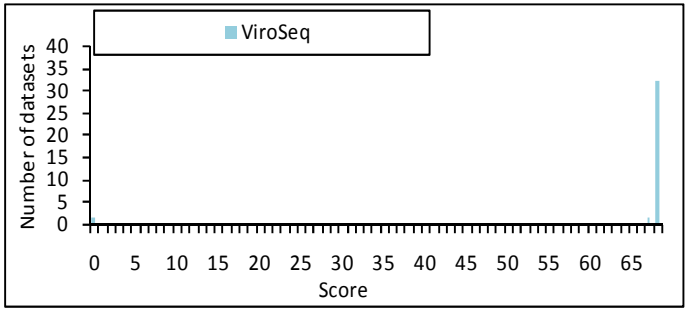




ENVA9-03





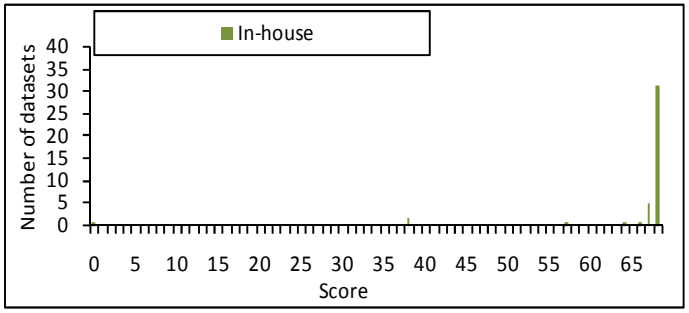


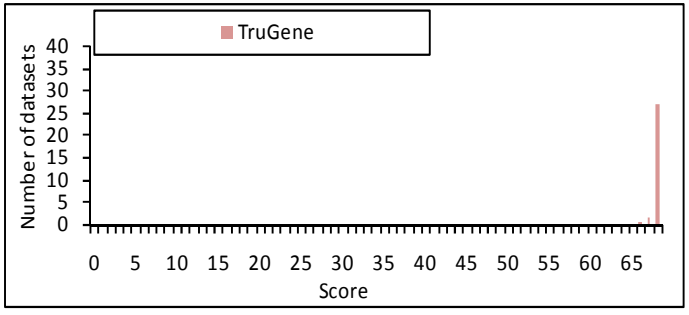
The maximum score achievable for all samples was 68.

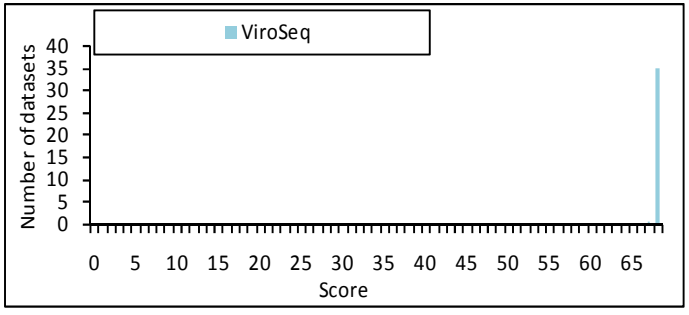
Datasets containing either a frame-shift or missing sequences resulted in a low performance score.

Figure 7(b): Summary of performance scores by panel sample

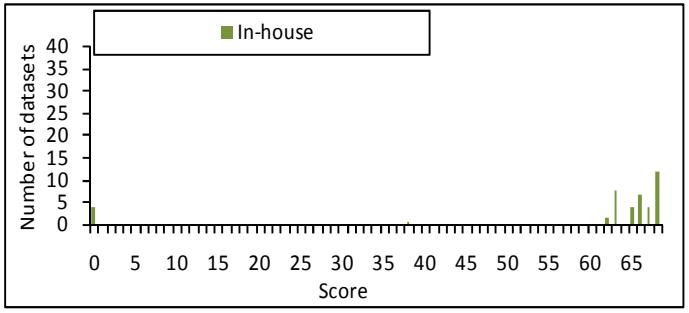
ENVA9-05

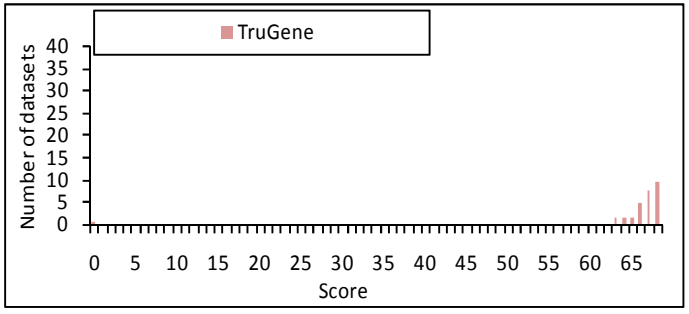


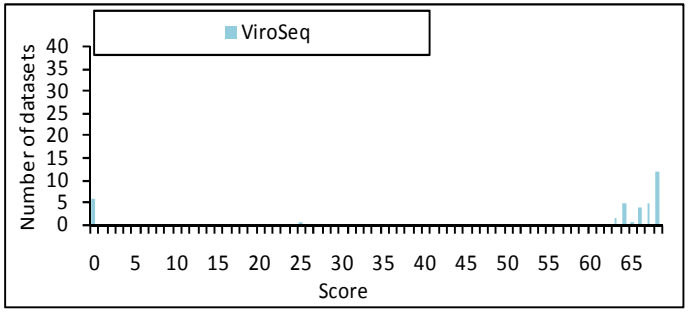




ENVA9-06





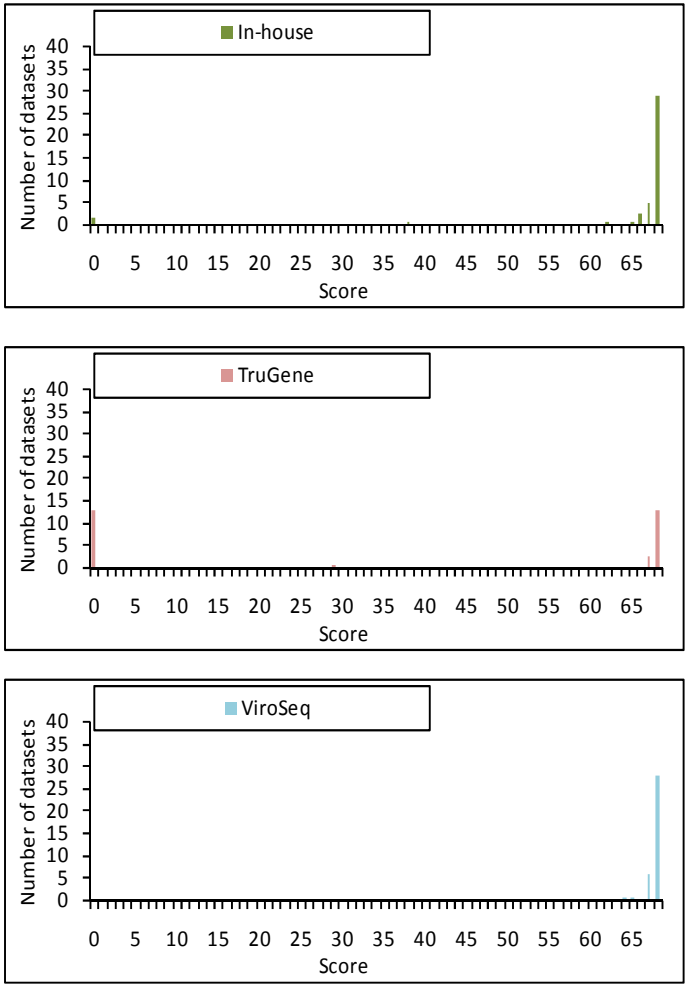


The maximum score achievable for all samples was 68.

Datasets containing either a frame-shift or missing sequences resulted in a low performance score.

Figure 7(c): Summary of performance scores by panel sample

ENVA9-07



The maximum score achievable for all samples was 68.

Datasets containing either a frame-shift or missing sequences resulted in a low performance score.

Comments

The ENVA9 EQA panel was chosen by the Europe HIV Resistance (EHR) working group on quality control. The panel consisted of human plasma samples spiked with cultured patient isolates or recombinant viruses. The viruses present in the panel were of HIV-1 subtype A, B, C or G.

- The consensus sequences calculated from the testing results were in agreement with the consensus sequences calculated from the results of all participant datasets submitted during the actual distribution.

- The majority of datasets were generated using commercial HIV drug resistance genotyping kits: 36 For ViroSeq (33.3%) and 30 for TruGene (27.8%). The remaining 42 datasets (38.9%) were generated with in-house genotyping assays (Table 3b).

- All technologies were successful in the sequence analysis of the panel samples. There were no systematic negative results with any of the panel samples. However :

    - The success rate for genotyping of ENVA9-07, harbouring an HIV-1 subtype A strain, was lower for TruGene than for other technologies. This observation will be investigated further in future EQA rounds.

    - The success rate for genotyping of ENVA9-06, harbouring an HIV-1 subtype C strain, was lower for Viroseq than for other technologies. The success rate for sample ENVA9-05, also harbouring an HIV-1 subtype C strain, was high. This observation will be investigated further in future EQA rounds.

- The number of complete datasets (covering both the Protease and Reverse transcriptase genes) for all samples was 72.2%. This was lower compared to the success rate in ENVA8 (84.3%). This difference maybe due to the different composition of the ENVA9 panel. In order to provide state of the art panels in each distribution, the ENVA panels are composed differently for each round. Therefore it is only possible to draw general conclusions on variations in success rates between distributions.

- The number of full datasets reported to QCMD was higher for the in-house assays (83.3%), compared to TruGene (50.0%) or ViroSeq (77.8%) (Table 3b). The lower rates for Trugene and ViroSeq were due to the lower success rates for samples ENVA9-06 and -07.

- Overall, the percentage of datasets recording over 99.0% of the maximum achievable score (340) was 53.7% (n=58/108) (Figure 6). The range of scores reported for those datasets that did not achieve 99.0% (of the maximum achievable score or greater) ranged from 38 to 335 points. Most of the lower scoring datasets were due to:

    - Missing results for complete genes (either PR or RT) or complete panel samples (Table 3a and Figure 6).

    - Reduced detection of mutant nucleotides in mixed wild type/mutant type codons in the RT gene of ENVA9-06 was observed, in particular for TruGene users. This specifically involved RT codons 62, 65, 69 and 75. This resulted in an underestimation of the prevalence of resistance mutations in this sample. The exact proportion of wildtype and mutant virus at these positions is unknown. Therefore, no conclusions can be drawn with regard to the absolute sensitivity of methods for the detection of mutant genotypes being part of mixed nucleotide populations. However, the results demonstrate that the capability for detecting specific nucleotides as part mixed populations may vary by technology.

Acknowledgements

QCMD would like to thank Celera Diagnostics (Alameda, USA), Siemens Clinical Laboratory (Berkeley, USA), the Institut fur Immunologie und Genetik (Kaiserslautern, Germany) and the Imperial College School of Medicine (London, United Kingdom) for performing testing.