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QUALITY CONTROL for MOLECULAR DIAGNOSTICS

Final Report

QCMD 2007 ENVA7 HIV Drug Resistance Typing Proficiency Programme

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on behalf of QCMD and its Scientific Advisory Board
November 2007

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 aim of this external quality assessment programme was 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 ENVA7	
Date programme distributed	31/07/2007
Number of participants	111
Number of countries	44
Number of respondents	95 (86%)
Number of non-respondents	16 (14%)
Number of datasets submitted	98

All participants who had not submitted results prior to the closing date for submission were contacted by e-mail. Seven participants withdrew from the programme and nine did not respond or submit results. In the previous HIV Drug Resistance Typing Proficiency Programme (ENVA6) 18 % of participants (18 laboratories) were non-respondents (QCMD 2006).

3. Panel composition

The ENVA7 panel consisted of five well-characterised samples. ENVA7-01 and ENVA7-02 and ENVA7-05 were derived from clinical isolates, ENVA7-03 and ENVA7-04 contained cultured recombinant viruses in human plasma. The sample materials were heat inactivated (30 minutes at 56 degrees centigrade) and aliquoted into 1ml aliquots. Viral loads of the material were determined before and after lyophilisation (Table 2).

Table 2: Panel composition & viral load determination

Sample	Matrix[1]	Subtype	Viral load (copies/ml) [2]	
			Pre-lyophilisation	Post-lyophilisation
ENVA7-01	Citrate Plasma	C	2.60×10^4	1.18×10^4
ENVA7-02*	Citrate Plasma	C	4.68×10^4	9.20×10^3
ENVA7-03	Citrate Plasma	B	4.60×10^3	3.29×10^3
ENVA7-04	Citrate Plasma	B	3.00×10^4	1.76×10^4
ENVA7-05	Citrate Plasma	F	2.60×10^4	2.23×10^4

1: All samples were diluted in HIV, HBV and HCV negative citrate plasma and lyophilised prior to distribution.

2. In-process quantitative analysis performed using the Roche Amplicor HIV-1 Monitor Test.

* Two insertions of 3 base pairs each after codon 67 in Reverse transcriptase.

4. Programme results

4a. Analysis of the Sequence Data

Table 3a: Summary of submitted results by individual dataset

		SAMPLE[1]									
		ENVA 7-01		ENVA 7-02		ENVA 7-03		ENVA 7-04		ENVA 7-05	
Lab. Code [2]	Method	PR1 [3]	RT1 [3]	PR2	RT2	PR3	RT3	PR4	RT4	PR5	RT5
AU008.SP	IH	■	■	■	■	■	■			■	■
BE007.CD	IH	■	■	■	■			■	■		
CA008.CD	IH	■	■	■	■	■	■	■	■	■	■
CH020.SP	IH	■	■	■	■	■	■	■	■	■	■
CY002.CD	IH	■	■	■	■	■	■	■	■	■	■
DE006.CD	IH	■	■	■	■	■	■	■	■	■	■
DE012.CD	IH	■	■	■	■	■	■	■	■	■	■
DE026.CD	IH	■	■	■	■	■	■	■	■	■	■
DE034.SP	IH	■	■	■	■	■	■	■	■		
ES036.CD	IH	■	■	■	■	■	■	■	■	■	■
FR002.CD	IH	■	■	■	■	■	■	■	■	■	■
FR003.CD	IH	■	■	■	■	■	■	■	■	■	■
FR039.SP	IH	■	■	■	■	■	■	■	■	■	■
GB003.2.CD	IH	■	■	■	■	■	■	■	■	■	■
GB006.CD	IH	■	■	■	■	■	■	■	■	■	■
GB007.CD	IH	■	■	■	■	■	■	■	■	■	■
GB015.CD	IH	■	■	■	■			■	■		
GB019.CD	IH	■	■	■	■	■	■	■	■	■	■
GB098.CD	IH	■	■	■	■	■	■	■	■	■	■
HK001.CD	IH	■	■	■	■	■	■	■	■	■	■
HU008.SP	IH	■	■	■	■			■	■	■	■
IL003.2.SP	IH	■	■	■	■	■	■	■	■	■	■
IT043.CD	IH	■	■	■	■	■	■	■	■	■	■
NO010.CD	IH	■	■	■	■	■	■	■	■	■	■
NZ001.CD	IH	■	■	■	■	■	■	■	■	■	■
RU012.SP	IH	■	■	■	■	■	■	■	■	■	■
SE001.CD	IH	■	■	■	■	■	■	■	■	■	■
SE002.SP	IH	■	■	■	■	■	■	■	■	■	■
SK005.SP	IH		■	■	■	■	■	■	■	■	■
UG002.CD	IH	■	■	■	■	■	■	■	■	■	■
US028.CD	IH	■	■	■	■	■	■	■	■	■	■
US048.CD	IH	■	■	■	■	■	■	■	■		
ZA014.CD	IH	■	■	■	■	■	■	■	■	■	■
ZA018.CD	IH	■	■	■	■	■	■	■	■	■	■
ZA039.CD	IH	■	■	■	■	■	■	■	■	■	■
AT002.CD	TG	■	■	■	■	■	■	■	■	■	■
BE001.SP	TG	■	■	■	■	■	■	■	■	■	■
BE002.2.CD	TG			■	■					■	■
BE002.CD	TG	■	■	■	■	■	■	■	■	■	■
BE005.CD	TG	■	■	■	■	■	■	■	■	■	■
BE020.CD	TG	■	■	■	■	■	■	■	■	■	■
BE026.CD	TG	■	■	■	■	■	■	■	■	■	■
BG002.SP	TG	■	■	■	■	■	■	■	■	■	■
DE003.CD	TG	■	■	■	■	■	■	■	■	■	■
DE027.SP	TG	■	■	■	■	■	■	■	■	■	■
ES014.CD	TG	■	■	■	■	■	■	■	■	■	■
ES018.CD	TG	■	■	■	■	■	■	■	■	■	■
ES028.CD	TG	■	■	■	■	■	■	■	■	■	■
FR053.CD	TG	■	■	■	■	■	■	■	■	■	■
GB003.SP	TG	■	■	■	■	■	■	■	■	■	■
GR013.CD	TG	■	■	■	■	■	■	■	■	■	■
HK020.SP	TG	■	■	■	■	■	■	■	■	■	■
HR004.SP	TG	■	■	■	■	■	■	■	■	■	■
IE002.CD	TG	■	■	■	■	■	■	■	■	■	■
IL003.SP	TG	■	■	■	■	■	■	■	■	■	■
LV005.SP	TG	■	■	■	■	■	■	■	■	■	■
PL010.CD	TG	■	■	■	■	■	■	■	■	■	■
TH001.CD	TG	■	■	■	■	■	■	■	■	■	■
US009.SP	TG	■	■	■	■	■	■	■	■	■	■
US051.CD	TG	■	■	■	■	■	■	■	■	■	■
US076.CD	TG	■	■	■	■	■	■	■	■		
ZA006.CD	TG	■	■	■	■	■	■	■	■	■	■
AT012.CD	VS	■	■	■	■	■	■	■	■	■	■
BE049.CD	VS	■	■	■	■	■	■	■	■	■	■
CZ001.CD	VS	■	■	■	■	■	■	■	■	■	■
DE004.CD	VS	■	■	■	■	■	■	■	■	■	■
DE014.CD	VS	■	■	■	■	■	■	■	■	■	■
DE020.CD	VS	■	■	■	■	■	■	■	■	■	■
DE023.SP	VS	■	■	■	■	■	■	■	■	■	■
DE051.CD	VS	■	■	■	■	■	■	■	■	■	■
DE084.CD	VS	■	■	■	■	■	■	■	■	■	■
DK009.CD	VS	■	■	■	■	■	■	■	■	■	■
FI001.CD	VS	■	■	■	■	■	■	■	■	■	■
GB001.CD	VS	■	■	■	■	■	■	■	■	■	■
GB010.CD	VS	■	■	■	■	■	■	■	■	■	■
GB011.CD	VS	■	■	■	■	■	■	■	■	■	■
GB016.CD	VS	■	■	■	■	■	■	■	■	■	■
GB020.CD	VS	■	■	■	■	■	■	■	■	■	■
GB064.CD	VS	■	■	■	■	■	■	■	■	■	■
IT011.CD	VS	■	■	■	■			■	■	■	■
IT012.CD	VS	■	■	■	■	■	■	■	■	■	■
IT037.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	■	■	■	■	■	■	■	■	■	■
PT008.CD	VS	■	■	■	■	■	■	■	■	■	■
SE003.CD	VS			■	■	■	■	■	■	■	■
SI006.CD	VS	■	■	■	■	■	■	■	■	■	■
US054.CD	VS	■	■	■	■	■	■	■	■	■	■
ZA003.CD	VS	■	■	■	■	■	■	■	■	■	■
ZA009.CD	VS	■	■	■	■	■	■	■	■	■	■
ZA030.CD	VS	■	■	■	■	■	■	■	■	■	■

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 system 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 (%)
ENVA7-01	95/98 (97%)	34/35 (97%)	26/27 (96%)	35/36 (97%)
ENVA7-02	98/98 (100%)	35/35 (100%)	27/27 (100%)	36/36 (100%)
ENVA7-03	93/98 (95%)	32/35 (91%)	26/27 (96%)	35/36 (97%)
ENVA7-04	96/98 (98%)	34/35 (97%)	26/27 (96%)	36/36 (100%)
ENVA7-05	90/98 (92%)	30/35 (86%)	26/27 (96%)	34/36 (94%)
ALL SAMPLES	80/98 (82%)	25/35 (71%)	23/27 (85%)	32/36 (89%)

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 ENVA7 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 ENVA7 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 28 for Reverse transcriptase (plus two codons for the 6 base pair insertion sequence in ENVA7-02). Therefore the maximum performance score that could be achieved was 66 points for panel members ENVA7-01, ENVA7-03, ENVA7-04 and ENVA7-05 and 68 points for ENVA7-02. This resulted in an overall maximum score achievable of 332 points.

Table 4: Genotypic composition of the ENVA7 samples at each of the IAS defined resistance codons

Amino Acid position	WT[1]	ENVA7				
		-01	-02	-03	-04	-05
PR-10	CTC	TTT		ATC	ATC	CTA
PR-11	GTC					
PR-13	ATA					
PR-16	GGG	GAG				
PR-20	AAG		AAA	ARG	ARR	AGG
PR-24	TTA					
PR-30	GAT	AAC				
PR-32	GTA					
PR-33	TTA	CTA				
PR-34	GAA					
PR-35	GAA	GAC		GAC	GAC	GAC
PR-36	ATG	GTA	ATA	ATA	ATA	ATA
PR-43	AAA			AMA	AMA	
PR-46	ATG			ATA	ATA	
PR-47	ATA					
PR-48	GGG	GGA	GGA			
PR-50	ATT		ATA			
PR-53	TTT					
PR-54	ATC			GTC	GTC	
PR-58	CAG		CAA			
PR-60	GAT		GAG			
PR-62	ATA	GTA		GTA	GTA	
PR-63	CTC	CTT	CCT	CCC	CCC	CTT
PR-64	ATA					
PR-69	CAT	AAA	AAA			CAC
PR-71	GCT		GCA	ACT	ACT	
PR-73	GGT	GGC				
PR-74	ACA	TCA				
PR-76	TTA					TTG
PR-77	GTA		GTG			
PR-82	GTC			GCC	GCC	
PR-83	AAC					
PR-84	ATA					
PR-85	ATT					
PR-88	AAT	GAT	AAC			AAC
PR-89	CTG	ATG	ATG	YTG	YTG	ATG
PR-90	TTG			ATG	ATG	
PR-93	ATT	CTT	CTT	CTT	CTT	

Amino Acid position	WT[1]	ENVA7				
		-01	-02	-03	-04	-05
RT-41	ATG	TTG				
RT-62	GCC					
RT-65	AAA	AAG	AAG			
RT-67	GAC	AAC	AAC			
RT-67-68	INSERT		TCT			
RT-68-69	INSERT		GAG			
RT-69	ACT	GAT	TCT			
RT-70	AAA	AAG	AAG			
RT-74	TTA					
RT-75	GTA					
RT-77	TTC	TTT				
RT-90	GTT					
RT-98	GCA	GGA				
RT-100	TTA					
RT-101	AAA		GAG			
RT-103	AAA					
RT-106	GTA	GTG	ATG			
RT-108	GTA					
RT-115	TAT					
RT-116	TTT					TTC
RT-151	CAG					CAA
RT-179	GTC	GTT		GTT	GTT	GTT
RT-181	TAC	TAT	TAT	TAT	TAT	
RT-184	ATG	GTA				
RT-188	TAT					
RT-190	GGA		GCA			GGG
RT-210	TTG	TGG	TGG			CTG
RT-215	ACC	TAC	TAC			ACT
RT-219	AAA	AAG	AAG			
RT-225	CCT	CCC	CCC			CCC

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.

These tables present the consensus sequences at the IAS resistance codons (Johnson *et al* 2007) for each of the ENVA7 panel samples, as determined by analysing all sequences submitted by participants for the Protease (PR) and Reverse transcriptase (RT) genes. HIV pNL4-3 is indicated as the reference sequence. Panel sample ENVA7-02 contained a 6 base pair insertion present after codon 67 of RT.

Johnson VA *et al* . Update of the Drug Resistance Mutations in HIV-1: Fall 2007. Topics in HIV Medicine 2007: 15(4); 119 – 125.

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

*: For ENVA07-02 (RT Gene), these two labs reported a single-nucleotide insertion at PR codon 9, which resulted in a frameshift in the downstream sequence.

†: The sequence of this lab could not be aligned because the sequence submitted lacked sufficient homology to the consensus sequence.

††: For ENVA07-03 and ENVA07-04 (PR Genes), in cases where a consensus sequence could not be defined (<60% homology), i.e. at codons 10, 20, 43 and 89 of PR, the mixed nucleotide was retained and scoring was done as per the ENVA scoring system. E.g. at codon 43 in ENVA07-03, the frequency of occurrence of nucleotide A (wild type) was 56% while the combined frequency of nucleotides C and mixed base M (mutant or mixture of wild type and mutant) was 44%; therefore the reporting of M or C were considered correct. A similar approach was taken for codons 10, 20, and 89 of PR for both of the above samples.

Figure 1: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA7-01

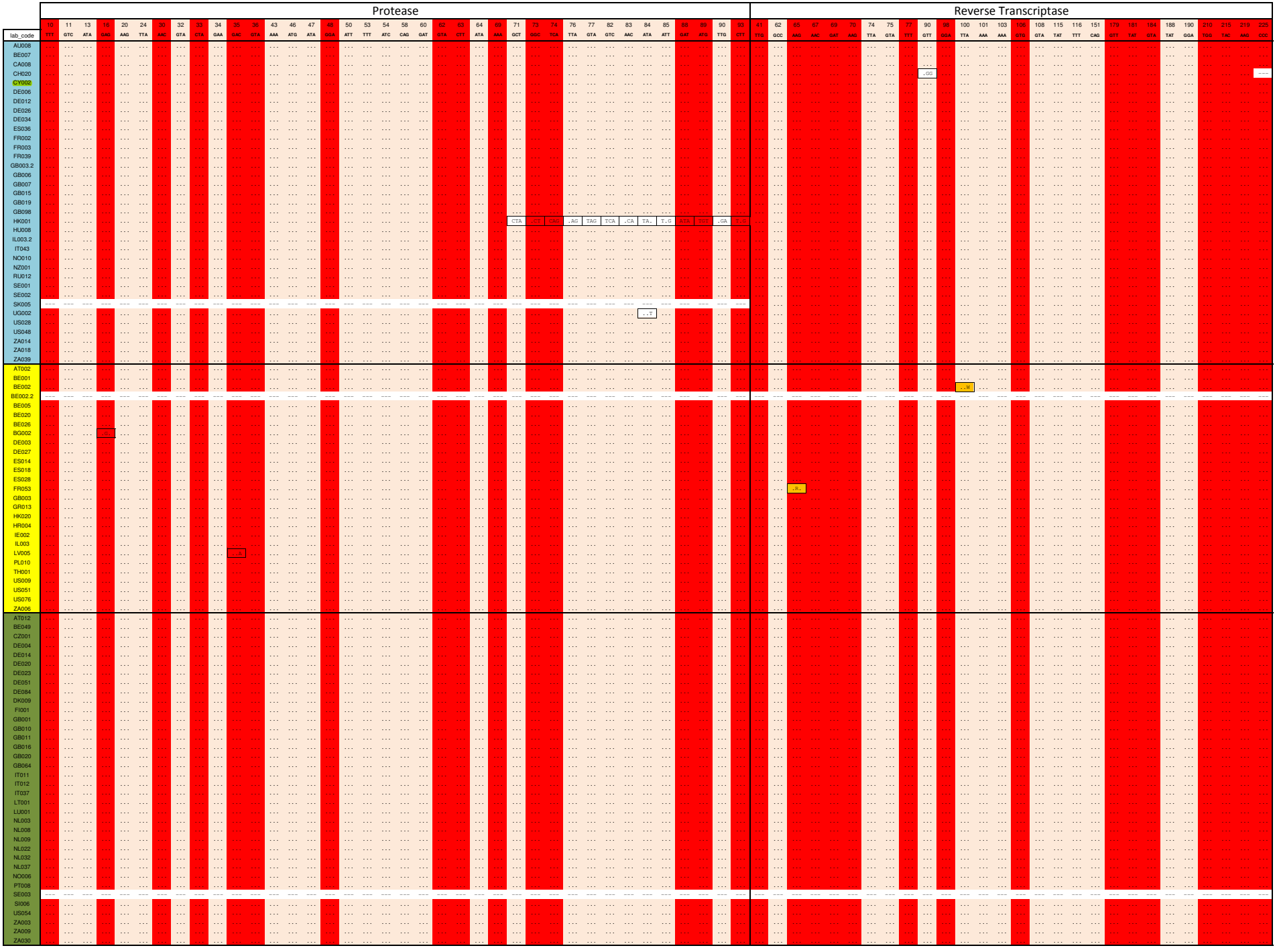


Figure 2: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA7-02

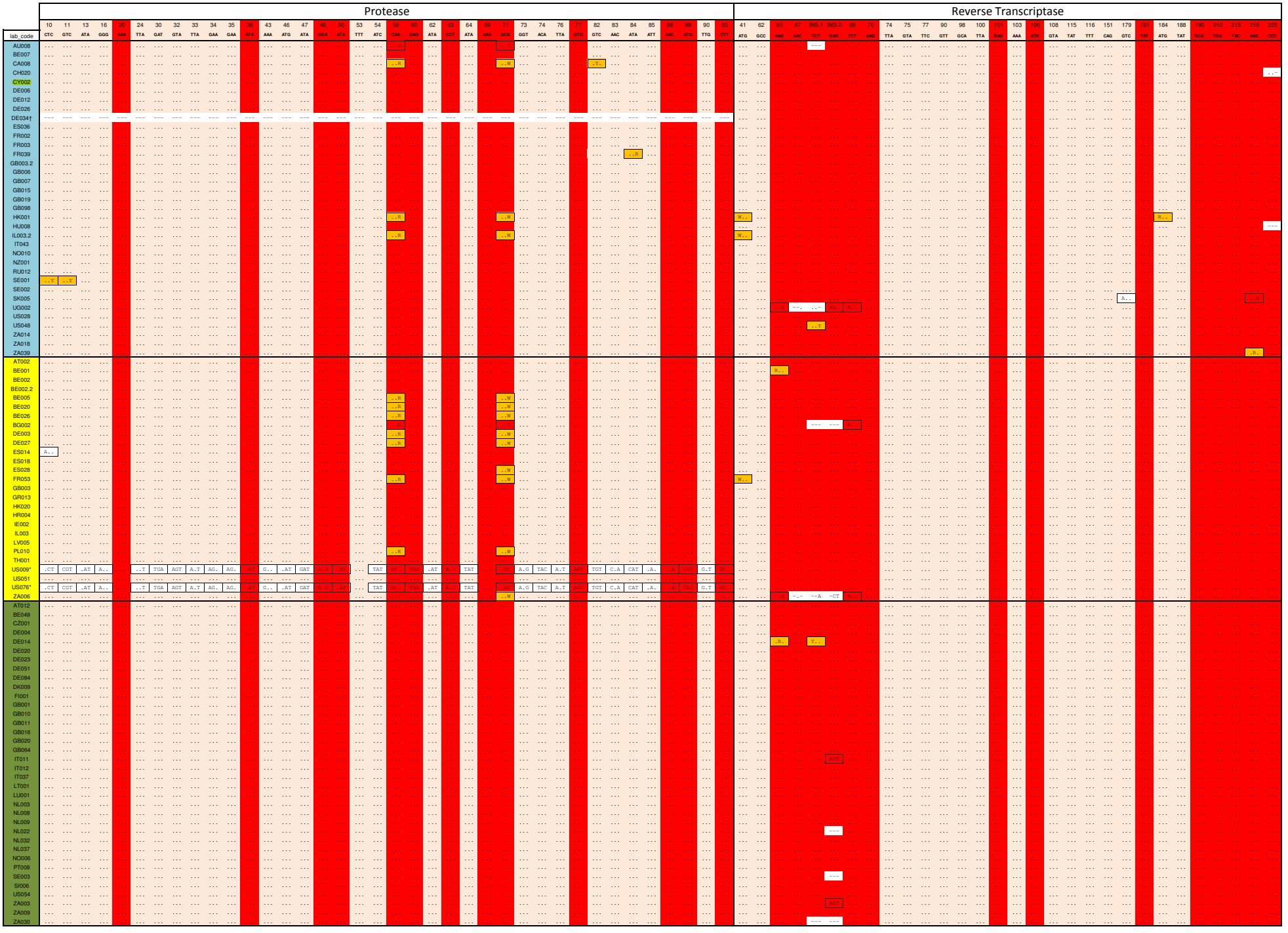


Figure 3: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA7-03††

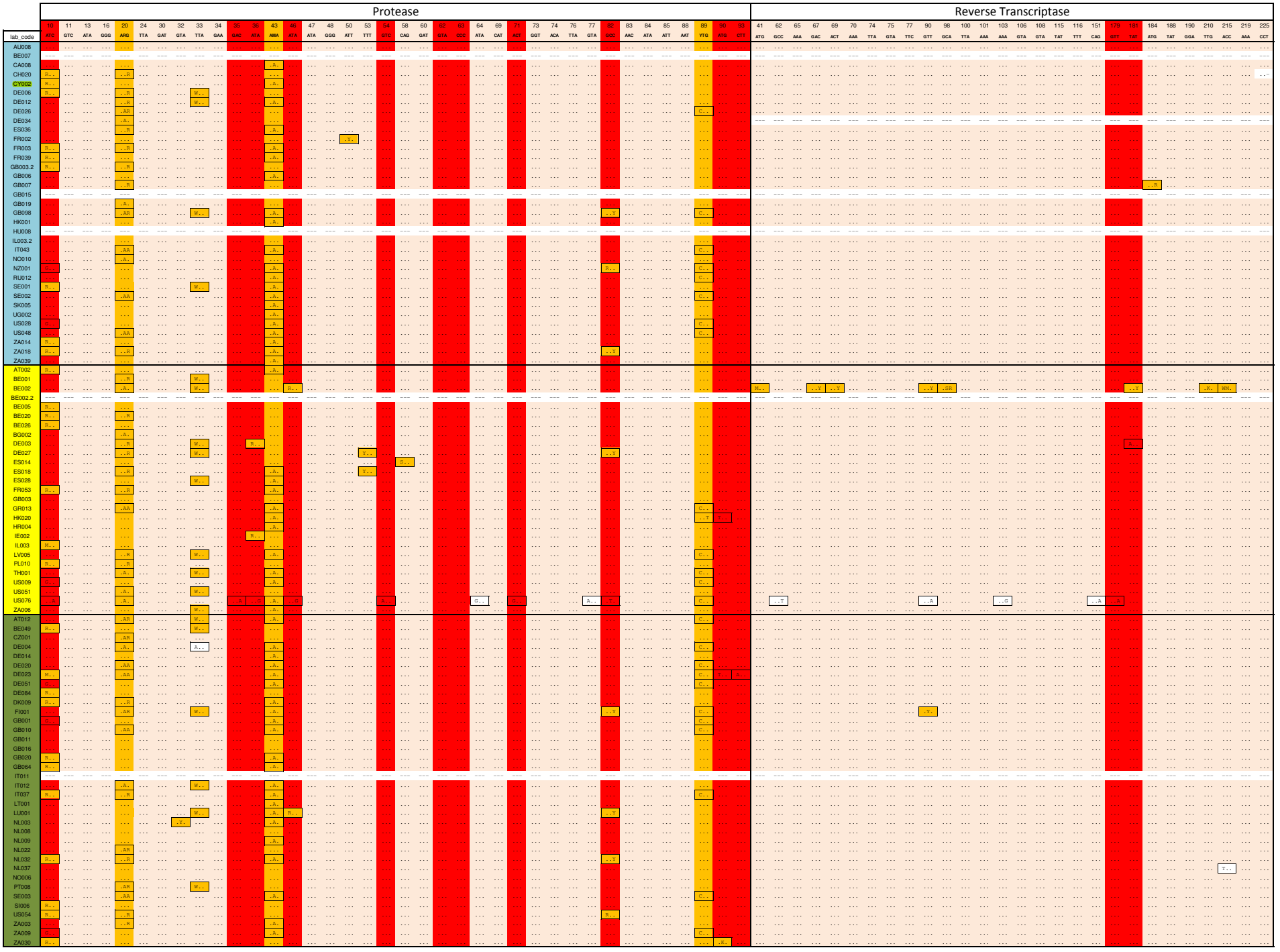


Figure 4: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA7-04††

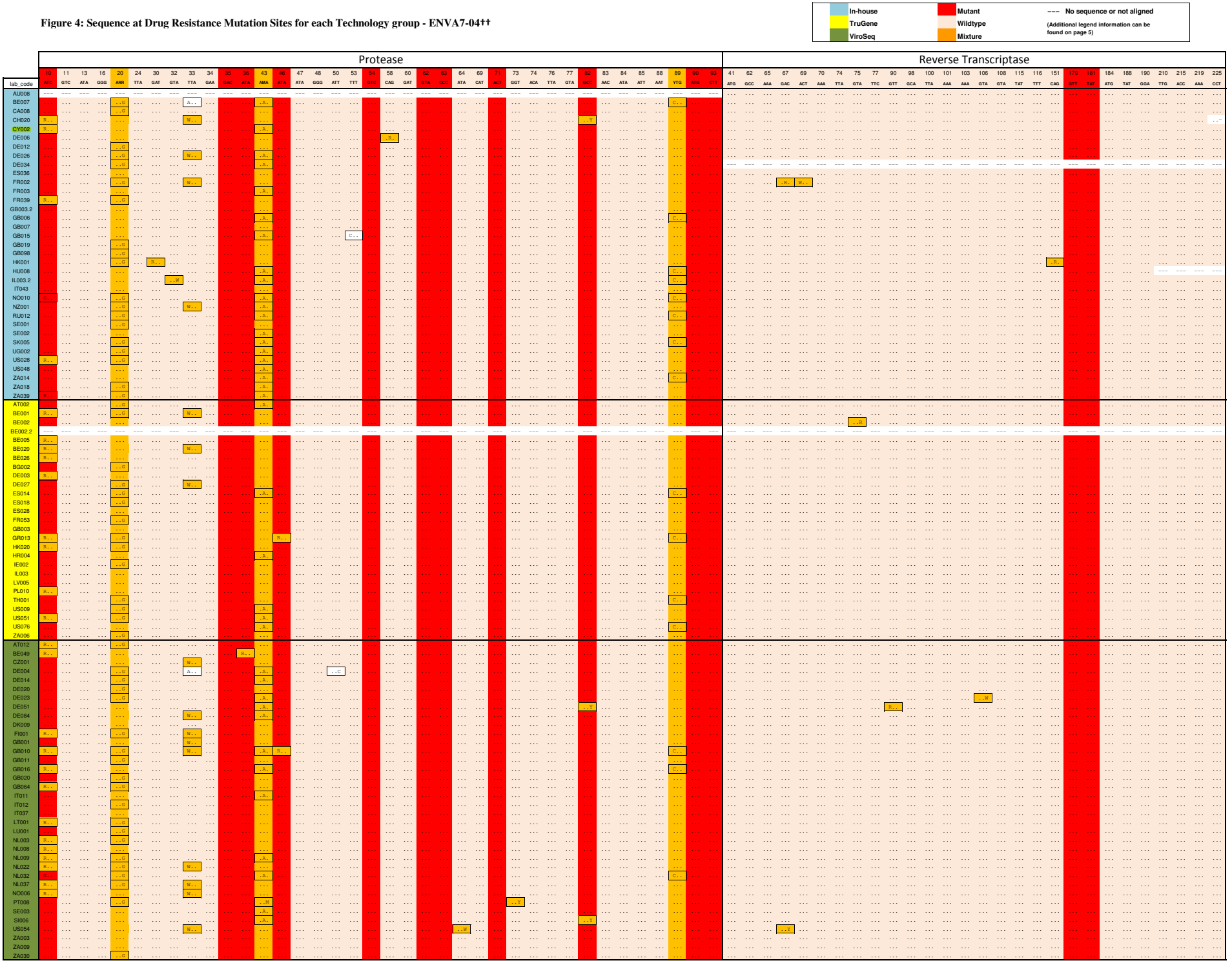


Figure 5: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA7-05

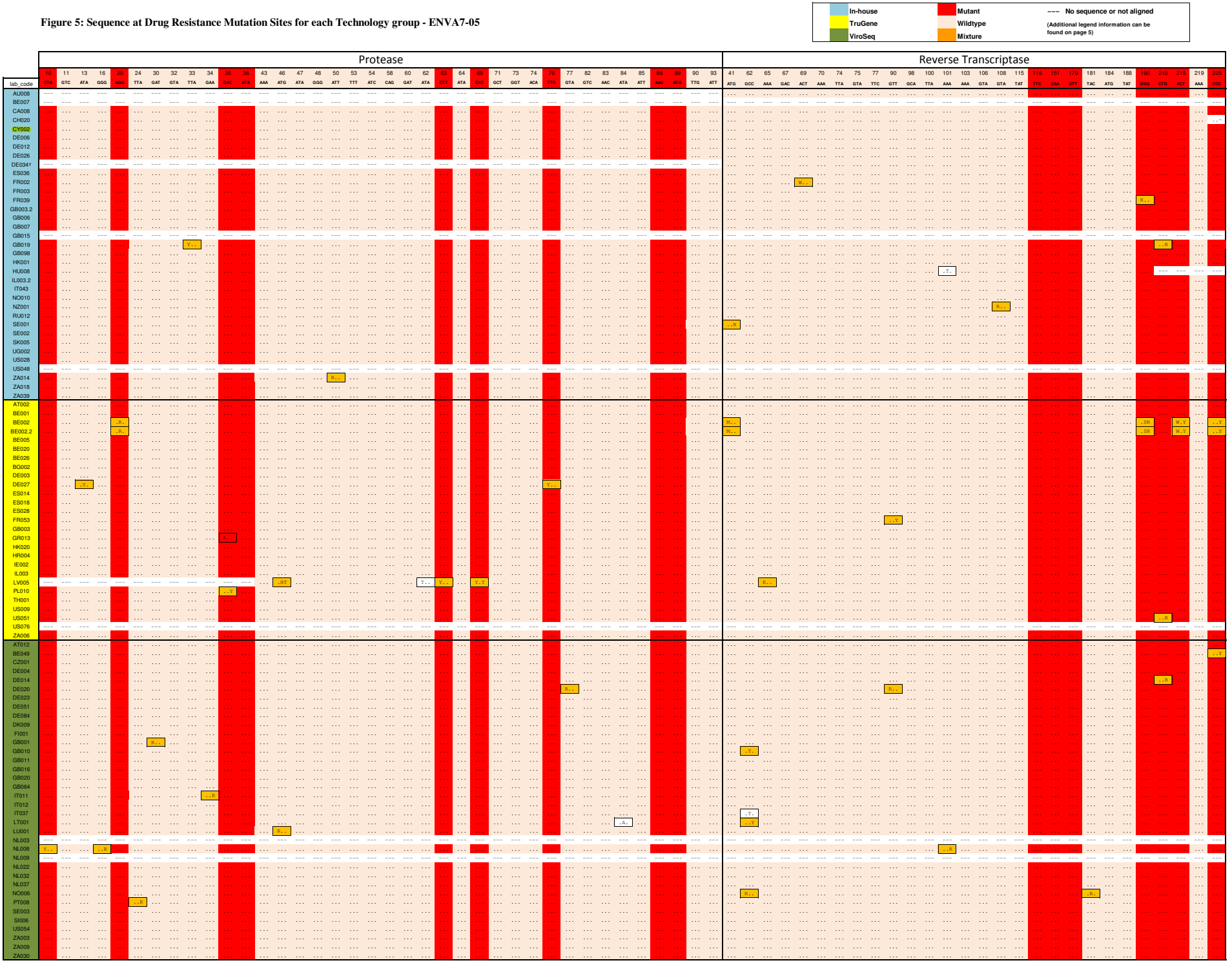
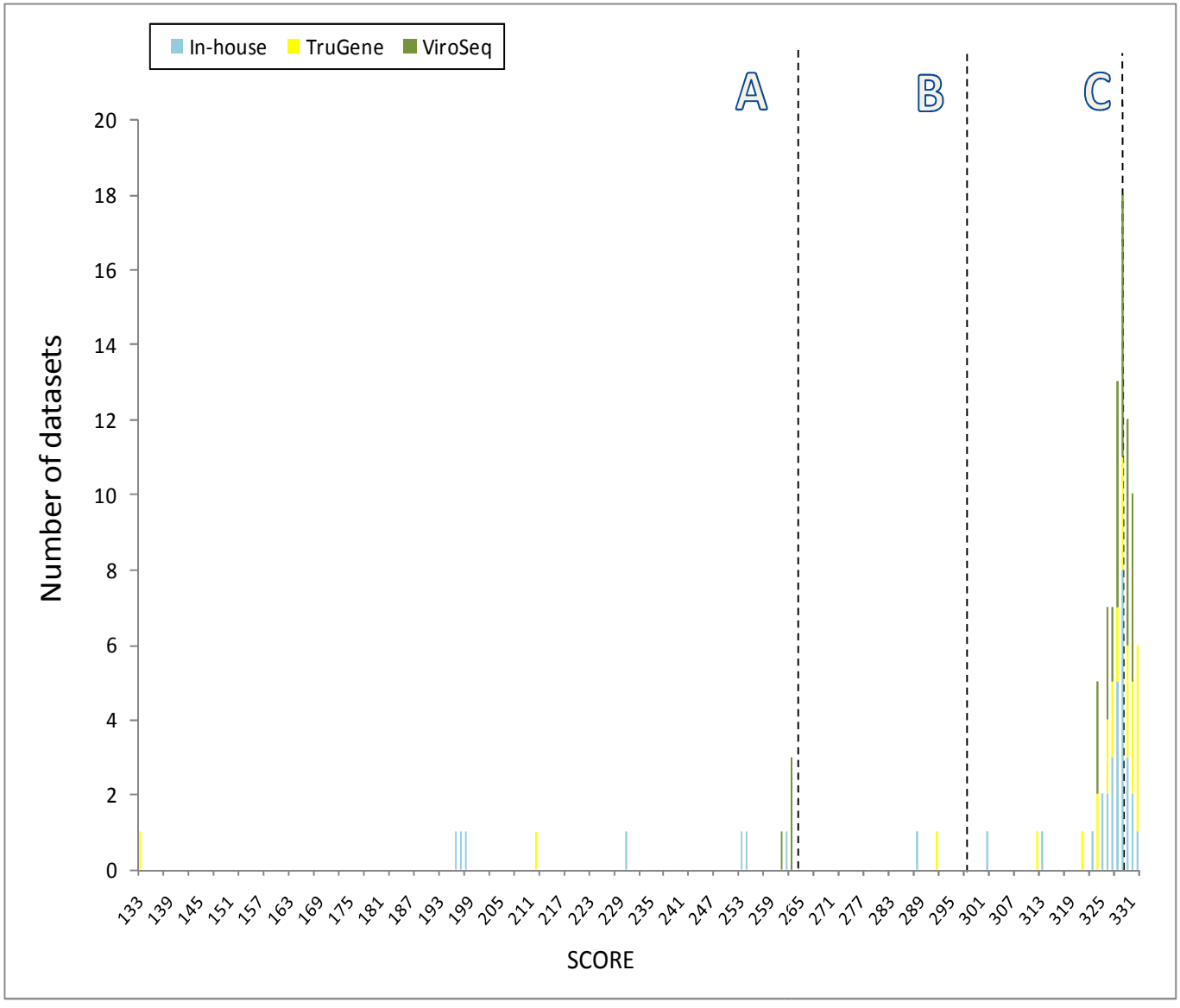


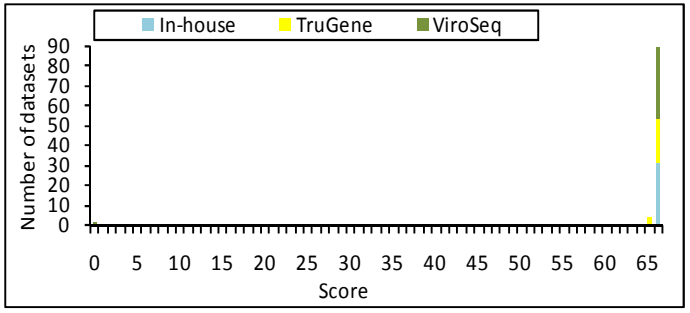
Figure 6: Summary of overall performance scores by technology



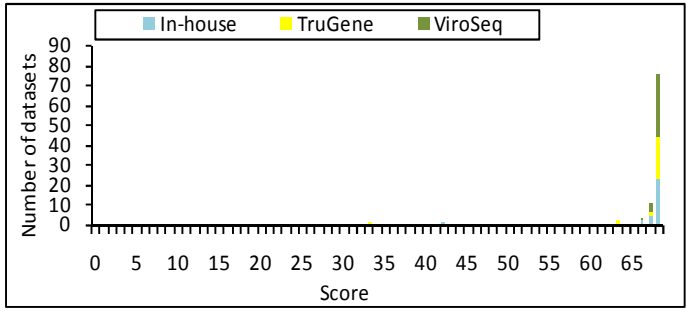
A: 80% (266) of maximum achievable score (332), B: 90% (299) of maximum achievable score (332), C: 99% (329) of maximum achievable score (332).

Figure 7: Summary of performance scores by panel sample

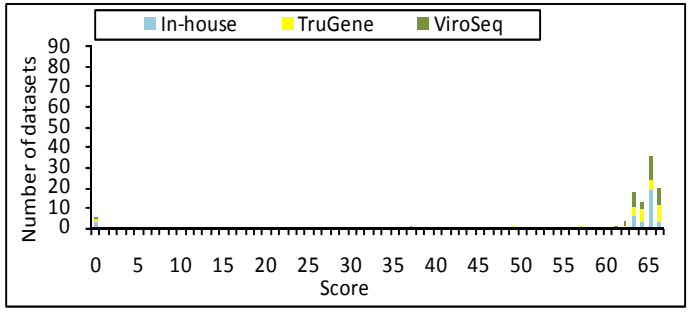
ENVA7-01



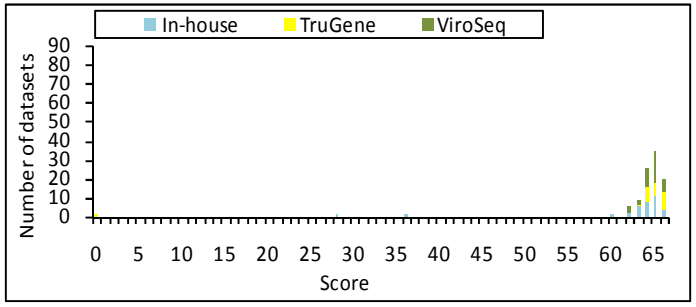
ENVA7-02



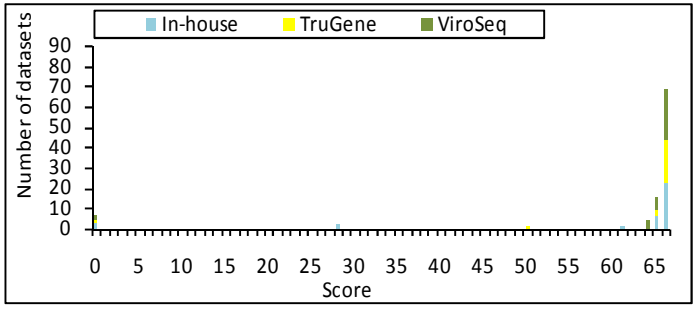
ENVA7-03



ENVA7-04



ENVA7-05



The maximum score achievable for ENVA7-01, ENVA7-03, ENVA7-04 and ENVA7-05 was 66 points each.
The maximum score achievable for ENVA7-02 was 68.

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

4b. Independent testing

Table 5: Summary of the independent testing results returned

Sample	Matrix	Subtype	Independent testing Sequencing summary		
			In-house dye primers (n=1)	Siemens TruGene HIV-1 Genotyping Assay (n=1)	Celera Diagnostics ViroSeq HIV-1 Genotyping System (n=3)
ENVA7-01	Citrate Plasma	C	1 (100 %)	1 (100 %)	3 (100 %)
ENVA7-02*	Citrate Plasma	C	1 (100 %)	1 (100 %)	3 (100 %)
ENVA7-03	Citrate Plasma	B	1 (100 %)	1 (100 %)	3 (100 %)
ENVA7-04	Citrate Plasma	B	1 (100 %)	1 (100 %)	3 (100 %)
ENVA7-05	Citrate Plasma	F	1 (100 %)	1 (100 %)	3 (100 %)

* Two insertions of 3 base pairs each were inserted after codon 67 in Reverse Transcriptase

This table summarises the sequencing data returned by the five independent testing laboratories. These data were used to construct the preliminary consensus sequences that were reported in the preliminary results letter. The sequence data from all five laboratories was in substantial agreement.

Comments

1. The ENVA7 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 B, C or F.

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

3. The majority of datasets were generated using commercial HIV drug resistance genotyping kits (36/98 for ViroSeq [37%] and 27/98 for TruGene [28%]). The remaining 35/98 datasets [36%] were generated with in-house genotyping assays.

4. All technologies were successful in the sequence analysis of the panel samples. There were no systematic negative results with any of the panel samples or technologies. For each of the panel samples the number of complete datasets (covering both the Protease and Reverse transcriptase genes) was comparably high. The number of full datasets was lower for in-house assays (71%), compared to TruGene (85 %) or ViroSeq (89 %). This difference was largely attributable to the lower rate of generating a result for sample ENVA7-05, i.e. the sample containing HIV-1 subtype-F.

5. No systematic errors were observed for the participants' results for any of the technologies, panel samples or resistance codons.

- It was noticed that for sample ENVA7-02, two participants reported a similar but incorrect mutational pattern for the PR gene. Upon in depth analysis of these results it was observed that these participants reported a single nucleotide insert at codon nine, that resulted in a frameshift in the downstream sequence of the PR gene.
- The six nucleotide insert associated with multi-drug resistance to NRTIs, which was present in sample ENVA7-02 after codon 67 was completely or partially missed in seven datasets. These omissions were reported in datasets generated using both the commercials kits as well as using in house assays.

6. Performance scores were high for each of the individual samples, in particular for samples ENVA7-01, ENVA7-02 and ENVA7-05. An overall performance score >99 % of the maximum score was achieved by 46 /98 (47%) of the datasets for the overall panel. This was lower than observed in 2006 for ENVA6 (68%). The lower rate for ENVA7 may (partially) be due to the high frequency of wild type nucleotide detection in the results for the mixed nucleotides present in PR codons 10, 20, 43 and 89 of samples ENVA7-03 and ENVA7-04.

7. The range of performance scores for datasets that did not achieve >99 % of the maximum score was wide and ranged from 133 to 328 (maximum performance score was 332). Low scores were mainly due to missing results for complete genes (PR and /or RT) in a given sample.

8. The lack of consensus in the results for samples ENVA7-03 and ENVA7-04, in particular at codons 10, 20, 43 and 89 of PR, indicates that issues remain in the detection of mixed nucleotides. The detection of resistance mutations as part of a genotype mixture is demanding and needs continuous attention.

9. Performance scores were comparable to those in ENVA6. The assays for genotypic HIV drug resistance determination have evolved into reliable procedures with limited intra-laboratory variation. Future distributions may include a low viral load sample given the current trend is towards genotyping at low viral load.

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