

## CHAPTER IV

### RESULTS

#### 1. Population study

Twenty-six specimens submitted for HCV genotyping to the Molecular Microbiology Laboratory, Ramathibodi Hospital by TRUEGENE HCV 5'NC Genotyping Kit (Visible Genetics, Canada) were consisted of 18 Thai male and 8 Thai female. Of these specimens, 3 specimens were genotype 1a, genotype 1b and 3a were 9 specimens each, 1 specimen was genotype 3b, 1 specimen was genotype 3d, 2 specimens could not classified the subtype in genotype 1 and 1 specimen could not classified the subtype in genotype 3 as shown in table 4.

**Table 4.** The numbers of HCV genotype specimens from Molecular Microbiology Laboratory, Ramathibodi Hospital.

Genotype	1	1a	1b	3	3a	3b	3d	Total
No. of samples	2	3	9	1	9	1	1	26

One hundred infected patients submitted for HCV genotyping to the Molecular Immunology Laboratory, King Chulalongkorn Memorial Hospital, Bangkok, Thailand were consisted of 60 Thai male and 40 Thai female. All of them are chronically infected and candidate for interferon treatment.

## 2. RT-PCR amplification

The 126 HCV RNA specimens were subjected to amplification and sequence analysis of 264 bp within 5' NCR. The amplified products of the first round of the in-house PCR using these two sets of primers would show the amplified products of 332 bp in some samples. For the nested round of the in-house PCR, the amplified products of 264 bp were presence in all specimens.



**Figure 5.** RT-PCR amplification results. The amplification products of HCV 5' NCR were separated on 2 % agarose gel. The first round PCR products were 332 bp and the nested PCR products were 264 bp. Lane 5 was the molecular size marker of 100 bp. Lane 1 and 6 were the negative control. Lane 4 and 9 were the positive control of the first and nested HCV in-house PCR, respectively. Lane 2 and 3 were amplification product of first round PCR. Lane 7 and 8 were HCV RNA positive.

### 3. PCR product purification

After purification by QIAGEN PCR product purification, the O.D. value was analyzed. The O.D. value at 260 nm. of all specimens was varies from 0.113 to 0.282 AU. Then, the concentration of HCV PCR products was varies from 28.25 ng/ul to 70.5 ng/ul. These step used to confirm the present of PCR product and used to calculate the amount of purified product that would be used in the sequencing reactions.

### 4. Sequencing and phylogenetic analysis

A phylogenetic analysis of the reference 5'NCR of HCV that retrieved from <http://hcv.lanl.gov/content/hcv-db/index> was performed to check the reliability of HCV genotype. The reference sequences of 5'NCR of HCV in each genotype were loaded to Clustal W program to construct the phylogenetic tree. Most of sequences were clearly demonstrated in their cluster. There were some references sequences such as 6a.VN.VN506, 6a.VN.VN538, 6a.VN.VN569, 6a.VN.VN571, 6b.TH.TH580, 6k.VN.VN405 showed the cluster that the branches of them were far from most sequence in their genotypes. However, this cluster was clearly separated from the other genotypes.

The extension products of each sample were loaded to ABI PRISM sequencer. After the sequences were analyzed, the data were copied into FASTA format with Chromas program before loading the sequences to Clustal W program. The FASTA format of each forward and reverse sequences were aligned and combined the sequence together. The final sequences were loaded to Clustal W program. The HCV genotypes were identified by grouping with the references clusters.

**Figure 6.** Phylogenetic tree deduced from 5' NCR of HCV reference sequence was presented in the next pages. The same genotypes from different geographic regions are clusters in the same branch and all subtypes are grouped in their respective genotypes.

Figure 6.

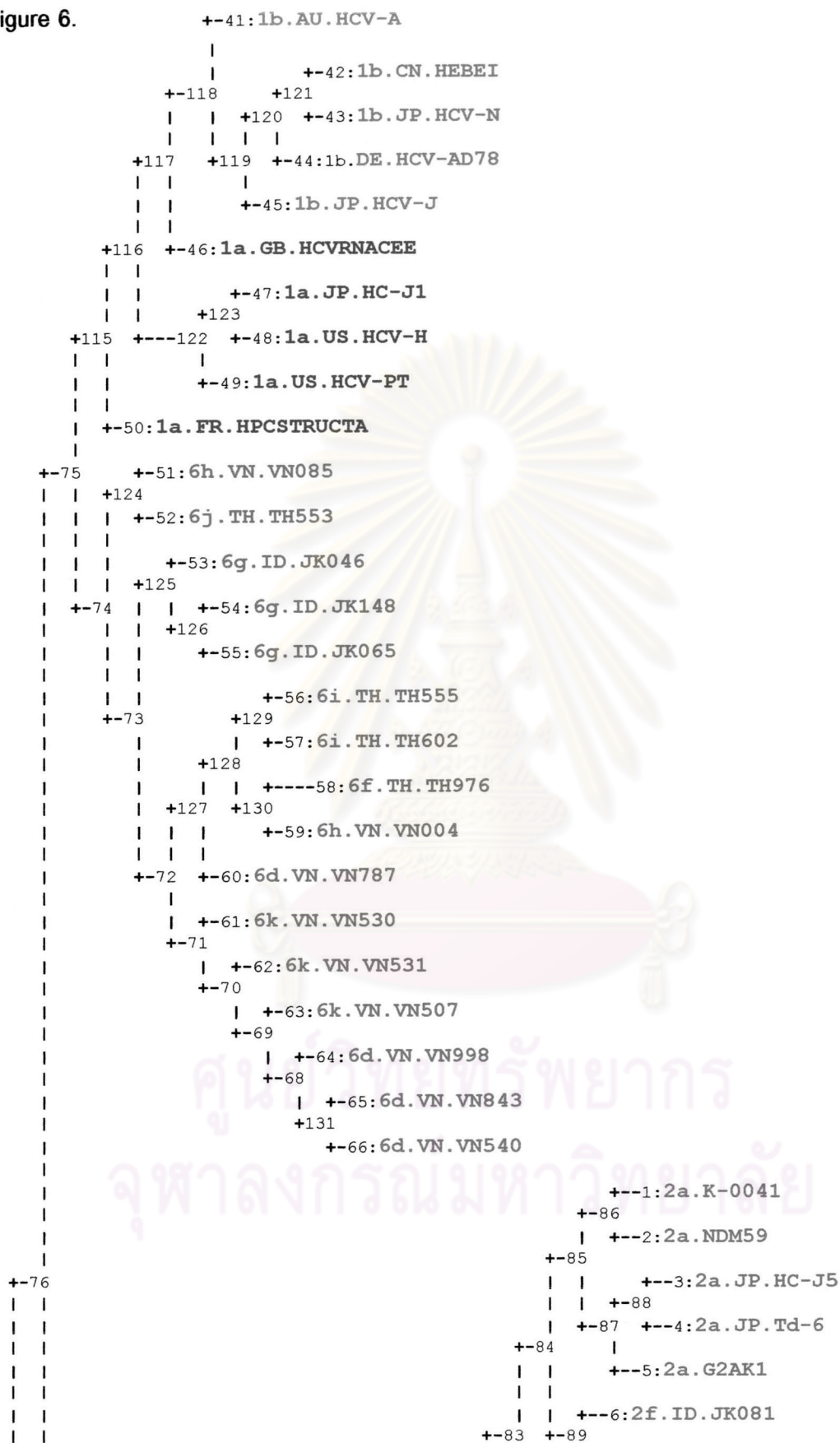
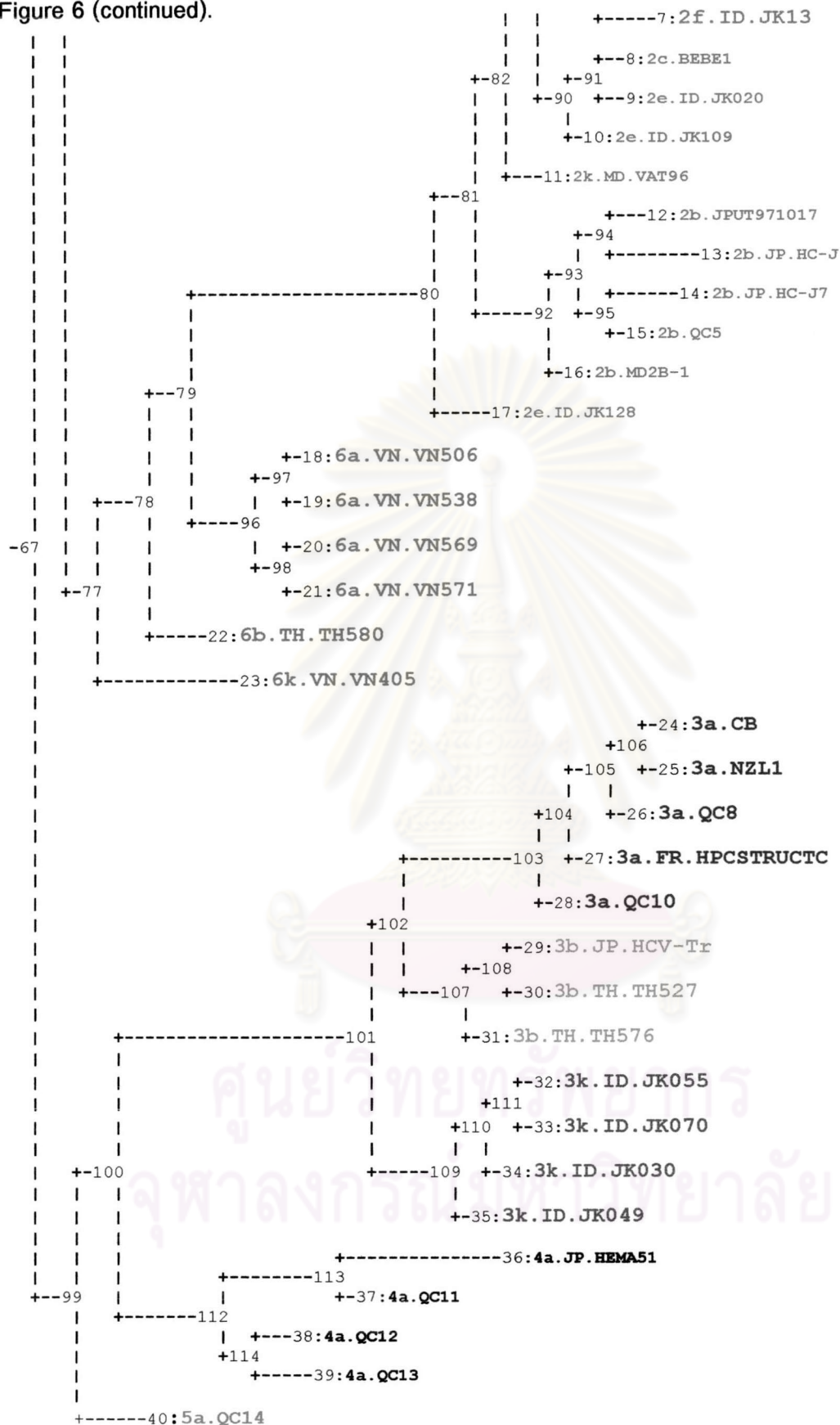


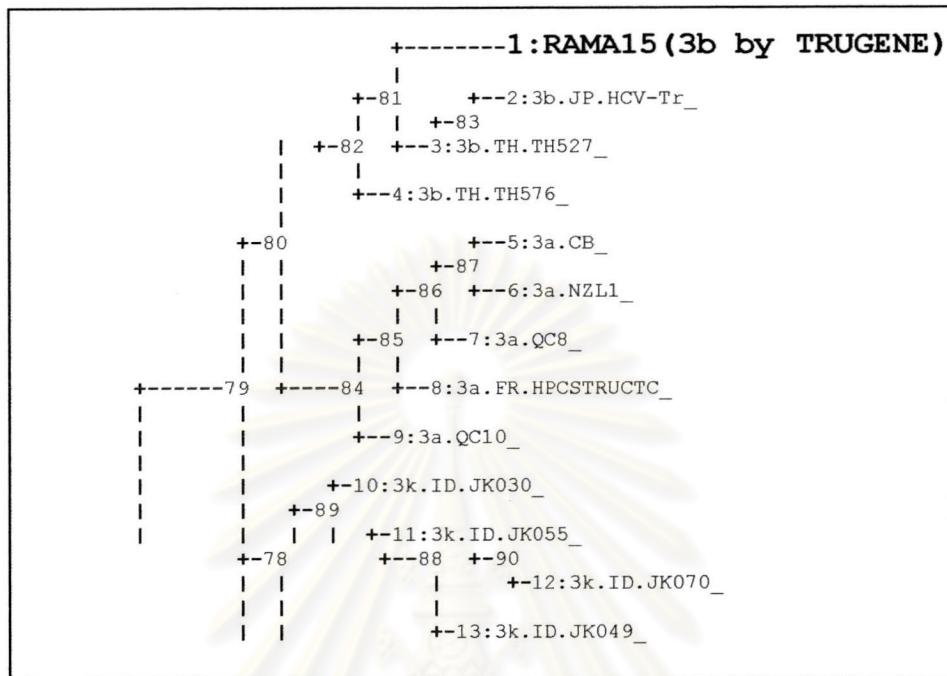
Figure 6 (continued).



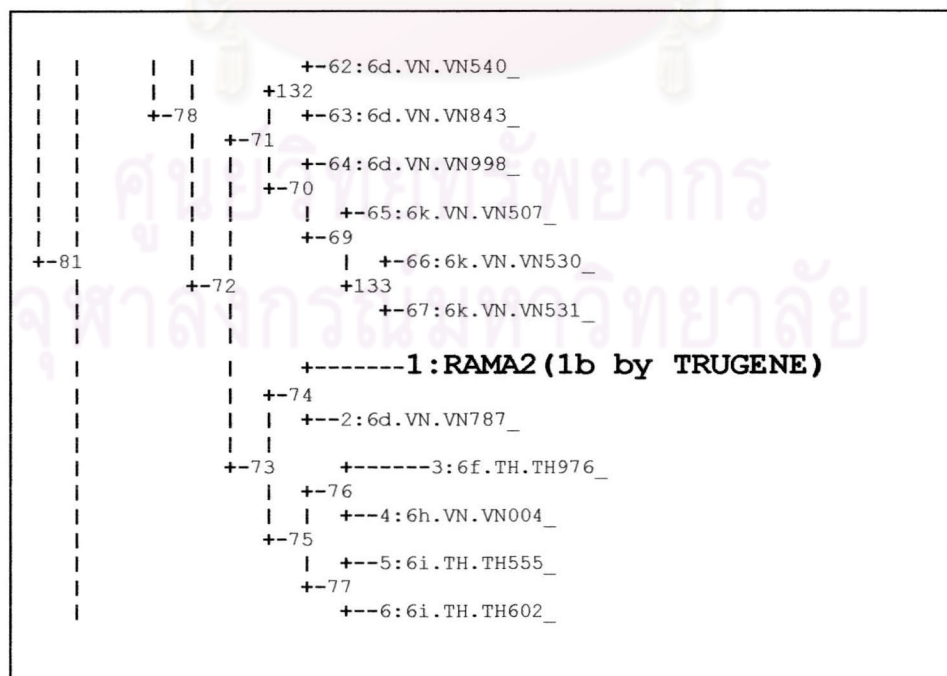




**Figure 10.** Phylogenetic tree of sample with HCV genotype 3b that determined by in-house 5' non coding region direct DNA sequencing assay. The Rama15 sequence is closely related to all 3b reference genotypes.



**Figure 11.** Phylogenetic tree of sample with HCV genotype 6 variants that determined by in-house 5' non coding region direct DNA sequencing assay. Rama2 is closely related to 6d. VN. VN787 and has been grouped in genotype 6 variants.





5. The reliable results of in-house HCV 5' NCR direct sequencing and sequence comparison by phylogenetic construction.

In order to validate the genotyping of in-house 5' NCR direct DNA sequencing assay, the assay was used to genotype the known genotype specimen from Ramathibodi Hospital. A total of 26 specimens were genotype by TRUEGENE HCV 5'NC Genotyping Kit and compared to our result as in table 6.

**Table 6.** Comparison of HCV genotyping results obtained by TRUEGENE HCV 5'NC Genotyping Kit and in-house 5' NCR direct DNA sequencing assay.

HCV genotype from in-house assay	HCV genotype from TRUEGENE assay		
	1	3	6
1	9	0	0
3	2	12	0
6	3	0	0

Typing results obtained by two methods were in complete concordance genotype in 80.77 % (21 of 26). All specimens that TRUEGENE assay identified as genotype 3 could be identified as genotype 3 by in-house 5' NCR genotyping assay. The genotype 1 could be showed the concordance genotype in 9 specimens or 64.29% (9 of 14). The different genotype assignment achieved in 5 specimens. Of these discordance genotype, two specimens were assigned as genotype 3 and three specimens were assigned as genotype 6 by in- house 5' NCR genotyping assay.

The results of two methods showed the concordance subtype in 65.38 %(17 of 26) of the cases. All specimen of genotype 1a were presented the concordance genotype. The nine specimens of genotype 1b presented the concordance result in only 4 cases. The

specimens that identified as genotype 3a or 3b by TRUGENE HCV 5'NC Genotyping kit were identified as genotype 3a or 3b respectively.

For the different subtype, 2 specimens that could not classified the subtype in genotype 1 by TRUGENE HCV 5'NC Genotyping kit were presented as genotype 1b in 1 case and genotype 1a in 1 case. The genotype 1b from TRUGENE HCV 5'NC Genotyping Kit were assigned different genotype in 5 cases: 3 of them are assigned genotype 6 and 2 of them are genotyping as 3a and 3b. The one specimen that could not classified the subtype in genotype 3 was identified as genotype 3a by this method. The one case of genotype 3d was classified into genotype 3a.

**Table 7.** Comparison of HCV subtyping results obtained by TRUGENE HCV 5'NC Genotyping Kit and in-house 5' NCR direct DNA sequencing assay.

HCV subtype from in-house assay (total)	HCV subtype from TRUGENE assay (total)							
	1 (2)	1a (3)	1b (9)	3 (1)	3a (9)	3b (1)	3d (1)	6 variants (0)
1a (4)	1	3	0	0	0	0	0	0
1b (5)	1	0	4	0	0	0	0	0
3a (12)	0	0	1	1	9	0	1	0
3b (2)	0	0	1	0	0	1	0	0
6variants (3)	0	0	3	0	0	0	0	0

**6. The prevalence of the HCV genotypes in patients from King Chulalongkorn Memorial Hospital.**

The in-house 5' non coding region (NCR) direct DNA sequencing assay are used to study the prevalence of HCV genotype in patients from King Chulalongkorn Memorial Hospital. The patients consist of 60 Thai male and 40 Thai female. HCV genotype 3a was the most predominant subtype found in study population with prevalence of about 44% (44 of 100), followed by genotype 1b about 21% (21 of 100), genotype 1a (16%), genotype 3b (12%) and genotype 6 variants (7%) as shown in table 8.

**Table 8.** Prevalence of HCV genotype in patients in King Chulalongkorn Memorial Hospital by in-house 5'NCR genotyping assay.

Genotype	Total (100)
1a	16
1b	21
3a	44
3b	12
6 variants	7

## 7. Increasing the efficiency of genotyping of 5' NCR by sequencing the longer template.

The alignment of 5' NCR based on sequences previously published and obtained in this study as reference were presented in Figure 11 by using Clustal X program. The previous position of the seven variable regions located inside the nested primer pairs in this study (primer 3 and primer 4). There were nucleotides -240 to -233, nucleotides -167 to -155, nucleotides -147 to -142, nucleotides -138 to -132, nucleotides -128 to -118, nucleotides -100 to -92 and nucleotides -81 to -70 that presented in boxes.

The two sets of primers in this study were presented other 3 variable regions at position -317 to -306, position -290 to -275 and position -5 to -1. These variable regions might be useful in genotyping of HCV.

The variations at position -317 to -306 could be used to assign the genotype. Genotype 2 and some of genotype 6 variants were found a G at position -317 while other genotype contained an A in this position. However, these genotype 6 variants were found a T at position -312 while other genotypes were an A. The other genotype 6 variants were presented the gap at this position. Moreover, the genotype 1a and 1b could be differentiated at position -312 to -311. The genotype 1a was found nucleotides GA while genotype 1b was an AG in all published sequence reference. Genotype 3a has GG at position -312 to -311 while genotype 3b was GA. Genotype 4, 5 and some strains of subtype 2a were presented a C at position -309. However, the different between genotype 4 and 5 was found at position -307. Genotype 4 was found a G while an A was found in genotype 5.

Genotype 3 was the only genotype that could be ascribed at position -290 to -275. This genotype obtained a T at position -290 and a G at positions -275 while all other genotypes were an A at this two positions.

The last variations region was found at position -5 to -1. The variation was found at position -2 only. Genotype 3 was found an A, genotype 6 variants were T. Genotype 1,4, 5, and some strains of genotype 2 were C. However, this region may not specific for genotype 2 because some strains were C or T.

**Figure 12.** The sequence alignment of HCV 5' NCR in all genotypes from different geographic regions were presented in the next pages. The seven variation regions between genotypes previously published were presented in boxes with black line. The other 3 variable regions at position -317 to -306, position -290 to -275 and position -5 to -1 presented in boxes with blue line. The two sets of primers in this study were presented in the bottom of the sequences. The primers of first PCR were red sequences and the nested PCR primers were blue sequences.



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	-320	-300	-280		
2a.JP.HC-J	G	C		55	
2a.JP.Td-6	G	C		55	
2a.G2AK1	G	C		55	
2a.K-0041	G			55	
2a.NDM59	G			55	
2k.MD.VAT9	G			55	
2f.ID.JK08	G			55	
2f.ID.JK13	G			55	
2b.MD2B-1	-----			38	
2b.JP.HC-J	G			55	
2b.QC5	-----			44	
2b.JP.HC-J	G			55	
2b.JPUT971	G			55	
2c.BEBE1	G			55	
2e.ID.JK02	G			55	
2e.ID.JK10	G			55	
2e.ID.JK12	G			55	
6a.VN.VN50	G	T	-	54	
6a.VN.VN53	G	T	-	54	
6a.VN.VN56	G	T	-	54	
6a.VN.VN57	G	T	-	54	
6b.TH.TH58		-		54	
6k.VN.VN40		-		54	
1a.JP.HC-J				55	
1a.US.HCV-				55	
1a.US.HCV-				55	
1a.FR.HPCS	---			52	
6g.ID.JK06		-		54	
6g.ID.JK14		-		54	
6j.TH.TH55		-		54	
6k.VN.VN53		-		54	
6k.VN.VN53		-		54	
6k.VN.VN50		-		54	
6g.ID.JK04		-		54	
6d.VN.VN99		-		54	
6d.VN.VN84		-		54	
6d.VN.VN54		-		54	
6f.TH.TH97		-		54	
6h.VN.VN00		-		54	
6i.TH.TH55		-		54	
6i.TH.TH60		-		54	
6d.VN.VN78		-		54	
6h.VN.VN08		-		54	
1a.GB.HCVR	-----	AG		44	
1b.JP.HCV-		AG		55	
1b.AU.HCV-		AG		55	
1b.CN.HEBE		AG		55	
1b.DE.HCV-		AG		55	
1b.JP.HCV-		AG		55	
5a.QC14	-----			43	
4a.QC11	-----	C.G		44	
4a.JP.HEMA	A	C.G	T	55	
4a.QC12	-----	C.G		44	
4a.QC13	-----	C.G		44	
3a.CB		G	T	G	55
3a.QC8	-----	G	T	G	44
3a.NZL1		G	T	G	55
3a.FR.HPCS	-----	G	T	G	47
3a.QC10	-----	G	T	G	44
3b.JP.HCV-			T	G	55
3b.TH.TH52		G	T	G	55
3b.TH.TH57			T	G	55
3k.ID.JK05			T	G	55
3k.ID.JK07			T	G	55
3k.ID.JK03			T	G	55
3k.ID.JK04			T	G	55

gacactcc coatga tCaCTCCCTGTGAGGAACTaCTGTCTTCACGCaGAAAG

-260

-240 R1

-220

2a.JP.HC-J	:	.....	A.	.....	C-	.....	:	109
2a.JP.Td-6	:	.....	A.	.....	C-	.....	:	109
2a.G2AK1	:	.....	A.	.....	C-	.....	:	109
2a.K-0041	:	.....	A.	.....	C-	.....	:	109
2a.NDM59	:	.....	A.	.....	C-	.....	:	109
2k.MD.VAT9	:	.....	A.	.....	C-	.....	:	109
2f.ID.JK08	:	.C.....	A.	.....	C-	.....	:	109
2f.ID.JK13	:	.C.....	A.	.....	C-	.....	:	109
2b.MD2B-1	:	.....	A.	.....	C-	.....	:	92
2b.JP.HC-J	:	.....	A.	.....	C-	.....	:	109
2b.QC5	:	.....	A.	.....	C-	.....	:	98
2b.JP.HC-J	:	.....	A.	.....	C-	.....	:	109
2b.JPUT971	:	.....	A.	.....	C-	.....	:	109
2c.BEBE1	:	.....	A.	.....	C-	.....	:	109
2e.ID.JK02	:	.....	A.	.....	C-	.....	:	109
2e.ID.JK10	:	.....	A.	.....	C-	.....	:	109
2e.ID.JK12	:	.....	A.	.....	C-	.....	:	109
6a.VN.VN50	:	.....		.....	C-	.....	:	108
6a.VN.VN53	:	.....		.....	C-	.....	:	108
6a.VN.VN56	:	.....	A.	.....	C-	.....	:	108
6a.VN.VN57	:	.....	A.	.....	C-	.....	:	108
6b.TH.TH58	:	.....		.....	C-	.....	:	108
6k.VN.VN40	:	.....		.....	-T	.....	:	108
1a.JP.HC-J	:	.....		.....	-	.....	:	109
1a.US.HCV-	:	.....		.....	-	.....	:	109
1a.US.HCV-	:	.....		.....	-	.....	:	109
1a.FR.HPCS	:	.....		.....	-	.....	:	106
6g.ID.JK06	:	.....		.....	-T	.....	:	108
6g.ID.JK14	:	.....		.....	-T	.....	:	108
6j.TH.TH55	:	.....		.....	-	.....	:	108
6k.VN.VN53	:	.....		.....	-	.....	:	108
6k.VN.VN53	:	.....		.....	-	.....	:	108
6k.VN.VN50	:	.....		.....	-	.....	:	108
6g.ID.JK04	:	.....		.....	-	.....	:	108
6d.VN.VN99	:	.....		.....	-	.....	:	108
6d.VN.VN84	:	.....		.....	-	.....	:	108
6d.VN.VN54	:	.....		.....	-	.....	:	108
6f.TH.TH97	:	.....		.....	-	.....	:	108
6h.VN.VN00	:	.....		.....	-	.....	:	108
6i.TH.TH55	:	.....		.....	-	.....	:	108
6i.TH.TH60	:	.....		.....	-	.....	:	108
6d.VN.VN78	:	.....		.....	-	.....	:	108
6h.VN.VN08	:	.....		.....	-	.....	:	108
1a.GB.HCVR	:	.....		.....	-	.....	:	98
1b.JP.HCV-	:	.....	T	.....	-	.....	:	109
1b.AU.HCV-	:	.....		.....	-	.....	:	109
1b.CN.HEBE	:	.....		.....	-	.....	:	109
1b.DE.HCV-	:	.....		.....	-	.....	:	109
1b.JP.HCV-	:	.....		.....	-	.....	:	109
5a.QC14	:	.....	AA	.....	-	.....	:	97
4a.QC11	:	.....	T	.....	-	.....	:	98
4a.JP.HEMA	:	.....	T.A	.....	-	.....	:	109
4a.QC12	:	.....	T.A	.....	-	.....	:	98
4a.QC13	:	.....	T	.....	-	.....	:	98
3a.CB	:	.C.....	C	.....	C-	.....	:	109
3a.QC8	:	.C.....	C	.....	-	.....	:	98
3a.NZL1	:	.C.....	C	.....	-	.....	:	109
3a.FR.HPCS	:	.C.....	C	.....	-	.....	:	101
3a.QC10	:	.C.....	C	.....	-	.....	:	98
3b.JP.HCV-	:	.....	C	.....	C-	.....T	:	109
3b.TH.TH52	:	.....	C	.....	C-	.....	:	109
3b.TH.TH57	:	.....	C	.....	C-	.....	:	109
3k.ID.JK05	:	.....	C	.....	C-	.....	:	109
3k.ID.JK07	:	.....	C	.....	C-	.....	:	109
3k.ID.JK03	:	.....	C	.....	C-	.....	:	109
3k.ID.JK04	:	.....	C	.....	C-	.....	:	109

CGtCTAGCCATGGCGTTAGTAtGAGTGTcGt CAGCCTCCAGG ccCCCCCTcC

	-200	-180	R2	
2a.JP.HC-J	:	:	G..A.	: 64
2a.JP.Td-6	:	:	G..A.	: 64
2a.G2AK1	:	:	G..A.	: 64
2a.K-0041	:	:	G..A.	: 64
2a.NDM59	:	:	G..A.	: 64
2k.MD.VAT9	:	:	G..A.	: 64
2f.ID.JK08	:	:	G..A.	: 64
2f.ID.JK13	:	:	G..A.	: 64
2b.MD2B-1	:	.A.	G.A.A.	: 47
2b.JP.HC-J	:	.A.	G.A.A.	: 64
2b.QC5	:	:	G.A.A.	: 53
2b.JP.HC-J	:	:	G.A.A.	: 64
2b.JPUT971	:	:	G.A.A.	: 64
2c.BEBE1	:	:	G..A.	: 64
2e.ID.JK02	:	:	G..A.	: 64
2e.ID.JK10	:	:	G..A.	: 64
2e.ID.JK12	:	:	G..A.	: 64
6a.VN.VN50	:	:	...T.	: 63
6a.VN.VN53	:	:	...T.	: 63
6a.VN.VN56	:	:	...T.	: 63
6a.VN.VN57	:	:	...T.	: 63
6b.TH.TH58	:	.A.	...T.	: 63
6k.VN.VN40	:	.A.	...T.	: 63
1a.JP.HC-J	:	:	...	: 64
1a.US.HCV-	:	:	...	: 64
1a.US.HCV-	:	:	...	: 64
1a.FR.HPCS	:	:	...	: 61
6g.ID.JK06	:	:	...	: 63
6g.ID.JK14	:	:	...	: 63
6j.TH.TH55	:	:	...	: 63
6k.VN.VN53	:	:	...	: 63
6k.VN.VN53	:	:	...	: 63
6k.VN.VN50	:	:	...	: 63
6g.ID.JK04	:	:	...	: 63
6d.VN.VN99	:	:	...	: 63
6d.VN.VN84	:	:	...	: 63
6d.VN.VN54	:	:	...	: 63
6f.TH.TH97	:	:	...T.	: 63
6h.VN.VN00	:	:	...T.	: 63
6i.TH.TH55	:	:	...	: 63
6i.TH.TH60	:	:	...	: 63
6d.VN.VN78	:	:	...	: 63
6h.VN.VN08	:	:	...	: 63
1a.GB.HCVR	:	-	...	: 52
1b.JP.HCV-	:	:	...	: 64
1b.AU.HCV-	:	:	...	: 64
1b.CN.HEBE	:	:	...	: 64
1b.DE.HCV-	:	:	...	: 64
1b.JP.HCV-	:	:	...	: 64
5a.QC14	:	:	G..T.	: 52
4a.QC11	:	.C.	G....	: 53
4a.JP.HEMA	:	.C.	G..T.	: 64
4a.QC12	:	.T..C.	...T.	: 53
4a.QC13	:	.T..C.	...T.	: 53
3a.CB	:	.C.	TG..GT.	: 64
3a.QC8	:	.C.	TG..GT.	: 53
3a.NZL1	:	.C.	TG..GT.	: 64
3a.FR.HPCS	:	.C.	TG..GT.	: 56
3a.QC10	:	.C.	TG..GT.	: 53
3b.JP.HCV-	:	.C.	G..T.	: 64
3b.TH.TH52	:	.C.	G..T.	: 64
3b.TH.TH57	:	.C.	G..T.	: 64
3k.ID.JK05	:	.C.	G..TT.	: 64
3k.ID.JK07	:	.C.	G..TT.	: 64
3k.ID.JK03	:	.C.	G..TT.	: 64
3k.ID.JK04	:	.C.	G..TT.	: 64

CGGGAGAGCCATAGTgGTCTGCGGAACCGGTGAGTaCACCgAAT gCc Gga G



-160 R2 R3 -140 R4 R5 -120

2a.JP.HC-J	T		A	A	T	C	CC		216
2a.JP.Td-6	T		A	A	T	C	TC		216
2a.G2AK1	T		A	A	T	C	CC		216
2a.K-0041	T		A	A	T	C	CC		216
2a.NDM59	T		A	A	T	C	CC		216
2k.MD.VAT9	T		A	A	T	C	CC		216
2f.ID.JK08	T		A	A	T	C	CC		216
2f.ID.JK13	T		A	A	T	C	CC		216
2b.MD2B-1	T		A	A	T	T.C	TC		199
2b.JP.HC-J	T		A	A	T	T.C	TC	AC	216
2b.QC5	T		A	A	T	T.C	TC		205
2b.JP.HC-J	T		A	A	T	T.C	TC		216
2b.JPUT971	T		A	A	T	T.C	TC		216
2c.BEBE1	T		A	A	T	C	TC		216
2e.ID.JK02	T		A	A	T	C	TC		216
2e.ID.JK10	T		A	A	T	C	C		216
2e.ID.JK12	T		A	A	T	C	C		216
6a.VN.VN50		CA							218
6a.VN.VN53		CA							218
6a.VN.VN56		CA							218
6a.VN.VN57		CA							218
6b.TH.TH58		CA							218
6k.VN.VN40					G				215
1a.JP.HC-J			A					C	216
1a.US.HCV-			A						216
1a.US.HCV-									216
1a.FR.HPCS			G						213
6g.ID.JK06									215
6g.ID.JK14									215
6j.TH.TH55									215
6k.VN.VN53									215
6k.VN.VN53									215
6k.VN.VN50									215
6g.ID.JK04									215
6d.VN.VN99									215
6d.VN.VN84									215
6d.VN.VN54									215
6f.TH.TH97			T		G				215
6h.VN.VN00			T						215
6i.TH.TH55			T						215
6i.TH.TH60			T						215
6d.VN.VN78			A						215
6h.VN.VN08									215
1a.GB.HCVR									204
1b.JP.HCV-									216
1b.AU.HCV-									216
1b.CN.HEBE									216
1b.DE.HCV-									216
1b.JP.HCV-									216
5a.QC14			A			C			204
4a.QC11			T			C	A		205
4a.JP.HEMA			T			C	A		216
4a.QC12							A		206
4a.QC13			T				A		206
3a.CB			A			A	CA	A	216
3a.QC8			A			A	CA	A	205
3a.NZL1			G			A	CA	A	216
3a.FR.HPCS			G			A	CA	A	208
3a.QC10			A			A	C	A	205
3b.JP.HCV-			A				C	A	216
3b.TH.TH52			A				C	A	216
3b.TH.TH57			A					A	216
3k.ID.JK05			A	C			C	A	216
3k.ID.JK07			A	T			C	A	216
3k.ID.JK03			A	T			C	A	216
3k.ID.JK04			A	T			C	A	216

AC GGGTCCTTTC TTGGat a ACCC CTC aTgcC gG ATTTGGGCgtGC

-100 R6

-80 R7

-60

2a.JP.HC-J	A	C	T	271
2a.JP.Td-6	A	C	T	271
2a.G2AK1	A	C	T	271
2a.K-0041	A	C	T	271
2a.NDM59	A	C	T	271
2k.MD.VAT9	A	C	T	271
2f.ID.JK08	A	C	T	271
2f.ID.JK13	A	C	T	271
2b.MD2B-1	A	C	T	254
2b.JP.HC-J	A	C	T	271
2b.QC5	A	C	T	260
2b.JP.HC-J	A	C	T	271
2b.JPUT971	A	C	T	271
2c.BEBE1	A	C	T	271
2e.ID.JK02	A	C	T	271
2e.ID.JK10	A	C	T	271
2e.ID.JK12	A	C	T	271
6a.VN.VN50	A	C	T	273
6a.VN.VN53	A	C	T	273
6a.VN.VN56	A	C	T	273
6a.VN.VN57	A	C	T	273
6b.TH.TH58	A	C	T	273
6k.VN.VN40	A	C	T	270
1a.JP.HC-J	A	C	T	271
1a.US.HCV-	A	C	T	271
1a.US.HCV-	A	C	T	271
1a.FR.HPCS	A	C	T	268
6g.ID.JK06	A	C	T	270
6g.ID.JK14	A	C	T	270
6j.TH.TH55	A	C	T	270
6k.VN.VN53	A	C	T	270
6k.VN.VN53	A	C	T	270
6k.VN.VN50	A	C	T	270
6g.ID.JK04	A	C	T	270
6d.VN.VN99	A	C	T	270
6d.VN.VN84	A	C	T	270
6d.VN.VN54	A	C	T	270
6f.TH.TH97	A	C	T	270
6h.VN.VN00	A	C	T	270
6i.TH.TH55	A	C	T	270
6i.TH.TH60	A	C	T	270
6d.VN.VN78	A	C	T	270
6h.VN.VN08	A	C	T	270
1a.GB.HCVR	A	C	T	259
1b.JP.HCV-	A	C	T	271
1b.AU.HCV-	A	C	T	271
1b.CN.HEBE	A	C	T	271
1b.DE.HCV-	A	C	T	271
1b.JP.HCV-	A	C	T	271
5a.QC14	A	C	T	259
4a.QC11	A	C	T	260
4a.JP.HEMA	A	C	T	271
4a.QC12	A	C	T	261
4a.QC13	A	C	T	261
3a.CB	A	C	T	271
3a.QC8	A	C	T	260
3a.NZL1	A	C	T	271
3a.FR.HPCS	A	C	T	263
3a.QC10	A	C	T	260
3b.JP.HCV-	A	C	T	271
3b.TH.TH52	A	C	T	271
3b.TH.TH57	A	C	T	271
3k.ID.JK05	A	C	T	271
3k.ID.JK07	A	C	T	271
3k.ID.JK03	A	C	T	271
3k.ID.JK04	A	C	T	271

CCCCGC AGActgCTAGCCgAGTAG GTTGGGT GCGAAaGGCctTGtGGTACTG

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2a.JP.HC-J	:	.....	C	321
2a.JP.Td-6	:	.....	C	321
2a.G2AK1	:	.....		321
2a.K-0041	:	.....	C	321
2a.NDM59	:	.....	C	321
2k.MD.VAT9	:	.....	C	321
2f.ID.JK08	:	.....	C	321
2f.ID.JK13	:	.....	T	321
2b.MD2B-1	:	.....		304
2b.JP.HC-J	:	.....		321
2b.QC5	:	.....		310
2b.JP.HC-J	:	.....	T	321
2b.JPUT971	:	.....	C	321
2c.BEBE1	:	.....		321
2e.ID.JK02	:	.....		321
2e.ID.JK10	:	.....		321
2e.ID.JK12	:	.....		321
6a.VN.VN50	:	.....		323
6a.VN.VN53	:	.....		323
6a.VN.VN56	:	.....		323
6a.VN.VN57	:	.....		323
6b.TH.TH58	:	.....	A	323
6k.VN.VN40	:	.....	A	320
1a.JP.HC-J	:	.....	C	321
1a.US.HCV-	:	.....	C	321
1a.US.HCV-	:	.....	C	321
1a.FR.HPCS	:	.....	C	318
6g.ID.JK06	:	.....		320
6g.ID.JK14	:	.....		320
6j.TH.TH55	:	.....		320
6k.VN.VN53	:	.....		320
6k.VN.VN53	:	.....		320
6k.VN.VN50	:	.....		320
6g.ID.JK04	:	.....		320
6d.VN.VN99	:	.....		320
6d.VN.VN84	:	.....		320
6d.VN.VN54	:	.....		320
6f.TH.TH97	:	.....		320
6h.VN.VN00	:	.....		320
6i.TH.TH55	:	.....		320
6i.TH.TH60	:	.....		320
6d.VN.VN78	:	.....		320
6h.VN.VN08	:	.....		320
1a.GB.HCVR	:	.....		309
1b.JP.HCV-	:	.....		321
1b.AU.HCV-	:	.....	C	321
1b.CN.HEBE	:	.....	C	321
1b.DE.HCV-	:	.....	C	321
1b.JP.HCV-	:	.....	C	321
5a.QC14	:	.....	C	309
4a.QC11	:	.....	C	310
4a.JP.HEMA	:	.....	C	321
4a.QC12	:	.....	C	311
4a.QC13	:	.....	C	311
3a.CB	:	.....	A	321
3a.QC8	:	.....	A	310
3a.NZL1	:	.....	A	321
3a.FR.HPCS	:	.....	C	313
3a.QC10	:	.....	A	310
3b.JP.HCV-	:	.....	A	321
3b.TH.TH52	:	.....	A	321
3b.TH.TH57	:	.....		321
3k.ID.JK05	:	.....	A	321
3k.ID.JK07	:	.....	A	321
3k.ID.JK03	:	.....	A	321
3k.ID.JK04	:	.....		321

CCTGATAGGGTGCCTTCCGAGTGCCTCCCGGAGGtCTCGTAGACCGTGCA c