Hepatitis B Virus Genotypes in Latvia

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Abstract: The aim of this study was to investigate the properties of HBV genomes isolated from patients with acute and chronic hepatitis, liver cirrhosis, and hepatic coma admitted to the Infectology Center of Latvia. HBV genotypes and HBsAg subtypes were determined by direct S-gene sequencing. Genotypes D (72.17%) and A (27.96%) were predominant. Only one case (0.87%) of HBV genotype E was found. Prevalence of genotype D over genotype A was more strongly pronounced in isolates from patients with acute hepatitis than from patients with chronic hepatitis– 74%-24% and 68%-32%, respectively. Number of previously described and some new mutations (Ser21Thr in preC region, Tyr134Lys, Phe134Leu, Ile110Met, Thr118Ile and Ser136Tyr in S-gene) were discovered by direct sequencing of PCR fragments corresponding to preS/S and preC/C regions and to a fragment of the X gene of HBV genome. The most frequently mutations were found in the region of the basal core promoter, preC and major immunodominant region of core protein and domain *a* of S protein. In general the rate of mutations discovered in chronic patients was just 1,23 times higher then in patients with acute hepatitis, at the same time the ratio for the hot-spot preC 1896 stop mutation was higher and reached 1,81. A possible role of discovered mutations in the HBV pathogenesis is discussed. The findings are relevant to diagnosis and prognosis of HBV-induced liver disease.

Keywords: Hepatitis B virus, genotype, subtype, variant, mutation.

INTRODUCTION

The clinical course of hepatitis B virus (HBV) infection varies from self-limited and asymptomatic acute to fulminant hepatitis. A definite population of patients with acute HBV infection (AH) develops chronic liver disease: chronic hepatitis (CH), liver cirrhosis (LC), and hepatocellular carcinoma (HCC). HBV is classified into 8 genotypes from A to H based on more than 8% sequence divergence of the entire genome sequences [1-3]. The HBV genotypes appear to be associated with particular geographic distribution, ethnicity, and possibly diagnoses and clinical outcome of the liver disease. At first, the HBV genotypes have distinct geographical distributions, e.g. A – Northwest Europe, North America, and Central Africa; D – Mediterranean area, Middle East, and India; E – sub-Saharan Africa. The HBV genotypes are divided into clusters or subgenotypes with 4 to 8% sequence divergence. Existence of two subgenotypes within the genotype A is described for South Africa [4]. These subgenotypes are assigned as A1 and A2 and the major difference between them is associated with to amino acid (aa) residues 207 and 209 in the HBs protein S (Asn/Leu and Ser/Val substitutions for A1 and A2, respectively). It has been pointed out that European HBV strains belong to the subgenotype A2, whereas Afro-Asian strains belong to the subgenotype A1. A subgenotype A3, found in Cameroon, is a recombinant of the genotypes A and E [5]. The most widespread in the world is the genotype D. This genotype is divided into four subgenotypes D1 to D4. The geographic distribution of these subgenotypes is less restricted than that for the genotype A, although the strains from the Middle East mainly belong to D1, from South Africa and Alaska to D3, and from Oceania and Somalia to D4 [6]. None of these subgenotypes is characterized with specific aa substitutions.

Traditionally, HBV is classified into 9 hepatitis B surface antigen (HBsAg) subtypes: *adw2, adw4, adrq*$^{+}$*, adrq*$^{-}$*, ayw1, ayw2, ayw3, ayw4* and *ayr* according to the phenotypic expression of the HBs-encoding part of the gene S of different HBV genotypes [7]. Epidemiological studies show that the prevalence of these subtypes also varies in different parts of the world and that they probably have a different clinical significance [8,9]. The *adw2* and *ayw1* subtypes usually belong to the genotype A, whereas the *ayw2, ayw3, ayw4* and *adw*3 subtypes belong to the genotype D. The *ayw4* subtype appears in the genotype E.

Despite the introduction of anti-HBV vaccination in 1997 and constant improvement of medical care in Latvia, the number of hepatitis B cases remains high. In previous papers we described appearance of HBV genotypes in Latvia [10,11] and their distribution in two Latvian clinics: the Kidney transplantation Centre and the Hematology Unit of the Children's Clinical University Hospital in Riga [12,13]. Due to the specific nature of the clinics obtained data represented exclusively the HBV genome distribution for different sorts of chronic HBV infection.

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Table 1. Distribution of Patients Admitted to Study by Sex, Age, Gender and Major HBV and HCV Markers

Desase	Total	Sex	Number	Age	HBV DNA +	HBV DNA -	a-HCV +
Acute hepatitis	89	m	47	34.82±17.42	42	5	8
		f	42	44.22±18.26	40	2	3
Chronic hepatitis	33	m	24	40.91±17.65	19	5	7
		f	9	36.00±19.87	6	3	0
Other liver damages	9	m	6	27.67±2.89	5	1	1
		f	3	69.50±7.78	3	0	0
Total	131	m	77	35.69±17.08	66	11	16
		f	54	44.25±18.92	49	5	3

The aim of this study is to evaluate a present epidemiological distribution of the HBV genotypes, subgenotypes, and subtypes among patients of the Infectology Center of Latvia (ICL), a major medical institution in this field in Latvia, whose main specialization is acute HBV infection. We present here the appearance of mutations within the HBV genome regions EnhII/BCP/X-preC/C and preS/S against the HBV genome prototypes. Obtained results shows that the distribution of the HBV genotypes in Latvia remains the same for all kinds of HBV-induced liver disease patients and diagnoses, and the profile of the medical institutions involved. More than 20-years long persistence of area-specific HBV strains with preserved “old” mutations reveals a clear predominance of “local” HBV genotypes, despite the rapid development and growing migration rate in Latvia.

MATERIALS AND METHODS

Patients

A total of 131 (54 female and 77 male) consecutive patients of the ICL from December 1st 1997 to December 31st 2006 were admitted to the study. Diagnosis: AH, CH, LC, autoimmune hepatitis, and hepatic coma (Table **1**). Diagnosis was based on traditional clinical, biochemical, and immunochemical hepatitis B marker tests. The mean age of the female and male patients was 44.25 ± 18.92 years and 35.69 ± 17.08, respectively. State agency "Infectology Center of Latvia" and the Scientific Council of the Biomedical Research and Study Centre approved the protocol of this study and appropriate research permission was obtained from the independent Ethics Committee of Latvia. Informed consent to participate into this study was obtained from all included patients.

Serological Tests

The serum samples were stored at -20 °C until assay was performed. All serum samples were tested for the presence of HBsAg (ETI-MAK-4, DiaSorin, Saluggia, Italy and Enzygnost HBsAg 5.0, Dade Behring, Marburg, Germany), total antibodies against hepatitis B core antigen (anti-HBc) (ETI-AB-COREK-2, DiaSorin, Saluggia, Italy), as well as for anti-HBc IgM, and anti-HBs. HBsAg positive samples were tested for hepatitis B e antigen (HBe) (ETI-EBK-2, DiaSorin, Saluggia, Italy) and antibodies against it (anti-HBe) (ETI-AB-EBK, DiaSorin, Saluggia, Italy). The study protocol was in agreement with rules of Latvian Ethics Committee.

PCR Amplification and Sequence Analysis

HBV DNA was extracted from 50 µl of serum with a commercially available DNA-RNA isolation kit based on

Table 2. PCR Primers Used in Study

Region of Analysis		Primer	Sequence
pres/S	2750-813 nt	17p	5’ TTATTTACATACTCTTTGGAAGGC 3'
		8.15	5’ AATGTATACCCAAAGACAG 3’
gene S (nested)	215-710 nt	S1p	5' TTGTTGACAAGAATCCTCACAATACC 3'
		S2p	5' GCCCTACGAACCACTGAACAAATGG 3'
preC/C	1741-2516 nt	M3	5' CTGGGAGGAGTTGGGGGA 3'
		p19	5' AGGTACTGTAGAGGAATAAAGCCC 3'
geneC (nested)	2045-2387nt	C1"	5' GTTCACCTCACCATACTGCACTCAGGC 3'
		C2'	5' GAGTTCTTCTTCTAGGGGACCTGCCTCG 3'
BCP/preC (nested)	1741-2114 nt	M3	5' CTGGGAGGAGTTGGGGGA 3'
		2.364	5’ CCCAGGTAGCTAGAGTCAT 3'
gene X	1505-1825 nt	21p	5' CGTTCCAGCCGACCACGGGGCGC 3'
		2-Sp	5' AAAAAGTTGCATGGTGCTGG 3'

phenol/chloroform extraction (Lyteh, Moscow, Russia). HBV genome fragments carrying the whole preS/S, preC/C regions, and a fragment of the X gene, which included the EnII/BCP region, were PCR-amplified with several primer sets (Table **2**), as previously described [13]. When amplification of the whole fragments failed, we used nested PCR with inner primers. Amplified HBV DNA fragments were sequenced in both directions directly using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction (Applied Biosystems, Foster City, Calif., USA) and electrophoregrams were obtained on an ABI Prism 377 sequencer (Applied Biosystems). In all cases PCR primers were used as sequencing primers. For sequencing of the complementary strand of the HBV preS region, an additional primer p14 – 5' CTGTAA CACGAGCAGGGGTCCTAG 3' was used [14]. The sequences were edited manually with the BioEdit Sequence Alignment Editor and subsequently aligned in the FASTA format (http://ngfnblast.gbf.de/docs/fasta.html). The phylogenetic tree was constructed by the MEGA program using the UPGMA algorithm.

RESULTS AND DISCUSSION

Sera of patients were divided into three groups: first group from 89 patients with AH, second one from 33 patients with CH, and third one from 9 patients with LC, autoimmune hepatitis, hepatic coma, and fulminant hepatitis (FH). The last group was named "other liver damage" (Table **1**). All AH sera patients were HBsAg and anti-HBc IgM positive. Sera of 19 patients contained anti-HCV antibodies. One serum of an AH patient contained anti-HAV antibodies and one of the CH patients was positive for HAV. Serological data concerning presence of HBsAg and HBe/anti-HBe patient status are listed in Table **3**.

All samples were tested for HBV DNA by PCR; HBV DNA was detected in sera from 115 of the 131 patients (87.79 %), 16 samples were negative (12.21 %) (Table **1**).

Phylogenetic Analysis of HBV Genotypes

The genotype of each HBV sequence was determined by comparison with sequences of known HBV genotypes A-H in the GenBank and EMBL nucleotide databases using the BLAST program. Phylogenetic tree was constructed for the HBV gene S (Fig. **1**). Reference sequences, which are shown with their accession numbers, were obtained from the EMBL/GenBank database. Despite the multinational society existing in Latvia and increasing population mobility during recent years, we found only genotypes A (26.96% of cases) and D (72.17% of cases) and only one case of a "foreign" genotype E (0.87%) in the studied patients. Phylogenetic analysis clearly showed similarity between sequences of the genotype A, whereas the genotype D sequences were more divergent. All genotype A isolates clustered together with A2 subgenotype sequences from the GenBank database and contained typical aa residue Ser at position 207 and Val at position 209. This means that the genotype A strains possess a rather low level of divergence and are "newcomers" to Latvia. The genotype D isolates had a more pronounced level of divergence and fell into three main clusters. The major cluster consisted of 48 isolates, which shared some similarity with the strains from the D2 subgenotype [6]. From this cluster, some subclusters were distinguished. Two isolates showed similarity with the strains from the D1

Table 3. Frequency of Specific Immunological Markers in Two Groups of Patients: AH, CH and other Liver Damages

Disease	Total	HBsAg+ HBeAg+	HBsAg+ a-HBe +	HBsAg+	HBsAg- a-HBe+
Acute hepatitis	89	27	26	1	13
Chronic hepatitis	33	16	8	3	5
Other liver damages	9	3	2	3	1

Table 4. Distribution of HVB Genotypes, Subgenotypes and HBsAg Subtypes between LIC Isolates. Number of Isolates with Mutations is Shown in Brackets

Disease	Total	Sex	Number	Genotypes, Subgenotupes and HBsAg Subtypes										
				D							A		E
				D1	D2		D3		D?	S gene PCR negative	A2	S gene PCR negative	ayw4
				ayw2	ayw3	ayw2	ayw2	ayw3			adw2		
Acute hepatitis	82	m	42(25)	0	22(13)		2(2)	1(1)	0	5(1)	10(8)	2	0
		f	40(23)	0	21(11)		3(1)		0	7(5)	6(4)	2(1)	1(1)
Chronic hepatitis	25	m	19(9)	1	1		1(1)		1(1)	8(4)	4(3)	3(2)	0
		f	6(4)	0	1	1	2(2)		0	1(1)	1(1)	0	0
Other liver damage	8	m	5(4)	0	2(2)			0	0	0	3(2)	0	0
		f	3(2)	1	1(1)			0	0	1(1)	0	0	0
Total	115	m	66(40)	1	25(15)		4(4)		1(1)	13(4)	17(13)	5(2)	0
		f	49(29)	1	24(12)		5(3)		0	9(7)	7(5)	2(1)	1(1)

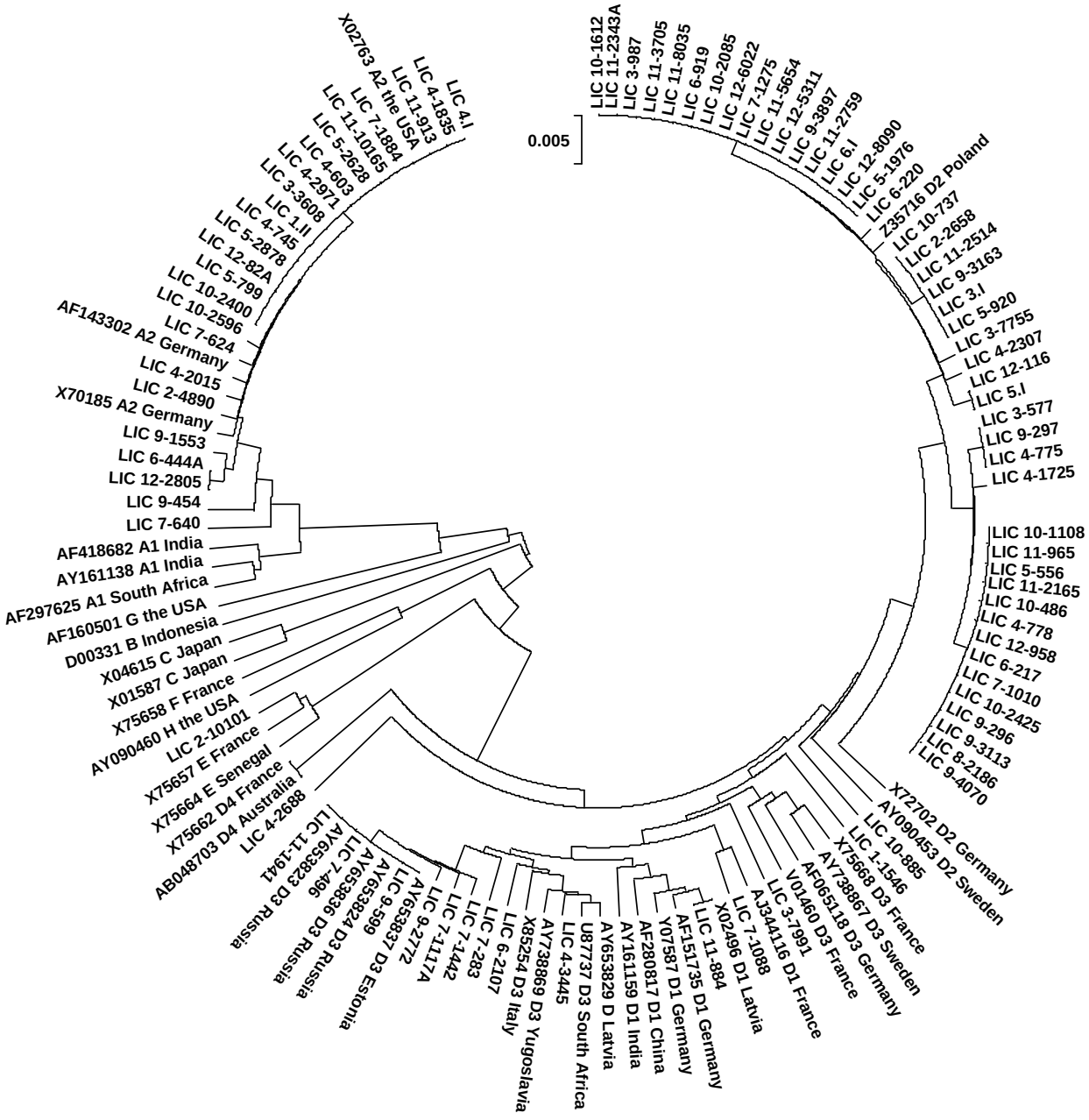

Fig. (1). The UPGMA phylogenetic tree based on sequences of fragments from HBV S-gene. Reference isolates are given specifying GeneBank access number, genotype and country of origin.

subgenotype. Nine isolates were similar to the subgenotype D3 genome.

Since monospecific subtype-determining antibodies were not available in Latvia, the HBV subtypes were determined by analysis of gene S sequences according to Magnius & Norder [7]. 26.32 % of our isolates belonged to the subtype *adw2*, and 10.53% and 47.37 % represented the subtypes *ayw2* and *ayw3*, respectively. In the case of one patient (1.32%), LIC1-1546, it was impossible to define subtype, since mutation affected aa 127 of HBsAg, which is subtype-specific. In 22 cases (13.16%) of the genotype D and 7 cases (3.95%) of the genotype A, it was impossible to amplify the S region and determine subtypes, probably due to mutations within regions adjacent to primers (Table **4**). All subgenotype A2 strains were of the *adw*2 subtype. All subgenotype D2 strains belonged to the *ayw*3 subtype. Two strains of the *ayw*2 subtype were of the D1 subgenotype. Eight strains with the subtype *ayw2* and one of the *ayw*3 subtype belonged to the D3 subgenotype. The distribution of genotypes and subtypes within different groups of patients is listed in Table **4**.

Sequence Variation in the HBV Genomes

It is known that mutations appear in different regions of the HBV genome, and probably each stage of hepatitis B infection may be affected by the appearance of specific mutations [15]. Thorough analysis of major HBV regions EnhII/BCP/X-preC/C and preS/S of three groups of patients

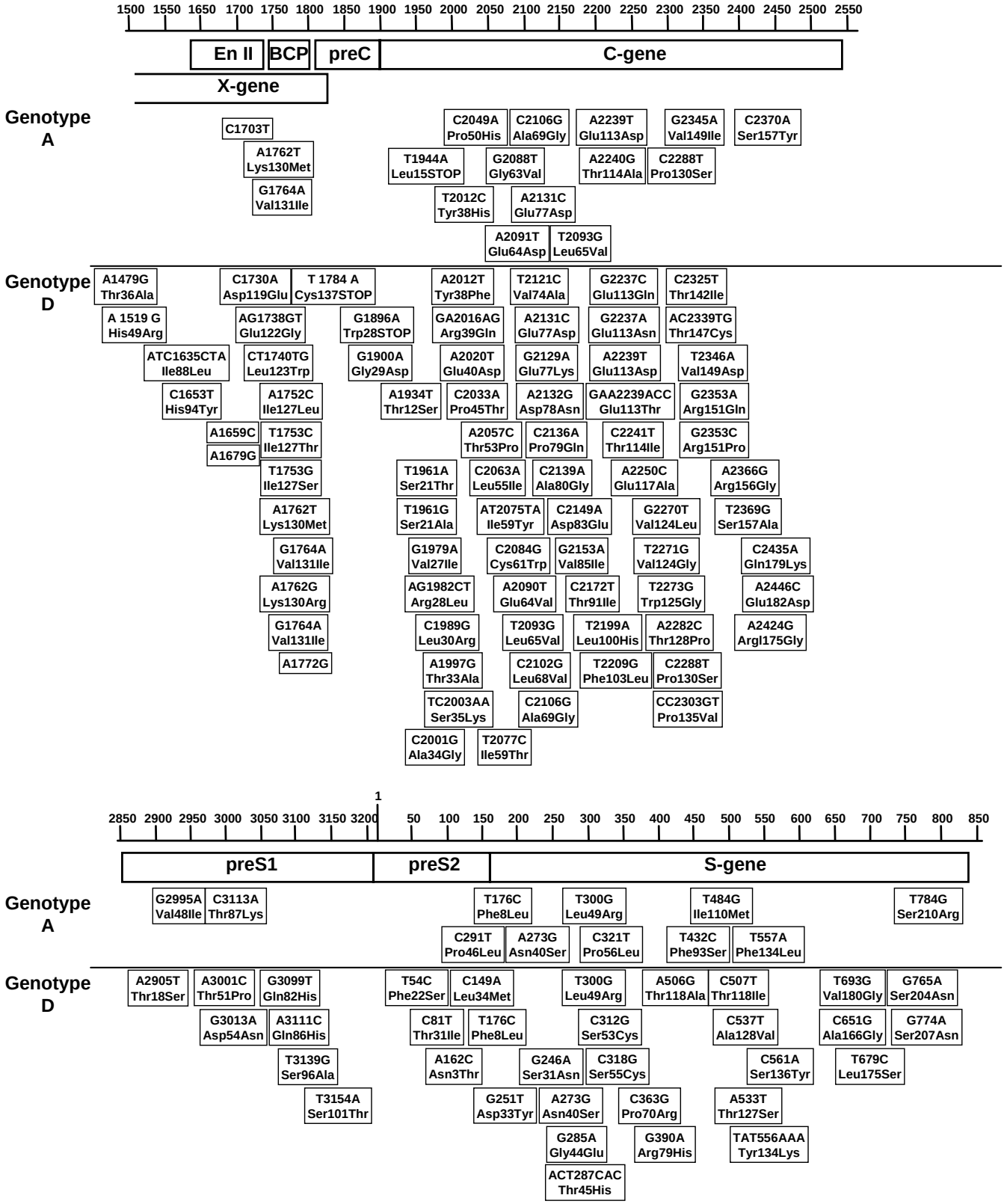

Fig. (2). Mutations discovered in tested HBV isolates.

was performed with the intention of revealing an association of the appearing mutations with the clinical course of HBV infection. Sequences of analyzed HBV regions compared with reference HBV sequences are given in Appendix. Detailed analysis of sequenced HBV genes is presented in Figs. (**2** and **3**). After genotype- and subtype-specific mutations, it reveals also the presence of silent and missense mutations.

Examining the distribution of mutations within the groups of patients and HBV genotypes (Fig. **3**), we may conclude that, in general, in groups of CH patients and patients with other liver damage, mutations occur more frequently than in groups of AH patients. On the other hand, strains of genotype D contain more mutations when compared to strains of genotype A.

The distribution of the mutations on the genome of HBV correlates well enough with arrangement of the functionally and immunologically important regions. On the diagram we can see some hot spots corresponding to BCP, preC, MIR region in C-gene, and domain *a* in S-gene.

Gene X/EnII/BCP

The BCP mutations A1762T and G1764A, most frequently observed in HBV-infected patients with CH, HCC, and FH, were found in one CH and six AH cases. Additional mutations at C1653T were found in two AH cases and T1753C in four AH and one CH case. These mutations may also be associated with the progression of liver disease to cirrhosis [16]. One AH isolate contained mutation at position T1753C in conjunction with the G1764A mutation. One CH isolate contained G1764T and C1765G mutations, which resulted in Val131Leu substitution in the gene X, in conjunction with the T1752C and T1753G mutations, which led to Ile127Thr substitution. The C1653T mutation led to the His94Tyr substitution in the immunodominant epitope of the protein X [17]. The A1752C mutation in patients with CH was reported also in conjunction with the 1762 and 1764 mutations and appeared to be associated with liver damage [18]. We found this mutation in five cases, and in all cases it was independent of other mutations. Two AH samples contained aa substitution Ile88Leu within the protein X. One AH isolate possessed T1784A mutation, which led to conversion of Cys137 to stop codon. Other individual AH isolates demonstrated A1479G mutation leading to the Thr36Ala substitution, A1519G mutation leading to His49Arg substitution, and C1730A mutations leading to Asp119Glu substitution. One AH isolate carried four mutations: AG1738GT and CT1740TG, which changed two aa residues: Glu122Gly and Leu123Trp.

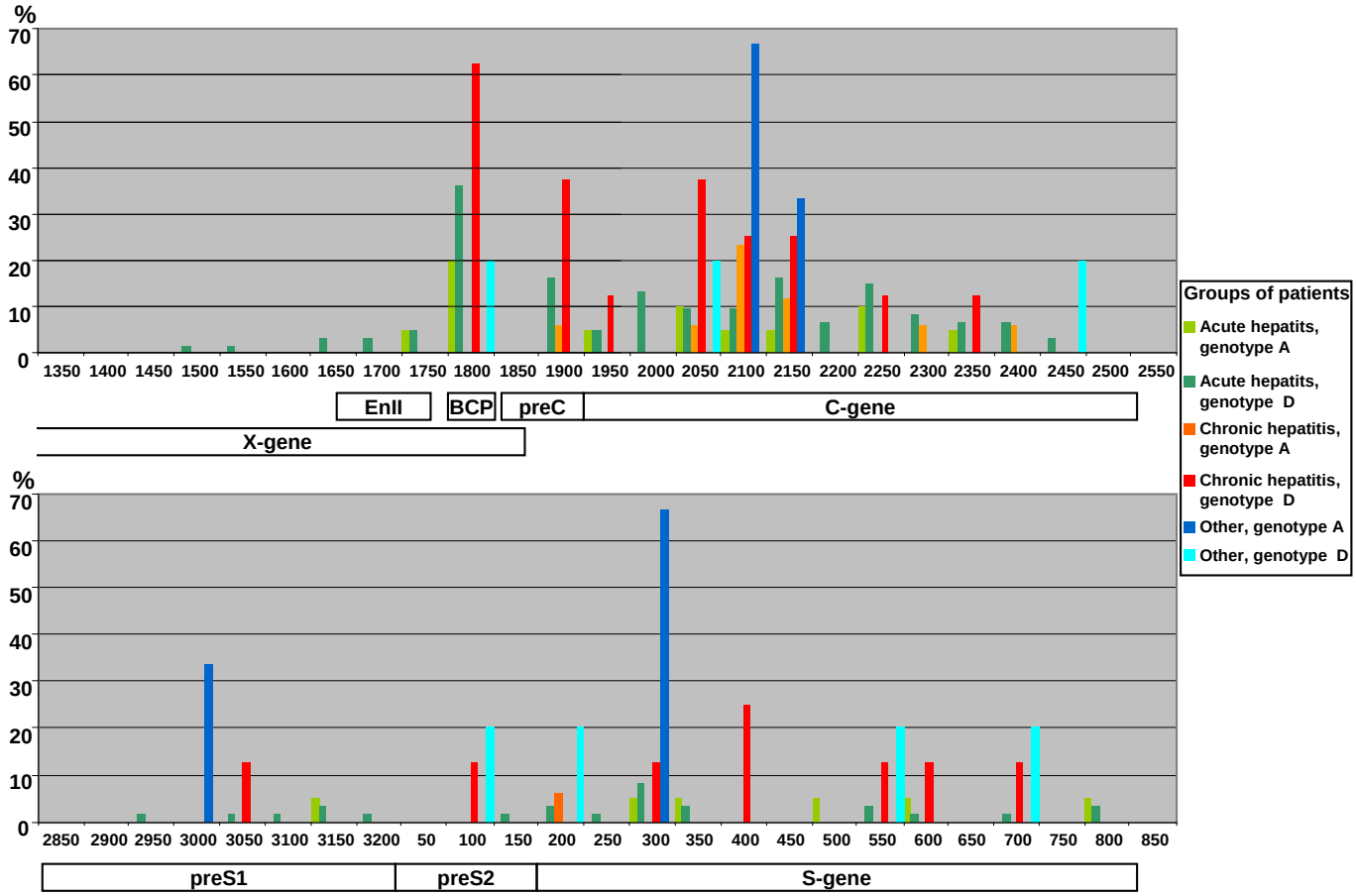

Fig. (3). Frequency of mutations discovered in HBV genome within groups of LIC patients and HBV genotypes.

It is interesting that the hepatic coma isolate possessed only few mutations, but two of them were located within the EnhII region.

PreC/C Gene

The preC stop codon mutation G1896A leading to substitution of the preC Trp -28 to UGA stop codon was detected in eight AH and three CH isolates. Absence of HBeAg in all except one patients' sera confirmed the observation that such mutations abort HBeAg expression and cause CH disease [19, 20]. The one CH and two AH isolates contained mutation at position 1900 (G→A), which changed aa 29 of the preC (Gly29Asp).

We detected numerous mutations within the HBc region 84–101, which overlaps the CTL epitopes (Fig. **2**). Such mutations accumulate usually during periods with frequent hepatitis exacerbation [21,22]. Numerous unique mutations were found in the region 48-60 (Fig. **2**), which were described as typical for FH [23-25] and severe exacerbation hepatitis B patients [26,27]. We detected also mutations in previously described mutation clustering regions at aa 21–34 [28] and 31–49 [29].

In two AH isolates we found substitutions within the HBc CTL epitope 18-27: Ser21Ala and Ser21Thr, which were not described before. HBc CTL epitope 18–27 mutations Ser21Asn/Ala/Val were found earlier in HLA-A2-positive CH patients [30]. In other HBc CTL epitopes: 63–71 and 141-149 [31], the following substitutions were found Gly63Val (twice in AH and CH patients), Glu64Asp (twice in LC and CH patients), and Glu64Val (once in AH patient). The Glu64Asp substitution was combined with two other mutations within the same epitope: Leu65Val and Ala69Gly. Mutations were found also within the C-terminal CTL epitope 141-149 (Fig. **2**).

We found Pro130Ser substitution in two isolates: one AH and one CH. The aa 130 belongs to both T helper and B cell epitopes, and the Pro130Ile/Thr/Ser substitutions could be associated with exacerbation of chronic hepatitis [32,33] and HCC [34]. Such substitutions were detected in patients with or without HBeAg [35]. In our case the AH patient had no HBeAg in his sera, whereas CH patient had it. The Arg151Gln substitution, which could be regarded as common, was found once as well as Arg151Pro substitution, both in AH patients. We found a set of mutations within the T helper epitope 50–69 and within the B cell epitope 74–83, previously detected in patients seroconverted from HBe to anti-HBe [36]: Thr53Pro/Ala was found in two cases in CH and AH patients with anti-HBe in sera. The AH patient sample contained also Cys61Trp substitution. The Glu77Lys/Asp substitution was found in four AH patients, one of them was anti-HBe positive, another one was however HBeAg positive. The latter patient possessed also Asp78Asn substitution. The Pro79Gln mutation was found in one AH and two CH isolates, but the Ala80Gly substitution was found in an AH isolate, two of them were anti-HBe positive. A large number of mutations was found within the α2a, α2b, and α5 helices of the HBc protein. The most frequent among them were: Tyr38His (two AH cases) and Tyr38Phe (one AH case), Glu40Asp (three AH and three CH cases), Glu113Gln (one AH and one CH case), Glu113Asp (four AH cases). The Glu113Asn substitution was found once in AH isolate, Glu113Thr also once in AH isolate. The Thr114Ala/Ile and ValGlu124Leu substitutions were found in two AH cases.

In contrast to earlier observations [37], in all our studies we did not find any HBc deletion variants.

PreS/S Gene

Two mutations were found in the HBV preS1 region five times: G2995A that leads to Val48Ile substitution and G3001C - to Asp51His substitution. These mutations appeared in *adw*2 patients, three times in AH and twice in CH patients. Single G2995A mutation was found in an LC isolate. One CH isolate possessed A3001C mutation leading to the Thr51Pro substitution. Besides this, the following individual mutations were observed: A2905T (Thr18Ser), G3013A (Asp54Asn), G3099T (Gln82His), A3111C (Gln86His), C3113A (Thr87Lys), T3139G (Ser96Ala), T3154A (Ser101Thr), all in AH isolates.

Only three mutations were found within the preS2 region: T54C (Phe22Ser) in a CH isolate, C81T (Thr31Ile) in a coma patient and C149A (Leu34Met) in an AH patient.

It was documented that chronic HBV carriers and HCC patients frequently have mutations encompassing residues 29–53 [38] of the S region. We found some mutations within this region, but only Leu49Arg substitution was found twice, but in AH isolates. Within the site of hypervariability located at position 134 [39] we found the following substitutions: Tyr134Phe/Asn/Trp/Ser or Phe134Tyr depending of the HBsAg subtype. At this position we found some novel substitutions - Tyr134Lys in a CH patient and Phe134Leu in an AH patient. Novel substitutions were also observed at positions Ile110Met, Ser136Tyr, and Thr118Ile, all in AH patients. Only Thr118Ala substitution was reported earlier [40]. We found the Leu175Ser substitution in the hepatic coma patient, although this mutation appeared before only in combination with a Phe179Tyr substitution [41].

HBV GENOTYPES IN LATVIA AND NEIGHBORING COUNTRIES

The present study is the first epidemiological attempt examining the prevalence of the HBV genotypes A and D and their mutational status in the wide group of patients admitted to the Infectology Center of Latvia. Our findings reflect the overall genotype and subtype distribution in the Latvian population: 71.52 % of genotype D and 27.81 % of genotype A, 0.87% of genotype E; 23.18 % of subtype *adw*2, 15.23 % of subtype *ayw*2, 39.07 % of subtype *ayw*3, 0.66% of subtype *ayw*4 and 1.99 *adw*3. In one case, it was impossible to establish subtype due to unique Thr127Ser substitution at the subtype-specific residue, which has not been described previously. In our neighbor country, Poland, the genotype A is prevalent (74.1%) [42], but the genotype D is on the second place (20.7%). Almost all HBV genotype A cases are of the *adw*2 subtype. As to the genotype D similar to major subtypes found in Latvia, they are represented by *ayw*3 and *ayw*2 variants. In Estonia, another neighboring country [43], the situation is similar to Latvia, with predominance of the genotype D and prevalence of the

subtypes *ayw*3 and *adw*2. In contrast to Latvia, both neighboring countries show only a few cases of the *ayw*4 subtype. Data from another neighbor, Belarus, show also prevalence of the D genotype (87.5%) over the A genotype (12.5%) [44]. The most widespread within the D genotype in Belarus is the D2 subgenotype (56.3%). In contrast to our data, the subgenotype D4 (3.1%) was also found. Our neighbor to the East, the Russian Federation, is very large and proportion of HBV genotypes varies strongly depending on geographic location [45]. Thus, the genotype D comprises 85 % among children in Moscow [45,46]. In Western Siberia, the genotype D constitutes even more than 96 % of the total. In contrast to Latvia, the *ayw*2 subtype was clearly predominant over the *ayw*3 subtype [47]. Our data also support the postulate, that genotype D is predominant in the Mediterranean region, Southern and Eastern Europe, in such countries as Hungary [48], Italy: 73% of the genotype D, and 26% of the genotype A [49,50], and Spain: 63,2% and 23.1%, respectively [51,52]. In most Western European countries, such as Belgium 54% of genotype A, and 31% of genotype D [53], the Netherlands 64%, and 21% respectively [54] genotype A dominates.

CONCLUSIONS

- This study indicates that the genotype D is a predominant genotype among patients in Latvia, as in most of the neighboring countries The genotype D isolates are more heterogeneous than those of the genotype A.
- PreC/C mutants are prevalent among patients of Latvia. A never previously reported preC substitution Ser21Thr was found. At the same time we found no evidence for the existence of preC deletion variants.
- Comparison of the HBV sequences from the preS/S region with the strain of the same genotype revealed no deletions and relatively low number of mutations. At the same time we discovered S protein substitutions Tyr134Lys and Phe134Leu not previously described. Novel substitutions were observed also at positions Ile110Met, Thr118Ile and Ser136Tyr.

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Appendix

Aligned nucleotide and protein sequences of different regions of Hepatitis B genome obtained during the study. Sequences are ranged by genotype and diagnosis of patients: AH – acute hepatitis, CH – chronic hepatitis, COMPL – other complications. As a reference sequences are given for genotype A - X02763, for genotype D - V01460 and X02496, for genotype E - X75657. Synonymous nucleotides are shown as dots.

Appendix Part A. Sequences of HBV region preC/C obtained during the study.

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                    1820      1830      1840      1850      1860      1870      1880      1890      1900      1910      1920      1930
                   .|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
X02763             ATGCAACTTTTTCACCTCTGCCTAATCATCTCTTGTACATGTCCCACTGTTCAAGCCTCCAAGCTGTGCCTTGGGTGGCTTTGGGGCATGGACATTGACCCTTATAAAGAATTTGGAGCT
                    M  Q  L  F  H  L  C  L  I  I  S  C  T  C  P  T  V  Q  A  S  K  L  C  L  G  W  L  W  G  M  D  I  D  P  Y  K  E  F  G  A
   AH 2-4890       ................................................................................................................................
                    M  Q  L  F  H  L  C  L  I  I  S  C  T  C  P  T  V  Q  A  S  K  L  C  L  G  W  L  W  G  M  D  I  D  P  Y  K  E  F  G  A
   AH 1.I          --------------------------------------------------------------------------------------------------------------------------

   AH 4-1835       --------------------------------------------------------------------------------------------------------------------------

   AH 4-2015       ..............................................................................................G.........................
                    M  Q  L  F  H  L  C  L  I  I  S  C  T  C  P  T  V  Q  A  S  K  L  C  L  G  W  L  W  G  M  D  I  D  P  Y  K  E  F  G  A
   AH 5-2878       ................................................................................................................................
                    M  Q  L  F  H  L  C  L  I  I  S  C  T  C  P  T  V  Q  A  S  K  L  C  L  G  W  L  W  G  M  D  I  D  P  Y  K  E  F  G  A
   AH 7-1884       ................................................................................................................................
                    M  Q  L  F  H  L  C  L  I  I  S  C  T  C  P  T  V  Q  A  S  K  L  C  L  G  W  L  W  G  M  D  I  D  P  Y  K  E  F  G  A
   AH 7-624        --------------------------------------------------------------------------------------------------------------------------

   AH 7-640        ..........................................T.....................................................G.........................
                    M  Q  L  F  H  L  C  L  I  I  S  C  T  C  P  T  V  Q  A  S  K  L  C  L  G  W  L  W  G  M  D  I  D  P  Y  K  E  F  G  A
   AH 8-1993       ................................................................................................................................
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   AH 10-2400      ................................................................................................................................
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   AH 10-2596      ................................................................................................................................
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   AH 11-2641      --------------------------------------------------------------------------------------------------------------------------

   AH 12-21        --------------------------------------------------------------------------------------------------------------------------

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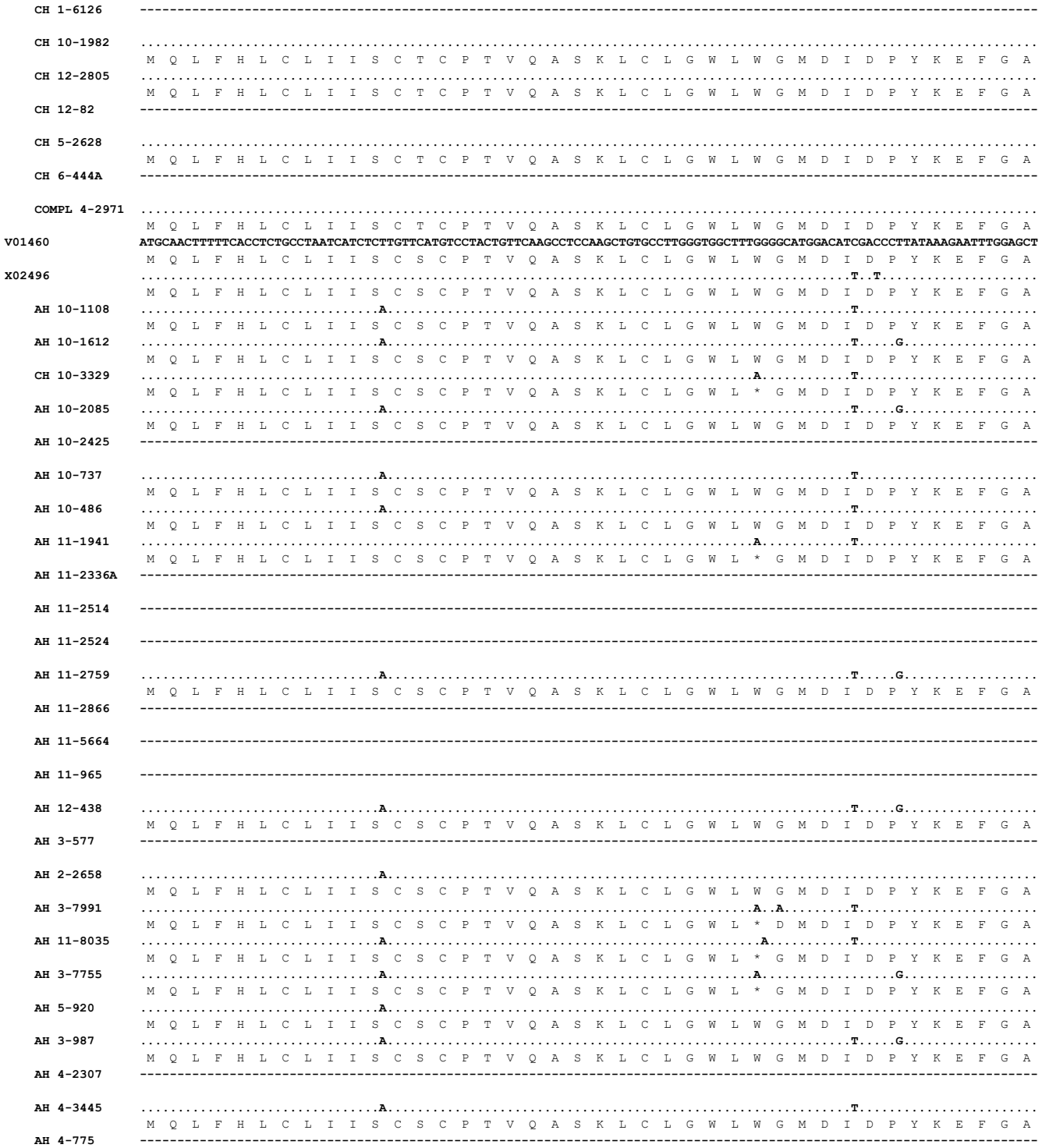
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CH 12-2805
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CH 12-82
CH 5-2628
M Q L F H L C L I I S C T C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
CH 6-444A
COMPL 4-2971
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V01460
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M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
X02496
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
AH 10-1108
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
AH 10-1612
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
CH 10-3329
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L * G M D I D P Y K E F G A
AH 10-2085
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
AH 10-2425
AH 10-737
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
AH 10-486
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
AH 11-1941
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L * G M D I D P Y K E F G A
AH 11-2336A
AH 11-2514
AH 11-2524
AH 11-2759
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
AH 11-2866
AH 11-5664
AH 11-965
AH 12-438
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
AH 3-577
AH 2-2658
M Q L F H L C L I I S C S C P T V Q A S K L C L G W L W G M D I D P Y K E F G A
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M Q L F H L C L I I S C S C P T V Q A S K L C L G W L * D M D I D P Y K E F G A
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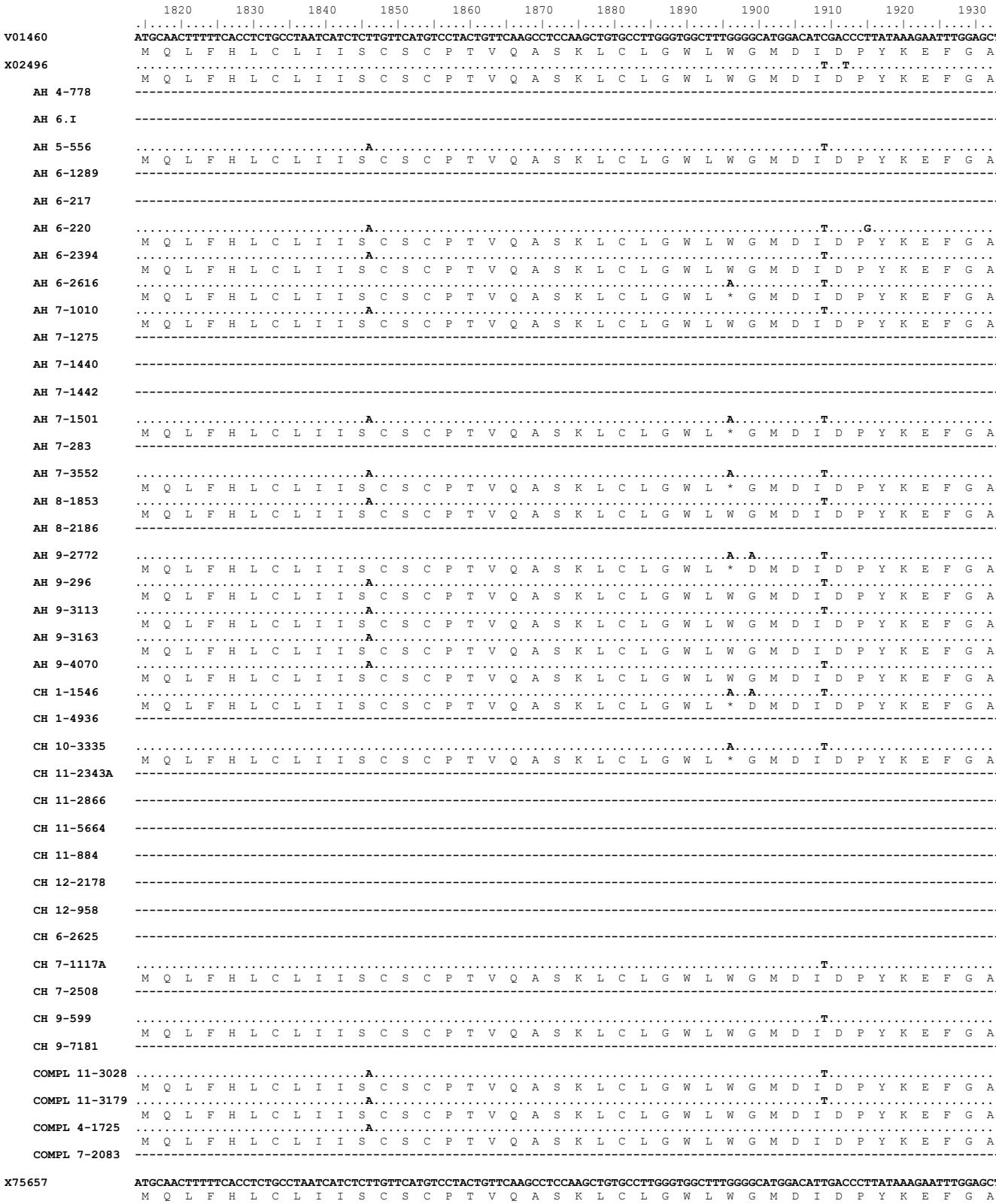
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X02496 ..T..T.....
AH 4-778
AH 6.I
AH 5-556 ..A..T.....
AH 6-1289
AH 6-217
AH 6-220 ..A..T.....G.....
AH 6-2394 ..A..T.....
AH 6-2616 ..A..T.....
AH 7-1010 ..A..T.....
AH 7-1275
AH 7-1440
AH 7-1442
AH 7-1501 ..A..A..T.....
AH 7-283
AH 7-3552 ..A..A..T.....
AH 8-1853 ..A..T.....
AH 8-2186
AH 9-2772 ..A..A..T.....
AH 9-296 ..A..T.....
AH 9-3113 ..A..T.....
AH 9-3163 ..A..T.....
AH 9-4070 ..A..T.....
CH 1-1546 ..A..A..T.....
CH 1-4936
CH 10-3335 ..A..T.....
CH 11-2343A
CH 11-2866
CH 11-5664
CH 11-884
CH 12-2178
CH 12-958
CH 6-2625
CH 7-1117A ..T.....
CH 7-2508
CH 9-599 ..T.....
CH 9-7181
COMPL 11-3028 ..A..T.....
COMPL 11-3179 ..A..T.....
COMPL 4-1725 ..A.....
COMPL 7-2083
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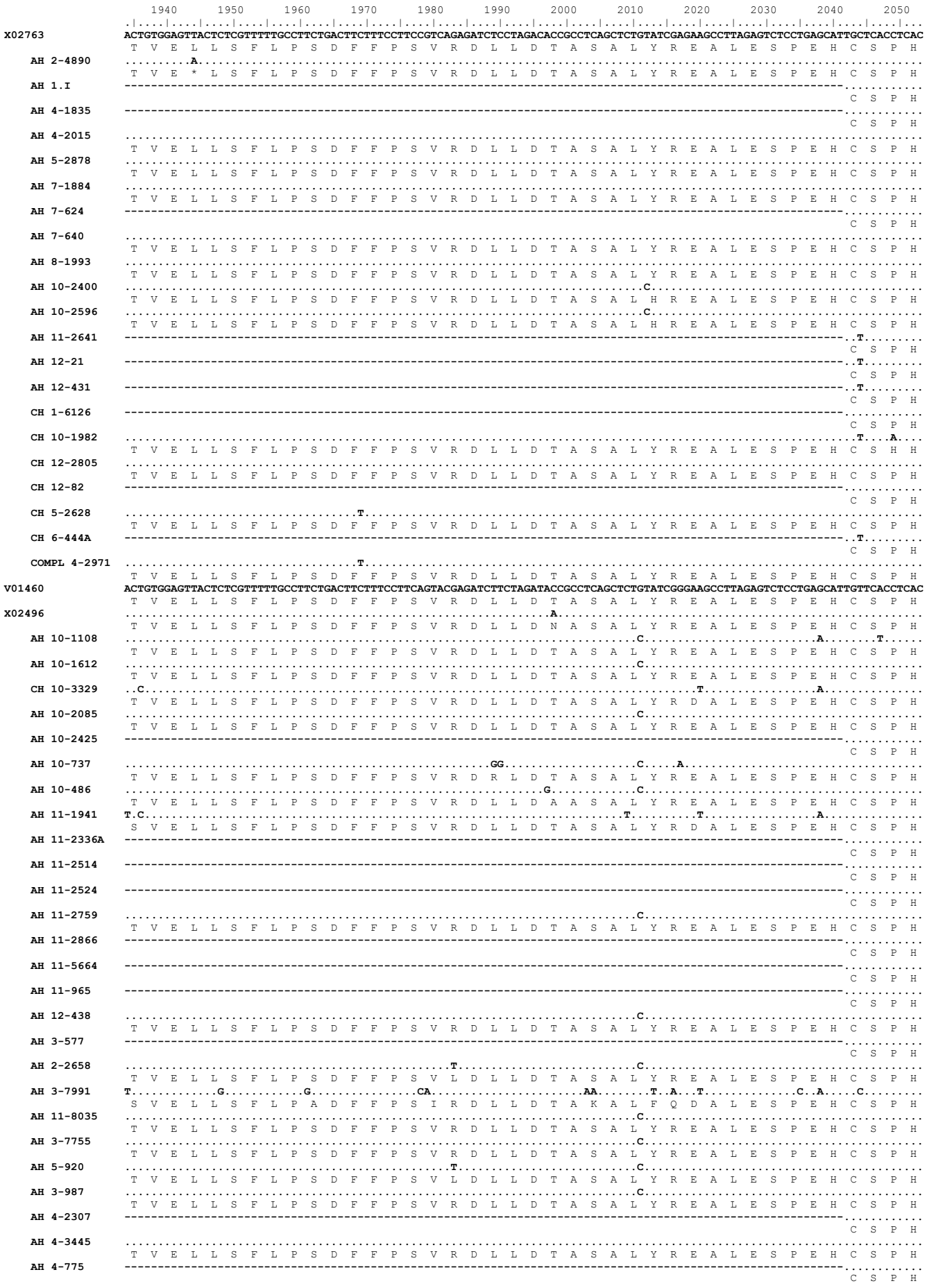
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X02763
AH 2-4890
AH 1.I
AH 4-1835
AH 4-2015
AH 5-2878
AH 7-1884
AH 7-624
AH 7-640
AH 8-1993
AH 10-2400
AH 10-2596
AH 11-2641
AH 12-21
AH 12-431
CH 1-6126
CH 10-1982
CH 12-2805
CH 12-82
CH 5-2628
CH 6-444A
COMPL 4-2971
V01460
X02496
AH 10-1108
AH 10-1612
CH 10-3329
AH 10-2085
AH 10-2425
AH 10-737
AH 10-486
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AH 12-438
AH 3-577
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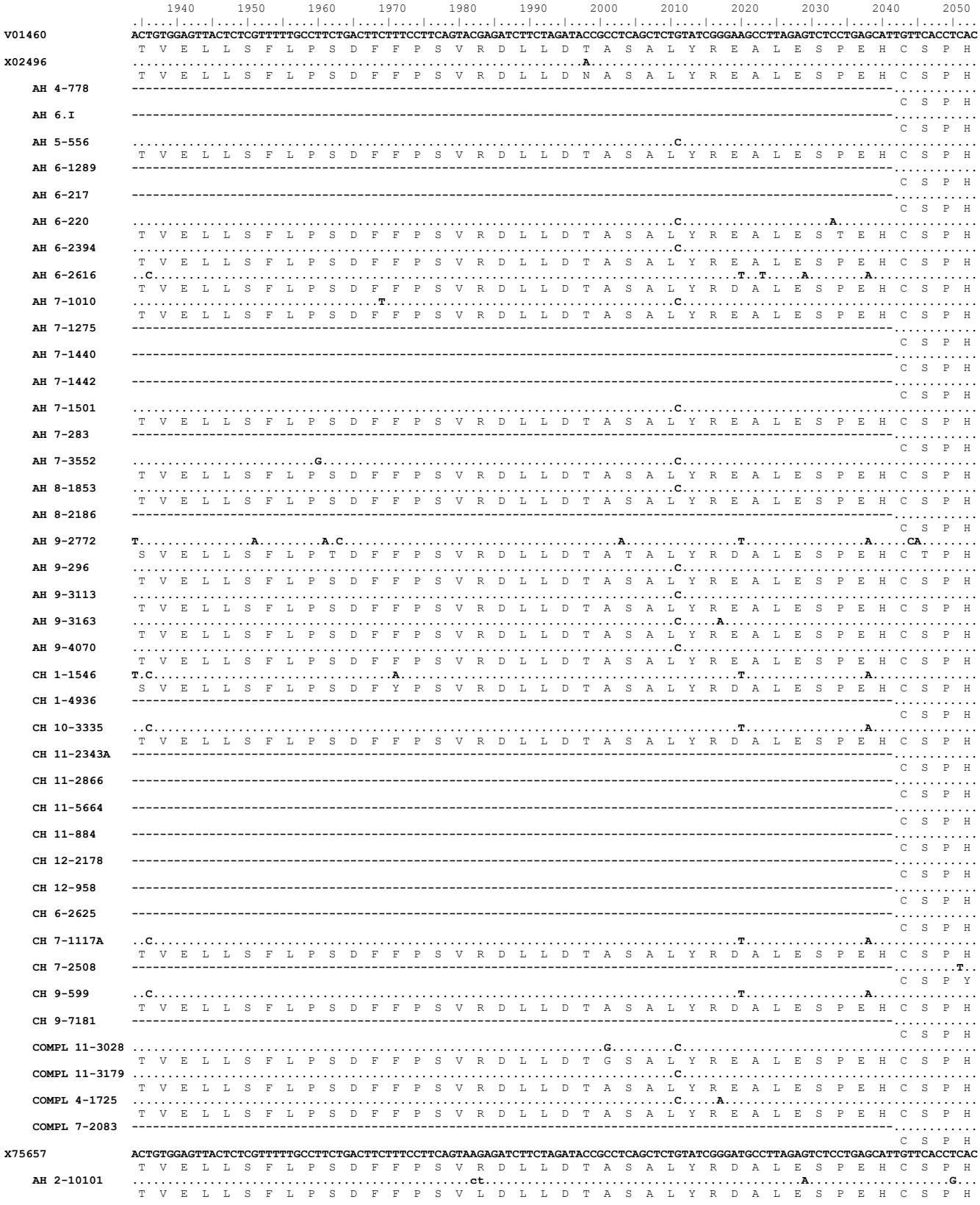
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T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S P E H C S P H
X02496 ....A..........
T V E L L S F L P S D F F P S V R D L L D N A S A L Y R E A L E S P E H C S P H
AH 4-778 ----------
C S P H
AH 6.I ----------
C S P H
AH 5-556 ....C..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S P E H C S P H
AH 6-1289 ----------
C S P H
AH 6-217 ----------
C S P H
AH 6-220 ..........C..........A..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S T E H C S P H
AH 6-2394 ....C..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S P E H C S P H
AH 6-2616 ..C..........T..T.....A.....A..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R D A L E S P E H C S P H
AH 7-1010 ..........T..........C..........
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AH 7-1275 ----------
C S P H
AH 7-1440 ----------
C S P H
AH 7-1442 ----------
C S P H
AH 7-1501 ....C..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S P E H C S P H
AH 7-283 ----------
C S P H
AH 7-3552 ..........G..........C..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S P E H C S P H
AH 8-1853 ....C..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S P E H C S P H
AH 8-2186 ----------
C S P H
AH 9-2772 T..........A.....A.C..........A..........T..........A.....CA.....
S V E L L S F L P T D F F P S V R D L L D T A T A L Y R D A L E S P E H C T P H
AH 9-296 ....C..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S P E H C S P H
AH 9-3113 ....C..........
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R E A L E S P E H C S P H
AH 9-3163 ..........C.....A..........
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AH 9-4070 ....C..........
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CH 1-1546 T.C..........A..........T..........A.....
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CH 1-4936 ----------
C S P H
CH 10-3335 ..C..........T..........A.....
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R D A L E S P E H C S P H
CH 11-2343A ----------
C S P H
CH 11-2866 ----------
C S P H
CH 11-5664 ----------
C S P H
CH 11-884 ----------
C S P H
CH 12-2178 ----------
C S P H
CH 12-958 ----------
C S P H
CH 6-2625 ----------
C S P H
CH 7-1117A ..C..........T..........A.....
T V E L L S F L P S D F F P S V R D L L D T A S A L Y R D A L E S P E H C S P H
CH 7-2508 ----------T..
C S P Y
CH 9-599 ..C..........T..........A.....
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CH 9-7181 ----------
C S P H
COMPL 11-3028 ..........G.....C..........
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C S P H
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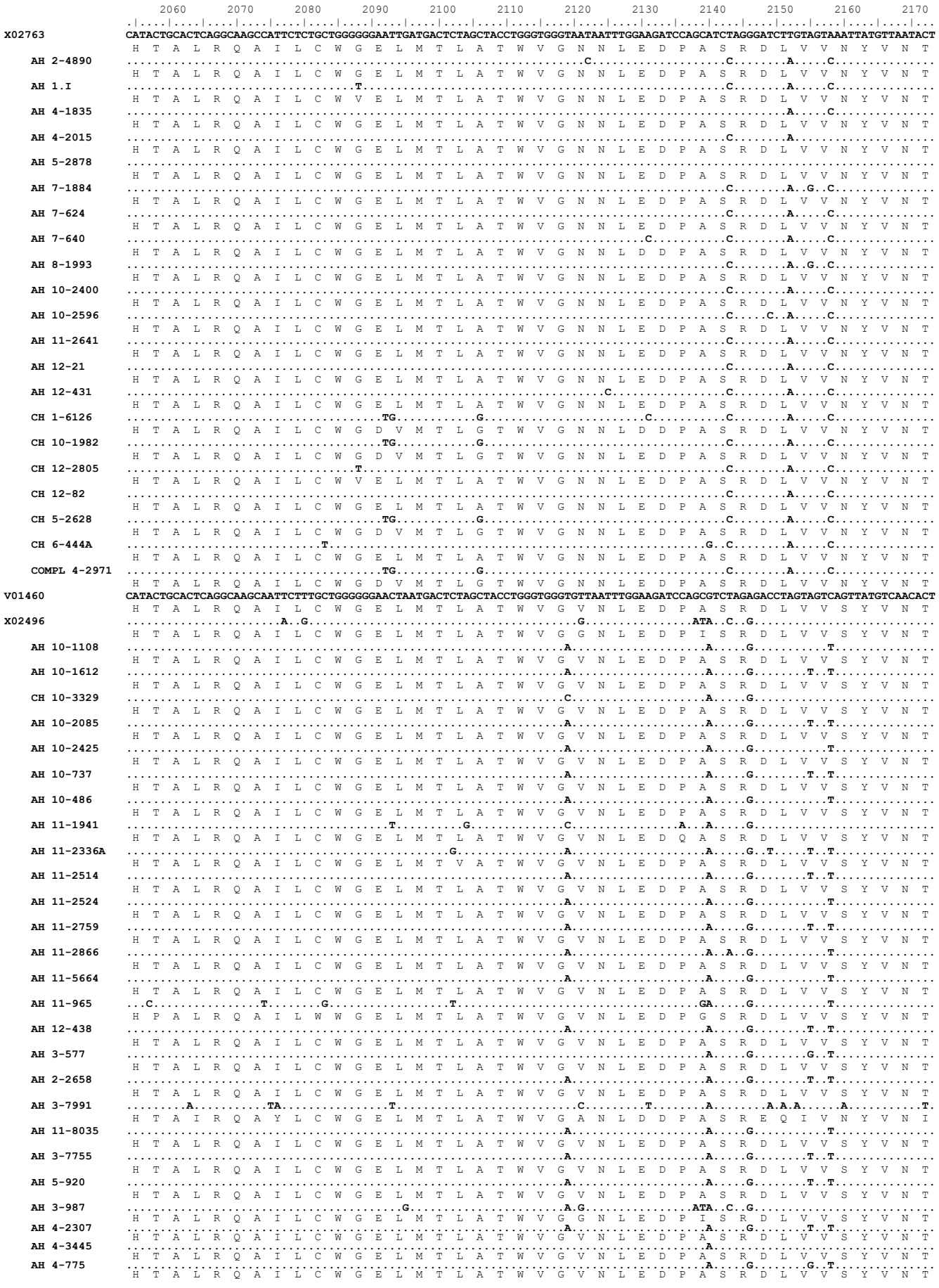
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AH 2-4890
AH 1.I
AH 4-1835
AH 4-2015
AH 5-2878
AH 7-1884
AH 7-624
AH 7-640
AH 8-1993
AH 10-2400
AH 10-2596
AH 11-2641
AH 12-21
AH 12-431
CH 1-6126
CH 10-1982
CH 12-2805
CH 12-82
CH 5-2628
CH 6-444A
COMPL 4-2971
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AH 10-1108
AH 10-1612
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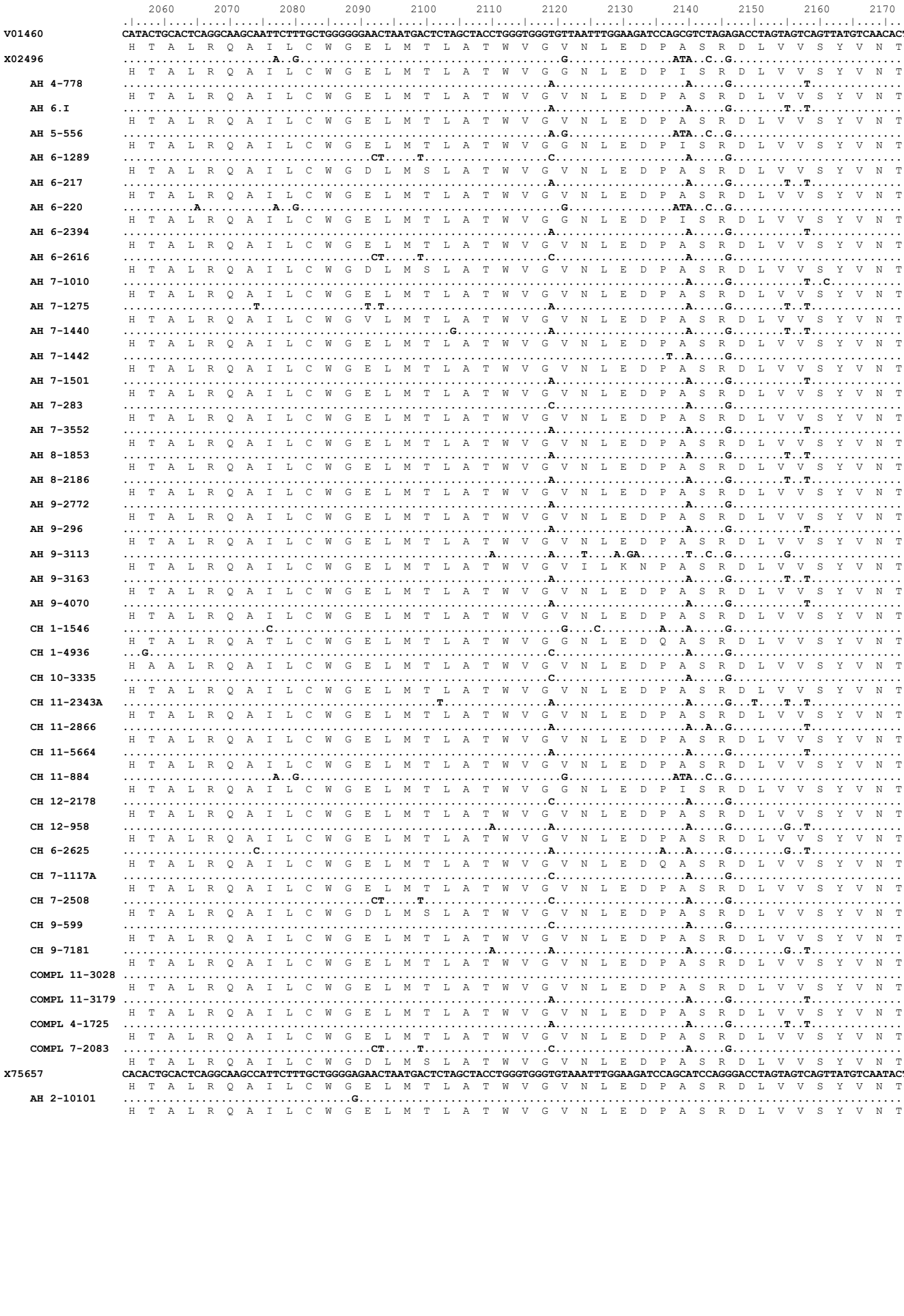
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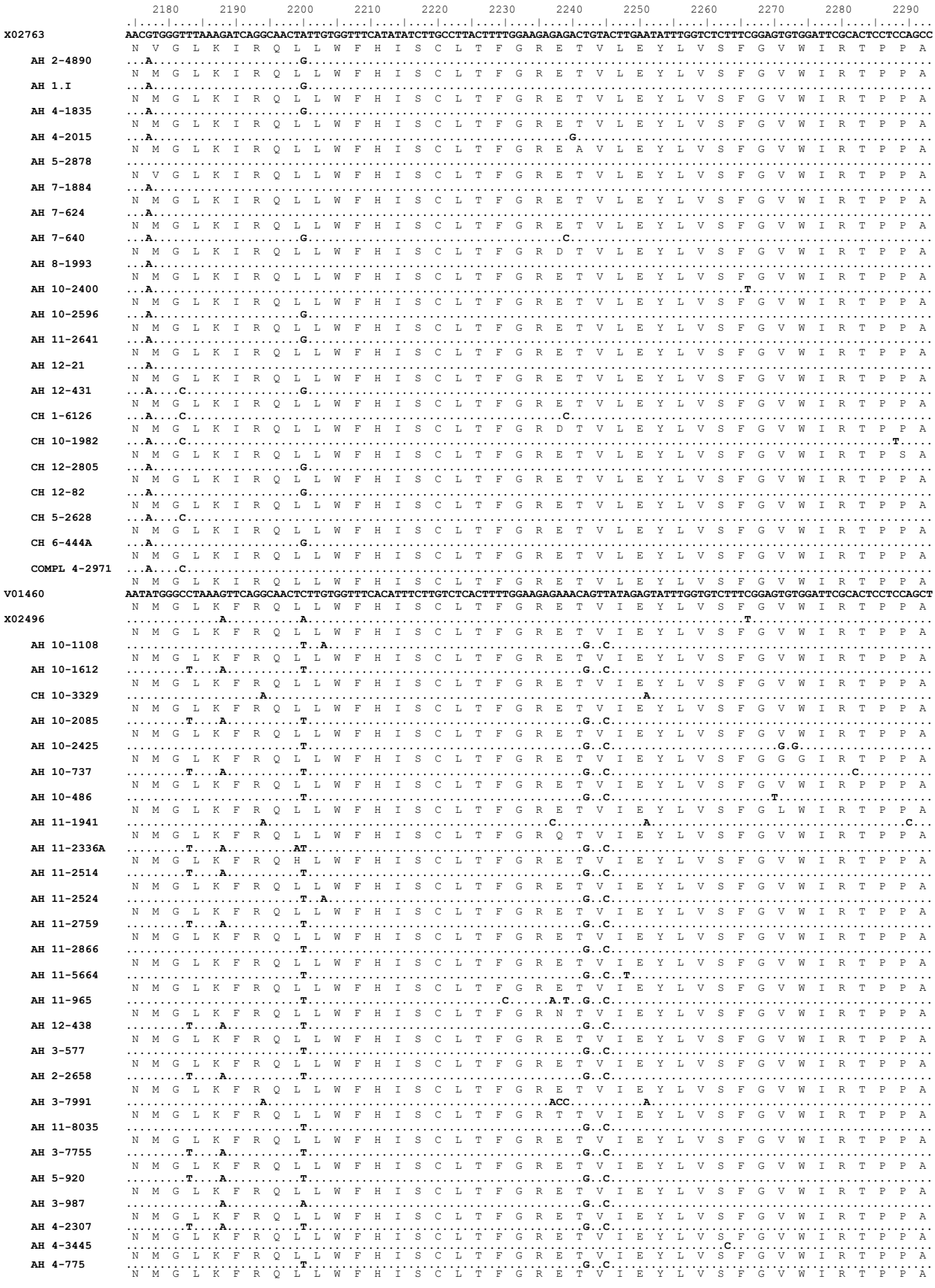
AH 2-10101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N V G L K I R Q L L W F H I S C L T F G R E T V L E Y L V S F G V W I R T P P A
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AH 1.I ...A.....G.....
AH 4-1835 ...A.....G.....
AH 4-2015 ...A.....G.....
AH 5-2878 .....
AH 7-1884 ...A.....
AH 7-624 ...A.....
AH 7-640 ...A.....G.....C.....
AH 8-1993 ...A.....
AH 10-2400 ...A.....T.....
AH 10-2596 ...A.....G.....
AH 11-2641 ...A.....G.....
AH 12-21 ...A.....
AH 12-431 ...A....C.....G.....
CH 1-6126 ...A....C.....C.....
CH 10-1982 ...A....C.....T.....
N M G L K I R Q L L W F H I S C L T F G R E T V L E Y L V S F G V W I R T P S A
CH 12-2805 ...A.....G.....
CH 12-82 ...A.....G.....
CH 5-2628 ...A....C.....
CH 6-444A ...A.....G.....
COMPL 4-2971 ...A....C.....
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N M G L K F R Q L L W F H I S C L T F G R E T V I E Y L V S F G V W I R T P P A
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CH 10-3329 .....A.....A.....
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AH 10-2425 .....T.....G..C.....G.G.....
AH 10-737 .....T....A.....T.....G..C.....C.....
AH 10-486 .....T.....G..C.....T.....
AH 11-1941 .....A.....C.....A.....C...
AH 11-2336A .....T....A.....AT.....G..C.....
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AH 11-965 .....T.....C.....A.T..G..C.....
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AH 3-577 .....T.....G..C.....
AH 2-2658 .....T....A.....T.....G..C.....
AH 3-7991 .....A.....ACC.....A.....
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AH 3-7755 .....T....A.....T.....G..C.....
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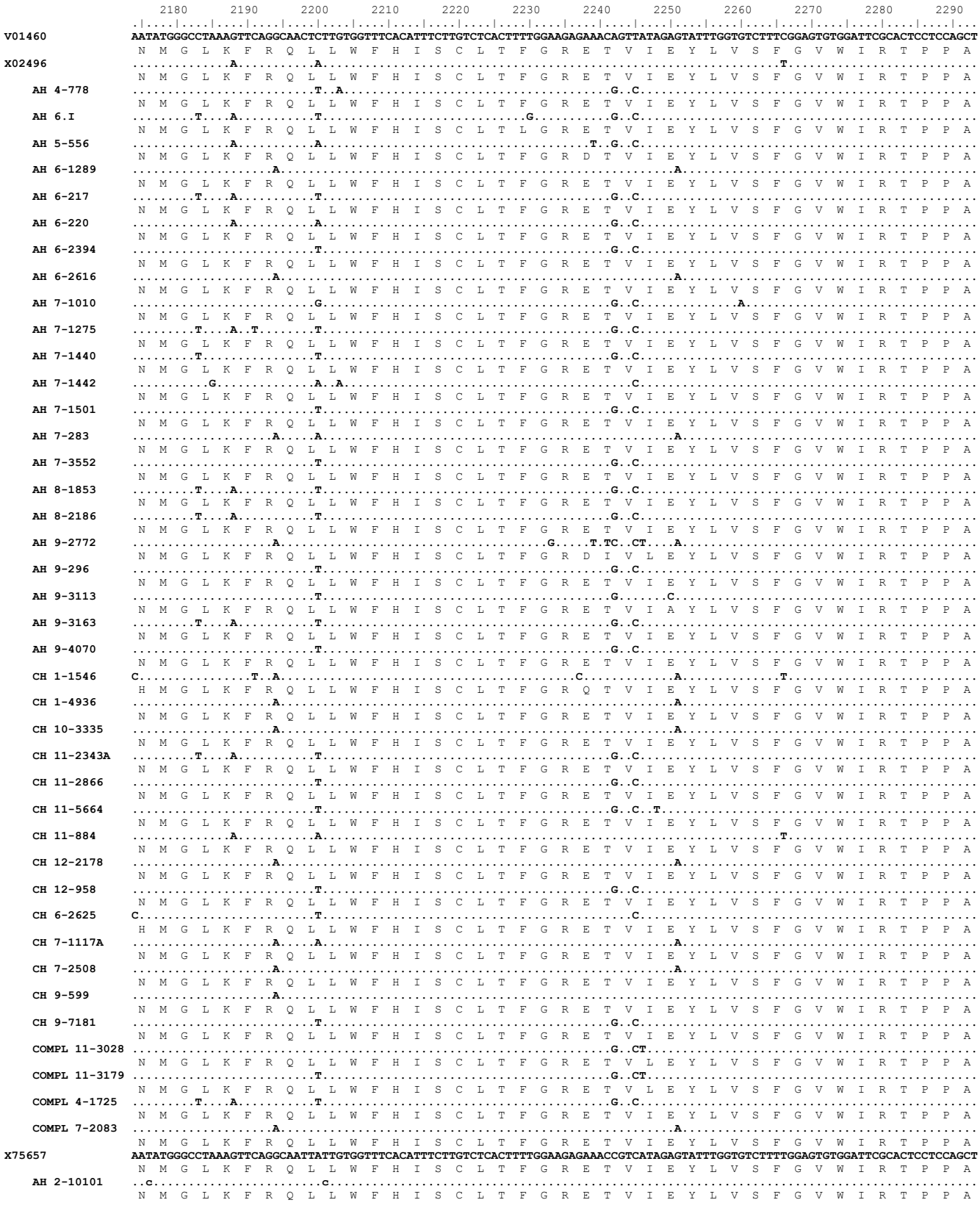
AH 4-775 .....T.....G..C.....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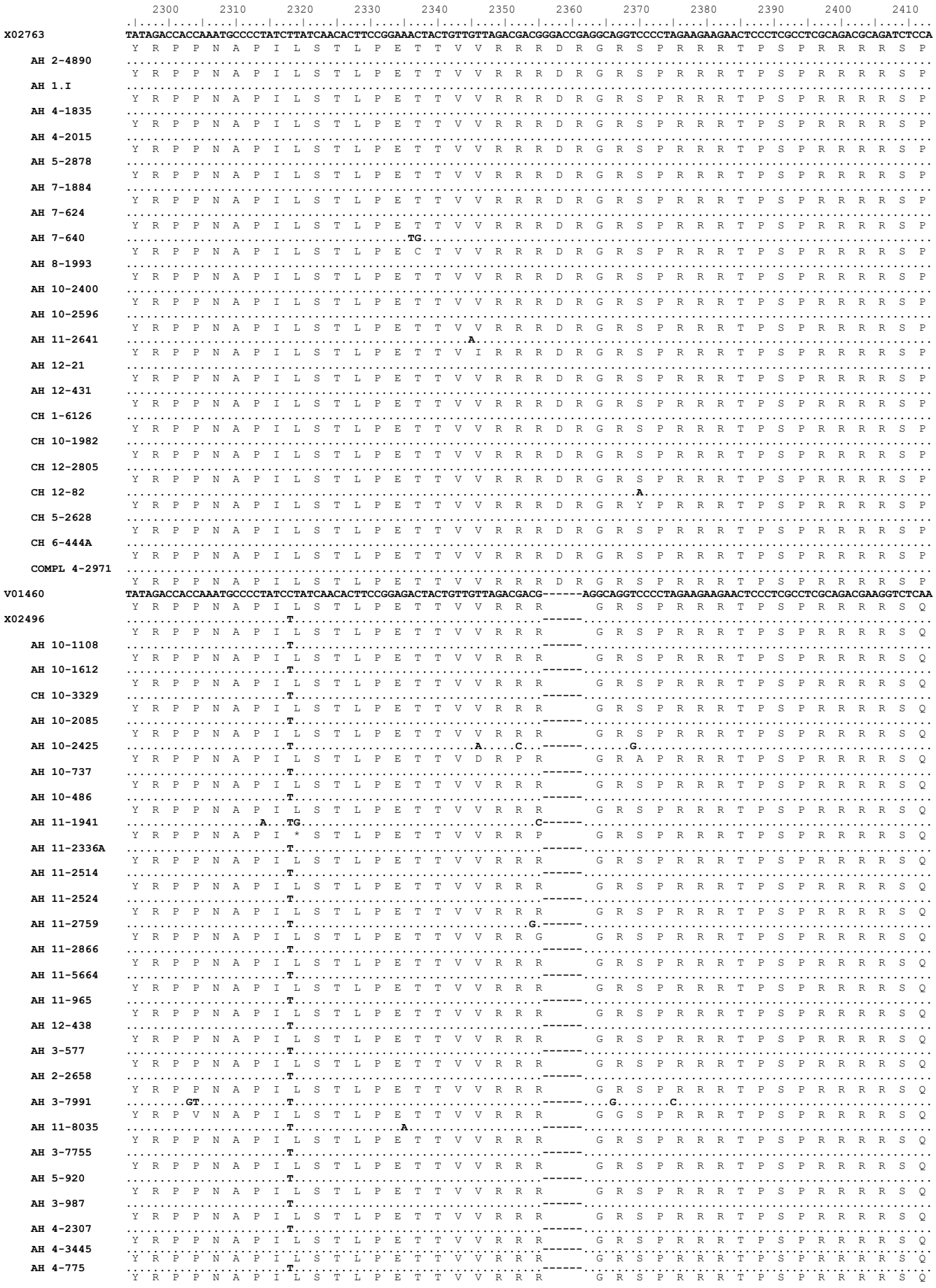
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AH 2-4890
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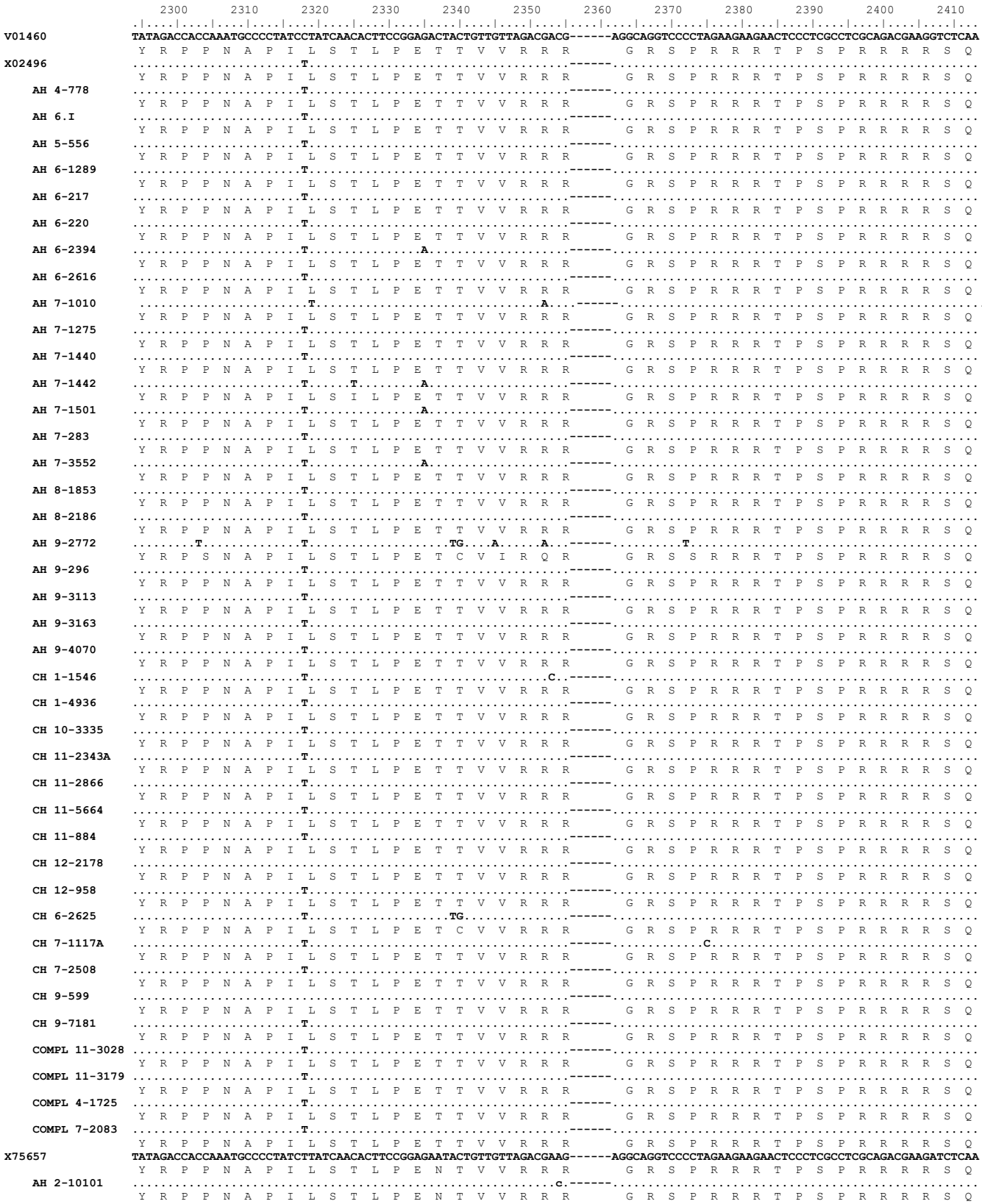
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AH 6.I
AH 5-556
AH 6-1289
AH 6-217
AH 6-220
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AH 6-2616
AH 7-1010
AH 7-1275
AH 7-1440
AH 7-1442
AH 7-1501
AH 7-283
AH 7-3552
AH 8-1853
AH 8-2186
AH 9-2772
AH 9-296
AH 9-3113
AH 9-3163
AH 9-4070
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CH 1-4936
CH 10-3335
CH 11-2343A
CH 11-2866
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CH 12-2178
CH 12-958
CH 6-2625
CH 7-1117A
CH 7-2508
CH 9-599
CH 9-7181
COMPL 11-3028
COMPL 11-3179
COMPL 4-1725
COMPL 7-2083
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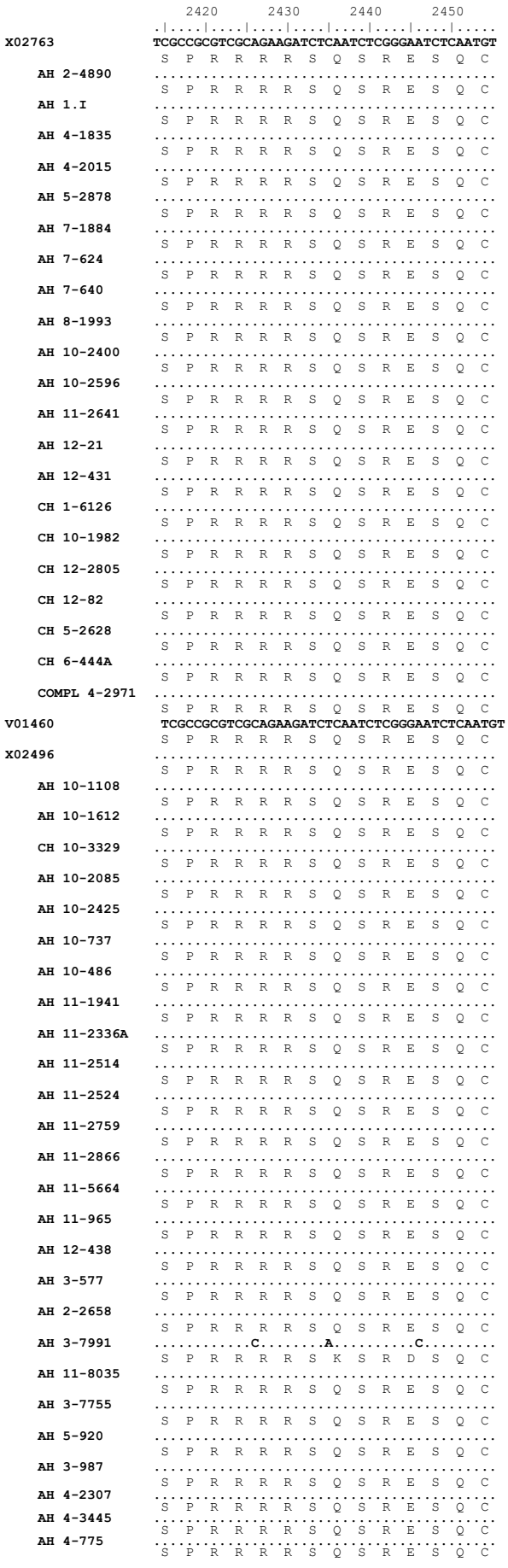
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AH 4-1835
AH 4-2015
AH 5-2878
AH 7-1884
AH 7-624
AH 7-640
AH 8-1993
AH 10-2400
AH 10-2596
AH 11-2641
AH 12-21
AH 12-431
CH 1-6126
CH 10-1982
CH 12-2805
CH 12-82
CH 5-2628
CH 6-444A
COMPL 4-2971
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X02496
AH 10-1108
AH 10-1612
CH 10-3329
AH 10-2085
AH 10-2425
AH 10-737
AH 10-486
AH 11-1941
AH 11-2336A
AH 11-2514
AH 11-2524
AH 11-2759
AH 11-2866
AH 11-5664
AH 11-965
AH 12-438
AH 3-577
AH 2-2658
AH 3-7991 .....C.....A.....C.....
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AH 3-987
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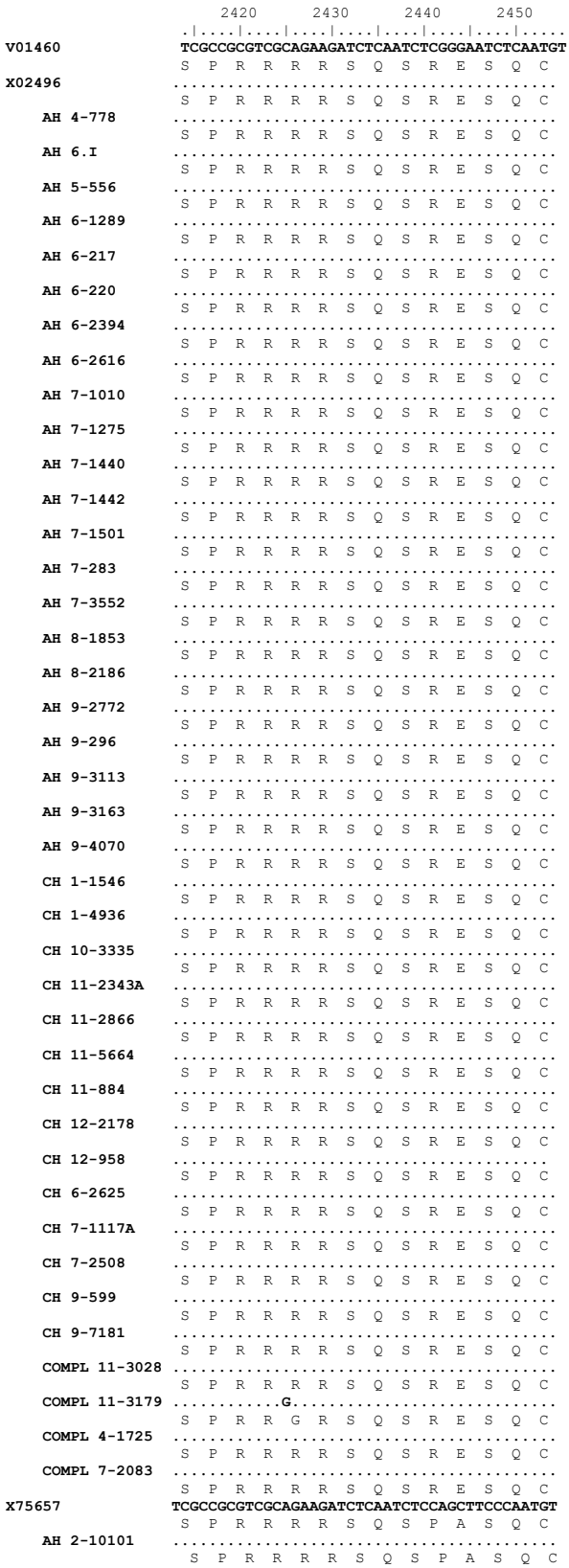
AH 4-3445
AH 4-775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AH 4-778 .....
AH 6.I .....
AH 5-556 .....
AH 6-1289 .....
AH 6-217 .....
AH 6-220 .....
AH 6-2394 .....
AH 6-2616 .....
AH 7-1010 .....
AH 7-1275 .....
AH 7-1440 .....
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CH 7-1117A .....
CH 7-2508 .....
CH 9-599 .....
CH 9-7181 .....
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COMPL 11-3179 .....G.....
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COMPL 7-2083 .....
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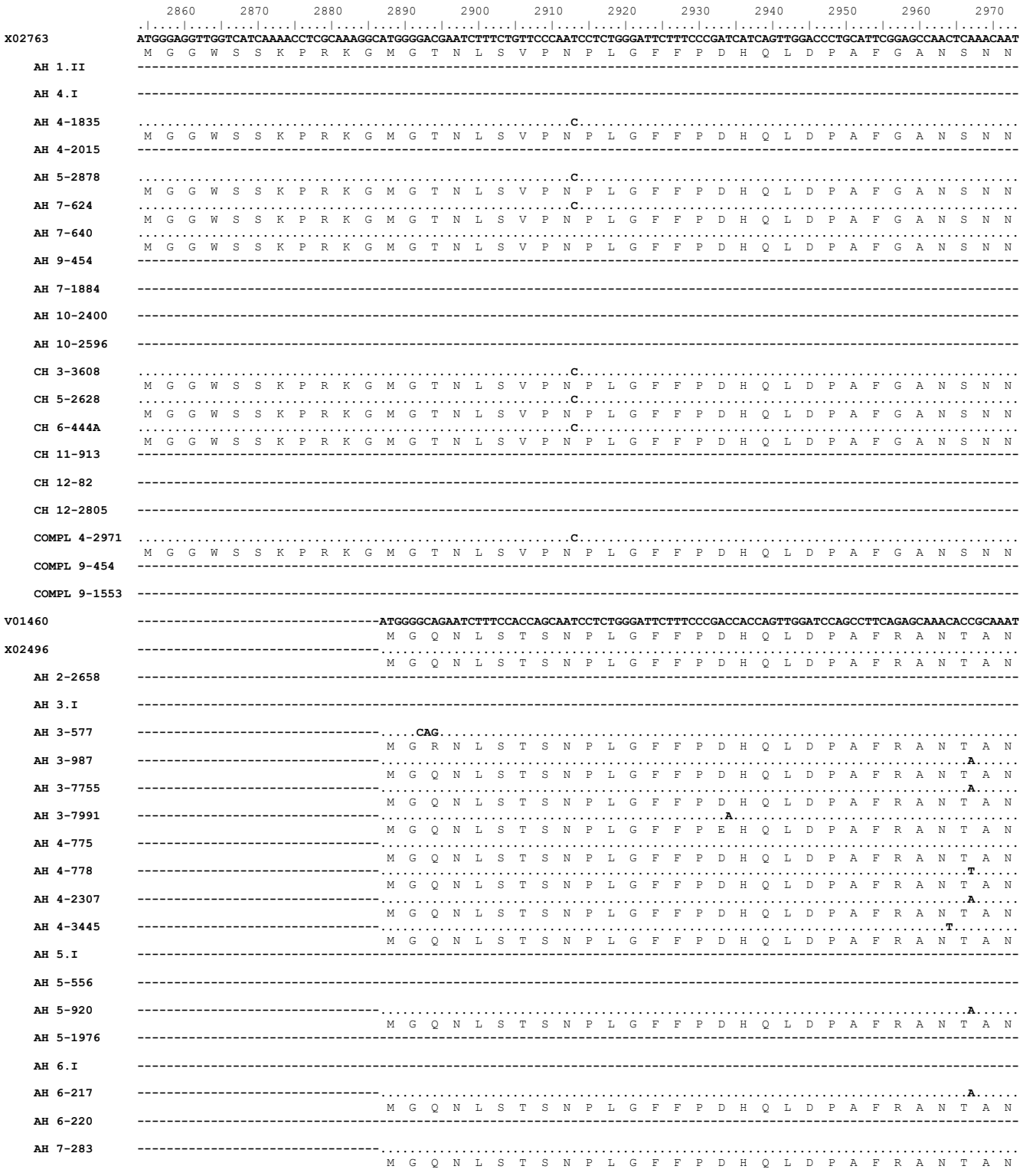
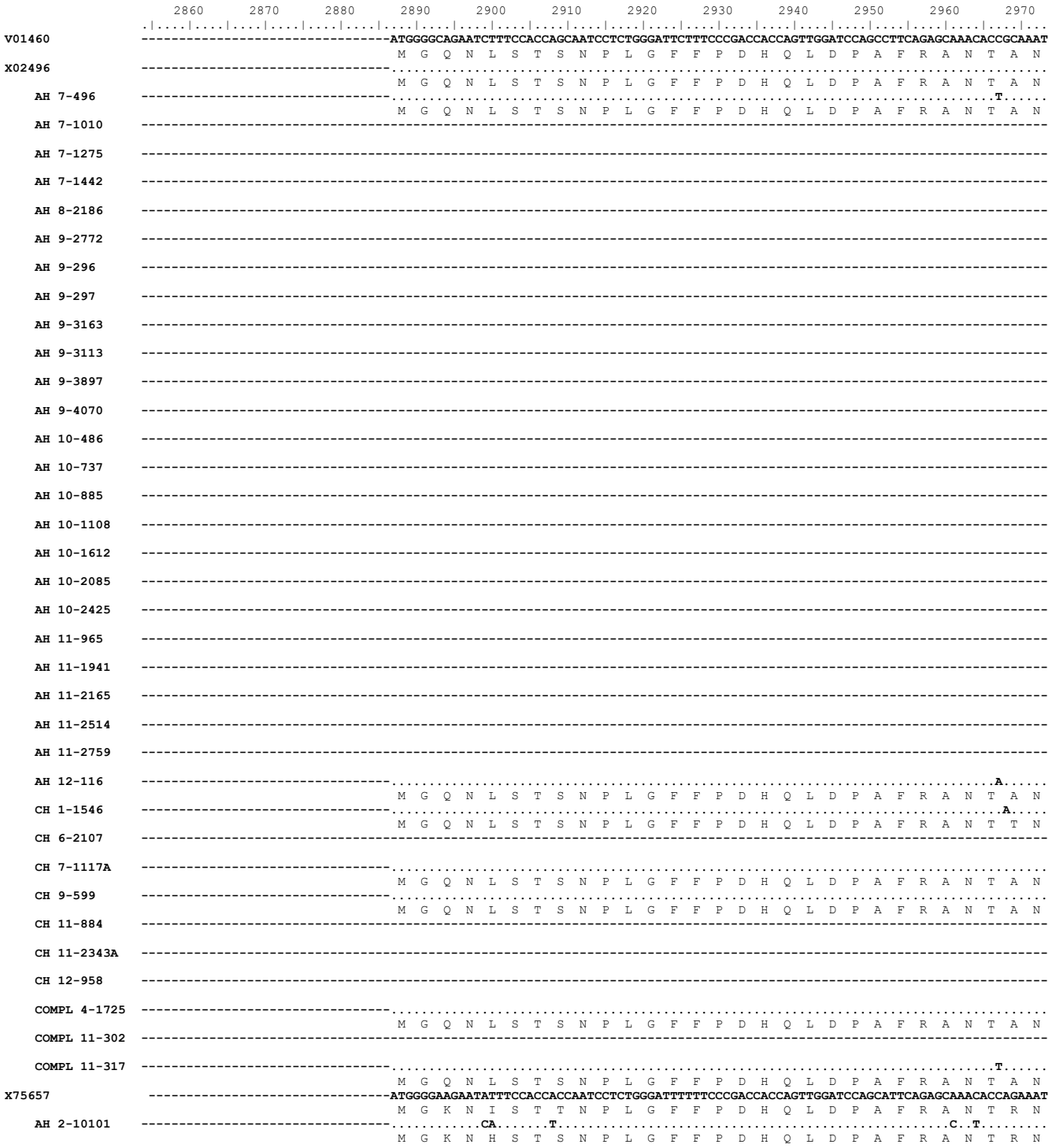
Appendix Part B. Sequences of HBV region preS/S obtained during the study.

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X02496
M G Q N L S T S N P L G F F P D H Q L D P A F R A N T A N
AH 7-496
M G Q N L S T S N P L G F F P D H Q L D P A F R A N T A N
AH 7-1010
AH 7-1275
AH 7-1442
AH 8-2186
AH 9-2772
AH 9-296
AH 9-297
AH 9-3163
AH 9-3113
AH 9-3897
AH 9-4070
AH 10-486
AH 10-737
AH 10-885
AH 10-1108
AH 10-1612
AH 10-2085
AH 10-2425
AH 11-965
AH 11-1941
AH 11-2165
AH 11-2514
AH 11-2759
AH 12-116
M G Q N L S T S N P L G F F P D H Q L D P A F R A N T A N
CH 1-1546
M G Q N L S T S N P L G F F P D H Q L D P A F R A N T T N
CH 6-2107
CH 7-1117A
M G Q N L S T S N P L G F F P D H Q L D P A F R A N T A N
CH 9-599
M G Q N L S T S N P L G F F P D H Q L D P A F R A N T A N
CH 11-884
CH 11-2343A
CH 12-958
COMPL 4-1725
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COMPL 11-317
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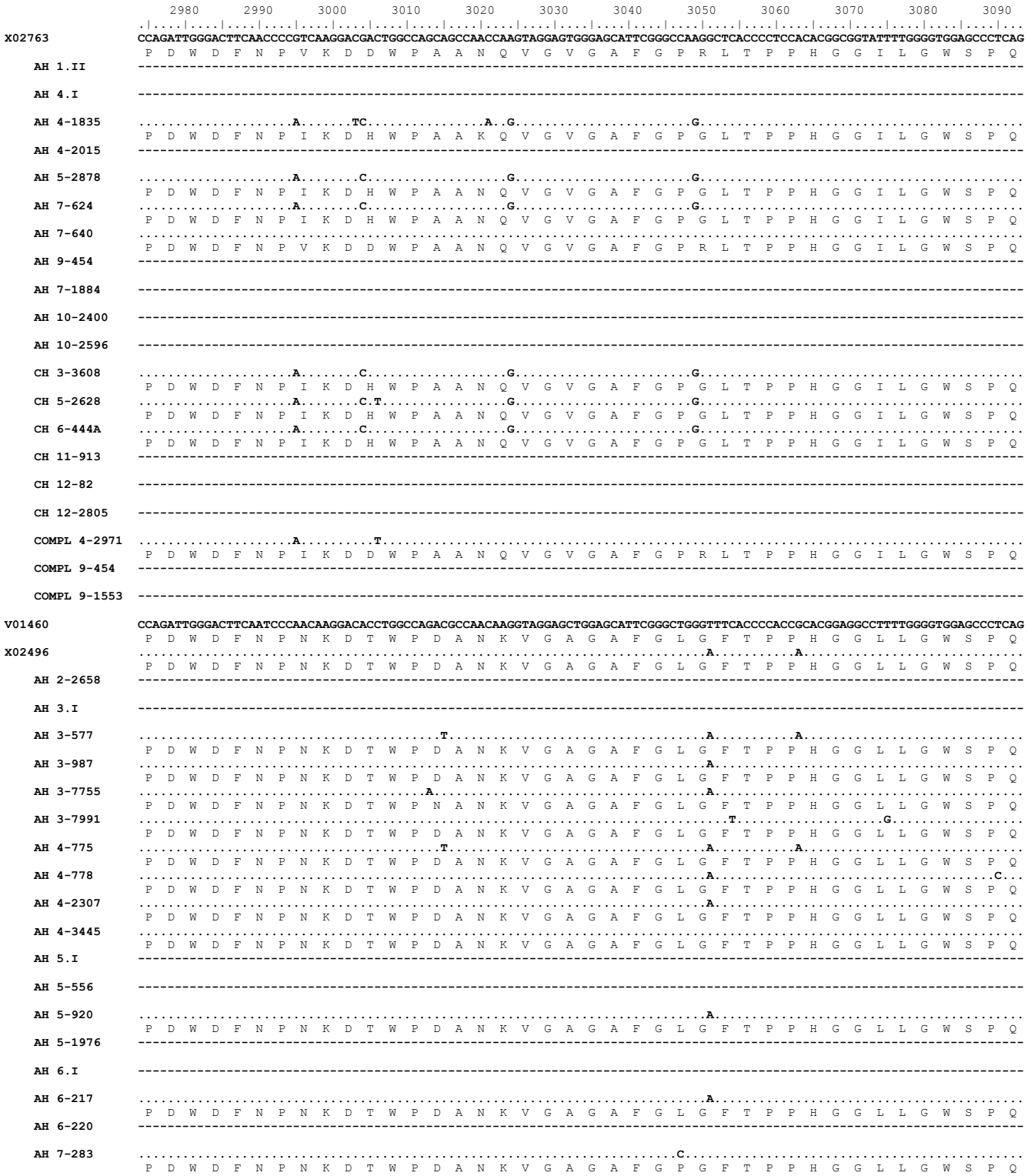
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AH 1.II
AH 4.I
AH 4-1835
P D W D F N P I K D H W P A A K Q V G V G A F G P G L T P P H G G I L G W S P Q
AH 4-2015
AH 5-2878
P D W D F N P I K D H W P A A N Q V G V G A F G P G L T P P H G G I L G W S P Q
AH 7-624
P D W D F N P I K D H W P A A N Q V G V G A F G P G L T P P H G G I L G W S P Q
AH 7-640
P D W D F N P V K D D W P A A N Q V G V G A F G P R L T P P H G G I L G W S P Q
AH 9-454
AH 7-1884
AH 10-2400
AH 10-2596
CH 3-3608
P D W D F N P I K D H W P A A N Q V G V G A F G P G L T P P H G G I L G W S P Q
CH 5-2628
P D W D F N P I K D H W P A A N Q V G V G A F G P G L T P P H G G I L G W S P Q
CH 6-444A
P D W D F N P I K D H W P A A N Q V G V G A F G P G L T P P H G G I L G W S P Q
CH 11-913
CH 12-82
CH 12-2805
COMPL 4-2971
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COMPL 9-454
COMPL 9-1553
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AH 2-2658
AH 3.I
AH 3-577
P D W D F N P N K D T W P D A N K V G A G A F G L G F T P P H G G L L G W S P Q
AH 3-987
P D W D F N P N K D T W P D A N K V G A G A F G L G F T P P H G G L L G W S P Q
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AH 4-775
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AH 4-778
P D W D F N P N K D T W P D A N K V G A G A F G L G F T P P H G G L L G W S P Q
AH 4-2307
P D W D F N P N K D T W P D A N K V G A G A F G L G F T P P H G G L L G W S P Q
AH 4-3445
P D W D F N P N K D T W P D A N K V G A G A F G L G F T P P H G G L L G W S P Q
AH 5.I
AH 5-556
AH 5-920
P D W D F N P N K D T W P D A N K V G A G A F G L G F T P P H G G L L G W S P Q
AH 5-1976
AH 6.I
AH 6-217
P D W D F N P N K D T W P D A N K V G A G A F G L G F T P P H G G L L G W S P Q
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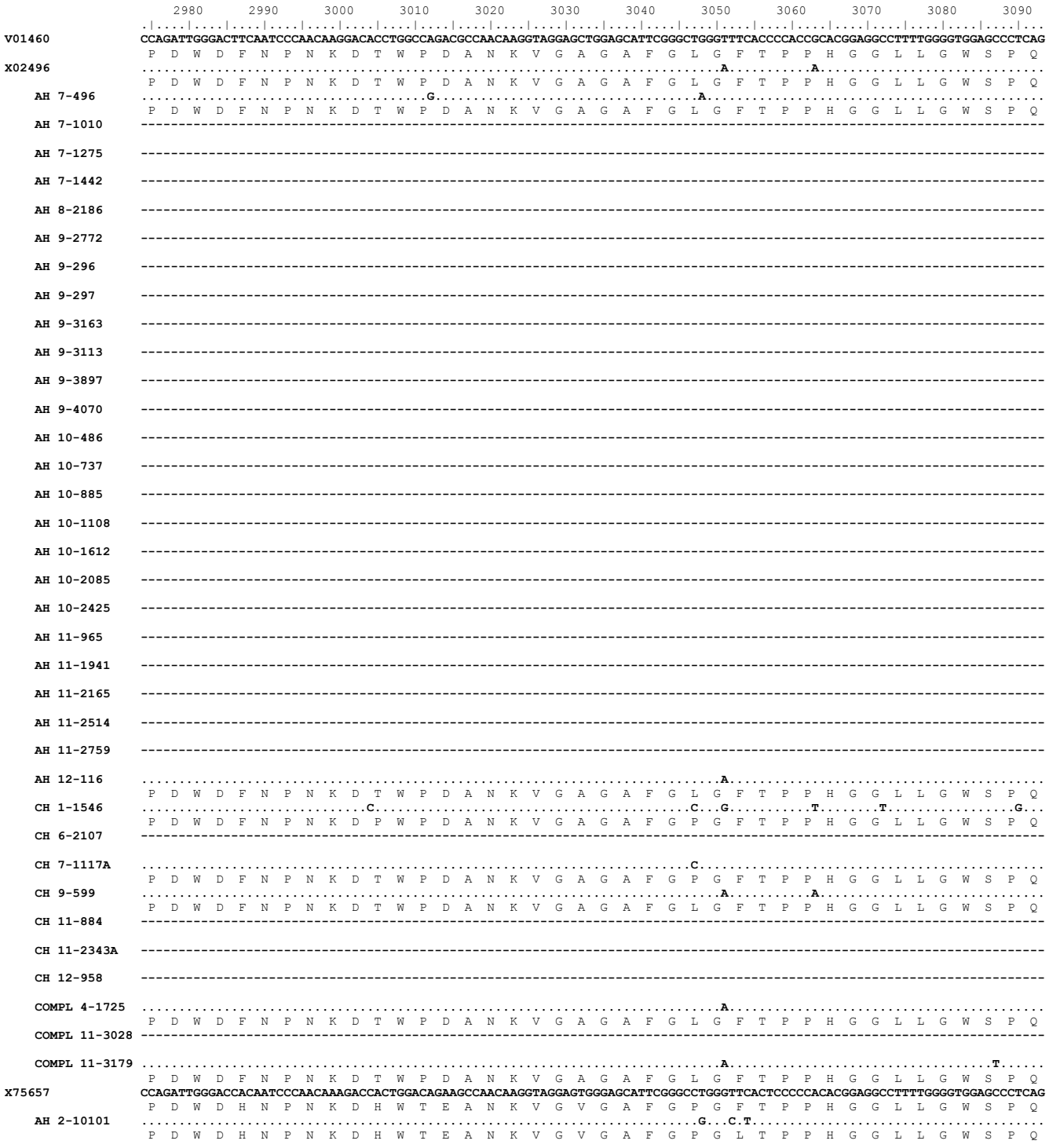
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AH 7-1010
AH 7-1275
AH 7-1442
AH 8-2186
AH 9-2772
AH 9-296
AH 9-297
AH 9-3163
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AH 9-3897
AH 9-4070
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AH 10-1612
AH 10-2085
AH 10-2425
AH 11-965
AH 11-1941
AH 11-2165
AH 11-2514
AH 11-2759
AH 12-116 .....A.....
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CH 1-1546 .....C.....C...G.....T.....T.....G...
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CH 6-2107
CH 7-1117A .....C.....
P D W D F N P N K D T W P D A N K V G A G A F G P G F T P P H G G L L G W S P Q
CH 9-599 .....A.....A.....
P D W D F N P N K D T W P D A N K V G A G A F G L G F T P P H G G L L G W S P Q
CH 11-884
CH 11-2343A
CH 12-958
COMPL 4-1725 .....A.....
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COMPL 11-3028
COMPL 11-3179 .....A.....T.....
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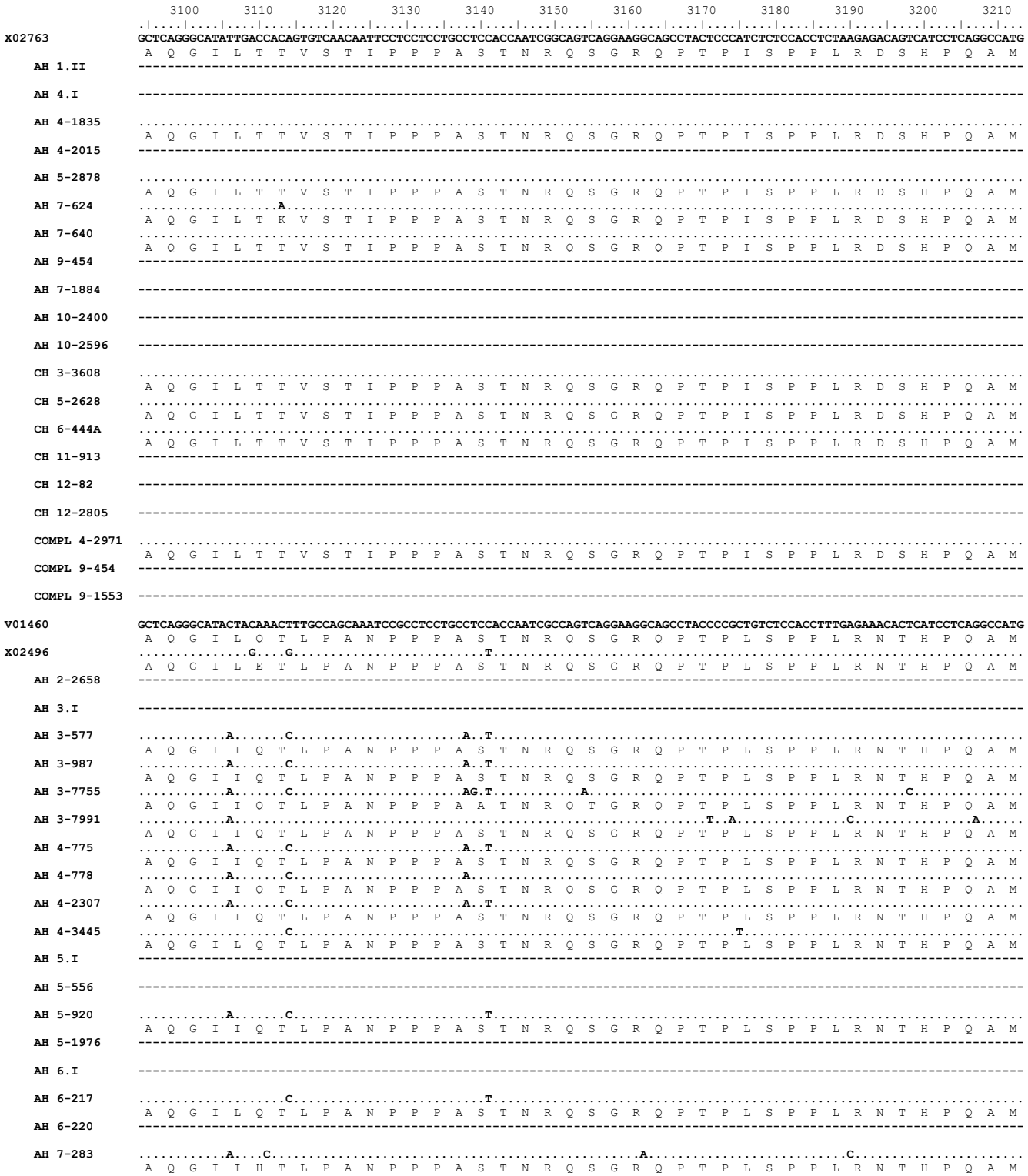
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AH 4-2015
AH 5-2878
AH 7-624
AH 7-640
AH 9-454
AH 7-1884
AH 10-2400
AH 10-2596
CH 3-3608
CH 5-2628
CH 6-444A
CH 11-913
CH 12-82
CH 12-2805
COMPL 4-2971
COMPL 9-454
COMPL 9-1553
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X02496
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AH 3.I
AH 3-577
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AH 4-3445
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AH 5-556
AH 5-920
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AH 5-1976
AH 6.I
AH 6-217
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AH 7-283
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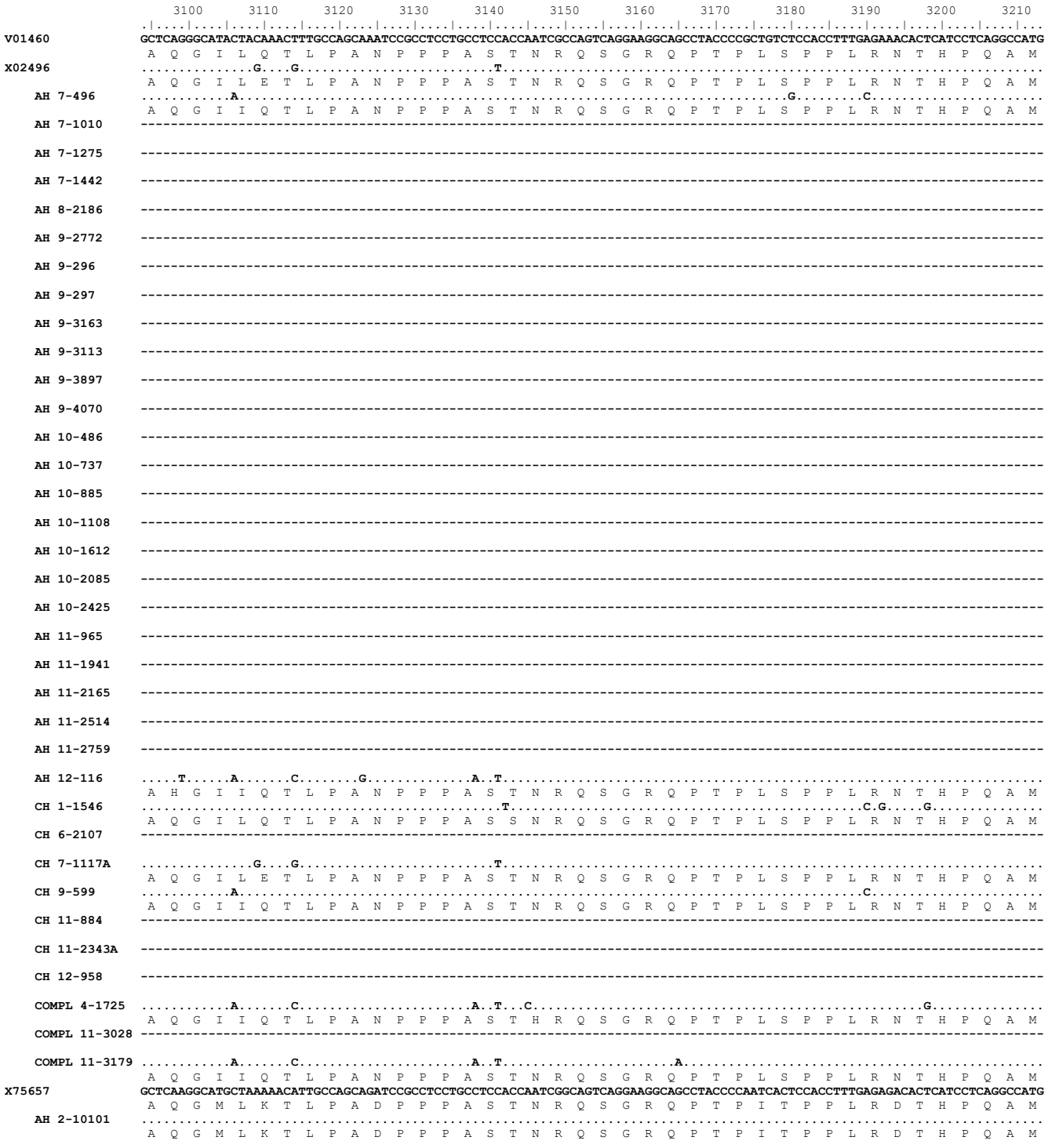
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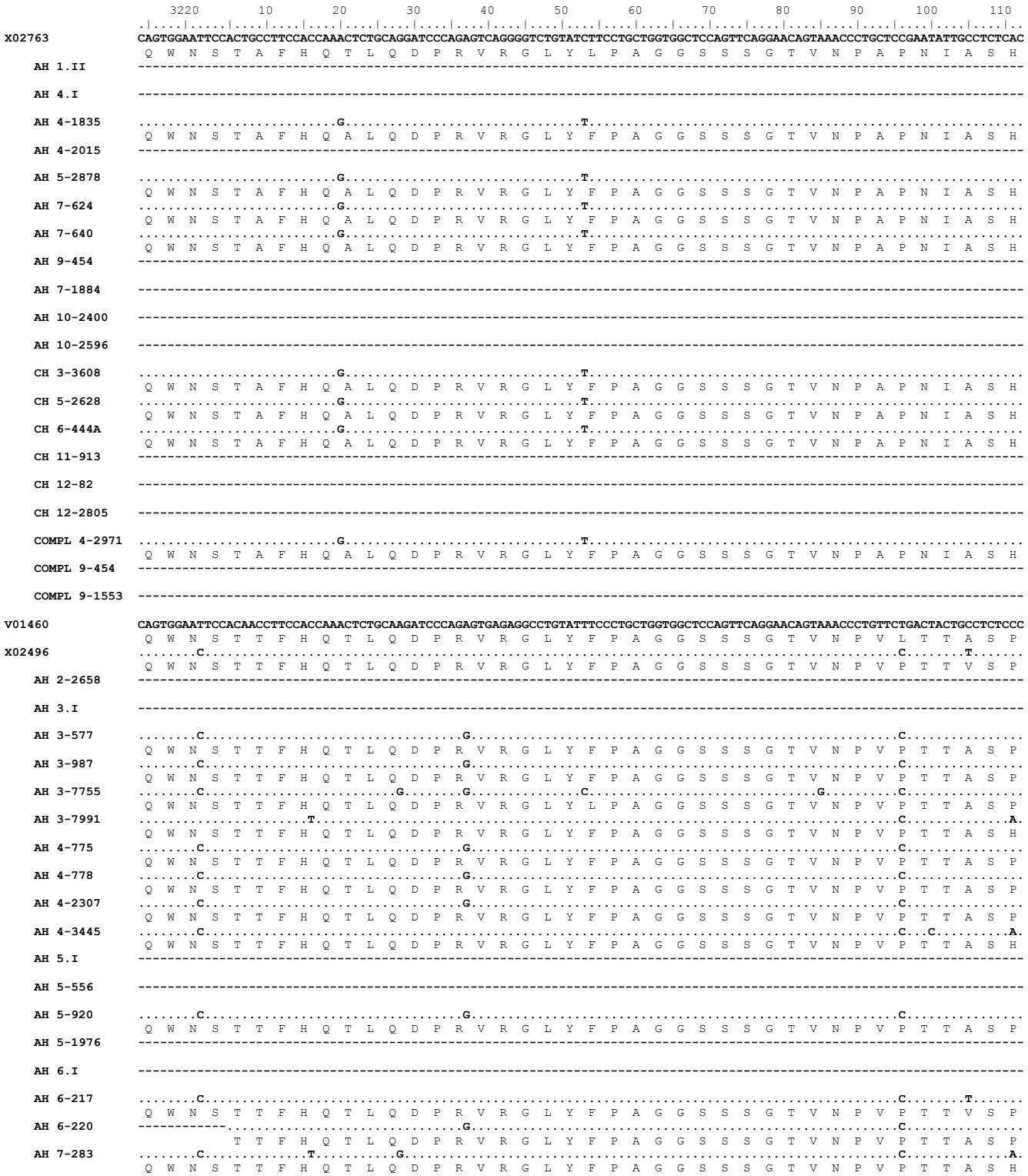
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AH 4.I
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AH 4-2015
AH 5-2878
AH 7-624
AH 7-640
AH 9-454
AH 7-1884
AH 10-2400
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CH 3-3608
CH 5-2628
CH 6-444A
CH 11-913
CH 12-82
CH 12-2805
COMPL 4-2971
COMPL 9-454
COMPL 9-1553
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X02496
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AH 3.I
AH 3-577
AH 3-987
AH 3-7755
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AH 4-778
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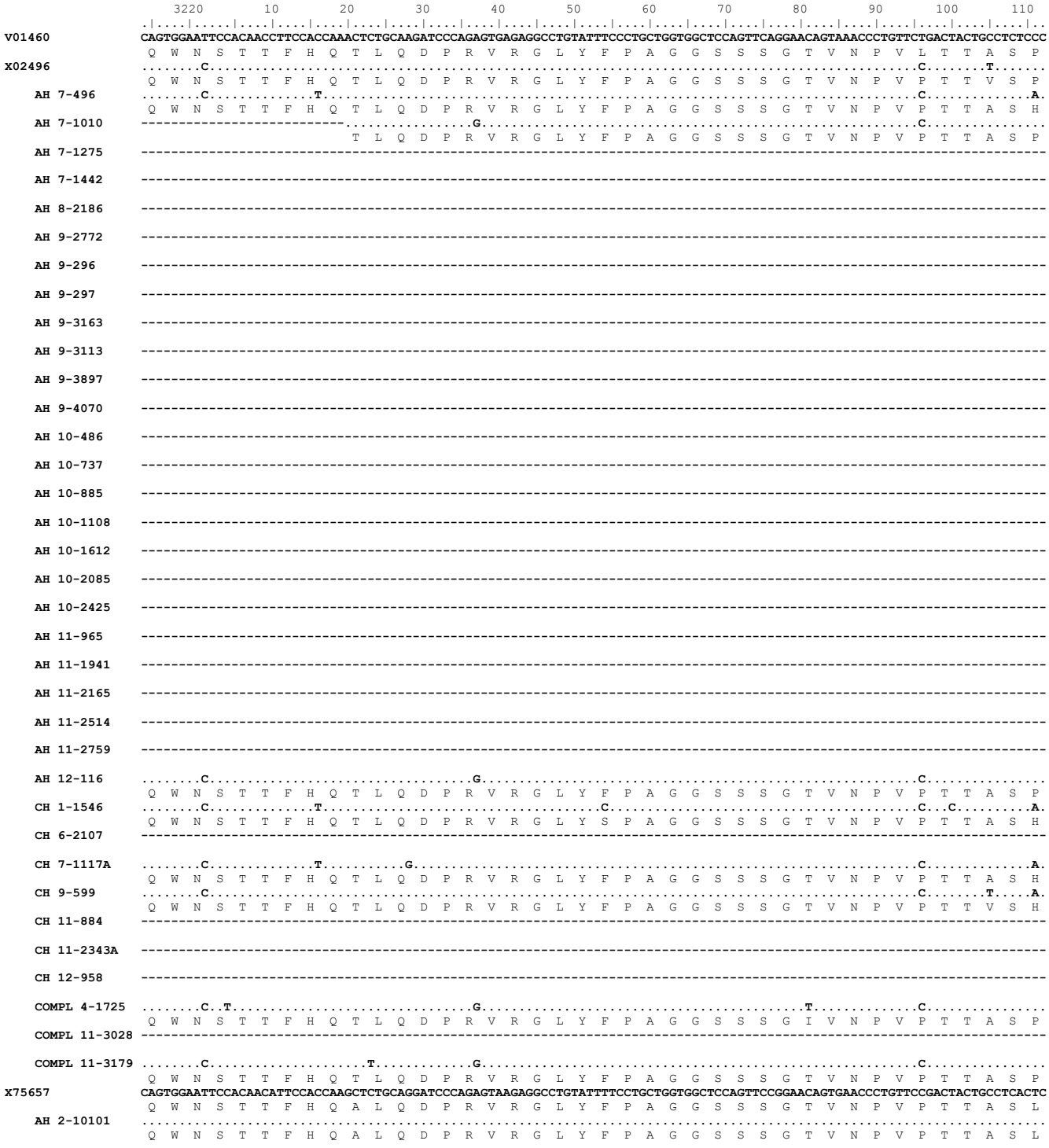
AH 7-283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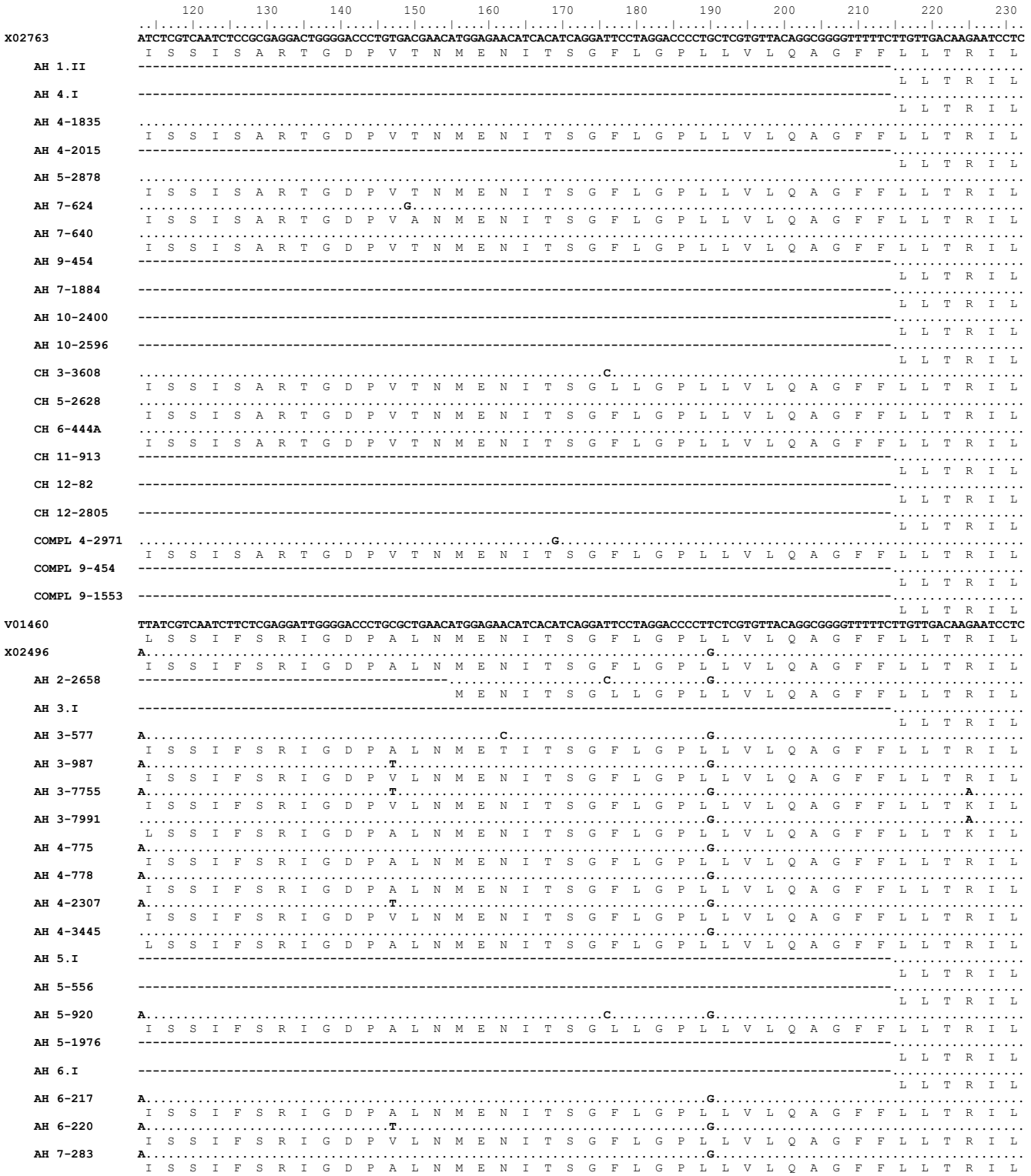
AH 2-10101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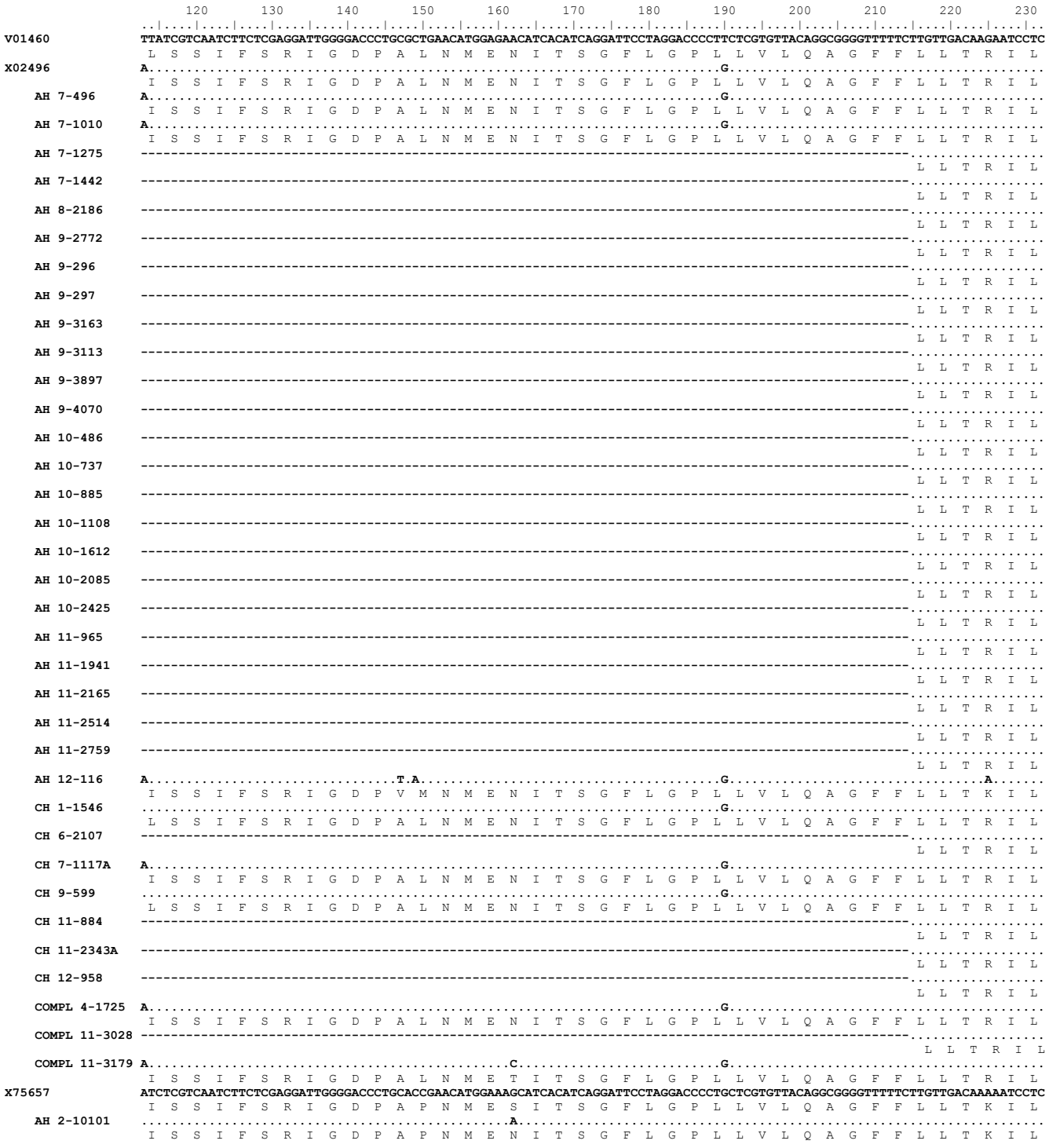
AH 1.II
L L T R I L
AH 4.I
L L T R I L
AH 4-1835
I S S I S A R T G D P V T N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 4-2015
L L T R I L
AH 5-2878
I S S I S A R T G D P V T N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 7-624
I S S I S A R T G D P V A N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 7-640
I S S I S A R T G D P V T N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 9-454
L L T R I L
AH 7-1884
L L T R I L
AH 10-2400
L L T R I L
AH 10-2596
L L T R I L
CH 3-3608
I S S I S A R T G D P V T N M E N I T S G L L G P L L V L Q A G F F L L T R I L
CH 5-2628
I S S I S A R T G D P V T N M E N I T S G F L G P L L V L Q A G F F L L T R I L
CH 6-444A
I S S I S A R T G D P V T N M E N I T S G F L G P L L V L Q A G F F L L T R I L
CH 11-913
L L T R I L
CH 12-82
L L T R I L
CH 12-2805
L L T R I L
COMPL 4-2971
I S S I S A R T G D P V T N M E N I T S G F L G P L L V L Q A G F F L L T R I L
COMPL 9-454
L L T R I L
COMPL 9-1553
L L T R I L
V01460 TTATCGTCAATCTTCTCGAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCACATCAGGATTCCTAGGACCCCTTCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTC
L S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
X02496
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 2-2658
M E N I T S G L L G P L L V L Q A G F F L L T R I L
AH 3.I
L L T R I L
AH 3-577
I S S I F S R I G D P A L N M E T I T S G F L G P L L V L Q A G F F L L T R I L
AH 3-987
I S S I F S R I G D P V L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 3-7755
I S S I F S R I G D P V L N M E N I T S G F L G P L L V L Q A G F F L L T K I L
AH 3-7991
L S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T K I L
AH 4-775
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 4-778
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 4-2307
I S S I F S R I G D P V L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 4-3445
L S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 5.I
L L T R I L
AH 5-556
L L T R I L
AH 5-920
I S S I F S R I G D P A L N M E N I T S G L L G P L L V L Q A G F F L L T R I L
AH 5-1976
L L T R I L
AH 6.I
L L T R I L
AH 6-217
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 6-220
I S S I F S R I G D P V L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 7-283
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L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L S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
X02496 A.....G.....
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 7-496 A.....G.....
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 7-1010 A.....G.....
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
AH 7-1275 ----------.....
L L T R I L
AH 7-1442 ----------.....
L L T R I L
AH 8-2186 ----------.....
L L T R I L
AH 9-2772 ----------.....
L L T R I L
AH 9-296 ----------.....
L L T R I L
AH 9-297 ----------.....
L L T R I L
AH 9-3163 ----------.....
L L T R I L
AH 9-3113 ----------.....
L L T R I L
AH 9-3897 ----------.....
L L T R I L
AH 9-4070 ----------.....
L L T R I L
AH 10-486 ----------.....
L L T R I L
AH 10-737 ----------.....
L L T R I L
AH 10-885 ----------.....
L L T R I L
AH 10-1108 ----------.....
L L T R I L
AH 10-1612 ----------.....
L L T R I L
AH 10-2085 ----------.....
L L T R I L
AH 10-2425 ----------.....
L L T R I L
AH 11-965 ----------.....
L L T R I L
AH 11-1941 ----------.....
L L T R I L
AH 11-2165 ----------.....
L L T R I L
AH 11-2514 ----------.....
L L T R I L
AH 11-2759 ----------.....
L L T R I L
AH 12-116 A.....T.A.....G.....A.....
I S S I F S R I G D P V M N M E N I T S G F L G P L L V L Q A G F F L L T K I L
CH 1-1546 .....G.....
L S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
CH 6-2107 ----------.....
L L T R I L
CH 7-1117A A.....G.....
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
CH 9-599 .....G.....
L S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
CH 11-884 ----------.....
L L T R I L
CH 11-2343A ----------.....
L L T R I L
CH 12-958 ----------.....
L L T R I L
COMPL 4-1725 A.....G.....
I S S I F S R I G D P A L N M E N I T S G F L G P L L V L Q A G F F L L T R I L
COMPL 11-3028 ----------.....
L L T R I L
COMPL 11-3179 A.....C.....G.....
I S S I F S R I G D P A L N M E T I T S G F L G P L L V L Q A G F F L L T R I L
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I S S I F S R I G D P A P N M E S I T S G F L G P L L V L Q A G F F L L T K I L
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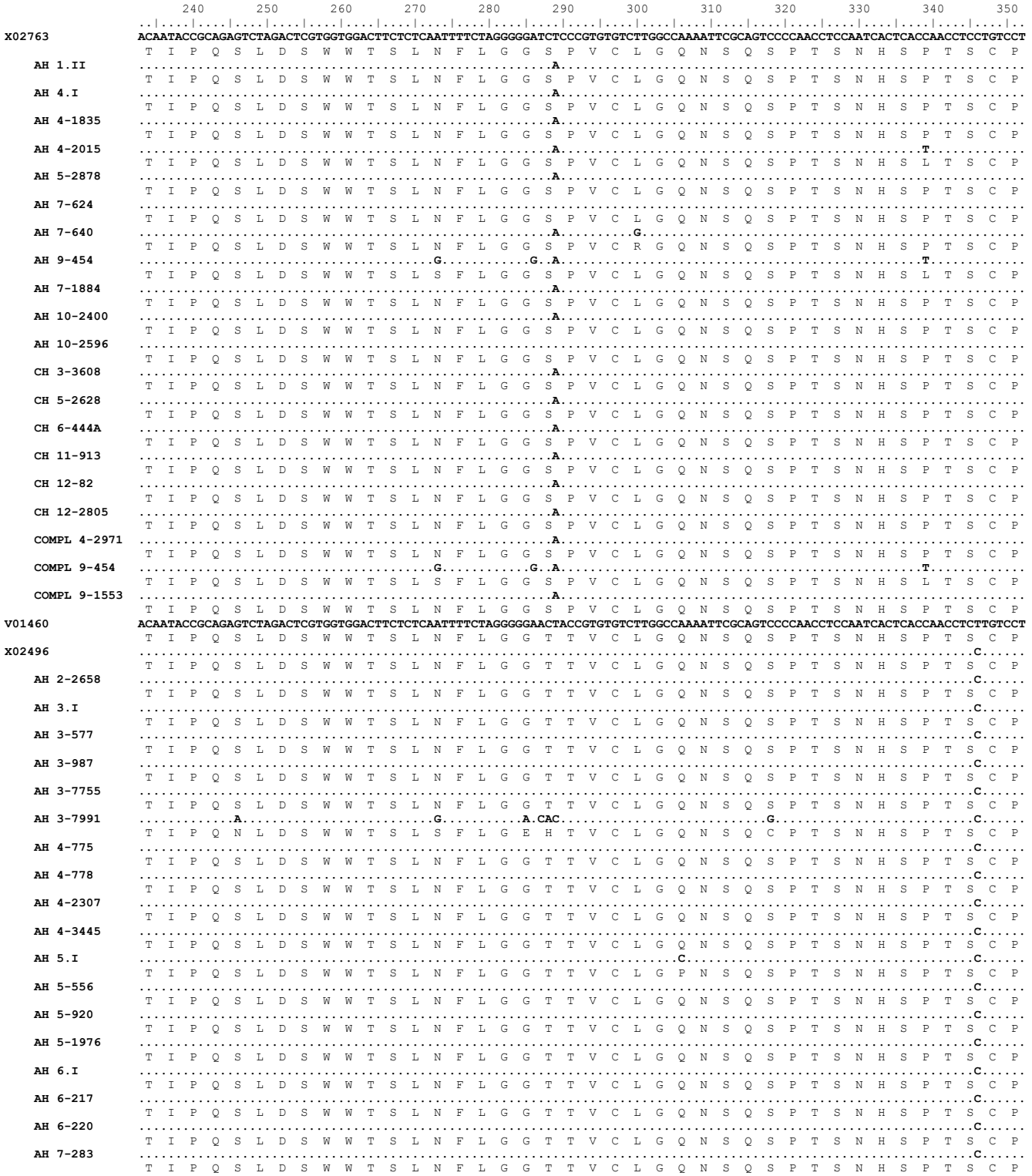
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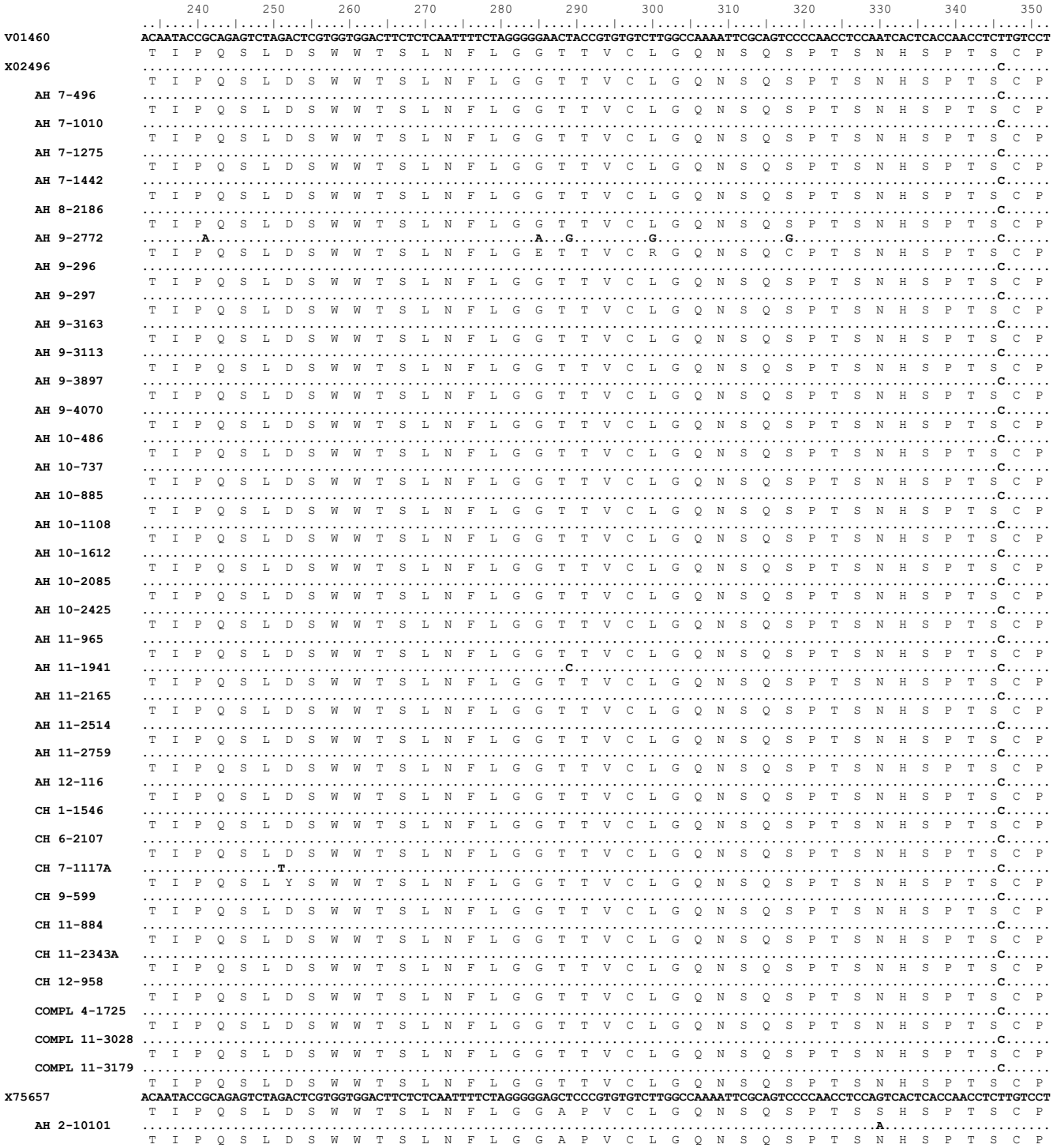
T I P Q S L D S W W T S L N F L G G S P V C L G Q N S Q S P T S N H S P T S C P
AH 1.II ....A.....
AH 4.I ....A.....
AH 4-1835 ....A.....
AH 4-2015 ....A.....T.....
AH 5-2878 ....A.....
AH 7-624
AH 7-640 ....A.....G.....
AH 9-454 ....G.....G..A.....T.....
AH 7-1884 ....A.....
AH 10-2400 ....A.....
AH 10-2596
CH 3-3608 ....A.....
CH 5-2628 ....A.....
CH 6-444A ....A.....
CH 11-913 ....A.....
CH 12-82 ....A.....
CH 12-2805 ....A.....
COMPL 4-2971 ....A.....
COMPL 9-454 ....G.....G..A.....T.....
COMPL 9-1553 ....A.....
V01460 ACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCCT
X02496 .....C.....
AH 2-2658 .....C.....
AH 3.I .....C.....
AH 3-577 .....C.....
AH 3-987 .....C.....
AH 3-7755 .....C.....
AH 3-7991 .....A.....G.....A.CAC.....G.....C.....
AH 4-775 .....C.....
AH 4-778 .....C.....
AH 4-2307 .....C.....
AH 4-3445 .....C.....
AH 5.I .....C.....C.....
AH 5-556 .....C.....
AH 5-920 .....C.....
AH 5-1976 .....C.....
AH 6.I .....C.....
AH 6-217 .....C.....
AH 6-220 .....C.....
AH 7-283 .....C.....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T I P Q S L D S W W T S L N F L G G T T V C L G Q N S Q S P T S N H S P T S C P
X02496 .....C.....
AH 7-496 .....C.....
AH 7-1010 .....C.....
AH 7-1275 .....C.....
AH 7-1442 .....C.....
AH 8-2186 .....C.....
AH 9-2772 .....A.....A...G.....G.....G.....C.....
T I P Q S L D S W W T S L N F L G E T T V C R G Q N S Q C P T S N H S P T S C P
AH 9-296 .....C.....
AH 9-297 .....C.....
AH 9-3163 .....C.....
AH 9-3113 .....C.....
AH 9-3897 .....C.....
AH 9-4070 .....C.....
AH 10-486 .....C.....
AH 10-737 .....C.....
AH 10-885 .....C.....
AH 10-1108 .....C.....
AH 10-1612 .....C.....
AH 10-2085 .....C.....
AH 10-2425 .....C.....
AH 11-965 .....C.....
AH 11-1941 .....C.....C.....
AH 11-2165 .....C.....
AH 11-2514 .....C.....
AH 11-2759 .....C.....
AH 12-116 .....C.....
CH 1-1546 .....C.....
CH 6-2107 .....C.....
CH 7-1117A .....T.....C.....
T I P Q S L Y S W W T S L N F L G G T T V C L G Q N S Q S P T S N H S P T S C P
CH 9-599 .....C.....
CH 11-884 .....C.....
CH 11-2343A .....C.....
CH 12-958 .....C.....
COMPL 4-1725 .....C.....
COMPL 11-3028 .....C.....
COMPL 11-3179 .....C.....
X75657 ACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAGCTCCCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAGTCACTCACCAACCTCTTGTCCT
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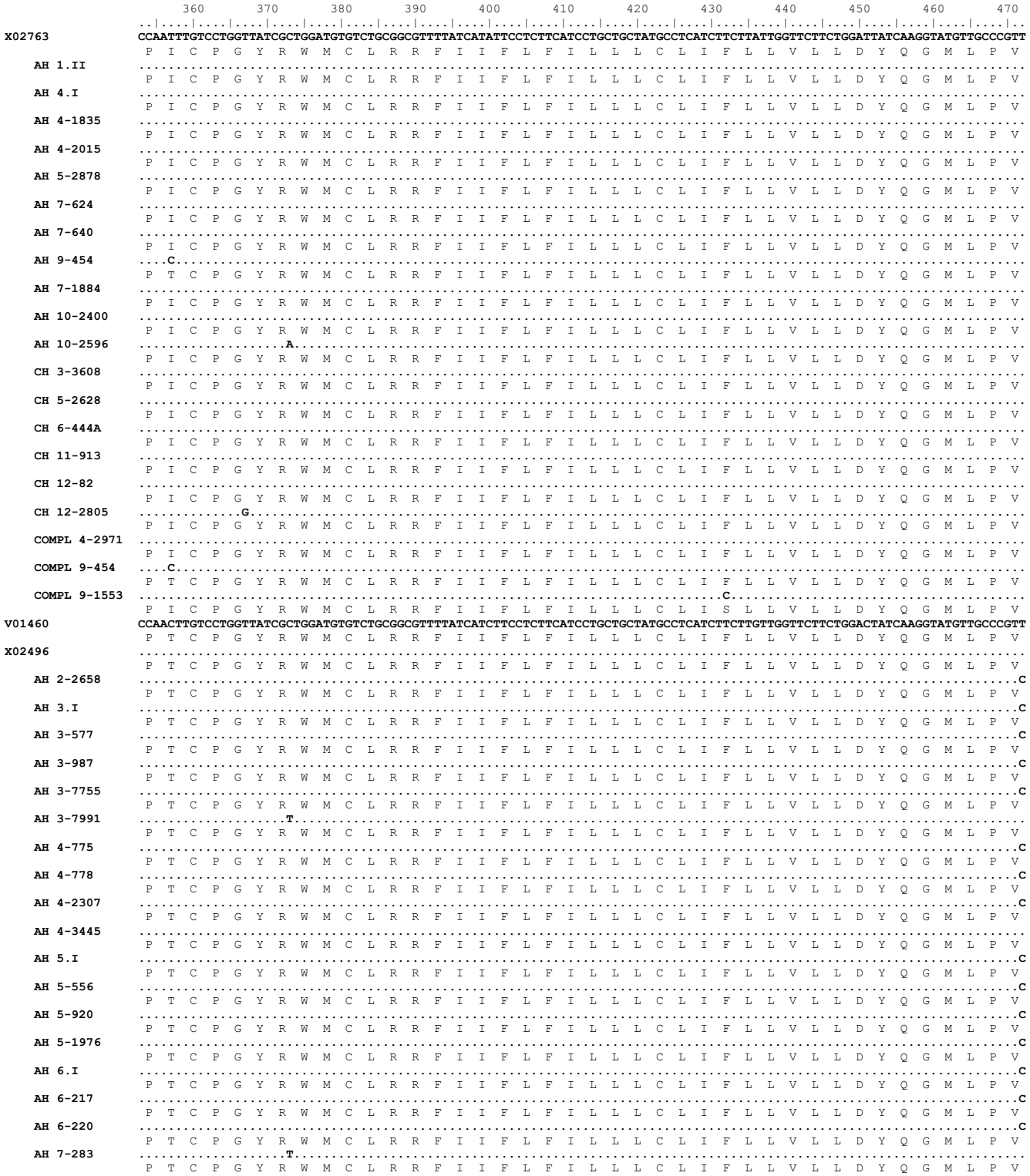
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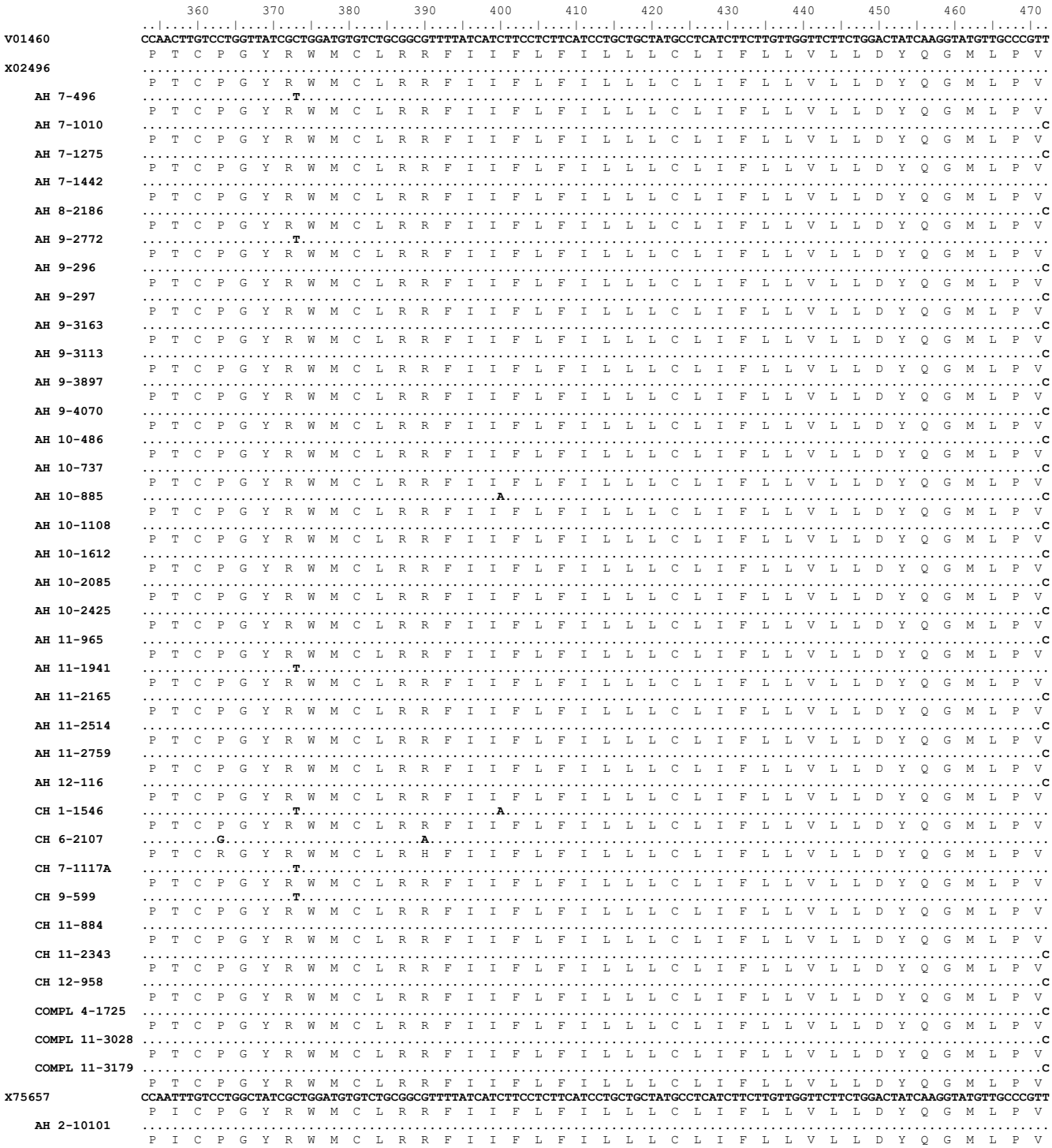
P I C P G Y R W M C L R R F I I F L F I L L L C L I F L L V L L D Y Q G M L P V
AH 1.II
AH 4.I
AH 4-1835
AH 4-2015
AH 5-2878
AH 7-624
AH 7-640
AH 9-454 C
P T C P G Y R W M C L R R F I I F L F I L L L C L I F L L V L L D Y Q G M L P V
AH 7-1884
AH 10-2400
AH 10-2596 .....A
CH 3-3608
CH 5-2628
CH 6-444A
CH 11-913
CH 12-82
CH 12-2805 .....G
COMPL 4-2971
COMPL 9-454 C
COMPL 9-1553 C
P I C P G Y R W M C L R R F I I F L F I L L L C L I S L L V L L D Y Q G M L P V
V01460 CCAACTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGCCCGTT
X02496
AH 2-2658 C
AH 3.I C
AH 3-577 C
AH 3-987 C
AH 3-7755 C
AH 3-7991 .....T.....
AH 4-775 C
AH 4-778 C
AH 4-2307 C
AH 4-3445
AH 5.I C
AH 5-556 C
AH 5-920 C
AH 5-1976 C
AH 6.I C
AH 6-217 C
AH 6-220 C
AH 7-283 .....T.....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P T C P G Y R W M C L R R F I I F L F I L L L C L I F L L V L L D Y Q G M L P V
X02496 .....
AH 7-496 .....T.....
AH 7-1010 C
AH 7-1275 C
AH 7-1442 .....
AH 8-2186 C
AH 9-2772 .....T.....
AH 9-296 C
AH 9-297 C
AH 9-3163 C
AH 9-3113 C
AH 9-3897 C
AH 9-4070 C
AH 10-486 C
AH 10-737 C
AH 10-885 .....A.....C
AH 10-1108 C
AH 10-1612 C
AH 10-2085 C
AH 10-2425 C
AH 11-965 C
AH 11-1941 .....T.....
AH 11-2165 C
AH 11-2514 C
AH 11-2759 C
AH 12-116 C
CH 1-1546 .....T.....A.....
CH 6-2107 .....G.....A.....
P T C R G Y R W M C L R H F I I F L F I L L L C L I F L L V L L D Y Q G M L P V
CH 7-1117A .....T.....
CH 9-599 .....T.....
CH 11-884 .....
CH 11-2343 C
CH 12-958 C
COMPL 4-1725 C
COMPL 11-3028 C
COMPL 11-3179 C
X75657 CCAATTTGTCCTGGCTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGCCCGTT
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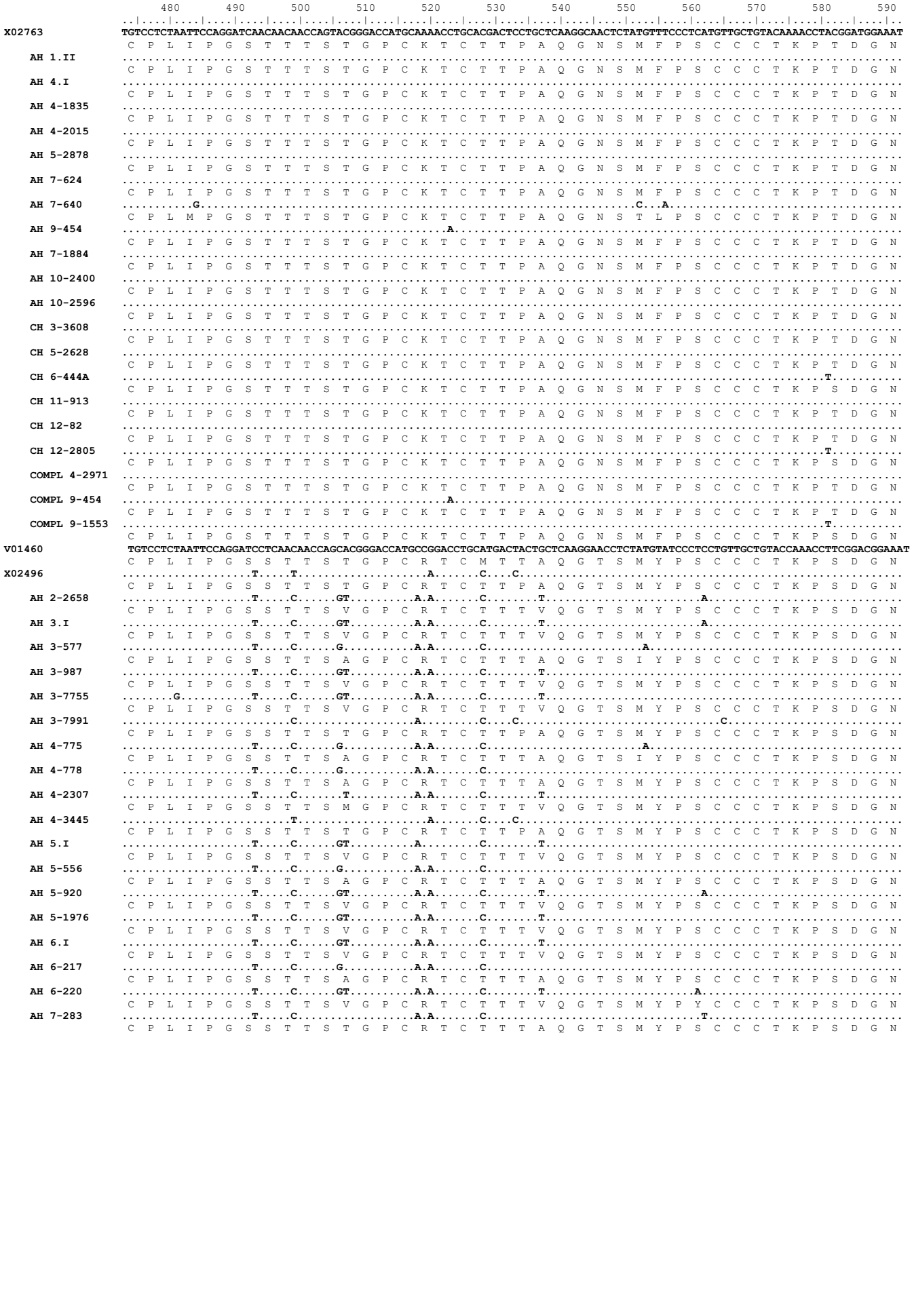
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AH 5-2878
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CH 3-3608
CH 5-2628
CH 6-444A
CH 11-913
CH 12-82
CH 12-2805
COMPL 4-2971
COMPL 9-454
COMPL 9-1553
V01460
X02496
AH 2-2658
AH 3.I
AH 3-577
AH 3-987
AH 3-7755
AH 3-7991
AH 4-775
AH 4-778
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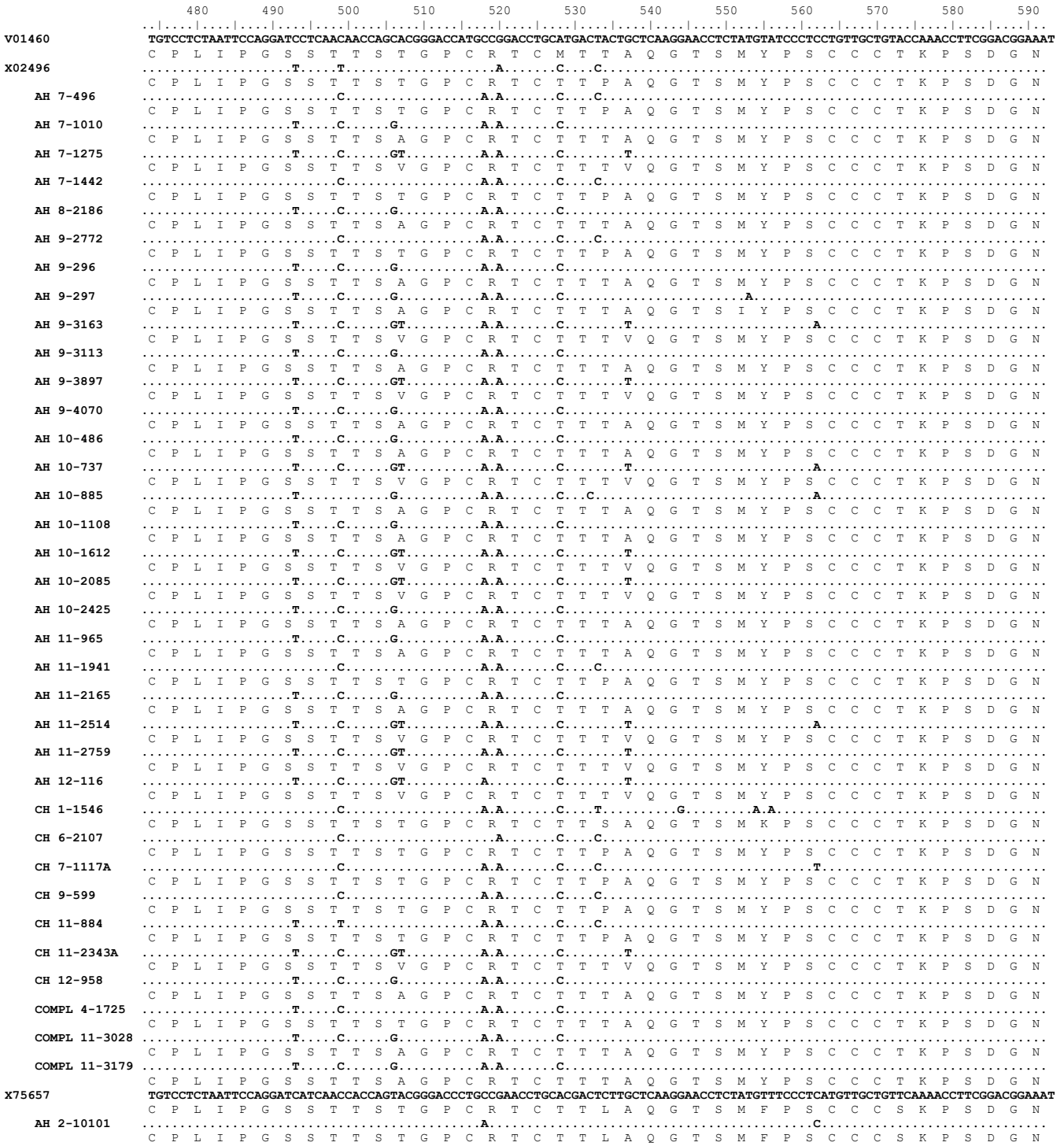
AH 7-283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C P L I P G S S T T S T G P C R T C M T T A Q G T S M Y P S C C C T K P S D G N
X02496 .....T.....T.....A.....C....C.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
AH 7-496 .....C.....A.A.....C....C.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
AH 7-1010 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 7-1275 .....T.....C.....GT.....A.A.....C.....T.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
AH 7-1442 .....C.....A.A.....C....C.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
AH 8-2186 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 9-2772 .....C.....A.A.....C....C.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
AH 9-296 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 9-297 .....T.....C.....G.....A.A.....C.....A.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S I Y P S C C C T K P S D G N
AH 9-3163 .....T.....C.....GT.....A.A.....C.....T.....A.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
AH 9-3113 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 9-3897 .....T.....C.....GT.....A.A.....C.....T.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
AH 9-4070 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 10-486 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 10-737 .....T.....C.....GT.....A.A.....C.....T.....A.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
AH 10-885 .....T.....G.....A.A.....C...C.....A.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 10-1108 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 10-1612 .....T.....C.....GT.....A.A.....C.....T.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
AH 10-2085 .....T.....C.....GT.....A.A.....C.....T.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
AH 10-2425 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 11-965 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 11-1941 .....C.....A.A.....C....C.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
AH 11-2165 .....T.....C.....G.....A.A.....C.....
C P L I P G S S T T S A G P C R T C T T T A Q G T S M Y P S C C C T K P S D G N
AH 11-2514 .....T.....C.....GT.....A.A.....C.....T.....A.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
AH 11-2759 .....T.....C.....GT.....A.A.....C.....T.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
AH 12-116 .....T.....C.....GT.....A.....C.....T.....
C P L I P G S S T T S V G P C R T C T T T V Q G T S M Y P S C C C T K P S D G N
CH 1-1546 .....C.....A.A.....C....T.....G.....A.A.....
C P L I P G S S T T S T G P C R T C T T S A Q G T S M K P S C C C T K P S D G N
CH 6-2107 .....C.....A.....C....C.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
CH 7-1117A .....C.....A.A.....C....C.....T.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
CH 9-599 .....C.....A.A.....C....C.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
CH 11-884 .....T.....T.....A.A.....C....C.....
C P L I P G S S T T S T G P C R T C T T P A Q G T S M Y P S C C C T K P S D G N
CH 11-2343A .....T.....C.....GT.....A.A.....C.....T.....
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CH 12-958 .....T.....C.....G.....A.A.....C.....
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COMPL 11-3028 .....T.....C.....G.....A.A.....C.....
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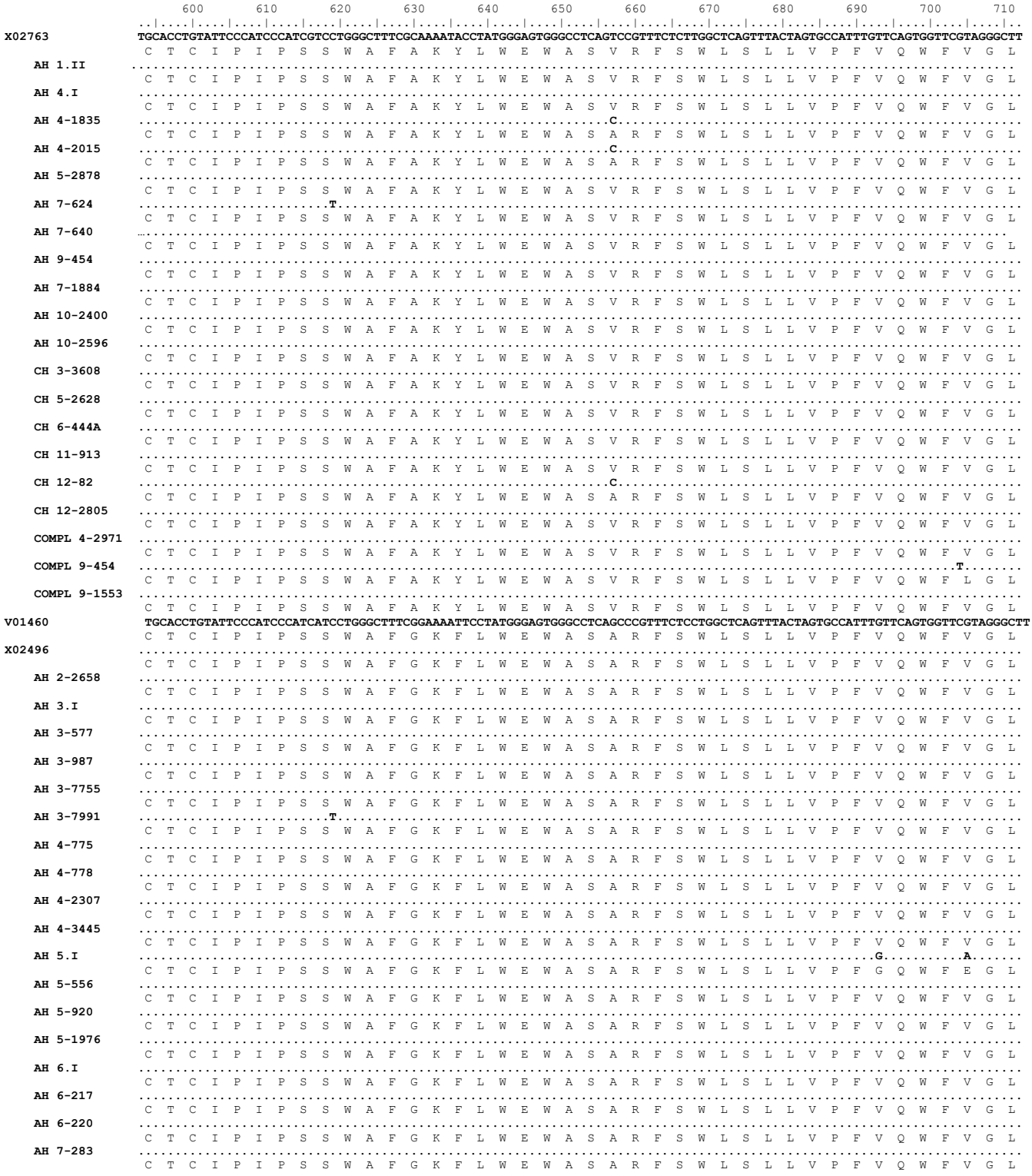
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AH 1.II
AH 4.I
AH 4-1835
AH 4-2015
AH 5-2878
AH 7-624
AH 7-640
AH 9-454
AH 7-1884
AH 10-2400
AH 10-2596
CH 3-3608
CH 5-2628
CH 6-444A
CH 11-913
CH 12-82
CH 12-2805
COMPL 4-2971
COMPL 9-454
COMPL 9-1553
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AH 3-577
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AH 6.I
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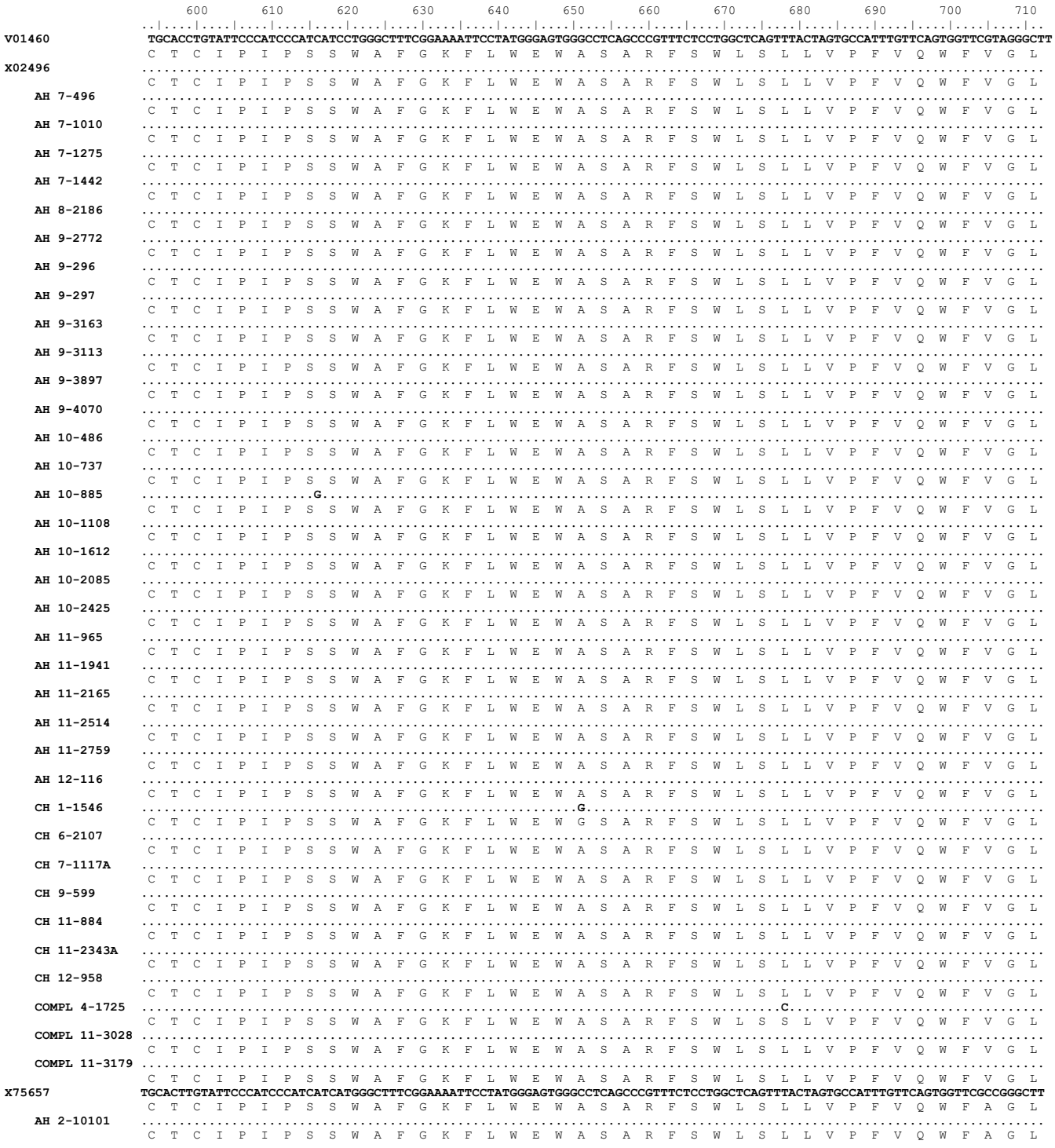
AH 7-283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AH 7-1275
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AH 9-3897
AH 9-4070
AH 10-486
AH 10-737
AH 10-885 .....G.....
AH 10-1108
AH 10-1612
AH 10-2085
AH 10-2425
AH 11-965
AH 11-1941
AH 11-2165
AH 11-2514
AH 11-2759
AH 12-116
CH 1-1546 .....G.....
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CH 7-1117A
CH 9-599
CH 11-884
CH 11-2343A
CH 12-958
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COMPL 11-3179
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AH 2-10101
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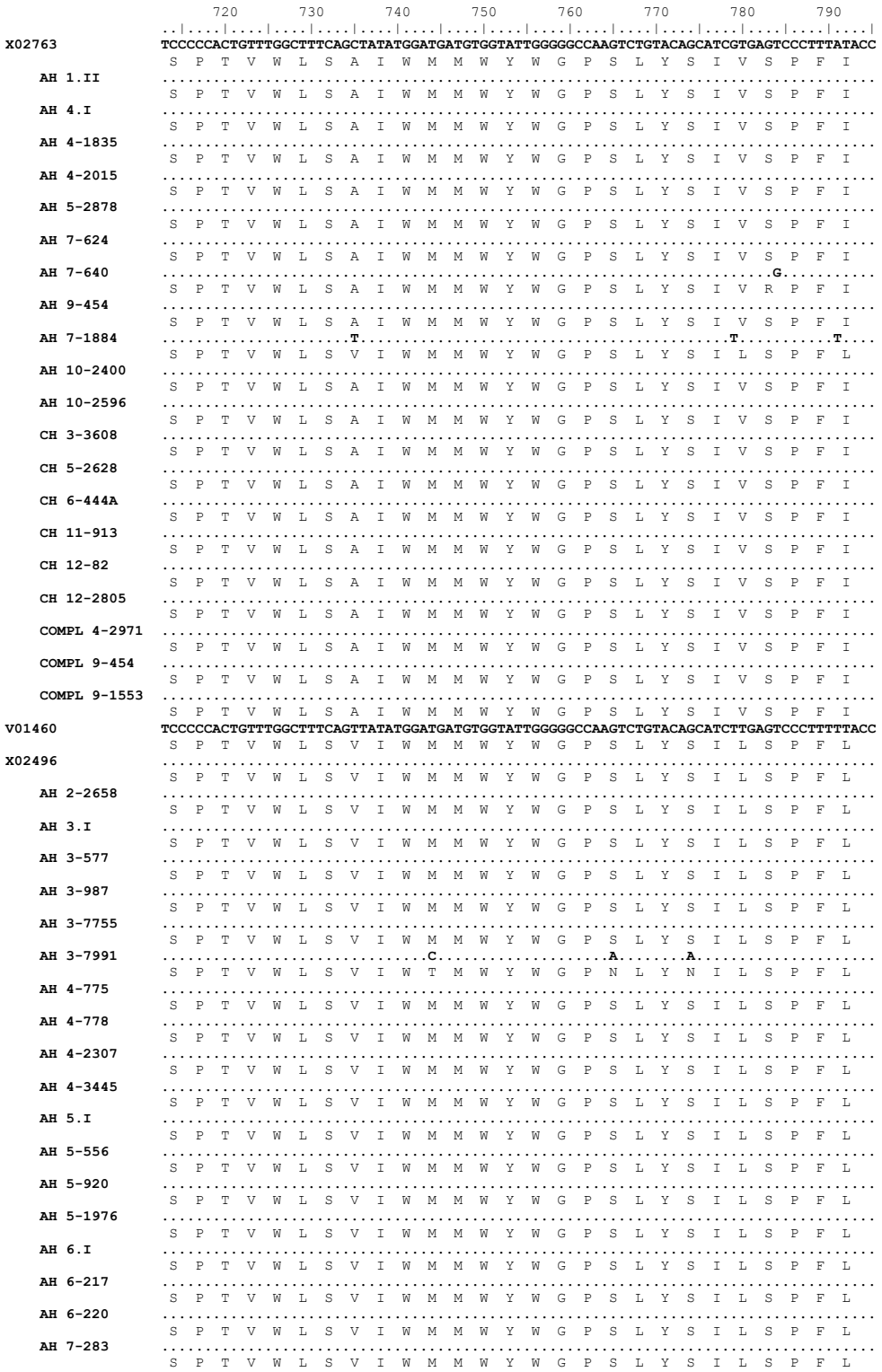
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AH 1.II
AH 4.I
AH 4-1835
AH 4-2015
AH 5-2878
AH 7-624
AH 7-640
AH 9-454
AH 7-1884
S P T V W L S V I W M M W Y W G P S L Y S I L S P F L
AH 10-2400
AH 10-2596
CH 3-3608
CH 5-2628
CH 6-444A
CH 11-913
CH 12-82
CH 12-2805
COMPL 4-2971
COMPL 9-454
COMPL 9-1553
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X02496
AH 2-2658
AH 3.I
AH 3-577
AH 3-987
AH 3-7755
AH 3-7991
S P T V W L S V I W T M W Y W G P N L Y N I L S P F L
AH 4-775
AH 4-778
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AH 5.I
AH 5-556
AH 5-920
AH 5-1976
AH 6.I
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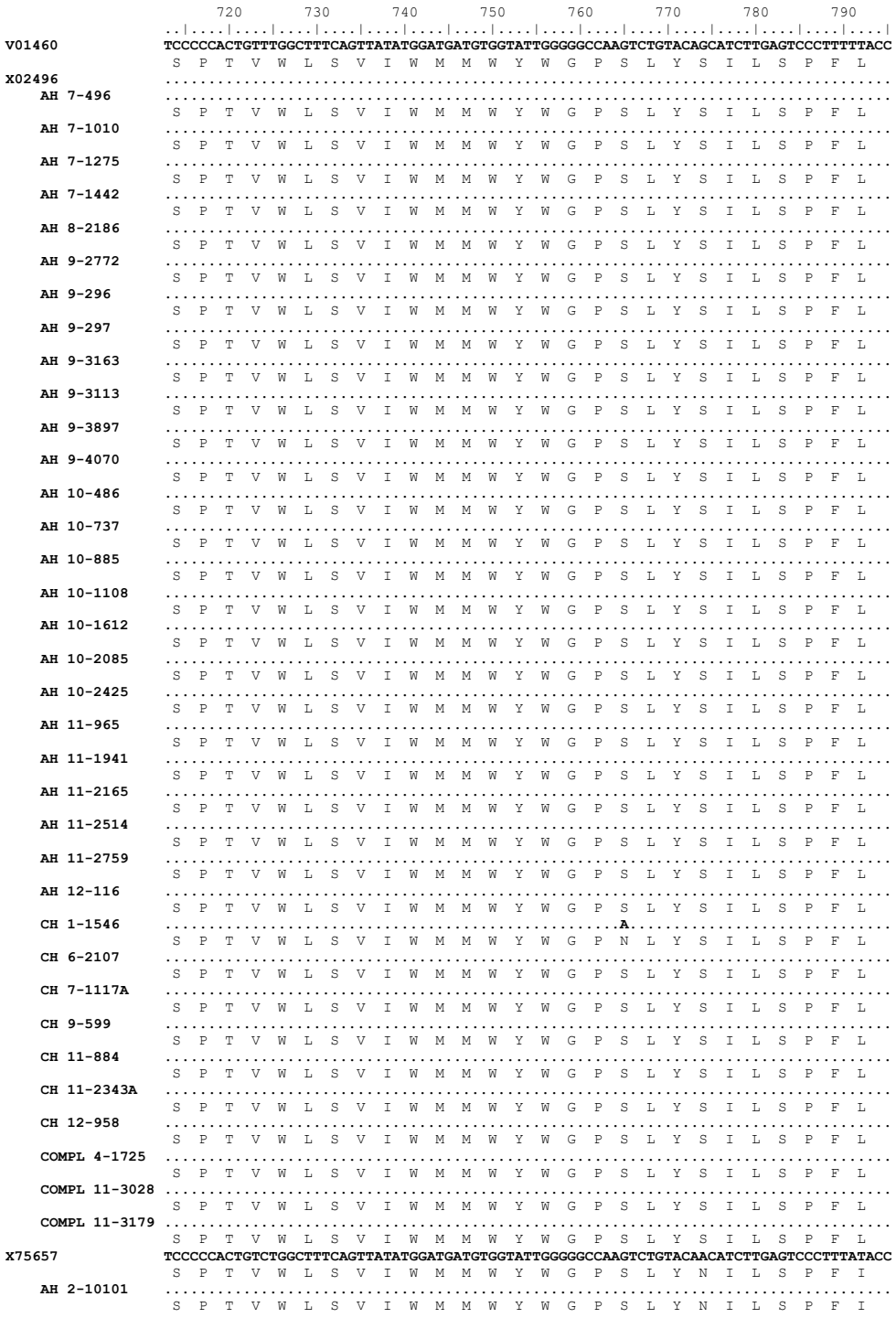
AH 7-283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CH 1-1546
A
S P T V W L S V I W M M W Y W G P N L Y S I L S P F L
CH 6-2107
CH 7-1117A
CH 9-599
CH 11-884
CH 11-2343A
CH 12-958
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COMPL 11-3028
COMPL 11-3179
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AH 2-10101

Appendix Part C. Sequences of gene X obtained during the study.

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   CH 12-2805     ................................................................................................................................
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   COMPL 4-2971   ................................................................................................................................
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X02496            ...T............................................................................................................A
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   AH 3-7755      ................................................................................................................................
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   AH 3-7991      ....................................C...........................................................................A...........
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   AH 3-987       ................................................................................................................................
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   AH 5-920       ................................................................................................................................
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   AH 6-2394      .....A.......G..................................................................................................................
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   AH 9-296       ................................................................................................................................
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   CH 7-1117A     ....................................C...........................................................................................
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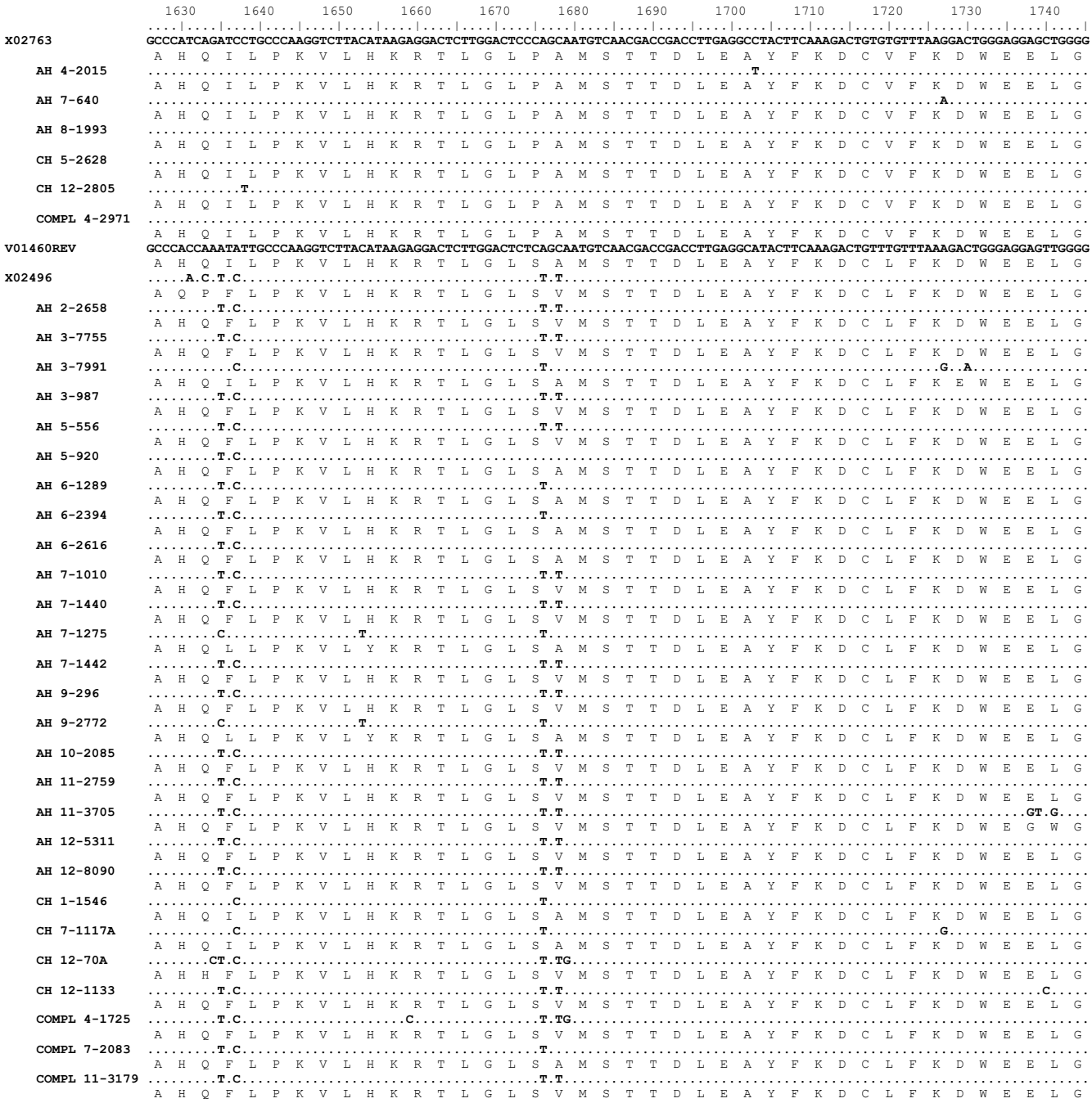
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A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 5-556 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 5-920 .....T.C.....
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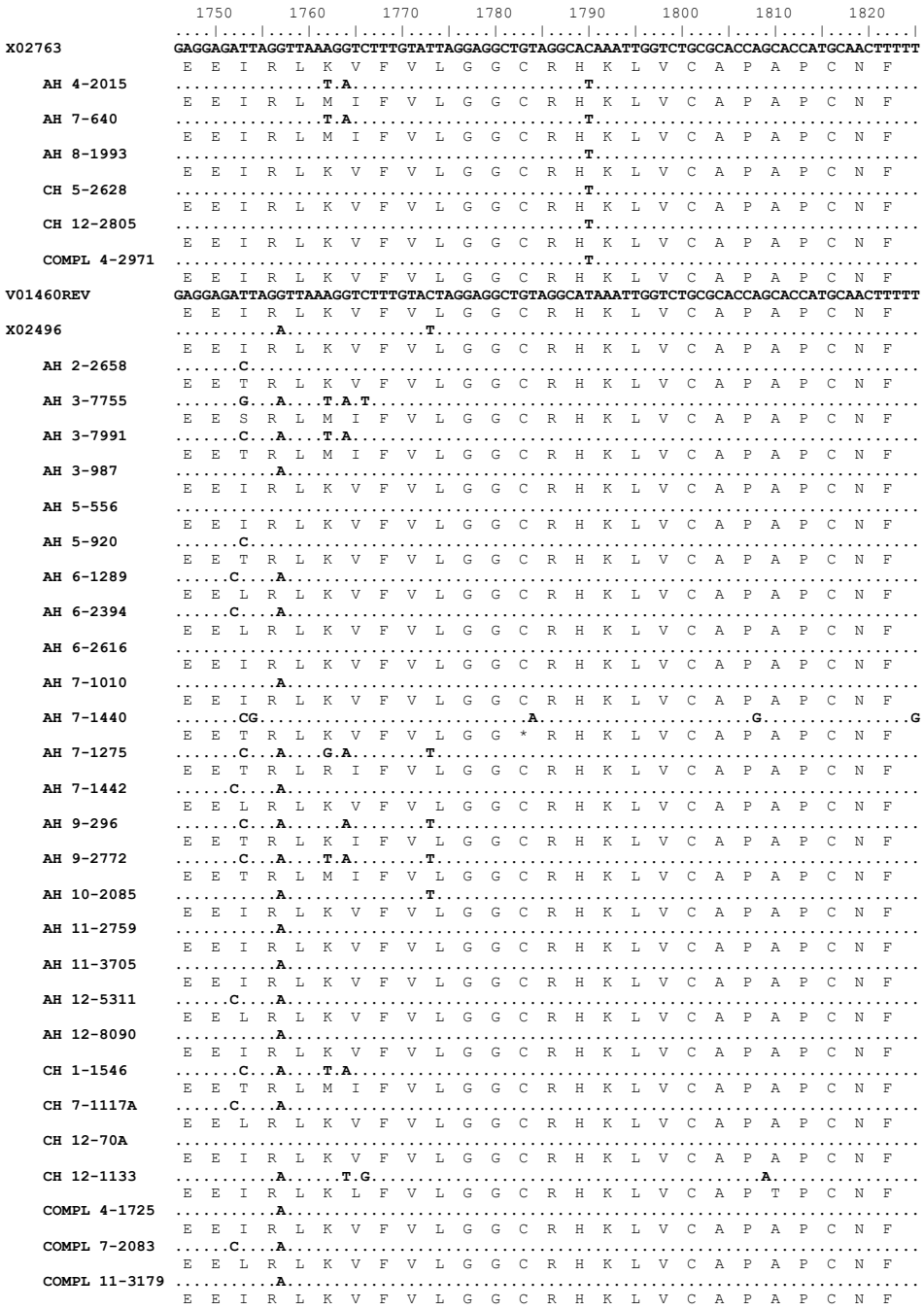
AH 7-1010 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 7-1440 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 7-1275 .....C.....T.....T.....
A H Q L L P K V L Y K R T L G L S A M S T T D L E A Y F K D C L F K D W E E L G
AH 7-1442 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 9-296 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 9-2772 .....C.....T.....T.....
A H Q L L P K V L Y K R T L G L S A M S T T D L E A Y F K D C L F K D W E E L G
AH 10-2085 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 11-2759 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 11-3705 .....T.C.....T.T.....GT.G....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E G W G
AH 12-5311 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
AH 12-8090 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
CH 1-1546 .....C.....T.....
A H Q I L P K V L H K R T L G L S A M S T T D L E A Y F K D C L F K D W E E L G
CH 7-1117A .....C.....T.....G.....
A H Q I L P K V L H K R T L G L S A M S T T D L E A Y F K D C L F K D W E E L G
CH 12-70A .....CT.C.....T.TG.....
A H H F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
CH 12-1133 .....T.C.....T.T.....C.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
COMPL 4-1725 .....T.C.....C.....T.TG.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G
COMPL 7-2083 .....T.C.....T.....
A H Q F L P K V L H K R T L G L S A M S T T D L E A Y F K D C L F K D W E E L G
COMPL 11-3179 .....T.C.....T.T.....
A H Q F L P K V L H K R T L G L S V M S T T D L E A Y F K D C L F K D W E E L G



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