

Compilation, alignment, and phylogenetic relationships of DNA polymerases

Dan K. Braithwaite and Junetsu Ito*

Department of Microbiology and Immunology, College of Medicine, Arizona Health Sciences Center, Tucson, AZ 85724, USA

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INTRODUCTION

This is an update of an earlier compilation and alignment of DNA polymerase sequences (Ito and Braithwaite, 1991). As in the previous compilation, we attempted to compile complete sequences, to facilitate the identification of conserved and viable regions of the DNA polymerases (1). This update includes, for the first time, three DNA polymerase sequences from *Archaea* (2); two new members of the Family A DNA polymerases; and 19 new members of the Family B DNA polymerases. In addition, we included nucleases that have related amino acid sequences to *E. coli* DNA polymerase I, and the sequence of *E. coli* DNA polymerase III (ϵ -subunit) was aligned to Family C due to its homology to *Bacillus subtilis* DNA polymerase III.

As in the previous compilation (1), Family A DNA polymerases are named for their homology to the product of the *polA* gene specifying *E. coli* DNA polymerase I; Family B DNA polymerases are named for their homology to the product of the *polB* gene encoding *E. coli* DNA polymerase II; and Family C DNA polymerases are named for their homology to the product of the *polC* encoding *E. coli* DNA polymerase III alpha subunit.

Table 1 summarizes the molecular weights and isoelectric points of each DNA polymerase and nuclease. Table 1 also serves as a reference guide to the sequences shown in Figures 1A, 1B, and 1C. Since no new sequences were published for the Family X DNA polymerases (β -like), we have excluded them from this compilation.

SEQUENCE ALIGNMENT

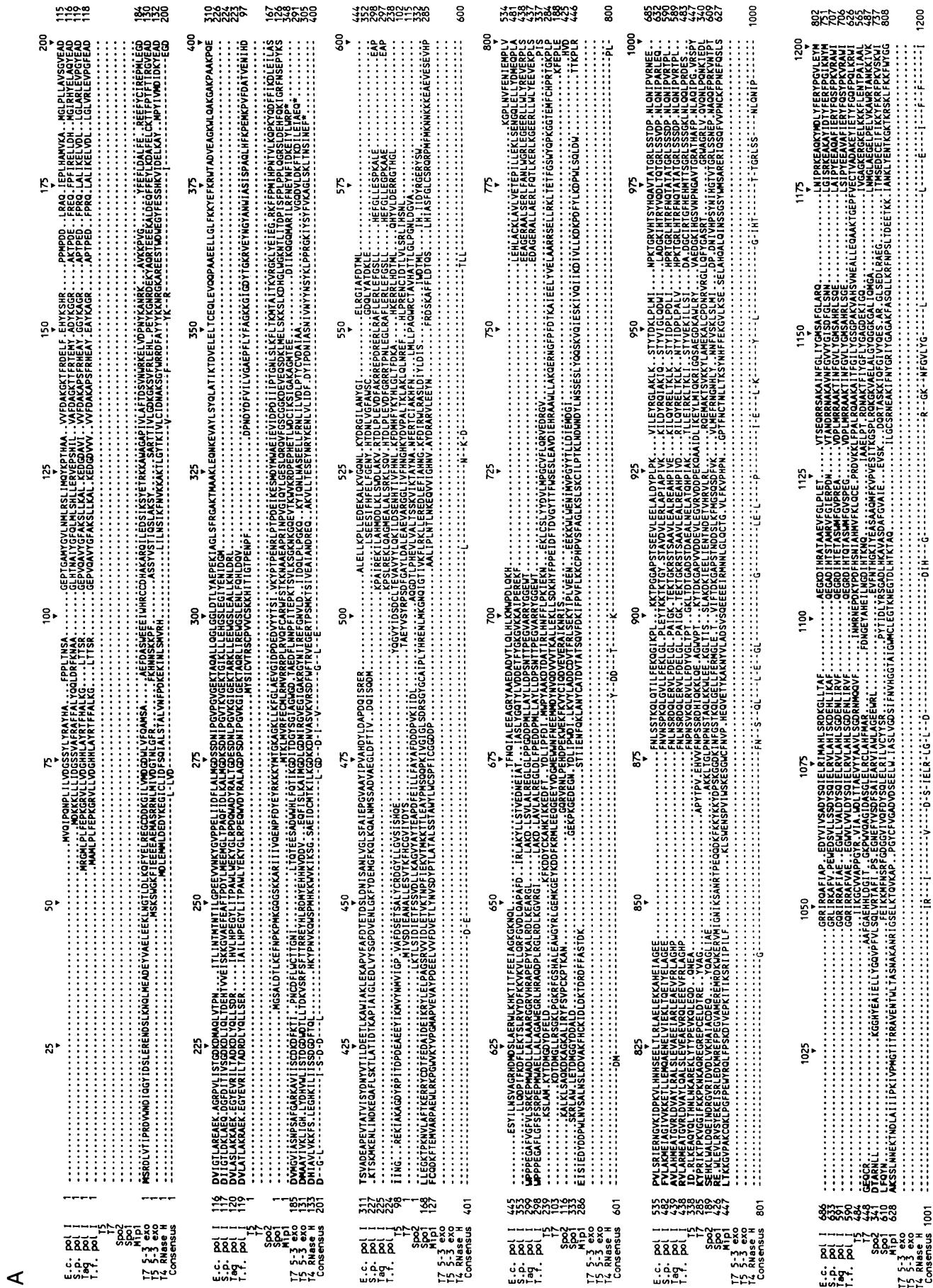
The multiple alignments of the amino acid sequences for this update were performed in most cases by merely adding on to our original alignments (1) where possible. Due to the large number of sequences added to the alignment for Family B we have changed the original alignment in some areas between obvious blocks of conserved sequences. The newer sequences were added by aligning each to the closest related sequence already aligned, or in many cases to the closest related group of sequences already aligned. A more recent addition to the UWGCG (University of Wisconsin Genetic Computer Group) program package, PILEUP, a multiple alignment program, was used extensively to try and locate significant homology in groups

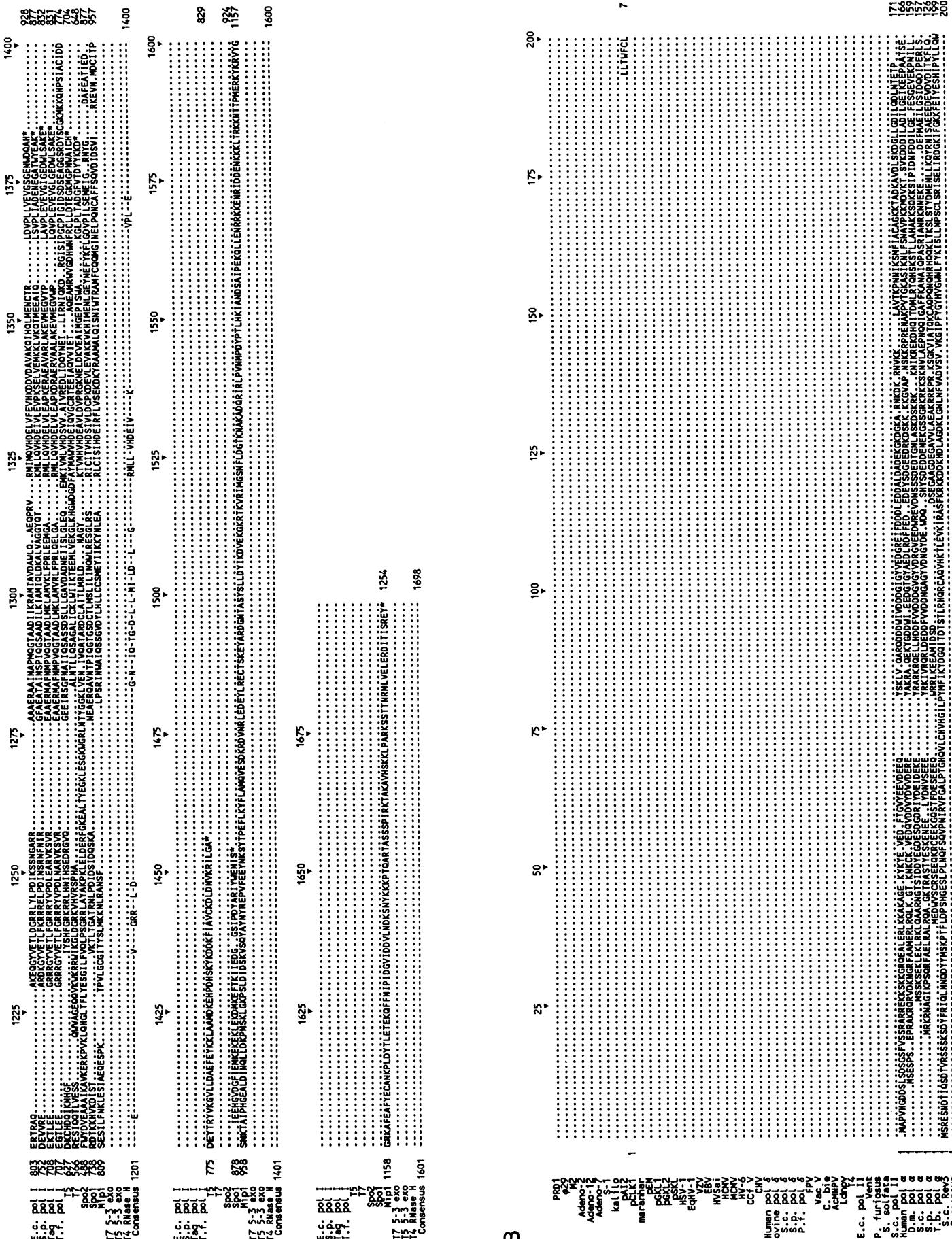
of closely related sequences. These newly formed groups of highly related sequences were then regapped to conform with the entire alignment based upon the previous alignment of those sequences in the new group from the original alignment. As in the previous paper, all the final adjustments had to be made by eye and, as stated above, in Family B the added sequences led to some improvements to the original alignment that became evident to the eye when they were being combined with the entire alignment by hand.

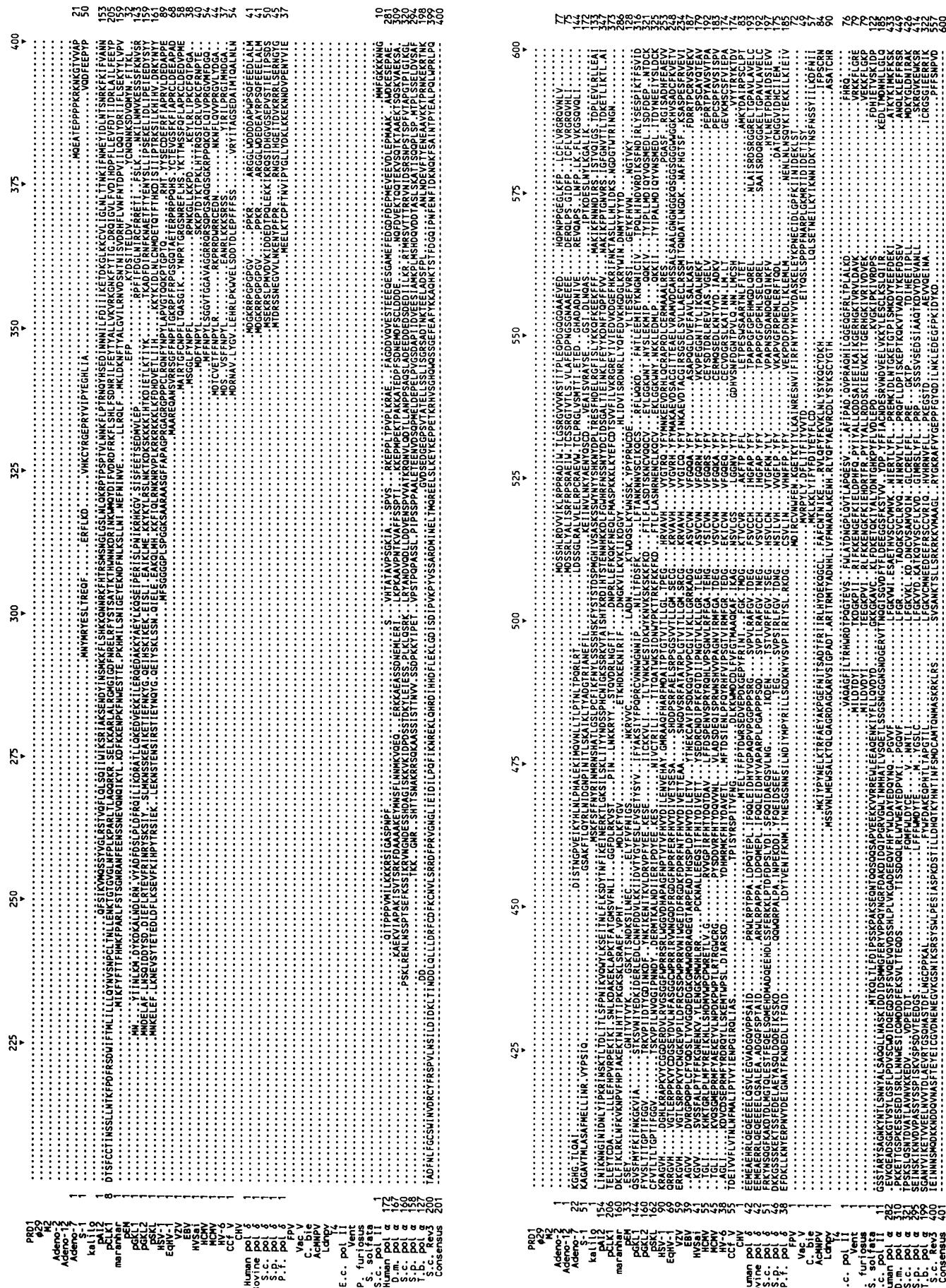
GENERATION OF PHYLOGENETIC TREES FOR THE DNA POLYMERASE DOMAINS

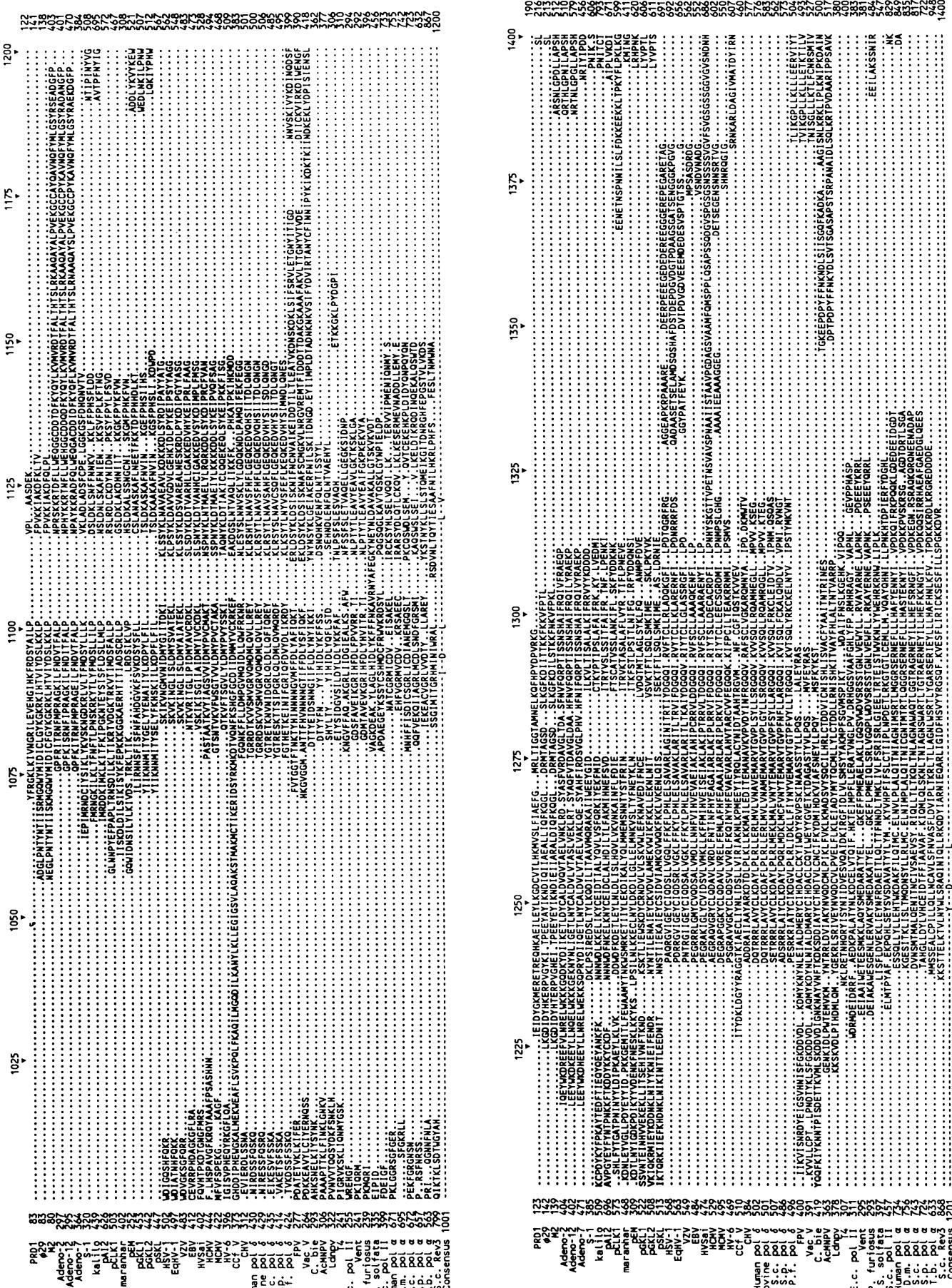
Using Felsenstein's PHYLIP program package (71), specifically the programs named in the outline below, we generated phylogenetic trees for the 9 Family A DNA polymerases (Figures 2A and 2B) and for the 47 Family B DNA polymerases (Figures 3A and 3B). The trees for Family A were created from the alignment in Figure 1A using the most conserved regions found at the following positions: 798 to 814, 877 to 998, 1047 to 1090, 1104 to 1123, 1131 to 1158, 1175 to 1206, 1236 to 1251, 1284 to 1305, 1322 to 1340, and 1365 to 1379. These conserved regions were recombined and 100 bootstrap samples were generated using SEQBOOT program. Using the DNADIST program, we turned the samples into distance matrices using the Kimura-2 parameter method. The resulting matrices were then input to the NEIGHBOR program using the UPGMA method to produce approximately 100 trees. Finally those trees were reduced to a single tree using the CONSENSE program. This final tree was then plotted for publication using two different methods. The trees in Figures 2A and 3A were created by the DRAWGRAM program setup to produce a phenogram type tree and the trees in Figures 2B and 3B were created by the DRAWTREE program. The trees for Family B were created from the alignment in Figure 1B, according to the same procedure, using the most conserved regions found at the following positions: 1407 to 1760, 1885 to 1901, 1956 to 1990, 2081 to 2100, 2181 to 2210, and 2280 to 2320. The Family B DNA polymerases can be subdivided into two subfamilies, the protein-primed DNA polymerase subfamily and the RNA-primed DNA polymerase subfamily.

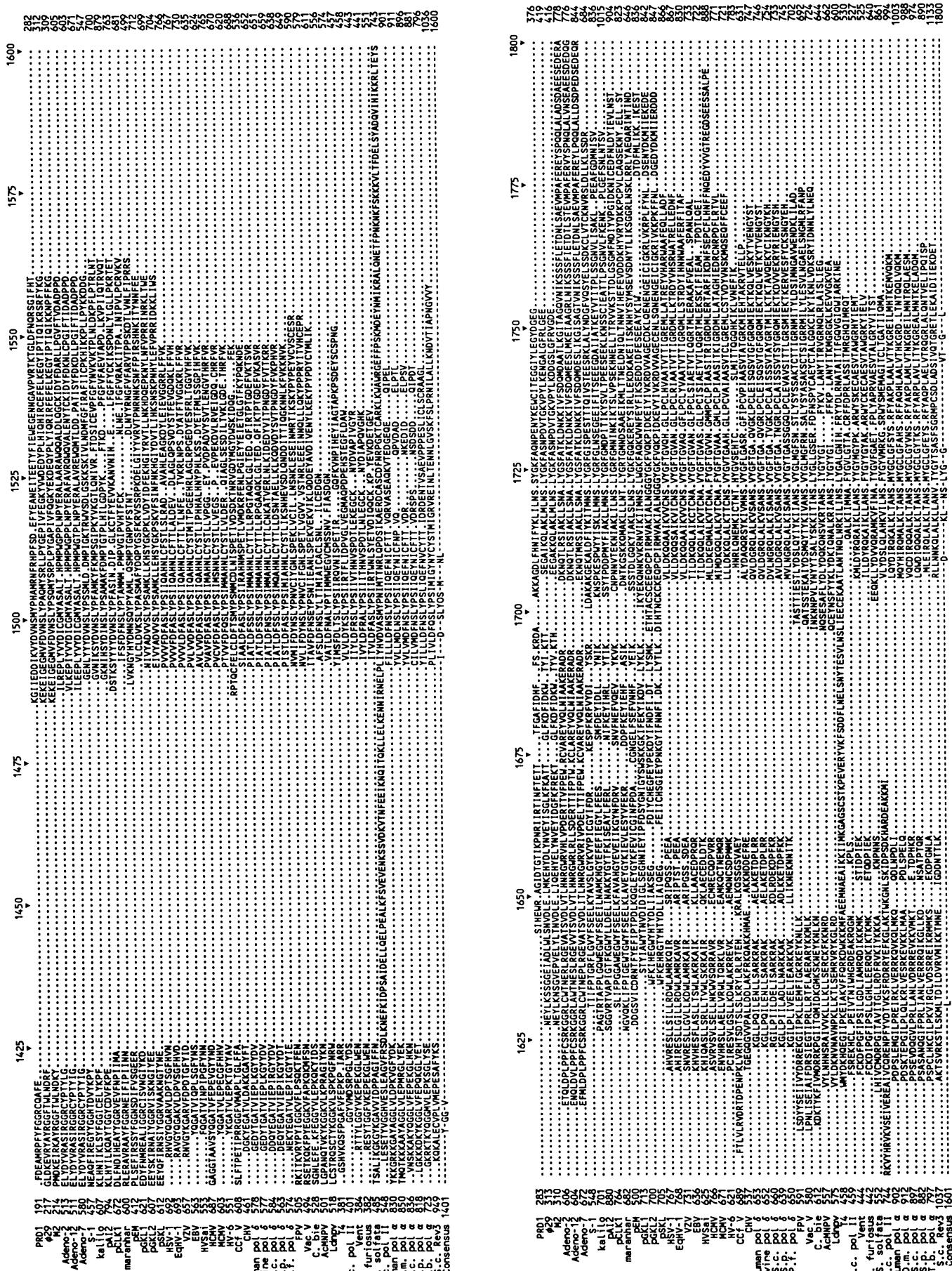
* To whom correspondence should be addressed

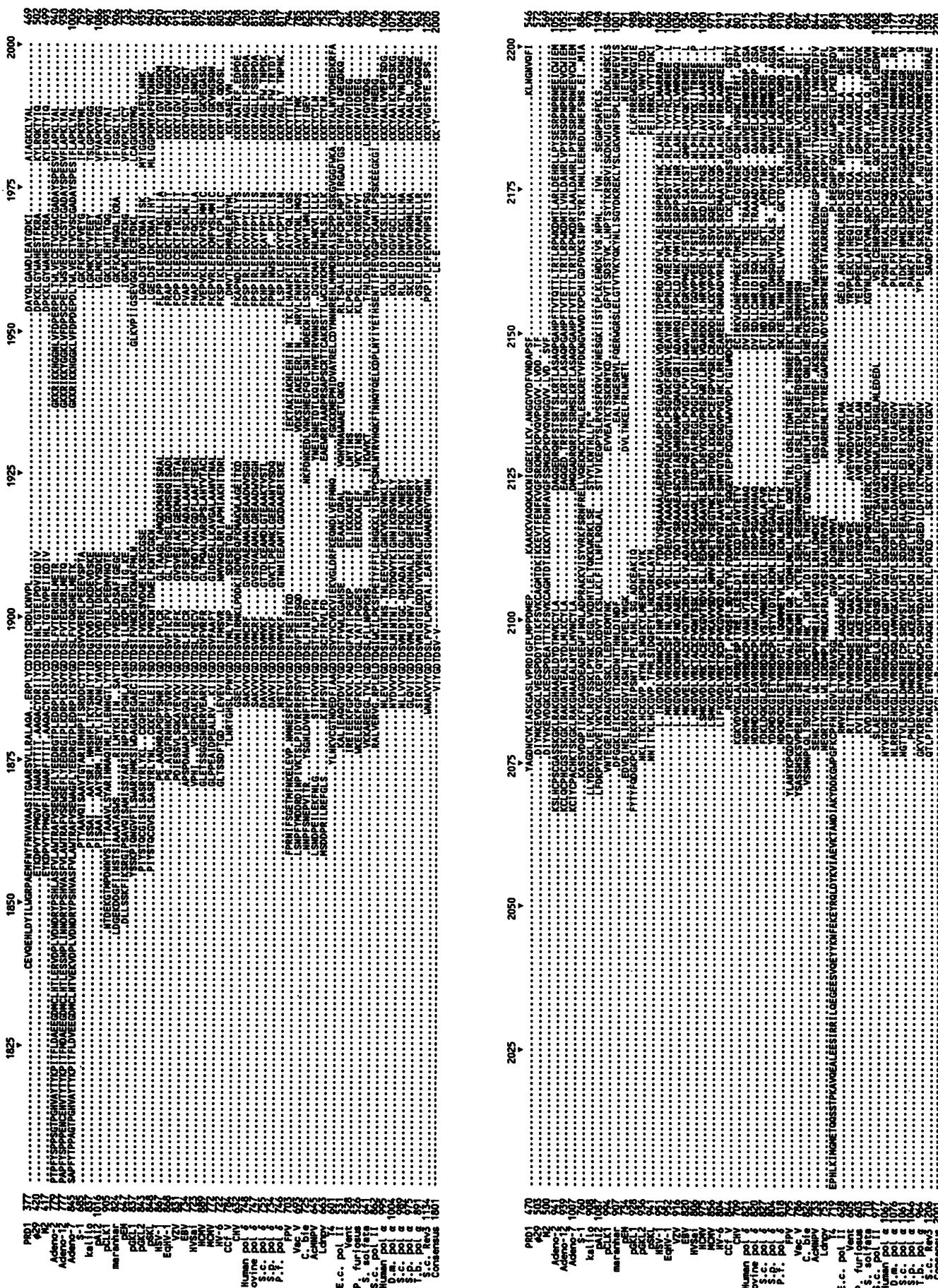


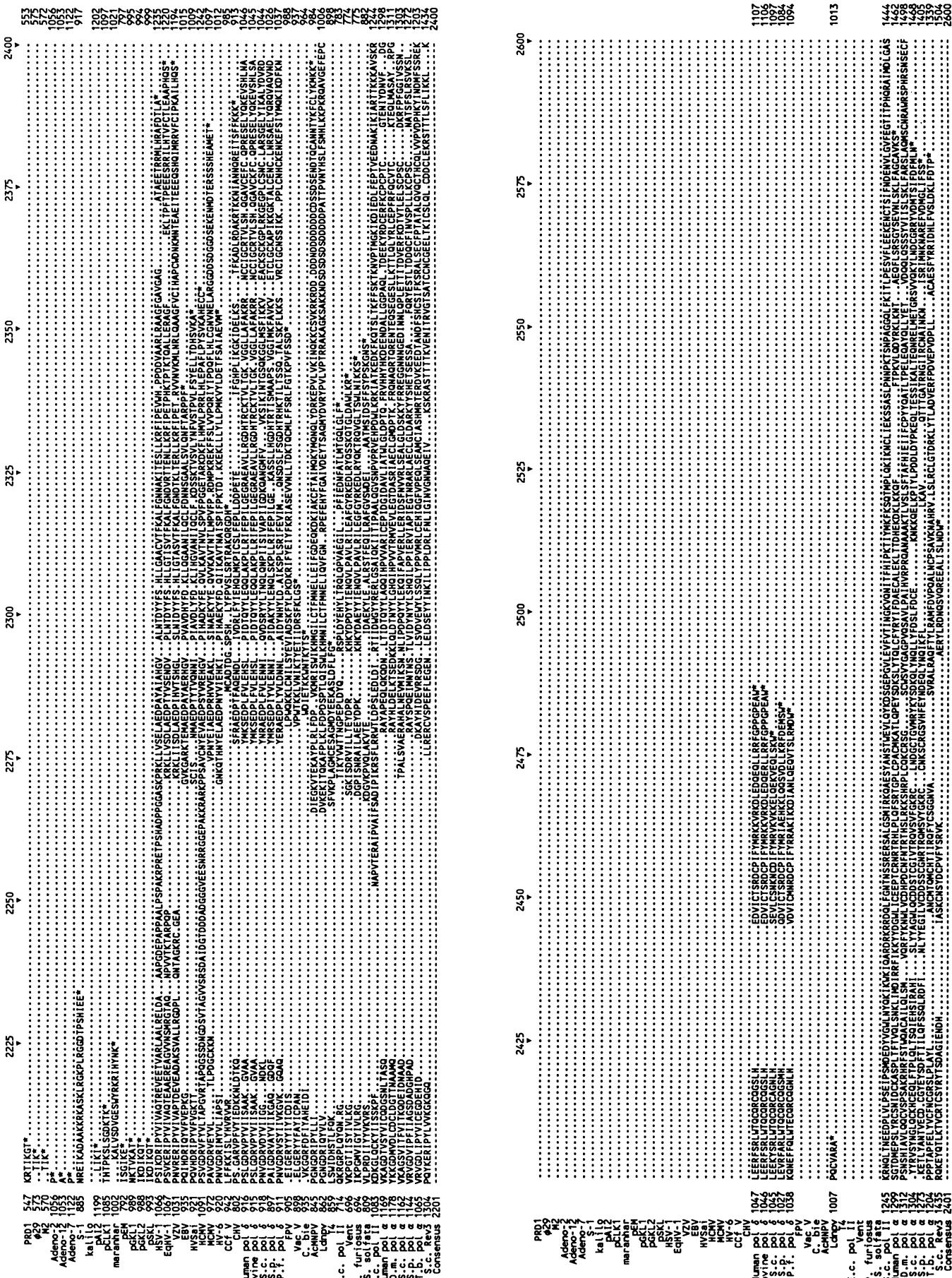


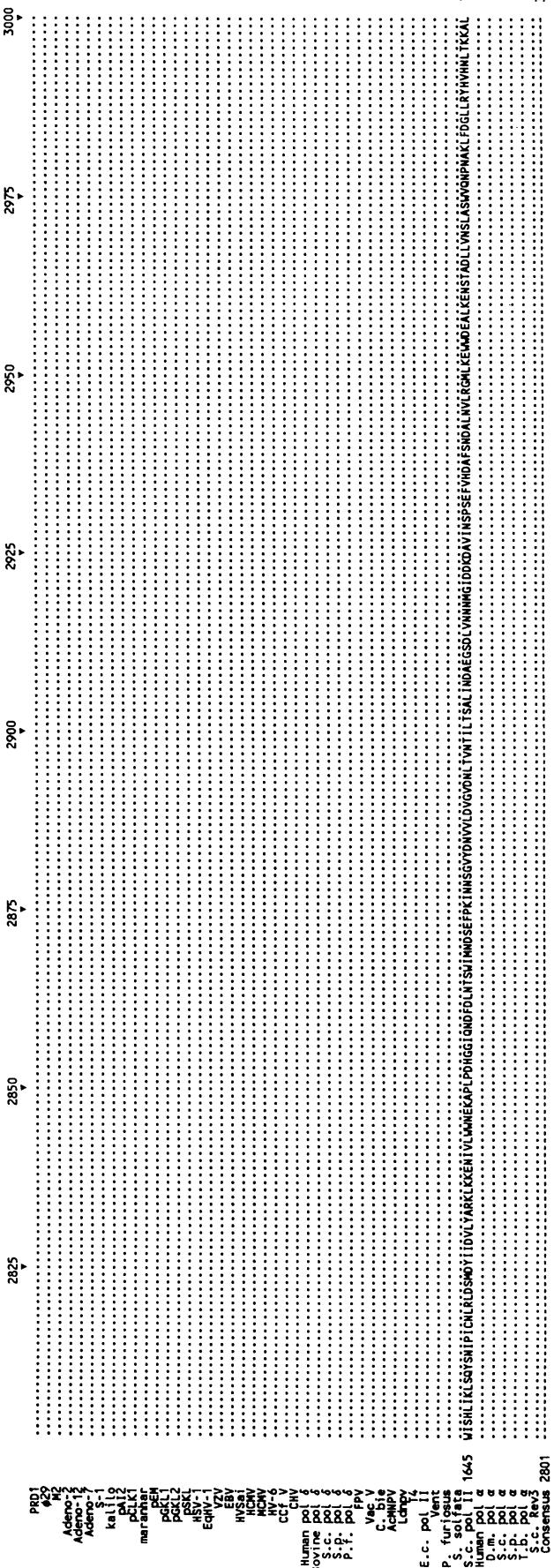
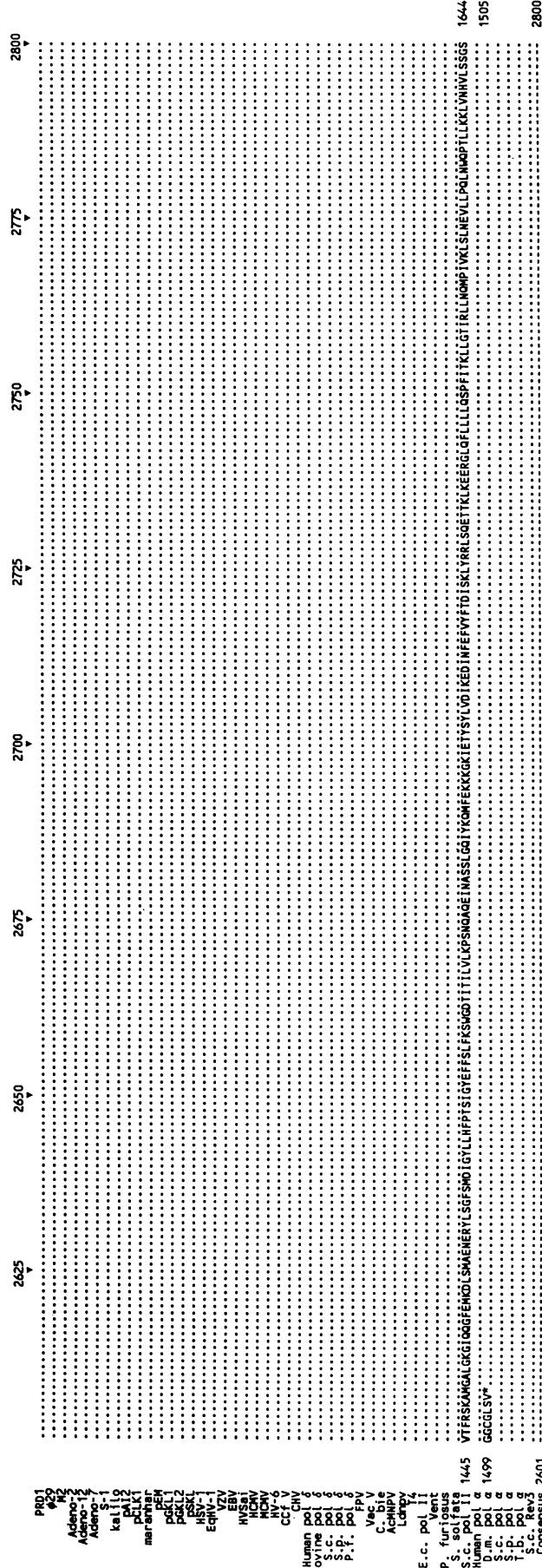


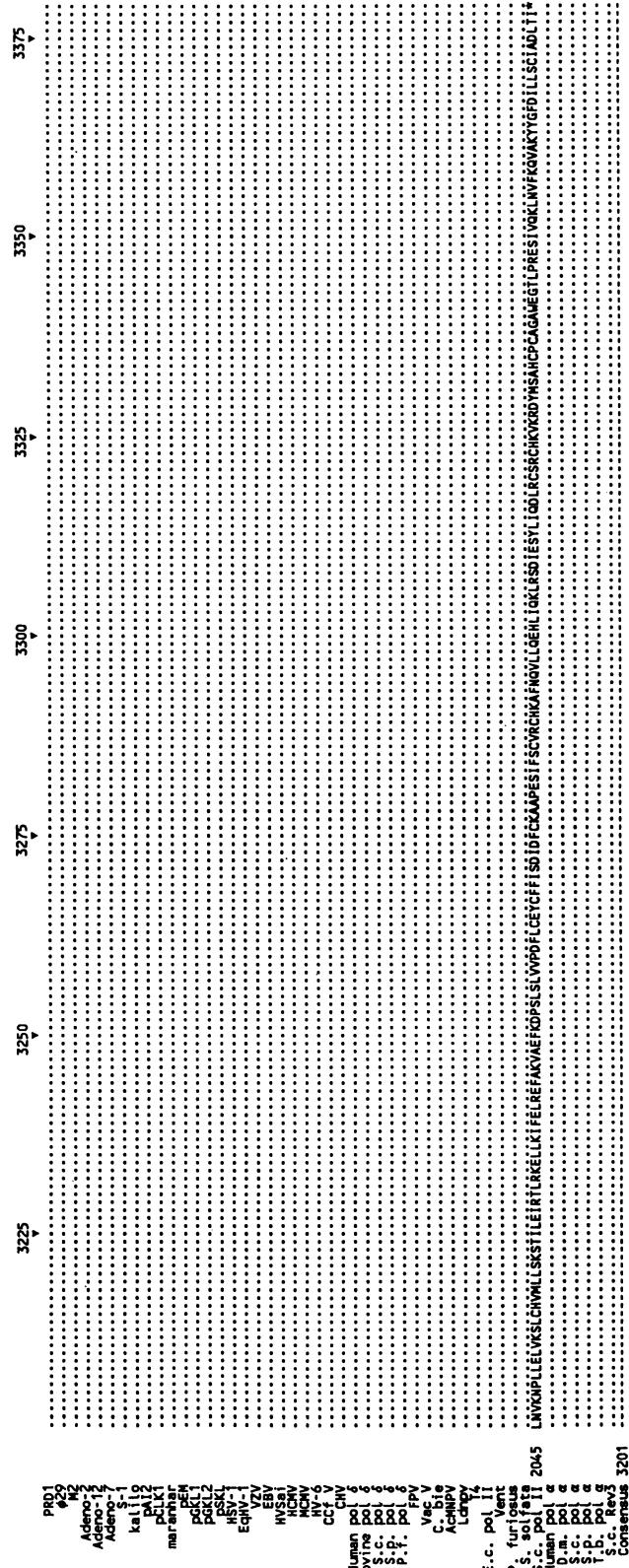
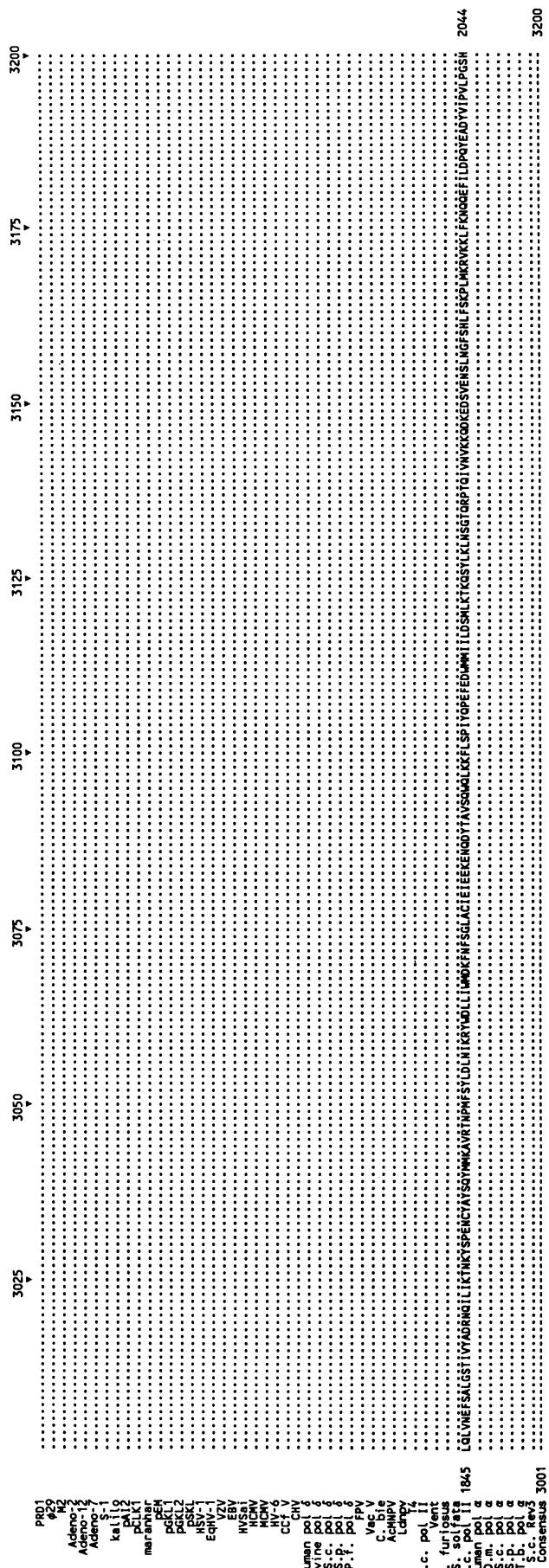












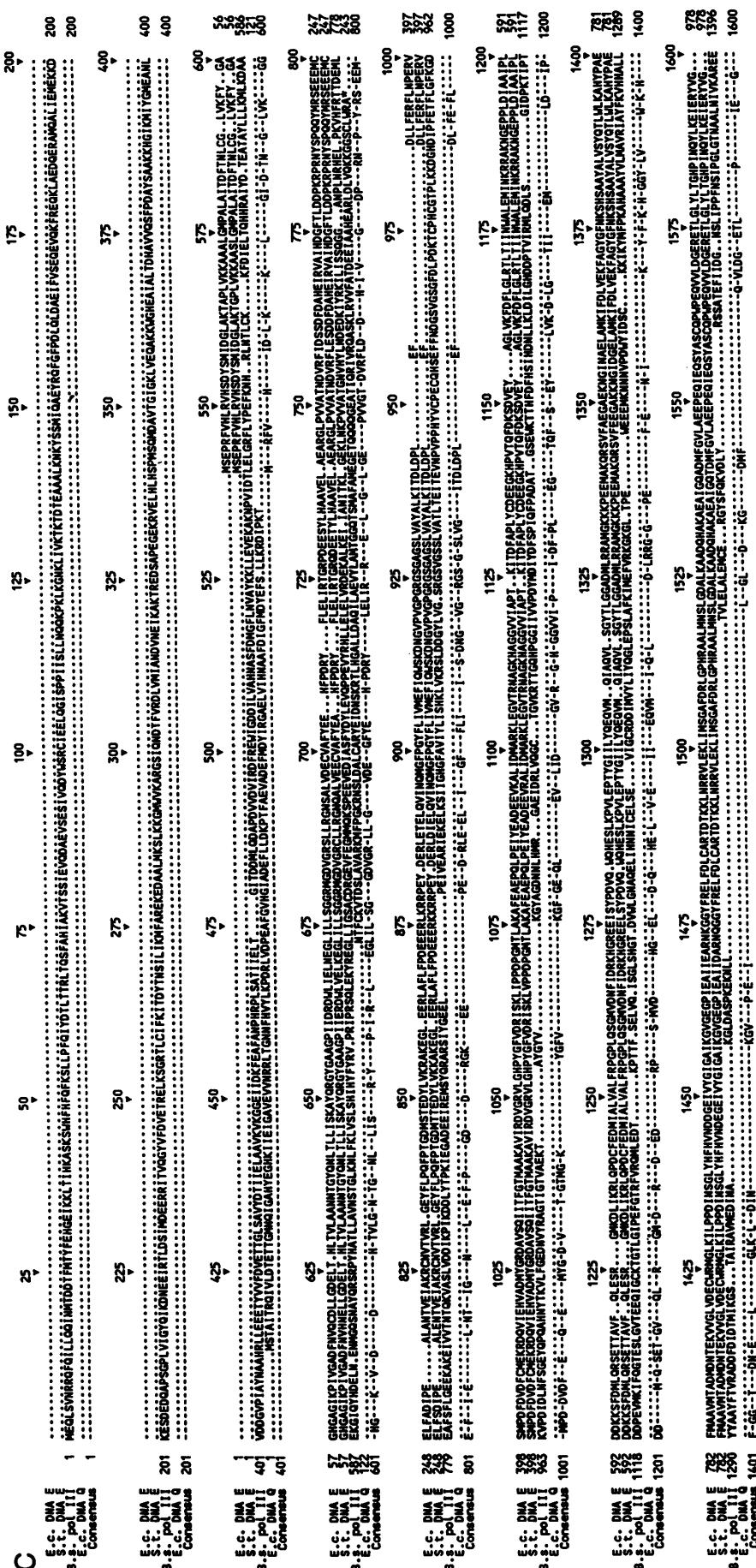


Figure 1A. Family A DNA polymerases—*E. coli* DNA pol. I (E.c. pol. D(3)), *Streptococcus pneumoniae* DNA pol. (S.p. pol. D(4)), *Thermus aquaticus* DNA pol. I (Tag pol. D(5)), *Thermus flavus* DNA pol. I (T.f. pol. D(6)), T5 DNA pol. (T7) T7 DNA pol. (8), Spol DNA pol. (9), Spol2 DNA pol. (10), yeast mitochondrial DNA pol. (11,12,13), T7 5'-3' exonuclease (gp 6)(4,8), T5 5'-3' exonuclease (gp 6)(4,8), T5 DNA pol. (14,15). **B.** Family B DNA polymerases—PRD1 DNA pol. (18,19), φ29 DNA pol. (20), M2 DNA pol. (21), Adenovirus type-2 DNA pol. (Adeno-2)(54), Adenovirus type-12 DNA pol. (Adeno-12)(56), Adenovirus type-7 DNA pol. (Adeno-7)(55), S-1 maize mitochondrial DNA pol. (S-1)(57), *kallilo neurospora intermedia* DNA pol. (kallilo)(58), *Ascobolus immersus* plasmid DNA pol. (pAD)(59), *Clinodiplosis purpurea* plasmid DNA pol. (cpKL1)(60), *Mazanaria neurospora crassa* cDNA pol. (mazanaria)(61), *Agaricus bisporus* DNA pol. (pEM)(62), *Kluyveromyces lactis* plasmid DNA pol. (pGKL1)(63), *Kluyveromyces lactis* plasmid DNA pol. (Kluyveromyces lactis)(64), *maranhami*(61), *Agaricus bisporus* DNA pol. (S-1)(57), *Varicella-zoster virus* plasmid DNA pol. (pSKL)(65), herpes simplex type-1 DNA pol. (HSV-1)(40), Equine herpes virus type-1 DNA pol. (EqHV-1)(40), *Varicella-zoster virus* plasmid DNA pol. (pGKL2)(64), *Marinimyces klyuyvei* plasmid DNA pol. (HCMV)(44), Murine cytomegalovirus DNA pol. (MCMV)(45), Human DNA polymerase delta (Human pol. δ)(31) (Note: another virus DNA pol. (V7V)(41), Epstein-Barr virus DNA pol. (EBV)(42), *Herpesvirus saimiri* DNA pol. (HVSai)(43), Human cytomegalovirus DNA pol. (HCMV)(44), Chlorella virus DNA pol. (CCF V)(47), Chlorella virus DNA pol. (NY-2A)(CHV)(48)). Human DNA polymerase III (delta) (S.c. pol. sequence for Hum. DNA pol. delta has been published with 5 amino acid differences(32)), Bovine DNA polymerase delta (Bovine pol. δ)(33), *Saccharomyces cerevisiae* DNA polymerase III (delta) (S.c. pol. sequence for Hum. DNA pol. delta has been published with 5 amino acid differences(32)), *Plasmodium falciparum* DNA polymerase delta (P.f. pol. δ)(36), Fowlpox virus DNA pol. (PPV)(49), Vaccinia virus DNA pol. (Vac δ)(34), *Schizosaccharomyces pombe* DNA polymerase (delta)(S.p. pol. δ)(35), *Lymantria dispar* nuclear polyhedrosis virus DNA polymerase (LdNPV)(53), *Y. (Yeast) 23*, *Pyrococcus furiosus* DNA pol. (P. furiosus)(24), *Saffoldirus softariarius* DNA pol. (S. softaria)(25), *Saccharomyces cerevisiae* DNA T4 DNA pol. (T4)(22), *E. coli* DNA pol. II (E.c. pol. II)(17), *Thermococcus litoralis* DNA pol. (C. bie)(51), Autographa californica nuclear polyhedrosis virus DNA pol. (AcMNPV)(52), *Drosophila melanogaster* DNA polymerase alpha (D.m. pol. α)(29), *Saccharomyces cerevisiae* DNA polymerase I (alpha)(Human pol. α)(26), *Trypanosoma brucei* DNA polymerase alpha (T.b. pol. α)(30), and *Salmonella typhimurium* DNA polymerase III α-subunit (S.t. DNA E)(65), *Bacillus subtilis* DNA polymerase III ε-subunit (E.c. DNA Q)(69,70).

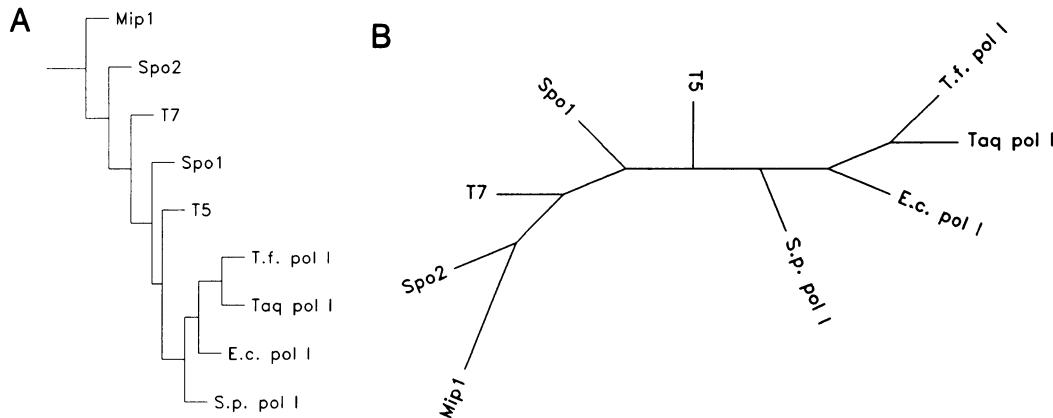


Figure 2A. Phylogenetic phenogram tree produced from the alignment of Family A DNA polymerases in Figure 1A using only the following conserved sequence blocks of the DNA polymerase domain: 798–814, 877–998, 1047–1090, 1104–1123, 1131–1158, 1175–1206, 1236–1251, 1284–1305, 1322–1340, and 1365–1379. **B.** Unrooted phylogenetic tree of the Family A DNA polymerases produced as in Figure 2A plotted by a different method.

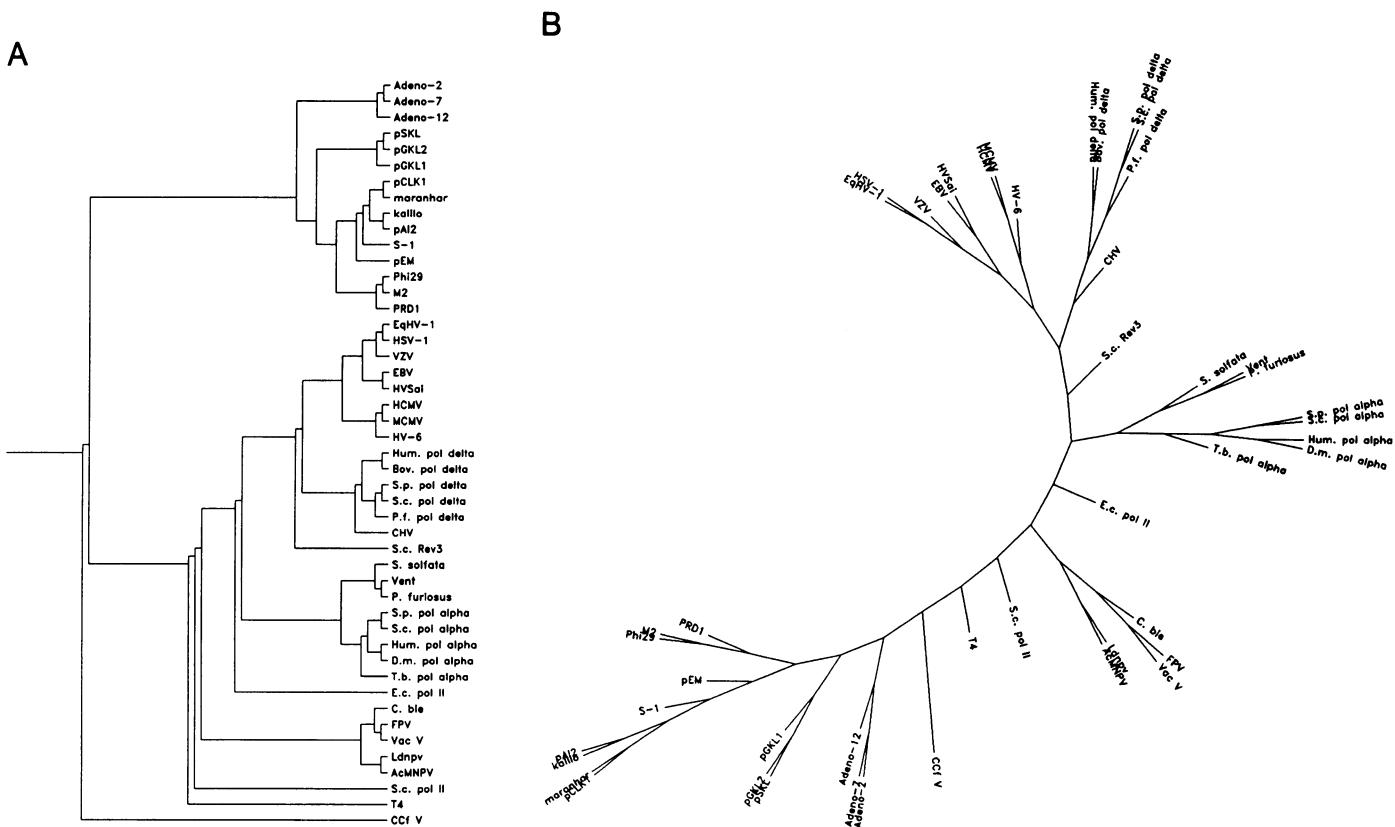


Figure 3A. Phylogenetic phenogram tree produced from the alignment of Family B DNA polymerases in Figure 1B using only the following conserved sequence blocks of the DNA polymerase domain: 1407–1760, 1885–1901, 1956–1990, 2081–2100, 2181–2210, and 2280–2320. **B.** Unrooted phylogenetic tree of the Family B DNA polymerases produced as in Figure 3A plotted by a different method.

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Table 1. The main families and subclassifications of DNA polymerases. The number of amino acids, molecular weight and isoelectric point of each DNA polymerase or nuclease were computed using the Proteinsort program from the UWGCG. Those Family B DNA polymerases marked with a star (*) are protein-primed DNA polymerases. The sequence marked with (+) pEM (62) is not a complete sequence missing some unknown number of amino acids on the N-terminus and so the numbers in the table only represent this short sequence.

| | Amino acids (No.) | Mol. Wt. | Isoelectric pt. | Reference |
|---|----------------------|----------|-----------------|------------|
| A. Family A DNA polymerases | | | | |
| 1. Bacterial DNA polymerases | | | | |
| a) <i>E.coli</i> DNA polymerase I | 928 | 103,117 | 5.37 | (3) |
| b) <i>Streptococcus pneumoniae</i> DNA polymerase I | 877 | 99,078 | 4.78 | (4) |
| c) <i>Thermus aquaticus</i> DNA polymerase I | 832 | 93,909 | 6.38 | (5) |
| d) <i>Thermus flavus</i> DNA polymerase I | 831 | 93,783 | 6.00 | (6) |
| 2. Bacteriophage DNA polymerases | | | | |
| a) T5 DNA polymerase | 829 | 94,410 | 6.19 | (7) |
| b) T7 DNA polymerase | 704 | 79,691 | 6.45 | (8) |
| c) Spo1 DNA polymerase | 924 | 106,808 | 5.34 | (9) |
| d) Spo2 DNA polymerase | 648 | 72,561 | 8.50 | (10) |
| 3. Mitochondrial DNA polymerase | | | | |
| Yeast mitochondrial DNA polymerase (MIP1) | 1254 | 143,479 | 9.23 | (11,12,13) |
| 4. 5' to 3' Exonucleases with homologous sequences to <i>E.coli</i> DNA polymerase I | | | | |
| a) T4 RNase H (gp 33.2) | 305 | 35,558 | 9.00 | (14,15) |
| b) T5 Exonuclease (gp D15) | 291 | 33,448 | 5.12 | (7,16) |
| c) T7 Exonuclease (gp 6) | 348 | 40,126 | 4.54 | (4,8) |
| B. Family B DNA polymerases | | | | |
| 1. Bacterial DNA polymerase | | | | |
| <i>E.coli</i> DNA polymerase II | 783 | 90,020 | 6.85 | (17) |
| 2. Bacteriophage DNA polymerases | | | | |
| a) PRD1 DNA polymerase* | 553 | 63,336 | 6.68 | (18,19) |
| b) φ29 DNA polymerase* | 575 | 66,714 | 8.83 | (20) |
| c) M2 DNA polymerase* | 572 | 66,423 | 7.69 | (21) |
| d) T4 DNA polymerase | 898 | 103,609 | 6.20 | (22) |
| 3. Archaeabacterial DNA polymerases | | | | |
| a) <i>Thermococcus litoralis</i> DNA polymerase (Vent) | 774 | 89,913 | 8.29 | (23) |
| b) <i>Pyrococcus furiosus</i> DNA polymerase | 775 | 90,112 | 7.92 | (24) |
| c) <i>Sulfolobus solfataricus</i> DNA polymerase | 882 | 101,332 | 9.72 | (25) |
| 4. Eukaryotic Cell DNA polymerases | | | | |
| (1) DNA polymerase alpha | | | | |
| a) Human DNA polymerase (alpha) | 1,462 | 165,859 | 5.71 | (26) |
| b) <i>S.cerevisiae</i> DNA polymerase I (alpha) | 1,468 | 166,776 | 6.14 | (27) |
| c) <i>S.pombe</i> DNA polymerase I (alpha) | 1,405 | 159,348 | 6.85 | (28) |
| d) <i>Drosophila melanogaster</i> DNA polymerase (alpha) | 1,505 | 171,167 | 8.22 | (29) |
| e) <i>Trypanosoma brucei</i> DNA polymerase (alpha) | 1,339 | 151,611 | 6.39 | (30) |
| (2) DNA polymerase delta | | | | |
| a) Human DNA polymerase (delta) | 1,107 | 123,634 | 6.94 | (31,32) |
| b) Bovine DNA polymerase (delta) | 1,106 | 123,707 | 7.52 | (33) |
| c) <i>S.cerevisiae</i> DNA polymerase III (delta) | 1,097 | 124,618 | 7.96 | (34) |
| d) <i>S.pombe</i> DNA polymerase III (delta) | 1,084 | 123,211 | 7.63 | (35) |
| e) <i>Plasmodium falciparum</i> DNA polymerase (delta) | 1,094 | 126,883 | 8.76 | (36) |
| (3) DNA polymerase epsilon | | | | |
| <i>S.cerevisiae</i> DNA polymerase II (epsilon) | 2,222 | 255,669 | 6.92 | (37) |
| (4) Other eukaryotic DNA polymerases | | | | |
| <i>S.cerevisiae</i> DNA polymerase Rev3 | 1,504 | 172,956 | 8.86 | (38) |
| 5. Viral DNA polymerases | | | | |
| a) Herpes Simplex virus type 1 DNA polymerase | 1,235 | 136,547 | 7.35 | (39) |
| b) Equine herpes virus type 1 DNA polymerase | 1,220 | 135,955 | 6.67 | (40) |
| c) Varicella-Zoster virus DNA polymerase | 1,194 | 134,047 | 7.80 | (41) |
| d) Epstein-Barr virus DNA polymerase | 1,015 | 113,417 | 7.38 | (42) |
| e) <i>Herpesvirus saimiri</i> DNA polymerase | 1,009 | 113,934 | 7.31 | (43) |
| f) Human cytomegalovirus DNA polymerase | 1,242 | 137,101 | 7.25 | (44) |
| g) Murine cytomegalovirus DNA polymerase | 1,097 | 123,573 | 6.68 | (45) |
| h) Human herpes virus type 6 DNA polymerase | 1,012 | 115,819 | 7.11 | (46) |
| i) Channel Catfish virus DNA polymerase | 985 | 113,468 | 7.98 | (47) |
| j) Chlorella virus DNA polymerase | 913 | 104,955 | 6.66 | (48) |
| k) Fowlpox virus DNA polymerase | 988 | 116,658 | 8.11 | (49) |
| l) Vaccinia virus DNA polymerase | 937 | 108,564 | 7.50 | (50) |
| m) Choristoneura bimaculata DNA polymerase | 964 | 114,818 | 7.95 | (51) |
| n) Autographa californica nuclear polyhedrosis virus (AcMNPV) DNA polymerase | 984 | 114,337 | 8.35 | (52) |
| o) Lymantria dispar nuclear polyhedrosis virus DNA polymerase | 1,013 | 115,921 | 9.08 | (53) |

| | | | | |
|---|-------|---------|-------|------|
| p) Adenovirus-2 DNA polymerase* | 1,056 | 120,431 | 6.65 | (54) |
| q) Adenovirus-7 DNA polymerase* | 1,122 | 128,648 | 6.73 | (55) |
| r) Adenovirus-12 DNA polymerase* | 1,053 | 120,863 | 6.86 | (56) |
| 6. Eukaryotic linear DNA plasmid encoded DNA polymerases | | | | |
| a) S-1 maize DNA polymerase* | 917 | 105,935 | 8.62 | (57) |
| b) <i>kalilo neurospora intermedia</i> DNA polymerase* | 970 | 112,902 | 9.71 | (58) |
| c) pAI2 <i>Ascochylus immersus</i> DNA polymerase* | 1,202 | 138,279 | 10.10 | (59) |
| d) pCLK1 <i>Claviceps purpurea</i> DNA polymerase* | 1,097 | 126,627 | 8.76 | (60) |
| e) <i>maranhar neurospora crassa</i> DNA polymerase* | 1,021 | 119,074 | 9.62 | (61) |
| f) pEM <i>Agaricus bitorquis</i> DNA polymerase* | †797 | †91,922 | †8.24 | (62) |
| g) pGKL1 <i>Kluyveromyces lactis</i> DNA polymerase* | 995 | 116,345 | 8.04 | (63) |
| h) pGKL2 <i>Kluyveromyces lactis</i> DNA polymerase* | 994 | 117,560 | 8.33 | (64) |
| i) pSKL <i>Saccharomyces kluyveri</i> DNA polymerase* | 999 | 117,544 | 9.79 | (65) |

C. Family C DNA polymerases**1. Bacterial replicative DNA polymerases**

| | | | | |
|--|------|---------|------|---------|
| a) <i>E.coli</i> DNA polymerase III α subunit | 1160 | 129,903 | 5.04 | (66) |
| b) <i>S. typhimurium</i> DNA polymerase III α subunit | 1160 | 130,118 | 5.05 | (67) |
| c) <i>Bacillus subtilis</i> DNA polymerase III | 1437 | 162,648 | 5.23 | (68) |
| 2. <i>E.coli dnaQ (MutD)</i> | | | | |
| <i>E.coli</i> DNA polymerase III ϵ subunit | 243 | 27,099 | 5.68 | (69,70) |

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