FIGURE 27.2. Designing PCR primers. Alignment of a region of the DNA polymerase genes from a sample of herpesviruses (abbreviations of the virus names given in leftmost column). The different amino acids present at each position in these samples (e.g., the first amino acid is Y in all of the viruses, the seventh varies among TLIV) are shown above the top horizontal bar. The amino acid abbreviations are as in Figure 2.23. An alignment of the DNA sequences encoding these proteins is shown with conserved nucleotides highlighted in blue and with each codon separated. Note that even related viruses frequently use different codons for the same amino acid. “Degenerate” PCR primers could be designed from this alignment that take into account the variation in codon usage and even the choice of amino acid. Such primers would include a mixture of all the possible sequences. Even when a protein’s amino acid sequence is 100% conserved between species, the degeneracy of the genetic code usually prevents the use of nondegenerate primers. This is an important consideration because PCR works better with less degenerate primers. (Modified from Rose T.M. Nucleic Acids Res. 26: 1628–1635, Fig. 2, © 1998 Oxford University Press.)

Evolution © 2008 Cold Spring Harbor Laboratory Press