

Supporting Text

Amino acid sequence analysis of multiple evolved and improved p40 and p35 subunits and corresponding human (h)IL-12 subunits revealed interesting insights that may explain the improved phenotype of the evolved IL-12 molecules. Alignment of the six most improved clones revealed a region in domain 2 (D2) of p40 that had undergone significant recombination. In each of these clones, the sequence found in hIL-12, QGVTCGAATLSAERVGRGDNKEYEYS, was replaced with a corresponding parental sequence from pig, goat or cow (Fig. 7). The clone exhibiting the highest level of expression and activity, sh-p40.1, contained a sequence from pig; this resulted in a shortening of β strand E and numerous charge reversals compared to the equivalent sequence in hIL-12. Whether shortening of β strand E has any influence on main chain conformation is presently unknown. Interestingly, sh-p40.4 contained the substitution, C252G, which resulted from a recombination event with the parental cat p40 sequence. This substitution, which is also present in pig p40, suggests that cysteine at position 252 in h-p40 is not required for expression or activity.