PowerPlex™ 1.2 (8 loci) used in combination with Promega’s FFFL kit (4 loci) resolves most routine trio and motherless paternity cases to a Combined Paternity Index (CPI) of 1,000 or greater which corresponds to a Probability of Paternity >99.9% calculated with a Prior Probability of 50%. Nonetheless, challenging cases are encountered where examination of the combined 12 loci in PowerPlex™ 1.2 + FFFL fails to yield a CPI of 1,000. PowerPlex™ 16 assesses a larger panel of loci that includes the 13 CODIS core loci plus Pentas D and E. The 15 loci examined by PowerPlex™ 16 encompasses the 8 in PowerPlex™ 1.2 plus 7 additional loci. The ability of PowerPlex™ 16 to resolve challenging paternity cases was evaluated in selected examples that had previously been evaluated with PowerPlex™ 1.2 or PowerPlex™ 1.2 + FFFL. DNA from buccal swabs or blood spotted to FTA® paper was amplified according to the instructions provided in the kits and quantified by gel electrophoresis using an ABI 373. In one example, a trio paternity case with a CPI of 245 assessed by PowerPlex™ 1.2 yielded a CPI of >1 million with PowerPlex™ 16. Likewise, another trio case that yielded a CPI of 816 with PowerPlex™ 1.2 + FFFL yielded a CPI of over 800,000 with PowerPlex™ 16. Thus, the seven additional loci examined in the PowerPlex™ 16 kit appear to be particularly useful in resolving challenging trio cases where the obligate paternal alleles for PowerPlex™ 1.2 loci occur with a high frequency in the relevant population. Six motherless cases that produced low CPI's when examined with PowerPlex™ 1.2 or PowerPlex™ 1.2 + FFFL were also re-evaluated. Several of these cases had CPI’s less than 10 as assessed with PowerPlex™ 1.2 + FFFL and all had CPI’s less than 310. PowerPlex™ 16 yielded CPI’s ranging from 3,800 to greater than 7 million in these six motherless cases. As above, the additional seven loci in the PowerPlex™ 16 kit proved very effective in achieving acceptable to high CPI’s. Another re-evaluation involved genetic reconstruction of an alleged father from his parents (the child’s paternal grandparents). PowerPlex™ 1.2 + FFFL yielded a CPI of 25 while a CPI greater than 7,000 was obtained with PowerPlex™ 16. The CPI obtained with PowerPlex™ 16 + FFFL was approximately 13,000. Cases involving exclusion of an alleged father at a single genetic locus are particularly problematic (challenging). In two examples where a single paternal exclusion occurred at the FESFPS locus, the CPI obtained with PowerPlex™ 1.2 + FFFL was less than 3. PowerPlex™ 16 produced a CPI greater than 10 million in both cases and the combination of PowerPlex™ 16 + FFFL yielding overall CPI’s of 42,300 and 401,700, respectively. Likewise, a trio paternity case with a single exclusion at the D16S539 locus that yielded a CPI of 1.2 with PowerPlex™ 1.2 + FFFL produced CPI’s of 498 and 2,050 with PowerPlex™ 16 and PowerPlex™ 16 + FFFL, respectively. There was perfect agreement between the alleles determined with PowerPlex™ 1.2 and PowerPlex™ 16 except in one instance. In one person, PowerPlex™ 16 failed to recognize a 10 allele at the D5S818 locus which was detected by both PowerPlex™ 1.2 and Profiler Plus™. The evaluation of previously identified challenging paternity cases with PowerPlex™ 16 proved to be very informative. The additional seven loci contained in PowerPlex™ 16 relative to PowerPlex™ 1.2 appear to be particularly powerful in resolving paternity in all types of scenarios. Overall, PowerPlex™ 16 appears to be especially useful in resolving both routine and challenging paternity cases.