CODIS-approved markers have worldwide acceptance for their application in forensic and paternity DNA profiling. The basis for this wide acceptance relates to their relative genetic stability, low mutational rates and di-allelic formation within each locus. The latter characteristic refers to his or her simple genetic inheritance from each parent. However, information regarding their behavior in genetic disease associated conditions is very limited. Therefore, the intent of this study is to examine the effect of disease-associated trisomy as in Down Syndrome patients on DNA profiles produced by CODIS-approved and non-approved markers. Down syndrome is a genetic disease, which has been linked to various clinical manifestations of mental retardation resulting from several chromosomal rearrangements at the molecular level. DNA profiling from children with established Down syndrome revealed the presence of significant levels of trisomy on several individual CODIS approved and non-approved genetic markers. In contrast, children with severe forms of the disease showed tri-allelic formation in about five CODIS-approved markers and one non-CODIS approved marker. The presence of these tri-allelic aneuploidies is likely to complicate mixture analysis in forensic casework and paternity casework analysis given the fact that the rate of occurrence of Down syndrome is approximately 1:800 to 1:1000 within living adults.