STR Allele Concordance Between Different Primer Sets—A Brief Summary

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The FBI has developed a national DNA databank called CODIS (Combined DNA Index System). CODIS operations enable: 1) improved assistance to investigators in the identification by DNA typing of suspects of violent crimes, and 2) augmented capabilities of forensic laboratories by providing software to conduct DNA casework and perform statistical calculations. Thus, an expanded use of DNA typing technology is anticipated.

CODIS is a hierarchical database (local, state and federal) of DNA identification records. The DNA records in CODIS contain limited information to enable profile searching, which generally includes 1) a laboratory identifier, 2) a specimen identifier, 3) DNA characteristics and 4) information to classify and review the integrity of the DNA record. To be an effective mechanism, CODIS must be a fully integrated network among local, state and federal crime laboratories. While computer software and hardware integration needs are focal points of such a database, it is imperative that the DNA markers used by the various CODIS-participating laboratories are compatible for data exchange.

A PCR-based technology/genetic marker system offers high sensitivity of detection and specificity, and with increased automation a concomitant decrease in assay time and labor is anticipated. Furthermore, many degraded DNA samples can be typed using PCR-based assays. To apply PCR-based technology to human identity testing for casework and for use in CODIS, defined polymorphic genetic markers, robust analytical techniques and population studies are required. The short tandem repeat (STR) loci are the most informative PCR-based genetic markers for attempting to individualize biological material (1,2). The STR loci are composed of tandemly repeated sequences (each of which is two to seven base pairs in length), are highly informative, and when amplified simultaneously in a multiplex PCR, can be extremely effective for individualizing a wide range of forensic samples.

After an extensive validation study (see reference 3), 13 STR loci were selected to form the core (i.e., required) genetic markers for CODIS. The 13 core loci are: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11. To take full advantage of the power of STR typing and to ensure compatibility for the searching of DNA profiles, typing all 13 STR loci is required for convicted felon samples, and typing of all 13 loci shall be attempted when analyzing casework.

High quality, commercially available kits have facilitated the implementation of STR loci typing in application-oriented laboratories. Indeed, there are commercially available kits for multiplex typing of STR loci from two manufacturers. Some of the available kits include: *GenePrint*® PowerPlex™ 1.1 System and *GenePrint*® PowerPlex™ 2.1 System (Promega Corporation), AmpF*l*STR® Profiler™, AmpF*l*STR® Profiler Plus™ and AmpF*l*STR® COfiler™ DNA typing systems (Perkin-Elmer Biosystems). No more than two PCR/STR kits are needed for typing the 13 core STR loci.

However, the kits from the different manufacturers use different primer sets. Potentially, differences in the sequence where the primer binds may destabilize the primer and inhibit primer extension during PCR. This phenomenon can lead to allele dropout when typing a particular sample using one primer set from one manufacturer's kit and not when using the other manufacturer's kit. If the allele dropout phenomenon occurs at a relatively high frequency, then comparing DNA profiles can be problematic between laboratories that use different primer sets per locus. In contrast, if allele dropout occurs at a very low frequency, then common sense and searching algorithms in CODIS can accommodate the matching requirements.

The different primer sets from the different manufacturers can be used to determine whether or not allele dropout occurs at a substantial frequency. If the same samples are typed with both primer sets, the degree of allele dropout can be determined. Previously, Budowle *et al.* (4) typed several hundred individuals for the loci D5S818, D7S820, D13S317, CSF1PO, TPOX and TH01. There were no typing discrepancies for any samples at the D5S818, D7S820, TPOX and TH01 loci. All typing results were concordant at the CSF1PO locus in Caucasians, except for one sample that demonstrated allele dropout. The level of allele dropout was low and well within acceptable performance tolerance. However, at the D13S317 locus, nine samples were typed as heterozygotes using the Profiler Plus™ kit, which appeared as homozygotes using the PowerPlex™ 1.1 kit. The Promega Corporation was contacted about the null alleles at the D13S317 locus, and the primers were modified in the PowerPlex™ 1.2 kit so that amplification of the variant alleles can be accomplished. Thus, the null allele frequency at the D13S317 locus using either kit now is extremely rare.

In addition, Kline *et al.* (5) typed 600 samples using both the PowerPlex™ 1.1 System and AmpF*l*STR Blue™ kit (Perkin-Elmer, Foster City, CA). Both kits contain primers to amplify the vWA locus. Only one sample was typed differently when using these kits. Thus, the presence of null alleles at the vWA locus is a rare occurrence and should not present a problem when comparing vWA profiles generated using different primer sets (6).

Recently, the FBI and the Promega Corporation carried out an additional allele dropout study. The use of the *GenePrint*® PowerPlex™ 2.1 System (Promega Corporation) enables multiplex amplification of the STR loci FGA, TH01, TPOX, vWA, D3S1358, D8S1179, D18S51, D21S11 and Penta E and provides an opportunity to perform primer pair testing on CODIS core STR loci not described in the aforementioned studies. Approximately 750 samples from African-Americans, Caucasians, Southwestern Hispanics and Bahamians that were typed previously using the AmpF*l*STR® Profiler Plus™ and AmpF*l*STR® COfiler™ DNA typing systems were typed using the *GenePrint*® PowerPlex™ 2.1 System. There were no differences in typing results using either manufacturer's primer sets for the core CODIS loci FGA, TH01, TPOX, vWA, D3S1358, DSS1179, D18S51 and D21S11. All allele calls were within acceptable measurement error ranges.

In conclusion, the current primer sets from the commercial manufacturers, Promega Corporation and Perkin-Elmer Biosystems, do not produce significant levels of allele dropout. Having different primer sets enabled validation of STR typing for CODIS purposes, and the knowledge of the primer sequences was not a requirement for carrying out the validation study. Either manufacturer's kit(s) can be used for STR typing and the data used in CODIS. As long as proper protocols are used, reliable results can be obtained. Additional concordance studies are underway. Finally, if new primer sets are developed for any of the 13 core STR loci, concordance studies, as described here, should be performed before the data can be considered acceptable for CODIS.

REFERENCES

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This is publication number 00-04 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation.