

Validation Studies Performed on STR Systems

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Validation Studies on STR Systems

Before a new STR system or STR multiplex may be routinely employed in human identity testing it should be extensively validated to insure reliability of results. We have included below many of the validation studies which have appeared in the literature. We hope this material will help future researchers design their validation studies and allow those who use common STR systems to review what studies have been performed previously.

Species Specificity

Crouse and Schumm {73} examined the species specificity of nine STR systems (CSF1PO, TPOX, TH01, HPRTB, FES/FPS, VWA, F13A1, and CSF1PO/TPOX/TH01 and HPRTB/FESFPS/VWA triplexes). They reported that no STR PCR products were obtained for 17 of the 23 species tested. Human, gorilla, chimpanzee, and orangutan DNAs were amplified with 8 of the 9 STR systems. Only

FES/FPS primers failed to amplify DNA fragments from non-human primates. Most of the STR PCR products migrated outside of the human allelic ladder fragment range and could not be typed with the PAGE silver stain method employed.

The British Forensic Science Service {332} studied six STR loci and the amelogenin sex-test. Among the non-primates no STR loci were amplified. However, a DNA fragment 2 bp shorter than the X allele was observed for 5 species (pig, cattle, sheep, fox, and badger). The STR loci were observed to amplify quite well among primates, particularly the chimpanzees. However, the allele sizes often fell outside those normally observed for humans {332}.

Microbial DNA templates from 30 microorganisms that may be found in forensic samples were examined with the STR systems HUMTH01, TPOX, and CSF1PO {238}. No PCR products were observed at these STR loci. On the other hand, D1S80 amplifications from six of the bacterial DNAs analyzed produced some nonspecific PCR products that were located within the range of human D1S80 alleles {238}.

Mixtures

DNA mixtures originating from multiple donors may exist in forensic samples, particularly from rape cases where the perpetrator=s DNA will be mixed with the victim=s. Usually, a mixture is identified by the presence of 3 or more bands at one or more STR loci {346}. If the components of the mixture have identical alleles at a particular locus, then the peaks will be higher (with a system that records peak height or area) as a result. The quantitative nature of allele identification with fluorescence detection facilitates the identification of mixed body samples which may occur in forensic cases {121}.

Useful studies to evaluate the ability of a detection system to recognize mixtures include {363}:

- examining mixtures of purified DNA in defined ratios
- examining peak height ratios between heterozygous alleles within an STR locus
- determining the range of stutter band percentage for each allele of each locus

Null Alleles

A null allele is a term for the failure to amplify DNA sequences which are present in a sample. Allele amplification failure may result if polymorphisms occur in the primer binding site. This condition was first predicted by Caskey and Edwards {121} (U.S. Patent #5,364,759, column 19) and was recently reported for the MBP-B {379} and the D19S253 {332} STR systems. The problem of failed PCR

amplifications was resolved by redesigning the primer sets to avoid the polymorphism in the former primer annealing region. In the case of D19S253, it was dropped from consideration in future multiplex STR systems {332}.

Contaminants

The higher sensitivity of PCR-based DNA typing assays make it imperative to separate laboratory procedures, such as DNA extraction and PCR amplification, into designated working areas. Potential sources of DNA contamination include: sample contamination with genomic DNA from the environment, contamination between samples during preparation, and contamination of a sample with amplified DNA from a previous PCR reaction {346}. The first source of contamination has been discussed in detail by Peter Gill {419} where he examines the utility of substrate controls (*Forensic Sci. Int.* (1997) 85:105-111). The last two may be controlled by appropriate laboratory procedures.

Materials which fluoresce in the visible region of the spectrum (~500-600 nm) may also interfere with DNA typing when using fluorescent scanners or one of the ABI PRISM systems by appearing as identifiable peaks in the electropherogram. Urquhart and coworkers {332} examined a number of fluorescent compounds and their apparent mobility when electrophoresed in a polyacrylamide gel. All of the compounds studied, which included antibiotics, vitamins, polycyclic aromatics, fluorescent brighteners, and various dyes, could be removed with an organic extraction (i.e., phenol/chloroform, as is commonly used to extract DNA from cells). The Chelex method of DNA extraction (Walsh, P.S., *et al.* (1991) *BioTechniques* 10:506-518) failed to remove all of the contaminating fluorescent peaks. However, these interfering peaks were usually wide and possessed a broader fluorescent spectrum which made it fairly easy to distinguish them from the fluorescent dye-labelled PCR products. Urquhart *et al.* {332} recommends comparing unamplified material to PCR-amplified sample when appropriate substrate controls are not present. They also describe four possible forensic scenarios where contaminants would be possible: a) body fluid stains on dyes materials from which the dye may leach during extraction; b) body fluid stains on plant material, from which chlorophyll may co-extract with DNA; c) blood or tissue samples from individuals with some pathological conditions, e.g., lead poisoning or some forms of porphyria, in which blood porphyrin levels are greatly elevated; d) bone or tooth samples from individuals who were treated with tetracycline-group antibiotics in their youth (growing bones and teeth are known to incorporate and accumulate these antibiotics) {332}.

Spurious PCR products may arise from non-templated nucleotide addition of adenine or from stutter bands and thus provide 'contaminating' peaks in an electropherogram.

Non-template Addition of Nucleotides

An extra adenine is often included at the 3' end of a PCR product resulting in what is commonly referred to as non-template addition (Clark, J.M. (1988) *Nucleic Acids Res.* 16:9677-9686). Oldroyd and coworkers {8} found that inconsistent addition of an extra base by Taq DNA polymerase following extension resulted in double peaks differing in size by 1 base for each allele at the D21S11 locus. The problem was

eliminated by switching the fluorescent label to the reverse primer. Caskey and Edwards {121} resolved this problem by digesting the PCR product with the restriction enzyme *MluI* after incorporating a restriction site through the reverse primer (U.S. patent, column 19-20).

Stutter Bands

Stutter bands or 'shadow bands' were first reported with dinucleotide repeat polymorphisms by Hauge and Litt {85}. This phenomenon results in minor peaks which appear one repeat unit shorter than the major allele peak. Hauge and Litt {85} proposed slipped strand mispairing during PCR as the major mechanism for generation of these shadow bands. Several commonly used STR systems exhibit stutter bands including VWA {106}. Using Taq DNA polymerase, stutter bands are typically less than 10% of the allele band {106}.

Linkage of STR Systems to Genetic Disease

From C. Kimpton, *et al. Forensic Sci. Int.* 71 (1995) 137-152:

It is likely that many or possibly most STRs will eventually be shown to be useful in following a genetic disease or other genetic trait within a family and therefore this possibility must be recognized at the outset of the use of such systems {66}.

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Summary of EDNAP Collaborative STR Exercises

First one (P. Gill, *et al. Forensic Sci. Int.* 1994, 65, 51-59)

examined HUMTH01 and SE33 (ACTBP2)

Result: good agreement with HUMTH01 but a diversity of responses for the more complex SE33 locus

Second one (C. Kimpton, *et al. Forensic Sci. Int.* 1995, 71, 137-152)

examined HUMTH01, VWA, F13A1, FES/FPS

Result: robust analysis with ABI 373 detection of dye-labelled primers but problems with allele designation at F13A1 and FES/FPS when alternative detection methods were used

Third one (J. Andersen, *et al. Forensic Sci. Int.* 1996, 78, 83-93)

examined HUMTH01 and VWA with DNA mixtures

Result: all participating laboratories successfully typed the DNA from five stains, including two of mixed origin; these results were obtained for HUMTH01 and VWA despite diverse amplification, electrophoresis, and detection conditions (no amplification reagents or protocols were provided or specified as part of the study)

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(Alphabetical order; see [complete STR reference listing](#))

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