

DNA Commission of the International Society of Forensic Genetics: recommendations on forensic analysis using Y-chromosome STRs

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Abstract

During the past few years, the DNA Commission of the International Society of Forensic Genetics has published a series of documents providing guidelines and recommendations concerning the application of DNA polymorphisms to the problems of human identification. This latest report addresses a relatively new area — namely, Y-chromosome polymorphisms, with particular emphasis on short tandem repeats (STRs). This report addresses nomenclature, use of allelic ladders, population genetics and reporting methods. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

The use of Y-chromosome STR polymorphisms has become commonplace in forensic laboratories. The application includes its use in deficiency paternity testing cases (e.g. where the father is not available for analysis and inferences are made by reference from relatives in the male lineage such as a brother) and especially to discriminate stains in forensic investigation when a male suspect is involved. Y-chromosome analysis can be particularly helpful to detect the male DNA fraction from sexual assault stains that comprise male–female mixtures, where the former is at low concentration [1–7].

During the last few years, in addition to the description of new Y-chromosome STR polymorphisms, there has been a large amount of population and sequencing data published. As a consequence changes in the nomenclature were proposed for some systems (i.e. DYS19, DYS389 or DYS390). However, this resulted in some confusion about the appropriate notation to use and subsequently problems were noted with some national proficiency testing trials [8].

A number of new Y-chromosome STR markers are being described [9,10] and more are expected in the near future. Although many of the recommendations previously outlined for STRs can be applied to the Y-chromosome, the purpose of this paper is to give specific guidance on how to report Y-chromosome polymorphisms.

2. Nomenclature of Y-chromosomal STRs

Recommendations for the nomenclature of STRs are summarised by Olaisen et al. [11]. Essentially, the recommendations regarding the utilisation of Y-chromosomal STRs follow exactly the same principles. In particular, the commission would like to emphasise the following.

1. The sequence is always read in the 5′–3′ direction and the DNA strand that is used will be the one that is originally described in the literature or the first public database entry, preferably GENBANK.
2. It is recommended that the nomenclature is standardised as far as possible by utilising the D#S# system. Provided that non-standard designations are in almost universal use then there is no point to change this. However, discoverers of new loci are strongly encouraged to register D#S# designations in order to assist with the process of standardisation. It is suggested that editors of scientific journals also have a part to play in assisting the process of standardisation in this respect.

3. Allele nomenclature

Recommendations on STR allele nomenclature are also described in detail by Olaisen et al [11]. They are briefly summarised as follows.

1. The designation system follows from the number of complete repeats. This is straightforward for simple repeating sequences such as DYS388 or DYS392.
2. If a partial repeat is present then it is designated according to the number of complete repeat motifs and, separated by a dot, followed by the number of bases in the incomplete repeat.
3. The majority of Y-STR polymorphisms previously described are tetranucleotide repeats that consist of both non-variant and variant sequences. The nomenclature of some loci has been based on the total number of repetitive units (non-variant plus variant; e.g. DYS19) whilst others have taken into account only the repetitive stretches of DNA that are variant (e.g. DYS391) [3,8].

If a nomenclature is already in use, it is recommended that it should be continued. However, to encourage consistency for newly reported STRs, it is recommended that alleles should be named according to the total number of the repeat units of the DNA that comprises both variant and non-variant repeats.

4. For very complex STRs typified by the autosomal ACTBP2 that comprise multiple repeats of different sizes, the designation of alleles is not as easy. Olaisen et al. [11] recommended that there should be a mathematical relationship to the length in bases of consensus alleles. Alternative nomenclatures may be possible, however. In this case, provided that the nomenclature follows ISFG guidelines, the default standard nomenclature will follow from the first publication or the first public database entry.
5. Occasionally, intermediate alleles can appear due to a single base insertion or deletion in the flanking region. This happens with DYS385 using the primers described in the GDB generating intermediate 0.1 and 0.2 alleles [12] which cannot be detected using primer sets amplifying shorter fragments [13]. With the alternative primer sets the mutation is outside the amplicon.

Amongst Y-STRs currently in common use in forensic analysis, DYS389I, DYS389II, DYS390, DYS391, DYS385, DYS437 and DYS438 are complex repeats.

6. Duplicated systems such as DYS385, where the observed fragments cannot be assigned unequivocally to a defined genetic locus have to be treated as genotypes and the alleles should be separated by a hyphen, e.g. “DYS385*11-14”.

4. References and web-sites

There have been extensive collaborative studies to characterise Y-chromosome STRs [3,9,10,14–18].

A list of loci are published on Peter de Knijff's web-site <http://www.medfac.leidenuniv.nl/fldo>. This will shortly be moved to <http://www.fldo.nl>. This web-site contains locus information, PCR protocols, recommended nomenclature,

procedures and other information on the following Y-chromosome specific microsatellites: DYS19, DYS388, DYS389, DYS390, DYS391, DYS392, DYS393.

DYS394 and DYS395 represent different primer sets of loci DYS19 and DYS393, respectively.

The YHRD site <http://ystr.charite.de> [19], which is maintained at the Institute of Legal Medicine, Humboldt University, Berlin, Germany, has databases, primer sequences for commonly used Y-chromosome STRs, locus characteristics, useful multiplexes and haplotype characteristics as well as selected references.

In addition, the STR database of NIST [20] (http://www.cstl.nist.gov/biotech/strbase/y_strs.htm) contains Y-chromosome STR fact-sheets and a list of references.

5. Allelic ladders

The principles of the use of allelic ladders with STR systems is described by Olaisen et al. [11]. The recommendations are the same for Y-chromosome STRs. To summarise,

1. allelic ladders should span the distance of known allelic variants within each locus;
2. the rungs of the ladder should be one repeat unit apart, wherever possible;
3. they should be sequenced and
4. they should be widely available.

6. Mutation rates

A moderately high mutation rate of $\sim 0.2\%$ per generation was reported by Heyer et al. [21], and is supported by a recent study of 5000 male germline transmissions from confirmed father–son pairs at 15 Y-STRs which demonstrated 14 mutations [22]. The overall average mutation rate for Y-STRs was estimated as 2.80×10^{-3} (95% CIL 1.72×10^{-3} to 4.27×10^{-3}) which is similar to that observed for autosomal STRs [23,24]. Mutations of Y-STRs have been found to favour single repeat versus multiple repeat changes, and always occur within homogeneous repetitive arrays of at least 11 repeats [22].

Mutations have been observed at multiple loci in the same individual for Y-STRs [22] and autosomal STRs [25] and may lead to a wrongful exclusion [26]. However, likelihood ratios that incorporate a method to take account of mutation rates across multiple loci can be used to analyse data with apparent exclusions caused by mutation events. Rolf et al. [27] use such an approach to calculate paternity probabilities.

Additional alleles, possibly generated by duplication polymorphisms followed by mutations leading to expansion or contraction of the number of repeats have been observed in the non-recombining part of the Y-chromosome [22]

which is especially rich in repetitive sequences [28]. For DYS19, the frequency of duplicated alleles presumably caused by segmental duplication within the Y-chromosome has been estimated to be 0.12% [22], which is probably an underestimate as many recent duplications may exhibit two identical alleles.

7. Population genetics and the collection of databases

A Y-STR haplotype reference database for US populations is online available at <http://www.ystr.org/usa>.

Within Europe, Y chromosomal diversity is clinal and influenced primarily by geography rather than language [29] and ϕ_{ST} values have been found to be <0.01 among many European populations [30].

A large number of databases are available on an Internet site at <http://ystr.charite.de>, where it is constantly updated by participating laboratories. It is recognised that these databases are not yet representative of the whole of Europe because the collection is incomplete. Considerable effort is underway to add to the collection. Contributions are strongly encouraged (see web-site for further details).

In the absence of a completed database of Europe, we recognise that it is unknown whether existing databases are representative of the whole of Europe. For this reason, we recommend that Y-chromosomal STR polymorphisms are reported by reference to a local database.

Databases are collected on the basis of unrelatedness, therefore, close relatives are excluded as far as is possible. Consequently, common haplotypes, because they come from large pedigrees within a certain population, may be only sampled once and, therefore, frequencies will be underestimated because of deliberate non-relatedness selection criteria [31,32]. In a random survey from a large cosmopolitan population with marked mobility, the chance of selecting two patri-lineage related people is low. However, in small isolated rural populations the effect may be important and must be considered if the frequency estimate from a general population is used to make inferences on the former. Unfortunately, it is not possible to offer an easy solution to this problem, other than to type samples from the specific (rural) population in question wherever possible.

Concerning database collection, as much information on source should be collected as possible, including recording details of the specific region from where samples were obtained.

8. Reporting guidelines

The absence of recombination means that within a population every individual will share a haplotype with an unknown number of others who are identical by descent even though they may be separated by several generations. However, at the same time individuals will become

dispersed throughout the population. Rapid dispersal within typical modern cosmopolitan populations is a reasonable assumption.

There are two ways to report Y-chromosome polymorphisms. Either the counting method may be used, where the number of observations may be declared in a relevant database, or a frequency (or likelihood ratio) may be used. The problem is outlined in the DNA Commission guidelines on mtDNA, where similar approaches could be used [33]. The Bayesian approach of Roewer et al. [30] which estimates a posterior frequency distribution of haplotypes has been suggested as a potential way forward. Any interpretation entails assumptions. The usual assumption is that the suspect, if innocent, has been selected from the population in a way that gives him only a random chance to be related to the perpetrator. If the defense alleges that the brother of the suspect is the perpetrator then the likelihood ratio is considerably reduced with autosomal STRs. With Y-chromosome markers, close relatives will share the same haplotype and cannot be distinguished (unless a mutation has occurred). However, this is only relevant to the extent that there is other non-DNA evidence to suggest that the suspect (if innocent) and perpetrator are indeed close relatives.

It is not valid to multiply together individual allele frequencies — the Y-chromosome STRs are considered to be a single haplotype and its frequency is assessed relative to the relevant population, based on reasonably large haplotype databases.

9. Additional issues related to the application of Y-chromosome STRs in forensic cases

9.1. Paternity testing

For paternity analysis, mutation rates must be taken into account, especially for cases involving kinship [27].

9.2. Analysis of the male component in male–female mixtures

Parts of the X- and Y-chromosomes show high sequence similarity. Consequently, even when high stringency conditions are used in the amplification, bands may be observed in females. This occurs with DYS391 and DYS393, where additional amplification products are present in males and can be typed in females [6,34]. In the case of the DYS391 system, this problem was solved by using newly designed Y-chromosome specific primers [6] but this is not always possible in other systems (e.g. DYS393).

9.3. Contamination risks

The profiles of male laboratory staff in direct contact with the analysis of Y-chromosomal material should be recorded

and compared against evidential material in order to guard against the possibility of contamination.

If increased (e.g. 34–40) PCR amplification cycles are used then there is an increased risk of contamination from a source that is unrelated to the crime. This must be taken into account when the evidence is interpreted. One way to do this is described by Gill et al. [35].

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