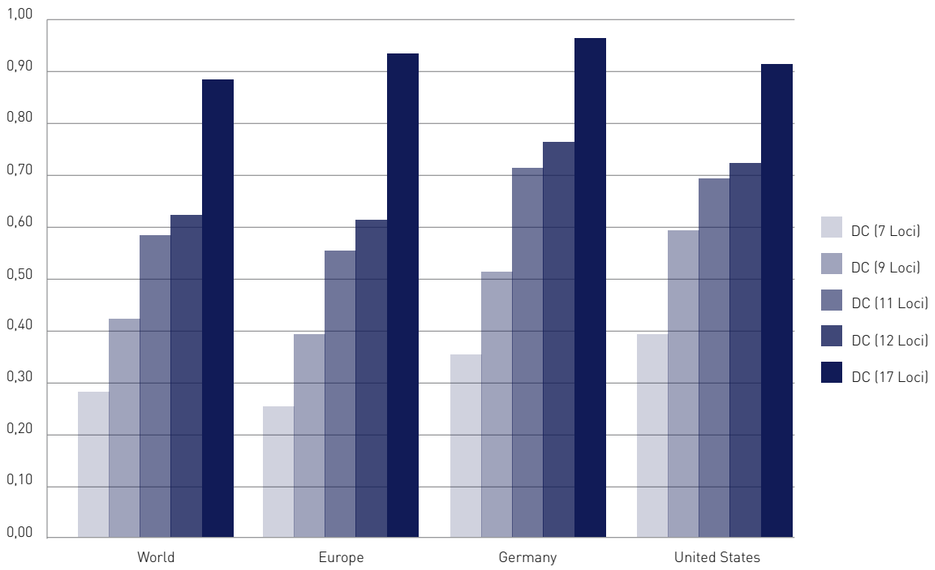
Professor Roewer said: "In the early days of the database, many people started with homemade protocols, but now most rely on commercial kits. We have set a standard of at least nine loci for all profiles included but, in reality, the overwhelming majority of laboratories who have submitted data in the last three years use the Yfiler® kit. This is largely because it is currently the commercial kit with the largest number of Y-STRs included, so it has the highest discrimination capacity. We showed this clearly in a recent comparison looking at Yfiler® against other lower resolution panels of 7-12 loci (Fig.2)." The discrimination capacity in Europe or the US is 60-70 % for 12 loci, but nearly 90 % for 17 loci.

Fig. 2: Discrimination capacity (DC) in different YHRD population sets (release 37)



A strong future ahead for Y chromosome analysis

Y chromosome analysis is now increasingly used routinely in sexual cases, and is mostly accompanied by several other lines of evidence. It is rare that Y chromosome evidence forms the centre of a case, but it is especially important in high profile cases not to lose any possible line of evidence, and it is often used by the police as a pointer to what population they are looking for, or as evidence of a serial offender, finding the same DNA profile from several crimes.

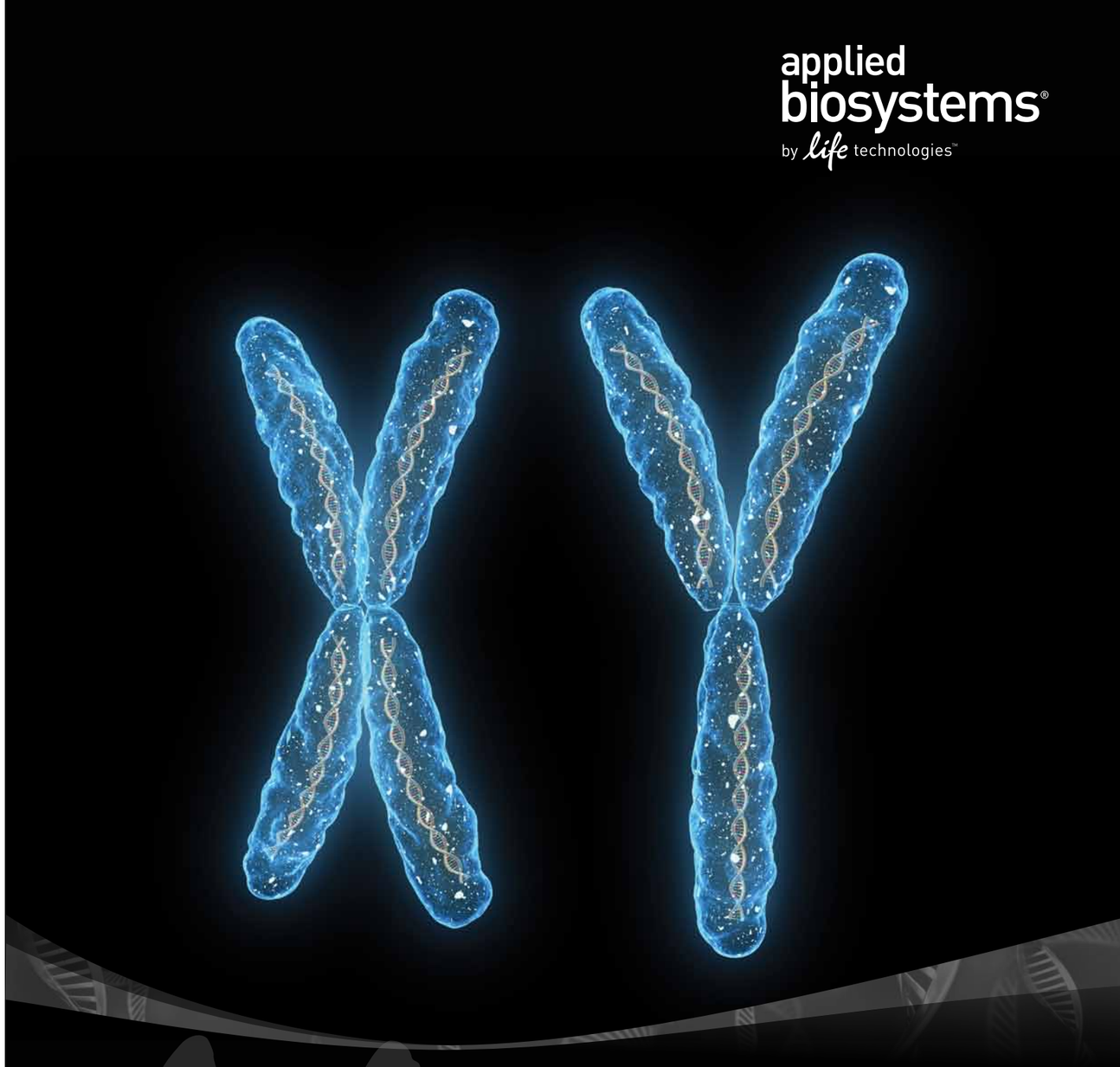
The International Society for Forensic Genetics has issued two recommendations on how to use Y chromosome analysis and nomenclature issues, and the YHRD database is a large enough resource to now give excellent confidence in the information it provides.

Professor Roewer concluded: "The combination of common standards and excellent reference data continues to improve credibility in the forensic information Y-STR analysis provides. The Group here in Berlin continues to work on ways to close the gap between autosomal and Y chromosome analysis with new markers, and I am confident that soon even first degree relatives will be able to be differentiated using this technique."

life technologies™

4ST 00658 CO23867

applied biosystems®
by life technologies™



Case Study: Y Testing at Court

AmpFℓSTR® Yfiler® drives the success of Y chromosomal analysis in forensic genetics

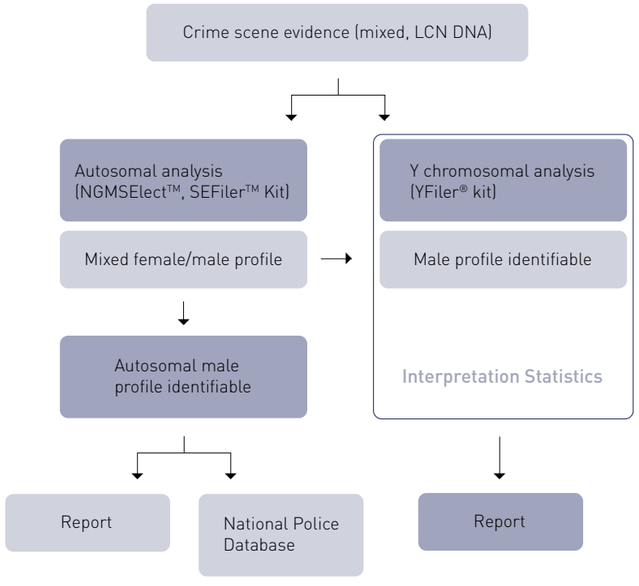
Case study: Y testing at court
AmpFℓSTR® Yfiler® drives the success of Y chromosomal analysis in forensic genetics

In 1992, a paper was published[1] describing the first case to be successfully resolved using the technique of Y chromosome analysis; in a murder case in Berlin, a male-female mixture could not be solved using autosomal analysis but analysis of just one locus on the Y chromosome was able to clearly exclude a male suspect.

Since then, the technique has grown to be an increasingly useful tool for identifying male-specific DNA in mixed stains for forensic genetics, as well as for tracing paternal lineages in genealogical and kinship testing. Professor Lutz Roewer, Head of the Department of Forensic Genetics at the Institute of Legal Medicine and Forensic Sciences of the Charité Medical Faculty in Berlin, has been heavily involved in the research of Y chromosome analysis since its emergence in the early nineties. His department performs around a third of the casework for the Berlin Police (Landeskriminalamt Berlin), including all DNA analysis and covering the whole spectrum of crime cases and now uses the technique routinely for all crimes of a sexual nature.

Professor Roewer explained: "We are a high throughput, accredited laboratory and so defining the workflow is very important to keep costs low and speed high. We have used Y-STR analysis on certain cases for some time – for example for murders – and have now added it to the standard workflow for all sexual crimes, where regularly applying this as a second kit makes most sense, scientifically and cost-effectively (Fig.1). All cases undergo fully automated DNA extraction and pipetting of the PCR plates, using two robots to process about 200 samples per day. After extraction, again nearly all samples are processed using an autosomal STR kit, with any successful results repeated before they are reported to the police and recorded on the database."

Fig. 1: Workflow sexual assault cases, Berlin Laboratory



"In a recent trial carried out internally, 10 out of 40 cases gave significant autosomal profiles and, of the 30 remaining negatives, we were able to identify 10 Y-chromosomal profiles, thereby increasing the number of informative profiles by 100 %. Autosomal STR analysis will, of course, always be the first line technique, not least in order to build on and make use of the excellent national databases that have been established over the years. However, to be able to give the police information about the existence of male DNA and/or the number of perpetrators is also a great advantage. Standard PCR always amplifies the major component of a mixture so if the male component is in very small amounts it is effectively hidden from autosomal analysis and the evidence is lost. In these cases, and also when the results from autosomal analysis are difficult to interpret, Y analysis can be much more sensitive, capable of finding just one sperm in 1,000 female cells."

The individuality of the male-specific part of the Y chromosome is analyzed using a set of highly variable STR markers approved by the forensic and scientific community, in the case of the Berlin laboratory using the AmpFℓSTR® Yfiler® PCR Amplification Kit from Life Technologies. Yfiler® coamplifies 17 Y chromosome STRs in a single PCR reaction, including the core set of loci defined as the European Minimal Haplotype, the loci recommended by the Scientific Working Group on DNA Analysis Methods, plus six additional highly polymorphic loci which increase the discrimination capacity even further.

Understanding lineage-specific inheritance

Despite excellent sensitivity, Y chromosome analysis is only now beginning to earn its place in routine forensic testing. Professor Roewer explained why: "Firstly, the forensic world has been used to a well-established system of autosomal STR typing backed by statistics that, in principle, give you overwhelming 100 % assurance that identifies one person. The Y chromosome in comparison is lineage-specific, so one profile might belong to more than one male; if the male in question has brothers, cousins, a father, they might all possess the same Y chromosome. This changes gradually with spontaneous mutations through the generations, so the further back a genealogy goes, the more differences will be found. The degree of differentiation also increases with more loci or with loci which have a higher mutation rate. This is also seen within populations, where there is a certain degree of interrelation across a territory or country. In some isolated regions with endogamous populations, there is strong identity in each group, although differences can still be seen if a higher number of loci is used. Conversely, in out-bred European or US populations, typing 100 random samples with just nine loci would result in perhaps 5-10 % similarity, but increasing the number of loci to 17 would bring this down to 1 %. Lineage information like this is very useful, as long as it is correctly interpreted and reported."

The fundamental genetic rules of statistics

"The second point is a simple matter of the frequency of the loci that are used as markers. In reality, nobody knows how often a single genotype might occur, however, fundamental genetic rules allow the multiplication of unlinked loci in autosomal analysis, but not linked loci in Y chromosomes. In autosomal analysis, 10 loci chosen as markers are most likely to be found on different chromosomes and inheritance is completely unlinked. From a reference population, it is possible to produce a frequency for each locus, and then the frequencies of each of the 10 loci can be extrapolated, giving a wonderful number of perhaps one in a billion, or even one in a trillion, for a single profile. The same is not true of the Y chromosome, because 10 loci on the same chromosome are fully linked – a haplotype inherited together. Instead of extrapolating the frequency of each loci, a reference database is required that gives the frequency of a certain haplotype, compared with every known variation of the same haplotype. This is fundamental; the weight of evidence is lower for a Y profile than it is for an autosomal profile, and this cannot be solved. We can expect that, with a certain number of loci, we can differentiate all Y chromosomes in the world, which is then one in 6 billion, but we cannot now."

"In a global Y-STR database (YHRD) we have now collected 100,000 chromosomes from 750 populations from different parts of the world and looked for clusters of frequent Y chromosomes in populations. Groups of related haplotypes have been identified, in Western Europe, for instance, which are completely different from those in Japan, North Africa and even Eastern Europe. This has a lot to do with social practices; in central Asia, in countries like Kazakhstan, there is a very high excess of certain haplotypes, with clans of men – brothers, sons, etc. – living together in a certain territory; whereas in other groups, like Western Europe or American Caucasians, there is a great deal of migration and flexibility in inter-cultural marriages and haplotypes are 'diluted'. We can use this additional information to say that an individual is probably from a certain region or population of the world. This serves rather as intelligence than the path of identification, but you can indeed give intelligent information on the likely origin of the person, and there is no better genetic system that shows these subtle differences around the world."

Establishing a global database

This element of population genetics was the driving force behind the establishment of an internet-based Y-STR Haplotype Reference Database (YHRD) in 2000 (www.yhrd.org). Over 350 forensic groups from around the World have submitted their original data to feed the database and, at the same time, have the benefit of access to the frequency and the global distribution of Y-STR profiles.

[1] Roewer L and Epplen JT(1992) For Sci International 53: 163-171