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SORT TANDEM REPEAT (STR) KITS AND ITS FORENSIC APPLICATIONS

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ABSTRACT

Short tandem repeats (STRs) are short DNA sequences that are repeatedly repeated at particular loci in the human genome. These sequences are usually 2–6 base pairs. Individuals typically vary in the number of repetitions they carry at each STR locus due to the significant polymorphism of these loci. STRs provide a strong genetic fingerprint for identification because they are common and vary widely among individuals. Although STRs do not impact health and are frequently found in noncoding areas, their diversity makes them perfect indicators. In actuality, each examined locus's allele (repeat count) is listed in a forensic STR profile, and a DNA match necessitates that the profiles match at every locus.

STR kits are commercial systems that amplify multiple STR loci simultaneously by multiplex polymerase chain reaction (PCR). DNA Polymerase and PCR buffer, primer mixes for each target STR locus (with distinct fluorescent labels), and allelic ladders—sets of DNA fragments of specified sizes—that act as internal standards for allele calling are all included in a typical STR kit. Reagents for quantification and control DNA samples may also be included in kits. To optimize discrimination, modern forensic STR kits target numerous loci in a single reaction, frequently 20 or more. For instance, several popular kits amplify all of the essential U.S. CODIS loci in addition to other markers for higher power. These kits can generate a highly discriminative DNA profile from small or degraded materials by creating a profile from many loci in a single response.

Keywords: Animal hair, dog hair, forensic investigation, microscope, microscopic examination, etc

INTRODUCTION

Reliable DNA profiling in forensic settings is the goal of forensic STR kits. Important elements and characteristics consist of:

- Primer Mixes: A panel of STR loci's primers are included in each kit. In order to differentiate the amplified fragments of each locus in a single reaction, primers border the repeat region and contain fluorescent dyes.
- PCR Master Mix: Taq polymerase (often in a hot-start form), deoxynucleotide triphosphates (dNTPs), magnesium chloride, and a high-sensitivity buffer are all included in this reaction mix. Low-copy or partially degraded DNA can be amplified with the use of strong enzyme formulations.
- Allelic Ladder: The common allele sizes at each locus are represented by a collection of reference DNA pieces. Allele designations are assigned during capillary electrophoresis by comparing sample peaks to the ladder.
- Internal Size Standard: To achieve precise sizing

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and calibrate the electrophoretic separation, a DNA size standard (a combination of fragments of known lengths) is run with every sample.

- Controls: Positive control A negative (no-DNA) control looks for contamination, while DNA with a known profile confirms that amplification was successful.

Multiplex PCR, which amplifies numerous STR loci in a single tube, is made possible by these kits. A single PCR reaction can produce data for 20–30 loci thanks to multiplexing, which significantly boosts throughput and preserves material. All loci are selected based on their technological robustness and high population heterozygosity. Tetranucleotide STRs (repeat units of four bases) are used in the majority of forensic kits because they yield clear, easily interpreted peaks with few stutter artifacts. When more markers are required, some kits may include pentanucleotide or trinucleotide loci.

Before being used, STR kits undergo extensive validation. Each kit is tested by manufacturers and forensic labs for sensitivity (the lowest quantity of DNA that produces a profile), repeatability, stability with inhibitors (which are frequently found in forensic samples), and mixture resolution capability. Validation adheres to regulations set forth by organizations like international ISO standards or the FBI's Quality Assurance Standards. In casework, only kits that successfully complete these demanding tests are used.

APPLICATIONS OF SHORT TANDEM REPEATS (STR)

1. Applications in Forensic Science

a. Human Identification in Criminal Cases

. In criminal investigations, STR kits are the standard method for identifying people. DNA is taken and amplified using a STR kit to create a DNA profile when biological evidence (blood, semen, saliva, hair, or skin cells) is discovered at a crime scene. DNA samples from suspects and victims are handled similarly. The forensic scientist can be extremely certain that the STR profiles came from the same person if they match at every locus. In reality, when 15–20 STR loci are compared, the match probability—the likelihood that two unrelated individuals share the profile—is frequently one in several billions. Because of this, STR profiling is significantly more discriminating than previous techniques (such as blood group or protein assays).

. Mixed DNA sample analysis is also made possible by STR kits. DNA from several individuals may be found in crime scene evidence (e.g., mixed blood or sperm/epithelial cell combinations in sexual assault instances). By recognizing several allele peaks at each locus, modern STR tests and software methods can frequently deconvolute simple mixes. The kit's large number of loci aids in the statistical separation of contributors. Specialized interpretation techniques are employed for very complicated mixes, and often only a partial match is feasible.

b. Paternity and Kinship Testing

. STR kits are frequently used for parentage testing in addition to forensic investigations. At each STR locus, a child receives one allele from each parent. Analysts can ascertain parentage by comparing the child's profile with that of the purported father (and frequently the mother). Each allele in the child must be present in one of the parents. As a result, STR profiles can determine paternity or maternity with probability that frequently surpass 99.99%. Because these loci are so informative, multiplex STR assays for relationship testing usually employ similar loci as forensic kits. Paternity indices or likelihood ratios are produced by statistical computations (using population allele frequencies).

. In complicated kinship situations (grand parentage, sibling testing, missing parents), STR analysis is also utilized. In these situations, autosomal STRs can be supplemented with other specialized markers to boost confidence, such as X-chromosome STRs for maternal lineages or Y-chromosome STRs for paternal lineages. Due to their increased accuracy and efficiency, STR kits have generally supplanted older techniques (such as HLA or RFLP) in familial relationship testing.

c. Specialized STR Kits: Autosomal, Y-Chromosome, X-Chromosome

. There are several classes of STR kits tailored to different forensic needs:

- **Autosomal STR Kits:** The most popular type of STR kits are autosomal ones, which amplify sites on non-sex chromosomes inherited from both parents. They offer strong personal identification. 15–31 autosomal loci, including key database markers, are usually targeted by current commercial kits (such as PowerPlex, GlobalFiler, Identifiler and Human ID HID 31 STR loci systems).

- **Y-Chromosome STR Kits:** These kits amplify 17–27 markers on the Y-chromosome, which is only found in males. Y-STR profiles are

the same among patrilineal relatives because the Y chromosome is essentially unaltered from father to son. When a sample contains a combination of male and female DNA, such as in cases of sexual assault with extra female DNA, Y-STR kits are helpful for determining paternal lineages. A Y-STR profile can verify male identity or link several male suspects to evidence if the profile of a male contributor cannot be identified autosomally.

- **X-Chromosome STR Kits:** These amplify STRs on the X chromosome, which is found in both sexes but has two copies in females and one copy in males. In complicated kinship instances involving female lineages, X-STR profiles can be useful (e.g., to aid identify between maternal relatives).

d. DNA Databases and Mass Disaster Identification

- STR profiles of arrested individuals, convicted criminals, and unidentified samples are kept in national DNA databases. For instance, millions of profiles based on particular STR loci are stored in the U.S. Combined DNA Index System (CODIS) and comparable databases elsewhere. Commercial STR kits incorporate the necessary core loci in order to be interoperable with these databases. A forensic lab can search the database after creating a profile from the evidence using a standardized kit. An unknown sample can be instantly connected to a person on file or to other evidence from the crime scene via a match, or "hit." When it comes to identifying missing people and major disasters, STR kits are essential. Bodies may be burned, broken, or decomposed in situations like airline crashes, natural disasters, and conflict areas. From little bone or teeth samples, STR analysis frequently produces a profile. Using the same STR loci, these profiles can then be compared to ante-mortem personal DNA samples (such as blood cards, toothbrushes, and hospital tissue) or reference samples from the victims' family. Large-scale STR genotyping helped identify thousands of people by connecting remains to families in high-profile cases (e.g., World Trade Center or tsunami victims, and victims of genocide in Rwanda, former Yugoslavia). In these situations, STR kits' capacity to function on damaged DNA—particularly those that amplify tiny amplicons—is a crucial benefit.

2. Applications in Anthropology and Population Genetics

- The history and variety of the human population are also studied using STR variation. STR loci are helpful for analyzing recent genetic links between groups since they mutate somewhat quickly. To evaluate allele frequencies across ethnic or geographic populations, population geneticists have employed panels of STR markers, which are comparable to or identical to forensic kits. These investigations can uncover genetic structure, mixing, and migration patterns. For instance, historical migrations and ties between tribes or ethnic groupings have been traced using paternal-lineage Y-STR profiles.

  STR markers are used in anthropological genetics to reconstruct pedigree patterns and family relationships in distant or historical populations without written records. DNA from anthropological samples can be genotyped and compared to reference datasets by geneticists. STR profiling is occasionally used in forensic anthropology on skeletal or ancient remains; if enough DNA is preserved in the bones, the profile can be compared to databases of living relatives or the community. Due to its sensitivity and the size of the current STR data pool, classical STR analysis is still useful for some forensic anthropological cases, even if modern research increasingly uses single-nucleotide polymorphisms (SNPs) and next-generation sequencing for deep ancestry.

  Furthermore, even though STRs are essentially identification markers, forensic investigators can use STR data in conjunction with statistical models to determine a person's physical characteristics or biogeographic heritage. In anthropological work, STR kits are essentially the same; researchers only utilize them to help with the legal repatriation of remains or to genotype samples for population studies.

3. Applications in Medical and Biological Research

Beyond forensics and anthropology, STR analysis has important roles in medical genetics and biological research:

- **Genetic Disease Testing:** Expansions of STR sequences inside genes are responsible for a number of inherited illnesses. Examples include myotonic dystrophy (CTG repeats in DMPK), fragile X syndrome (CGG repeat in FMR1 gene), Huntington's illness (an increase of CAG repeats in the HTT gene), and others. To identify or forecast certain illnesses, clinical diagnostic kits amplify the

particular STR locus and measure its size (number of repetitions). These assays use the same technique of STR amplification and sizing as forensic kits, however they only target one locus at a time.

- **Cell Line and Sample Authentication**: STR profiling is the gold standard for confirming the authenticity of human cell lines and tissue samples in scientific research and biotechnology. Because cross-contamination of cell cultures is a frequent issue, labs frequently create a genetic fingerprint of a cell line using STR kits, which are typically a smaller forensic panel of 16–20 loci. To verify validity, this fingerprint is compared to a database (such the ATCC STR database) or a reference sample. The sex-identifying amelogenin marker is typically included in authentication kits. In order to guarantee the authenticity of research, several journals and funding organizations increasingly demand STR verification of cell lines.
- Transplantation and Chimerism: Patients who have bone marrow or stem cell transplants develop mixed donor and recipient cells, a condition known as chimerism. By comparing patient profiles over time or against donor profiles, STR profiling can measure the percentage of donor vs. recipient DNA in blood or marrow samples. This aids in tracking relapse or engraftment success.
- Cancer and Pathology: Panels of STR markers can be used in cancer research to identify loss of heterozygosity or microsatellite instability, which is a characteristic of some malignancies. Furthermore, STR profiles are helpful in pathology because they can confirm that two tumor samples (primary and metastatic, for example) originated from the same patient.
- Wildlife and Conservation Genetics: STR kits, also known as microsatellite kits, are utilized in animals for paternity testing, breed identification, and tracking illicit wildlife trade, even though they are outside the purview of humans. STR kits for cattle or wildlife species are sold by numerous businesses.

In all these fields, the concept of using short tandem repeats for identification remains the same as in forensic cases: PCR amplification and allele sizing by electrophoresis.

TECHNOLOGICAL ASPECTS AND VALIDATION OF STR KITS

The design of STR kits reflects both the biological properties of STRs and the practical needs of forensic labs. Important technical features include:

- Multiplex PCR: Several primer pairs are included in a single reaction with STR kits. Every primer pair has a distinct fluorescent label and is intended to amplify a distinct STR locus. When sample quantity is constrained, multiplexing is essential because it lowers the quantity of DNA and the number of reactions needed. In order to prevent interference and achieve balanced amplification of every locus, the primers are meticulously adjusted.
- Fluorescent Labeling and Detection: Various fluorescent dyes are used to label PCR primers. Following amplification, capillary electrophoresis is used to mix and separate all of the PCR products, or amplicons. As DNA pieces pass, a laser picks up the fluorescent signals, creating an electropherogram. Each peak represents an allele at a certain locus; the locus and allele number are determined by the combination of color and size.
- Allelic Ladders and Size Standards: By matching fragment lengths, the software can assign an allele designation to each peak using allelic ladders, which are reference fragments for every potential allele at every locus. A ruler for fragment sizing in each sample is provided by the internal size standard. Determining precise repeat counts would be impossible without this.
- Optimized Chemistry: A kit's amplification mix is designed to be robust. To get over typical PCR inhibitors (such hemoglobin or humic acids), it could use additions (BSA, proprietary boosters). The enzymes are frequently designed to have great efficiency and fidelity. Newer kits may offer direct amplification or rapid cycling techniques to expedite processing.
- Loci Selection: Core loci in STR kits are selected based on their high heterozygosity and compliance with database or legal criteria. Current kits include international standard loci in addition to all core CODIS loci. To increase compatibility or matching strength across areas, more loci are frequently added.

Each STR kit goes through a developmental validation process, which involves rigorous testing with known samples, mixes, and difficult situations, prior to forensic usage. For instance, repeated dilutions

of control DNA are amplified to assess sensitivity. The capacity to identify minor contributors is tested through mixture studies. Drop-in/drop-out rates and stutter, a frequent PCR artifact, are described. A kit is only used once it has been shown to be dependable and repeatable. The proper use of the kits in forensic casework is ensured by standard operating procedures and training.

STR analysis is continually being improved by emerging technologies. There are currently more advanced kits that can examine up to 31 loci at once, and mini-STR kits use shorter amplicons (around 70–150 bp) for extremely damaged DNA. Massively parallel sequencing (next- generation sequencing) of STR loci has been used in certain labs to improve mixture deconvolution and identify sequence differences within repeats. With little hands-on time, automated "rapid DNA" devices can complete multiplex STR amplification and analysis in a matter of hours, allowing for almost instant identification (e.g., booking a suspect in jail).

DIFFERENT TYPES OF STR KITS

STR kits are classified according to the biological systems they examine, the particular loci they target, and their intended uses. Although amplifying polymorphic short tandem repeat loci is the basic objective of all STR kits, the following main kinds are frequently employed in forensic and research contexts:

1. Autosomal STR Kits

These have loci on autosomes (non-sex chromosomes) and are the most commonly utilized. With the exception of identical twins, they are intended to offer a thorough DNA profile that is specific to each individual. The foundation of human identity, autosomal STR kits are frequently utilized in DNA databasing, paternity testing, and forensic investigations. Examples consist of:

- **GlobalFiler™ (Thermo Fisher Scientific)**: Covers 21 autosomal STR loci plus amelogenin (for sex determination), designed to meet international database standards.
- **PowerPlex® Fusion and Fusion 6C (Promega):** Amplify up to 27 loci, including all CODIS and European standard markers.
- **Human ID HID 31 STR loci reagent kit (Syntol LLC):** Amplification of 31 STR loci which covers 26 autosomal STR loci and 5 sex loci (Amelogenin, Yindel, SRY, DYS391, DYS392), includes all CODIS (Combined DNA Index System) and ESS (European Standard Set) database loci, as well as SE33, Penta D, Penta E loci.

2. Y-Chromosome STR (Y-STR) Kits

Loci on the Y chromosome, which is inherited from father to son, are the focus of Y-STR kits. These kits are helpful for analyzing male-specific DNA, particularly in mixes where female DNA predominates, such evidence of sexual assault. All male paternal relatives will share the same Y-STR haplotype because the Y chromosome does not recombine.

- **Yfiler™ Plus (Thermo Fisher Scientific):** Amplifies 27 Y-STR loci with high discrimination capacity among male individuals.
- **PowerPlex® Y23 System (Promega):** Offers a robust 23-locus Y-STR profile suitable for kinship analysis and human migration studies.

3. X-Chromosome STR (X-STR) Kits

X chromosomal loci are the focus of X-STR kits. These are very useful for doing intricate kinship analysis, including determining the ties between half-siblings, aunts, uncles, nieces, and nephews, or when reconstructing the female lineage. Specialized statistical analysis is needed to understand their inheritance patterns.

- **Mentype® Argus X-8/X-12/X-16 QS Kits (Biotype):** Available in versions amplifying 8, 12, or 16 X-STR loci.
- **Investigator® Argus X-12 Kit (QIAGEN):** Commonly used in forensic and paternity testing involving female-related lineages.

4. Mini-STR Kits

Mini-STR kits are perfect for examining deteriorated materials, such as those from burned tissues, aged bones, or evidence exposed to the environment, because they are made to amplify shorter DNA fragments, usually less than 150 base pairs. The primers are moved closer to the repetition regions by these kits.

- **MiniFiler™ (Thermo Fisher Scientific):** Targets nine autosomal loci optimized for degraded DNA.
- **PowerPlex® 16 HS and MiniSTR kits** (Promega): Include shortened amplicons for better performance with compromised DNA.

5. 5. Rapid STR Kits

In less than two hours, non-experts may create STR profiles thanks to integrated systems that include rapid DNA kits. These kits are utilized in booking stations and in field-forward applications like military or disaster victim identification (DVI).

- **RapidHIT® ID System Kits (Thermo Fisher**

Scientific): Compatible with rapid DNA instruments for real-time STR analysis.

- **ANDE Rapid DNA Kits (ANDE Corporation)**: FDA-approved kits used in forensic booking applications.

6. Specialized STR Kits for Research and Quality Control

These include kits for animal genotyping, chimerism monitoring, and cell line verification. They are designed for certain biological systems and are based on the same STR principles, however they are not usually meant for forensic use.

- **GenePrint® 10 System (Promega):** Used for human cell line authentication and sample tracking in research.
- **StemElite™ ID System:** Used in stem cell research and chimerism testing post- transplantation.
- **Canine or Equine STR Kits:** Used for breed verification and individual identification in veterinary forensics or wildlife management.

CONCLUSION

In conclusion, STR kits—which include size standards, fluorescence primers, and multiplex PCR reagents—are essential resources in contemporary genetics. They are essential in forensic science for kinship testing, criminal case resolution, and human identification due to their high sensitivity and multiplex capacity. Because STR kits are standardized, they provide uniformity between labs and compatibility with national DNA databases, which has transformed genetic identification and crime solving. STR genotyping is widely used in anthropology, population genetics, clinical diagnostics (for repeat expansion illnesses), and biological sample quality control in addition to forensics. Larger marker sets and platform integration are two further ways that STR kits are developing as technology progresses. The fundamental idea is still the same, though: short tandem repeat analysis yields a distinct DNA profile that has amazing capacity to personalize samples in a variety of scientific and legal contexts.

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