DEVELOPMENT OF A COMPUTATIONAL TOOL FOR FORENSIC DNA ANALYSIS

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THESIS ABSTRACT

Development of a computational tool for Forensic DNA Analysis

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Forensic DNA analysis uses repetitive sequences in the human genome called Short Tandem Repeats (STRs) for human identification. This study has contributed to the building, design, and development of a fast and easy to use software package to calculate the \textit{a posteriori} probability (APP) on the number of contributors in an STR profile and to calculate the likelihood ratio (LR), a statistic that conveys the strength of a match for a given suspect, as well as the distribution of the LR over random non-contributors. This research specifically deals with the (1) design and implementation of an algorithm for MatchIt (the component that calculates the LR and its distribution); (2) optimization the code to reduce running time; and (3) development of a user-friendly interface.
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Introduction

Starting from mid 90s, Short Tandem Repeats (STRs) have been used in the field of human identification for forensic purposes [1]. STRs are repetitive sequences that are 1–7 base pairs in length and scattered throughout the human genome. An STR DNA profile developed from a biological sample (like saliva, semen, blood, etc.) collected at a crime scene is either compared with that of a person of interest (POI) or run against a database to check for a match. The Scientific Working Group on DNA Analysis Methods (SWGDAM) recommends that forensic reports include a statement regarding the assumption made about the number, or the minimum number of contributors, to the sample being investigated [2].

The number of contributors to a crime scene sample is generally unknown and must be estimated by the analyst based on the electropherogram obtained.

An assumption about the number of contributors is needed when determining whether a known should be excluded as a contributor to an item of evidence. Changing the number of contributors could lead to different conclusions about whether to include or exclude an individual as a contributor to the sample. Further, an assumption about the number of contributors to a sample is needed to calculate a match statistic, called the Likelihood Ratio [3]. A statistic that is commonly used internationally and is gaining acceptance in the United States.

The Likelihood Ratio (LR) is defined as:

\[
LR = \frac{Pr(E|H_p, n_p)}{Pr(E|H_d, n_d)},
\]
where $E$ is the evidence in the form of the electropherogram (epg); $H_p$ and $H_d$ are the hypotheses specified by the prosecution and the defense, respectively; and $n_p$ and $n_d$ are the number of contributors specified by the prosecution and the defense, respectively. The numerator is the probability of observing the evidence given the prosecution’s hypothesis and the denominator is the probability of observing the evidence given the defense’s hypothesis. The evidence shows support for the prosecution’s hypotheses if $LR > 1$; if $LR < 1$ the defense’s hypothesis is supported by the evidence. The calculation of a Likelihood Ratio depends upon an assumption about the number of contributors both in the numerator as well as the denominator, making it essential to have a good estimate about the number of contributors to accurately calculate a statistic that represents the information captured in the signal. Thus, utilizing a number of contributors that is not representative of the actual number that gave rise to a sample may affect the interpretation of the sample's profile. The p-value for the suspect is defined as the probability that a randomly picked person from the population would give rise to an LR at least as large as the one observed for the suspect.

$$p - value(s) = (LR(R) \geq LR(s))$$
Purpose

The purpose of the current study was to develop a software package that estimates the number of contributors to an evidence sample and subsequently calculates a LR for person of interest based on the given DNA sample and the number of contributors in an accurate, reliable and efficient manner. This work is specifically focused on the following three aspects of mixture interpretation:

1. Design and implementation of an algorithm for MatchIt
2. Code optimization to reduce running time
3. User interface design and development
Design and Implementation of MatchIt Algorithm

3.1 Introduction

The software is divided mainly in three parts as 1) Calibration, 2) NOCIt, and 3) MatchIt.

Characterization of the peak heights is done by using single source calibration profiles with known genotypes obtained from samples amplified from a wide range of input DNA masses. Absolute DNA (extracted from 28 individuals) quantification was performed using real-time PCR and the Quantifiler® Duo™ Quantification kit according to the manufacturer’s recommended protocol and one external calibration curve [4, 5]. The extracted DNA was amplified using the manufacturer’s recommended protocol for AmpFISTR® Identifiler® Plus Amplification Kit (Life Technologies, Inc.) [6]. A fragment analysis was performed using GeneMapper IDX v1.1.1 (Life Technologies, Inc.) and an RFU threshold of one. A threshold of 1 RFU was used in order to capture all peak height information (i.e., the allelic peaks, baseline noise and stutter peaks) in the signal. Known artifacts such as pull-up, spikes, -A, and artifacts due to dye dissociation were manually removed while generating the sample data. Refer to figure 1 for a sample electropherogram with peak heights at each allele. For a detailed description of how the calibration samples were created, refer to [7].
Figure 1 Sample electropherogram with peak heights at each allele

NOCIt is a computational tool that calculates the APP on the number of contributors to a DNA sample [7].

MatchIt uses single source samples with known genotypes and calculates a LR and a $p$-value for a specified POI on a question sample. It is a fully continuous method that works by modeling the peak heights observed in a calibration data set consisting of single source samples with known genotypes. It accounts for dropout and stutter (both reverse and forward), two common artifacts observed in low template samples [8]. Additionally, MatchIt also computes a $p$-value for the LR by sampling a large number of random genotypes from the population.

3.2 MatchIt Algorithm

The Likelihood Ratio (LR) is defined as:

$$LR = \frac{Pr(E|H_p, n_p)}{Pr(E|H_d, n_d)}.$$
In practice, \( n_p \) and \( n_d \) can be chosen by the prosecution and the defense to maximize their respective probabilities and there is no necessity for \( n_p \) to be equal to \( n_d \). However, we have developed MatchIt to use the same number of contributors in both the numerator and denominator to calculate the LR. It should be noted that the method could be extended to work on different assumptions on the number of contributors. Moving forward, we omit the notation \( n \) for the sake of brevity. We note that for purposes of this work, \( n_p = n_d \) in all cases presented herein, and we use the known, and thus the true \( n \) to test the capabilities of MatchIt.

For this study, we use the following hypotheses for \( H_p \) and \( H_d \):

\( H_p \): The evidence is a mixture of the genotype profile of a suspect (\( s \)) and the profiles of \( n - 1 \) other unknown, unrelated contributors, whom we term for the purpose of this paper as “the interference contributors”.

\( H_d \): The evidence is from \( n \) unknown individuals unrelated to the suspect.

In most cases, the value of the LR is very large (or very small) and it is easier to work with \( \log(\text{LR}) \). Hence we have:

\[
\log(\text{LR}(s)) = \log(Pr(E|R = s, U^{n-1})) - \log(Pr(E|U^n)),
\]

where \( U^i = \{U_1, ..., U_i\} \) are the random genotypes of \( i \) contributors and \( R \) is the random genotype of a single contributor, whether it be a true contributor, or non-contributor.

### 3.2.1 LR numerator calculation

Our algorithm assumes a constant mixture ratio at all the loci. The mixture ratio specifies the proportion of the total template mass contributed by each contributor to the sample. The
underlying mixture ratio of an evidence sample is unknown and needs to be described by a model in order to compute a continuous LR. A constant mixture ratio model assumes that the mixture ratio is the same at all the markers, whereas a variable mixture model accounts for the possibility of the mixture ratio being different at the various markers. Both models are reasonable and are used in existing continuous methods to compute the LR. Perlin et al [9] assign a uniform prior probability for the template mixture weight and construct its probability distribution by drawing individual locus weights using a multivariate normal distribution. Cowell et al [10] and Puch-Solis et al [11] use a constant mixture ratio model and implement a discrete approximation over the interval (0,1) by assigning a uniform prior. Taylor et al [12] use the variable model and assume the mixture weights to be independent across the loci. Since we adopt the constant mixture ratio approach, we integrate over all possible mixture ratios to calculate the probability of observing the evidence:

\[ \Pr(E|R = s, U^{n-1}) = \int_{\theta \in \Delta^{n-1}} \Pr(E|\theta = \theta, R = s, U^{n-1}) f_\theta(\theta), \]

where \( \theta \) is the vector with components \( \theta_i \), the mixture proportion of each contributor \( i \in \{1, \ldots, n_{\text{max}}\} \); \( \Delta^{n-1} = \{ (\theta_1, \ldots, \theta_n) \in \mathbb{R}^n | \sum_{i=1}^{n} \theta_i = 1, \theta_i > 0 \forall i \} \) is the unit \( n-1 \) simplex; and \( f_\theta \) is the probability density function of \( \theta \), which we assume to be uniform over \( \Delta^{n-1} \). For \( n = 1 \), \( \Delta^{n-1} \) consists of the single element \{1\}. For mixtures, we implement the integration over \( \Delta^{n-1} \) by dividing it into equal-sized subsets and representing each subset with its centroid, resulting in a discrete sum.

To do this, we performed k-means clustering in Python (Python Software Foundation, Beaverton, Oregon). k-means clustering is an algorithm used to partition observations into a set of clusters by repeated minimization of the distance from an observation to the centroid
of its cluster [13]. For \( n = 2 \), the space was divided into 9 equally sized clusters, while for \( n = 3 \), 12 clusters were used.

Let \( L \) be the set of all loci in the evidence sample, \( E_l \) be the evidence at locus \( l \), \( U_l^{n-1} \) be the genotype of the interference contributors at locus \( l \) and \( s_l \) be the genotype of the suspect at locus \( l \). The STR loci used for forensic DNA analysis are assumed to be in linkage equilibrium and independent of each other [14]. Hence we obtain:

\[
\Pr(E|\Theta = \theta, R = s, U^{n-1}) = \prod_{l \in L} \Pr(E_l|\Theta = \theta, R_l = s_l, U_l^{n-1}).
\]

The prosecution’s hypothesis states that the profile is made of the suspect’s contribution plus the contribution from \( n - 1 \) other random, unrelated contributors. Since there are many possibilities for the genotype of these interference contributors at each locus and going over each case would take a large amount of time, we calculate

\[
\Pr(E_l|\Theta = \theta, R_l = s_l, U_l^{n-1})
\]

using importance sampling.

Importance sampling is a Monte Carlo sampling algorithm in which, instead of sampling directly from the target distribution, samples are generated from a different distribution that is easier to sample from [15]. To take into account the fact that the samples have come from the ‘wrong’ distribution, weights are introduced to adjust the ‘importance’ of each sample. For the problem at hand, instead of sampling using the allele frequency distribution, we generate samples of the interference genotypes using the peak height distribution observed at the locus. The reason for sampling from the peak height distribution is that this method is faster and requires fewer samples for convergence than the method that samples from the allele frequency distribution.
Let $J$ be the number of interference samples used. Now we obtain:

$$Pr(E_l | \theta = \theta, R_l = s_l, U_l^{n-1}) = \sum_{i=1}^{J} Pr(E_l | U_l^{n-1} = u_l^{n-1}, \theta = \theta, R_l = s_l) w_i \sum_{i=1}^{J} w_i,$$

where $w_i = P(U_l^{n-1}) / Q(U_l^{n-1})$ is the weight of sample $i$; $P(U_l^{n-1})$ is the probability of the interference genotypes under the allele frequency distribution; and $Q(U_l^{n-1})$ is the probability of the interference genotypes under the peak height distribution. Since $u_l^{n-1}$ and $s_l$ establish the true peaks in the signal (and by extension the stutter and noise peaks), $Pr(E_l \lor U_l^{n-1} = u_l^{n-1}, \theta = \theta, R_l = s_l)$ is calculated using the parameters from the calibration data.

### 3.2.2 LR distribution and p-value calculation

Since the denominator of the LR is the same for all the random genotypes $R$, it is sufficient if we compare the numerator of the LR for $R$ and $s$.

$$p-value(s) = Pr(Pr(E|R, U^{n-1}) \geq Pr(E|R = s, U^{n-1})).$$

During testing of MatchIt, we observed that because of floating-point precision, $Pr(E \lor R, U^{n-1})$ evaluated to 0 for many of the random genotypes $R$ that fit the data poorly. As a result, we were able to eliminate those genotypes from the p-value calculation as a preliminary step. Formally, let $R$ be the set of all genotypes. We define $R_1 = \{ r \in R \lor Pr(E_l | R_l = r_l) \neq 0 \text{ for all loci } l \}$ and $R_2 = \{ r \in R \lor \exists \text{ locus } l \text{ s.t.} Pr(E_l | R_l = r_l) \approx 0 \}$, where $\approx 0$ means “evaluates to 0 using double-precision 64-bit floating-point arithmetic”.

Thus, we have $R = R_1 \cup R_2 \land R_1 \cap R_2 = \emptyset$. We see that for all $r \in R_2$, $Pr(E | R = r) \approx 0$.

We omit the notation on $U^{n-1}$ for the sake of brevity. We have:
\[ p\text{-value}(s) = Pr(R \in R_1) \sum_{r \in R_1} Pr(Pr(E|R = r) \geq Pr(E|R = s)) Pr(R = r| R \in R_1) \]

\[ + Pr(R \in R_2) \sum_{r \in R_2} Pr(E|R = r) \geq (Pr(E|R = s) Pr(R = r| R \in R_2) \]

We see that the second term is 0, provided \( Pr(E \lor R = s) \) is greater than 0. Hence we get:

\[ p\text{-value}(s) = Pr(R \in R_1) \sum_{r \in R_1} 1(Pr(E|R = r) \geq Pr(E|R = s)) Pr(R = r| R \in R_1), \]

where

\[ 1(Pr(E|R = r) \geq Pr(E|R = s)) = \begin{cases} 1, & \text{if } Pr(E|R = r) \geq Pr(E|R = s), \\ 0, & \text{otherwise}. \end{cases} \]

We have:

\[ Pr(R \in R_1) = \prod_{l \in L} \sum_{r_l \in \{r_l | Pr(E_l|R_l = r_l) \neq 0\}} Pr(R_l = r_l). \]

We compute the \textit{p-value} using Monte Carlo simulation. We generate \( M \) random genotypes \( r^1, ..., r^M \) according to the distribution \( Pr(R \lor R \in R_1) \) and calculate the p-value as:

\[ p\text{-value}(s) = Pr(R \in R_1) \frac{\sum_{i=1}^{M} 1(Pr(E|R = r^i) \geq Pr(E|R = s))}{M} \]

Increasing the value of \( M \) increases the accuracy of the p-value computed, but this also increases the run time and hence a tradeoff has to be achieved between the two. In this study, we have used 1 billion or 10^9 random genotypes to compute the p-value.

In order to facilitate the computation of the p-value, as an initial step

\[ Pr(E_l|\theta = \theta, R_l = g_l, U_l^{n-1}) \] is computed for all possible genotypes \( g_l \) at all loci \( l \) for all
values of $\theta$. Once this is done, for the p-value computation, 109 genotypes $r^I$ are generated based on the allele frequencies. Since we know $\Pr(E_l|R_l = r^I_l)$ for all loci $l$, we can compute $\Pr(E|R = r^I)$ as:

$$\Pr(E|R = r^I) = \int_{\theta \in \Delta} \prod_{l} \Pr(E_l|\theta = \theta, R_l = r^I_l, U_{l-1}) f_\theta(\theta).$$

### 3.2.3 LR denominator calculation

Let $\bar{R}$ be the genotype of an unknown contributor in the defense’s hypothesis. The denominator of the LR can be written as:

$$\Pr(E|U^n) = \sum_{\bar{r}} \Pr(E|\bar{R} = \bar{r}, U^{n-1}) \Pr(\bar{R} = \bar{r})$$

Since the number of possible values that $\bar{R}$ can take is large and summing over all of them is computationally intensive, we utilize the random genotypes $r^I$ that are sampled for the p-value computation to compute the denominator of the LR as follows:

$$\Pr(E|U^n) = \Pr(R_1) \frac{\sum_{l=1}^{M} \Pr(E|R = r^I_l)}{M}.$$
Figure 2 Calibration data of true peak mean at locus D7S820, distribution ax+b, R square value .9929

Figure 3 Calibration data of reverse stutter peak mean at locus D7S820, distribution a*e^{bx}+c, R square value .9956
We found that the amount of template DNA from the contributor had an impact on the LR – small LRs were associated with contributors having low template amounts and high levels of dropout and stutter. Since we used $10^9$ samples to calculate the $p$-value, the lowest possible $p$-value that can be achieved is $10^9$, and this was obtained in all the cases where the LR was greater than $10^8$.

All the graphs shown below in Figure 5, 6 and 7 were tested for various sample files with varying DNA mass. Each sample was tested for actual contributors and 3 other non-contributors. And results were more or less as expected, which is for actual contributor $p$-value is $-9$ (as we are considering one billion samples) and for non-contributors it is close to zero.
Figure 5 Graph showing results for MatchIt using 1p samples

Figure 6 Graph showing results for MatchIt for 2p sample
As the number of contributors increases chances of deflection in the correct also increases. This could also be seen in Figure 7, but the values are clear enough and do not hinder the integrity of the result.
Code Optimization using various techniques

4.1 Introduction

In general, a software could be optimized in three different aspects 1) reduce the running time, 2) use less memory or resources, and 3) draw less power [16]. In this paper our main focus was to reduce the running time of the software. As other two aspects are not that relevant in correspondence to the software. Memory or resources requirements and power usage for this software are as less as to what a personal computer is equipped with these days, which could also be phrased as – 2.3 GHz central processing unit, 1GHz RAM. Software requirements are - windows 7 or later, macintosh X or later, java version 8. There is a widely accepted “rule of thumb”[17] in speed optimization known as the Pareto Principle [18] phrasing that almost 90% of the execution time is spend executing only 10% of the code.

An optimization technique known as “Profile-guided optimization technique” [19] has been used to find the part of code, which is consuming maximum running time. This optimization technique is based on profiling the code, where a dynamic program analysis is done to measure the space (memory), running time of a program, or frequency and duration of function calls [20]. Software was run using a code profiler called “JProfiler” to analyze the running time (CPU cycles) of each class, routine, and line of code during the execution of the software. Then, various optimization techniques (mentioned in Appendix_A) were applied to the code, prioritizing the functions that had longer running times. Changes were accepted if they resulted in an improvement in the runtime performance and discarded otherwise.
4.2 Example of one code snippet

The following function was called around 100,000 times in the software in one execution.

```java
public double[] calcSlopeValue(String locusName, double massValue,
HashMap<String, double[]> meanSlope, HashMap<String, double[]> stdDevSlope) {
    try {
        double a_mean = meanSlope.get(locusName)[0];
        double b_mean = meanSlope.get(locusName)[1];
        double a_stddev = stdDevSlope.get(locusName)[0];
        double b_stddev = stdDevSlope.get(locusName)[1];
        double mean = (a_mean * massValue) + b_mean;
        double stddev = (a_stddev * massValue) + b_stddev;
        return new double[] {mean, stddev};
    } catch (Exception e) {
        return null;
    }
}
```

We changed the input parameters from HashMaps to Arrays, which saved approximately 2% of the total running time (CPU cycles). Code became easy to understand and simple to use.

Modified code snippet is as follows:
public double[] calcSlopeValue(double[] aMean, double[] aStdDev, double massValue) {

    try {

        double mean = (aMean[0] * massValue) + aMean[1];

        double stddev = (aStdDev[0] * massValue) + aStdDev[1];

        return new double[] {mean, stddev};

    } catch (Exception e) {

        return null;

    }

}

4.3 Results

Created Allele class which saved 70% of auto-boxing conversions from primitive data type to Wrapper class. After this implementation, saved 21.5% in running time.

Converted few HashMaps(get function) to Arrays which reduced overall running time by 14.2%.

Converted few HashMaps(put function, indexing) to ArrayLists which reduced overall running time by 7%.

Changed the implementation of two base methods (calcTwoExpValue and calcSlopeValue and calcExpValue), which had HashMap parameters. This technique implementation saved 10% of the overall running time.

Histogram shown in Figure 8 shows the reduction in time for the core calculation of the program after each optimization technique mentioned in the Table 1. There is an approximately 40% improvement in the overall running time for the core calculation.
Figure 8 Reduction in running time of base code snippet with each optimization

Graph shown in Figure 9 shows the percent gain in the overall run time of the software.

Figure 9 Software efficiency percent gain in different versions
User interface design and development

5.1 Introduction

Having an interface, which makes it easier for the user to handle the software, is an important part of a software package. For example, in calculating curve fitting data during the calibration process (see Section 3.2), it is important to develop a user interface that allows users to easily see the results of the calibration and to modify it interactively. One part of this work was to come up with such an easy-to-use interface for the software, and to display calculated calibration data in a proper manner.

5.2 Design and Implementation

Various versions of the software were developed, paying attention to several principles in user design [21]. The software interface is divided in three different tabs: (1) Calibration, (2) NOCI, and (3) MatchIt. The calibration tab helps the user to input the data files and parameters. The next window of the calibration tab can be used to input the parameters used for curve fitting. After the calculation, the user can easily navigate using a tree table on the left, and can see the data in an easy to read graphical form on the right side of the window.

Similarly, for the NOCI and MatchIt tabs, the user can input parameters for the calculation and select the corresponding calibration data generated in the previous step. Based on the parameters, the result is displayed in an easy to understand graphical form, which is displayed in the lower window of the respective tab.

5.3 Results

Calibration is the tab from which our software starts with, as it is usually the first step in the
whole process. Figure 8 shows start of the software where user can input the parameters and data files required to generate calibration data.

![Image of software interface](image)

**Figure 10 First tab of software - Calibration**

After inputting the parameters on first window of the calibration tab, user is supposed to click on the “Next” button. This button will take the user to next window of calibration tab as shown below in Figure 9.

User can select parameters and values associated with each of the marked fields in the table and then have to click on the “Calibrate” button to start the calculation. After a few seconds, calculated calibration data will be displayed in a tabular form. User can select any row from the displayed tree table which will display the corresponding graph on the right.
Once the calibration data is generated, user can either save it or can directly go to any of the next two tabs NOCIt and MatchIt. Below Figure 10 shows the NOCIt tab of the software where user can select any number of rows, each with the appropriate parameters to start NOCIt calculation. Result will be shown as a histogram on the bottom of the window.
MatchIt tab is very similar to NOCIt tab where user can select various rows with appropriate parameters and will get the result in the form of a histogram on the bottom of the window.

Figure 11 shows the MatchIt tab without a result.

![MatchIt tab](image)

**Figure 13** MatchIt tab
### Appendix A

<table>
<thead>
<tr>
<th>Optimization Technique</th>
<th>Example</th>
<th>Reason and Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common sub-expression elimination</td>
<td>a = b * c + g; d = b * c * e; To tmp = b * c; a = tmp + g; d = tmp * e;</td>
<td>Saves various operations</td>
</tr>
<tr>
<td>Code motion</td>
<td>for(int x : array) { value += func(3) * x; To tmp = func(3); for(int x : array) { value += tmp * x;</td>
<td>Saves multiple function calls</td>
</tr>
<tr>
<td>Use arrays instead of HashMaps</td>
<td>HashMap&lt;Integer,Double&gt; valueMap = new HashMap&lt;&gt;(); To double[] valueMap = new double[size];</td>
<td>Saves conversion from primitive data-type to corresponding object. Overall calculation becomes much more efficient.</td>
</tr>
<tr>
<td>Unrolling loops</td>
<td>for (int j=0; j&lt; 2-l; j++) { s = &quot;0&quot;+s; } TO for (int j=0; j&lt; 2-l; j+=2) {</td>
<td>Saves multiple integer operations</td>
</tr>
<tr>
<td>Method</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Removal of dead or unreachable code</td>
<td>Helps in decreasing memory utilization Saves unnecessary memory loads</td>
<td></td>
</tr>
<tr>
<td>Constant folding</td>
<td>$x = 2.0 \times x \times 4.0$ to $x = 8 \times x$ Saves a floating point operation</td>
<td></td>
</tr>
</tbody>
</table>
| Loop invariant optimization                  | While $x > 0$  
|                                             | $x = x - (y+z)$; to $t = y+z$; while $(x > 0)$ $x = x - t$; Saves multiple integer addition operations |
| Dead-variable elimination                    | Checked, analyzed and removed unnecessary dead variables Saves a memory operation |
| Changed LinkedInHashMap<Integer,Integer> to ArrayList<Integer> | LinkedInHashMap<Integer,Integer> value = new LinkedInHashMap<>(); To ArrayList<Integer> value = new ArrayList<>(); Saves conversion from primitive data-type to corresponding object. Overall calculation becomes much more efficient. |
| Changed ArrayLists to Arrays                | ArrayList<Integer> value = new ArrayList<>(); To int[] value = new int[size]; Saves conversion from primitive data-type to corresponding object. Overall calculations becomes much more efficient. |
REFERENCES


2. SWGDAM Interpretation Guidelines for Autosomal STR Typing


