Final Report for the NIJ Grant:

Capillary Electrophoresis for Forensic STR Analysis:

Validation and Cost Effectiveness

Grant # 96-IJ-CX-0076

This is the final report in our evaluation of Capillary Electrophoresis for forensic STR Analysis. We have made some progress in a number of areas since our previous report. An outline of the progress made in implementing the goals of our grant follow.

1) Preparation of laboratory space:

The modification of a room within our laboratory to accommodate the CE had been delayed due to problems within the State Buildings Division of General Services. This division is involved in the design of the ventilation system intended as part of the modifications required for the "CE room". This ventilation system has been completed and the bench work has been installed and the electrical and plumbing will be finished by the end of the grant period.

The CE system appears to be exquisitely sensitive and validation efforts have revealed some interesting results. Consequently, it has taken us longer than expected to implement the sex typing system amelogenin for casework. However, we expect to go on-line for amelogenin casework by the end of November 1997. We have found that there is a considerable amount of variability in the quantity of amplified X and Y products derived from male samples. This ratio of the products is an important factor in determining whether or not samples are a mixture of male and female DNA. Our findings indicate that one could expect a Y to X ratio of 0.65 to 1.10 in normal single source samples. Values outside of this range could indicate mixtures although more study on this may be required. In addition, we have found that the amplification process can

impact the ratios obtained, necessitating reamplification in some instances. We have completed a rough draft of our findings concerning the validation of the amelogenin system for use in capillary electrophoresis for publication. We anticipate that this should be ready to submit for publication by mid November. The draft is included with this report and any manuscript will be forwarded as soon as review process is completed.(See Attachments)

2) Comparable Technologies and Parallel Testing:

As described in our previous report, we have made an extensive evaluation of the ability of the CE to obtain results that were comparable to gel-based technologies. As described in the paper that was included in our previous report, all allelic calls were the same as those obtained by a variety of gel-based systems. We feel very confident that we will be able to report alleles in the same manner as those who use gel-based systems. This comparative analysis will be instrumental in getting CE past the "Frye" or "Daubert" standard for court. This work, in addition to the other validation studies currently underway here and in other laboratories, will show that CE analysis is an appropriate analytical tool for forensic DNA analysis.

We have also started parallel testing in conjunction with casework. The work on the amelogenin system is essentially complete and work on the STR system CTTA has started. Much work will be needed to develop the body of knowledge required to correctly interpret mixtures. The analysis of casework will assist in this process, along with validation efforts using known mixtures. Before we put the STR systems on line,

we will need to develop extensive guidelines based on our data and experience for the interpretation of mixtures. These guidelines are still in development.

3) Technique limitations:

Capillary electrophoresis is a recent development for forensic DNA analysis and we have only begun the study of the limitations of the technique. We have established fluorescence linearity for the system and reconciliation for those samples falling outside these parameters. We have accumulated some data that evaluates resolution versus fragment length. This data indicates that resolution is profoundly effected by sample preparation. Poorly de-ionized formamide can drastically effect the resolution seen on the CE, where as this may not effect gel-based systems as seriously. Some of this data was detailed in our paper (included with previous report). The resolution studies are shown on a table included with this report. (See Attachments)

4) Validation Testing:

We have collect some data on the validation of the AmpFlSTR Blue and Green I systems. The validation of the amelogenin system is almost complete. We have accumulated STR data on about 100 individuals for a population data base for the AmpFlSTR Blue system and the AmpFlSTR Green I system. The same individuals have previously been evaluated in the DQA1 and Polymarker system and we plan to have our data analyzed to see if it would be appropriate to use all systems in conjunction for the

statistical evaluation of DNA profiles. There is much published data on the CTT system. Using this published data in conjunction with the in-house validation testing that we are currently pursuing with CTTA, we should be able to offer this simple multiplex STR system for casework analysis in the near future. As discussed before, mixtures will be a larger part of the validation studies that we will undertake. We have done some of these studies with more to follow. We have examined stutter bands in the "Blue" system and have to do the same with the "Green" system. Our findings indicate that stutter is locus dependent and may be found as high as 13% of the true allele in some instances.

5) Labor Savings:

The labor savings issue may be harder to determine than originally planned. The decisions that are made concerning the use of CE or another technology is often due to sample through-put constraints. Capillary electrophoresis is based on sequential analysis of one sample at a time. This may not be suitable for high production facilities where a large number of samples must be analyzed. However, once samples are processed and loaded, CE is essentially a "walk-away" system. Pouring of gels and maintenance of gel apparatus is eliminated, thus saving time, space and effort. We have also found that it can meet the needs of a small laboratory and since it costs considerably less than a sequencer, some laboratories may consider the purchase of two of these instruments to accommodate the additional sampling demands.

6) Reporting Results:

Since capillary electrophoresis is a relatively new technology in forensics, we have attempted to disseminate our results to the forensic community as quickly as possible. We have accomplished the following to date:

1) Capillary Electrophoresis STR analysis: Comparison of Gel-Based Systems, Buel, E., Schwartz, M. LaFountain, M. A., Journal of Forensic Sciences, in press.

2) Presentation, Eight International Symposium on Human Identification Scottsdale, Arizona 1997, "Evaluation of Capillary Electrophoresis for the Forensic Analysis of Short Tandem Repeats," Buel, E., Herrin, G., LaFountain, M., and Schwartz, M. B.

3) Presentation, Northeastern Association of Forensic Scientists, White Plains, New York 1997, "Validation of the Amelogenin Locus for Capillary Electrophoresis," LaFountain, M., Schwartz, M. B., Cormier, J., and Buel, E.

4) Presentation, Northeastern Association of Forensic Scientists, White Plains, New York 1997, "Closer than Cousins but not Quite Brothers," LaFountain, M., and Schwartz, M. B., and Buel, E.

Attachments:

1) Draft of Amelogenin manuscript

2) Abstracts of Presentations

3) Resolution Table

Draft of Amelogenin manuscript

VALIDATION OF CAPILLARY ELECTROPHORESIS FOR ANALYSIS OF THE X-Y HOMOLOGOUS AMELOGENIN GENE

Abstract:

Capillary electrophoresis is a versatile technology with tremendous potential applications in forensic science. A refinement of the traditional slab-gel, separation occurs in a liquid polymer contained within a capillary. The capillary electrophoresis unit is computer-driven, semi-automating the loading and analysis of samples.
As with any new technology, a full performance assessment is required before it may be implemented with confidence. This paper focuses on validation of CE technology for analysis of a portion of the X-Y homologous gene used in gender determination. Typical Y/X ratios of peak heights for male samples were determined. Instrument linearity, sample resolution and reproducibility were examined. Samples subjected to contamination, extreme environmental conditions or extracted from a variety of substrates were also tested by CE. All samples correctly typed. Genetic material from a number of common non-primate animals was amplified with amelogenin primers. Some species yielded no product. Products derived from the animal samples that did amplify produced peaks on CE analysis readily distinguishable from those of human origin.

Introduction:

Capillary electrophoresis is a separation technology that uses the basic principles of traditional gel-based methods. The separation occurs within a polymer-filled capillary monitored by a laser. Fluorescently-tagged PCR products are detected as they migrate past a window within the capillary. Features such as semi-automated sample loading and analysis offer some key advantages over the current gel-based technologies. Repeat runs or modifications of run conditions are quickly initiated and do not require the pouring of additional gels. Internal sizing standards run with each sample insure accurate sizing and help to compensate for minor run-to-run variations. Allelic ladders provide a reference allowing for comparisons with other methods and laboratories.

Forensic samples offer challenge to nearly any analytical method. Sample age or origin may be undetermined and available sample volume small. Material submitted for

testing may be deposited on any imaginable surface, or it may be incidentally or intentionally contaminated or obliterated. Biological material brought to the forensic lab may have come from more than one individual. Additionally, the outcome of forensic testing can have serious consequences. With these considerations in mind, validation of methods or instrumentation for forensic employment must be particularly rigorous using samples comparable to those found in the field.

What follows is a description of the validation work for CE analysis of amelogenin amplicons.

Materials and Methods:

Samples for the studies were extracted using proteinase K (Life Technologies, Gaithersberg, MD) digestion with subsequent extraction with phenol/chloroform with the aqueous extract subjected to Microcon-100 (Amicon Inc., Beverly, MA) dialysis ( ). Samples were quantitated by mini-gel electrophoresis employing the DNA dye DAPI ( ). For low concentration extracts that fell below the four to eight nanogram detectable level for DAPI, the mini-gel was placed in a gel buffer solution containing a 1:10,000 dilution of SYBR™ Green I (Molecular Probes Inc., Eugene, Oregon) and gently rocked for twenty minutes. DNA was visualized using UV light and evaluated by comparison of relative fluorescence with a series of human concentration standards ranging from 125 to 0.12 nanograms per lane. Sensitivity of this SYBR Green I technique was approximately 0.25 nanograms.

Amplifications were performed using amelogenin primers generously provided by PE Applied Biosystems. These primers are identical to those supplied in the multiplex

STR kit AmpFlSTR™ Green I (PE Applied Biosystems), which is designed to amplify three STR loci in addition to the amelogenin locus. Approximately two nanograms of extracted DNA were added to eight picomoles of forward and reverse primers, 0.5 microliters of AmpliTaq Gold™ polymerase and AmpFlSTR™ PCR reaction mix for a final volume of twenty-five microliters. The amplifications were performed using a GeneAmp® PCR System 9600 thermocycler (PE Applied Biosystems) using the following conditions:

95° C for 11 minutes then:

29 cycles at:

94° C for 1 minute

59° C for 1 minute

72° C for 1 minute

followed by a 30 minute hold at 60° C

The capillary electrophoresis unit employed to evaluate the amelogenin amplicons was an ABI PRISM™ 310 Genetic Analyzer (PE Applied Biosystems). An uncoated 50 um ID capillary (Perkin-Elmer), 47 cm in length with a 37 cm read length (distance from injection end to laser), was used for the separation. The separation medium was the proprietary polymer product Performance Optimized Polymer 4 (PE Applied Biosystems). The run buffer was a 1:10 dilution of 310 Genetic Analyzer Buffer with EDTA (PE Applied Biosystems). Samples were routinely electrokinetically injected at 15 kV for 5 seconds, followed by a 24 minute run at a constant voltage of 15 kV and a constant temperature of 60° C. Prior to each injection the column was back flushed with new polymer by the instrument.

Amplified amelogenin products were prepared for CE analysis, as was an allelic ladder from the AmpFlSTR™ Green I kit, by mixing one microliter with 0.5 microliter of Genescan® 350 ROX internal lane standard (PE Applied Biosystems) and twelve microliters of deionized formamide. Formamide (Amresco, Solon, OH) was deionized by using a molecular grade mixed bed resin (Sigma, St. Louis, MO).

Validation testing:

Stains for testing or for reference samples were prepared from saliva and liquid EDTA blood spotted on cotton cloth or suitable substrate and allowed to air dry. Stains that were used for contamination studies had the appropriate contaminant added directly to the stain. Those samples that were subjected to degradative environmental conditions were placed in a sunny window or heated for the designated time prior to testing. Animal blood dried on cotton or paper or from muscle tissue was used for the non-human study. Tissues from a single donor were used to determine tissue specificity. Reproducibility was assessed by multiple testings of the same samples. Male and female samples were combined for the mixing studies.

The average base pair size, range of sizes and standard deviations were obtained from the data set resulting from the analysis of 107 male and 49 female samples. The variability in quantity of the X and Y products derived from males was determined by examining eighty-two amplified, standard, male blood samples and calculating the Y/X ratio for each. To identify some of the variables which could influence this ratio, approximately two nanograms of male DNA were amplified in five separate reactions. Each of the five resulting amplifications was split to make two samples that were then

processed separately for CE analysis. Processing involved addition of formamide and the internal lane standard followed by heat denaturation, as described above. Finally, these samples were each run three times through the capillary column.

The linearity of the system was examined by varying the concentration of template DNA and also by examining fluorescence versus injection time. Concentrations tested ranged from 0.06 to 10 nanograms of template DNA. Injection times examined were from one to ten seconds.

Resolution achieved for the amelogenin system was calculated using the following equation as detailed by Luckey et al., 1993 ( ):

$$R = [2 \ln 2]^{1/2} * [(t2-t1)/(hw1+hw2)].$$

The resolution in base pairs was determined by dividing the calculated resolution value by the difference between the base pair sizes obtained for the X and Y amplicons.

Results and Discussion:

Validation studies for the CE were structured to establish instrument limits such as fluorescence linearity and resolution as well as to develop acceptance guidelines based on data gathered from samples collected from individuals of known gender.

At amplification, fluorescently-tagged primers are incorporated into the PCR product. Consequently, the quality and concentration of the template material at amplification and the injection time of the sample at the time of CE analysis affect the level of fluorescence detected by the CE. The linear fluorescence range is detailed in Figure 1. Linearity was seen up to a peak height of approximately 5000 fluorescence units when the concentration of template DNA was varied from 0.6 to 10 nanograms/microliter. Samples

falling in the linear fluorescence range at the standard injection time of five seconds were amplified from templates of approximately one nanogram of DNA.

The linearity of the system was also examined using injection time as the variable, as seen in Figures 2.1 and 2.2. As mentioned before, standard injection time is five seconds, however, this may be increased or decreased to compensate for weak or overly strong fluorescence. When the injection time was varied from one to ten seconds and plotted against either peak height or peak area, the range observed was linear.

Resolution was examined by analyzing the data generated from running ten allelic ladders from the AmpFlSTR™ Green I amplification kit. The ladders were run on different days and on different capillaries. Resolution was found to be 0.931 basepairs for the amelogenin system. The average resolution of the system is a useful measure of the performance of the system and allows tracking to determine when a capillary may need to be replaced or may indicate other issues that must be addressed. For example, amplified PCR products processed for CE analysis using inferior deionized formamide displayed extremely poor resolution. Reprocessing of these samples using formamide deionized with a different resin bed markedly improved resolution. (Data not shown.) Resolution measurements also allow system performance comparisons between laboratories.

From sequencing data, the expected size of the X and Y amplicons are 107 and 113 basepairs respectively, which includes the addition of a terminal nucleotide to the PCR product ( ). The average basepair size for the X and Y amplicons seen using CE is 103.15 and 108.80 basepairs. Table 1 summarizes the results of the basepair size study. Amelogenin also has a six basepair size difference in the sequences copied from X and Y chromosomes. Though close to the expected six basepair difference, the 5.65 average

basepair difference reflects the uncertainty that may be seen in the actual values obtained by CE analysis.

Table 1 also shows the variability of the Y/X ratio found in male samples. Data from the eighty-two male samples with peak heights below 5000 RFU, the observed upper limit of fluorescence linearity, was examined. The Y/X peak height ratio of these samples ranged from 0.65 to 1.10, with an average value of 0.88, showing that the X amplicon typically predominates.

The aim of the work detailed in Diagram 1 was to determine which step, the amplification, the denaturation process, or the electrophoresis, most influenced Y/X ratio variability. First, approximately ten nanograms of DNA extracted from a male subject was evenly distributed to five tubes and amplified. Each amplification product was then split in half and processed separately. Finally, each processed product was run three times on the capillary column.

The findings of this experiment, summarized in Table 2, indicate that most of the variability demonstrated by these samples comes from the amplification step. Little change was seen between samples when electrophoresis was the only variable. For example, Sample 1A had an average Y/X height ratio of 0.842, and showed a difference of only 0.016 for separate electrophoretic runs. With separate processings included in the variables, this difference rose to 0.093. Finally for Sample 1, the difference in ratios after all of the amplifications, processings and runs on the CE, was 0.615. This change was significantly greater than those observed when processing and electrophoresis, or electrophoresis alone, were the variables. Amplification, therefore, introduces more of the variability seen in the Y/X height ratio of these samples, than does any of the other steps.

During the analysis of the data, monomorphic peaks were identified. These were sized as approximately 91 and 96 basepairs. These were found regardless of the sex of the DNA donor, but were typically less than 20% of the X or Y amplicon. The 91 peak was usually larger in height than the 96 peak. Since these peaks are not included in the range examined for amelogenin, further studies were not undertaken.

Crime scene materials are often exposed to degradative environmental conditions or to contaminating substances. These may interfere with DNA amplification and subsequent analysis either by degrading DNA or by inhibiting amplification. To evaluate the ability of the CE to produce reliable results from potentially compromised samples, three sets of samples were set up to simulate a variety of conditions and treatments. Blood was patched to cotton fabric and exposed to differing conditions involving light, temperature and exposure times. Common contaminants were applied directly to bloodstained cotton. Blood was also allowed to dry on typically encountered substrates. Extracted DNA from these stains was amplified and analyzed by CE and compared to untreated reference standards. As seen in Table 3, the results of all samples tested matched those obtained from the reference samples.

Blood, hair, skin and saliva sample sets were taken from two individuals. Amplification of the hair samples failed, but all other materials amplified and typed accurately. Subsequently, other hair samples have been successfully amplified. Correct typing of these samples was obtained by CE analysis.

A panel of non-primate animal samples representing both common domestic and wild species was amplified using the amelogenin primers. Coding for an integral protein of mammalian tooth enamel, the amelogenin gene has been studied in a number of species

and is found to have large areas in both the coding and non-coding regions which have been conserved throughout evolution ( ). Five of the sixteen samples tested yielded no amplified product. The successful amplifications each exhibited a peak in the range of 98.59 to 99.64 basepairs. This distinctive peak could not be confused with either the X or the Y peak or with the monomorphic peaks sometimes seen in human samples. These findings dismiss concerns that material derived from non-primate animals could be mistakenly matched to human samples and indicate amelogenin primers are of some use for distinguishing animal and human samples. (See Table 4.)

Data derived from the mixed male and female sample studies indicates that a sample within the linear fluorescence range, and containing a 108 basepair peak above the background fluorescence, possesses a male component. Male:female mixtures of 1:20 to 1:30 yielded a 108 basepair peak with fluorescence three times background. As would be expected in mixes such as this in which the female fraction is much larger than the male, the X peak is significantly larger than the Y peak.

More difficult to evaluate were mixes in which the male and female fractions approached equal amounts or the female component was the lesser. As mentioned earlier, the Y/X ratio for standard male samples ranged from 0.65 to 1.10 with an average of 0.88. While a Y/X ratio less than 0.65 may be suggestive of a mix, any sample with a Y/X ratio deviating from the average should be scrutinized and interpreted with care.

Conclusions:

Tandem use of fluorescently-tagged amelogenin primers and CE technology is a convenient and reliable methodology for gender determination of forensic samples.

Amplified samples from simulated field conditions and from standards were shown to type correctly. Additionally, this method allows unambiguous distinction between human and non-human animal samples.

The validation work presented here established operating parameters for our CE instrumentation and criteria for linearity and resolution. Also established was a range for the Y/X ratio of standard male samples. More work must be done to fully realize the usefulness of the Y/X ratio for interpreting mixes of male and female samples.

Figure 1: DNA Concentration vs. Peak Heights of X and Y

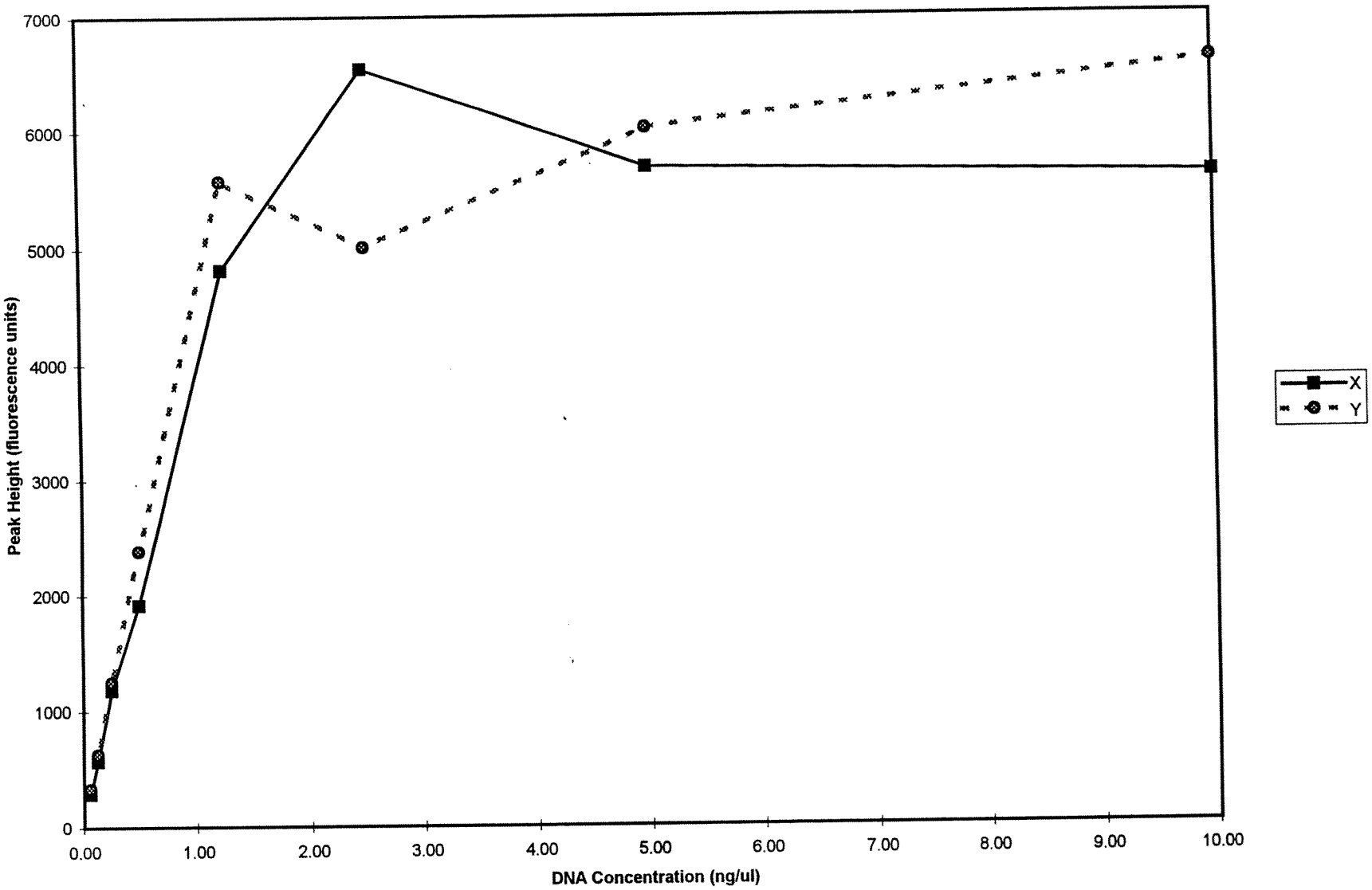


Figure 2.1: Injection Time vs. X and Y Peak Heights

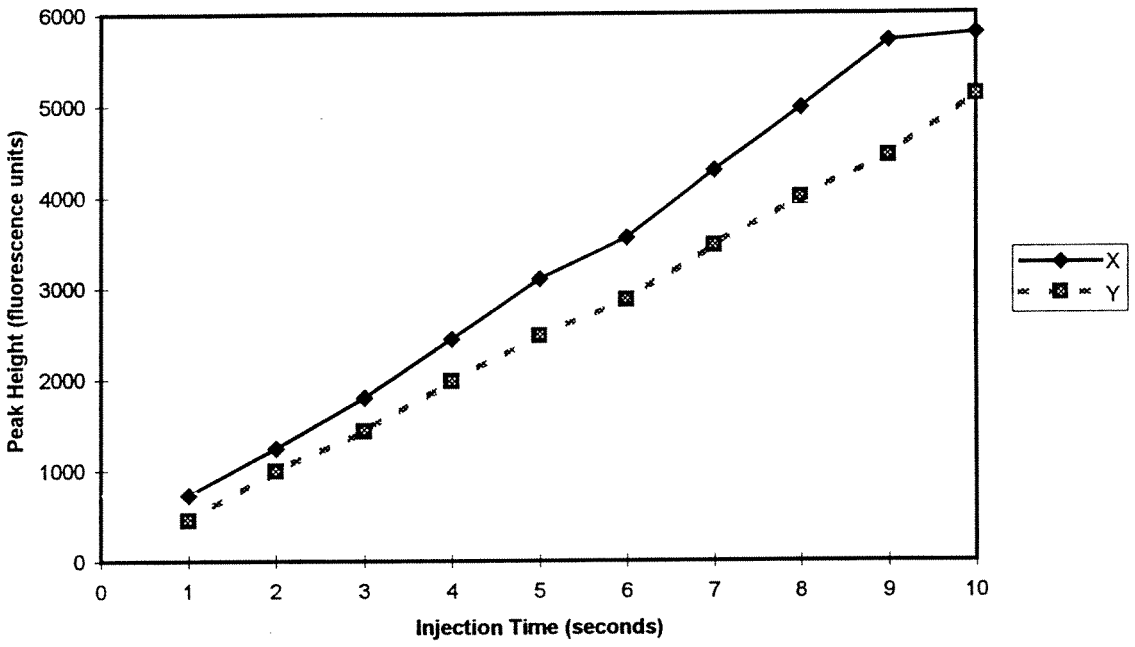


Figure 2.2: Injection Time vs. X and Y Peak Areas

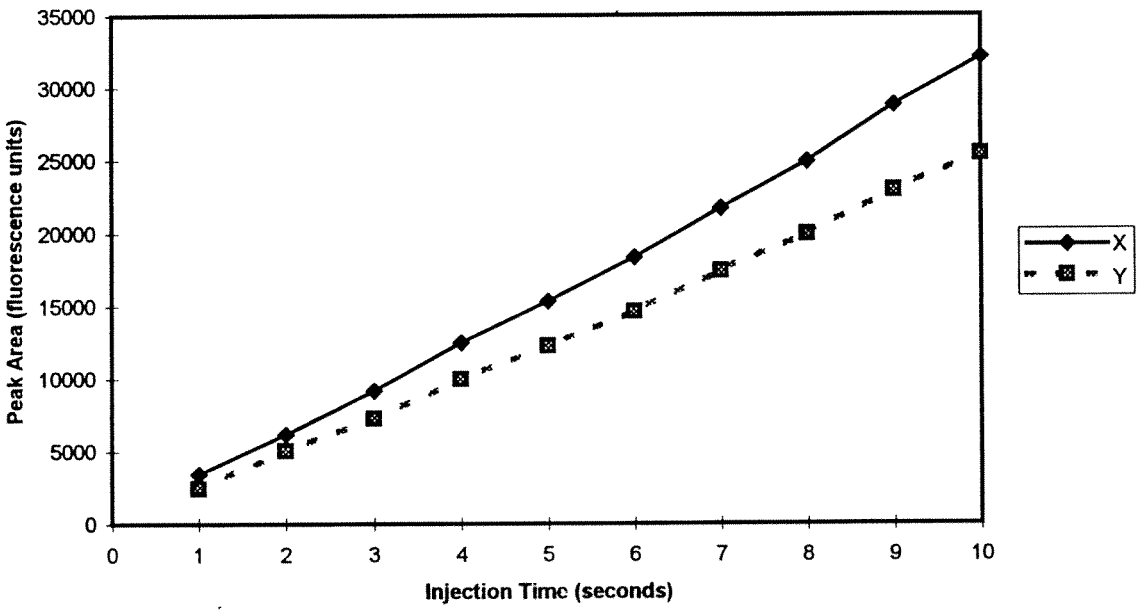
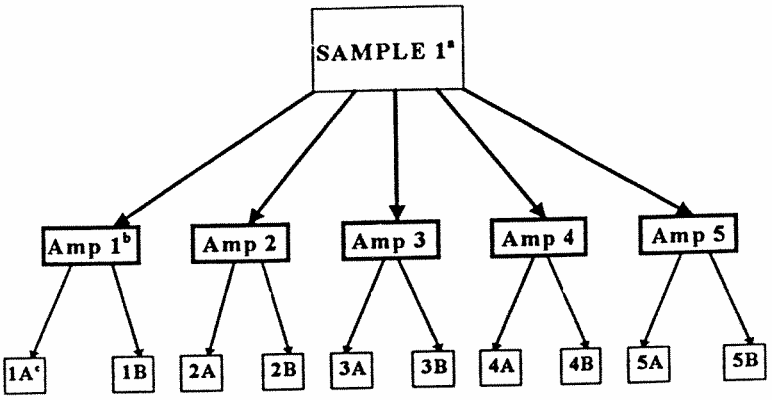


Diagram 1: Design of the "Controlling Variable" Experiment



a. The sample was amplified five times.
b. Each amplification product (Amp 1-5) was split in half and processed separately.
c. In addition, each processing (1A-5B) was run through the column three times.

Table 2: The Effects of Amplification, Processing, and Electrophoresis on the Y/X Height Ratio of a Sample

Sample	Variables	Average Y/X Height Ratio	Minimum Ratio	Maximum Ratio	Range
Sample 1	Amplification, Processing, & Electrophoresis	0.870	0.500	1.115	0.615
Amp 1	Processing & Electrophoresis	0.839	0.791	0.884	0.093
Amp 2	"	1.039	1.022	1.071	0.049
Amp 3	"	0.608	0.500	0.651	0.151
Amp 4	"	0.787	0.628	0.865	0.237
Amp 5	"	1.077	1.038	1.115	0.077
1A	Electrophoresis	0.842	0.834	0.849	0.016
1B	"	0.836	0.791	0.884	0.093
2A	"	1.032	1.022	1.038	0.016
2B	"	1.047	1.033	1.071	0.038
3A	"	0.586	0.500	0.642	0.142
3B	"	0.629	0.617	0.651	0.034
4A	"	0.773	0.628	0.865	0.237
4B	"	0.801	0.780	0.835	0.054
5A	"	1.068	1.038	1.115	0.077
5B	"	1.085	1.075	1.096	0.021

TABLE 1

Table 1: Average X and Y Base Pair Sizes and Y/X Peak Height Ratios as Measured on a Capillary Electrophoresis System

	Number of Samples	Average	Standard Deviation	Minimum Value	Maximum Value
X bp size	156	103.14	0.19	102.78	103.92
Y bp size	107	108.80	0.17	108.47	109.42
Y/X ratio	107	0.90	0.11	0.65	1.31
Y/X ratio (peaks < 5000)	82	0.88	0.08	0.65	1.10

Table 3 Samples subjected to chemical contaminants or deposited on substrates followed by amplification with amelogenin primers and CE analysis

Treatment	Amelogenin
CONTROL (uncontaminated)	XY
5% Bleach	XY
Cardboard (Substrate)	XY
0.02 M $CuCl_2$	XY
Denim (Substrate)	XY
5% Detergent	XY
10% EDTA	XY
Glass (Substrate)	XY
3% H_2O_2	XY
Metal (Substrate)	XY
0.02 M $MgCl_2$	XY
0.02 M $MnCl_2$	XY
0.02 M $NiCl_2$	XY
Sun, room temp., 4 weeks	XY
Sun, room temp., 3 months	XY
37° C, 4 weeks	XY
Dark, room temp., 3 months	XY
Dark, 37° C, 3 months	XY

Table 4 Animal samples amplified with amelogenin primers and analyzed by CE

ANIMAL	PEAK SIZE
Bear	99.04
Bobcat	99.29
Cat	no amplified product
Chicken	no amplified product
Cow	98.59
Dog	98.94
Deer	98.71
Goat	99.06
Horse	98.71
Human (male)	102.92 /108.61
Moose	98.60
Porcupine	no amplified product
Rabbit	no amplified product
Raccoon	99.29
Sheep	98.94
Swine	99.64
Turkey	no amplified product

Abstracts of Presentations

NORTHEASTERN ASSOCIATION
OF
FORENSIC SCIENTISTS

TWENTY-THIRD ANNUAL MEETING
PROGRAM BOOKLET

WHITE PLAINS, NEW YORK
OCTOBER 15 - 18, 1997

ABSTRACTS: POSTER SESSION

CLOSER THAN COUSINS, BUT NOT QUITE BROTHERS

M. J. LaFountain, M. Schwartz, E. Buel, Vermont Forensic Lab

Courtroom testimony regarding the probability of a match is fundamental to the DNA analysis process. Juries may have a difficult time evaluating statistics which employ numbers beyond reasonable comprehension. Often concrete examples provide the link that assists juries to understand the statistical value of DNA analysis. We have examined double first cousins in an attempt to present an example of the power of DNA analysis. In concept, it is easy to understand that the probability of a close relative having the same DNA type is significant. If closely related individuals can be distinguished by a particular system and the magnitude of the DNA variation demonstrated, then the statistics presented for unrelated individuals are more understandable. We have typed eight double first cousins and their parents using the PM/DQA1, AmpFlSTR Blue, and AmpFlSTR Green I PCR-based multiplex systems as well as five RFLP probes. The ability of these systems, singly and in combination, to distinguish closely related individuals will be discussed.

POWERPOINT '97 AND THE INTERNET

L. Fuller, Chemistry Dept., SUNY-Oswego

Microsoft's Powerpoint presentation software allows one to produce colorful and animated slides for computer presentations. The release of an add-on utility that will convert a slide presentation in an Internet html file makes it relatively painless to create a presentation for posting on the world wide web. One can also conveniently printout min-slides of the presentation or distribute the presentation in outline format.

I have coupled these features with Microsoft's FrontPage '97 web publishing program to create an entire college course that is available to my students, and others, on the Internet at the address http://chem.oswego.edu/chem209. The use of Microsoft's Personal Web Server, that is included with FrontPage, was installed my office P-166 personal computer that has an active Internet LAN connection.

The use these tools to create and disseminate information may be the wave of the future for educational information distribution. My computer system will be setup to demonstrate the building of a website along with the actual course web that I first used in teaching a summer 1997 college course.

QUANTITATIVE BENZOYLECGONINE ANALYSIS EXTRACTED FROM MUMMIFIED REMAINS

C. Salerno, B.S.[1], R. Dettling, M.S.[1], J. Bidanset, Ph.D.[2], Dr. Fredrick Zugibe[3], Dr. E. Zappi[3], J. Costello[3], J. Segelbacher[3], M. Breithaupt[3], 1-Forensic Associates, 2-St. John's University, 3-Rockland County M. E. Office

TIMELY COLLECTION OF SUSPECT'S RAPE KIT YIELDS DNA RESULTS FROM FEMALE VICTIM

C. A. Scherczinger, Ph.D., **J. C. Reho, M.S.**, C. Ladd, Ph.D., M. T. Bourke, Ph.D., M. S. Adamowicz, Ph.D., H. C.Lee, Ph.D., Connecticut State Police Forensic Science Laboratory

A female prison inmate reported that she had been repeatedly sexually assaulted by a male prison gaurd over the course of a year. In the incident reported here, the gaurd allegedly forced the inmate to perform oral sex on him. He ejaculated in her mouth, and she spat into a handkerchief which he provided. The gaurd put this handkerchief and a number of other items into a gym bag, which he then stored in his locker. The inmate reported the assault to prison authorities, and a rape kit was collected at the prison hospital approximately four hours after the incident. The kit included oral swabs, oral smears, and a known blood sample from the victim. A search warrant was executed, and a salive sample, genital swab, and a known blood sample were collected from the prison guard. In addition, several handkerchiefs and other pieces of cloth were seized from his gym bag.

ABSTRACTS: FORENSIC BIOLOGY TECHNICAL SESSION

We also chose to validate two new STR multiplexes (ABD AmpflSTR Blue and Green) for the capillary only. This entails all of the above, plus validation of the amplification conditions and testing a series of different DNA extracts. Validating the systems for only one instrument type saves the work for repeating the precision, stutter and other electrophoresis based tests.

The capillary units are robust and require less hands on time. They are quite expensive to operate: based on a capillary life of a hundred samples, it costs $4.50 to run one sample. It also takes a long time to finish a 48 sample run. It is possible though to start analyzing the finished samples while still collecting data for the others. The fact that different from a 373 gel, where all samples have to stay together, the separate sample runs can be recombined in a "project" caused the need to set up new requirements for the controls of a given set of samples that were amplified and run together.

VALIDATION OF THE AMELOGENIN LOCUS FOR CAPILLARY ELECTROPHORESIS

M. J. LaFountain, M. Schwartz, J. Cormier, E. Buel, Vermont Forensic Laboratory

The amelogenin locus has been used extensively for the sex-typing of forensic samples. The analysis of the PCR products derived from the amplification of the X-Y homologous gene has traditionally been by gel-based methods. We have validated the use of capillary electrophoresis (CE) instrumentation for analysis of fluorescently-tagged amplicons derived from the PCR amplification of a portion of the amelogenin gene. Using an ABI 310 Genetic Analyzer, we determined the average base pair sizes for the amplified X and Y alleles to be 103 and 109 respectively. Typing was unfailingly reliable even in samples subjected to a variety of environmental insults and extracted from a number of substrate materials. Comparisons of the X and Y allele peak heights of male samples, however, showed significant variability. Differences in the Y/X ratio seemed most influenced by the amplification step, while CE results between samples were notably consistant. Peak size resolution for the X and Y alleles of amelogenin was also examined and was found to be 0.91 base pairs. Some factors found to adversely affect resolution were high template concentrations, use of inadequately deionized formamide in processing samples for CE analysis and sample injection times <2 seconds or >9 seconds. Resolved by CE technology were difficulties associated with traditional gel-based differention of human and non-primate animal samples. Animal samples amplified using the amelogenin primer set and run on the analyzer produced a peak at approximately 99 base pairs or no peak at all, thus providing a ready means for distinguishing human from animal amplification products. We have found CE analysis of amelogenin amplicons to be fast, trustworthy, and easier to evaluate and less labor intensive than traditional gel-based methods.

INTERNAL VALIDATION STUDIES OF A MULTIPLEX STR PROCEDURE

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The Technical Working Group on DNA Analysis Methods (TWGDAM) provides nationally recognized guidelines for the internal validation of DNA analysis procedures. Internal validation studies based on the TWGDAM guidelines were performed to evaluate the accuracy, precision and reproducibility of AmpFlSTR Blue and AmpFlSTR Green I PCR amplification kits (Perkin Elmer) for use in forensic case work. The Perkin Elmer kits allow multiplexing of short tandem repeat (STR) loci. The AmpFLSTR Blue kit is used to amplify D3S1358, vWA and FGA; the AmpFlSTR Green I kit amplifies Amelogenin, THO1, TPOX and CSF1PO. All PCR products were run on an ABI PRISM 377 DNA Sequencer. The internal validation studies included: 1) testing known samples, 2) establishing match criteria, 3) demonstrating that laboratory procedures do not introduce contamination which would lead to errors in typing, 4) evaluating mixed body fluid samples, and 5) testing non-probative evidence samples. The result of these studies demonstrate that AmpFlSTR Blue and AmpFlSTR Green I can be utilized in forensic case work.

Eighth International Symposium on Human Identification

Scottsdale, Arizona

Poster Abstracts

Presentations:

Thursday, September 18
3:30-5:00 pm (odd numbered posters)

Friday, September 19
3:40-5:00 pm (even numbered posters)

The Plaza

8. Effect of Time and Environmental Factors on Postmortem Stability of DNA in Egypt

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The applicability of DNA polymorphism to the individualization of human blood and tissues have been demonstrated. DNA may degrade rapidly in cadaver's blood and tissues even in the early post mortem period. This might be a consequence of rapid bacterial growth in decomposing bodies. High humidity conditions and temperatures are factors that affect the degradation rate of DNA. Our study aimed at evaluating the DNA integrity of muscle tissue in different environmental conditions in Egypt within different time intervals. DNA extraction was done using Wizard™ Genomic DNA purification system. Restriction enzyme digestion of DNA were EcoRI, SmaI, Hind III (Boehringer Mannheim).

DNA gel electrophoresis with ethidium bromide was used to visualize DNA pattern followed by Southern Blotting application and hybridization. Our results and photos will be presented and discussed in full detail.

9. Evaluation of Capillary Electrophoresis for the Forensic Analysis of Short Tandem Repeats

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Capillary electrophoresis is emerging as an important tool for forensic laboratories. The technique employs a capillary instead of the traditional gel-based technology to analyze STRs, semi-automating the analytical process. The use of CE for STR analysis is relatively new in forensics. In this study we establish that CE provides the same allelic designation as that determined by laboratories using a variety of gel-based methods. Eighty samples were amplified using either one or both of the multiplex systems AmpF*l*STR Blue and AmpF*l*STR Green I, amplifying D3S1358, vWA, FGA, or CSF1PO, TH01, TPOX and amelogenin respectively. The amplicons were analyzed by CE, and the results compared to those previously obtained by the contributing laboratory. Over all, 80 samples, providing 532 allelic identifications, were analyzed using CE. All identifications with CE were consistent with those of the contributing laboratory. Further studies with the AmpF*l*STR Blue multiplex were conducted to validate the use of this multiplex for casework as well as the use of CE for the analysis of this multiplex. Portions of the TWGDAM validation studies conducted will be presented. Characteristics of the instrument including resolution, standard deviation for the loci studied, and column longevity will be discussed.

Resolution Table

Resolution in Basepairs

LOCUS	AVERAGE RESOLUTION	STD. DEVIATION
AMELOGENIN	0.910	0.076
THO1	1.047	0.085
TPOX	1.303	0.036
CSF1PO	1.579	0.143