Rapid Microfluidic Human Specific DNA Quantitation

Eugene Tan

NetBio, 830 Winter Street, Waltham, MA 02451

This project was supported by Grant Number NIJ 2008-DN-BX-K009 awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice.

Abstract

The goal of this research program was to develop a human-specific DNA quantitation module for incorporation into NetBio's fully integrated microfluidic instrument and biochip in development. NetBio is applying microfluidic technology to develop an STR analysis instrument that will perform DNA purification, multiplexed PCR amplification, and electrophoretic separation and detection. This fully integrated biochip and instrument will generate STR profiles in 45 minutes without user intervention.

Commercially available STR typing kits allow the effective generation of highly accurate STR profiles, but only when the input DNA template falls within a narrowly optimized range. The DNA advisory board to the Federal Bureau of Investigation has recommended the use of human-specific DNA quantitation prior to PCR amplification of casework samples as there is the potential for sample contamination from non-human sources including non-human mammalian, bacterial, and fungal DNA. Accordingly, a fully-integrated microfluidic system for

forensic human identification should perform human-specific DNA quantitation in order to determine precisely the amount of DNA template to be subjected to STR amplification.

This research consisted of three elements: (I) selection and optimization of a DNA quantitation assay, (II) design and development of instrumentation and microfluidic biochip for DNA quantitation, and (III) testing of the microfluidic quantitation system using forensic samples. Based on this research, a microfluidic biochip endpoint PCR assays was developed and evaluated based on assay performance (including time to completion, sensitivity, reproducibility, and dynamic range), ease of implementation in a microfluidic format, and cost. This research represents an important step towards the development of a fully-integrated STR instrument that can be put to widespread use in forensic laboratories.

Table of Contents

Introduction

Purpose, Goals, and Objectives

Commercial kits and instruments used for performing STR analysis place strict limits on the amount of template DNA, specifically human genomic DNA, that can be used to generate profiles acceptable for STR analysis. For a standard STR reaction, an input human genomic template range of between 0.5 ng and 2.0 ng is generally required. When too little template is applied to the assay, artifacts including allele peak height imbalance and allele drop-out can occur. When too much template is applied, artifacts including increased stutter, non-specific band creation, incomplete non-template addition, and pull-up peaks resulting from incomplete color separation can occur. These artifacts lead to difficulties in interpretation of an STR profile. For evaluation of casework samples, the DNA Advisory Board to the FBI recommends the use of human-specific quantification rather than total DNA quantification (DNA Advisory Board (2000)). This practice ensures that an appropriate amount of human DNA is subjected to amplification, even if bacterial and fungal or other non-human DNA is present in a given DNA sample.

Accordingly, any fully-integrated system for processing casework samples, beginning with the isolation of DNA and resulting in an STR profile, should incorporate a human-specific DNA quantitation step. In a fully-integrated system, the instrument would then be capable of determining the precise amount of DNA to be amplified following quantitation. NetBio has completed development of a microfluidic biochip-based DNA separation and detection instrument, Genebench-FX™ Series 100, microfluidic chip-based multiplex PCR amplification

module, and purification module. The work reported here focused on the development of a microfluidic human-specific DNA quantitation module suitable for incorporation into a fully-integrated microfluidic instrument for STR analysis.

The process flow of the fully integrated. STR analysis system is shown in Figure 1. A forensic sample is collected using a swab-based sample collection device and placed into a Smart Cartridge. Within the cartridge, cells are lysed and DNA is purified and transferred to the microfluidic biochip. On the biochip, the purified DNA is quantified using the methodologies reported here. Following quantitation, an appropriate aliquot of the remaining DNA is transferred to the PCR chamber for STR amplification. The DNA fragments are then subjected to separation and detection, resulting in an STR profile.

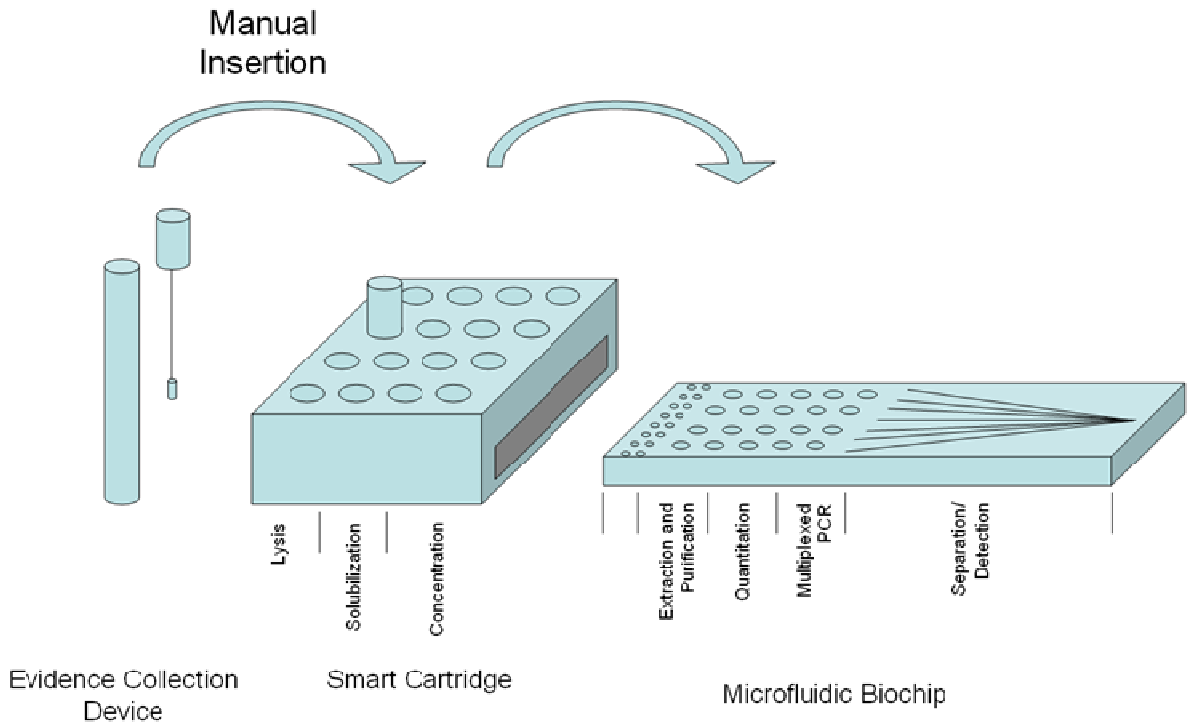

Figure *1. Process flow for sample-in to results-out STR analysis of casework samples in the fully-integrated system.*

NetBio Sample-in to Results-out Components

Microfluidic Separation and Detection

Genebench FX™ is a rapid, high resolution, and high sensitivity DNA fragment sizing and sequencing instrument for laboratory and field use. The instrument separates DNA based on fragment size by electrophoresis on microfluidic biochips, and excitation and detection of labeled DNA fragments is accomplished by laser-induced fluorescence detection. Genebench FX™ can be operated in both the forensic laboratory and in the field, has low power consumption, and is CE marked under the Low Voltage Directive 73/23/EEC. Separation of the DNA fragments take place within a microfluidic biochip that is filled with a sieving matrix. The biochip accepts 16 samples to allow for simultaneous analysis of multiple samples and required control reactions.

Rapid Multiplex PCR Amplification in a Microfluidic Chip

Rapid multiplexed STR amplification was accomplished by focusing on two major areas: rigorous optimization of all reaction mix components and cycling parameters and the development of instrumentation to allow rapid and highly-controlled temperature transitions. The custom thermal cycler shown in Figure 2 is designed with a high output thermoelectric cooler/heater mounted to a high efficiency heat sink, together referred to as the heatpump. This instrument accepts a 16-chamber microfluidic biochip (Figure 3) which is coupled to the heatpump by applying a compressive pressure with a clamping mechanism. Each PCR chamber is 500 µm deep and approximately 1 mm wide and holds 7 µl of PCR reaction solution. The 16 PCR reaction solutions are placed into individual chambers of the microfluidic biochip. The

thermal cycler has the ability to heat and cool a reaction solution at rates of 15.8°C/s and 15.4°C/s respectively, much faster than commercially available cyclers. Appropriate selection of an enzyme with a high extension rate and a fast hot start activation, along with the optimization of the cycling protocols, allows a highly multiplexed PCR reaction to be performed in as little as 17 minutes (Figure 4) (Giese 2009). Figure 5 shows a representative fast STR profile using the NetBio thermal cycler and separated and detected on Genebench FX. The fast PCR profiles generated using this approach meet forensically relevant requirements including signal strength, stutter, peak-height ratio, incomplete non-template nucleotide addition, and inter locus balance (Giese 2009).

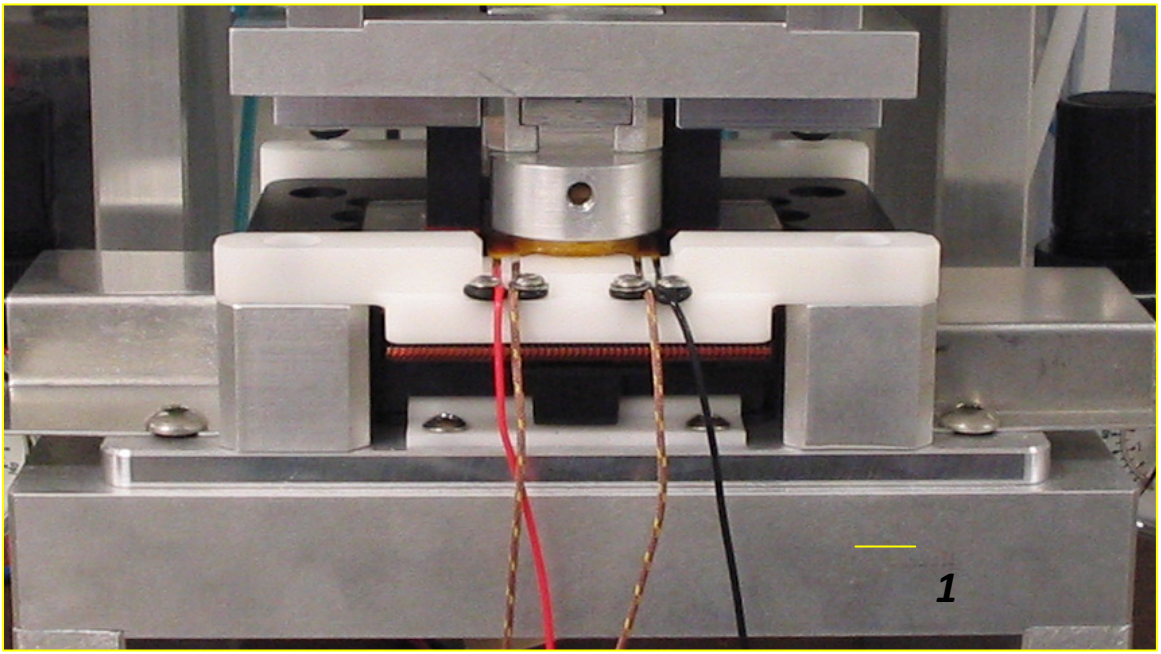

Figure 2. NetBio fast thermal cycler.

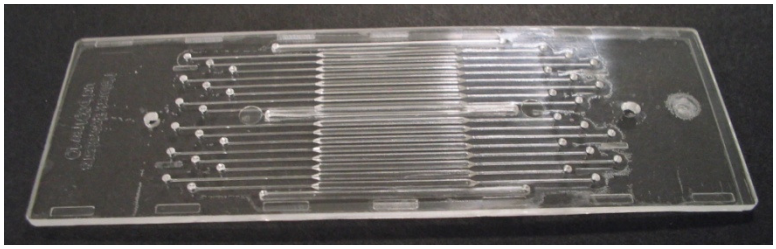

Figure 3. 16-sample PCR Biochip for rapid highly-multiplexed amplification in NetBio's fast thermal cycler. Biochip dimensions are 25 mm x 75 mm.

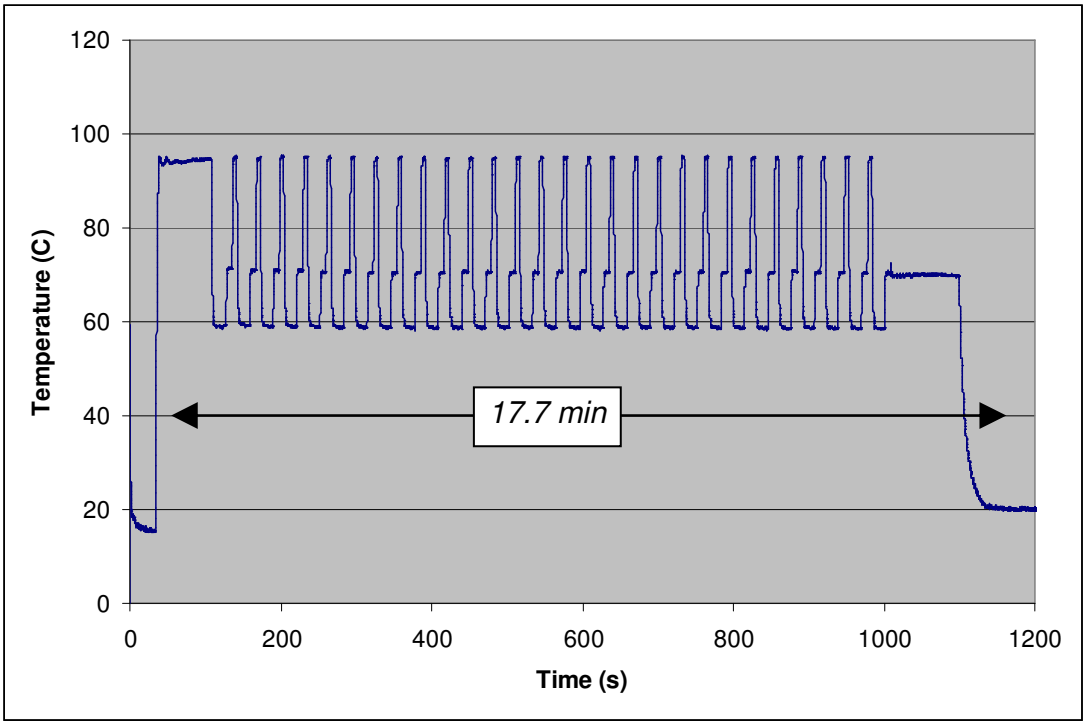

Figure 4. Thermal profile for fast multiplexed amplification of STRs in the fast thermal cycler.

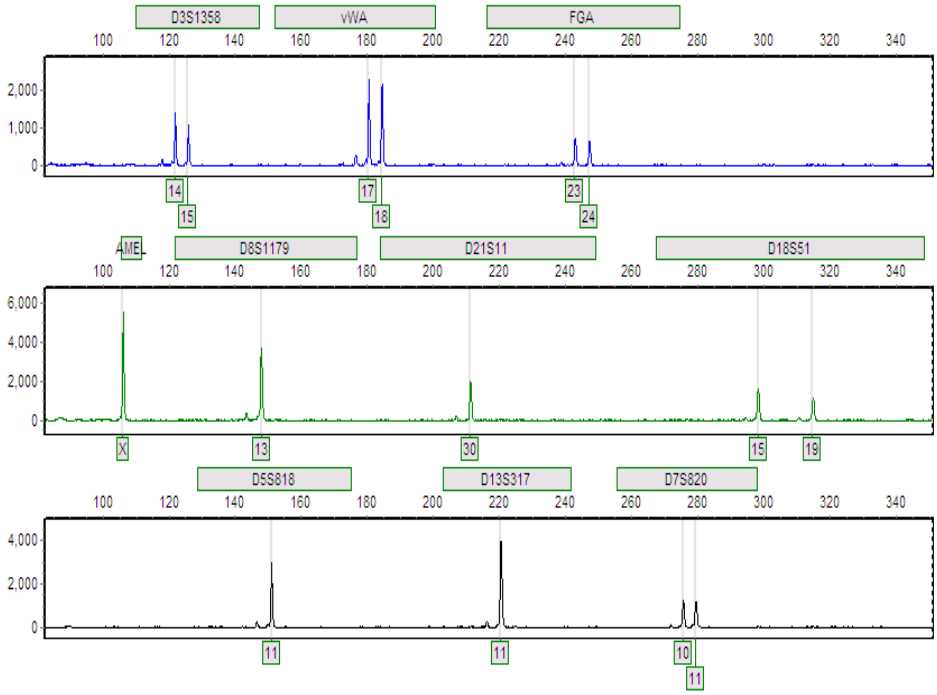

Figure 5. STR profile for 0.5 ng DNA (9947) amplified in chip under fast thermal cycling conditions with primers from the AmpFlSTR Profiler Plus ID PCR primer set.

Microfluidic DNA Purification

Development of the microfluidic biochip-based purification module is completed and this work has been funded in part by NIJ Award 2007-DN-BX-K184. For blood, lysis is performed with a guanadium hydrochloride (GuHCl) based-lysis buffer, and DNA in the lysate is bound to a silica membrane. Figure 6 shows that the human genomic DNA purified on the biochip has an average length of approximately 50 kb, a size appropriate for STR amplification. Finally, DNA extracted using this microfluidic biochip amplifies effectively and generates STR profiles (Figure 7) that are indistinguishable from those generated using DNA purified using conventional tube-based reactions.

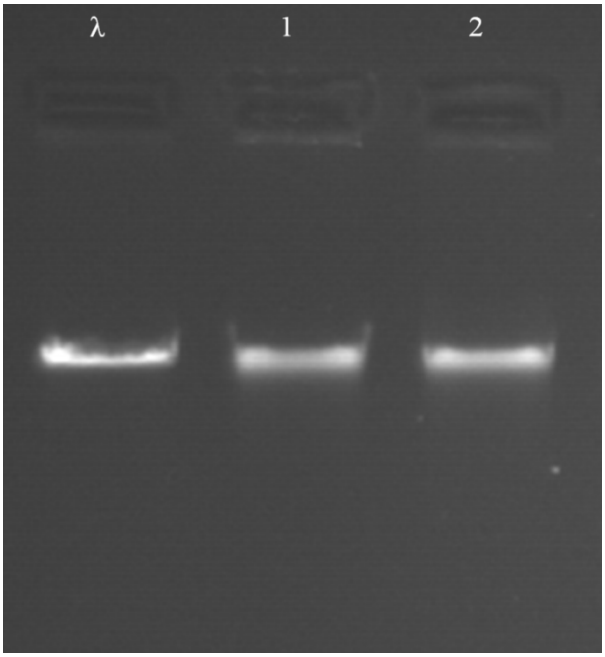

Figure *6. Agarose gel analysis of DNA extracted from whole blood with microfluidic biochip. (1:Biochip extaction, 2:Qiagen control extraction, λ:50kbp lambda DNA)*

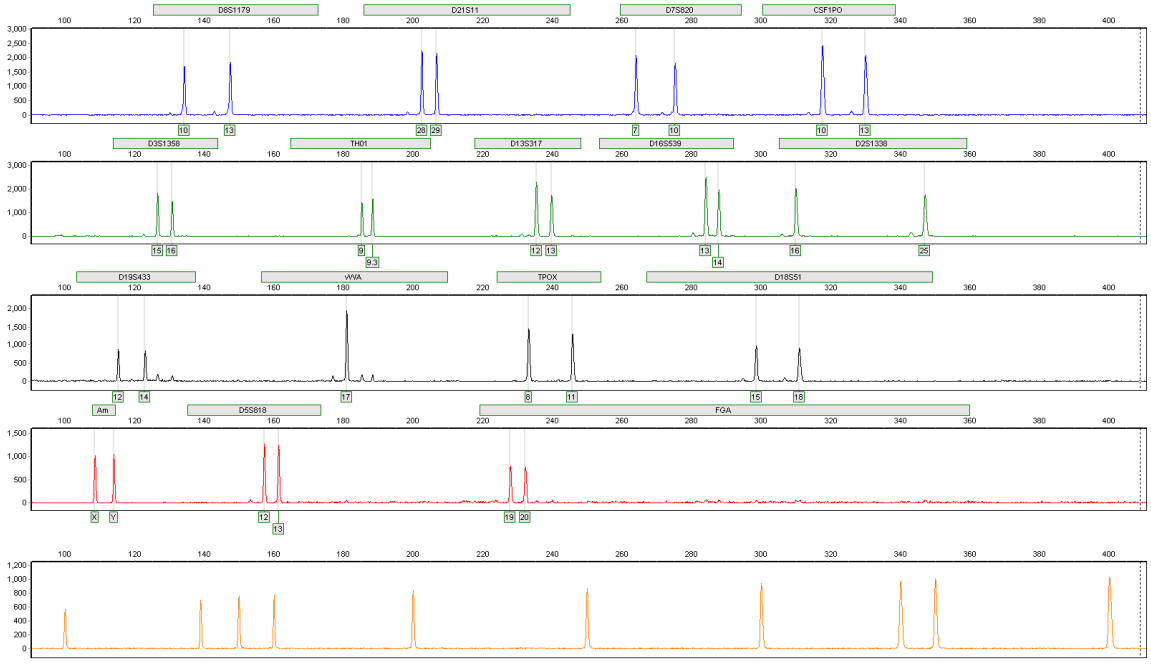

Figure *7. STR profile from DNA extracted and purified from whole blood with microfluidic biochip.*

Taken together, these results show the developmental progress of the modules that make up a fully-integrated, sample-in to results-out forensic DNA analysis system. The quantitation module developed as described herein will accept DNA transferred from the DNA extraction and purification module and provide critical human-specific quantification information for normalizing the template DNA level in the subsequent STR amplification module. This research program is based on a quantitation instrument that incorporates the rapid thermal cycling module discussed above.

Review of Relevant Literature

Accurate DNA quantification is an essential step in the generation of STR profiles for forensic identification for two related reasons. In general, multiplexed PCR assays are most reliable within a narrowly defined range of input DNA and, in particular, STR assays are most informative when the amount of human DNA falls within that range. For commercially available STR typing kits, the amount of input DNA required to generate accurate STR profiles with balanced peak heights is between 0.5 and 2 ng of human DNA (LaFountain 2001; Moretti 2001; Krenke 2002). When too little template is applied to the assay, artifacts including allele peak height imbalance and allele drop-out can occur. When too much template is applied, artifacts including increased stutter, non-specific band creation, incomplete non-template addition, and pull-up peaks resulting from incomplete color separation can occur. These artifacts lead to difficulties in interpretation of an STR profile (Tomsey 2001; Kline 2003; Butler 2005).

Forensic samples have the potential to be contaminated with non-human mammalian, bacterial, or fungal DNA which, when present, contributes to the total DNA in the sample.

Accordingly, for evaluation of crime scene samples, the DNA Advisory Board to the FBI recommends the use of human-specific quantification rather than total DNA quantification (DNA Advisory Board 2000). This practice ensures that an appropriate amount of human DNA is subjected to amplification even if bacterial and fungal or other non-human DNA is present in the DNA extracts.

Several methods for DNA quantification have been developed. Perhaps the simplest is to measure the optical density of a DNA solution at a wavelength of 260 nm using a spectrophotometer. Similarly, gel electrophoresis to separate DNA fragments and subsequent quantification of band fluorescence from intercalating dyes and the direct quantification of fluorescence from solutions containing DNA and intercalating dyes are commonly utilized techniques. Though these methods were widely utilized in the early days of forensic DNA analysis (Van Dyke and Szustkiewicz 1968; Hopwood 1997; Singer 1997), they are much less useful today as they are not specific for human DNA. Other limitations of these nonspecific methods are lack of sensitivity, the need for relatively large amounts of sample, and a lack of specificity for double and single stranded DNA and RNA. Intercalating fluorescent dyes such as Hoechst dyes, PicoGreen, and ultrasensitive cyanine dyes (YOYO-1, TOTO-1, YO-PRO-1) show a significantly improved sensitivity and have the ability to selectively quantify double stranded DNA. Using Hoechst dyes DNA, concentrations down to approximately 10 ng/mL can be determined (Tanious 1992; Ausubel 1998). Cyanine dyes and PicoGreen enable detection of DNA down to the picogram level (Rye 1993; Singer 1997; Haugland 2002). PicoGreen in particular, has found widespread application in microplate assays for sensitive quantitation of double stranded DNA over an extended quantitative range (25 pg/ml to 1 µg /ml (Singer 1997;

Romppanen 2000)). Nonetheless, though these assays have their utility, they do not satisfy the requirement for human-specific quantification.

To determine the amount of human-specific DNA in a forensics sample, several techniques have been developed and efforts to improve the performance of existing assays and design new assays are ongoing. An early method for human specific quantification method was slot blot hybridization (Walsh 1992) and was commercially available as the Quantiblot™ Human DNA quantification kit from Applied Biosystems (Foster City, California). The technique is based on the hybridization of a probe complementary to the primate-specific alpha satellite DNA sequence D17Z1 to genomic DNA immobilized on a nylon membrane and subsequent chemiluminescent or colometric detection. The procedure can detect down to 150 pg DNA but is time-consuming and labor-intensive and, most importantly, lacks accuracy (Budowle 2001; Duewer 2001). A related technique, developed by Promega (Madison, WI), is the AluQuant assay which probes highly abundant Alu sequences in the human genome without the necessity to immobilize the DNA. Hybridization of the probe triggers enzymatic reactions resulting in the production of light that can be measured using a luminometer. The dynamic range of this assay is between 0.1 and 50 ng (Mandrekar 2001; Hayn 2004).

Several PCR-based methods have been developed as alternatives to hybridization approaches to specific human DNA quantification. The advantage of these procedures is that they are somewhat less labor intensive than hybridization methods and are highly sensitive, accurate, and reproducible. Furthermore, they can determine the effective amount of

amplifiable human DNA, of particular importance if the DNA sample contains inhibitors or is highly degraded.

In real-time PCR assays, amplification progress is monitored after each cycle as a fluorescent output signal from a specific probe. Several real-time PCR assays have been designed and optimized to target single or multiple human-specific sequences. Among those targeted sequences are highly repetitive Alu sequences scattered throughout the genome (Nicklas and Buel 2003; Shewale 2007), sequences on X and Y chromosomes (Alonso 2004), and loci used in STR typing kits such as TH01 and CSF1PO (Swango 2006). Chemistries applied for real time PCR detection are SYBR Green (Wittwer 1997), fluorogenic probes (TaqMan probes; Holland 1991; Lee 1993) and molecular beacons (Tyagi 1996; Nazarenko 1997; Whitcombe 1999; Tyagi 2000). Real-time PCR is highly sensitive for human-specific DNA quantification (detection of even single copy DNA is possible) and the dynamic range of detection readily spans 3 orders of magnitude (30 pg to 30 ng; Swango 2006).

A simpler PCR-based approach for DNA quantification that is less expensive than real-time PCR is endpoint PCR. The method relies on amplification of defined human sequences with specific primers and subsequent detection of the amplified DNA with fluorescent dyes such as PicoGreen. Alternatively, amplification can be performed in the presence of a fluorescent dye. DNA concentrations are determined using a series of known DNA standards. Amplified regions described in the literature include STR loci TH01 (Fox 2003) and Alu sequences (Sifis 2002; Nicklas and Buel 2003). Dynamic ranges of 200 pg-40 ng (Fox 2003), 2.5 pg-100pg (Sifis 2002) and 10 pg-10 ng (Nicklas and Buel 2003) have been reported.

Microfluidics offers the potential for significant improvements in forensics DNA analysis including decreased process time and costs and increased sensitivity, accuracy, and throughput. Progress has been reported using microdevices for DNA extraction, PCR amplification, and separation and detection. The first reports of micro-quantitative real-time PCR (Northrup 1998 and Ibrahim 1998) demonstrated the feasibility of real time PCR in a microfluidic format. A real time assay in a PDMS microfluidic device with a 12 nL reaction volume has been reported, but DNA quantification was not shown (Liu 2002). In general, the application of microfluidics to human-specific DNA quantification for forensic application has not been an active area of research and significantly more developmental work is necessary in order to bring microfluidic human DNA quantification to the forensic laboratory.

Implications for Criminal Justice Policy and Practice

The availability of an STR typing instrument that combines DNA extraction and purification, amplification, and separation into a single, easy to operate instrument would represent a substantial advance in forensic DNA analysis. Significant burdens in setting up and operating a forensic DNA analysis laboratory are the costs of dedicated rooms to prevent PCR contamination, automating the procedures (either through robotics or dedicated technicians), and validating and re-validating individual instruments and the entire series of laboratory processes. A fully-integrated instrument has the potential to be faster, more sensitive, less susceptible to contamination, less costly, and less labor-intensive than currently available technologies.

Human-specific DNA quantification is required for STR analysis of forensic samples and, as such, must be incorporated into any fully-integrated microfluidic STR typing system. The research conducted under this grant award represents an important step towards making fully-integrated forensic DNA analysis a reality. Finally, a fully-integrated microfluidic STR typing instrument would offer forensic scientists new capabilities not possible with conventional instrumentation.

Research Plan

Overview. The goal of the research program was to develop a microfluidic human-specific DNA quantification system suitable for incorporation into a fully-integrated forensic STR typing system. The system has two components, (1) a microfluidic biochip capable of accepting 16 forensic DNA samples and the DNA samples for generation of a standard curve and (2) the instrumentation to perform thermal cycling and excitation and detection. The requirements of the quantitation system are:

Specificity. The system must detect only human (or higher order primate) DNA.

Sensitivity. The system must be sensitive, accurate, and reproducible.

Integration. All materials and reagents must be compatible with transfer using microfluidic principles.

The specific objective of this research were to:

Develop a microfluidic chip-based human-specific DNA quantitation module.

Design and build instrumentation for the quantitation assay.

Evaluate the quantitation module for forensic use.

Methods and Materials

Biological Samples

Human Samples (Mock casework Samples)

Fresh Whole Blood. Wet blood samples on swabs were prepared by pipetting 100 µL of blood solution onto a ceramic tile and then wiped off with swab collection head. Extraction using QIAamp protocol was done immediately after. Dried blood sample on swab was prepared similarly but allowed to dry for overnight inside the desiccated swab storage

*Saliva***.** Saliva was collected by expectorating into a 50 ml falcon tube. 100 µL is pipetted out and placed onto a ceramic surface and then wiped off with a cotton swab..

Epithelial Cells (Palm). Epithelial cellular samples were collected by rubbing the swab head on the palm and in between the fingers of a human subject.

Rapid PCR Amplification in Biochip

Rapid PCR reactions were performed with the 7 µL PCR reaction and cycling protocol as described in (Giese 2009). Amplification of 16-samples in the microfluidic biochip was completed in approximately 17 minutes. PCR product were either stored in 4˚C until ready for assay or processed immediately.

PicoGreen Staining of DNA.

1 µL of the PCR product was added to 9 µL of a 1:200 dilution in TE buffer of Quant-iT™ Picogreen® reagent (Invitrogen). The solution was mixed by pipetting up and down, allowed to incubate at room temperature for 2 minutes and transferred, by pipetting, into the quantitation biochip.

PCR Amplification with thermostable intercalating dye.

Rapid PCR reactions were performed with the 7 µL PCR reaction and cycling protocol as described in (Giese 2009). Amplification of 16-samples in the microfluidic biochip was completed in approximately 17 minutes and the amplified products were manually retrieved from the individual PCR.

UV Absorbance Quantification

NanoDrop spectrophotomer blanked with elution buffer was used to measure the concentration of DNA extracts by pipetting out 1.5 µL of a homogenized solution onto the instrument pedestal. At least duplicate measurements were done.

NetBio's Microfluidic DNA Quantitation

The biochip chambers were filled with amplicon-dye mixture and placed under the excitation and detection plate.

STR Amplification Reaction.

Multiplex PCR reactions were performed with the AmpFℓSTR® Identifiler® PCR Amplification primer set (Applied Biosystems, Foster City, CA). The 7 µL PCR reaction and

cycling protocol was prepared as previously described (Giese 2009). Amplification of 16-sample in the microfluidic biochip was completed in approximately 17 minutes and the amplified products were manually retrieved from the individual lanes.

STR separation and detection

Amplified products were separated and detected using NetBio's Genebench-FX™ Series 100.

Development of Instrumentation and Biochip for Human-Specific DNA Quantitation

Endpoint PCR was performed by thermal cycling a reaction mix and exciting and detecting the fluorescent probes present in the reaction solution. NetBio has previously developed instrumentation for rapid thermal cycling (Giese 2009) and the separation and detection of fluorescently labeled DNA (see "NetBio Sample-in to Results-out Components," above).

Optical Train, and Excitation and Detection Sources

The optical train is a series of optical elements that couple the light from the excitation source to the optical interrogation chamber and the emitted fluorescence from the optical interrogation chamber to the optical detector. It is comprised of optical elements including lenses, mirrors, and filters.

Excitation and detection parameters for quantitation

Initial excitation and detection parameters were developed for use with the quantitation system. A master mix of DNA and picoGreen intercalating dye were prepared and loaded into the manual quantitation biochip (see "Microfluidic Biochip Design," below) and

used for excitation and detection with the quantitation instrument. Figure 8 shows that signal strength increases with increasing PMT gain with 3 orders of amplification, from 10 RFU to 10,000 RFU, over the range of PMT gain settings.

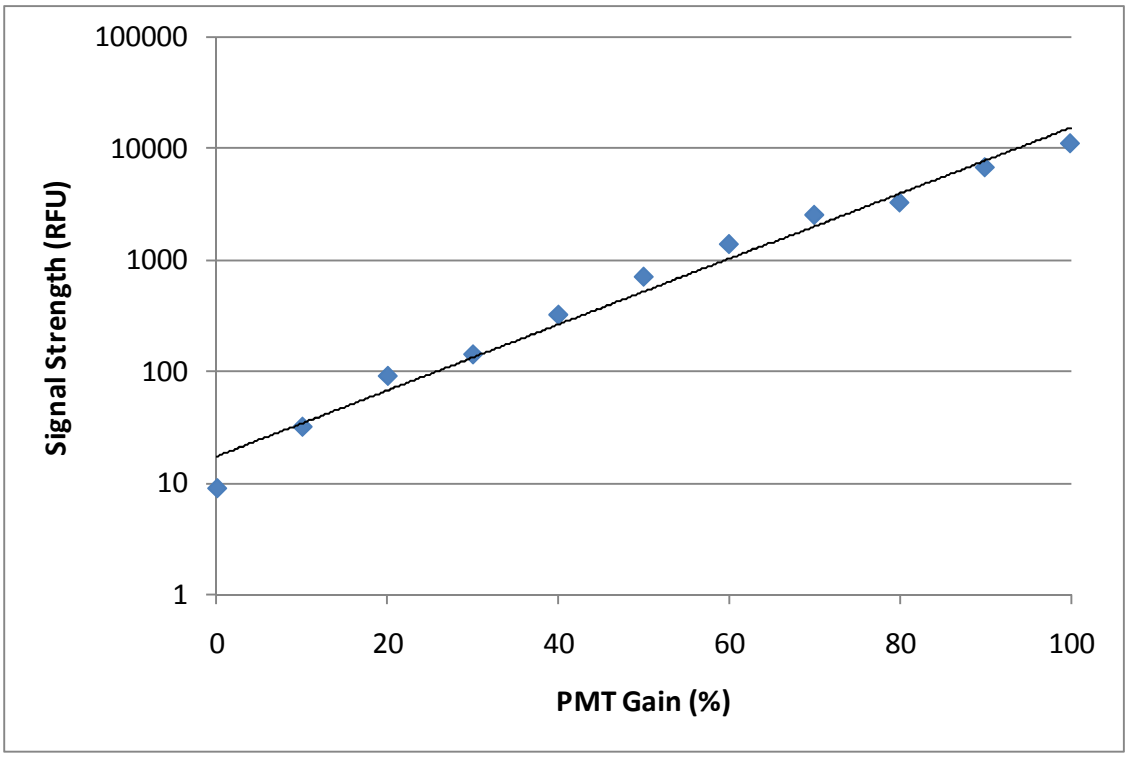

Figure *8. Signal strength as a function of PMT gain.*

Microfluidic biochip design

The quantitation assays were conducted in 16-lane microfluidic biochips designed to be compatible with NetBio's thermal cycling instrumentation for rapid PCR amplification. The quantitation biochips were also designed to allow for fluidic transport in cases where separate thermal cycling and optical interrogation chambers are utilized.

An initial 16-sample biochip based on the design of that used for rapid multiplexed PCR and optimized for optical interrogation was developed. The process flow for quantitation is shown in Figure 9. The biochip allows PCR reagents, intercalating dyes, and DNA to be inserted into the sample reservoir, mixed, and amplified. Fluids within the biochip are pneumatically-driven under computer control following an automated script.

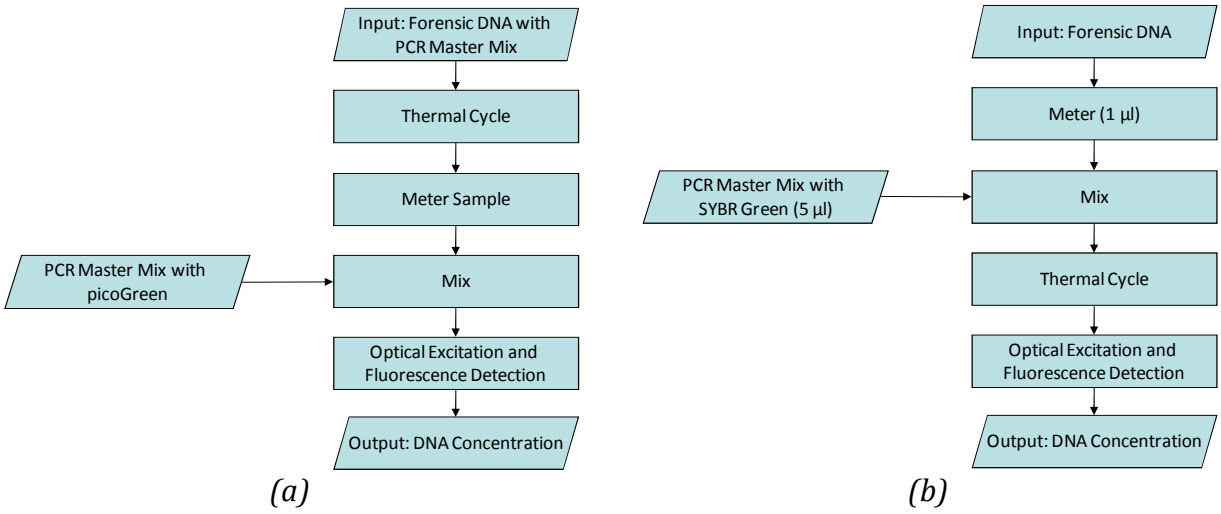

Figure *9. Process flow for DNA quantitation.*

Quantitation with microfluidic biochip and excitation and detection system

An initial quantitation experiment was performed in the biochip and quantitation instrument. In this experiment, PCR reaction product from amplification with 0, 1, 5, 10, and 30 ng of 9947A DNA was mixed with picoGreen and loaded into the lanes of the biochip. Each lane in the biochip was excited and fluorescence signal was recorded. Average signals were taken from 5 different positions in each lane. Figure 10 illustrates an increase in the raw fluorescence signal with DNA template. This results shows that the instrument and associated biochip are suitable for DNA quantitation.

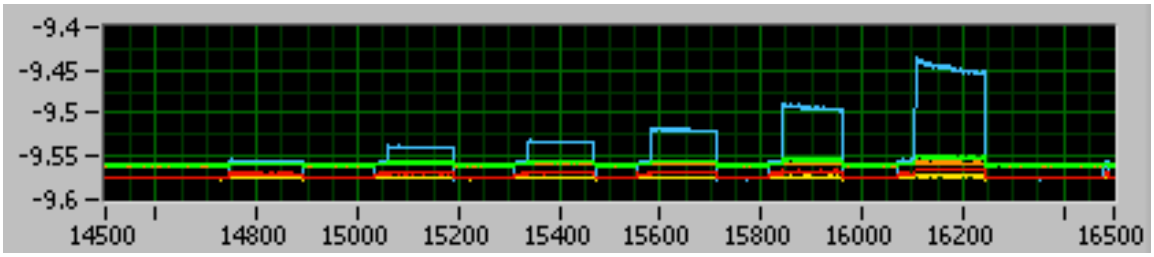

Figure *10. Analysis of picoGreen Stained PCR product in biochip. Signal from left to right: plastic background, 0- , 1- , 5- , 10- and 30- ng 9947A DNA.*

Microfluidic Biochip-based Human Specific DNA Quantitation

Endpoint PCR quantification was performed by amplifying a DNA sample with human-specific primers in the presence of intercalating dyes. In principle, intercalating dyes bind to double-stranded amplicons to generate a fluorescent signal that is significantly enhanced over the unbound dye. This approach to quantitation was evaluated by using single-copy human-specific primers for the human-specific Th01 gene. In addition, primers for human Alu repeats were also tested.

Rapid biochip PCR with the Th01 and Alu primers was performed with NetBio's custom biochip thermal cycler using a 28-cycle protocol consisting of a hot start activation of 60 seconds at 93˚C followed by 28-cycles of [93˚C for 4 seconds, 58˚C for 15 seconds, 70˚C or 7 seconds] and final extension for 90 seconds at 70˚C. This protocol takes approximately 17 minutes to complete. Agarose gel analysis (Figure 11) of the amplified products shows that the product fragment sizes for TH01 and Alu amplification correlate with the expected sizes. The gel intensity analysis also shows that more DNA was generated with Alu primers compared with TH01 with the same amount of input DNA to the PCR.).

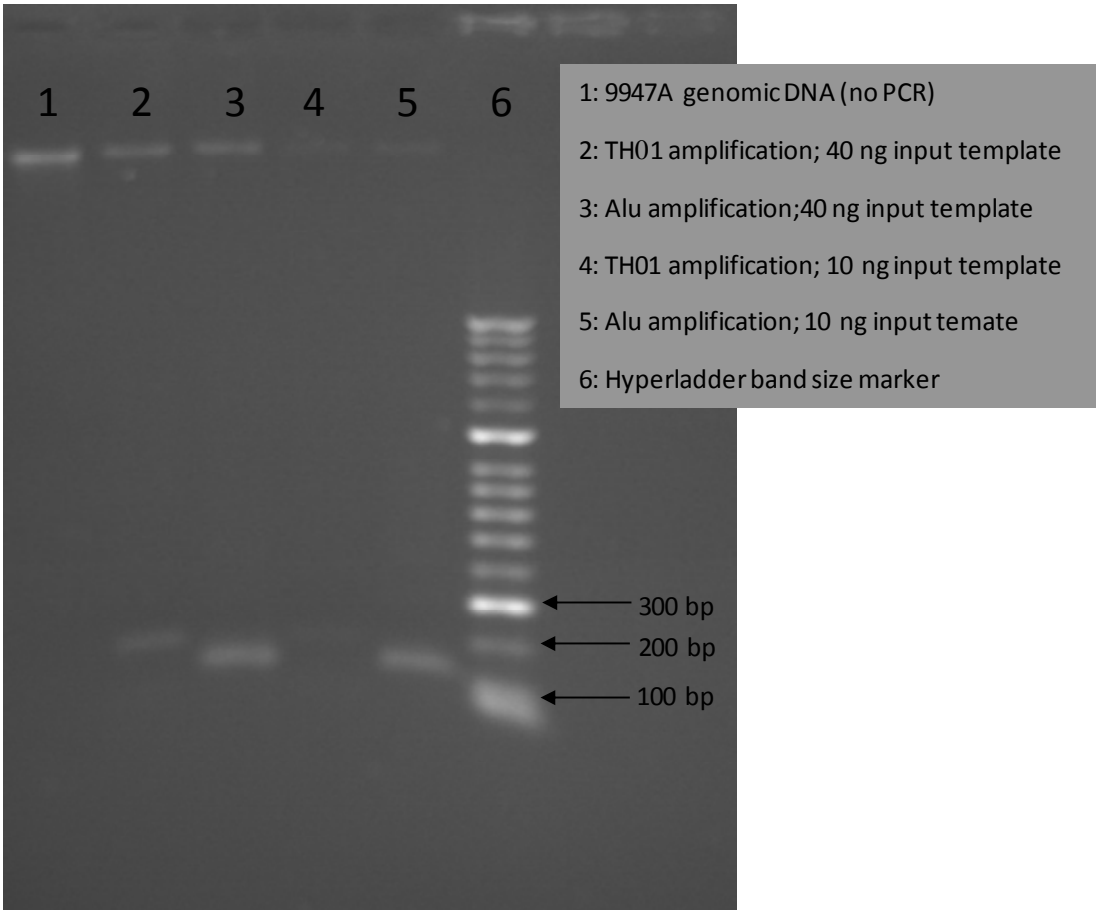

Figure *11. Gel analysis of Th01 and Alu primer sequences.*

The PCR product, amplified in the biochip with the Th01 primers, was mixed with picoGreen reagent and analyzed on a plate reader. The input DNA versus RFU from plate reader has R^2= 0.9991 correlation (Figure 12). Taken together, these results demonstrate that the end-point PCR assay has the potential to be fast when rapid biochip amplification protocols are implemented.

A switch from picoGreen reagent to SYBR Green, a thermally stable intercalating dye, eliminates post-PCR prep time since the latter can be incorporated into the PCR mixture. A reduction in the number of PCR cycles from 28 to 15 and 10 cycles showed a reduction in the

total cycle time for sample preparation to 8 and 6 minutes; respectively. These data demonstrate that microfluidic implementation of endpoint PCR utilizing NetBio's technology for fast multiplexed PCR amplification results in significant reduction in the assay time.

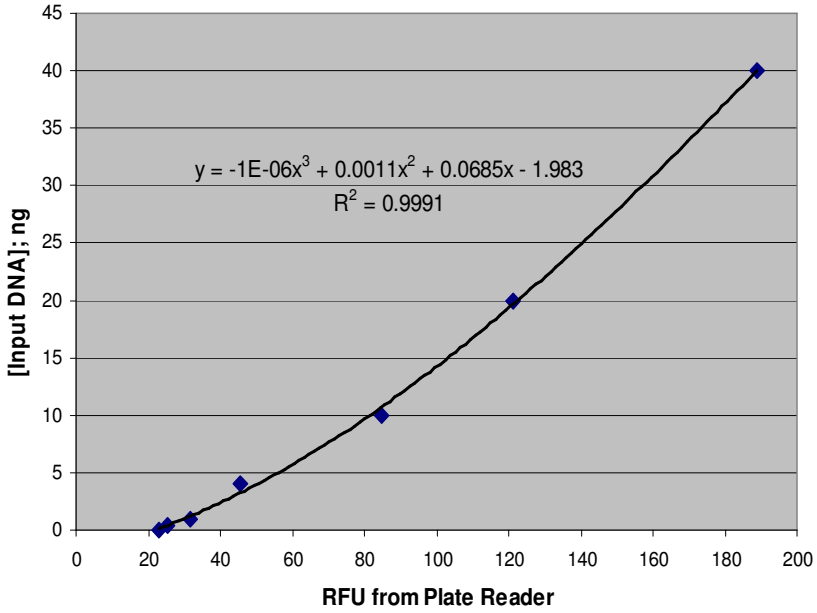

Figure *12. Correlation between input DNA and signal from plate reader for PCR product from rapid biochip-based amplification with Th01 primers.*

Quantification of Casework Samples.

A set of mock casework samples were prepared and purified following both microfluidic biochip and Qiagen protocols. DNA concentrations were measured using absorbance at 260 nm. 1 µL of the quantified DNA solution was amplified together with a set of 9947A standards. Table 1 summarizes the results of both quantification methods. Full STR profiles were generated for each of the mock casework samples using the quantitation of the end-point assay. The ability to generate full profiles from a variety of sample types and level of DNA demonstrates the compatibility of this assay for forensic application.

Samples Tested	From Absorbance	From Picogreen Assay
Dry Whole Blood	19 ng	22 ng
Wet Whole Blood 1	12 ng	13-16 ng
Wet Whole Blood 2	20 ng	15-16 ng
Cellular	4 ng	5-7 ng
Touch	9 ng	9 ng
Saliva	18 ng	26-28 ng
Adult Buccal	10 ng	8-14 ng
Baby Buccal	10 ng	5-14 ng

Table 1. Quantitation of mock casework samples with the NetBio's microfluidic quantitation assay

Conclusions

The results demonstrate the successful development of microfluidic approaches to DNA quantitation capable of human specific quantitation from a variety of sample types and substrates relevant to the forensics sciences community. The successfully developed quantitation module can be incorporated into a fully integrated instrument that will generate an STR profile in 45 minutes from sample introduction with minimal operating requirements. The microfluidic DNA quantitation system will be easy to operate and compatible with both forensic and microfluidic requirements.

Dissemination Strategy.

Presentations. The findings from this work have been presented in part as a poster and demonstration entitled "Microfluidic Purification of DNA from Forensic Samples and Human-Specific DNA Quantitation" at the NIJ Conference in Arlington, VA on June 15-17, 2009. In addition, this work was disseminated in an oral presentation entitled "Rapid Microfluidic Human Specific DNA Quantitation" at the American Academy of Forensics Scientists 62nd Annual Scientific Meeting in Seattle, WA on Feb 22-27, 2010.

References

Alonso, A., P. Martin, C. Albarran, P. Garcia, O. Garcia, L. F. de Simon, J. Garcia-Hirschfeld, M. Sancho, C. de La Rua and J. Fernandez-Piqueras (2004). "Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies." Forensic Sci Int 139(2-3): 141-9.

Ausubel, F. M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, Fj.G., Smith, J.A., Struhl, K. (1998). Current protocols in molecular biology. New York, Wiley.

DNA Advisory Board (2000). "Quality assurance standards for forensic DNA testing laboratories." Forensic Sci Commun 2(3).

Budowle, B., W. R. Hudlow, S. B. Lee and L. Klevan (2001). "Using a CCD camera imaging system as a recording device to quantify human DNA by slot blot hybridization." Biotechniques 30(3): 680-5.

Butler, J. M. (2005). Forensic DNA typing. Biology, technology, and genetics of STR markers. Burlington, MA, Elsevier Academic Press.

Duewer, D. L., M. C. Kline, J. W. Redman, P. J. Newall and D. J. Reeder (2001). "NIST mixed stain studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples." J Forensic Sci 46(5): 1199-210.

Fox, J. C., C. A. Cave and J. W. Schumm (2003). "Development, characterization, and validation of a sensitive primate-specific quantification assay for forensic analysis." Biotechniques 34(2): 314-8, 320, 322.

Giese, H., R. Lam, et al. (2009). "Fast multiplexed polymerase chain reaction for conventional and microfluidic short tandem repeat analysis." J Forensic Sci 54(6): 1287-96.

Haugland, R. P. (2002). "Handbook of fluorescent probes and research products." 9th edn. Molecular Probes, Eugene, OR: chapter 8.3, pp 300-308.

Hayn, S., M. M. Wallace, M. Prinz and R. C. Shaler (2004). "Evaluation of an automated liquid hybridization method for DNA quantitation." J Forensic Sci 49(1): 87-91.

Holland, P. M., R. D. Abramson, R. Watson and D. H. Gelfand (1991). "Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of Thermus aquaticus DNA polymerase." Proc Natl Acad Sci U S A 88(16): 7276-80.

Hopwood, A., N. Oldroyd, S. Fellows, R. Ward, S. A. Owen and K. Sullivan (1997). "Rapid quantification of DNA samples extracted from buccal scrapes prior to DNA profiling." Biotechniques 23(1): 18-20.

Ibrahim, M. S., R. S. Lofts, P. B. Jahrling, E. A. Henchal, V. W. Weedn, M. A. Northrup and P. Belgrader (1998). "Real-time microchip PCR for detecting single-base differences in viral and human DNA." Anal Chem 70(9): 2013-7.

Kline, M. C., D. L. Duewer, J. W. Redman and J. M. Butler (2003). "NIST Mixed Stain Study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity." Anal Chem 75(10): 2463-9.

Krenke, B. E., A. Tereba, S. J. Anderson, E. Buel, S. Culhane, C. J. Finis, C. S. Tomsey, J. M. Zachetti, A. Masibay, D. R. Rabbach, E. A. Amiott and C. J. Sprecher (2002). "Validation of a 16-locus fluorescent multiplex system." J Forensic Sci 47(4): 773-85.

LaFountain, M. J., M. B. Schwartz, P. A. Svete, M. A. Walkinshaw and E. Buel (2001). "TWGDAM validation of the AmpFlSTR Profiler Plus and AmpFlSTR COfiler STR multiplex systems using capillary electrophoresis." J Forensic Sci 46(5): 1191-8.

Lee, L. G., C. R. Connell and W. Bloch (1993). "Allelic discrimination by nick-translation PCR with fluorogenic probes." Nucleic Acids Res 21(16): 3761-6.

Liu, J., M. Enzelberger and S. Quake (2002). "A nanoliter rotary device for polymerase chain reaction." Electrophoresis 23(10): 1531-6.

Mandrekar, M. N., A. M. Erickson, K. Kopp, B. E. Krenke, P. V. Mandrekar, R. Nelson, K. Peterson, J. Shultz, A. Tereba and N. Westphal (2001). "Development of a human DNA quantitation system." Croat Med J 42(3): 336-9.

Moretti, T. R., A. L. Baumstark, D. A. Defenbaugh, K. M. Keys, J. B. Smerick and B. Budowle (2001). "Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples." J Forensic Sci 46(3): 647-60.

Nazarenko, I. A., S. K. Bhatnagar and R. J. Hohman (1997). "A closed tube format for amplification and detection of DNA based on energy transfer." Nucleic Acids Res 25(12): 2516-21.

Nicklas, J. A. and E. Buel (2003). "Development of an Alu-based, QSY 7-labeled primer PCR method for quantitation of human DNA in forensic samples." J Forensic Sci 48(2): 282-91.

Nicklas, J. A. and E. Buel (2003). "Development of an Alu-based, real-time PCR method for quantitation of human DNA in forensic samples." J Forensic Sci 48(5): 936-44.

Northrup, M. A., B. Benett, D. Hadley, P. Landre, S. Lehew, J. Richards and P. Stratton (1998). "A miniature analytical instrument for nucleic acids based on micromachined silicon reaction chambers." Anal Chem 70(5): 918-22.

Romppanen, E. L., K. Savolainen and I. Mononen (2000). "Optimal use of the fluorescent PicoGreen dye for quantitative analysis of amplified polymerase chain reaction products on microplate." Anal Biochem 279(1): 111-4.

Rye, H. S., J. M. Dabora, M. A. Quesada, R. A. Mathies and A. N. Glazer (1993). "Fluorometric assay using dimeric dyes for double- and single-stranded DNA and RNA with picogram sensitivity." Anal Biochem 208(1): 144-50.

Shewale, J. G., E. Schneida, J. Wilson, J. A. Walker, M. A. Batzer and S. K. Sinha (2007). "Human genomic DNA quantitation system, H-Quant: development and validation for use in forensic casework." J Forensic Sci 52(2): 364-70.

Sifis, M. E., K. Both and L. A. Burgoyne (2002). "A more sensitive method for the quantitation of genomic DNA by Alu amplification." J Forensic Sci 47(3): 589-92.

Singer, V. L., L. J. Jones, S. T. Yue and R. P. Haugland (1997). "Characterization of PicoGreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation." Anal Biochem 249(2): 228-38.

Swango, K. L., M. D. Timken, M. D. Chong and M. R. Buoncristiani (2006). "A quantitative PCR assay for the assessment of DNA degradation in forensic samples." Forensic Sci Int 158(1): 14-26.

Tan, E., H. S. Giese, et al. (2009). "Microfluidic DNA Extraction and Purification from Forensic Samples: Towards Rapid, Fully Integrated STR Analysis." National Criminal Justice Reference Service, NCJ 226810, (http://www.ncjrs.gov/app/Publications/abstract.aspx?ID=248809).Tanious, F. A., J. M. Veal, H. Buczak, L. S. Ratmeyer and W. D. Wilson (1992). "DAPI (4',6-diamidino-2-phenylindole) binds differently to DNA and RNA: minor-groove binding at AT sites and intercalation at AU sites." Biochemistry 31(12): 3103-12.

Timken, M. D., Swango, K. L., Orrego, C., Buoncristiani, M. R. (2005). "A duplex real-time qPCR assay for the quantification of human nuclear and mitochondrial DNA in forensic samples: implications for quantifying DNA in degraded samples." J Forensic Sci 50(5): 1044-60.

Tomsey, C. S., M. Kurtz, F. Kist, M. Hockensmith and P. Call (2001). "Comparison of PowerPlex 16, PowerPlex1.1/2.1, and ABI AmpfISTR Profiler Plus/COfiler for forensic use." Croat Med J 42(3): 239-43.

Tyagi, S. and F. R. Kramer (1996). "Molecular beacons: probes that fluoresce upon hybridization." Nat Biotechnol 14(3): 303-8.

Tyagi, S., S. A. Marras and F. R. Kramer (2000). "Wavelength-shifting molecular beacons." Nat Biotechnol 18(11): 1191-6.

Van Dyke, K. and C. Szustkiewicz (1968). "Automated systems for the fluorometric determination of nucleic acids by the ethidium bromide technique." Anal Biochem 23(1): 109-16.

Walsh, P. S., J. Varlaro and R. Reynolds (1992). "A rapid chemiluminescent method for quantitation of human DNA." Nucleic Acids Res 20(19): 5061-5.

Whitcombe, D., J. Theaker, S. P. Guy, T. Brown and S. Little (1999). "Detection of PCR products using self-probing amplicons and fluorescence." Nat Biotechnol 17(8): 804-7.

Wittwer, C. T., M. G. Herrmann, A. A. Moss and R. P. Rasmussen (1997). "Continuous fluorescence monitoring of rapid cycle DNA amplification." Biotechniques 22(1): 130-1, 134-