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TECHNICAL NOTE

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Quantitation of Human Genomic DNA Through Amplification of the Amelogenin Locus*

ABSTRACT: An alternate method for quantitation of human genomic DNA is presented. Quantitative template amplification technology (abbreviated "Q-TAT") estimates the quantity of human DNA present in an extract by comparing fluorescence in X and Y amplicons produced from unknowns with fluorescence in a standard curve amplified from known quantities of reference DNA. Q-TAT utilizes PCR and electrophoresis with fluorescent detection/quantitation, precluding the need for new instrumentation, methodology, or quality assurance associated with slot-blot or real-time PCR. In a comparison study incorporating shared samples, Q-TAT was found to be more sensitive than widely used slot-blot methods but somewhat less sensitive than real-time PCR. Among samples containing DNA concentrations ranging from 100 pg/μL to 2–4 ng/μL, Q-TAT produced DNA concentration estimates that agreed reasonably well with either Quantiblot® or real-time PCR. Q-TAT was reproducible with a typical coincidence of variation of about 35%. Quantitation of human DNA in this study involved summing fluorescence in X and Y amplicons in unknowns and quantitation standards. However, analyzing fluorescence in X and Y amplicons individually could allow estimates of male and female DNA present in mixtures to be made. Moreover, since X and Y amplicons exhibit sizes of 210 and 216 bp, respectively, the integrity as well as the concentration of the genomic DNA template can be assessed. Q-TAT represents an alternate method useful for the quantitation of human genomic DNA prior to amplification of STR loci used for identity testing purposes. The method uses existing equipment and procedures in conjunction with a well-characterized DNA standard to produce concentration estimates for unknowns that reliably produce STR profiles suitable for analysis.

KEYWORDS: forensic science, DNA quantitation, capillary electrophoresis, PCR amplification, amelogenin locus, small tandem repeat (STR) loci

It is common practice for forensic laboratories to quantitate the amount of human genomic DNA recovered from evidentiary biological samples. Motivations for the quantitation of PCR template to be used for multiplex amplification of STR loci include minimizing the amplification of partial DNA profiles, minimizing allele dropout or imbalance if template amounts are too low, or off-scale data, allelic/locus imbalance, or other spurious artifacts when the input template is too high (1–4). In addition, the laboratory is required to consume only what is needed to produce a result and retain the remaining evidence and/or DNA for independent testing by a different laboratory, should the court so order.

Standard 9.3 in the Quality Assurance Standards for Forensic DNA Testing Laboratories mandates that forensic DNA typing laboratories determine the amount of human genomic DNA recovered from samples (5). Any laboratory desiring accreditation/certification by accrediting agencies using these or other similar standards must quantitate human DNA recovered from biological evidence that is to be used for DNA typing.

Current nonspecific methods available for DNA quantitation include the absorbance of ultraviolet light at 260 nm, quantitation of fluorescence in genomic DNA co-electrophoresed with known amounts of DNA in a "yield gel," and quantitation of fluorescence produced by intercalating fluorescent dyes (6). Specific methods for quantitating human chromosomal DNA include quantitative hybridization of human DNA probes to slot blots or dot blots of dilutions of DNA from unknowns and quantitation standards (7), quantitative measurement of fluorescence produced by dyes intercalating in Alu sequences amplified from human genomic DNA (8,9), and fluorescence produced by accumulating DNA product measured after each extension step in a PCR cycling program (i.e., real-time PCR) (10–13). Real-time PCR methods incorporate primers that target specific genomic sequences in human DNA whose accumulation during repeated rounds of amplification can be measured and is proportional to the amount of input DNA template. Included among human loci targeted for real-time PCR are Alu sequences scattered throughout the genome (8) or sites on the X and Y chromosomes (11) whose amplification can provide data not only on DNA quantity, but also on the sex of the sample and the possible existence of male:female mixtures in extracts. Of the methods described above, only those that use of human-specific probes or primers will meet the intent of the quality assurance standards in that they will produce an estimate of human DNA quantity.

A quantitative template amplification methodology (called Q-TAT) for reliably quantitating the amount of human DNA extracted from biological samples is described here. The method involves quantitation of fluorescence incorporated into amplicons from the X and Y chromosomes produced from samples with unknown amounts of DNA that are compared with fluorescence in X

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and Y amplicons amplified from known amounts of reference DNA template and used to create a standard curve. To perform the Q-TAT assay, a commercially available human sex typing kit, a thermal cycler, and a genetic analyzer are all that are required. The Q-TAT method will detect as little as 20–30 pg of human DNA and has a dynamic range that extends to about 500 pg. In addition, because the primers direct the amplification of products 210 and 216 bp from the X and Y chromosomes, respectively, the assay also provides information regarding the integrity of the DNA template.

In summary, the Q-TAT assay for the quantitation of human genomic DNA is both sensitive and reproducible, and enables a forensic DNA typing lab to use existing technology and instrumentation to estimate accurately the amount of human genomic DNA recovered from biological evidence. Incorporating the Q-TAT method into the normal workflow in the lab may be more attractive to some laboratories than making the commitment to add real-time PCR technology, which will involve funding, space allocation, training, and other quality assurance/quality control (QA/QC) issues.

Materials and Methods

DNA Extraction

DNA was extracted from nonprobative forensic samples used in this study with standard methods using SDS and proteinase K digestion followed by phenol:$CHCl_3$:isoamyl alcohol (9:0.96:0.04 vol/vol) extraction (14). Reference samples were extracted using the same methods, or, using inorganic extraction methods based on the original work of Miller et al. (15). The male and female DNA used to produce the standard curves were extracted from blood samples supplied by lab workers, and were dispensed in aliquots in 10 mM Tris-Cl pH 8.3+0.1 mM EDTA (TE-4) and stored at $-20^{\circ}C$ for repetitive use. Quantities of DNA in these reference standards were established by absorbance at 260 nm as well as through the use of a yield gel, comparing ethidium bromide fluorescence produced by a range of known concentrations of lambda DNA co-electrophoresed with dilutions of the human genomic DNA samples to be used as quantitation standards. Concentration estimates of the human standards were further evaluated by amplifying what was calculated to be a 1 ng aliquot of reference DNA (based upon the A_{260} and yield gel estimates) with the Profiler Plus STR typing kit (Applied Biosystems, Foster City, CA) according to recommendations from the supplier. The peak relative fluorescence unit (rfu) measurements of alleles amplified from the reference template exhibited average peak heights of 1000–3000 rfu, which is consistent with expectations when using 1 ng of a good-quality DNA template.

PCR Amplification and Genetic Analysis

Samples were amplified by PCR using either a PTC-200 (MJ Research, Reno, NV) or an ABI 9700 thermal cycler (Applied Biosystems, Foster City, CA). Profiler Plus amplifications were set up following the instructions supplied with the STR typing kit (Applied Biosystems, Fullerton, CA).

Amplification of X and Y products using the amelogenin, sex typing kit (Promega Corp., Madison, WI) was performed as directed by the kit instructions, except that Ampli-Taq Gold DNA polymerase (Applied Biosystems, Foster City, CA) and GoldSTR reaction buffer (Promega Corp.) were substituted in place of the reagents recommended or supplied with the kit. In addition, 12.5 μL reaction volumes were substituted for the 25 μL volumes specified in the manufacturer's protocol. The primers in this kit are labeled with fluorescein and are reasonably specific for primate DNA, although small amounts of product whose size differs from the human products can be produced with the genomic DNA from other species as well (16).

Amplification products produced either with the Amelogenin or Profiler Plus kits were mixed with formamide (Applied Biosystems, Foster City, CA) containing GS350 or GS500 size standards (Applied Biosystems, Foster City, CA) according to the recommendations of the supplier. The samples were then subjected to electrophoresis and fluorescent analysis with the aid of an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Each Q-TAT assay incorporated the creation of a standard curve produced by quantitating fluorescence in X or XY amplicons produced with 0, 31.25, 62.5, 125, 250, or 500 pg of human reference DNA template. The standard curve was produced as follows: DNA serving as the human quantitation standard was diluted to 500 pg/μL in dH_2O initially and then serially diluted twofold to establish the concentration range. One microliter aliquots of each dilution were then used as templates for amplification of X, or X and Y alleles in a 12.5 μL PCR reaction. Each assay also included a 100 pg aliquot of positive control DNA contained within the Profiler Plus STR typing kit (Applied Biosystems, Foster City, CA) to serve as an internal quantitation control.

Data Analysis

For estimation of the DNA concentrations in unknowns, the relative fluorescence in the X (and possibly Y) amplicons was compared with the fluorescence in XY amplified from known amounts of reference DNA template using the following strategy: the area under the curves of the X/Y products in each dilution of reference DNA was summed and normalized for the different injections through comparison with the total rfu contained within the 200 bp standard present in the GS350 or GS500 size standards. Since the size standard mixture is present in constant amounts in each sample (due to the use of a master mix of formamide/size standard that is prepared and aliquoted into the sample tubes needed for each assay), variation in the fluorescence in X/Y amplicons resulting from subtle injection fluctuations or laser/CCD camera variability can be normalized across the run. Normalized fluorescence in X/Y amplicons per input ng of reference DNA could then be computed from the slope of the standard curve. Fluorescence in X/Y products from unknowns was simply plotted on the standard curve to estimate their respective template concentrations.

DNA Quantitation Using Quantiblot® (QB) and Real-Time PCR

Estimates of DNA concentration in samples processed by the Tulsa Police Laboratory were produced using blotting/hybridization methods incorporated into the QB kit available from Applied Biosystems (Foster City, CA). QB estimates were made following colorimetric visualization of probe hybridization to quantitation standards and unknowns slot-blotted onto a nylon membrane according to manufacturer's instructions. The highest concentration of quantitation standard on the slot blot contained 8 ng/μL (the QA++ standard) of human genomic DNA supplied with the QB kit. Serial twofold dilutions were made of this DNA sample and blotted on the membrane with the least concentrated sample corresponding to 0.0325 ng/μL (the QG sample). In estimating the DNA concentration in unknowns, however, any sample producing

a color fainter than the 0.125 ng/μL standard (the QE standard) was labeled as <QE and no attempt was made by the analyst to estimate the concentration more accurately.

DNA samples quantitated using real-time PCR were kindly provided by Dr. Arthur Eisenberg, Center for Health Sciences, University of North Texas State, Fort Worth, TX. DNA was quantitated in those samples using a 7000SDS thermal cycler and the Quantifiler kit (both obtained from Applied Biosystems, Foster City, CA).

Results

The strategy for quantifying human genomic DNA in extracts was to generate a standard curve of fluorescence in amelogenin amplicons produced from known quantities of input human template DNA. Highly reproducible standard curves could be produced using either male or female reference human genomic DNA (Fig. 1). Substituting female rather than male DNA as the quantitation standard had no significant effect on the concentration estimates for unknowns (not shown). This is likely due to the comparable efficiency of amplification of the amelogenin gene on the X and Y chromosomes. Thus, each copy of the amelogenin gene on each of the two X chromosomes in a female would produce the same amount of total amplicon fluorescence as the product amplified from one copy of the gene on X and the other located on Y in the male.

Several features of the Q-TAT method are apparent from Fig. 1. The sensitivity of the method for reliably detecting human DNA is operationally about 20–30 pg. This level of sensitivity should adequate for most forensic applications. It is also apparent from Fig. 1 that one limitation of the assay is the dynamic range, which extends only over a 10–20-fold span of concentration before the curve begins to plateau, possibly due to depletion of primers or other reactants in the amplification reaction, or for as yet undefined reasons as discussed by Morrison and Gannon (17). Reducing the number of cycles could help alleviate this problem, but sensitivity could be compromised as a consequence. As an alternative approach, a small dilution series of an extract would likely produce a result within the acceptable range of the standard curve. The PCR conditions chosen therefore represent a good working balance between sensitivity and usable concentration range for input DNA.

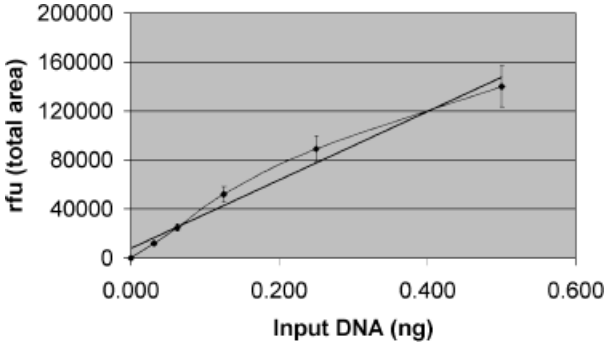

FIG. 1—*Standard curves produced using male reference DNA and quantitative template amplification technology in 10 independent assays. Standard curves were produced by amplifying 0, 31.25, 62.5, 125, 250, and 500 pg aliquots of male reference DNA using the sex typing kit as described in Materials and Methods. At each data point, the mean fluorescence incorporated into the X and Y amplicons from 10 separate assays is shown along with error bars reflecting a coincidence of variation of approximately 25% at all concentrations of input reference DNA. A best fit of the data from the 10 separate assays ($R^2 = 0.9751$) is also shown.*

The true test of any new method is whether it passes validation through comparison to existing, accepted methods. As part of the Q-TAT validation process, Q-TAT was used to quantitate DNA in a cohort of samples supplied by other labs that had been subjected to prior quantitation using Quantiblot® (QB) or real-time PCR. The results of the comparisons are shown in Figs. 2 and 3. Comparison between DNA concentration estimates by QB and Q-TAT are shown as scatter plots in Fig. 2A. Most samples showed reasonable agreement in the estimates made by the two methods, and there was no indication of a possible bias inherent in either method for estimating DNA concentration. As might be expected however, very low or very high concentrations of DNA exhibited the greatest disagreement between the two methods. Part of the explanation for the variation in estimates could be due to the more subjective nature of estimation using the QB method, which directs an analyst to try and match the color intensity of an unknown to one of the twofold dilutions of the control DNA, a process very much like yield gel methods. If an unknown contained a DNA concentration lying in the middle of a twofold range bracketed by

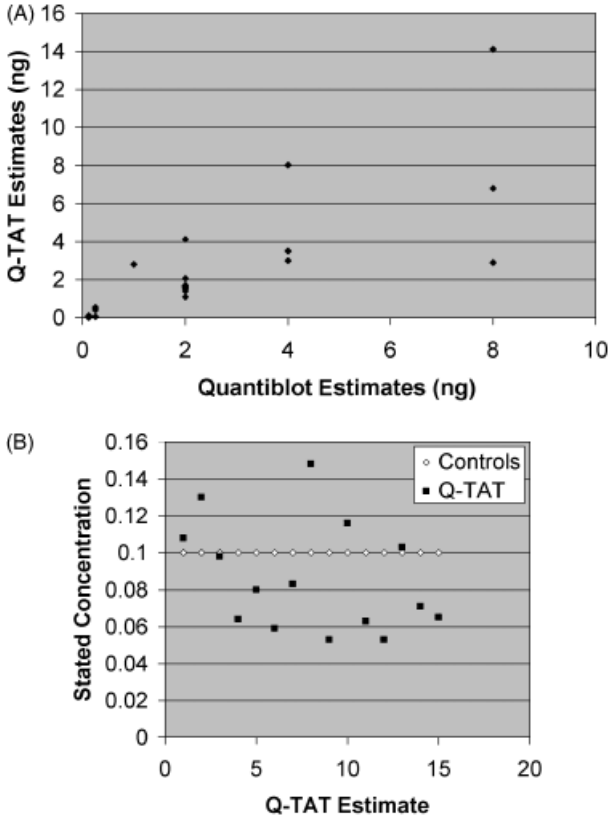

FIG. 2—*(A) Comparison of Quantiblot® and quantitative template amplification technology (Q-TAT) quantitation methods with shared human DNA samples. A group of 30 nonprobative human DNA samples extracted from suspects, victims, and evidentiary items were quantitated with both quantiblot (QB) and Q-TAT. Estimates of DNA concentration produced for each sample with the two methods were then plotted as a scatter plot. DNA concentration estimates were made using QB by an analyst with several years experience using the method and consisted of assigning unknowns to concentration categories spanning a range from QA++ (8.0 ng/μL) to <QE (<0.125 ng/μL). The analyst did not incorporate the QF (0.06 ng/μL) and QG (0.03 ng/μL) categories suggested in the instructions provided with the QB kit into concentration estimates of unknowns. (B) Estimates of DNA quantity in the positive control sample in Profiler Plus and Identifiler STR typing kits. The vial of positive control DNA sample supplied with STR typing kits from Applied Biosystems was used as a quantitation control in each Q-TAT assay. Quantity estimates obtained by Q-TAT were plotted along with the quantity of DNA indicated on the vial in the STR typing kit.*

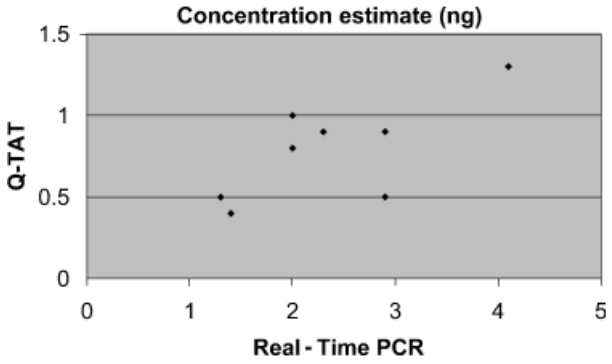

FIG. 3—*Comparsion of real-time PCR and quantitative template amplification technology (Q-TAT) quantitation methods with shared human DNA samples. A group of 10 DNA samples previously quantitated using real-time PCR (with the Quantifiler kit from Applied Biosystems, Inc.) was subjected to quantitation using Q-TAT.*

the quantitation standards, more variation might be expected in estimating the concentration of that sample. Moreover, unknowns exhibiting either very low or very high intensities of color would be expected to exhibit the greatest variability in concentration estimate in the same way as estimates made from yield gels.

An alternate approach to evaluate the accuracy of the Q-TAT method is to quantify DNA contained within known samples. As a normal component of every Q-TAT assay, a 100 pg aliquot of positive control DNA supplied with Profiler Plus or Identifiler STR typing kits was included as a "quantitation control." Results from 15 separate assays are shown in Fig. 2B. The average Q-TAT estimate of DNA in these assays was 86.2 pg and the coincidence of variation was about 34%. Assuming that the concentration of DNA present in the positive control supplied with the STR typing kits is accurate, Q-TAT may exhibit a bias to underestimate the amount of human DNA in a sample.

DNA concentration comparisons from real-time PCR and Q-TAT are shown in Fig. 3. As was observed above, both methods produced concentration estimates that agree reasonably well. Interestingly, whereas concentration estimates from QB and Q-TAT exhibited variation that was random demonstrating no obvious bias, real-time PCR consistently yielded an estimate that was higher than Q-TAT (Fig. 3).

It is also imperative for any method used for DNA quantitation to be reproducible. The coincidence of variation calculated for repeated measurements of DNA in a total of 10 samples (40 measurements) containing approximately 0.5–1.0 ng/μL using Q-TAT and seven samples (28 measurements) in the 0.1 ng/μL range was about 35%. This is comparable to the reproducibility exhibited by Quantiblot® technology (not shown).

Discussion

Quality assurance standards upon which audits by the American Society of Crime Lab Directors, Laboratory Accreditation Board and the National Forensic Science Training Center are based mandate the quantitation of human DNA recovered from any evidentiary items subjected to STR analysis in the forensic laboratory. The reasons for the mandate revolve around ensuring the quality of the DNA–STR profiles produced since currently available multiplexes are affected by the quantity of input human DNA template (1–3). Since most items of biological evidence will serve as a growth substrate for micro-organisms, DNA quantitation methods that are not specific for human DNA will not accurately quantify the amount of human template present in an extract that may also contain non-human DNA. An additional reason to quantify human DNA in an extract relates to the preservation of as much template as possible for repeat testing by another laboratory, should this be requested by the court.

Current methods for quantifying human DNA include slot blot/probe hybridization available currently in the Quantiblot® kit. This technology is one that stands alone in the forensic laboratory, requiring specialized methods and equipment not used for other procedures in a DNA section. Sensitivities for Quantiblot® technology range from approximately 150 pg to 10 nanograms (package insert from the Quantiblot® kit), which is less sensitive than the Q-TAT technology, but has a much broader dynamic range.

An approach to quantifying human DNA very similar to Q-TAT was reported by Sifis et al. in 2002 (9). Rather than using fluorescence incorporated into amelogenin amplicons, these authors measured fluorescence incorporated into amplicons amplified from the *Alu* family of short interspersed repeats. Like the amelogenin locus, the *Alu* sequences are primate specific, thereby making the assay useful for quantifying human DNA. The assay reported by Sifis et al. (9) exhibited a useful range of 2.5–100 pg of human genomic DNA. This level of sensitivity is higher than the sensitivity reported here, although the upper limit of DNA that can be estimated is slightly higher in the Q-TAT assay. The principal difference between the two assays, and one advantage of Q-TAT, is that the technique quantitates fluorescence in PCR products from the X and Y chromosomes, which creates the potential for identifying male:female mixtures of DNA present in extracts from forensic evidence. At a minimum, Q-TAT can suggest whether a male:female mixture of DNA is present in an extract, and with sufficient validation, it may be possible to use Q-TAT to estimate the relative proportions of male and female DNA in a mixed sample.

A new and rapidly growing technology for the quantitation of human DNA is real-time PCR (8,10–12). Primers exist for several loci, including the X and Y chromosome, that make the amplification specific for primate DNA, and the incorporation of intercalating dyes into PCR product at the end of each PCR cycle, or the cleavage of quenched fluors attached to primers through a Taq-man strategy allows for quantitation of product buildup (8,10–12). The sensitivity of real-time PCR is better than Q-TAT, and the dynamic range is much higher because quantity estimates are in part based upon the slope of the rate of increase in PCR product with each successive round of amplification. Therefore, in amplifications containing a high concentration of DNA template, early rounds of amplification would demonstrate a dramatic rate of increase in PCR product, and an accurate estimate of template quantity could be made after early rounds of the cycling program before primers and other reactants become limiting. Q-TAT technology, on the other hand, requires all cycles to be completed before quantitation occurs. For samples with a high concentration of DNA, reactants could become limiting, making accurate quantitation impossible. Analysts must therefore examine concentration estimates from extracts demonstrating a high concentration of DNA carefully and perhaps requantitate that sample(s) using a dilution series to obtain an accurate estimate of the DNA present. While this may appear to be a significant limitation of the Q-TAT methodology, in our experience, analysts can incorporate features of an evidentiary sample being extracted into a theoretical estimate of how much DNA will be recovered from that sample and bracket a useful template input initially using several dilutions of the unknown to obtain a reliable estimate of DNA concentration. In fact, a lab may wish to amplify 2–3 dilutions of every unknown as a standard procedure. Amplifying a

TABLE 1—*Considerations among three methods for DNA quantitation.*

Method	Quantiblot®	RT-PCR	Q-TAT
Start-up	≈ $2,000	≈ $50,000	Existing equipment
Reagent costs	≈ $100/48 samples	≈ $400/96 samples	≈ $150/200 samples (at 12.5 μL)
QA	15 reagents plus one kit tracked; three instruments maintained; waterbath vacuum pump, slot-blot apparatus	One to two kits tracked (X or Y)	One new kit tracked (X and Y)
		Three new instruments: ABI Prism7000, computer, 96-well plate spinner	No new reagents
			No new instruments
QC	Built in with each use	Five additional monthly checks: Tungston lamp life, 7000 function Test background assay, RNaseP verification	Built in with each use

Q-TAT, quantitative template amplification technology.

small dilution series can also reveal the presence of PCR inhibitors in evidentiary samples, the logic being that more diluted samples would reveal the presence of greater amounts of DNA than less diluted or undiluted samples in the series.

The Q-TAT, Quantiblot®, and real-time PCR quantitation methods all rely on comparing the amount of DNA in an unknown to some well-characterized (and quantitated) reference standard. In the case of Q-TAT, a standard curve consisting of fluorescence contained within amelogenin products amplified from different amounts of reference DNA was used to estimate amounts in unknowns. Although the work reported here was performed using a male DNA sample to prepare the standard curve, use of a female reference sample produced comparable concentration estimates as long as the area under both the X and Y products was summed before comparison with the standard curve prepared from female DNA. This is not surprising since single copies of the amelogenin locus reside on the X and Y chromosomes and are amplified with roughly equal efficiency. Hence, the amelogenin locus on the two copies of the X chromosome in a female exhibit molar equivalence to amelogenin loci on a single copy of X and a single copy of Y in the male.

The Q-TAT assay may also enable an estimate of male and female DNA in a mixed sample from a sexual assault by plotting on the fluorescence under the Y amplicon with comparison with Y amplicon fluorescence in a standard curve prepared from male DNA. Such a validation is being planned and would further enhance the utility of Q-TAT as a quantitation tool in a DNA typing laboratory.

Comparison of concentration estimates between real-time PCR and Q-TAT indicated a consistently higher estimate made using real-time PCR. Likewise, real-time PCR consistently produces estimates that are higher on samples also tested using QB methods (Dr. Arthur Eisenberg, University of North Texas State, personal communication). In contrast, comparisons between Q-TAT and QB exhibited a random pattern of variation in concentration estimates among the shared samples. These results may indicate a bias of real-time PCR to overestimate human DNA concentrations, or an underestimation bias characteristic of QB and Q-TAT. In this regard, replicate measurements of the positive control supplied with STR typing kits may suggest that Q-TAT underestimates DNA quantities. Ultimately, the requirement for any quantitation assay is reliability. If a quantitation method underestimates or overestimates human DNA, the bias can be identified through validation and considered when amplifying a template for STR results. As long as the amount of DNA template amplified reliably produces a DNA profile that is of sufficient quality to interpret, the limitations of the assay used to quantitate DNA in a sample are not so critically important.

Some considerations related to the use of QB, real-time PCR, and Q-TAT are summarized in Table 1. Included among the considerations are start-up costs, sample processing costs, and QA/QC costs for each of the three technologies. Among the methods, Q-TAT appears to be the least expensive technology to set up and maintain (Table 1). Q-TAT is performed using existing equipment and essentially a methodology identical to that in place for STR analysis. In contrast, real-time PCR appears to be the most expensive to set-up and maintain with an initial capital expense of almost $50,000 and ongoing costs associated with newly added QA/QC (Table 1). Although grants are being made available to crime labs for the purchase of the instrumentation required for real-time PCR technology, there are going to be cost, space, and maintenance issues for some labs that will not be covered by a grant. Q-TAT does not require any new equipment or additional space in the DNA typing laboratory. Implementation of the Q-TAT method relies upon the use of existing thermal cyclers and DNA analysis instrumentation to perform the assay. A second advantage of Q-TAT is that it is the same technology used routinely in the forensic lab for processing casework. Thus, technologists do not need to be trained for a different technology beyond that used for routine DNA typing. In a crime lab already strained to keep up with casework due to personnel limitations, this can be a significant advantage. Additionally, the procedures used for Q-TAT are also amenable to automation in the same way as high throughput STR typing. PCR setup, sample preparation, and analysis can all be automated allowing maximal efficiency for processing casework. Finally, Q-TAT in a single PCR reaction provides information regarding the sex of the sample donor(s). In cases of sexual assault in which mixtures are anticipated, Q-TAT affords the opportunity to identify mixtures of male and female DNA and the possibly to estimate the relative contributions of DNA from each gender to an evidentiary sample.

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References

1. The Perkin Elmer Corporation. AmpFlSTR® profiler plus, PCR amplification kit, user's manual. San Jose, CA: The Perkin Elmer Corporation; 1998.
2. Applied Biosystems. AmpFlSTR® identifiler PCR amplification kit, user's manual. Foster City, CA: Applied Biosystems; 2001.
3. Kline MC, Duewer DL, Redman JW, Butler JM. NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. Anal Chem 2003;75:2463–9.
4. Butler JM. Forensic DNA typing. Biology, technology, and genetics of STR markers. 2nd ed. Burlington, MA: Elsevier Academic Press; 2005.
5. DNA Advisory Board, Federal Bureau of Investigation. Quality assurance standards for forensic DNA testing laboratories. Washington, DC: Federal

Bureau of Investigation; 2000. Accessible at: http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm#Introduction.
6. Singer VL, Jones LJ, Yue ST, Haugland RP. Characterization of PicoGreen reagent and development of a fluorescent-based solution assay for double stranded DNA quantitation. Anal Biochem 1997;249:228–38.
7. Walsh P, Varlaro J, Reynolds R. A rapid chemiluminescent method for quantitation of human DNA. Nucl Acids Res 1992 Oct 11;20(19):5061–5.
8. Nicklas JA, Buel E. Development of an Alu based, real-time PCR method for quantitation of human DNA in forensic samples. J Forensic Sci 2003;48:936–44.
9. Sifis ME, Both K, Burgoyne LA. A more sensitive method for the quantitation of genomic DNA by *Alu* amplification. J Forensic Sci 2002;47:589–92.
10. Nicklas JA, Buel E. Development of an Alu based, QSY7-labeled primer PCR method for quantitation of human DNA in forensic samples. J Forensic Sci 2003;48:282–91.
11. Alonso A, Marten P, Albarran C, Garcia P, Garcia O, Fernandez de Simon L. Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies. For Sci Int 2004;139:141–9.
12. Walker JA, Kilroy GE, Xing J, Shewale J, Sinha SK, Batzer MA. Human DNA quantitation using Alu element based polymerase chain reaction. Anal Biochem 2003;315:122–8.
13. Andreasson H, Gyllensten U, Allen M. Real-time PCR quantification of nuclear and mitochondrial DNA in forensic analysis. Biotechniques 2002;33:402–11.
14. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1982.
15. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
16. Micka KA, Amiott EA, Hockenberry TL, Sprecher CJ, Lius AM, Rabbach DR. TWGDAM validation of a nine-locus and a four-locus fluorescent STR multiplex system. J Forensic Sci 1999;44:1243–57.
17. Morrison C, Gannon J. The impact of the PCR plateau phase on quantitative PCR. Biochim Biophys Acta 1994;121:493–8.

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