Visual Automated Fluorescence Electrophoresis Provides Simultaneous Quality, Quantity, and Molecular Weight Spectra for Genomic DNA from Archived Neonatal Blood Spots

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Sudden unexpected death in epilepsy (SUDEP) is the most common cause of mortality in people with seizure disorders, and the mechanisms of SUDEP are incompletely understood. Candidate molecular risk factors include defects in ion channel genes linked to long QT syndrome,1 epilepsy,2 and genes of the serotonergic pathways involved in arousal and respiration.3 Precision molecular autopsy4,5 and diagnostics,6,7 as well as personalized or familial risk prediction8–11 based on candidate gene profiling, are critically dependent on the availability of representative high-quality tissue samples.12 However, procurement of postmortem biological specimens often poses logistical, ethical, or cultural obstacles.13–15

The Guthrie 903 card archived dried blood spots (DBSs) are a unique but terminal resource amenable for individual and population-wide genomic profiling. The limited amounts of DBS-derived genomic DNA (gDNA) can be whole genome amplified, producing sufficient gDNA for genomic applications, albeit with variable success; optimizing the isolation of high-quality DNA from these finite, low-yield specimens is essential. Agarose gel electrophoresis and spectrophotometry are established postextraction quality control (QC) methods but lack the power to disclose detailed structural, qualitative, or quantitative aspects that underlie gDNA failure in downstream applications. Visual automated fluorescence electrophoresis (VAFE) is a novel QC technology that affords precise quality, quantity, and molecular weight of double-stranded DNA from a single microliter of sample. We extracted DNA from 3-mm DBSs archived in the Swedish Neonatal Repository for >30 years and performed the first quantitative and qualitative analyses of DBS-derived DNA on VAFE, before and after whole genome amplified, in parallel with traditional QC methods. The VAFE QC data were correlated with subsequent sample performance in PCR, sequencing, and high-density comparative genome hybridization array. We observed improved standardization of nucleic acid quantity, quality and integrity, and high performance in the downstream genomic technologies. Addition of VAFE measures in QC increases confidence in the validity of genetic data and allows cost-effective downstream analysis of gDNA for investigational and diagnostic applications. (J Mol Diagn 2013, 15: 283–290; http://dx.doi.org/10.1016/j.jmoldx.2013.01.003)

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The Guthrie 903 card archived dried blood spots (DBSs) are an established enduring medium frequently collected by neonatal registries and forensic laboratories.16,17 They are a unique resource amenable for individual and population-wide...
genomic profiling,\textsuperscript{18\textendash}21 considering the easy and cost-effective specimen collection, simple and stable long-term storage, and straightforward processing in nucleic acid extraction.\textsuperscript{15,22} In cases of sudden death among otherwise healthy individuals, the DBSs collected at birth may become the sole high-quality genomic sample to allow molecular autopsy, personalized diagnostics, and familial risk prediction.\textsuperscript{13,23} Because the DBSs represent a nonrenewable reserve, they require judicious allocation to projects of considerable public health impact with well-informed methods that maximize the source utility.\textsuperscript{17,18,22}

Currently, the actual DBS-derived DNA performance often fluctuates disproportionately to the qualitative and quantitative values predicted by the established methods of gel electrophoresis and spectrophotometry, respectively.\textsuperscript{15,22,24} Moreover, the visual estimate of DNA integrity derived from agarose gel is imprecise and consumes much of the precious original specimen. A new visual automated fluorescence electrophoresis (VAFE) technology used by the Agilent 2200 TapeStation (Agilent Technologies, Inc., Santa Clara, CA) enables precise and visible postextraction quality control (QC) of nucleic acids, including high-molecular-weight genomic DNA (gDNA). It allows qualitative and quantitative standardization of nucleic acids before their terminal commitment to downstream diagnostic or profiling technologies while requiring a negligible amount of DNA for accurate molecular characterization of the template. This translates into cost-effective use of the analytical platforms and increased confidence in the validity of the final genomic data.

We successfully extracted serial gDNA samples from 16 individual 3-mm DBSs collected on Guthrie 903 cards and deposited at room temperature in the Swedish Repository for \(\geq 30\) years. We performed the first systematic combined quantitative and qualitative analysis of aged archived DBS-derived DNA with VAFE before and after whole genome amplification (WGA)\textsuperscript{25,26} and in parallel to our extant, traditional methods—based QC paradigm (Figure 1A).\textsuperscript{15} Finally, we correlated the novel postextraction DNA QC with sample performance in PCR, sequencing, and a high-density comparative genome hybridization array.

**Materials and Methods**

**DBS Samples**

The Swedish Repository of DBS samples collected on Guthrie 903 cards for neonatal screening of inborn diseases provided 2 \(\times\) 3-mm blood spot punches in deidentified sterile microfuge tubes. There were total of 16 samples representing two decades: the 1970s (1975, 1976, 1977, and 1978) and the 1980s (1980, 1982, 1984, and 1986).

**gDNA Extraction from 3-mm Blood Spot**

A single 3-mm DBS from each sample was transferred to a fresh 1.5-mL microfuge tube. Using the QiaAmp Mini Spin Kit (Qiagen, Hilden, Germany), gDNA was extracted according to the manufacturer’s protocol for DBSs. In brief, the DBS was incubated for 10 minutes at 85°C in buffer ATL and then proteinase K solution was added. The sample was vortexed and incubated for 1 hour at 56°C to facilitate enzymatic digestion of the sample. Buffer AL was then added, the sample was incubated at 70°C for 10 minutes, absolute ethanol was added, and the sample was vortexed thoroughly to mix. The supernatant solution was applied to the QIAamp mini spin column and was bound to the column via a 1-minute centrifugation at 6000 \(\times\) g. Filter was discarded, and the sample was washed on the column by the application of buffer AW1, followed by a 1-minute 6000 \(\times\) g spin where the filtrate was discarded. Buffer AW2 was then applied, the sample was spun at 20,000 \(\times\) g for 3 minutes, the filtrate was discarded, and the spin column was centrifuged again for 1 minute at 20,000 \(\times\) g to remove all trace ethanol. The DNA-containing column was then moved to a clean 1.5-mL microcentrifuge tube, and 50 \(\mu\)L of DNAase/RNAase free water (Qiagen) prewarmed to 55°C was applied to the column. The columns were incubated with water for 2 minutes and collected by centrifugation. The 50-\(\mu\)L eluant was immediately reapplied to the column and collected by centrifugation to maximally recover and concentrate the gDNA in a small end volume.

**Whole Genome Amplification**

In the Repli-g Ultrafast WGA mini kit (Qiagen) a total input of 10 ng of DNA from each DBS was used per reaction according to the manufacturer’s protocol. Denaturation buffer D1 was added to template gDNA, vortexed to mix, and centrifuged to collect. The samples were incubated at room temperature (approximately 22°C) for 3 minutes. The reaction was then neutralized by the addition of buffer N1. A master mix containing reaction buffer and DNA polymerase was applied to the gDNA and incubated for 1.5 hours at 30°C, followed by a heat inactivation step of 3 minutes at 65°C. Samples were then moved into quality analysis.

In the Genomex WGA Kit (Sigma-Aldrich, St. Louis, MO) template DNA was fragmented by applying the 10× fragmentation buffer followed by an incubation at 94°C for 4 minutes. The library preparation buffer and library stabilization solution were added to the fragmented gDNA template, vortexed to mix, and centrifuged to collect, followed by 2-minute incubation at 95°C. The sample was cooled on ice and the library preparation enzyme was added. The reactions were placed in a PCR thermocycler and subjected to a cycle of 20 minutes at the following temperatures in succession: 16°C, 24°C, and 37°C, followed by 5-minute 72°C final elongation step. Amplification was performed using the WGA DNA polymerase and amplification master mix provided. The reactions were cycled 14 times in a PCR thermocycler after denaturation at 95°C for 3 minutes, denaturation at 94°C for 15 seconds, and a 65°C 5-minute anneal and extension step. Samples were moved into quality analysis.
Quantification and Integrity Analysis of Genomic Samples

Sample quantification was performed in parallel using the traditional QC algorithm versus a fluorescence-based VAFE method.

The Traditional QC Algorithm

Two microliters of pre- and post-WGA DNA from each sample was quantified using a 260/280 absorbance ratio on a Nanodrop1000 (ThermoFisher, Waltham, MA). After quantification, 2 µL of DNA was stored in a 0.2-mL sterile PCR tube for visualization on a slab gel. To assess gDNA integrity before and after WGA, 1% agarose/ethidium bromide gel using Tris-acetate-EDTA buffer was prepared. Samples reserved from quantification were prepared by the addition of 10 µL of sterile water and 3 µL of loading dye. Samples were loaded pairwise onto the gel with two known concentrations (275 and 12 ng) of reference gDNA extracted from venous blood. A total of 5 µL of Hyperladder I...
(Bioline, Taunton, MA) was used to identify samples of high (>10 kb) molecular weight.

The VAFE QC Algorithm

A fresh 1-μL aliquot of the sample was added to 10 μL of sample buffer, quantified, visualized, and qualitatively assessed on the Agilent 2200 TapeStation. The DNA molecular weight spectra were reviewed, and all data were saved and stored for future reference and comparative analysis.

PCR Analysis

Pre and post-WGA gDNA from DBSs of all ages was assessed by quality PCR amplification. Standard 50-μL PCRs using Apex Taq Polymerase (Genesee Scientific, San Diego, CA) were performed with oligonucleotide primers targeting known polymorphisms that contained exons from ion channelopathy genes. Amplicons ranging from 300 to 1000 bp in size were generated using 1 μL of input DNA (concentration range, 0.6 to 12.5 ng/μL) using a PCR program with 10-minute initial denaturation at 94°C, followed by 40 cycles of 94°C for 30 seconds, 56°C for 1 minute, 72°C for 1 minute, and a final elongation step of 10 minutes at 72°C. A positive control reaction using 12.5 ng/μL of reference DNA extracted from venous blood was included for comparison. Reactions were size resolved on a 2% agarose/ethidium bromide Tris-acetate-EDTA gel using a 10-kb ladder for size comparison.

Sanger Sequencing

The individual PCRs that yielded a single 1000-bp amplicon from the RYR2 gene were purified using the QiaQuick PCR Purification Spin Kit (Qiagen). Templates were quantified according to the specifications of the vendor on the Nanodrop1000, and approximately 10 to 15 ng of template was submitted for commercial Sanger sequencing (GeneWiz, South Plainfield, NJ). The resulting traces were visually validated, and chromatograms were aligned pairwise with pre- and post-WGA DBS data and compared across all samples.

Comparative Genome Hybridization Copy Number Variant Analysis

We assessed the utility of post-WGA DBS samples for comparative genomic hybridization (CGH) arrays for detection of genomic copy number variation (CNV) using our custom-designed, high-resolution epilepsy candidate gene array (Agilent 019033). The array has even coverage along the whole genome backbone and additional high-density exon probe coverage (approximately 3 to 5 probes per exon) across roughly 250 ion channel genes, along with additional monogenic epilepsy genes with variable density coverage of intronic probes. All CGH experiments were performed using the manufacturer’s recommended protocol and the Agilent Genome Enzymatic Label Kit. Briefly, 1.125 μg of each post-WGA sample and 1.125 μg of control female reference gDNA were digested with RsaI and AluI restriction enzymes for 2 hours at 37°C, followed by heat inactivation at 65°C for 20 minutes. The complete digestion was confirmed visually by running 2 μL of each sample on a 1% agarose/ethidium bromide Tris-acetate-EDTA gel. The remaining digested DNA was subsequently purified and labeled with either Cy-3-dUTP (reference DNA) or Cy-5-dUTP (post-WGA DNA). DNA was hybridized for 40 hours at 65°C under constant rotation in a light impermeable hybridization oven (Agilent Technologies). Arrays were then washed and stabilized before image acquisition on the SureScan D Scanner (Agilent Technologies). The resulting image was imported into Agilent Feature Extraction Software version 10.7.3.1 (Agilent Technologies) and analyzed for fluorescent signal log ratio generating a feature extraction file for each individual genome profile. The feature extraction files were analyzed in Genome Workbench (Agilent Technologies) using the built-in ADM-2 detection settings and the default aberration filter version 2. Data for this experiment and corresponding platform files can be viewed at http://www.ncbi.nlm.nih.gov/geo (accession number GSE44249).

Results

VAFE Provides Superior Accuracy in the Measurement of DBS-Derived DNA Concentration

The total DNA extracted from each DBS was quantified in parallel using a spectrophotometer (ThermoFisher Nanodrop1000) and a fluorescence-based system (Agilent 2200 TapeStation) (Figure 1A). Both methods detected measurable amounts of nucleic acids in all samples, and there was no definite correlation between the sample age and the DNA concentration, which is in agreement with data reported previously.17 However, a statistically significant difference was found in the total DNA per blood spot as measured by individual platforms (40 to 384 ng per the Nanodrop1000 versus 12 to 49 ng per the Agilent 2200 TapeStation; t = 3.68, P = 0.002) (Figure 1B). We confirmed measurement accuracy by using high-molecular-weight gDNA standards of known concentrations on both systems. We found that both performed well when quantifying high-quality double-stranded DNA (dsDNA). The sample detection limit was >1 ng/μL by the Nanodrop1000 and >0.5 ng/μL by VAFE (Supplemental Figure S1A). Using high-molecular-weight quantitative PCR standards, we found that the minimal concentration necessary for fluorescence-based reliable visual profiling of gDNA molecular-weight spectra was ≥2 ng/μL (Supplemental Figure S1B). These results support our earlier observation15 that the quantification based on absorbance ratio tends to overestimate DNA concentration in suboptimal or degraded samples. The ratiometric measure provides an estimate of sample purity, but it is blind to the nucleic acid integrity, length, or proportional representation of single- versus double-stranded helices, both of which can
act as a template for WGA amplification. In contrast, the VAFE sample buffer contains a SYBR green-based fluorophore, which intercalates with dsDNA molecules, giving an accurate measure of dsDNA below the concentration of <100 ng/μL.

VEFE Measures of Quantity and Quality Outperform Traditional Platforms and Facilitate Systematic WGA Amplification of gDNA from Archived Neonatal DBS Samples

A single 3-mm DBS averaged 26.3 ± 13.1 ng of total gDNA, an amount insufficient for many commercial or diagnostic high-throughput whole genome or whole exome sequencing or structural variant profiling assays, which typically require >1 μg of high-molecular-weight DNA to interrogate the entire genome. To overcame this limitation, we performed WGA on the archived DBSSs using 10 ng of extracted blood spot DNA as a template. The resulting amplified DNA samples were VAFE quantified, and all demonstrated an increase in total DNA amount (Figure 1C). Most samples (7 of 8) showed a 20 to 50 times increase, resulting in approximately 1 μg of total DNA content. A single sample dated 1982 relatively under-performed, and the final total amount was 154 ng of gDNA. Although sufficient sample quantity is important, sample utility critically depends on the proportional representation and availability of DNA fragments of usable length. To assess the integrity and molecular weight of the samples, pairs of the pre- and post-WGA samples were run on a traditional agarose slab gel in parallel with a 10-kb ladder and reference DNA extracted from venous blood (Figure 1D). As expected, the DBS-extracted original gDNA was not visible on the slab gel because of the low concentration (approximately 1 to 3 ng/μL) of DNA in these samples. However, the post-WGA samples were clearly observed and ran at the same apparent molecular weight as the reference venous blood—derived DNA. Notably, none of WGA blood spots showed any discernible signs of degradation smear at the lower size range.

The identical sample set was run on VAFE, which uses microelectrophoresis of the DNA sample through a precast gel tape using a high-molecular-weight ladder for size comparison. We then compared the results between the two systems. Unlike the slab gel, VAFE clearly shows a high-molecular-weight band >15 kb in size in all of the pre-WGA DNA extractions from decades-old blood spots. Analysis of the post-WGA sample confirmed that not only did the amplification produce substantially more dsDNA, but it also occurred preferentially in the high-molecular-weight range, and the post-WGA samples uniformly resembled the venous blood—derived DNA (Figure 1E). Individual samples were then analyzed with regard to the size profile of the DNA fragments (Figure 1F). Unlike the agarose slab gel, the VAFE clearly discriminated small but individually variable and age-independent degradation in all samples, as evidenced by the presence of lower-molecular-weight DNA fragments. The relative abundance of the high- versus lower-molecular-weight fragments was also variable between samples, suggesting that the pre- and post-amplification sample quality is better evaluated on an individual basis. In summary, the integrated quantity and quality measures from VAFE analysis define the integrity of archived DBS-derived gDNA, providing a QC parameter that is predictive for yielding high-molecular-weight gDNA after WGA.

VEFE-Established Sample Quality Confirms Utility in PCR, Sanger Sequencing, and CGH

Polymerase Chain Reaction

Our current QC paradigm for gDNA integrity involves an experimental assessment of DNA utility because samples may be variably visible on a traditional slab gel and the established 260/280 absorbance ratio—based quantification method may overestimate the content and integrity of the specimen. In contrast, VAFE can assess quality, quantity, and integrity of a gDNA sample simultaneously using a minimal volume of a discrete sample. This is extremely important when samples are limited and is critical in retrospective archival postmortem molecular diagnostic autopsy or research on genomic causality of sudden unexpected death. We successfully PCR amplified all pre- and post-WGA samples except for two samples that failed to amplify two different amplicons. A 1975 DBS sample failed to amplify the 300-bp amplicon of the 32nd exon of the RYR2 gene encoding the cardiac ryanodine receptor (Supplemental Figure S2). However, we saw the 1000-bp amplicon for exon 37 in the same gene in this sample, suggesting that the WGA DNA from 1975 was of sufficient quality and quantity to act as a template across the gene region. Similarly, the WGA sample from the 1986 blood spot failed to amplify the 1000-bp exon in HTR2A while producing a faint 1000-bp amplicon for RYR2 exon 37 and a robust 300-bp amplicon for exon 32 in RYR2 (Supplemental Figure S2). Our quality PCR standard operating procedure requires that any failed amplicons be repeated with the failed DNA sample to differentiate failure due to intrinsic (sample quality) versus extrinsic (technical flaw) factors (Figure 1A). All initially failed amplicons amplified on successive quality PCR assays, thus eliminating concerns regarding sample quality. It was reassuring to know that the inherently low concentration of the freshly extracted gDNA is sufficient to produce robust PCR amplification. This ensures that genetic variation identified in the WGA samples can be validated in the original blood spot gDNA to remove any potential false-positive variants that result from the WGA.

Sanger Sequencing

The 1000-bp fragment from RYR2 exon 37 was the template for commercial Sanger sequencing reactions (Supplemental Figure S3). All pre- and post-WGA blood spot samples were successfully sequenced with clear bp calls from the chromatogram. We consistently observed higher background
signal in pre-WGA samples in the <250-bp range; however, this background noise was insufficient to prevent the identification of heterozygous half height chromatogram peak reductions. In summary, our work supports the literature\(^\text{10–12,28}\) in that gDNA from decades-old infantile blood spots can be used successfully in traditional medical genetic resequencing studies to detect single-nucleotide polymorphism (SNP) variants that possibly contribute to the risk of epilepsy and SUDEP.

## Comparative Genomic Hybridization

The enzymatic digestion of the post-WGA DNA was successful, showing fragment size and distribution similar to the digested reference DNA (Supplemental Figure S4A), with the notable absence of high-molecular-weight undigested DNA. After purification and labeling, all of the post-WGA samples met QC requirements for the microarray, and hybridization was performed. The results were visualized (Supplemental Figure S4B), and all 8 individual arrays underwent successful feature extraction. The QC of the array data revealed that all arrays had a moderate background signal and a signal to noise ratio requiring visual assessment of probe distribution. Seven of the eight arrays passed QC. The 1978 post-WGA sample had an unacceptably high number of nonuniform outlier probes. Using the ADM-2 default algorithm in Genomic Workbench (Agilent), CNVs were detected in all samples. Because we performed our comparative hybridization blind to sex using a female reference DNA, we were able to use the CNV algorithm to identify male samples by comparing the copy number of the X chromosome. Female samples with XX genotype showed discreet small CNVs across the chromosome, whereas male XY samples showed a relative loss of X chromosome consistent with their XY genotype (Supplemental Figure S4C).

These results support the literature that post-WGA amplified gDNA from DBS sources is amenable to high-throughput array-based technologies.\(^\text{19,20,24,28}\) Unequal whole genome amplification needs to be considered, especially in degraded samples.\(^\text{25}\) However, previous studies comparing pre- and post-WGA gDNA samples from neonatal blood spots similar to ours reported >99% SNP concordance between amplified and native samples.\(^\text{24}\)

## Failed VAFE QC Informs Suboptimal gDNA Extractions from DBS

The previously extracted suboptimal samples that underperformed in our traditional QC paradigm were simultaneously analyzed with VAFE to compare and define the informative value of each platform and the reasons for sample failure. The 260/280 absorbance—based measurement suggested 6.8 to 11.8 ng/μL of gDNA across the four samples. However, the low concentration of the initial template was below the limit of visualization on the traditional agarose gel, indicating a lower gDNA concentration than indicated by the absorbance-based quantification. We performed WGA using two separate systems using different amplification strategies. The Repli-g Ultrafast WGA kit uses a multidisplacement amplification method, resulting in long DNA fragments (>10 kb), whereas the Genomeplex WGA kit uses the omniplex method producing short (500 bp) DNA fragments. The WGA kit using the Repli-g system augmented the total gDNA amount (Supplemental Figure S5A), and subsequent slab gel visualization indicated the existence of the high-molecular-weight gDNA in the presence of some degradation as evidence by smear (Supplemental Figure S5B). The Genomeplex WGA system only produced a moderate increase in DNA content (71.6 ± 29.3 ng total, \(n = 4\)) compared with the Repli-g system (1024 ± 239 ng total, \(n = 4\)) when using the same input template. Following our traditional QC protocol, the quality PCR was performed targeting amplicons ranging in size from 250 to 1000 bp in parallel on pre- and post-WGA samples. However, it failed across all samples except for the 250-bp fragment of the \(R\)YR2 gene generated on the pre-WGA blood spot extracted DNA (Supplemental Figure S5C).

Examination of the suboptimal samples pre- and post-WGA by VAFE revealed lesser than traditionally measured quantity of the starting template. Importantly, the electropherograms produced by the fluorescent signal clearly showed the substantial degradation of the initial gDNA template (Supplemental Figure S5, D and E). Both WGA systems tested increased the total gDNA amount after amplification. As predicted by the kit method, the Genomeplex WGA samples were composed of <500-bp DNA fragments. In contrast, most DNA molecules produced using the Repli-g system remained of low molecular weight, ranging in size from 400 to 4000 bp, indicating a lesser quality, fragmented input template from suboptimal DBS gDNA extractions (Supplemental Figure S5E). These smaller post-WGA fragments subsequently failed in downstream applications as demonstrated by the failed quality PCR. This outcome is also in agreement with our earlier observations.\(^\text{16}\) Analysis of the suboptimal samples showed that VAFE clarifies important parameters related to sample quality, quantity, and integrity to be considered before proceeding with further costly and time-consuming downstream genomic investigation.

## Discussion

The DBSs collected within the framework of neonatal screening program are an attractive alternative source of gDNA because of their low cost and ease of collection and long-term storage.\(^\text{17,18,22,29}\) The DBSs preserved and protected in the neonatal biobanks represent a multigenerational archive of populations and offer an unparalleled opportunity to assess both personal and population-based profiles of genetic variation.\(^\text{20,21,30}\) Moreover, they may be the only resource for a personal genomic profile in cases of sudden unexpected death.\(^\text{4,5,12,13,15,18,23,31}\) However, DBSs are a finite reserve. They need to be judiciously allocated to projects and used with utmost efficiency to maximize and extend the possibilities for use of individual samples.\(^\text{8,10,29}\)
The VAFE is an efficient QC technology that is ideally suited for projects that work with samples of minimal quantity or uncertain quality. Our data indicate that VAFE delivers reliable quantification of precious, much diluted gDNA samples extracted from decades-old DBS samples. Additionally, it is capable of providing complementary information simultaneously through the visualization of sample quality, integrity, and molecular-weight spectrum from a single microliter of gDNA. This platform demonstrated that gDNA extracted from decades-old infantile blood spots is composed of small fragmented DNA (<200 bp) and high-molecular-weight (>15 kb) molecules (Figure 1F), and if it is sufficient in quality and integrity, it is amenable to WGA amplification, resulting in large amounts (>1 µg) of predominantly high-molecular-weight DNA. The VAFE-based QC can identify suboptimal samples early in sample processing, and our follow-up experiments confirmed and correlated the failed QC with their limited utility in downstream applications, including the imprecise PCR (Supplemental Figure S4C). We also observed that the quality or utility of the DNA did vary across individual blood spots, indicating the need for systematic integration of reliable, efficient, and highly informative QC into sample preparation and assessment to maximize the quality and reliability of final genomic data and use of resources.

We have previously demonstrated a working effective QC paradigm that uses commonly available, albeit indirect and somewhat imprecise, methods for characterization of the gDNA properties extracted from alternative tissues, including DBSs. However, visual automated fluorescent electrophoresis represents a new-generation QC that delivers direct visualization and accuracy and that considers the imperative for minimal sample consumption. Results of this study will be applicable for projects that work with limited and fine DNA samples expected to deliver diagnostically accurate genomic data.

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**Supplemental Data**

Supplemental material for this article can be found at http://dx.doi.org/10.1016/j.jmoldx.2013.01.003.

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