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METHODS AND PROTOCOLS

Evaluation of different sources of DNA for use in genome wide studies and forensic application

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Abstract In the field of epidemiology, Genome-Wide Association Studies (GWAS) are commonly used to identify genetic predispositions of many human diseases. Large repositories housing biological specimens for clinical and genetic investigations have been established to store material and data for these studies. The logistics of specimen collection and sample storage can be onerous, and new strategies have to be explored. This study examines three different DNA sources (namely, degraded genomic DNA, amplified degraded genomic DNA and amplified extracted DNA from FTA card) for GWAS using the Illumina platform. No significant difference in call rate was detected between amplified degraded genomic DNA extracted from whole blood and amplified DNA retrieved from FTA™ cards. However, using unamplified–degraded genomic DNA reduced the call rate to a mean of 42.6% compared to amplified DNA extracted from FTA card (mean of 96.6%). This study establishes the utility of FTA™ cards as a viable storage matrix for cells from which DNA can be extracted to perform GWAS analysis.

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Introduction

The collection of biological samples on paper matrices is a common and routine practice. For example, use of Guthrie spots on filter papers to store and transport samples of blood from newborns by a heel prick method is standard practise. With advances in molecular techniques, specific preservatives and novel extraction chemicals have been developed to enhance papers. Flinders Technology Associates (FTA™) was developed to simplify the collection, shipment and archiving of a wide variety of biological specimens. It comprises a cellulose-based matrix containing chemicals (formamide, citrate and Trizma-base) for cell lysis and nucleic acid preservation (Moscoso et al. 2004). Chemical activation occurs when a biological fluid comes into contact with the FTA™ surface. The preservatives on the FTA™ matrix inactivate bacteria and viruses, thus protecting the biological samples from microbial growth and contamination. Further, users collecting biological specimens for DNA are protected from hazardous microbes that may be present in the specimen. FTA™ technology has also been used in a number of animal tissue culture applications. For example, it has been used to safely transport samples infected by foot-and-mouth disease virus (FMDV) (Muthukrishnan et al. 2008). FTA™ paper also provides the advantage of sample storage at ambient room temperatures.

FTA™ paper has been commonly used as a matrix for DNA storage in a number of disciplines, particularly in the pharmaceutical sector (Martins et al. 2002; Tolunay et al. 2006), law enforcement groups (Raina and Dogra 2002; Tack et al. 2007), agriculture (Crabbe 2003; Ndunguru et al.

2005) and regulatory agencies. In the field of forensic science, FTA™ technology has excelled (Harvey 2005; Yoshihiko and Shin-ichi 2006). The simplicity of the collection technique, its adaptability to the range of biological specimens encountered at potential crime scenes and the ease of storage has made the technology the preferred evidence collection method. It has been shown that specimens stored on FTA™ paper have long shelf life, with DNA samples recovered from FTA™ stored over 17 years used for reliable human identification (Ndunguru et al. 2005).

On the forensics front, the use of microsatellite short tandem repeats (STR) for DNA profiling has been invaluable (Gill et al. 1985). However, with advances in genome science, new opportunities continue to be considered (Foster et al. 1998). The large amount of data from Genome-Wide Association Studies (GWAS), once a hindrance to applied work such as criminal profiling, will become more manageable with the development of analysis, visualisation and interrogation software. Here, we have shown that FTA™ archived DNA can be used in GWAS. Consequently, current DNA storage procedures in forensics are acceptable in the event GWAS or similar genomic methods are adopted for criminal profiling.

In 2008, the Hunt BioSciences study in Norway, which commenced in 1984, used FTA™ technology for storage of DNA. The study is comprised of population-based epidemiological health studies which have focused on factors that predispose to diabetes and breast cancer. There are some 75,000 participants, with a participation rate of 88%. In their third ongoing survey, HUNT3, in which 10,000 samples were collected, biological specimens were collected and preserved on FTA™.

In this study, the suitability of DNA stored on FTA™ was assessed for more sophisticated DNA analysis techniques, namely GWAS. GWAS applications have led to a proliferation in the number of biobanks or biological sample repositories to provide the necessary biological resource for these substantial genome-wide studies. Considerable effort has been put into collecting blood and tissue samples and matching these to patient information ranging from demographic data to specific clinical histories. Over the years, associations between these phenotypes and genetic polymorphisms have revealed a plethora of genetic associations.

For GWAS studies, the FTA™ Elute system, when used in combination with whole genome amplification (WGA) technologies can create a virtually unlimited supply of nucleic acid template. Valuable biological samples can be archived or banked at ambient laboratory temperatures, replacing the need for expensive, space-consuming and energy-demanding freezer banks. In GWAS studies, the investigation of large groups is necessary because genetic factors involved in the cause of multifactorial diseases can only ever supply partial explanations. There is only a certain probability that genetic factors will result in a given multifactorial disease, and as the sample number increases, the probability becomes more precise and accurate. However, current storage systems are relatively limited and require significant infrastructure (e.g. −80 °C freezers) and support. Consequently, more convenient alternatives have to be considered. It is expected that the dissection of genetic factors that predispose to disease and which explain the etiology of the complex multi-factorial disorders will be the key to preventative strategies, as well as the development of targeted therapeutic modalities. The development and assessment of technologies including FTA™ that facilitate large-scale genomic efforts are critical to these outcomes.

Material and methods

Sample set

Peripheral blood was drawn and collected in EDTA tubes from three healthy unrelated individuals (denoted S1, S2 and S3) after receiving ethical approval from the Ministry of Health in the United Arab Emirates. These three samples were used in each set of the experiments mentioned below. Four drops of blood from each sample were transferred to a FTA™ paper (Whatman, Maidstone, Kent, UK) and stored at ambient room temperature (20 °C).

Preparation of genomic DNA for GWAS analysis

Three different sets of DNA templates were prepared and used in the present study.

Set 1: DNA was extracted from blood embedded in FTA™ (abbreviated PCR-FTA) and then amplified. DNA samples were purified from FTA by placing a 3-mm disk in a microcentrifuge tube. The disk was rinsed in TE^{-1} (10 mM Tris–HCI, 0.1 mM EDTA, pH 8) buffer twice and left to stand for 5 min at room temperature (20 °C). The buffer was subsequently removed and fresh TE^{-1} buffer was added. The disk was left to stand in elution buffer for 20 min at room temperature. This step was repeated twice. Subsequently, the elution buffer was removed and the disk was dried at room temperature for 1 h. At the end of the drying process, a complete WGA step was performed by thermal Cycler GeneAmp PCR system 9700 (Applied Biosystems, Lincoln Centre

Drive, Foster City, CA, USA) on all three samples separately using Sigma's Genomeplex® kit (Sigma #WGA4) according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). Prior to GWAS analysis, the PCR products were cleaned using a Promega Kit (Promega, Madison, WI, USA) according to the protocol provided.

Set 2: DNA was extracted from whole blood using standard methods and amplified (referred to as PCR-dgDNA). The quantity and purity of the three DNA samples used were determined by absorbance measurements using a NanoDrop ND-1000 Spectrophotometer (NanoDrop, Wilmington, DL, USA). A total of 10 ng/μl of each DNA sample was amplified using Sigma's Genomelex® kit, with PCR clean up performed using the PCR purification Kit of Promega using thermal Cycler GeneAmp PCR system 9700 (Applied Biosystems).

Set 3: DNA was extracted from whole blood using standard methods without further amplification (dgDNA). Three DNA samples at concentrations of 50 ng/μl were prepared for GWAS analysis.

All sample sets were qualified for GWAS analysis, with DNA ratios (A260:A280) of 1.9 and the average DNA concentrations of 200 ng/μl used for the study. All samples were diluted to a concentration of 50 ng/μl in Tris EDTA (TEKnova, Hollister, CA, USA).

GWAS assay

A genome-wide study was performed on all three sets of DNA with the Human660W-Quad BeadChip (Illumina, San Diego CA, USA), which contains 660,000 SNPs derived from the International HapMap Project. The genotype assays for the three sets of DNA were performed according to the manufacturer's recommendations. In brief, 200 ng of DNA template was subjected to whole-genome amplification at 37 °C for 20 to 24 h. Products were degraded, precipitated, and re-suspended in hybridisation buffer. The re-suspended samples were denatured at 95 °C for 20 min, loaded onto the BeadChips, and placed in a 48 °C hybridisation chamber for 16 to 20 h. After hybridisation, non-hybridised DNA was washed away from the BeadChips. An allele-specific single-base extension of the oligonucleotides on the BeadChip was performed in a 48-position GenePaintTM Slide Chamber Rack (Tecan, Männedorf, Switzerland) using labelled deoxynucleotides and the captured DNA as a template. After staining of the extended DNA, BeadChips were washed and scanned on an I-Scan apparatus (Illumina), and genotypes were called using the BeadStudio software version 3.0 (Illumina).

Statistical analysis

Statistics on the data generated were carried out with one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison tests.

Results

The integrity of degraded genomic DNA is critical when used as template for GWAS studies. The call rates for degraded DNA can be variable, which compromises the integrity of the study. By way of illustrating this in Fig. 1, when GWAS assays were performed using DNA templates that were degraded, the ratio of “calls” to “no calls” can be highly variable. The efficiency of the assay is low, with call rates as low as one in five (or 20%) achieved.

The use of a WGA step prior to GWAS analysis can improve the call rate to around 96% (call rates for three samples under the PCR-degraded gDNA category in Fig. 1). In the same study, the use of FTA™ as a DNA collection and storage media was assessed with call rates of 96% and higher achieved (PCR-FTA Fig. 1).

In Fig. 2, the quality of the base calling function is illustrated. Specifically, in Fig. 2c, clustering of the plots shows that genotypes are not assigned when using degraded DNA templates. The results for three separate samples, S1, S2 and S3, fall outside the ‘call zone’. There is some improvement, when the degraded genomic DNA is processed with an amplification step prior to GWAS analyses (Fig. 2b). Interestingly, amplified genomic DNA collected using FTA™ cards generated the best results (Fig. 2a), suggesting that this simple method of specimen collection and nucleic acid purification could be a suitable prelude for GWAS studies.

In the 20 selected SNPs on chromosome 18, it is clear the genotypes are not called when degraded genomic DNA is used for analysis. Examples of the types of missed calls and no calls are specifically shown in Fig. 3. The three different DNA templates (PCR-FTA, PCR-dgDNA and dgDNA) for all three individuals (S1, S2 and S3) were compared and the range of examples of call scenarios is presented. There are three examples of SNP positions where there is concordance between all three DNA templates shown, rs10083985 and rs10163808 for S1 and rs1010360 for S3. In sample S1, the only example of a no call observed for all three DNA templates used can be seen at SNP rs10163736.

Importantly, the type of DNA template used can give rise to erroneous results. These errors are compounded and generally missed due to the large amount of data that is generally associated with GWAS studies. An example of a miscall genotype is shown at position rs1008899 in sample

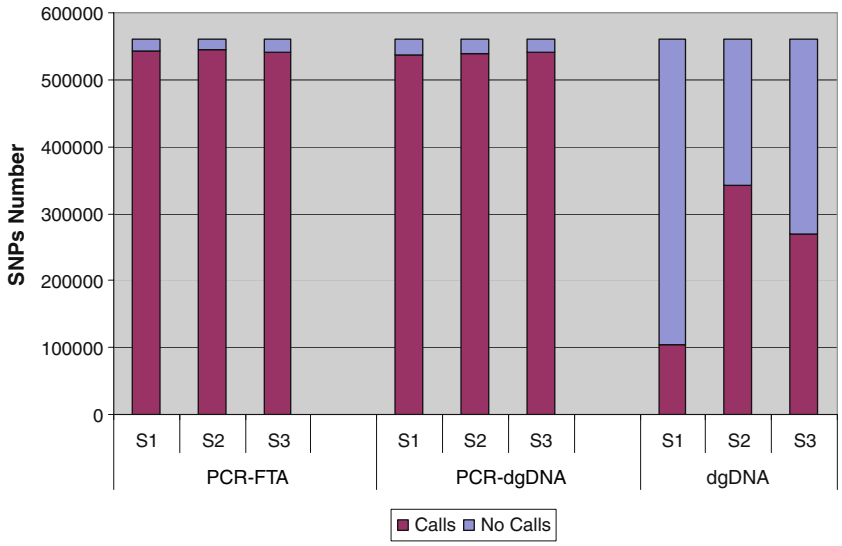

Fig. 1 Summary of called genotype and no genotype calls of 657,366 SNPs across 23 chromosomes using three sources of DNA: PCR-FTA, PCR-dgDNA and dgDNA. For each source of DNA, three independent samples were collected (S1, S2 and S3) for testing and comparison

S1 when using degraded genomic DNA as a template for GWAS assays.

To provide a chromosome-wide perspective of the data selected for Fig. 3, the same data for all the SNPs analysed for chromosome 18 is presented using Illumina's Chromosome Browser (Fig. 4). The density of genotypes called when using DNA templates collected by FTA™ paper is higher when compared to amplified degraded genomic DNA. The number of genotypes called and accuracy of the calls with degraded genomic DNA was poor. These results were consistent with the quality control step using box plots to represent the log R ratio recommended by Illumina (see Fig. 5). The log R values when using amplified DNA template from FTA™ were typically 0.1 to 0.25 for all three samples studied, the range for a good call (Fig. 5a). The average score for amplified degraded genomic DNA was acceptable (Fig. 5b); however, SNPs were not as tightly clustered as seen with amplified templates from FTA™. As expected, the scores reflected the poor quality of results obtained using degraded DNA (Fig. 5c).

In summary, for three subjects studied (S1, S2 and S3), the call rates were variable when using degraded DNA as a template (19%, 61% and 48%, respectively, Table 1).

While collecting blood in the conventional fashion for S1, S2 and S3, blood spots were also collected on FTA™ paper. The DNA was harvested and subjected to a genome-wide amplification step prior to the GWAS assay. The call rates using these DNA templates were equivalent to (96%) or better than (97%) the assays that used amplified degraded genomic DNA as templates (Table 1).

Results from one-way ANOVA (Table 2) shows pairwise comparisons of the three sources of DNA. Overall, there is a significant difference (p=0.0027) between the call rates observed for degraded genomic DNA (dgDNA) when compared to PCR amplified degraded genomic DNA and DNA sourced from FTA™. The call rates of the latter two were similar (mean of 96.0% and 96.6%), respectively. These call rates above 95% are above the optimal rates used for conventional GWAS using pristine quality DNA.

Discussion

DNA collected for SNP analysis needs to be of sufficient quality to ensure high genotype call rates. Association studies investigating the underlying factors of complex diseases increasingly require sustainable high-quality DNA resources for large-scale single-nucleotide polymorphism (SNP) genotyping (Paynter et al. 2006).

While venous blood is often considered the optimal source for DNA, the invasiveness and cost of obtaining venous blood samples can be prohibitive, especially for large-scale human studies or those that deal with livestock and wild animals. Additionally, fresh samples collected in the field may experience degradation before they can be processed. Previous research has shown that multiple genomic sources, including lymphocytes (Dictor et al. 2007), buccal cells (Milne et al. 2006), sperm (Yoshihiko and Shin-ichi 2006) and fingernails (Nakashima et al. 2008), can be used to generate high-density SNP data provided the DNA sample is of adequate quality and quantity (Jasmine et al. 2008). The ease of collection, transportation, storage and protection from degradation of samples stored on FTA™ cards provides a possible solution. McClure et al. (2009) used DNA extracted from cells on FTA™ cards to study SNPs on Illumina's I-select Bead Ship, which contains 54,122 SNPs (McClure et

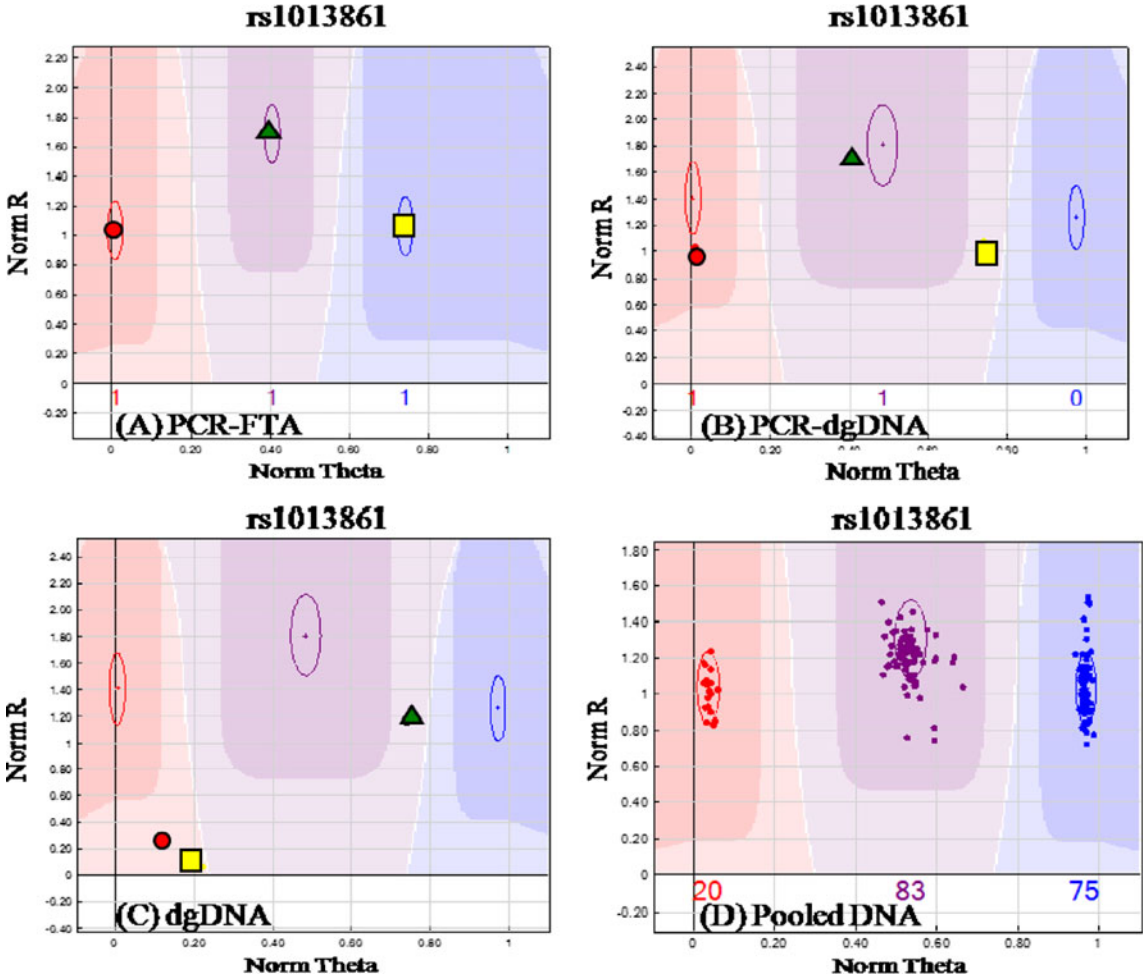

Fig. 2 Examples of clustering plots showing the accuracy of calling for SNP rs1013861 on chromosome 18 using different sources of DNA. **a** High call rate for the three PCR-FTA samples, *squares* S1, *circles* S2 and *triangles* S3, with the genotype called correctly. **b** When using PCR amplified degraded DNA, there were two correct calls (S2 and S3) and one no call (S1). **c** The genotypes of all three samples of degraded DNA could not be assigned due to poor call rates. **d** A typical clustered SNP clustering pattern in 178 samples with all genotypes being correctly called. *Norm R*, normalised intensity; *Norm Theta*, angle of the centre of cluster in normalised polar coordinates. *Dark shaded area*, the call zone for AA (*right*), AB (*middle*) and BB (*left*) genotypes

al. 2009). This study expands on McClure et al.'s (2009) study and assessed three different sources of DNA as suitable templates in a genome-wide study (GWAS) using Illumina's human 660 W-Quand Bead Chip, which contains 660,000 SNP markers.

In this study, three different types of DNA templates (PCR-FTA, PCR-dgDNA and dgDNA see methodology) were used for GWAS. A call rate of greater than 95% may be obtained for GWAS studies of a good-quality DNA on Illumina's Infinium Array. On the other hand, poor-quality DNA, such as degraded DNA, can result in low call rates as a result of polymorphisms that were called erroneously (miss call) or SNPs that were not called (no call). Figure 1 shows the ratio of "calls" to "no calls" can be highly variable among the three templates. For instance, degraded DNA (dgDNA) shows a low number of SNP calls, which affected the call rate (mean of 42.6%), where the use of an amplification step on degraded DNA (PCR-dgDNA) prior to GWAS improved the call rate (mean of 96.0%). It would appear that the use of FTA™ as a DNA collection method also increased the call rate of the samples (mean of 96.6%).

In order for a SNP to be called or genotyped correctly, the SNP should fall in the call zone (middle of darker shade) of the designated AA, AB or BB regions (see Fig. 2). Poor-quality DNA can result in the SNP falling outside the dark shaded area, which results in a "no call" for the marker. Where an amplification step was used before GWAS, the SNPs fall within the call zone and were genotyped correctly. Moreover, when using DNA from FTA™, the highest call rate results were obtained. This suggests the possibility of using this simple specimen, relatively inexpensive collection and nucleic and purification technology as a convenient method of collection and storage of blood samples before embarking on GWAS studies.

Chromosome		18																			
SNPs		rs1000055	rs1004403	rs10083961	rs10083985	rs1008899	rs1009819	rs1010360	rs1010444	rs1011947	rs1013861	rs10153405	rs1015460	rs10163657	rs10163736	rs10163808	rs10164009	rs1017252	rs1019989	rs1021599	rs10221443
S1	PCR-FTA	AB	AB	AA	BB	AB	AA	AA	AA	AA		AB	AA	BB		AA	AA	BB	AB	BB	AB
	PCR-dgDNA	AB	AB	AA	BB	AB	AA	AA	AA	AA		AB	AA	BB		AA	AA	BB	AB	BB	AB
	dgDNA				BB	AA										AA					
S2	PCR-FTA	AB	AA	AA	BB	AB	AA	AB	AB	BB	AA	AA	AA	BB	AB	AA	AA	AB	AB	AB	BB
	PCR-dgDNA	AB	AA	AA	BB	AB	AA	AB	AB	BB	AA	AA	AA	BB	AB	AA	AA	AB	AB	AB	BB
	gDNA																				
S3	PCR-FTA	AB	AB	AA	AB	AB	AA	AA	BB	BB	AB	AA	AA	BB	AA	AA	AA	AA	AB	AB	AA
	PCR-dgDNA	AB	AB	AA	AB	AB	AA	AA	BB	BB	AB	AA	AA	BB	AA	AA	AA	AA	AB	AB	AA
	dgDNA							AA													

Fig. 3 Examples of correct calls, miscalls and no calls in three samples (S1, S2 and S3) in a comparison between PCR amplified DNA from blood sample collected on FTA (*PCR-FTA*), whole genome amplified from degraded DNA (*PCR-dgDNA*) and degraded genomic DNA (*dgDNA*). Twenty SNPs on chromosome 18 were randomly selected from the 660,000 SNPs available for all three subjects. At each SNP, the genotypes were either (1) called correctly: see dgDNA genotype of rs10083985 for S1, (2) miscalled: see dgDNA genotype of rs1008899 or (3) not called: see genotype of all three sources of DNA for rs10163736

A further problem when dealing with poor quality of DNA is the miscalled genotype (or mistakenly called) effect. Figure 3 shows an example of miss call for SNP rs1008899 in S1 when using degraded DNA. The SNP was genotyped AA, with the call falling outside call zone and between AA and AB areas. When the sample was amplified and subsequently genotyped, the SNP called AB. The genotype called was in the middle of the shaded area for AB. Further, the same SNP from the sample sourced from FTA™ confirmed the call was indeed AB.

One of the advantages of using the Illumina platform is the ability to study the loss of heterozygosity (or LOH). Figure 4 shows the effect of poor-quality DNA on the call rate. The result for degraded DNA is scattered throughout the plot, and it is difficult to distinguish whether the call of the SNP is AA, AB or BB. Whereas in PCR-amplified degraded DNA, the call rate or efficiency for SNPs improved. The use of DNA sourced from FTA™ also gave rise to a high call rate with SNPs genotyped correctly.

Strategies to recover degraded DNA samples for GWAS analyses have previously been used (Ballantyne et al. 2007), one of which is based on an amplification step prior to the pre-amplification step that occurs during the GWAS assay (Ryo et al. 2007). In this study, 10 ng/μl of each degraded DNA sample was amplified using Sigma's Genomelex® kit, followed by a clean-up step performed using Promega's PCR purification kit. This additional amplification step before the GWAS assay step proper improved the call rates from 19% to 96% in the first sample (S1 in Table 1). The call rates in S2 and S3 also improved to 96% from 61% and 48%, respectively (see Table 1).

Quality control (QC) algorithms for GWAS have been incorporated in the analysis process to assess, evaluate and guarantee the quality of genotyping. The bead studio analysis software package provides several convenient QC modules, such as the Box Plot, a useful tool to quickly visualise the variation within an array and between arrays. A “log of R ratio” provides a measure of noise in the data. The typical values associated with high-quality data ranges from 0.1 to 0.25. Figure 5 shows results generated from DNA extracted from FTA™ had the least noise of the three templates. This provides some degree of confidence that DNA from biological samples collected and stored on this matrix can be used for genome-wide studies. The p value of 0.0027 obtained from ANOVA shows significant difference between the three templates. A Bonferroni pair-wise comparison was also performed and showed there were significant differences (Table 2) between both PCR amplified degraded DNA and PCR amplified DNA from FTA™ when compared to degraded DNA. Although the three samples discussed to this point show a call rate of 96%, analysis was performed across 23 samples with an average call rate of 99% (data not shown) when using DNA from FTA™.

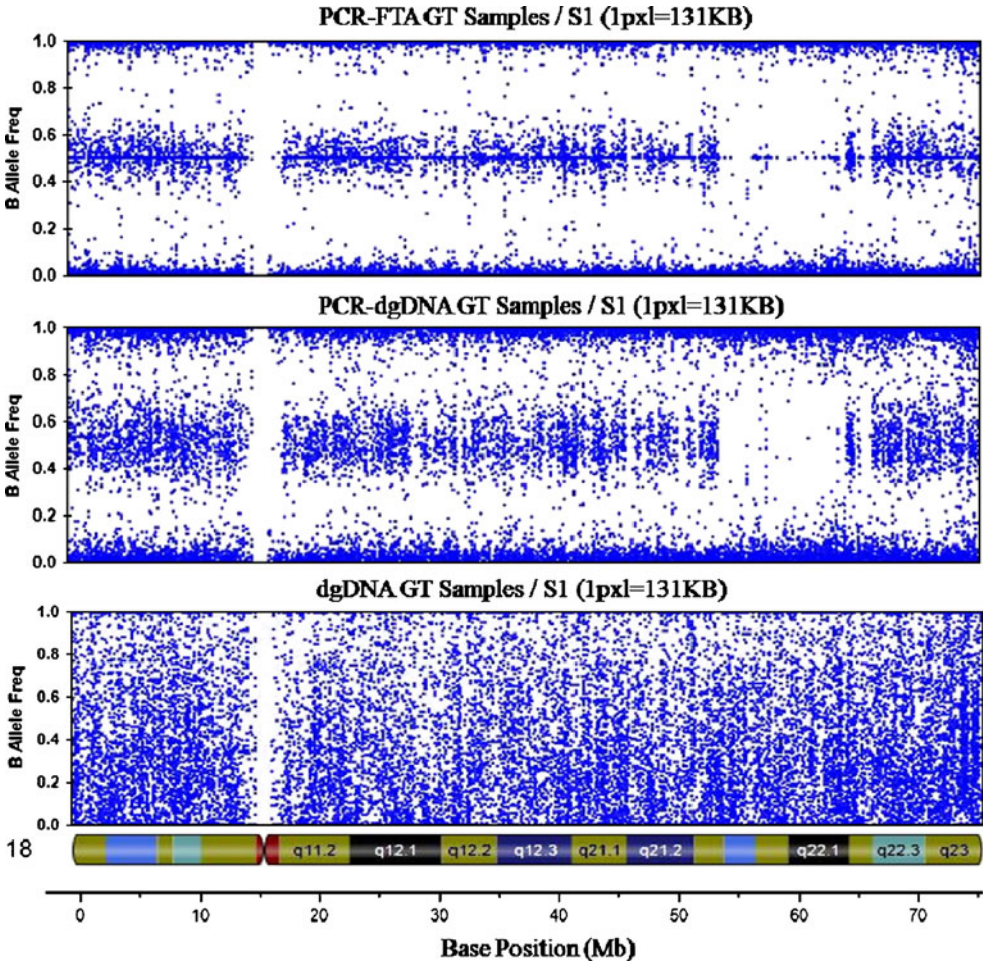

Fig. 4 The Illumina Chromosome Browser (ICB) features a plot of the B allele frequencies along the chromosome 18 in sample 1. The *horizontal axis* denotes the physical position of SNPs (scale in megabases, *Mb*), and the *vertical axis* denotes the estimated the B allele frequency. **a** Ninety six percent of SNPs were called and genotyped as AA, AB or BB using PCR-FTA as a source of DNA. **b** Ninety five percent of SNPs were called and genotyped using PCR-dgDNA as a source of DNA. **c** Eighteen percent of SNPs were called and genotyped using dgDNA. **a**, **b** There is a deletion in 55 to 65 Mb, where, in **c**, due to poor-quality DNA, the deletion was not obvious

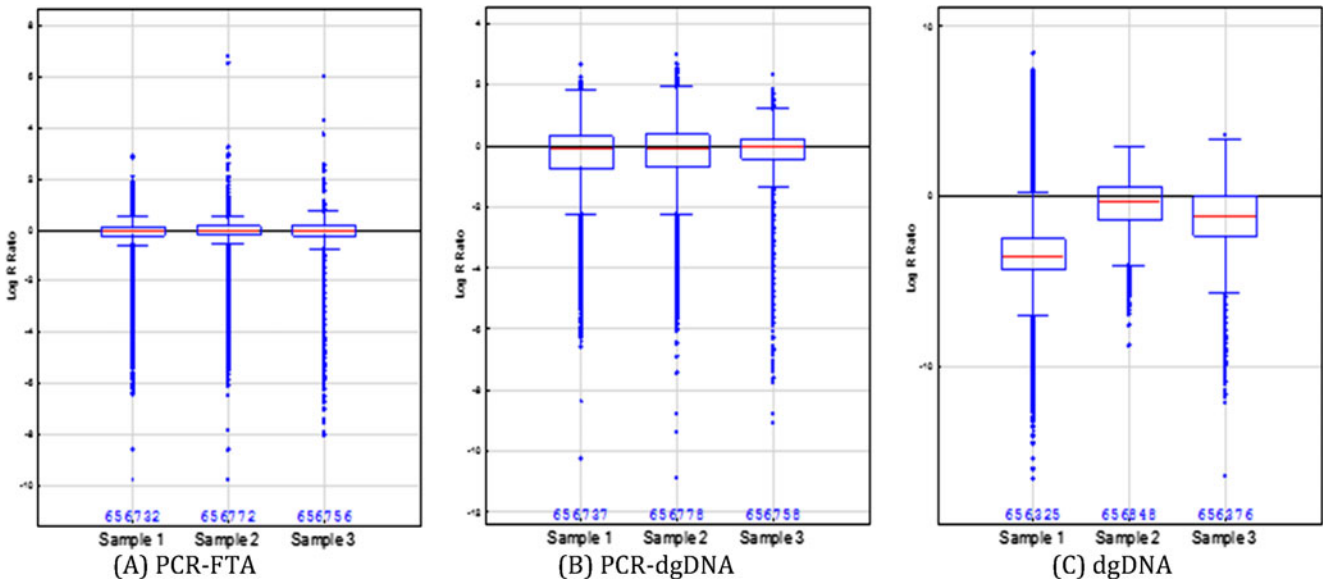

Fig. 5 A box plot representing the distribution of log R ratio in all three samples using three different sources of DNA. The log R ratio provides a measure of the noise in the data. Typical values associated with high-quality data are 0.1 to 0.25. **a** A log R ratio is shown using PCR-FTA. **b** The log R ratio was not as tightly grouped when using PCR-dgDNA. **c** Good-quality log R ratio was observed due to a poor DNA quality when using dgDNA

Table 1 Summary of number of "calls" and "no calls", call rate, allele frequencies for the AA, AB and BB genotypes, minor allele frequency and percentile of Gen Call on 657,366 Loci for PCR-FTA, PCR-dgDNA and dgDNA

DNA sources	Sample	#No calls	#Calls	Call_rate	A/A freq	A/B freq	B/B freq	Minor freq	50% GC_score	10% GC_score
PCR-FTA Loci= 657,366	S1	18,494	542,996	0.9671	0.3312	0.2923	0.3765	0.4773	0.4396	0.2867
	S2	17,446	544,044	0.9689	0.315	0.3261	0.359	0.478	0.4396	0.2861
	S3	20,156	541,334	0.9641	0.3259	0.3032	0.3709	0.4775	0.4396	0.2861
PCR-dgDNA Loci= 657,366	S1	25,007	536,483	0.9555	0.3285	0.2956	0.376	0.4763	0.8741	0.5439
	S2	23,455	538,035	0.9582	0.3131	0.327	0.3599	0.4766	0.8795	0.5534
	S3	20,041	541,449	0.9643	0.324	0.3055	0.3706	0.4767	0.8915	0.6483
dgDNA Loci= 657,366	S1	457,583	103,907	0.1851	0.5549	0.2823	0.1628	0.3039	0.6957	0.2085
	S2	218,313	343,177	0.6112	0.2174	0.4559	0.3268	0.4453	0.7994	0.2781
	S3	292,182	269,308	0.4796	0.299	0.3517	0.3493	0.4749	0.7853	0.2594

Furthermore, there have been studies that have shown that blood spots on FTA™ cards are a more efficient source of DNA for studying genetic polymorphisms including STR analysis (Guangyun et al. 2005). DNA from neonatal blood that has been stored over 10 years on Guthrie cards have been successfully extracted using modified FTA™ technology known as GenSolve for whole genome microarray analysis. In contrast, the traditional procedures of strong alkali or heat treatment used for DNA extraction compromised the physical and chemical integrity of nucleic acid (Hardin et al. 2009).

FTA™ has received considerable interest from other sectors of bioscience, such as forensics, due to its non-invasive and cost-effective means for obtaining DNA in large-scale studies. FTA™ cards have also been shown to be compatible with virtually all cell types (McClure et al. 2009). While early studies have shown that DNA harvested from FTA™ cards were suitable for genotyping 1,516 SNPs on the Illumina Golden Gate platform and 10,000 SNPs on the Affymetrix 10 K GeneChip, more recently, FTA™ cards have been shown to be suitable for high-throughput genotyping on the Illumina iSelect platform, which currently assays up to 200,000 SNPs. McClure et al. (2009) concluded that FTA™ cards provide an excellent medium for harvesting DNA from multiple cell types, and that, when assayed using the Illumina iSelect technology, yield high-genotype call rates and reproducibility, particularly when the DNA is extracted using the GenSolve kit (McClure et al. 2009). DNA from FTA™ cards has been used in Illumina Golden Gate Bead Array Assay in ovarian cancer studies to assess its performance in multiple displacement association WGA studies performed by Cunningham et al.(2008) (Cunningham et al. 2008). In this study, DNA from FTA™ was successfully used on Illumina's chip containing 660,000 SNPs and showed the highest accurate call rates in comparison to other DNA sources, amplified and not-amplified genomic DNA.

In conclusion, FTA™ cards capture nucleic acid in one easy step. Captured nucleic acid is ready for downstream applications in less than 30 min. Nucleic acids collected on FTA™ cards are stable for years at room temperature. FTA™ cards are stored at room temperature before and after sample application, reducing the need for laboratory freezers. They are suitable for virtually any cell type and any genotyping platform. FTA™ cards come with a built-in indicator that changes colour upon sample application to facilitate handling

Table 2 Bonferroni's multiple test shows that the call rates for genomic DNA extracted from FTA (96.6%) and PCR amplified genomic DNA (average=96.0%) are significantly higher when compared to degraded genomic DNA (42.6%) (p=0.0027)

Bonferroni's multiple comparison test					
Test	Mean difference	t	Significance (p<0.05)	95% Cl of difference	
FTA-PCR vs PCR-dgDNA	0.006	0.065	No	−0.33 to 0.34	
FTA-PCR vs dgDNA	0.540	5.326	Yes	0.21 to 0.87	
PCR-dgDNA vs dgDNA	0.533	5.260	Yes	0.20 to 0.87	
ANOVA (one-way analysis of variance)					
Test	Sum of squares	Degrees of freedom	Mean squares	F ratio	p value
Three DNA templates	0.570	2	0.30	18.68	0.0027
Call rate	0.090	6	0.02		
Total	0.660	8			

of colourless samples. They are available in a variety of configurations to meet application requirements. They have been widely used in the fields of forensics, transgenics, transfusion medicine, plasmid screening, food and agriculture testing, drug discovery, genomics, STR analysis, animal identification, diagnostics, pharmacogenomics and molecular biology. Thus, FTA™ Cards are a routine and cost-effective technology that provide a simple method for preservation of biospecimens, amenable to high-throughput DNA extraction, all the attributes required to undertake successful GWAS in an efficient manner.

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Conflict of interest All authors declare that they have no conflict of interest.

References

Ballantyne KN, van Oorschot RA, Mitchell RJ (2007) Comparison of two whole genome amplification methods for STR genotyping of LCN and degraded DNA samples. Forensic Sci Int 166:35–41

Crabbe MJ (2003) A novel method for the transport and analysis of genetic material from polyps and zooxanthellae of scleractinian corals. J Biochem Biophys Meth 57:171–176

Cunningham JM, Sellers TA, Schildkraut JM, Fredericksen ZS, Vierkant RA, Kelemen LE, Gadre M, Phelan CM, Huang Y, Meyer JG, Pankratz VS, Goode EL (2008) Performance of amplified DNA in an Illumina Golden Gate Bead Array assay. Cancer Epidemiol Biomark Prev 17:1781–1789

Dictor M, Skogvall I, Warenholt J, Rambech E (2007) Multiplex polymerase chain reaction on FTA cards vs. flow cytometry for B-lymphocyte clonality. Clin Chem Lab Med 45:339–345

Foster EA, Jobling MA, Taylor PG, Donnelly P, de Knijff P, Mieremet R, Zerjal T, Tyler-Smith C (1998) Jefferson fathered slave's last child. Nature 396:27–28

Gill P, Jeffreys AJ, Werrett DJ (1985) Forensic application of DNA 'fingerprints'. Nature 318:577–579

Guangyun S, Ritesh K, Prodipto P, Michael W, Diane S, Hong C, Mei L, Ranajit C, Li J, Ranjan D (2005) Whole-genome amplification: relative efficiencies of the current methods. Leg Med (Tokyo, Japan) 7:279–286

Hardin J, Finnell RH, Wong D, Hogan ME, Horovitz J, Shu J, Shaw GM (2009) Whole genome microarray analysis, from neonatal blood cards. BMC Genet 10:38

Harvey ML (2005) An alternative for the extraction and storage of DNA from insects in forensic entomology. J Forensic Sci 50:627–629

Jasmine F, Ahsan H, Andrulis IL, John EM, Chang-Claude J, Kibriya MG (2008) Whole-genome amplification enables accurate genotyping for microarray-based high-density single nucleotide polymorphism array. Cancer Epidemiol Biomark Prev 17:3499–3508

Martins S, Trigo F, Azevedo L, Silva MJ, Guimaraes JE, Amorim A (2002) Haplotype study of microsatellites flanking the t(15;17) breakpoint in acute promyelocytic leukemia patients from North Portugal. Leukemia 16:1353–1357

McClure M, McKay S, Schnabel R, Taylor J (2009) Assessment of DNA extracted from FTA(R) cards for use on the Illumina iSelect BeadChip. BMC Res Notes 2:107

Milne E, van Bockxmeer FM, Robertson L, Brisbane JM, Ashton LJ, Scott RJ, Armstrong BK (2006) Buccal DNA collection: comparison of buccal swabs with FTA cards. Cancer Epidemiol Biomark Prev 15:816–819

Moscoso H, Thayer SG, Hofacre CL, Kleven SH (2004) Inactivation, storage, and PCR detection of mycoplasma on FTA filter paper. Avian Dis 48:841–850

Muthukrishnan M, Singanallur NB, Ralla K, Villuppanoor SA (2008) Evaluation of FTA cards as a laboratory and field sampling device for the detection of foot-and-mouth disease virus and serotyping by RT-PCR and real-time RT-PCR. J Virol Meth 151:311–316

Nakashima M, Tsuda M, Kinoshita A, Kishino T, Kondo S, Shimokawa O, Niikawa N, Yoshiura K (2008) Precision of high-throughput single-nucleotide polymorphism genotyping with fingernail DNA: comparison with blood DNA. Clin Chem 54:1746–1748

Ndunguru J, Taylor NJ, Yadav J, Aly H, Legg JP, Aveling T, Thompson G, Fauquet CM (2005) Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. Virol J 2:45

Paynter RA, Skibola DR, Skibola CF, Buffler PA, Wiemels JL, Smith MT (2006) Accuracy of multiplexed Illumina platform-based single-nucleotide polymorphism genotyping compared between genomic and whole genome amplified DNA collected from multiple sources. Cancer Epidemiol Biomark Prev 15:2533–2536

Raina A, Dogra TD (2002) Application of DNA fingerprinting in medicolegal practice. J Indian Med Assoc 100:688–694

Ryo I, Takamitsu T, Chinatsu S, Mitsugi I, Kazunari U (2007) Simple and rapid detection of the porcine reproductive and respiratory syndrome virus from pig whole blood using filter paper. J Virol Meth 141:102

Tack LC, Thomas M, Reich K (2007) Automated forensic DNA purification optimized for FTA card punches and identifiler STR-based PCR analysis. Clin Lab Med 27:183–191

Tolunay B, Raymond KB, Robert JC (2006) Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations. Proc Natl Acad Sci USA 103:1699–1704

Yoshihiko F, Shin-ichi K (2006) Application of FTA® technology to extraction of sperm DNA from mixed body fluids containing semen. Leg Med (Tokyo, Japan) 8:43–47