Identification of *P. Falciparum* in DNA recovered from FTA paper using a SNP-based molecular barcode

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Abstract

Novel methods of single nucleotide polymorphism (SNP) genotyping are facilitating identification and tracking of the Plasmodium falciparum parasite. Whatman FTA® paper is an easy means of collecting and storing blood samples for later analysis such as SNP genotyping; however, attempting to recover DNA from FTA paper often results in low yields or poor quality DNA. Here, we report that DNA may be efficiently recovered from blood samples preserved on FTA paper using GenTegra's GenSolve method.

Introduction

A novel method of single nucleotide polymorphism (SNP) genotyping called a molecular barcode has recently been developed that addresses the challenge of parasite identification[1]. A molecular barcoding assay is a quick, convenient and low-cost method of parasite identification. Uses of molecular barcoding assays include in-lab quality control, detecting new outbreaks of a quiescent disease and distinguishing from re-infection, as well as for examining the parasite distribution of a patient population during drug treatment regimens. Parasite isolate identification in blood samples stored on FTA paper is a challenging process due to the difficulty in identifying all varieties of a particular parasite and the inability to recover sufficient amounts of high quality DNA from FTA paper. To overcome the obstacle of recovering DNA from blood stored on FTA paper, GenTegra has recently developed GenSolve, which facilitates the recovery of DNA from Guthrie cards, FTA paper and newborn screening cards. Unlike other recovery methods, which often result in low yields of DNA that is single-stranded or fragmented, GenSolve allows recovery of high molecular weight, double-stranded DNA. The high-quality, high-purity DNA recovered with GenSolve is suitable for applications such as SNP genotyping, PCR, STR analysis, HLA typing and common genotyping assays including Illumina and Affymetrix. Here, we report the use of a molecular barcoding assay developed for P. falciparum to successfully genotype DNA recovered from FTA paper using GenSolve.

Materials and Methods

DNA extraction

DNA was extracted from whole blood samples that had been preserved on FTA® paper (Whatman, Florham Park, NJ) using either the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) or the GenSolve Kit for Whole blood DNA recovery (GenTegra, Pleasanton, CA) using three 3-mm punches for each method.

DNA Quantitation

The total DNA yield in the recovered samples was quantitated using a PicoGreen dsDNA quantitation assay (Invitrogen); using the protocol with a 1:20 dilution of sample into a prepared dye solution. The interpolated results were calculated using Prism (GraphPad Software, La Jolla, CA).

TaqMan-MGB assays (Applied Biosystems, Foster City, CA) were also used to differentiate between DNA derived from human and P. falciparum sources in DNA samples recovered from FTA paper-preserved blood samples. For quantitation of malaria DNA, TaqMan-MGB primers and reporter sequence probes were designed from a highly conserved region of PF07_0076, a 519 bp coding sequence on chromosome 7 encoding a protein of unknown function in the malaria genome. HB3 genomic DNA was used as control in a series of 8 different dilutions: 30 ng, 10 ng, 3 ng, 1 ng, 0.3 ng, 0.1 ng, 0.03 ng, and 0.01 ng of DNA per 5 µl reaction. Human DNA was quantified using RNase P Control Reagent (Applied Biosystems) with a standard curve generated using human genomic DNA (Bioline, Taunton, MA) at the concentrations described above. All quantification of both human and P. falciparum DNA was done in triplicate. DNA was diluted 1:10 and 1 µl per 5 µl reaction was used. TaqMan assays were run on an AB 7900HT (Applied Biosystems) and run for Absolute Quantification (50°C for 2 minutes, 95°C for 10 minutes, 95°C for 15 seconds, 60°C for 1 minute, repeat steps 2-4 for 50 cycles). Results were analyzed using SDS 2.3 software (Applied Biosystems).

Barcoding assay

Recovered DNA samples were quantified as described and normalized for concentration. For each reaction, template and water in a total volume of 2.5 µl were added to a 2.5 µl mix made up of 0.125 µl 40x SNP assay and 2.5 µl Master Mix (Applied Biosystems) for a total reaction volume of 5 µl. Reactions were performed in an ABI 7900HT using the standard PCR Protocol (95°C for 10 minutes, 95°C for 15 seconds, 60°C for 1 minute, repeating steps 2-4 for 50 cycles). Following amplification, the samples were analyzed using Allelic Discrimination and Absolute Quantitation functions within the SDS 2.3 software (Applied Biosystems).

Results

Blood samples collected and stored on FTA paper are of interest in many areas of research, and frequently only a small amount of material is available from which to recover DNA. Thus, it is essential to recover the highest possible yield of DNA from these limited samples. In this study, DNA was recovered from eight unique samples, using three 3-mm punches of FTA paper for each sample. We compared two different methods of extracting DNA from FTA paper; the QIAamp blood mini kit (Qiagen) and the GenSolve kit (GenTegra). The total yield of double-stranded DNA determined by PicoGreen was higher for each of the eight samples examined when the GenSolve kit was used for recovery (Figure 1).

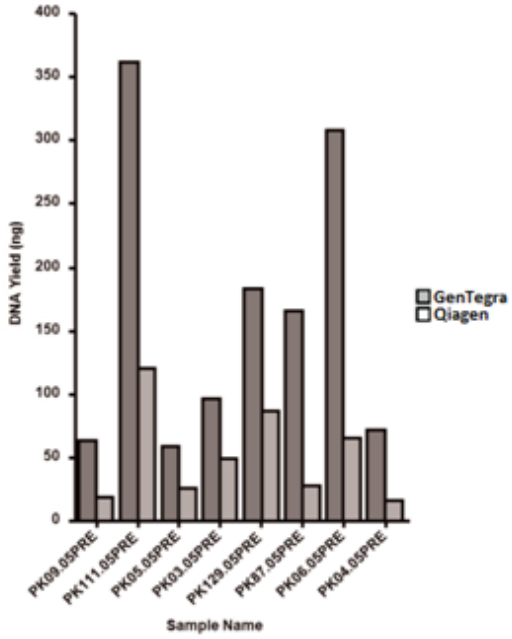


Figure 1. Comparison of total DNA yield in samples recovered from blood stored on FTA paper using GenSolve or QIAamp protocols. Total DNA yield was calculated using a PicoGreen dsDNA quantitation assay.

The samples were also quantitated using TaqMan assays to determine the quantity of human and P. falciparum DNA present in each sample (Figure 2).

In addition to recovering sufficient yields of DNA for analysis, it is essential that the recovered DNA is of high quality and purity. Following quantitation, the samples were subjected to SNP genotyping to determine the molecular barcodes of the samples using an assay developed for P. falciparum. All samples recovered using either the GenSolve or QIAamp kits were successfully genotyped using this assay (Table 1), and the same genotype was obtained for each unique sample regardless of the kit used.

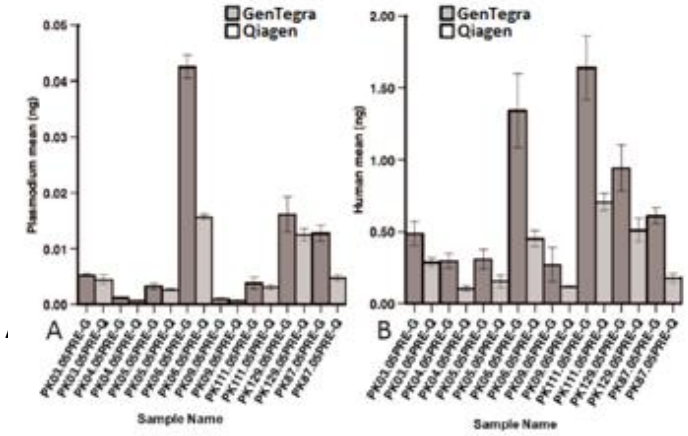
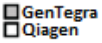


Figure 2. Comparison of yield plasmodium (A) and human (B) DNA (ng) in samples recovered from blood stored on FTA paper using GenSolve or QIAamp protocols. Total DNA yield for each species was calculated using separate TaqMan assays.



Sample	Molecular Barcode
PK09.05PRE	TACTGGGGATTGCACCCXAGCTTG
	TACTGGGGATTGCACCCXAGCTTG
PK111.05PRE	TACTNGNGNNTNNACATAAGCTTG
	TACTNGNGNNTNNACATAAGCTTG
PK05.05PRE	TACTGCGGATCGCCAATAAGCTTG
	TACTGCGGATCGCCAATAAGCTTG
PK03.05PRE	TACTCGAGTCTACCCCCAAACTTG
	TACTCGAGTCTACCCCCAAACTTG
PK129.05PRE	TATTCCGGTCXACCCACTAGCCTG
	TATTCCGGTCXACCCACTAGCCTG
PK87.05PRE	TATTCGAGACCGTACCCACGCTTT
	TATTCGAGACCGTACCCACGCTTT
PK06.05PRE	CATTCGGGTCXACCACTAAGATTG
	CATTCGGGTCXACCACTAAGATTG
PK04.05PRE	TACTCGAGTCTACCCCCXAACTTG
	TACTCGAGTCTACCCCCXAACTTG

Table 1. Molecular barcodes of samples recovered from blood stored on FTA paper using GenSolve or QIAamp protocols.

Conclusion

In many cases, the only data available from a particular malaria patient is a small blood spot preserved on FTA paper. Thus, it is essential to recover the largest quantity of DNA possible that is of acceptable quality for downstream analysis. In the present study, a sufficient yield of DNA for use in the molecular barcoding assay was recovered using the GenSolve kit. The recovered DNA is double-stranded, high quality and free of inhibitors of PCR, as evidenced by the success of TaqMan and SNP genotyping assays. GenSolve enabled retrieval of increased quantities of high quality, high molecular weight DNA from blood samples stored on FTA paper, compared with the Qiagen DNA Blood Mini Kit. The samples were successfully genotyped in a molecular barcoding assay, demonstrating the high quality of recovered DNA and allowing identification of the unique P. falciparum present in the samples.

References

1.A general SNP-based molecular barcode for Plasmodium falciparum identification and tracking. Rachel Daniels, Sarah K Volkman, Danny A Milner, Nira Mahesh, Daniel E Neafsey, Daniel J Park, David Rosen, Elaine Angelino, Pardis C Sabeti, Dyann F Wirth and Roger C Wiegand. Malaria Journal 2008, 7:223doi:10.1186/1475-2875-7-223.