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Performance of DNAStable® with the Illumina® Human 610 BeadChip™

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Introduction:

Molecular diagnostic assays performed on genomic DNA samples freshly isolated from patients are relatively straightforward. In the cases where it is necessary to use stored genomic DNA, preserving sample integrity becomes paramount. Biomatrica has developed DNAStable®, a proprietary technology which stabilizes DNA dry at room temperature to protect against degradation and subsequent sample loss. DNA purified from clinical samples will often be used in large-scale genome-wide association studies to elucidate the underlying genetic basis of disease phenotypes. Illumina® genotyping assays are the lead platform for many of these studies to determine SNPs that have statistically significant alleles or genotypes that are over-represented in disease patients when compared to control samples with no disease. DNAstable was assessed for compatibility with the Illumina Infinium assay, employed on the Human610 Genotyping BeadChip™. This study was undertaken to rigorously evaluate the integrity, quality and purity of DNA stored dry in DNAstable for use in whole-genome SNP genotyping using Illumina's BeadArray technology.

Materials and Methods:

DNA Purification and Storage: DNA was purified from frozen human whole blood (0.2 ml) from four individuals using the QIAamp® DNA Blood Mini Kit (Qiagen) and QIAcube™ automated purification system. Four 500 ng aliquots and two 1000 ng aliquots of each sample were applied to DNAstable tubes and allowed to dry overnight in a laminar flow hood, following manufacturer's guidelines. Dried samples were stored at room temperature (RT) for 14 days or at 37°C for the identical time period to simulate accelerated aging conditions equivalent to 28 days at RT. Aliquots of each sample were also stored at -20°C as reference samples. Samples were recovered DNAstable by rehydrating with water and used directly for analysis without further purification; rehydrated samples were stored at 4°C until ready for use. *Quantitation and Analysis:* DNA quantification was determined by spectrophotometer using a NanoDrop® 1000 (Thermo Scientific, Waltham, MA). The integrity and molecular weight of the recovered DNA was assessed after electrophoresis; 50 ng of each sample was electrophoresed on a 0.8% agarose gel stained with ethidium bromide. *Long-range PCR:* Aliquots of rehydrated DNA were included as templates in long-range PCR reactions using primers specific for target region of interest. *SNP Detection*: The Illumina Human610 Genotyping BeadChip (610,901 SNPs) was used for genotyping analysis using 200 ng DNA from each sample. The Human610 BeadChip utilizes the Infinium assay as described in Steemers et al., 2006 (*Nat. Methods.* Jan; 3(1):31-3). The fluorescent intensity generated from the beads was detected using the Illumina iScan Reader, and genotypes were called using the BeadStudio software. The final list of 590,622 SNPs used in this study was determined previously from a study of over 1,200 samples subjected to genotype analysis.

Results:

The integrity of recovered DNA samples (C00362, C00120, C00146 and C00217) following storage in DNAstable at RT and 37°C, as well as the control sample stored at -20˚C, was assessed using gel electrophoresis. Visualized results for all samples show bands, without smearing, corresponding to 40 kb, indicating no sample degradation (Figure 1). Protection against degradation during dry storage is present even at elevated temperatures.

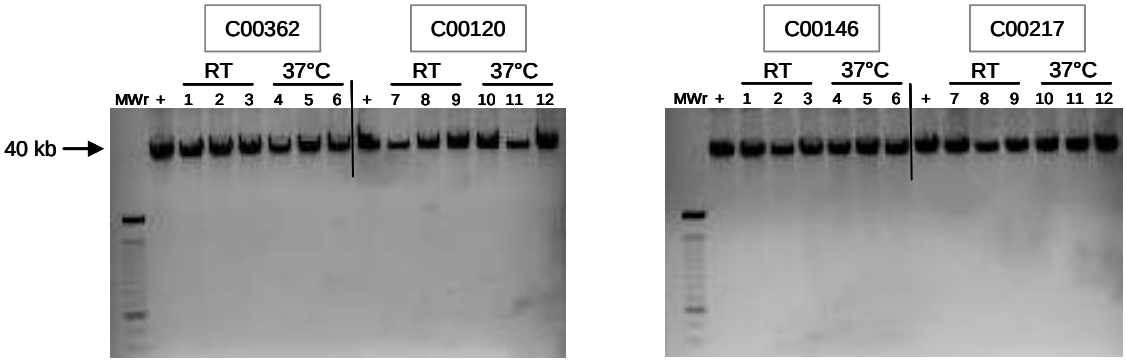

Figure 1. Recovery of intact DNA following storage in DNAstable. Triplicate aliquots (50 ng) of genomic DNA recovered following dry storage at room temperature (RT) or 37°C for 14 days were run on a 0.8% agarose gel. MWr: 100 kb ladder; (+): reference sample stored at -20°C. Arrow indicates band corresponding to 40 kb.

Successful genotyping analysis was performed on all native genomic samples using the Illumina 610 Genotyping BeadChip. The metrics used to assess DNA quality were: 1) call rate (the proportion of all SNPs called across each sample), 2) genotype concordance between DNA samples stored in DNAstable and at -20C, and 3) standard deviation Log R ratio (a parameter based on the normalized intensity values and an important metric for studies of copy number variants). The average call rate for all samples was >99.954% and the genotype concordance between all was >99.999% (Table 1). Samples stored dry at RT or 37°C exhibited lower standard deviation Log R ratios (0.1734 and 0.1783, respectively) than reference samples kept at -20°C (0.1809) indicating higher sample purity and quality following storage in DNAstable.

Table 1. Results of Human610 BeadChip (590,622 SNPs) Genotyping Analysis

Sample	Condition	Call Rate	Frozen-Dry GT concordance	Standard Deviation Log R Ratio
C00362	Frozen	99.9492%	NA	0.1693
C00362	25°C - dry	99.9590%	99.9992%	0.1668
C00362	37°C - dry	99.9709%	9.9992%	0.1724
C00120	Frozen	99.9485%	NA	0.1796
C00120	25°C - dry	99.9462%	99.9994%	0.1800
C00120	37°C - dry	99.9594%	99.9993%	0.1969
C00146	Frozen	99.9511%	NA	0.1747
C00146	25°C - dry	99.9360%	99.9991%	0.1715
C00146	37°C - dry	99.9656%	99.9992%	0.1725
C00217	Frozen	99.9555%	NA	0.2000
C00217	25°C - dry	99.9428%	99.9997%	0.1752
C00217	37°C - dry	99.9687%	99.9997%	0.1714
Average (4 samples)	Frozen	99.9511%	NA	0.1809
Average (4 samples)	25°C - dry	99.9460%	99.9994%	0.1734
Average (4 samples)	37°C - dry	99.9661%	99.9993%	0.1783

Compatibility of DNAstable with common strategies used to pre-select and amplify genomic regions of interest was assessed by performing long-range PCR using rehydrated DNA as template for reactions (Figure 2). Results demonstrate that the integrity of genomic DNA subsequently used for SNP genotyping is maintained during dry storage in DNAstable.

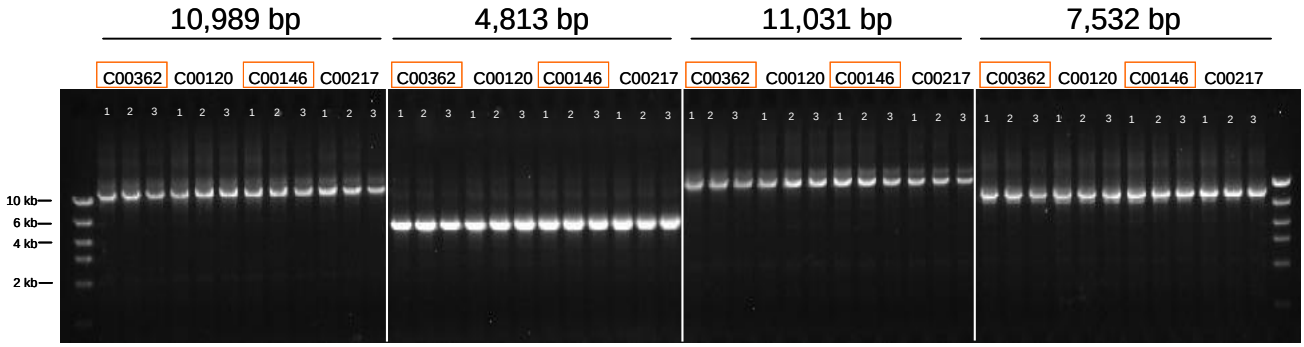

Figure 2. Long-range PCR. Aliquots of genomic DNA recovered from storage at (1) -20°C; (2) RT; and (3) 37°C were used as templates in long-range PCR reactions. Amplicon size (bp) noted above.

Conclusions and Discussion:

Human genomic DNA samples purified from whole human blood were stored dry and protected in DNAstable for 14 days at room temperature or for the equivalent of 28 days at room temperature (conditions simulated by accelerated aging through storage at 37°C for 14 days). Following recovery using a simple one-step rehydration protocol, samples were used directly, without further purification or concentration, in downstream applications to assess the purity, integrity and stability of recovered DNA samples, as well as the compatibility with the Infinium genotyping assay.

Results indicate recovery of intact genomic DNA following dry storage in DNAstable, even at elevated temperatures with no difference in the quality or quantity as compared to reference samples stored at -20°C. Genotyping of the samples on the Human610 BeadChip produced genotype calls in over 590,000 SNPs. All samples were successfully genotyped regardless of storage temperature. There was no difference in the call rate between samples stored at -20°C or dry in DNAstable and the >99.999% concordance rate indicates successful SNP analysis following dry storage. The lower standard deviation Log R ratios obtained for dry stored samples is an important and sensitive criterion for accurate CNV analysis.

All of these results indicate that the presence of DNAstable in the recovered samples does not interfere with or inhibit the analysis and is compatible with the Illumina Infinium assay that employs the Human610 Genotyping BeadChip. DNAstable provides a convenient alternative to traditional freezer storage of precious genomic DNA samples destined for genotyping analysis.

DNAstable has been validated for stabilizing DNA purified from human blood for over 2 years at ambient temperatures. DNA stability has also been rigorously assessed by storage at extremely high temperatures in order to simulate accelerated aging conditions and results indicate sample stability equivalent to 30 years storage at room temperature. DNA stored in DNAstable can be stored, archived or shipped at ambient temperatures without the need for conventional cold storage and sample integrity is maintained for downstream applications.