Performance of DNAstable® with the Nanogen® Genotyping System

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Introduction:

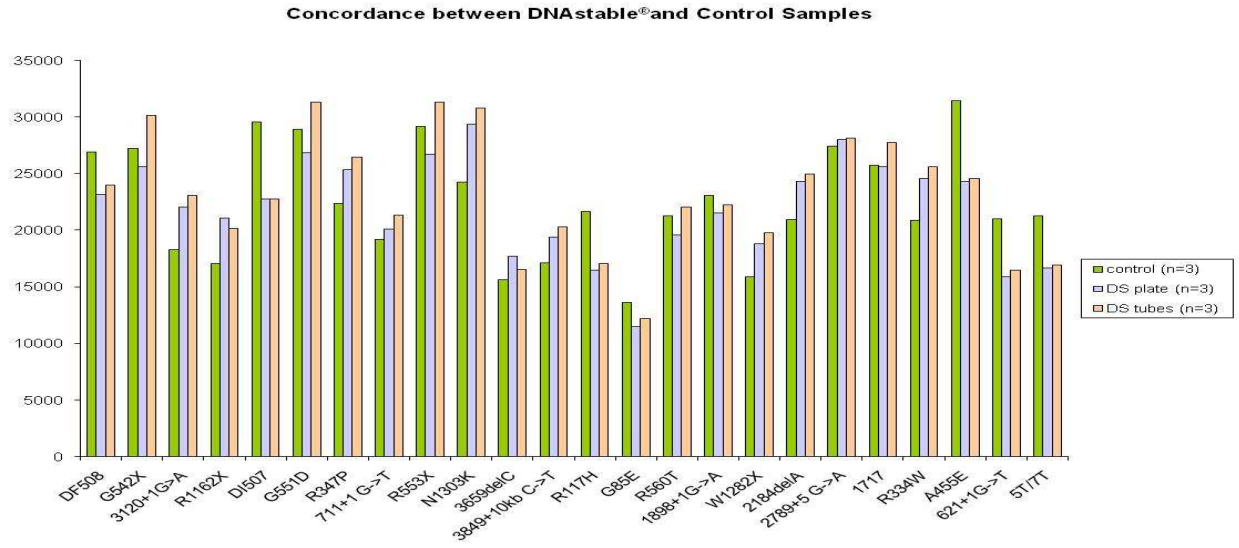
Molecular diagnostics assays are typically performed on genomic DNA samples freshly isolated from patient specimens; however, in cases where stored genomic DNA is used, preserving sample integrity becomes critical. Biomatrica® has developed a novel synthetic chemistry based on the principles of anhydrobiosis (meaning "life without water") for room temperature storage of complex biological samples, including nucleic acids. Biomatrica's DNAstable® storage technology was assessed for compatibility with the amplification and detection of mutations in the Cystic Fibrosis Transmembrane Regulator gene using Nanogen® reagents*. The assay detects and identifies a panel of mutations in the CFTR gene in DNA isolated from blood specimens. The 23 ACOG/ACMG recommended CFTR mutations detected by the Nanogen reagents are listed below:

1	ΔF508	7	R347P	13	R117H	19	2789+5 G->A
2	G542X	8	711+1 G->T	14	G85E	20	1717
3	3120+1G>A	9	R553X	15	R560T	21	R334W
4	R1162X	10	N1303K	16	1898+1G->A	22	A455E
5	ΔI507	11	3659delC	17	W1282X	23	621+1G->T
6	G551D	12	3849+10kb C->T	18	2184delA		

*For Investigational Use Only; The performance of these reagents have not been established. This product is currently under FDA review

Materials and Methods:

Human whole blood was used for the extraction of genomic DNA using the QIAamp® DNA Blood Mini Kit (Qiagen). The Nanogen CFTR genotyping reagents have been tested with template inputs ranging from 25 ng to 2000 ng per amplification reaction. In order to test under the most rigorous conditions, a total of 20 µl at 5 ng/µl of genomic DNA were stored dry at room temperature in DNAstable® 96-well plates and 1.7 ml standard microfuge tube formats for 2 months. A control genomic DNA sample was stored at 4ºC for an identical interval. DNAstable stored samples were re-hydrated with 20 µl of water and used directly without any further purification. PCR reactions were set up using 5 µl of genomic DNA from each storage condition: control (n=3); DNAstable plate (n=3); and DNAstable tubes (n=3) for a total of 9 reactions. Amplified DNA was tested according to the manufacturer's protocol. The performance of DNAstable stored DNA was compared to control DNA (4ºC).



Results and Discussion:

There was no difference observed in the performance of DNA stabilized in DNAstable (plates or tubes) or cold stored control. All storage conditions gave unequivocal genotyping results for all 23 markers. Furthermore, there was virtually no difference in the fluorescent signal intensities seen per marker between samples stored in DNAstable and control DNA. Dehydration and storage of genomic DNA for up to 2 months in DNAstable is compatible with downstream processes involving amplification (PCR) and genotype detection on the Nanogen platform. As little as 25 ng of re-hydrated genomic DNA could be processed without any loss in performance when compared to an equivalently stored genomic DNA sample maintained at 4ºC. These results indicate that DNAstable does not exhibit any interference or inhibition when used for storage and stabilization of genomic DNA for detection of CFTR markers on the Nanogen platform.