

## Assessment of the Integrity of DNA after Long-term Storage in the PGDNABank

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### Introduction

Knowledge of human genetics and genetic variants has expanded rapidly over the last decade due to advances in next generation (NextGen) sequencing technologies and the steady march of human genetics research. Despite this rapid increase in knowledge, the DNA sequence variants which contribute to disease are not completely understood. The curation of a family's medical history, as well as appropriate genetic testing of affected and unaffected individuals can aid in reaching an accurate molecular diagnosis. Often, testing of many family members is required to reach a definitive answer.

DNA Banking is a means by which an individual's genetic material is preserved for decades or even centuries. This allows for future clinical testing and potential benefits to descendants and other living family members. For example, approximately half of the children and siblings of patients with autosomal dominant hereditary cancer syndromes will have a significantly increased risk of cancer. Preservation of DNA allows for DNA testing in a family decades after an affected family member has died (Quillin et al. 2011. PubMed ID: 21886501).

**PGDNABank** is a service offered by PreventionGenetics for the long-term storage of DNA samples for future clinical testing. PreventionGenetics has a long history of offering high-quality genetic testing. DNA banking has been part of our service since 1996. To assess the effectiveness of our current long-term DNA storage practices, we conducted a series of multi-year experiments assessing DNA quality under a variety of storage conditions. The results of these studies are reported here.

### What Happens to DNA in the PGDNABank?

After receiving blood, saliva, or tissue samples from patients, genomic DNA is extracted and purified using standard clinical procedures. In order to ensure their integrity and safety, these extracted genomic DNA samples are stored under two different conditions: DNA dissolved in Tris-EDTA buffer (TE) is stored at -80°C at the main PreventionGenetics **PGDNABank**, whereas wicked DNA (DNA dried onto a paper card) or more recently, DNA absorbed unto trehalose is stored dry at -20°C at an offsite location. The offsite DNA serves as a backup for the frozen DNA in the event of fire or other disaster.

## What is the Quality of DNA after 20 Years of Storage in the PGDNABank?

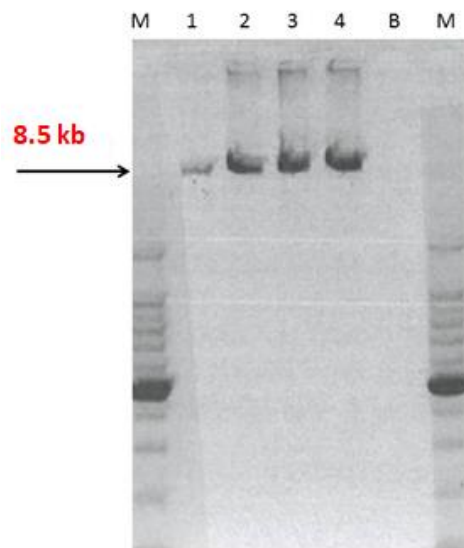
The aim of this retrospective study was to investigate the stability and integrity of DNA samples stored at PreventionGenetics since 1996. Three 20-year-old control DNA samples (wicked DNA, DNA dissolved in TE and stored at 4°C, and DNA dissolved in TE and stored at -80°C) as well as one fresh DNA sample acting as a control were selected for the following experiments. All samples were obtained from the same individual.

Thirteen autosomal DNA microsatellite markers were used to generate a “genotyping panel” on each of the DNA samples. The three DNA samples from the various treatment conditions showed similar intensity of DNA polymerase chain reaction (PCR) fragments ranging in size from about 100-350 base pairs (bp) when compared to fresh DNA samples (data not shown). The same microsatellite alleles were identified in each sample.

### Amplification of Small-, Medium-, and Long-Range PCR

For small- and medium-range PCR, all four DNA samples were amplified using a Roche Expand Long Template System (Sigma; St. Louis, MO). In this assay, four sets of PCR amplicons sized at 1,577, 2,272, 3,715, and 6,883 bp were successfully produced (data not shown).

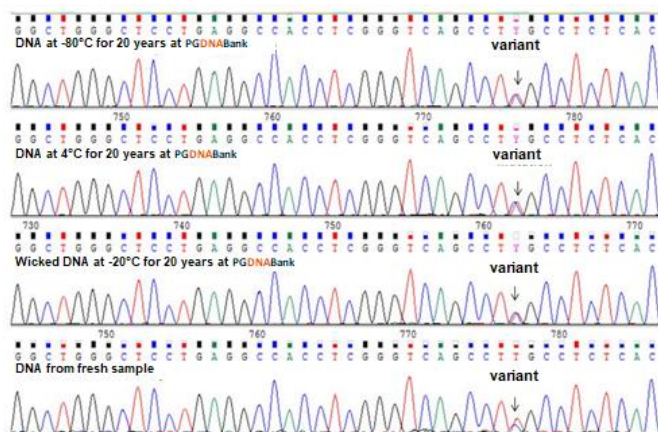
For long-range PCR, all four DNA samples were amplified using a Roche Expand Long Range System (Sigma; St. Louis, MO). PCR successfully generated the expected 8.5 kilobase (kb) amplicon in the *CYP21A2* gene for each sample. However, the yield of the PCR amplification from wicked DNA was markedly less than that of DNA stored under other conditions (Figure 1).



**Figure 1:** Long-range PCR amplicons encompassing the *CYP21A2* gene (8.5 kb). Lane 1, -20°C wicked DNA; Lane 2, 4°C DNA in TE; Lane 3, -80°C DNA in TE; Lane 4, fresh control DNA; Lane M, DNA size markers; Lane B, blank control. While PCR from DNA stored in each condition did result in the formation of an 8.5 kb amplicon, the yield of PCR amplification from wicked DNA was markedly less than that of DNA stored under other conditions.

## DNA Sanger Sequencing

PCR amplicons were subjected to standard Sanger dideoxy sequencing methods on a 3730xl capillary electrophoresis instrument (Applied Biosystems/Hitachi). Forward and reverse sequences were generated. High quality DNA sequence was obtained in both the forward and reverse directions for DNA stored under all conditions assessed (Figure 2).



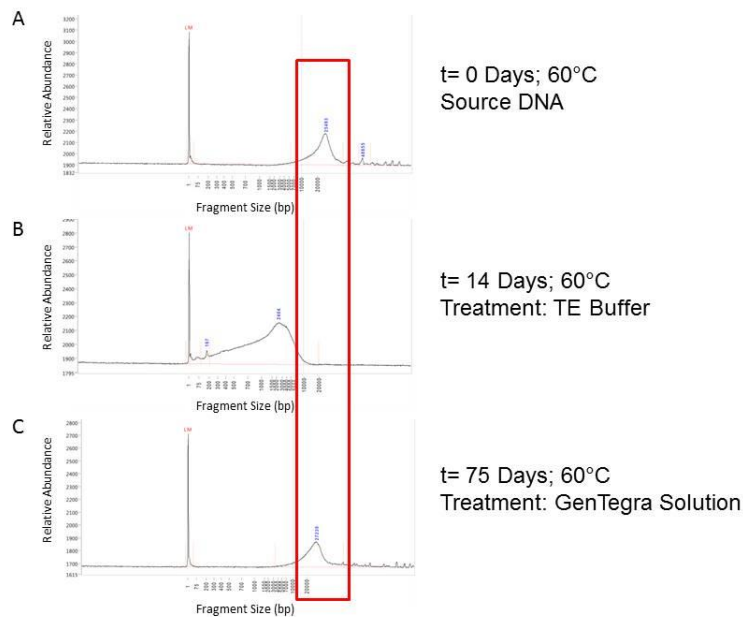
**Figure 2:** Sanger sequencing of 20 year-old DNA stored at -80°C, 4°C, and wicked DNA stored at -20°C compared to a fresh sample of the same DNA. High quality DNA sequence was obtained in both the forward and reverse directions for DNA stored under all conditions assessed.

## Long-Term Storage of DNA with GenTegra

While the DNA samples stored on-site continue to be dissolved in TE buffer and frozen at -80°C , we recently decided to replace the wicked DNA storage method with a new technique utilizing the protective effects of trehalose on nucleic acid stabilization (Clermont et al. 2014. PubMed ID: 24955733; Smith and Morin. 2005. PubMed ID: 16225214). We used a trehalose product known as GenTegra (GenTegra LLC; Pleasanton, CA) to store a portion of the DNA dry at -20°C.

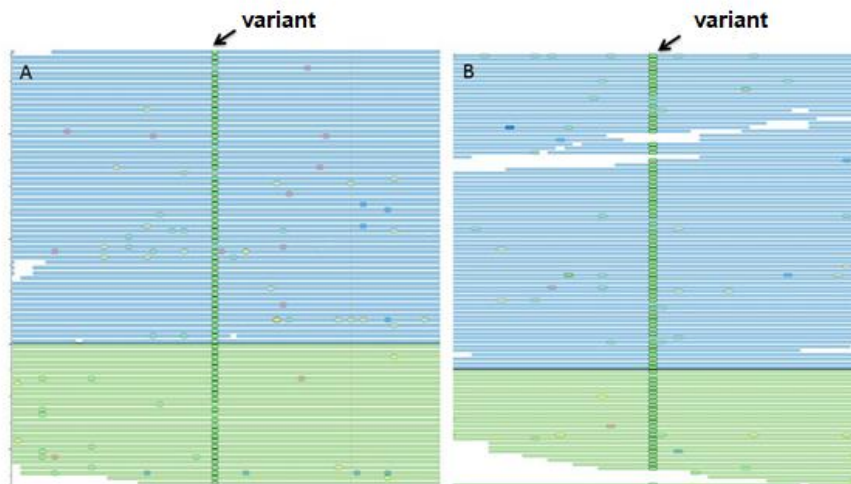
Storage of samples under extreme environmental stress, such as heat, can accelerate sample aging (Lee et al. 2012. PubMed ID: 21324769). In order to simulate long-term storage conditions and the effectiveness of this new process, GenTegra and TE buffer-treated DNA were incubated in a 60°C oven for 8 months, with data collected at various time points.

DNA was extracted using standard procedures and confirmed to be of sufficient quality, with the majority of the sample having a molecular weight of over 20 kb (Figure 3A). The amount of high molecular weight DNA was assessed using an Advanced Analytical Fragment Analyzer (Advanced Analytical; Ankeny, IA). After just 2 weeks, the DNA in solution showed a marked decrease in molecular weight, with an average fragment size of ~2.4 kb (Figure 3B). In contrast, after 2.5 months (75 days) at 60°C, samples treated with the GenTegra product had no significant shift in the average molecular weight (Figure 3C).



**Figure 3:** A. Confirmation of high molecular weight DNA using the PreventionGenetics extraction protocol was assessed on a Fragment analyzer. DNA was then treated with either GenTegra solution or TE buffer and heat-treated at 60°C for various time points. B. DNA treated with TE Buffer only underwent significant evaporation in only two weeks, and a marked decrease in the average molecular weight of the sample was observed. C. DNA treated with GenTegra solution did not experience severe evaporation or degradation, as apparent by a similar average molecular weight observed in the source DNA and the treated DNA.

After 8 months, the heat treated GenTegra mixtures were evaluated by DNA Fingerprint, small-medium- and long-range PCR, and Sanger and NextGen sequencing using fresh DNA samples as a control. No difference was observed between heat-treated and fresh DNA samples. The NextGen panel of Parkinson's disease was selected for NGS sequencing validation (Figures 4A and B).



**Figure 4:** A. DNA-GenTegra mixture samples were subjected to NextGen sequencing after being heat treated in a 60°C oven for 8 months. B. Fresh DNA samples were subjected to NextGen sequencing.

## Conclusion

DNA stored for 20 years in the PreventionGenetics DNA Bank retained its integrity as assayed by PCR amplification of small-, medium-, and long-range amplicons, Sanger sequencing, and NextGen sequencing. DNA stored dry after treatment with trehalose was stable at 60°C for 8 months. TE buffer alone was not sufficient to maintain DNA quality under heat stress. This (in part) retrospective study confirms the high quality and integrity of DNA samples in **PGDNABank**.

## References

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