Application Note

GenTegra

Storage in GenTegra™ DNA Tubes Preserves Quality and Integrity of Bison DNA for use in PCR-Based Assays

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Abstract

Dry-state, room temperature storage of DNA in GenTegra™ DNA Tubes is an innovative method of preserving DNA quality, purity and integrity. It is easy to recover DNA from GenTegra™ DNA tubes, and no further purification is required prior to downstream applications such as mitochondrial DNA assays.

Introduction

Researchers at Texas A&M University have developed PCR-based assays to detect the presence of domestic cattle crossbreeding within populations of bison and wild cattle and improve conservation efforts for these species. In the present study, genomic DNA was purified from blood samples collected from wild American bison. Following genetic analysis, the remaining purified DNA is saved for use in future studies. However, current methods of DNA storage in freezers are a costly and inefficient manner of preserving the increasing number of purified DNA samples. Recently, GenTegra LLC has developed GenTegra™ DNA, an innovative
technology which provides a dry state, room temperature environment for DNA storage. GenTegra™ DNA tubes contain an inert chemical matrix that allows storage of DNA in a "bone dry", water-free environment, which protects DNA samples from hydrolysis and oxidation. The GenTegra™ matrix ensures integrity, stability, and quantitative recovery of DNA samples. The purpose of this study was to evaluate the integrity, quality and purity of DNA stored in GenTegra™ DNA Tubes and its applicability for use in American bison genetic studies.

Materials and Methods

DNA Purification

Whole blood samples were collected in 2000 from American bison (Bison bison) from a herd maintained by the National Park Service. Genomic DNA was extracted from buffy coats using the Super Quik-Gene DNA Isolation Kit (AGTC, Denver, CO). Purified DNA samples were stored at -80ºC. A mitochondrial PCR assay and numerous nuclear microsatellite multiplexes were performed on the samples from 2000-2002, and the resultant genotypes were stored in a database for comparative purposes.

Application, Storage and Recovery of DNA

In August 2008, four samples (A-D) were thawed at 4ºC and concentrations were determined via spectrophotometry on a NanoDrop 1000. A 50 ng/µl aliquot was prepared for each sample. The presence of high molecular weight DNA was confirmed via agarose gel electrophoresis. A mitochondrial PCR assay was performed on each sample to confirm the DNA was still viable for PCR. Four 1 µg aliquots of each sample were applied to GenTegra™ DNA Tubes and dried overnight according to the manufacturer's instructions. After drying, the GenTegra™ tubes were stored at room temperature for 14 days (real-time study) or 37°C for 14 days (accelerated study, equivalent to 28 days). An aliquot of each sample was also stored at -20°C. DNA was recovered from GenTegra™ tubes in a volume of 20 µl according to the manufacturer's instructions and stored at 4°C thereafter.

DNA Quantitation and Gel Electrophoresis

DNA was quantitated using a NanoDrop 1000 (Thermo Scientific). The molecular weight of the recovered DNA was examined by running 50 ng of unamplified DNA directly on a 0.8% agarose gel.

PCR Analysis

Two multiplexed PCR assays were performed on each sample. For each target, one of the primers was labeled with a fluorescent tag (6-FAM, NED, or VIC). Amplification was performed in 5-µL volumes with 1-µL of DNA. PCR products were separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems). A ROX-labeled internal size standard (Mapmarker LOW, Bioventures, Inc.) was utilized for inter-assay standardization. Allele identification and comparison was performed with the fragment analysis program GeneMapper 3.7 (Applied Biosystems).

Results

The quantity and quality of recovered DNA was evaluated with a NanoDrop 1000 spectrophotometer by measuring the absorbance at 260 nm, the 260/280 ratio (as a measure of DNA quality), and the estimated concentration of DNA (ng/µl). A 1.5 µl aliquot of each sample was examined, from which the maximum expected concentration was 50 ng/µl (1 µg DNA in 20 µl recovery solution). For comparison, an identical aliquot of the original DNA, which had been stored at -20ºC during the testing period, was also evaluated. The estimated concentration of the samples ranged from 35 - 112 ng/µl, while A260/280 ratios ranged from 1.75 - 2.04. No differences in DNA quantity or quality were detected for samples stored at room temperature or 37ºC compared with those stored at -20 ºC.

The molecular weight of the recovered DNA was examined by gel electrophoresis, revealing a band of at least 21.2 kb for all samples with minimal smearing. There was no difference in the molecular weight of samples stored in GenTegra™ DNA Tubes at either room temperature or 37°C and samples stored at -20°C (Figure 1).

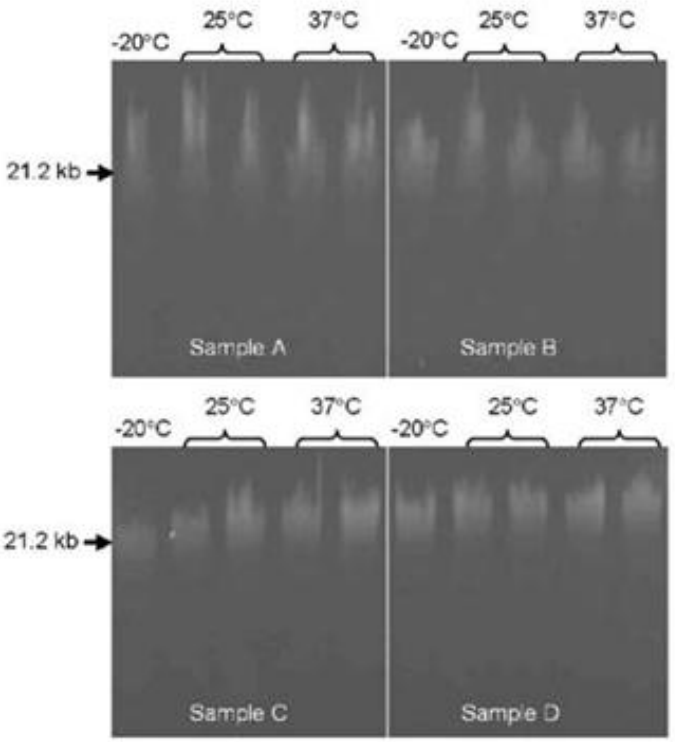

Figure 1. DNA recovered from GenTegra™ DNA Tubes and frozen controls, 50 ng of each sample was run on a 0.8% agarose gel.

The quality of the recovered DNA for PCR was evaluated using two multiplexed assays developed and are routinely utilized at Texas A&M University. The mtDNA assay was designed to differentiate bison and domestic cattle mtDNA haplotypes (Ward et al. 1999), and targets two regions of the mtDNA genome. One set of primers amplifies part of the 16S gene and is used as an internal PCR control; these primers were designed in a conserved region of the 16S gene and, therefore, are expected to produce amplicons across most mammalian species. The second set of primers targets a degenerative region of the D-loop and is expected to amplify a product only when domestic cattle mtDNA is present. The second multiplexed assay amplifies five nuclear microsatellites which are part of a panel developed for parentage analysis in bison and domestic cattle (Schnabel et al. 2000).

The 16S target in the mtDNA assay was successfully amplified for each sample. Large amounts of amplification were detected in each case. The mtDNA haplotypes for the test samples matched the originally-collected haplotypes (bison mtDNA type). An example of the mtDNA assay results for Sample B is shown in Figure 2.

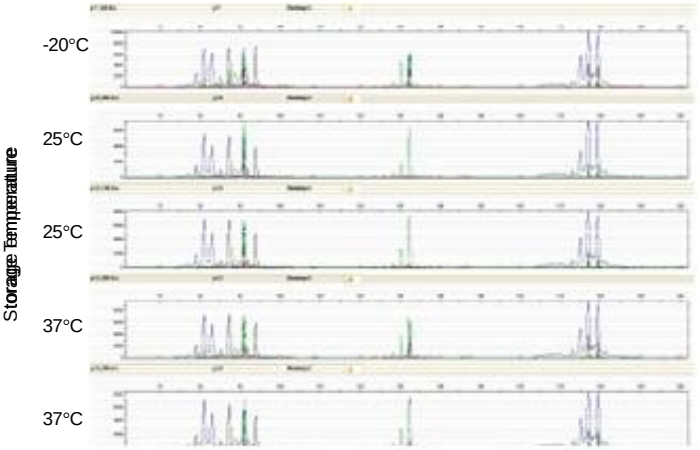

Figure 2. mtDNA assay results for duplicate aliquots of Sample B recovered from GenTegra™ DNA Tubes after storage at 25ºC or 37ºC for 14 days and an identical sample stored at -20ºC.

In the nuclear microsatellite assay, successful amplification for each of the five markers was detected in all samples. An example of the assay results for Sample B is shown in Figure 3. Genotypes were highly consistent across treatments, and were 100% congruent with previously-collected genotypic data.

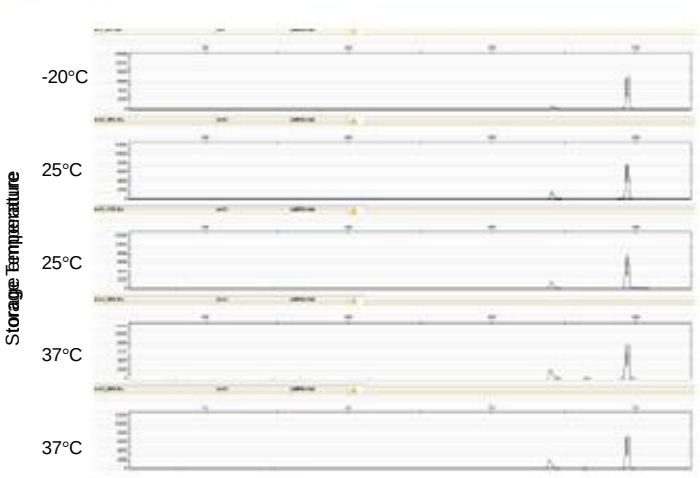

Figure 3. Nuclear microsatellite assay results for duplicate aliquots of Sample B recovered from GenTegra™ DNA Tubes after storage at 25ºC or 37ºC for 14 days and an identical sample stored at -20ºC.

Conclusions

Purified DNA samples were stored in a dry state in GenTegra™ DNA tubes for 14 days at room temperature. Additionally, the same samples were stored in GenTegra™ DNA tubes for 14 days at 37°C, which is equivalent to 28 days of real-time storage. Following recovery, the purity, integrity and stability of DNA samples was assessed. Agarose gel analysis revealed that the recovered DNA was high molecular weight with no evidence of degradation at either storage temperature. No differences in DNA quality or integrity were observed between DNA stored frozen and DNA stored in GenTegra™ DNA tubes, indicating that GenTegra™ DNA Tubes preserved DNA integrity during the storage period.

The inert chemical matrix in GenTegra™ DNA Tubes does not interfere with UV absorbance in the range of DNA and, thus, has no effect on downstream applications, including quantitation. Samples recovered from GenTegra™ DNA Tubes were of high quality and purity, with A260/280 ratios ranging from 1.75 - 2.04. Additionally, no further purification is required for samples recovered from GenTegra™ DNA tubes prior to use in downstream applications. In the present study, the recovered DNA was used directly for multiplexed PCR and fluorescent fragment analysis. Storage of DNA in GenTegra™ DNA Tubes had no effect on the results of either the of the evaluated assays. In the mtDNA assay, the 16S target gene was successfully amplified in all samples, regardless of storage temperature, and the mtDNA haplotypes matched the previously-collected haplotypes in each case. In the PRTG1 assay, all five markers were detected, and the results were 100% congruent with the original genotypic data. GenTegra™ DNA Tubes provide an innovative method of storing DNA safely at room temperature. Shipping samples at room temperature in GenTegra™ DNA Tubes is cost-effective and eliminates concern about shipping delays that can result in loss of frozen samples. In conclusion, GenTegra™ DNA Tubes preserve DNA integrity and pure, high molecular weight DNA is easily recovered for immediate use in downstream applications.

References

Ward TJ, Bielawski JP, Davis SK, Templeton JW, Derr JN (1999) Identification of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap. Animal Conservation 2, 51-57.

Schnabel RD, Ward TJ, Derr JN (2000) Validation of 15 microsatellites for parentage testing in North American bison, Bison bison and domestic cattle. Animal Genetics 31, 360-366.