INTRODUCTION

Advances in recombinant technology and completion of the Human Genome Project paved the way for identification and detection of genetic markers of disease. DNA, though considered a relatively stable macromolecule, is susceptible to hydrolysis. DNases, radiation, free radicals and a number of destabilizing conditions (John GB, 2008). Availability of high quality DNA is essential for incidence and epidemiological studies. The increasing trend to study disease and drug response at the genetic level has focused attention on DNA as a precious resource (Jennier Joiner, 2002). Degradation of DNA has a major effect on the results generating errors that are both quantitative and qualitative. Reduction in DNA size may have an effect on downstream applications such as PCR-based and hybridization assays. For Whole Genome Amplification it is critical that the DNA is of high molecular weight so the amplified product has low level of focus or allelic bias (Laikene et al. 2003).

Therefore, determination of efficient storage methods is critical to maintain the quality of isolated DNA. Several storage conditions were evaluated to determine the best method to store genomic DNA without compromising quality.

In this study, high quality genomic DNA was extracted from whole blood using the Autopure Workstation. The DNA was dissolved in TE buffer and stored at various conditions: room temperature (RT), 4°C, -20°C and -80°C. Real time and stress stability studies were performed. DNA quality was evaluated by agarose gel electrophoresis, PCR amplification of an indicator housekeeping gene (β-globin), and SNP assays on various platforms.

MATERIALS & METHODS

DNA Extractions: Genomic DNA from whole blood was extracted using GeneTorrent’s Autopure LS work station. The DNA was dissolved in TE buffer, and the yields were quantitated by OD measurements. DNA samples were then moved to the respective test conditions for the study (Table 1). All the testing was performed in triplicates.

Analysis of Extracted DNA for Quality Control: Quality of the DNA is determined by performing agarose gel analysis and PCR amplification on the extracted DNA. The presence of high molecular weight DNA with no smear on the gel suggests that the DNA is of high quality. PCR amplification was performed on 5ng of purified DNA by using the β-globin primer pair that amplifies a ~536 bp DNA fragment. Successful amplification suggests that the extracted DNA does not contain any PCR inhibitors.

SNP Analysis: DNA from various test conditions were tested for Single nucleotide polymorphisms (SNPs) using ABI’s TTHF_A1026C SNP assay and Factor II G20210A on ABI 7500 Sequence Detector System (Applied Biosystems, Inc., Foster City, CA, USA).

RESULTS

Table 1: Parameters to analyse the stability of genomic DNA

<table>
<thead>
<tr>
<th>DNA Concentration</th>
<th>Test Parameters</th>
<th>Time Interval</th>
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</thead>
<tbody>
<tr>
<td>30 μg/mL and 20 μg/mL</td>
<td>PCR</td>
<td>0, 7, 14, 21, 28D, 3M</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>4, 6, 8, 10, 12, 15 and 18D</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>3, 5 8, 11, 12, 15 and 18D</td>
</tr>
</tbody>
</table>

CONCLUSIONS

DNA stored in dry state at room temperature showed degradation more rapidly then other storage conditions. For short term studies genomic DNA can be stored at 4°C or even RT without degradation, but samples should be monitored for DNA concentration and evaporation.

REFERENCES