Stability of Genomic DNA at Various Storage Conditions

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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 for 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 (Jennifer 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 locus or allelic bias (Lasken 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 Gentra System's Autopure LS work station. The DNA was dissolved in TE buffer, and the yields were quantitated by OD reading at 260 nm using the SpectraMax Plus Spectrophotometer (Molecular Devices) and Picogreen quantitation was performed using Quant-iT™ PicoGreen® dsDNA Assay Kit From Molecular Probes (Invitrogen). DNA was normalized to 2 concentrations, 100+/-20µg/mL and 20+/-5µg/mL. The normalized DNA was aliquoted into multiple tubes at 50µL volume. The tubes 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 smearing on the gel suggests that the DNA is of high quality. PCR amplification was performed on 50ng 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 amplification inhibitors.

SNP Analysis: DNA from various test conditions were tested for Single nucleotide polymorphisms (SNP's) using ABI's MTHF_A1298C 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

DNA Concentration	Test Parameters	Time Interval
100 µg/mL and 20 µg/mL	RT	0, 7, 14, 21, 28D, 3M, 6M, 9M and 12M
	4°C	
	-20°C	0, 9, 12, 24, 36, 48 and 60M
	-80°C	
	Freeze Thaws	1, 3, 5, 8, 10, 12, 15 and 19FT

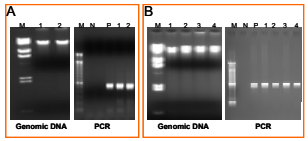

Figure 1: Stability of Genomic DNA at -20°C and -80°C

Panel A: Agarose gel electrophoresis and PCR amplification (536 bp) of Genomic DNA at zero timepoint
Lane M: DNA Marker
Lane N: Negative control
Lane P: Positive Control
Lane 1: -20C
Lane 2: -80C

Panel B: Agarose gel electrophoresis and PCR amplification (536 bp) of Genomic DNA after 24 months of storage at -20°C and -80°C
Lane M: DNA Marker
Lane N: Negative control
Lane P: Positive Control
Lane 1&2: -20C
Lane 3&4: -80C

Panel C: Genomic DNA stored at -20°C and -80°C remains stable for 24 months (studies still ongoing). Concentration of DNA had no effect on the stability.

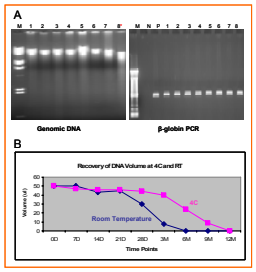

Figure 2: Stability of Genomic DNA at Room Temperature and 4°C

Panel A: Agarose gel electrophoresis and PCR amplification (536 bp) of Genomic DNA at various time points

Lane M: DNA Marker
Lane N: Negative control
Lane P: Positive Control

Lane 1: 4C- 0D
Lane 2: 4C- 3M
Lane 3: 4C- 6M
Lane 4: 4C 9M
Lane 5: RT- 0D
Lane 6: RT- 3M
Lane 7: RT- 6M
Lane 8: RT- 9M
*Degradation of genomic DNA

Panel B: The volume of DNA recovered at RT and 4°C. DNA stored at RT and 4°C showed varying levels of evaporation

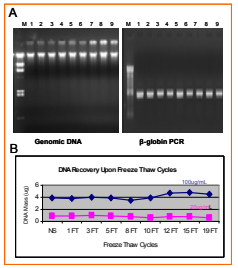

Figure 3: Stability of Genomic DNA Upon Multiple Freeze Thaw Cycles

Panel A: Agarose gel electrophoresis and PCR amplification (536 bp) of Genomic DNA after multiple freeze thaw cycles

Lane M: DNA Marker

Lane 1: NS
Lane 2: 1FT
Lane 3: 3FT
Lane 4: 5FT
Lane 5: 8FT
Lane 6: 10FT
Lane 7: 12FT
Lane 8: 15FT
Lane 9: 19FT

Panel B: Recovery of genomic DNA was not effected upon multiple freeze thaw cycles.

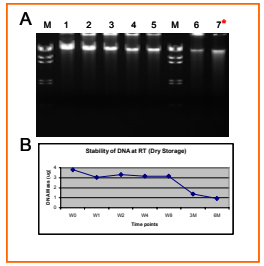

Figure 4: Stability of Genomic DNA at RT in Dry State

Panel A: Agarose gel electrophoresis of Genomic DNA stored in dry state at RT

Lane M: DNA Marker

Lane 1: W0
Lane 2: W1
Lane 3: W2
Lane 4: W4
Lane 5: W8
Lane 6: 3M
Lane 7: 6M
*Degradation of genomic DNA

Panel B: Genomic DNA stored at room temperature under dry state shows loss of DNA recovery after 8 weeks.

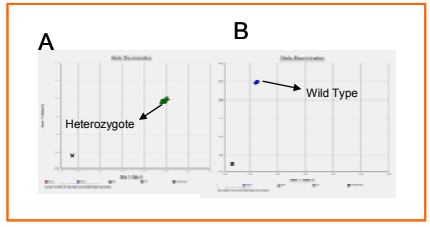

Figure 5: SNP Analysis of genomic DNA stored at 4°C and RT for 12 months

SUMMARY

Genomic DNA aliquots stored at -20°C and -80°C were stable for over 24 months (real time stability still ongoing) (Figure 1). Multiple freeze-thaw cycles up to 19 freeze thaws showed no detectable DNA degradation as assessed by agarose gels, PCR amplification and genotyping (Figure 3). DNA samples stored at 4°C and RT showed varying degrees of evaporation but DNA was stable for up to 12 months at 4°C. Samples stored at room temperature totally evaporated by 6 months (Figure 2). At RT, DNA degradation was seen at 9 months. DNA stored in dry state at room temperature showed degradation at 3 months of storage (Figure 4).

Table 2: Summary of DNA storage conditions on DNA stability

Test condition	Results
-20° and -80°C	Stable up to 24M, studies ongoing for 5 years
4°C	Stable up to 12 M
Freeze Thaws	Multiple Freeze Thaws (19FT) from -80C stable
Room temp (TE Buffer)	Degradation observed from 6M
Room temp (Dry state)	Degradation observed from 3M

CONCLUSIONS

- Genomic DNA stored at -20°C and -80°C was of good quality, and these samples withstood multiple freeze-thaw cycles.
- 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.
- DNA stored in dry state at room temperature showed degradation more rapidly then other storage conditions.

REFERENCES

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