

# 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 ( $\beta$ -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<sup>TM</sup> PicoGreen<sup>®</sup> dsDNA Assay Kit From Molecular Probes (Invitrogen). DNA was normalized to 2 concentrations, 100 $\mu$ g/mL and 20 $\mu$ g/mL. The normalized DNA was aliquoted into multiple tubes at 50 $\mu$ L volume. The tubes were then moved to the respective test conditions for the study (Table 1). All the testing was performed in triplicates.

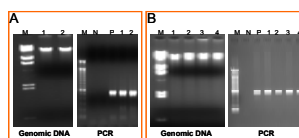
**Analysis of Extracted DNA for Quality Control:** Quality of the DNA is determined by performing agarose gel analysis and PCR amplification on the extracted DNA. The presence of high molecular weight DNA with no smearing on the gel suggests that the DNA is of high quality. PCR amplification was performed on 50ng of purified DNA by using the  $\beta$ -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 $\mu$ g/mL and 20 $\mu$ g/mL	RT	0, 7, 14, 21, 28D, 3M,
	4°C	6M, 9M and 12M
	-20°C	0, 9, 12, 24, 36, 48 and
	-80°C	60M
	Freeze Thaws	1, 3, 5, 8, 10, 12, 15 and 19FT

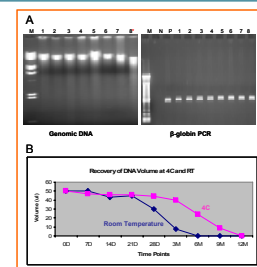


**Figure 1: Stability of Genomic DNA at -20°C and -80°C**

**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: -20C  
Lane 2: -80C

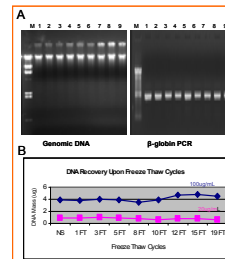
**Panel C:** Genomic DNA stored at -20°C and -80°C remains stable for 24 months (studies still ongoing). Concentration of DNA had no effect on the stability.



**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  
Lane 9: RT-12M  
Lane 10: RT-15M  
Lane 11: RT-18M  
Lane 12: RT-21M  
Lane 13: RT-24M  
Lane 14: RT-27M  
Lane 15: RT-30M  
Lane 16: RT-33M  
Lane 17: RT-36M  
Lane 18: RT-39M  
Lane 19: RT-42M  
Lane 20: RT-45M  
Lane 21: RT-48M  
Lane 22: RT-51M  
Lane 23: RT-54M  
Lane 24: RT-57M  
Lane 25: RT-60M  
Lane 26: RT-63M  
Lane 27: RT-66M  
Lane 28: RT-69M  
Lane 29: RT-72M  
Lane 30: RT-75M  
Lane 31: RT-78M  
Lane 32: RT-81M  
Lane 33: RT-84M  
Lane 34: RT-87M  
Lane 35: RT-90M  
Lane 36: RT-93M  
Lane 37: RT-96M  
Lane 38: RT-99M  
Lane 39: RT-102M  
Lane 40: RT-105M  
Lane 41: RT-108M  
Lane 42: RT-111M  
Lane 43: RT-114M  
Lane 44: RT-117M  
Lane 45: RT-120M  
Lane 46: RT-123M  
Lane 47: RT-126M  
Lane 48: RT-129M  
Lane 49: RT-132M  
Lane 50: RT-135M  
Lane 51: RT-138M  
Lane 52: RT-141M  
Lane 53: RT-144M  
Lane 54: RT-147M  
Lane 55: RT-150M  
Lane 56: RT-153M  
Lane 57: RT-156M  
Lane 58: RT-159M  
Lane 59: RT-162M  
Lane 60: RT-165M  
Lane 61: RT-168M  
Lane 62: RT-171M  
Lane 63: RT-174M  
Lane 64: RT-177M  
Lane 65: RT-180M

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



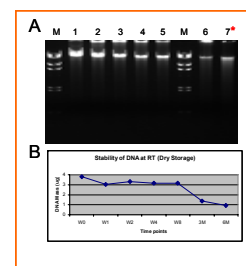
**Figure 3: Stability of Genomic DNA Upon Multiple Freeze Thaw Cycles**

**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: 18FT  
Lane 10: 20FT

**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  
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