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Effects of Storage Buffer and Temperature on the Integrity of Human DNA

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In this study, we have examined the effects of the storage time and temperature on DNA quality and have also studied the effects of the hydration buffer in which DNA is dissolved. This study was performed using 160 human blood samples collected with informed consent from 2007 to 2008 in the hospital where this cohort study was performed. The DNA extracted was dissolved using distilled water (DW) or Tris-EDTA (TE) buffer, and stored in the deep freezer or refrigerator for up to 10 weeks at −70℃, −20℃, 4℃, and 25℃, respectively. DNA integrity was determined by the degree of smearing of DNA on the gel. After four weeks, all of the 20 DNA samples dissolved in DW and stored at 25℃ were entirely degraded. After 10 weeks, 6 of the 20 DNA samples dissolved in TE buffer and stored at 25℃ were fairly degraded, and 4 of the 20 DNA samples dissolved in DW and stored at 4℃ were fairly degraded. The 20 DNA samples dissolved in TE buffer and stored at 4℃ were stable for 10 weeks. DNA samples stored at −20℃ and −70℃ did not appear to degrade in either DW or TE buffer, even at the 10-week point. We suggest that TE buffer should use for DNA elution, in order to protect against degradation and to preserve DNA for a long period of time, and the samples should be stored at −20℃ or −70℃.

Key Words : Storage Buffer, Storage Temperature, Human DNA

INTRODUCTION

In recent years, analytics of DNA has been increasingly important for the study of genomes, for the detection of the carrier state of genetic diseases, and for advances in personalized medicine (Diamandis, *et al.*, 2010). A cohort study is a form of longitudinal study (a type of observational study) used in medicine, social sciences, actuarial sciences, and ecology (Porta, 2008). These cohort studies require analysis of a large number of DNA samples, which must be of sufficient quality for DNA analysis.

Human DNA which is colle cted and banked for the cohort study, is widely used for microarray analyses such as single-nucleotide polymorphisms (SNPs), copy-number variations (CNVs), and genome-wide association studies (GWAS) (Stenson, *et al.*, 2009; Redon, *et al.*, 2006). Long-term storage of DNA is often required for the number of these genetic studies. We have performed DNA banking for this cohort study, a long-term, national project. However, one portion of the DNA banking was degraded over a long period of time. Optimal conditions for long-term storage of DNA have not yet been clearly defined. In this study, to investigate the cause of DNA degradation, we have focused on the effects of storage time and temperature ranging from −70℃ to +25℃ in the quality of DNA

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extracted from human blood. Moreover, we have investigated the effects of the hydration buffer in which the DNA is dissolved.

The aim of our study is to determine whether these temperatures could be used as an alternative long-term storage option, assessing various temperatures' impact on the DNA samples.

MATERIALS AND METHODS

1. Subjects and specimens

This study was performed using 160 human blood samples collected with informed consent from 2007 to 2008 in the hospital where this cohort study was performed.

2. DNA extraction and estimation of DNA in the variation of storage temperature

Genomic DNA from whole blood was extracted by the salting out method (Miller, *et al.*, 1998). The concentration of DNA was measured by NanoDrop (ND-1000, Thermo Fisher Scientific, Wilmington, DE, USA). The DNA extracted was dissolved using distilled water or Tris-EDTA (TE, 10 mM Tris-HCl + 1 mM EDTA, pH 8.0) buffer, divided into equivalent aliquots of 50 μg each, and stored in the deep freezer or refrigerator for up to 10 weeks at −70℃, −20℃, 4℃, and 25℃ in capped 1.5 mL microtubes in batches of 20.

3. Assessment of DNA integrity by gel electrophoresis

The DNA stored at each different temperature was subsequently analyzed by gel electrophoresis. One microliter of solution containing 50 ng of DNA and stored at each of the above temperatures, was loaded on a 1% agarose gel for 2 h at 55 V. Gel photo was taken by Gel Documentation system (Gel logic integrated illumination cabinet, Kodak, Rochester, NY, USA). DNA integrity was determined by the degree of smearing of DNA on the gel (Fig. 1).

RESULTS

We have examined the effects of storage time and temperature on DNA quality and have studied the effect of the hydration buffer in which the DNA is dissolved.

First, the DNA extracted was dissolved using distilled water (pH 7.0) or TE (10 mM Tris-HCl + 1 mM EDTA, pH 8.0) buffer divided with equivalent aliquots, and stored in the deep freezer or refrigerator for up to 10 weeks at −70℃, −20℃, 4℃, and 25℃, respectively. The DNA was subsequently analyzed by gel electrophoresis. The degree of degradation is described below. In the beginning stage, DNA smearing appears slightly on the gel (degradation 1+); in the middle stage, DNA smearing appears fairly on the gel (degradation 2+); and in the last stage, DNA smearing appears entirely on the gel (degradation 3+) (Fig. 1).

In this study, DNA stored at each of the four temperatures remained intact at a high molecular weight in the first week and showed no differences between the different temperatures (Fig. 1). Two of the 20 DNA samples (10%) dissolved in distilled water and stored at 25℃ showed early signs of degradation after two weeks (degradation, 〉1+), and 18 of the 20 DNA samples (90%) were severely degraded (degradation, 〉3+) after three weeks. After four weeks, all of the DNA samples (100%) were entirely degraded (degradation, 〉3+), (Table 1) (Fig. 2 A).

On the other hand, the 20 DNA samples (100%) dissolved in TE buffer and stored at 25℃ did not show any degradation after five weeks. After six weeks, however, five of the 20 DNA samples (25%) started to degrade rapidly (degradation, 〉1+). After that, the DNA samples progressively degraded. After ten weeks, 6 of the 20 DNA samples (30%) were fairly degraded (degradation, 〉2+), (Table 2) (Fig. 2 B).

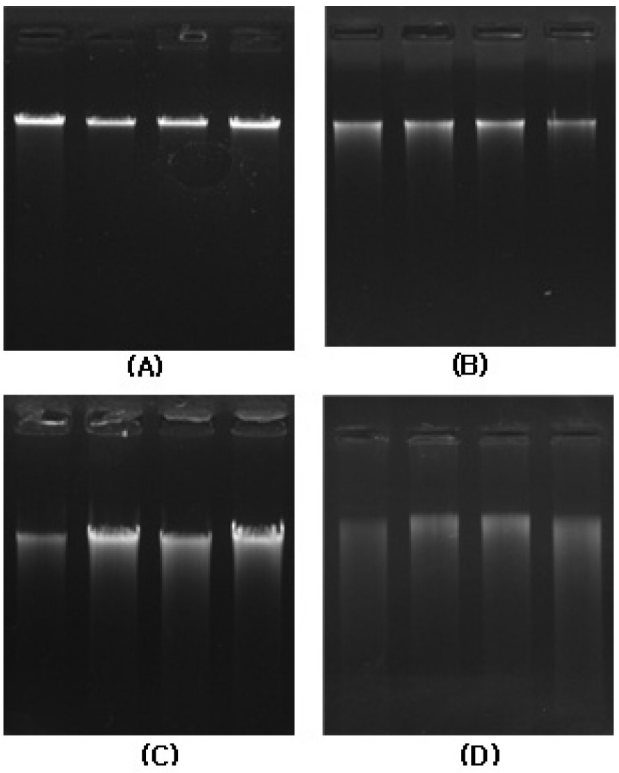

Fig. 1. Electrophoretic pattern of degree of DNA degraded. (A) Normal DNA, (B) The beginning stage, DNA smearing appears slightly on the gel (degradation 1+), (C) the middle stage, DNA smearing appears fairly on the gel (degradation 2+), (D) the last stage, DNA smearing appears entirely on the gel (degradation 3+). DNA samples were separated by electrophoresis in 1% agarose gel and stained with ethidium bromide before photography.

Table 1. DNA degradation depending on storage temperature and distilled water

Storage time (weeks)	Proportion of DNA degradation depending on storage temperature in hydration buffer (distilled water)							
	25℃ (n=20)		4℃ (n=20)		-20℃ (n=20)		-70℃ (n=20)	
	No. of degradation (%)	degree of degradation	No. of degradation (%)	degree of degradation	No. of degradation (%)	degree of degradation	No. of degradation (%)	degree of degradation
1	0	0	0	0	0	0	0	0
2	2 (10)	〉2+	0	0	0	0	0	0
3	18 (90)	〉3+‡	0	0	0	0	0	0
4	20 (100)	〉3+	1 (5)	〉1+*	0	0	0	0
5	20 (100)	〉3+	1 (5)	〉2+†	0	0	0	0
6	20 (100)	〉3+	1 (5)	〉2+	0	0	0	0
7	20 (100)	〉3+	3 (15)	〉2+	0	0	0	0
8	20 (100)	〉3+	4 (20)	〉2+	0	0	0	0
9	20 (100)	〉3+	4 (20)	〉2+	0	0	0	0
10	20 (100)	〉3+	4 (20)	〉2+	0	0	0	0

* 〉1+; degradation 1 stage, DNA smearing appears slightly on the gel.
† 〉2+; degradation 2 stage, DNA smearing appears fairly on the gel.
‡ 〉3+; degradation 3 stage, DNA smearing appears entirely on the gel.

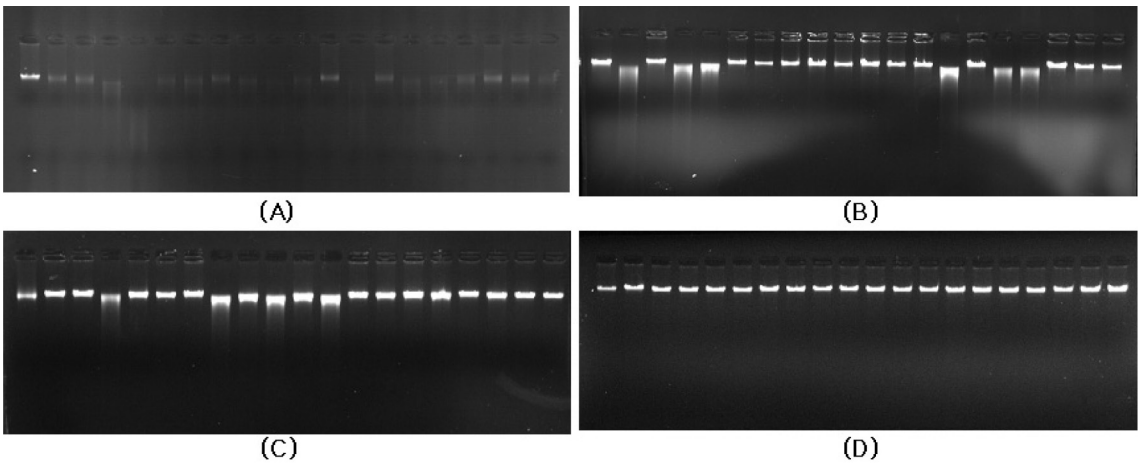

Fig. 2. The effect of storage in the variation of temperature for 10 weeks on DNA in solution. (A) DNA dissolved in distilled water and stored at 25℃ ; All of the 20 DNA samples were entirely degraded (degradation 3+), (B) DNA dissolved in TE buffer and stored at 25℃ ; six of the 20 DNA samples were degraded (degradation 2+), (C) DNA dissolved in distilled water and stored at 4℃ ; four of the 20 DNA samples were degraded (degradation 2+), (D) DNA dissolved in TE buffer and stored at 4℃ ; All of the 20 DNA samples did not show degradation even after 10 weeks. Each lane 1 is the λ DNA marker. DNA samples were separated by electrophoresis in 1% agarose gel and stained with ethidium bromide before photography.

Meanwhile, the 20 DNA samples dissolved in distilled water and stored at 4℃ were stable for three weeks. After four weeks, one of the 20 DNA samples (5%) started to degrade (degradation, 〉1+), and after seven weeks, three of the 20 DNA samples (15%) were degraded (degradation, 〉2+). After that, the DNA samples progressively degraded, and after 10 weeks, 4 of the 20 DNA samples (20%) were fairly degraded (degradation, 〉2+), (Table 1) (Fig. 2 C). However, the 20 DNA samples (100%) dissolved in TE buffer and stored at 4℃ were stable for 10 weeks. (Table 2) (Fig. 2 D).

Meanwhile, DNA samples stored at −20℃ and −70℃ did not appear to degrade in either distilled water or TE buffer, even at the 10−week point. In this study, we found that DNA stored at 25℃ (room temperature) and 4℃ degraded much faster than DNA stored at −20℃ and −70℃ (freezing temperatures), and purified DNA samples are stable at −20℃ and −70℃ for at least three months.

DISCUSSION

The application of genetics both to study human disease and to analyze gene function *in vivo* depend upon the quality of DNA samples (Cushwa and Medrano, 1993). The extraction of DNA, particularly from human leukocytes, and its recovery after long−term storage of this DNA is very important in studying genetic diseases (Mardisen, *et al.*, 1987). Intact, good−quality DNA is also essential for the study of polymorphisms. There are various factors which affect the integrity of DNA, including storage temperature and hydration buffer. Among them are storage temperature and the hydration buffer. Here we presented both a method for preparing DNA for banking and the results of the effects of various temperatures of storage on the gel electrophoretic patterns. The results of this study indicate that both storage buffer and temperature influence DNA stability.

TE buffer is intended to protect DNA during storage by "buffering" against low pH and EDTA−inhibiting nucleas-

Table 2. DNA degradation depending on storage temperature and Tris-EDTA buffer

Storage time (weeks)	Proportion of DNA degradation depending on storage temperature in hydration buffer (Tris-EDTA buffer)							
	25℃ (n=20)		4℃ (n=20)		-20℃ (n=20)		-70℃ (n=20)	
	No. of degradation (%)	degree of degradation	No. of degradation (%)	degree of degradation	No. of degradation (%)	degree of degradation	No. of degradation (%)	degree of degradation
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	5 (25)	〉1+*	0	0	0	0	0	0
7	5 (25)	〉2+†	0	0	0	0	0	0
8	6 (30)	〉2+	0	0	0	0	0	0
9	6 (30)	〉2+	0	0	0	0	0	0
10	6 (30)	〉2+	0	0	0	0	0	0

* 〉1+; degradation 1 stage, DNA smearing appears slightly on the gel.
† 〉2+; degradation 2 stage, DNA smearing appears fairly on the gel.

es. DNA samples remained intact and undegraded for longer periods of time when the DNA was dissolved in higher concentrations of EDTA; EDTA added to the buffer inhibits this degradation (Lahiri and Schnabel, 1993). DNA samples that were dissolved in distilled water showed a double band on the gel in the first day, whereas DNAs dissolved in TE buffer did not show a double band. The results of these experiments are shown in Figure 3. It seems that due to the pH of distilled water (pH 5-6) being lower than the pH of the TE buffer (pH 8.0); DNA cannot be dissolved in distilled water easily, because distilled water is not buffered and can be slightly acidic. Therefore, the agglomerative DNA that could not be dissolved in distilled water is shown by the double band on the gel. We think the DNA degradation may be caused by the agglomerative DNA. Although future studies will be required to ascertain this phenomenon, it is determined that extracted DNA must be dissolved in TE buffer.

In recent years, due to storage temperatures, we experienced a ghastly error when we tried to maintain DNA integrity. It was a serious event that spoiled our cohort study which is part of the national project. In the analysis, the cause of the error was determined to be the long-term storage (more than three months) of DNA at 4℃. Also, we found that long-term storage of DNA at 4℃ as well as 25℃ has a severe and detrimental impact on the yield and the integrity of the DNA in this study. Meanwhile, long-term DNA storage undertaken at −70℃ and −20℃ has been shown to have only a small impact on DNA yield or quality. Therefore, storage of DNA at 4℃ as well as 25℃ offers unacceptable alternative to frozen storage. There have been no reported studies on the impact of long-term storage at 4℃ (conventional refrigerator) on DNA integrity. Although many studies have focused on the temperature effects of DNA storage, there have been no detailed studies to evaluate DNA stored at 4℃, because in the past there was no interest in storing DNA at 4℃ or 25℃. We also confirmed that DNA of analyzable quality could be

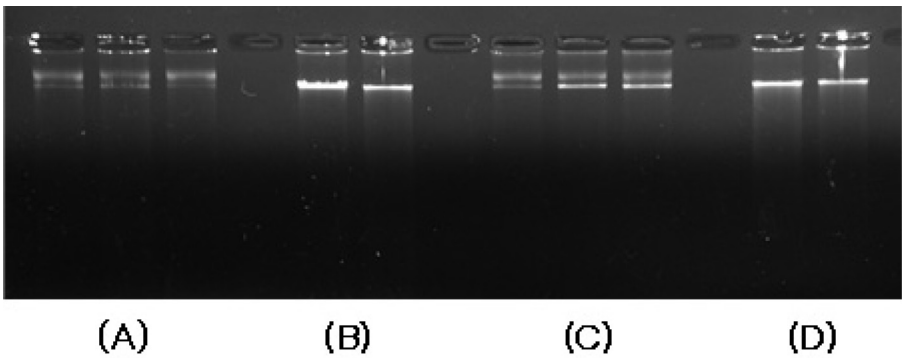

Fig 3. Electrophoretic pattern of band of DNA in solution. (A) (C) ; DNA dissolved in distilled water showed a double band on the gel. (B) (D) ; DNA dissolved in TE buffer did not show a double band on the gel. It seems that due to the pH of water (pH 5–6) being lower than the pH of the TE buffer (pH 8.0); DNA cannot be dissolved in water easily. Therefore, the agglomerative DNA that could not be dissolved in water is shown by the double band on the gel.

obtained when blood has been held at 4℃ or 25℃ for a week or more. The stored blood at 4℃ was stable for one month, but blood stored at 25℃ was only stable for two days in yield and quality (data not shown). Declines in yield occurred within three days whether whole blood or packed cells were stored (Towne and Devor, 1990). Thus, we extracted the genomic DNA within two days after blood-collecting. Interestingly, DNA degradation was not related to ratio (260/280, 260/230) and DNA yield. In other words, although DNA was degraded, DNA yield and ratio was appeared at normal values in the NanoDrop. For this reason, in order to avoid this faulty analysis, we must perform gel electrophoresis for analysis of DNA degradation. DNA from blood samples that had undergone more than four freeze-thaw cycles was found to be partially degraded [8]. We alternatively performed four freeze-thaw cycles of DNA at −70℃ and 25℃, but the DNA was not degraded (data not shown).

In this study, we found that the effect of storage temperature and hydration buffer is crucial in order to maintain the integrity of DNA from whole blood. In particular, the role of the DNA hydration buffer is very important at these temperatures. In other words, DNA eluted in TE buffer can be stored at 4℃ for more than two months, but the DNA eluted in distilled water must be stored at −20℃ or −70℃ because the DNA stored in distilled water at 4℃ can be degraded during prolonged storage.

In conclusion, we recommend that in order to preserve DNA for a long period of time, TE buffer should be used for elution to protect against degradation and the samples should be stored at −20℃ or −70℃. We experienced that DNA which is generally believed to be stable for long periods of time, can actually be easily degraded by the effects of temperature and hydration buffers. Therefore, in order to provide this experience to other researchers, we have investigated this study. This study, the first investigation of its kind published in Korea, will be useful in studies that use DNA stored for very long periods of time.

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