Direct PCR from Whole Blood

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Abstract

Reliable PCR assay results were obtained for the human hemochromatosis gene, for the human apo E gene, which has a G+C content of 75%, and for multiplex assays for the cystic fibrosis transmembrane regulator (CFTR) using the FailSafe™ PCR System to amplify genomic DNA in one microliter of heparin-treated whole blood that was stored for up to six months.

Introduction

Researchers often purify DNA from blood samples prior to performing PCR because it is believed that blood constituents and the reagents commonly used to preserve blood samples (e.g., anticoagulants) interfere with PCR.[1] In order to save the time and expense required for template purification, several methods have been reported for direct PCR of blood samples. These include microwave irradiation[2], hydrogen peroxide treatment[3], and boiling in NaOH.[4] Here, we report PCR amplification results using the FailSafe PCR System to amplify several human genes from unpurified DNA in untreated whole human blood and in whole blood stored for up to six months at +4°C, -20°C, or -70°C in the presence of the commonly-used anticoagulants sodium or lithium heparin, sodium citrate, and EDTA.

Methods and Results

Blood collection

Blood samples were collected using standard blood draw procedures and placed in various types of collection tubes corresponding to the respective method tested. In a limited number of cases, a portion of a collected sample was used for PCR within 5 minutes of collection without any treatment. The remaining blood of each sample was stored in a Vacutainer™ tube with Hemogard™ closure (Becton Dickinson, NJ) containing one of four different commonly-used anticoagulants to prevent clotting: sodium heparin; lithium heparin; 3.2% sodium citrate; or 7.5% K_3EDTA. The different preserved blood samples were then stored at +4°C, -20°C, or -70°C for various times before use.

PCR Conditions

PCR primers and PCR temperature cycling conditions were as described elsewhere by researchers working on the respective templates. The optimal FailSafe PCR PreMix (which contains PCR buffer, $MgCl_2$, dNTPs, and FailSafe™ PCR Enhancer), was determined for each template and primer pair as described in the FailSafe PCR PreMix Selection Kit protocol using purified human genomic DNA as a template; the same optimal FailSafe PCR PreMix was then used to amplify the respective gene from whole untreated or anticoagulant-treated blood.

Analysis of the effect of different blood anticoagulants on PCR of the human hemochromatosis and apo E genes

Blood samples are generally stored in anticoagulants until use. In order to test whether PCR amplifications would be successful using whole blood samples stored in various anticoagulants without DNA extraction, the hemochromatosis gene was amplified from blood samples stored at +4°C in four common anticoagulants - sodium heparin, lithium heparin, sodium citrate, and EDTA. Each 50-µl reaction included 1 X FailSafe™ PCR PreMix D, 50 pmoles of the forward and reverse primers, 2.5 U of the FailSafe™ PCR Enzyme Mix, and 1 µl of the whole blood. Cycling conditions were 5 minutes at 95°C, followed by 38 cycles of 30 seconds at 96°C, 30 seconds at 55°C, and 1 minute at 72°C, then 7 minutes at 72°C. One nanogram of purified human genomic DNA was also amplified under the same conditions as a positive control, while the negative control reaction included everything except for the template.

The ability to amplify difficult sequences, such as sequences with high G+C content, can be crucial for some studies. To test whether the FailSafe™ PCR systems can be used to successfully amplify a G+C-rich sequence directly from blood samples stored in various anticoagulants, PCR was carried out for the apo E gene, which has a G+C content of 75%. Each 50-µl reaction included 1 X FailSafe™ PCR PreMix J, 25 pmoles of the forward and reverse primers, 2.5 U of the FailSafe™ PCR Enzyme Mix, and 1 µl of an anticoagulant-treated whole blood sample. Cycling conditions were 5 minutes at 94°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 1 minute at 72°C. One nanogram of purified human genomic DNA was also amplified under the same conditions as a positive control, while the negative control reaction included everything except the template.

Multiplex PCR of CFTR in heparin-treated whole blood

Multiplex PCR is useful for many assay situations, such as for genetic linkage studies using microsatellite markers or for detection of multiple potential pathogens. In order to determine whether unpurified whole blood samples could be used for multiplex PCR, blood samples stored at -20°C in sodium heparin were used to amplify a five-band multiplex PCR of the cystic fibrosis transmembrane regulator (CFTR). Five exons (4, 10, 11, 20, and 21) of CFTR were amplified using the FailSafe PCR System. Each 50 µl reaction included 1 X FailSafe™ PCR PreMix C, 25 pmoles of each of all 5 sets of the forward and reverse primers, 2.5 U of the FailSafe™ PCR Enzyme Mix, and 1 µl of whole blood sample stored in sodium heparin at -20°C. Cycling conditions were 2 minutes at 94°C, followed by 30 cycles of 10 seconds at 94°C, 10 seconds at 53°C, and 10 seconds at 74°C, then 5 minutes at 74°C.

Effect of storage temperature and time of storage on the ability of heparin-treated whole blood to be used for PCR

Whole blood samples treated with sodium heparin were assayed as described above for their ability to be used for PCR amplification following storage for up to six months at +4°C, -20°C, or -70°C.

Analysis of PCR products

Approximately 10% of each reaction was electrophoresed on a 2% agarose gel and visualized by staining with SyberGold™ (Molecular Probes, OR), unless otherwise stated.

Results and Discussion

Satisfactory PCR amplifications of the hemochromatosis and apo E genes were obtained using freshly drawn untreated blood samples tested (i.e., without any anticoagulants) (data not shown). However, due to the difficulty of working with untreated blood, samples are typically treated with an anticoagulant prior to use or storage.

Our studies indicated that treatment of whole blood with heparin is preferable to treatment with sodium citrate or EDTA if the sample is to be used for PCR amplification. PCR assays for the hemochromatosis gene, the apoE gene, and the CFTR multiplex were always successful for all of the heparin-treated samples tested (Figure 1, Panel A). On the other hand, although amplification was sometimes successful using sodium citrate- or EDTA-treated blood (data not shown), PCR consistency was poor, with an estimated PCR failure rate of about 50%. The consistency of PCR was 100% in sodium heparin-treated blood for the target templates and samples tested. However, it should be noted that this result was based on using only 1 µl of blood per 50-µl PCR reaction. It is possible that use of greater amounts of blood would result in inhibition of PCR, but this was not tested. If PCR of a heparin-treated blood sample fails, the author recommends first increasing the number of PCR cycles in preference to increasing the amount of blood used.

Our data (Figure 1, Panel B) also showed that sodium heparin-treated whole blood can be used successfully to amplify target DNA templates with high G+C content using the FailSafe™ PCR System, such as the apoE gene, which has a G+C content of 75%.

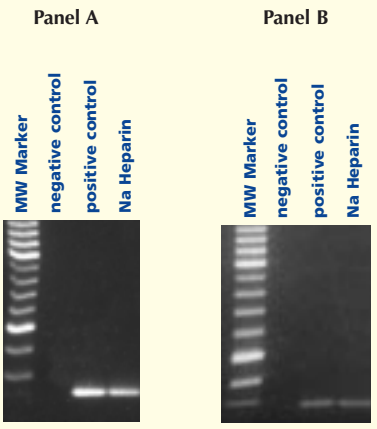

Figure 1. Amplification of hemochromatosis and apoE gene using blood samples stored in sodium heparin.

Panel A. A 200 bp region of hemochromatosis gene was amplified with blood samples stored at 4°C in sodium heparin.

Panel B. A 268 bp region of apo E gene was amplified with blood samples stored at 4°C in sodium heparin.

The positive and negative controls were also included.

Further, the 5 bands representing 5 exons of the CFTR gene were all amplified from DNA in heparin-treated whole blood (Figure 2), indicating that even difficult multiplex PCR targets can be amplified in blood using the FailSafe™ PCR System.

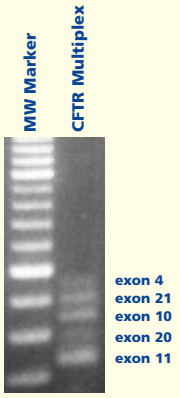

Figure 2. Five-band CFTR multiplex PCR with blood. Blood samples stored in sodium heparin at -20°C were used to amplify CFTR exons, 4, 10, 11, 20, and 21.

Blood samples are generally stored at +4°C, -20°C, or -70°C. Successful direct PCR amplifications were obtained from heparin-treated blood samples stored at all of these temperatures for up to 6 months. The amount of PCR product appeared to be the same from sodium heparin-treated blood samples stored at all three temperatures for the hemochromatosis and apo E genes. For example, Figure 3, Panel B illustrates successful PCR amplifications of hemochromatosis and apo E genes from blood samples stored in sodium heparin for 6 months at -70°C. However, since the intensity of the PCR product bands appeared to decrease somewhat for multiplex PCR of CFTR in sodium heparin-treated blood samples stored for 6 months at +4°C, the author recommends keeping heparin-treated blood samples in a freezer for long-term storage whenever possible. No decrease in the amount of PCR product was observed following multiplex PCR of sodium heparin-treated blood stored for 6 months at -20°C (Figure 3, Panel A).

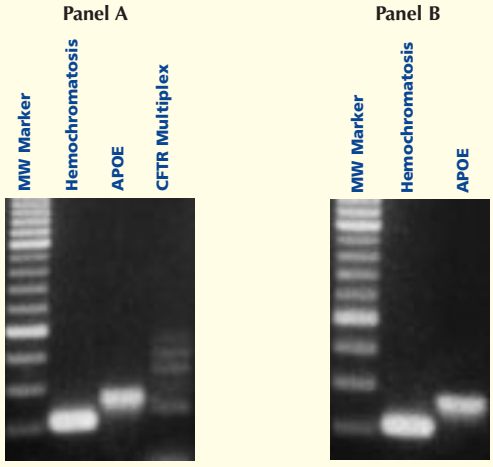

Figure 3. PCR of blood samples stored at -20°C or -70°C. Blood samples in sodium heparin stored at either -20°C (Panel A) or -70°C (Panel B) were used to PCR amplify hemochromatosis and apo E genes. CFTR multiplex PCR was also done with samples stored at -20°C. Approximately 25% of each reaction was electrophoresed on a 2% agarose gel.

Tips for Successful PCR of Whole Blood

1. Use sodium heparin as the anticoagulant. Lithium heparin was just as successful for the assays tested, but fewer assays were performed with this anticoagulant.
2. Use only 1 µl of mixed whole blood per 50-µl PCR assay if possible.
3. If necessary, use more PCR cycles rather than more blood sample to improve amplification efficiency.
4. For short-term storage (i.e., up to about 2 weeks), store blood samples at +4°C. However, for long-term storage, store at -20°C or -70°C since some degradation of DNA, which led to reduced PCR efficiency, was seen with blood samples stored at +4°C compared to those stored at the freezer temperatures.
5. The FailSafe PCR System provides a simple and fast way to find the optimal PCR conditions for any template and primer pair. With every new set of primers tested, obtain the most optimal PCR conditions by using the FailSafe™ PCR PreMix Selection Kit with purified DNA. After the optimal FailSafe™ PCR PreMix is chosen, then use this PreMix for direct PCR from whole blood.
6. While the data presented in this report were obtained for PCR assays using 1 µl of well-mixed whole blood, using 1ml of the buffy coat in the PCR reactions should provide similar results.
7. Use of whole blood in PCR reactions will result in a reddish color in the final PCR products. This does not interfere with the detection of PCR products via electrophoresis.

Conclusion

Consistent successful PCR amplifications were achieved from untreated or heparin-treated unpurified whole blood using the FailSafe™ PCR System. Reliable PCR results were obtained for difficult templates, including multiplex templates and templates with high G+C content. Consistent PCR results were obtained for all targets tested using heparin-treated whole blood samples that were stored for at least 2 weeks at +4°C, or for at least 6 months at -20°C or -70°C.

References

1. Wang, J-T, *et al.* (1992) *J. Clin. Microbiol.* **30**, 750.
2. Ihhara, M, *et al.* (1994) *BioTechniques* **17** (4), 726.
3. Rudbeck, L. and Dissing, J. (1998) *BioTechniques* **25** (4), 588.
4. M. I. Queipo-Ortuna, M. A., *et al.* (1999) *BioTechniques* **27** (2), 248.

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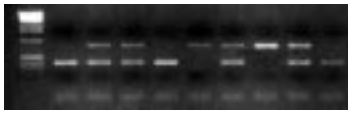
"The FailSafe System worked beautifully! … I'm responsible for providing PCR data trustworthy enough to make breeding decisions for each line. Our experiments require screening of the animals' genotypes. We are now using the FailSafe System to screen two knockout lines (multiplex) and four transgenic (single PCR product) lines of mice. Soon we will be using the FailSafe PreMix Selection Kit again when we start working with a new line of knockout mice."

— Jessica Otte,
Center of Neurovirology & Cancer Biology,
Temple University, Philadelphia, PA



PCR results obtained using the FailSafe™ PCR System to screen for mouse Knockout Gene P. PCR reactions with mouse genomic DNA and two forward primers with one shared reverse primer (0.1 µg each) were incubated for 30 cycles of 90°C for 15 sec., 55°C for 15 sec., and 72°C for 1 min., followed by 72°C for 10 minutes. (Data courtesy of Jessica Otte).

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F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ø80d*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara, leu*)7697 *gal*U *gal*K λ- *rps*L *nup*G

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Table 1. The average transformation efficiency of eight independent transformations of TransforMax™ EC100™ Electrocompetent *E. coli* with pUC vector was 9.2 X 10^9. All values are in cfu/µg DNA.

	Transformation efficiency
TransforMax EC100 *E. coli*	9.2 X 10^9
Competitor S	5 X 10^9
Competitor I	4 X 10^9
Competitor B	3 X 10^9

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