

Extract-N-Amp™: A Novel Approach for Rapidly Extracting and Amplifying Genomic DNA from Blood and Tissue

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Abstract

Standard methods for purifying DNA from blood and tissue can be laborious and time consuming. For this reason, two novel systems, Extract-N-Amp Blood and Extract-N-Amp Tissue have been developed for the rapid extraction and subsequent amplification of genomic DNA from blood and animal tissues. An extraction treatment has been designed that releases sufficient genomic DNA from blood or tissue for direct use in PCR applications. This extraction system eliminates time-consuming steps such as organic extraction, overnight enzyme digestions, column purification, filtration, and alcohol precipitation. A specially formulated PCR reaction mixture includes a hot start antibody for highly specific amplification of genomic DNA, allowing for amplification directly from the extract. The extraction procedures are extremely rapid, with samples being processed in 8 to 15 minutes. To meet the high-throughput requirements for the genetic analysis of blood, an automated method has been developed for the Extract-N-Amp Blood system. Multiplex and quantitative PCR data shown here demonstrate that these procedures extract genomic DNA of sufficient yield and quality to allow for the amplification of target genes from a wide variety of blood and tissue sources. Data shown here also demonstrates the ability to sequence the PCR products generated by this method. In addition the genomic DNA extracts are stable when stored at 4 °C for a minimum of 6 months, allowing for multiple reassays.

Automated Extract-N-Amp Blood PCR Kit Results

Automated Method for the Extract-N-Amp PCR Blood Kit: PCR Analysis Blood Samples

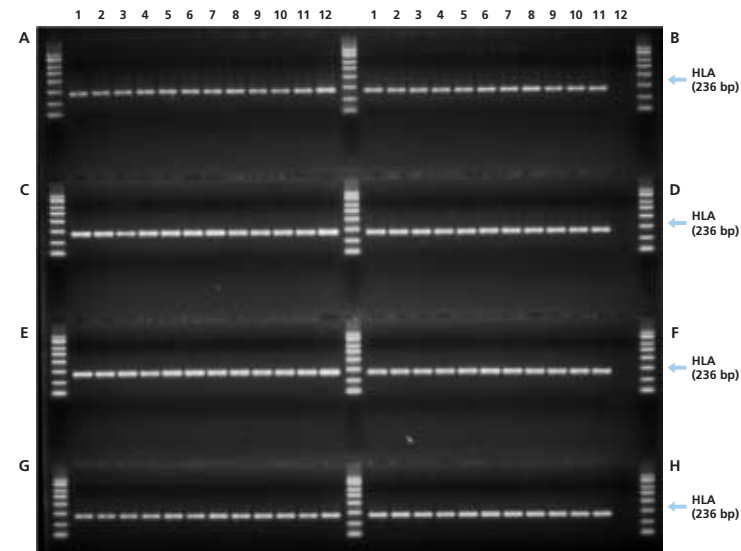


Figure 1: Agarose gel analysis of 96 PCR samples. DNA was extracted from 88 samples of human whole blood (5 µl) from a single donor using the automated Extract-N-Amp™ Blood PCR procedure on the Sciclone ALH 3000. Amplification of 236 bp fragment of the HLA gene followed using 2 µl of extracted template and 2 µl of human genomic DNA controls in a 20 µl PCR reaction incorporating the 2X PCR Ready Mix. 6 µl of each reaction was analyzed on a 2% Agarose gel.

Cross-contamination Analysis

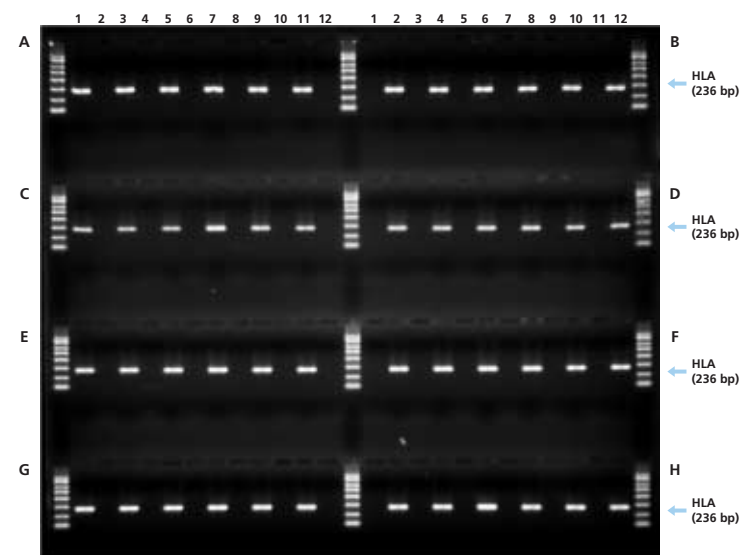


Figure 2: Agarose gel analysis of cross-contamination. 5 µl samples of blood or PBS were placed in alternating wells of an Extraction plate. The 96-well plate was processed using the automated Extract-N-Amp Blood PCR procedure on the Sciclone ALH 3000. All samples were then subjected to amplification and 6 µl of the resultant products were electrophoresed on a 2% Agarose gel. No PCR products were detected in the samples containing PBS controls.

Automated Extract-N-Amp Blood PCR Kit: Quantitative PCR Results

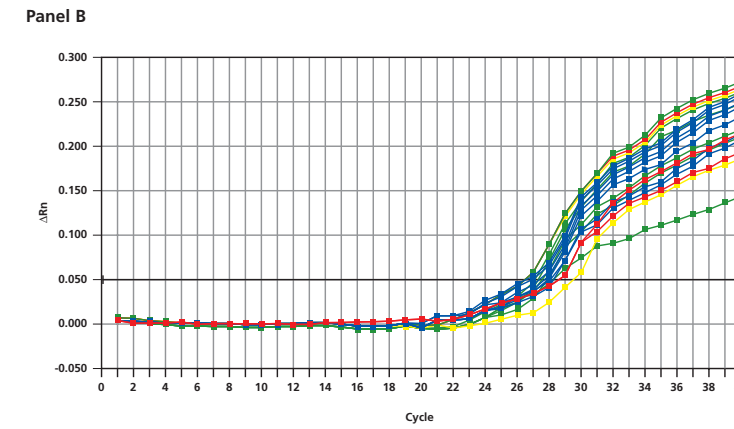
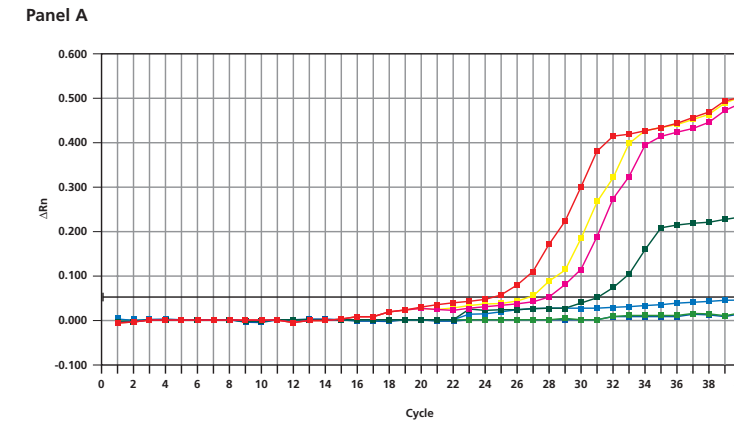


Figure 3: Quantitative PCR results of human blood genomic DNA extracted on Sciclone ALH 3000. Eighty-eight human blood samples were extracted using Extract-N-Amp Blood PCR Kit following the automated procedures. Panel A shows the amplification of the standards used to quantify the DNA in the blood extracts (Red: 3.2 µg/ml; Yellow: 1.6 µg/ml; Pink: 0.8 µg/ml; Green: 0.2 µg/ml). Panel B shows the amplification of 88 blood extracts. Reaction analyses were performed on an ABI Prism® 7700 Sequence Detection System.

Stability of Blood Extracts at 37 °C

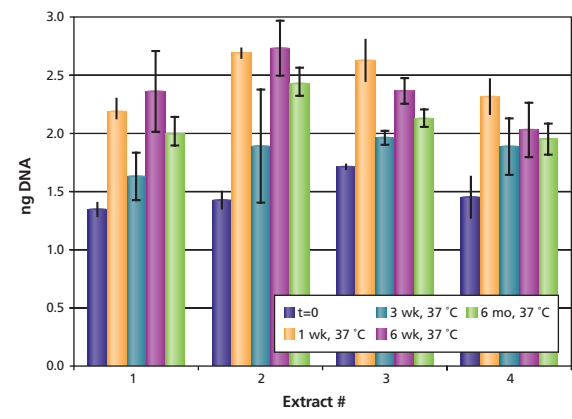


Figure 4: Extracts were stored at 37 °C instead of the recommended 4 °C to test accelerated storage and stability. Samples were removed at various time intervals for testing. Stability was determined by monitoring yield for quantitative PCR using SYBR® Green on an ABI PRISM® 7700 instrument. Robust signals were obtained even after storage at 37 °C for 6 months.

Extract-N-Amp Tissue PCR Kit

PCR Analysis of Genomic DNA Extracted from Various Samples Using Sigma's Extract-N-Amp™ Tissue PCR Kit

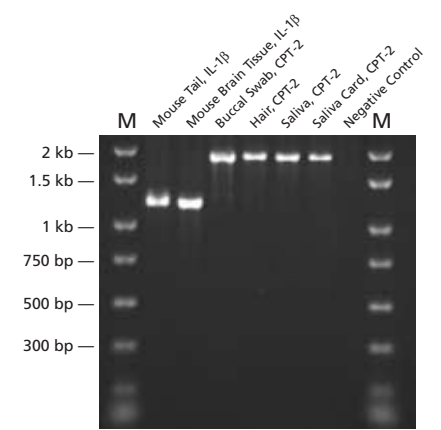


Figure 5: The Extract-N-Amp Tissue PCR Kit was used to isolate genomic DNA from various mouse and human samples. The extracts were then used to generate PCR products ranging from 1200 bp-1800 bp using the specially formulated Hot Start PCR Mix included in the kit. The PCR products generated are 2 distinct genetic markers.

Sequence Determination for 1181 bp Interleukin-1β (IL-1β) Mouse Tail PCR Product

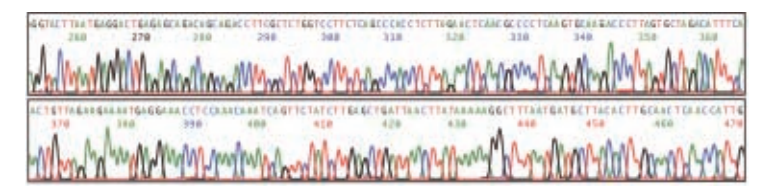


Figure 6: PCR product was purified with the GenElute™ PCR Clean-Up Kit (Product Code NA1020). The DNA extraction and PCR were performed using Sigma's Extract-N-Amp™ Tissue PCR Kit. The sequence was obtained using ABI BigDye™ Terminator Chemistry and the same primers as for the original PCR. Reaction products were resolved on an ABI PRISM® 377 DNA Sequencer.

Conclusions

- The Extract-N-Amp Blood and Tissue PCR kits contain all the reagents necessary to rapidly extract genomic DNA.
- Data shows that you can extract from a variety of sources and amplify a variety of genes.
- The automated protocol for the Extract-N-Amp Blood PCR kit provides walk-away automation, enabling high-throughput genomic DNA extractions in just 25 minutes.
- Automation protocol development is underway for the Extract-N-Amp Tissue and Plant PCR kits.

Combined Automated Blood and Tissue Protocol

