Extract-N-Amp™ PCR Kits: A Novel and High-throughput Approach for Rapidly Extracting and Amplifying Genomic DNA from Animal Tissues

Danhui Wang, Carol Kreader, Scott Weber, Derek Douglas, Jessica Copeland, Jennifer Van Dinther, and Rafael Valdes-Camin

Sigma-Aldrich Corporation
2909 Laclede Ave., St. Louis, MO 63103

Abstract

Standard methods for purifying DNA from animal tissues can be laborious, time consuming, and not amenable to automation. The Extract-N-Amp™ Tissue PCR Kit is a high-throughput system for the rapid extraction and subsequent amplification of genomic DNA from mouse-tail clippings and other animal tissues. This novel extraction treatment has been designed to release genomic DNA from various animal tissues in minutes for direct use in PCR applications, without further cleanup of DNA. To further simplify the procedure, the Extract-N-Amp Tissue PCR Kit includes a specially formulated PCR ReadyMix™, which virtually eliminates any PCR optimization.

To meet the high-throughput requirements for genomic screening of animal samples, an automated method has been developed for the kits. Homogenization and overnight enzymatic digestion of tissue samples prior to extraction is unnecessary, making the entire process fully automated and rapid. In only 35 minutes, DNA can be extracted from 96 tissue samples. In addition, the genomic DNA extracts are stable when stored at 4 °C for a minimum of 6 months, allowing for multiple reassays.

Data is shown to demonstrate that extracted genomic DNA is of sufficient yield and quality to allow for the amplification of target genes from a wide variety of animal tissue sources.

Materials

Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma-Aldrich (St. Louis, MO). The PCR primers were obtained from Sigma-Genosys (Woodlands, TX). Extract-N-Amp™ Tissue PCR Kit (P/N: XNATR or XNAT2R) was used for genomic DNA isolation from mouse tissues and PCR reactions setup. GenElute™ Mammalian Genomic DNA Miniprep Kit (P/N: G1N10) was used to isolate genomic DNA from mouse tail as positive controls. SYBR® Green I Dye (P/N: S9430) and Reference Dye (P/N: R4526) were used for quantitative PCR.

Methods

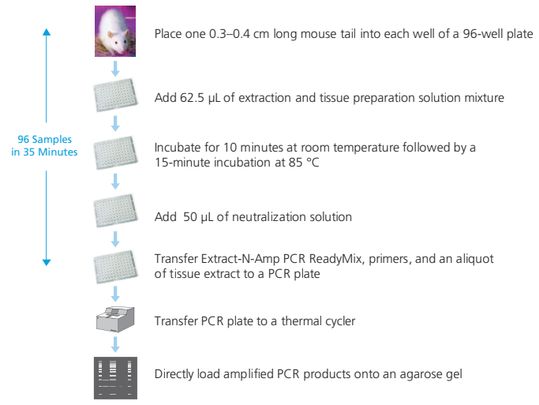
Genomic DNA Extraction:
A 0.3 to 0.4 cm long mouse tail or 3–5 mg of other mouse tissue sample was placed into each well of a 96-well PCR plate and kept on ice until use. DNA was extracted with the Extract-N-Amp Tissue PCR Kit utilizing the automated procedure developed for the Biomek® FX Workstation.

PCR Amplification:
The Beckman Biomek® FX Liquid Handling Workstation was used to set up 96 reactions with a volume of 20 µL. These reactions included DNA extracts (4 µL) from mouse tissue samples or mouse genomic DNA controls (4 µL), as well as the 2x REDExtract-N-Amp™ PCR ReadyMix and Interleukin-1ß primers. Cycling was performed in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA).

Quantitative PCR:
The Biomek® FX Liquid Handling Workstation was used to set up 96 reactions for quantitative PCR. Reactions included DNA extracts (4 µL) from mouse tissue samples or mouse genomic DNA controls (4 µL), 2x Extract-N-Amp™ PCR ReadyMix, Interleukin-1ß primers, SYBR® Green dye, and Reference Dye for a total reaction volume of 20 µL. Quantitative PCR was performed in an ABI PRISM® 7700 Sequence Detection System (Applied Biosystems, Foster City, CA).

Agarose Gel Analysis:
4 µL of each PCR reaction was analyzed on a 1% agarose gel.

Automated Extract-N-Amp Tissue Protocol



Automated Extract-N-Amp Tissue PCR Kit Results

PCR Analysis of Mouse Tail Samples

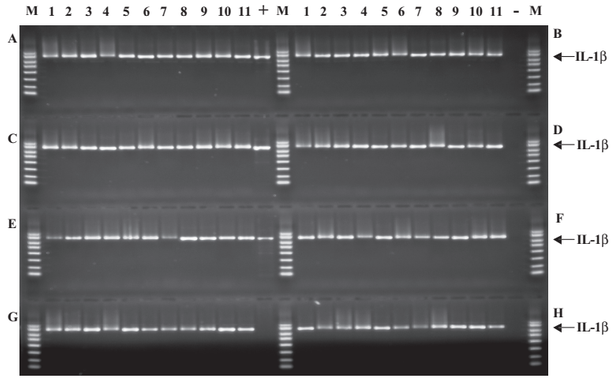


Figure 1: Agarose gel analysis of 96 PCR samples. Genomic DNA was extracted from 88 samples of mouse tail and PCR amplified as described in the Methods. Amplification of 1181bp fragment of the IL-1ß gene is indicated by the arrow.
M = PCR marker. **+** = mouse genomic DNA control. **-** = no DNA template control.

Cross-contamination Analysis

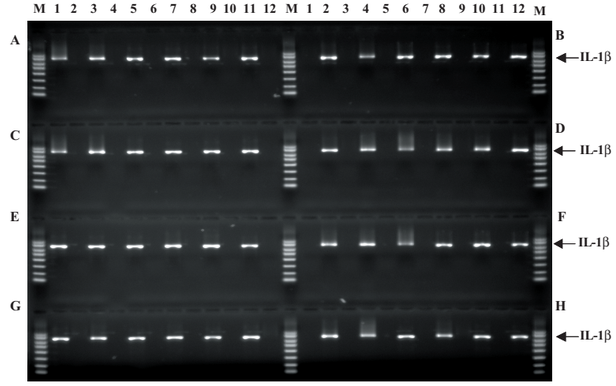


Figure 2: Agarose gel analysis of cross-contamination test. Mouse tails were placed in alternating wells of a 96-well plate. Genomic DNA extraction, PCR amplification, and agarose gel analysis were performed as described in the Methods. No PCR products were detected in the wells containing no tissue samples. **M** = PCR marker.

PCR Analysis of Different Tissue Types

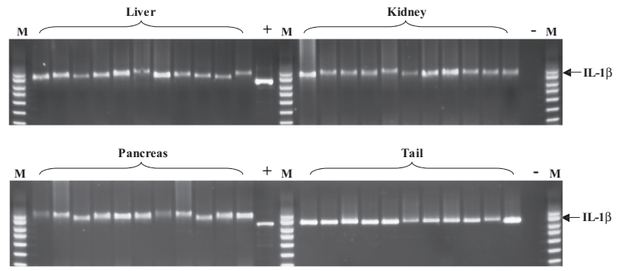


Figure 3: Agarose gel analysis of 44 PCR samples from different tissue extracts. Genomic DNA was extracted from mouse liver, mouse kidney, mouse pancreas, and mouse tails, and subsequently PCR amplified as described in the Methods. Amplification of 1181bp fragment of the IL-1ß gene is indicated by the arrow.
M = PCR marker. **+** = mouse genomic DNA control. **-** = no DNA template control.

Quantitative PCR Analysis of Mouse Tail Samples

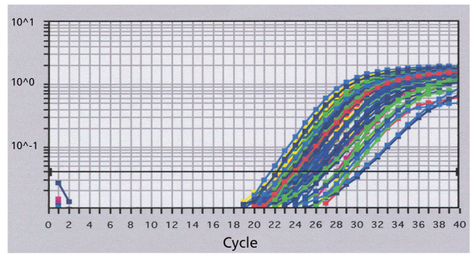


Figure 4: Quantitative PCR analysis of mouse genomic DNA extracted from mouse tails. Genomic DNA was extracted from 88 samples of mouse tail and subsequently analyzed by quantitative PCR as described in the Methods.

Stability Study of Tissue Extract

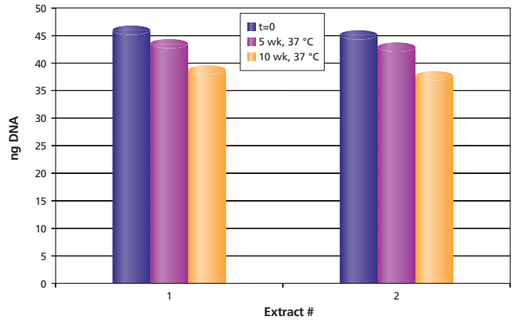


Figure 5: Stability of DNA in mouse tail extracts. Two mouse tails were extracted using the Extract-N-Amp Tissue PCR Kit. Mouse tails were removed from samples prior to storage. The extracts were stored at 37 °C (accelerated) and quantitative PCR was repeated after 5 and 10 weeks with the extracts.

Conclusions

- Data demonstrates the effectiveness of the Extract-N-Amp Tissue PCR Kit in the isolation and subsequent amplification of target genes from a variety of animal tissue sources.
- Data presented here demonstrates that genomic DNA extracts are stable when stored at 4 °C for a minimum of 6 months, allowing for multiple reassays.
- The walk-away automated protocol for Extract-N-Amp Tissue PCR Kit enables high-throughput genomic DNA extractions required for genotyping studies.
- The entire process is automated from tissue sample through PCR reaction setup.
- There is no requirement for homogenization or overnight enzymatic digestions prior to the start of the method.
- This method is rapid—96 samples can be processed on the Biomek® FX in 35 minutes.

Trademarks

00000-021104