Applications

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Automated Protocol for Sigma's Extract-N-Amp™ Tissue PCR Kits using the Eppendorf ep*Motion*® 5075 VAC

Clyde Brown, Steve Michalik, Sigma-Aldrich Biotech, St. Louis, MO, USA
Renate Fröndt, Eppendorf AG, Hamburg, Germany

Abstract

This Application Note describes a fast and easy-to-use automated protocol using Sigma's Extract-N-Amp Tissue PCR Kits on the ep*Motion* 5075. The Extract-N-Amp Tissue PCR Kits have been developed for use as a high-throughput system for the integrated extraction and amplification of genomic DNA in a 96-well format from mouse-tails, hair, buccal swabs, saliva and other animal tissues. The Extract-N-Amp Tissue PCR Kits provide a novel DNA extraction system, eliminating the need for long enzymatic digestions and homogenization steps that are not amenable to automation.

Introduction

Extract-N-Amp PCR Reaction Mix is an optimized reagent that includes a 2X reaction mixture of buffer, salts, dNTPs and *Taq* polymerase. The reaction mix uses Sigma's antibody mediated hot start polymerase, JumpStart *Taq* polymerase, for highly specific amplification of genomic DNA directly from the DNA extract. The XNAT and XNATR kits include REDExtract-N-Amp PCR Reaction Mix that also contains an inert dye for convenient direct loading of the PCR reactions onto an agarose gel, where as the XNAT2 and XNAT2R kits are compatible with TaqMan probes and other fluorescent-labelled probe chemistries since they do not contain dyes. Finally, XNATG and XNATRG kits contain SYBR Green which allows for qPCR via the fluorescent signal afforded by the binding of SYBR Green to double stranded DNA.

Materials and Methods

The extraction and amplification of genomic DNA from animal tissues is accomplished in 4 easy steps.

1) The Extraction and Tissue Preparation Solution is added to the tissue samples and incubated at room temperature for 10 minutes.
2) The extracts are incubated for 15 minutes at 85 °C.
3) A Neutralization Solution is added to the extract. (Neutralized extracts can be stored at 4 °C for at least 6 months.)
4) PCR reactions are set up using 4 µL of the extracts. In just 50 minutes the Eppendorf ep*Motion* 5075 can complete the extraction and PCR setup for 96 tissue samples.

Sigma Extract-N-Amp Tissue PCR Kit Components:
Extraction Solution
Tissue Preparation Solution
Neutralization Solution B
Extract-N-Amp PCR ReadyMix, Extract-N-Amp SYBR Green PCR ReadyMix or REDExtract-N-Amp PCR ReadyMix
Eppendorf ep*Motion* 5075 VAC equipped with:
Thermal Module
Dispensing Tools TM300-8 and TM50-8
Reservoir Rack
Thermoadapter for 96-well DWP
Thermoblock for 96-well PCR Plates
Eppendorf Consumables:
Reagent Reservoirs: 30 mL and 100 mL
epT.I.P.S Motion Filtertips 50 µL and 300 µL
Deep Well Plate 96/1000 µL
Other User Supplied Materials:
96-well PCR Polypropylene Plate
Animal Tissues
Small dissecting scissors
Forceps (small to medium in size)
Primers for genes of interest
Thermal Cycler for PCR

Tissue Preparation

For Fresh or Frozen Mouse Tails
Rinse scissors and forceps in 70 % ethanol prior to use and between different samples. Place a 0.3-0.4 cm piece of mouse-tail clip (cut end down) into a 96-well PCR plate ensuring that each sample is centered down into the bottom of each well.
Chill the plate at 2-8 °C until needed. Place the plate in Deck Position B2 when ready to start the Eppendorf ep*Motion* 5075 VAC.
Other Animal Tissues
Rinse scissors and forceps in 70 % ethanol prior to use and between different samples. Place a 4-6 mg piece of tissue into a 96-well PCR plate ensuring that each sample is centered down the bottom of each well. Chill the plate at 2-8 °C until needed. Place the plate in Deck Position B2 when ready to start the Eppendorf epMotion 5075 VAC.

Reagent Preparation

Extraction and Tissue Preparation Solution Mixture:
Pre-mix the Extraction and Tissue Preparation Solutions at a ratio of 4:1 (e.g. combine 12 mL of Extraction Solution with 3 mL Tissue Preparation Solution). This mixture can be stored up to 2 hours before use. To process a single plate of 96 samples, add 15 mL of the mixture to Reagent Position 1 of the reservoir rack.

Neutralization Solution:
To process a single plate of 96 samples, add 15 mL of Neutralization Solution to Reagent Position 2 of the reservoir rack.
PCR Master Mix:
To prepare the PCR Master Mix, add water and primers (forward and reverse) to the Extract-N-Amp PCR ReadyMix as described in Table 1 below. If using Extract-N-Amp SYBR Green PCR ReadyMix, it may be necessary to supplement reference dye into the PCR Master Mix if required by your real-time PCR instrument. Place the resulting PCR Master Mix in Reagent Position 3 of the reservoir rack.

Master Mix (2.42 mL)	
Water	0.9 mL
Extract-N-Amp PCR ReadyMix (E3004, S4320 or R4775)	1.5 mL
Forward Primer (100 µM)	10 µL
Reverse Primer (100 µM)	10 µL

Table 1: PCR MasterMix

Automated Method Overview
The prepared samples and reagents are set up in the worktable layout as described.
Upon executing the run, if the number of samples is defined as variable, then a window opens to allow entry of the numbers of samples to be processed.
The ep*Motion*'s robotic carrier will begin to monitor for labware and reagent levels via an optical sensor.
The thermal module (TEMP1) will begin to preheat to 85 °C. The 96 Thermoadapter DWP located on TEMP1 enables complete heat transfer to the samples.
125 µL of the Extraction and Tissue Preparation Solution (Worktable Position B1-Reagent Position 1) is aspirated and dispensed to the tissue samples (Worktable Position B2).
Incubation at ambient temperature occurs for 10 minutes.
The samples are incubated for 15 minutes at 85 °C. Upon completion of incubation, the Gripper moves the samples back to Position B2.
100 µL of Neutralization Solution (Worktable Position B1-Reagent Position 2) is added and mixed with the sample extracts in worktable Position B2.
16 µL of the PCR Master Mix (Worktable Position B1-Reagent Position 3) is aspirated and then multi-dispensed in the PCR plate located in the 96 PCR Plate Thermoblock (Worktable Position C3).
4 µL of the tissue extract (Worktable Position B2) is aspirated, dispensed and mixed in the PCR plate (Worktable Position C3) containing the PCR Master Mix.
All 96 samples are transferred to the PCR plate.
The samples are now ready for PCR amplification.

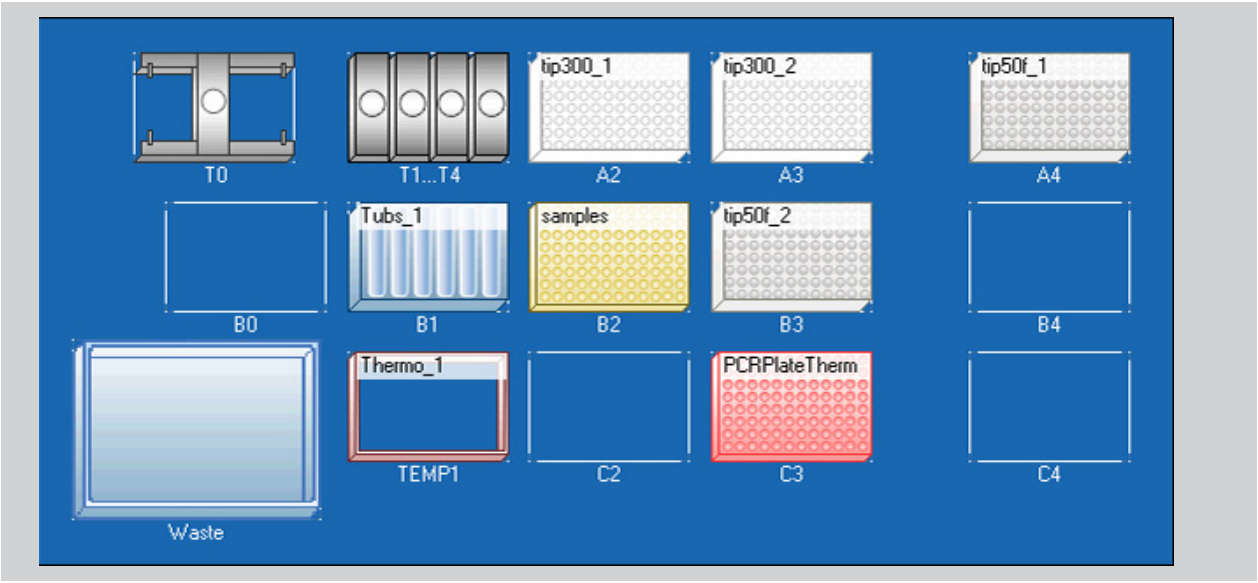

Fig. 1: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the Extract-N-Amp Tissue PCR.

Worktable Position	Labware
A2	300 µL epTips
A3	300 µL epTips
A4	50 µL epTips, Filtertips
B1	Reservoir Rack with 3 Reagent Reservoirs 30 mL
B2	Tissue Sample Plate 96 DWP
B3	50 µL epTips, Filtertips
B4	empty
TEMP1	Thermoadapter for 96 DWP
C3	Thermoblock with PCR Plate
C4	empty

Table 2: ep*Motion* worktable allocation for Extract-N-Amp Tissue PCR Kit

Step	Temperature	Time	Cycles
Intial Denaturation	94-96 °C	3 minutes	1
Denaturation	94-96 °C	0.5-1 minute	30-40
Annealing	45-68 °C	0.5-1 minute	
Extension	72 °C	1-2 minutes (~kb/min)	
Final Extension	72 °C	7 minutes	1
Hold	4 °C	Indefinitely	

Table 3: : Incubation program for PCR

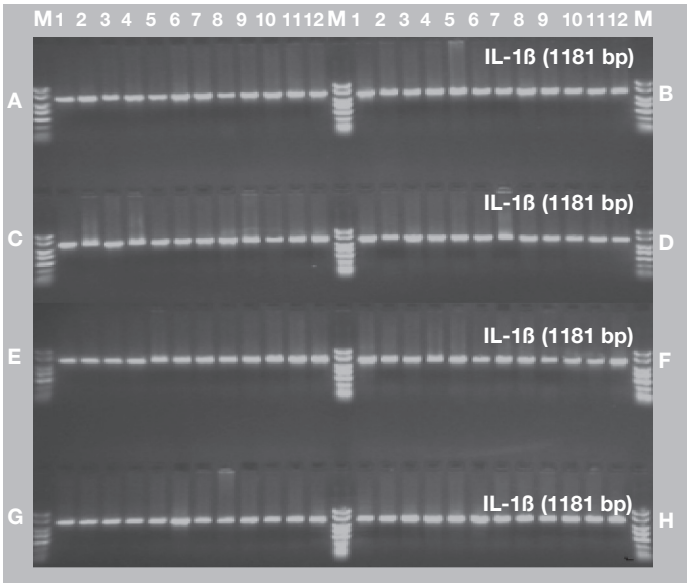

Fig. 2: Agarose gel analysis of 96 PCR samples. DNA was extracted from 96 mouse-tails (0.3-0.4 cm) using the automated Extract-N-Amp Tissue procedure on the Eppendorf ep*Motion*. Amplification of the 1181 bp of the IL-1ß gene was accomplished by adding 4 µL of the extracted template in a 20 µL PCR reaction incorporating the 2X PCR Reaction Mix. 6 µL of each reaction was analyzed on a 1 % agarose gel.

Results and Summary

The automated method was created and validated for use on the Eppendorf ep*Motion*. This procedure provides a walk-away protocol for all aspects of the Extract-N-Amp Tissue PCR kit.

Figure 2 shows an agarose gel of amplicons (1,181 bp IL-1ß gene) generated from processing 96 different mouse-tails using the ep*Motion* according to the protocol outlined in this document.

Ordering information Eppendorf

Article	Order no. international	Order no. North America
ep*Motion*® 5075 VAC, 230 V	5075 000.016	N/A
ep*Motion*® 5075 VAC, 120 V	N/A	960020014
ep*Motion*® 5075 LH, 230 V	5075 000.008	N/A
ep*Motion*® 5075 LH, 120 V	N/A	960020006
Dispensing tool TM 300-8	5280 000.231	960001052
Dispensing tool TM 50-8	5280 000.215	960001044
Reservoir rack	5075 754.002	960002148
Thermoadapter for DWP	5075 751.054	960002391
epMotion Reservoir 30 mL	0030 126.505	960051009
epT.I.P.S® Motion 300 µL, Filter	0030 003.977	960050061
epT.I.P.S® Motion 50 µL, Filter	0030 003.950	960050029
Thermoblock for 96 PCR Plates	5075 766.000	960002083

Ordering information Sigma

Article	Reactions	Order no.
REDExtract-N-Amp™ Tissue PCR Kit	100	XNAT
REDExtract-N-Amp™ Tissue PCR Kit	1000	XNATR
Extract-N-Amp™ Tissue PCR Kit	100	XNAT2
Extract-N-Amp™ Tissue PCR Kit	1000	XNAT2R
SYBR® Green Extract-N-Amp™ Tissue PCR Kit	100	XNATG
SYBR® Green Extract-N-Amp™ Tissue PCR Kit	1000	XNATRG

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Eppendorf AG · 22331 Hamburg · Germany · Tel: +49 40 53801-0 · Fax: +49 40 538 01-556 · E-mail: eppendorf@eppendorf.com
Eppendorf North America, Inc. · 102 Motor Parkway · Hauppauge, N.Y. 11788-5178 · USA
Tel: +1 516 334 7500 · Toll free phone: +1 800-645-3050 · Fax: +1 516 334 7506 · E-mail: info@eppendorf.com

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