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ProductInformation

Automated Protocol for Extract-N-Amp™ Tissue PCR Kits

Using the Biomek® FX (Beckman Coulter)

Extract-N-Amp Tissue Product Codes **XNATR and XNAT2R**

Automation Guide

I. Description

The Extract-N-Amp™ Tissue PCR Kits (XNATR and XNAT2R) have been developed for use as a high-throughput system for the rapid extraction and subsequent amplification of genomic DNA in a 96-well format from mouse tails 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. The XNAT2R Kit includes a specially formulated Extract-N-Amp PCR ReadyMix™ reagent that includes a 2X reaction mixture of buffer, salts, dNTPs, and *Taq* polymerase. The ReadyMix reagent also contains Sigma's antibody mediated hot start polymerase, JumpStart™ *Taq* polymerase, for highly specific amplification of genomic DNA directly from the extract. The XNATR Kit includes the REDExtract-N-Amp™ PCR ReadyMix that also contains an inert tracking dye for convenient direct loading of the PCR reactions onto an agarose gel for analysis.

The automated method created and validated for use on the Biomek FX Liquid Handling Workstation from Beckman Coulter provides a walk-away protocol for all aspects of the Extract-N-Amp Tissue PCR Kits.

Extraction and amplification of genomic DNA from animal tissues is accomplished in 4 easy steps:

1. The Extraction and Tissue Preparation Solution mixture is added to tissue samples and incubated at room temperature for 10 minutes
2. Extracts are incubated for 15 minutes at 85 °C
3. A Neutralization Solution is added to the extract
   a. Once the Neutralization Solution has been added, 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 35 minutes, the Biomek FX can complete DNA extraction of and PCR reaction setup for 96 tissue samples.

II. Product Components

Reagents Provided	Product Code	Extract-N-Amp Tissue XNAT2R	REDExtract-N-Amp Tissue XNATR
	Package Size	1,000 extractions 1,000 amplifications	1,000 extractions 1,000 amplifications
Extraction Solution	E7526	240 ml	240 ml
Tissue Preparation Solution	T3073	30 ml	30 ml
Neutralization Solution B	N3910	240 ml	240 ml
Extract-N-Amp PCR Ready Mix or REDExtract-N-Amp PCR Ready Mix	E3004 (for XNAT2R) R4775 (for XNATR)	12 ml	12 ml

III. Storage

The Extract-N-Amp Tissue PCR Kits can be stored at 2 - 8 °C for up to 3 weeks. For long-term storage, store at –20 °C. Do not store in a frost-free freezer.

IV. Materials to Be Supplied by the User

1. Animal Tissues
2. Small dissecting scissors
3. Forceps (small to medium in size)
4. Primers for genes of interest
5. Molecular biology grade water (Sigma, W4502)
6. 96-well PCR plates, with full skirt (Sigma, P4616)
7. 96-well PCR plates, with half skirt (ABgene, AB-1100)
8. Lid, universal (Fisher, 07200694)
9. Ultra clear cap strip (ABgene, AB-0866)
10. Corning plate holder (Corning, 6525)
11. Sealing film, SealPlate (Sigma, Z369659)
12. Microcentrifuge tubes (1.5 ml, 2 ml screw cap)
13. 24 position Eppendorf® IsoTherm System (Fisher, 05-405-22)
14. 12 column reagent reservoir with low profile (Innovative Microplates, S30028)
15. 96-well reservoir with low profile and pyramidal bottom (Innovative Microplates, S30018)
16. (Optional) High profile 12 column reagent reservoir (Innovative Microplates, S30019)
17. (Optional) High profile 96-well reservoir with pyramidal bottom (Innovative Microplates, S30014)
18. Thermal Cycler
19. Thermometer (Fisher, 15-077-26)

V. Instrument Requirements for the Biomek FX Workstation

Part Description	Qty	Ordering Information
Orbital Shaker	1	Contact Beckman Coulter
Peltier ALP	1	Contact Beckman Coulter
Multichannel Pod (96 Mandrel 200 µl Head)	1	Contact Beckman Coulter
Span-8 Pod (1 ml Syringe)	1	Contact Beckman Coulter
Gripper	1	Contact Beckman Coulter
Tip Loader	1	Contact Beckman Coulter
Span-8 Tip Trash	1	Contact Beckman Coulter
Span-8 Tip Wash	1	Contact Beckman Coulter
Standard Passive ALPs (One by Three)	4	Contact Beckman Coulter
Standard Passive ALPs (One by One)	3	Contact Beckman Coulter
AP96 P250 Barrier Tips, Sterile	2	BK717253 (Beckman Coulter)
AP96 P20 Barrier Tips, Sterile	1	BK717256 (Beckman Coulter)
Span-8 P250 Barrier Tips, Sterile	1	BK379503 (Beckman Coulter)
Span-8 P20 Barrier Tips, Sterile	1	BK379506 (Beckman Coulter)

VI. Tissue Preparation

For Fresh or Frozen Mouse Tails:

1. 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 tip (cut end down) into a 96-well PCR plate ensuring that each sample is centered down into the bottom of each well.
2. Chill the plate at 2-8 °C until needed.

Other Animal Tissues:

1. 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 into the bottom of each well.
2. Chill the plate at 2 - 8 °C until needed.

VII. Reagent Preparation

1. *Extraction and Tissue Preparation Solution Mixture*: Pre-mix the Extraction and Tissue Preparation Solutions at a ratio of 4:1. This mixture can be stored for up to 2 hours before use. To process a single plate of 96 samples, add 18 ml of the mixture to the 96-well reservoir located at position P4 (see Section IX for deck layout). If it is desired to process more than 12 plates of samples, the high-profile reservoir (S30014) is required.

2. *Neutralization Solution*: To process a single plate of 96 samples, add 20 ml of Neutralization Solution to the 96-well reservoir located at position P8 (see Section IX for deck layout). If it is desired to process more than 12 plates of samples, the high-profile reservoir (S30014) is required.

3. *PCR Master Mix*: The Extract-N-Amp Tissue PCR ReadyMix is a 2X reaction mixture containing buffer, salts, dNTPs, and *Taq* polymerase. To prepare a PCR Master Mix add water and primers (forward and reverse) to the Extract-N-Amp Tissue PCR ReadyMix as described in table below.

Stock	Water	PCR ReadyMix (E3004)	Forward Primer (100 μM)	Reverse Primer (100 μM)
PCR Master Mix (2.4 ml)	0.9 ml	1.5 ml	12 μl	12 μl

   To set up 20 μl PCR reactions in one 96-well plate, add 2.4 ml of PCR Master Mix to the first column of the 12-column reservoir located at position P13 (see Section IX for deck layout). If setting up more than 3 plates of samples for PCR, it will be necessary to use reservoir S30019.

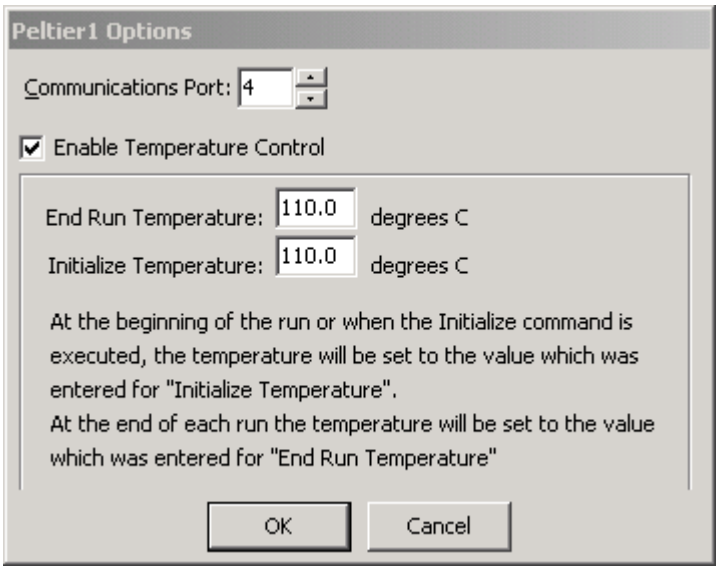
4. *No-template Control (optional)*: Add water to four 2 ml screw cap tubes and place in column 2 (positions 5-8) of the 24-position tube rack.

5. *DNA Controls (optional)*: Prepare genomic DNA controls for quantification of tissue DNA extracts. Prepare 4 tubes containing 10 ng/μl, 5 ng/μl, 1.25 ng/μl, and 0.31 ng/μl samples of genomic DNA and place in column 1 (positions 1-4) of the 24-position tube rack.

VIII. Temperature Control Device (Watlow) Setup

Prior to the first run of the automated method, verify the performance of the Peltier ALP. Manually set the temperature control device to the setting of 110 °C with an offset of –4 °C (refer to the User's Manual). Place a PCR plate on the Peltier ALP and measure the temperature inside the wells using thermometer probes. Verify that the temperature in the wells is at a minimum of 85 °C after 3 minutes. If well temperature does not reach a minimum of 85 °C, it may be necessary to adjust the offset (refer to User's Manual).

Approximately one hour prior to running the automated method, manually turn on the temperature control device and verify that the temperature display on the controller has reached the desired reading. In Biomek software, set both Initialize and End Run Temperature settings at 110 °C by selecting the Configuration Options for the Peltier ALP from the Device Editor menu as shown below:



IX. Automated Method Description

This overview describes the general liquid handling steps required to execute the automated Extract-N-Amp Tissue PCR method and can be customized to a variety of applications. To customize applications, see Section XI.

A. Getting Started

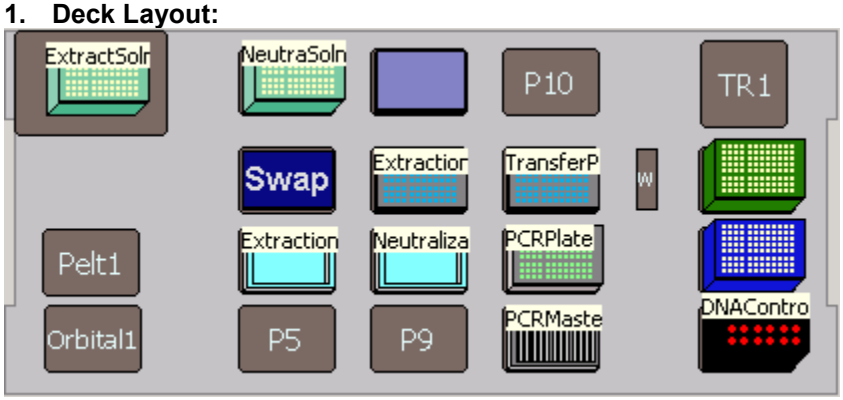
1. Turn on temperature control device.
2. Set up deck layout: place the tip boxes, plates, tube rack, and reservoirs at the appropriate positions on the deck as described in Deck Layout Section.
3. Add reagents to the appropriate reservoirs as described in Section VI.
4. Run the method using Biomek Software Version 3.1.
5. At the completion of the method, place cap strips onto the PCR plate, vortex to mix the solution, and briefly centrifuge. The PCR plate is now ready to be placed into a thermal cycler.
6. Seal the PCR plate containing tissue extracts with a sealing film. Tissue extracts can be stored for up to 6 months at 4 °C.

B. Biomek Methods:

1. *Extract-N-Amp_Tissue_PCRSetup*: Performs all of the steps necessary to extract DNA from 96 tissue samples and setup PCR reactions. The 96 channel head is used to prepare extracts, and the Span-8 is used to prepare PCR reactions from extracts and control DNA samples. To perform PCR reaction setup, there is a step in the method that calls up the *PCR_Setup (with controls)* method.
2. *PCR_Setup (with controls)*: Performs PCR reaction setup for 88 tissue samples and 8 controls using a Master Mix and transfers tissue DNA extracts using Span-8. This method may be used if it is desired to perform additional amplification experiments from the tissue extracts.
3. *PCR_Setup (no controls)*: Performs PCR reaction setup for 96 samples using a Master Mix and transfers tissue DNA extracts. The Span-8 is used to transfer the Master Mix to the PCR plate, and the 96 channel head is used to transfer extracts to the PCR plate. This method may be used if it is desired to perform amplification experiments from the whole plate of tissue extracts without preparing PCR controls. This method can also be called up in the *Extract-N-Amp Tissue_PCRSetup* method if it is desired to transfer extracts with the 96 channel head.

C. Description of the Extract-N-Amp_Tissue_PCRSetup Method

1. **Deck Layout:**



Deck Position	Equipment
TL1	AP96 P250 Barrier Tips, Sterile
P2	AP96 P250 Barrier Tips, Sterile
P3	Swap
P4	96-well reservoir for the mixture of Extraction and Tissue Preparation Solution
P6	Lid
P7	96-well PCR plate with full skirt containing tissue samples
P8	96-well reservoir for Neutralization Solution
P11	96-well PCR plate with full skirt for transferring neutralized tissue extracts
P12	96-well PCR plate with half skirt for PCR reaction setup (seated into a plate holder)
P13	12 column reservoir for PCR Master Mix
P14	Span-8 P250 Barrier Tips
P15	Span-8 P20 Barrier Tips
P16	24 position Eppendorf IsoTherm system (DNA Control)

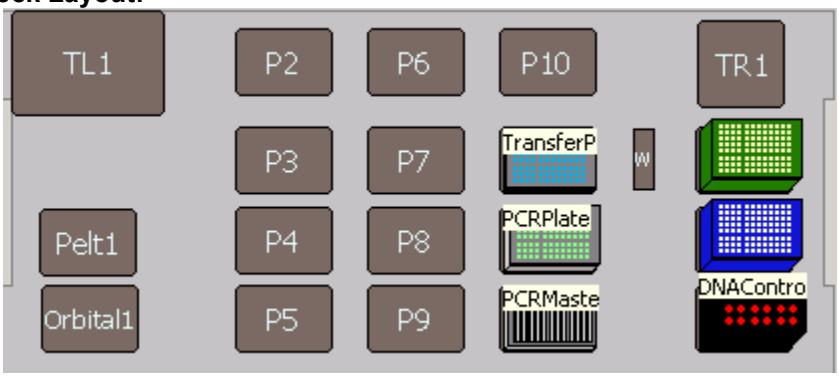
2. Method Overview

Below is a summary of the Extract-N-Amp Tissue automated method. For complete program details download automation program at www.sigmaaldrich.com/automation

1. 62.5 µl of Extraction and Tissue Preparation Solution mixture is aspirated from a reservoir and dispensed into multiwell plate containing tissue samples by the 96 channel head.
2. Gripper tool is used to move the plate containing tissue extract to the shaker.
3. Shaker is activated to begin mixing plate with tissue extracts for 30 seconds at 750 rpm.
4. Pause Shaker for a 10 minute incubation at room temperature.
5. Gripper tool is used to move plate containing tissue extracts from shaker to Peltier ALP.
6. Pause Peltier ALP for a 15 minute incubation at 85 °C.
7. The lid is removed from the plate containing tissue extracts and placed at P6.
8. Gripper tool is used to move plate containing tissue extracts from Peltier ALP to P7.
9. 50 µl of Neutralization Solution is aspirated from a reservoir and dispensed into the multiwell plate with the tissue extracts by the 96 channel head.
10. The 96 channel head is used to pipette-mix the extracts for 8 cycles and then transfer 80 µl of tissue extract to a new plate for the storage.
11. A command calls up and performs all steps of the PCR_Setup (with controls) Method. See below for explanation of the method.

D. Description of PCR_Setup (with controls) Method

1. Deck Layout:



Deck Position	Equipment
P11	96-well PCR plate with tissue DNA Extracts
P12	96-well PCR amplification plate (seated into a plate holder)
P13	12 column reservoir for PCR Master Mix
P14	Span-8 P250 Barrier Tips
P15	Span-8 P20 Barrier Tips
P16	24 position Eppendorf IsoThem system (DNA Control)

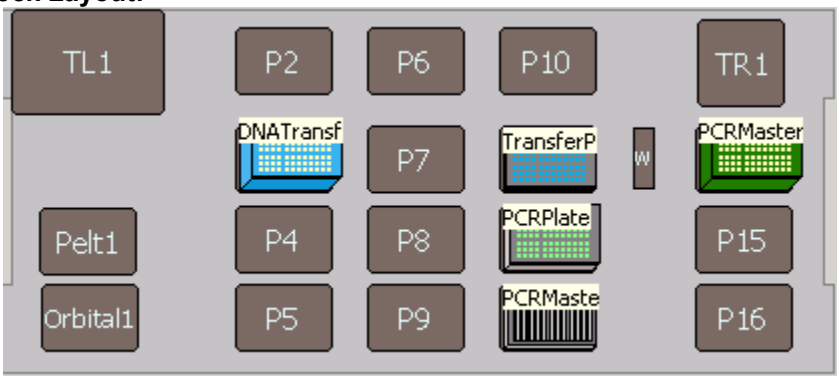
2. Method Overview

Below is a summary of the PCR Setup method using Span-8 to transfer 4 µl of DNA extracts. For complete program details, download automation program from www.sigmaaldrich.com/automation

1. Wash the Span-8 dispense head with 2 ml of system fluid.
2. 200 µl barrier disposable tips are loaded onto the Span-8 dispense head.
3. PCR Master Mix is aspirated from the 12 column reservoir using the Span-8 dispense head. The Span-8 is acting like a bulk reagent dispenser, and is aspirating enough reagents to dispense to a quarter of the plate.
4. 16 µl of PCR master mix is multi-dispensed to the PCR amplification plate using the Span-8 dispense head.
5. Steps 3 and 4 are repeated 3 more times until the Span-8 has dispensed 16 µl of PCR master mix to all 96-wells of the PCR amplification plate.
6. 200 µl barrier tips are removed from the Span-8 dispense head.
7. 4 µl of tissue extract is aspirated from the multiwell plate containing tissue extracts with Span-8 dispense head.
8. Tissue extract is dispensed into the PCR amplification plate.
9. Because the Span-8 dispense head can only perform operations eight wells at a time, a loop is created to account for all samples. Steps 7-10 are repeated 10 times or the number of times as needed. New 20 µl barrier disposable tips are used for each transfer.
10. 20 µl barrier disposable tips are removed from the Span-8 dispense head.
11. 4 µl of control DNA samples are aspirated from four microcentrifuge tubes and dispensed to wells of A12, C12, E12, G12 of the PCR amplification plate using the Span-8 dispense head with tips 5, 6, 7, and 8. Refresh 20 µl barrier disposable tips.
12. 4 µl of water are aspirated from four microcentrifuge tubes and dispensed to wells of B12, D12, F12, H12 of the PCR amplification plate using the Span-8 dispense head with tips 5, 6, 7, and 8. Refresh 20 µl barrier disposable tips.

E. Description of PCR_Setup (no controls) Method

1. Deck Layout:



Deck Position	Equipment
P3	AP96 P20 Barrier Tips, Sterile
P11	96-well PCR plate with tissue DNA Extracts
P12	96-well PCR amplification plate (seated into a plate holder)
P13	12 column reservoir for PCR Master Mix
P14	Span-8 P250 Barrier Tips

2. Method Overview

Below is a summary of the PCR Setup method using 96 channel head to transfer 4 µl of DNA extracts. For complete program details, download automation program from www.sigmaaldrich.com/automation

1. Wash the Span-8 dispense head with 2 ml of system fluid.
2. 200 µl barrier disposable tips are loaded onto the Span-8 dispense head.
3. PCR Master Mix is aspirated from the 12-column reservoir using the Span-8 dispense head. The Span-8 is acting like a bulk reagent dispenser, and is aspirating enough reagents to dispense to a quarter of the plate.
4. 16 µl of PCR Master Mix is multi-dispensed to PCR amplification plate using the Span-8 dispense head.
5. Steps 3 and 4 are repeated 3 more times until the Span-8 has dispensed 16 µl of PCR Master Mix to all 96-wells of the PCR amplification plate.
6. 200 µl barrier tips are removed from the Span-8 dispense head.
7. 4 µl of tissue extract is aspirated from the multiwell plate containing tissue extracts using 96 channel head.
8. Tissue extract is dispensed into the PCR amplification plate.

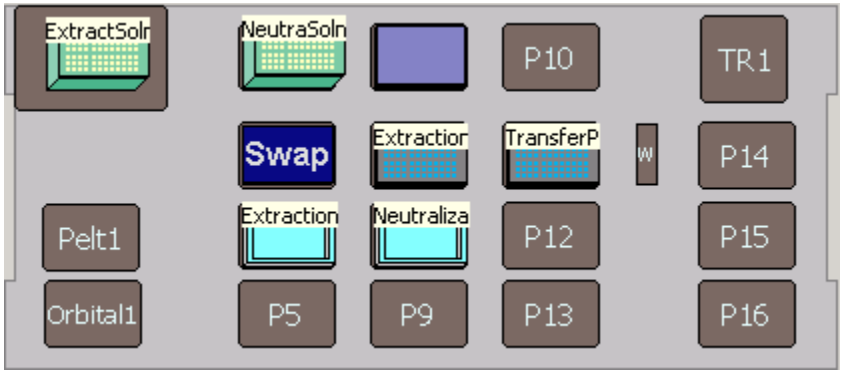
X. Recommended Parameters for PCR Amplification:

Step	Temperature	Time	Cycles
Initial Denaturation	94-96 °C	3 minutes	1
Denaturation	94-96 °C	0.5-1 minute	
Annealing	45-68 °C	0.5-1 minute	30-40
Extension	72 °C	1-2 minutes (~1 kb/min)	
Final Extension	72 °C	10 minutes	1
Hold	4 °C	Indefinitely	

XI. Method Customization

Performing extraction without subsequent amplification

Tissue samples may be subjected to extraction without subsequent amplification. To account for this modification, step 11 in the Method Overview Section of *Extract-N-Amp_Tissue_PCRSetup* method should be deleted and the deck layout in the Instrument Setup step needs to be updated as following:

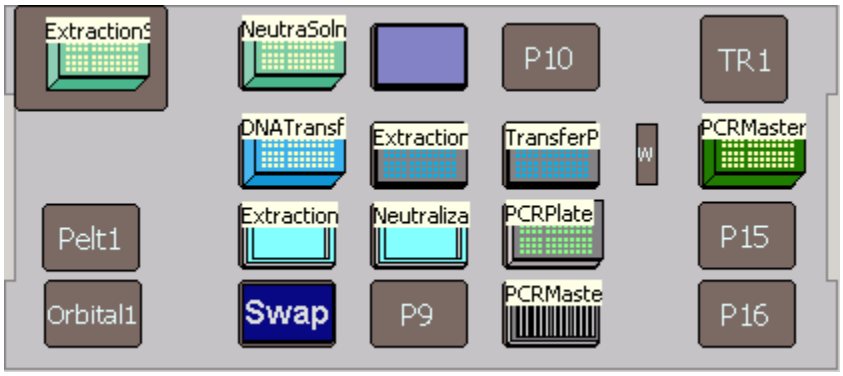


Deck Position	Equipment
TL1	AP96 P250 Barrier Tips
P2	AP96 P250 Barrier Tips
P3	Swap
P4	96-well reservoir for the mixture of Extraction and Tissue Preparation Solution
P6	Lid
P7	96-well PCR plate with full skirt containing tissue samples
P8	96-well reservoir for Neutralization Solution
P11	96-well PCR plate for transferring neutralized tissue extracts for long-term storage

Preparing 96 tissue extracts for PCR

It may be desired to extract DNA from 96 tissue samples and set up all samples for PCR in a single 96-well PCR plate. Two changes need to be made in the *Extract-N-Amp_Tissue_PCRSetup* method.

1. Click on the Run PCR_Setup (with controls) step of the *Extract-N-Amp Tissue_PCRSetup* method. Use the drop down arrow next to File Name to select *PCR_Setup (no controls)* method.
2. Update the deck layout in the Instrument Setup step of both *Extract-N-Amp Tissue_PCRSetup* and *PCR_Setup (no controls)* methods as following:



Deck Position	Equipment
TL1	AP96 P250 Barrier Tips, Sterile
P2	AP96 P250 Barrier Tips, Sterile
P3	AP96 P20 Barrier Tips, Sterile
P4	96-well reservoir for the mixture of Extraction and Tissue Preparation Solution
P5	Swap
P6	Lid
P7	96-well PCR plate with full skirt containing tissue samples
P8	96-well reservoir for Neutralization Solution
P11	96-well PCR plate for transferring neutralized tissue extracts for long-term storage
P12	96-well PCR amplification plate (seated into a plate holder)
P13	12 column reservoir for PCR Master Mix
P14	Span-8 P250 Barrier Tips

PCR setup only

Tissue extracts may be subjected to additional amplifications. The *PCR_Setup (with controls)* or *PCR_Setup (no controls)* method described in Section IX may be used for this purpose.

Use of a different PCR plate

The automated method was created using the 96-well PCR amplification plates with half skirt from Abgene. Other PCR plates including 384 well plates may be used in this method, but may require the creation of a new labware in the Biomek software.

PCR setup using multiple primer sets

To amplify genomic DNA from the tissue extract with different primer sets, primers can be added to microcentrifuge tubes and placed on the 24 position tube racks or added to the PCR ReadyMix and placed on different columns of 12 column reservoir S30028. Additional steps will need to be added to the corresponding *PCR_Setup* method to account for the primer addition or aspirating PCR Master Mix from a different column position.

Transfer of tissue extracts to a new plate

For long-term storage of tissue extracts it is desirable to transfer them to a new plate. To avoid clogging of the pipette tips with tissue samples it may be necessary to adjust the height of aspiration in step 10 described in the method overview for the *Extract-N-Amp_Tissue_PCRSetup* method. In some instances, manual transfer of the extracts to a new plate may be required.

When extracting DNA from tissue samples other than tail clips, small pieces of tissue samples may float in the prepared extracts. To avoid clogging of the pipette tips with tissue samples, it may be required to centrifuge down the extracts in the Extraction plate prior to transfer to a new plate.

XII. Performance Characteristics

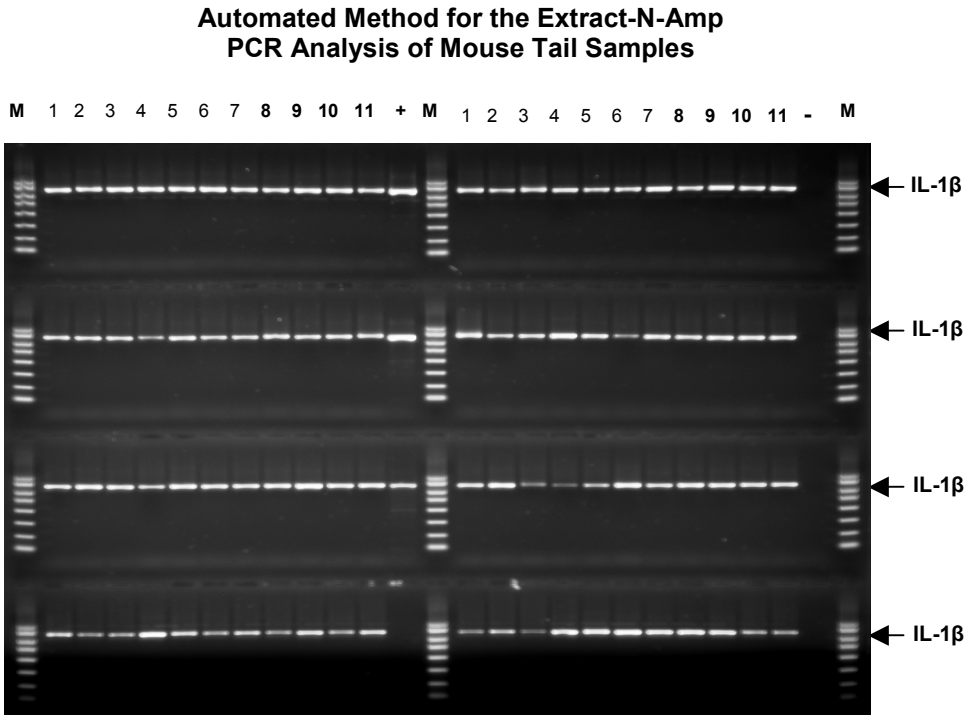


Figure 1. DNA was extracted from 88 samples of mouse tails (0.3 – 0.4 cm) using the automated Extract-N-Amp Tissue PCR procedure on the Biomek FX workstation. Amplification of the 1181 bp of the IL-1β gene followed using 4 µl of extracted template or 4 µl of human genomic DNA controls in a 20 µl PCR reaction incorporating the 2× PCR ReadyMix. 6 µl of each reaction was analyzed on a 1% Agarose gel.

Cross-Contamination Analysis

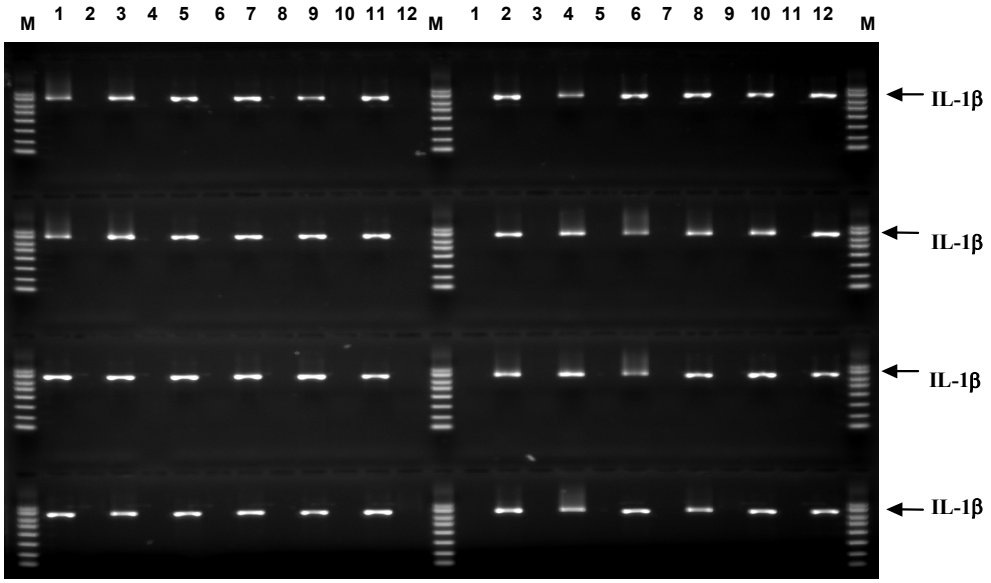


Figure 2. Mouse tails were placed in alternating wells of the extraction plate. The extraction plate was processed using the automated Extract-N-Amp Tissue PCR procedure on the Biomek FX workstation. All samples were amplified and 6 μl of the resultant products were electrophoresed on a 1% agarose gel. No PCR products were detected in the wells without tissue samples.

Multiple Tissue Samples

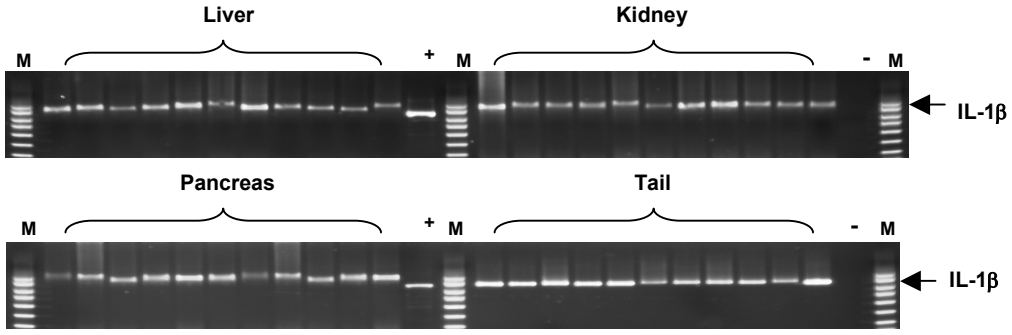


Figure 3. DNA was extracted from mouse liver, kidney, pancreas, and tails using the automated Extract-N-Amp Tissue PCR procedure on the Biomek FX workstation. Amplification of the 1181 bp fragment of the IL-1β gene followed using 4 μl of extracted template DNA or 4 μl of human genomic DNA controls in a 20 μl PCR reaction incorporating the 2× PCR ReadyMix. 6 μl of each reaction was loaded on a 1% agarose gel.

XIII. Troubleshooting

Problem	Cause	Solution
Little or no PCR product is detected.	A PCR component is missing or degraded.	Run a positive control to ensure components are functioning.
	No tissue extract is added to the PCR reactions.	Check the performance of liquid handler. Prime the system if needed. Adjust the aspiration distance of the pipettors in the extraction plate.
	PCR reaction is inhibited due to contaminants in the tissue extract.	Use less extract or dilute the extract with 50:50 mix of Extraction and Neutralization Solutions and repeat PCR.
	PCR reaction is inhibited due to the presence of a precipitate that may form in the tissue extract.	Centrifuge the plate containing tissue extracts before adding the extracts to PCR amplification plate.
	The mixing of Neutralization Solution with tissue DNA extract is not sufficient due to inefficient mixing by the Liquid Handler and/or the clogging of the pipette tip by the tissue.	Increase the aspiration and dispensing speed and/or cycle times in the mixing steps. Decrease the aspiration distance of the pipette tips in the mixing steps to avoid sucking up the tissue by the pipettors.
	Genomic DNA is sheared when the solution is mixed with the pipettor.	Reduce the aspiration and dispensing speed and/or cycle times in the mixing steps. It is critical for amplifying the large genomic DNA fragments.
	Too few cycles are performed.	Increase the number of cycles (5-10 additional cycles at a time).
	Others	Refer to the Technical Bulletin of Extract-N-Amp™ Tissue PCR Kits.
Negative control shows a PCR product or “false positive” results are obtained.	Reagents are contaminated.	Use new labware and new batch of reagents. Test a reagent blank without DNA template to determine if the reagents used in extraction or PCR are contaminated.

XIV. Contact Information

Technical Service
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Email: techserv@sial.com

Customer Service
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(800) 588-9160
www.sigma-aldrich.com/order

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