



Product Information

Automated Protocol for Extract-N-Amp™ Blood PCR Kits Using the Biomek® FX Workstation (Beckman Coulter)

Extract-N-Amp Blood Product Codes **XNABR** and **XNAB2R**

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Automation Guide

I. Description

The Extract-N-Amp Blood PCR Kit has been developed for use as a high throughput system for the rapid extraction and subsequent amplification of genomic DNA from whole blood in a 96-well format. The Extract-N-Amp Blood PCR Kits provide a novel extraction system that eliminates the need for any type of purification, organic extraction, centrifugation, heating, filtration, or alcohol precipitation. Included in the kit is a specially formulated Extract-N-Amp PCR ReadyMix™ reagent that is a 2x reaction mixture of buffer, salts, dNTPs, and *Taq* polymerase. It also contains Sigma's antibody mediated hot start mechanism, JumpStart™ *Taq* polymerase, for highly specific amplification of genomic DNA directly from the extract. There is also a second formulation of the ReadyMix, REExtract-N-Amp™ PCR ReadyMix reagent that contains a 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 walk-away protocol for all aspects of the Extract-N-Amp Blood PCR kit.

Extraction and amplification of genomic DNA from whole blood is accomplished in 3 easy steps:

1. 10 µl of Lysis Solution is added to 5 µl of whole blood and incubated at room temperature for 5 minutes.
2. 90 µl of Neutralization Solution is added to the extract. Extracts are now stable for at least 6 months if stored at 4 °C.
3. PCR reactions are set up using 2 µl of the extracts.

In just 13 minutes, the Biomek FX workstation can complete extraction and PCR reaction setup of 96 whole blood samples.

II. Product Components

Reagents Provided	Product Code	Extract-N-Amp Blood XNAB2R	REExtract-N-Amp Blood XNABR
	Package Size	1000 extractions 1000 amplifications	1000 extractions 1000 amplifications
Lysis Solution for Blood	L 3289	25 ml	25 ml
Neutralization Solution for Blood	N 9784	250 ml	250 ml
Extract-N-Amp PCR Ready Mix or REExtract-N-Amp PCR Ready Mix	P 8115 (for XNAB2R) P 8240 (for XNABR)	12 ml	12 ml

III. Storage

The Extract-N-Amp Blood PCR Kits can be stored at 2-8 °C for up to 3 weeks. For long-term storage, store at -20 °C in a frost-free freezer.

IV. Materials to Be Supplied by the User

1. Whole blood
2. Primers for genes of interest
3. Molecular biology grade water (Sigma, W4502)
4. 96-well polypropylene, round bottom plates (Sigma, P6866)
5. 96-well PCR amplification plates, with half skirt (ABgene, AB-1100)
6. Ultra clear cap strip (ABgene, AB-0866)
7. Corning plate holder (Corning, 6525)
8. Sealing film, SealPlate (Sigma, Z369659)
9. Microcentrifuge tubes (1.5 ml, 2 ml screw cap)
10. 24-position Eppendorf® IsoTherm System (Fisher, 05-405-22)
11. 12 column reagent reservoir with low profile (Innovative Microplates, S30028)
12. 96-well reservoir with low profile and pyramidal bottom (Innovative Microplates, S30018)
13. (Optional) 12 column reagent reservoir with high profile (Innovative Microplates, S30019)
14. (Optional) 96-well reservoir with high profile and pyramidal bottom (Innovative Microplates, S30014)
15. Thermal Cycler

V. Instrument Requirements for the Biomek FX Workstation

Part Description	Qty	Ordering Information
Orbital Shaker	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
AP96 P250 Tips, Non-sterile	2	BK717251 (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. Blood Collection

Observe Universal Precautions when handling blood or blood products.

1. Collect blood into tubes containing EDTA, sodium citrate, or sodium heparin. The best results may be obtained with EDTA or sodium citrate. Mix thoroughly by inverting collection tubes two to three times.
2. Chill the tubes at 2-8 °C until needed.
3. Carefully aliquot 5 µl of each blood sample into individual wells of a 96-well polypropylene plate ensuring that each sample is centered down into the bottom of each well. Seal plate with sealing film until needed.

VII. Reagent Preparation

1. *Lysis Solution*: To process a single plate of 96 samples, add 20 ml of Lysis Solution to 96-well reservoir S30018 located at position P2 (see section VIII for deck layout).
2. *Neutralization Solution*: To process a single plate of 96 samples, add 30 ml of Neutralization Solution to 96-well reservoir located at position P5 (see section VIII for deck layout). This reservoir has a maximum capacity of 80 ml and if processing more than 5 plates of samples it may be necessary to use a larger reservoir S30014.
3. *PCR Master Mix*: The Extract-N-Amp Blood PCR ReadyMix is a 2x reaction mixture containing buffer, salts, dNTPs, and Taq polymerase. To prepare a Master Mix, add water, and forward and reverse primers to the Extract-N-Amp Blood PCR ReadyMix as described in table below.

	Water	PCR ReadyMix	Forward Primer	Reverse Primer
Stock		P 8115	100 μ M	100 μ M
Master Mix (2.7 ml)	1.2 ml	1.5 ml	12 μ l	12 μ l

To set up one multiwell plate of 20 μ l PCR reactions, 1.8 ml of PCR Master Mix should be placed in the first column of reservoir S30028 located at position P9 (see section VIII 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 into four 2 ml screw cap tubes and place in column 2 (position 5-8) of the 24-position tube rack located at position P10 (see section VIII for deck layout).
5. *DNA Controls (optional)*: Prepare genomic DNA controls for quantification of the blood DNA extracts. Solutions of 3.2 ng/ μ l, 1.6 ng/ μ l, 0.8 ng/ μ l, and 0.2 ng/ μ l of genomic DNA were prepared and placed on column 1 (position 1-4) of the 24-position tube rack located at position P10 (see section VIII for deck layout).

VIII. Automated Method Description

This section describes the setup and protocol for the automated Extract-N-Amp Blood methods. Three methods are available depending upon your throughput needs and instrument configuration.

A. General Procedures:

1. 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.
2. Add reagents to the appropriate reservoirs as described in section VI.
3. Run the method using Biomek Software Version 3.1.
4. At the completion of the method, place the cap strips onto the PCR plate, vortex to mix the solution and briefly centrifuge. The PCR plate is now ready for placement into a thermal cycler.
5. Blood extracts can be stored for up to 6 months at 4 $^{\circ}$ C.

B. Biomek Methods:

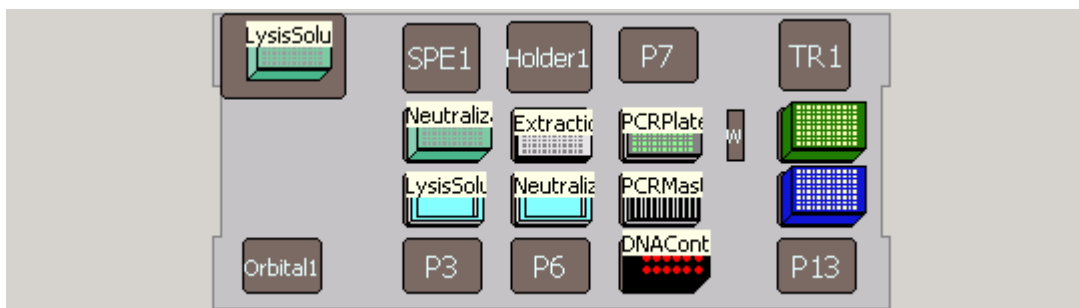
1. *Extract-N-Amp_Blood_PCRSetup*: Performs all of the steps necessary to extract DNA from 96 blood samples and setup PCR reactions. The multichannel head is used to prepare extracts and the Span-8 is used to prepare PCR reactions from extracts and control DNA samples. To perform PCR reaction setup, 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 blood samples and 8 controls using a Master Mix and transfers blood DNA extracts using Span-8. This method may be used if it is desired to perform additional amplification experiments from the blood extracts.

3. *PCR_Setup (no controls)*: Performs PCR reaction setup for 96 samples using a Master Mix and transfers blood DNA extracts. The Span-8 is used to transfer the Master Mix to the PCR plate and the multichannel 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 blood extracts without preparing PCR controls. This method can also be called up in the *Extract-N-Amp Blood_PCRSetup* method if it is desired to transfer extracts with the multichannel head.

C. Description of the Extract-N-Amp_Blood_PCRSetup Method

1. Deck Layout



Deck Position	Equipment
TL1	AP96 P250 Tips (Lysis Solution)
P1	AP96 P250 Tips (Neutralization Solution)
P2	96-well reservoir for Lysis Solution
P3	Swap
P4	96-well polypropylene plate with blood samples
P5	96-well reservoir for Neutralization Solution
P8	96-well PCR amplification plate (seated into a plate holder)
P9	12 column reservoir for PCR Master Mix
P10	24 position Eppendorf IsoThem system
P11	Span-8 P250 Barrier Tips
P12	Span-8 P20 Barrier Tips

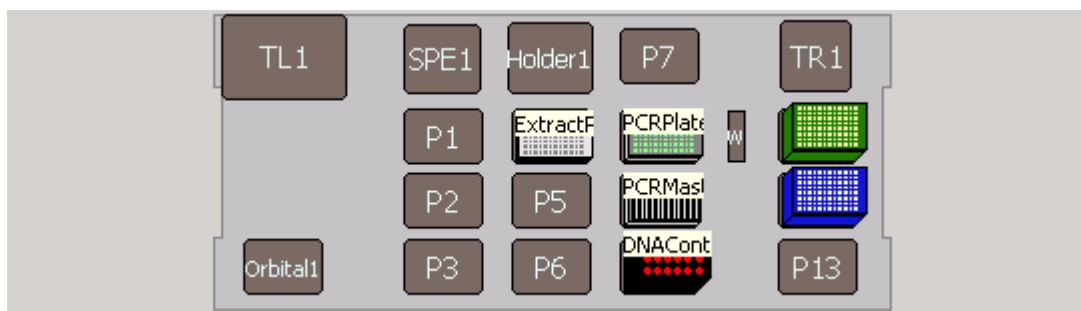
2. Method Overview

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

- 10 μ l of Lysis Solution is aspirated from a reservoir and dispensed into a multiwell plate containing blood samples by the 96 channel head.
- The 96 channel head is used to pipette-mix blood samples to prepare extracts. 10 cycles of mixing are performed.
- Gripper tool is used to move the plate containing blood extract to the shaker.
- Shaker is activated to begin mixing plate with blood extracts for 30 seconds at 750 rpm.
- Gripper tool is used to move plate containing blood extracts from shaker to P4.
- Pause for a 5 minute incubation at room temperature.
- 90 μ l of Neutralization Solution is aspirated from a reservoir and dispensed into the multiwell plate with the blood extracts by the 96-channel head.
- The 96-channel head is used to pipette-mix the extracts for 8 cycles.
- Gripper tool is used to move the plate containing blood extract to the shaker.
- Shaker is activated to begin mixing plate with blood extracts for 30 seconds at 750 rpm.
- Gripper tool is used to move plate containing blood extracts from shaker to P4.
- 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
P4	96-well polypropylene plate with DNA Extracts
P8	96-well PCR amplification plate (seated into a plate holder)
P9	12 column reservoir for PCR Master Mix
P10	24 position Eppendorf IsoThem system
P11	Span-8 P250 Barrier Tips
P12	Span-8 P20 Barrier Tips

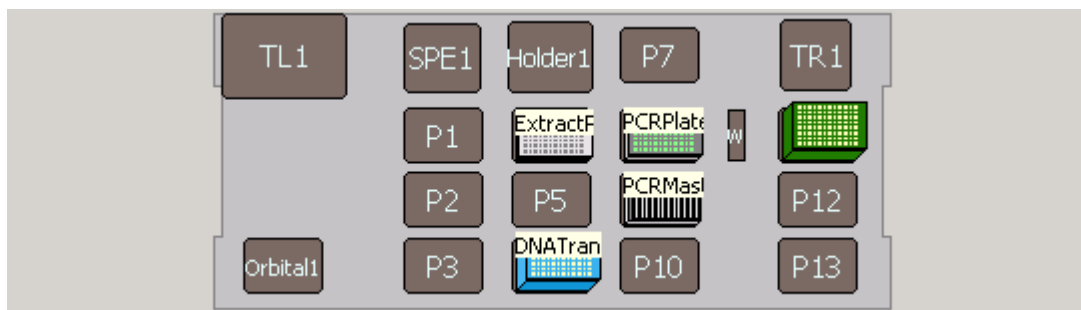
2. Method Overview

Below is a summary of the PCR Setup method using Span-8 to transfer 2 μ l 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 μ l 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 acts like a bulk reagent dispenser and aspirates enough reagents to dispense to a quarter of the plate.
4. 18 μ l 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 18 μ l of PCR master mix to all 96 wells of the PCR amplification plate.
6. 200 μ l barrier tips are removed from the Span-8 dispense head.
7. 2 μ l of blood extract is transferred to the PCR amplification.
8. Because the Span-8 dispense head can only perform operations eight wells at a time, a loop is created to account for all samples. Step 7 is repeated 10 times or as needed. New 20 μ l barrier disposable tips are used for each transfer.
9. 20 μ l barrier disposable tips are removed from the Span-8 dispense head.
10. 2 μ l of control DNA is aspirated from four microcentrifuge tubes and dispensed to wells of A12, C12, E12, and G12 of the PCR amplification plate using the Span-8 dispense head. Refresh 20 μ l barrier disposable tips.
11. 2 μ l of water is aspirated from four microcentrifuge tubes and dispensed to wells of B12, D12, F12, and H12 of the PCR amplification plate using the Span-8 dispense head. Refresh 20 μ l barrier disposable tips.

E. Description of PCR_Setup (no controls) Method

1. Deck Layout:



Deck Position	Equipment
P4	96-well polypropylene plate with DNA Extracts
P6	AP96 P20 Barrier Tips, Sterile
P8	96-well PCR amplification plate (seated into a plate holder)
P9	12 column reservoir for PCR Master Mix
P11	Span-8 P250 Barrier Tips

2. Method Overview

Below is a summary of the PCR Setup method using 96-channel head to transfer 2 μ l 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 μ l 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 acts like a bulk reagent dispenser and aspirates enough reagents to dispense to a quarter of the plate.
4. 18 μ l 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 18 μ l of PCR Master Mix to all 96 wells of the PCR amplification plate.
6. 200 μ l barrier tips are removed from the Span-8 dispense head.
7. 2 μ l of blood extracted is aspirated from the multiwell plate containing blood extracts using 20 μ l barrier disposable tips
8. Blood extract is dispensed into the PCR amplification plate.

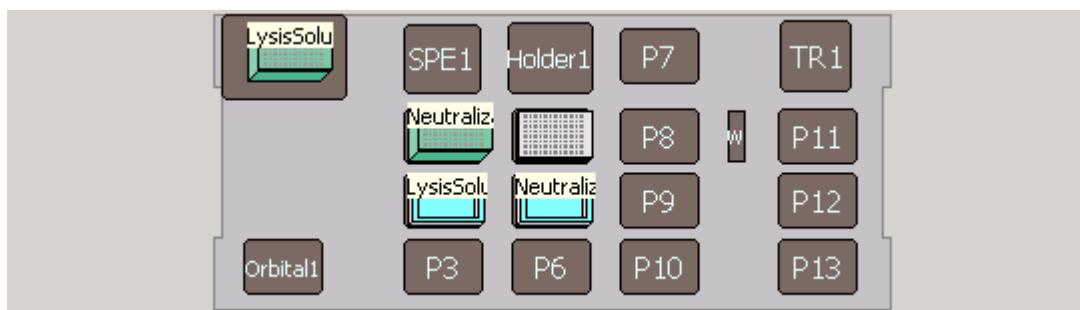
IX. Recommended Parameters for PCR Amplification:

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	

X. Method Customization

Performing extraction without subsequent amplification

Blood samples may be subjected to extraction without subsequent amplification. To account for this modification, delete the last two steps of the *Extract-N-Amp_Blood_PCRSetup* method that correspond to PCR reaction setup. The deck layout in the Instrument Setup step needs to be updated as following:

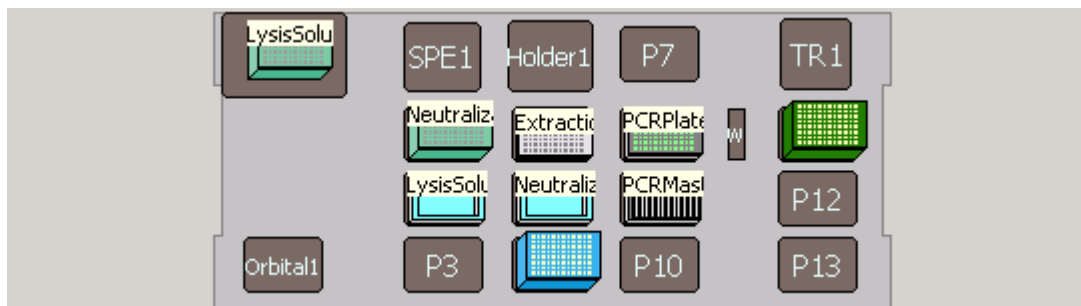


Deck Position	Equipment
TL1	AP96 P250 Tips (Lysis Solution)
P1	AP96 P250 Tips (Neutralization Solution)
P2	96-well reservoir for Lysis Solution
P3	Swap
P4	96-well polypropylene plate with blood samples
P5	96-well reservoir for Neutralization Solution

Preparing 96 blood extracts for PCR

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

1. Click on the Run PCR_Setup (with controls) step of the *Extract-N-Amp_Blood_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_Blood_PCRSetup* and *PCR_Setup (no controls)* methods as following:



Deck Position	Equipment
TL1	AP96 P250 Tips (Lysis Solution)
P1	AP96 P250 Tips (Neutralization Solution)
P2	96-well reservoir for Lysis Solution
P3	Swap
P4	96-well polypropylene plate with blood samples
P5	96-well reservoir for Neutralization Solution
P6	AP96 P20 Barrier Tips, Sterile
P8	96-well PCR amplification plate (seated into a plate holder)
P9	12 column reservoir for PCR Master Mix
P11	Span-8 P250 Barrier Tips

PCR setup only

Blood extracts may be subjected to additional amplifications. The PCR Setup methods 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 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 96 blood extracts 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 reservoir S 30028 located at position P9. 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.

XI. Performance Characteristics

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

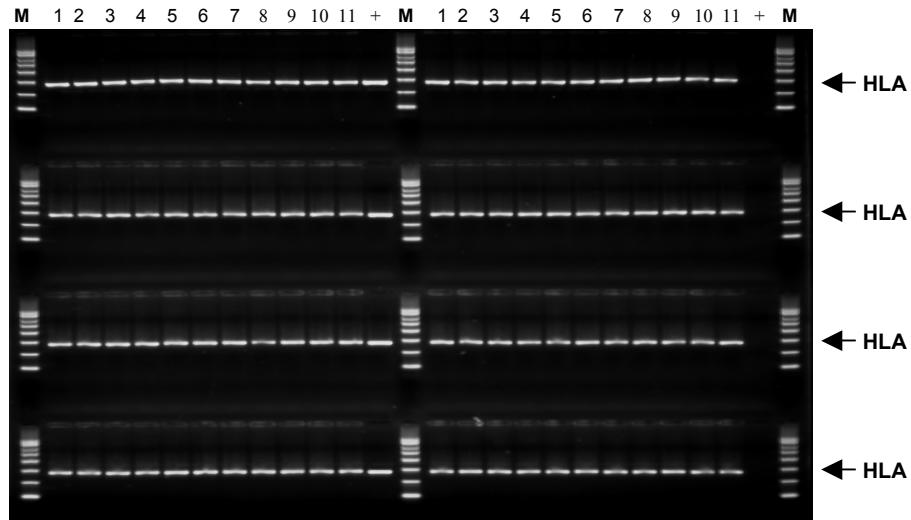


Figure 1. Agarose gel analysis of 96 PCR samples. DNA was extracted from 88 samples of human whole blood (5 μ l) from a single donor using the automated Extract-N-Amp Blood PCR procedure on the Biomek FX. Amplification of 236 bp fragment of the HLA gene followed using 2 μ l of extracted template and 2 μ l of human genomic DNA controls in a 20 μ l PCR reaction incorporating the 2X PCR ReadyMix. 6 μ l of each reaction was analyzed on a 2% agarose gel.

Cross-Contamination Analysis

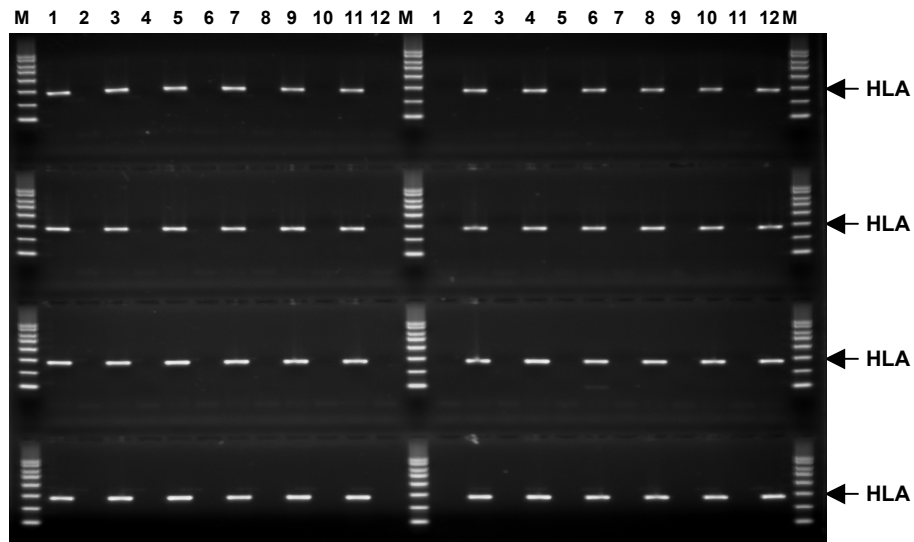


Figure 2. Cross-contamination analysis. 5 μ l samples of blood or PBS were placed in alternating wells of an extraction plate. The 96-well plate was processed using the automated Extract-N-Amp Blood PCR procedure on the Biomek FX. All samples were then subjected to amplification and 6 μ l of the resultant products were electrophoresed on a 2% agarose gel. No PCR products were detected in the samples containing PBS controls.

XII. 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 blood 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 blood extract.	Use less extract or dilute the extract with water and repeat PCR.
	The extraction of blood DNA is not sufficient, due to inefficient mixing by the FX during lysis step.	Lower aspiration and dispensing height in the extraction plate, increase the aspiration and dispensing speed, and/or cycle times in the mixing steps. Observe the color change (from dark red to dark brown) of blood sample after thorough mixing with Lysis Solution.
	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 Blood PCR Kits.
Negative control shows a PCR product or “false positive” results are obtained.	Reagents are contaminated.	Use new labware and new batches of reagents. Test a reagent blank without DNA template to determine if the reagents used in extraction or PCR are contaminated.

III. Contact Information

Technical Service
(800) 325-5832
E-mail: techserv@sial.com

Customer Service
(800) 325-3010
(800) 588-9160
www.sigma-aldrich.com/order

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