



Biomek Automated Genomic Sample Prep Accelerates Research

Biomek i-Series Automation of the Beckman Coulter Agencourt Genfind v2 Blood & Serum DNA Isolation Kit

Introduction

The Agencourt Genfind v2 kit isolates genomic DNA from fresh or frozen whole blood and serum containing Citrate, EDTA, or Heparin anticoagulants. The kit produces a high recovery of DNA for downstream applications such as agarose gel analysis, PCR amplification, restriction enzyme digestion, human identity testing, membrane hybridizations (e.g., Southern and dot/slot blots), AFLP, RFLP, RAPD, microsatellite and SNP analyses (for genotyping, fingerprinting, etc.). The purification protocol first uses lysis buffer and Proteinase K to rupture cell membranes and digest proteins. DNA is then immobilized on Agencourt's patented SPRI (Solid Phase Reversible Immobilization) paramagnetic beads by the addition of a magnetic binding reagent. This differential binding of DNA enables separating DNA with a magnetic field, followed by rinsing away the contaminants using a simple washing procedure, leaving the genomic DNA ready for elution. SPRI also enables fast separation, easy manipulation and simple automation compared to the traditional vacuum filtration and centrifugation technologies. In this technical note, we demonstrate the automated performance of Agencourt Genfind v2 kit on the Biomek i-series Genomics Workstations.

When compared to manual operations, the Agencourt Genfind v2 kit automated on Biomek platform provides:

- Reduced hands-on-time and increased throughput
- Option to run the method end-to-end with only setup and tear-down touch points
- Reduction in pipetting errors
- Standardized workflow for improved results
- Quick implementation with demonstrated methods
- Knowledgeable support for reagents, automation and methods all from a single vendor

Spotlight: Biomek i5 Multichannel 96 Genomics Workstation

System features deliver reliability and efficiency to increase user confidence and walk-away time compared to manual operation.

- 300 uL or 1200 uL Multichannel head with 1-300 uL or 1-1200uL pipetting capability
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers optimizes access to high density decks
- High deck capacity by 25 positions and separate locations for trash
- Orbital Shakers, peltiers and 96 channel tip washing for controlling sample processing



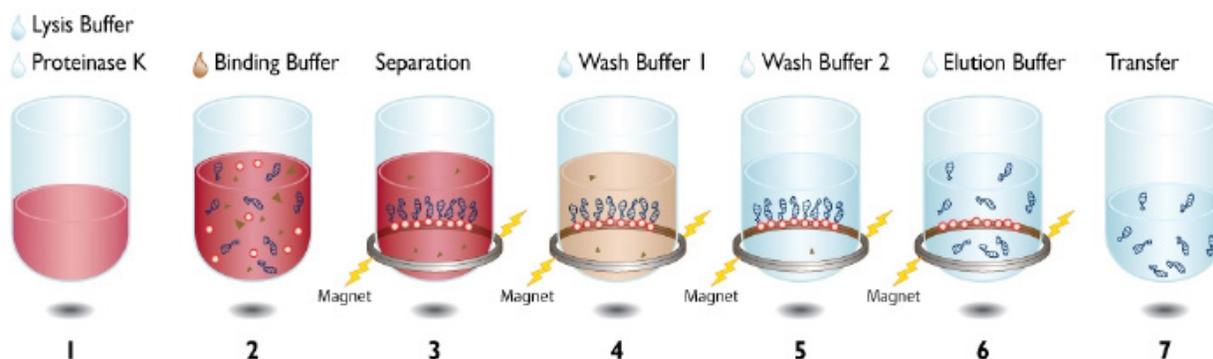


Figure 1. Beckman Coulter Agencourt Genfind v2 kit protocol

Automated method

We automated the Beckman Coulter Agencourt Genfind v2 kit protocol on Biomek i-series, incorporating on-deck lysis. In general, the automated protocol enables DNA extraction from 1-96 blood and serum samples up to 400 μ L, in approximately 2 hours (Table 1). The data for this technical note has been generated using i5/i7 MC (Multichannel) method.

The handling of blood and blood waste require special precautions and waste disposal procedures. Therefore, the automated Genfind method isolates blood waste (e.g. supernatants, tip wash waste) into waste plates, facilitating proper disposal of biohazardous waste.

Procedure	i5/i7 MC method: 1-96 samples, Enhance Selective Tip Pipetting	i7 MC method: 2 full plates
Reagent and instrument preparation*	15 mins	15 mins
Agencourt Genfind v2 method	1 hr 46 mins	2 hrs 28 mins
Total	2 hrs 1 mins	2 hrs 43 mins

*: Timing does not include reagent and sample thawing, blood/serum collection, homogenization or dissection.

Table 1. Estimated run times for Agencourt Genfind v2 kit protocol on the Biomek i-series (Sample volume 400 μ L, with detergent protocol). Note: A modified Genfind v2 method that uses heated buffers is also available.

Demonstrated Method Interface (DMI)

The automated Genfind v2 protocol was performed through the DMI that provided the user full instructions to setup the method. It consists of three modules.

1. Biomek Method Launcher (BML)

BML is an add-on for Biomek software that provides a secure interface for new users to run a method, without affecting method integrity (Figure 2). BML allows remote monitoring of the run progress, through milestones. The manual control option in BML provides the opportunity to interfere if needed.

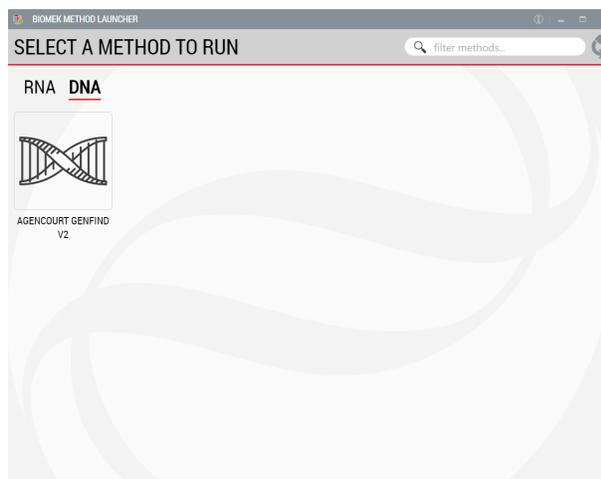


Figure 2. Biomek Method Launcher provides an easy interface to run the method

2. Method Options Selector (MOS)

MOS enables selection of sample number and processing options to suit the needs of the user (Figure 3). For instance the MOS of 1-96 sample automated protocol enables selecting 1-96 samples of in the volume range of 40-400 μL , for processing. The user has the ability to run the Genfind v2 supplementary protocol (e.g. Detergent protocol), if preferred. Based on the available volume of reagents (e.g. Bind buffer) the user can select the type of labware, to minimize the dead volume. In addition, depending on the required concentration of DNA output, the user can either increase or decrease the elution volume. This flexibility allows the users to utilize the extracted DNA directly in downstream applications, without further processing to adjust the concentration.

The screenshot shows the 'Method Options' section of the Beckman Coulter AGENCOURT GENFIND v2 DNA isolation protocol software. The interface is titled 'Optimized for Biomek i-Series' and 'Automated by Beckman Coulter, Inc'. It includes several input fields and dropdown menus: 'Enter Number of Samples' (set to 6), 'Enter the Sample Volume in Microliters' (set to 200), 'Select Sample Type' (set to Blood), 'Perform Detergent Protocol?' (set to Yes), 'Select a Labware Type for Bind Buffer' (set to Plate (Low dead volume)), and 'Enter the Elution Volume in Microliters' (set to 200). At the bottom, there are 'Start run' and 'Abort' buttons, and the Beckman Coulter logo is visible in the bottom right corner.

Figure 3. Biomek Method Options Selector indicates sample number and processing options

3. Guided Labware Setup (GLS)

GLS is generated based on user selected options in the MOS. It provided specific text and graphical setup instructions with reagent volume calculation and step by step instructions to prepare reagents (Figure 4).

The screenshot shows the 'Guided Labware Setup (GLS)' interface. The main window is titled 'SET UP BIOMEK DECK' and has two tabs: 'Prepare labware' (selected) and 'Place labware'. The 'Prepare labware' tab displays 'Setup notes: Preparation of Wash Buffer 2:' with instructions for adding ethanol to Agencourt Genfind v2 plate kits and tube kits. Below the notes, there is a large blue box with the text 'Wash Buffer 2 212 ml'. On the left side, there is a list of reagents and tips, including 'Transfer_Tips', 'Supernatant_Removal_tips', 'Elute_Tips', 'Detergent_Buffer', 'Wash_Buffer_1_1', 'Wash_Buffer_1_2', 'Wash_Buffer2', 'Elute_Buffer', 'Bind_Buffer_Plate', and 'Lysis_Mix_Plate'. At the bottom, there are 'Cancel', 'Back', and 'Next' buttons. The Windows taskbar is visible at the bottom of the screen, showing the date and time as 11:38 AM 7/14/2017.

Figure 4. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup

Experimental design

Frozen human blood preserved in Citrate, EDTA and Heparin was used in the experiment. DNA was extracted using both manual and automated (i5/i7 MC method, 1-96 samples) Genfind protocols (200 μ L blood samples, 2 replicates from each preservative category) for comparison purposes. The quantity and the quality of the DNA samples were assessed using NanoDrop 2000™ (Thermo Fisher Scientific), Agilent TapeStation 2200 (with Agilent High Sensitivity D5000 ScreenTape system) and qPCR (KAPA SYBR® Fast One-Step qPCR Master Mix (2x), Beta Actin primer, reactions done in duplicates for each preservative category).

Results

The quality and the quantity of DNA extracted from the automated protocol was comparable to the manual protocol (Table 2). DNA Integrity Numbers (DIN) calculated by TapeStation provided another estimate of DNA quality. Both methods enable extraction of high quality DNA, as indicated by the excellent DIN scores (Figure 5).

Both manually and automatically extracted DNA samples amplified in the range of Ct 15-20 (Figure 6). Certain anticoagulants such as Heparin act as powerful PCR inhibitors. Our results show that both manual and automated protocols is able to remove these inhibitors successfully (Figure 6). This indicates that the extracted DNA is suitable for downstream applications such as PCR and genotyping.

Method	Sample	Total concentration (μ g)	260/280
Manual	1. Sample 1 Citrate	5.256	1.45
Manual	2. Sample 1 Citrate	1.266	1.23
Manual	3. Sample 1 EDTA	2.878	1.60
Manual	4. Sample 1 EDTA	6.84	1.44
Manual	5. Sample 1 Heparin	4.406	1.66
Manual	6. Sample 1 Heparin	6.322	1.57
Automated	1. Sample 1 Citrate	3.664	1.58
Automated	2. Sample 1 Citrate	4.928	1.55
Automated	3. Sample 1 EDTA	5.49	1.76
Automated	4. Sample 1 EDTA	2.754	1.55
Automated	5. Sample 1 Heparin	7.122	1.66
Automated	6. Sample 1 Heparin	6.836	1.72

Table 2. The yield and the purity of DNA as indicated by NanoDrop 2000

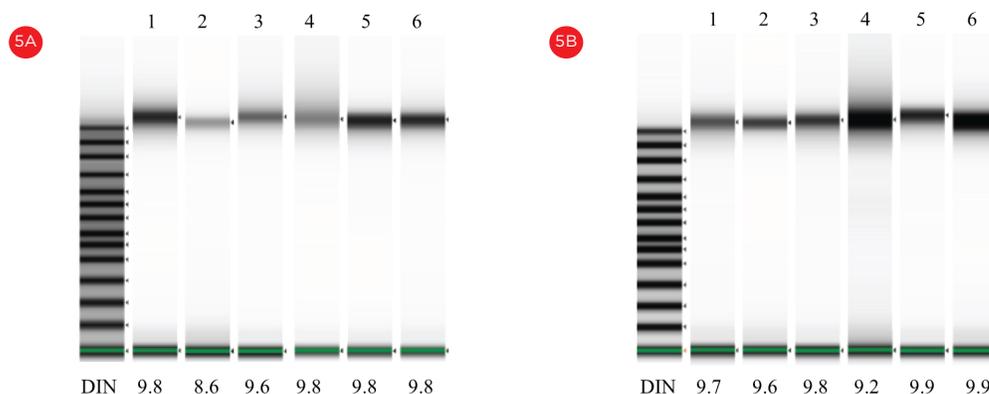


Figure 5. Manual (A) and automated (B) DNA samples analyzed on Agilent TapeStation 2200

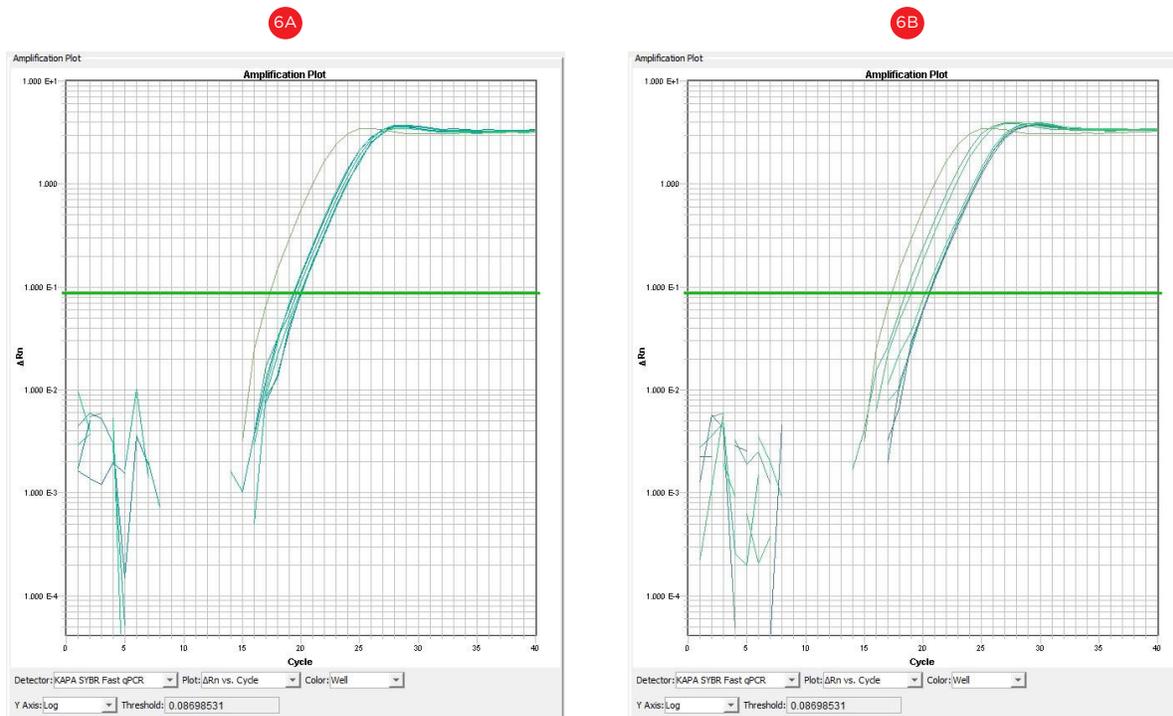


Figure 6. qPCR amplification plots (cycle number vs. fluorescence) corresponding to manual (A) and automated (B) DNA templates. DNA template concentration 10 ng/uL; X: positive control, 10 ng/uL Promega gDNA

Summary

We have demonstrated automation of Agencourt Genfind v2 on the Biomek i-series Genomics Workstations. The quality and quantity of DNA extracted by the automated protocol was comparable to manually extracted DNA and suitable for downstream applications. The automated method provided several sample processing options and input/output volume options to better suit the needs of the customers. The method was implemented through Biomek Method Launcher that provided a convenient interface to run the method, without affecting the method integrity.

Data obtained during development

Beckman Coulter Life Sciences Biomek Automated Workstations and genomic reagent kits are not intended or validated for use in the diagnosis of disease or other conditions.

© 2017 Beckman Coulter, Inc. All rights reserved. Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries. All other trademarks are the property of their respective owners.

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at beckman.com

AAG-292608.17