AUTOMATED PROTOCOL

Automated DNA IQ™ System Protocol for the Beckman Coulter Biomek® 3000

Instructions for Use of Products
DC6701 and DC6700

EP033
Revised 1/11

Automated DNA IQ™ System Protocol for the Beckman Coulter Biomek® 3000

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Automated Protocol.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

This document describes automation of the DNA IQ™ System[a] on the Beckman Coulter Biomek® 3000 laboratory automation workstation.

Please contact the Promega Genetic Identity team (genetic@promega.com) prior to implementing this method on your workstation. To obtain information about other methods for human identification applications, visit:
www.promega.com/applications/hmnid/automation/

Note: All Promega Technical Manuals are available at: **www.promega.com/protocols/**

2. Requirements and Product Storage Conditions

PRODUCT	SIZE	CAT.#
DNA IQ™ System	**100 reactions***	**DC6701**
	400 reactions*	**DC6700**

*Cat.# DC6701 contains sufficient reagents to process one 96-well plate of up to 100µl for aqueous samples or up to 200µl for lysis samples; DC6700 has sufficient reagents for four 96-well plates. When processing larger sample volumes, you will need to purchase additional DNA IQ™ Lysis Buffer. Processing four 96-well plates of 400µl aqueous or lysis samples requires an additional 70ml of DNA IQ™ Lysis Buffer.

Storage Conditions: Store all components at room temperature (22–25°C).

Items Available Separately

PRODUCT	SIZE	CAT.#
DNA IQ™ Resin	**50ml**	**A8251**
Lysis Buffer	**150ml**	**A8261**
2X Wash Buffer	**70ml**	**A8271**
Elution Buffer	**50ml**	**A8281**

Not for Medical Diagnostic Use.

3. Materials to be Supplied by the User

- DNA IQ™ System (if you are processing more than 100µl of each aqueous sample or 200µl of each lysis sample, you will need to purchase additional DNA IQ™ Lysis Buffer; see Section 2)
- 99% isopropyl alcohol
- 95–100% ethanol
- DTT (Cat.# V3151 for 5g, Cat.# V3155 for 25g)

See Sections 5.A and 5.B for instrumentation requirements and labware requirements, respectively.

4. Before You Begin

4.A. Preparation of Solutions

Prior to beginning the automated DNA IQ™ System method, prepare the following solutions:

Prepared Lysis Buffer

1. Add 1µl of 1M DTT for every 100µl of Lysis Buffer.
2. Mix by inversion several times.
3. Mark and date label to record addition of DTT.

This solution can be stored at room temperature for up to one month if the bottle is closed tightly.

DNA IQ™ Resin Solution (prepared Lysis Buffer + resin)

1. Thoroughly mix the DNA IQ™ Resin by inversion for several minutes.
2. Make the prepared Lysis Buffer as described above (i.e., add 1µl of 1M DTT for every 100µl of Lysis Buffer).
3. Prepare the DNA IQ™ Resin Solution by combining 860µl + (# samples × 43µl) of prepared Lysis Buffer and 140µl + (# samples × 7.0µl) of DNA IQ™ Resin.

   For example, when processing 96 samples, combine 860µl + (96 × 43µl) = 4,988µl of prepared Lysis Buffer with 140µl + (96 × 7.0µl) = 812µl of DNA IQ™ Resin for a total volume of 5,800µl.

   Note: Prepare the DNA IQ™ Resin Solution fresh before each run. Do not store.
4. Mix thoroughly by inversion several times.

1X Wash Buffer

1. Add ethanol and isopropyl alcohol directly to the 2X Wash Buffer (15ml of 95–100% ethanol and 15ml of 99% isopropyl alcohol for DC6701; 35ml of 95–100% ethanol and 35ml of 99% isopropyl alcohol for DC6700).
2. Replace the cap, and mix by inversion several times.
3. Mark label as 1X Wash Buffer, and indicate addition of alcohols.
4. Store at room temperature (22–25°C). Make sure bottle is closed tightly to prevent evaporation.

4.B. Sample Processing Before Automated Processing (Optional)

For samples on solid supports, preprocessing must be performed prior to the start of the automated method. For more information about sample preprocessing, refer to the *DNA IQ™ System—Small Sample Casework Technical Bulletin* #TB296 or *DNA IQ™ System—Database Protocol Technical Bulletin* #TB297 or contact Promega Technical Services.

5. Automated Processing Requirements for the Biomek® 3000 Workstation

This section lists the instrumentation and labware requirements for the automated DNA IQ™ System method on the Biomek® 3000.

5.A. Instrumentation Requirements for the Automated DNA IQ™ System Method on the Biomek® 3000

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required for the automated DNA IQ™ System method on a Biomek® 3000.

Part Description	Quantity	Beckman Coulter Part Number
Biomek® 3000 Workstation, 50/60Hz, 100–240V	1	986120
Biomek® 3000 Automation Controller XP and Monitor with Biomek® System Software	1	A16170
Biomek® 3000 Left Side Module	1	987264
Biomek® 3000 Right Side Module	1	987263
Extra Serial Port	1	977092
Teleshake Driver	1	custom item
Gripper Tool Kit for Biomek® 3000	1	A09053
MP200 Eight-Tip Tool	1	986146
Black Tip Rack Holder	4[1]	391910
AP96 P250 Tips, Barrier	1–3 per run	717253
Gray Labware Holder	7[1]	609120
Modular Frame for Reservoirs	1	372795
Quarter Reservoir (case of 48)	1	372790
Quarter Reservoir, Divided by Length (case of 48)	1	372788
Half Reservoir (case of 24)	1	372786
Large Disposal Option (optional)	1	609751

[1]These quantities represent the total number of items required. Two tip rack holders and three gray labware holders are supplied with the Biomek® 3000 Workstation.

5.B. Labware Requirements for the Automated DNA IQ™ System Method on the Biomek® 3000

The following is a list of Promega labware parts and their corresponding part numbers that are required for the automated DNA IQ™ System method on a Biomek® 3000.

Part Description	Quantity	Promega Cat.#
MagnaBot® 96 Magnetic Separation Device	1	V8151
1/4 inch Foam Spacer	1	Z3301
2.2ml, Square-Well Deep Well Plate or Slicprep™ 96 Device (for preprocessed solid samples)	1 per run 1 per run	V6781 V1391
1.2ml, Round-Bottom Deep Well Plate	2 per run	V6771
Shaker Integration Plate	1	V3691
VARIOMAG® Teleshake (110V, For North American Use Only)	1	V6751
V&P Scientific Heating Block (110V, for North America use only)	1	V6761
Deep Well Heat Transfer Block	1	V6741
96-well PCR plate or strip tubes on plate stand	1 per run	(user-selected)

5.C. Biomek® 3000 Initial Deck Configuration

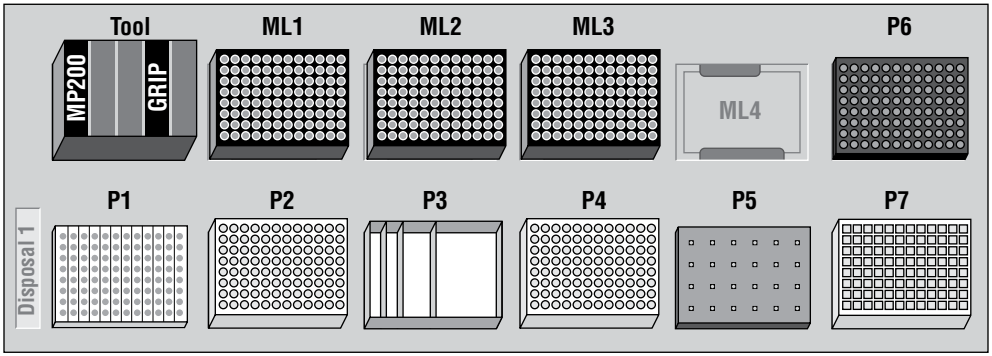

Figure 1. Biomek® 3000 initial deck configuration.

Position Tool	Tool rack containing MP200 and gripper tools
Position ML1	Tip rack holder, AP96 P250 tips (required for each run)
Position ML2	Tip rack holder, AP96 P250 tips when processing up to 88 samples
Position ML3	Tip rack holder, AP96 P250 tips when processing up to 96 samples
Position ML4	Tip rack holder
Position P6	Gray labware holder, V&P Scientific Heating Block, Deep Well Heat Transfer Block
Position P1	Gray labware holder, 96-well PCR plate or strip tubes on plate stand (Elution Plate)
Position P2	Gray labware holder, empty 1.2ml, Round-Bottom Deep Well Plate (Purification Plate 2)
Position P3	Gray labware holder, frame for reserviors, reservoirs with reagents (see Figure 2 for configuration)
Position P4	Gray labware holder, empty 1.2ml, Round-Bottom Deep Well Plate (Purification Plate)
Position P5	Gray labware holder, MagnaBot® 96 Magnetic Separation Device, 1/4 inch Foam Spacer
Position P7	Shaker Integration Plate, VARIOMAG® Teleshake, 2.2ml, Square-Well Deep Well Plate containing samples (Sample Plate) Samples should be arranged in columns across the plate and will be processed from left to right using the 8-channel MP200 tool.
Disposal 1	The large disposal option (optional)

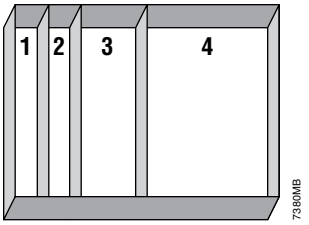

Figure 2. Configuration of troughs at deck position P3. The Quarter Reservoir, Divided by Length, creates positions 1 and 2. Position 3 is one Quarter Reservoir. Position 4 is one Half Reservoir.

You will be prompted to enter a number of user-defined variables at the start of the automated method. These variables are defined by the user based on processing needs. Variables can be found by selecting the Start icon.

Overridable	Prompt	Variable Name	Value
☐	☑	A_Sample_Volume	400
☐	☑	Add_Lysis_Buffer	N
☐	☑	Elution_Volume	50
☐	☑	End_Column	6
☐	☑	First_Tip_Column	1
☐	☐	LysisWash	Y
☐	☑	Start_Column	1
☐	☐	Tip_Waste	N

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A_Sample_Volume

The A_Sample_Volume variable corresponds to the volume of the starting sample (i.e., 400µl = 400). The starting sample volume must be the same for all samples processed on the plate. Note that the method accommodates up to 400µl of samples that do not contain any DNA IQ™ Lysis Buffer. For samples that already contain Lysis Buffer (from preprocessing steps), up to 800µl may be accommodated.

Add_Lysis_Buffer

The Add_Lysis_Buffer variable can be set to Y (yes) or N (no), depending on the preprocessing procedure for the starting samples. You can direct the robot to add prepared Lysis Buffer to the samples (i.e., select Y) or not add Lysis Buffer (i.e., N) if the preprocessing procedure already includes addition of Lysis Buffer.

5.C. Biomek® 3000 Initial Deck Configuration (continued)

Elution_Volume

The Elution_Volume variable corresponds to the final volume of Elution Buffer added to each sample well (i.e., 40–100µl). If a volume outside of this range is entered, the default volume of 40µl or 100µl will be used. Note that the recovered elution volume in the Elution Plate at the end of the method will be less than the volume of Elution Buffer added due to evaporation on the heater. As much as 8–10µl can be lost.

Start_Column

The Start_Column variable indicates the first column of wells that contain samples (i.e., Column 1 = 1).

End_Column

The End_Column variable indicates the last column of wells that contain samples (i.e., Column 6 = 6).

First_Tip_Column

The First_Tip_Column variable indicates the first usable column of tips in the first tip box at deck position ML1 (i.e., Column 1 = 1). This allows the robot to load tips from a partial box of tips, economizing tip usage and enabling you to make use of partial tip boxes.

Note: The Lysis Wash and Tip_Waste variables can be configured during method installation. Contact Promega for more information.

5.D. Biomek® 3000 Reagent Dispense Volumes

Prior to beginning the run, dispense the reagents as described below. A series of user prompts at the beginning of the method directs you to add the appropriate volume of each reagent based on the user-defined variables entered previously. See Section 4.A for solution preparation.

Trough 1	DNA IQ™ Elution Buffer: Dispense 2,500µl + (# of samples × desired elution volume µl) into the trough. For example, for 96 samples with 100µl elution, the volume is 2,500µl + (96 × 100µl) = 12,100µl = 12.1ml.
Trough 2	DNA IQ™ Resin Solution: Use the full volume prepared in Section 4.A.
Trough 3	1X Wash Buffer (with ethanol and isopropyl alcohol added): Use 1,500µl + (# of samples × 300µl). For 96 samples = 1,500µl + (96 × 300µl) = 30,300µl = 30.3ml.
Trough 4	Prepared Lysis Buffer (see Section 4): Use 2,000µl + (# of samples × 100µl). For example, for 96 samples, the volume is 2,000µl + (96 × 100µl) = 11,600µl = 11.6ml.

! At the beginning of the automated method, there are a series of user prompts that provide the minimum required volume of each solution per trough.

Number of Samples	DNA IQ™ Elution Buffer[1]	DNA IQ™ 1X Wash Buffer	DNA IQ™ Lysis Buffer containing DTT
96	12.1ml	30.3ml	11.6ml
80	10.5ml	25.5ml	10.0ml
64	8.9ml	20.7ml	8.4ml
48	7.3ml	15.9ml	6.8ml
32	5.7ml	11.1ml	5.2ml
16	4.1ml	6.3ml	3.6ml

[1]These Elution Buffer volumes are specifically calculated for 100µl of elution buffer.

6. Description of the Automated DNA IQ™ System Method

This overview describes the general liquid-handling steps required for the automated DNA IQ™ System method.

1. **Lysis Buffer Addition (optional, depending on starting sample).** The liquid-handling robot adds prepared Lysis Buffer to each sample in the Sample Plate. The volume added depends on the starting volume of aqueous samples.
2. **DNA IQ™ Resin Solution Addition.** The liquid-handling robot adds 50µl of DNA IQ™ Resin Solution to each sample in the Sample Plate.
3. **DNA Binding.** The Sample Plate is subjected to a series of shaking and incubation steps (30-second shake, 1-minute incubation; repeated three times and followed by a final 30-second shake) to allow DNA binding to the DNA IQ™ Resin.
4. **Volume Transfer.** The Sample Plate contents are transferred to the Purification Plate atop the MagnaBot® 96 Device, which collects the resin at the sides of each well.
5. **Lysis Buffer Removal.** The supernatant (prepared Lysis Buffer) is removed to the Sample Plate, which will now serve as the Lysate Waste Plate.
6. **Lysis Buffer Wash.** The liquid-handling robot adds 100µl of prepared Lysis Buffer to each sample well of the Purification Plate. The plate then is moved to the shaker, and the resin is washed by shaking for 30 seconds.
7. **Lysis Buffer Wash Removal.** The Purification Plate is moved back onto the MagnaBot® 96 Magnetic Separation Device, and the supernatant (prepared Lysis Buffer) is removed to the Lysate Waste Plate.
8. **1X Wash Buffer Addition #1.** The liquid-handling robot adds 100µl of 1X Wash Buffer containing alcohols to each sample well of the Purification Plate. The plate is placed on the shaker, and the resin is washed by shaking for 30 seconds.

6. Description of the Automated DNA IQ™ System Method (continued)

9. **Plate Transfer.** Purification Plate 2 is moved onto the MagnaBot® 96 Device. The resin and Wash Buffer are transferred from the first Purification Plate to Purification Plate 2.
10. **1X Wash Buffer Removal #1.** The supernatant (1X Wash Buffer) is removed from Purification Plate 2 and returned to the first Purification Plate, which will now serve as the Alcohol Wash Waste Plate.
11. **Washes #2 and #3 with 1X Wash Buffer.** The 1X Wash Buffer addition and removal steps are repeated twice for a total of three washes.
12. **Heated Drying.** Purification Plate 2 is moved onto the heater. The system pauses for 2.5 minutes to allow evaporation of any Wash Buffer in the sample wells.
13. **Elution Buffer Addition.** The liquid-handling robot adds the desired volume (e.g., 100µl) of DNA IQ™ Elution Buffer to each sample in Purification Plate 2. Purification Plate 2 is placed on the shaker and heater in a series of three 30-second shakes and two 2.5-minute heated incubation steps to elute DNA from the DNA IQ™ Resin into the Elution Buffer.
14. **Elution.** Purification Plate 2 is moved onto the MagnaBot® 96 Device, and the supernatant (Elution Buffer containing purified DNA) is removed to the Elution Plate.
15. **Method Ends.** The automated DNA IQ™ System method is now complete. The purified DNA samples in the Elution Plate may be processed immediately or stored at 4°C.

7. Important Considerations

1. Use aerosol-resistant tips to minimize cross-contamination, particularly for casework samples.
2. Thoroughly resuspend the DNA IQ™ Resin before use by shaking vigorously. Prior to combining the resin and Lysis Buffer, turn the resin bottle upside-down to ensure that no clumps of resin remain at the bottom of the bottle.
3. Be sure to turn on the heater, and set it to 85°C before running the automated method.
4. The heater set and display temperatures may differ by ~1°C. This is not uncommon for the heater. A difference of ~1°C at the 85°C set temperature will not affect elution efficiency. During heated elution, samples should reach a temperature of approximately 65°C to achieve complete elution. If necessary, adjust temperature or calibration settings for the heater control unit to ensure that proper temperatures are reached. At the appropriate calibration and temperature settings, the surface of the Heat Transfer Block should be ~70–72°C.
5. The recovered elution volume in the Elution Plate at the end of the method may be less than the volume of DNA IQ™ Elution Buffer added due to evaporation on the heater.
6. This version of the automated DNA IQ™ System method should not be used with samples that have been processed using the automated Differex™ System method. For those samples, refer to automated protocol #EP032, *Automated Differex™ System Protocol for the Beckman Coulter Biomek® 3000*, for instructions for the Automated DNA IQ™ After Differex™ System Method on the Biomek® 3000 workstation.

[a]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756, European Pat. No. 1 204 741, Mexican Pat. No. 209436 and other patents pending.