

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

Automated Protocol #EP033

DESCRIPTION OF THE DNA IQ™ SYSTEM METHOD FOR THE BECKMAN COULTER BIOMEK® 3000.

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1. Description

This document describes 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 Bulletins are available at: www.promega.com/tbs/

2. Product Requirements and Storage Conditions

Product	Size	Cat.#
DNA IQ™ System	1 × 96 reactions*	DC6701
	4 × 96 reactions*	DC6700

For Laboratory Use.

*If processing fewer than 96 samples per run, you will need purchase additional reagents. See a list of Items Available Separately below.

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 (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. 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.
4. This solution can be stored at room temperature for up to one month if sealed, or alternatively, the prepared Lysis Buffer can be made fresh for each run.

Total Volume (ml) of Lysis Buffer to Prepare with DTT (to make Prepared Lysis Buffer).

The volume of Prepared Lysis Buffer shown in the table below includes sufficient volume for preparation of the DNA IQ™ Resin Solution. That is, part of the Prepared Lysis Buffer volume shown will be used to create the DNA IQ™ Resin Solution, and the remaining volume will be used for the method.

	Number of Samples											
	96	88	80	72	64	56	48	40	32	24	16	8
Swab Method	16.1	15	13.9	12.7	11.6	10.4	9.3	8.1	7	5.8	4.7	3.6
Aqueous Method	34.5	31.8	29.1	26.5	23.8	21.1	18.5	15.8	13.2	10.5	7.8	5.2

4. Preparation of Solutions (continued)

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., adding 1µl of 1M DTT for every 100µl of Lysis Buffer).
3. Prepare the DNA IQ™ Resin Solution by combining the volumes of prepared Lysis Buffer and DNA IQ™ Resin calculated below based on the method of processing starting samples.

a. Swab Method

Volume of prepared Lysis Buffer = $860\mu\text{l} + (\# \text{ samples} \times 43\mu\text{l})$

Volume of DNA IQ™ Resin = $140\mu\text{l} + (\# \text{ samples} \times 7.0\mu\text{l})$

For example, 96 samples requires:

Prepared Lysis Buffer = $860\mu\text{l} + (96 \times 43\mu\text{l}) = 4,988\mu\text{l} \cong 5.0\text{ml}$

DNA IQ™ Resin = $140\mu\text{l} + (96 \times 7.0\mu\text{l}) = 812\mu\text{l}$

Total volume = $5,800\mu\text{l} = 5.8\text{ml}$

b. Aqueous Method

Volume of prepared Lysis Buffer = $942\mu\text{l} + (\# \text{ samples} \times 113\mu\text{l})$

Volume of DNA IQ™ Resin = $58\mu\text{l} + (\# \text{ samples} \times 7.0\mu\text{l})$

For example, 96 samples requires:

Prepared Lysis Buffer = $942\mu\text{l} + (96 \times 113\mu\text{l}) = 11,790\mu\text{l} \cong 11.8\text{ml}$

DNA IQ™ Resin = $58\mu\text{l} + (96 \times 7.0\mu\text{l}) = 730\mu\text{l}$

Total volume = $12,520\mu\text{l} \cong 12.5\text{ml}$

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.

5. Automated Processing Requirements for the Biomek® 3000 Workstation

This section lists the instrument 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
Gripper Tool Kit for Biomek® 3000	1	A09053
MP200 Eight-Tip Tool	1	986146
Tip Rack Holder	4	391910
AP96 P250 Tips, Barrier	1–4 per run	717252
Gray Labware Holder	6	609120
Frame for Reservoirs	1	372795
Quarter Reservoir	2	372790
Quarter Reservoir, Divided by Length	2	372788

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	1 per run	V6781
1.2ml, Round-Well Deep Well Plate	1 per run	V6771
Shaker Integration Plate	1	V3691
VARIOMAG® Teleshake (110V, for North America 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	(customer-selected)

5.C. Biomek® 3000 Initial Deck Configuration

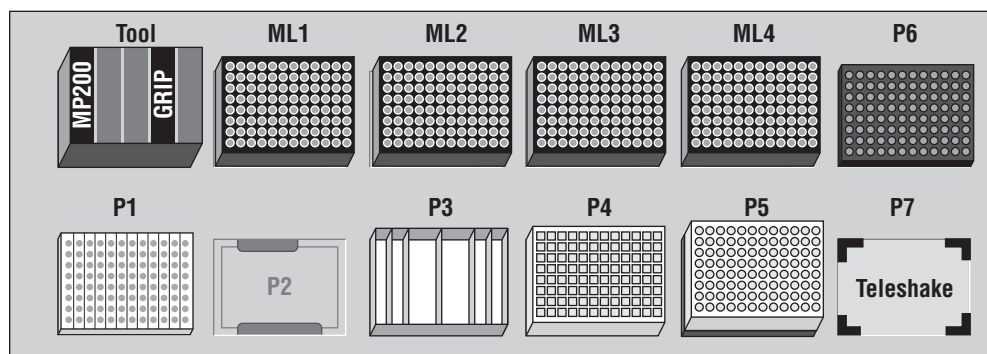


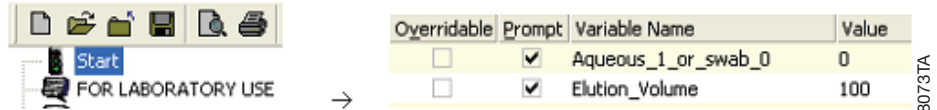
Figure 1. Biomek® 3000 initial deck configuration.

Position Tool	Tool rack containing MP200 and gripper tools
Position ML1	Tip rack holder, P250 tips (required for each run)
Position ML2	Tip rack holder, P250 tips (include when processing up to 48 samples)
Position ML3	Tip rack holder, P250 tips (include when processing up to 88 samples)
Position ML4	Tip rack holder, P250 tips (include when processing a full plate)
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
Position P3	Gray Labware holder, frame for reservoirs, reservoir with reagents (see Figure 2 for configuration)
Position P4	Gray Labware holder, 2.2ml, Square-Well Deep Well Plate containing samples (Sample Plate)
Position P5	Gray Labware holder, MagnaBot® 96 Magnetic Separation Device, 1/4 Inch Foam Spacer, Empty 1.2ml Round-Well Deep Well Plate (Working Plate)
Position P7	Shaker Integration Plate, VARIOMAG® Teleshake

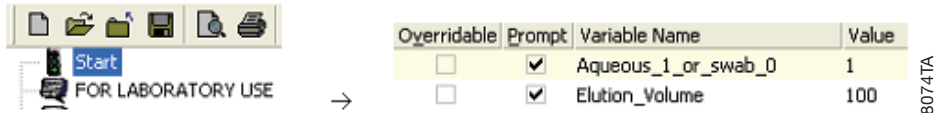
5.C. Biomek® 3000 Initial Deck Configuration (continued)

This automated method can be used to process two types of starting samples: swab samples or aqueous samples.

Swab samples are solid substrate samples that have been pre-processed by heating in the Lysis Buffer, such as pre-processed buccal swabs or pre-processed blood punches. The maximum starting volume of each swab sample is 400µl. When running this automated method to purify DNA from swab samples, be sure to enter “0” (corresponding to swab samples) when prompted, or highlight “Start” and enter “0” next to the Variable Name “Aqueous_1_or_swab_0” as shown below.



Aqueous samples are those that have been pre-processed by heating in a buffer other than the Lysis Buffer, such as a proteinase K-digested liquid blood sample. The maximum starting volume per well of each aqueous sample is 100µl. This is because the sample requires two volumes of Lysis Buffer per one volume of aqueous sample to effectively bind DNA to the DNA IQ™ Resin. When running this automated method to purify DNA from aqueous samples, be sure to enter “1” (corresponding to aqueous samples) when prompted, or highlight “Start” and enter “1” next to the Variable Name “Aqueous_1_or_swab_0” as shown below.



Elution volume. A 100µl elution volume is required.

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 also provides the calculation of the volume of each solution per trough.

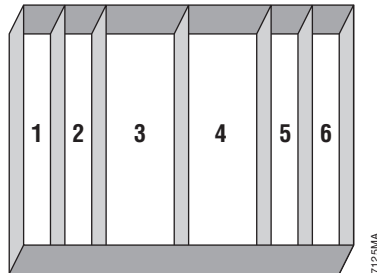


Figure 2. Configuration of troughs at position P3. Troughs 1–2 and 5–6 each use Quarter Reservoirs, Divided by Length. Troughs 3 and 4 each use Quarter Reservoirs.

Trough 1 DNA IQ™ Elution Buffer

Swab or Aqueous Method: Volume of DNA IQ™ Elution Buffer = $1,200\mu\text{l} + (\# \text{ of samples} \times \text{desired elution volume } \mu\text{l})$.

For example, 96 samples with $100\mu\text{l}$ elution = $1,200\mu\text{l} + (96 \times 100\mu\text{l}) = 10,800\mu\text{l} = 10.8\text{ml}$.

Trough 2 Empty

Trough 3 1X Wash Buffer (see Section 4)

Swab or Aqueous Method: Volume of DNA IQ™ Wash Buffer = $1,500\mu\text{l} + (\# \text{ of samples} \times 300\mu\text{l})$.

For example, 96 samples = $1,500\mu\text{l} + (96 \times 300\mu\text{l}) = 30,300\mu\text{l} = 30.3\text{ml}$.

Trough 4 Prepared Lysis Buffer (see Section 4)

Swab Method: Volume of prepared Lysis Buffer = $1,500\mu\text{l} + (\# \text{ of samples} \times 100\mu\text{l})$.

For example, 96 samples = $1,500\mu\text{l} + (96 \times 100\mu\text{l}) = 11,100\mu\text{l} = 11.1\text{ml}$.

Aqueous Method: Volume of prepared Lysis Buffer = $1,500\mu\text{l} + (\# \text{ of samples} \times 220\mu\text{l})$.

For example, 96 samples = $1,500\mu\text{l} + (96 \times 220\mu\text{l}) = 22,620\mu\text{l} = 22.62\text{ml}$.

Trough 5 DNA IQ™ Resin Solution (see Section 4; use total volume)

Swab Method: Use total volume.

For example, 96 samples requires $5,800\mu\text{l} = 5.8\text{ml}$.

Aqueous Method: Use total volume.

For example, 96 samples requires $12,500\mu\text{l} = 12.5\text{ml}$.

Trough 6 Empty

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. **(Aqueous Method Only) Lysis Buffer Addition.** The liquid-handling robot adds 115µl of prepared Lysis Buffer to each sample in the Sample Plate.
2. **Resin Solution Addition.**
(Swab method) The liquid-handling robot adds 50µl of DNA IQ™ Resin Solution to each sample in the Sample Plate.
(Aqueous method) The liquid-handling robot adds 120µl of DNA IQ™ Resin Solution to each sample in the Sample Plate.
3. **DNA Binding.** The Sample Plate is placed on the shaker followed by 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 binding of DNA to the DNA IQ™ Resin.
4. **Volume Transfer.** The contents of the Sample Plate are transferred to the Working Plate atop the MagnaBot® 96 Device in which the resin collects near the magnetic sides of each well.
5. **Lysis Buffer Removal.** The supernatant (Lysis Buffer) is removed to the Sample Plate, which will now serve as a Waste Plate.
6. **Lysis Buffer Wash.** The liquid-handling robot adds 100µl of prepared Lysis Buffer to each sample well of the Working Plate. Then the plate is moved to the shaker, and the resin is washed by shaking for 30 seconds.
7. **Lysis Buffer Wash Removal.** The Working Plate is moved back onto the MagnaBot® 96 Device, and the supernatant (Lysis Buffer) is removed to the Waste Plate.
8. **1X Wash Buffer Addition #1.** The liquid-handling robot adds 100µl per well of 1X Wash Buffer containing alcohols to each sample well of the Working Plate. The plate is placed on the shaker, and the resin is washed by shaking for 30 seconds.
9. **1X Wash Buffer Removal #1.** The Working Plate is moved onto the MagnaBot® 96 Device, and the supernatant (1X Wash Buffer) is removed to the Waste Plate.
10. **Washes #2 and #3 with 1X Wash Buffer.** Steps 8 and 9 are repeated twice for a total of three 1X alcohol washes.
11. **Drying.** The system pauses for 5 minutes to allow evaporation of any remaining Wash Buffer in the sample wells.
12. **Elution Buffer Addition.** The liquid-handling robot adds the desired volume (e.g., 100µl) of DNA IQ™ Elution Buffer to each sample in the Working Plate. The Working Plate is placed on the shaker and heater in a series of three 30-second shakes and two 2.5-minute heated incubation steps (85°C) to elute DNA from the DNA IQ™ Resin into the Elution Buffer.
13. **Elution.** The Working Plate is moved onto the MagnaBot® 96 Device, and the supernatant (Elution Buffer containing purified DNA) is removed to the Elution Plate.
14. **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 dispensing, turn the resin bottle upside-down to ensure that no clumps of resin remain attached to the bottom of the bottle.
3. Be sure to turn on the heater to 85°C when 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 have any impact on elution efficiency. The optimal elution temperature is ~65°C. Be sure the liquid inside the Working Plate wells atop the heater reaches this temperature by the end of the elution step.
5. The recovered elution volume in the Elution Plate at the end of the method will be less than the volume of DNA IQ™ Elution Buffer added due to evaporation on the heater. The average loss is ~8–10µl.
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. Rather, refer to electronic 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.

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