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

1. Description

This document describes automation of the DNA IQ™ System(a) on the Beckman Coulter Biomek® NXP 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/products/genetic-identity/automation-for-genetic-identity/

Note: All Promega Technical Manuals are available at: www.promega.com/protocols/
2. Requirements and Product Storage Conditions

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat.#</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA IQ™ System</td>
<td>100 reactions*</td>
<td>DC6701</td>
</tr>
<tr>
<td></td>
<td>400 reactions*</td>
<td>DC6700</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat.#</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA IQ™ Resin*</td>
<td>50ml</td>
<td>A8251</td>
</tr>
<tr>
<td>Lysis Buffer*</td>
<td>150ml</td>
<td>A8261</td>
</tr>
<tr>
<td>2X Wash Buffer*</td>
<td>70ml</td>
<td>A8271</td>
</tr>
<tr>
<td>Elution Buffer*</td>
<td>50ml</td>
<td>A8281</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>100mg</td>
<td>V3021</td>
</tr>
<tr>
<td>DTT, Molecular Grade (Dry Powder)</td>
<td>25g</td>
<td>V3155</td>
</tr>
</tbody>
</table>

*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.
4.A. 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., add 1µl of 1M DTT for every 100µl of Lysis Buffer).
3. Prepare the DNA IQ™ Resin Solution by combining $860\mu$l + ($\#$ samples × $43\mu$l) of prepared Lysis Buffer and $140\mu$l + ($\#$ samples × $7.0\mu$l) of DNA IQ™ Resin.
   
   For example, when processing 96 samples, combine $860\mu$l + (96 × $43\mu$l) = 4,988µl of prepared Lysis Buffer with $140\mu$l + (96 × $7.0\mu$l) = 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.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® NXP Workstation**

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

### 5.A. Instrumentation and Beckman Coulter Labware Requirements for the Automated DNA IQ™ System Method on the Biomek® NXP

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® NXP.

<table>
<thead>
<tr>
<th>Part Description</th>
<th>Quantity</th>
<th>Beckman Coulter Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomek® NXP® Span-8 Laboratory Automation Workstation (w/Gripper)</td>
<td>1</td>
<td>A31840</td>
</tr>
<tr>
<td>Configuration should be set up with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Span-8 disposable tips</td>
<td></td>
<td>719811</td>
</tr>
<tr>
<td>Biomek® Software</td>
<td></td>
<td>719349</td>
</tr>
<tr>
<td>Biomek® Syringes 500µl</td>
<td></td>
<td>719815</td>
</tr>
<tr>
<td>Biomek® Controller</td>
<td></td>
<td>987820</td>
</tr>
<tr>
<td>Monitor</td>
<td></td>
<td>978062</td>
</tr>
<tr>
<td>Biomek® NXP®/NX Span-8 4x3 ALP Kit</td>
<td>1</td>
<td>989839</td>
</tr>
<tr>
<td>Biomek® NXP®/NX Span-8 P50/P200 Shuck To Box ALP</td>
<td>1</td>
<td>Contact Beckman</td>
</tr>
<tr>
<td>Biomek® Shaking ALP (Orbital), Single-Position</td>
<td>1</td>
<td>379448</td>
</tr>
<tr>
<td>Biomek® NXP®/NX Half Trash ALP Kit</td>
<td>1</td>
<td>989778</td>
</tr>
<tr>
<td>Span-8 P250 Tips, Barrier (Case of 10)</td>
<td>1–2 per run</td>
<td>379503</td>
</tr>
<tr>
<td>Span-8 P1000 Tips, Barrier (Case of 5)</td>
<td>1 per run</td>
<td>987925</td>
</tr>
<tr>
<td>Modular Frame for Reservoirs</td>
<td>1</td>
<td>372795</td>
</tr>
<tr>
<td>Quarter Reservoir (case of 48)</td>
<td>1</td>
<td>372790</td>
</tr>
<tr>
<td>Quarter Reservoir, Divided by Length (case of 48)</td>
<td>1</td>
<td>372788</td>
</tr>
<tr>
<td>Half Reservoir (case of 24)</td>
<td>1</td>
<td>372786</td>
</tr>
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</table>

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

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® NXP.

<table>
<thead>
<tr>
<th>Part Description</th>
<th>Quantity</th>
<th>Promega Cat.#</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagnaBot® 96 Magnetic Separation Device</td>
<td>1</td>
<td>V8151</td>
</tr>
<tr>
<td>1/4 Inch Foam Spacer</td>
<td>1</td>
<td>Z3301</td>
</tr>
<tr>
<td>2.2ml, Square-Well Deep Well Plate or Slicprep™ 96 Device (for preprocessed solid samples)</td>
<td>1 per run</td>
<td>V6781</td>
</tr>
<tr>
<td>1.2ml, Round-Bottom Deep Well Plate</td>
<td>2 per run</td>
<td>V6771</td>
</tr>
<tr>
<td>V&amp;P Scientific Heating Block (110V, for North America use only)</td>
<td>1</td>
<td>V6761</td>
</tr>
<tr>
<td>Deep Well Heat Transfer Block</td>
<td>1</td>
<td>V6741</td>
</tr>
<tr>
<td>96-well PCR plate or strip tubes on plate stand</td>
<td>1 per run</td>
<td>(user-selected)</td>
</tr>
</tbody>
</table>
5.C. Biomek® NXP Initial Deck Configuration

Position STB1  Span-8 P50/P200 Shuck to Box ALP with Span-8 P250 Barrier Tips
Position P1    Span-8 P1000 Barrier Tips
Position P2    Frame for reservoirs, reservoirs with reagents
                (see Figure 2 for configuration)
Position P3    Empty (may be used for additional Span-8 P1000 Barrier Tips)
Position P4    1.2ml Round Bottom Deep Well Plate (Processing Plate) on
                MagnaBot® 96 Magnetic Separation Device, ¼ Inch Foam spacer
Position P5    2.2ml, Square-Well Deep Well Plate containing samples
                (Sample Plate)
Position P6    Empty 1.2ml Round Bottom Deep Well Plate (Processing Plate 2)
Orbital1     Empty Orbital Shaker
Position P7    Empty
Position P8    Empty
Position P9    Empty
Position P10   V&P Scientific Heating Block with Deep Well Heat Transfer Block
Position P11   Span-8 P250 Barrier Tips
Position P12   PCR Plate or Strip Tubes in Strip Tube Holder (Elution Plate) for eluted samples
Position TR1   Beckman Half Tip Trash

Figure 1. Biomek® NXP initial deck configuration.

Figure 2. Configuration of troughs at deck position P2. The Quarter Reservoir, Divided by Length, creates positions 1 and 2. Position 3 is one Quarter Reservoir. Position 4 is one Half Reservoir.
5.C. Biomek® NXP Initial Deck Configuration (continued)

You will be prompted to enter the number of used tip columns in the Span-8 P1000 and Span-8 P250 tip boxes at the start of the automated method. These variables are defined by the user based on the state of the system at run time. The user will be prompted to enter these variables upon beginning the run, but the declaration of these variables can be found by selecting the Start icon in the automated method script.

<table>
<thead>
<tr>
<th>Overridable</th>
<th>Prompt</th>
<th>Variable Name</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>✅</td>
<td>UsedP1000TipCols</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>✅</td>
<td>UsedP200TipCols</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VolRed_P200_1_or_P1000_2</td>
<td>1</td>
</tr>
</tbody>
</table>

**UsedP1000TipCols**

The UsedP1000TipCols variable corresponds to the number of columns of Span-8 P1000 tips that are missing from the tip box at position P1. Each run of the method requires the use of at least 3 columns of Span-8 P1000 tips. If volume reduction during the method has been set to use the Span-8 P1000 tips (i.e., the VolRed_P200_1_or_P1000_2 variable declared in the Start step has been set to a value of 2), more of these tips will be required. If a sufficient number of tips to perform this run are not available in a partial tip box, the user will be prompted to place an additional box or two full boxes of Span-8 P1000 tips on the deck.

**UsedP200TipCols**

The UsedP200TipCols variable corresponds to the number of columns of Span-8 P250 tips that are missing from the tip box on the Shuck To Box ALP (position STB1) and at P11. Equal numbers of tips will be used from each box during each run. Each run of the method requires the use of as many Span-8 P250 tips from this box as there are samples in the run. If a sufficient number of tips to perform this run are not available in partial tip boxes, the user will be prompted to replace these tip boxes with full boxes of Span-8 P250 tips.
5.D. DNA IQ™ User Interface

The DNA IQ™ User Interface is a graphical method for users to enter information about the samples that will be processed in this DNA IQ™ run.

Selecting Sample Wells: To select sample wells to populate on the plate, click the well. You can select a consecutive range of wells to populate with the same sample type information by pressing Left Click on the mouse for the first well and pressing Shift-Left Click on the mouse for the last well in the range to be populated. Nonconsecutive wells can be populated with the same sample information by pressing Ctrl-Left Click on the mouse for each well to be populated with the same information. It is not necessary that the samples on the plate be in consecutive wells; blank wells are allowed on the plate.

Populating Sample Wells: Once a well or range of wells has been selected, it can be populated with information about the sample type, sample volume, elution volume and desire to perform a lysis buffer wash.

Sample Type: There are two choices for sample type: aqueous and lysis. Aqueous samples are those that have been preprocessed using a nonguanidine-containing lysis buffer (e.g., Proteinase K digest of samples). During processing, DNA IQ™ Lysis buffer will be added to aqueous samples. Lysis samples are those that have been preprocessed by heating in DNA IQ™ Lysis Buffer. No additional DNA IQ™ Lysis Buffer will be added to these samples during processing. Sample type does not have to be consistent across the plate, column or row.

Sample Volume: Acceptable sample volume for any given sample or range of samples will depend on sample type. For aqueous samples, volumes can be 20–400µl. For lysis samples, volumes can be 200–800µl. Sample volumes do not have to be consistent across the plate, column or row.
5.D. User Interface (continued)

**Elution Volume:** Sample elution volumes can vary between 25µl and 200µl. Elution volumes do not need to be consistent across the plate, column or row.

**Perform Lysis Buffer Wash?** This option allows users to choose whether the DNA IQ™ Lysis Buffer wash should be performed. The default status is to perform the lysis buffer wash. However, for certain sample types, it may not be necessary to perform this wash. This option can be selected for individual wells and does not need to be consistently applied across the plate.

**Enter:** Once the sample type, sample volume, elution volume and lysis buffer wash status have been selected, press Enter to set these values and assign a color coding for the selected well(s).

**Clear Selections:** To remove the settings for a well or group of wells, select the well(s), and click the Clear Selections button.

**Finished:** After the plate map has been populated, click the Finished button to accept the values and create an output file that will be used to direct the processing performed by the Biomek® NXP DNA IQ™ Automated Method. The user interface will then display the reagent trough setup screen.
5.D. User Interface (continued)

Reservoir Volumes: The Reservoir Volumes screen displays the minimum volumes of each reagent required to process the samples entered on the previous screen. Each volume shown is calculated based on the sample type, sample volume, elution volume and lysis buffer wash status for the specified sample setup. In addition, the reagents are displayed as they will be dispensed to the reagent reservoirs placed on the deck of the robot.

Close Window: Once the appropriate volumes of reagents are dispensed into the reagent reservoirs, select the Close Window button to close the DNA IQ™ User Interface, and proceed with the automated method.

6. Description of the Automated DNA IQ™ System Method

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

1. DNA IQ™ Resin Solution Addition. The liquid-handling robot adds 50μl of DNA IQ™ Resin Solution to each sample in the Sample Plate.

2. 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.

3. DNA Binding. The Sample Plate is subjected to a series of shaking and incubation steps (7-second shake, 30-second incubation; repeated ten times) to allow DNA binding to the DNA IQ™ Resin.
6. Description of the Automated DNA IQ™ System Method (continued)

4. **Volume Transfer.** The Sample Plate contents are transferred to the Processing Plate atop the MagnaBot® 96 Device, which collects the resin at the sides of each well. This step is performed with either Span-8 P250 tips or Span-8 P1000 tips, depending on the setting for the variable VolRed_P200_1_or_P1000_2 (see Section 5.C).

5. **Lysis Buffer Removal.** The supernatant (prepared Lysis Buffer) is removed to the Sample Plate, which will now serve as the Lysate Waste Plate. This step is performed with either Span-8 P250 tips or Span-8 P1000 tips, depending on the setting for the variable VolRed_P200_1_or_P1000_2 (see Section 5.C).

6. **Lysis Buffer Wash (optional depending on sample settings).** The liquid-handling robot adds 100µl of prepared Lysis Buffer to each sample well of the Processing Plate. The plate then is moved to the shaker, and the resin is washed by shaking for 10 seconds followed by a 60-second pause and an additional 5-second shake.

7. **Lysis Buffer Wash Removal (optional depending on sample settings).** The Processing 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 Processing Plate. The plate is placed on the shaker.

9. **Clean Plate Transfer.** Processing Plate 2 is moved onto the MagnaBot® 96 Device. After shaking for 5 seconds per column to be transferred, the resin and Wash Buffer are transferred from the first Processing Plate to Processing Plate 2. The system is paused for 7 seconds to allow for resin capture to the magnet and then the 1X Wash Buffer is transferred back to the first Processing Plate. Twice more, the 1X Wash Buffer is transferred from the first Processing Plate to Processing Plate 2, the system is paused for 7 seconds, and the 1X Wash Buffer is transferred back to the first Processing Plate. The Sample Plate is moved to position P6 and the Processing Plate is moved to position P5.

10. **1X Wash Buffer Removal #1.** The supernatant (1X Wash Buffer) is removed from Processing Plate 2 and returned to the first Processing Plate, which will now serve as the Alcohol Wash Waste Plate.

11. **Washes #2 and #3 with 1X Wash Buffer.** One hundred microliters of 1X Wash Buffer is added to Processing Plate 2. This plate is moved to the shaker followed by shaking for 5 seconds. Processing Plate 2 is then moved onto the MagnaBot® 96 Device, and the system is paused for 10 seconds to allow for resin capture. The 1X Wash Buffer is then transferred to the first Processing Plate. The addition and removal process described in this step is repeated for a total of three washes.

12. **Heated Drying.** Processing 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 Processing Plate 2. Processing Plate 2 is placed on the shaker for a 30-second shake, then is twice cycled between the heater for a 2.5-minute heated incubation step and the shaker for a 20-second shake to elute DNA from the DNA IQ™ Resin into the Elution Buffer.
6. Description of the Automated DNA IQ™ System Method (continued)

14. **Elution.** Processing 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.

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(a) U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, European Pat. No. 1 204 741 and other patents pending.

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