AUTOMATED PROTOCOL

Identity Automation™ DNA IQ™ System Protocol for the Hamilton Microlab® STAR Line of Liquid-Handling Workstations

Instructions for Use of Products
DC6701 and DC6700

Revised 6/19
EP063

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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: genetic@promega.com

1. Description

This document describes automation of the DNA IQ™ System[a] on the Hamilton Microlab® STARlet laboratory automation workstation.

Please contact the Promega Genetic Identity team (**genetic@promega.com**) prior to implementing this method on your workstation. For additional information about Identity Automation™ methods for human identification applications, visit: **www.promega.com/idautomation/**

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

2. Product Requirements and Storage Conditions

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

Not for Medical Diagnostic Use.

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. Cat.# DC6700 has sufficient reagents for four 96-well plates of up to 100µl for aqueous samples or up to 200µl for lysis samples. 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 (15–30°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 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

We recommend that you wear gloves when processing Identity Automation™ DNA IQ™ samples for the Hamilton Microlab® STARlet.

4.A. Preparation of Solutions

Prior to beginning the Identity Automation™ 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.

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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 Cat.# DC6701; 35ml of 95–100% ethanol and 35ml of 99% isopropyl alcohol for Cat.# 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 (15–30°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 Hamilton Microlab® STARlet Workstation

This section lists the instrumentation and labware requirements for the Identity Automation™ DNA IQ™ System method on the Hamilton Microlab® STARlet.

5.A. Instrumentation Requirements

The following is a list of Hamilton Robotics parts and their corresponding part numbers that are required for the automated DNA IQ™ System method on a Microlab® STARlet.

Part Description	Quantity	Hamilton Part#
Microlab® STARlet instrument (or larger) configured with (8) 1ml channels CO-RE Gripper and Venus Two Instrument Software[1]	1	Contact Hamilton
Tip Carrier for 5 Tipracks (landscape) integration	2	182085
Multiflex carrier base (landscape orientation)	2	188039
Reagent Reservoir Carrier with Barcode Window–5 positions	1	194057
Multiflex reagent module, 5ml and 2ml tubes	1	188307
Sample carrier for holding 32 × 2ml screw cap tubes (set of 3)[2]	1	173410
SMP_INS_2ml_32, tube carrier inserts [2]	3	188102
Multiflex DWP module[2]	5	188042
Multiflex PCR plate module 96[2]	2	188049
Multiflex Tip rerack module[2]	1	92882-01
Request to mount on LT1 Baseplate MFX	1	63629-01
Hamilton Heater Shaker (HHS) 3mm orbit[3]	1	199034
Nunc 2.0ml heat plate adapter[3]	1	199028

[1]Add the Autoload bar code scanner with raster scanner to use sample and plate bar coding features.

[2]Carriers and quantity may differ based on throughput and desired labware.

[3]These items may be purchased together as Hamilton Part# 199039.

The consumables below are required for the Identity Automation™ DNA IQ™ method for the Hamilton Microlab® workstation. When automating additional products, refer to the appropriate Automated Protocol for a list of Promega items required for that kit and your platform.

Consumables Required

Supplier	Cat.#	Description	Number Required (per plate)
Hamilton	235940	100µl Conductive Disposable Tips, Filtered, Sterile	3 racks
Hamilton	235938	300µl Conductive Disposable Tips, Filtered, Sterile	3 racks
Hamilton	235979	50µl Conductive Disposable Tips, Filtered, Sterile	1 rack
Hamilton	187297	Reagent Containers, 50ml	4 per run

5.B. Labware Requirements

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 Hamilton Microlab® STARlet.

Part Description	Quantity	Cat.#
MagnaBot® FLEX 96 Magnetic Separation Device	1	VA1290
Nunc 2.0ml Deep Well Plate	2	AS9307
Elution plate	1	User selected
2.2ml, Square-Well Deep Well Plate	1	V6781

5.C. Hamilton Microlab STARlet Deck Configuration

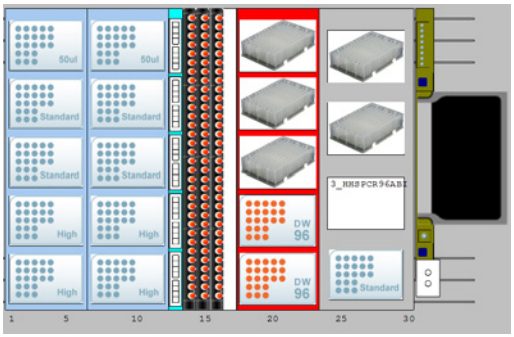

Figure 1. Hamilton Microlab® STARlet deck configuration for DNA IQ™. The layout shown supports DNA extraction and is shown as an example only; the automated method can be adapted to any worktable layout as long as this hardware or equivalent is present. Note that additional labware positions are required to run the maximum number of plates for each protocol.

Tracks 1 and 7: Tip Carrier, Landscape. In each carrier:

Site 1 50µl tips
Site 2 300µl tips
Site 3 300µl tips
Site 4 1,000µl tips
Site 5 1,000µl tips

Track 13: Multiflex Reagent Trough Module with 4 reagent troughs

Tracks 14–16: 32-position sample tube racks

Track 18: Multiflex Carrier

Site 1 Waste Plate
Site 2 Processing Plate
Site 3 Processing Plate 2
Site 4 Empty
Site 5 PCR Elution Plate

Track 24: Multiflex Carrier

Site 1 Hamilton Heater Shaker
Site 2 Magnet
Site 3 Empty
Site 4 Tip Rerack position

5.D. Microlab® STARlet 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. If samples are preprocessed using 400µl of aqueous extraction buffer, 400µl of additional Prepared Lysis Buffer per sample is required for DNA binding. For example, for 96 samples, the volume is 2,000µl + (96 × 100µl) + (96 × 400µl) = 50.0ml.

6. Description of the Identity Automation™ 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® Flex Magnetic Separation 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® Flex Magnetic Separation Device, and the supernatant (prepared Lysis Buffer) is removed to the Lysate Waste Plate.

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

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.

9. **Plate Transfer.** Purification Plate 2 is moved onto the MagnaBot® Flex Magnetic Separation 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® Flex Magnetic Separation Device, and the supernatant (Elution Buffer containing purified DNA) is removed to the Elution Plate.

15. **Method Ends.** The Identity Automation™ 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.

[a] European Pat. No. 1 204 741 and Japanese Pat. No. 4425513.