AUTOMATED PROTOCOL

Identity Automation™ Differex™ System Protocol for the Beckman Coulter Biomek® 4000

Instructions for Use of Products
A8501 and A8511

EP060
7/15

Identity Automation™ Differex™ System Protocol for the Beckman Coulter Biomek® 4000

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

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1. Description

Sections 1–7 of this document describe the Identity Automation™ Differex™ System on the Beckman Coulter Biomek® 4000 Automated Laboratory Workstation. General automation guidelines for adaptation to other liquid-handling platforms are provided in Section 7. To troubleshoot chemistry issues, please refer to the *Differex™ System Technical Bulletin* #TBD020.

Section 8 describes the Identity Automation™ DNA IQ™ After Differex™ System method on the Beckman Coulter Biomek® 4000 Automated Laboratory Automation Workstation. This automated method directly follows the use of the Identity Automation™ Differex™ System on the Biomek® 4000 (Sections 1–7; see Section 8.I). We recommend using this DNA IQ™ After Differex™ System method to obtain optimal results in downstream applications (e.g., qPCR or STR amplification).

Section 9 of this document provides the automated processing requirements for full workflow on the Biomek® 4000 Workstation.

To obtain information about methods for human identification applications, visit:
www.promega.com/idautomation/

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

2. Product Requirements and Storage Conditions

Note: The Differex™ System was initially developed for use in a manual format. The reagent volumes in this document have been adjusted for the automated method. However, the Differex™ System[a] (Cat.# DC6801, DC6800) does not provide the listed number of sample isolations in the automated format. We recommend that you purchase the reagents as standalone products. The following table lists the standalone reagents required for 4 × 48 (full plate) automated differential extractions. The Identity Automation™ Differex™ System method works in concert with the DNA IQ™ System for genomic DNA purification from differentially extracted samples. DNA IQ™ Resin is **required** for automated differential extraction of samples and is available as part of the DNA IQ™ System.

PRODUCT	SIZE	CAT.#	QTY. REQUIRED FOR 4 × 48 AUTOMATED DIFFERENTIAL EXTRACTIONS
Differex™ Digestion Buffer*	**150ml**	**A8501**	**1**
Differex™ Separation Solution*	**40ml**	**A8511**	**1**
Nuclease-Free Water**	**50ml**	**P1193**	**1**
	150ml	**P1195**	**2**
DNA IQ™ System*	**400 reactions**	**DC6700**	**1 (DNA IQ™ Resin component)**

*Not for Medical Diagnostic Use.
**For Laboratory Use.

Storage Conditions: Store Differex™ Digestion Buffer, Differex™ Separation Solution, DNA IQ™ Resin and Nuclease-Free Water at 15–30°C.

Items Available Separately

PRODUCT	SIZE	CAT.#
Slicprep™ 96 Device**	10 pack	V1391
2.2ml, Square-Well Deep Well Plate	50/case	V6781
MagnaBot® Flat Top Magnetic Separation Device	1 each	V6041
Proteinase K	100mg	V3021
DTT	5g	V3151
	25g	V3155
DNA IQ™ Resin**	50ml	A8251
Lysis Buffer**	150ml	A8261
2X Wash Buffer**	70ml	A8271
Elution Buffer**	50ml	A8281

*For Laboratory Use.
**Not for Medical Diagnostic Use.

3. Materials to be Supplied by the User

- Centrifuge capable of 1,500 × *g*, fitted with 96-well plate adapters
  (for a list of centrifuges compatible with the Slicprep™ 96 Device, visit:
  www.promega.com/products/pm/genetic-identity/slicprep-96-centrifuge-compatibility/
- Proteinase K (Cat.# V3021)
- DTT (Cat.# V3151, V3155)
- DNA IQ™ Resin (either from DNA IQ™ System [Cat.# DC6701, DC6700] or as a standalone reagent [Cat.# A8251])

See Sections 5.A and 5.B for a list of instrumentation and labware required for the Identity Automation™ Differex™ System on the Beckman Coulter Biomek® 4000 Workstation.

4. Before You Begin

We recommend that you wear gloves when processing Identity Automation™ Differex™ samples for the Beckman Coulter Biomek® 4000.

4.A. Preparation of Solutions

Prior to beginning the Identity Automation™ Differex™ System procedure, prepare the following solutions.

Proteinase K Solution
Dilute Proteinase K to 20mg/ml with Nuclease-Free Water. Freeze unused portions in single-use aliquots at –30 to –10°C for up to 2 months.

4.A. Preparation of Solutions (continued)

Digestion Solution

Prepare Digestion Solution just before use. For each sample, you will need 400µl of Digestion Solution, which consists of 25µl of Proteinase K Solution and 375µl of Digestion Buffer. For example, if there are 40 samples, prepare 40 × 400µl = 16ml of Digestion Solution by mixing 40 × 25µl = 1ml of Proteinase K Solution with 40 × 375µl = 15ml of Digestion Buffer. Include an additional 75µl of Proteinase K and 1,125µl of Digestion Buffer for the necessary dead volume for all sample quantities used.

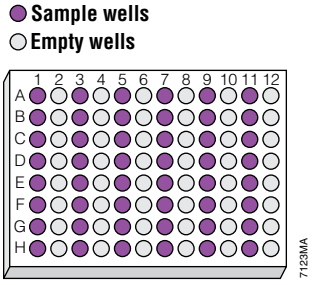
Resin Solution

Prepare Resin Solution prior to running the Identity Automation™ Differex™ System method. Each sample requires 100µl of Resin Solution, which consists of 14µl of DNA IQ™ Resin and 86µl of Nuclease-Free Water. In addition, a 1,500µl dead volume (210µl DNA IQ™ Resin and 1,290µl Nuclease-Free Water) is needed in the trough. That is:
Resin volume = (# samples × 14µl of DNA IQ™ Resin) + 210µl dead volume
Nuclease-Free Water volume = (# samples × 86µl) + 1,290µl dead volume

For example, if there are 40 samples:
Resin volume = (40 × 14µl of DNA IQ™ Resin) + 210µl dead volume = 770µl
Nuclease-Free Water volume = (40 × 86µl) + 1,290µl dead volume = 4,730µl

Prepare this solution, and mix thoroughly before pouring into the trough.

4.B. Sample Preparation Before Automated Processing



Prepare a Slicprep™ 96 Device by removing the U-Shaped Collar and pushing the 96 Spin Basket into the 96 Deep Well Plate. Sexual assault swabs should be placed in columns 1, 3, 5, 7, 9 and 11 of a 96 Spin Basket. To each sample, add 400µl of Digestion Solution, and seal the top of the device with a plate sealer (e.g., Seal & Sample Aluminum Foil Lids Beckman Cat.# 538619). Place the Slicprep™ 96 Device in a water bath or incubator at 56°C for 1.5 hours. After digestion, raise the 96 Spin Basket, and insert the U-Shaped Collar between the plate and 96 Spin Basket. Start the automated method while spinning the Slicprep™ 96 Device at 1,500 × *g* for 10 minutes.

5. Automated Processing Requirements for the Biomek® 4000 Workstation

This section lists the instrument and labware requirements for the Identity Automation™ Differex™ System method on the Biomek® 4000.

5.A. Beckman Coulter Products Required for the Identity Automation™ Differex™ System on the Biomek® 4000

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required for the Identity Automation™ Differex™ System method on a Biomek® 4000.

Part Description	Quantity	Beckman Coulter Part Number
Biomek® 4000 Basic Liquid Handling Package: Includes Biomek® 4000 Laboratory Automated Workstation, Biomek® Software Version 4.x with Windows® 7 Automation Controller, Monitor and Mouse, P200L Single Channel Pipette Tool with LLS, MP200 Eight Channel Pipette Tool, Accu Frame Autoframing Tool, Tip Rack Holder (Qty 2), Labware Holder (Qty 3), Tool Holder, and Starter Kit with assorted BCI Labware, nasic on-site training, basic application support and complete system installation	1	B22867
Biomek® 4000 Integration Deck	1	A95573
Module Accessory, Left Side, Biomek® 3000/4000	1	987264
Gripper Tool System, Biomek® 3000/4000: Includes Gripper Tool, Gripper Tool Rack, Calibration Plate, Disposal Option, Disposal Bags and spare Gripper Pads	1	986129
Holder, Tip Rack	2	391910
Holder, Labware, Gray	2	609120
Standard Single-Position ALP	1	719357
Orbital Shaker ALP	1	379448
Frame for Reservoirs	1	372795
Quarter Reservoir (sold in case of 48)	2	372790
Quarter Reservoir, Divided by Length (sold in case of 48)	1	372788
AP96 P250 Barrier Tips (sold in case of 10)	1	717253

5.B. Promega Products Required for Identity Automation™ Differex™ System on the Biomek® 4000

Part Description	Quantity	Promega Cat.#
MagnaBot® Flat Top Magnetic Separation Device	1	V6041
Slicprep™ 96 Device	1	V1391
2.2ml, Square-Well Deep Well Plate	2–3	V6781

5.C. Biomek® 4000 Initial Deck Configuration

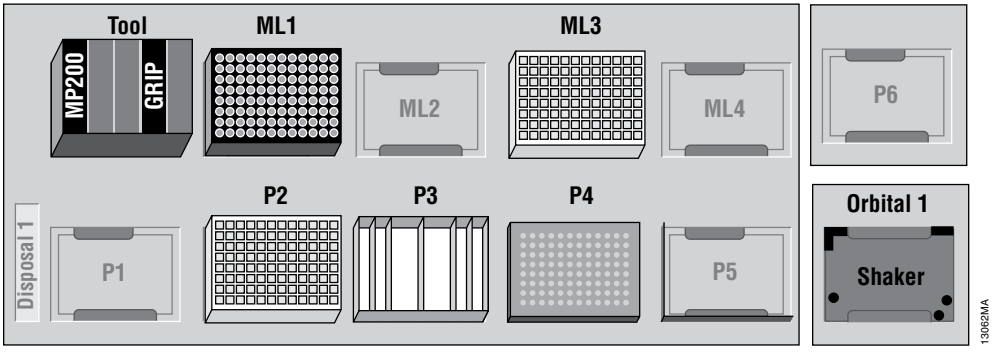


Figure 1. Biomek® 4000 initial deck configuration.

Position Tool	Tool rack containing MP200 and Gripper tools
Position ML1	Tip rack holder, Biomek® AP96 P250 Tips
Position ML3	Tip rack holder, empty 2.2ml, Square-Well Deep Well Plate (“Archival/Waste Plate”)
Position P2	Gray labware holder, empty 2.2ml, Square-Well Deep Well Plate (“Wash Plate”)
Position P3	Gray labware holder, frame for reservoir, reservoirs with reagents (see Figure 2 for configuration)
Position P4	Gray labware holder, MagnaBot® Flat Top Magnetic Separation Device
Position Orbital1	Biomek® Orbital Shaker

5.D. Biomek® 4000 Reagent Dispense Volumes

Prior to beginning the run, dispense Differex™ System reagents in the following configuration:

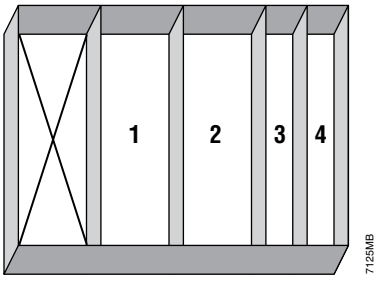


Figure 2. Configuration of troughs at position P3. Troughs 1 and 2 are each Quarter Reservoirs. Troughs 3 and 4 are a single Quarter Reservoir, Divided by Length.

Trough 1	Nuclease-Free Water (if processing more than 24 samples). Fill with 1,600µl per sample (total samples over 24) plus 2ml dead volume (e.g., for 40 samples, use 40 – 24 = 16 samples × 1,600µl = 25.6ml + 2ml dead volume = 27.6ml fill volume).
Trough 2	Nuclease-Free Water (up to 24 samples). Fill with 1,600µl per sample plus 2ml dead volume (e.g., for 40 samples, use 24 samples total × 1,600µl = 38.4ml + 2ml dead volume = 40.4ml fill volume).
Trough 3	Resin Solution. Fill with 100µl per sample plus 1.5ml dead volume (e.g., for 40 samples use 40 × 100 = 4ml + 1.5ml dead volume = 5.5ml total). See Section 4.A for solution preparation.
Trough 4	Separation Solution. Fill with 125µl per sample plus 2ml dead volume (e.g., for 40 samples, use 40 × 125µl = 5ml + 2ml dead volume = 7ml fill volume).

6. Description of the Identity Automation™ Differex™ System Method

This overview describes the general liquid-handling steps required for the Identity Automation™ Differex™ System and can be adapted to a variety of automated liquid-handling robots. For additional information for adaptation to liquid-handling robots other than the Beckman Coulter Biomek® 4000 Laboratory Automation Workstation, see Section 7.

1. **Wash Plate Preparation.** The liquid handler transfers 125µl of Separation Solution from a reservoir to every well of the 96-well, 2.2ml Square-Well Deep Well Plate (Wash Plate, position P2) for each column of samples to be processed (half the plate). Then it transfers 1,600µl of Nuclease-Free Water from a reservoir to each well of the 96-well, 2.2ml Square-Well Deep Well Plate for every column of samples to be processed (second half of the plate). During this step, the Slicprep™ 96 Device can be centrifuged. After the Wash Plate is prepared, the automated method pauses for placement of the Sample Plate (the 96-Well Deep Well Plate from the Slicprep™ 96 Device after removing the Basket and Collar) on the Orbital 1 Shaker position.
2. **Pellet Capping.** The liquid handler mixes then transfers 50µl of Resin Solution to each well of empty, even-numbered columns of the Sample Plate. The liquid handler then caps the sperm pellet by slowly adding 25µl of Resin Solution to each well containing samples. Then the gripper tool is used to move the Sample Plate to the MagnaBot® Flat Top Magnetic Separation Device.
3. **Removal of Epithelial Fraction.** The liquid handler transfers 75µl of epithelial fraction from each sample well to the adjacent well in the next column. The remaining epithelial fraction volume is transferred to the Archival/Waste Plate, leaving a depth of ~2.5mm in the bottom of the sample well. If desired, remove and save the Archival/Waste Plate containing the remaining epithelial fraction volume.

   Note: The Archival/Waste Plate can be stored at 2–10°C for up to 24 hours.

   If the Archival/Waste Plate is removed, it must be replaced with an empty 2.2ml, Square-Well Deep Well Plate to be used for waste. Otherwise, the Archival/Waste Plate will be used as a waste plate.
4. **Wash #1.** The liquid handler dispenses 400µl of Nuclease-Free Water to each sperm-fraction-containing sample well of the Sample Plate. The wash is removed to the Waste Plate, leaving liquid to a depth of ~2.5mm in each sample well.
5. **Wash #2.** The liquid handler repeats Step 4 for the second wash. Then the gripper tool is used to move the plate from the MagnaBot® Flat Top Magnetic Separation Device to the Orbital Shaker.
6. **Wash #3 Addition.** The liquid handler rapidly adds 400µl of Nuclease-Free Water to each sample well to disrupt the sperm pellet. The Orbital Shaker shakes the plate to resuspend sperm pellet and release any trapped epithelial material. The method pauses for manual removal of the Sample Plate to centrifuge it for 10 minutes at 1,500 × *g*. After centrifugation, place the Sample Plate back onto the Orbital Shaker.
7. **Pellet Capping.** The gripper tool moves the Sample Plate onto the MagnaBot® Flat Top Magnetic Separation Device. The liquid handler mixes the Resin Solution and then slowly adds 25µl to each sample well to cap the sperm pellet.

8. **Underlaying of Separation Solution.** The liquid handler slowly adds 115µl of Separation Solution just above the sperm pellet. This will float remaining epithelial material and allow removal of as much epithelial solution as possible.

9. **Wash #3 Removal.** The liquid handler removes the Nuclease-Free Water added in Step 6 from the sample wells, leaving the Separation Solution layer intact.

10. **Wash #4.** The liquid handler dispenses 400µl of Nuclease-Free Water to each sample-containing well of the Sample Plate. Then it removes the wash and Separation Solution to the Waste Plate, leaving liquid to a depth of ~2.5mm in the bottom of the well.

11. **Method Ends.** The Identity Automation™ Differex™ System method is now complete, and the original samples have been separated into epithelial and sperm fractions. The separated samples now can either be processed by a DNA isolation method (e.g., DNA IQ™ System) or may be stored at 2–10°C for up to 24 hours.

    Note: The sperm cells are still intact at the end of the method.

7. Important Considerations

The Resin Solution used for this purification settles rapidly. Ensure that the resin is completely resuspended in the trough before dispensing into processing plates.

The Aspiration and Dispense speeds as well as pipetting heights are critical to the success of this method. Wash solution removal is performed at 66µl/second for the first two aspirations and 10µl/second for each additional aspiration. To dispense 400µl of Nuclease-Free Water (for washes 1, 2 and 4), the addition speeds for each 100µl of liquid are 5µl/second, 10µl/second, 25µl/second and 66µl/second, respectively. The dispensing speed for wash #3 is 100µl/second. All wash dispensing is performed above the level of the liquid in the well. Add Separation Solution at 5µl/second and 3mm above the sperm pellet.

8. Identity Automation™ DNA IQ™ After Differex™ System Method for the Beckman Coulter Biomek® 4000

8.A. Description

This section describes the Identity Automation™ DNA IQ™ After Differex™ System method on the Beckman Coulter Biomek® 4000 Laboratory Automation Workstation. This automated method directly follows the use of the Identity Automation™ Differex™ System on the Biomek® 4000 (Sections 1–7; see Section 8.I). We recommend using this DNA IQ™ After Differex™ System method to obtain optimal results in downstream applications (e.g., qPCR or STR amplification). To obtain information about methods for human identification applications, visit: **www.promega.com/idautomation/**

8.B. Materials to be Supplied by the User

- DNA IQ™ System (Cat.# DC6701, DC6700)
- 2.2ml, Square-Well Deep-Well Plate containing Differex™ samples
- 1.2ml Round-Well Deep-Well Plates (2; Cat.# V6771)
- 96-well PCR plate or strip tubes in plate holder or base (1)
- 99% isopropyl alcohol
- 95–100% ethanol
- DTT (Cat.# V3151, V3155)

We recommend that you wear gloves when processing Identity Automation™ DNA IQ™ After Differex™ System samples for the Beckman Coulter Biomek® 4000.

8.C. Preparation of Solutions

Prior to beginning the Identity Automation™ DNA IQ™ After Differex™ System method, prepare the following solutions.

DNA IQ™ Lysis Buffer
Add 6µl of 1M DTT for every 100µl of Lysis Buffer. Mix by inverting several times. Mark and date label to record addition of DTT. This solution can be stored at room temperature for up to one month if sealed, or alternatively, Lysis Buffer plus DTT can be made fresh for each run.

DNA IQ™ 1X Wash Buffer
Add ethanol and isopropyl alcohol directly to the 2X Wash Buffer as directed on the bottle. Replace the cap and mix by inversion several times. Mark label as 1X Wash Buffer to indicate addition of alcohols. Store at room temperature.

8.D. Beckman Coulter Products Required for the Identity Automation™ DNA IQ™ After Differex™ System Method on the Biomek® 4000

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required for the Identity Automation™ DNA IQ™ After Differex™ System method on a Biomek® 4000.

Part Description	Quantity	Beckman Coulter Part Number
Biomek® 4000 Basic Liquid Handling Package: Includes Biomek® 4000 Laboratory Automated Workstation, Biomek® Software Version 4.x with Windows® 7 Automation Controller, Monitor and Mouse, P200L Single Channel Pipette Tool with LLS, MP200 Eight Channel Pipette Tool, Accu Frame Autoframing Tool, Tip Rack Holder (Qty 2), Labware Holder (Qty 3), Tool Holder, and Starter Kit with assorted BCI Labware, basic on-site training, basic application support and complete system installation	1	B22867
Biomek® 4000 Integration Deck	1	A95573
Module Accessory, Left Side, Biomek® 3000/4000	1	987264
Gripper Tool System, Biomek® 3000/4000: Includes Gripper Tool, Gripper Tool Rack, Calibration Plate, Disposal Option, Disposal Bags and spare Gripper Pads	1	986129
Holder, Tip Rack	2	391910
Holder, Labware, Gray	2	609120
Standard Single-Position ALP	1	719357
Orbital Shaker ALP	1	379448
Frame for Reservoirs	1	372795
Quarter Reservoir (sold in case of 48)	1	372790
Quarter Reservoir, Divided by Length (sold in case of 48)	1	372788
Half Reservoir (sold in case of 24)	1	372786
AP96 P250 Barrier Tips (sold in case of 10)	1–3	717253

Promega

8.E. Promega Products Required for the Identity Automation™ DNA IQ™ After Differex™ System Method on the Biomek® 4000

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 (containing Differex™ samples)	1	V6781
1.2ml, Round-Well Deep Well Plate	1	V6771
V&P Scientific Heater	1	V6761
Deep Well Heat Transfer Block	1	V6741
96-well plate or strip tubes on plate stand	1	customer-selected

8.F. Biomek® 4000 Initial Deck Configuration

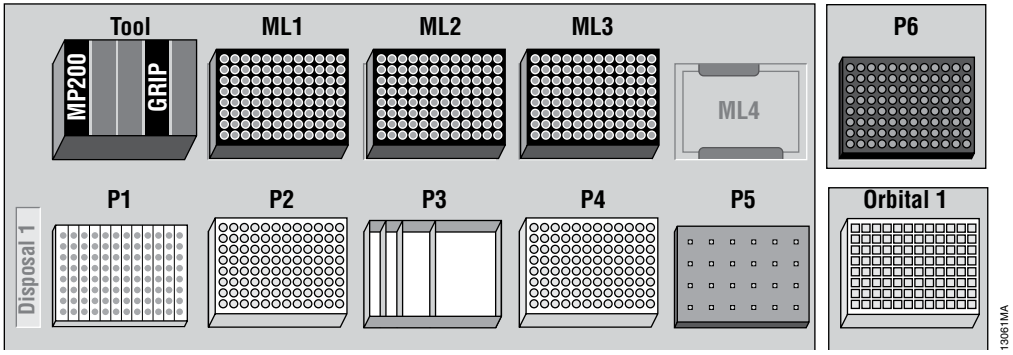


Figure 3. Biomek® 4000 initial deck configuration.

Position Tool	Tool rack containing MP200 and Gripper tools
Position ML1	Tip rack holder, Biomek® AP96 P250 Tips (required for each run)
Position ML2	Tip rack holder, Biomek® AP96 P250 Tips as needed
Position ML3	Tip rack holder, Biomek® AP96 P250 Tips as needed
Position P6	Single-Position ALP, V&P Scientific Heating Block, Deep Well Heat Transfer Block
Position P1	Gray labware holder, 96-well PCR plate or strip tubes (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 reservoirs, 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 Orbital1	Biomek® Orbital Shaker, 2.2ml, Square-Well Deep Well Plate containing Differex™ samples.
Disposal 1	Large Disposal Option (optional)

8.F. Biomek® 4000 Initial Deck Configuration (continued)

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
☐	☐	E_Fraction_Volume	125
☐	☑	Elution_Volume	50
☐	☑	End_Column	10
☐	☑	First_Tip_Column	1
☐	☐	Lysis_Wash	Y
☐	☐	P_Fraction_Volume	50
☐	☐	Start_Column	1
☐	☐	Tip_Disposal	N
☐	☐		

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E_Fraction_Volume

The E_Fraction_Volume variable corresponds to the starting volume of the Epithelial Fractions from an Identity Automation™ Differex™ System automated method run. This variable should not be edited.

P_Fraction_Volume

The P_Fraction_Volume variable corresponds to the starting volume of the Pellet Fractions from an Identity Automation™ Differex™ System automated method run. This variable should not be edited.

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 the use of partial tip boxes.

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

8.G. Biomek® 4000 Reagent Dispense Volumes

Prior to beginning the run, dispense Identity Automation™ DNA IQ™ After Differex™ System reagents in the following configuration:

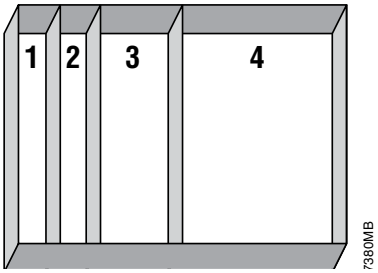

Figure 4. Configuration of troughs at position P3. The Quarter Reservoir, Divided by Length, creates positions 1 and 2. Position 3 is one Quarter Reservoir. Position 4 is one Half Reservoir.

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 8.C for solution preparation.

Trough 1	DNA IQ™ Elution Buffer Dispense 2,500µl + (# of samples × desired elution volume) into the trough. For example, for 96 samples with 100µl elution, the volume is 2,500µl + (96 × 100µl) = 12,100µl = 12.1ml.
Trough 2	Empty
Trough 3	1X Wash Buffer (with ethanol and isopropyl alcohol added) Use 1,500µl + (# of samples × 300µl). For example, for 96 samples = 1,500µl + (96 × 300µl) = 30,300µl = 30.3ml.
Trough 4	Prepared Lysis Buffer (see Section 8.C) Use 2,000µl + (# of Pellet Fractions × 500µl) + (# of Epithelial Fractions × 375µl). For example, for 40 Differex™ samples separated into 40 Pellet and 40 Epithelial fractions the volume is 2,000µl + (40 × 500µl) + (40 × 375µl) = 37,000µl = 37.0ml.

8.H. Description of the Identity Automation™ DNA IQ™ After Differex™ System Method

This overview describes the general liquid-handling steps required for the Identity Automation™ DNA IQ™ After Differex™ System method.

1. **Lysis Buffer Addition.** The liquid-handling robot adds prepared Lysis Buffer to each sample in the Sample Plate. Sperm fractions receive 400µl of prepared Lysis Buffer; epithelial fractions receive 275µl.
2. **DNA Binding.** The Sample Plate is subjected to a series of shaking and incubation steps to allow DNA to bind to the DNA IQ™ Resin.
3. **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.
4. **Lysate Removal.** The supernatant is removed to the Sample Plate, which now serves as the Lysate Waste Plate.
5. **Lysis Buffer Wash.** The liquid-handling robot adds 100µl of prepared Lysis Buffer to each sample well of the Purification Plate. The plate is moved to the shaker, and the resin is washed by shaking.
6. **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.
7. **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.
8. **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.
9. **1X Wash Buffer Removal #1.** The supernatant (1X Wash Buffer) is removed from Purification Plate 2 and returned to the first Purification Plate, which now serves as the Alcohol Wash Waste Plate.
10. **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.
11. **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.
12. **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.
13. **Method Ends.** The Identity Automation™ DNA IQ™ After Differex™ System method is now complete. The purified DNA samples in the Elution Plate may be processed immediately or stored at 2–10°C.

8.I. Important Reminders

It is critical to run the Identity Automation™ DNA IQ™ After Differex™ System method after the Identity Automation™ Differex™ System method (instead of after the Identity Automation™ DNA IQ™ System method) for two reasons:

1. This method uses a sixfold greater amount of DTT in the Lysis Buffer to properly lyse the sperm cells for purification (i.e., 6µl of 1M DTT per 100µl of Lysis Buffer). Other versions of the DNA IQ™ System method only use 1µl of 1M DTT per 100µl of Lysis Buffer, which is insufficient to lyse the sperm cells.
2. This method does not add more DNA IQ™ Resin to the samples as the wells already contain a sufficient amount of DNA IQ™ Resin carried over from the Identity Automation™ Differex™ System method.

Be sure to add DTT to the Lysis Buffer at a ratio of 6µl of 1M DTT per 100µl of Lysis Buffer when using the Identity Automation™ DNA IQ™ After Differex™ System method.

9. Automated Processing Requirements for Full Workflow on the Biomek® 4000 Workstation

Full Workflow Requirements

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required for full workflow automation on the Biomek® 4000 workstation (Differex™ System, DNA IQ™ System, Plexor® HY System and DNA Normalization and PowerPlex® Setup methods).

Description	Quantity	Beckman Coulter Part Number
Biomek® 4000 Basic Liquid Handling Package: Includes Biomek® 4000 Laboratory Automated Workstation, Biomek® Software Version 4.x with Windows® 7 Automation Controller, Monitor and Mouse, P200L Single Channel Pipette Tool with LLS, MP200 Eight Channel Pipette Tool, Accu Frame Autoframing tool, Tip Rack Holder (Qty 2), Labware Holder (Qty 3), Tool Holder, and Starter Kit with assorted BCI Labware, basic on-site training, basic application support and complete system installation	1	B22867
Biomek® 4000 Integration Deck	1	A95573
Module Accessory, Left Side, Biomek® 3000/4000	1	987264
Gripper Tool System, Biomek® 3000/4000: Includes Gripper Tool, Gripper Tool Rack, Calibration Plate, Disposal Option, Disposal Bags, and spare Gripper Pads	1	986129
Holder, Tip Rack	2	391910
Holder, Labware, Gray	2	609120
Standard Single-Position ALP	1	719357
Orbital Shaker ALP	1	379448

9. Automated Processing Requirements for Full Workflow on the Biomek® 4000 Workstation (continued)

Additional Hardware, Software, Labware and Consumables Required

The following additional items are required for Identity Automation™ full workflow processing on a Biomek® 4000 workstation.

Additional Promega Hardware and Software Required for Full Workflow (Differential Extraction, DNA Purification, DNA Quantitation, Normalization and STR Analysis)

		Number Required for the Indicated Automated Method			
Cat.#	**Description**	**Differex™ Method**	**DNA IQ™ Method**	**Plexor® HY Method**	**PowerPlex® Normali-zation**
V6041	MagnaBot® Flat Top Magnetic Separation Device	1			
A2661	Heat Block Adapter	1			
V6761	V&P Scientific Heating Block (110V, for North America use only)		1		
V8151	MagnaBot® 96 Magnetic Separation Device		1		
Z3301	1/4 inch Foam Spacer		1		
V6741	Deep Well Heat Transfer Block		1		
DG1820	STR Normalization Manager™ Software				1
V1601	Four-Position Tube Holder			2	2
V8251	Plate Clamp 96 (for use with nonskirted plates and strip tubes)			1–2 (optional)[1]	1–2 (optional)[1]
V8261	Plate Stand (for use with nonskirted plates and strip tubes)			1–2 (optional)[1]	1–2 (optional)[1]

[1]The Plate Clamp 96 and Plate Stand are optional for securing nonskirted 96-well plates or MicroAmp® Strip Tubes on the worktable. The Applied Biosystems MicroAmp® 96-Well Base, Part Number N801-0531, or other base also may be suitable.

Additional Consumables Required for Full Workflow Automation (Differential Extraction, DNA Purification, DNA Quantitation, Normalization and STR Analysis)

			Number Required (Per Plate Processed) for the Indicated Automated Method(s)			
Supplier	**Cat.#**	**Description**	**Differex™ Method**	**DNA IQ™ Method**	**Plexor® HY Method**	**PowerPlex® Normalization**
Beckman Coulter	717253	Biomek® AP96 P250 Tips, Pre-sterile with Barrier (case of 10)	1 box	2 boxes	<¼ box	<¼ box
Beckman Coulter	A21586	Biomek® P50 Tips, Pre-sterile with Barrier (case of 10)			1 box	1–3 boxes
Beckman Coulter	372786	Half Reservoir (case of 24)		1		1
Beckman Coulter	372788	Quarter Reservoir, Divided by Length (case of 48) (for preparing PCR amplification mix volumes of up to 20ml)	1	1		1[1]
Beckman Coulter	372790	Quarter Reservoir (case of 48)	2	1		1[1]
Promega	V6771	1.2ml, Round-Bottom Deep Well Plate		2		
Promega	V6781	2.2ml, Square-Well Deep Well Plate	2–3	1[2]		
Promega	V1391	Slicprep™ 96 Device	1	1[2]		
Promega	V6821	1.1ml, Square-Well, V-Bottom Deep Well Plate				2
User-selected		96-well PCR plate or strip tubes for PowerPlex® amplification				1
User-selected		96-well PCR plate or strip tubes for Plexor® HY amplification			1	
User-selected		96-well PCR plate or strip tubes for standard curve preparation			1	
User-selected		96-well PCR plate or strip tubes for purified DNA samples	1			
User-selected		1.5ml microcentrifuge tube			2	1[1]

[1]Only one tube or reservoir type is required per run; the type depends on the PCR amplification mix volume and user choice.

[2]The 2.2ml, Square Well Plate or SlicPrep™ 96 Device may be used; both are not required for the DNA IQ™ method. Samples processed using the Differex™ System do not require an additional plate for DNA purification using the DNA IQ™ System.

[a]U.S. Pat. No. 7,320,891.

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