AUTOMATED PROTOCOL

Automated Protocol for the Identity Automation™ DNA Normalization and PowerPlex® Setup Method on the Hamilton Microlab® STAR Line of Liquid-Handling Workstations

Instructions for Use of Product **DG1820**

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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: techserv@promega.com

1. Description

This document describes the automated protocol for the Identity Automation™ DNA Normalization and PowerPlex® Setup method on the Hamilton Microlab® STAR line of liquid-handling workstations [e.g., STARlet or larger (STARlet, STAR and STARplus)]. For additional information about Identity Automation™ methods for human identification applications, please visit: **www.promega.com/idautomation/**

For troubleshooting PowerPlex® chemistry issues, please refer to the appropriate PowerPlex® Technical Manual. All Promega Technical Manuals are available at: **www.promega.com/protocols/**

This automated method for DNA normalization and PowerPlex® reaction setup is compatible with all Promega PowerPlex® Systems that include a master mix containing *Taq* DNA polymerase (i.e., PowerPlex® 16 HS System or newer). The automated method supports amplification of extracted DNA (Normalization Protocol), direct amplification of swabs extracted with SwabSolution™ Reagent (Swab Protocol), direct amplification of nonFTA punches pretreated with PunchSolution™ Reagent (Punch Protocol) and direct amplification of FTA® punches (FTA® Protocol). In addition, the automated method enables multiple amplification plates to be prepared during a single automated method run, supporting 1–2 plates for the Normalization Protocol, 1–4 plates for the Swab Protocol and 1–8 plates for the Punch and FTA® Protocols. For more information visit: **www.promega.com/products/genetic-identity/str-analysis/**

Note: STR Normalization Manager™ Software is required for use of this automated method.

2. Product Requirements

PRODUCT	SIZE	CAT.#
STR Normalization Manager™ Software	**3 CD-ROM**	**DG1820**

Not for Medical Diagnostic Use.

Instrumentation requirements and lists of the labware and consumables required to run this method are provided in Section 5.

3. Materials to be Supplied by the User

Normalization Protocol

- TE^{-4} buffer [10mM Tris (pH 8.0), 0.1mM EDTA] or Water, Amplification Grade (Cat.# DW0991), for sample dilution

Swab Protocol

- SwabSolution™ Kit (Cat.# DC8271)

Punch Protocol

- PunchSolution™ Kit (Cat.# DC9271)

Note: The AmpSolution™ Reagent is supplied with the SwabSolution™ and PunchSolution™ Kits and is a required component of the PCR amplification mix for some PowerPlex® systems. During method installation, you or the installer should specify within the configuration settings whether AmpSolution™ Reagent is required. When the method is run, you will be reminded as to whether AmpSolution™ Reagent is required for the PowerPlex® system you are using.

4. Before You Begin

4.A. Sample Considerations

Samples can be presented to the system in 96-well format (96-well plate or strip tubes) or in individual tubes within the appropriate sample tube rack.

Amplification of Extracted DNA

Centrifuge extracted DNA samples briefly to remove any air bubbles that might be present in the wells, as air bubbles can interfere with sample aspiration. If quantification data are imported into the STR Normalization Manager™ Software, the well locations noted in your data file must match the sample well layout.

Direct Amplification of DNA from Swabs

If swab heads are present in the deep-well plate during automated sample transfer, be sure that the substrate is completely covered. The liquid handler will aspirate a small volume of swab extract from near the top of the liquid. If the disposable tips strike the swab head, the tips may fail to aspirate sample. Whole swabs should be cut or snapped off uniformly and close to the head to prevent these issues.

Direct Amplification of DNA from Storage Card Punches

Static may be problematic when adding punches to amplification plate wells. For FTA® card punches, adding PCR amplification mix or amplification-grade water to the well before adding the punch may help alleviate static problems. For nonFTA card punches, adding PunchSolution™ Reagent to the well before adding the punch during pretreatment may help alleviate static problems.

4.B. Preparation of Solutions

PCR amplification reagents (e.g., primer pair mix and master mix) should be completely thawed and vortexed well prior to use. The automated method provides the option to robotically prepare PCR amplification mix at volumes less than 5ml in a 5ml tube. Larger volumes of PCR amplification mix must be prepared manually and placed into an appropriate tube or trough. Manually prepared PCR amplification mix must be vortexed thoroughly (several 5- to 10-second pulses) prior to placing the mix on the worktable.

5. Automated Processing Requirements for the Hamilton Microlab® STAR Line of Liquid-Handling Workstations

Confirm that you have the required instrument and labware listed in Sections 5.A and 5.B for use of the Identity Automation™ DNA Normalization and PowerPlex® Setup method on the Hamilton Microlab® STAR line of liquid-handling workstations.

5.A. Instrumentation Requirements

Minimum Installation Requirements

The following is a list of Hamilton Robotics parts and their corresponding part numbers that are suggested for the Identity Automation™ DNA Normalization and PowerPlex® Setup method on the Hamilton Microlab® STAR line of liquid-handling workstations. Differing deck carriers can be used, but the minimum labware capacity requirements must be met.

Note: A Microlab® STARlet worktable provides sufficient space for Identity Automation™ DNA normalization and PowerPlex® setup.

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
Multiflex reagent trough module	1	188047
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
Muliflex PCR plate module 96[2]	2	188049

[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.

Table 1. Labware Positions Required on the Worktable by Protocol and Number of Sample Plates.

Protocol	Number of Sample Plates That can be Processed	Labware Positions Required
Normalization Protocol	1–2	4 or 8 (4 per plate processed)
Swab Protocol	1–4	2–8 (2 per plate processed)
Punch Protocol	1–8	1–8 (1 per plate processed)
FTA® Protocol	1–8	1–8 (1 per plate processed)

5.B. Labware and Consumables Required

The following additional items are the minimum requirements for the Identity Automation™ DNA Normalization and PowerPlex® Setup method on the Hamilton Microlab® STAR line of liquid-handling workstations.

Consumables

Consumable Supplier	Cat.#	Description	Number Required (Per Plate Processed)		
			Normalization Protocol	Swab Protocol	Punch and FTA® Protocols
Hamilton	235940	1000µL Conductive Disposable Tips, Filtered, Sterile	<¼ rack	<¼ rack	<¼ rack
Hamilton	235938	300µL Conductive Disposable Tips, Filtered, Sterile	<¼ rack	<¼ rack	<¼ rack
Hamilton	235979	50µL Conductive Disposable Tips, Filtered, Sterile	1–2 racks	1–2 racks	1 rack
Hamilton	187297	Reagent Containers, 50mL	one per run	one per run	one per run
Thermofisher	5000-0050	Nalgene, 5mL Tube, Polypropylene, Sterile	one per run	one per run	one per run
Promega	V6821	1.1ml Square-Well,V-Bottom Deep Well Plate	1–2		
Promega	V6781	2.2ml, Square-Well Deep Well Plate (for pretreatment with SwabSolution™ Reagent)		1	
User-selected		96-well PCR plate for PCR amplification	1	1	1
User-selected		96-well PCR plate or set of sample tubes containing DNA samples	1		

5.C. Microlab® STARlet Deck Configuration

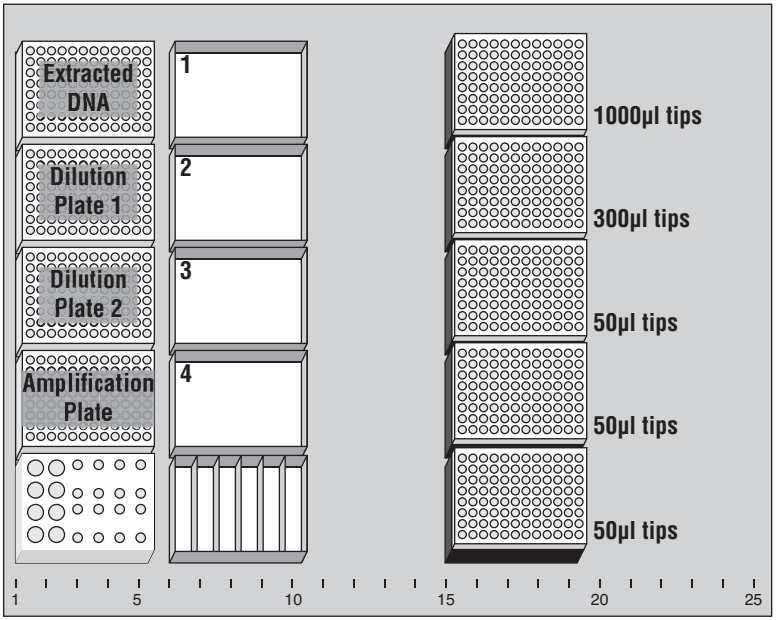
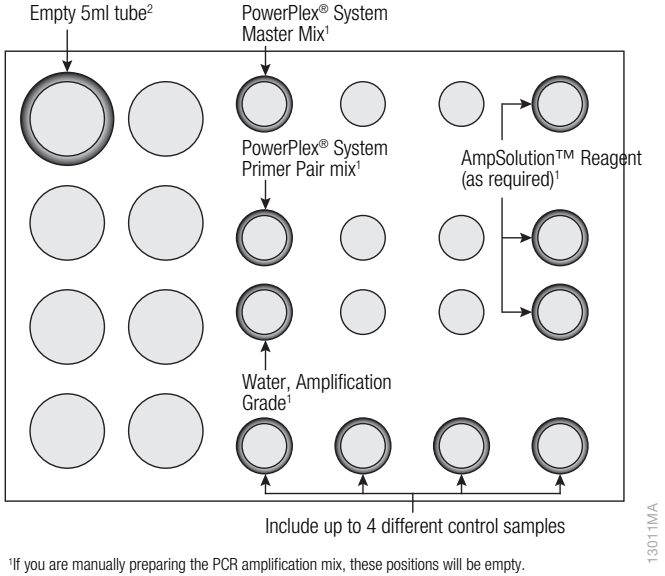


Figure 1. Microlab® STARlet deck configuration. For Tracks 1 and 6, the minimum requirements are shown for installation of the DNA Normalization and PowerPlex® Setup method; the maximum number of tip racks is shown in Track 15. The layout shown supports extracted DNA normalization and is shown as an 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.

Track 1 Multiflex Carrier
- Site 1 DNA sample plate
- Site 2 Empty 1.1ml Square-Well, V-Bottom Deep Well Plate (first dilution plate)
- Site 3 Empty 1.1ml Square-Well, V-Bottom Deep Well Plate (second dilution plate)
- Site 4 PCR amplification plate
- Site 5 Multiflex Reagent Module, 5mL and 2mL Tubes (PCR mix and components)

Track 6 Multiflex Carrier
- Site 5 Multiflex Reagent Trough Module, 50mL Trough (Sample Diluent)

Track 15 Tip Carrier, Landscape
- Site 1 1000µL Conductive Disposable Tips, Filtered, Sterile
- Site 2 300µL Conductive Disposable Tips, Filtered, Sterile
- Site 3 50µL Conductive Disposable Tips, Filtered, Sterile
- Site 4 50µL Conductive Disposable Tips, Filtered, Sterile
- Site 5 50µL Conductive Disposable Tips, Filtered, Sterile



1If you are manually preparing the PCR amplification mix, these positions will be empty.
2Optional: Manually prepared PCR amplification mix.

Figure 2. Configuration of PowerPlex® reagents and tubes within the Multiflex Reagent Module Track 1, Site 5. The minimum volume for each reagent is determined by the number of samples processed. The instrument will prompt you to add the appropriate minimum volume and number of tubes of these reagents.

STR Normalization Manager™ Software

The Promega STR Normalization Manager™ Software is the user interface for entering information about the samples that will be processed in the Identity Automation™ DNA Normalization and PowerPlex® Setup method run. Information about sample number, sample well locations, dilution strategy, PCR amplification mix preparation and dispense, and incorporation of amplification controls are automatically exported for use by the Hamilton method.

For information about on-site method installation by Promega, which includes training, installation and initial setup of the STR Normalization Manager™ Software for your laboratory, visit: **www.promega.com/a/idautomation/overview.html**

6. Description of the Identity Automation™ DNA Normalization and PowerPlex® Setup Method

This overview describes the general liquid-handling steps required for the Identity Automation™ DNA Normalization and PowerPlex® Setup method on the Hamilton Microlab® STAR line of liquid-handling workstations.

Note: The Identity Automation™ DNA Normalization and PowerPlex® Setup method has an option for automated transfer of SwabSolution™ Reagent or PunchSolution™ Reagent and on-deck heated incubation for direct-amplification sample preprocessing.

1. **Run and Deck Setup:** Upon method launch, the user will be prompted to sign in to the STR Normalization Manager™ Software and configure the run. After configuration, the user will be prompted for deck setup.
2. **Sample and Plate Bar Coding (Optional):** The liquid handler scans the sample plate or tube bar code(s) as well as the amplification plate bar code.

6. Description of the Identity Automation™ DNA Normalization and PowerPlex® Setup Method (continued)

3. **Diluent Transfer:** The liquid handler transfers diluent to one or two dilution plates per plate processed. The number of dilution plates used and the volumes transferred depend on the dilution required for the samples being processed. If no samples require dilution, this step will be skipped. Automation for direct amplification of DNA from storage card punches and swabs does not require diluent transfer or dilution plates.

4. **Preparation of PCR Amplification Mix:** If the user chooses to use the liquid handler to prepare the PCR amplification mix, the required volume of each reaction component will be transferred to a 5ml tube and tip-mixed. Alternatively, manually prepared PCR amplification mix can be placed on the worktable in a 5ml tube.

5. **Dispense of PCR Amplification Mix:** The liquid handler transfers the PCR amplification mix to appropriate wells of each amplification plate or set of strip tubes, dispensing by column from column 1 to column 12 for each set of 96 amplification wells. PCR amplification mix may be transferred using 50µl or 300µl tips, depending on method settings and end-user input during the run. When possible, PCR amplification mix is aspirated in bulk and dispensed to multiple wells of the amplification plate to minimize run time. Programming is provided for changing tips between wells containing sample punches (Punch and FTA® Protocols) to prevent cross-contamination. When working with multiple amplification plates, the workstation dispenses reagents in the order that the plates were configured in the STR Normalization Manager™ Software.

6. **Sample Dilution and Transfer to the Amplification Plate**

Extracted DNA Normalization Protocol: If samples are presented in plate format, samples and controls are processed by column (column 1 to column 12) based on the final amplification plate layout. If samples are presented in bar coded tubes, the liquid handler will process the samples and controls according to the bar coded order of the amplification plate layout. The liquid handler processes each DNA sample and control through one of three dilution pathways to achieve the desired template amount in the final PCR amplification.

Direct Transfer: Samples and diluent are aspirated separately and transferred directly to the amplification wells.

One Dilution: Samples are diluted through a single dilution plate; up to a 100-fold dilution can be achieved.

Two Dilution: Samples are diluted through two dilution plates; up to 100-fold dilution can be achieved in each dilution plate for a maximum 10,000-fold dilution.

Table 2 shows how each dilution pathway achieves the desired template amount in each PCR amplification reaction.

Swab Protocol: If samples are presented in plate format, extracts prepared using the SwabSolution™ Reagent and controls are processed by column (column 1 to column 12) based on the final amplification plate layout. If samples are presented in bar coded tubes, the liquid handler will process the samples and controls according to the bar coded order of the amplification plate layout. The specified volume of each sample and control is aspirated and dispensed to the appropriate amplification well. In addition, an option for a definable sample dilution exists.

Punch and FTA® Protocols: Controls are processed by column from column 1 to column 12 based on the final amplification plate layout. The specified volume of each control is aspirated and dispensed to the appropriate amplification well.

Table 2. Direct-Transfer, One-Dilution and Two-Dilution Strategies Applied Across a Range of Starting Sample Dilutions. All volumes are in microliters (µl).

Sample Concentration (ng/µl)	Dilution Pathway	Volume into First Dilution	Volume of Diluent into First Dilution	Volume into Second Dilution	Volume of Diluent into Second Dilution	Volume into Final Plate	Volume of Diluent into Final Plate	Template DNA Amplified (ng)
0.01	Direct	0	0	0	0	17.5	0	0.18
0.02	Transfer	0	0	0	0	17.5	0	0.35
0.03		0	0	0	0	17.5	0	0.52
0.04	Direct	0	0	0	0	17.5	0	0.70
0.05	Transfer	0	0	0	0	17.5	0	0.88
0.075		0	0	0	0	13.33	4.17	1.0
0.1	Direct	0	0	0	0	10	7.5	1.0
0.25	Transfer	0	0	0	0	4	13.5	1.0
0.5		0	0	0	0	2	15.5	1.0
0.6	One	0	0	2.38	22.62	17.5	0	1.0
0.7	Dilution	0	0	2.04	22.96	17.5	0	1.0
0.8		0	0	2	26	17.5	0	1.0
1	One	0	0	2	33	17.5	0	1.0
2	Dilution	0	0	2	68	17.5	0	1.0
4		0	0	2	138	17.5	0	1.0
6	Two	2	198	45	2.25	17.5	0	1.0
8	Dilution	2	198	45	18	17.5	0	1.0
10		2	198	45	33.75	17.5	0	1.0
20	Two	2	198	45	112.5	17.5	0	1.0
30	Dilution	2	198	38.1	161.9	17.5	0	1.0
50		2	198	22.86	177.14	17.5	0	1.0
75	Two	2	198	15.25	184.76	17.5	0	1.0
100	Dilution	2	198	11.43	188.57	17.5	0	1.0
250		2	198	4.57	195.43	17.5	0	1.0
500	Two	2	198	2.29	197.71	17.5	0	1.0
600	Dilution	2	198	16.67	183.33	2	15.5	1.0
750		2	198	2	198	13.33	4.17	1.0
1,000	Two	2	198	2	198	10	7.5	1.0
2,500	Dilution	2	198	2	198	4	13.5	1.0
5,000		2	198	2	198	2	15.5	1.0
6,000	Two Dilution	2	198	2	198	2	15.5	1.2

7. Important Considerations

1. Always use aerosol-resistant tips to minimize the risk of cross-contamination.
2. All PowerPlex® System reagents must be thoroughly mixed by vortexing before placing them on the deck for a run. This includes manually prepared PCR amplification mix, which contains DNA polymerase; vigorous mixing will ensure homogeneity and will not harm performance. Mix as directed in the appropriate PowerPlex® System technical manual.
3. The calculations for PCR amplification mix preparation include excess reagent to ensure that enough PCR amplification mix is prepared for all amplification wells.
4. Liquid Classes must be calibrated to ensure accurate volume handling for both samples and amplification reagents. Calibration checks are performed as part of Promega standard installation service.
5. The Promega STR Normalization Manager™ Software is integrated into methods for the Identity Automation™ DNA Normalization and PowerPlex® Setup on the Hamilton Microlab® STAR, Tecan Freedom EVO®, Beckman Coulter Biomek® 3000, Biomek® NX^{P} and Biomek® FX^{P} platforms. For more information or to inquire about the potential to integrate this software onto other instruments, please visit: **www.promega.com/a/idautomation/overview.html**