Promega Direct Amp STR Setup of Swabs Method Technical Manual

Instructions for Use of Products AS9100, AS9101, AS9200, AS9201

Use in combination with the Amplification Setup Methods for the Maxprep™ Liquid Handler Technical Manual, #TM526



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1. Description

The Promega Direct Amp STR Setup of Swabs method for the Maxprep[™] Liquid Handler is designed to automate the preparation of short tandem repeat (STR) direct amplification reactions, including master mix preparation, placement of controls, transfer of master mix and transfer of swab extracts to an amplification plate. Swab extracts are prepared by pretreating swabs with the SwabSolution[™] Kit (Cat.# DC8271) as described in the *SwabSolution[™] Kit Technical Manual*, #TMD037. Administrators can create variant methods in the Maxprep[™] software that specify reaction setup options to meet the needs of the laboratory.

2. Materials to Be Supplied by the User

- amplification plate (user-specified)
- 1.5ml tubes (e.g., ClickFit Microtube, 1.5ml; Cat.# V4741)
- (optional) amplification plate base (e.g., ThermoFisher MicroAmp® 96-well base, Cat.# N8010531)
- Maxprep[™] 50µl Conductive Disposable Tips, Filtered (Cat.# AS9301)
- Maxprep[™] 300µl Conductive Disposable Tips, Filtered (Cat.# AS9302)



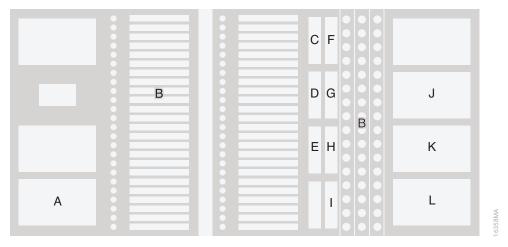
3. Run-Specific Information

The first screen of the method requests information regarding general method run parameters that must be entered prior to amplification setup, such as input labware containing samples in SwabSolution, destination amplification labware and STR Kit.

- 1. A popup screen is presented for STR Kit selection.
 - a. Touch the text box below the "Scan STR Kit Lot" title to scan the bar code on your STR kit and filter the list of STR kits available for this run.
 - b. Scanning the STR Kit Lot may be required by the administrator.
 - c. Select an STR kit to use for this direct amplification setup using the drop-down menu near the bottom of the screen.
 - d. After selecting the STR kit to use, touch the Exit button at the bottom of the popup screen.
- 2. Use the drop-down menus to specify the Input Labware and Destination Labware to be used.
 - a. Swab extracts are placed on the system in the selected Input Labware.
 - b. The amplification reaction is prepared in the Destination Labware.
 - c. Amplification plates or strip tubes can be used as Destination Labware options.
- 3. At the top right corner of the screen is an indication of the Maximum Number of Samples allowable within this amplification setup run. The number displayed is based on:
 - a. The number of sample positions in the Input Labware
 - b. The number of sample positions in the Output Labware
 - c. The number of control wells specified
 - d. The number of ladder wells specified
- 4. To view the administrator-defined settings for this method, touch the **Variant Information** button.
- 5. To change the STR kit that should be used for this run, touch the **Select STR Kit** button to open the STR kit selection popup screen described in Step 1.

4. Instrument Setup Instructions

The figure and table below indicate the general layout of the MaxprepTM Liquid Handler and the positions of all required labware for this method.



Position	Reagent/Labware	Notes
А	Amplification Plate	User-specified amplification plate
B ¹	Input Sample Positions	Samples in a plate (plate position on deck tray carrier) or samples in tubes
С	Reaction Mix Preparation; up to 3 empty 1.5ml tubes	Maxprep [™] 3-Position Reagent Tube Holder
\mathbf{D}^1	Amplification Controls; up to 3 individual tubes	Maxprep [™] 3-Position Reagent Tube Holder
E^1	Up to 3 tubes of AmpSolution	Maxprep [™] 3-Position Reagent Tube Holder
F	Up to 3 tubes of Promega STR Master Mix	Maxprep [™] 3-Position Reagent Tube Holder
G	Up to 3 tubes of Promega STR Primer Pair Mix	Maxprep [™] 3-Position Reagent Tube Holder
H^{1}	Up to 3 tubes of Enzyme	Maxprep [™] 3-Position Reagent Tube Holder
Ι	Up to 3 tubes of Amplification-Grade Water	Maxprep [™] 3-Position Reagent Tube Holder
J	300µl Conductive Disposable Tips, Filtered	Partial or full rack
K	50µl Conductive Disposable Tips, Filtered	Partial or full rack
L	50µl Conductive Disposable Tips, Filtered	Full rack

¹Optional labware positions based on variant settings and STR Kit selection.

Note: Swab heads must be entirely submerged in SwabSolution within the input labware. Damage to the Maxprep[™] Instrument may result if swab heads are exposed.



5. Promega Direct Amp STR Setup of Swabs Protocol

The Maxprep[™] Liquid Handler will prepare direct amplification reactions as indicated by the method type and variant selected. The following steps are performed by the Maxprep[™] Liquid Handler:

- 1. Amplification reaction master mix is prepared in one or more 1.5ml tubes for all samples and controls:
 - a. Promega STR Primer Pair Mix volume specified by the selected STR Kit
 - b. Enzyme volume specified by the selected STR Kit
 - c. Promega STR Master Mix volume specified by the selected STR Kit
 - d. AmpSolution volume specified by the selected STR Kit
 - e. Amplification Grade Water volume specified by the selected STR Kit
- 2. Amplification reaction master mix is transferred to the amplification plate.
- 3. Sample is transferred to the amplification plate.
- 4. Controls are transferred to the amplification plate.
- 5. Method is complete. Open the instrument door, remove and seal or cap the amplification plate. Centrifuge at $500 \times g$ for 30 seconds to remove any bubbles. Prepare the plate as per your amplification protocol. Remove primary samples, plates and used tips from the waste bin and discard as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.



Consumables for Maxprep[™] methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

6. Variant Method Variables

Administrators should create laboratory-specific variants of the Promega Direct Amp STR Setup of Swabs method to define the settings for the amplification reactions. Below is a list of the variables that can be adjusted by administrators for the Promega Direct Amp STR Setup of Swabs method. None of the variable values can be adjusted by users at run time.

Controls

These variables define the controls to place on the amplification plate. Up to three different controls can be used within a run, and multiple well locations can be defined for each control. Options within the table below are available for each of three control types; within the table, X indicates the value 1, 2, or 3.

Note: Placement of controls on the plate occurs sequentially. If the same well location is specified for controls 1, 2 and 3, only control 3 will be placed at that location.

Setting	Details
Control #X Concentration	Concentration value of the first control to be used. Set the concentration value to 0 to specify a negative control. Amplification Grade Water will be added as the sample for negative controls. Any control with concentration greater than zero will have 1µl of control added to the specified well.
Control X Name	Name of the first control; identifies the control during instrument setup.
Control #X Well Numbers ¹	Well locations where control #X should be placed. For multiple control wells, specify the well locations as a comma-delimited list (e.g., 1, 17).
Empty Ladder Wells	Well locations that should receive no sample or reaction mix. Ladder wells are intended to simplify the process of manual CE setup.

¹Well numbering proceeds down columns and across the plate. For example, A1=1, B1=2, A2=9, etc. A well numbering diagram is displayed in the Appendix of the *Amplification Setup Methods for the Maxprep*[™] *Liquid Handler Technical Manual*, #TM526.

STR Kit

These variables define the requirements for STR kit lot information and the tip usage during reaction mix distribution.

Setting	Details
1. Is STR kit lot required?	Determines whether the user is required to enter STR kit lot information during method setup.
2. Must STR kit be within expiration?	Specify whether the STR kit must be within expiration date to proceed with the method setup.



It is the manufacturer's responsibility to provide equipment electromagnetic compatibility information to the customer or user.

It is the user's responsibility to ensure that a compatible electromagnetic environment for the equipment can be maintained in order that the device will perform as intended.

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