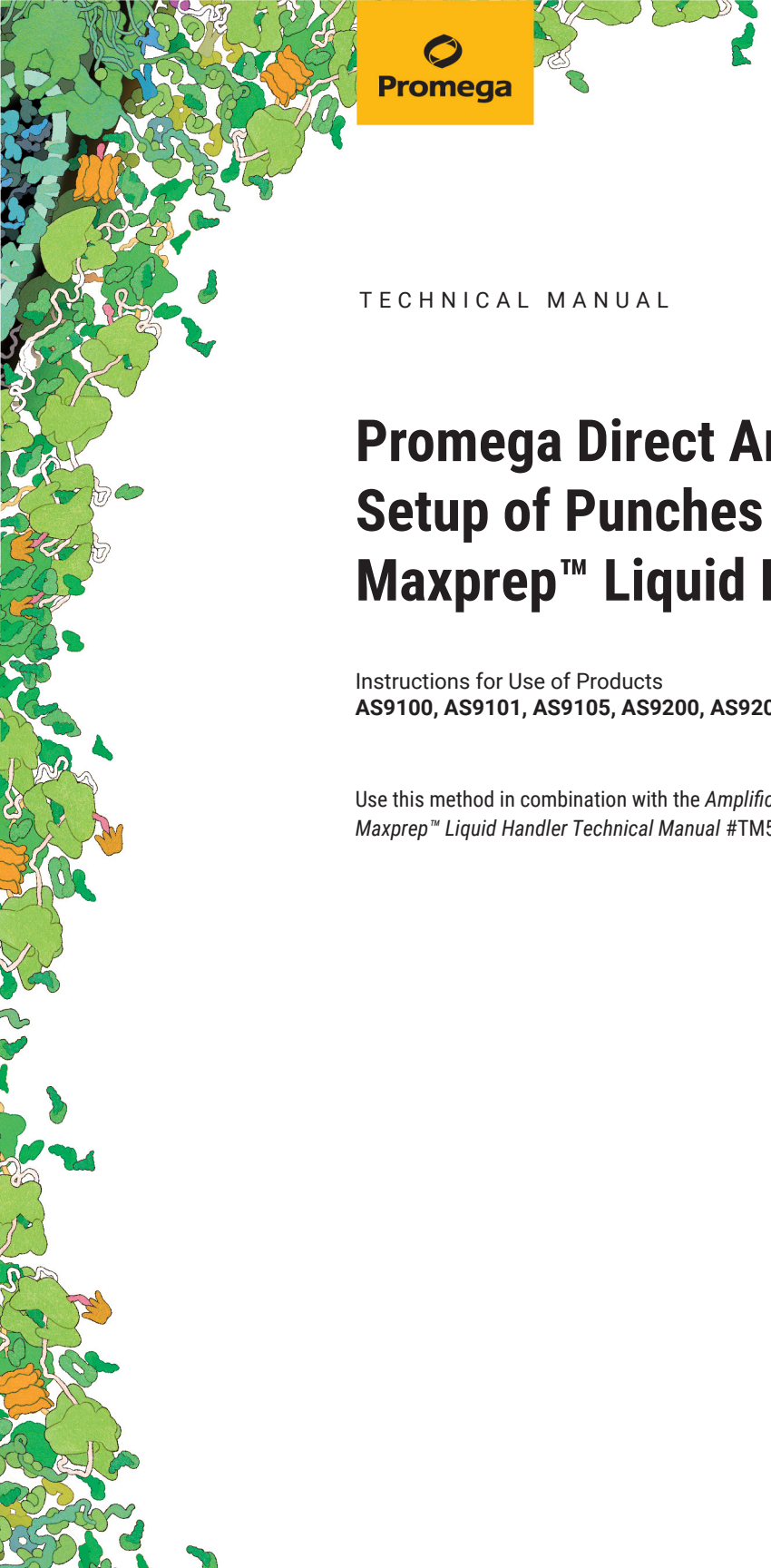


TECHNICAL MANUAL

Promega Direct Amp STR Setup of Punches Method for the Maxprep™ Liquid Handler

Instructions for Use of Products
AS9100, AS9101, AS9105, AS9200, AS9201 and AS9205

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

Revised 2/24
TM585

Promega Direct Amp STR Setup of Punches Method for the Maxprep™ Liquid Handler

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Maxprep™ Promega Direct Amp STR Setup of Punches method is designed to automate the preparation of STR direct amplification reactions including master mix preparation, placement of controls and transfer of master mix to plate wells containing lytic or nonlytic storage card punches. Administrators can create variant methods in the Maxprep™Liquid Handler 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)
- Maxprep™ 50µl Conductive disposable Tips, Filtered (Cat.# AS9301)
- Maxprep™ 300µl Conductive disposable Tips, Filtered (Cat.# AS9302)
- **optional:** amplification plate base (e.g., ThermoFisher MicroAmp® 96-well base, Cat.# N8010531)
- **optional:** Maxprep™ Reagent Reservoir, 50ml (Cat.# AS9304)

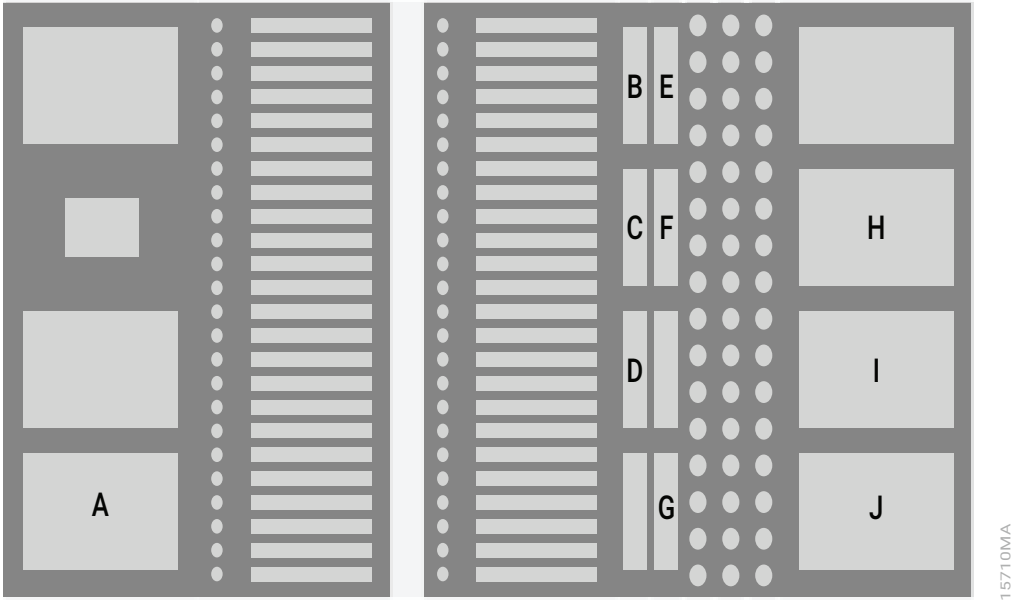
3. Run-Specific Information

The first screen of the method requests information regarding general method run parameters, such as amplification labware containing storage card punches and STR kit, that must be entered prior to amplification setup.

1. Use the drop-down menus to specify the Amplification Labware to be used for this run. Punches are placed on the system in the Amplification Labware, and the amplification reaction is prepared in this labware. Amplification plates or strip tubes can be used as amplification labware options.
2. Select an STR kit to use for this direct amplification setup using the drop down menu near the bottom of the screen. Touch the text box below the "Scan STR Kit Lot" title to filter the list of STR kits available for this run. Scanning the STR kit Lot may be required by the administrator.
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:
    - The number of sample positions in the amplification labware
    - The number of control wells specified
4. To view the administrator-defined settings for this method, press the **Variant Information** button.

4. Instrument Setup Instructions

Below is an image and table indicating the general layout of the Maxprep™ Liquid Handler and the positions of all labware that could be used for this method. Depending on the Promega STR kit being used and whether controls are being placed in the amplification plate, not all reagents or labware may be required. Deck setup instructions will indicate what labware and reagents to place on the sstem for your specific run settings.



Pos.	Reagent/Labware	Notes
A	Amplification Plate	User-specified amplification plate containing lytic or nonlytic storage card punches
B	Reaction Mix Preparation; up to 3 empty 1.5ml tubes, as prompted	Maxprep™ 3-Position Reagent Tube Holder
C[1]	Amplification Controls; up to 3 individual tubes	Maxprep™ 3-Position Reagent Tube Holder
D[1]	Up to 3 tubes of 5X AmpSolution™ Reagent	Maxprep™ 3-Position Reagent Tube Holder
E	Up to 3 tubes of Promega STR Master Mix	Maxprep™ 3-Position Reagent Tube Holder
F	Up to 3 tubes of Promega STR Primer Pair Mix	Maxprep™ 3-Position Reagent Tube Holder
G	Up to 3 tubes of Water, Amplification Grade	Maxprep™ 3-Position Reagent Tube Holder
H	300µl Conductive Disposable Tips, Filtered	Partial or full rack
I	50µl Conductive Disposable Tips, Filtered	Partial or full rack
J	50µl Conductive Disposable Tips, Filtered	Full rack

[1]Optional labware positions based on variant settings.

5. Promega Direct Amp STR Setup of Punches Protocol

The Maxprep™ Liquid Handler will prepare direct amplification reactions as indicated by the method 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 (Reaction Master Mix Volumes are determined by the selected STR kit):
    a. Promega STR Primer Pair Mix
    b. Promega STR Master Mix
    c. 5X AmpSolution™ Reagent
    d. Water, Amplification Grade
2. Transfer of amplification reaction master mix to the amplification plate.
3. Transfer of controls to the amplification plate.
4. Method is complete. Open the instrument door, remove the amplification plate and centrifuge at 500 × *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 Punches method for each amplification reaction they wish to process. Below is a list of the variables that can be adjusted by administrators for the 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 control types can be used within a run, and for each of these a number of replicates can be specified. Controls are placed on the amplification plate consecutively in unassigned wells. For each control replicate, 1µl is transferred from the corresponding control tube to the reaction. Options within the following table are available for each of the three control types. Within the table, X indicates the value 1, 2 or 3.

Setting	Details
1. Control X Name	Name used to identify the control during instrument setup.
2. Control X Concentration	Concentration of the control. Only used for reporting purposes.
3. Control X Replicates	Number of replicate wells to create for the control.

Sample

Variables in the sample section are used to specify how much water may have been preloaded into the sample wells and how to identify ladder wells on a plate map.

1. Volume of water already in sample wells. It is a common practice in some laboratories to add water to wells that will receive sample punches. Use this variable to specify the volume of water that had been placed in the amplification wells prior to punching samples into those wells. This volume will be subtracted from the water required in the amplification reaction. In cases where no water has been preloaded into punch wells or when solution has been evaporated from sample wells prior to amplification setup, the value of this variable should be set to zero. Be aware that it is possible to specify a water volume that is incompatible with the amplification parameters for a direct amplification STR kit. Confirm that the volume entered in this field is compatible with the amplification reaction setup parameters specified in the technical manual for the direct amplification STR chemistry being used.
2. Text to identify ladder wells. Ladder wells can be identified in the input sample set based on text present in the SampleID. Reaction mix will not be added to any well in the sample set that contains the text in this field within the sample identifier.

STR

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.

7. Summary of Changes

The following changes were made to the 2/24 revision of this document:

1. Added new Cat.# AS9105 and AS9205.
2. Updated font and cover image.
3. Made minor text edits.