PIPETMAX®: Automation of the Illumina® Nextera® XT DNA Library Preparation Kit

SETUP GUIDE FOR APPLICATION NOTE AN1011

OVERVIEW

Please refer to this setup guide when configuring PIPETMAX®, preparing bed elements, and running scripts in TRILUTION® micro for *Application Note AN1011: PIPETMAX®: Automation of the Illumina® Nextera® XT DNA Library Preparation Kit*.

Description

The Illumina® Nextera® XT DNA Library Preparation Kit is used to prepare libraries for next-generation sequencing (NGS). Five scripts have been created and tested for PIPETMAX. Each script is a module used to help researchers carry out a portion of the Nextera XT workflow. The process diagram below (Figure 1) shows the typical progression of the five PIPETMAX script modules described in this document. Some scripts include off-bed steps accomplished through user intervention, such as the centrifugation of a microplate between liquid handling steps.

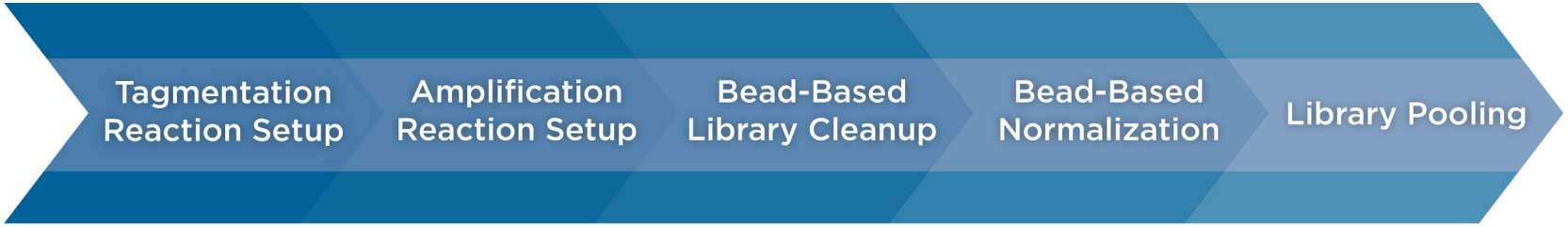

Figure 1
Illumina® Nextera® XT System Process Diagram

Prerequisites

Read the *PIPETMAX® 268 User's Guide* (available at www.gilson.com) and technical resource documents for the Illumina® Nextera® XT DNA Library Preparation Kit (available at www.illumina.com) before setting up the application. Read all hardware, software, and safety instructions for equipment, reagents, and materials before performing the application. This setup guide is provided as a general resource and without warranty. Email Gilson technical support (techsupport@gilson.com) for additional assistance.

GILSON®

SETUP

To run the application, you will need to configure PIPETMAX with pipette heads (MAX8x20 and MAX8x200), tip waste chute, bed riser, and underbed tip waste bin.

Required PIPETMAX Configuration

DESCRIPTION	P/N	QTY.	DETAILS
PIPETMAX with Cover Cutouts	32100001	1	Cover cutouts are required to accommodate on-bed devices with cabling that extend beyond the instrument.
TRILUTION® micro on Tablet	32000321		Only one copy of the control software (for tablet or PC) is required.
TRILUTION® micro for PC	32000320	1	
MAX8x200 Pipette Head	FC10021	1	8-channel head (20–200 µL).
MAX8x20 Pipette Head	FC10022	1	8-channel head (1–20 µL).
PIPETMAX Tray, 384 Well	32000091	1	Removable tray with nine bed positions that includes clips for securing microplates.

Required User Materials

DESCRIPTION	P/N	QTY.	DETAILS
Storage Plate	Bio-Rad HSP-9601	1	96-well, skirted microplate
Reaction Plate	Eppendorf 128.575	1	96-well, semi-skirted microplate
Sample Plate	Greiner 659101	1	96-well clear V-bottom polystyrene low binding microplate
Strip Tubes	Thermo AB-0451	5	0.2 mL strip tubes.
2 mL Flip Cap Tubes	Greiner 623201	2	2 mL flip cap tubes.
RoboRack-96	Micronic MPW51001BC3PK	1	Rack to hold Illumina TruSeq Index primers for Amplification script.
12-Column Reservoir	Agilent Seahorse 201256-100	1	12-column reservoir.
Midi Plate	Thermo AB-0859	2	96-well deep well microplate.
Centrifuge	N/A	1	Compatible with microplates.
Thermal Cycler	N/A	1	Compatible with Eppendorf 128.575.
AMPure XP Beads	Beckman Coulter A63880 OR A63881 OR A63882	1	Magnetic beads for library cleanup and normalization.

Required Materials from Illumina

DESCRIPTION	PART NUMBER	DETAILS
Nextera XT DNA Library Preparation Kit	FC-131-1096	Consult www.illumina.com for detailed instructions
Nextera XT Index Kit	FC-131-1001	

Required PIPETMAX Accessories

DESCRIPTION	P/N	QTY.	DETAILS
PIPETMAX Tip Adapter Block	32000175	3	Reusable support racks for holding pipette tips.
Riser Kit for Off-Bed Tip Disposal	32000177	1	Includes on-bed tip waste chute and under-instrument tip waste bin.
Magnetic Bead Separator Rack	SPL-2294F-HDW	1	Base for magnetic bead separator rack.
Magnetic Insert	SPL-2294E-HDW	1	Magnetic insert for the magnetic bead separator rack.
PIPETMAX Tip Block Clip Set	32000311	3	The thumbscrew, reload block clip, and washer secure the magnetic bead separator rack in place on the bed.
Orbital Shaker	32000199	1	Big Bear Automation Shaker for PIPETMAX®.
PIPETMAX® On-Bed Device Cable Guide	32000247	1	Required for use with orbital shaker.
Rack for Flip Cap Tubes, 1.5–2.0 mL, Code 424	32000198	1	Rack for flip cap tubes.
Portrait Adapter Rack	SPL-2141C-HDW	1	Adapter that holds labware in “portrait” orientation, instead of “landscape.” Occupies two bed positions in the tray.
MicroAmp Short 96-Well PCR Tube Rack	32000303	2	Solid base that holds the semi skirted PCR plate.
DSF30ST DIAMOND Tips in Blister Packaging	F172313	1	Sterile filter tips with 30 µL capacity.
DSF200ST DIAMOND Tips in Blister Packaging	F172513	1	Sterile filter tips with 200 µL capacity.
PIPETMAX Scripts	Custom	N/A	The PIPETMAX run files mentioned in this setup guide are available upon request from techsupport@gilson.com

NEXTERA XT SCRIPTS OVERVIEW

Figure 2
Schematic representation of the five PIPETMAX® protocols for automation of the Nextera® XT DNA Library Kit workflow. Each blue rounded rectangle represents one PIPETMAX protocol, each of which corresponds to a portion of the Nextera XT workflow. Some protocols include repositioning the plate or off-bed steps accomplished through user intervention, such as centrifugation of a microplate between liquid handling steps.

SCRIPT: NEXTERA XT_TAGMENTATION_96

Overview

The tagmentation script will prepare genomic DNA samples for the tagmentation reaction. The custom PIPETMAX scripts mentioned in this setup guide are available from techsupport@gilson.com.

Bed Layout and Preparation

There are nine positions on the bed of the PIPETMAX® instrument. Each position can hold an item that conforms to the footprint dimensions defined in ANSI SLAS 1-2004 (R2012), such as a 96-well microplate. The images below show a schematic of each position occupied by specific labware. The convention in this setup guide is that the tip waste is always in position 1, which is in the left rear position when the user is facing the instrument.

Automation Steps

1. Aliquot 10 µL of Tagment DNA Buffer (TD) from the strip tubes to the reaction plate.
2. Transfer 5 µL of gDNA sample(s) from 96-well plate to the reaction plate.
3. Transfer 5 µL of Amplicon Tagment Mix (ATM) from the strip tubes to reaction plate.
4. PROMPT FOR OFF-BED ACTION: Centrifuge.
5. OFF-BED ACTION: Thermocycle.
6. Transfer 5 µL of NT from strip tubes to the reaction plate.
7. PROMPT FOR OFF-BED ACTION: Centrifuge.
8. Incubate at room temperature for five minutes.

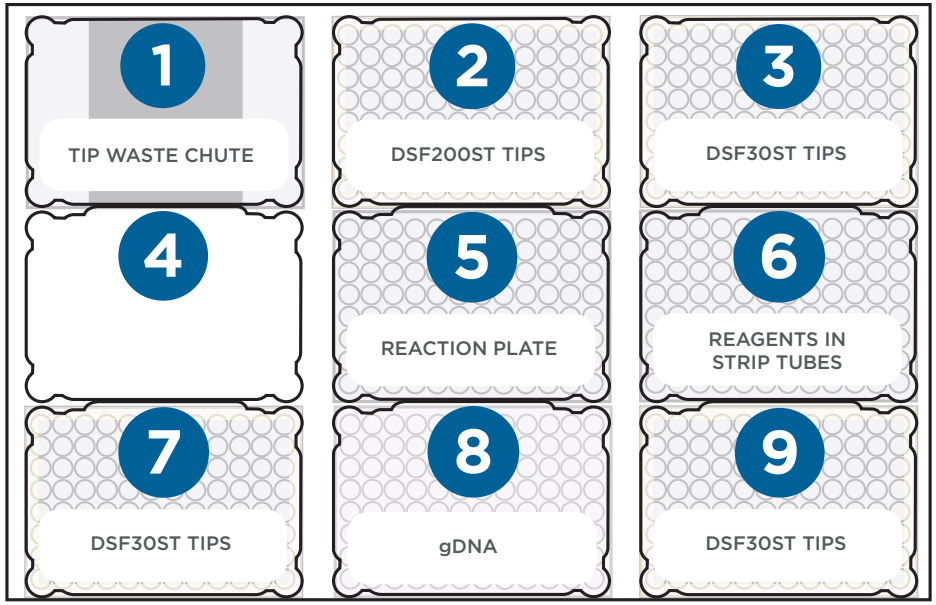

Figure 3
PIPETMAX® bed layout for Illumina® Nextera® XT System.

Bed Layout

POSITION	BED ELEMENT		PART NUMBER	INITIAL VOLUMES	DETAILS
1	Tip Waste Chute			N/A	Waste chute, underbed bin
2	DSF200ST Tips in Tip Adapter Block			N/A	Sterile filter tips
3, 7, and 9	DSF30ST Tips in Tip Adapter Block			N/A	Sterile filter tips
4	Not used		N/A	N/A	N/A
	Reaction Plate		Eppendorf 128.575		
5	MicroAmp® Base		32000303	0 µL	Reaction plate
	Reagents in Strip Tubes	Tagment DNA Buffer (TD) in A1:H1		140 µL	
		Amplicon Tagment Mix (ATM) in A2:H2		80 µL	
		Neutralize Tagment Buffer (NT) in A3:H3	Thermo AB-0451	80 µL	
6	MicroAmp® Base		32000303		Reagents
8	Genomic DNA		Greiner 651901		gDNA (0.2 ng/ µL)

SCRIPT: NEXTERA XT_AMPLIFICATION_96

This procedure prepares samples for amplification using the Illumina® TruSeq® index primers.

Overview

The Amplification Plate Setup script will dispense reagents into the tagmentation reaction plate prepared in the previous script. In the first step, i7 primers are dispensed into the reaction plate, which is held in the portrait orientation using an adapter (part number SPL-2141C-HDW). The user then repositions the reaction plate into the standard landscape orientation and the i5 primers are dispensed, creating a matrix of primers as required by the Nextera XT workflow. The Nextera PCR Master Mix (NPM) reagent is then added and the plate is ready for amplification on a thermal cycler.

Automation Steps

1. Position the reaction plate in the portrait orientation rack.
2. Transfer 5 µL each i7 primer to proper position in reaction plate.
3. Move reaction plate to bed position 5 (landscape orientation).
4. Transfer 5 µL of each i5 primer to proper position in reaction plate.
5. Add 15 µL of NPM to each well containing index adapters.
6. Mix by pipetting.
7. OFF-BED ACTION: Centrifuge reaction plate.
8. OFF-BED ACTION: Thermocycle the amplification reaction.

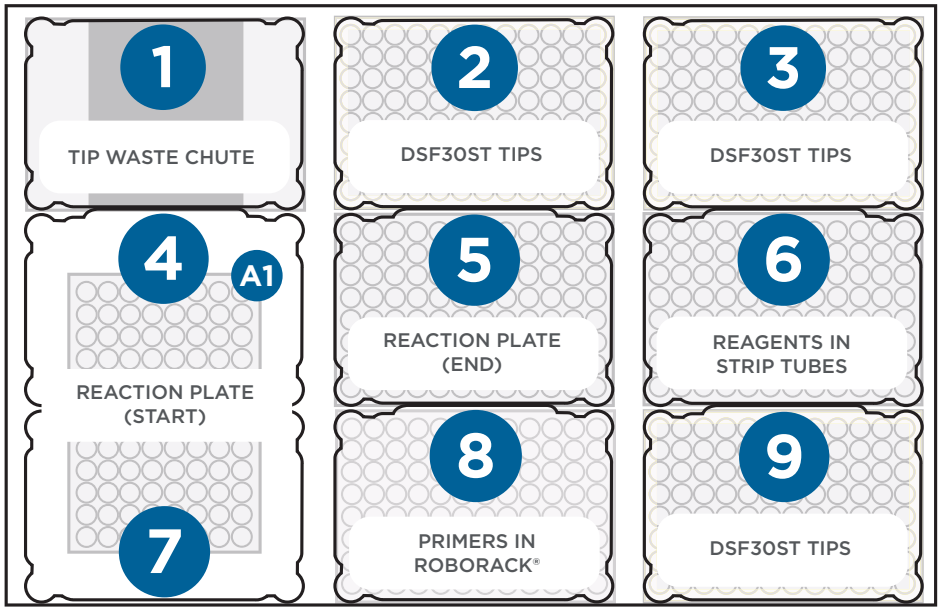

Figure 4
PIPETMAX® bed layout for Amplification Plate Setup

Bed Layout

POSITION	BED ELEMENT	PART NUMBER	INITIAL VOLUMES	DETAILS
1	Tip Waste Chute		N/A	Waste chute, underbed bin
2, 3, and 9	DSF30ST Tips in Tip Adapter Block			Sterile filter tips
4 and 7	Portrait Adapter Rack Reaction Plate MicroAmp® Base	SPL-2141C-HDW Eppendorf 128.575 32000303	25 µL	The reaction plate from the tagmentation procedure is placed here at the beginning of the amplification plate setup protocol.
5	Reaction Plate MicroAmp® Base	Eppendorf 128.575 32000303	30 µL	When prompted, the user moves the reaction plate to this position.
6	Reagents in Strip Tubes MicroAmp® Base	Thermo AB-0451 32000303	200 µL	NPM reagent in A4:H4
8	Illumina® TruSeq Index Primers	Illumina® FC-131-1001	Primer remaining volume information to be provided by user at run time.	i5 primers in A1:H1 i7 primers in A11:H11 and A12:D12

SCRIPT: NEXTERA XT_CLEAN UP LIBRARIES_96

This procedure cleans up the amplified libraries, using AMPure XP magnetic beads.

Overview

The Clean Up Libraries script will clean up the amplified libraries that were prepared in the previous script. This script makes use of the magnetic bead separator rack for PIPETMAX, an on-bed device. The script allows PIPETMAX to automatically engage or disengage the magnets to capture or release the magnetic beads.

Script Variables

DESCRIPTION	DEFAULT VALUE
Library Wells to be Processed	A1:H12
Library Volume	40 µL
AMPure XP Beads Transfer Volume	24 µL
Wash Transfer Volume	200 µL
RSB Transfer Volume	42 µL
Incubation Time	5 Minutes
First Bead Pelleting Wait Time	5 Minutes
Post Wash Bead Pelleting Wait Time	2 Minutes
Air Dry Time	15 Minutes
Wash Steps	2

DESCRIPTION	DEFAULT VALUE
Pellet Resuspend Mix Cycles	4
Change Tips for Supernatant Removal	True
Change Tips for Wash Dispense	False
Change Tips for RSB Dispense	True
Change Tips for RSB Elution Mix	True
Elute Library to New Plate	True
Elution Transfer Volume	20 µL
RSB Tubes to Use	1
Source Library Wells	A1:H12

Bed Layout and Preparation

POSITION	BED ELEMENT		PART NUMBER	INI. VOL.	DETAILS
1	Tip Waste Chute			N/A	Waste chute, underbed bin.
2, 3, 7, and 8	DSF200ST Tips in Tip Adapter Block			N/A	Sterile filter tips.
4	Amplified NGS Libraries MicroAmp® Base		Eppendorf 128.575 32000303	50 µL	The reaction plate from the amplification procedure is placed here at the beginning of the clean up libraries procedure.
5	Empty Midi Plate		Thermo AB-0859	0 µL	Libraries will be eluted into this plate during the script.
6	12-Column Reservoir	AMPure® XP in Column 1 80% Ethanol in Column 2 80% Ethanol in Column 3 Resuspension Buffer (RSB) in Column 4	Seahorse 201256-100	3.5 mL 21 mL 21 mL 5.04 mL	Reagents
9	Empty Midi Plate Magnetic Bead Rack		Thermo AB-0859 SPL-2294F-HDW and SPL 2294E-HDW	0 µL	Bead cleanup location.

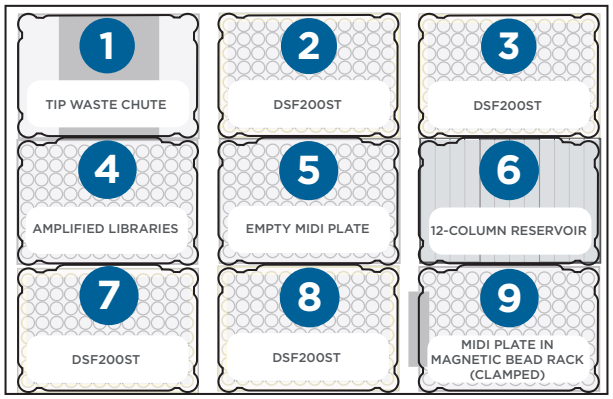

Figure 5
PIPETMAX® bed layout for Amplification Plate Setup

Automation Steps

1. OFF-BED ACTION: Centrifuge plate.
2. Disengage magnets (move magnetic bead separator rack to home position).
3. Transfer 24 µL AMPure beads to positions A1:H12 in Midi plate on magnetic bead separator rack.
4. Transfer 40 µL of amplified libraries from A1:H12 of twin.tec™ to A1:H12 of the MIDI plate on the magnetic bead separator rack.
5. Wait. Engage magnets on magnetic bead separator rack. Wait. Remove supernatant.
6. Add 200 µL 80% Ethanol wash. Wait. Remove supernatant.
7. Add 200 µL 80% Ethanol wash. Wait. Remove supernatant.
8. Wait. Disengage magnets on magnetic bead separator rack.
9. Add 42 µL resuspension buffer RSB.
10. Mix libraries to elute.
11. Engage magnets on magnetic bead separator rack. Wait.
12. Transfer eluent to new Midi Plate.

Optional: after completion of the Bead Cleanup script, you may wish to carry out BioAnalyzer analysis of 1 µL from one or more libraries.

NOTE Prepare the reagents and bed elements for bead normalization during the library cleanup run.

SCRIPT: NEXTERA XT_NORMALIZE LIBRARIES_96

This procedure normalizes the amount of material in each library, using the procedure and reagents provided by Illumina®.

Overview

The normalize libraries script will normalize the amount of material in the cleaned up libraries that were prepared in the previous script. This script makes use of the magnetic bead separator rack for PIPETMAX® as well as the orbital shaker for PIPETMAX. The script allows PIPETMAX to automatically engage or disengage the magnets in order to capture or release the magnetic beads, and to control the speed and time of shaking of the orbital shaker. The script prompts the user to reposition the plate from magnetic bead separator rack to orbital shaker and vice versa, as required. Please refer to the *Orbital Shaker Setup Guide* (LT375095) for more information on setup and use of the shaker, including setting up the COM port.

Bed Layout and Preparation

POSITION	BED ELEMENT		PART NUMBER	INI. VOL.	DETAILS
1	Tip Waste Chute		N/A	N/A	Waste chute, underbed bin
2, 3, and 8	DSF200ST Tips in Tip Adapter Block		N/A	N/A	Sterile filter tips.
4	12-Column Reservoir	LNA1/LNB1 in Column 1	Seahorse 201256-100	5.7 mL	Reagents.
		LNW1 in Column 2		10 mL	
		0.1N NaOH in Column 3		4 mL	
		LNS1 in Column 4		3.5 mL	
5	Storage Plate		Bio-Rad HSP-9601	0 µL	Storage plate.
7	Orbital Shaker		32000199	N/A	Orbital shaker for PIPETMAX.
9	NGS Libraries Magnetic Bead Separator Rack		SPL-2294F-HDW and SPL2294E-HDW	0 µL	Cleaned up libraries from previous script in magnetic bead separator rack.

Script Variables

DESCRIPTION	DEFAULT VALUE
COM Port for Shaker	COM6
Library Wells to be Processed	A1:H12
Library Wells Initial Volume	20 µL
LNA1 LNB1 Transfer Volume	45 µL
LNW1 Transfer Volume	45 µL
NaOH Transfer Volume	30 µL

DESCRIPTION	DEFAULT VALUE
Final Elution Volume	20 µL
Bead Incubation Shake Time	30 minutes
Pre Wash Bead Pull Down Time	2 minutes
After Wash Bead Pull Down Time	1 minute
NaOH Shake Time	4 minutes

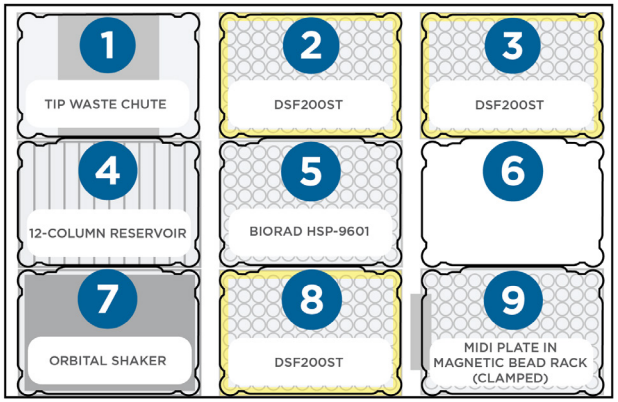

Figure 6
PIPETMAX® bed layout for Normalization Libraries Setup

Automation Steps

1. Check COM port
2. OFF-BED ACTION: Centrifuge plate.
3. Disengage magnets (move magnetic bead separator rack to home position)
4. Transfer 45 µL from LNA1/LNB1 to the libraries in the Midi plate on the magnetic bead separator rack.
5. PROMPT FOR OFF-BED ACTION. Manually move the plate to the orbital shaker. Shake at 1800 rpm for 30 minutes.
6. Manually move plate to magnetic bead separator rack. Engage magnets. Wait. Remove the supernatant.
7. Add 45 µL of LNW1 wash. Wait. Remove supernatant.
8. Add 45 µL of LNW1 wash. Wait. Remove supernatant.
9. Aliquot LNS1 to SGP plate.
10. Add 30 µL of 0.1 N NaOH to NGS libraries in Midi plate
11. Prompt. Manually move plate to orbital shaker. Shake 1800 rpm for four minutes.
12. Prompt. Move plate back to magnetic bead separator rack. Wait.
13. Transfer supernatant to the SGP plate
14. OFF-BED ACTION: Centrifuge 1000 x g for one minute.

SCRIPT: NEXTERA XT_POOL LIBRARIES_96

This procedure pools the libraries together in preparation for sequencing.

Overview

The pool libraries script will pool the normalized libraries that were prepared in the previous script. The libraries are pooled from a 96-well plate first into a strip tube, and finally into a 2 mL microfuge tube. Libraries generated using the PIPETMAX® automated Nextera® XT DNA Library Kit scripts are ready for downstream Illumina® sequencing.

Automation Steps

1. OFF-BED ACTION: Centrifuge 1000 x g, 20C for one minute.
2. Transfer 10 µL from the storage plate to the 8-tube strip in the MicroAmp base.
3. Transfer 15 µL from each tube of 8-tube strip to the 2 mL tube in the 424 rack.
4. Mix.
5. After the pooling script is complete, proceed to sequencing with an Illumina MiSeq® instrument.

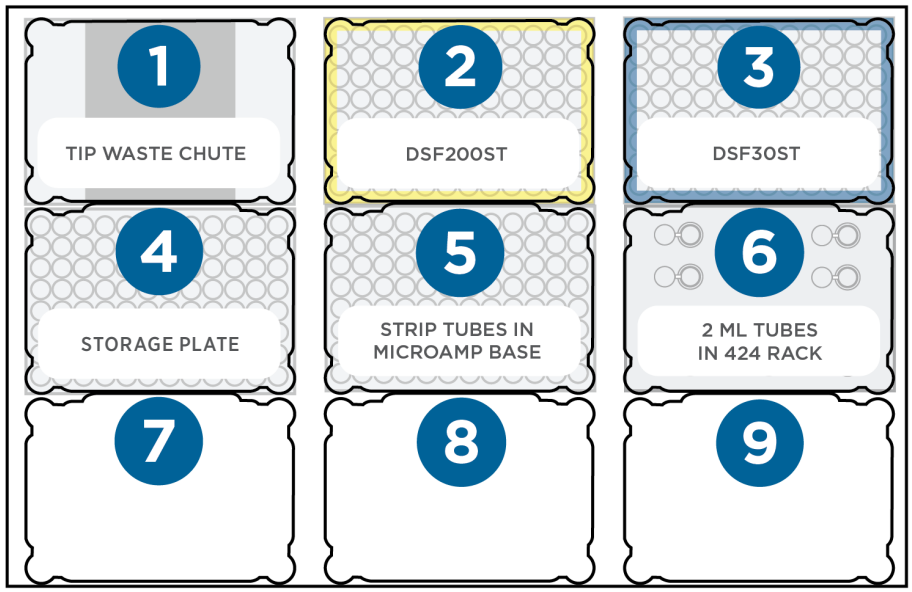

Figure 7
PIPETMAX® bed layout for Pool Libraries Setup

Bed Layout and Preparation

POSITION	BED ELEMENT	PART NUMBER	INI. VOL.	DETAILS
1	Tip Waste Chute	N/A	N/A	Waste chute, underbed bin
2	DSF200ST Tips in Tip Adapter Block	N/A	N/A	Sterile filter tips
3	DSF30ST Tips in Tip Adapter Block	N/A	N/A	Sterile filter tips
4	Storage Plate	Bio-Rad HSP-9601	20 µL	Storage plate
5	Intermediate pool	Thermo AB-0451 (0.2 ml strip tubes) in MicroAmp® base 32000303	0 µL	Reaction plate
6	Pooled libraries	2 mL Greiner flip-cap tube 623201 in Code 424 rack	0 µL	N/A
7, 8, and 9	N/A	N/A	N/A	N/A

FREQUENTLY ASKED QUESTIONS (FAQ)

Q: The instructions specify particular labware for the compound plate, reagent plate and assay plate. Can I use some different labware that I have on hand in the lab?

A: No, this script has been validated with specific labware. Any deviation from the specified plates and rack may result in incorrect pipetting or crashes. Contact technical support for further assistance if alternate labware is required (techsupport@gilson.com).

Q: Can the PIPETMAX® accurately dispense small volumes?

A: Yes, the PIPETMAX® exceeds the Maximum Permissible Errors for piston operated multichannel pipettes (ISO 8655) at 1 µL on the MAX8x20 pipette head (see table below).

		PIPETMAX® MAX8X20	ISO 8655
1 µL	Systematic Error	±0.08 µL (8%)	±0.10 µL (10%)
1 µL	Random Error	±0.05 µL (5%)	±0.10 µL (10%)

Q: What is the procedure for placing the Portrait Adapter Rack (SPL-2141C-HDW) onto the PIPETMAX® tray?

A: Remove the spring clips from the tray in the two positions that will be occupied by the portrait adapter rack. Place the adapter rack in the removable tray, with the spring clip that is built into the adapter rack toward the rear of the instrument. Clamp the adapter rack in place using two of the thumbscrew clips that are typically used to secure the tip adapter blocks.

Q: How many tips are needed? Can I use the partial tip box from one run in a second run?

A: The number of tips required will depend on which script is being run and how many samples are being processed. Partial tip boxes may be used in a subsequent run; to do this simply enter the number of missing tips when prompted during Tip setup in the step-by-step wizard.

Q: Do I always need to place all of the indicated boxes of tips on the bed even if my script requires fewer tips?

A: When processing 24 samples in the Tagmentation module only 72 tips (DF30ST) are consumed, which is less than one full box. It is possible to only use one box of tips in this scenario; however, the user will need to go through the Tip setup in the Step-by-step wizard to indicate that the other boxes are empty (i.e., 96 tips missing) in order to ensure that the instrument is aware that a box is missing.

Gilson, Inc.
3000 Parmenter Street · PO Box 620027 · Middleton, WI 53562, USA
T 608-836-1551 or 800-445-7661 · F 608-831-4451

Gilson S.A.S.
19 avenue des Entrepreneurs · 95400 Villiers-le-Bel, France
T +33 (0) 1 34 29 50 00 · F +33 (0) 1 34 29 50 20

www.gilson.com/ContactUs