

User Manual

Version 1.1 - UMMW4160INT



Evercode™ MAX WT v4

Library Prep

16 Reactions on INTEGRA

ASSIST PLUS

For use with
ECMW4160



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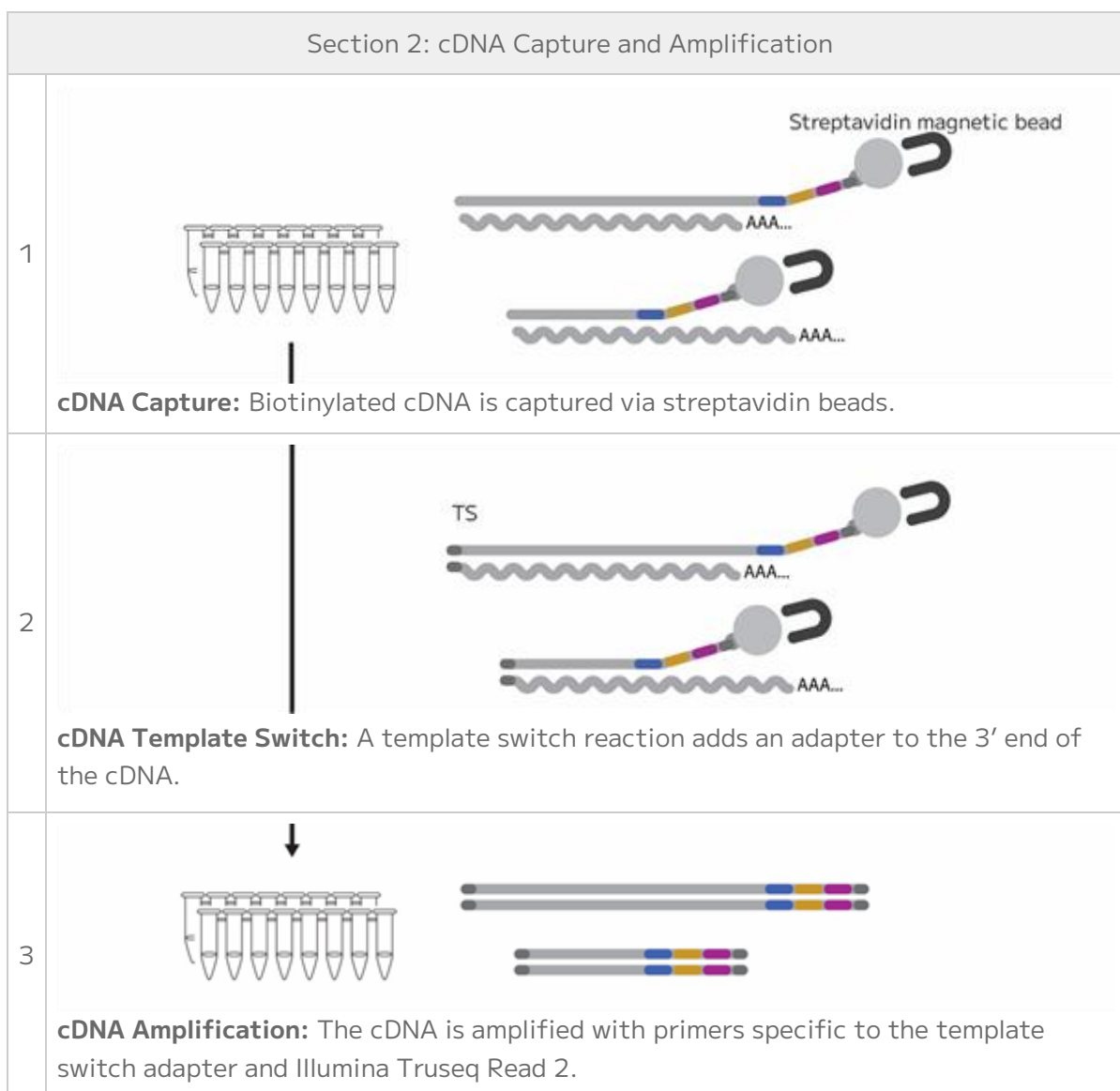
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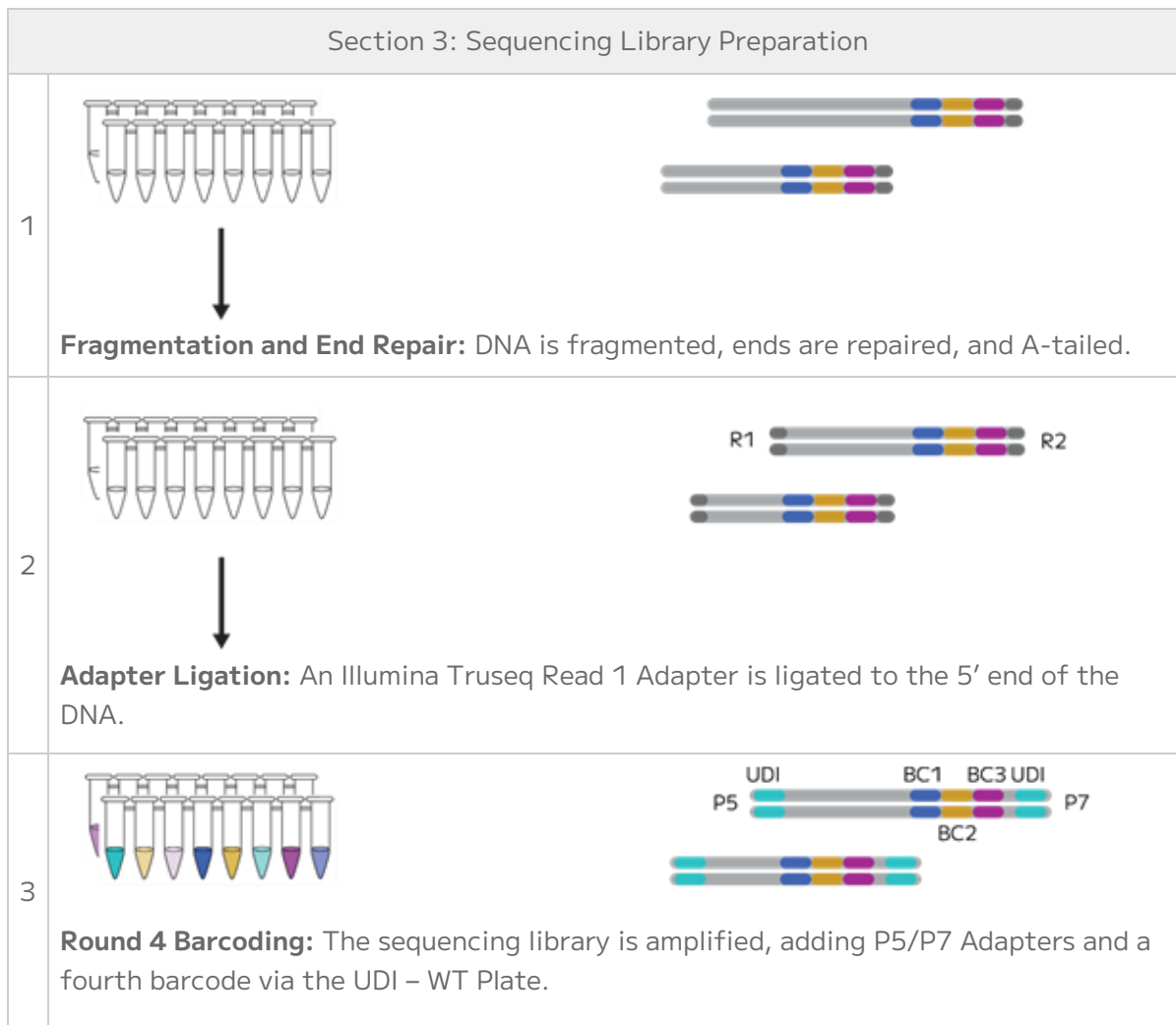
Overview

Workflow

This guide covers sublibrary preparation only and should be completed after the Evercode MAX WT v4 Cell Barcoding workflow. This user guide can be used to prepare between 16 sublibraries concurrently for sequencing.

The Sublibrary Preparation portion of the Evercode MAX WT v4 workflow starts with capturing the cDNA lysed at the end of Section 1 workflow.





Important Guidelines

The following guidelines provide additional information to obtain optimal performance with the Evercode MAX WT v4 L with INTEGRA ASSIST PLUS barcoding workflow.

For further information on the experimental or automation workflow, please contact support@parsebiosciences.com. Please contact support-us@integra-biosciences.com for any questions regarding the INTEGRA ASSIST PLUS instrument.

Avoiding RNase Contamination

- Take standard precautions to avoid introducing RNases into samples or reagents throughout the workflow. Always wear proper laboratory gloves and use aseptic technique.
- Although RNases are not inactivated by ethanol or isopropanol, they are inactivated by products such as RNaseZap™ RNase Decontamination Solution (Thermo Fisher Scientific®). These can be sprayed on benchtops and pipettes. The Assist Plus Base Unit and hardware can also be wiped down with RNase Zap to inactivate RNases.
- Low retention, filtered pipette tips should be used to reduce RNase contamination from pipettes.

Kit Selection and Run Size

- The INTEGRA ASSIST PLUS workflow described in this guide requires exactly 16 reactions per run. Other reaction counts are not supported.
- The 16-reaction Library Prep kit does not contain sufficient reagent volume for two separate 8-reaction runs on the INTEGRA ASSIST PLUS. If planning two separate 8-reaction runs, we recommend purchasing two 8-reaction Library Prep kits.

Indexing Primers

- The Evercode MAX WT v4 Library Prep kit requires the UDI Plate - WT. The UDI Plate - WT box is included in the INTEGRA bundles but will need to be separately purchased if necessary for subsequent orders.
- The UDI Plate - WT is a 96-well plate containing 48 unique dual indexing (UDI) primers. Each well is a single-use reaction sufficient for one Barcoding Round 4 PCR reaction for a single sublibrary. Thus, the UDI Plate - WT can be used for multiple Evercode kits.

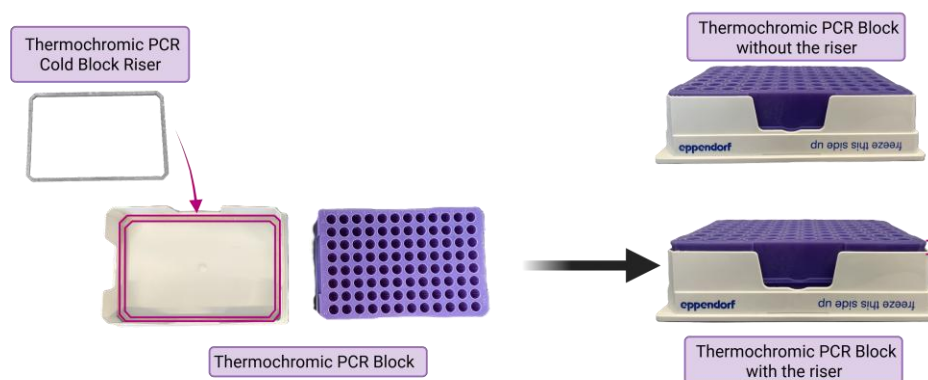
- The UDI Plates are sealed with a pierceable foil to minimize cross-contamination. The plate seal should be wiped with 70% ethanol and pierced with a new pipette tip immediately prior to use. Avoid splashing or mixing the liquid between individual wells. Once a well has been used, it should not be resealed or reused.
- We recommend selecting UDIs column-wise from left to right (starting with indices 1-8). UDI sequences can be found in Appendix A.

Plate Sealing

- When sealing or unsealing 96 well plates, do not splash liquid onto the PCR plate seal or between wells. Securing plates in PCR tube racks will minimize this occurrence.
- PCR plate seals may be difficult to remove. Carefully peel off the PCR plate seal while applying downward pressure on the edges of the plate to keep it in the PCR plate rack. Avoid touching the top of the open wells.

Thermochromic PCR Cold Block

- The Thermochromic PCR Cold Block changes color from dark purple to bright pink (too warm) when the block temperature exceeds 7°C. If greater than 30% of the block is bright pink, replace the Thermochromic PCR Cold Block with a fully frozen dark purple block.
- To maintain optimal performance and minimize temperature fluctuations, store the Thermochromic PCR Cold Blocks in a -20°C freezer when not in use. The blocks should be stored upside down to prevent warping.
- The Thermochromic PCR Cold Block Riser needs to be installed in the Thermochromic PCR Cold Block prior to use as illustrated below. The Riser does not need to be removed when freezing.



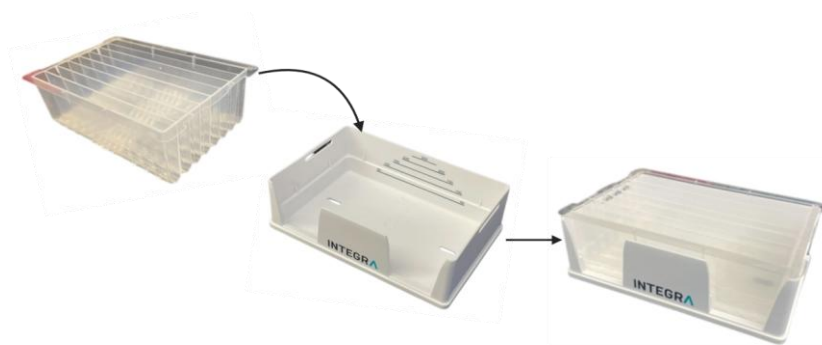


Note: Uneven freezing of the liquid inside the Thermochromic PCR Block can cause slanting of the cooling position. The Thermochromic PCR Cold Block Riser reduces the variability caused by the freezing process. If the PCR Cold Block riser is not used, there will be extra liquid left in the wells of the PCR plate during aspiration steps.

- Tip pinching may occur when using a fully frozen Thermochromic PCR Cold Block for a plate pooling step. To minimize tip pinching, a fully frozen block should be warmed slightly by a few degrees by leaving it at room temperature for 10 minutes before using it on the INTEGRA ASSIST PLUS Deck.
- To ensure a secure positioning within the Thermochromic PCR Cold Block, apply firm pressure on the PCR plates until they are securely seated. Avoid touching the top of the open wells.

Bases and Reservoirs

- Both the 300 mL Reservoir Base and the 8-Row Polystyrene Reservoir are consumables that must be purchased from INTEGRA. The 300 mL Reservoir Base is designed to securely hold the 8-Row Reservoir in place during use.



Fixation 8 Row Reservoir Adapter with Cooling Adapter Base.

INTEGRA ASSIST PLUS Pipetting Programs

- Ensure that the VIALAB program is installed on the same Windows computer that will be used to communicate with the D-ONE Pipetting Module.
- Ensure that the Evercode workflow precheck script has been confirmed prior to running the INTEGRA + Parse workflow. This can be done by running the Evercode MAX v4 with INTEGRA ASSIST PLUS Precheck Scripts available on the Customer Support Suite.
- Automated pipetting programs are set up and can be selected using the pipette's user interface. In the INTEGRA + Parse workflow, prompts are built into the programs as checkpoints. When the prompt is followed by a double dash, "--", the program will

continue. If the prompt does not contain a double dash, "--", it is followed by another prompt.

Deck Loading

- Always ensure the tip box placed on the deck clicks when put into place. Remove the tip box lid prior to starting the program.
- To prevent malfunctions during the procedure, ensure that all unnecessary labware is cleared from the deck before initiating a new program on the INTEGRA ASSIST PLUS.

Parse Reagents

The Evercode kit includes the following boxes. Safety Data Sheets for these reagents can be provided upon request

-20°C Reagents Store at 4°C, PN XMW160

LABEL	ITEM	PN	FORMAT	QTY
	Wash Buffer A	MG143	5 mL tube	1
	Bead Wash Buffer	MG114	5 mL tube	1
	Binding Buffer	MG118	1.5 mL tube	1
	Wash Buffer B	MG144	5 mL tube	1
	Template Switch Buffer	WT148	2 mL tube	4
	Template Switch Enzyme	MG121	1.5 mL tube	1
	Template Switch Primer	MG122	1.5 mL tube	1
	PCR Additive	MG150	1.5 mL tube	1
	cDNA Amp Mix	MG123	1.5 mL tube	1
	cDNA Amp Primers	MG124	1.5 mL tube	1
	Fragm/End Prep Buffer	MG125	1.5 mL tube	1
	Fragm/End Prep Enzymes	MG126	1.5 mL tube	1
	Ligation Adapter	MG127	1.5 mL tube	1

LABEL	ITEM	PN	FORMAT	QTY
	Adapter Ligation Buffer	MG128	1.5 mL tube	1
	Adapter Ligation Enzyme	MG129	1.5 mL tube	1
	Library Amp Mix	MG130	1.5 mL tube	1

4°C Reagents. Store at 4°C, PN XMW170

LABEL	ITEM	PN	FORMAT	QTY
	Streptavidin Beads	PT201	2 mL tube	1

Parse-Provided Equipment

The following is a list of equipment provided by Parse Biosciences, required to successfully perform the Evercode workflows on the INTEGRA ASSIST PLUS.

The equipment listed should be stored at -20°C when not in use and taken out when instructed throughout the protocol.

ITEM	PN	QTY	CHECK LIST
Thermochromic PCR Cold Block	NTAC1102	2	☐
Thermochromic PCR Cold Block Riser	NTAC1103	2	☐
Parse Metal Cold Block	NTAC1107	1	☐

INTEGRA Components

The following are required INTEGRA components needed to run the Evercode assay on the INTEGRA and should not be substituted.

ITEM	ITEM TYPE	PN	QTY	CHECK LIST
Pipette Communication Module for VIAFLO / VOYAGER Pipettes	Accessory	4222	2	☐
Tip Deck for D-ONE Pipetting Module	Base	4535	1	☐
ASSIST PLUS Labware Pedestal (+24 mm) for D-ONE, portrait, for 10 mL, 25 mL, 100 mL, reservoirs and plates/reservoirs in SLAS/ANSI format (24 mm Labware Pedestal)	Adapter	4551	1	☐
D-ONE Pipetting Module 1-Ch, 5-1250 µL	Pipette	4532	1	☐
VOYAGER Pipette 8-Channel, 5 - 125 µL	Pipette	4722	1	☐
ASSIST PLUS Base Unit	Main	4505	1	☐
Communication/Charging Cable for VIAFLO	Accessory	4226	1	☐
HEATMAG Module	Module	4901	1	☐
96 Well Adapter for HEATMAG module	Adapter	4906	1	☐
300 mL Reservoir Bases	Accessory	6305	1	☐

Equipment

The following equipment is required to perform the protocol but are not provided by INTEGRA or by Parse Biosciences. Note that this list does not include standard laboratory equipment, such as freezers.

Equipment

ITEM	SUPPLIER	PN	NOTES	CHECK LIST
Centrifuge with Swinging Bucket Rotors	Various Suppliers	Varies	Compatible with 96 well plates and capable of reaching 4°C.	☐
Microcentrifuge	Various Suppliers	Varies	Compatible with 1.5 mL tubes and PCR 8-strip tubes.	☐
6-Tube Magnetic Separation Rack	New England Biolabs®	S1506S	Or an equivalent magnetic rack for 1.5 mL tubes.	☐
Plate Seal Applicator	Various Suppliers	Varies	Capable of adhering plate sealing films to 96 well plates.	☐
T100 Thermal Cycler	Bio-Rad Laboratories®	1861096	Or an equivalent thermocycler compatible with semi-skirted 96 well plates, 0.2mL PCR tubes with up to 100 µL of sample volume, sample heating and cooling from 4-98°C, and a heated lid capable of 30-105°C.	☐
Water bath	Various Suppliers	Varies	Or equivalent thermomixer or heat block capable of holding temperature at 37°C.	☐
Vortex-Genie 2®	Scientific Industries®	SI-0236	Or alternative vortex and adapter for 96 well plates, or a thermomixer or alternative shaker that can be set to 800-1000 RPM for 30 minutes.	☐
6-inch Platform	Scientific Industries	146-6005-00		
Microplate Foam Insert	Scientific Industries	504-0235-00		
Qubit™ Flex Fluorometer	Thermo Fisher Scientific	Q33327	Or an equivalent fluorometer.	☐
2100 Bioanalyzer	Agilent®	G2939BA	Choose one.	☐
4200 TapeStation System	Agilent	G2991BA		

Consumables and Reagents

The following is a list of consumables and reagents required to successfully perform Parse Evercode workflows on the INTEGRA ASSIST PLUS.

INTEGRA Consumables

ITEM	PN	QTY	CHECK LIST
8 Row Reagent Reservoirs, Polystyrene, standard footprint, with SUREFLO design (a.k.a. 8 Row Reservoir Insert)	6373	2	☐
1250 µL Pipette Tips	6545	1	☐
125 µL Pipette Tips	6565	5	

Other Consumables

ITEM	SUPPLIER	PN	QTY	CHECK LIST
1.5 mL Microtube	Genesee Scientific	21-257	3	☐
PCR Strip Tubes	USA Scientific®	1402-4700	2	☐
2 mL Microtubes	Genesee Scientific	21-255	6	☐
twin.tec® Semi-Skirted PCR Plate 96 LoBind® Semi-skirted 96 well plates	Eppendorf®	E951020362	3	☐
SealPlate®	Excel Scientific	100-SEAL-PLT	as needed	☐
TempPlate Sealing® Foil	USA Scientific	2923-0100	as needed	☐
Pipette Tips RT LTS 20 µL FL 960A/10 RT LTS 200 µL FL 960A/10 RT LTS 1000 µL FL 768A/8	Rainin®	30389226 30389240 30389213	as needed	☐

Reagents

ITEM	SUPPLIER	PN	NOTES	CHECK LIST
UDI Plate - WT	Parse Biosciences	UDI1001	Each 96 well plate contains 48 unique single-use reactions sufficient for multiple Evercode kits (48 sublibraries). UDI Plate - WT can be purchased separately or bundled with Evercode kits.	☐
AMPure® XP Reagent	Beckman Coulter®	A63880 (5 mL) A63881 (60 mL)	Choose one. We do not recommend substituting other magnetic beads.	☐
SPRIselect Reagent	Beckman Coulter	B23317 (5mL) B23318 (60mL)		
KAPA® Pure Beads	Roche®	KK8000 (5 mL) KK8001 (30mL)		
RNaseZap™ RNase Decontamination Solution	Thermo Fisher Scientific®	AM9780	Or equivalent RNase decontamination solution.	☐
Ethyl alcohol, Pure	Sigma-Aldrich®	459844	Or equivalent 100% non-denatured ethanol.	☐
Nuclease-Free Water	Sigma-Aldrich	W4502	Or equivalent nuclease-free water.	☐
Qubit dsDNA HS (High Sensitivity) Assay Kit	Thermo Fisher Scientific	Q33230 (100 assays) Q33231 (500 assays)	Or equivalent DNA quantifier.	☐
High Sensitivity DNA Kit	Agilent®	5067-4626	Choose one that corresponds to the chosen Bioanalyzer or Tapestation.	☐
High Sensitivity D5000 ScreenTape and Reagents	Agilent	5067-5592 (screen tape) 5067-5593 (sample buffer and ladder)		

Reading and Understanding Visuals and Diagrams

Understanding the figures in this user manual is essential for accurately interpreting the information presented.

While the figures are different, they share consistent formats, use standardized visual elements and follow common conventions.

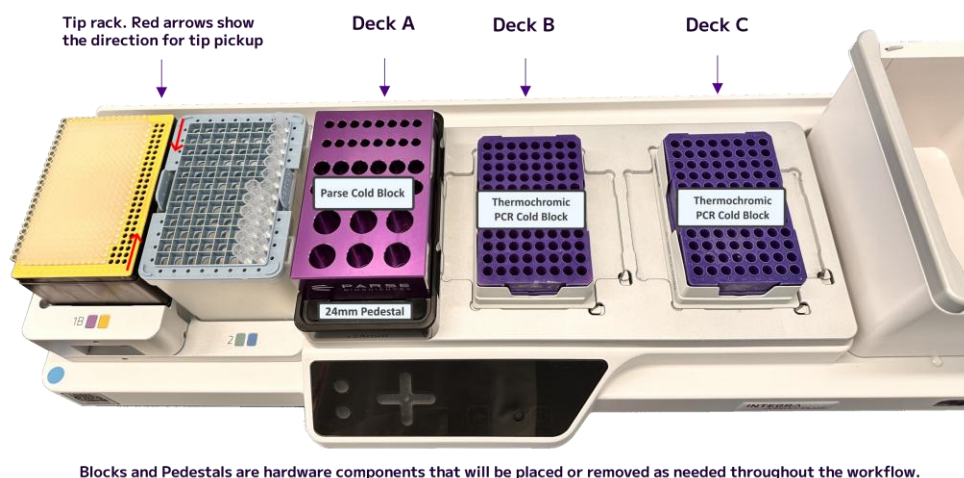
There are three types of figures in this manual.

- Photos of the hardware configurations, including tip rack, and Decks A, B, and C.
- Visual representation of Decks A, B, and C where consumable labware, reagents, and samples are placed.
- Diagrams of the pipette illustrating the steps to select the appropriate program.

We recommend reviewing this section and the illustrations throughout the manual to familiarize yourself with the visual language used.

Understanding the Hardware Configurations

The hardware configurations appear at the beginning of each subsection and show how to set up the INTEGRA platform to complete the associated steps. This photo displays the tip racks, Deck A, Deck B, and Deck C. Red arrows indicate the order in which tips are picked up by the INTEGRA platform, and required hardware is highlighted with text boxes.



Reading and Understanding the Deck Configurations

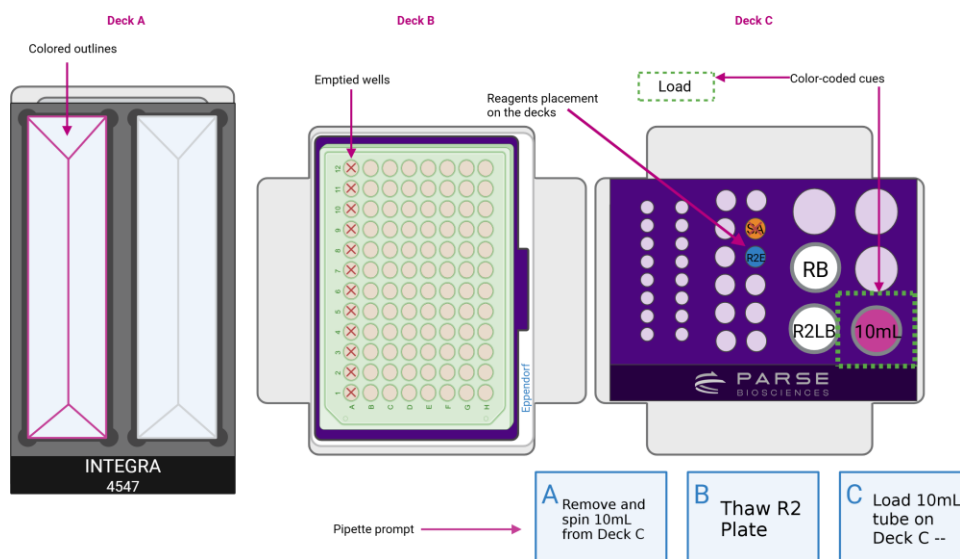
Each subsection includes multiple deck configurations that illustrate the steps executed by the program. These configurations display the exact placement of reagents and samples on the

decks, list the consumables required at each step, and highlight pipette prompts for manual intervention.

Graphical representation of basins and/or tubes with colored outlines show where specific reagents will be added. Each outline color matches the color of the reagent's label.

Color-coded cues guide the user through actions such as loading, moving, or removing hardware, reagents, and consumables, while also indicating the specific deck locations where these actions should occur.

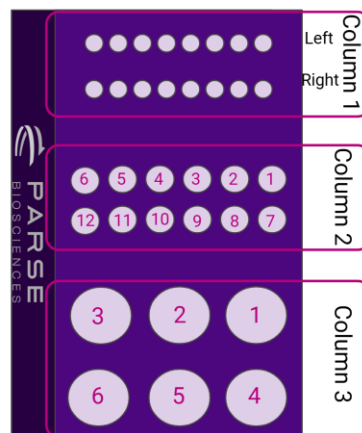
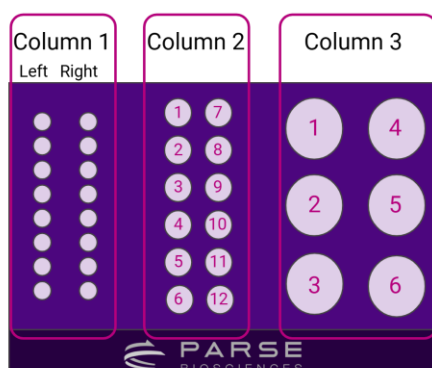
Letter-coded pipette prompts indicate the specific action the robot is about to perform when initiated. A double dash ("--") at the end of a prompt signifies that all prompts have been entered, and the robot will begin executing the programmed movements immediately after.



The Parse Metal Cold Block

It is critical to place reagents, samples, and consumables in the correct positions on the Parse Metal Cold Block.

The Parse Metal Cold Block is organized into three main columns, 1, 2 and 3, each containing two sub-columns (left and right) with slots of varying sizes to accommodate different tube types.



Color-Coded Cues

This workflow is semi-automated and still requires user interaction and input. Prompts will appear on the pipette display, instructing the user to perform tasks such as loading, moving, removing, or replacing hardware, reagents, and consumables, as well as mixing and pipetting specific reagents. In this user guide, each prompt is color-coded and shown in the deck configurations illustrating the required actions. Additional explanatory text accompanies each figure to provide further detail on the steps involved.



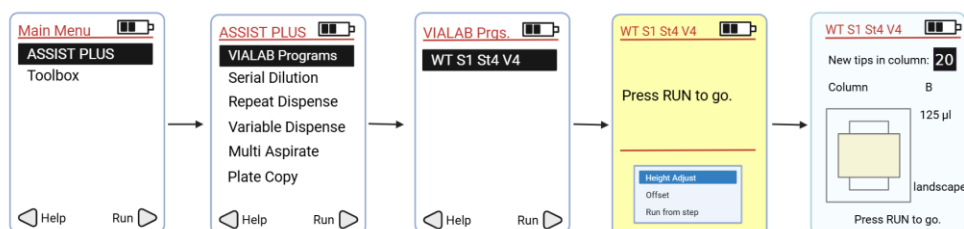
- **Load:** Indicates labware and/or reagents that need to be placed or reloaded onto the deck.
- **Remove:** Identifies labware that should be taken off the deck, either for incubation, disposal, or because it is not needed in the upcoming step.
- **Replace:** Highlights labware that has been used and needs to be swapped out for clean or new consumables.
- **Pipette:** Prompts the user to manually dispense reagents into designated basins.
- **Mix:** Instructs the user to mix specific reagents.
- **Move:** Indicates labware that should be relocated between deck positions.
- **X Emptied:** Reminds that the content has been used and the vessel is now empty.



Note: The position of the color-coded cues on top of the figures does not necessarily correspond to the location where the action should be taken.

Pipette Programs Diagram

At various points in the procedure, the user will need to switch pipettes and tip racks. In each subsection, the pipette is pre-programmed to carry out specific tasks defined by a designated script. The correct program must be loaded onto the pipette before beginning each set of steps. A diagram showing the path to load the appropriate program is provided in every subsection.



Section 2: cDNA Capture and Amplification

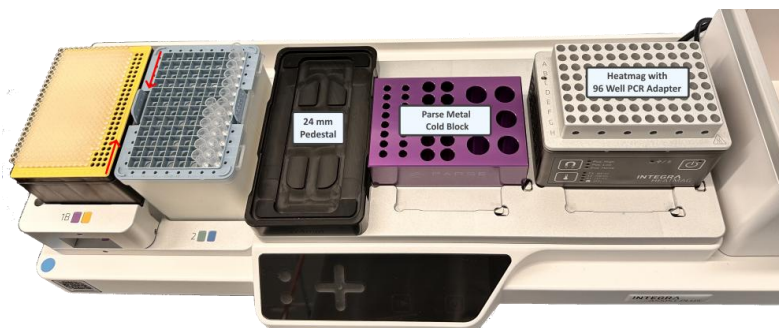
2.1. Reagents Plating

SPRI beads, Streptavidin Beads, Bead Wash and Binding Buffer are dispensed into a **Reagent Plate**. The Wash A and Wash B Buffers are dispensed into the INTEGRA 8 Row Reservoir on Deck A.

1. If continuing directly from Section 1, store lysates on ice until prompted.
2. Fill an ice bucket.
3. Gather the following items and handle as indicated below.

ITEM	SOURCE	QTY	HANDLING AND STORAGE
D-ONE Pipetting Module 1-Ch, 5-1250 µL	INTEGRA Component	1	
Tip Deck for D-ONE Pipetting Module	INTEGRA Component	1	
125 µL Tip Rack	INTEGRA	1	
1250 µL Tip Rack	INTEGRA	1	
24 mm Labware Pedestal	INTEGRA Component	1	
Parse Metal Cold Block	Parse	1	Keep at room temperature.
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
8 Row Reservoir Insert	INTEGRA	1	Individually wrapped consumable
300 mL Reservoir Base	INTEGRA	1	
96 well Semi-Skirted Plate	Consumables	1	
● Streptavidin Beads	4°C Reagents	1	Keep at room temperature.
● Binding Buffer	-20°C Reagents	1	Thaw at room temperature then store on ice. Mix by inverting 3x.
○ Bead Wash Buffer	-20°C Reagents	1	
○ Wash Buffer A	-20°C Reagents	1	
○ Wash Buffer B	-20°C Reagents	1	

4. Place the 24 mm Labware Pedestal on Deck A.
5. Place the Parse Metal Cold Block on Deck B.
6. Place the HEATMAG with 96 Well PCR Adapter (INTEGRA logo oriented to the front) on Deck C. Ensure HEATMAG power is on for the entire duration of Section 2.



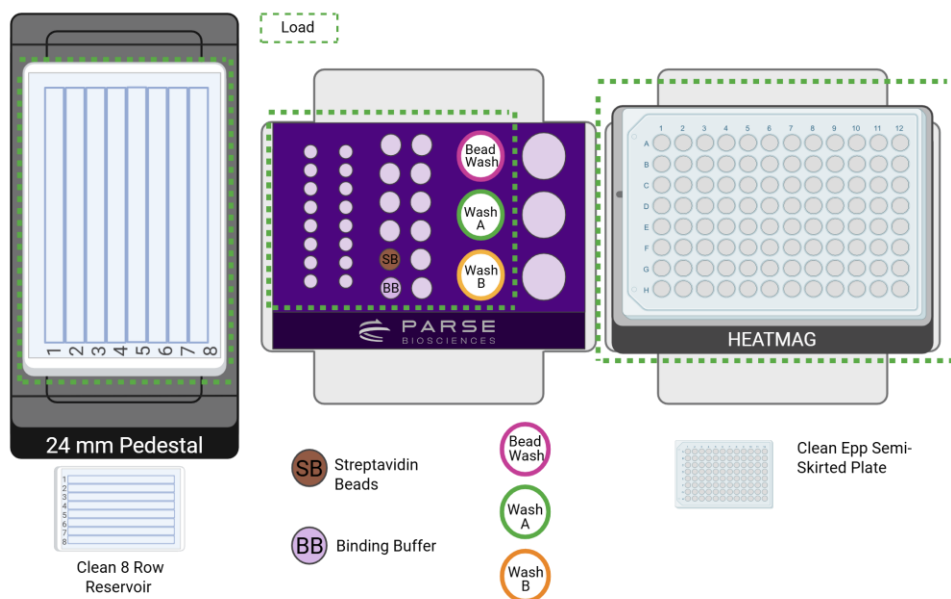
7. Attach D-ONE Pipetting Module 1-Ch, 5-1250 μ L and the corresponding tip rack. Ensure the Tip Deck and tip boxes are securely positioned by pressing them down firmly until they click into place. Remove the tip box lids prior to starting the program.
8. On the 24 mm Pedestal, place a clean INTEGRA 8 Row Reservoir Insert with the 300 mL Reservoir Base with column 1 on the left.
9. Vortex the ● Streptavidin Beads.



Note: Ensure that the Streptavidin Beads are thoroughly mixed before placing them on the deck. Beads can settle if the program isn't started immediately after loading all reagents.

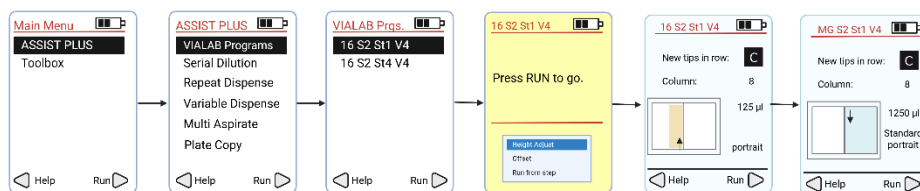
10. Invert Binding Buffer and wash buffers tubes 5 times and briefly centrifuge.
11. Load the following reagents and consumables to their respective positions on the Decks A, B and C:
 - a. Deck A: clean 8 Row Reservoir Insert in a 300mL Reservoir Base.
 - b. Deck B, column 2:
 1. Pos 5: Fully resuspended ● Streptavidin Beads.
 2. Pos 6: ● Binding Buffer.
 - c. Deck B, column 3:
 1. Pos 1: ○ Bead Wash Buffer.

2. Pos 2: ○ Wash Buffer A.
 3. Pos 3: ○ Wash Buffer B.
- d. Deck C: Place a clean Eppendorf 96-well semi-skirted plate on the HEATMAG and label it as the **Reagent Plate**.



12. Remove all reagent tube caps.

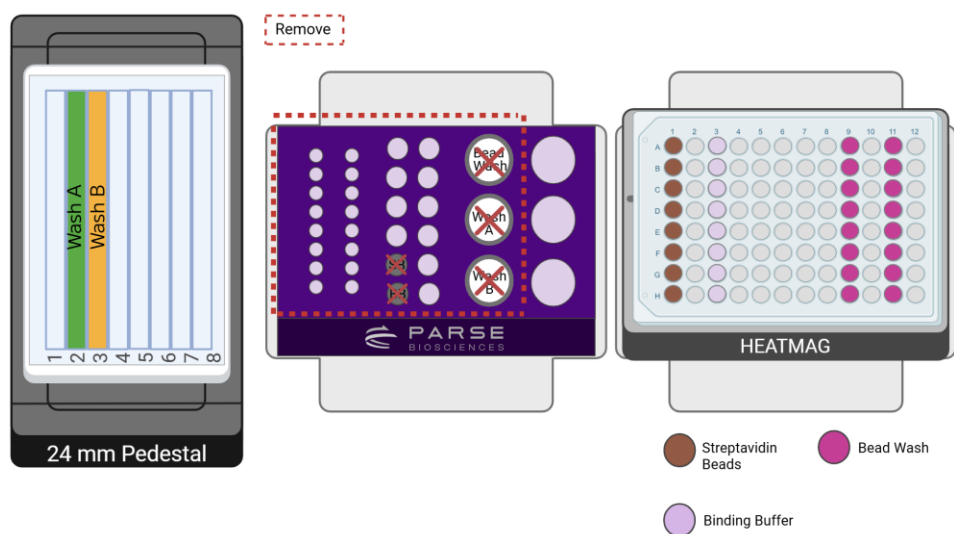
13. On the D-ONE Pipette select and run the program **16 S2 St1 V4_0** following the diagram below.



14. At the conclusion of the run:

- a. Confirm that the Streptavidin Beads are evenly distributed across all wells in column 1 of the **Reagent Plate** on Deck C.
- b. Verify that the Binding Buffer is evenly distributed across all wells in column 3 of the **Reagent Plate** on Deck C.

- c. Verify even Bead Wash Buffer distribution within columns 9 and 11. Columns 9 and column 11 may have different target volumes, but all wells within each column should be equal.
- d. Confirm that the full volume of Wash Buffer A has been transferred to column 2 of the 8-row reservoir.
- e. Confirm that the full volume of Wash Buffer B has been transferred to column 3 of the 8-row reservoir.
- f. Remove the Parse Metal Cold Block from Deck B.
- g. Discard the empty tubes and proceed immediately to Section 2.2.



2.2. cDNA Capture

This program will prepare 16 sublibraries. In these steps Streptavidin Beads are washed, then cDNA is captured with streptavidin-coated magnetic beads. The program controls the temperature and strength of the HEATMAG with 96 Well Adapter on Deck C.

To capture the cDNA:

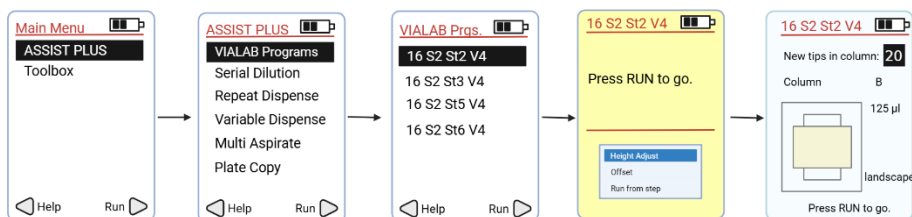
1. Gather the following items and set up the deck as shown.

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VOYAGER Pipetting Module 8-Ch 5-125 µL	INTEGRA Component	1	
Tip Deck for VOYAGER Pipetting Module	INTEGRA Component	1	
125 µL Tips	INTEGRA	1	
Thermochromic PCR Cold Block	Parse	1	Ensure the Thermochromic PCR Cold Block with riser is at room temperature before use.
Thermochromic PCR Cold Block Riser	Parse	1	
Vortex Plate Adapter	Equipment	1	
SealPlate®	Consumables	As needed	

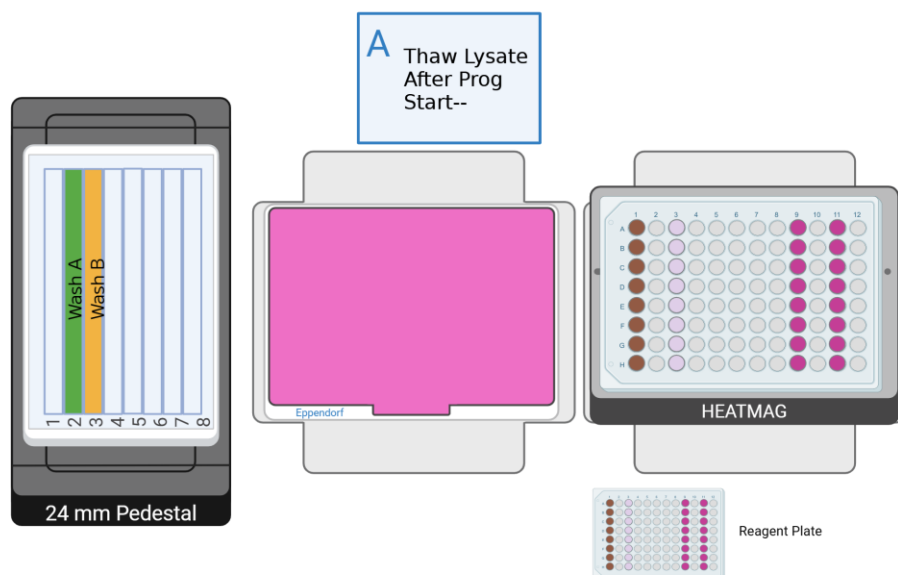
2. Set up the deck following the Deck Configuration below.



3. Load the following:
 - a. Place the room temperature Thermochromic PCR Cold Block with Riser on Deck B with A1 on the top left.
4. If continuing from the previous steps, the following should already be loaded:
 - a. Deck A: 8 Row Reservoir with 300 mL Reservoir Base on the 24 mm Riser
 - i. 8 Row Reservoir with Wash Buffer A and Wash Buffer B in columns 2 and 3.
 - b. Deck C: Place the **Reagent Plate** on the HEATMAG with A1 on the upper left corner.
5. Change the pipette that is connected to the INTEGRA ASSIST PLUS:
 - a. Remove D-ONE Pipetting Module 1-Ch, 5-1250 μ L and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.
 - b. Replace it with VOYAGER 8-Ch 5-125 μ L Pipette and corresponding Tip Deck. Ensure the Tip Deck and Tip Box are securely positioned by pressing them down firmly until they click into place. Remove the tip box lid prior to starting the program.
6. Select and run the program **16 S2 St2 V4_0** following the diagram below.



7. Follow the program prompts for manual intervention:



- a. If lysates are previously frozen, when prompted, incubate the lysates in a thermocycler at 37°C for **5 minutes** to thaw them. Press "Run" on the pipette to continue the program while the lysates are thawing.

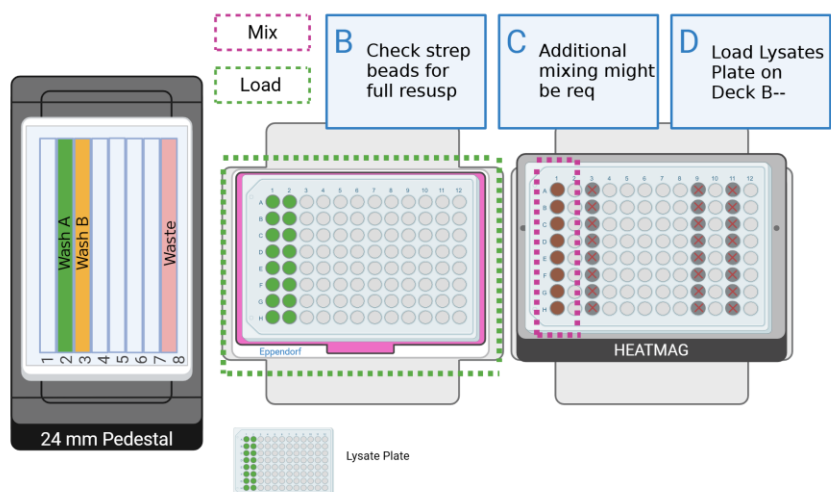
THAW LYSATE		
Run Time	5 minutes	
Lid Temperature	40°C	
Sample Volume	55 µL	
Step	Time	Temperature
1	5 min	37°C
2	Hold	4°C

8. Label the lysate plate as **Lysate Plate** if it has not been labeled already.
9. When the lysates finish thawing, briefly centrifuge **1 minute** at 100 x g at 4°C and store on ice.



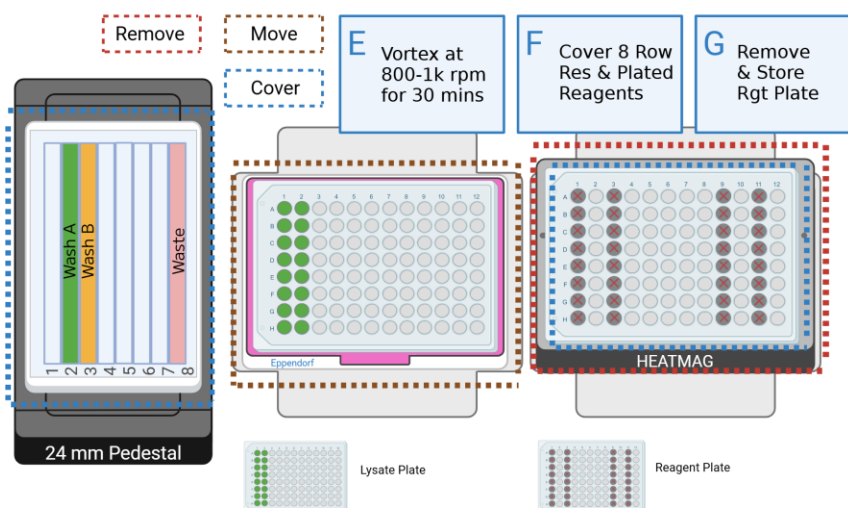
Note: When thawing the lysate, ensure there is no precipitate before proceeding. Otherwise, an additional 5 minutes at 37°C should be sufficient.

10. Follow the program prompts for manual intervention:



- Check streptavidin beads for full resuspension.
- If ● Streptavidin Beads are not fully resuspended in the ● Binding Buffer, seal the plate with foil and mix by vortexing at maximum speed. Briefly centrifuge for **10 seconds** at 100 x g before proceeding. Place back on Deck C, remove the seal, and continue.
- On Deck B, place the **Lysate Plate** onto the room-temperature Eppendorf Thermochromic Cold Block with well A1 positioned in the upper-left corner. Press "Run" to continue.

11. Follow the program prompts for manual intervention:



12. Seal **Lysate Plate** with a foil plate seal.

- e. Vortex at 800-1000 rpm for **30 minutes**.
- f. While the **Lysate Plate** is vortexing, cover the 8 Row Reservoir on Deck A and the **Reagent Plate** on Deck C with plastic seals to reduce contamination.
- g. Remove the **Reagent Plate** and store it at room temperature for later use.



Note: To ensure the beads are being mixed sufficiently, check that the beads are in solution 10 minutes into the vortexing. If settled, increase the vortex mixing speed to keep the beads in solution.

13. Press "Run" to complete the program.

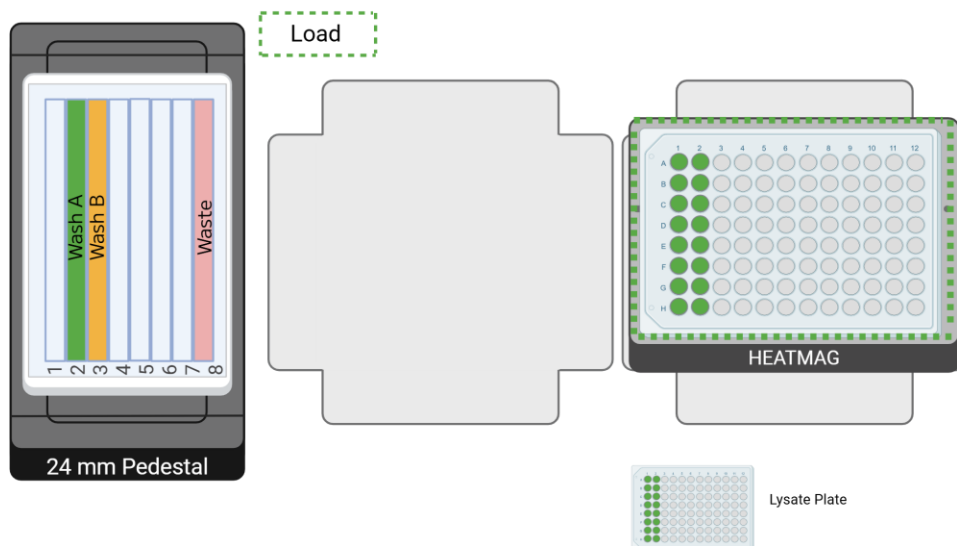
2.3. cDNA-Bound Bead Wash

The streptavidin beads with cDNA samples are washed to remove cellular debris.

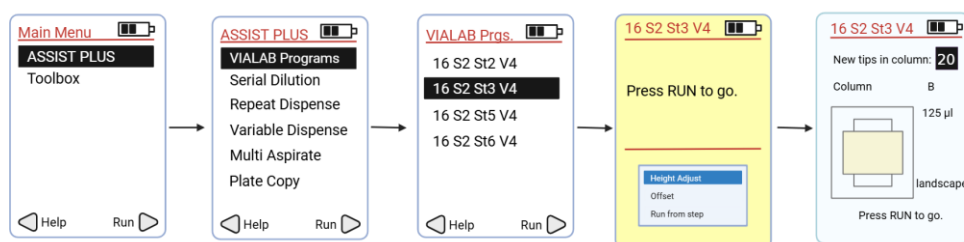
1. Gather the following reagents:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VOYAGER Pipetting 8-Ch 5-125 µL	INTEGRA Component	1	
Tip Deck for VOYAGER Pipetting Module	INTEGRA Component	1	
24 mm Labware Pedestal	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Ensure the Thermochromic PCR Cold Block with riser is at room temperature before use.
Thermochromic PCR Cold Block Riser	Parse	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
300 mL Reservoir Base	INTEGRA Component	1	
125 µL Tip Rack	INTEGRA	1	
SealPlate	Consumables	As needed	

2. After completion of the 30 minute vortexing, briefly centrifuge the captured **Lysate Plate** for **30 seconds** at 100 x g at 4°C.
3. Unseal and place the **Lysate Plate** back on the HEATMAG with 96 Well Adapter on Deck C with A1 on the upper- left corner.
4. Uncover the **8 Row Reservoir** on Deck A. The deck layout should correspond to the configuration below.

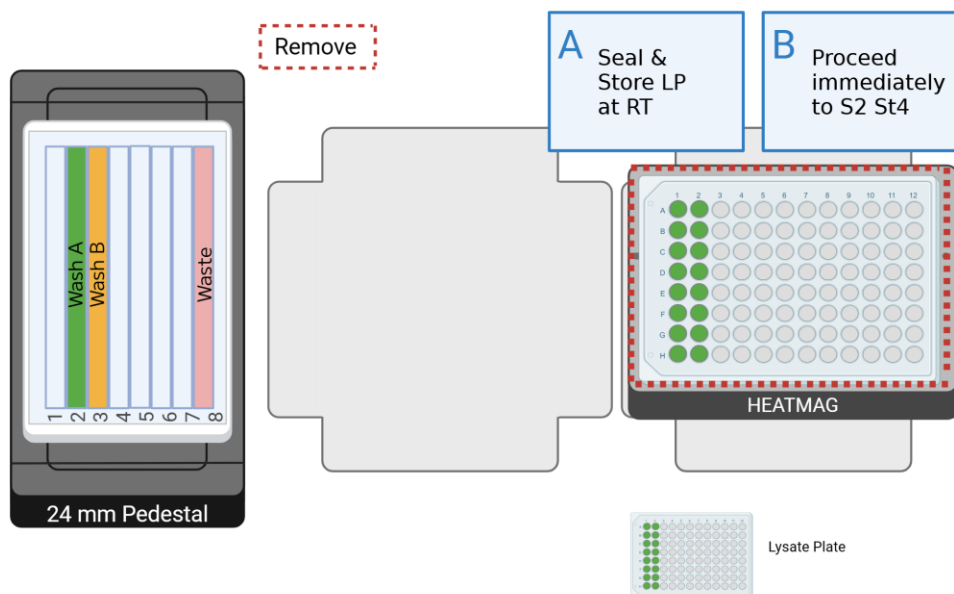


5. Select and run the program **16 S2 St3 V4_0** following the diagram below.



6. At the conclusion of the run:

- Remove the **Lysate Plate**, seal it with a plastic seal, and store it at room temperature for use in later steps.
- Proceed immediately to Section 2.4.



2.4. Master Mixes Preparation

The program prepares the Template Switch Master Mix and the cDNA Amplification Master Mix for the template switch and the cDNA amplification reactions.



CRITICAL! Manually combine the contents of two Template Switch Buffer tubes into one 2 mL tube before loading onto the INTEGRA ASSIST PLUS. This step is required to ensure sufficient master mix volume.

To prepare reagents:

1. Gather the following items and handle as indicated below:

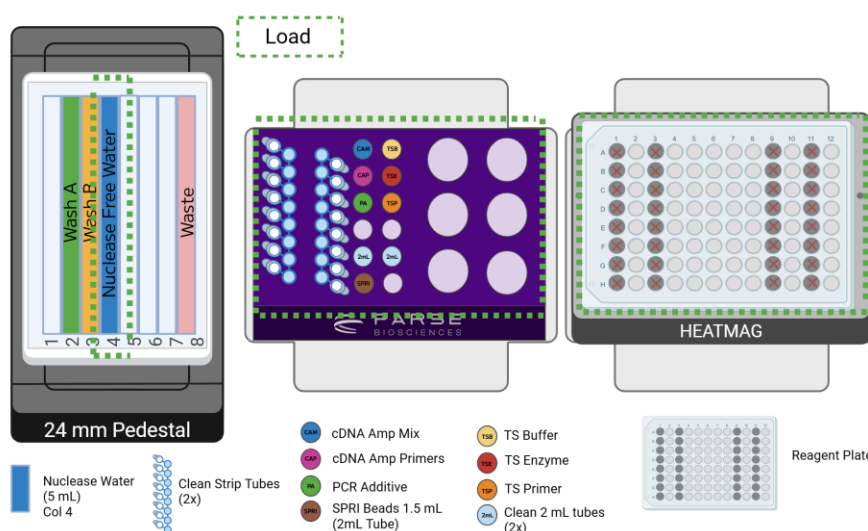
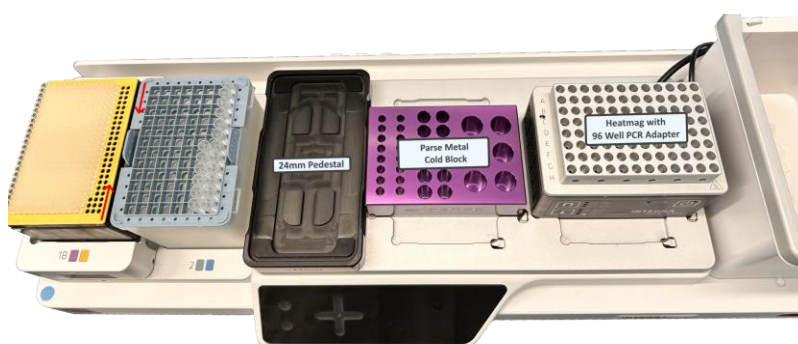
ITEM	SOURCE	QTY	HANDLING AND STORAGE
D-ONE Pipetting Module 1-Ch, 5-1250 μ L	INTEGRA Component	1	
Tip Deck for D-ONE Pipetting Module	INTEGRA Component	1	
Parse Metal Cold Block	Parse	1	
24 mm Labware Pedestal	INTEGRA Component	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
300 mL Reservoir Base	INTEGRA Component	1	
8-count PCR strip tubes	Consumables	2	
2 mL tubes	Consumables	3	
125 μ L Tip Rack	INTEGRA	1	
1250 μ L Tip Rack	INTEGRA	1	
● Template Switch Buffer	-20°C Reagents	2	Thaw at room temperature then place on ice. Mix by inverting 3x. Briefly centrifuge before use. We recommend combining them in a 2 mL tube.
● Template Switch Primer	-20°C Reagents	1	Thaw at room temperature then place on ice. Mix by inverting 3x. Briefly centrifuge before use.

ITEM	SOURCE	QTY	HANDLING AND STORAGE
● Template Switch Enzyme	-20°C Reagents	1	Keep on ice. Briefly centrifuge before use.
● cDNA Amp Mix	-20°C Reagents	1	Thaw at room temperature then place on ice. Mix by inverting 3x. Briefly centrifuge before use.
● cDNA Amp Primers	-20°C Reagents	1	
● PCR Additive	-20°C Reagents	1	
SPRI Beads	Reagents	As needed	Equilibrate at room temperature
Nuclease-free water	Reagents	As needed	



Note: Ensure that there is no precipitate in the ● Template Switch Buffer before proceeding.

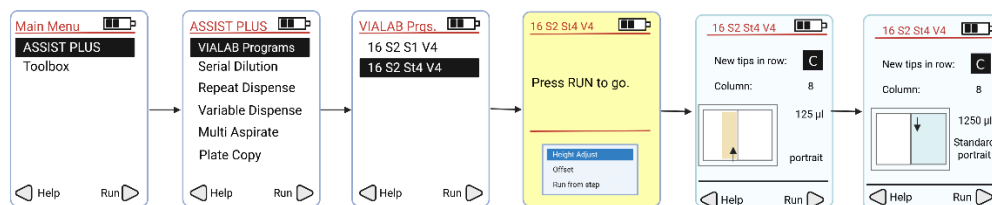
- Set up the deck following the Deck Configuration below.



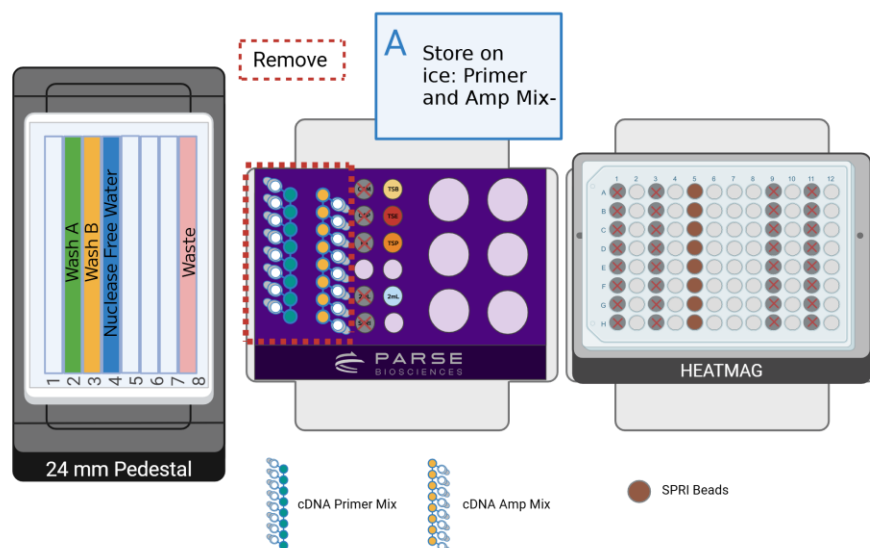
3. Deck A:
 - a. Fill column 4 of the 8-row reservoir with 5 mL of nuclease-free water.
4. On Deck B, on the Parse Metal Cold Block, place the following consumables and reagents using the deck configuration below:
 - a. Column 1 left and right: two clean 8-count PCR strip tubes with the following labels:
 - i. Left: **cDNA Primer Mix**
 - ii. Right: **cDNA Amp Mix**
 - b. Column 2:
 - i. Pos 1: ● cDNA Amp Mix.
 - ii. Pos 2: ● cDNA Amp Primers.
 - iii. Pos 3: ● PCR Additive.
 - iv. Pos 5: a clean 2 mL tube.
 - v. Pos 6: 1.5 mL of SPRI Beads (2 mL tube).
 - vi. Pos 7: ● Template Switch Buffer (combine two supplied TS Buffers in a 2 mL tube).
 - vii. Pos 8: ● Template Switch Enzyme.
 - viii. Pos 9: ● Template Switch Primer.
 - ix. Pos 11: a clean 2 mL tube.
5. Deck C: Remove the seal from the **Reagent Plate**, then place the plate on the HEATMAG with the 96-well PCR adapter, with well A1 positioned in the upper-left corner.
6. Change the pipette that is connected to the INTEGRA ASSIST PLUS:
 - a. Remove the VOYAGER 8-Ch 5-125 µL Pipette and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.
 - b. Replace it with D-ONE Pipetting Module 1-Ch, 5-1250 µL and corresponding Tip Deck. Ensure the tip deck and tip boxes are securely positioned by pressing them

down firmly until they click into place. Remove the tip box lids prior to starting the program.

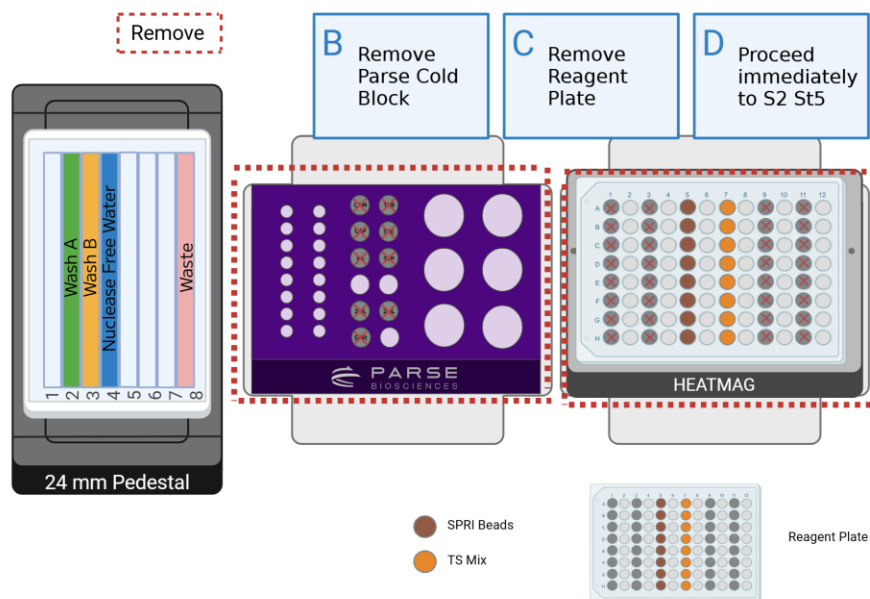
7. Remove the reagent caps, select and run the program **16 S2 St4 V4_0** following the diagram below.



8. Press "Run" to continue the program. Follow the program prompts for manual intervention:



- a. Remove, cap, and store cDNA Primer Mix and cDNA Amp Mix on ice for later use. Press "Run" to continue.
9. At the conclusion of the run:
 - b. Remove the **Reagent Plate** on Deck C and place on ice. It will be used in the next step.
 - i. Verify visually that the SPRI bead volume in column 5 of the **Reagent Plate** is even (~ 160 µL per well).
 - ii. Verify visually that the Template Switch Master Mix volume in column 7 of the **Reagent Plate** is even (~ 210 µL per well).



- c. Remove the Parse Metal Cold Block on Deck B and discard used 1.5 mL and 2 mL reagent tubes, column 2.
- b. Proceed immediately to Section 2.5.

2.5. Template Switch and cDNA Amplification

The Template Switch Master Mix is added to the captured cDNA and incubated for 30 minutes. The template switch reaction adds a 5' adapter to the cDNA.

To perform template switch:

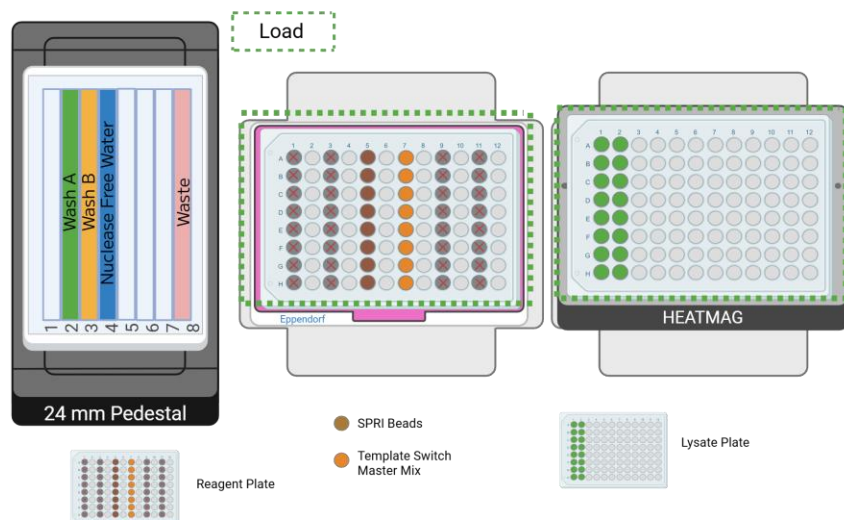
1. Gather the following items and set up the deck indicated:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VOYAGER 8-Ch 5-125 μ L	INTEGRA Component	1	
Tip Deck for VOYAGER Pipetting Module	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Ensure the Thermochromic PCR Cold Block with riser is at room temperature before use.
Thermochromic PCR Cold Block Riser	Parse	1	
24 mm Labware Pedestal	INTEGRA Component	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
300 mL Reservoir Base	INTEGRA Component	1	
125 μ L Tip Rack	INTEGRA	1	
SealPlate	Consumables	As needed	

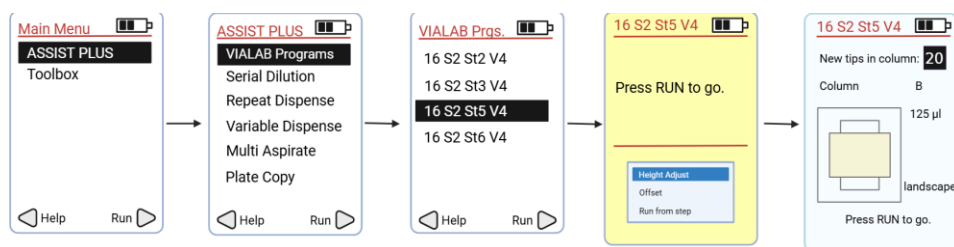
2. Set up the deck following the Deck Configuration below.



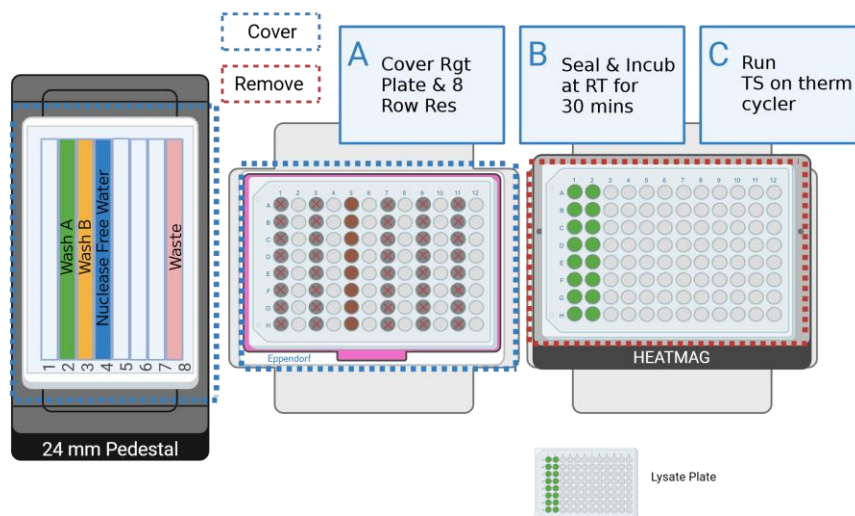
3. Ensure the following are loaded following the deck configuration below:



- Deck B: **Reagent Plate** on a room temperature Thermochromic PCR Cold Block with Riser with A1 on the upper-left corner.
 - Deck C: **Lysate Plate** on the HEATMAG with 96 Well PCR Adapter with A1 on the upper-left corner.
4. Change the pipette that is connected to the INTEGRA ASSIST PLUS:
- Remove D-ONE Pipetting Module, 5-1250 μ L and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.
 - Replace it with VOYAGER 8-Ch 5-125 μ L Pipette and corresponding Tip Deck. Ensure the Tip Deck and Tip Box are securely positioned by pressing them down firmly until they click into place. Remove the tip box lid prior to starting the program.
5. On the VOYAGER Pipette select and run the program **16S2 St5 V4_0** following the diagram below.



6. Press "Run" to continue the program. Follow the program prompts for manual intervention:

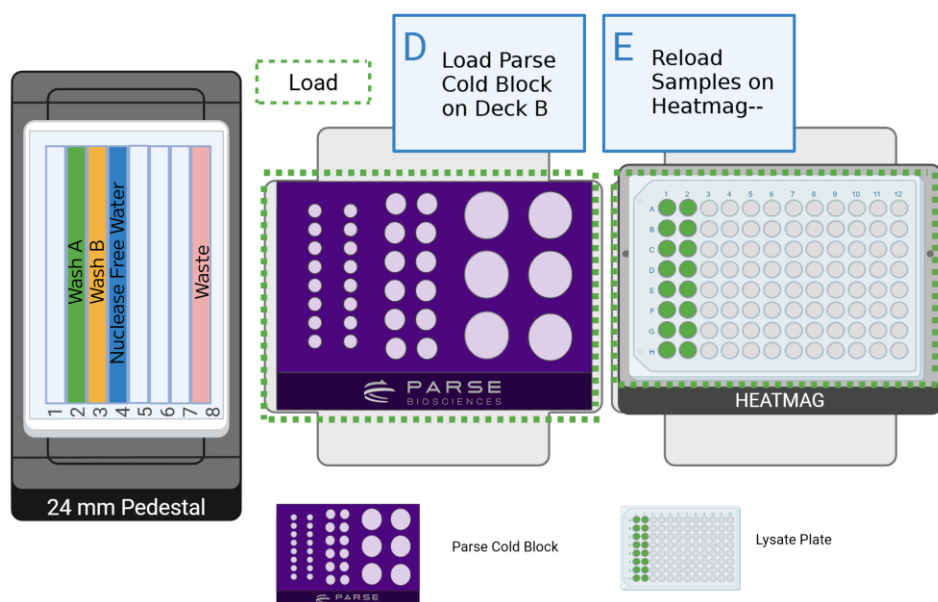


- Cover the **Reagent Plate** on Deck B and 8 Row Reservoir on Deck A with plastic seals to prevent contamination.
- Seal with a plastic seal and incubate the **Lysate Plate** on Deck C at room temperature for **30 minutes**.
 - After the 30 minute incubation, visually inspect the **Lysate Plate** for settled beads. If beads have settled, manually resuspend the samples by mixing 5x with a multichannel P200 pipette set to 80 μ L.
 - Reseal the **Lysate Plate** with a plastic plate seal.

- c. Place the **Lysate Plate** into a thermocycler. Run the following program.

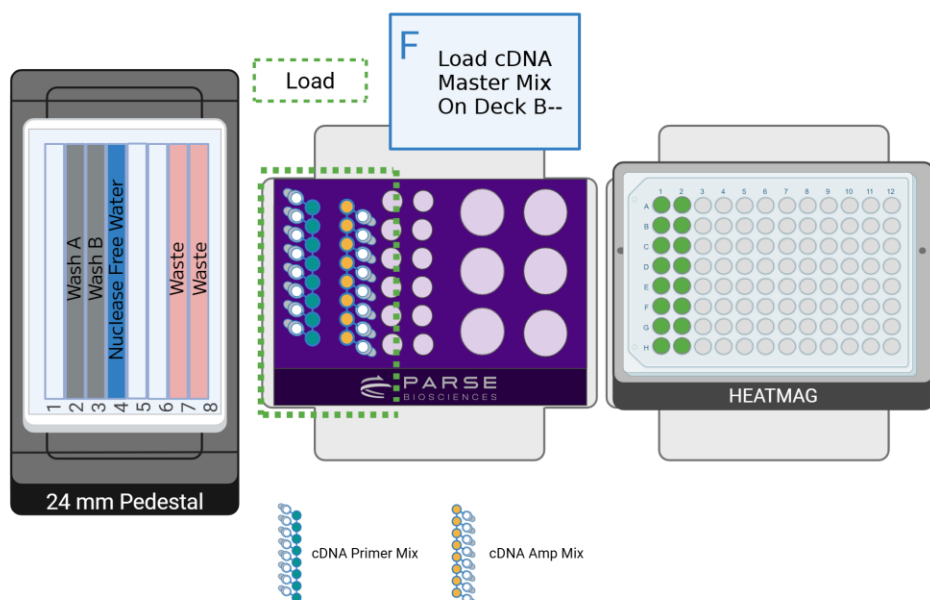
TEMPLATE SWITCH		
Run Time	60 minutes	
Lid Temperature	70°C	
Sample Volume	100 µL	
Step	Time	Temperature
1	60 min	42°C
2	Hold	4°C

7. While the Template Switch thermocycler is running, press "Run" on the pipette to continue the program on the Assist Plus. Follow the program prompts for manual intervention:

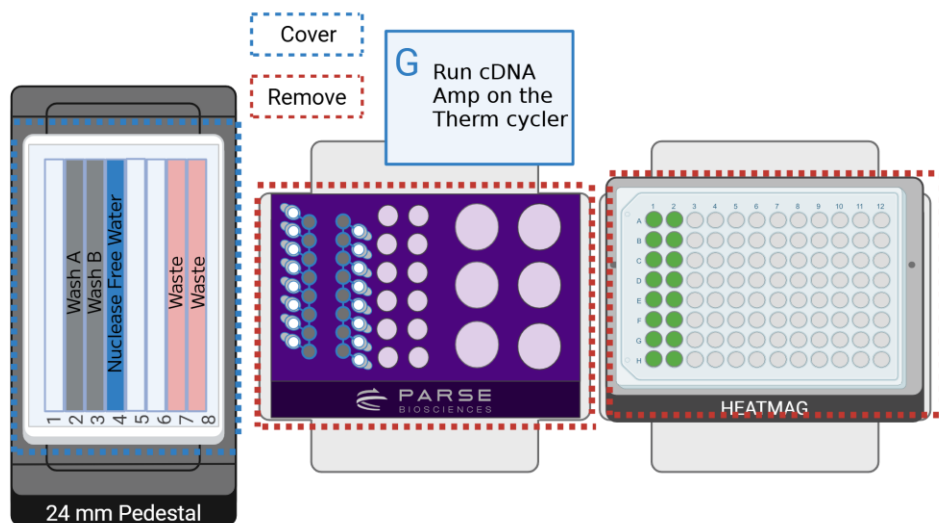


- d. Remove the Thermochromic Cold Block and load the Parse Metal Cold Block on Deck B with the PCR tubes on the left.
- e. When the Template Switch thermocycling program has completed:
- i. Remove the plate seal from the 8 Row reservoir on Deck A.

- ii. Reload the **Lysate Plate** on the HEATMAG on Deck C with A1 in the upper-left corner. Remove the plate seal.
8. Press "Run" to continue the program. Follow the program prompts for manual intervention:



- f. On Deck B, Column 1:
 - i. Left: uncap and load **cDNA Primer Mix**.
 - ii. Right: uncap and load **cDNA Amp Mix**.
9. Press "Run" to continue the program. Follow the program prompts for manual intervention:



10. Remove and seal the **Lysate Plate** with a plastic seal on Deck C.
11. Use the guidelines below to amplify the cDNA samples on the **Lysate Plate**.
12. Determine the number of PCR cycles required for cDNA amplification based on the table below. Although these recommendations are appropriate for many cell types, the number of cycles may need to be optimized for each sample type.

NUMBER OF PCR CYCLES			
Cells/nuclei in the Sublibrary	High RNA content such as cell lines	Low RNA content such as PBMCs	Nuclei
200-1,000	11	13	12
1,000-2,000	9	11	10
2,000-4,000	7	9	8
4,000-8,000	6	8	7
8,000-16,000	5	7	6
16,000-32,000	4	6	5
32,000-64,000	3	5	4

cDNA AMPLIFICATION			
Run Time		50-70 minutes	
Lid Temperature		105°C	
Sample Volume		100 µL	
Step	Time	Temperature	Cycle
1	3 min	95°C	1
2	20 sec	98°C	5
3*	45 sec	65°C*	
4	3 min	72°C	
5	20 sec	98°C	Variable, see above
6*	20 sec	67°C*	
7	3 min	72°C	
8	5 min	72°C	1
9	Hold	4°C	1



CRITICAL! If processing sublibraries with different numbers of cells or nuclei, amplify them in separate thermocyclers according to the recommendations above. Transfer samples requiring higher cycle numbers to a separate plate, maintaining the same well positions. After thermal cycling, transfer the samples back into their corresponding wells in the original **Lysate Plate**.



Note: Annealing steps 3* and 6* have different time and temperature settings. Ensure these are correct before starting the program.

13. While the thermocycler program is running:

- Remove the Parse Metal Cold Block and discard used strip tubes on Deck B.
- Cover the 8 Row Reservoir with a plastic seal during this time to reduce contamination.
- If not stopping, proceed to Section 2.6 while the cDNA Amp thermocycler program is running.



Safe stopping point: **Amplified cDNA** can be stored at 4°C for up to 18 hours. Covered 8 Row Reservoirs and the **Reagent Plate** can also be stored at room temperature for up to 18 hours.

2.6. Post-Amplification Purification

Amplified cDNA is purified with a 0.8x SPRI bead cleanup.

To purify the cDNA:

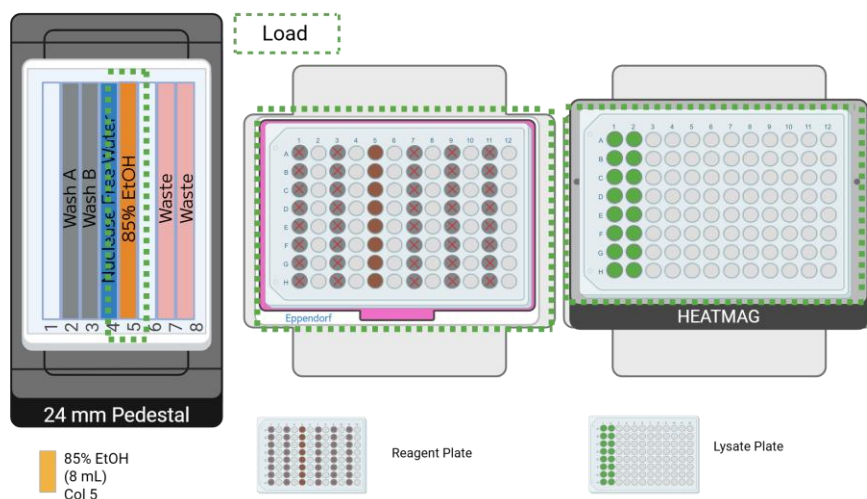
1. Gather the following items and set up the deck as indicated:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VOYAGER 8-Ch 5-125 μ L	INTEGRA Component	1	
Tip Deck for VOYAGER Pipetting Module	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Ensure the Thermochromic PCR Cold Block with riser is at room temperature before use.
Thermochromic PCR Cold Block Riser	Parse	1	
24 mm Labware Pedestal	INTEGRA Component	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
300 mL Reservoir Base	INTEGRA Component	1	
125 μ L Tip Rack	INTEGRA	1	
96 well Semi-Skirted Plate	Consumables	1	
SealPlate®	Consumables	As needed	
TempPlate Sealing® Foil	Consumables		
Ethanol, 85%	Reagents	1	Prepare 85% Ethanol by using 200 proof Ethanol and nuclease-free water. Make fresh the day it is needed.
Nuclease free water	Reagents	1	

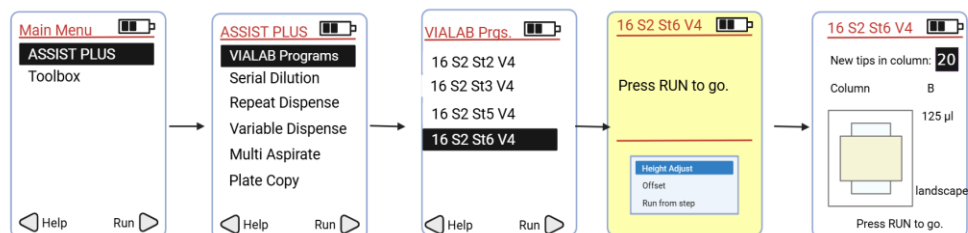
- Set up the deck following the Deck Configuration below.



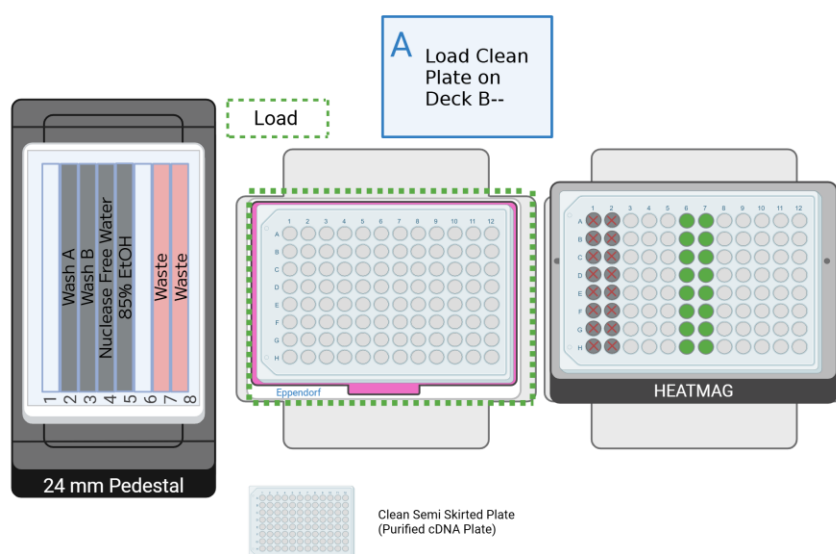
- Prepare at least **8 mL** 85% ethanol with nuclease-free water.
- Remove the seal and add **8 mL** 85% ethanol in column **5** of the 8 Row Reservoir on Deck A.
- Remove the seal and load the **Reagent Plate** onto the room temperature Thermochromic PCR Cold Block with Riser on Deck B, with well A1 positioned in the upper-left corner.
 - Visually inspect the Reagent Plate for settled SPRI beads. If beads have settled, manually resuspend the beads by mixing 5x with a multichannel P200 pipette set to 60 μ L.
- Once the cDNA Amplification thermocycler program is complete, unseal and load the **Lysate Plate** on the HEATMAG with the 96-well PCR adapter on Deck C, with well A1 positioned in the upper-left corner.



7. Select and run program **16S2 St6 V4_0** following the diagram below.

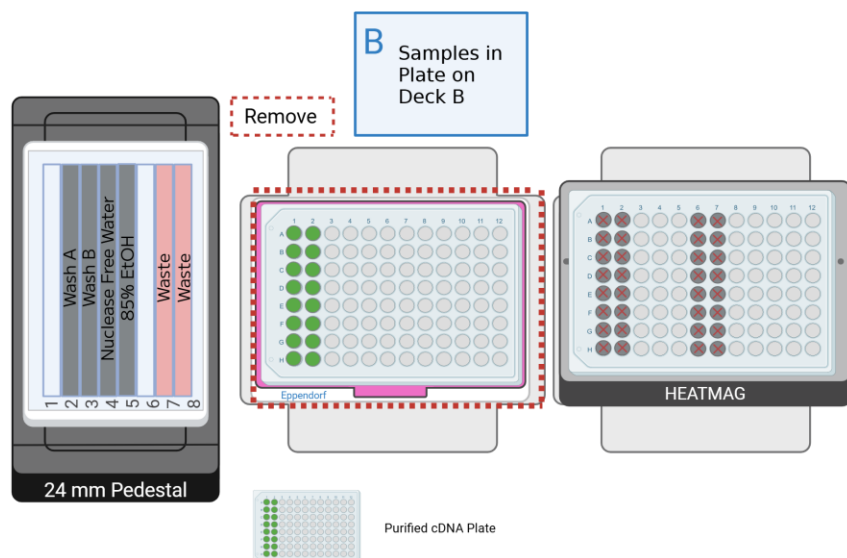


8. Press "Run" to continue the program. Follow the program prompts for manual intervention:



- Remove the **Reagent Plate** from Deck B.
- Load a clean Eppendorf semi-skirted plate labeled "**Purified cDNA**" onto the room temperature Thermochromic PCR Cold Block with Riser on Deck B.
- Press "Run" to continue the program.

9. At the conclusion of the run:



10. The **Purified cDNA** will be on Deck B. Seal the plate with a foil plate seal, then add a plastic plate seal on top of the foil plate seal. Alternatively, proceed directly to Section 2.7 to quantify the cDNA before continuing to Section 3. Seal the **Purified cDNA** plate after taking aliquots to QC.



Safe stopping point: **Purified cDNA** can be stored at 4°C for up to 48 hours or at -20°C for up to 3 months.



CRITICAL! In Section 3, you will need two ThermoChromic PCR Cold Blocks with ThermoChromic PCR Cold Block Risers. One block should remain cold throughout the process, while the other should be at room temperature. We recommend either leaving one block at room temperature overnight to thaw before starting, or running it under warm water to thaw quickly on the morning of Section 3.

2.7. cDNA Quantification

The concentration and size distribution of the cDNA are measured with fluorescent dyes and capillary electrophoresis. The cDNA is then stored at 4°C for up to 48 hours or at -20°C for up to 3 months.

To quantify the cDNA:

1. Measure the concentration of each tube of purified cDNA from Section 2.6 with the Qubit dsDNA HS (High Sensitivity) Assay Kit according to the manufacturer's instructions. Record the concentrations, which will be used in Section 3.
2. Assess the size distribution of each tube of purified cDNA with a High Sensitivity DNA Kit on the Agilent Bioanalyzer System or High Sensitivity D5000 ScreenTape and Reagents on the Agilent TapeStation System according to the manufacturer's instructions.



Safe stopping point: purified cDNA can be stored at 4°C for up to 48 hours or at -20°C for up to 3 months. Otherwise, proceed immediately to Section 3.

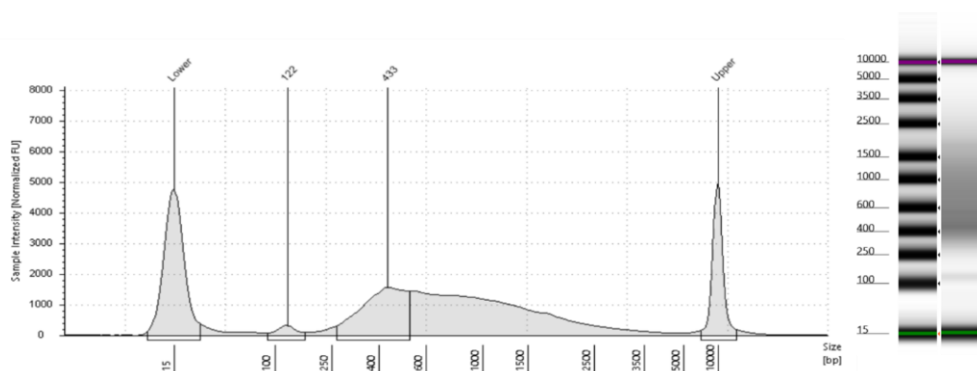


Figure 1: Expected post-amplification sublibrary cDNA size distribution. Example trace of Human cDNA run on a TapeStation.

Note: Samples may need to be diluted to be within the manufacturer's recommended concentration range. Typically, between a 1:3 to 1:10 dilution or 3-5 ng are appropriate.



Note: The traces above are representative of typical TapeStation cDNA traces. The shape and prominence of the trace is dependent on cell type, sublibrary size, and amount of DNA loaded into the TapeStation. Sublibraries with minor deviations can still produce high quality data.

Section 3: Sequencing Library Preparation

3.0. cDNA Normalization (Optional)

Prior to starting Section 3, cDNA can optionally be normalized to ensure that all sublibraries fall within the same amplification condition. It is advantageous to have all sublibraries use the same amplification condition to remove the need for splitting between multiple thermal cyclers during amplification. The program uses nuclease free water on Deck A to normalize amplified cDNA on Deck B.

If choosing NOT to normalize amplified cDNA:

Manually prepare Diluted cDNA in columns 1 and 2 of a Semi-Skirted 96 Well PCR Plate as follows to a final volume of 35 μ L and store on ice. Label this plate as your cDNA Sample plate and store on ice.

DILUTED cDNA	
Purified cDNA	10 μ L
Nuclease-free water	25 μ L
Total Volume	35 μ L

1. The remaining cDNA samples from the **Purified cDNA** plate can be stored at -20°C . Seal the plate with a foil plate seal, add a plastic plate seal on top of the foil plate seal, then place at -20°C .

If choosing to normalize amplified cDNA:

1. Gather the following items and handle as indicated below:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
D-ONE Pipetting Module 1-Ch, 5-1250 μ L	INTEGRA Component	1	
Tip Deck for D-ONE Pipetting Module	INTEGRA Component	1	
Parse Metal Cold Block	Parse	1	
Thermochromic PCR Cold Block	Parse	1	Pull the Thermochromic PCR Cold Block with riser from

ITEM	SOURCE	QTY	HANDLING AND STORAGE
Thermochromic PCR Cold Block Riser	Parse	1	the -20°C freezer and leave them at room temperature for 10 minutes prior to use.
24 mm Labware Pedestal	INTEGRA Component	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
Semi-Skirted 96 Well PCR Plate	Consumables	1	
125 µL Tip Rack	INTEGRA	1	
1250 µL Tip Rack	INTEGRA	1	
2 mL tube	Consumables	1	
SealPlate®	Consumables	As needed	
TempPlate Sealing® Foil	Consumables		
Nuclease free water	Reagents		

2. If frozen, thaw the **Purified cDNA** plate and store it on ice.
3. Download the Parse Biosciences Evercode WT Mega v4 Integra Normalization file. The most current version can be found on the [Parse Biosciences Customer Support Suite](#).
4. Obtain recorded cDNA concentrations from Section 2.7.
5. Fill out the following cells of the Parse Biosciences Evercode Mega/WT Integra Normalization v4.0.xlsm. Target sample volume should be around 10 µL.
 - a. Sample
 - b. Source Well
 - c. Concentration (ng/µL)
 - d. Library Input (ng)



Note: If any parameter is out of range, the cells will turn pink in color and/or an error message will appear. Be sure to address and adjust worksheet input values appropriately before continuing.

Parse biosciences
700 Dexter Ave
Suite 600
Seattle, WA 98109



Support Suite: support.parsebiosciences.com
Email: support@parsebiosciences.com
WT Mega - Version 2.0

Evercode WT cDNA Normalization Loading Table

- For more details on using this cDNA Normalization Loading Table, see the Important Guidelines section of the User Manual

This sheet should be filled out prior to starting Section 3.

Step	Instructions
1	Ensure Macros are enabled.
4	Input your sample names.
6	Input source wells location.
6	Input destination well location.
6	Input cDNA concentration in ng/μL.
7	Input total ng library prep input.
8	CRITICAL: Ensure Sample Volume (μL) is between 4 to 25 μL. Larger sample volume leads to higher sublibrary complexity.
9	Open the "Integra Loading Table" sheet. Click on the "Generate a cDNA Normalization Worklist for Import into VIALAB" to generate the worklist file.

Number of PCR Cycles	
cDNA Input (ng)	PCR Cycles
10 - 24	13
25 - 49	12
50 - 99	11

Number of PCR Cycles	
cDNA Input (ng)	PCR Cycles
100 - 299	10
300 - 999	8
1,000 or more	7

Sample	Source Well	Destination Well	Conc. (ng/μL)	Library Input (ng)	Sample Volume (μL)	Diluent Volume (μL)
a	A1	A1	10.28	339	33.0	2.0
b	B1	B1	9.64	100	10.4	24.6
c	C1	C1	3.96	100	25.3	9.7
d	D1	D1	7.26	100	13.8	21.2
e	E1	E1	3.82	100	26.2	8.8
f	F1	F1	8.78	100	11.4	23.6
g	G1	G1				
h	H1	H1				
i	A2	A2	7.74	100	12.9	22.1
j	B2	B2	7.02	100	14.2	20.8
k	C2	C2	5.18	100	19.3	15.7

Figure 2: Evercode WT Mega cDNA normalization loading table.

- Navigate to the "INTEGRA Loading Table" tab on the excel sheet, and click on "Generate a cDNA Normalization Worklist for Import". Save the generated CSV file (called Section3NormMGWorksheet_xxxxxxx_xxxxxxx.csv) (Figure 3).

SampleID	SourceDeckPosition	SourceWell	TargetDeckPosition	TargetWell	TransferVolume (μL)	Tip Type
a	B2	F2	C1	A1	2	125
b	B2	F2	C1	B1	24.6	125
c	B2	F2	C1	C1	9.7	125
d	B2	F2	C1	D1	21.2	125
e	B2	F2	C1	E1	8.8	125
f	B2	F2	C1	F1	23.6	125
g	B2	F2	C1	A2	22.1	125
h	B2	F2	C1	B2	20.8	125
i	B2	F2	C1	C2	15.7	125
j	B2	F2	C1	D2	27.7	125
k	B1	A1	C1	A1	33	125
b	B1	B1	C1	B1	10.4	125
c	B1	C1	C1	C1	25.3	125
d	B1	D1	C1	D1	13.8	125
e	B1	E1	C1	E1	26.2	125
f	B1	F1	C1	F1	11.4	125
g	B1	A2	C1	A2	12.9	125
h	B1	B2	C1	B2	14.2	125
i	B1	C2	C1	C2	19.3	125
j	B1	D2	C1	D2	7.3	125

Generate a cDNA
Normalization
Worklist for Import

Figure 3: Generated cDNA normalization worklist.

- Open the VIALAB program **16 S3 St0 V4_0 DONE** and navigate to the "Method" section. In the "O2 Worklist", under the "Worklist and Volumes" tab, import the "Section3Norm16Worksheet_xxxxxxx_xxxxxxx.csv" worklist file generated in the previous step. The "Import" button is located in the upper left of the Worklist and Volumes tab (Figure 4).

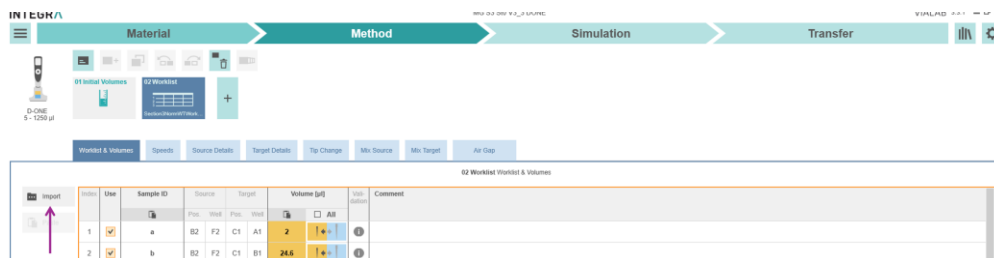
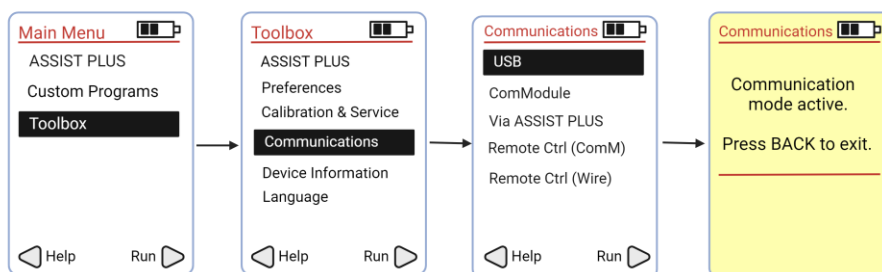


Figure 4: VIALAB worklist generation for diluent volumes using O2 Worklist.

8. Save and transfer your program to your D-ONE Pipetting Module (1-Ch, 5-1250 µL) as follows:
 - a. Connect your computer with the D-ONE Pipette using Communication/Charging.
 - b. Follow the instructions on the pipette menu, as shown in the diagram below:



- c. In the VIALAB software on your computer, select "Transfer".
- d. Confirm that the pipette has been recognized by the software. Click "Send to pipette". An audible signal will indicate the successful upload of the **16 S3 St0 V4_0** program to the D-ONE Pipette.
- e. Disconnect the cable and proceed with the protocol on the INTEGRA ASSIST PLUS platform.
- f. A program named **16 S3 St0 V4_0** will be found on your pipette.



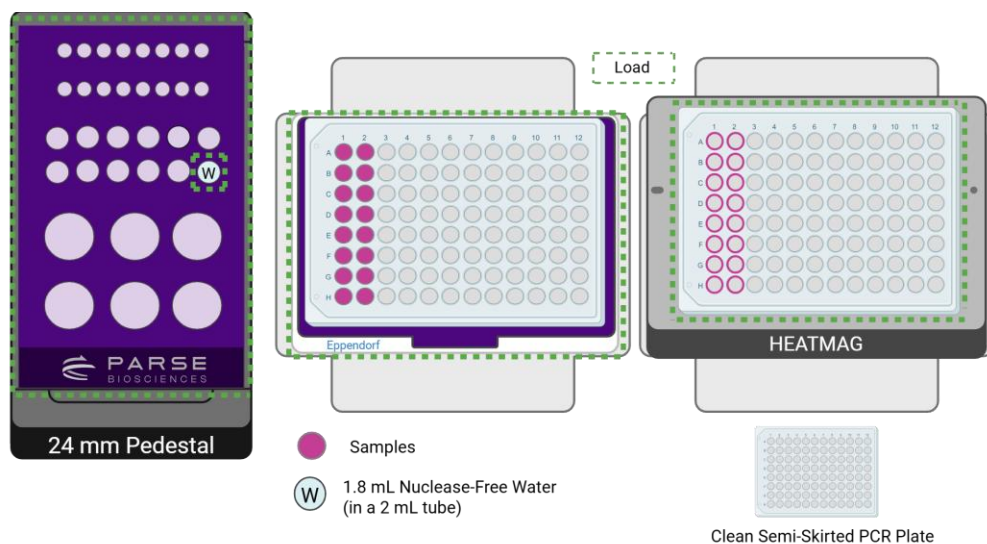
CRITICAL! Delete any previous scripts from the D-ONE pipette to avoid running incorrect scripts.

9. Set up the deck following the deck configuration below.



10. Set up the deck layout following the Deck Configuration below:

- a. Deck A: Parse Metal Cold Block on 24mm Labware Pedestal
 - i. Column 2, position 7: **1.8 mL** nuclease-free water in a 2 mL tube
- b. Deck B: Briefly vortex and centrifuge the **Purified cDNA** plate, remove the seal, and place it on the Thermochromic PCR Cold Block with A1 in the upper left corner, pressing firmly to ensure the plate is fully seated.
- c. Deck C: Place a clean semi-skirted plate on the HEATMAG with 96 Well Adapter with A1 in the upper left corner, pressing firmly to ensure the plate is fully seated. Label the plate as **cDNA Sample** plate.

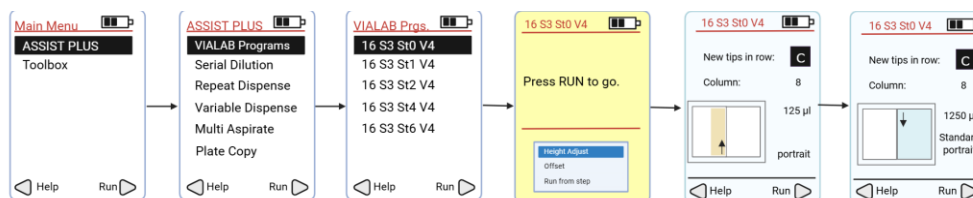


11. If needed, change the pipette:

- a. Remove the VOYAGER 8-Ch 5-125 μ L Pipette and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.

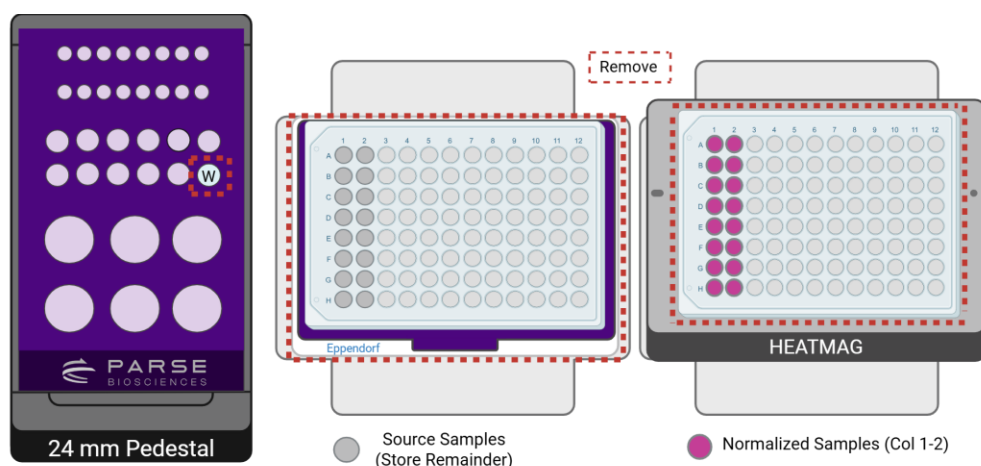
- b. Replace it with D-ONE Pipetting Module 1-Ch, 5-1250 µL Pipette and corresponding Tip Deck. Ensure the tip deck and tip boxes are securely positioned by pressing them down firmly until they click into place. Remove the tip box lids prior to starting the program.

12. Select and run program **16 S3 St0 V4_0** following the diagram below.



13. At the conclusion of the run:

- a. The remaining cDNA samples from the **Purified cDNA** plate on Deck B can be stored at -20°C. Seal the plate with a foil plate seal, add a plastic plate seal on top of the foil plate seal, then place at -20°C.
- b. Seal the **cDNA Sample** plate on Deck C with a plastic plate seal and store on ice. It will be used in Section 3.2.



3.1. SPRI Bead Plating

The SPRI beads are aliquoted to be used throughout all of Section 3. Aliquots can then be quickly accessed via multichannel pipette when needed. SPRI beads should be kept at room temperature.

1. Gather the following components and reagents:

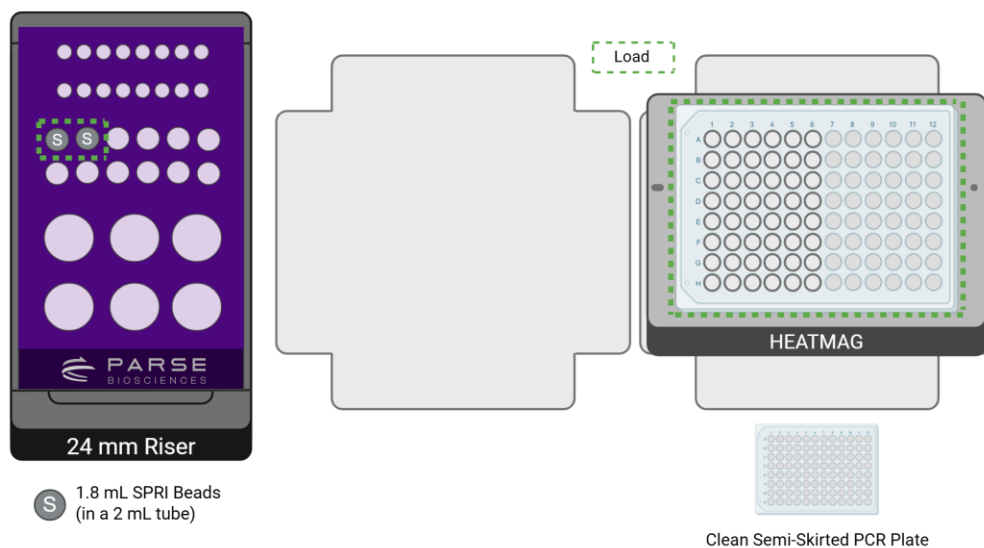
ITEM	SOURCE	QTY	HANDLING AND STORAGE
D-ONE Pipetting Module 1-Ch, 5-1250 μ L	INTEGRA Component	1	
Tip Deck for D-ONE Pipetting Module	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Pull the Thermochromic PCR Cold Block with riser from the -20°C freezer and leave them at room temperature for 10 minutes prior to use.
Thermochromic PCR Cold Block Riser	Parse	1	
Parse Metal Cold Block	Parse	1	
24 mm Labware Pedestal	INTEGRA Component	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
Semi-Skirted 96 Well PCR Plate	Consumables	1	
2 mL tube	Consumables	2	
125 μ L Tip Rack	INTEGRA	1	
1250 μ L Tip Rack	INTEGRA	1	
SealPlate	Consumables	As needed	
SPRI Beads	Reagents		

2. If not connected already, connect the D-ONE Pipetting Module 1-Ch, 5-1250 μ L Pipette and corresponding Tip Deck. Ensure the tip deck and tip boxes are securely positioned by pressing them down firmly until they click into place. Remove the tip box lids prior to starting the program.
3. Load the 24 mm Labware Pedestal on Deck A.

4. If not connected already, connect the HEATMAG on Deck C.
5. Move the Parse Metal Cold Block onto the 24 mm Labware pedestal on Deck A. The deck layout should correspond to the configuration below:



6. Ensure the following are loaded, using the deck configuration below:
 - a. Deck A column 1:
 - i. Pos 5 and 6: two 2 mL tubes with **1,800 μ L** fully resuspended SPRI beads each. Visually inspect the 2 mL tube for settled SPRI beads. If beads have settled, manually resuspend the beads by mixing 5x with a multichannel P1000 pipette set to 1000 μ L prior to loading them on the Parse Metal Cold Block.
 - b. Deck C: clean semi-skirted 96 well PCR plate.



7. Select and run the program **16 S3 St1 V4_0** following the diagram below.

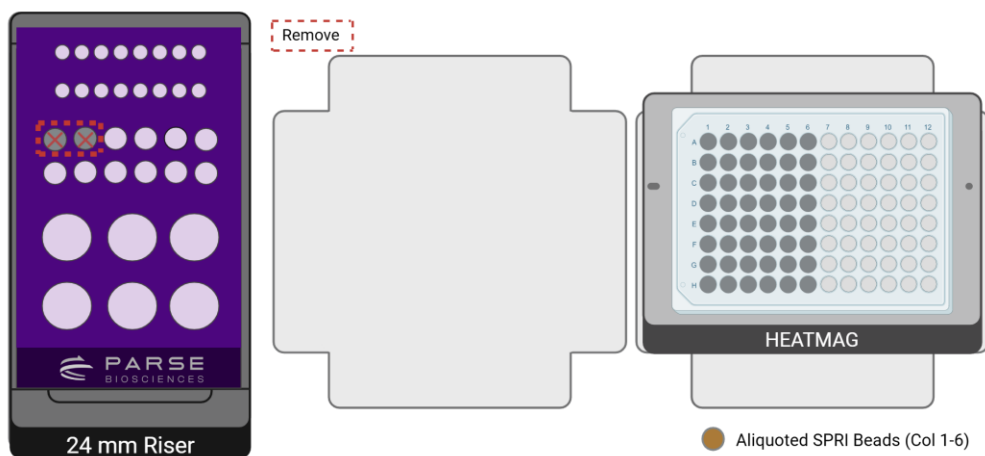


8. Wait for the program to complete.

9. After pressing "Run" remove a Thermochromic PCR Cold Block with riser from the -20°C to thaw at room temperature for 10 minutes. This will be used in Section 3.2.

10. At the conclusion of the run:

- Leave the D-ONE pipette connected to the instrument.
- Label the 96-well plate on Deck C as **Reagent Plate S3**. If proceeding immediately to Section 3.2, leave the **Reagent Plate S3** on the HEATMAG. Otherwise, remove the **Reagent Plate S3**, seal with a PCR plate seal, and keep it aside at room temperature.
- Discard the used tubes on Deck A. Remove and store the Parse Metal Cold Block on ice.



3.2. Fragmentation Mix Creation and Plating

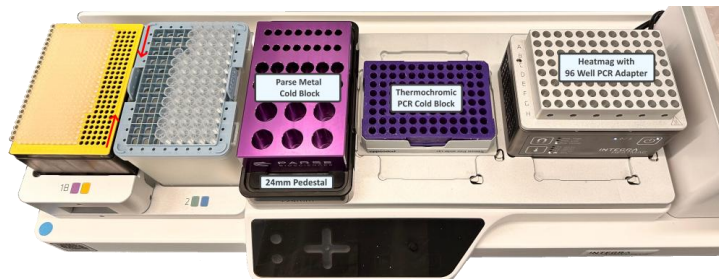
The program makes the Fragmentation Mix and aliquots out the mix such that it can be easily pipetted into sublibraries using the multichannel pipette.

1. Gather the following components and reagents:

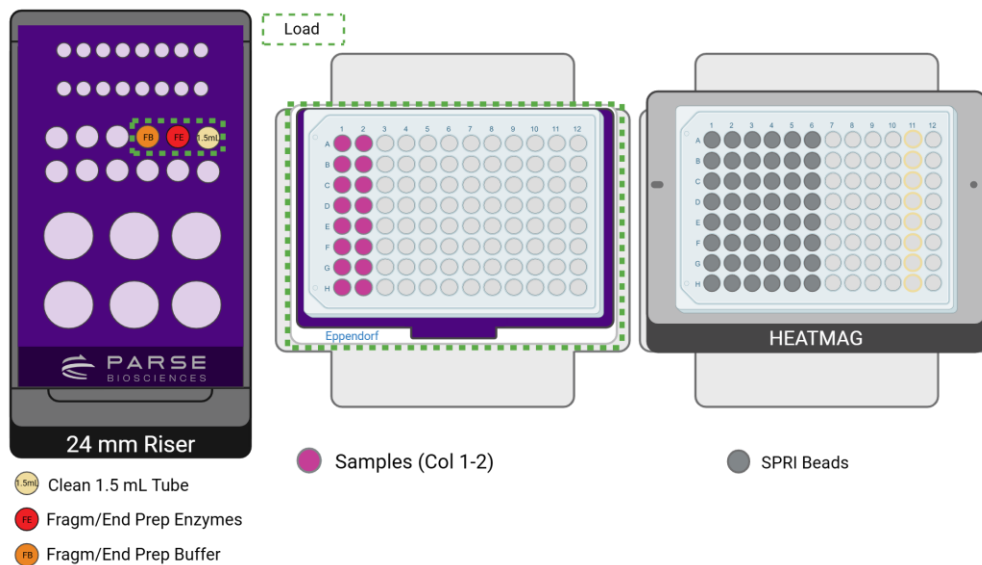
ITEM	SOURCE	QTY	HANDLING AND STORAGE
D-ONE Pipetting Module 1-Ch, 5-1250 μ L	INTEGRA Component	1	
Tip Deck for D-ONE Pipetting Module	INTEGRA Component	1	
Parse Metal Cold Block	Parse	1	
24 mm Labware Pedestal	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Pull the Thermochromic PCR Cold Block with riser from the -20°C freezer and leave them at room temperature for 10 minutes prior to use.
Thermochromic PCR Cold Block Riser	Parse	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
125 μ L Tip Rack	INTEGRA	1	
1250 μ L Tip Rack	INTEGRA	1	
1.5 mL tube	Consumables	1	
● Fragm/End Prep Buffer	-20°C Reagents	1	Thaw at room temperature then store on ice.
● Fragm/End Prep Enzymes	-20°C Reagents	1	Place directly on ice. Briefly centrifuge before use.

2. If not already connected, connect the D-ONE Pipetting Module 1-Ch, 5-1250 μ L Pipette and corresponding Tip Deck. Ensure the tip deck and tip boxes are securely positioned by pressing them down firmly until they click into place. Remove the tip box lids prior to starting the program.
3. If not done already, remove both Thermochromic PCR Cold Block with Riser from -20°C and thaw at room temperature for **10 minutes**.

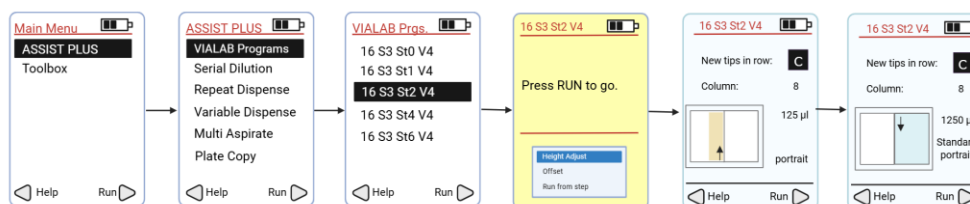
4. Place the Thermochromic PCR Cold Block with Riser on Deck B.
5. Place the cooled Parse Metal Cold Block on the 24 mm Labware Pedestal on Deck A. The deck layout should correspond to the configuration below:



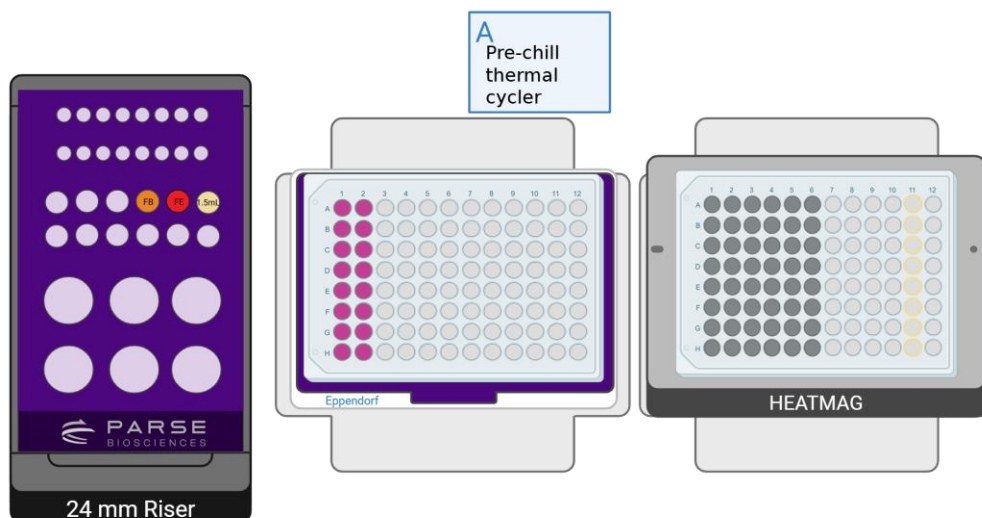
6. Place the following components using the deck configuration below:
 - a. Deck A, column 1:
 - i. Pos 1: a clean 1.5 mL tube.
 - ii. Pos 2: ● Fragm/End Prep Enzymes.
 - iii. Pos 3: ● Fragm/End Prep Buffer.
 - b. Transfer the **cDNA Sample** plate stored on ice from Section 3.0 to the Thermochromic PCR Cold Block on Deck B with A1 in the upper left corner, pressing firmly to ensure the plate is fully seated. If you have not generated a **cDNA Sample** Plate, please refer back to Section 3.0.
 - c. If it was set aside, place the **Reagent Plate S3** (containing SPRI beads) back on Deck C.



7. Remove the reagent caps, then select and run the program **16 S3 St2 V4_0** following the diagram below.



8. Follow the program prompts for manual intervention:

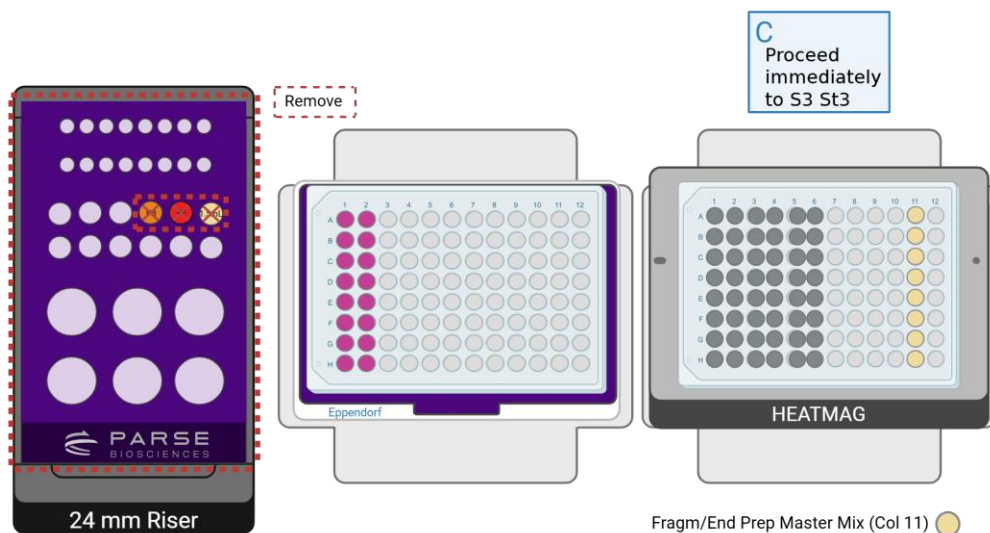


- a. Start the Fragmentation and End Prep program on the thermocycler to pre-chill, ensuring that Step 1 of the cycling program is set to 4°C, so it will be at the

correct temperature when needed during Section 3.3.6. Press "Run" on the pipette to continue.

FRAGMENTATION AND END PREP		
Run Time	40 min	
Lid Temperature	70°C	
Sample Volume	50 µL	
Step	Time	Temperature
1	Hold	4°C
2	10 min	32°C
3	30 min	65°C
4	Hold	4°C

9. At the conclusion of the run:
 - a. Discard the tubes on Deck A.
 - b. Keep all labware on Decks B and C as they will be used in the next step.
 - c. Remove the Parse Metal Cold Block from the Deck A, place it on ice, and proceed immediately to Section 3.3.



3.3. Fragmentation Mix Addition and Post-Fragmentation SPRI Cleanup

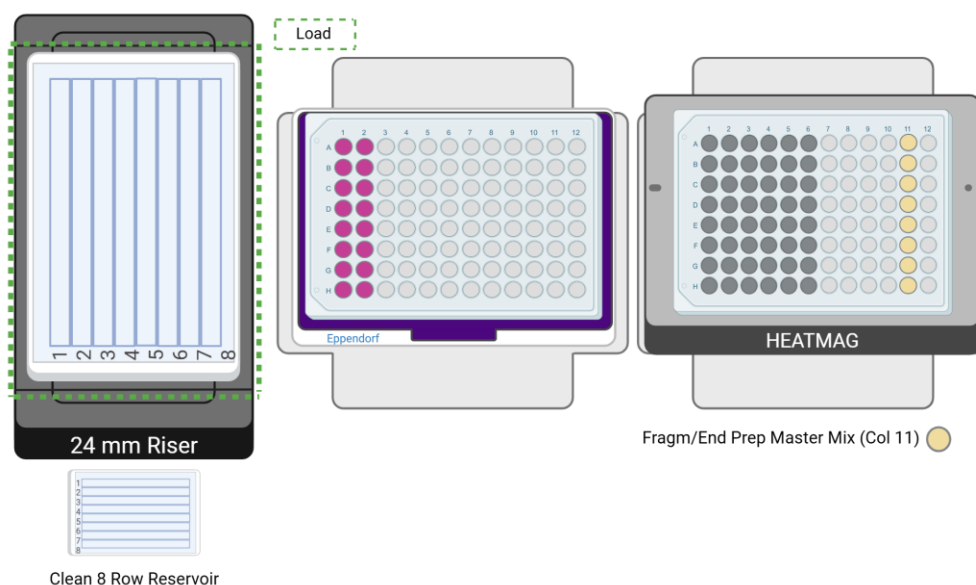
The program uses the VOYAGER multichannel pipette to add Fragmentation Mix to sublibraries. After the user runs the Fragmentation thermal cycling program, sublibraries are loaded back onto the ASSIST PLUS and a double-sided SPRI cleanup is carried out.

1. Gather the following components and reagents and set up the deck as indicated below:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VOYAGER Pipetting Module 8-Ch, 5-125 µL	INTEGRA Component	1	
Tip Deck for VOYAGER Pipetting Module	INTEGRA Component	1	
24 mm Labware Pedestal	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	2	Pull the Thermochromic PCR Cold Block with riser from the -20°C freezer. Thaw one at room temperature for 10 minutes prior to use. Allow the second one to reach room temperature.
Thermochromic PCR Cold Block Riser	Parse	2	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
300 mL Reservoir Base	INTEGRA	1	
8 Row Reservoir Insert	INTEGRA	1	
125 µL Tip Rack	INTEGRA	1	
SealPlate	Consumables	as needed	
Ethanol	Reagents		Prepare 85% Ethanol by using 200 proof Ethanol and nuclease-free water. Make fresh the day it is needed.
Nuclease-free water	Reagents		

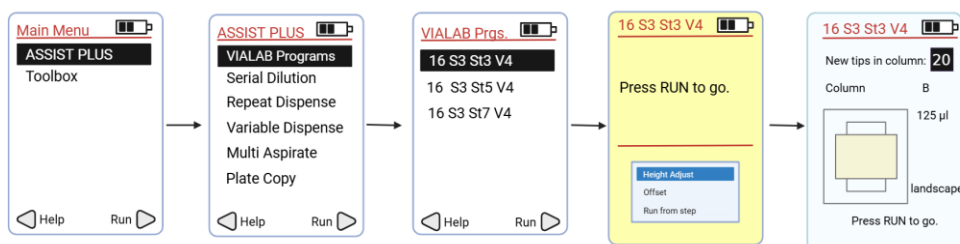


2. Prepare at least **8 mL** of 85% ethanol with nuclease-free water.
3. Place a clean 8 Row Reservoir Insert on the 300 mL Reservoir Base on the 24 mm Labware Pedestal on Deck A. The deck layout should correspond to the configuration below.

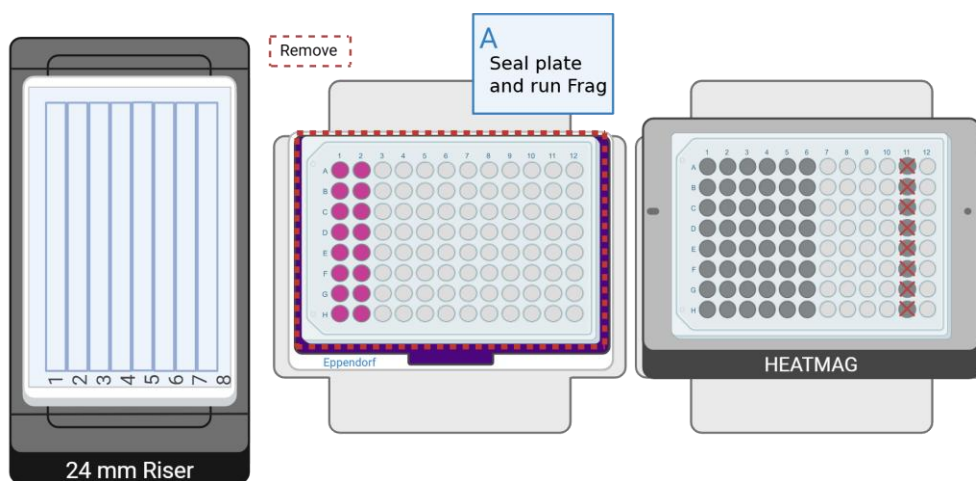


4. Change the pipette that is connected to the INTEGRA ASSIST PLUS:
 - a. Remove D-ONE Pipetting Module 1-Ch, 5-1250 μ L and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.
 - b. Replace it with VOYAGER 8-Ch 5-125 μ L Pipette and corresponding Tip Deck. Ensure the Tip Deck and tip box are securely positioned by pressing them down firmly until they click into place. Remove the tip box lid prior to starting the program.

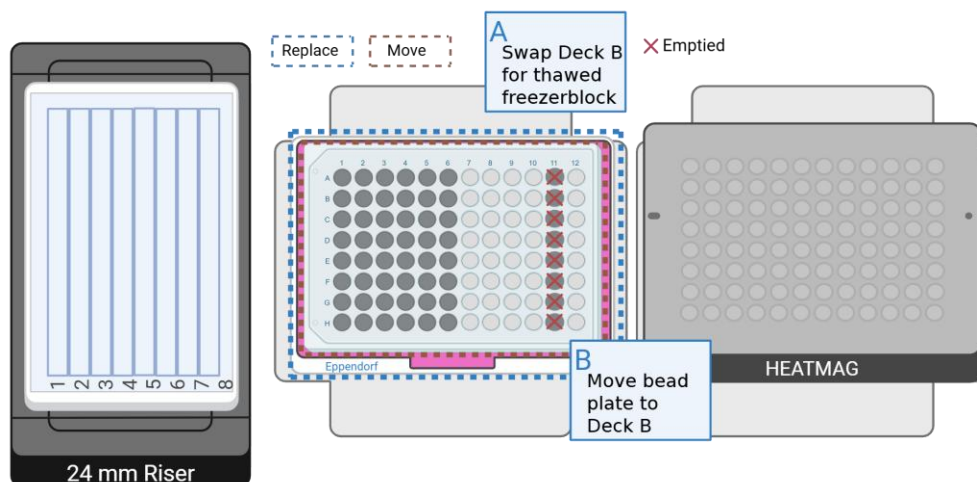
5. Select and run the program **16 S3 St3 V4_0** following the diagram below.



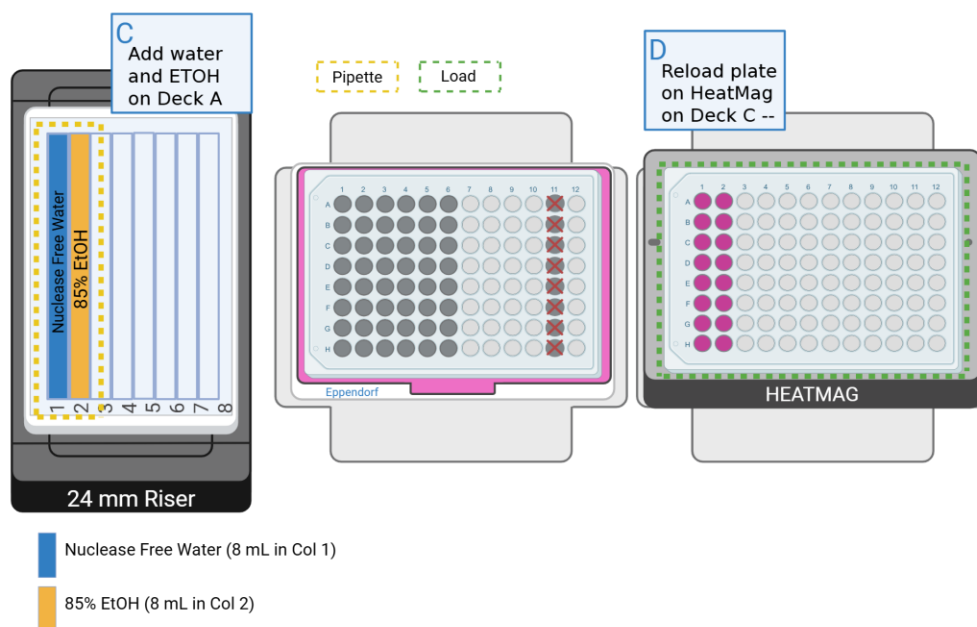
6. Press "Run" to continue the program. Follow the program prompts for manual intervention:
- Remove the **cDNA Sample** plate from Deck B and seal with a PCR plate seal. Place the sealed plate into the pre-cooled thermocycler from Section 3.2.8a.



- Ensure the thermocycler is cool prior to use and start the Fragmentation and End Prep program in the thermocycler pre-cooled in Section 3.2.8a. Skip the 4°C hold in Step 1 so the thermocycler proceeds to Step 2 of the Fragmentation and End Prep program.
- While the thermocycler is running, press "Run" on the pipette to continue the program. Follow the program prompts for manual intervention:



- Replace the frozen Thermochromic PCR Cold Block with riser on Deck B with the one that has been brought to room temperature. A fully thawed Thermochromic PCR Cold block should look pink in color. Press "Run" to continue.
- Move the **Reagent Plate S3** on Deck C onto the fully thawed Thermochromic PCR Cold Block on Deck B with A1 in the upper left. Press "Run" on the pipette to continue.



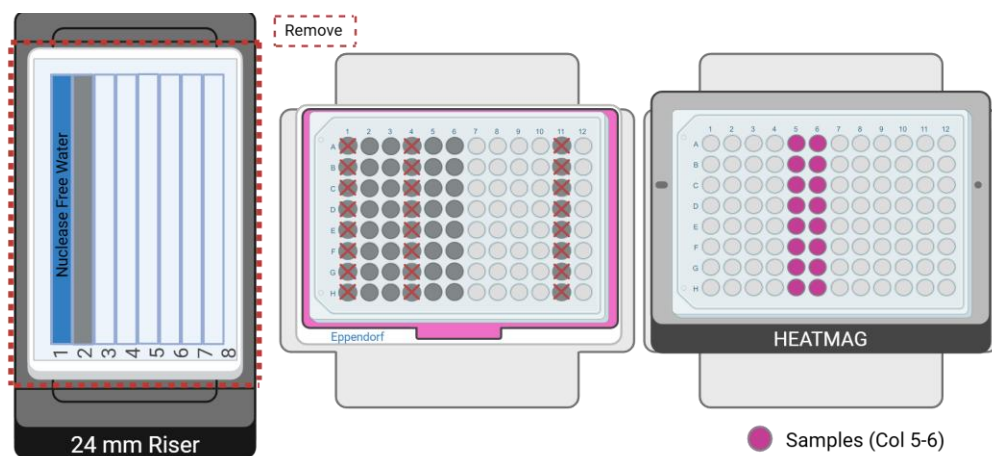
- With a P1000 pipette set to 1,000 μ L or a serological pipette add:
 - 8 mL** nuclease free water in lane **1** of the 8 Row Reservoir on Deck A.

ii. **8 mL** 85% Ethanol in lane **2** of the 8 Row Reservoir on Deck A. Press "Run" to continue.



CRITICAL! Ensure the nuclease free water and 85% Ethanol are in the correct locations. Incorrect placement of these reagents will lead to an assay failure.

- d. When Fragmentation has completed and when prompted, load the **cDNA Sample** plate onto the HEATMAG on Deck C with A1 in the upper left corner. Ensure the nuclease free water and the 85% Ethanol are evenly distributed within their rows. Press "Run" to continue.
9. At the conclusion of the run:
 - a. Cover with a plastic plate seal and remove the 8 Row Reservoir on Deck A. Store at room temperature for later use.
 - b. Keep all labware on Decks B and C. They will be used for the next step.
 10. When the program is complete, continue to Section 3.4.



Safe stopping point: The size-selected fragmented and end prepped **cDNA Sample** plate can be stored at 4°C for up to 18 hours or at -20°C for up to 2 weeks. The **Reagent Plate S3** on Deck B can be sealed with a plastic plate seal and stored at room temperature.

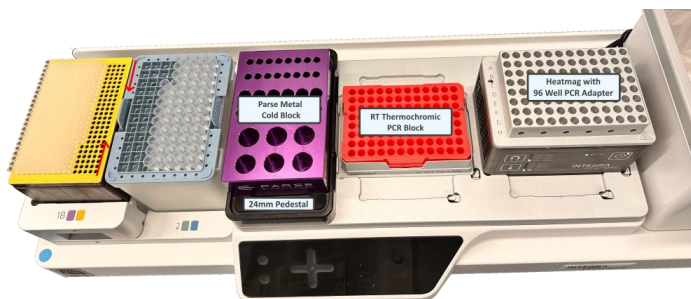
3.4. Ligation Mix Creation and Plating

The program prepares the Ligation Mix in a clean 1.5 mL tube, then dispenses it into column 10 of the **Reagent Plate S3** on Deck B, allowing for easy pipetting into sublibraries using the multichannel pipette.

1. Gather the following components and reagents:

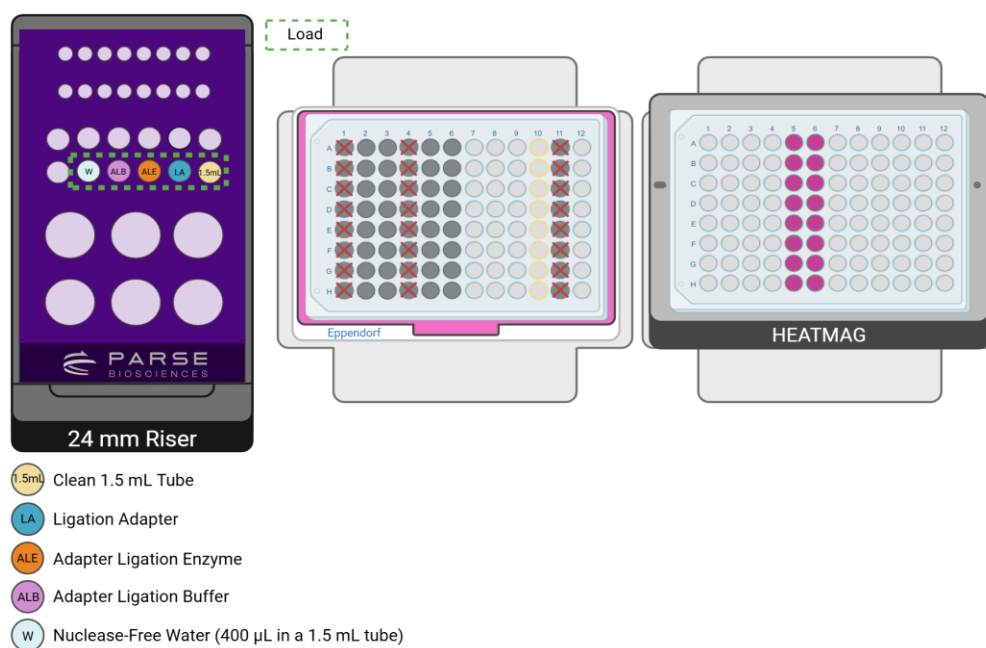
ITEM	SOURCE	QTY	HANDLING AND STORAGE
D-ONE Pipetting Module 1-Ch, 5-1250 µL	INTEGRA Component	1	
Tip Deck for D-ONE Pipetting Module	INTEGRA Component	1	
Parse Metal Cold Block	Parse	1	
24 mm Labware Pedestal	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Ensure the Thermochromic PCR Cold Block with riser is at room temperature before use.
Thermochromic PCR Cold Block Riser	Parse	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
125 µL Tip Rack	INTEGRA	1	
1250 µL Tip Rack	INTEGRA	1	
1.5 mL tube	Consumables	2	
● Ligation Adapter	-20°C Reagents	1	Thaw at room temperature then place on ice. Mix by inverting 3x. Briefly centrifuge before use.
● Adapter Ligation Buffer	-20°C Reagents	1	
● Adapter Ligation Enzyme	-20°C Reagents	1	Place directly on ice. Briefly centrifuge before use.
Nuclease-free water	Reagents		

2. Place the Parse Metal Cold Block on Deck A. The deck layout should correspond to the configuration below.



3. On the Parse Metal Cold Block place these components following the deck configuration below:

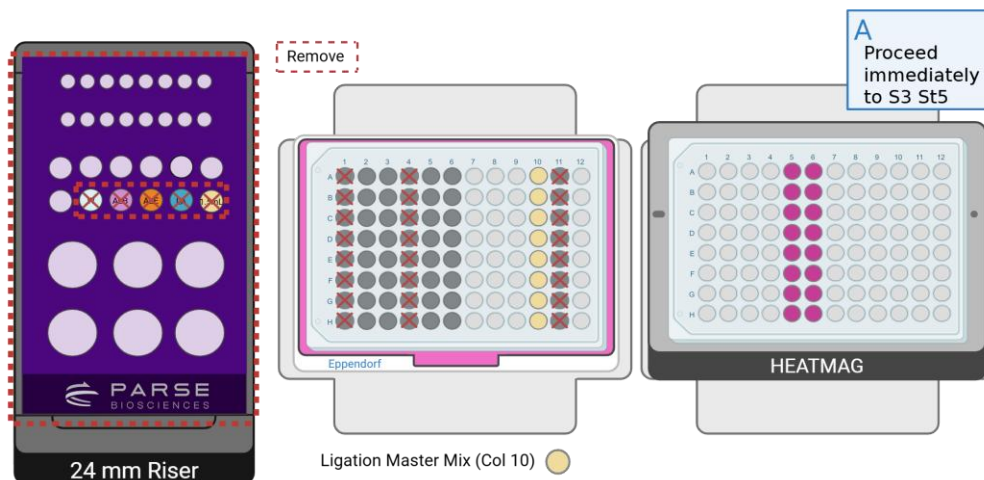
- a. Column 2:
 - i. Pos 7: a clean 1.5 mL tube.
 - ii. Pos 8: ● Ligation Adapter.
 - iii. Pos 9: ● Adapter Ligation Enzyme.
 - iv. Pos 10: ● Adapter Ligation Buffer.
 - v. Pos 11: a 1.5 mL tube filled with **400 μ L** of nuclease free water.



4. Change the pipette that is connected to the INTEGRA ASSIST PLUS:
 - a. Remove the VOYAGER 8-Ch 5-125 μ L Pipette and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.
 - b. Replace it with D-ONE Pipetting Module, 5-1250 μ L and corresponding Tip Deck. Ensure the tip deck and tip boxes are securely positioned by pressing them down firmly until they click into place. Remove the tip box lid prior to starting the program.
5. Remove the reagent caps, select and run the program **16 S3 St4 V4_0** following the diagram below.



6. At the conclusion of the program:
 - a. Remove the Parse Metal Cold Block from Deck A. Discard all the tubes.
 - b. Keep all labware on Decks B and C. Proceed immediately to section 3.5.



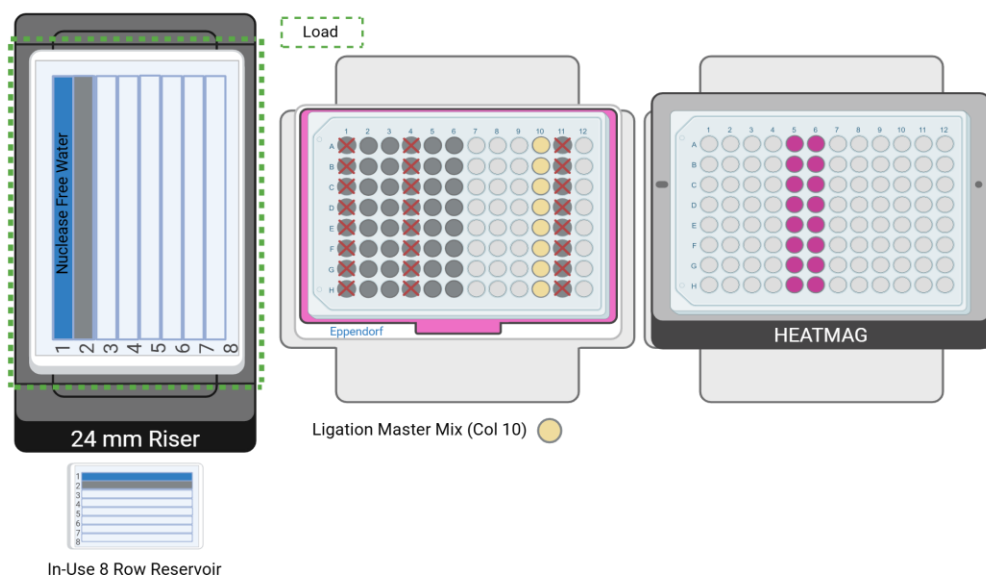
3.5. Ligation Master Mix Addition and Post-Ligation SPRI Cleanup

The program uses the VOYAGER multichannel pipette to add Ligation Mix to sublibraries. After the user runs the Ligation thermocycling program, sublibraries are loaded back onto the ASSIST PLUS and a single-sided SPRI cleanup is carried out.

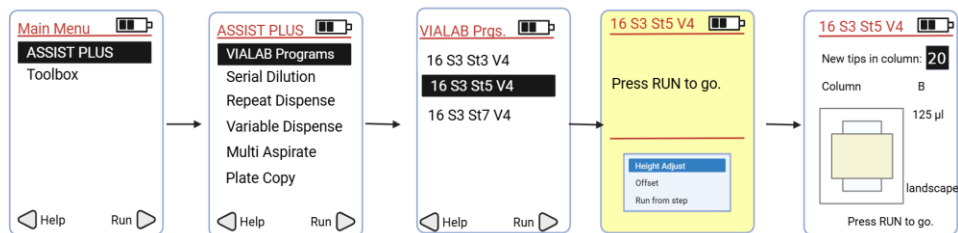
1. Gather the following components and reagents:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
VOYAGER Pipetting Module 8-Ch, 5-125 µL	INTEGRA Component	1	
Tip Deck for VOYAGER Pipetting Module	INTEGRA Component	1	
24 mm Labware Pedestal	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Ensure the Thermochromic PCR Cold Block with riser is at room temperature before use.
Thermochromic PCR Cold Block Riser	Parse	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
300 mL Reservoir Base	INTEGRA Component	1	
125 µL Tip Rack	INTEGRA	1	
SealPlate	Consumables	as needed	
Ethanol	Reagents		Prepare 85% Ethanol by using 200 proof Ethanol and nuclease-free water. Make fresh the day it is needed.
Nuclease-free water	Reagents		

2. Prepare at least **8 mL** of 85% ethanol with nuclease free water.
3. Load the 8 mL Reservoir stored from Section 3.3 onto a 300 mL Reservoir Base and place on the 24 mm Labware Pedestal on Deck A. Remove the plastic plate seal. The deck layout should correspond to the configuration below.



4. Change the pipette that is connected to the INTEGRA ASSIST PLUS:
 - a. Remove the D-ONE Pipetting Module, 5-1250 μ L and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.
 - b. Replace it with VOYAGER 8-Ch 5-125 μ L Pipette and corresponding Tip Deck. Ensure the tip deck and tip box are securely positioned by pressing them down firmly until they click into place. Remove the tip box lid prior to starting the program.
5. Navigate to the VIALAB programs on the Voyager Pipetting Module, select and run the program **16 S3 St5 V4_0** following the diagram below.



6. Press "Run" to continue the program. Follow the program prompts for manual intervention:

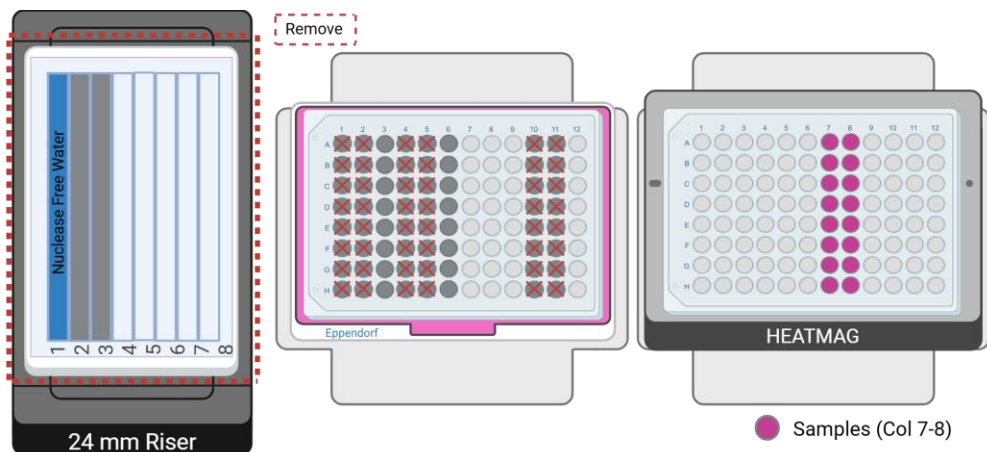
- a. Seal the **cdNA Sample** plate with a PCR plate seal on Deck C and place it into a thermocycler. Run the following program. Proceed to the next step while the program is still running.

ADAPTER LIGATION		
Run Time	15 minutes	
Lid Temperature	30°C*	
Sample Volume	100 µL	
Step	Time	Temperature
1	15 min	20°C
2	Hold	4°C



Note: *If the thermocycler's lid can't reach the recommended temperature of 30°C, turn the lid heating off.

- b. With a P1000 pipette set to 1000 µL, add **8 mL** 85% Ethanol to lane **3** of the 8 Row Reservoir on Deck A.
 - c. Upon thermocycling completion reload the **cdNA Sample** plate onto the HEATMAG with 96 Well Adapter located on Deck C and remove the seal.
7. At the conclusion of the program:
- a. Remove the reagent reservoir on Deck A and cover with a plastic plate seal. Store at room temperature for later use.
 - b. Keep all labware on Decks B and C. Proceed to Section 3.6.



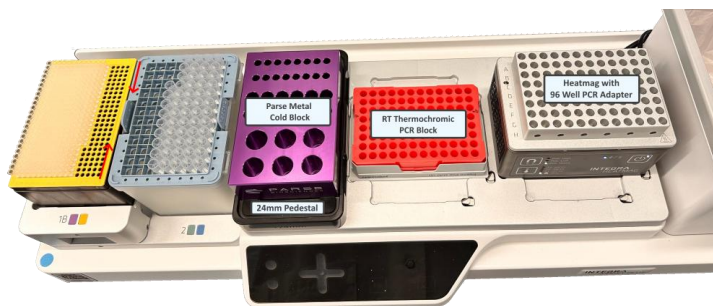
3.6. Barcoding Round 4

Program uses the pipette to aliquot out the Library Amp Mix such that it can be easily pipetted into sublibraries using the multichannel pipette. During this program, the user will manually add UDIs to sublibraries.

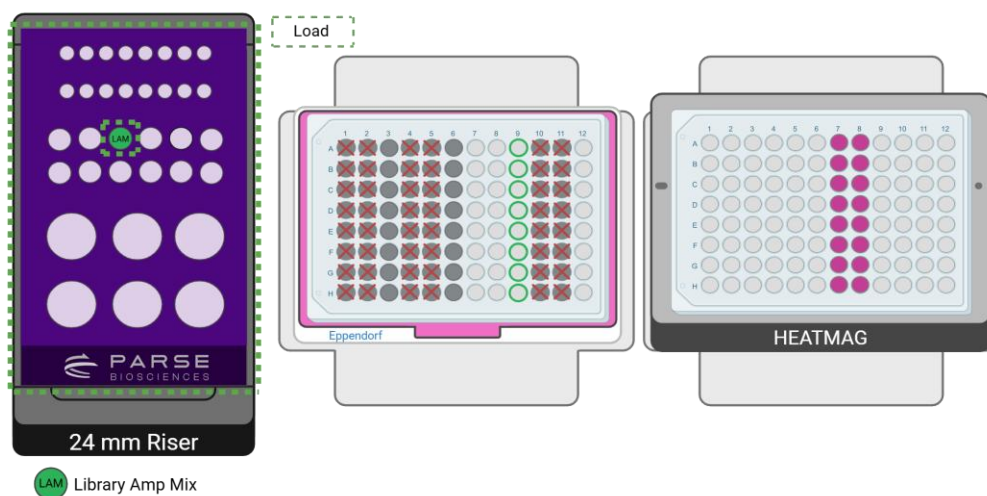
1. Gather the following components and reagents:

ITEM	SOURCE	QTY	HANDLING AND STORAGE
D-ONE Pipetting Module 1-Ch, 5-1250 µL	INTEGRA Component	1	
Tip Deck for D-ONE Pipetting Module	INTEGRA Component	1	
Parse Metal Cold Block	INTEGRA Component	1	
24 mm Labware Pedestal	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Ensure the Thermochromic PCR Cold Block with riser is at room temperature before use.
Thermochromic PCR Cold Block Riser	Parse	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
125 µL Tip Rack	INTEGRA	1	
1250 µL Tip Rack	INTEGRA	1	
● Library Amp Mix	-20°C Reagents	1	Thaw at room temperature then place on ice. Mix by inverting 3x. Briefly centrifuge before use.
UDI Plate - WT	Parse reagents	1 sealed well per sublibrary	Thaw at room temperature then place on ice.

2. Load the Parse Metal Cold Block on the 24 mm Labware Pedestal on Deck A. The deck layout should correspond to the configuration below.



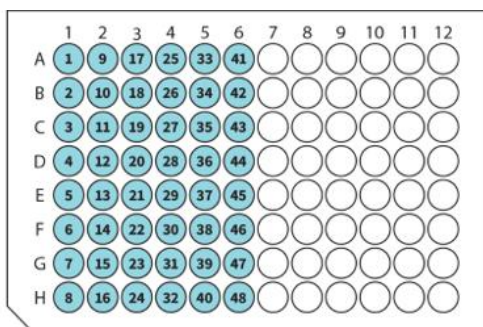
3. Place the ● Library Amp Mix in the Parse Metal Cold Block, column 1, position 4. The deck layout should correspond to the configuration below.



4. Change the pipette that is connected to the INTEGRA ASSIST PLUS:
 - a. Remove the VOYAGER 8-Ch 5-125 μ L Pipette and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.
 - b. Replace it with D-ONE Pipetting Module, 5-1250 μ L and corresponding Tip Deck. Ensure the tip deck and tip boxes are securely positioned by pressing them down firmly until they click into place. Remove the tip box lids prior to starting the program.
5. Uncap the reagents caps, select and run the program **16 S3 St6 V4_0** following the diagram below.



6. While program is running, centrifuge the thawed UDI Plate - WT at 100 x g for **1 minute**.
7. Wipe the surface of the UDI Plate - WT with 70% ethanol and allow it to dry.
8. Orient the UDI Plate - WT with the notch on the bottom left as pictured below. For each sublibrary being processed, choose one unused well of the UDI Plate - WT and **record the well position and number for each sublibrary**.



9. With a multichannel P20, manually pierce the seal of the chosen wells of the UDI Plate - WT.
10. **When prompted**, with a multichannel P20 and new tips, manually mix by pipetting 5x then immediately transfer **4 µL** from a chosen unused well of the UDI Plate - WT to its corresponding sample well in columns 7 and 8 on Deck C.

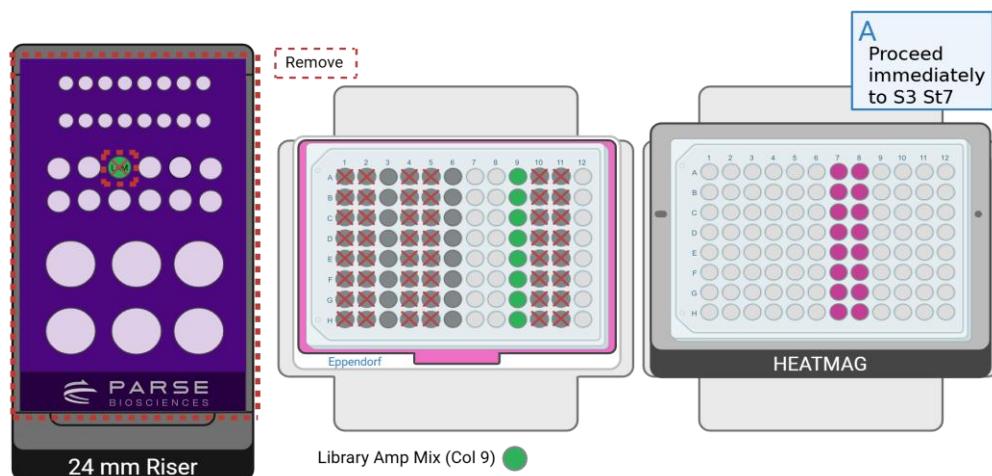


Note: Record which UDI wells were used for each sample well.



CRITICAL! Only transfer primers from 1 well of the UDI Plate - WT to 1 well of adapter ligated DNA.

11. If any unused wells remain in the UDI Plate - WT, store the plate at -20°C. Do not reuse pierced wells.
12. Press "Run" to continue the program. At the conclusion of the run:
 - a. Remove all labware from Deck A.
 - b. Proceed immediately to Section 3.7.



3.7. Library Amp Mix Addition and Size Selection

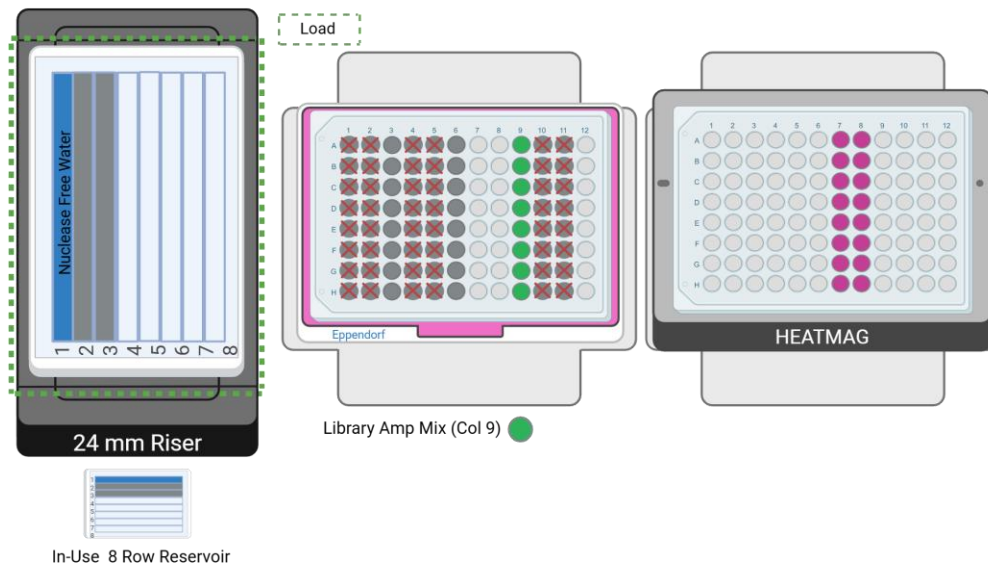
The program uses the VOYAGER multichannel pipette to add Library Amp Mix to the sublibraries. After the user runs the Indexing PCR thermocycling program, sublibraries are loaded back onto the ASSIST PLUS and a double-sided SPRI cleanup is carried out.

1. Gather the following components and reagents:

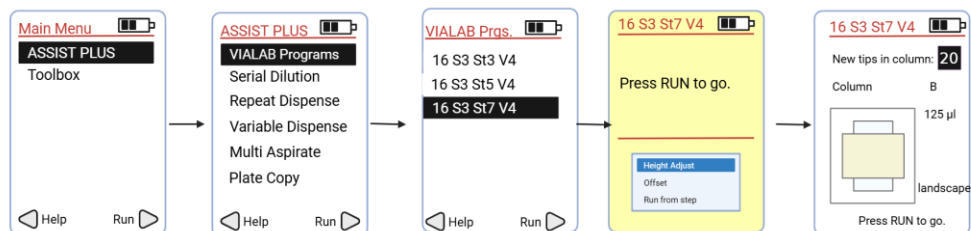
ITEM	SOURCE	QTY	HANDLING AND STORAGE
VOYAGER Pipetting Module 8-Ch 5-125 µL	INTEGRA Component	1	
Tip Deck for VOYAGER Pipetting Module	INTEGRA Component	1	
24 mm Labware Pedestal	INTEGRA Component	1	
Thermochromic PCR Cold Block	Parse	1	Ensure the Thermochromic PCR Cold Block with riser is at room temperature before use.
Thermochromic PCR Cold Block Riser	Parse	1	
300 mL Reservoir Base	INTEGRA Component	1	
HEATMAG with 96 Well Adapter	INTEGRA Component	1	
125 µL Tip Rack	INTEGRA	1	
SealPlate	Consumables	as needed	
Ethanol	Reagents		Prepare 85% Ethanol by using 200 proof Ethanol and nuclease-free water. Make fresh the day it is needed.
Nuclease-free water	Reagents		



2. Prepare **8 mL** of 85% ethanol with nuclease-free water.
3. Load the 8 mL Reservoir stored from Section 3.5 onto a 300 mL Reservoir Base and place on the 24 mm Labware Pedestal on Deck A. Remove the plastic plate seal. The deck layout should correspond to the configuration below.



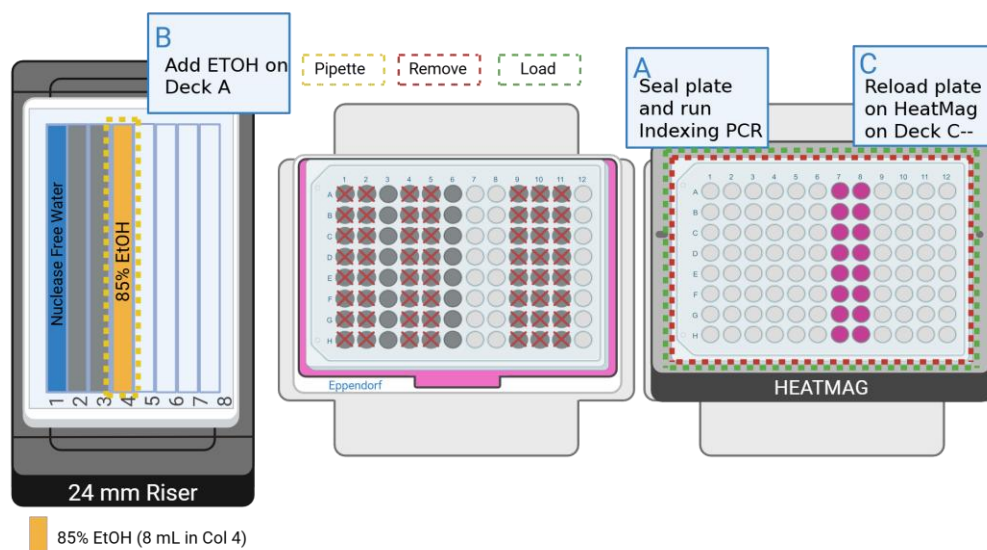
4. Change the pipette that is connected to the INTEGRA ASSIST PLUS:
 - a. Remove D-ONE Pipetting Module, 5-1250 μ L and corresponding Tip Deck. Ensure it is disconnected by pressing the back button until the main menu is displayed.
 - b. Replace it with VOYAGER 8-Ch 5-125 μ L Pipette and corresponding Tip Deck. Ensure the tip deck and tip box are securely positioned by pressing them down firmly until they click into place. Remove the tip box lid prior to starting the program.
5. Select and run the program **16 S3 St7 V4_0** following the diagram below.



- While the program is running, determine the number of PCR cycles required for the Indexing PCR based on the amount of cDNA added to the fragmentation and end prep reaction.

NUMBER OF PCR CYCLES	
cDNA Input (ng)	PCR Cycles
10-24	13
25-49	12
50-99	11
100-199	10
200-399	9
400-999	8
1,000 or more	7

- Press "Run" on the pipette to continue the program. Follow the program prompts for manual intervention:



- a. Remove the **cDNA Sample** plate from the INTEGRA ASSIST PLUS, seal the sample plate on Deck C with a plastic plate seal and place it into a thermocycler. Run the following program. When the Indexing PCR thermocycling program is complete, press "Run" on the Assist Plus to continue.

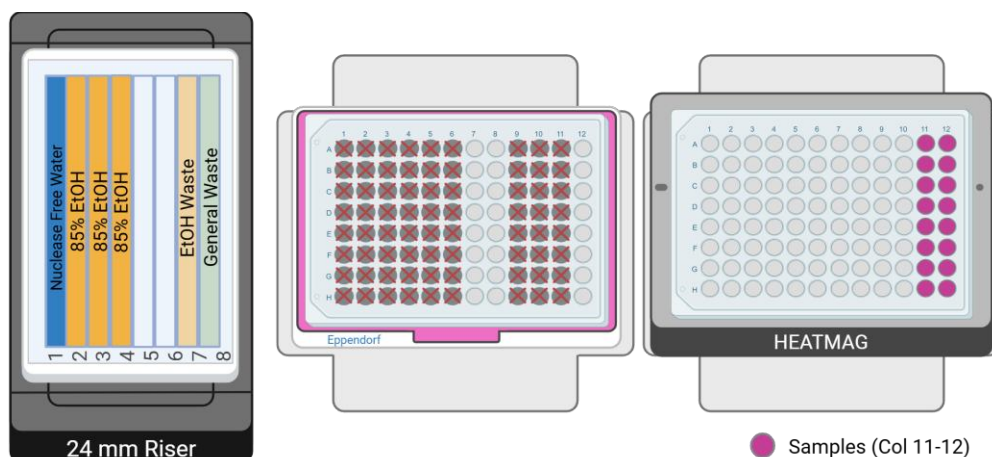
INDEXING PCR			
Run Time		~30 minutes	
Lid Temperature		105°C	
Sample Volume		50 µL	
Step	Time	Temperature	Cycles
1	3 min	95°C	1
2	20 sec	98°C	Varies, see table above
3	20 sec	67°C	
4	1 min	72°C	
5	5 min	72°C	1
6	Hold	4°C	1

- b. With a P1000 pipette, add **8 mL** 85% Ethanol to row **4** of the 8 Row Reservoir on Deck A. Press "Run" to continue.

- c. Reload the **cDNA Sample** plate onto the HEATMAG with 96 Well Adapter on Deck C.
8. At the conclusion of the run:
 - a. The sequencing libraries will be in columns 11 and 12 in the **cDNA Sample** plate on Deck C. Sequencing libraries can be stored at -20°C for up to 3 months. Seal the plate with a foil plate seal, then add a plastic plate seal on top of the foil plate seal. Alternatively, proceed directly to Section 3.8 to immediately quantify the Final Libraries. Seal the cDNA Sample plate after taking aliquots to QC.
 - b. Discard the used 8 Row Reservoir on Deck A and their content.
 - c. Discard the used **Reagent Plate S3** on Deck B.



Safe stopping point: the **cDNA Sample** plate can be stored at -20°C for up to 3 months.



3.8. Sequencing Library Quantification

The concentration and size distribution of the sequencing libraries are measured with fluorescent dyes and capillary electrophoresis. Sequencing libraries can be stored at -20°C for up to 3 months.

To quantify the sequencing libraries:

1. Measure the concentration of each purified sequencing library from Section 3.7 with the Qubit dsDNA HS (High Sensitivity) Assay Kit according to the manufacturer's instructions.
2. Assess the size distribution of each purified sequencing library with a High Sensitivity DNA Kit on the Agilent Bioanalyzer System or High Sensitivity D5000 or D1000 ScreenTape and Reagents on the Agilent TapeStation System according to the manufacturer's instructions.

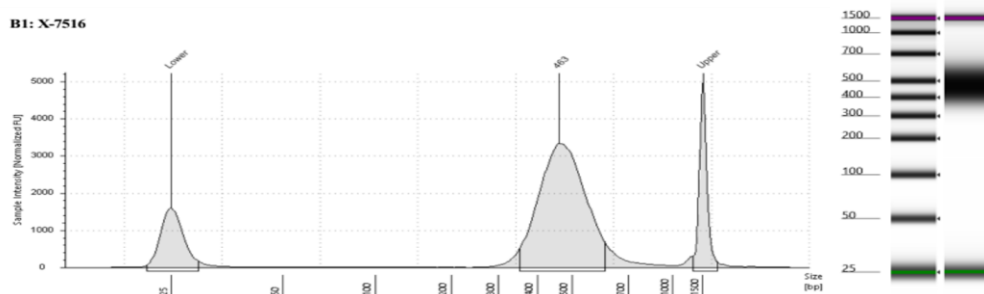


Figure 5: Expected Size Distribution before Illumina Sequencing. Example trace of Human DNA from indexed sublibraries run on a TapeStation.

Note: Samples may need to be diluted to be within the manufacturer's recommended concentration range. Typically, between a 1:3 to 1:10 dilution is appropriate.

Note: The traces above are representative of typical TapeStation of DNA from indexed sublibraries. There should be a peak between 300-450 bp. The prominence of the trace is dependent on the amount of DNA loaded into the TapeStation. Sublibraries with minor deviations can still produce high quality data.

Note: If using a Bioanalyzer, there may be an additional peak present. This typically occurs if products are overamplified, but it should not impact sequencing or data quality (assuming there is still a peak present at 300-450 bp). Do not use this additional peak when estimating amplicon size.



Appendices

Appendix A: Sequencing Information

We recommend a minimum sequencing depth of 10,000 reads per cell for whole transcriptome libraries. However, ideal sequencing depth is dependent on the sample type and experimental goals. For example, if trying to capture rare cell types in a heterogenous population, deeper sequencing is required. Alternatively, analyzing a homogenous population, more shallow sequencing may be appropriate.

Whole transcriptome sequencing libraries should be diluted and denatured according to the manufacturer's instructions for the relevant sequencing instrument. If using the Illumina platform, we strongly recommend adding 5% PhiX for optimal sequencing quality.

Details of the final whole transcriptome sequencing library structure are below.

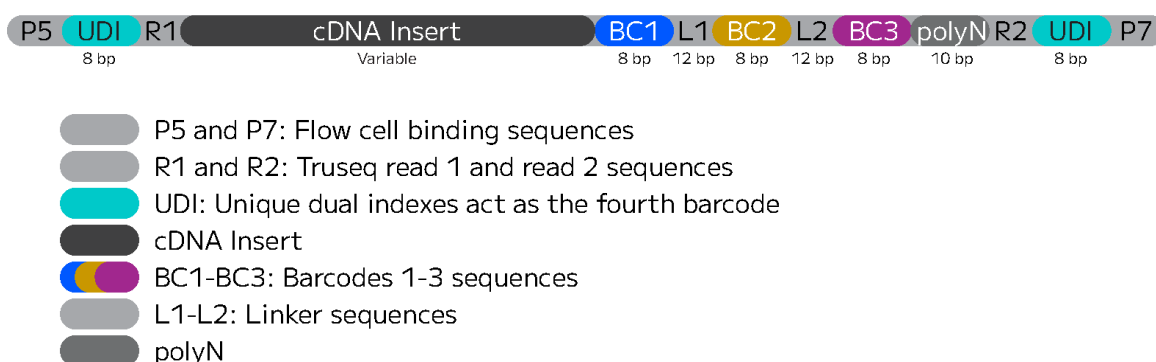


Figure 6: Whole transcriptome sequencing library structure.

Libraries should be sequenced with paired reads using the read structure in the table below. Read lengths longer than those recommended below are acceptable, but additional bases in read 2 are trimmed by the Parse Analysis Pipeline.

READ	FUNCTION	INSERT
Read 1	cDNA Insert	64
i7 Index (Index 1)	Barcode 4 / UDI	8
i5 Index (Index 2)	Barcode 4 / UDI	8
Read 2	Barcodes 1-3	58

The fourth barcode that tags each sublibrary acts as a standard Illumina i7 and i5 indexes. Please refer to the following table to demultiplex the whole transcriptome libraries.

Sublibrary Index ID	Well Position	i7 Forward Sequence	i5 Reverse Complement Sequence	i5 Forward Sequence
UDI_Plate_WT_1	A1	CAGATCAC	ATGTGAAG	CTTCACAT
UDI_Plate_WT_2	B1	ACTGATAG	GTCCAACC	GGTTGGAC
UDI_Plate_WT_3	C1	GATCAGTC	AGAGTCAA	TTGACTCT
UDI_Plate_WT_4	D1	CTTGTAAT	AGTTGGCT	AGCCAACT
UDI_Plate_WT_5	E1	AGTCAAGA	ATAAGGCG	CGCCTTAT
UDI_Plate_WT_6	F1	CCGTCCTA	CCGTACAG	CTGTACGG
UDI_Plate_WT_7	G1	GTAGAGTA	CATTCATG	CATGAATG
UDI_Plate_WT_8	H1	GTCCGCCT	AGATACGG	CCGTATCT
UDI_Plate_WT_9	A2	GTGAAACT	TACAGACT	AGTCTGTA
UDI_Plate_WT_10	B2	TCATTCCT	AATGCCTG	CAGGCATT
UDI_Plate_WT_11	C2	GGTAGCAT	TGCTTGCC	GGCAAGCA
UDI_Plate_WT_12	D2	ACTTGATC	TTTGGGTG	CACCCAAA
UDI_Plate_WT_13	E2	ATGAGCAT	GAATCTGA	TCAGATTC
UDI_Plate_WT_14	F2	GCGCTATC	CGACTGGA	TCCAGTCG
UDI_Plate_WT_15	G2	TGACCAGT	ACATTGGC	GCCAATGT
UDI_Plate_WT_16	H2	TATAATCA	ACCACTGT	ACAGTGGT
UDI_Plate_WT_17	A3	CAAAAGTC	CGGTTGTT	AACAACCG
UDI_Plate_WT_18	B3	CGATGTCA	CATGAGGA	TCCTCATG
UDI_Plate_WT_19	C3	CTCAGAGT	TGGAGAGT	ACTCTCCA
UDI_Plate_WT_20	D3	TAATCGAC	TGACTTCG	CGAAGTCA
UDI_Plate_WT_21	E3	CATTTTCT	GGAAGGAT	ATCCTTCC
UDI_Plate_WT_22	F3	CTATACTC	TGTTTCGAG	CTCGAACA
UDI_Plate_WT_23	G3	CACTCACA	AAGGCTGA	TCAGCCTT
UDI_Plate_WT_24	H3	CTCGAACA	CTCGAGTG	CACTCGAG

Sublibrary Index ID	Well Position	i7 Forward Sequence	i5 Reverse Complement Sequence	i5 Forward Sequence
UDI_Plate_WT_25	A4	CTCTATCG	ATCGGTGG	CCACCGAT
UDI_Plate_WT_26	B4	TCCTCATG	AGGTCTTG	CAAGACCT
UDI_Plate_WT_27	C4	AACAACCG	AGGAAGCG	CGCTTCCT
UDI_Plate_WT_28	D4	GCCAATGT	ACATGTGT	ACACATGT
UDI_Plate_WT_29	E4	TGGTTGTT	ATACAGTT	AACTGTAT
UDI_Plate_WT_30	F4	TCTGCTGT	ATCGCCTT	AAGGCGAT
UDI_Plate_WT_31	G4	TTGGAGGT	TTCGACGC	GCGTCGAA
UDI_Plate_WT_32	H4	TCGAGCGT	TGTCGTTC	GAACGACA
UDI_Plate_WT_33	A5	TGCGATCT	TCCATAGC	GCTATGGA
UDI_Plate_WT_34	B5	TTCCTGCT	TAAGTGTC	GACACTTA
UDI_Plate_WT_35	C5	TTCCATTG	CTGGCATA	TATGCCAG
UDI_Plate_WT_36	D5	TAACGCTG	CTGAGCCA	TGGCTCAG
UDI_Plate_WT_37	E5	TTGGTATG	CTCAATGA	TCATTGAG
UDI_Plate_WT_38	F5	TGAACTGG	CGCATACA	TGTATGCG
UDI_Plate_WT_39	G5	TCCAGTCG	CCGAAGTA	TACTTCGG
UDI_Plate_WT_40	H5	TGTATGCG	CCAGTTCA	TGAACTGG
UDI_Plate_WT_41	A6	TGGCTCAG	CAGCGTTA	TAACGCTG
UDI_Plate_WT_42	B6	TATGCCAG	CAATGGAA	TTCCATTG
UDI_Plate_WT_43	C6	GGTTGGAC	ATCCTGTA	TACAGGAT
UDI_Plate_WT_44	D6	GACACTTA	AGCAGGAA	TTCCTGCT
UDI_Plate_WT_45	E6	GAACGACA	ACGCTCGA	TCGAGCGT
UDI_Plate_WT_46	F6	AAGGCGAT	ACAGCAGA	TCTGCTGT
UDI_Plate_WT_47	G6	ATGCTTGA	ACAAGCTA	TAGCTTGT
UDI_Plate_WT_48	H6	AGTATCTG	CATCAAGT	ACTTGATG

Appendix B: Pipetting Programs

Section 2.1. Reagents Plating

16 S2 St1 V4_0 DONE

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Strep Beads (132 μ L/well)	1 min 3 sec
3	Binding Buffer (120 μ L/well)	1 min 2 sec
4	Volume Change	0
5	Bead Wash Col 9 (260 μ L/well)	1 min 12 sec
6	Bead Wash Col 11 (130 μ L/well)	1 min 3 sec
7	Wash A (1000 μ L/well x 5)	1 min 18 sec
8	Wash B (1000 μ L/well x 5)	1 min 18 sec
9	Proceed to next step	0

Section 2.2. cDNA Capture

16 S2 St2 V4_0 VOYAGER

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Thaw Lysates	0
3	Raise Magnet	0 min 10 sec
4	DELAY	2 min 0 sec
5	Remove Supernatant 1 (75 μ L/well x 2)	1 min 7 sec

STEPS	ACTION	DURATION
6	Release Magnet	0 min 10 sec
7	Bead Wash 1x (120 µL/well)	1 min 44 sec
8	Raise Magnet	0 min 10 sec
9	2 min delay	2 min 0 sec
10	Remove Supernatant 1 (125 µL/well)	0 min 46 sec
11	Release Magnet	0 min 10 sec
12	Bead Wash 2x (120 µL/well)	1 min 32 sec
13	Raise Magnet	0 min 10 sec
14	2 min delay	2 min 0 sec
15	Remove Supernatant 1 (125 µL/well)	0 min 46 sec
16	Release Magnet	0 min 10 sec
17	Bead Wash 3x (120 µL/well)	1 min 32 sec
18	Raise Magnet	0 min 10 sec
19	2 min delay	2 min 0 sec
20	Remove Supernatant 1 (125 µL/well)	0 min 46 sec
21	Release Magnet	0 min 10 sec
22	Binding Buffer 1 (110 µL/well)	1 min 31 sec
23	Mix Binder Beads 1	1 min 20 sec
24	Check Strep Bead Resuspension	0
25	Additional Mixing Required	0

STEPS	ACTION	DURATION
26	Load Lysates	0
27	Add Binder Beads (50 µL/well)	3 min 5 sec
28	Vortex at 800 - 1000 RPM	0
29	Cover Reagents	0
30	Remove and Store Rgt Plate	0

Section 2.3. cDNA-Bound Beads Wash

16 S2 St3 V4_0 VOYAGER

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Raise Magnet	0 min 10 sec
3	2 min Delay	2 min 0 sec
4	Remove Supernatant 1 (125 µL/well)	0 min 46 sec
5	Remove Supernatant 2 (125 µL/well)	0 min 46 sec
6	Release Magnet	0 min 10 sec
7	Add Wash A.1 (120 µL/well)	1 min 51 sec
8	Slow Wash A.1	0 min 30 sec
9	Add Wash A.2 (120 µL/well)	1 min 51 sec
10	Slow Wash A.2	0 min 30 sec
11	Raise Magnet	0 min 10 sec
12	2 min Delay	2 min 0 sec

STEPS	ACTION	DURATION
13	Volume Change	0
14	Remove Supernatant 1 (125 µL/well)	0 min 46 sec
15	Remove Supernatant 2 (125 µL/well)	0 min 46 sec
16	Release Magnet	0 min 10 sec
17	Add Wash B.1 (120 µL/well)	1 min 51 sec
18	Slow Wash B.1	0 min 30 sec
19	Add Wash B.2 (120 µL/well)	1 min 51 sec
20	Slow Wash B.2	0 min 30 sec
21	Proceed to next step	0

Section 2.4. Master Mixes Preparation

16 S2 St4 V4_0 DONE

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Dispense SPRI Beads (160 µL/well)	1 min 6 sec
3	cDNA Amp Primer (978 µL/well)	0 min 37 sec
4	cDNA PCR Add (42.5 µL/well)	0 min 36 sec
5	Mix cDNA MM	1 min 15 sec
6	Dispense cDNA MM 1.1 (115 µL/well)	1 min 1 sec
7	cDNA Amp Enz (115 µL/well)	1 min 4 sec
8	Store cDNA MM on Ice	0

STEPS	ACTION	DURATION
9	TS Buffer 1.1 (731 μ L/well x 2)	1 min 12 sec
10	TS Enz 1.1 (100.8 μ L/well)	0 min 48 sec
11	TS Primer 1.1 (50.4 μ L/well)	0 min 38 sec
12	TS Mix 1.1	2 min 10 sec
13	Dispense TS MM 1.1 (190 μ L/well)	1 min 23 sec
14	Remove Parse Cold Block	0
15	Remove Reagent Plate	0
16	Proceed to next step	0

Section 2.5. Template Switch and cDNA Amplification

16 S2 St5 V4_0 VOYAGER

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Raise Magnet	0 min 10 sec
3	DELAY	2 min 0 sec
4	Remove Supernatant 1 (125 μ L/well)	0 min 37 sec
5	Remove Supernatant 2 (125 μ L/well)	0 min 37 sec
6	Release Magnet	0 min 10 sec
7	Add Water 1 (20 μ L/well)	0 min 42 sec
8	Add Water 2 (20 μ L/well)	0 min 42 sec
9	Add TS 1 (80 μ L/well)	1 min 24 sec

STEPS	ACTION	DURATION
10	Add TS 2 (80 µL/well)	1 min 24 sec
11	Cover Rgt Plate and 8 Row Reservoir	0
12	Incubate 30 mins	0
13	Run TS on Therm	0
14	Load Cold Block	0
15	Reload Samples	0
16	Labware Change	0
17	Volume Change	0
18	Raise Magnet	0 min 10 sec
19	DELAY	2 min 0 sec
20	Remove TS Sup 1 (125 µL/well)	0 min 37 sec
21	Remove TS Sup 2 (125 µL/well)	0 min 37 sec
22	Release Magnet	0 min 10 sec
23	Add Wash A1 (120 µL/well)	1 min 14 sec
24	Add Wash A2 (120 µL/well)	1 min 14 sec
25	Volume Change	0
26	Raise Magnet	0 min 10 sec
27	DELAY	2 min 10 sec
28	Remove Wash A1 (125 µL/well)	0 min 37 sec
29	Remove Wash A2 (125 µL/well)	0 min 37 sec

STEPS	ACTION	DURATION
30	Release Magnet	0 min 10 sec
31	Add Wash B1 (120 μ L/well)	1 min 14 sec
32	Add Wash B2 (120 μ L/well)	1 min 14 sec
33	Load cDNA	0
34	Volume Change	0
35	Raise Magnet	0 min 10 sec
36	DELAY	2 min 10 sec
37	Remove Wash B1 (125 μ L/well)	0 min 37 sec
38	Remove Wash B2 (125 μ L/well)	0 min 37 sec
39	Release Magnet	0 min 10 sec
40	Add cDNA Primer (50 μ L/well)	1 min 31 sec
41	Add cDNA Enz (50 μ L/well)	2 min 9 sec
42	Run cDNA on Therm	0

Section 2.6. Post-Amplification Purification

16 S2 St6 V4_0 VOYAGER

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Mix Samples	0 min 40 sec
3	Raise Magnet	0 min 10 sec
4	Mix SPRI Beads	1 min 8 sec

STEPS	ACTION	DURATION
5	DELAY	0 min 30 sec
6	Transfer 90uL 1 (90 µL/well)	0 min 33 sec
7	Transfer 90uL 2 (90 µL/well)	0 min 33 sec
8	Release Magnet	0 min 10 sec
9	Add SPRI Beads 1 & 2 (72 µL/well)	3 min 25 sec
10	DELAY	5 min 0 sec
11	Raise Magnet	0 min 10 sec
12	DELAY	6 min 0 sec
13	Volume Change	0
14	Remove Sup 1 (100 µL/well x 2)	1 min 14 sec
15	Remove Sup 2 (100 µL/well x 2)	1 min 14 sec
16	Add EtOH 1 (90 µL/well x 2)	0 min 51 sec
17	Add EtOH 2 (90 µL/well x 2)	0 min 51 sec
18	Volume Change	0
19	Remove Sup 1 (100 µL/well x 2)	1 min 14 sec
20	Remove Sup 2 (100 µL/well x 2)	1 min 14 sec
21	Add EtOH 1 (90 µL/well x 2)	0 min 51 sec
22	Add EtOH 2 (90 µL/well x 2)	0 min 51 sec
23	Volume Change	0
24	Remove Sup 1 (100 µL/well x 2)	1 min 14 sec

STEPS	ACTION	DURATION
25	Remove Sup 2 (100 µL/well x 2)	1 min 14 sec
26	DELAY	2 min 0 sec
27	Release Magnet	0 min 10 sec
28	Elute (25 µL/well)	1 min 38 sec
29	DELAY	5 min 0 sec
30	Raise Magnet	0 min 10 sec
31	DELAY	2 min 0 sec
32	Volume Change	0
33	Load New Plate	0
34	Elution Transfer (30 µL/well)	0 min 31 sec
35	Elution Transfer (30 µL/well)	0 min 31 sec
36	Release Magnet	0 min 10 sec
37	Samples on Deck B	0

Section 3.0. cDNA Normalization

16 S3 St0 V4_0 DONE

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Section3NormWTWorksheet	12 min 30 sec

Section 3.1. SPRI Bead Plating

16 S3 St1 V4_0 DONE

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Lower Magnet	0 min 10 sec
3	Plate out beads (60 μ L/well)	1 min 11 sec
4	Plate out beads (95 μ L/well)	1 min 20 sec
5	Plate out beads (50 μ L/well)	1 min 8 sec
6	Plate out beads (60 μ L/well)	1 min 11 sec
7	Plate out beads (95 μ L/well)	1 min 20 sec
8	Plate out beads (50 μ L/well)	1 min 8 sec

Section 3.2. Fragmentation Mix Creation and Plating

16 S3 St2 V4_0 DONE

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Pre-chill thermal cycler	0
3	Mix	0 min 24 sec
4	Distribute	0 min 7 sec
5	Plate out master mix (30 μ L/well)	1 min 51 sec
6	Proceed immediately to S3 St3	0

Section 3.3. Fragmentation Mix Addition and Post-Fragmentation SPRI Cleanup

16 S3 St3 V4_0 VOYAGER

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Stamp master mix into samples (15 μ L/well)	0 min 42 sec
3	Stamp master mix into samples (15 μ L/well)	0 min 42 sec
4	Run Frag	0
5	Replace Deck B with thawed freezer block	0
6	Move bead/reagent plate to Deck B	0
7	Add water and ETOH	0
8	Reload Plate	0
9	Reset volumes to reflect swapped plates	0
10	Ensure magnet is in Low position	0 min 10 sec
11	Mix beads	0 min 52 sec
12	Mix beads	0 min 52 sec
13	Add beads to sublibraries (30 μ L/well)	0 min 54 sec
14	Add beads to sublibraries (30 μ L/well)	0 min 54 sec
15	5 Minute RT Incubation	5 min 0 sec
16	Activate Magnet	0 min 10 sec
17	2 Minute Bead Immobilize	2 min 0 sec

STEPS	ACTION	DURATION
18	Transfer Supernatant (75 µL/well)	0 min 49 sec
19	Transfer Supernatant (75 µL/well)	0 min 49 sec
20	Deactivate Magnet	0 min 10 sec
21	Add beads to sublibraries (20 µL/well)	0 min 53 sec
22	Add beads to sublibraries (20 µL/well)	0 min 53 sec
23	5 Minute RT Incubation	5 min 0 sec
24	Activate Magnet	0 min 10 sec
25	3 Minute Bead Immobilize	3 min 0 sec
26	Allow for Over-Aspiration	0
27	Discard Supernatant (90 µL/well)	0 min 51 sec
28	Discard Supernatant (90 µL/well)	0 min 51 sec
29	EtOH Addition 1 (90 µL/well)	0 min 26 sec
30	EtOH Addition 1 (90 µL/well)	0 min 26 sec
31	EtOH Addition 1 (90 µL/well)	0 min 26 sec
32	EtOH Addition 1 (90 µL/well)	0 min 26 sec
33	1 Minute RT Incubation	1 min 0 sec
34	Discard EtOH (95 µL/well)	0 min 47 sec
35	Discard EtOH (95 µL/well)	0 min 47 sec
36	Discard EtOH (95 µL/well)	0 min 47 sec
37	Discard EtOH (95 µL/well)	0 min 47 sec

STEPS	ACTION	DURATION
38	EtOH Addition 2 (90 µL/well)	0 min 26 sec
39	EtOH Addition 2 (90 µL/well)	0 min 26 sec
40	EtOH Addition 2 (90 µL/well)	0 min 26 sec
41	EtOH Addition 2 (90 µL/well)	0 min 26 sec
42	1 Minute RT Incubation	1 min 0 sec
43	Discard EtOH (95 µL/well)	0 min 47 sec
44	Discard EtOH (95 µL/well)	0 min 47 sec
45	Discard EtOH (95 µL/well)	0 min 47 sec
46	Discard EtOH (95 µL/well)	0 min 47 sec
47	Deactivate Magnet	0 min 10 sec
48	Add Water to Resuspend (50 µL/well)	0 min 21 sec
49	Add Water to Resuspend (50 µL/well)	0 min 21 sec
50	Mix to Resuspend	0 min 34 sec
51	Mix to Resuspend	0 min 27 sec
52	Mix to Resuspend	0 min 27 sec
53	Mix to Resuspend	0 min 34 sec
54	Mix to Resuspend	0 min 27 sec
55	Mix to Resuspend	0 min 27 sec
56	5 Minute RT Incubation	5 min 0 sec
57	Activate Magnet	0 min 10 sec

STEPS	ACTION	DURATION
58	2 Minute Bead Immobilize	2 min 0 sec
59	Transfer Eluate (50 μ L/well)	0 min 41 sec
60	Transfer Eluate (50 μ L/well)	0 min 41 sec
61	Deactivate Magnet	0 min 10 sec

Section 3.4. Ligation Mix Creation and Plating

16 S3 St4 V4_0 DONE

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Mix	1 min 9 sec
3	Distribute	0 min 7 sec
4	Try to reduce tip losses	0 min 58 sec
5	Plate out master mix (104 μ L/well)	1 min 23 sec
6	Proceed immediately to S3 St5	0

Section 3.5. Ligation Mix Addition and Post-Ligation SPRI Cleanup

16 S3 St5 V4_0 VOYAGER

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Stamp master mix into samples (50 μ L/well)	1 min 2 sec
3	Stamp master mix into samples (50 μ L/well)	1 min 2 sec

STEPS	ACTION	DURATION
4	Run Adapter Ligation	0
5	Add ETOH	0
6	Reload Plate	0
7	Ensure magnet is in Low position	0 min 10 sec
8	Mix beads	0 min 47 sec
9	Mix beads	0 min 47 sec
10	Add beads to sublibraries (80 µL/well)	1 min 28 sec
11	Add beads to sublibraries (80 µL/well)	1 min 28 sec
12	5 Minute RT Incubation	5 min 0 sec
13	Activate Magnet	0 min 10 sec
14	5 Minute Bead Immobilize	5 min 0 sec
15	Allow for Over-Aspiration	0
16	Discard Supernatant (93 µL/well)	0 min 28 sec
17	Discard Supernatant (93 µL/well)	0 min 43 sec
18	Discard Supernatant (93 µL/well)	0 min 28 sec
19	Discard Supernatant (93 µL/well)	0 min 43 sec
20	EtOH Addition 1 (90 µL/well)	0 min 26 sec
21	EtOH Addition 1 (90 µL/well)	0 min 26 sec
22	EtOH Addition 1 (90 µL/well)	0 min 26 sec
23	EtOH Addition 1 (90 µL/well)	0 min 26 sec

STEPS	ACTION	DURATION
24	1 Minute RT Incubation	1 min 0 sec
25	Discard EtOH (95 µL/well)	0 min 47 sec
26	Discard EtOH (95 µL/well)	0 min 47 sec
27	Discard EtOH (95 µL/well)	0 min 47 sec
28	Discard EtOH (95 µL/well)	0 min 47 sec
29	EtOH Addition 2 (90 µL/well)	0 min 26 sec
30	EtOH Addition 2 (90 µL/well)	0 min 26 sec
31	EtOH Addition 2 (90 µL/well)	0 min 26 sec
32	EtOH Addition 2 (90 µL/well)	0 min 26 sec
33	1 Minute RT Incubation	1 min 0 sec
34	Discard EtOH (95 µL/well)	0 min 47 sec
35	Discard EtOH (95 µL/well)	0 min 47 sec
36	Discard EtOH (95 µL/well)	0 min 47 sec
37	Discard EtOH (95 µL/well)	0 min 47 sec
38	Air Dry Beads	1 min 0 sec
39	Deactivate Magnet	0 min 10 sec
40	Resuspend Beads in Water (23 µL/well)	0 min 30 sec
41	Resuspend Beads in Water (23 µL/well)	0 min 30 sec
42	5 Minute RT Incubation	5 min 0 sec
43	Activate Magnet	0 min 10 sec

STEPS	ACTION	DURATION
44	2 Minute Bead Immobilize	2 min 0 sec
45	Transfer Eluate (21 µL/well)	0 min 32 sec
46	Transfer Eluate (21 µL/well)	0 min 32 sec
47	Deactivate Magnet	0 min 10 sec

Section 3.6. Barcoding Round 4

16 S3 St6 V4_0 DONE

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Plate out Amp Mix (55 µL/well)	1 min 2 sec
3	Add UDIs	0
4	Proceed immediately to S3 St7	0

Section 3.7. Library Amp Mix Addition and Size Selection

16 S3 St7 V4_0 VOYAGER

STEPS	ACTION	DURATION
1	Initial Volumes	0
2	Stamp Amp Mix into samples (25 µL/well)	0 min 44 sec
3	Stamp Amp Mix into samples (25 µL/well)	0 min 44 sec
4	Run Indexing PCR	0
5	Add EtOH	0

STEPS	ACTION	DURATION
6	Reload Plate	0
7	Ensure magnet is in Low position	0 min 10 sec
8	Mix beads	0 min 36 sec
9	Mix beads	0 min 36 sec
10	Mix beads	0 min 36 sec
11	Mix beads	0 min 36 sec
12	Add beads to sublibraries (30 μ L/well)	0 min 54 sec
13	Add beads to sublibraries (30 μ L/well)	0 min 54 sec
14	5 Minute RT Incubation	5 min 0 sec
15	Activate Magnet	0 min 10 sec
16	2 Minute Bead Immobilize	2 min 0 sec
17	Transfer Supernatant (75 μ L/well)	0 min 49 sec
18	Transfer Supernatant (75 μ L/well)	0 min 49 sec
19	Deactivate Magnet	0 min 10 sec
20	Add beads to sublibraries (10 μ L/well)	0 min 49 sec
21	Add beads to sublibraries (10 μ L/well)	0 min 49 sec
22	5 Minute RT Incubation	5 min 0 sec
23	Activate Magnet	0 min 10 sec
24	3 Minute Bead Immobilize	3 min 0 sec
25	Allow for Over-Aspiration	0

STEPS	ACTION	DURATION
26	Discard Supernatant (90 µL/well)	0 min 51 sec
27	Discard Supernatant (90 µL/well)	0 min 51 sec
28	EtOH Addition 1 (90 µL/well)	0 min 26 sec
29	EtOH Addition 1 (90 µL/well)	0 min 26 sec
30	EtOH Addition 1 (90 µL/well)	0 min 26 sec
31	EtOH Addition 1 (90 µL/well)	0 min 26 sec
32	1 Minute RT Incubation	1 min 0 sec
33	Discard EtOH (95 µL/well)	0 min 47 sec
34	Discard EtOH (95 µL/well)	0 min 47 sec
35	Discard EtOH (95 µL/well)	0 min 47 sec
36	Discard EtOH (95 µL/well)	0 min 47 sec
37	EtOH Addition 2 (90 µL/well)	0 min 26 sec
38	EtOH Addition 2 (90 µL/well)	0 min 26 sec
39	EtOH Addition 2 (90 µL/well)	0 min 26 sec
40	EtOH Addition 2 (90 µL/well)	0 min 26 sec
41	1 Minute RT Incubation	1 min 0 sec
42	Discard EtOH (95 µL/well)	0 min 47 sec
43	Discard EtOH (95 µL/well)	0 min 47 sec
44	Discard EtOH (95 µL/well)	0 min 47 sec
45	Discard EtOH (95 µL/well)	0 min 47 sec

STEPS	ACTION	DURATION
46	Deactivate Magnet	0 min 10 sec
47	Resuspend Beads in Water (20 µL/well)	0 min 19 sec
48	Resuspend Beads in Water (20 µL/well)	0 min 19 sec
49	Mix to Resuspend	0 min 26 sec
50	Mix to Resuspend	0 min 23 sec
51	Mix to Resuspend	0 min 23 sec
52	Mix to Resuspend	0 min 26 sec
53	Mix to Resuspend	0 min 23 sec
54	Mix to Resuspend	0 min 23 sec
55	5 Minute RT Incubation	5 min 0 sec
56	Activate Magnet	0 min 10 sec
57	2 Minute Bead Immobilize	2 min 0 sec
58	Transfer Eluate (20 µL/well)	0 min 32 sec
59	Transfer Eluate (20 µL/well)	0 min 32 sec
60	Deactivate Magnet	0 min 10 sec

Appendix C: Thermocycling Programs

Section 2: cDNA Capture and Amplification

THAW LYSATES		
Run Time	5 minutes	
Lid Temperature	40°C	
Sample Volume	55 µL	
Step	Time	Temperature
1	5 min	37°C
2	Hold	4°C

TEMPLATE SWITCH		
Run Time	60 minutes	
Lid Temperature	70°C	
Sample Volume	100 µL	
Step	Time	Temperature
1	60 min	42°C
2	Hold	4°C

cDNA AMPLIFICATION			
Run Time		50-70 minutes	
Lid Temperature		105°C	
Sample Volume		100 µL	
Step	Time	Temperature	Cycle
1	3 min	95°C	1
2	20 sec	98°C	5
3*	45 sec	65°C*	
4	3 min	72°C	
5	20 sec	98°C	Variable, see above
6*	20 sec	67°C*	
7	3 min	72°C	
8	5 min	72°C	1
9	Hold	4°C	1

Section 3: Sequencing Library Preparation

FRAGMENTATION and END PREP		
Run Time		40 minutes
Lid Temperature		70°C
Sample Volume		50 µL
Step	Time	Temperature
1	Hold*	4°C
2	10 min	32°C
3	30 min	65°C
4	Hold	4°C

ADAPTER LIGATION		
Run Time	15 minutes	
Lid Temperature	30°C*	
Sample Volume	100 µL	
Step	Time	Temperature
1	15 min	20°C
2	Hold	4°C

INDEXING PCR			
Run Time		~30 minutes	
Lid Temperature		105°C	
Sample Volume		50 µL	
Step	Time	Temperature	Cycles
1	3 min	95°C	1
2	20 sec	98°C	Varies, see table above
3	20 sec	67°C	
4	1 min	72°C	
5	5 min	72°C	1
6	Hold	4°C	1

Appendix D: Troubleshooting

Error warning during the execution of a program

- In case of an error warning, abort the program. Do not press "RUN". Scroll down "Run from step". You can then continue from the error location. This allows to resume directly from the point of error.

Tip Pinching

- Tip pinching may occur when using a newly frozen PCR freezer block for a plate pooling step. Ensure that your PCR Freezer Block has been thawed at room temperature for at least 10 minutes.
- Ensure to apply sufficient pressure when placing the plate onto the frozen PCR freezer block.

Incorrect tip location inputted

- The D-ONE Pipette will check the pipette tips before the step starts. The ASSIST PLUS will display an error warning if a pipette tip is incorrectly detected. Abort and restart the program with the correct tip location input.
- The VIAFLO will check the pipette tip after the step is finished. The ASSIST PLUS will display an error warning if a pipette tip is incorrectly detected. Abort and restart the program with the correct tip location input.

Appendix E: Revision History

Version	Description	Date
1.0	Initial release	June 2026
1.1	Section 2.4: added buffer pooling recommendation.	October 2026

Appendix F: Acknowledgements

All graphics were created with [BioRender.com](https://www.biorender.com).



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