

Oct 22, 2025

Version 1

Setting up Illumina DNA flex library prep on Sciclone (Perkin Elmer) V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.81wgbw2w1gpk/v1>

Christopher Duda¹, Kathryn Judy², Padmini Ramachandran¹, Christopher Grim²

¹FDA; ²US FDA

GenomeTrakr

Tech. support email: genomeTrakr@fda.hhs.gov



Christopher Duda

FDA

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.81wgbw2w1gpk/v1>

Protocol Citation: Christopher Duda, Kathryn Judy, Padmini Ramachandran, Christopher Grim 2025. Setting up Illumina DNA flex library prep on Sciclone (Perkin Elmer). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.81wgbw2w1gpk/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: In development

We are still developing and optimizing this protocol

Created: September 30, 2025

Last Modified: October 22, 2025

Protocol Integer ID: 228481

Keywords: illumina dna flex library prep on sciclone, sciclone robot for the illumina dna, illumina dna flex library prep, sciclone robot, sciclone, illumina dna, flex library preparation, perkin elmer, flex library prep, detailed instruction, laboratory

Abstract

PURPOSE: This document provides detailed instructions on how to set up the Perkin Elmer's Sciclone robot for the Illumina DNA flex library preparation.

SCOPE: This protocol is intended for any laboratory who has Perkin Elmer's Sciclone robot, and for setting up Illumina DNA flex library prep.



Materials

Product name	- Samples	- Catalog number
--------------	-----------	------------------

Illumina DNA Prep, (M) Tagmentation	- (24 samples, IPB)	- 20060060
-------------------------------------	---------------------	------------

Illumina DNA Prep, (M) Tagmentation	- (96 samples, IPB)	- 20060059
-------------------------------------	---------------------	------------

Illumina DNA/RNA UD Indexes Set A, Tagmentation	(96 indexes, 96 samples)	20091654
---	--------------------------	----------

Illumina DNA/RNA UD Indexes Set B, Tagmentation	(96 indexes, 96 samples)	20091656
---	--------------------------	----------

Illumina DNA/RNA UD Indexes Set C, Tagmentation	(96 indexes, 96 samples)	20091658
---	--------------------------	----------

Illumina DNA/RNA UD Indexes Set D, Tagmentation	(96 indexes, 96 samples)	20091660
---	--------------------------	----------

Pipette Tips, Sterile, Filtered (P20, P200, P1000), any manufacturer

HardShell PCR Plate 96-well, blue-shell, clear wells (Revvity Cat# 6008870), 10 per run

StorPlate 96-well x 450 μ L V-bottom (Revvity Cat# 6008290), 2 per run

150 μ L Barrier Sterile 96 Rack Tips (Revvity Cat# 6070441), 19 boxes per run

Universal plate lids (Revvity Cat# 6000030), 1 per run

Disposable ODTs lids (Revvity Cat# CLS151919), 1 per run

96 $\times$ 2 mL V-bottom Deep-Well Storage Plate (Revvity Cat# 6008880), 1 per run

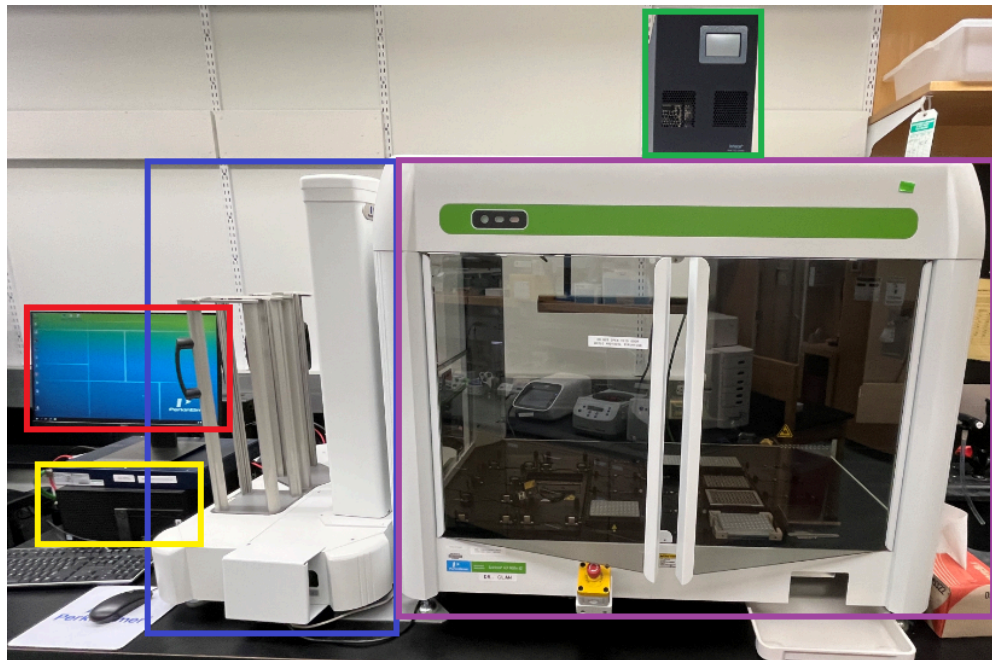
Deep-Well Reservoir, single reservoir or 12 column (Revvity Cat# 6008730 or 6008700), 1 per run

2.0 mL Eppendorf tubes (Eppendorf Cat# 022431048 or equivalent)

Reagent Reservoirs (Thermo Fisher Scientific Cat# 13681504 or equivalent)

Section 1A Machine Parts (Sciclone 1)

1



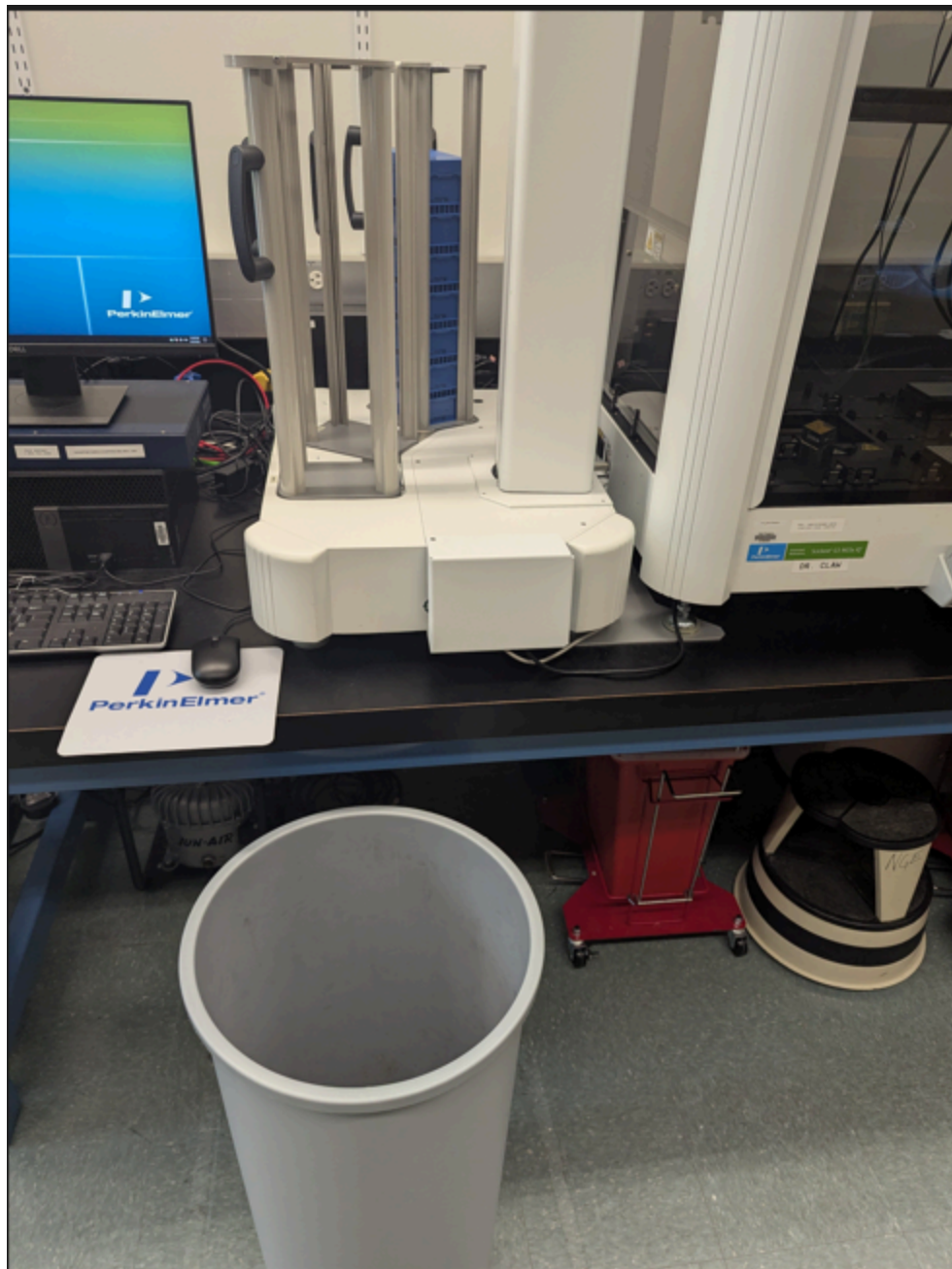
Purple: Main Sciclone platform/unit: Main part of the Sciclone that houses the machinery and where reagent plates are placed.

Blue: Twister 3 Sciclone attachment: Feeds pipette boxes to the Sciclone robotics and discards spent ones.

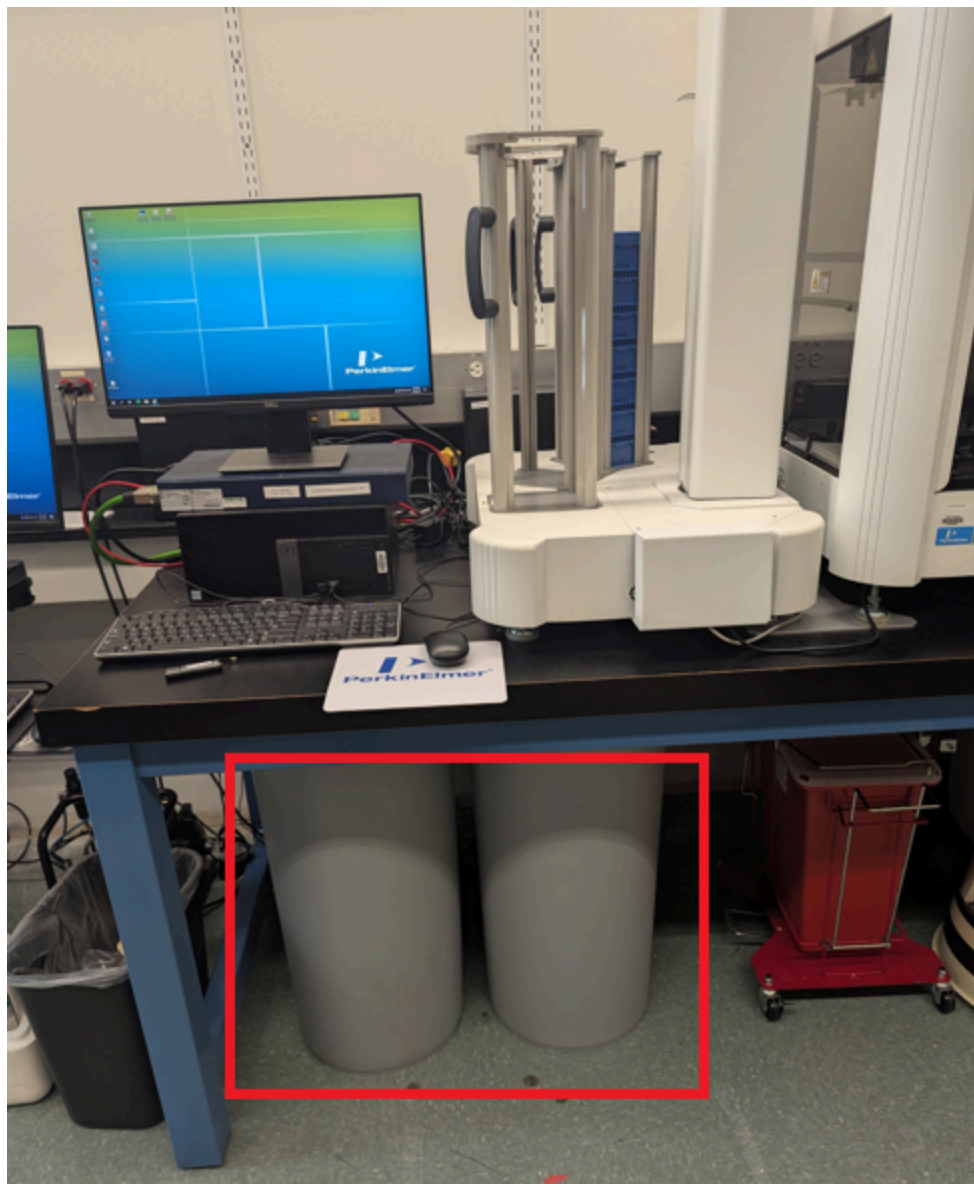
Red: Monitor: Needed for interfacing with the Sciclone. It is where programs are selected and how to find the volume of reagents for running DNA library prep.

Yellow: ODT power and control unit: Needed for operating the Sciclone.

Green: CPAC controller: Needed for operating the Sciclone.

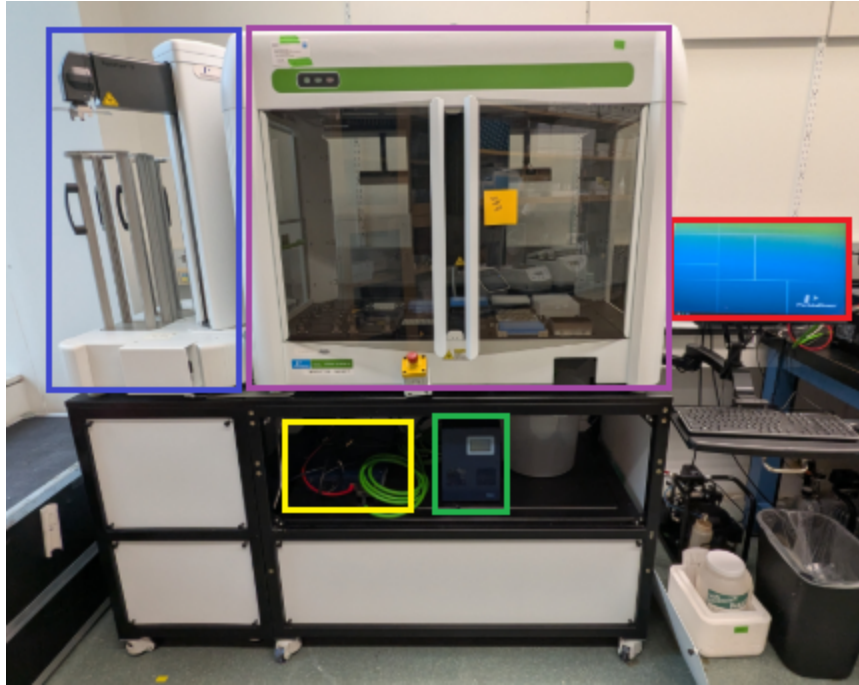


There is also a tall grey bucket that needs to be placed below the Twister 3 arm for discarding of empty pipette tip boxes and used plates. The buckets are located underneath the table that Sciclone 1 sits on.



Section 1B: Machine Parts (Sciclone 2)

2



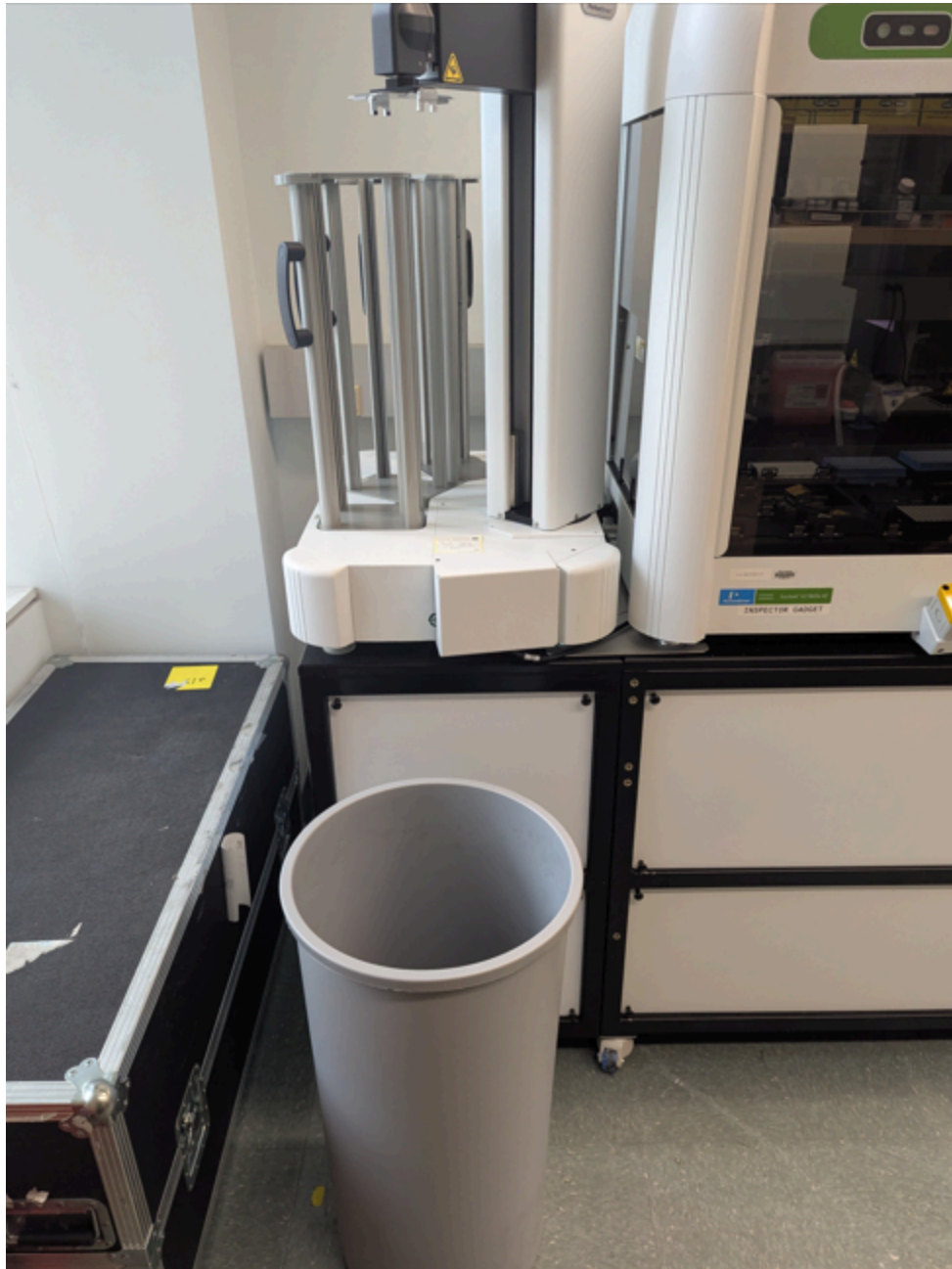
Purple: Main Sciclone platform/unit: Main part of the Sciclone that houses the machinery and where reagent plates are placed.

Blue: Twister 3 Sciclone attachment: Feeds pipette boxes to the Sciclone robotics and discards spent ones.

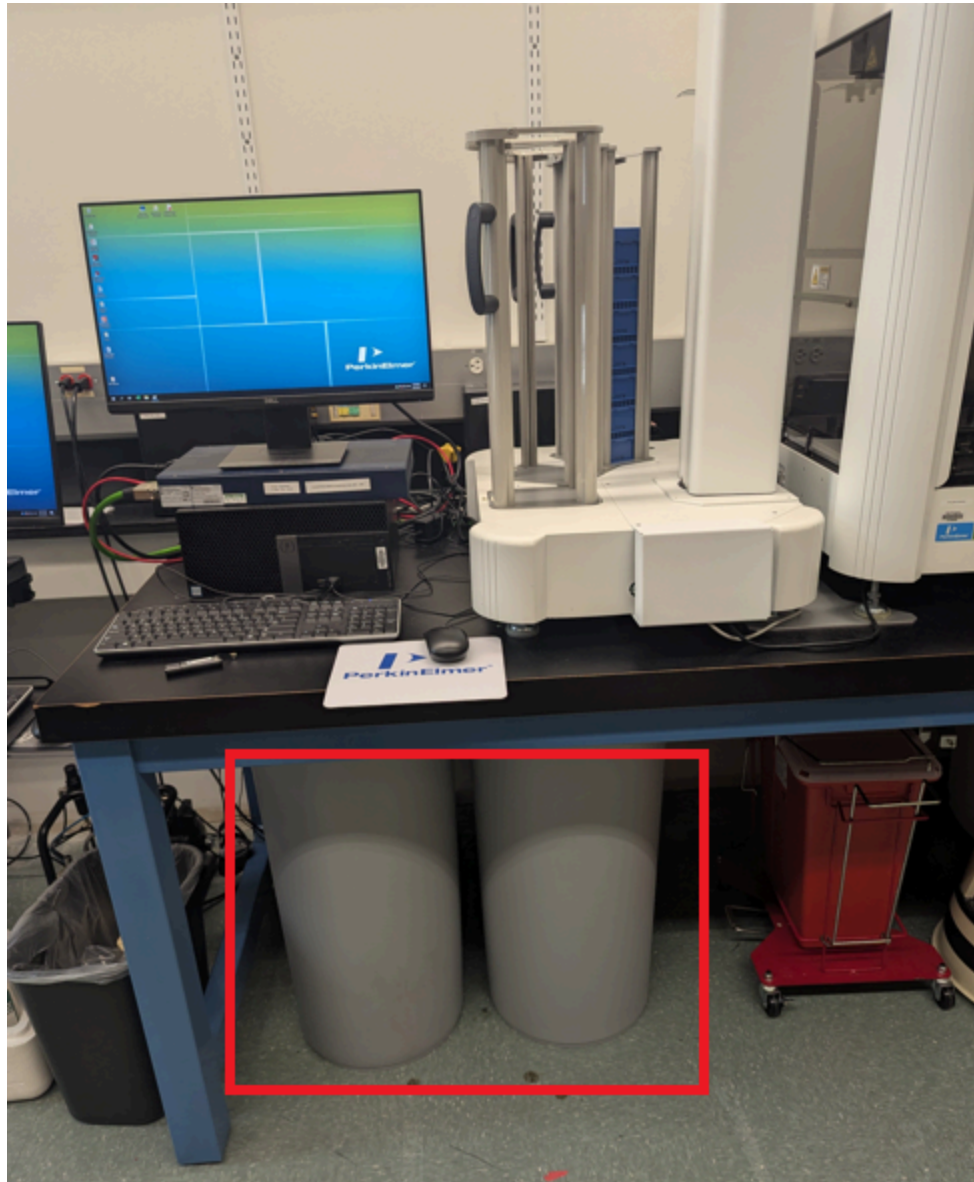
Red: Monitor: Needed for interfacing with the Sciclone. It is where programs are selected and how to find the volume of reagents for running DNA library prep.

Yellow: ODTN power and control unit: Needed for operating the Sciclone.

Green: CPAC controller: Needed for operating the Sciclone.

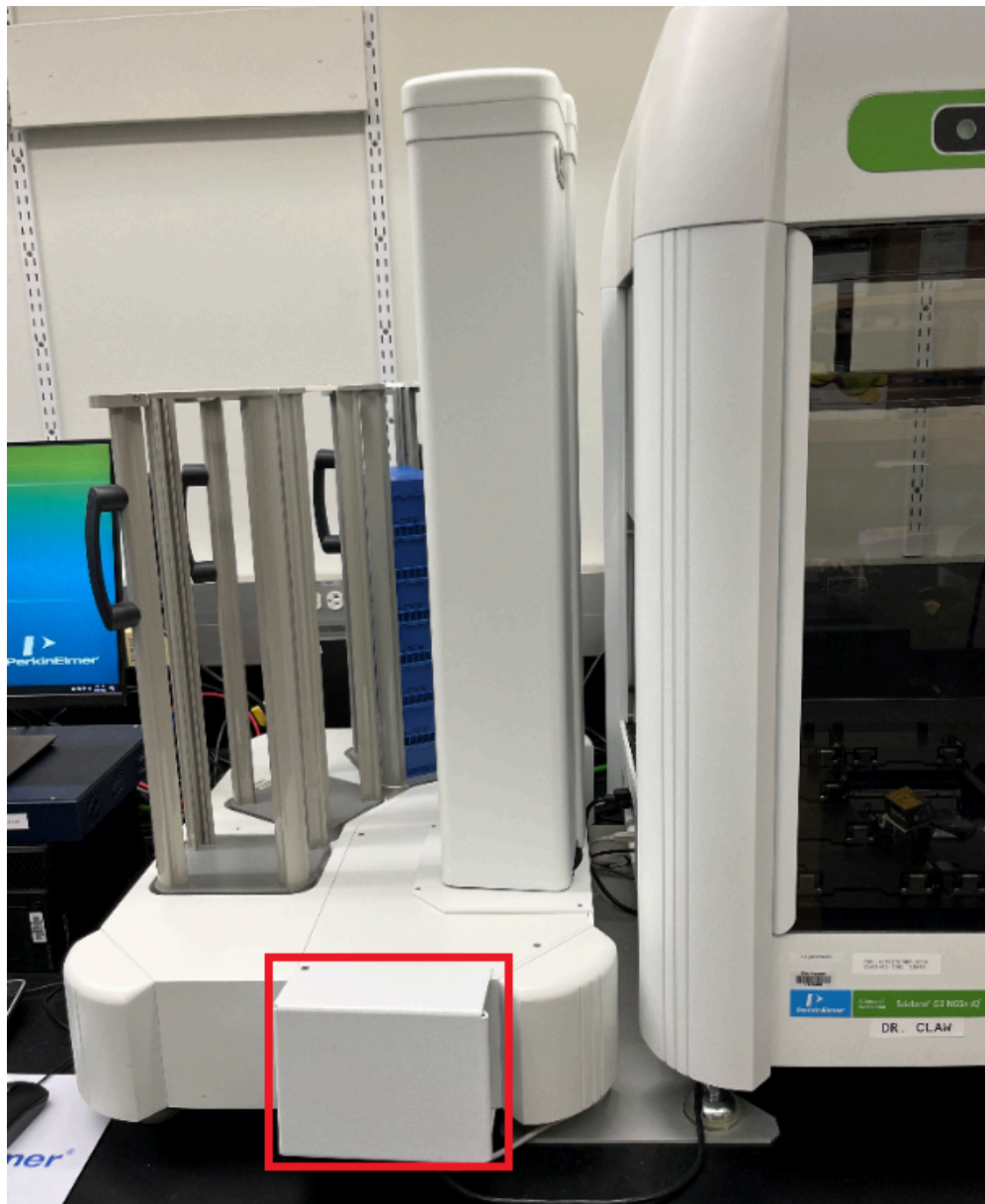


There is also a tall grey bucket that needs to be placed below the Twister 3 arm for discarding of empty pipette tip boxes and used plates. The buckets are located underneath the table that Sciclone 1 sits on.



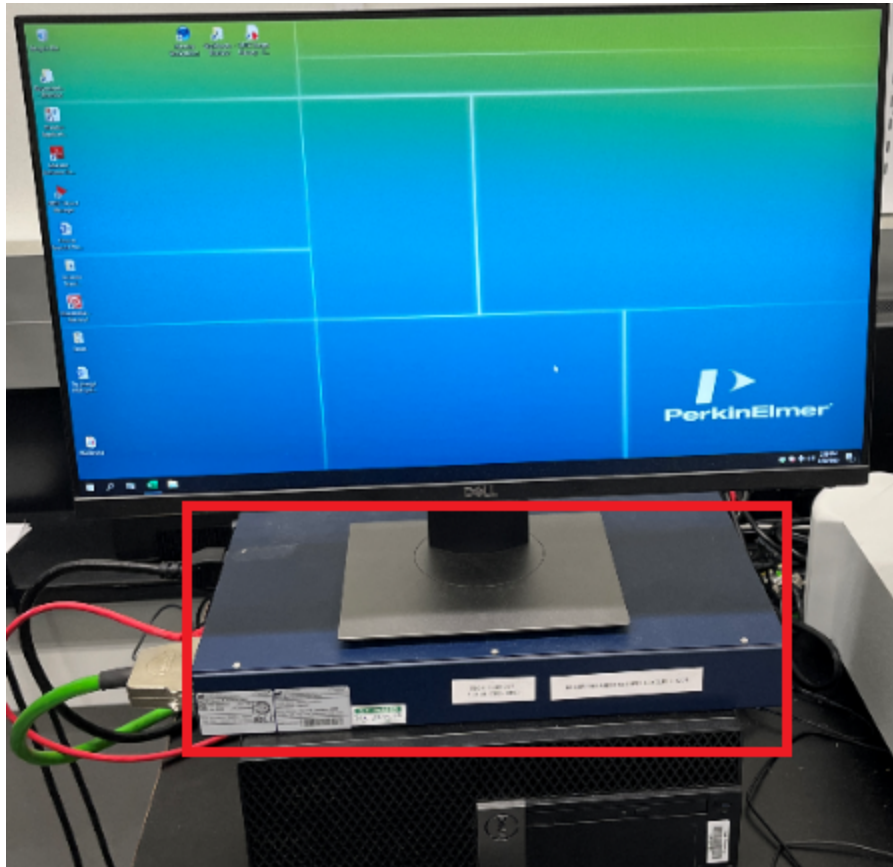
Section 2A: Prepping The Sciclone (Sciclone 1)

- 3 Turn the following on in this order *Important*
 1. Turn on the monitor attached to the Sciclone.
 2. Turn on the Twister 3 by opening the small door at the base of the tower and toggling on the power switch.



The door is located at the base of the tower.

- 4 4. Turn on the ODT controller by toggling on the power switch.



The ODT is located below the monitor



The power button is located on the left side of the ODT .

- 5 3. Turn on the CPAC control unit by switching on the power switch.

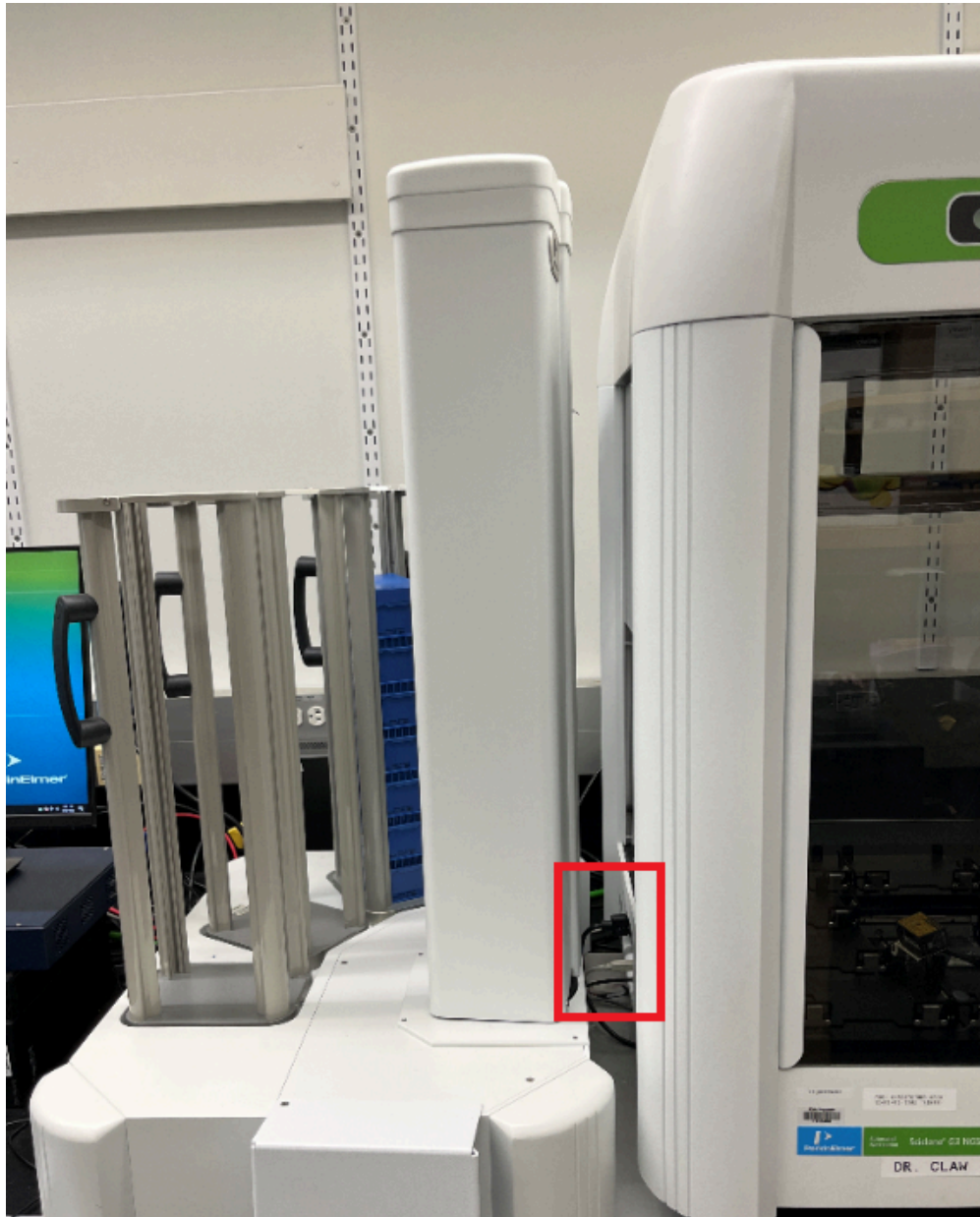


The CPAC is located on top of the Sciclone main unit.



The power button is located on the back of the CPAC unit.

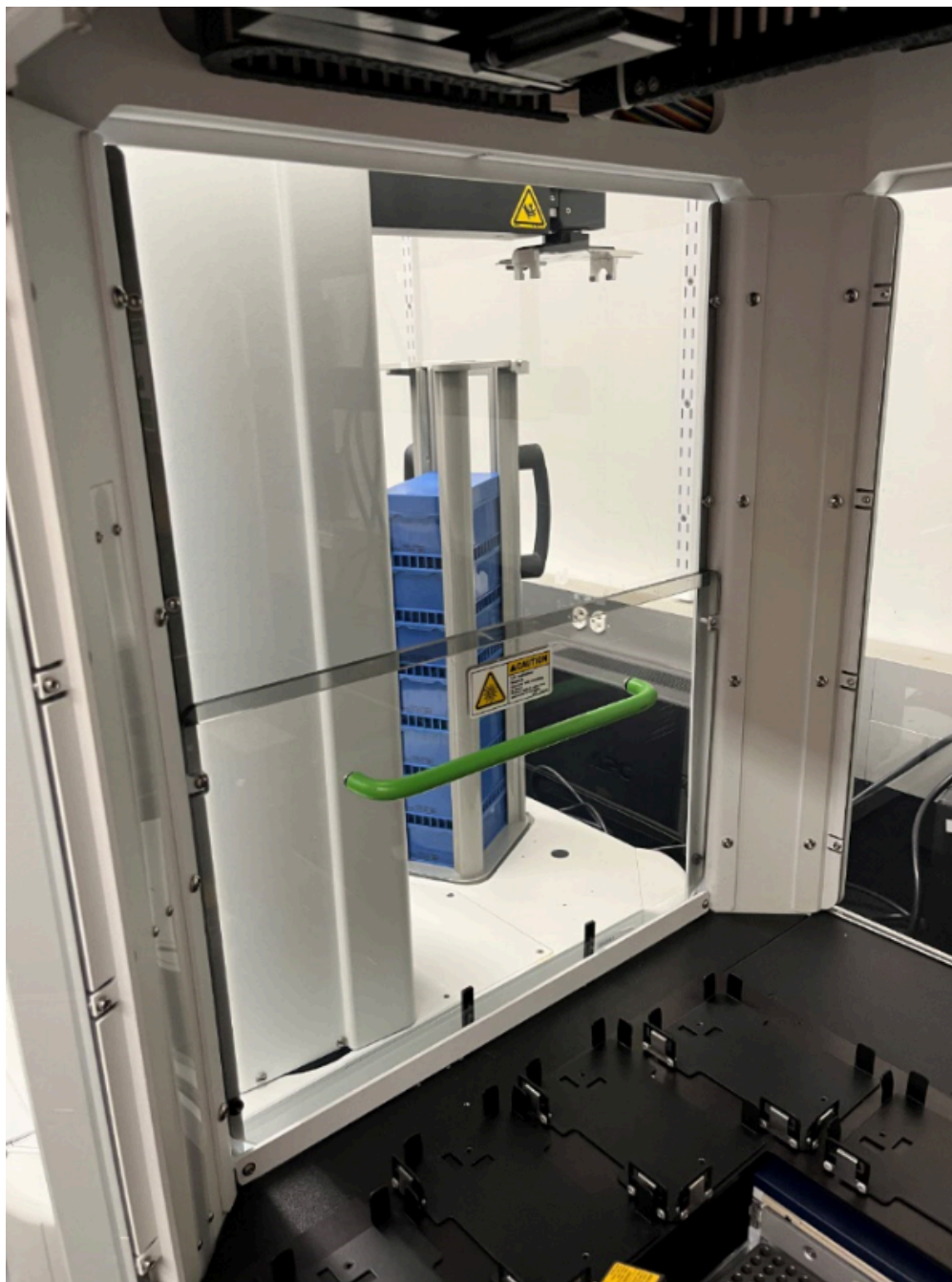
- 6 5. Turn on the Main Sciclone Unit by toggling the power switch



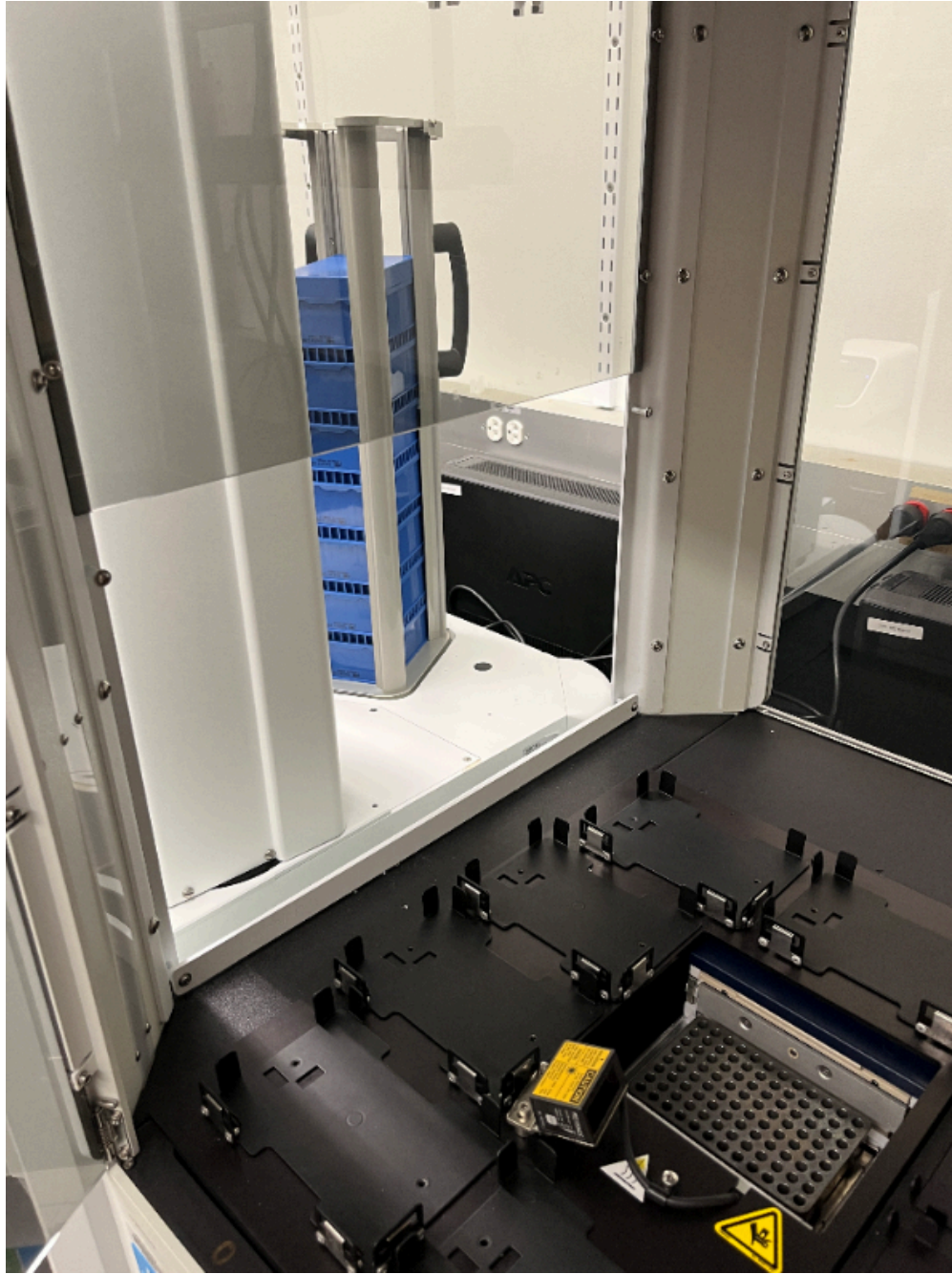
The power button is located between the Twister 3 and Main Sciclone Unit.



- 7 Remove the UV Shield by using the green handle. It is inside the main Sciclone unit on the same side as the Twister 3. This will prevent the Twister 3 from operating if left attached.



UV shield on



UV shield off.

Section 2B: Prepping The Sciclone (Sciclone 2)

- 8 Turn the following on in this order *Important*
 1. Turn on the monitor attached to the Sciclone.
 2. Turn on the Twister 3 by opening the small door at the base of the tower and toggling on the power switch.



The door is located at the base of the tower.



The power switch is located in the door panel.

- 9 4. Turn on the ODC controller by toggling on the power switch.



Power button is located in the front of the controller, highlighted in red.

- 10 3. Turn on the CPAC unit by switching on the power switch.



The CPAC unit is located under the main Sciclone unit

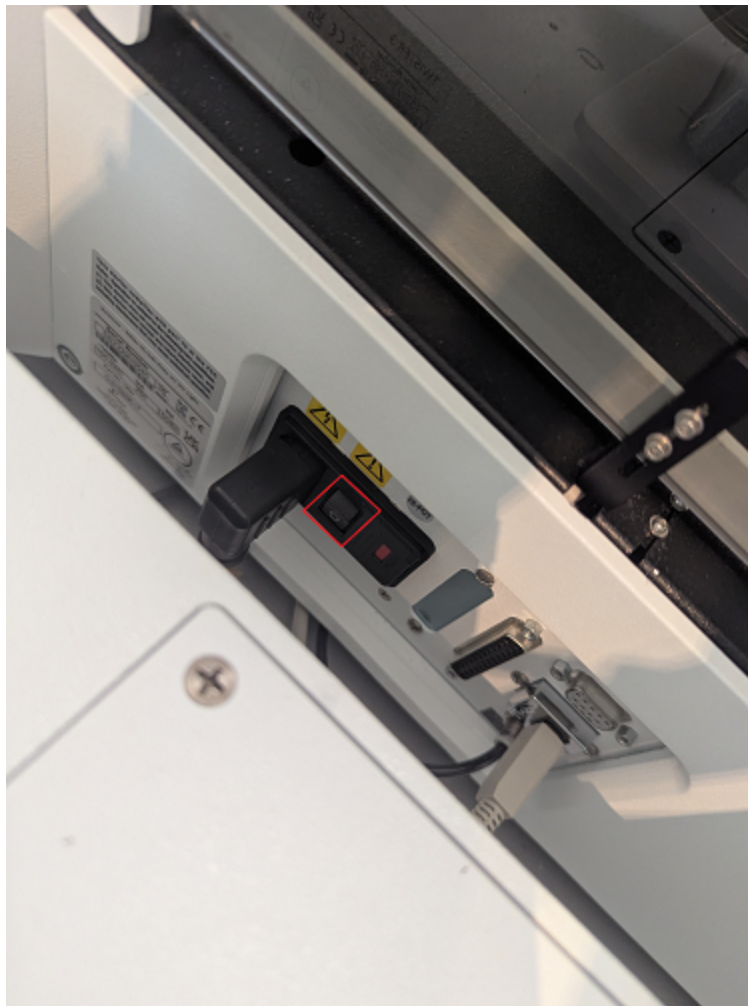


The power switch is located on the top of the back of the CPAC unit.

- 11 5. Turn on the Main Sciclone Unit by toggling the power switch

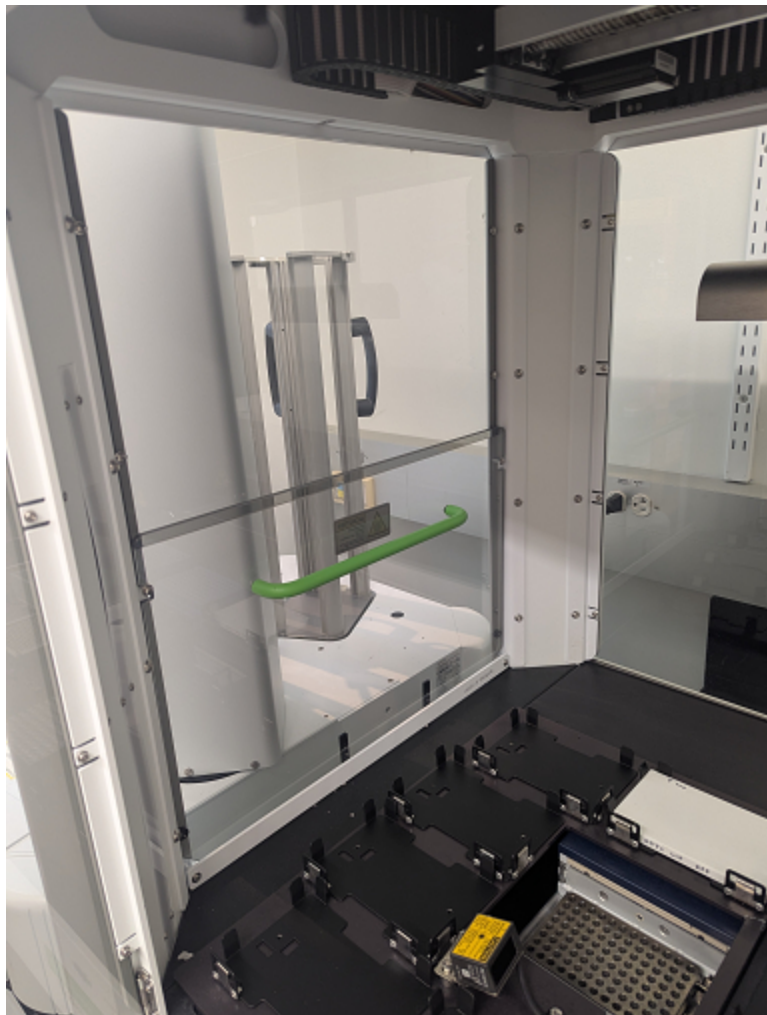


The power button is located in between the Sciclone and Twister 3 units. highlighted in red

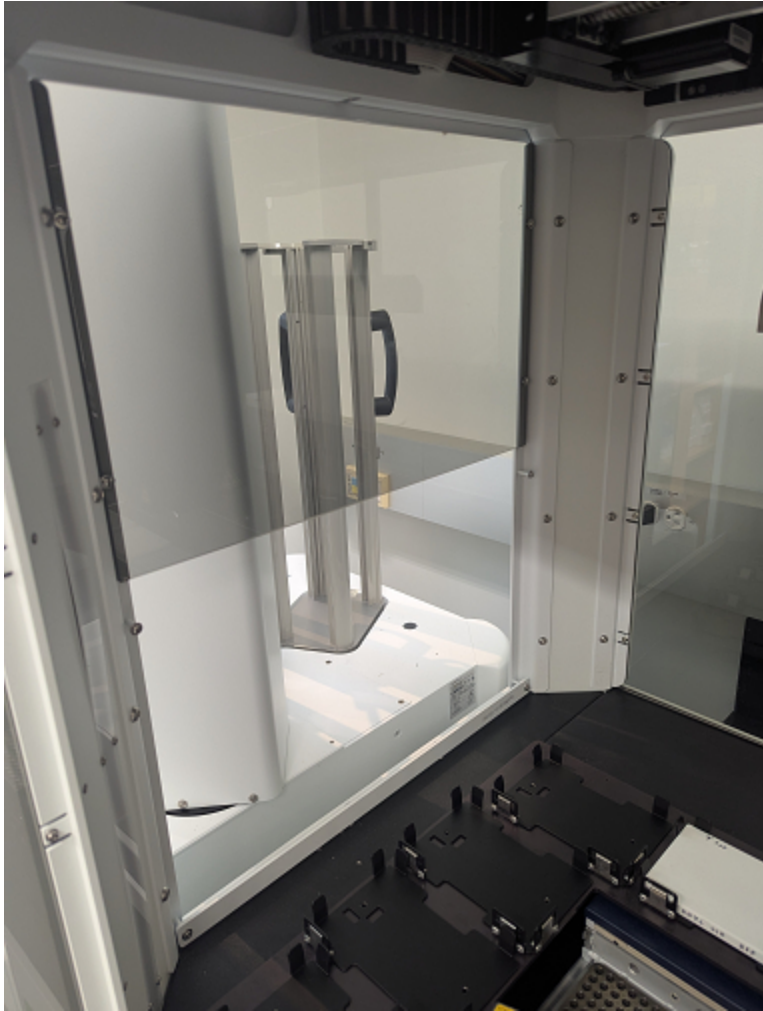


Power button highlighted in red

- 12 Remove the UV Shield by using the green handle. It is inside the main Sciclone unit on the same side as the Twister 3. This will prevent the Twister 3 from operating if left attached.



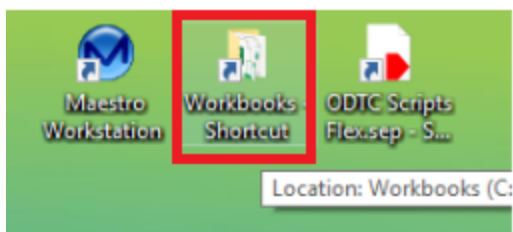
UV Shield on



UV Shield off

Opening the Nextera Flex Library Prep Workbook

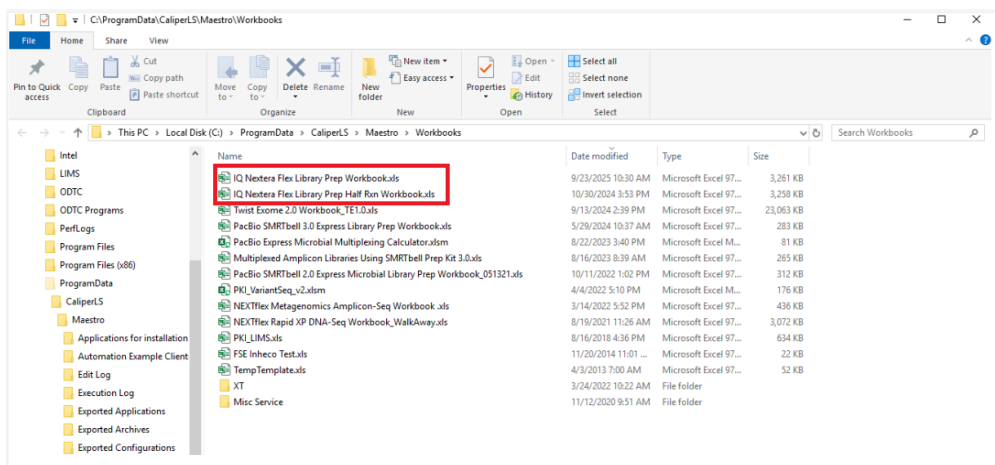
- 13 Once the Sciclone is turned on, go to the monitor and find the workbook shortcut on the desktop.



For Sciclone 2, The shortcut is on the left side of the desktop with the same name.

- 14 Once in the workbooks folder, select the "IQ Nextera Flex Library Workbook" or the "IQ Nextera Flex Library Prep Half RXN workbook" This protocol shows the "IQ Nextera Flex Library Workbook"

Be sure to open the correct workbook. Mismatching workbooks and Sciclone programs will cause libraries to fail



This will open an excel file that will guide you in how to set up the plates.

- 15 *The following screenshots are from the "IQ Nextera Library Flex DNA Prep Workbook".
If using a half reaction, the layout will be similar, but the reagent volumes will be different.

The screenshot shows the 'Nextera™ DNA Flex Library Prep' workbook interface. It features a grid for sample volumes across 12 columns and 96 rows (A-H x 1-12). The 'Number of Columns' is set to 12. A summary table on the right indicates the total number of samples (96) and the total volume of reagents (2,763,664). The grid is color-coded by row: A-H (blue), I-L (orange), M-P (green), and Q-T (red).

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	250	250	250	250	250	250	250	250	250	250	250	250
B	250	250	250	250	250	250	250	250	250	250	250	250
C	250	250	250	250	250	250	250	250	250	250	250	250
D	250	250	250	250	250	250	250	250	250	250	250	250
E	250	250	250	250	250	250	250	250	250	250	250	250
F	250	250	250	250	250	250	250	250	250	250	250	250
G	250	250	250	250	250	250	250	250	250	250	250	250
H	250	250	250	250	250	250	250	250	250	250	250	250

This is how the workbook should look for a full plate (12 columns, 96 samples).

The screenshot shows the 'Nextera™ DNA Flex Library Prep' workbook interface. It features a grid for sample volumes across 12 columns and 96 rows (A-H x 1-12). The 'Number of Columns' is set to 12. A summary table on the right indicates the total number of samples (96) and the total volume of reagents (2,763,664). The grid is color-coded by row: A-H (blue), I-L (orange), M-P (green), and Q-T (red).

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	30	30	30	30	30	30	30	30	30	30	30	30
B	30	30	30	30	30	30	30	30	30	30	30	30
C	30	30	30	30	30	30	30	30	30	30	30	30
D	30	30	30	30	30	30	30	30	30	30	30	30
E	30	30	30	30	30	30	30	30	30	30	30	30
F	30	30	30	30	30	30	30	30	30	30	30	30
G	30	30	30	30	30	30	30	30	30	30	30	30
H	30	30	30	30	30	30	30	30	30	30	30	30

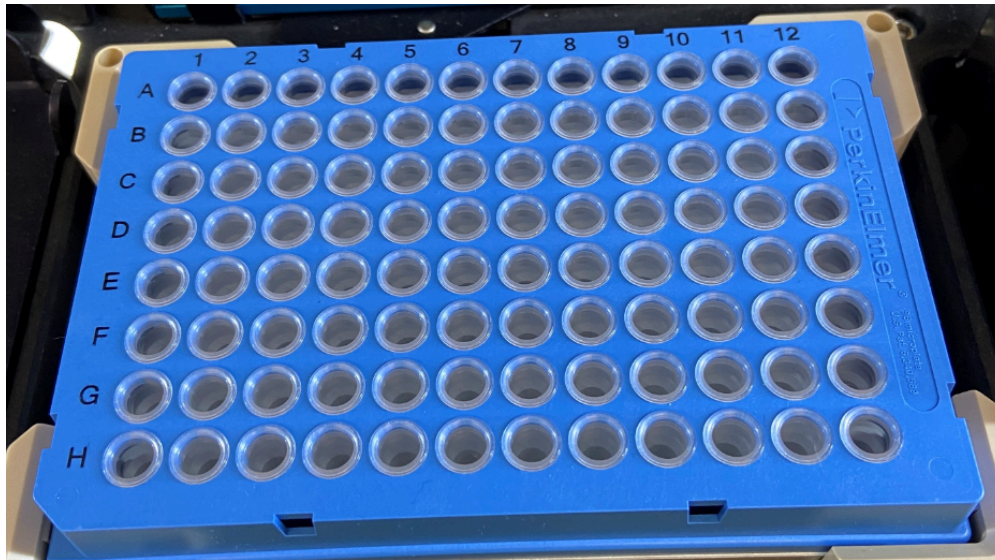
The way this workbook works is based on sample numbers in multiples of 8. The machine executes based on full columns, not total samples. It is advised that library prep with the Sciclone be done with a full 96 samples to not waste reagents. In the event that you do not have enough samples for a whole column, the missing samples can be left out or have the template DNA be replaced with water.

All library reagents will be consumed by unused wells

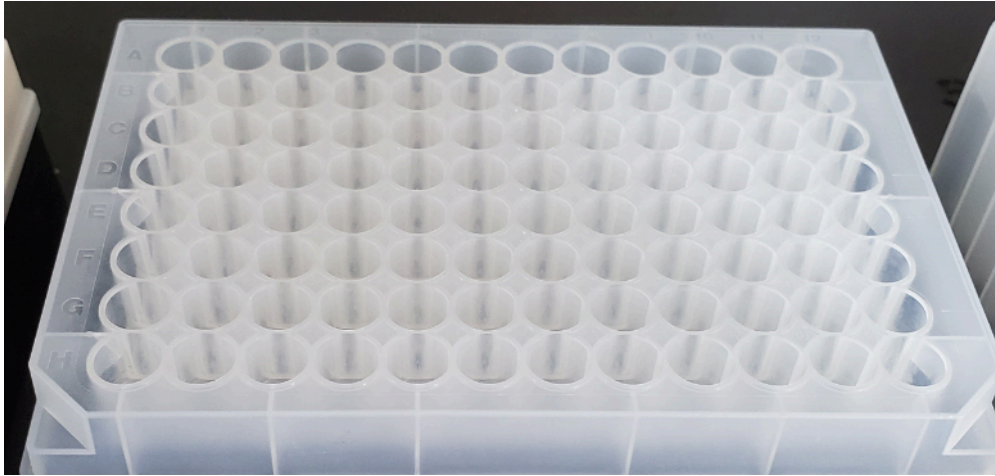
- 16 Once the number of columns has been input, enter the date in cell "B1" and save the document ("Ctrl+S")
- There are 7 total plates that need to have reagents added.
- This excel document displays the type of plate, reagent/s to be added, where to add reagents, volume of reagents, where to place the plate in the Sciclone, and any additional requirements for the plates i.e lids.
- It is recommended to label all plates with their location before adding reagents to avoid any mix-ups.

Plate Creation

- 17 While the excel sheet explains each plate and where they go, the process will be highlighted here.
- To start we will display each type of plate and what they look like.



Bio-Rad HSP-96 PCR Plate: Most of the plates are 96-well PCR plates.



Perkin Elmer 450 V-Bottom StorPlate: has a wide V shaped bottom with an increased volume. Used in washing steps.



Ethanol reservoir: Used to store a large volume of ethanol.

- 18 Use the workbook for your intended reaction to create the plates. A few notes before starting:
- The volume listed for all plates already has extra volume to account for pipetting error, and additional volume is not necessary.
 - The order of plate creation is arbitrary, except for the "Reagent Plate".
 - The only reagent that needs to be mixed before being added is the BL/TB1 master mix.

** The ratios for the master mix are found in the blue box to the right of the reagent plate in the excel workbook.8 **



- The last thing you should make is the BL/TB1 master mix. The beads will settle and have poor DNA binding if added too early. Vortex before adding right before starting the Sciclone.

19 Before adding reagents, thaw the following:

- Resuspension Buffer (RSB)
- Tagmentation Buffer 1 (TB1)
- Enhanced PCR Mix (EPM)
- Bead-Linked Transposome (BLT)
- Extracted DNA (If frozen)

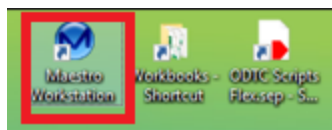
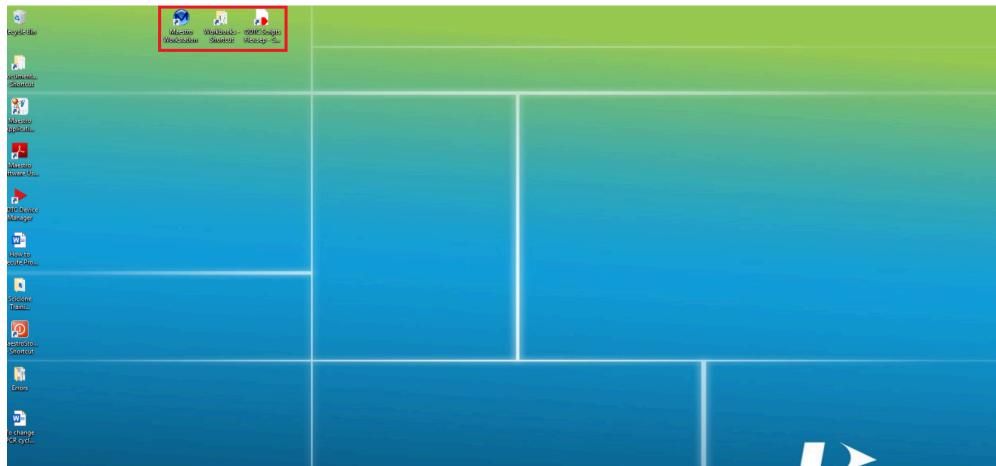
20 Dilute extracted DNA to the desired concentration. The sample plate is loaded with 30 μ l of sample DNA.

.	Hold at 10°C
Total DNA Input (ng)	Number of PCR Cycles (X)
1–9	12
10–24	8
25–49	6
50–99	5
100–500	5
Blood/Saliva	5

- 21 Create all sample plates in accordance with the excel workbook for your program and sample count and place them in the Sciclone. Also place the ethanol reservoir and all non-reagent containing plates in the specified location in the Sciclone. (See Step 22). Add the "Reagent Plate" last.
- For the "Reagent Plate":
1. Add the Enhanced PCR Mix (EPM) and Tagment Stop Buffer (TSB).
 3. Make the BL/TB-1 master mix in accordance with the blue table in the excel workbook.
 4. Vortex the master mix directly before adding it to the plate.
 5. Place the "Reservoir Plate" onto the Sciclone directly before starting.

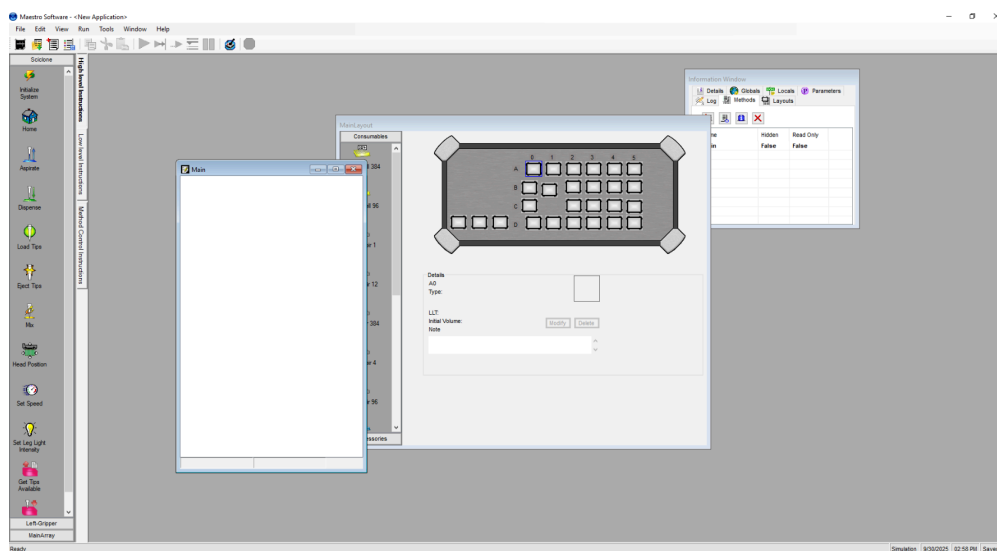
Running the Sciclone

- 22 The maestro software will tell you where the plates go. To open the software, double click the shortcut on the desktop.



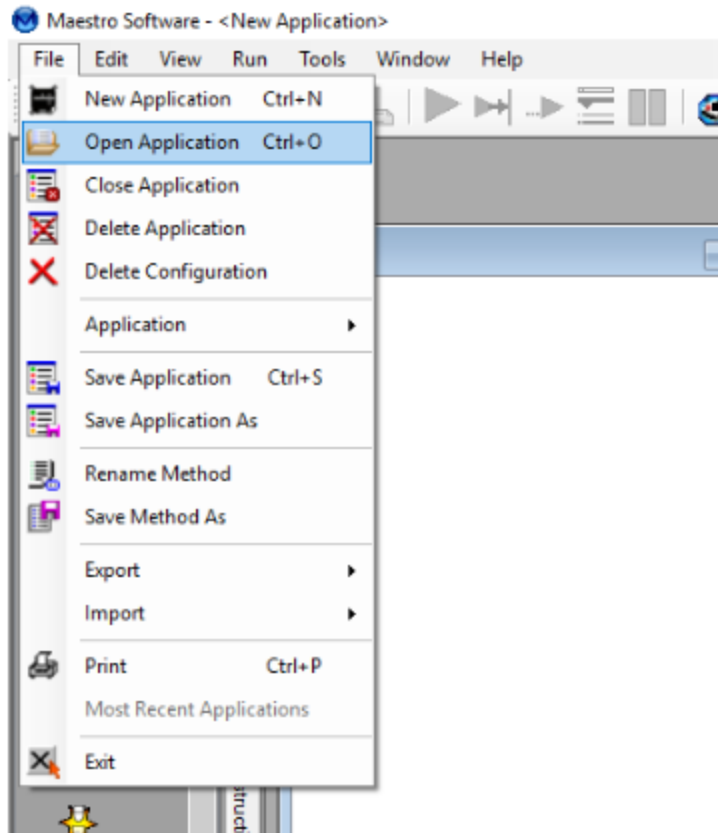
For Sciclone 2, The shortcut is on the left side of the desktop with the same name.

- 23 Once the Maestro software launches, it will attempt to communicate with the Sciclone and its peripherals. If they were all turned on correctly, this interface will open.



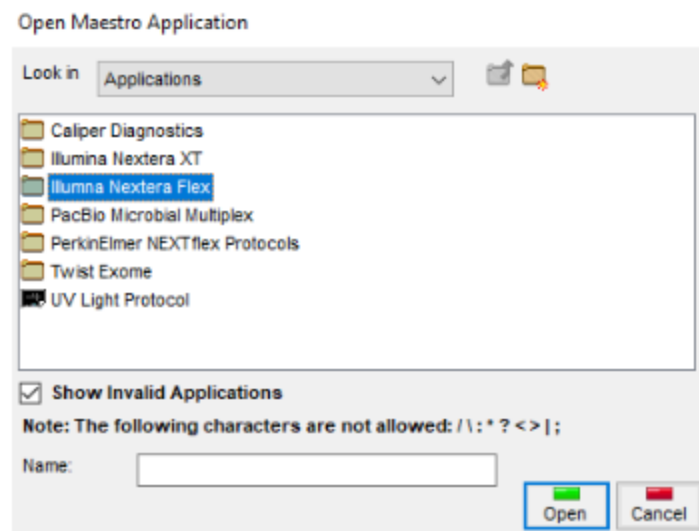
Open Maestro Software

- 24 Navigate to and click on the "file" tab in the top left of the screen. A dropdown menu will appear. Select the "Open Application" from the menu.

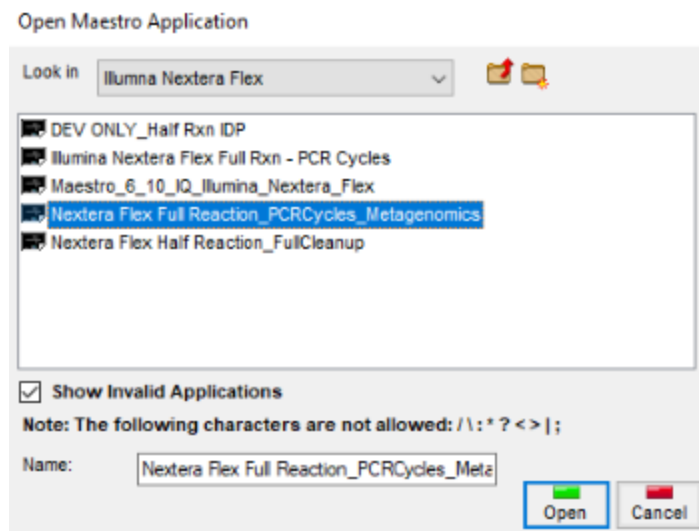


Navigate to "Open Application"

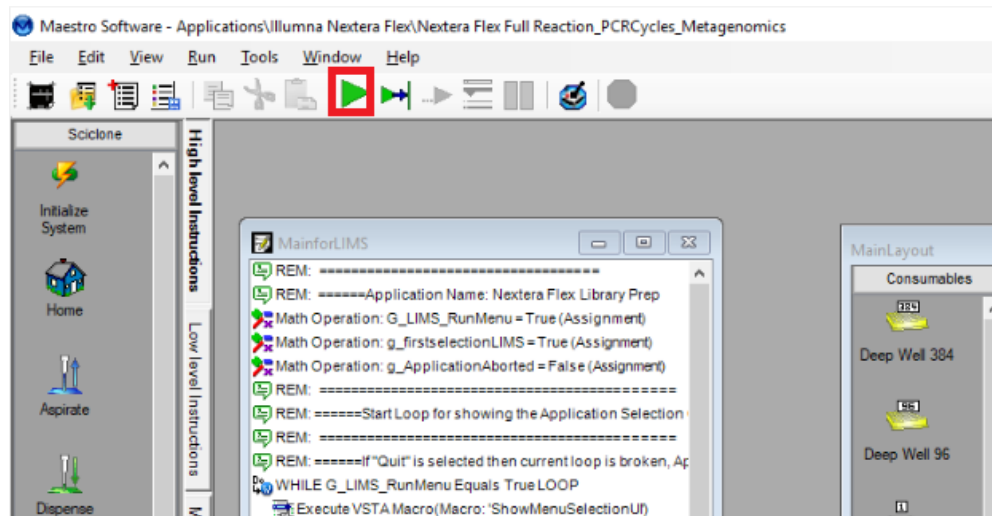
- 25 In the window that opens, select "Illumina Nextera Flex"



Select the desired program by clicking it, then click the "Open" button with the green bar. This protocol is demonstrating "Nextera Flex Full Reaction_PCR Cycles_Metagenomics".



- 26 This will open the program on the Maestro interface. After some communication with the Sciclone, the green play button "Execute" will become clickable. Once clickable, click it.

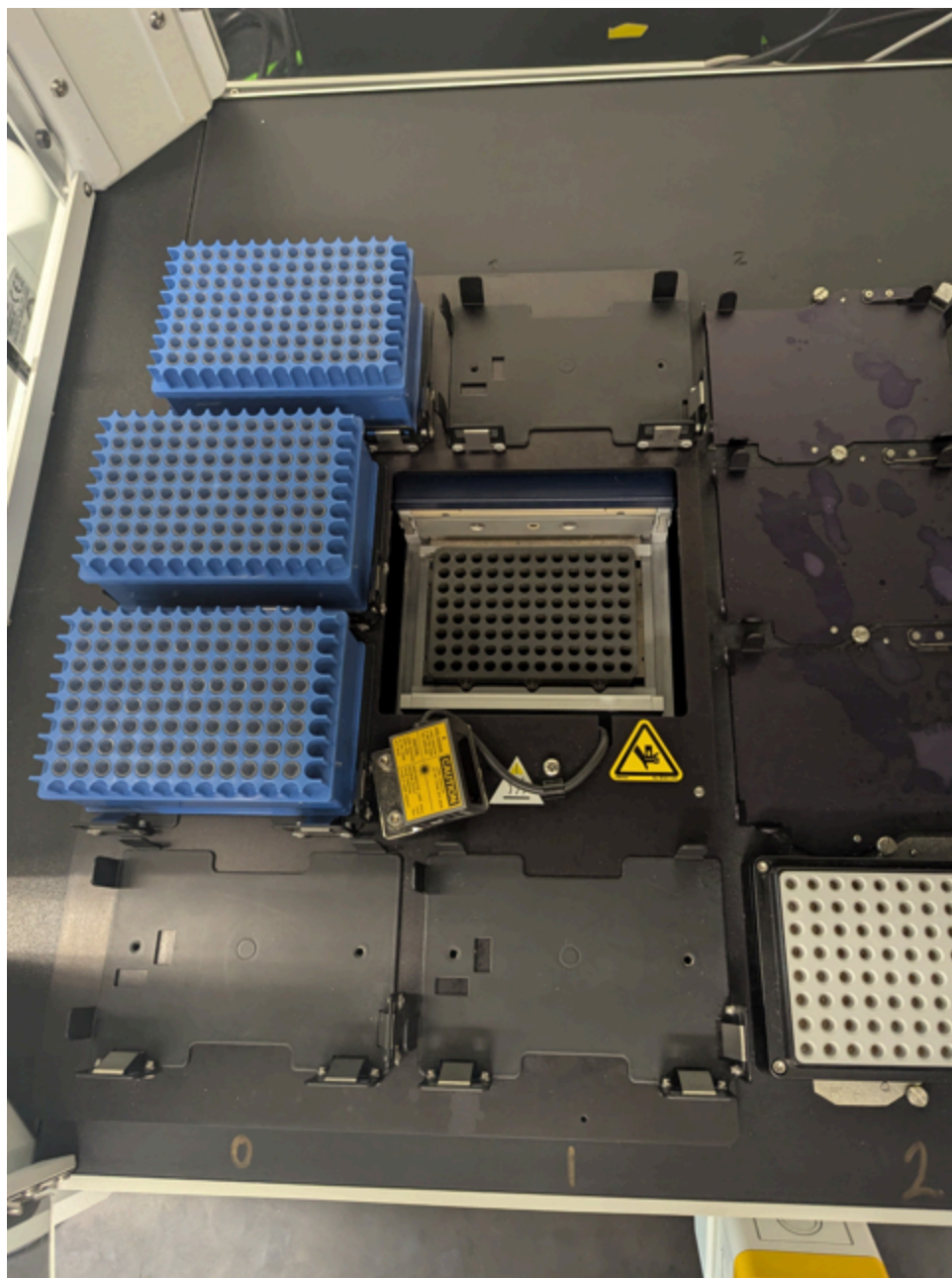


This will lead to more communicating with the Sciclone, and the plate set up guide will appear.

- 27 The setup wizard will tell you where to place all plate into the Sciclone. The images and instructions from the setup wizard is below:
- 28 Fill all 3 racks on the Twister with a total of 27 Full tip boxes.

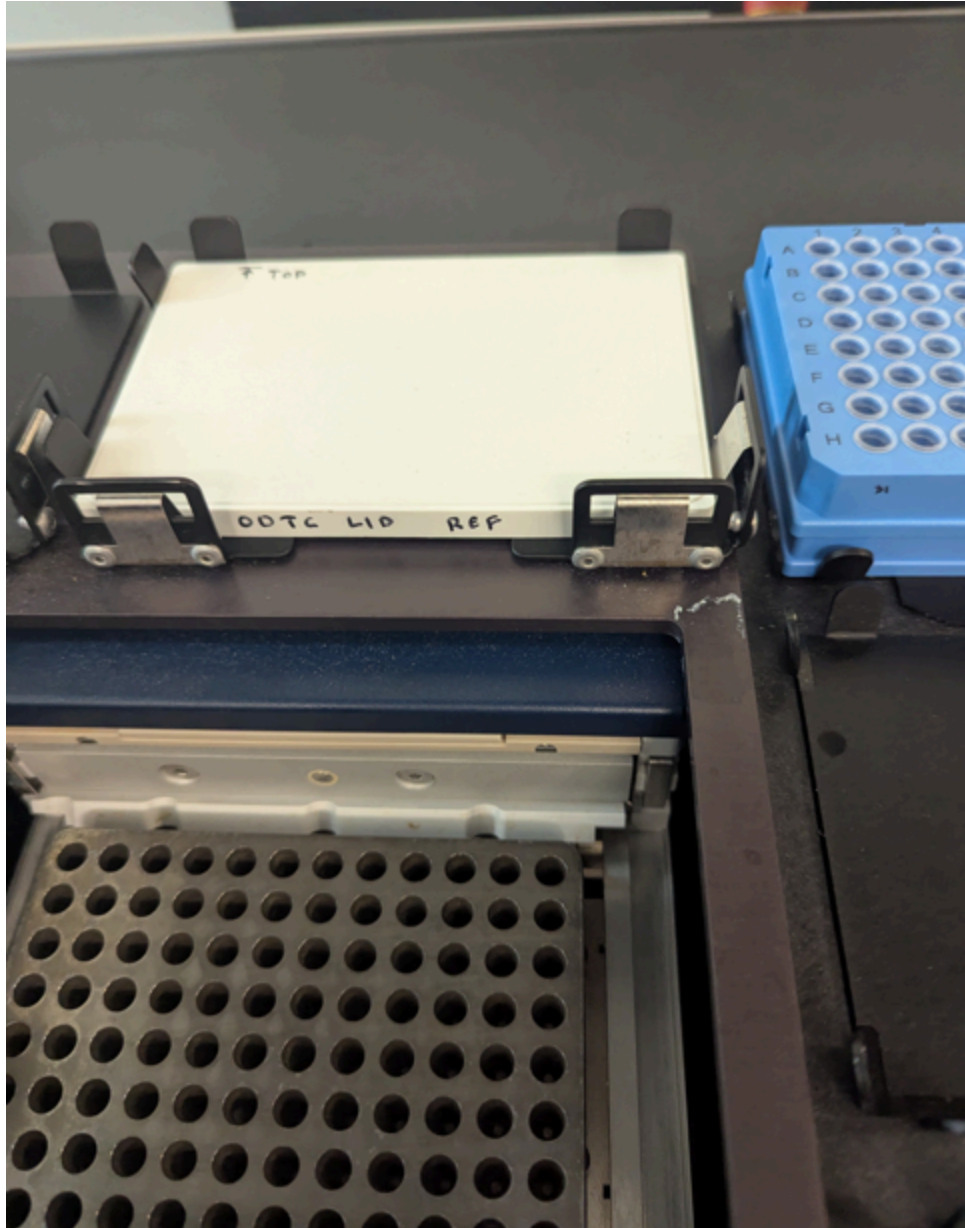
29

Place Full Tip boxes at positions A0, B0 and C0.



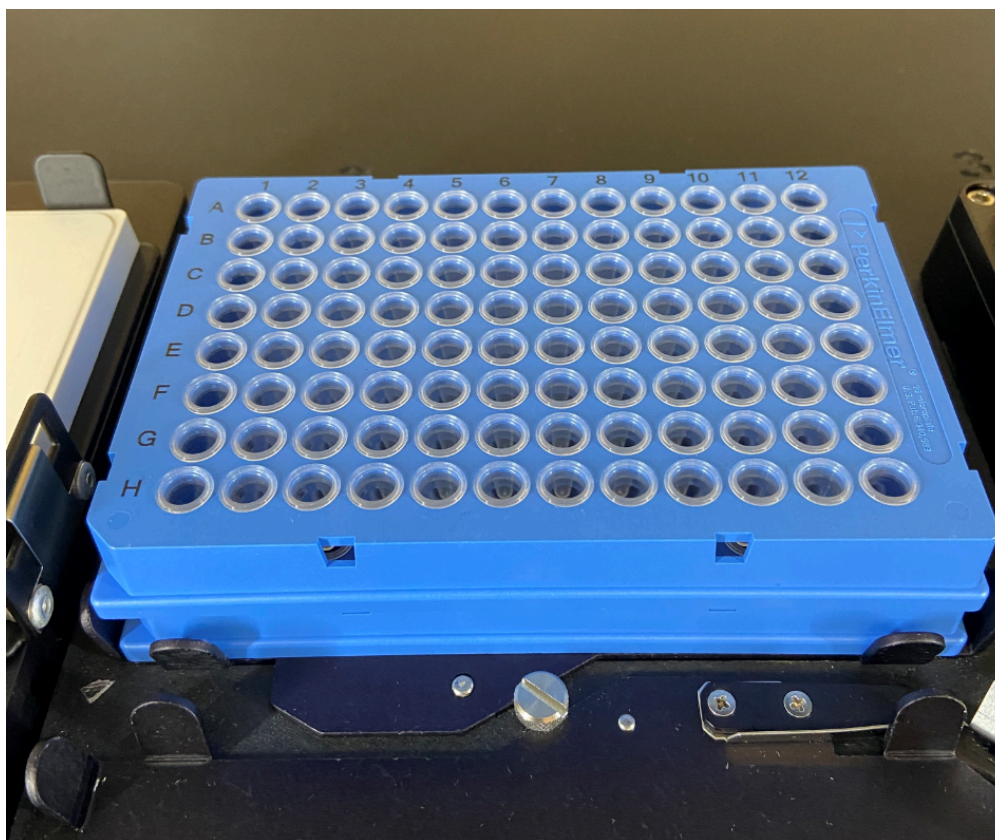
0	1	2	3	4	5	
						A
						B
					WASTE	C
						D

30 Place an ODT Lid at position A1.



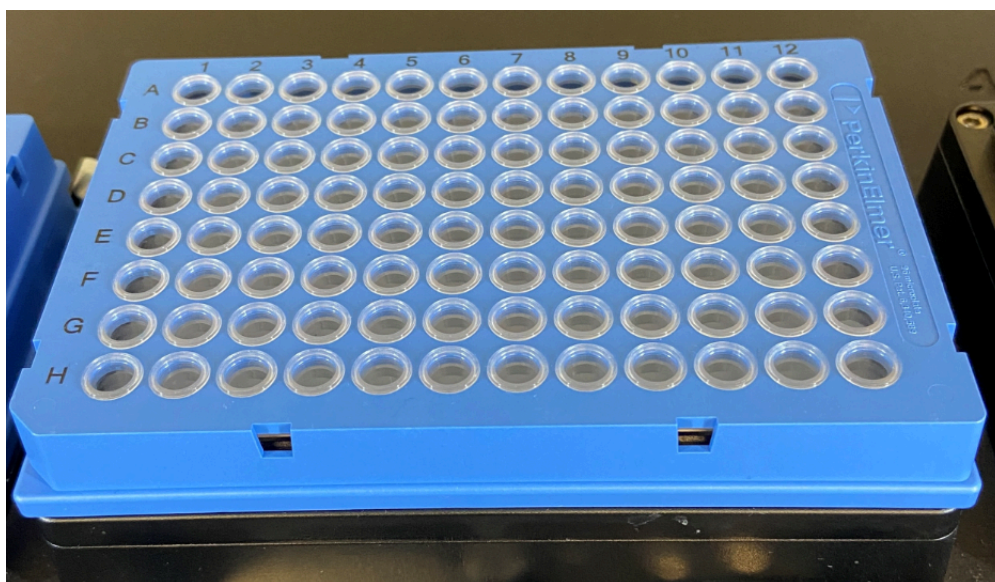
0	1	2	3	4	5	
						A
						B
					WASTE	C
						D

- 31 Place a stack of 2 clean plates at A2.



0	1	2	3	4	5	
						A
						B
						C
						D

32 Place a clean plate on the CPAC at A3.



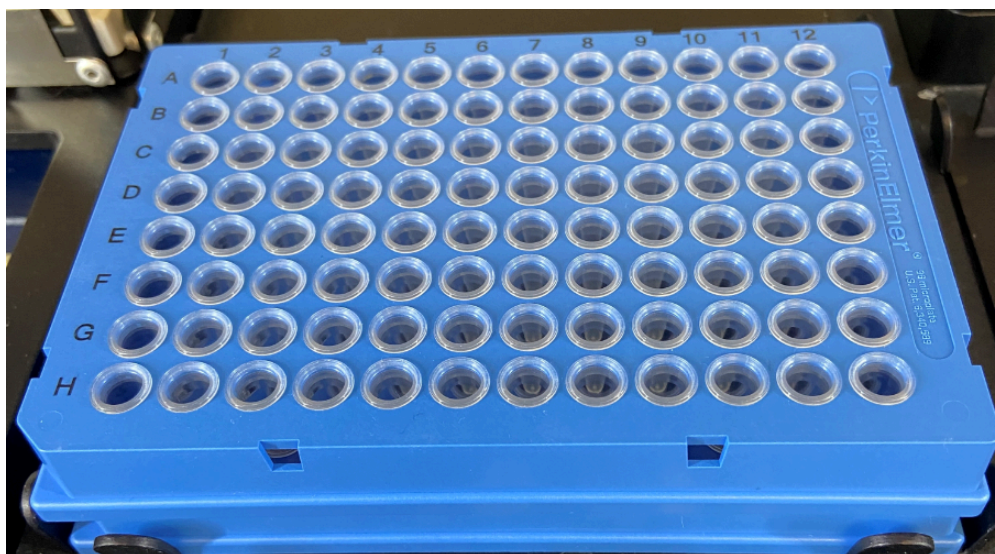
0	1	2	3	4	5	
						A
						B
						C
						D

33 Place the liquid waste at A5 (Empty Reservoir).



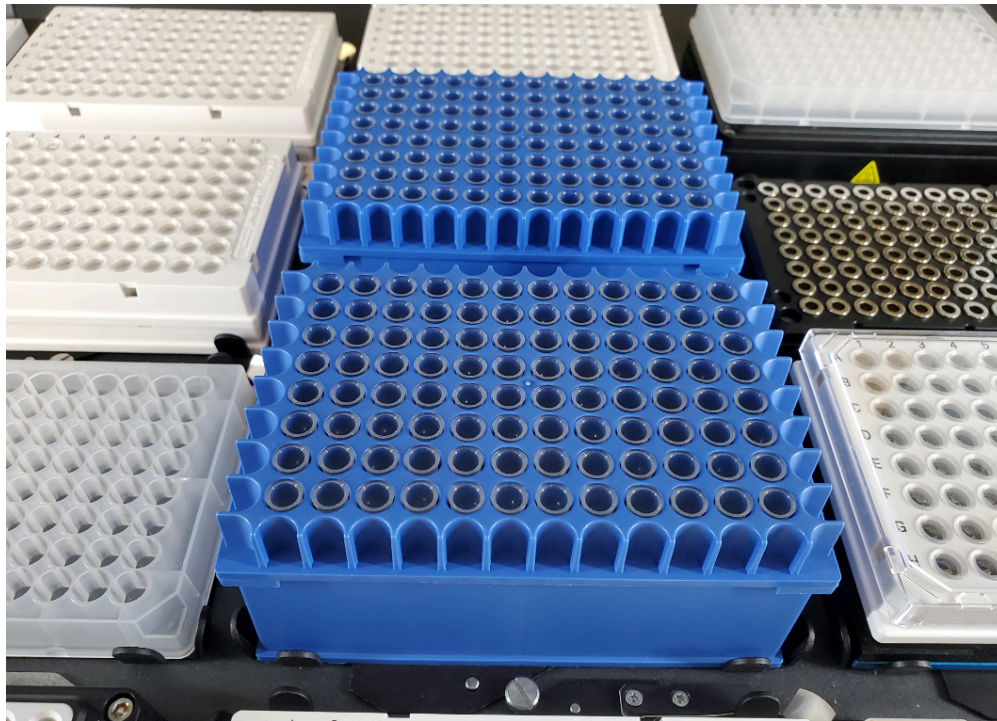
0	1	2	3	4	5	
						A
						B
						C
						D

- 34 Place a stack of 2 plates at B2.
Top plate is resuspension buffer (RSB).
Bottom plate is purification beads (SPB).



0	1	2	3	4	5	
						A
						B
						C
						D

35 Place Full tip boxes at B3 and C3.



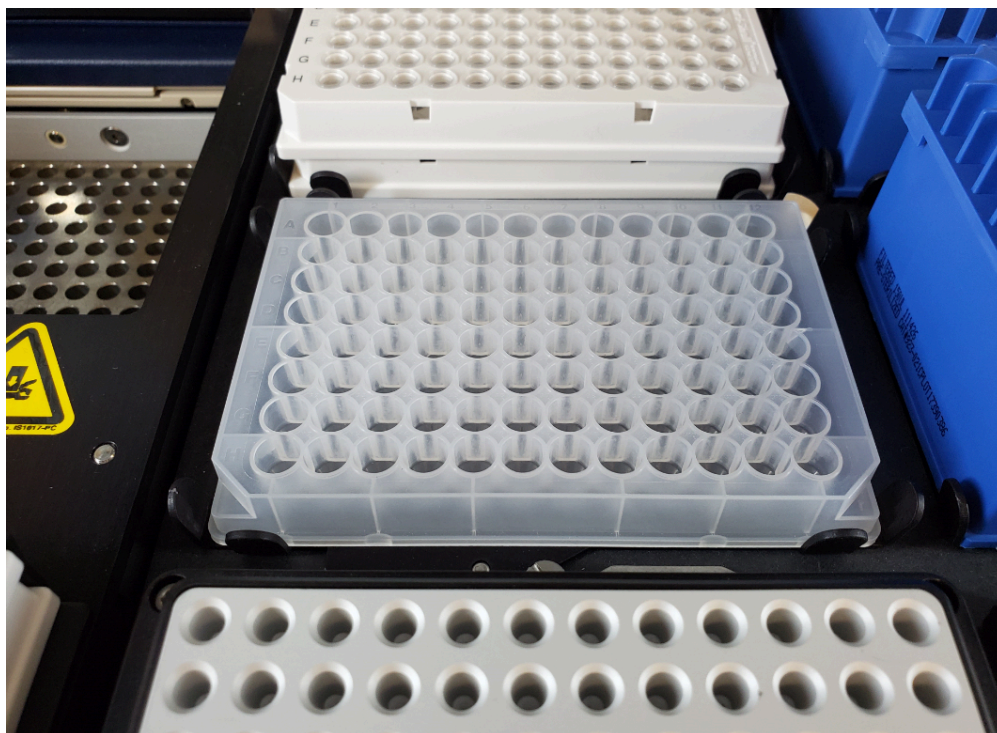
0	1	2	3	4	5	
						A
						B
						C
						D

36 Place a deep well trough with 80% Ethanol at B5.



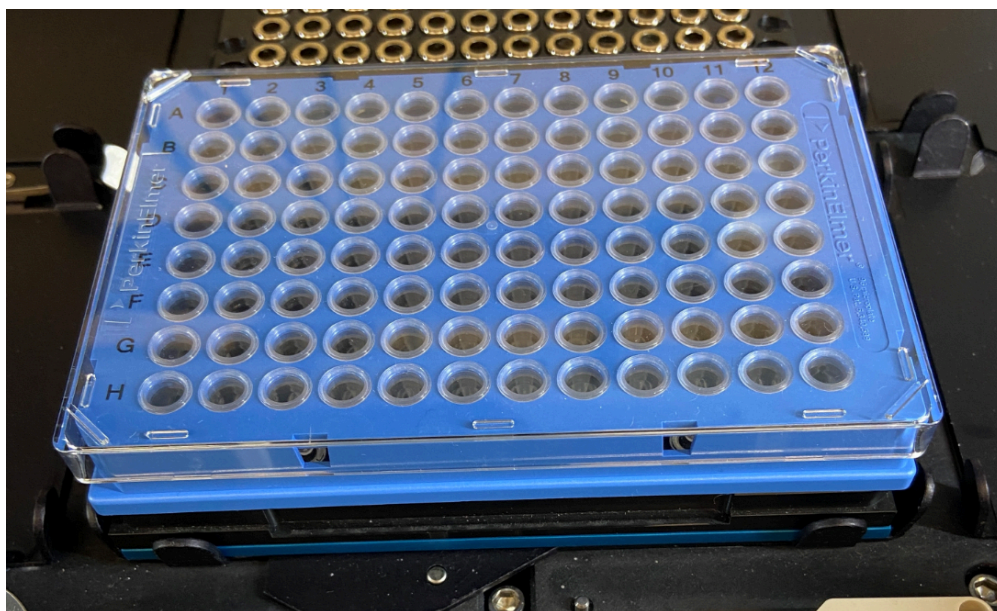
0	1	2	3	4	5	
						A
						B
						C
						D

37 Place a StorPlate with TWB in location C2



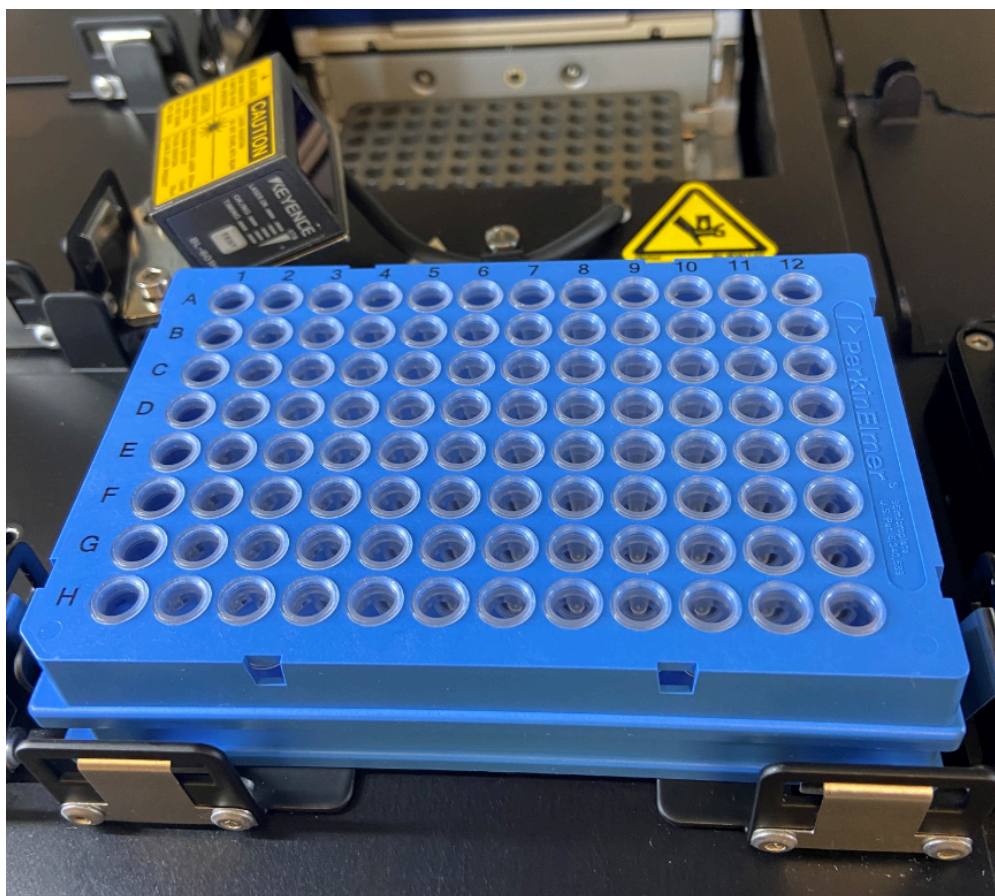
0	1	2	3	4	5	
						A
						B
						C
						D

38 Place a plate with the arrayed indexes at C4 on magnet with spacer and a Lid.



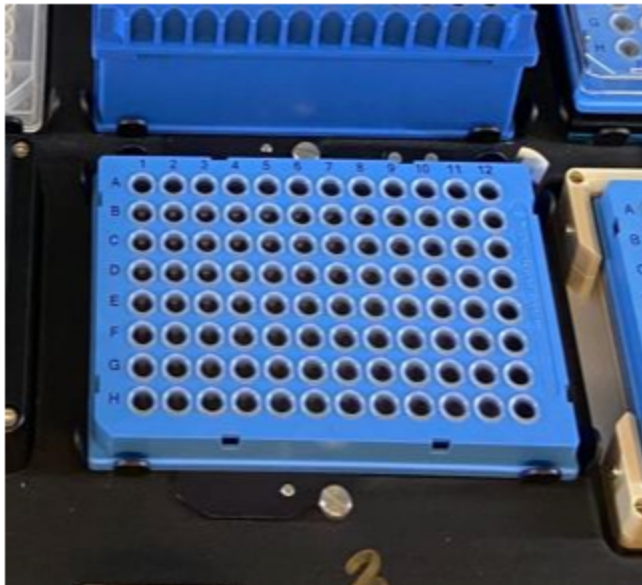
0	1	2	3	4	5	
						A
						B
						C
						D

39 Place 2 clean plates at D1.



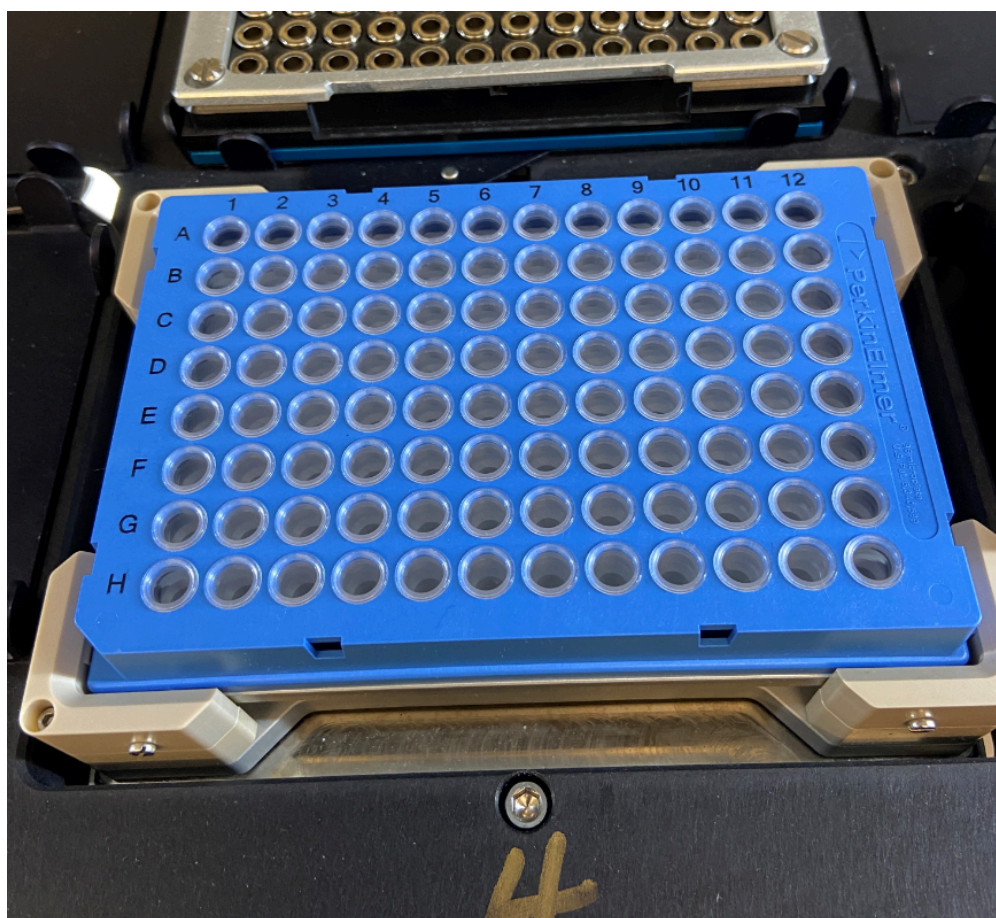
0	1	2	3	4	5	
						A
						B
					WASTE	C
						D

40 Place the water plate at D3.



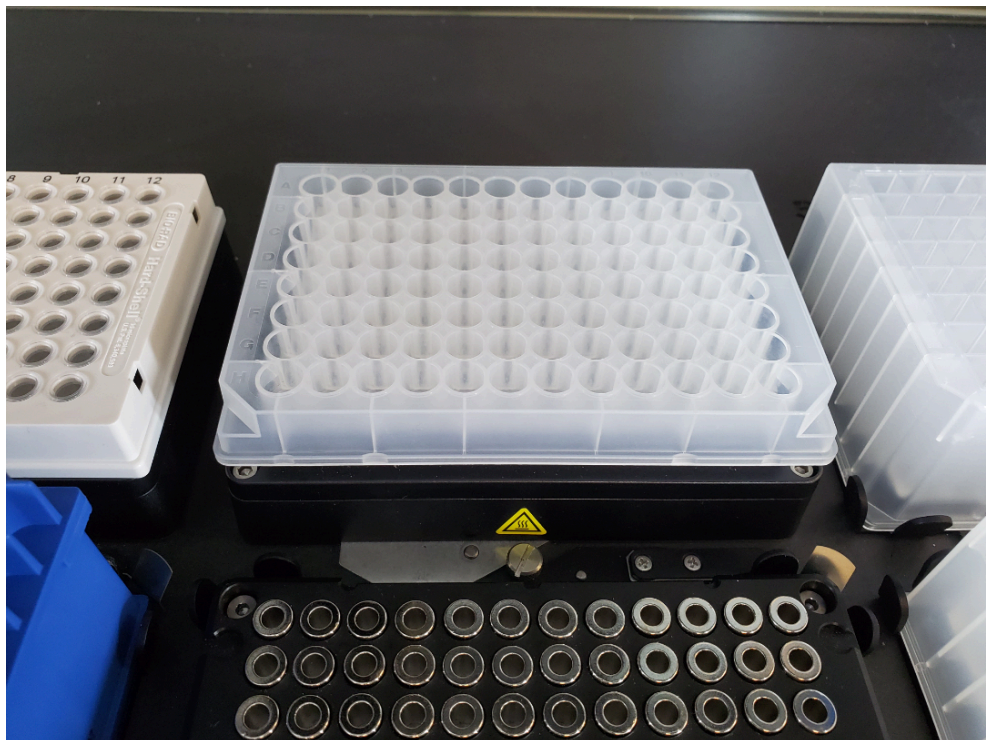
0	1	2	3	4	5	
						A
						B
						C
						D

41 Place the sample plate at D4.



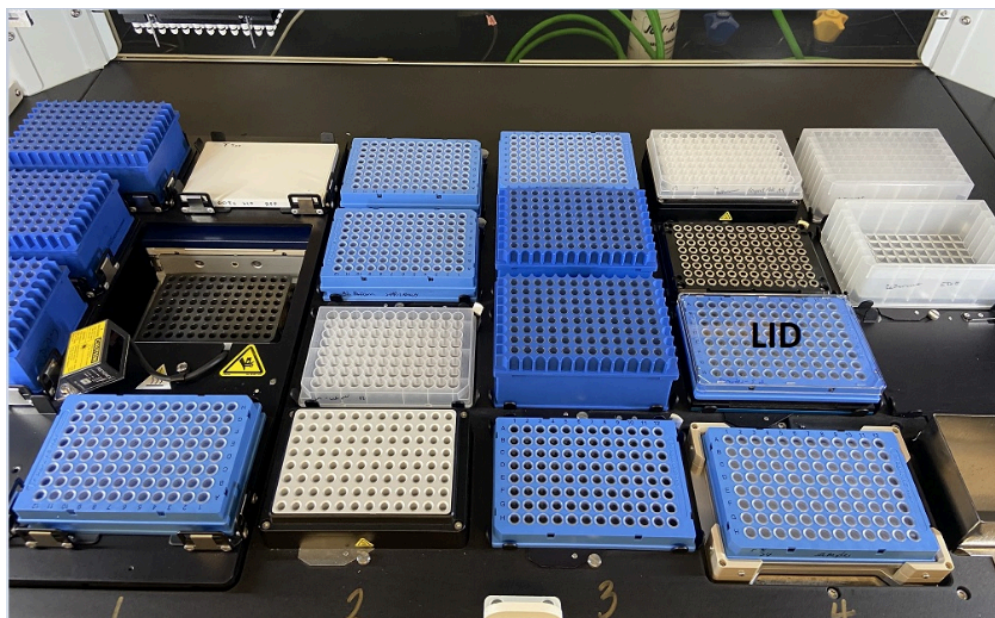
0	1	2	3	4	5	
						A
						B
						C
						D

42 Place the 450ul StorPlate with Reagent at A4.



0	1	2	3	4	5	
						A
						B
						C
						D

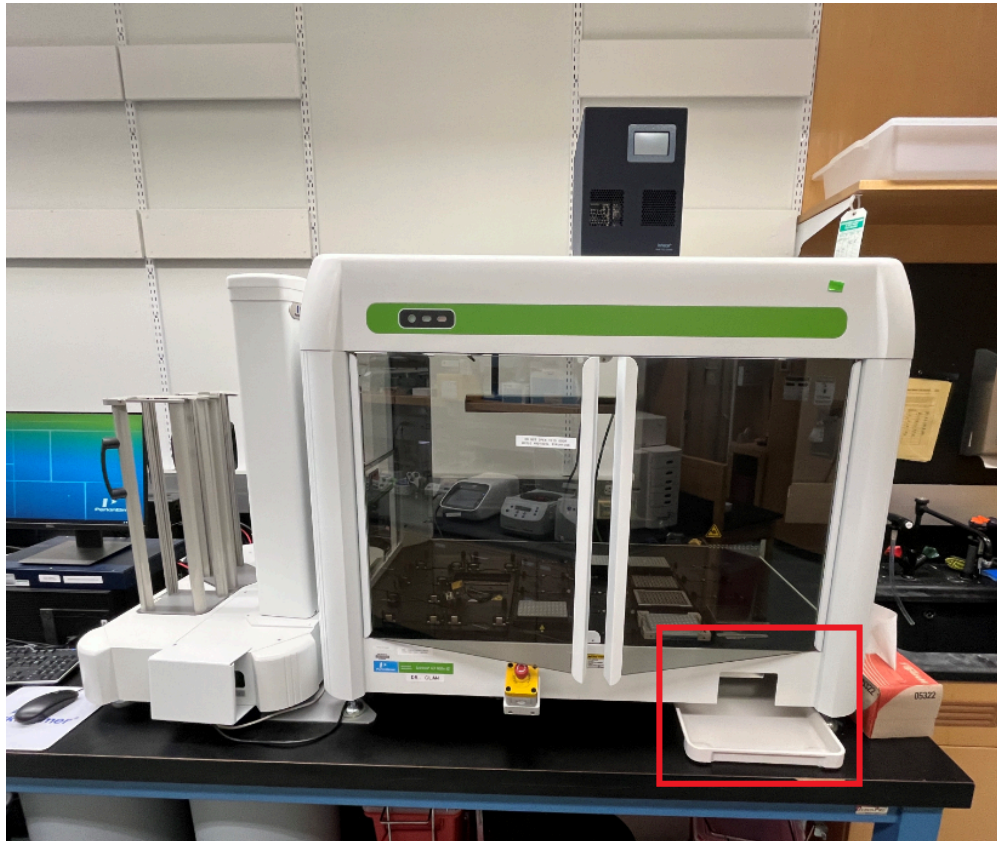
- 43 Make sure the deck layout matches the picture before starting the run. Plate at C4 has a LID on it.



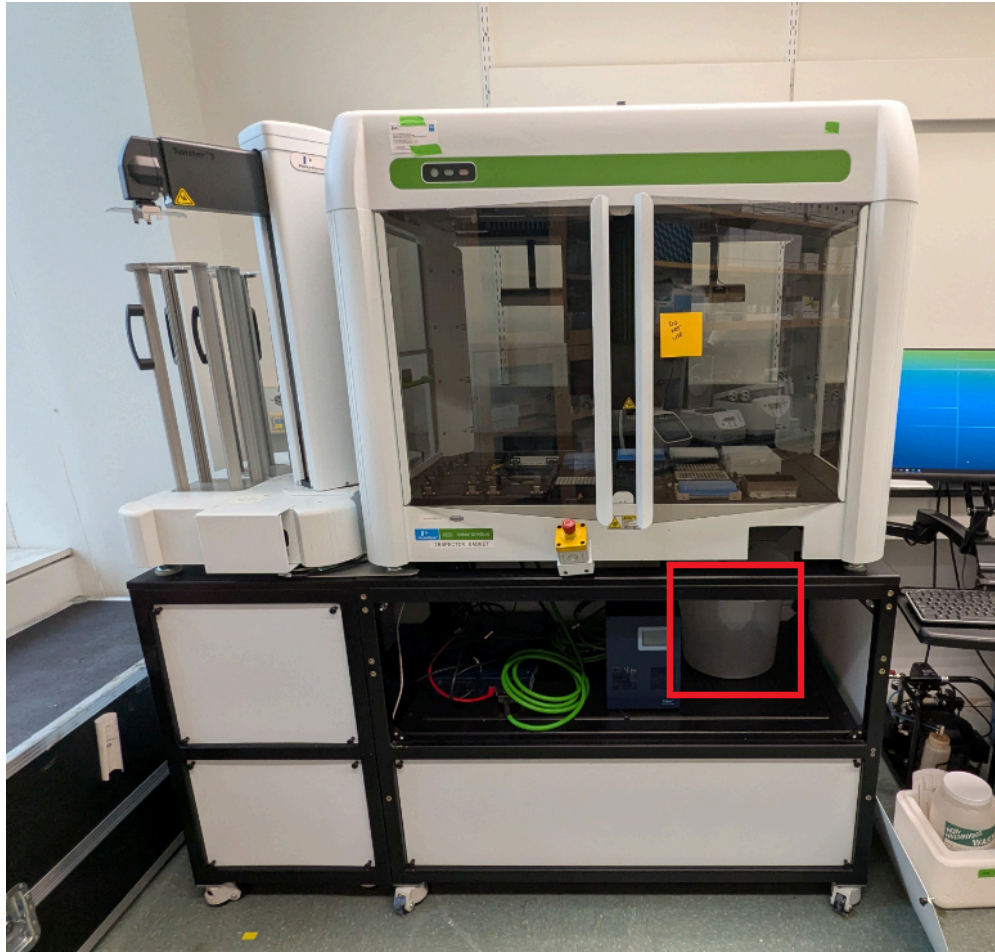
- 44 The run will begin after clicking Finish but there may be a delay for ~5 minutes while ODT block and lid execute Temperature Initialization.
The Sciclone takes HOW LONG DOES THE SCICLONE RUN?
Once temperature is reached the run will continue automatically.
NEED PICTURE OF THE MAESTRO WITH FINNSIH

Sciclone Cleanup

- 45 When the run completes, the prepared libraries will be in an unlabeled plate at position D4 with a clear lid on the plate. Remove the plate, seal with foil tape, and store at 4 °C until quantified, normalized, and sequenced
- 46 Empty the used pipette tips into a biohazard sharps container



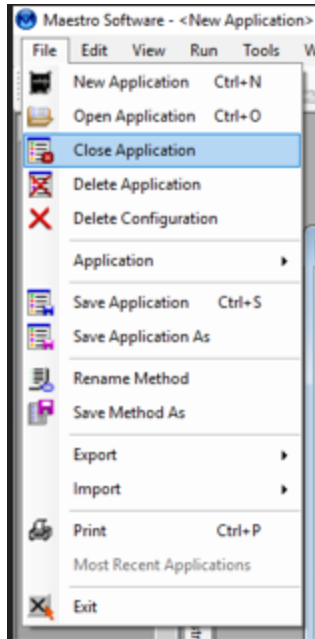
Where the used pipette tips are stored in Sciclone 1



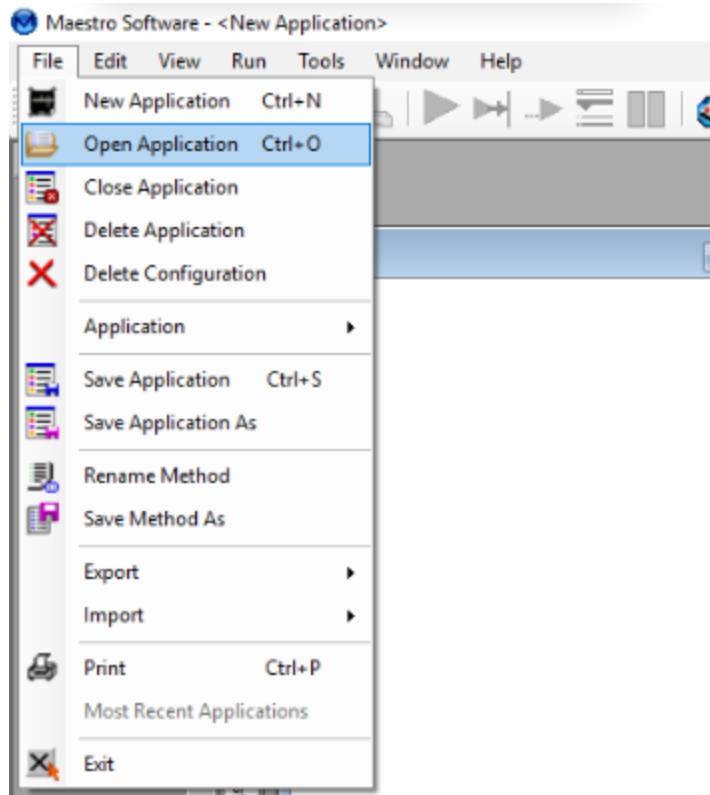
Where used pipette tips are stored in Sciclone 2

- 47 Discard the used ODC lid and all used plates (PCR and V bottom plates), including the liquid waste and alcohol reservoirs. PCR and V-bottom plates discarded from the deck during the run are in the gray Sciclone waste container and should be moved to biohazard trash. WHERE IS THIS CONTAINER?
- 48 Discard all empty pipette tip boxes (on deck and in the gray Sciclone waste container) in the laboratory trash bins.
- 49 Remove all pipette tip boxes from the deck, consolidate partial boxes, and replace lids.
- 50 Cover the topmost pipette boxes on the Twister 3 with lids.
- 51 Wipe down the deck with DNA/RNA-ExitusPlus™, especially position A1 (where the ODC lid was placed) and the tip waste chute at position D5.

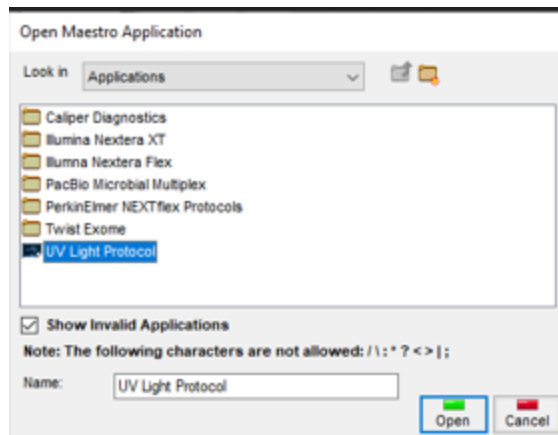
- 52 Insert the UB shield between the Twister 3 and Sciclone deck. Insert from the deck side with the green handle facing inside the deck. The shield has two tabs on the bottom that fit within grooves on the deck edge, and two indents that hook over two screws on the deck side to hold the shield in place. If the UV shield is not seated correctly the UV protocol will throw an error.
- 53 From the Maestro software, click Close Application to quit the DNA preparation protocol



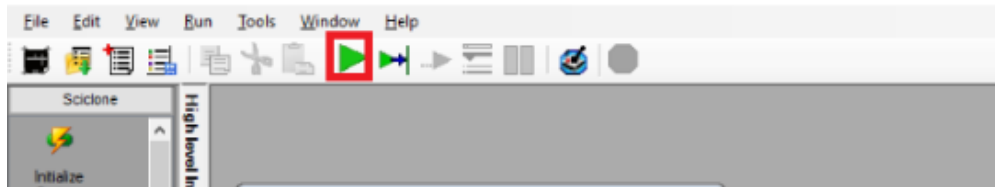
- 54 From the Maestro software menu, click File and then Open Application



- 55 From the file structure tree, select UV Light Protocol and open the file. This protocol may be a level up from the previous protocol.

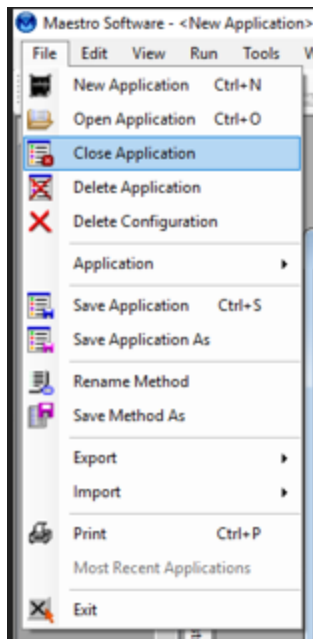


- 56 On the top of the screen, click the green arrow icon ("Execute").



If an error appears indicating the UV shield is not in place, reseal the UV shield, ensuring the tabs depress the sensor switches within the grooves on the deck edge, and select to Retry Instruction.

- 57 After the UV light protocol is complete, close the protocol by clicking Close Application from the Maestro software.



- 58 Close the Maestro software using the X in the top right corner.
- 59 Turn off all four components of the Sciclone instrument listed in Section 8.2. Do not turn off the computer or its monitor. (See section 1A or 1B).