Operation instructions

October 7, 2023

1. Prerequisites

Before you can modify the source code, you will need to install the following software:

1.1 Front-End

Visual Studio 2022 with .NET SDK 6.0+ Visual Studio 2022 Community Edition – Download Latest Free Version (microsoft.com).

If .NET cannot be found as in the error below:

Figure 1: .NET SDK cannot be found

Try the following fix: c# - Microsoft Visual Studio 2019: The project file cannot be opened. Unable to locate the .NET SDK - Stack Overflow

1.1.1 Firmware

Arduino IDE

1.2 Preparation

Before running experiments, it is important to ensure the following steps have been performed

- The red switch on the side of the power supply is set to 115V
- The BioCloneBot power supply is plugged in and turned on
- The MKS GEN L V2.1 mother board is connected to your computer using the provided USB cable
- You have uploaded the latest version of the BioCloneBot firmware to the mother board
- The device has been calibrated (steps follow in the following section)

To home the BioCloneBot, remove everything from the platform and remove any tip attached to the syringe pump. Once the platform is clear, select the "Home Device" button underneath the Protocol Queue. The device will now home the x, y, z, and pump axes in order.

1.2.1 Calibration

Before running any experiments, it is crucial to calibrate the BioCloneBot. If you do not calibrate the device, it will miss the target during the experiments.

Here are the major calibration positions:

(1) The (x,y) homed position

(2) The top left corner location for the labware slots on the platform

(3) The (x,y) location for the center of the top left reservoir or tip

(4) The distance between the center of the reservoirs or tips

(5) The max height in the z-axis

(6) The height of the labware

Each calibration position can be set by using the Manual Control Form of the BioCloneBot software. Open the Manual Control Form by going to Settings - Manually move pump.

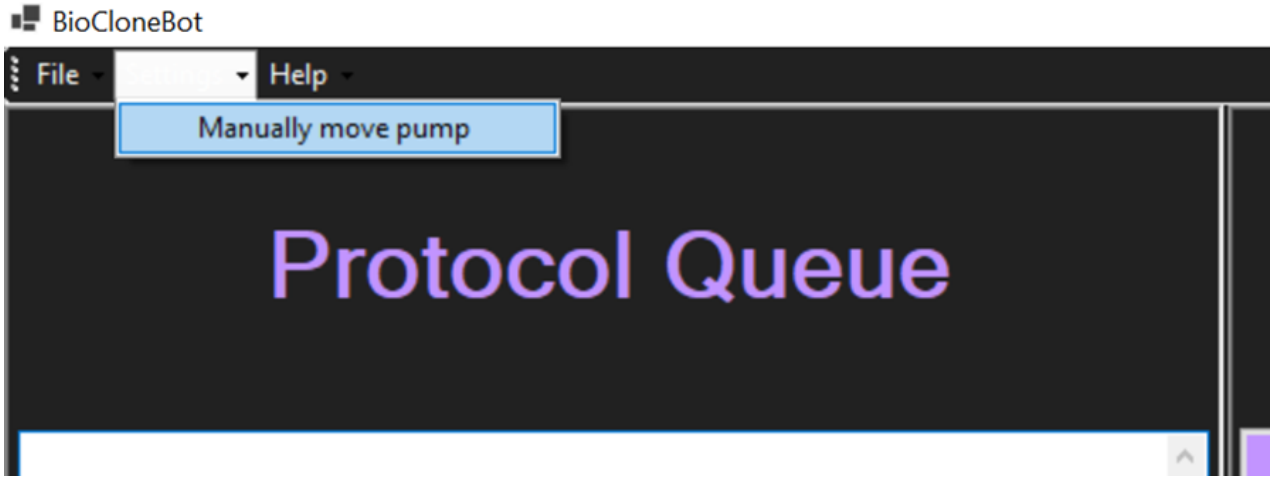

Figure 2: Opening the Manual Control Form

Carefully read the message pop-up then click OK.

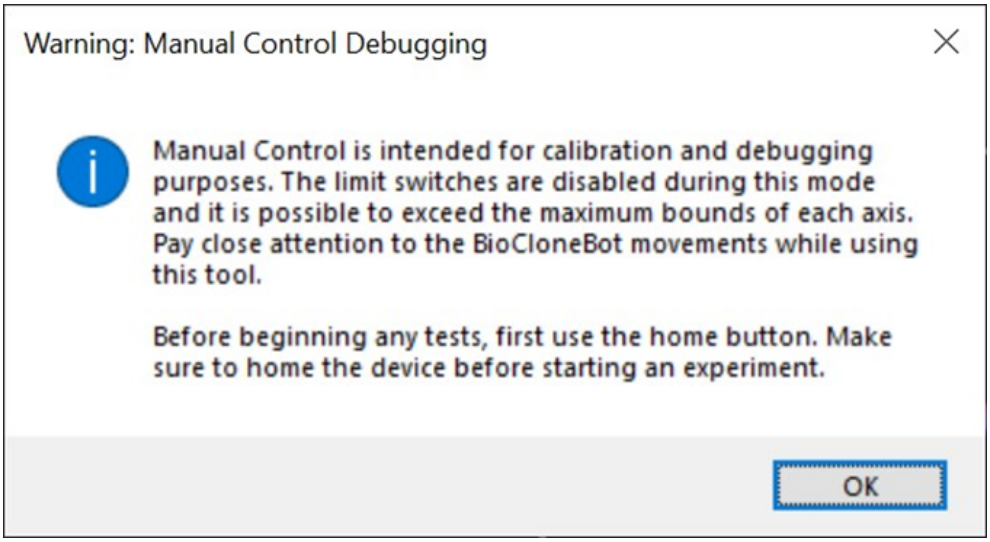

Figure 3: Manual Control Form warning pop-up

This will open the Manual Control Form

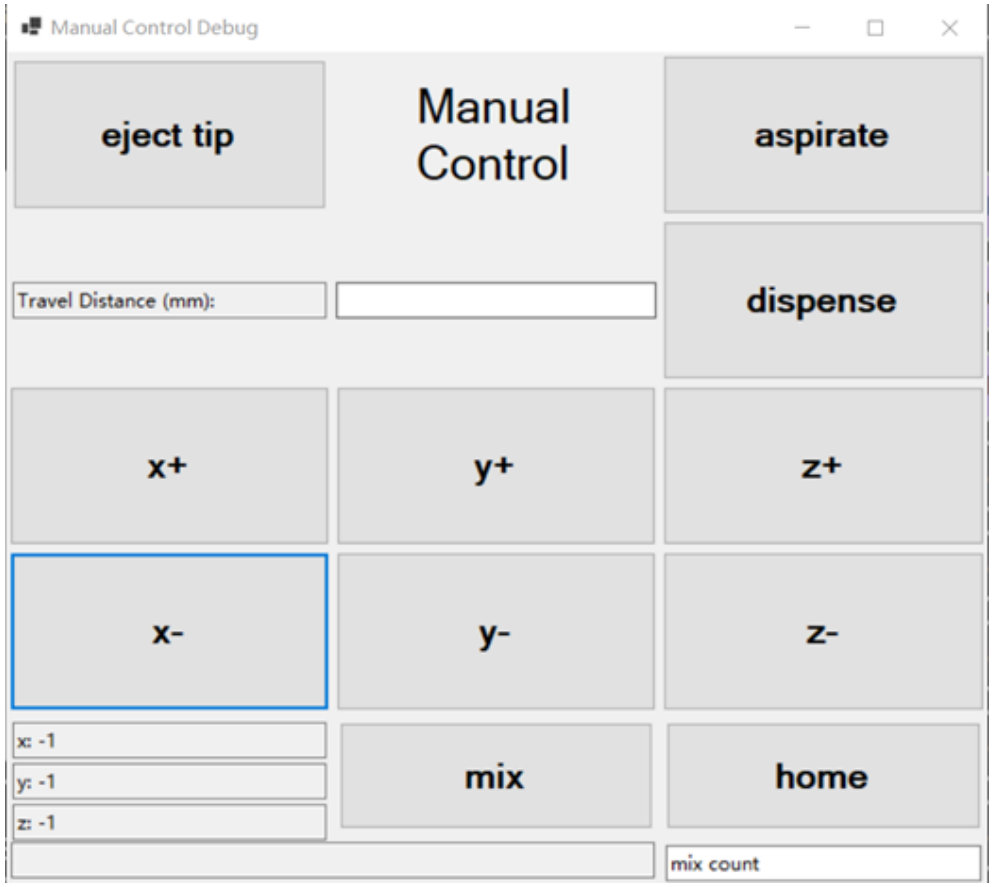

Figure 4: BioCloneBot Manual Control Form

To move a specific axis, enter a value into the "Travel Distance/Volume" text box, then press the corresponding button for the axis you would like to move. The + buttons move away from the limit switch of the axis and the - buttons move away from the limit switch of the axis. The aspirate and dispense buttons move the volume entered into the text box.

1.2.2 The (x,y) Homed Position

During the homing procedure, each axis starts by pressing the limit switch of that axis, then backing off until the switch is no longer pressed. Once finished, the center of the syringe pump tip will align with the bottom left of the platform. This refers to the (0, 0) position of the x-axis and the y-axis.

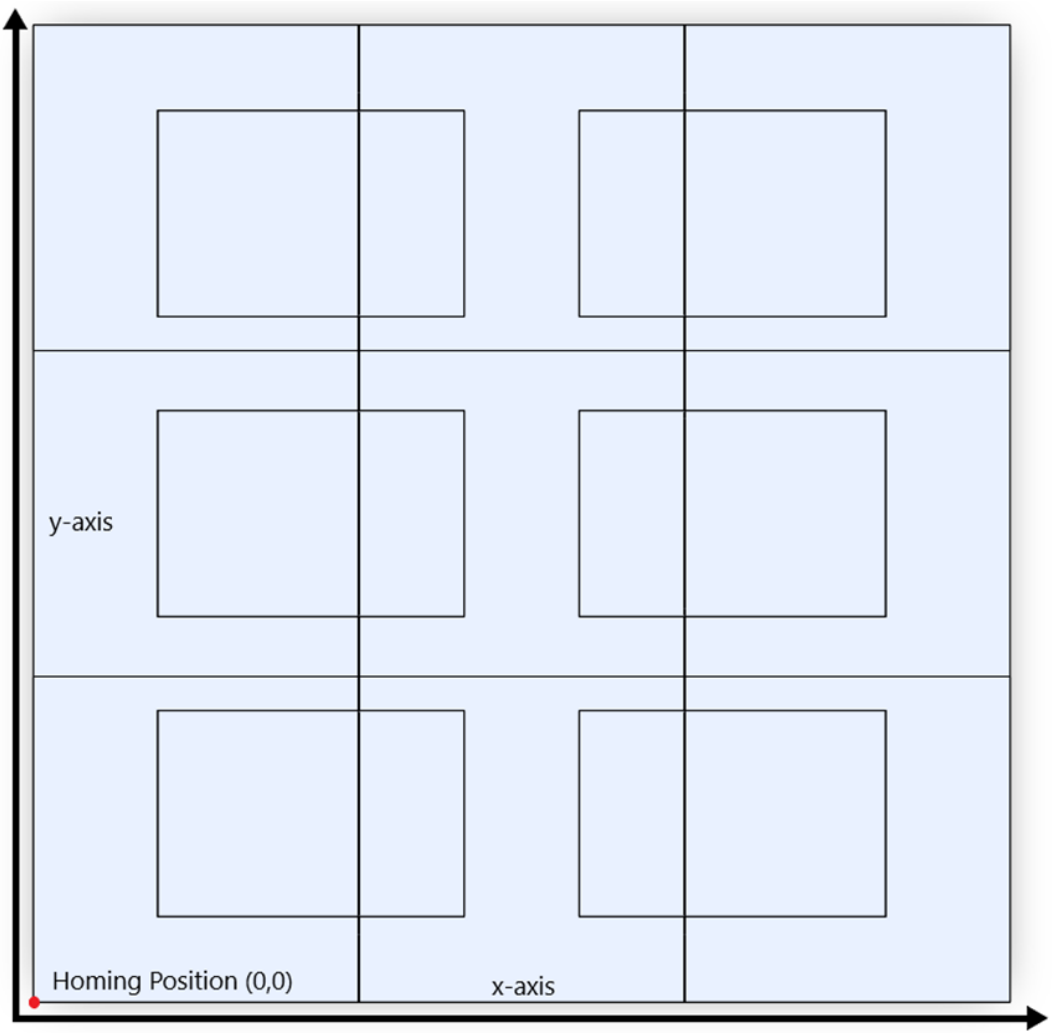

Figure 5: BioCloneBot platform with homed position in bottom-left corner

Press the home button and wait for the procedure to finish. Using the z+ button, move the pump until the pump is a couple cm above the platform. If the center of the syringe pump tip does not line up with the corner of the platform, open up the firmware file located inside the BioCloneBot code folder "BioCloneBot\Firmware\DeviceControllerV4\DeviceControllerV4.ino".

You can update the (x,y) homing location by modifying line 452.

```
452  movePump('1', '1', '0', 012.00, 033.00, 0.0);
453  //sets homing to 0 re-enabling the normal functionality of the limit switches as emergency stops
454  //and sets carriage location to (0.0, 0.0, 0.0) and syringe volume to 0
455  homing = 0;
```

Figure 6: Homing position inside of BioCloneBot firmware. Depending on the version of the code, the line is subject to change.

012.00 refers to x-location and 033.00 refers to the y-location. Make sure to maintain the XXX.XX and YYY.YY format. The third number, 0.0 can be ignored as it refers to the point on the z-axis.

1.2.3 Top Left Corner of Labware Slots

The BioCloneBot uses the top left corner of each labware slot as the reference point when moving the pump to perform an operation. These locations can be seen below:

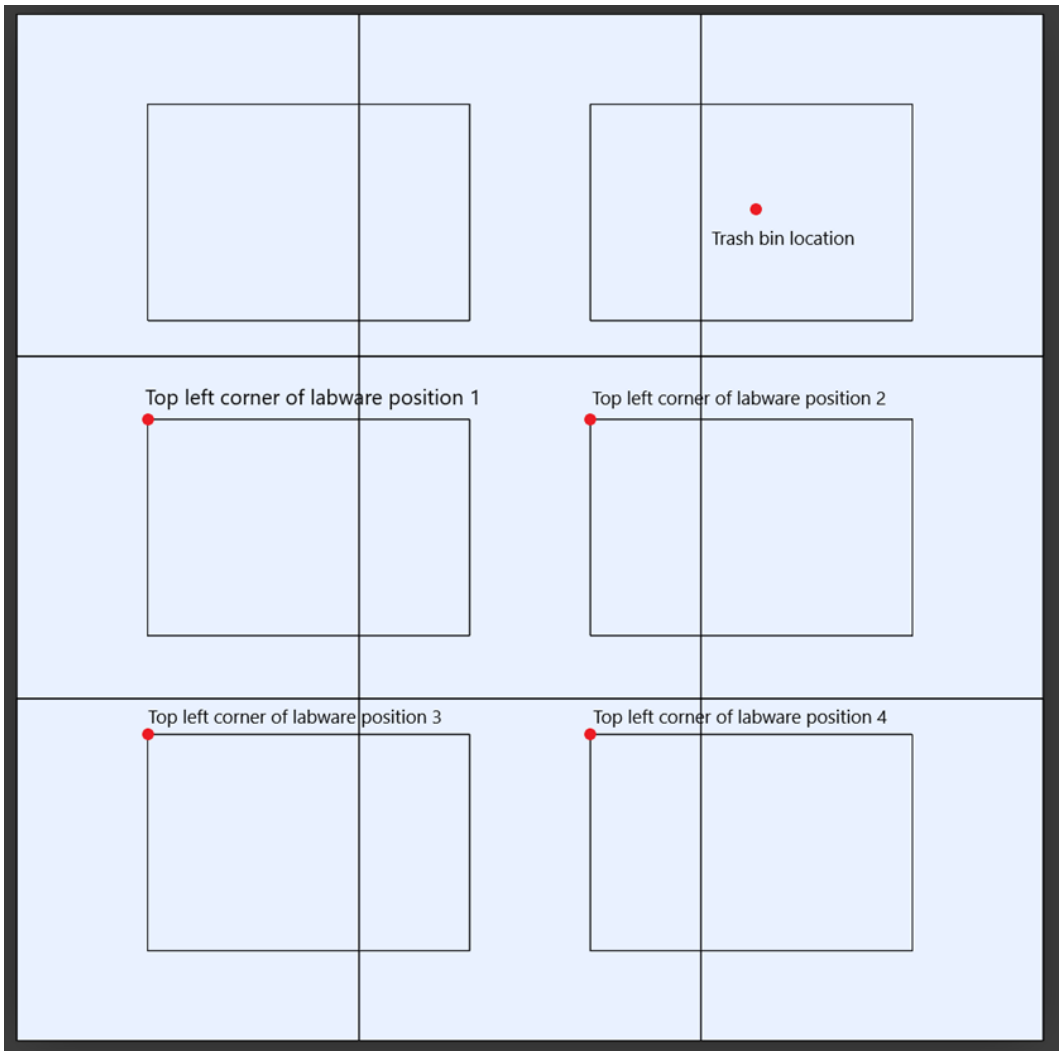

Figure 7: Top left reference corner for each labware slot on the BioCloneBot platform

These positions can be modified inside of the Platform.cs file located in "BioCloneBot\BioCloneBot\Platform.cs" The positions can be modified in lines in the AddLabware function of the Platform.cs class. topLeft- Corner[0] refers to the x-location and topLeftCorner[1] refers to the y-location.

1.2.4 (x,y) of Top Left Reservoirs

The next position to calibrate is the distance from the top left corner of the labware slot to the center of the corresponding labware reservoir.

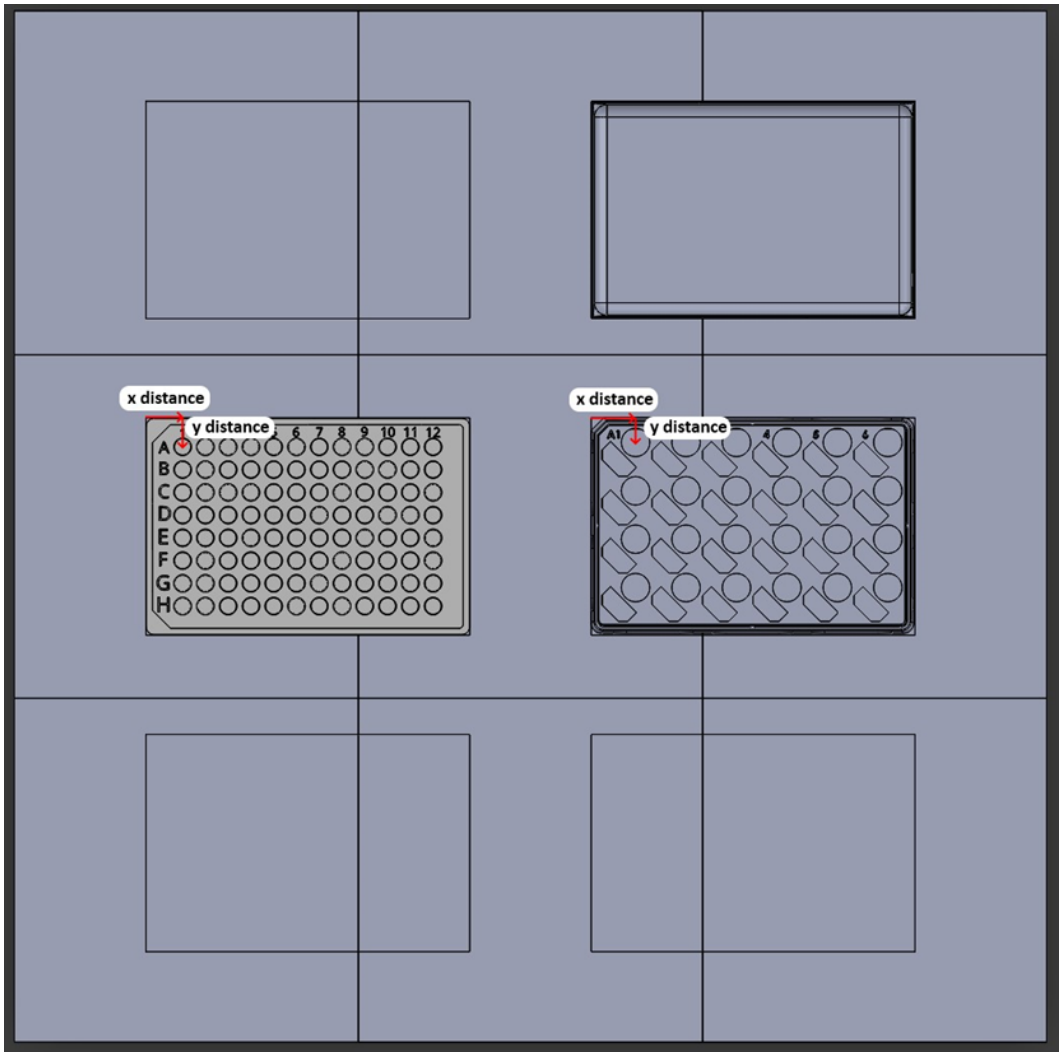

Figure 8: X and y distance from the top left corner of the labware position to the center of the top left reservoir.

These can be be modified by updating the ”startLocation” variable inside of the Wellplate.cs, Tubestand.cs, and Tipbox.cs classes. The classes can be found inside the “BioCloneBot\BioCloneBot\” folder. The first value is the x-distance and the second value is the y-distance.

1.2.5 Reservoir Distances

Now that we have calibrated the top left corner of the labware locations and the start location of the first reservoir, we need to calibrate the distance between the reservoirs.

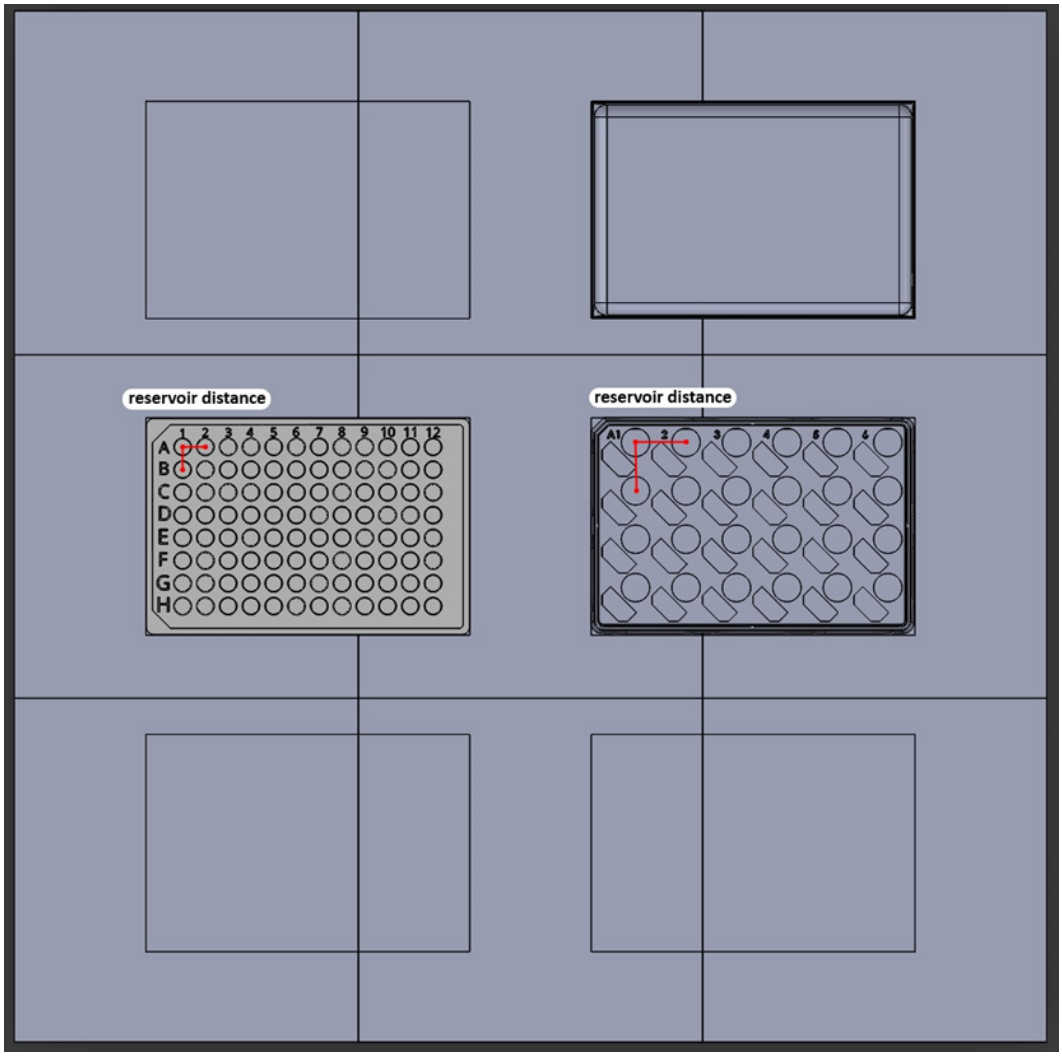

Figure 9: x and y reservoir distance

This can be updated inside of the class file for each labware: Wellplate.cs, Tubestand.cs, andTipbox.cs located in "BioCloneBot\BioCloneBot\". Here are the variable names for each class: "wellDistance" inside of Wellplate.cs "tubeDistance" inside of Tubestand.cs "tipDistance" inside of Tip- box.cs

1.2.6 Set Z-Max Value

The BioCloneBot uses Z-Max (the maximum travel distance in the z-axis) to help target labware during an operation. To calibrate Z-Max, home the device without a pipette tip attached.

Once homed, lower the pump until the tip adapter is touching the platform, but not pushing it down. This is the Z-Max value. This can be updated inside the Platform class file located at "BioCloneBot\BioCloneBot\Platform.cs" inside of the Platform constructor function.

1.2.7 Height of Labware

The labware height refers to the target location of the pump depending on the labware to target. If you are trying to attach a tip, assume that there is no tip attached. If you are targeting a wellplate or tube reservoir, attach a pipette tip to the syringe pump by hand to account for the extra distance added by the tip. You will need to use the Manual Control Form to figure out these values. You will need to move the pump in each axis until it is in the desired location. The final value for the z-location is the value to input.

Pipette Tip

Move the pump until the tip adapter is located over the center of a pipette tip. Lower the pump until the adapter is inside the tip, but it is not snug. The tip attachment process automatically lowers the syringe pump after it is located inside of the tip to ensure a snug fit.

Wellplate or tubestand

Move the pump until the pipette tip is located over the center of the reservoir. Lower the pipette tip until it has touched the bottom of the reservoir then back up around 0.5 mm. This will ensure the pipette tip is not blocked when trying to aspirate or dispense.

The labware height can be updated inside of the class file for each labware: Wellplate.cs, Tubestand.cs, and Tipbox.cs located in "BioCloneBo\BioCloneBot\". The third number in the "dimensions" variable refers to the measured height.

1.3 Setting Up an Experiment

Now that the labware has been defined and initialized, we can start building our experiment. There are currently 6 operations available:

- Home Device: homes the x, y, z, and pump motors
- Get Tip: gets a tip from a selected position on an available tip box
- Remove Tip: unloads tip into the trash bin
- Aspirate: aspirates a volume of µL into the pipette tip
- Dispense: dispenses a volume + 25µL to a destination reservoir
- Mix: mixes a volume a specified number of times in a destination well

Though the device should already be homed, it is best practice to start a protocol with a home Device Operation. Select the Home Device Operation and note that it fills the protocol queue.

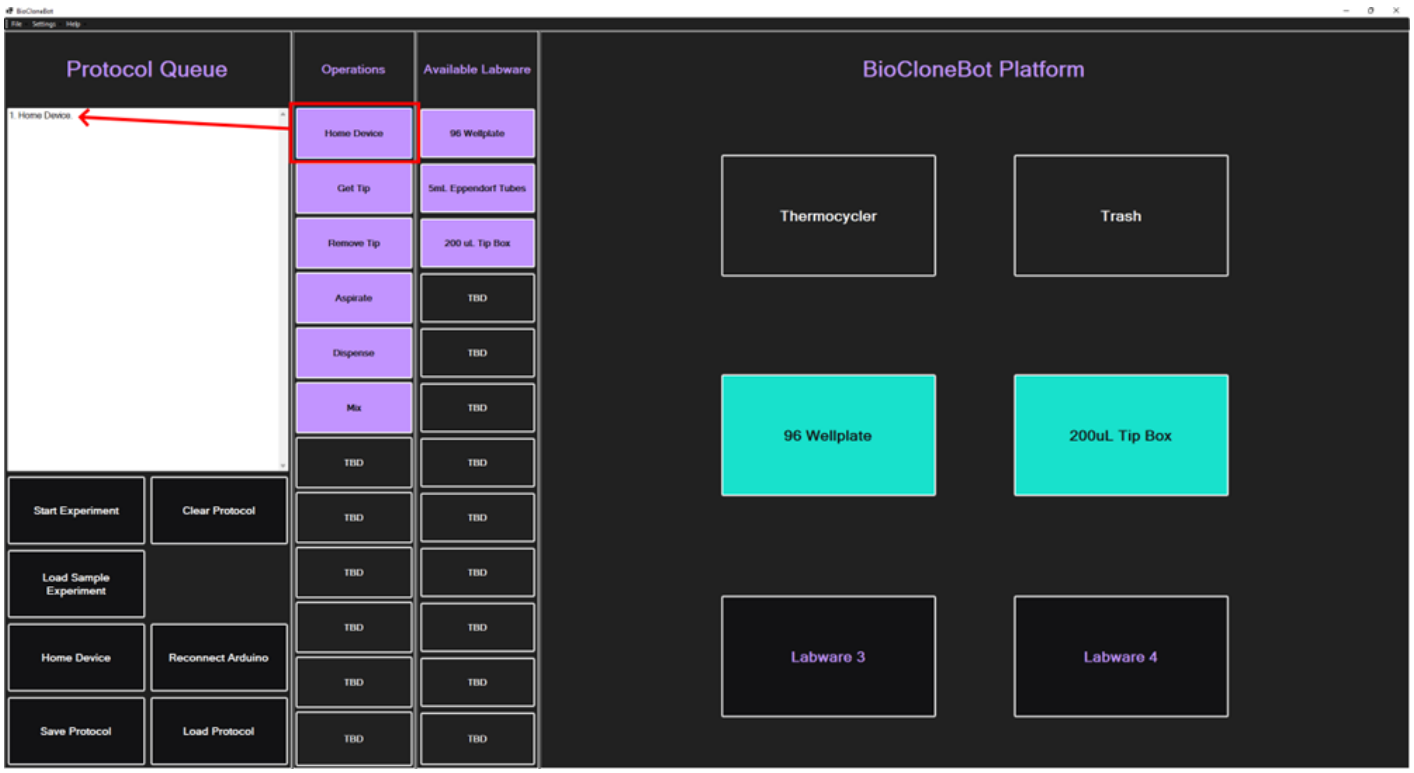

Figure 10: Add Home Device operation

Before we can aspirate or mix, we need to attach a pipette tip to the pump. Select the “Get Tip” operation then select the 200µL Tip Box on the platform

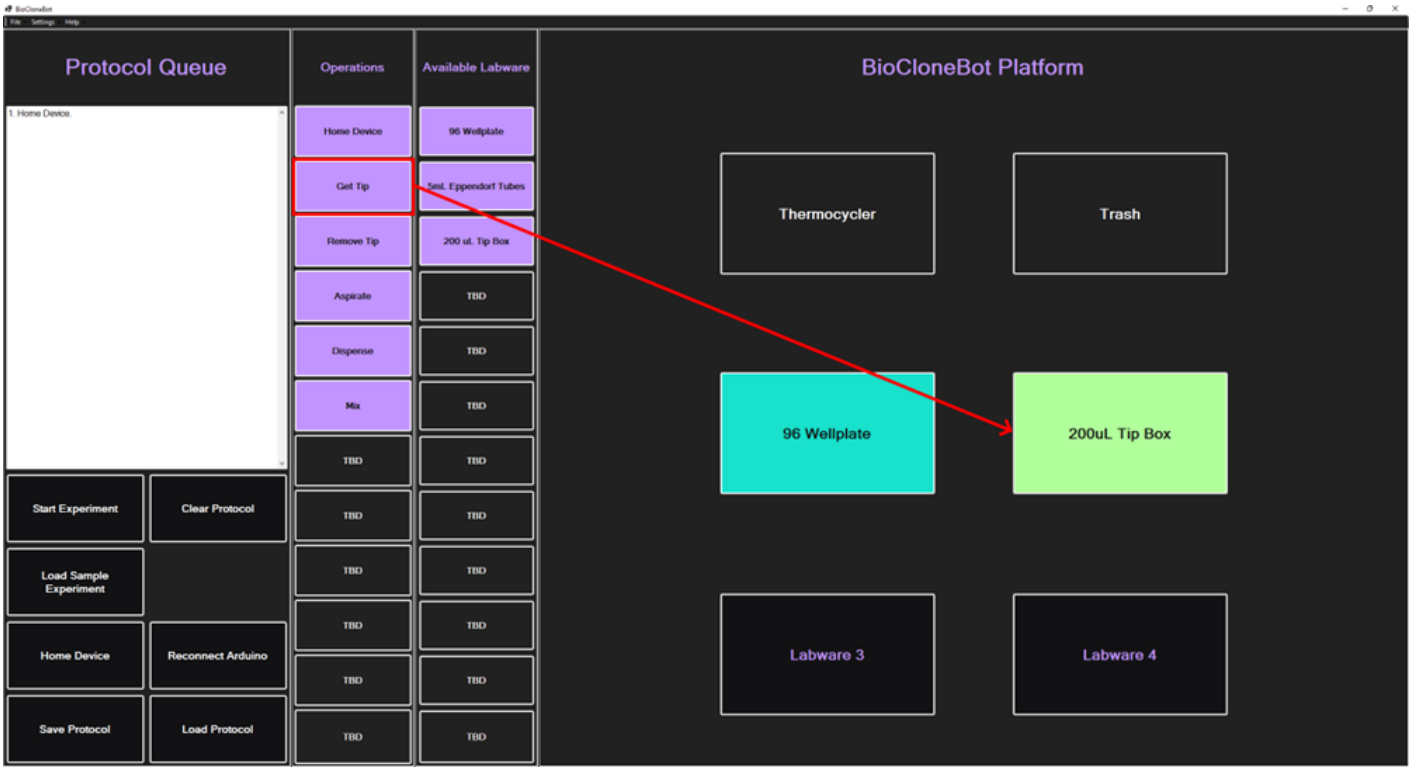

Figure 11: Add Get Tip Operation

The Attach Tip Operation form will now open.

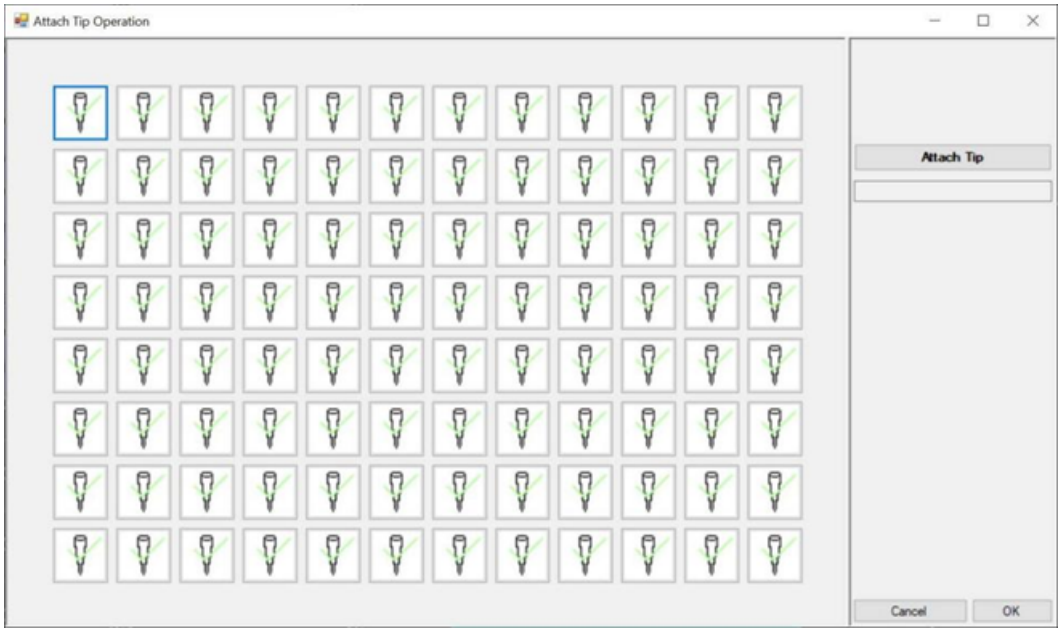

Figure 12: Get Tip Operation Form opened

Select the top left tip, then click the Attach Tip button.

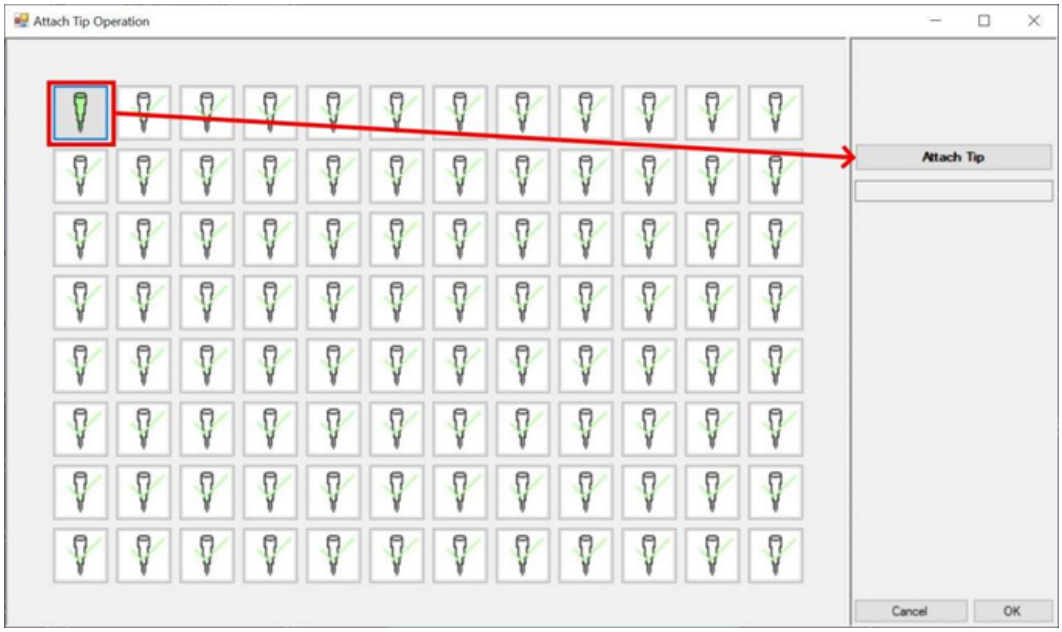

Figure 13: Select tip and click Attach Tip to attach the selected tip

Notice that the tip is no longer available for selection.

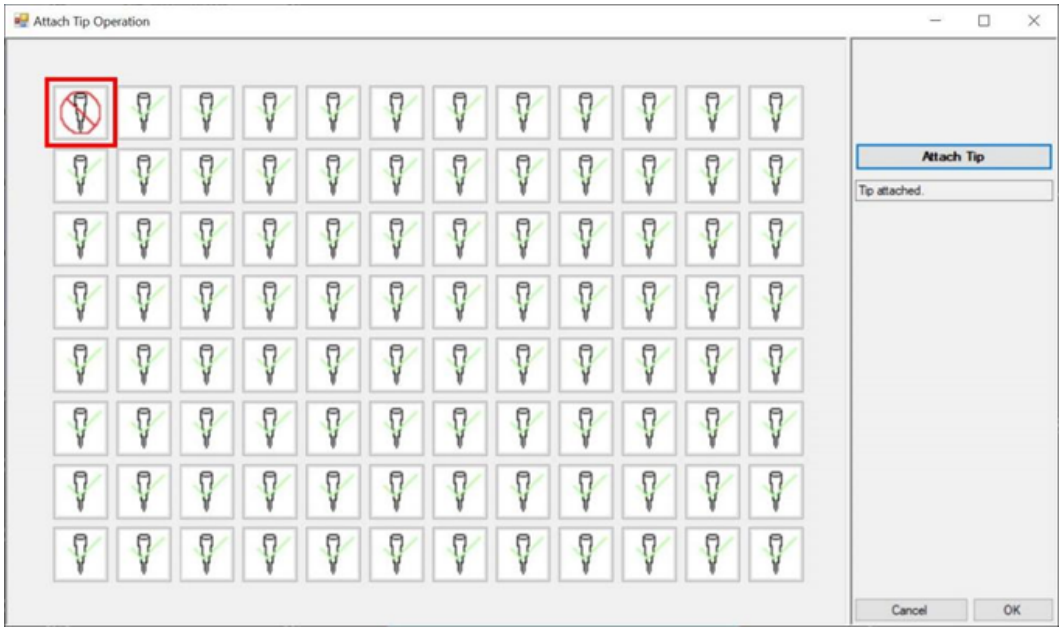

Figure 14: Tips are no longer available after being attached

Press OK to go back to the platform.

Now we can see the second step in the Protocol Queue “Get Tip from tipbox in position 2.” 5.

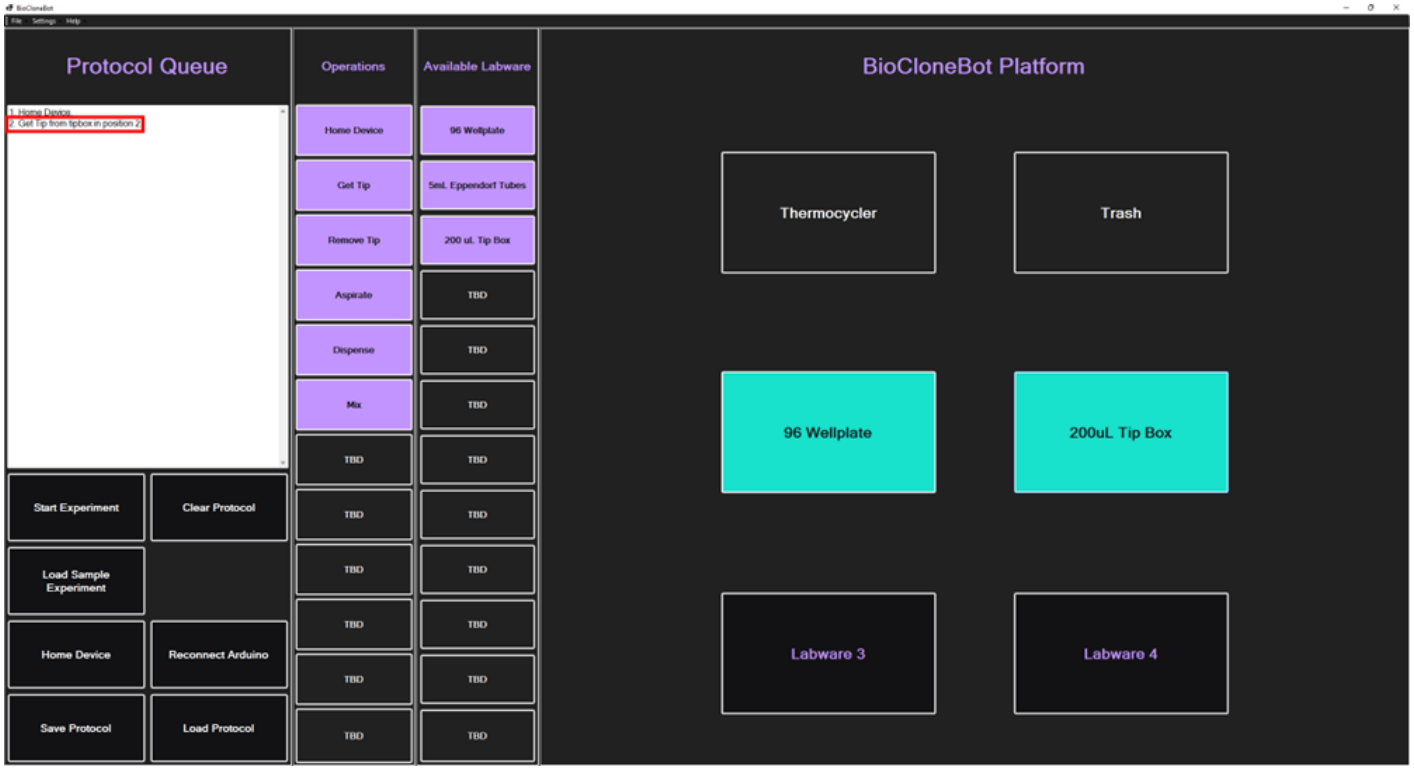

Figure 15: The Get Tip Operation is added to Protocol Queue after confirming

Next, select the Aspirate operation then select the 96 wellplate.

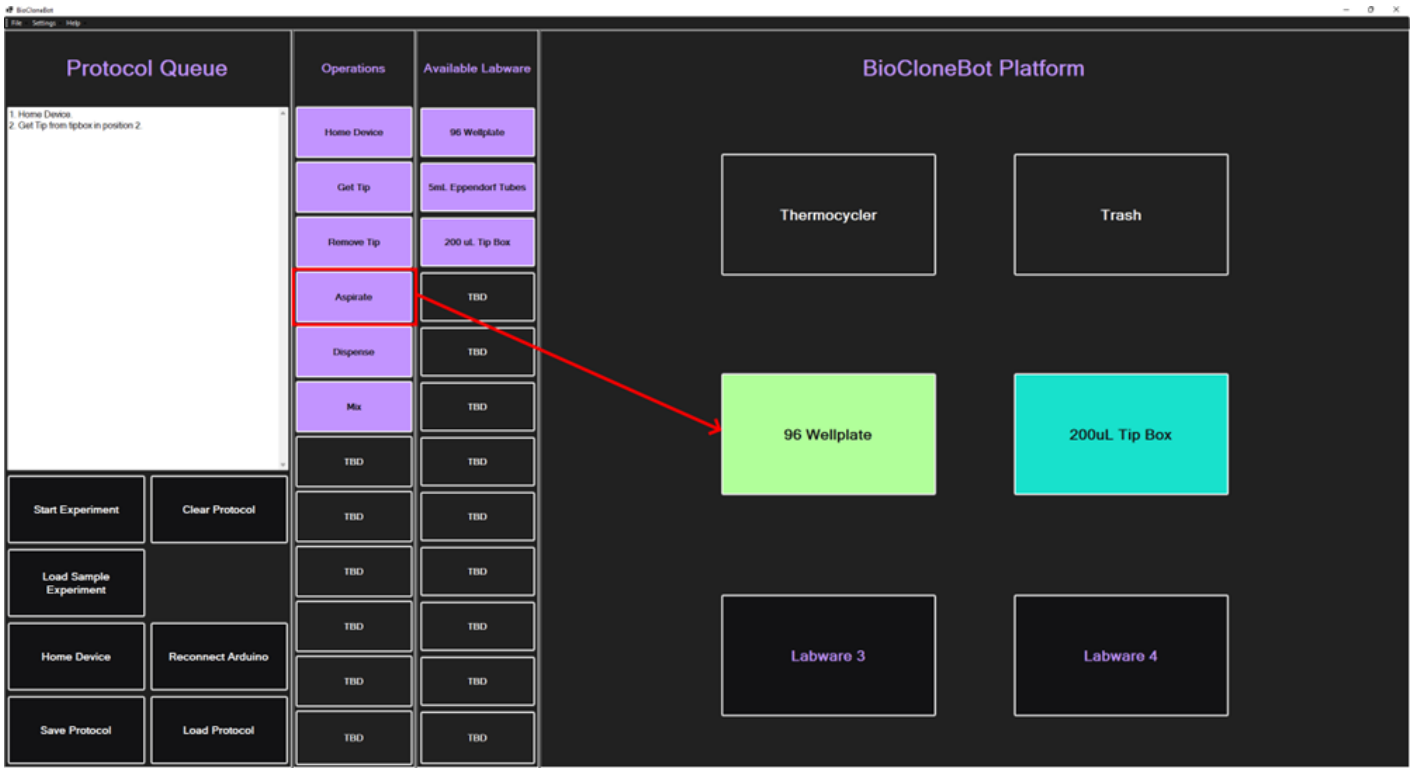

Figure 16: After clicking the Aspirate Operation button, the wellplate lights up

This will open the Aspiration Form.

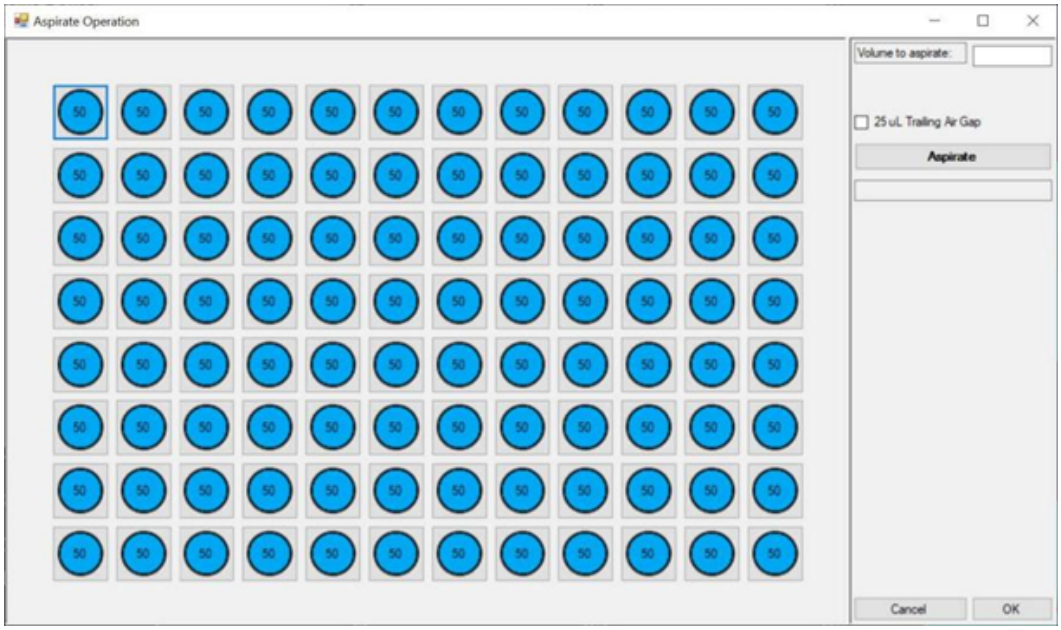

Figure 17: Aspiration Operation form

Select the top left well and enter in 50 into the Volume to aspirate text box, and click Aspirate.

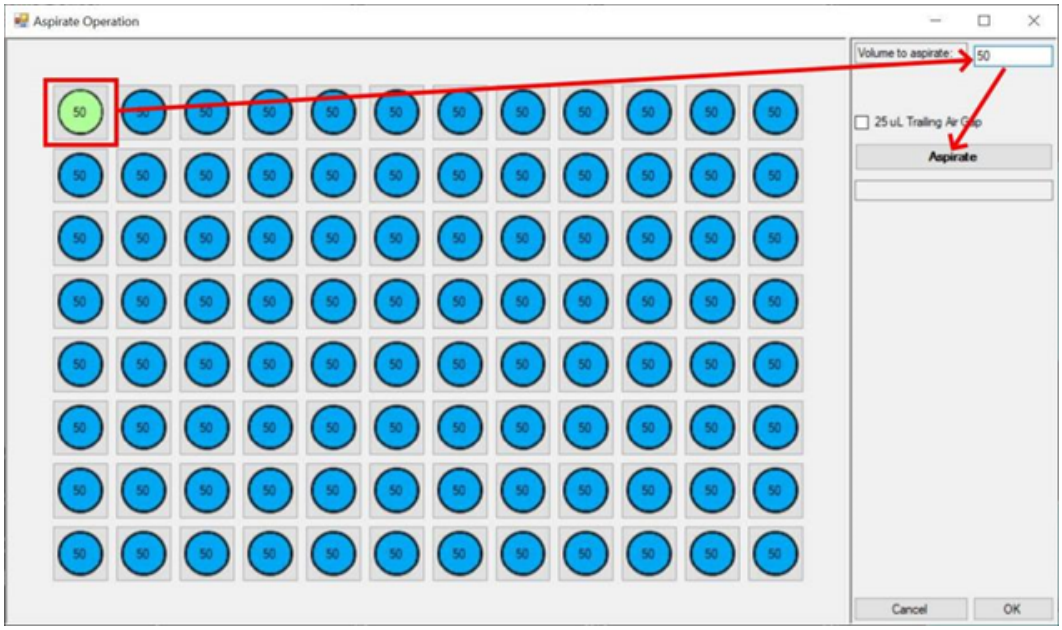

Figure 18: Select the well, enter in 50µL, then click Aspirate to confirm the Operation.

We can see that the operation was confirmed and that 50µL was removed from the well.

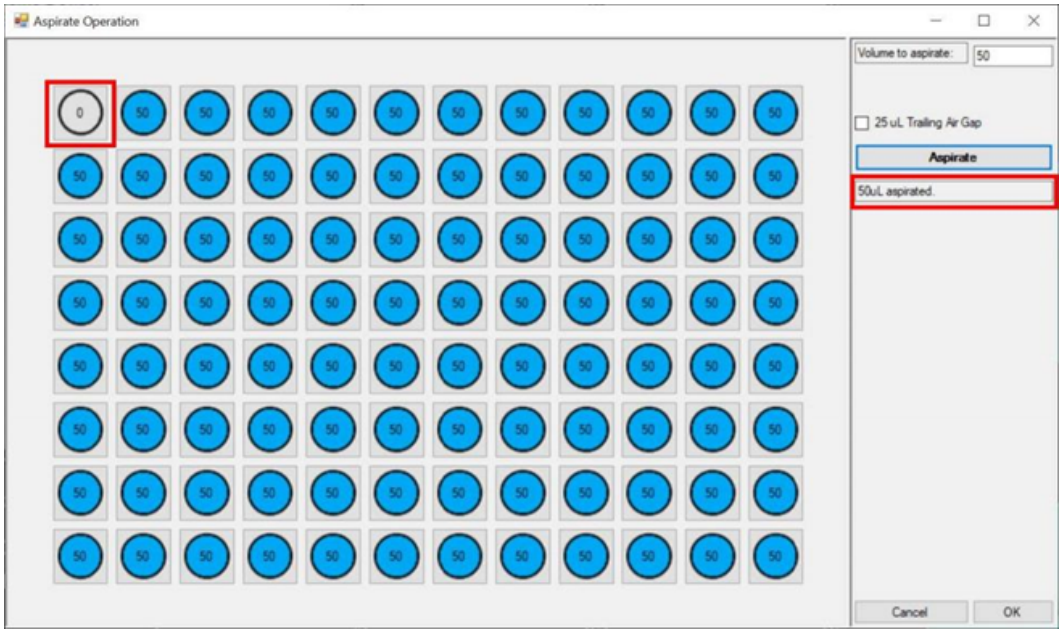

Figure 19: The well automatically updates to 0µL after confirming the operation

Note: You can use the 25µL Trailing Air Gap check box to aspirate 25.0µL of air before aspirating the designated volume from the well. This 25.0µL can then be used to simulate the double press action of a pipette where a larger volume is dispensed than picked up to guarantee all the liquid is ejected from the pipette tip. In this case, we will leave it blank.

Click OK and we will now see that the aspiration step has been added.

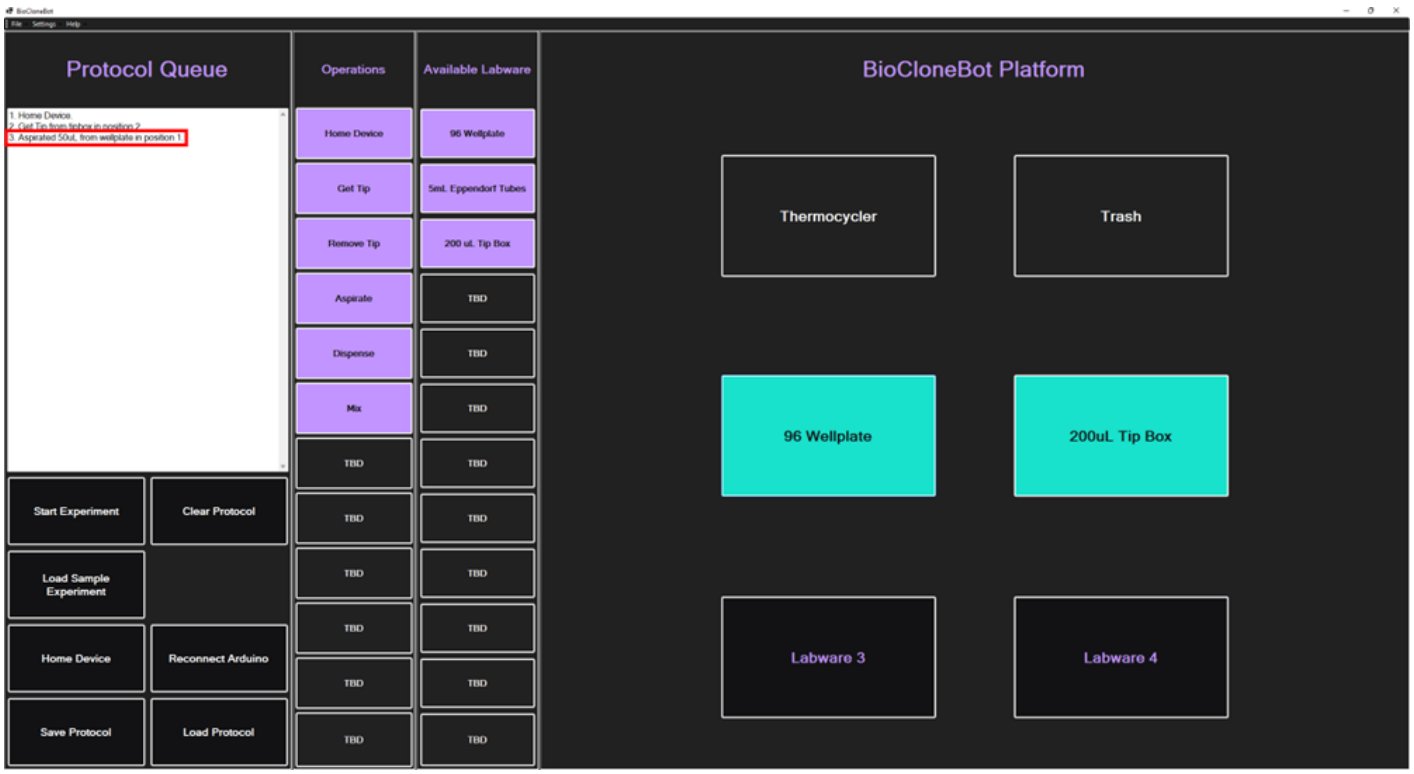

Figure 20: The Aspiration Operation was added to the Protocol Queue

Next we will dispense the 50µL. Select the Dispense operation then select the 96 wellplate.

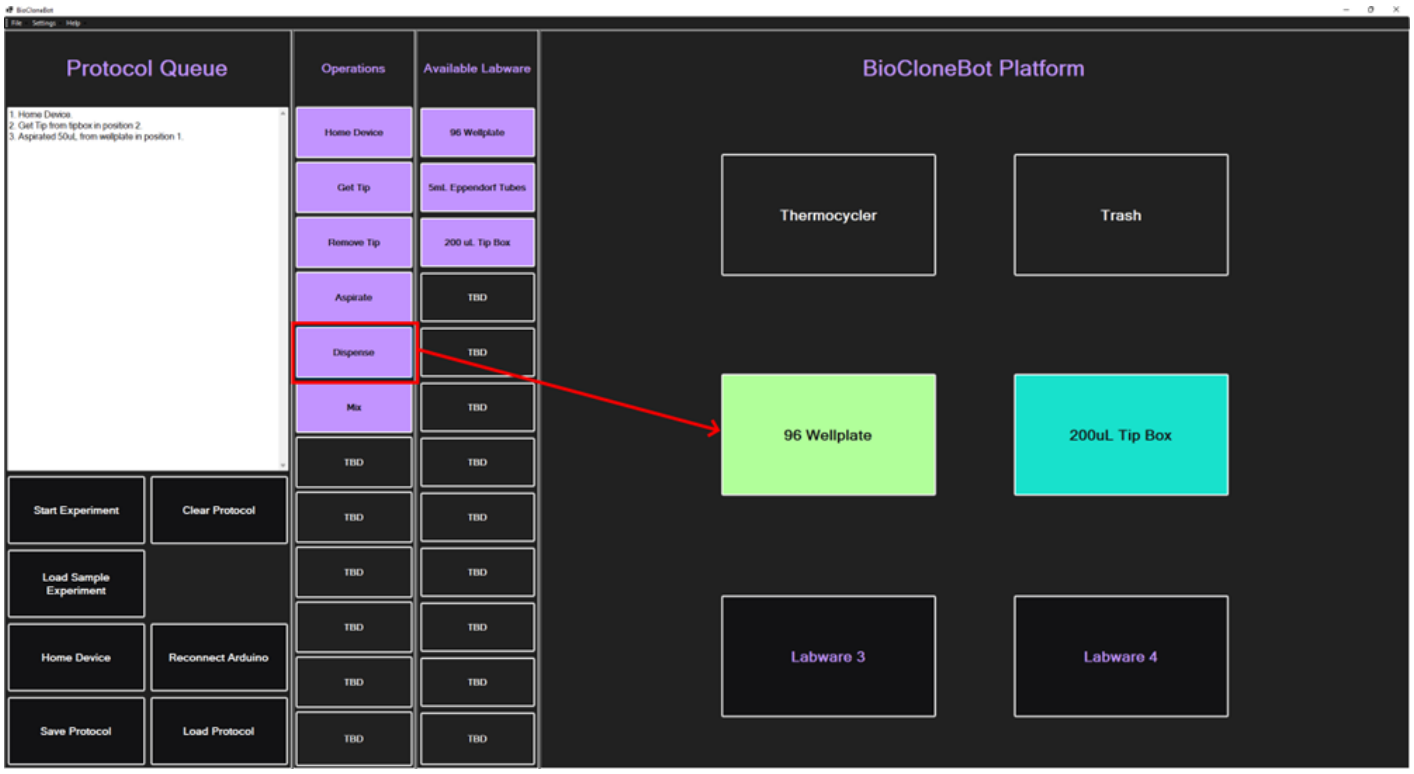

Figure 21: When selected the Dispense Operation, the wellplate lights up

This will open the dispense operation form.

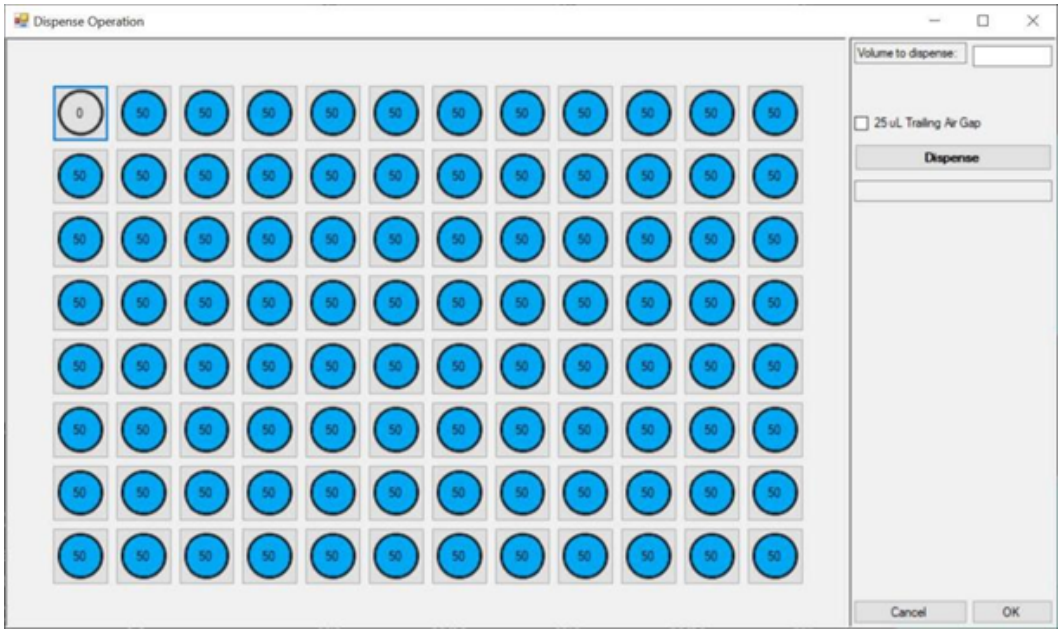

Figure 22: Dispense Operation Form

Select the top right well, enter 50 into the Volume to dispense text box, and click Dispense.

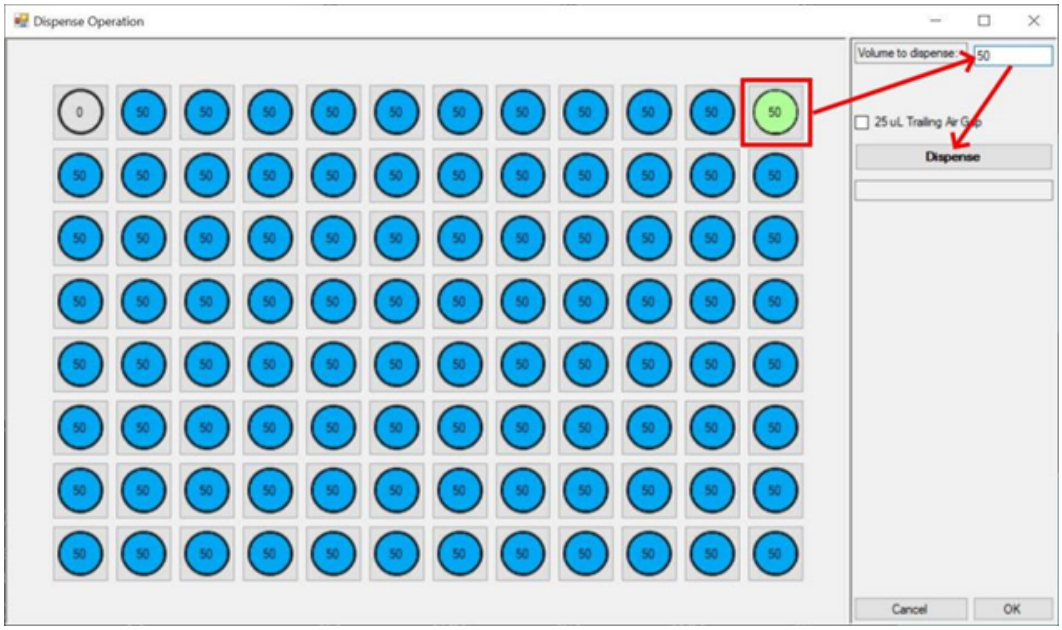

Figure 23: Select the well, enter in 50µl, then click dispense

Now we can see that 50µL was dispensed to the top right well.

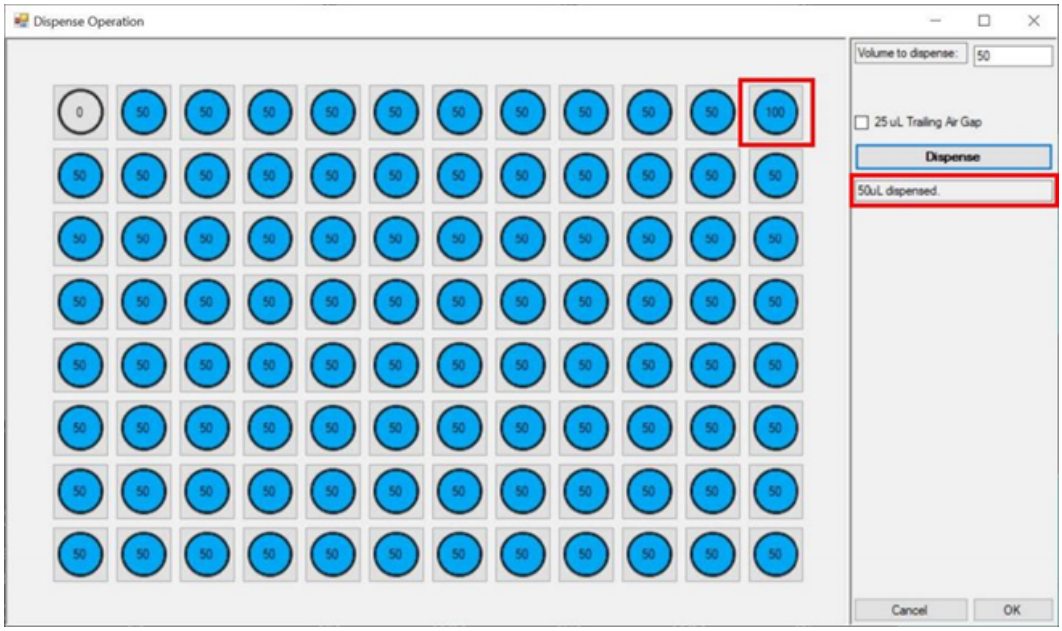

Figure 24: The well updates to 100µL after clicking Dispense

Click OK and we will see the dispense operation is added to the Operations Queue.

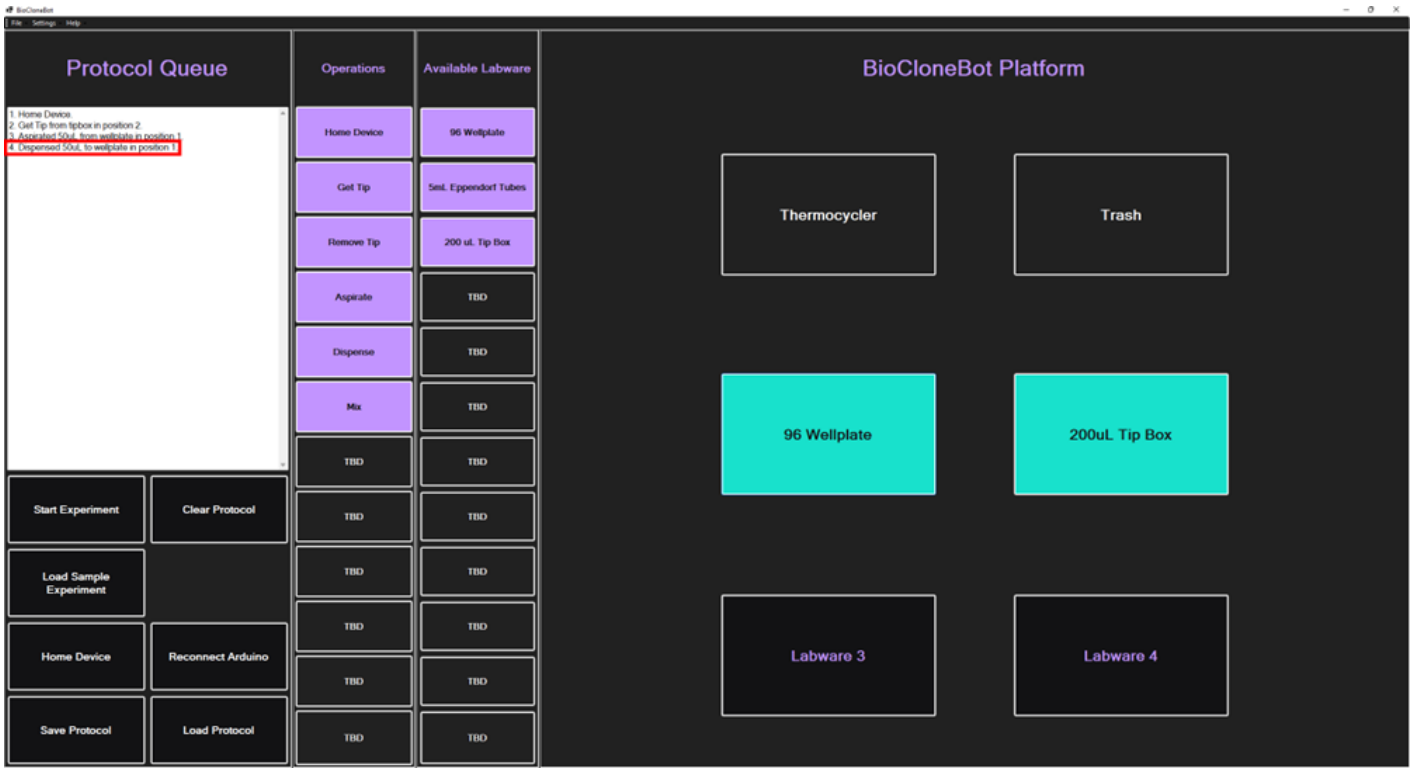

Figure 25: The Dispense Operation was added to the Protocol Queue

Next, select the Remove Tip operation. The software knows where the trash bin is so you do not need to select anything additional.

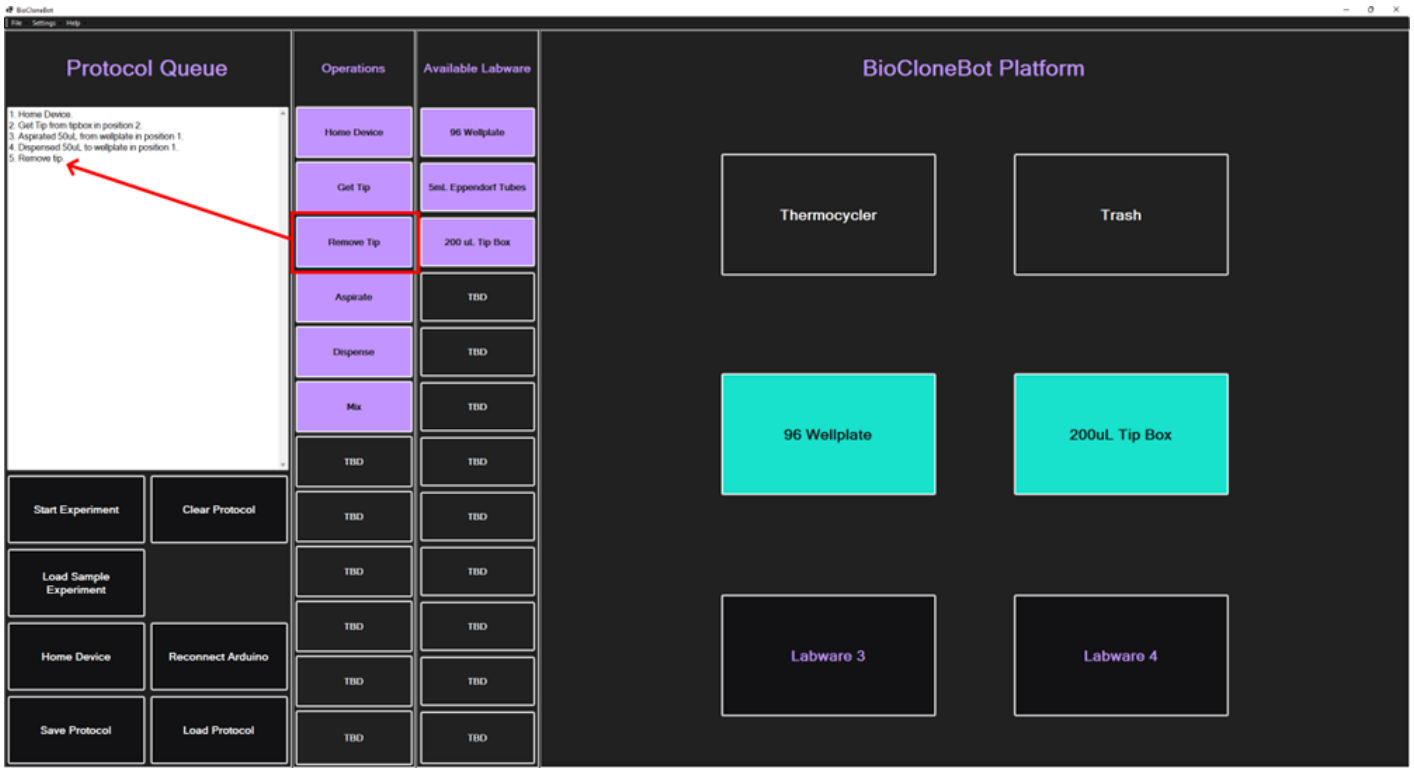

Figure 26: Pressing the Remove Tip Operation button adds a second Remove Tip to the Protocol Queue

Select the Get Tip operation and select the 200µL Tip Box.

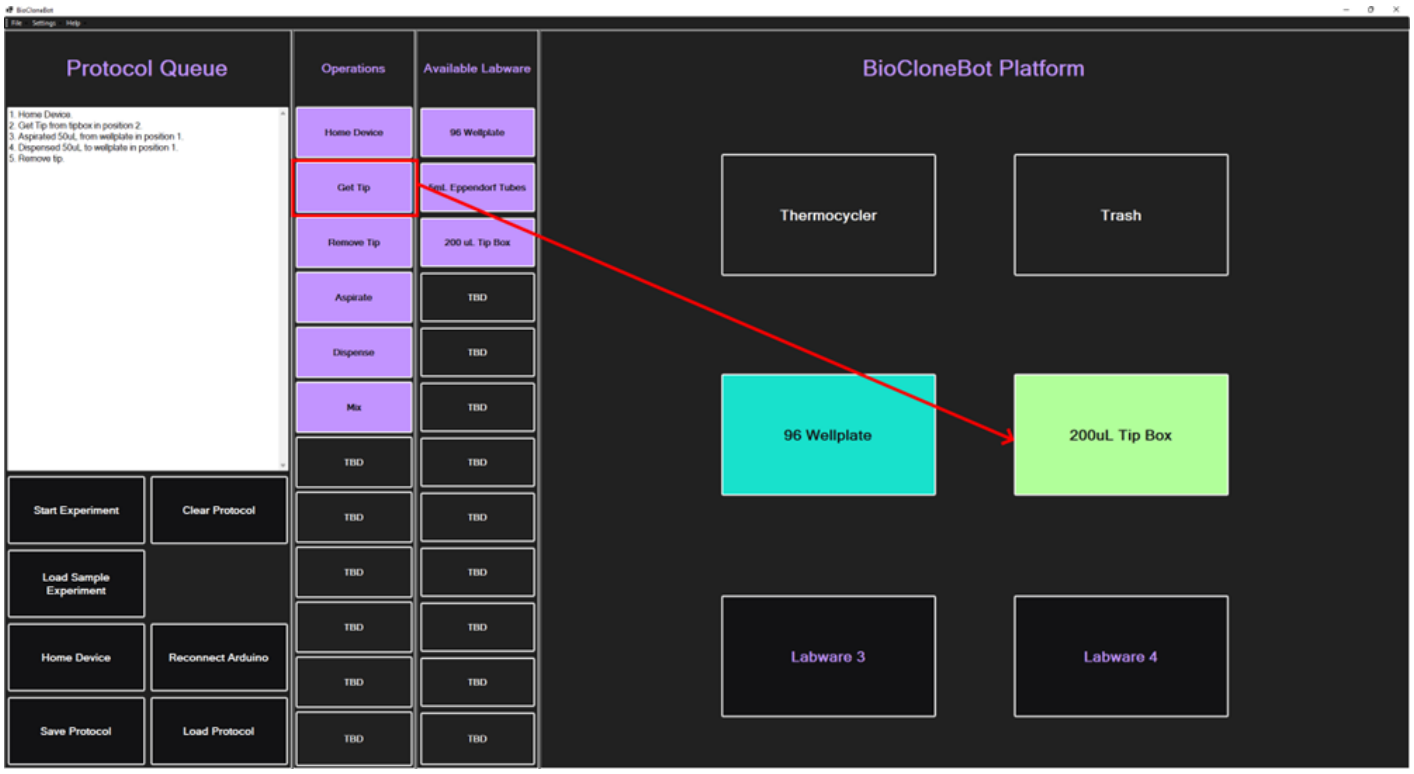

Figure 27: Select Get Tip to attach a fresh tip to the pump

Select the tip to the right of the used tip location and select Attach Tip.

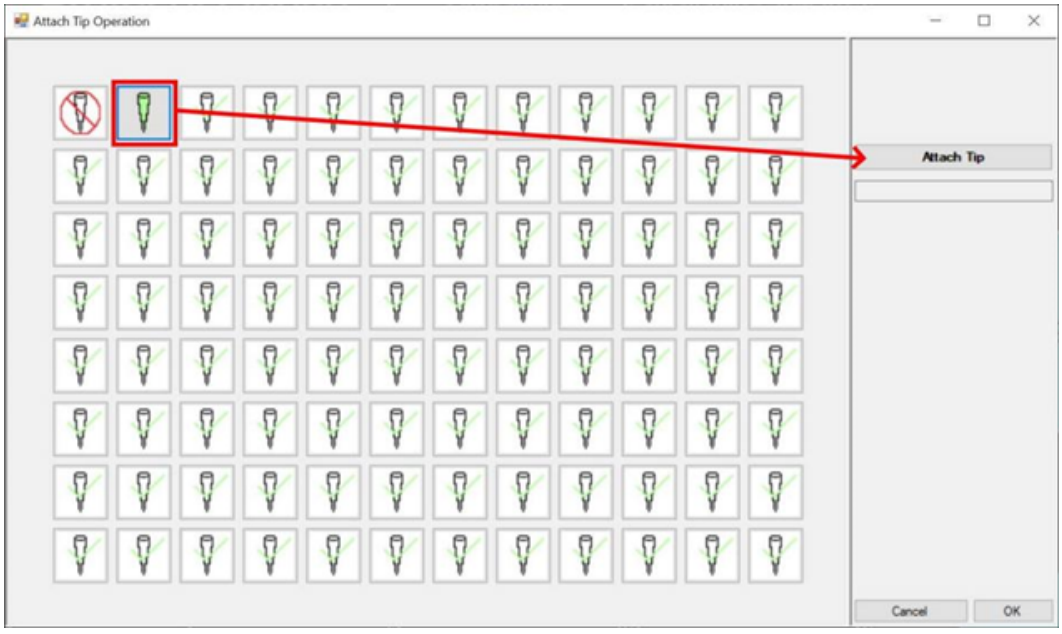

Figure 28: Select the second tip and click Attach Tip

We can see the second tip is removed and the new tip is attached.

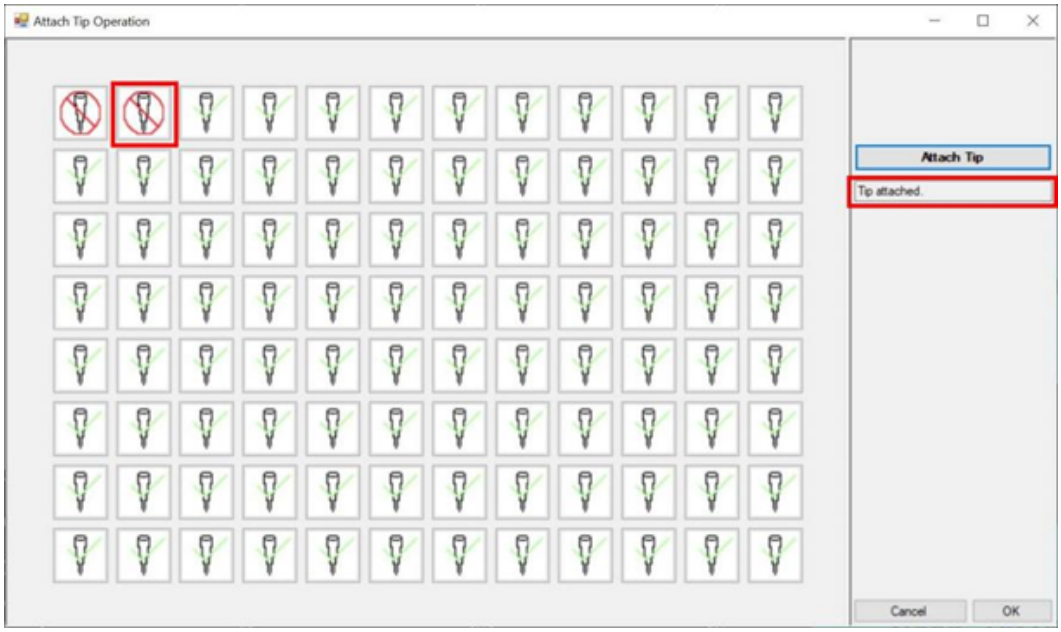

Figure 29: The second tip is no longer available

Click OK to return back to the Control Form. We can see the get tip operation was successful.

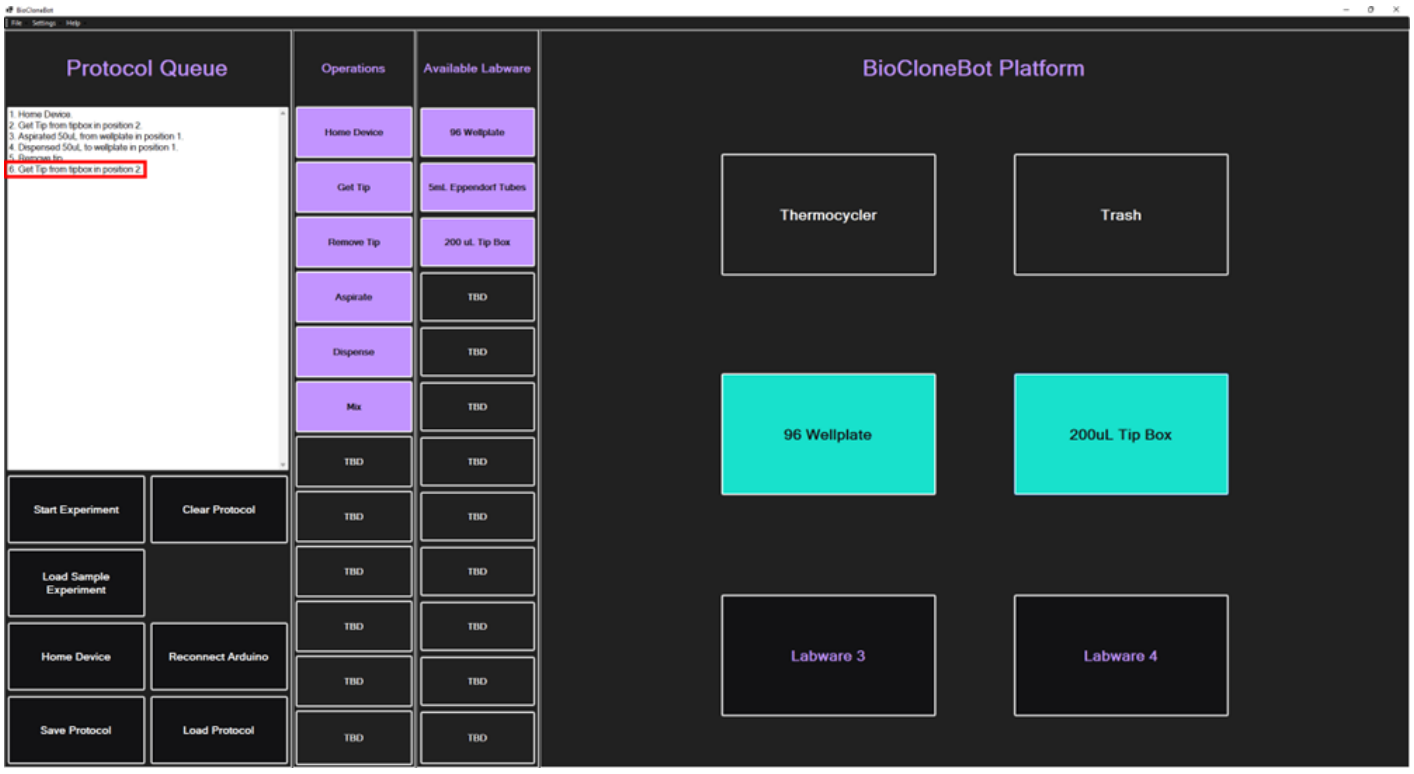

Figure 30: The second Get Tip Operation is added to the Protocol Queue

Next we will select the mix operation. Click Mix and select the 96 wellplate.

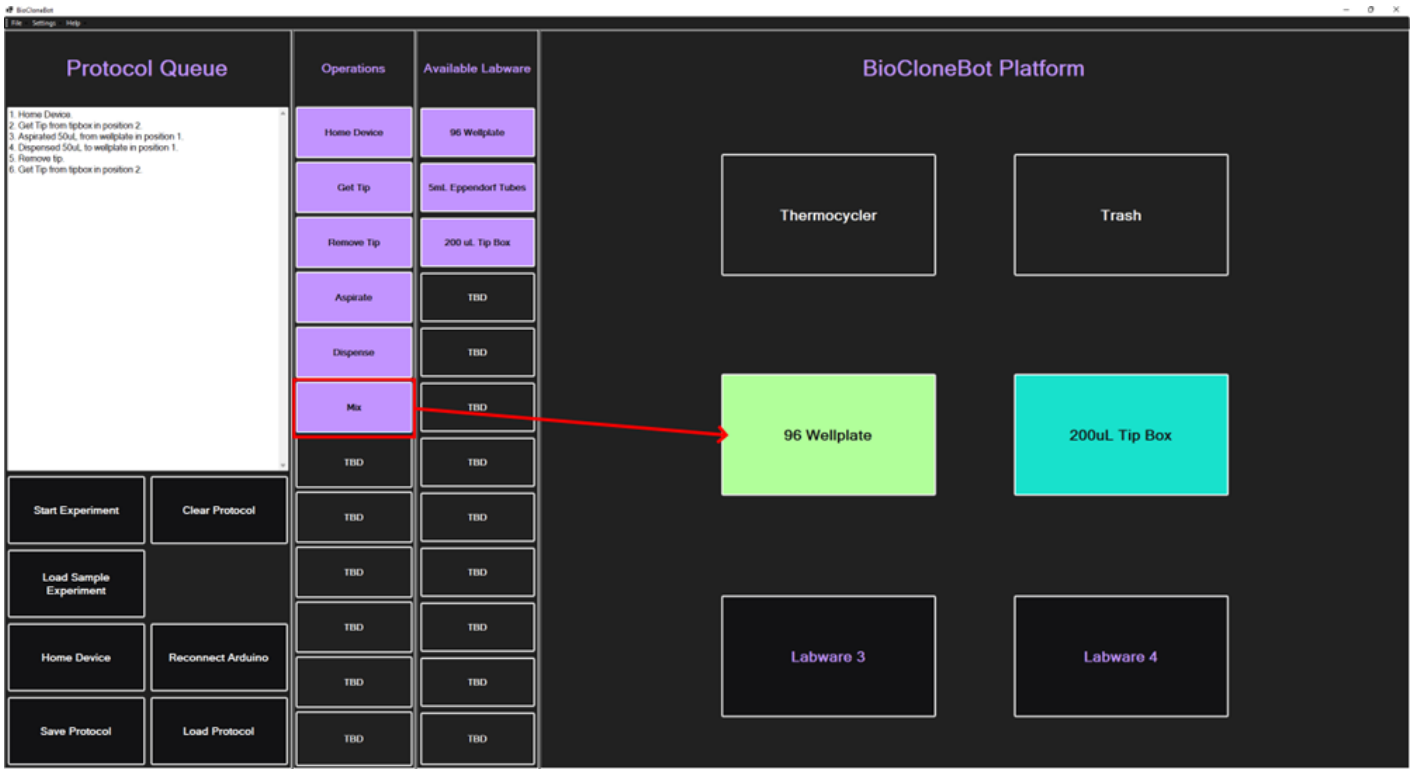

Figure 31: Selecting the Mix Operation highlights the 96 wellplate

The mix operation form will open.

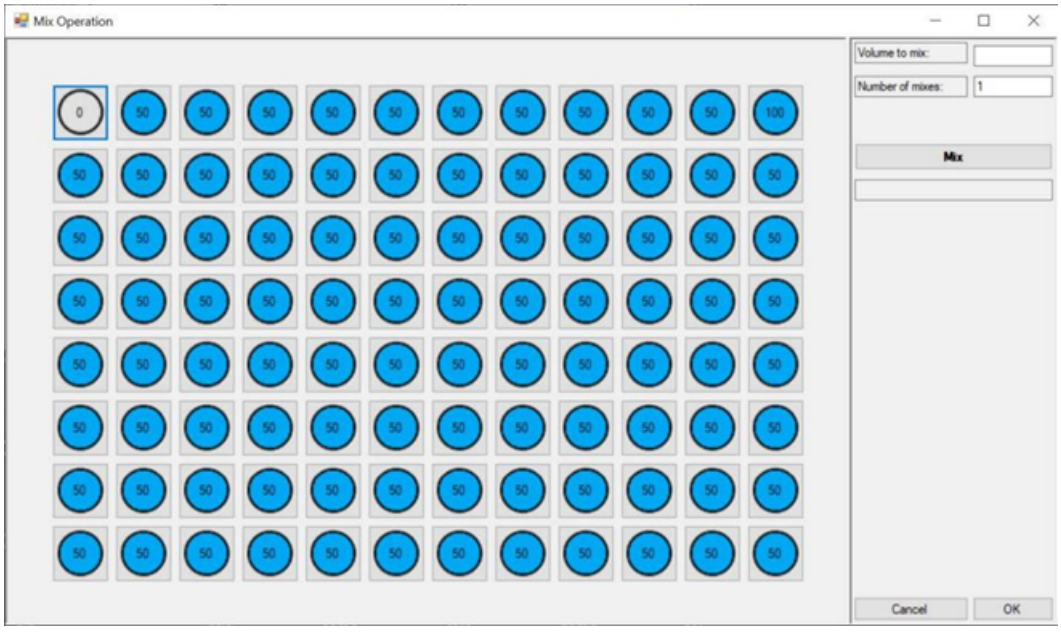

Figure 32: The Mix Operation Form

Select the top right well, enter in 25 into the Volume to mix text box, enter 5 into the number of mixes text box, and click Mix.

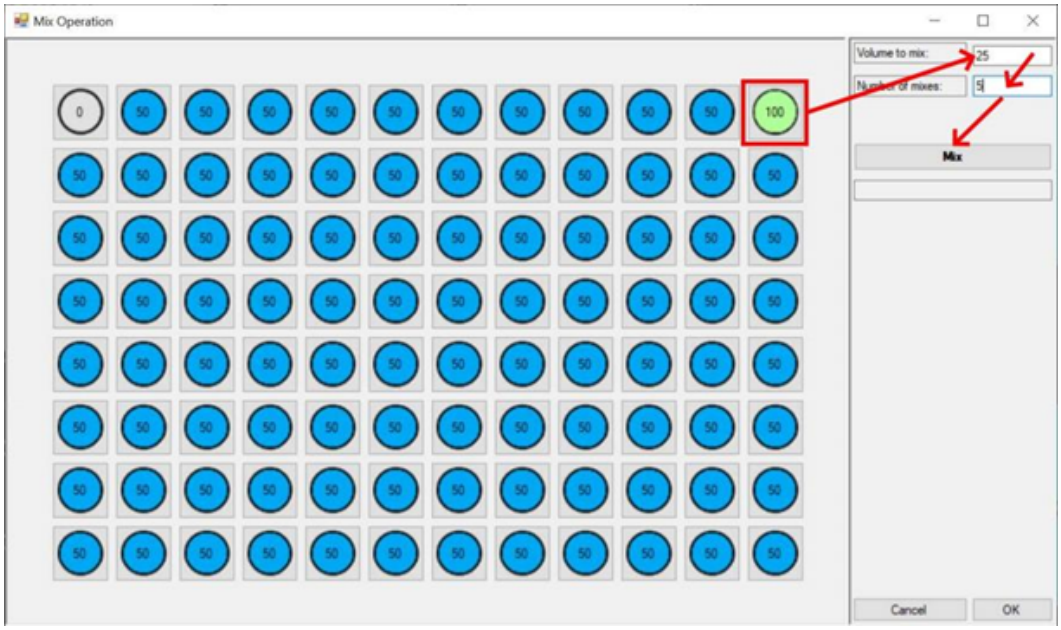

Figure 33: 25µL will be mixed 5 tips from the well containing 100µL

The Mix operation is now confirmed. This means the pump will aspirate then dispense 25µL 5 times into the selected well.

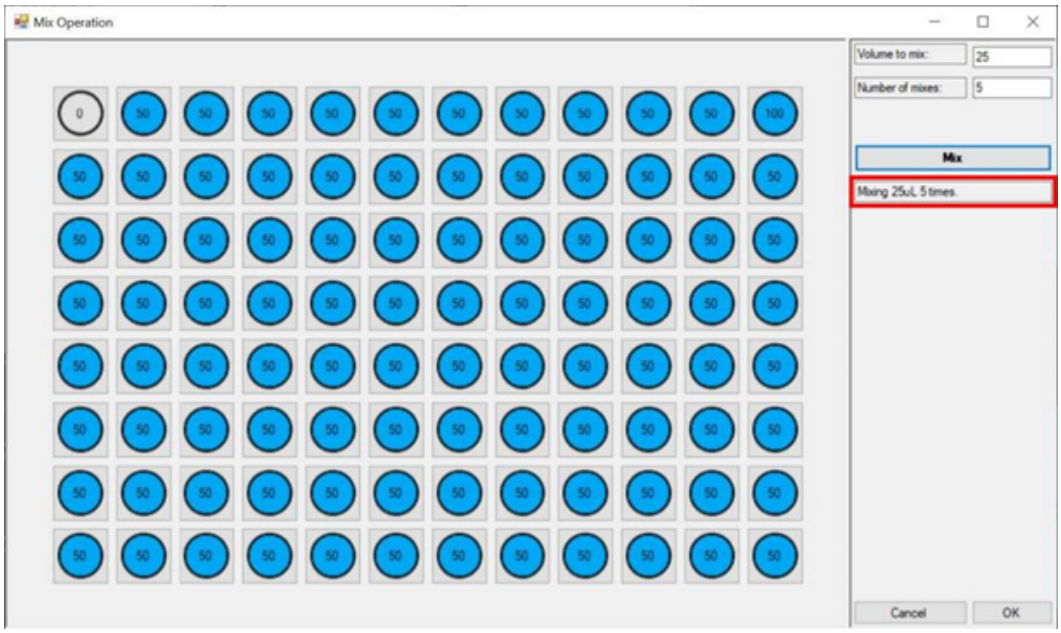

Figure 34: The Mix Operation of 25µL is confirmed

Click OK and we will see that the mix operation has been successfully added.

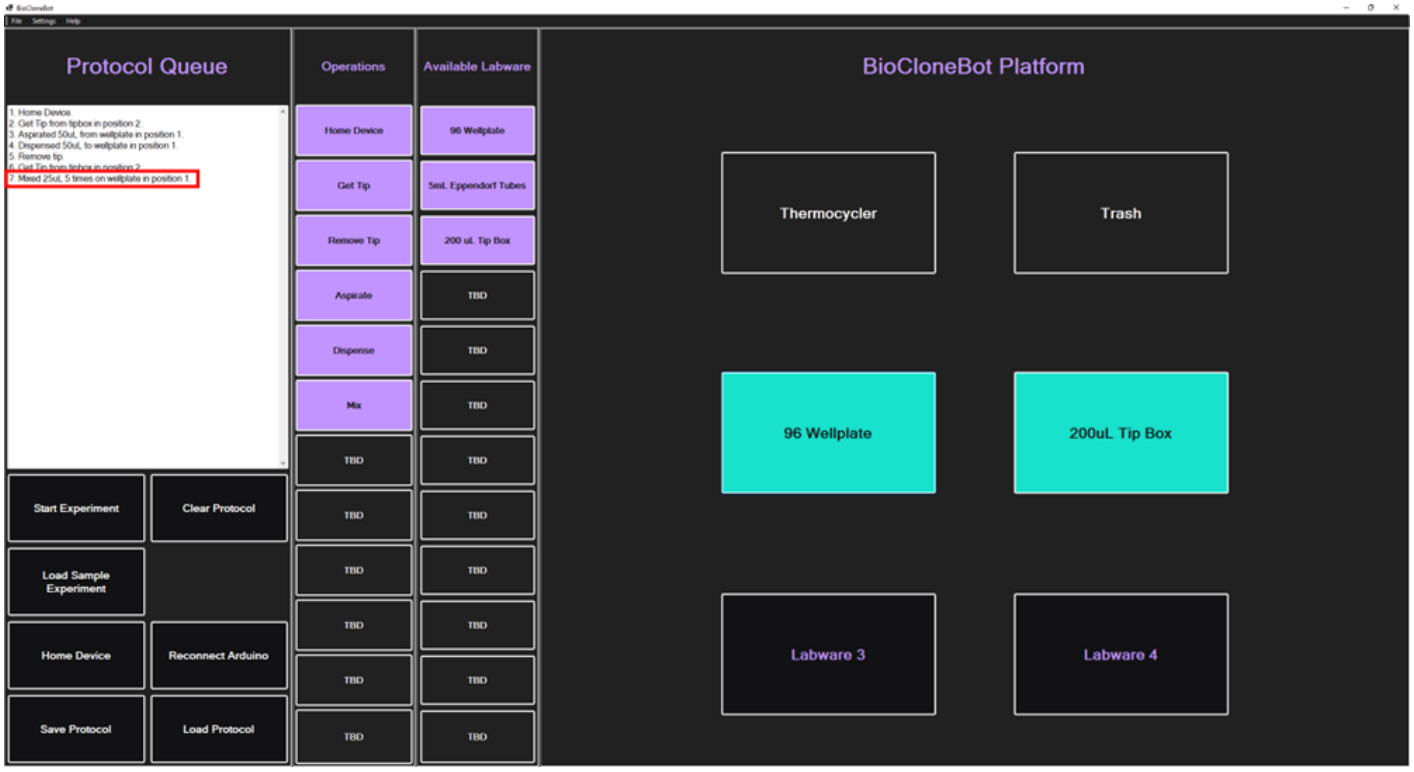

Figure 35: The Mix Operation is added to the Protocol Queue

Next we will add one final Remove Tip operation. Click Remove Tip to add the final operation.

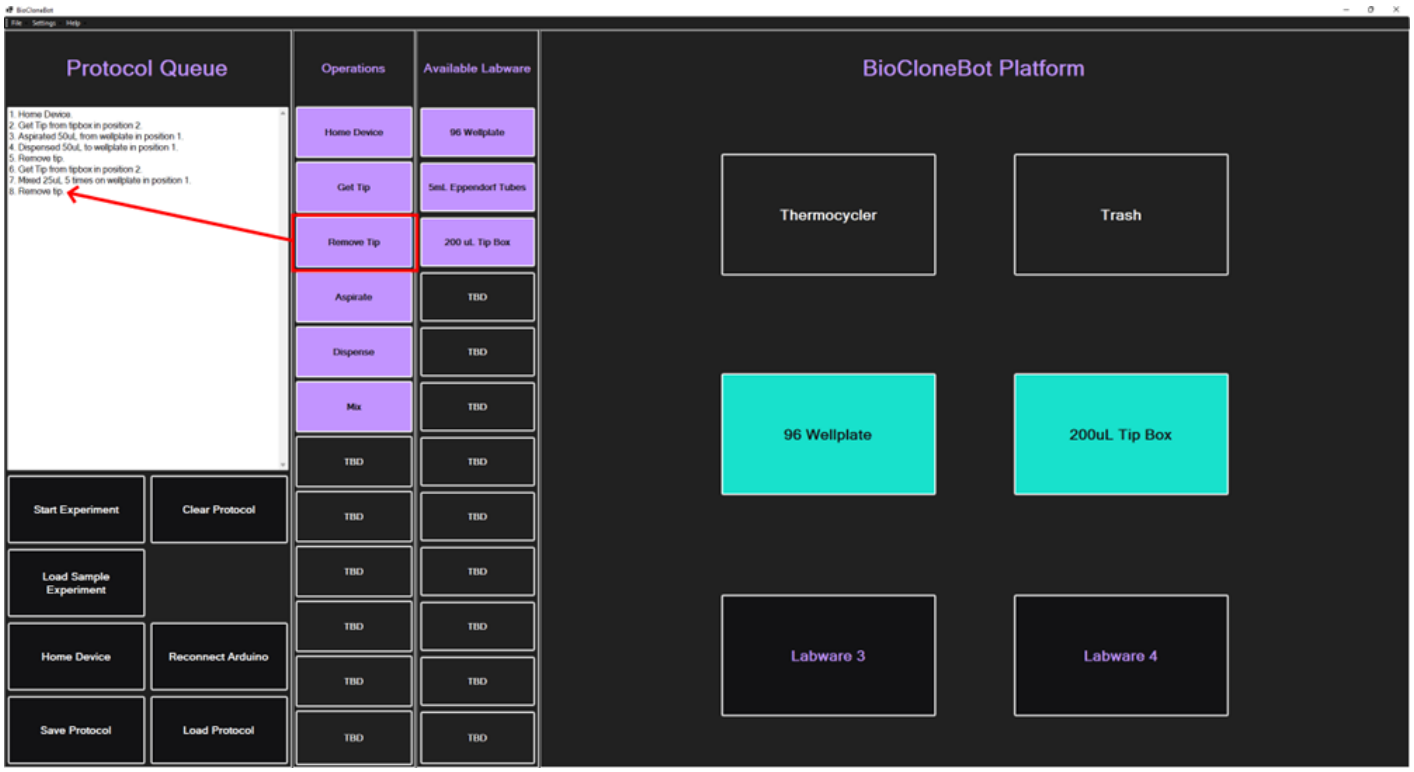

Figure 36: BCB/Images/Selecting Remove Tip adds a second Remove Tip Operation to the Protocol Queue

Our protocol is finally complete.

1.4 Running, Saving, Loading, and Clearing Protocols

Running a Protocol

Now that our experiment has been set up from the previous section, we can add the labware to the platform. Go ahead and load a 96 wellplate into Position 1 and a 200µL tip box into Position 2 on the physical BioCloneBot. Fill the top left well in the wellplate full of water. This will be the water used for the test.

Click the Start Experiment button.

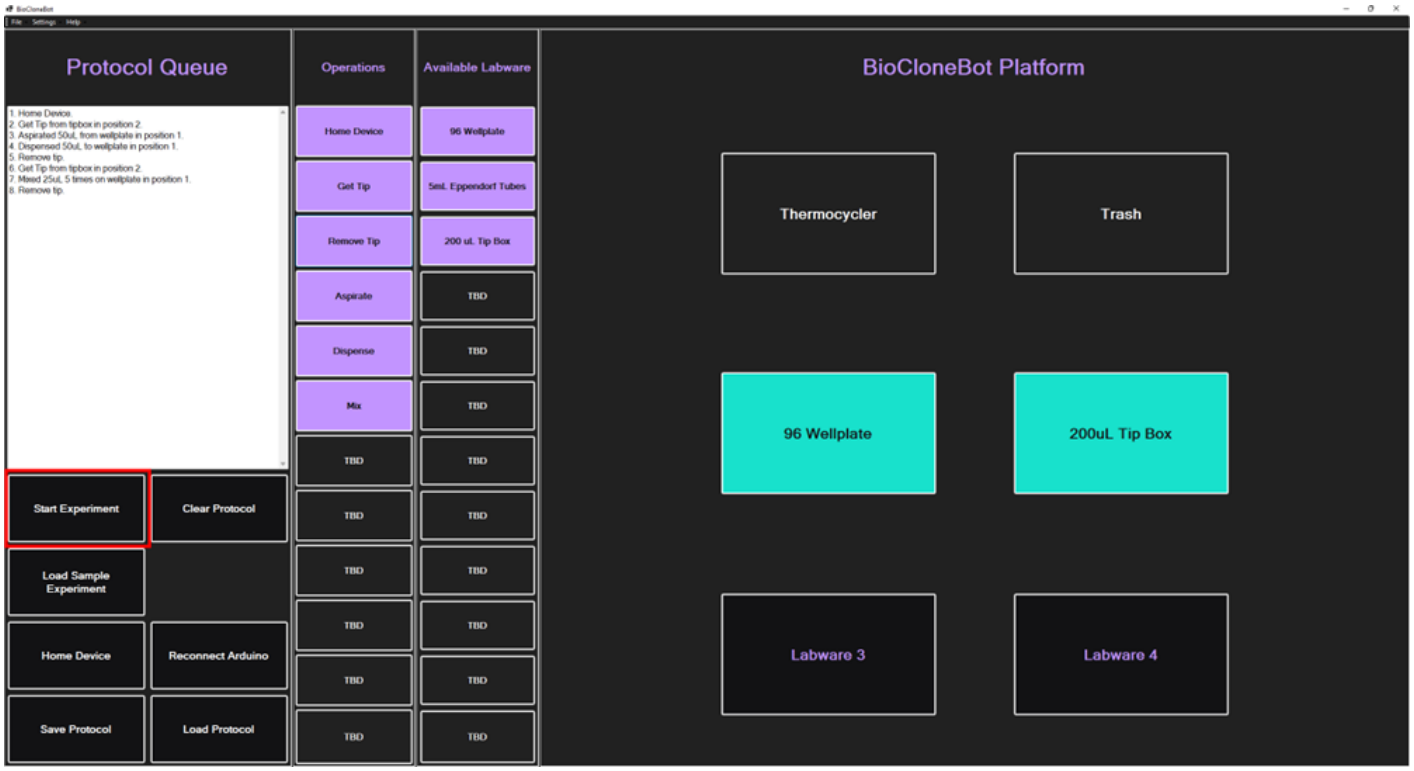

Figure 37: Pressing the Start Experiment button will begin the protocol

This will result in the following pop-up:

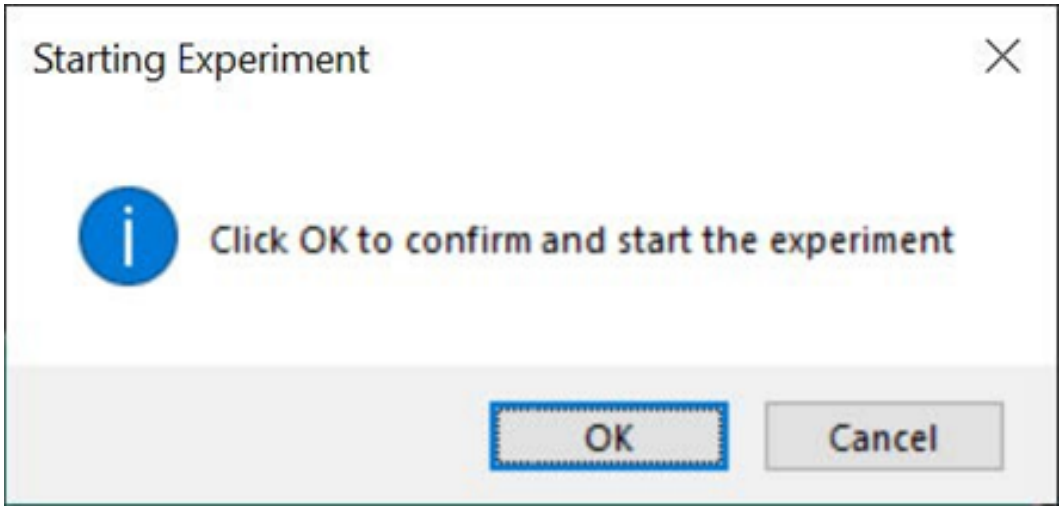

Figure 38: Start Experiment confirmation window

Once you are sure that your labware and protocol is properly correct, click OK to start the protocol. The front-

end generates a list of commands while setting up the protocols. The following loop runs until all commands have been completed:

1. Send current command to Arduino
2. Wait for Arduino to send response indicating the completion of the command
3. Repeat until all commands have been run

Once the experiment is done, we will receive the following pop-up:

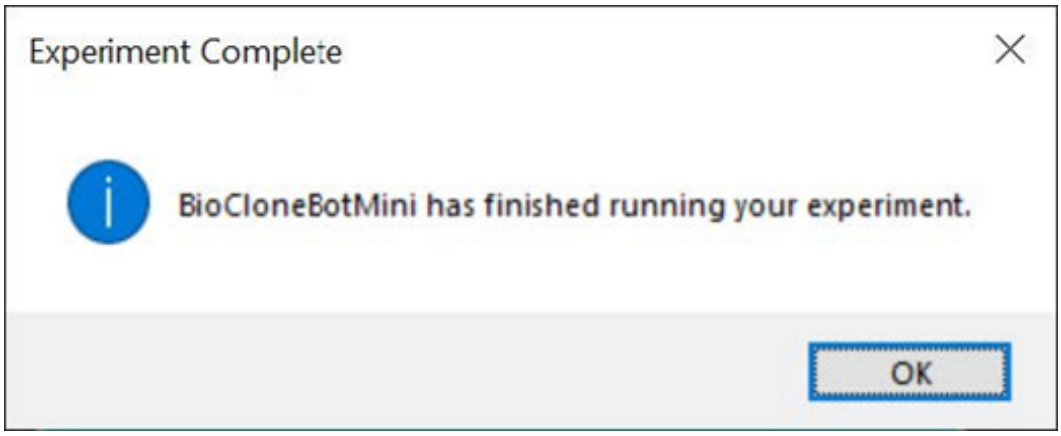

Figure 39: Experiment finished pop-up

Click OK to finish the experiment.

Saving a Protocol To save time and prevent repeating protocol steps over and over, saving has been implemented.

Once you have finished designing a protocol, click the Save Protocol button in the bottom left.

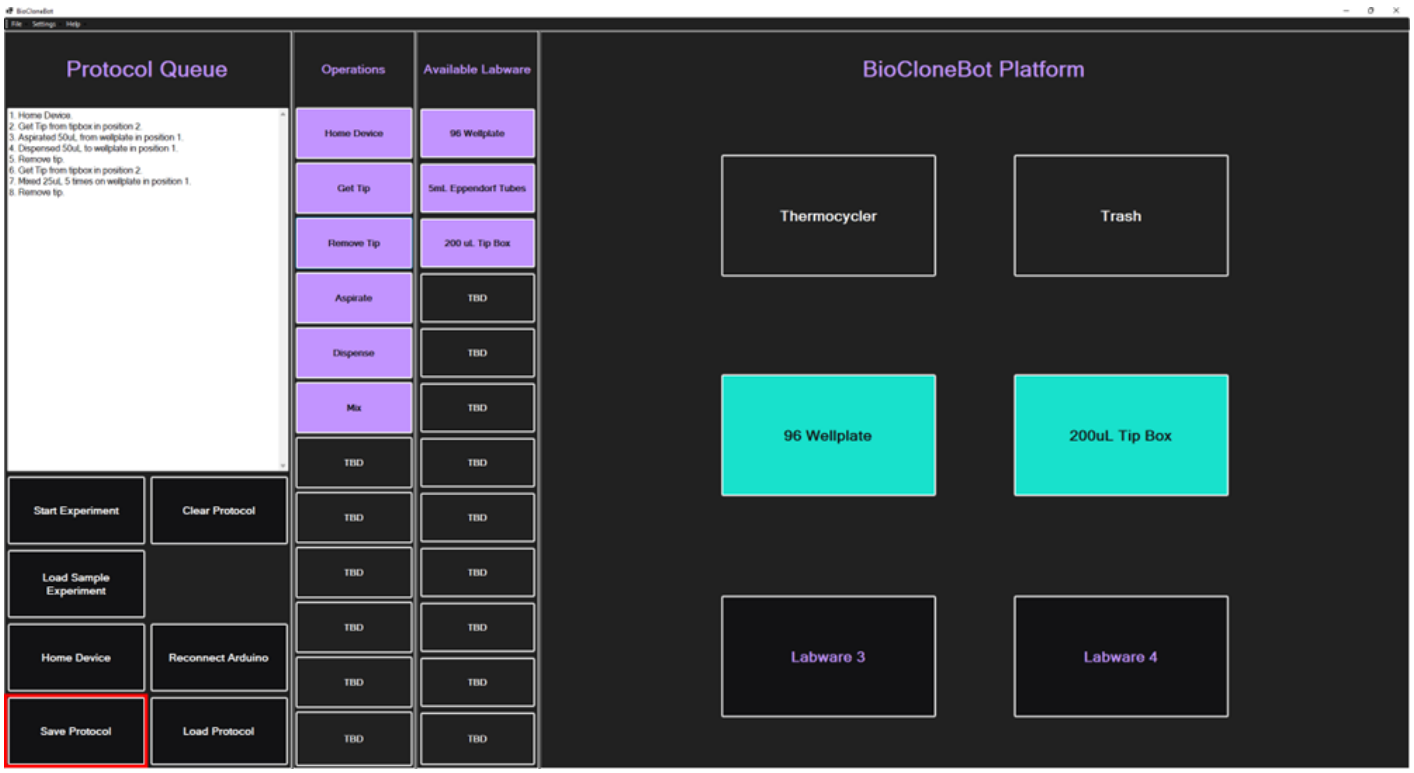

Figure 40: Press the Save Protocol button to save your protocol to a JSON file

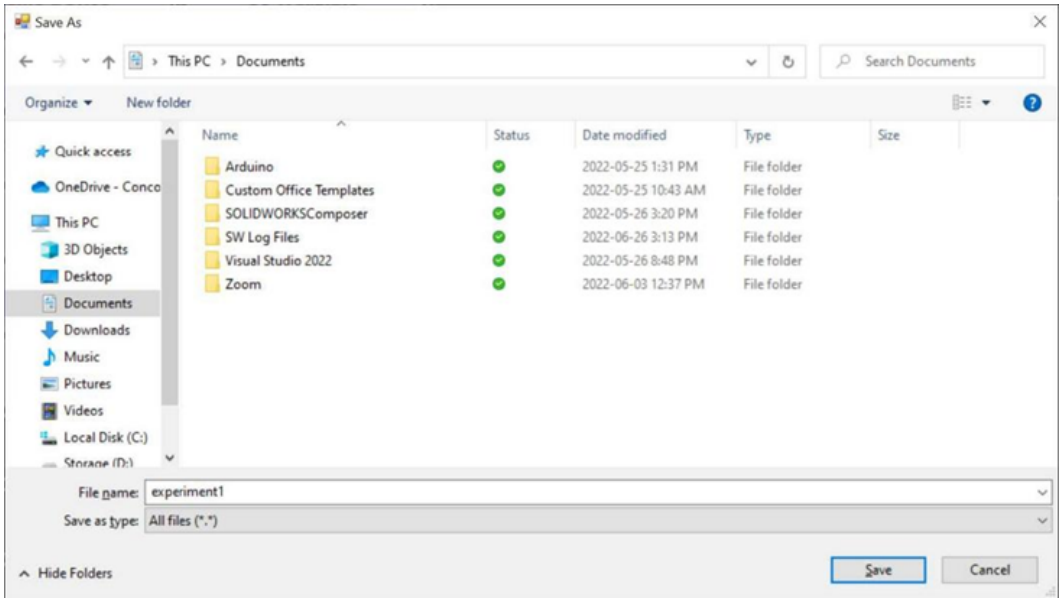

Figure 41: Save Protocol Window

A dialog window will open.

Select your save location, enter in a name for your experiment, and click Save.
This will generate a file containing your labware, the protocol, and the final conditions of the labware after all the protocol is run.

Loading a Protocol Click the Load Protocol button in the bottom left.

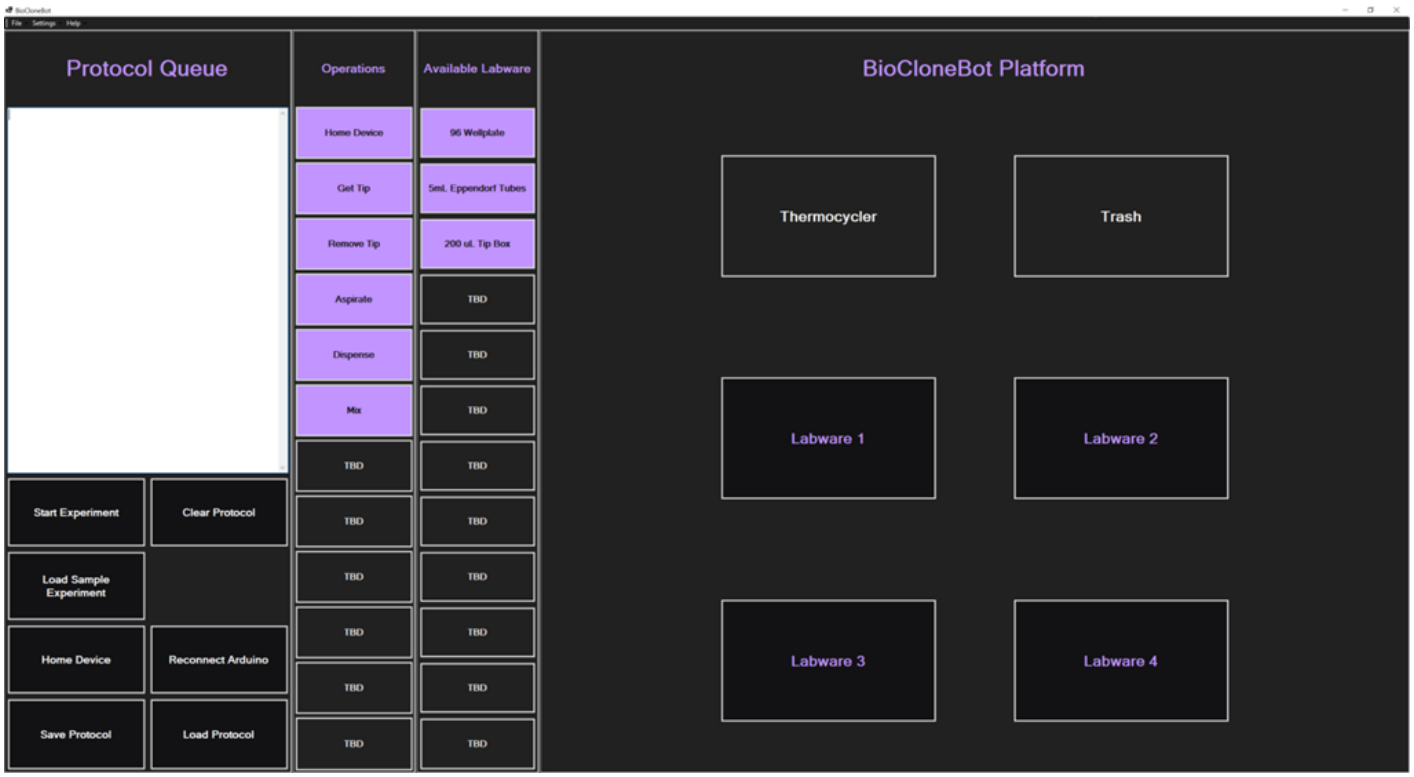

Figure 42: Press the Load Protocol button to load a protocol from a JSON file

A selection window will open. Navigate to the location of your protocol and press Open. Your protocol will

now load into the software.

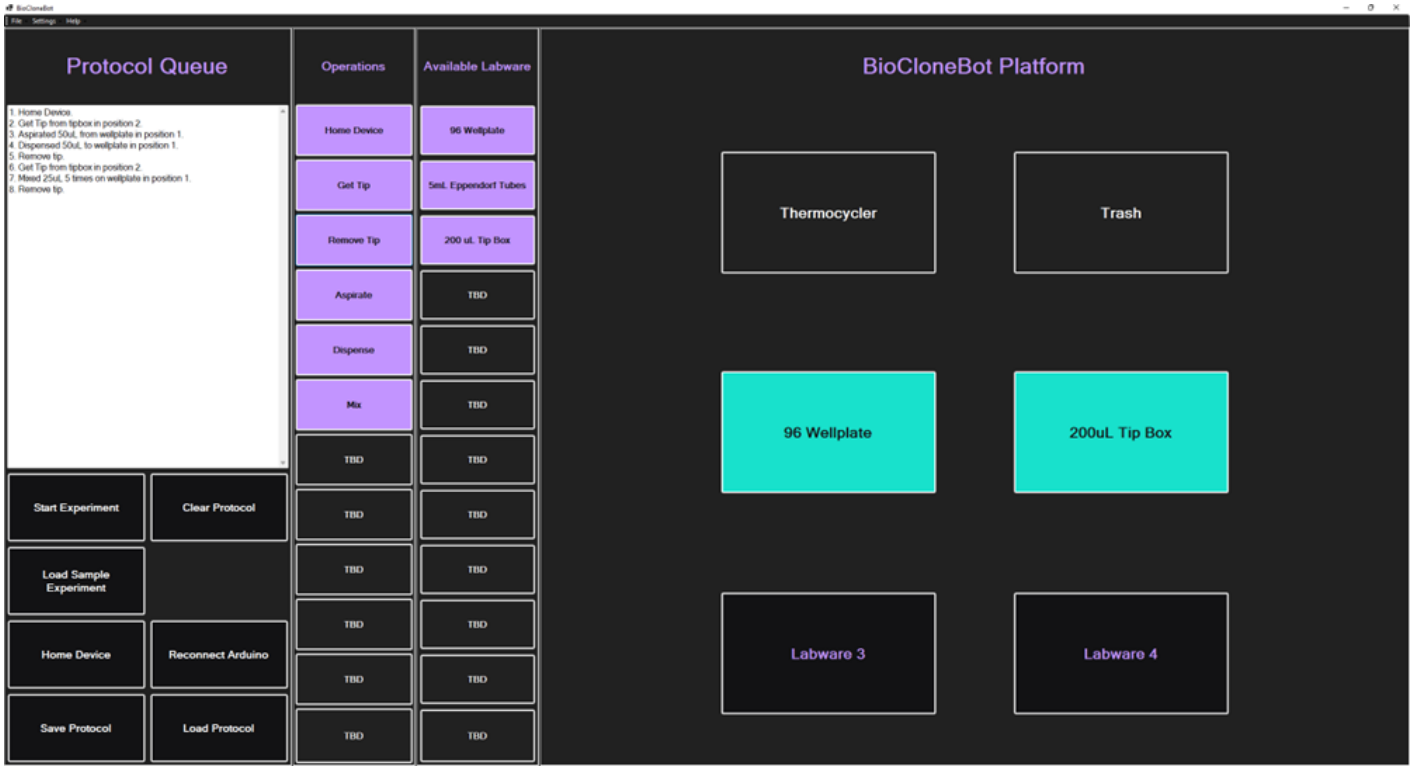

Figure 43: The protocol is loaded into the BioCloneBot software