Bravo Assisted Chromium Genome Assay Instructions

Version 1.1

Top View of Bravo Deck

This Document serves as working instructions for the use of the Bravo Automated liquid handling platform in the Chromium Genome Assay. Safety guidelines must be strictly followed when operating the Bravo to avoid serious injury and/or damage to the system.

The Work Instructions (WI) breaks up the Library Prep component of Chromium Genome Assay into a series of Bravo deck setups – Deck 1 through 13. Each deck setup consists of various labware that must be arranged on the deck by the user, and a Bravo Protocol associated with the deck setup. Labware is assigned deck positions, locations 1 through 9, on the bravo deck and reagents are assumed to be loaded from left to right unless otherwise instructed.

Reagents preparation and storage parameters are defined in this WI and should be followed precisely. Each reagent must be prepared by the user and loaded into labware as defined in the WI. Failure to follow labware placement instructions or reagent loading instructions may result in catastrophic failure and/or damage to the Bravo.

Safety Note: Keep hands and foreign objects away from the Bravo deck when a protocol is running (Figure 1). The bravo may cause serious injury. Use the red emergency stop (Figure 2), which is placed on the bench in front of the Bravo deck, to power down the Bravo in an emergency. Practice using the emergency stop before running protocols on the Bravo.

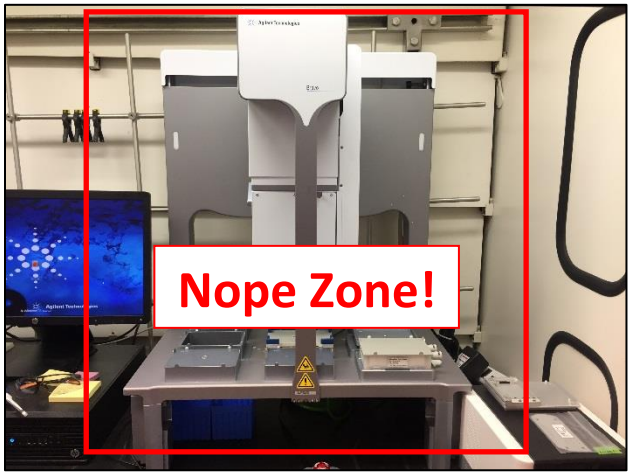


Figure 1: Danger area indicated by red.

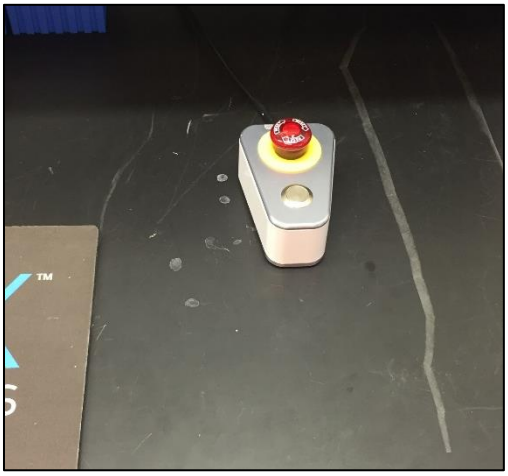
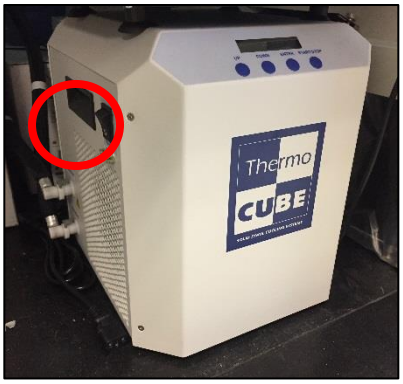
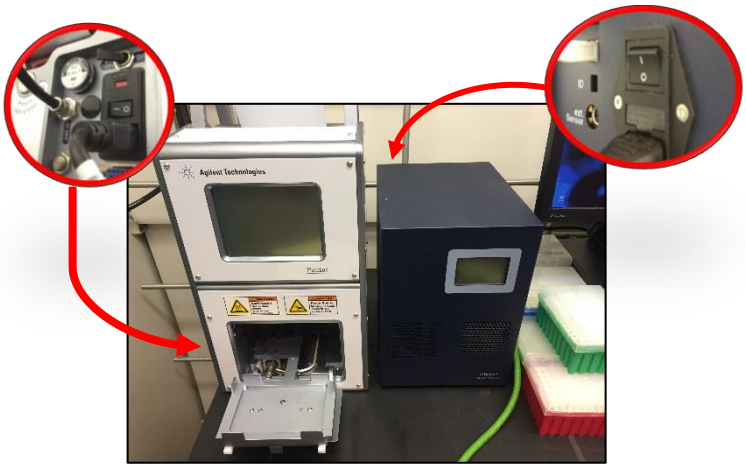


Figure 2: Emergency Stop

Bravo System Initialization:

Turn on microtiter chiller located below the bench, the Inheco CPAC Controller, the plate sealer, and the Bravo liquid handler.





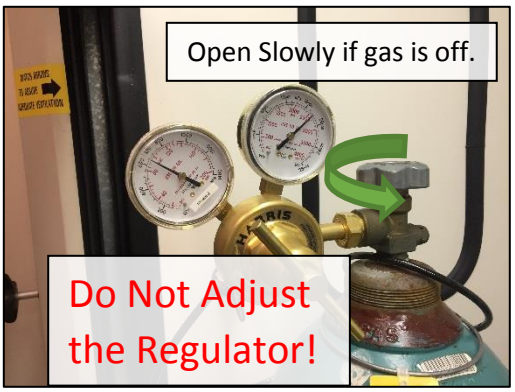


Figure 3, 4, 5, and 6 (Clockwise from top left): Power on devices and open gas reservoir.

Turn on the workstation and login with user name “10x” – there is no password. Double left click on the VWorks icon on the desktop. Login to VWorks with user name “a” – there is no password.

Figure 7: VWorks Desktop Icon

To begin the Bravo workflow, open the standard device file located at “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/”. Use the “10x Bravo” device Profile. Select initialize all devices in the device file. During initialization, follow the instructions as they are displayed in pop-up dialog boxes.

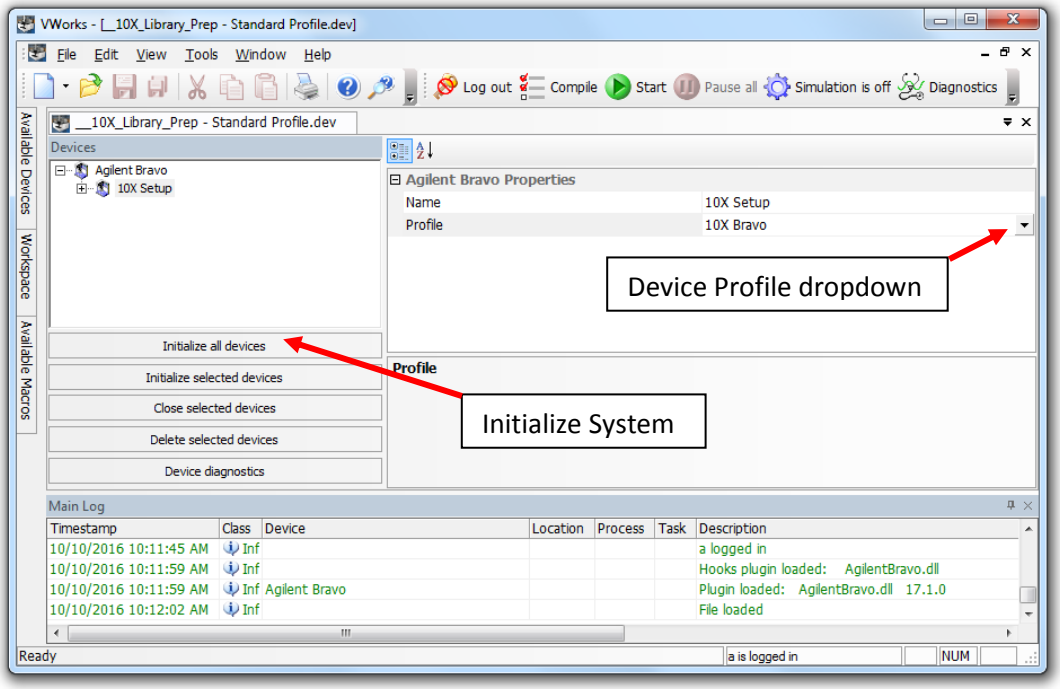


Figure 8: Check the Profile before initializing the device.

The “10x Bravo” profile will be used for deck set-ups 1 through 5. Deck set-ups 6 through 13 use the “10x Bravo – ALPAQUA Mag Block” profile. It is important to switch profiles before starting deck setup 6 to avoid crashing and possibly damaging the robot. It is also important to note that the Mag Block profile requires the ALPAQUA Mag Block to be placed at location 2 and a retaining block to be placed at location 8. These two locations will use this hardware for all protocols while the Mag Block profile is initialized.

To change profiles, go to the device tab and click on “Close selected devices”. The text in the dropdown menu on the right will change from grey to black. Use the drop down menu to select the “10x Bravo – ALPAQUA Mag Block” Profile. Initialize all devices and follow prompts in pop-up dialog boxes.

Reagents for Deck Setup 1 through 5:

Room Temp Reagents	Reagents on ice
Picogreen Quant TI Kit	Genome Enzyme Mix
Qiagen EB	
Genome Master Mix	
Additive A	
Denaturing Reagent	
Partitioning Oil V2	

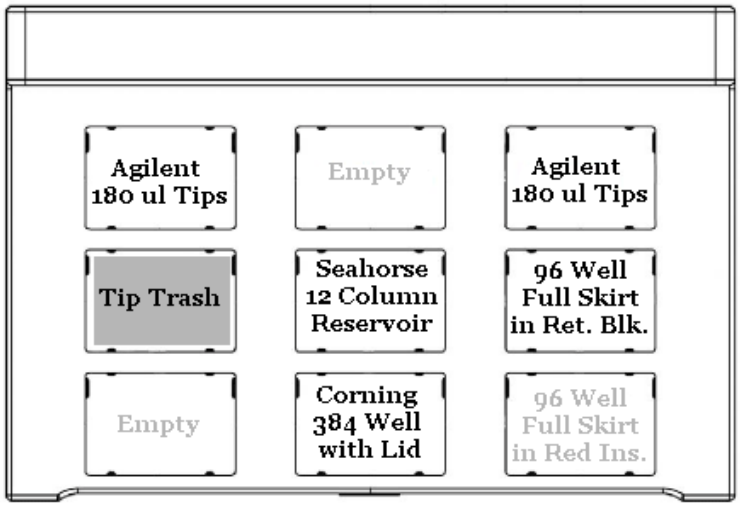
Table 1: Reagents for Bravo deck setup 1 through 5.

Bravo Deck 1 Setup:

Prepare Fresh Picogreen Reagent.

Reagent:	8 Samples	48 Samples
PicoGreen 20x Buffer	250 µl	750 µl
PicoGreen 200x Reagent	25 µl	75 µl
Nuclease Free Water	4.725 ml	14.175
Total	5 ml	15 ml

Table 2: Picogreen Reagent for quant.



1_Quant.pro Deck Setup

After reagents have acclimated to room temperature, Add Picogreen Quant-TI buffer to a new 15 ml Falcon tube. Add Picogreen reagent, then Add nuclease free water up to the final volume. Vortex thoroughly, then cover tube in foil or remove from light until use.

Add 2.1 mL of Picogreen reagent to each column of a Seahorse 12 Column Reservoir as needed. For example, one reservoir trough is filled for the standards and one reservoir trough is filled for each column of samples. Reservoir troughs must be filled in sequence such that there are no empty columns between Picogreen reservoirs.

Add Standards 1 through 8 to column 12 of an Eppendorf 96 well Twin.tec PCR plate. In the same PCR plate, add samples to columns 1 through 6. The dynamic range of the quant normalization method is 0.4 ng/µl to 20 ng/µl. Note that the sample volume needed varies depending on the sample concentration. If the sample concentration is greater than 2.5 ng/µl, only 20 µl is required. 40 µl is required if the sample is at 0.5 ng/µl. It is recommended that the user aliquot 40 µl of sample per well if the input concentration is unknown. If input sample concentrations are greater than 20 ng/µl it is required that the user dilute samples manually prior to the first quant step.

Figure 9: Samples and standards are loaded into a common Eppendorf 96 well Twin.tec PCR plate.

The loaded plate may be covered with pierceable foil.

Labware Locations:

- Place Agilent 180 µl tip racks at **locations 1 and 3**. Remove the tip lids before starting.
- Place a new 96 well Twin.tec PCR plate at **location 9** on a red aluminum insert **with a lid**.
- Place the 96 well PCR plate with standards and samples at **location 6** in a retaining block.
- Place the Seahorse 12 column reservoir with picogreen on the Bravo Deck at **location 5**.
- Place a 384 well clear bottom plate quant plate at **location 8 with a lid**.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “1_Quant.pro”

Double check the labware by inspecting the “configure” process in the startup protocol.

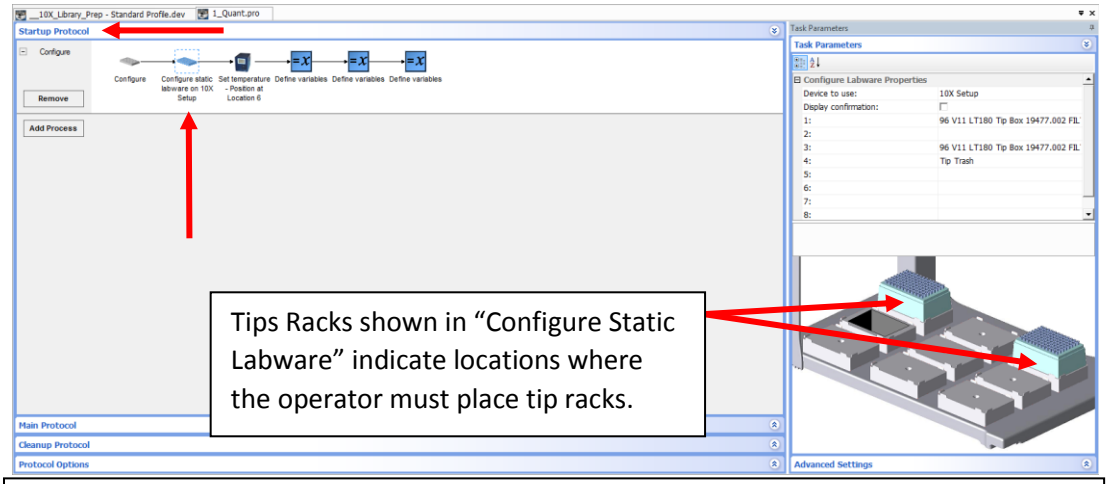


Figure 10: Static labware is defined in the Startup Protocol. Left click on the icon to view.

Non-static labware will be listed in the main protocol. Note the plate type and placement before starting the protocol.

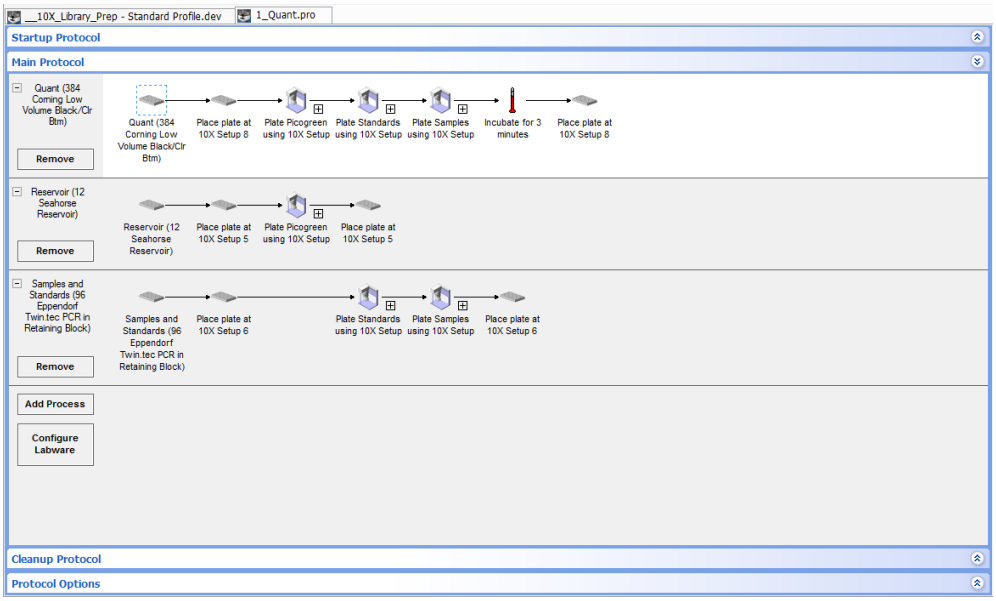


Figure 11: Non-static labware is defined in the Main Protocol. Left click on a process definition to view detail like lid status and plate type. Plate placement is defined by the “Place Plate” field.

Make sure the new tab “1_Quant.pro” is open before hitting start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

1

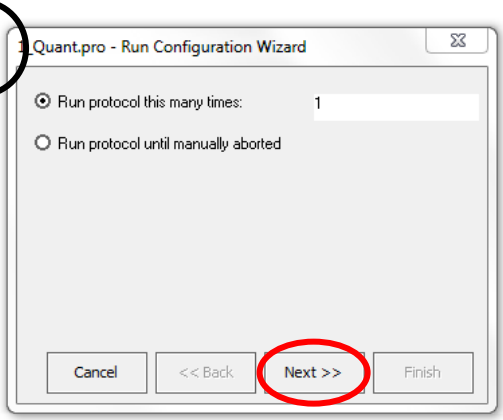


Figure 12: Dialog box 1 defined how many times the protocol should be run. This value will be “1” for all Chromium Genome Protocol.

2

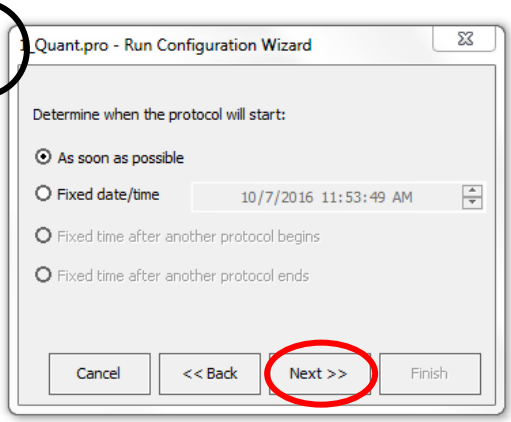


Figure 13: Dialog box 2 defines when the scheduler starts the protocol. The default option is correct for all Chromium Genome Protocols.

3a

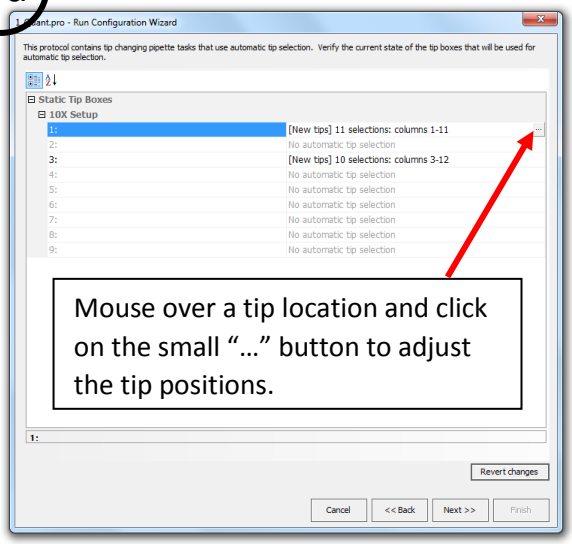


Figure 14: Pause at the tip state dialog box to confirm the information is correct. Incorrect tip states can cause catastrophic failure.

3b

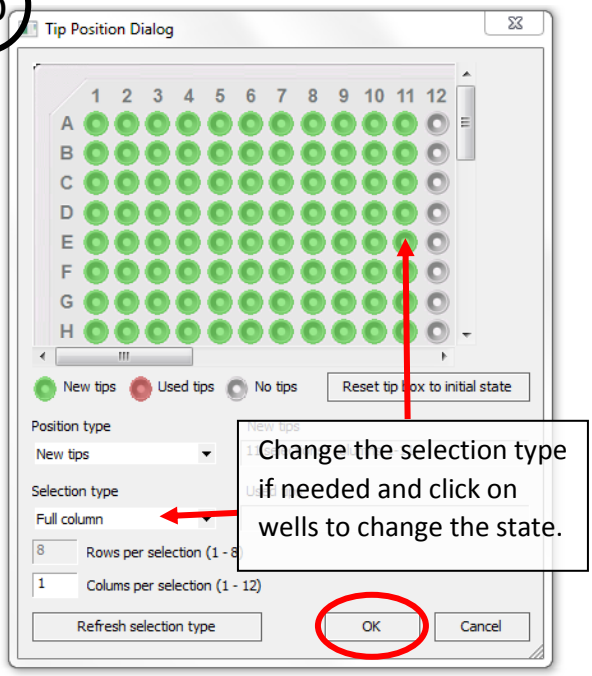


Figure 15: Tip states can be changed by left clicking on a position in the tip rack illustration. The selection type may be changed to correctly edit the tip states.

4

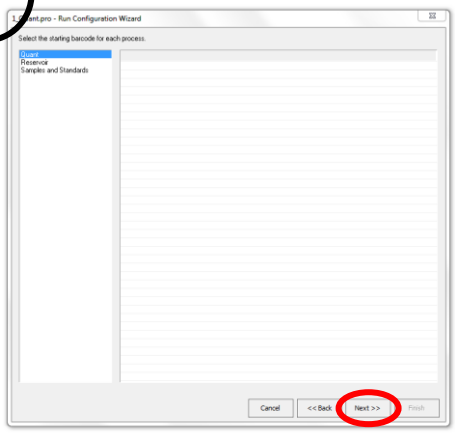


Figure 16: No user input is required in dialog box 4. Left-click on next to proceed.

5

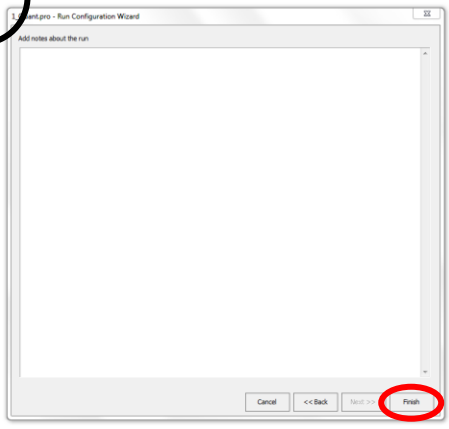


Figure 17: No user input is required in dialog box 5. Left-click on next to proceed.

When the protocol begins the user will be prompted for variable values. Input the appropriate value for the specific deck setup arranged by the user.

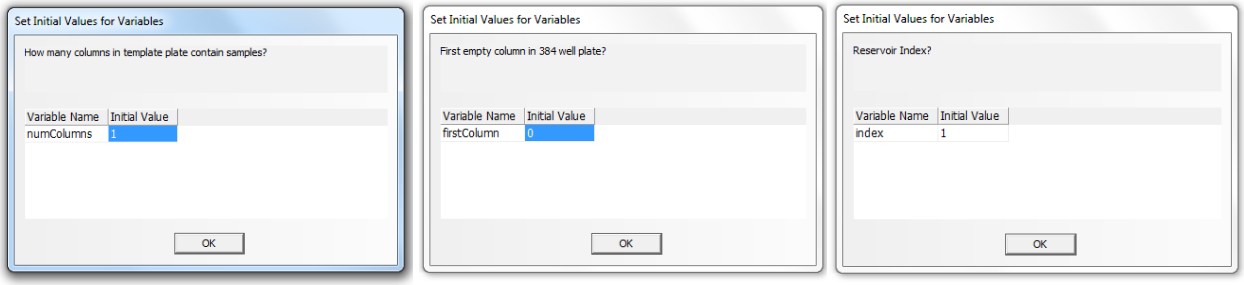


Figure 18: Variable input fields for user input.

Allow 10 minutes for the protocol to complete.

Remove the 384 well plate from position 8 after the Bravo concludes 1_Quant.pro and place the plate in the plate reader.

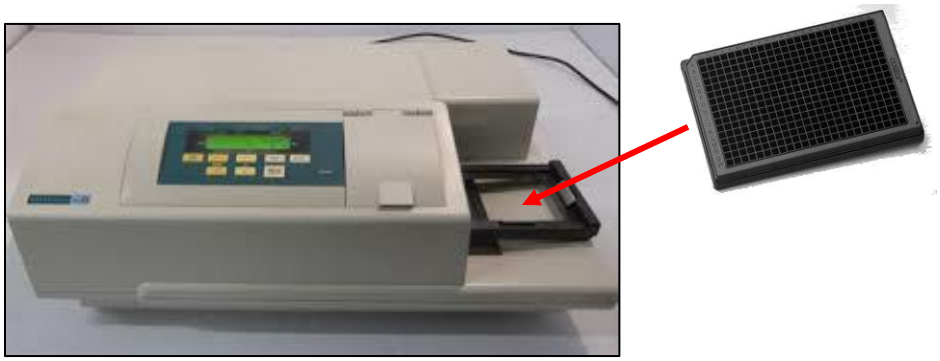


Figure 19: Plate reader showing drawer access

Make a reading by opening the “pg_standards” method in SoftMax Pro. The method file “pg_standards.sda” can be found on the workstation desktop if it is not in recent documents.

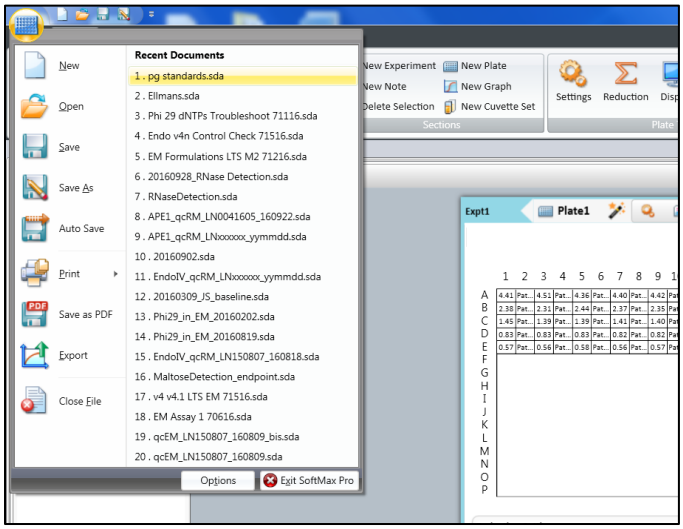


Figure 20: Open the “pg_standards.sda” method in recent documents.

Select the read area in the setup menu. Accept the changes and click on the start button.

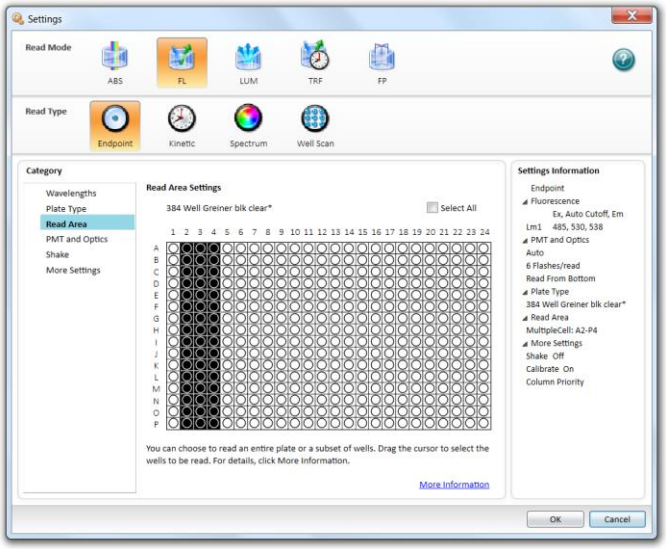


Figure 21: Click and drag to select the read area.

Export the data as a text file after the reading and save to a USB storage device.

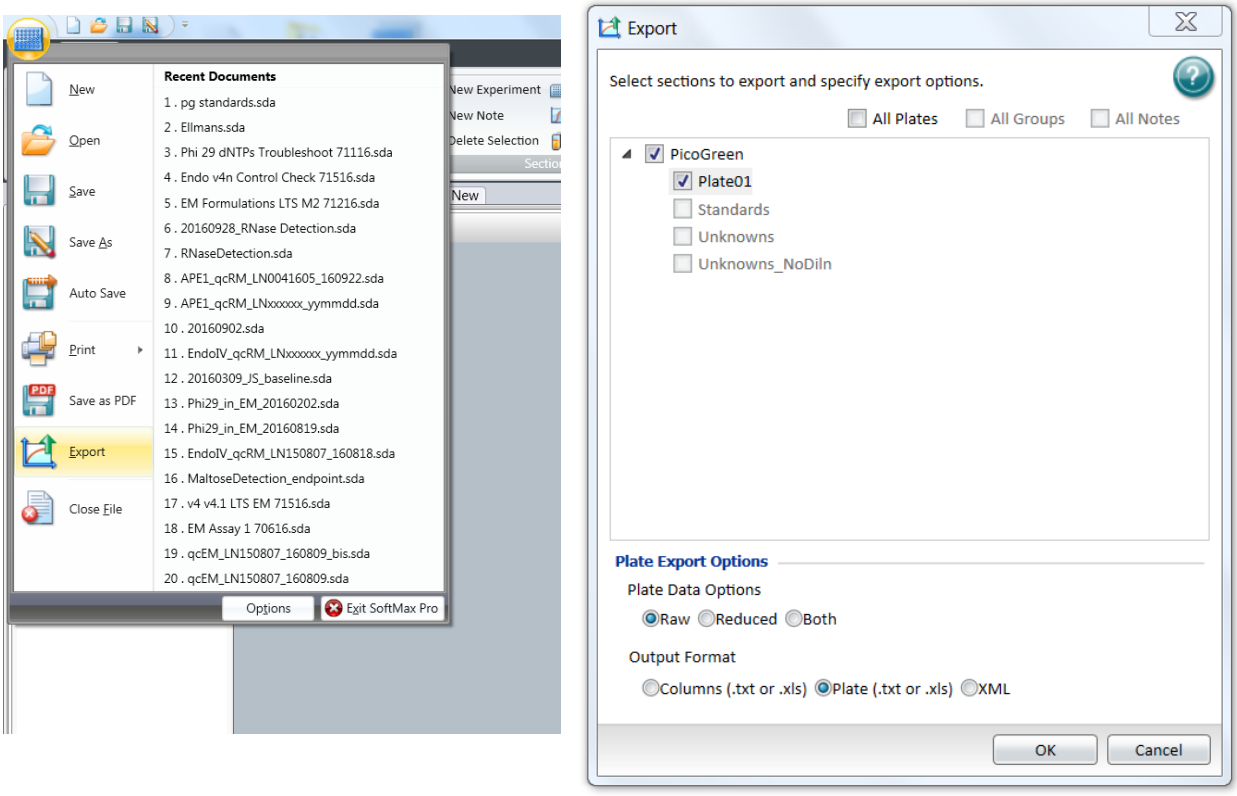
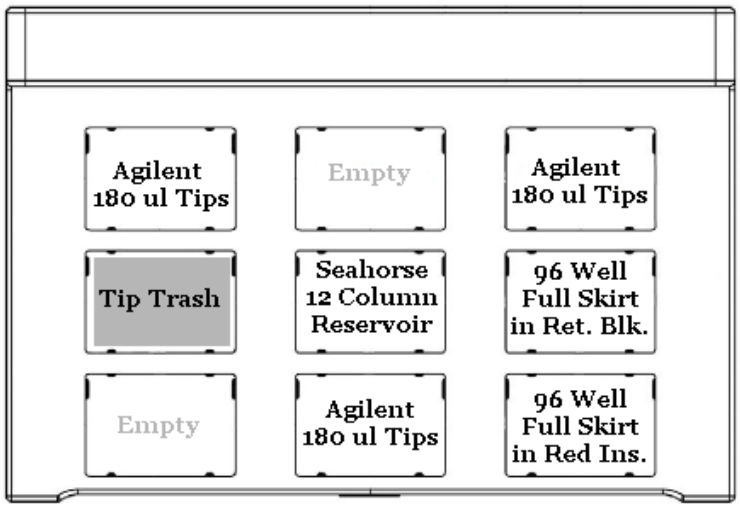


Figure 22: Export the plate data. Left: Export button in menu. Right: Export options.

Prepare an input file for normalization using Microsoft Excel or other validated software. The Normalization CSV file consists of a header followed by location and volume data. The volume is calculated based on the volume of diluent needed to adjust 5 µL of sample to 0.5 ng/µl.

Save the CSV file to the Bravo workstation and record the full file path for the next step.

Bravo Deck 2 Setup:



2_Normalize.pro Deck Setup

Double check to make sure the tip rack at **position 3** has at least 48 tips. Consolidate or exchange if necessary.

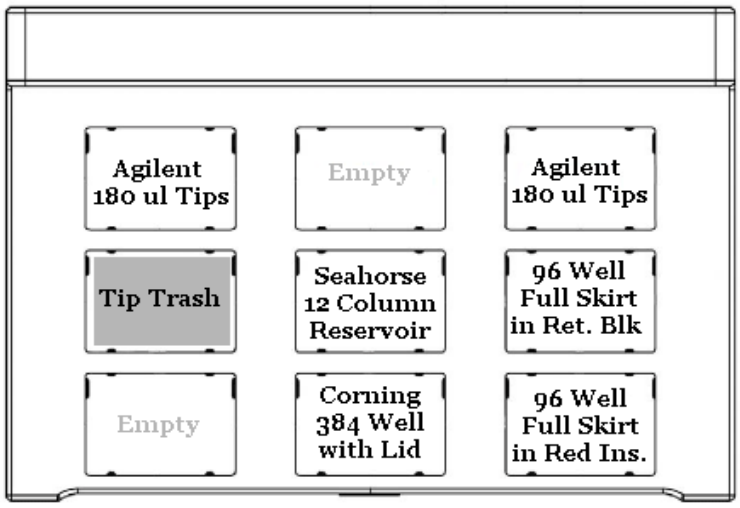
Place a rack of 180 µl tips at **position 8** with at least 49 tips.

Add Qiagen EB to a fresh trough in the Seahorse 12 column reservoir at **position 5**.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “2_Normalization.pro”

Start the normalization protocol and input the file path for the CSV file produced after the first quant step. Note that the volumes to be used are printed to the log after the normalization file is input by the user. Some values may be different than the input CSV due to the script correcting for low volume samples. Samples with less than 25 µl after normalization will be handled by the bravo to ensure the user has enough volume to move forward with the assay.

Bravo Deck 3 Setup:



3_Quant.pro Deck Setup

Prepare a fresh batch of picogreen reagent following Table 1.1 volumes.

Add 2.1 mL of Picogreen reagent to each fresh column of a Seahorse 12 Column Reservoir as needed. For example, one column is filled for the standards and one column is filled for each column of samples. Columns must be filled in sequence such that there are no empty columns between Picogreen reservoirs.

Place the same 384 well plate from Deck Setup 1 back on the deck at **position 8 with a lid**.

Move the normalization plate to **position 6** in the retaining block.

Move the Sample and Standards plate to **location 9** on the red aluminum insert.

Double check to ensure that tip racks at Locations 1 and 3 have enough tips to complete the run. Location 3 must be full and location 1 must have 9 columns for 48 samples.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “3_Quant.pro”

In the new tab “3_Quant.pro” hit start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

Remove the 384 well plate from position 8 after the Bravo concludes the 3_Quant.pro and follow the microplate reader instructions detailed in “1_Quant.pro.”

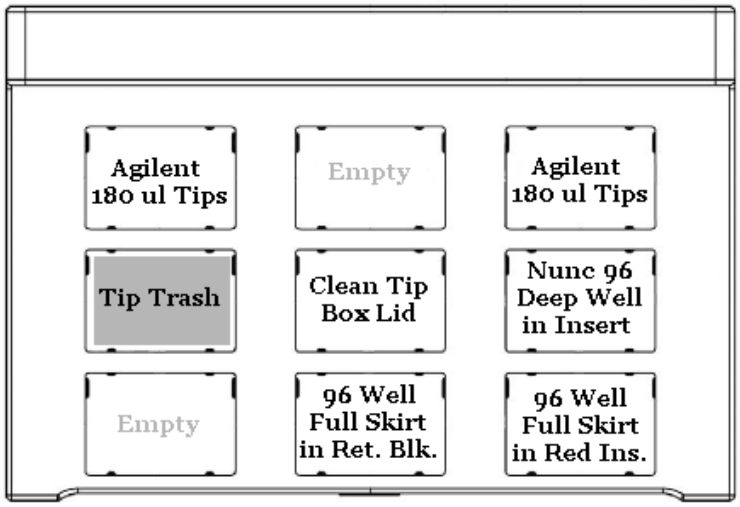
Double check that the normalization was effective by importing the saved text file to a preconfigured Excel workbook or other validated software.

Bravo Deck 4 Setup:

Place a Nunc 96 Deep Well plate in a silver insert at **position 9**. Allow this plate to acclimate to 4 °C.

Master Mix	8 Samples	48 Samples
Genome Master Mix	787.6	4725.6
Additive A	26.4	158.4
Genome Enzyme Mix	44	264
Total	858	5148

Table 3: Genome Master Mix



4_Make_Plate.pro Deck Setup

Thoroughly rinse a tip box lid with milli-Q water. Carefully dry the outside of the lid with a kimwipe, but do not dry the inside of the lid to avoid contamination with particles. Add 50 ml of denaturing reagent or prepare 0.2 M NaOH and filter through a 0.2 µm Polystyrene filter.

Place tip box lid with denaturing reagent at **position 5**. The volume should be sufficient to completely cover the bottom of the lid.

Aliquot equal amounts of Master Mix to a fresh column of the Nunc Deep Well plate at **position 6**.

Place Normalized plate from Deck 3 at **position 8** in a retaining block.

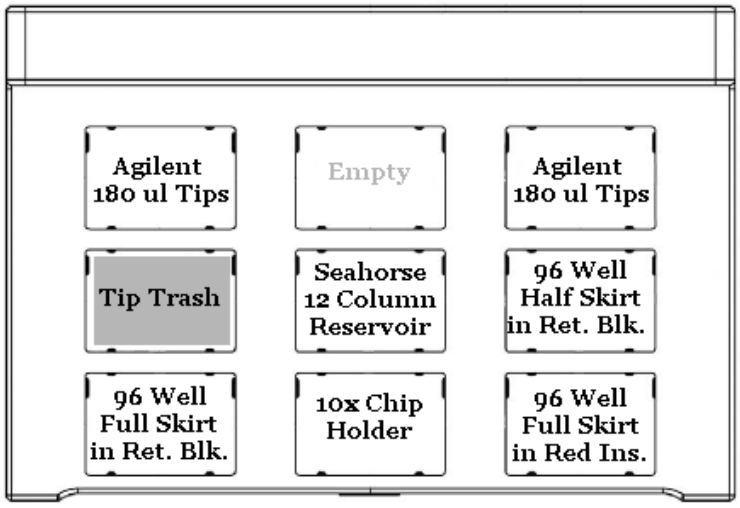
Place a fresh Eppendorf Twin.tec PCR plate at **position 9** in a red aluminum insert **with a lid**.

Ensure that the tip racks at position 1 has at least 48 tips and location 3 has at least 56 tips.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “4_Make_Plate.pro”

Start protocol and follow popup dialog prompts.

Bravo Deck 5 Setup:



5_Chromium_Controller.pro Deck Setup

Place 10x Beads at **location 7 in a retaining block**.

Place a 10x Genome V2 chip in a 10x Chip Holder, then use the adapter to place the chip at **location 8**.

Place Sample plate at **location 9 in a red aluminum insert**.

Place a Seahorse 12 Column reservoir at **position 5** and add 1 tube of partitioning oil to a fresh trough. Each additional sample requires a new aliquot of partitioning oil. The same trough may be reused.

Place a fresh Eppendorf Twin.tec Half Skirt plate at **position 6 in a red aluminum insert with a plate lid**.

Ensure tip racks at positions 1 and 3 are full.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “5_CrG_Chip.pro”

In the new tab “5_CrG_Chip.pro” hit start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

Note that the protocol prompts the user for index information before loading the chip and before transferring GEMs to the PCR plate. The user may skip either portion of the protocol by inputting index 0 when prompted. This pause for data input is also used to delay the bravo while the chip is removed from the deck and placed in a 10x Chromium Controller.

When prompted for an output plate index, leave the input as “0” until further instructed. Remove the 10x Chip holder at position 8 and attach a fresh gasket. Run the chip in a Chromium Controller.

After the Chromium controller finishes, Remove the chip assembly and discard the gasket. Place the Chip holder with the chip on the adapter at location 8.

Input the appropriate output plate index after the deck setup is restored, and the deck is clear of any foreign objects.

Repeat this protocol as many times a needed to load all samples into the output plate.

Use the Agilent plate sealer to cover the output plate.

Incubate the GEMs in a thermalcycler for 3 hours:

Step	Temperature	Time
1	30 °C	3 hours
2	65 °C	10 min
3	4 °C	∞

Table 4: incubation conditions for Landlord protocol.

Bravo device profile change:

The Bravo Device Profile must be changed before starting protocol 6, “6_GEM_Break.pro”. Open the current device file, then close selected device. Change the profile to “10x Bravo – ALPAQUA Mag Block” in the devices profile drop down menu. Select initialize all devices, then follow the popup prompts.

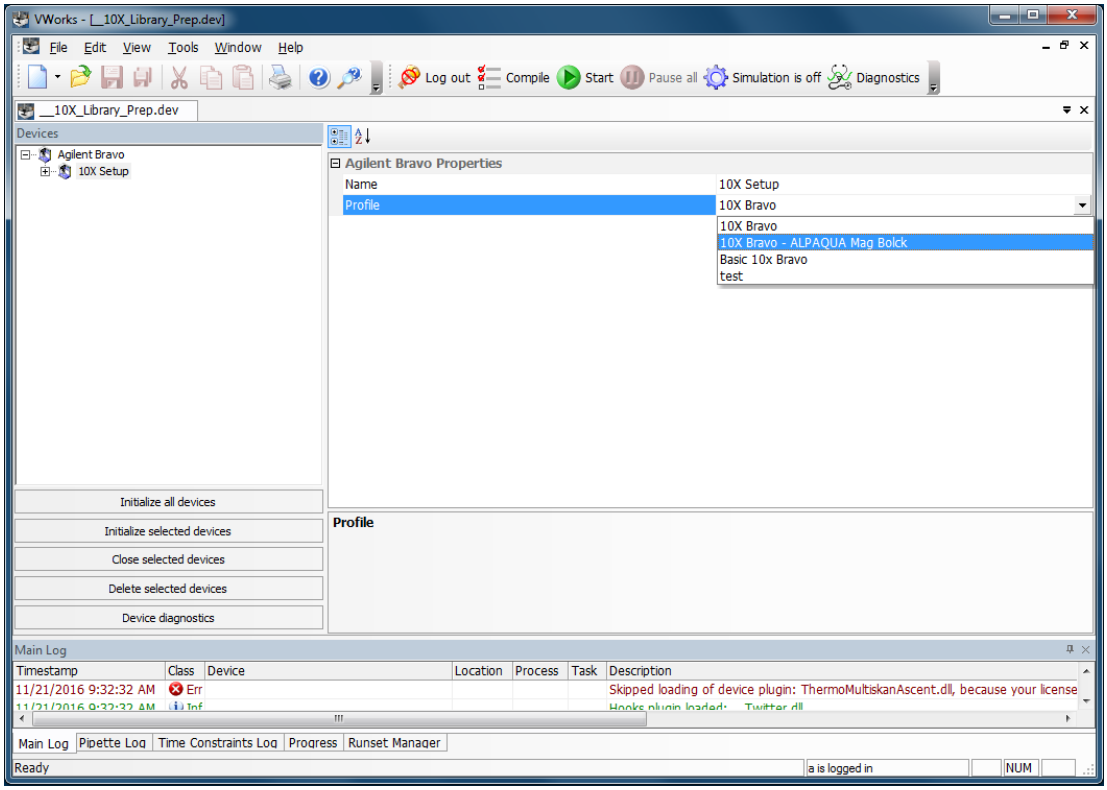


Figure 23: The modified device profile must be selected before protocol 6 is run

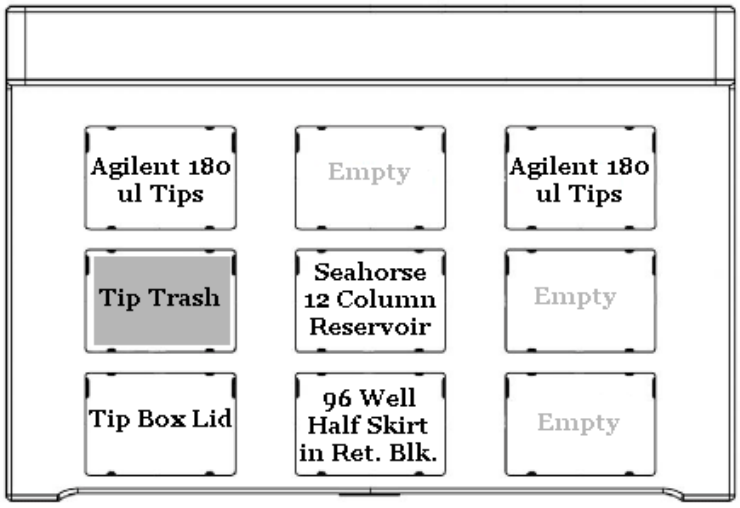
Note that the ALPAQUA Mag Block is always placed at location 2 and a retaining block base is always at location 8 when using the ALPAQUA Mag Block profile.

Reagents for deck setup 6 through 8:

Room Temperature Reagents	Heat to 65°C for 10 minutes, then Room Temp.
Recovery Reagent	Buffer for Sample Cleanup 1
DynaBead MyOne SILANE Beads	
SPRIselect Reagent	
Additive A	
Ethanol	
Nuclease Free Water	

Table 5: Reagents for GEM Cleanup

Bravo Deck 6 Setup:



6_GEM_Break.pro Deck Setup

Place a pipette box lid at **location 7** to collect Oil Waste.

Remove the GEMs from the thermalcycler and place the PCR plate in the retaining block at **location 8**. Note that the labware listed is in a red aluminum insert however, the retaining block is correct.

Place a Seahorse 12 Column Reservoir at **location 5**. Add 1.4 ml to consecutive troughs for each column of GEMs.

Confirm that location 1 has at least 48 tips.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “6_Break_GEMs.pro”

In the new tab “6_Break_GEMs.pro” hit start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

Dispose of waste oil in the appropriate waste receptacle After the protocol has concluded.

Bravo Deck 7 Setup:

DynaBead Cleanup Mix	8 Samples	48 Samples
Buffer for Sample Cleanup 1	1197 µl	7182 µl
DynaBeads MyOne SILANE	70.4 µl	422.4 µl
Additive A	52.8 µl	316.8 µl
Total	1320.2 µl	7921.2 µl

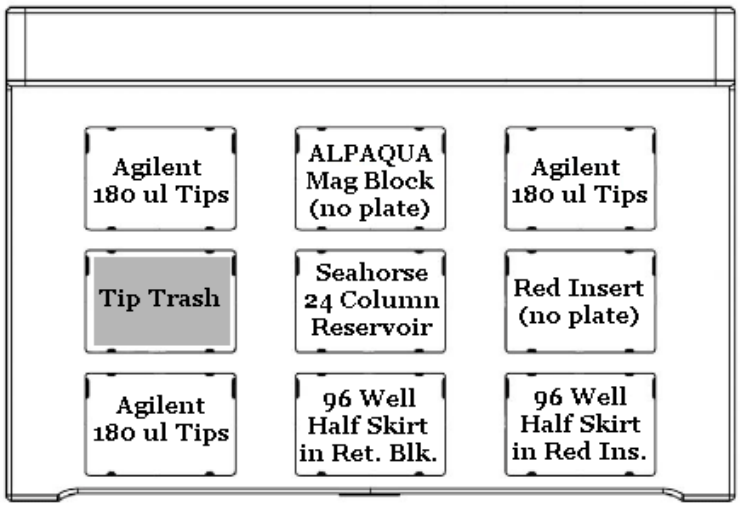
Table 6: DynaBead Mix for Cleanup 1

Elution Solution 1	8 Samples	48 Samples
10 mM Tris-Cl, pH 8.5	890 µl	5340 µl
10% Tween-20	10 µl	80 µl
Additive A	100 µl	600 µl
Total	1000 µl	6000 µl

Table 7: Elution Solution 1

80% Ethanol	8 Samples	48 Samples
Ethanol	3 ml	16 ml
Nuclease Free Water	750 µl	4 ml
Total	3.75 ml	20 ml

Table 8: Ethanol for Cleanup 1



7_DynaBead_Cleanup.pro Deck Setup

Place Full pipette tip boxes at **locations 1, 3, and 7**.

Place a red aluminum insert at **location 6** with no microplate.

Place the Sample plate from deck layout 6 at **location 8** on a retaining block without the retaining lid.

Place a fresh Eppendorf 96 well Half Skirt Twin.tec PCR plate at **location 9** on a red aluminum insert.

Place a Seahorse 24 column reservoir at **location 5**.

a. Fill every other trough, up through trough 11, with 1.3 ml of Bead Cleanup Buffer 1.
b. Fill every other trough, up through trough 12, with 1.0 ml of Elution Buffer 1.
c. Fill every other trough, from 13 on, with 3.3 ml of 80% EtOH.
d.

The reagent reservoir can be setup per column and reused by setting up reagents in fresh troughs at a different index. The spacing between reagents, the number of troughs between the elution buffer and the EtOH, must remain unchanged.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “7_DynaBead_Cleanup.pro”

In the new tab “7_ DynaBead_Cleanup.pro” hit start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

Dispose of waste in the appropriate waste receptacle After the protocol has concluded.

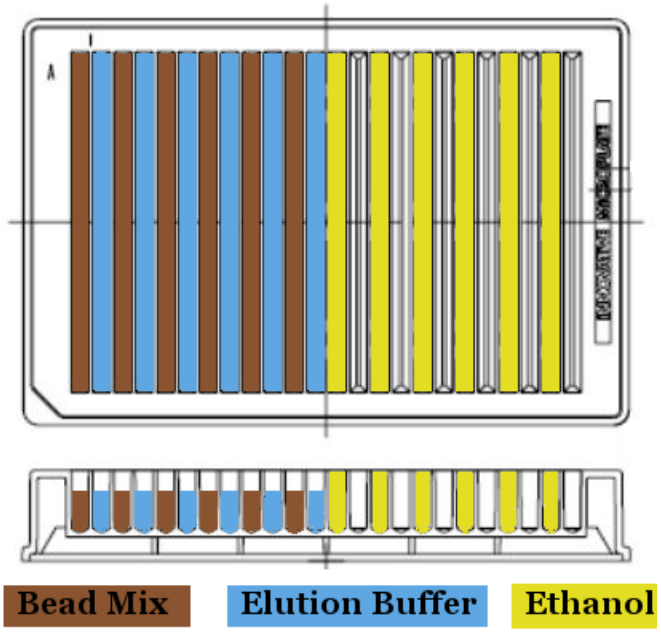


Figure 24: An illustration of the Seahorse 24 Column Reservoir at Location 5 showing reagent loading locations.

Bravo Deck 8 Setup:

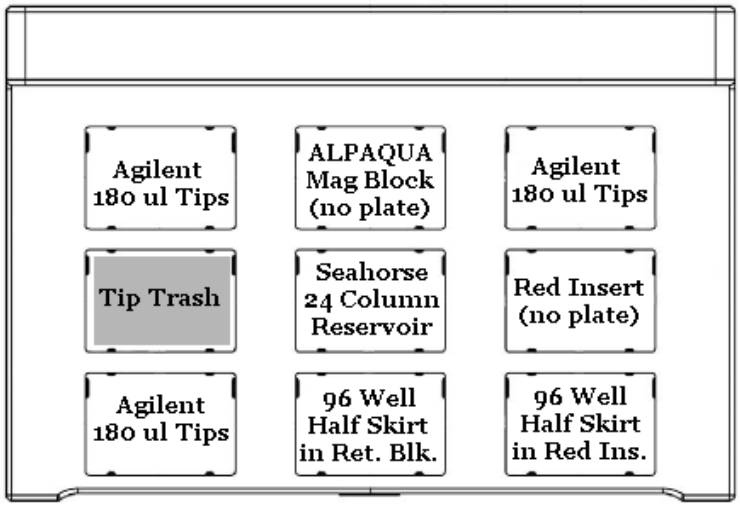
Prepare the following reagents and bring a tube of SPRIselect reagent up to room temperature.

Elution Solution 2	8 Samples	48 Samples
10 mM Tris-Cl, pH 8.5	980 µl	5880 µl
Additive A	20 µl	120 µl
Total	1000 µl	6000 µl

Table 9: Elution Solution 2

80% Ethanol	8 Samples	48 Samples
Ethanol	3 ml	16 ml
Nuclease Free Water	750 µl	4 ml
Total	3.75 ml	20 ml

Table 10: Ethanol for Cleanup 2



8_SPRIselect_Cleanup.pro Deck Setup

Place Full pipette tip boxes at **locations 1, 3, and 7**.

Place a red aluminum insert at **location 6** with no microplate.

Place the Output plate from deck layout 7 at **location 8** on a retaining block without the retaining lid.

Place a fresh Eppendorf 96 well Half Skirt Twin.tec PCR plate at **location 9** on a red aluminum insert.

Place a Seahorse 24 column reservoir at **location 5**.

a. Fill every other trough, up through trough 11, with 1.0 ml of SPRIselect Reagent.
b. Fill every other trough, up through trough 12, with 1.0 ml of Elution Buffer 2.
c. Fill every other trough, from 13 on, with 3.3 ml of 80% EtOH.

*Refer to Figure 8 for further clarification.

Note that the reagent reservoir can be setup per column and reused if running 24 or fewer samples. Input the new index of the SPRIselect when prompted during the protocol.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “8_SPRIselect_Cleanup.pro”

In the new tab “8_ SPRIselect_Cleanup.pro” hit start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

Dispose of waste in the appropriate waste receptacle After the protocol has concluded.

Run Bioanalyzer QC 1 at this point. Samples may be stored for up to 72 hours at 4 °C.

Reagents for deck setup 9 through 13:

Room Temperature Reagents	Reagents on Ice
End Repair and A-Tailing Buffer	End Repair and A-Tailing Enzyme
Ligation Buffer	DNA Ligase
Adapter Mix	PCR Master Mix
Chromium Genome Sample Index Plate	
Forward PCR Primer	
SPRIselect Reagent	

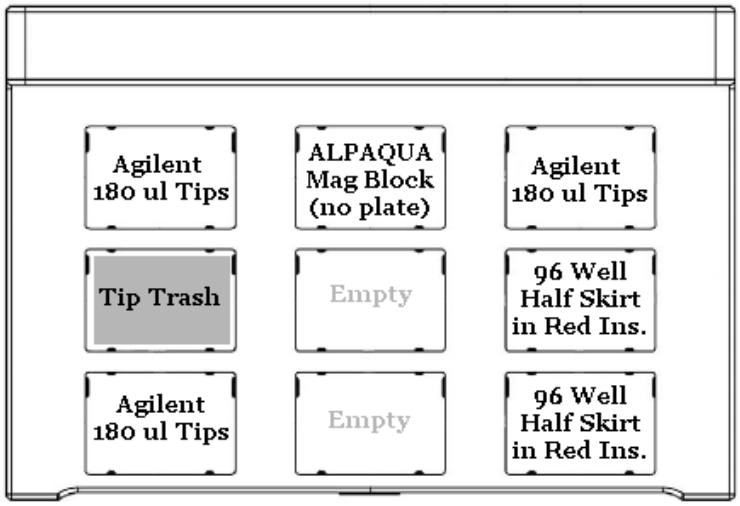
Table 11: Reagents for Library Construction

Bravo Deck Setup 9:

Pre-chill an Eppendorf 96 Well Twin.tec Half Skirt PCR Plate at location 9 on the bravo deck. The auxiliary CPAC can also be used as a pre-chilling location if manually set to 4 °C.

End Repair and A-Tailing Mix	8 Samples	48 Samples
Nuclease Free Water	22 µl	132 µl
End Repair and A-Tailing Buffer	66 µl	396 µl
End Repair and A-Tailing Enzyme	132 µl	792 µl
Total	220 µl	1320 µl

Table 12: End Repair and A-Tailing Master Mix



9_End_Repair_and_A-Tailing.pro

Assemble the End Repair and A-Tailing Master Mix. Evenly aliquot the master mix into one column of the pre-cooled PCR plate.

Place samples from deck 8 at **location 6** in a red aluminum insert.

Place the pre-chilled PCR plate with enzyme at **location 9** on a red aluminum insert.

Ensure that locations 1 and 3 have at least 48 tips.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “9_End_Repair_and_A-Tailing.pro”. In the new tab “9_ End_Repair_and_A-Tailing.pro” hit start. Follow the prompts in the pop-up dialog boxes.

Incubate samples, currently 75 µl, for 1 hour under conditions in table 13.

Step	Temperature	Time
1	20 °C	30 min
2	65 °C	30 min
3	4 °C	∞

Table 13: Incubation Conditions for End Repair and A-Tailing.

Note that the pre-chilled PCR plate at location 9 can be reused in deck setup 10.

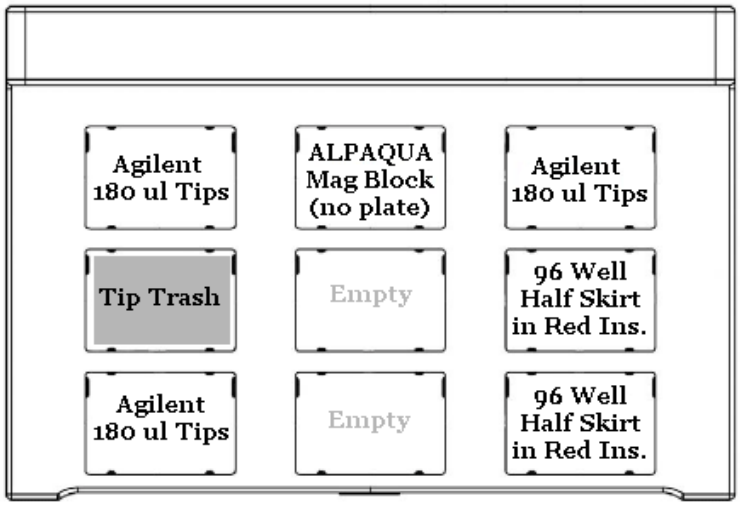
Bravo Deck Setup 10:

Pre-chill an Eppendorf 96 Well Twin.tec Half Skirt PCR Plate at location 9 on the bravo deck.

Assemble Ligation Reaction Mix and store at 4 °C.

Adapter Ligation Mix	8 Samples	48 Samples
Ligation Buffer	194 µl	1164 µl
DNA Ligase	97 µl	582 µl
Adaptor Mix	22 µl	132 µl
Total	313	1878 µl

Table 14: Ligation Reaction Mix



10_Adapter_Ligation.pro

Dispense equal volumes of Ligation Reaction Mix into one column of wells in the pre-chilled PCR plate.

Place the PCR plate from the thermalcycler at **location 6** in a retaining block with the retainer cover.

Place the pre-chilled PCR plate with Ligation Mix at **location 9**.

Ensure that locations 1 and 3 have at least 48 tips.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “10_Adapter_Ligation.pro”

In the new tab “10_ Adapter_Ligation.pro” hit start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

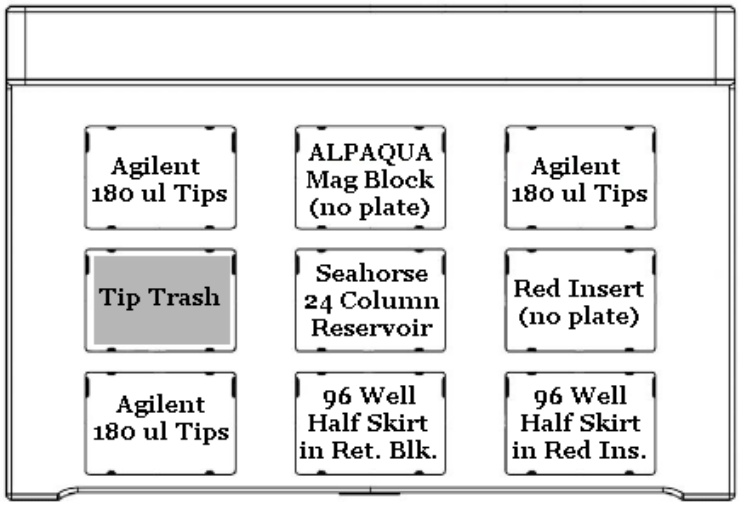
Note that the ligation protocol runs for approximately 30 minutes. The Post Ligation SPRIselect step should be setup and run immediately after ligation.

Bravo Deck Setup 11:

Prepare 80% Ethanol and bring a tube of SPRIselect reagent up to room temperature.

80% Ethanol	8 Samples	48 Samples
Ethanol	3 ml	16 ml
Nuclease Free Water	750 µl	4 ml
Total	3.75 ml	20 ml

Table 15: Ethanol for Post Ligation SPRIselect.



11_Post_Ligation_SPRIselect.pro

Place Full pipette tip boxes at **locations 1, 3, and 7**.

Place a red aluminum insert at **location 6** with no microplate.

Place the Output plate from deck layout 10 at **location 8** on a retaining block without the retaining lid.

Place a fresh Eppendorf 96 well Half Skirt Twin.tec PCR plate at **location 9** on a red aluminum insert.

Place a Seahorse 24 column reservoir at **location 5**.

a. Fill every other trough, up through trough 11, with 1.0 ml of SPRIselect Reagent.
b. Fill every other trough, up through trough 12, with 1.0 ml of 10 mM Tris-Cl, pH 8.5.
c. Fill every other trough, from 13 on, with 3.3 ml of 80% EtOH.
   *Refer to Figure 8 for further clarification.

Note that the reagent reservoir can be setup per column and reused if running 24 or fewer samples. Input the new index of the SPRIselect when prompted during the protocol.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “11_Post_Ligation_SPRIselect.pro”

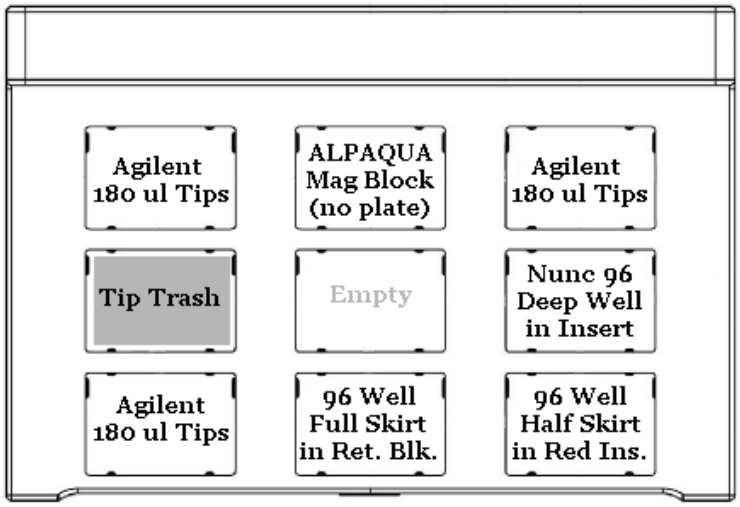
In the new tab “11_Post_Ligation_SPRIselect.pro” hit start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

Bravo Deck Setup 12:

Pre-chill a Nunc 96 Deep Well Plate in a silver insert. Location 9 or the auxiliary CPAC can be used. Prepare Sample Index PCR Mix and store at 4 °C.

Sample Index PCR Mix	8 Samples	48 Samples
Library Amplification Mix	440 µl	2640 µl
Forward PCR Primer	44 µl	264 µl
Total	484 µl	2904 µl

Table 16: Sample Index PCR Mix



12_Sample_Index_PCR.pro

Aliquot equal amounts of PCR Master Mix into one column of the pre-chilled Nunc Deep Well plate. Then, Place the Nunc Deep Well plate at **location 9**.

Place the output plate from Deck Setup 11 at **location 6** on a red aluminum insert.

Place a Chromium Genome Sample Index plate at **location 8** in a retaining block with retaining cover.

Ensure that **locations 1 and 3** have at least 48 tips.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “12_Sample_Index_PCR.pro”. In the new tab “12_Sample_Index_PCR.pro” hit start.

Run the thernalcycler protocol below in Table 17 after the Bravo run concludes.

Stopping Point: Store samples at 4 °C for up to 72 hours or proceed directly to SPRIselect cleanup.

Step	Temperature	Time
1	98 °C	45 sec
2	98 °C	20 sec
3	54 °C	30 sec
4	72 °C	20 sec
5	Go to Step 2, 9x	
6	72 °C	1 min
7	4 °C	∞

Table 17: PCR Conditions for Sample Index PCR

Bravo Deck Setup 13:

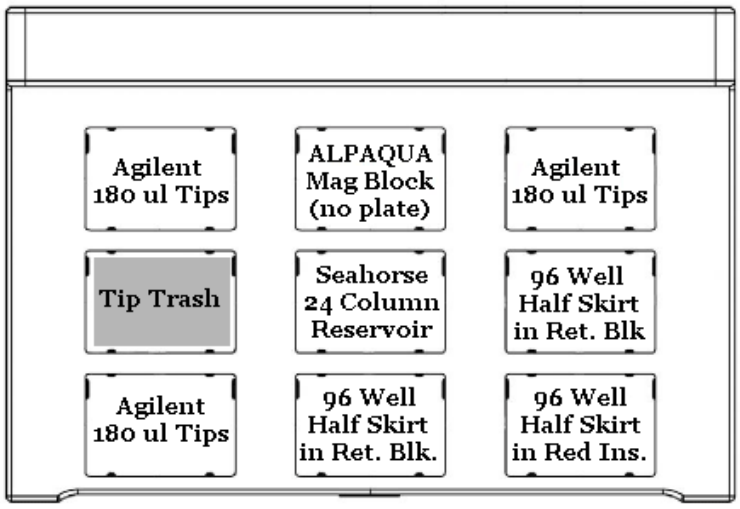
Prepare the following reagents and bring a tube of SPRIselect reagent up to room temperature.

Elution Solution 2	8 Samples	48 Samples
10 mM Tris-Cl, pH 8.5	980 µl	5880 µl
Additive A	20 µl	120 µl
Total	1000 µl	6000 µl

Table 9: Elution Solution 2

80% Ethanol	8 Samples	48 Samples
Ethanol	3 ml	16 ml
Nuclease Free Water	750 µl	4 ml
Total	3.75 ml	20 ml

Table 10: Ethanol for Cleanup 2



13_Post_SI-PCR_SPRIselect.pro

Place Full pipette tip boxes at **locations 1, 3, 6 and 7**.

Place the Output plate from deck layout 12 at **location 6** in a retaining block **with the retaining lid.**

Place a fresh Eppendorf 96 well Half Skirt Twin.tec PCR plate at **location 8** on a retaining block **without the retaining lid.**

Place a fresh Eppendorf 96 well Half Skirt Twin.tec PCR plate at **location 9** on a red aluminum insert.

Place a Seahorse 24 column reservoir at **location 5**.

a. Fill every other trough, up through trough 11, with 1.0 ml of SPRIselect Reagent.
b. Fill every other trough, up through trough 12, with 1.0 ml of 10 mM Tris-Cl, pH 8.5.
c. Fill every other trough, from 13 on, with 3.3 ml of 80% EtOH.
   * Refer to Figure 8 for further clarification.

Note that the reagent reservoir can be setup per column and reused if running 24 or fewer samples. Input the new index of the SPRIselect when prompted during the protocol.

Navigate to “C://VWorks Workspace/Protocol Files/Genome Library Prep SR_v005/” and select “13_Post_SI-PCR_SPRIselect_Cleanup.pro”

In the new tab “13_Post_SI-PCR_SPRIselect_Cleanup.pro” hit start. Follow the prompts in the pop-up dialog boxes. Make sure the pipette tip states are accurate to avoid malfunctions during the run.

Dispose of waste in the appropriate waste receptacle After the protocol has concluded.