# Vantage Kapa DNA Library Creation

Version Number: V1.6
Production Start Date: 2/22/24
Version 1.0 Date: 02/01/22

## Summary

The purpose of this procedure is to generate Illumina gDNA libraries using the Hamilton Vantage robot and the Kapa Hyper Prep Kit method. This method can process from 1 to 4, 96 well, plates at a time. This is a 1-2 day process, with the hyper prep program being broken into three separate sub processes for convenience. Step1 involves shearing samples with a Covaris sonicator, followed by double SPRI size selection. In STEP 2 the samples are processed through library creation (end repair, a-tailing, and 30 minute adapter ligation), followed by a cleanup step. Lastly for STEP 3 an additional Single SPRI adapter clean up may be done.

## Materials & Reagents

| **Materials/Reagents/Equipment** | **Vendor** | **Stock Number** |
|---|---|---|
| ***Consumables*** | | |
| Thermo Matrix 4309 Clear 384 Well V-Bottom | Thermo Fisher | 50823638 |
| E&K Seahorse Reagent Reservoir 290ml #201250-100 | ISC Bioexpress | Ek-2033 |
| StorPlate-96V, PP, 96 well, V-bottom, (V), 450?L | Perkin Elmer | 6008299 |
| BioRad-Plates, 96 Well, Hard-Shell Thin-Wall, Skirted PCR, Blue Shell, Clear Well | Bio Rad | HSP-9631 |
| E+K-Seahorse LID UNIVERSAL, CLEAR, PS | E & K Seahorse | EK-2079 (mfg 200856-100 ) |
| 96 microTUBE-50 AFA Fiber Plate | Covaris | 520168 |
| 384 Well u-bottom microplate | Perkin Elmer | 6008890 |
| 50ul Conductive Filter Tips | Hamilton | 235948 |

| | | |
|---|---|---|
| 150ul Piercing Tips, Filtered, Sterile | Hamilton | 235649 |
| Seahorse, 96well, 2ml, square well, Agilent #201240-100 | Agilent Tech | EK2076 |
| **Reagents** | | |
| Kapa Hyper Prep Kit | Kapa | KK8505 |
| Ethanol | Pharmco Aaper | 111000200CSPP |
| Mag-Bind® TotalPure NGS, Omega Bio-Tek beads | VWR | 75877-718 |
| HS NGS Fragment Analyzer kit (1- 6000bp) | AATI | DNF-474-1000 |
| Adapter plate sets 1-8, at 5uM | Perkin Elmer | |
| Nuclease Free Water | Growcells | NUPW -1000 |
| 1X Low EDTA-TE pH 8.0 | Quality Biologicals | 10128-588 |
| ***Equipment*** | | |
| Hamilton Vantage | Hamilton | |
| Microcentrifuge | VWR | - |
| Plate Centrifuge | Eppendorf | - |
| Fragment Analyzer | Agilent | - |
| Covaris LE220 | Covaris | - |
| Pipettes | Rainin LTS pipettes, manual and electronic | - |

## EH&S

JGI employees performing this procedure must wear a lab coat, gloves and safety glasses.

## Procedure

***NOTE***: All reagents/stock solutions should be defrosted on ice prior to the start of the procedure.

***NOTE*:** Take care to label <u>all</u> sample aliquot and Covaris plates when processing multiple plates together. Record which sample aliquot plate is number 1, 2, 3, 4.

1. **<u>Sonicator and DNA Sample Setup for 2X SPRI Size Selection</u>**

1.1. Obtain gDNA sample aliquots from Sample Management freezer.

a. Plates to be processed are listed in the Plate Availability/Schedule FY(current) GoogleDoc under the Scheduled tab. Choose the oldest plates first.

b. Highlight chosen plates to indicate in progress, and enter your initials in the library creator column. Note adapter plate choice if indicated.

c. Later fill in the reagent lot data for each plate in the gDNA tab of the gDoc

1.2. Defrost gDNA plates on ice. Each well should contain 200ng gDNA in 60ul. *Note: if volume is less than 60ul the well must be topped off to 60ul for successful shearing.*

1.3. Covaris Sonicator Preparation

a. Open the SONOLAB 7.3 software from the desktop icon on the Covaris computer.

b. Water bath should be filled up to level '0'on the run side marker. Fill the water bath with MilliQ water if low. ***Note:*** *Do not fill the bath higher than level '0'.*

c. Turn on the chiller and ensure that it is set to 6C. Use the start/stop button to turn on the chiller. There will be a (-) if the chiller is working and a (*) if it is not chilling. Working temperature should be between 4 – 8C.

d. Allow the system to degas for at least 1 hour prior to shearing any samples.

e. When loading plates in for shearing the instrument will adjust the plate to the correct level in the water bath.

1.4. DNA Sample Preparation

a. Label sample plates and corresponding Covaris plates 1 – 4 if processing more than 1.

b. Add 60ul nuclease free water to the aliquot plate(s) wells A12 and H1.

c. Add lambda control DNA, 200ng in 60ul, to well A1 and H12 of each aliquot the plate.

d. ***Important:*** *Add water to any blank wells in a column that will be sheared. Do not shear partial columns with empty wells.*

e. ***Important:*** *Ensure that every sample well contains 60ul. Shearing less than 55ul in the Covaris plate will result in poor shear results.*

2. **Reagent and Deck setup for 2X SPRI Size Selection**

a. Follow the recipe instructions to create SPRI reagent plate(s) described in the SOP **Reagent/Stock Preparation section,** or the printed Reagent Setup worksheet in the lab

b. Wait to fill the SPRI plate until all shearing is complete to reduce settling of beads. Fill the SPRI reagent plate only with the first SPRI bead volume defined in the workbook. Later in the program you will refill the SPRI source plate for the second SPRI addition.

c. Record barcode information for plates processed, lot data for SPRI and Kapa Hyper Kit, and other reagents used, on the printed Setup worksheet.

2.2 Open the Hamilton Run Control software on the desktop.

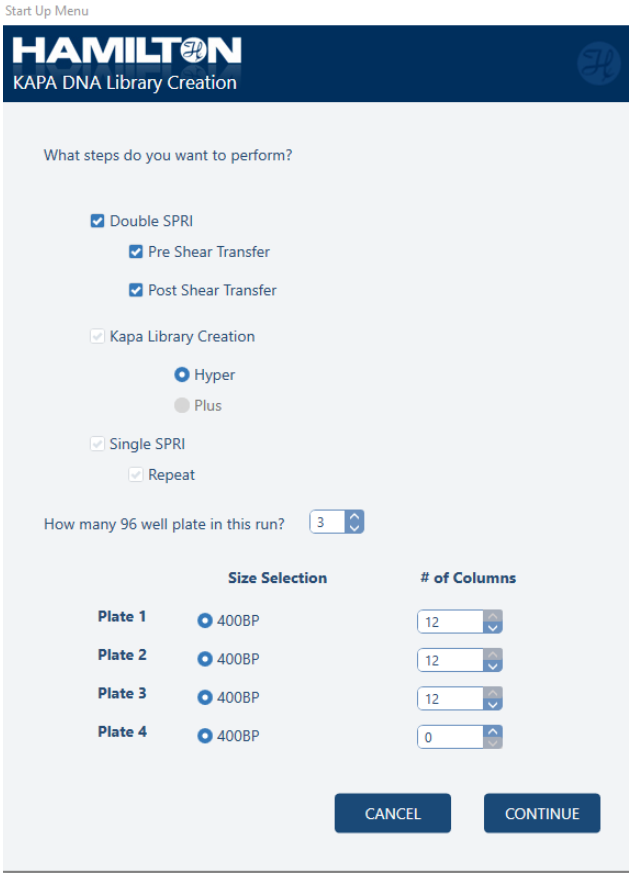
a. File > open > development > ***Kapa_Hyper_384_400bp_gDNA_V1.5_30minLigation.med***

b. Click the green arrow to start the program.

c. Select Double SPRI, Pre and Post shear transfer (each can be run separately)

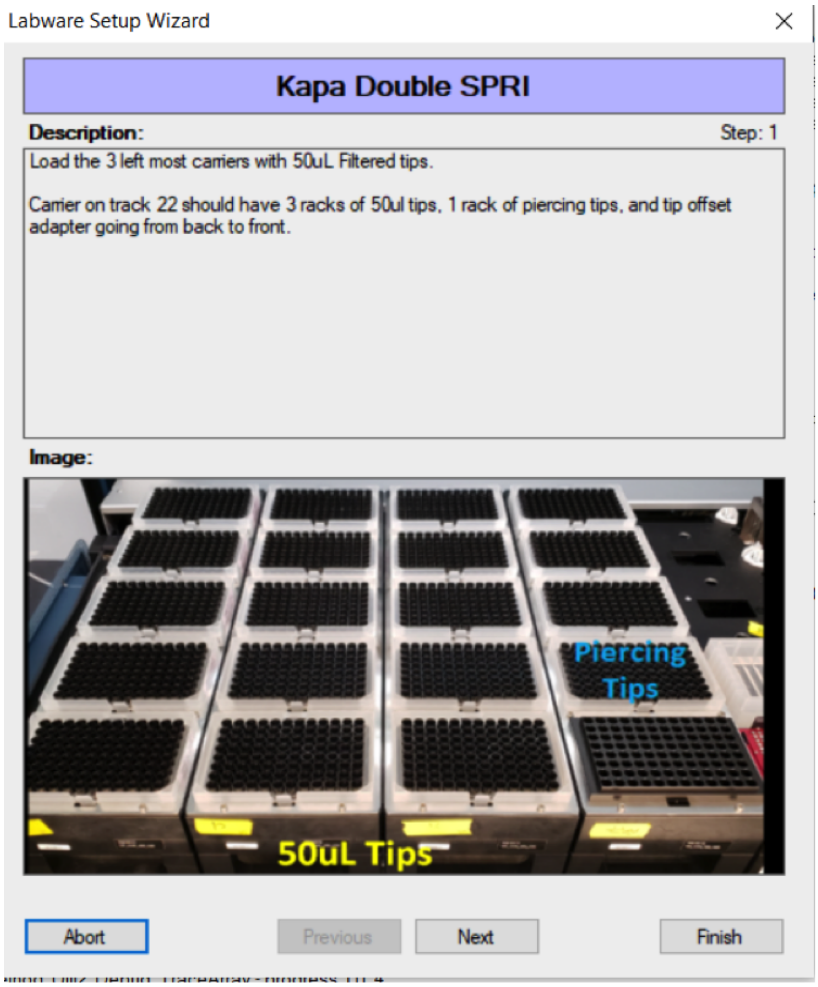
d. Enter Choose Plates and "number of columns" for each plate. Note: *Always enter the number of columns for each plate as* **12** *if running multiple plates. For a single plate, fewer than 12 columns can be processed see Figure 2*

*Figure 2*



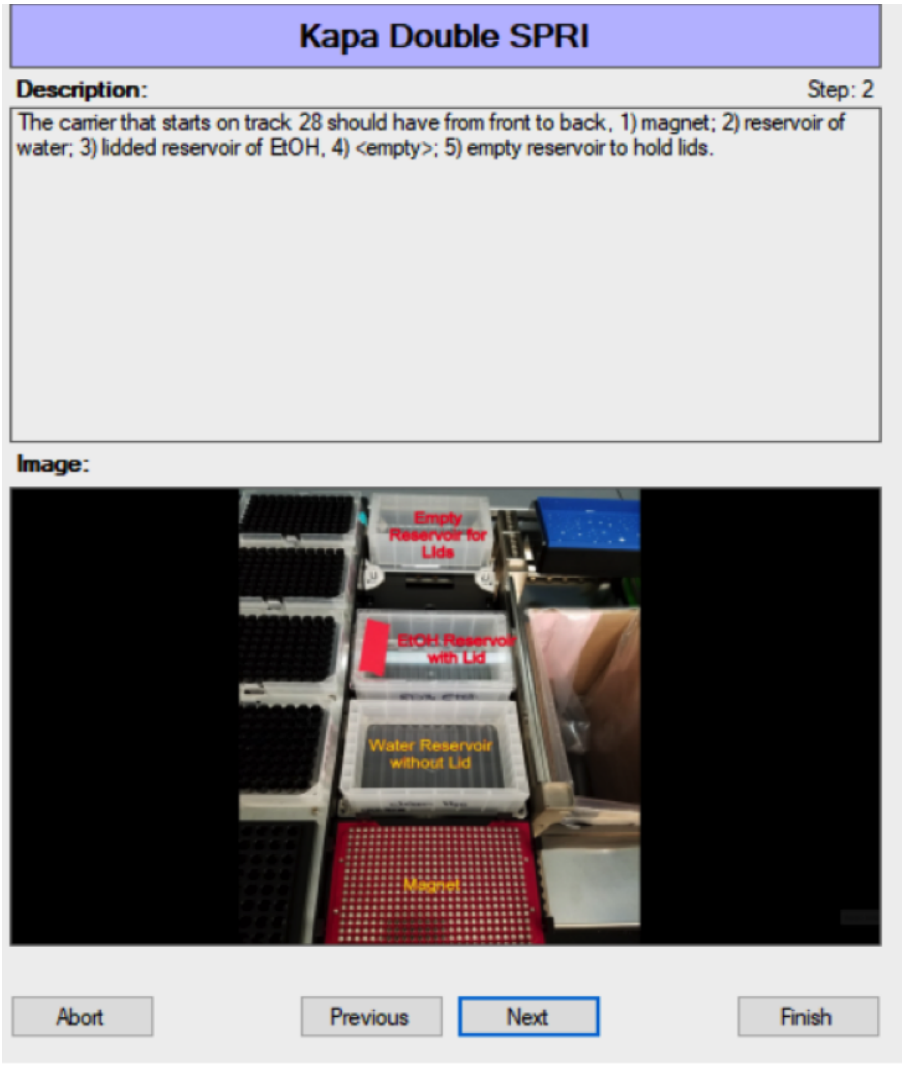

Load 50ul tips and one piercing tip rack onto the deck in rack positions 4 through 22. see Figure 3

*Figure 3*



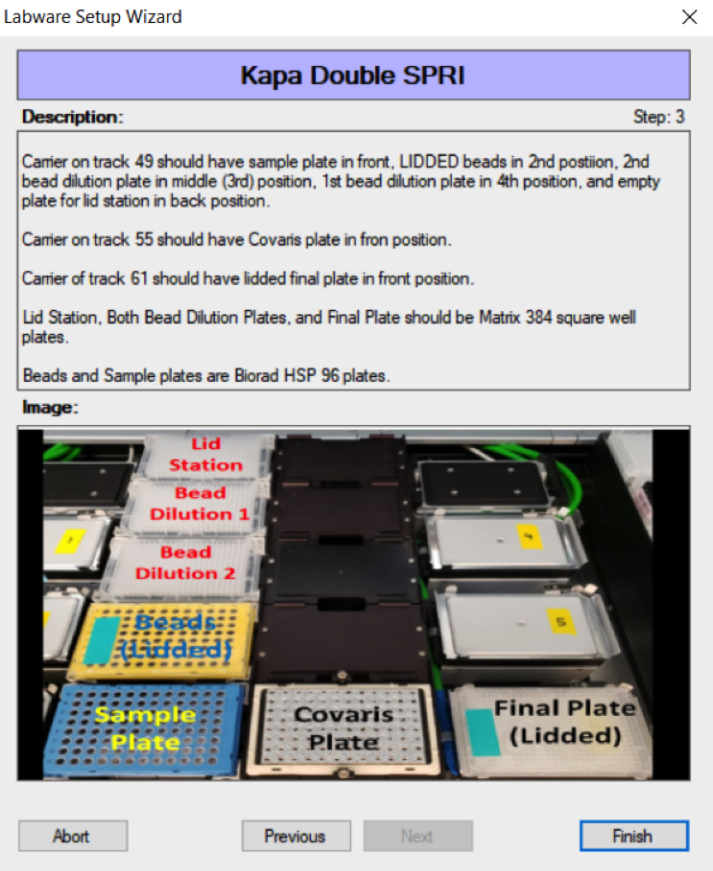

2.3 Load Elution buffer trough, 80% EtOH trough with lid, and lid park trough onto deck at position 28. see Figure 4.

*Figure 4*


Kapa Double SPRI
Description:
Step: 2
The carrier that starts on track 28 should have from front to back, 1) magnet; 2) reservoir of water; 3) lidded reservoir of EtOH, 4) <empty>; 5) empty reservoir to hold lids.
Image:
Empty Reservoir for Lids
EtOH Reservoir with Lid
Water Reservoir without Lid
Magnet
Abort
Previous
Next
Finish

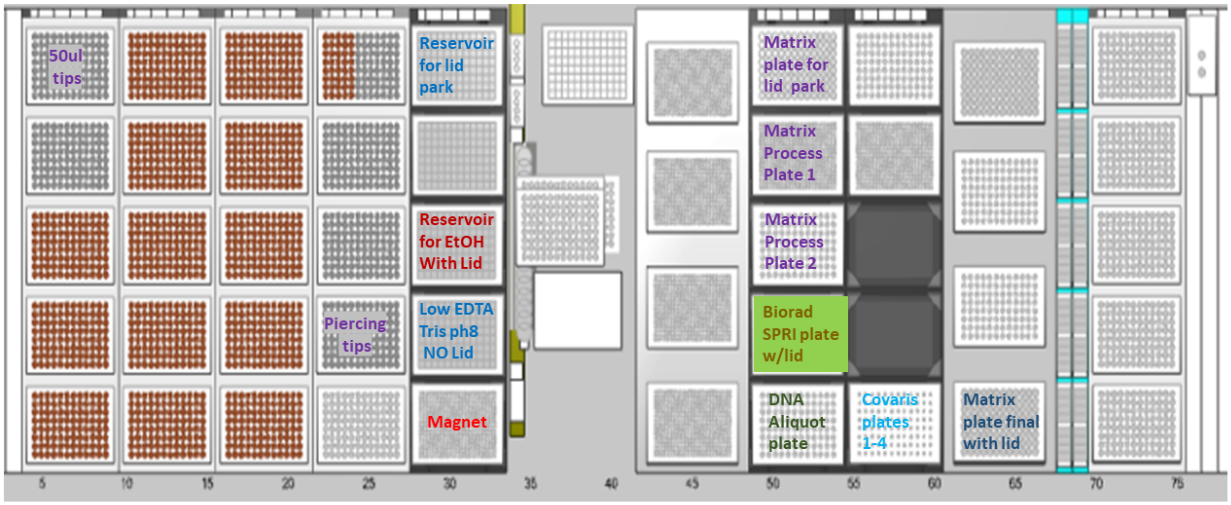
2.4 Load Matrix 384 well sample processing plates and final lidded plate as shown. Load Biorad SPRI bead plate (fill later, after shearing to reduce bead settling). Load the first sample plate onto the deck at position 49 as directed. Load the first Covaris plate into the APE Covaris carrier, to the right of the first sample plate. see Figure 5

*Figure 5*




2.5 Double check that the deck setup is correct using the deck setup placard card. See Figure 6. Also double check that deck carriers are pushed all the way in.

*Figure 6*




2.6 Click "Finish" to start the run and follow prompts for gDNA transfer to Covaris plate(s). The Vantage will first pierce the Covaris plate foil seals, then transfer DNA to the Covaris plate(s), and pause for shearing or next plate set loading.

2.7 After transfer check the surface of the Covaris plate for aliquot DNA drops on top of the Covaris plate foil. Also inspect the DNA plate for wells that did not transfer. Use a pipette to manually transfer missed samples into the Covaris plate.

2.8 *Important:* After DNA transfer into each Covaris plate DO NOT seal the plate until ready for shearing. Instead cover with a plastic plate cover and keep on ice. *If the seals are on the plate for too long they will be difficult to remove.*

2.9 Just before shearing, seal evenly and spin for 30seconds at 1000rpm to remove air bubbles. *Note: Seal evenly and securely, but do not use excessive pressure when sealing or the seal will be difficult to remove.*

## 3. DNA Sonication in Covaris LE220

3.1. Operating the Covaris for shearing

a. In the software window, click "OPEN", then select the method "**Vantage 55sec 400bp**' for Vantage shearing. This program will shear 12 columns. If running fewer than 12 columns in a plate, pause and abort the run when it completes the last column in the plate containing samples .

b. Check to make sure that the following settings are correct for 400bp insert shearing. Dithering settings are under the "motion" tab, all others under the "treatment" tab. see Figure 7

*Figure 7*

| **Program target size** | **400 bp** |
|---|---|
| Duration | 55 sec |
| Peak Power (PIP) | 450 |
| Acoustic Duty Factor | 15% |
| Cycles/Burst | 1000 |
| Average Power | 67.5 |
| X & Y dithering | 0.5mm |
| X & Y dither speed | 10 mm/sec |

c. Under "Motion Control" move the stage to the "START" position .

d. Place the MicroTube plate into the stage, first tipping up one side to allow any air bubbles to escape from beneath the plate. A1 should be in the back right corner as indicated. The orientation is very important for partial plates.

e. Double check that there are no air bubbles beneath the plate. Close the Covaris door and click RUN.

f. ***Note:*** *Each column being sonicated must have sample or water in every well. Empty wells will disrupt sonication in neighboring sample wells.*

3.2. Sonication takes 12 min/full plate for a 400bp shear plate.

3.3. Immediately after shearing each plate, spin the plate for 1 min at **3000**rpm. ***Important****: spin speeds lower than 3000rpm will leave splashed microdroplets on the seal, which can cross contaminate samples when seals are removed.*

3.4. *Immediately* remove the seal after spinning at 3000rpm. Starting at one corner carefully peel off, pulling horizontally, not upward, to avoid any cross contamination. It is helpful to place a clear seal over the foil seal (after shearing ) to help peel up the seal evenly.

3.5. ***Important****: Allowing the seal to sit on the plate for longer than necessary can make it very difficult to remove, risking cross contamination.* Use a clean hard plastic cover to protect the plate while waiting for the next step. *Important: Do not apply a new sticky seal to avoid cross contamination!*

*3.6. Operator tips for the transfer process to and from Covaris plates:*

a. Cut the barcode sticker from the Covaris plate before loading on the deck.

b. Organize the transfer and shearing time to minimize wait times.

c. Shear in the same order plates were transferred to Covaris plates, and mark them as sheared or spun, to avoid mixups.

d. Remove seal(s) as soon as shearing and 3000rpm spin down is complete. Leaving them on for an extended period can cause them to pull up the seal below, or even the well.

e. Always log out of the Covaris when not using. The instrument is set to automatically cycle chiller off at night and on again in the morning.

## 4. Continuation of the 2X SPRI process

4.1. Resume the 2XSPRI process. Place each Covaris plate on the Vantage deck when prompted to transfer sheared samples to the 384 well processing plate 1. ***Important:*** *Make sure plates are placed in correct numerical order and that foil seals are removed before transfer to 384 well processing plate 1.*

4.2. Plates 1-4 will be transferred, along with 29.5ul of SPRI beads, to the four quadrants of the processing plate in a Z pattern. eg: A2 P3 refers to well A2 of Plate 3. see Figure 8

*Figure 8*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A1 P1 | A1 P2 | A2 P1 | A2 P2 | A3 P1 | A3 P2 | A4 P1 | A4 P2 |
| A1 P3 | A1 P4 | A2 P3 | A2 P4 | A3 P3 | A3 P4 | A4 P3 | A4 P4 |
| B1 P1 | B1 P2 | B2 P1 | B2 P2 | B3 P1 | B3 P2 | B4 P1 | B4 P2 |
| B1 P3 | B1 P4 | B2 P3 | B2 P4 | B3 P3 | B3 P4 | B4 P3 | B4 P4 |

4.3. After transfer of each sheared plate check for missed wells and manually transfer and mix these if possible.

4.4. When all four plates are transferred, continue with the Double SPRI process.

*4.5.* ***Important:*** *Watch for prompts to add more SPRI beads, and reload tips during the cleanup process. The second SPRI bead addition is 12ul.*

4.6. After the Double SPRI process completes, the final 384 well plate may be foil sealed and stored at 4C or -20C until gDNA processing.

## 5. gDNA Library Creation - Reagent prep and Deck setup - (5.5 hr program).

**Includes End repair, A-tailing, Adaptor Ligation, and cleanup.**

5.1. Open a Kapa Hyper prep kit KK8805. Immediately place the enzyme tubes on ice, or store them in the freezer until ready for use. Thaw the buffers on the benchtop until all solids have been completely dissolved. Buffers may also be placed at 4c overnight to thaw.

5.2. *Important: reagents must be completely thawed before use. There should be no visible precipitates or performance will be affected. Do not place buffers back on ice or they will reprecipitate.*

5.3. For Reagent preparation instructions refer to the SOP **Reagent/Stock Preparation section 8,** or the printed Reagent Setup worksheet in the lab

a. Create the reagent mixes. Invert several times to mix well. Do not vortex.

b. *Important: Do not place reagents back on ice or they will reprecipitate.*

c. Dispense reagents immediately into a 96 well STOR plate and place on the deck until ready to start the run.

5.4. Thaw the required HPLC adapter plates containing 5ul of 10uM adapters. Adapters will be in Q1 of each 384 well Biorad HS plate.

5.5. Spin down and store the adapter plates on ice until prompted to load onto the deck after the End Repair/ A-tail incubation.

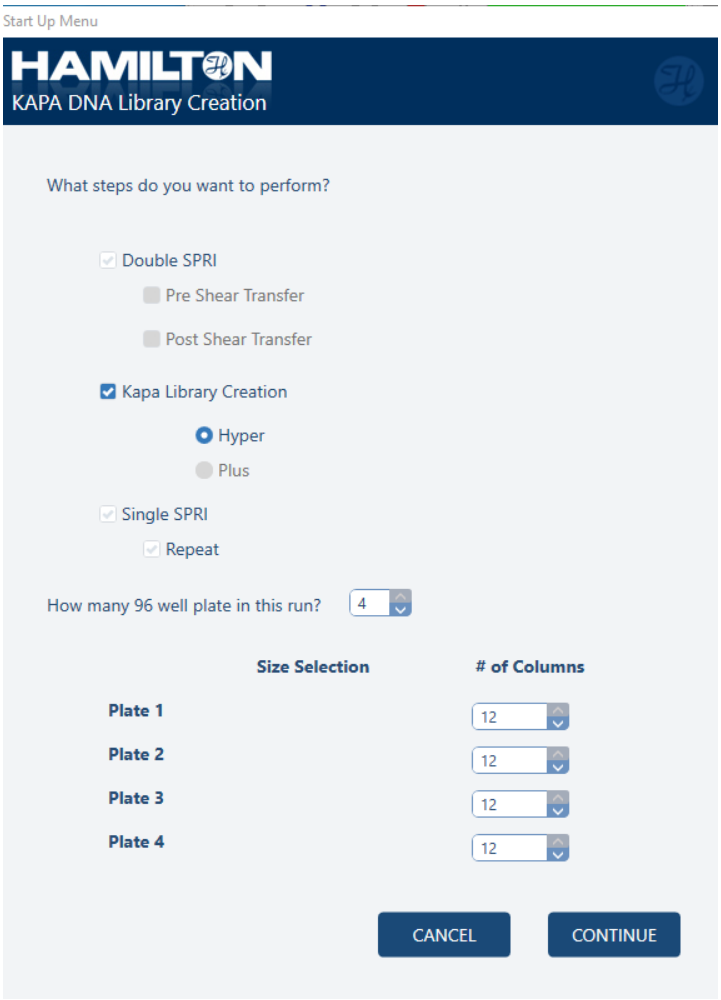
5.6. Open the Hamilton Run Control file:
***Kapa_Hyper_384_400bp_gDNA_V1.5_30minLigation.med***.

a. Select Kapa Library Creation , and Hyper. (Plus is for chemical shear method)

b. Select number of plates and number of columns (12) for each plate. Figure 10

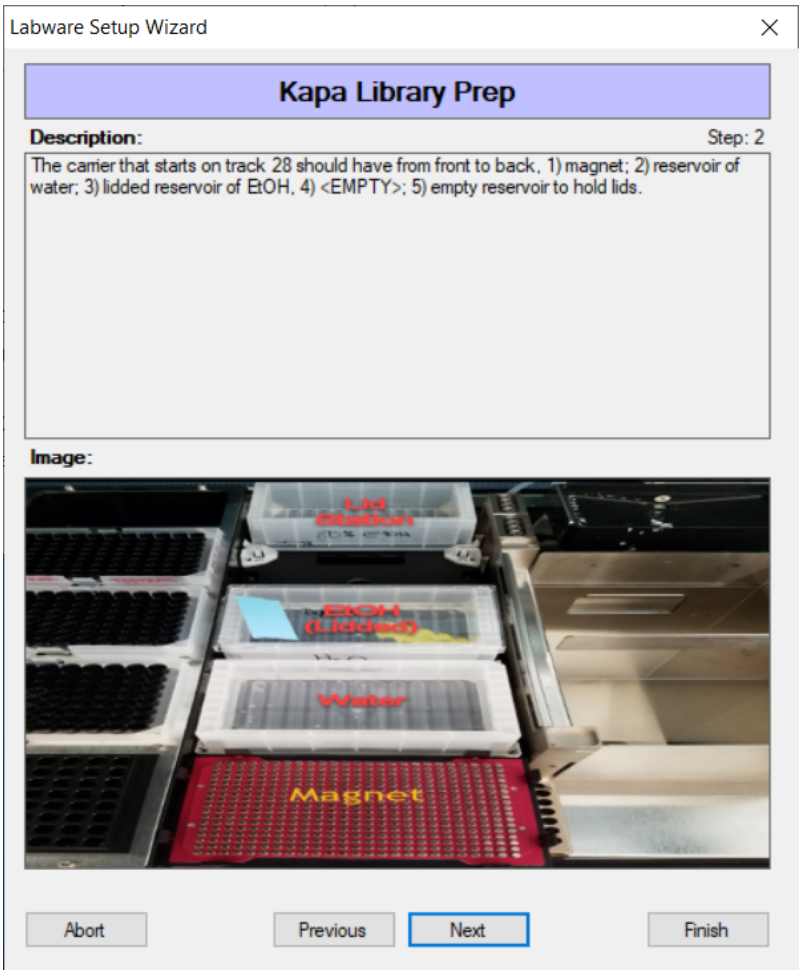
c. *Caution: Do Not enter less than 12 columns for each plate unless you are only processing a single plate. Tip counting errors will occur and the run will likely error out at some point.*

*Figure 10*

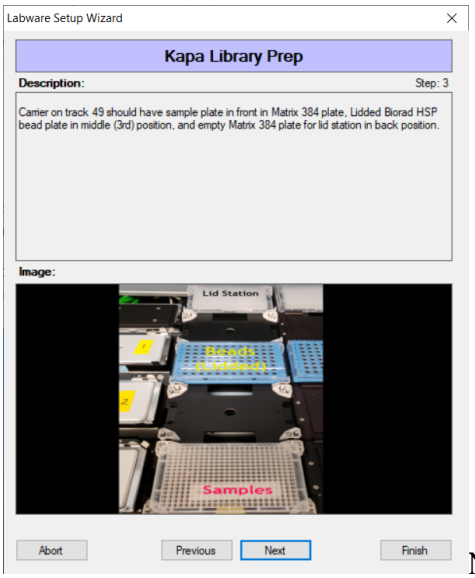

Start Up Menu
HAMILTON
KAPA DNA Library Creation
What steps do you want to perform?
Double SPRI
Pre Shear Transfer
Post Shear Transfer
Kapa Library Creation
Hyper
Plus
Single SPRI
Repeat
How many 96 well plate in this run?
4
Size Selection
# of Columns
Plate 1
12
Plate 2
12
Plate 3
12
Plate 4
12
CANCEL
CONTINUE

5.7. Follow deck setup prompts for tips, reagent troughs and plates. See Figures 11-15

*Figure 11*



5.8. *Figure 12*




No Lid on Sample plate!

*Figure 13*

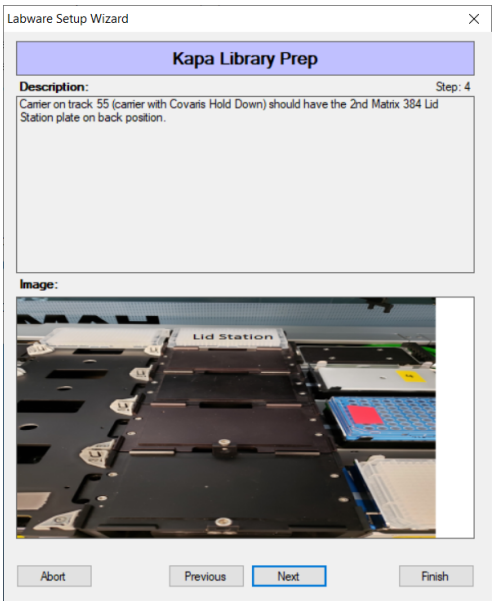



*Figure 14*

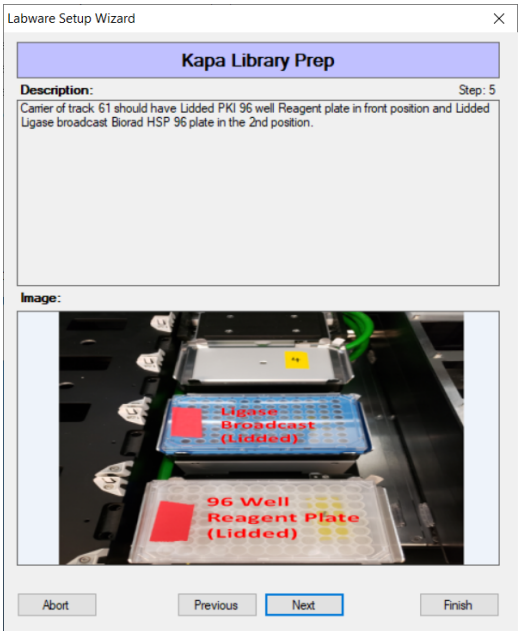
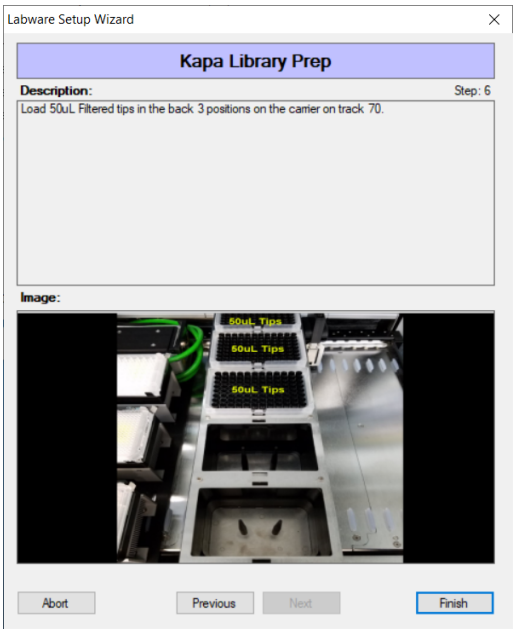


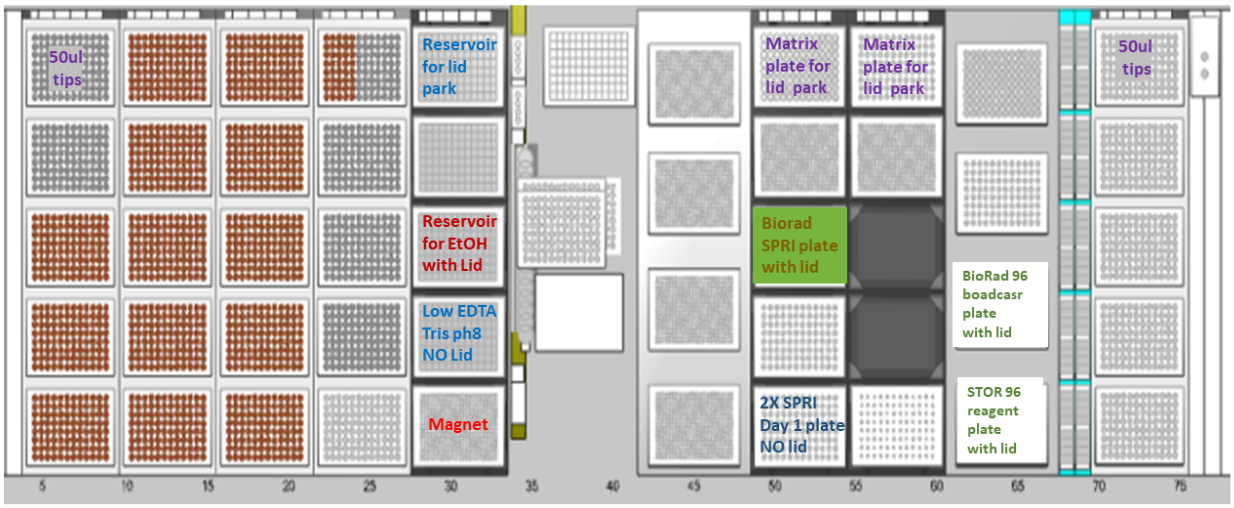

*Figure 15*




Load 1 full rack of tips for each full plate, plus one extra full rack .

5.9. Use the Deck setup placard to confirm that your deck is set up correctly. see Figure 16

*Figure 16*




5.10. After the End Repair A-tail reagent broadcast to the DNA plate (75 minutes for 4 full plates), at the prompt seal with a PCR seal, and place the plate in the flat plate Eppendorf cycler for End Repair A-tail. (1 hour) *Note: ensure that the plate 'clicks" into place on the cycler deck.*

5.11. Run the "erat" incubation program.

5.12. Continue the vantage program for concurrent broadcast of Ligase

5.13. When the EndRepair ATail program is complete, transfer the plate back to the Vantage deck (with lid), into the same deck position (CPAC 2), and continue the program.

5.14. The program will then add ligation mix and prompt for placement of adapter plates, one at a time, in the middle position at deck location 55.

5.15. Transfer the adapter plate label(s) to your setup worksheet as a QC check for later data entry.

5.16. The program will then prompt for final 384 well plate placement on CPAC 6.

5.17. After a 30 minute adapter ligation on deck, the robot will pause for the operator to load 80% Ethanol, SPRI beads, and water for elution, and on the deck.

5.18. The process will continue with a SPRI clean-up. ***Important:*** *Watch for several prompts to replace tips during the process.*

5.19. After the library creation step is complete the final 384 well process plate may be foil sealed and stored at 4C or -20C for later processing, or the operator may proceed with Single SPRI adapter clean up.

## 6. Single SPRI adapter cleanup (1 hr program)

6.1. Label a Biorad 96 well HS plate for each final plate being processed with date, initials, plate name and process type..

6.2. Open the 384 Kapa Single SPRI clean up Workbook in the Setup Workbooks folder.

a. Enter the number of columns for each plate quadrant (cells E3 – H3).

b. Follow excel workbook instructions to create reagent plate(s).

6.3. In Hamilton Run Control: File > open > development > ***Kapa_Hyper_384_400bp_gDNA_V1.5_30minLigation.med***.

a. Select Single SPRI, and select the number of plates as before.

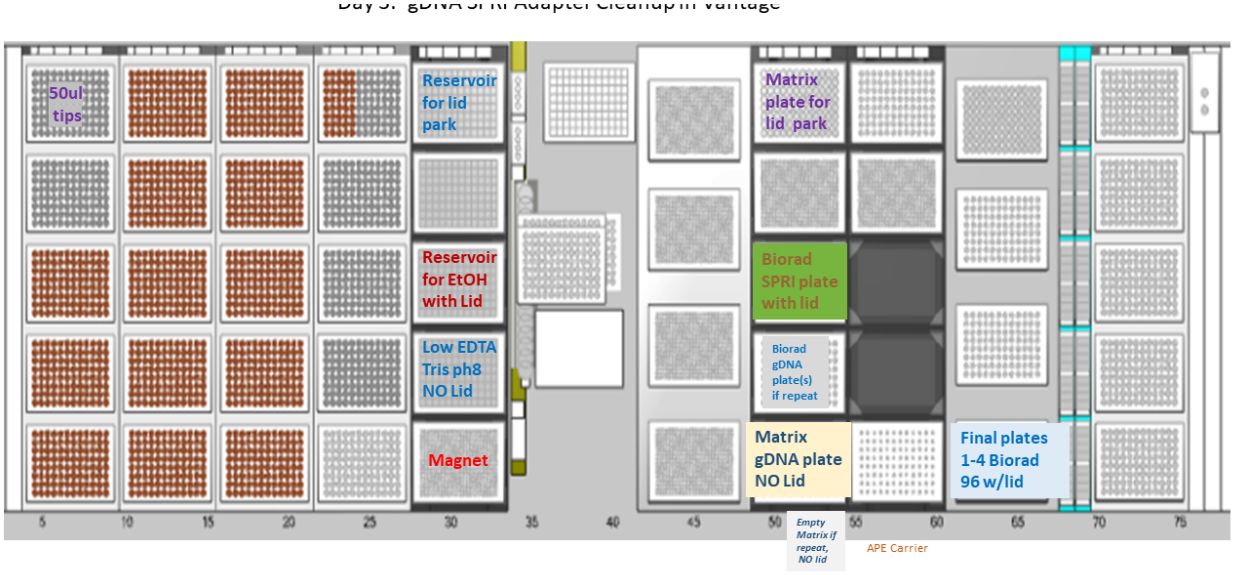
b. Place the matrix library plate from the previous process into the 5th position on deck location 49 for SPRI cleanup processing. See setup images.

c. If repeating the Single SPRI process, select “repeat” for samples that are in Biorad 96 well plate(s). In this case the Biorad 96 well plates will be placed sequentially in the 4th position, of deck location 49 when prompted. *Important: You must still place a matrix plate in the 5th position for processing.*

d. Follow set up instructions as for previous processes. Double check deck setup using the deck setup placard. see Figure 17

*Figure 17*

e. When complete, run the Fragment Analyser QC step, foil seal final 96 well plates, and store at -20c.




## 7. Run a Fragment Analyzer QC to Check Results

7.1. **Important:** When setting up a run you must import the Clarity library names for each plate. This will require that you claim plates from the Clarity database before proceeding with the QC step. See section 8 for how to do this.

7.2. Use the High Sensitivity NGS Fragment Analysis Kit, 1bp-6,000bp, from Advanced Analytical.

7.3. Prepare sample QC plate(s) according to the manufacturer user guide:

a. Dispense 22ul Diluent Marker into a BioRad 96 well plate(s) for sample wells in each library plate, and for ladder in well H12.

b. Dispense 24ul Blank Solution to all unused wells. Do not leave any empty wells.

c. For transfer of 2ul of each sample open file Sciclone > File > open application > production methods > Fragment_Anlayser_QC_setup, and follow process instructions. *Note:Manually add ladder to well H12*

d. Spin down the QC plate before loading on the Fragment Analyzer.

e. Follow Fragment Analyser instructions for running the QC plate(s)

*f. Important: Remember to import sample names prior to starting a run.*

7.4. After QC run completes, open the ProSize Software. File > open saved run file.

a. Set smear analysis for 125 - 1200 bp and apply to all wells.

b. Select file > generate report. Save as a PDF and name file with plate name.

c. File > export data. Choose to export all and use the PNG format.

d. Save the run folder in the Current Fragment Analyzer folder.

e. See Figure 8 for an example of a successful library trace.

f. See Figure 18 for an example of a successful library gel image.

Figure 18

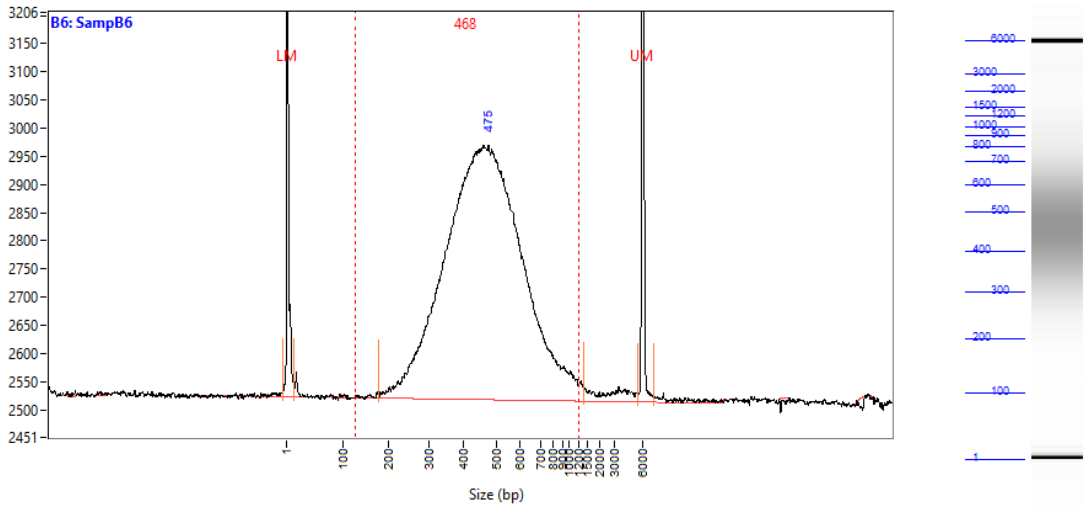



Figure 9:

## 8. Clarity gDNA Library Submission for qPCR

8.1. Claiming samples in Clarity (https://clarity-prd01.jgi-psf.org/clarity/login/auth).

a. Click on the LAB VIEW icon to show the AVAILABLE WORK list.
b. Select a plate from appropriate workflow, ex: LC Illumina Regular Fragment, 600bp, Plates
c. Click on Step 1>> LC Library Creation
d. Select the sample plate and click on Add Group to add into the ICE Bucket
e. Click on the green colored VIEW ICE BUCKET
f. Click on the green BEGIN WORK
g. In the new window, the samples were placed in a new plate. Then click on the green colored RECORD DETAILS
h. Open the Clarity worksheet and save it to the current library creation worksheets folder in Octopus/ prodseq/ Automated Library Creation.
i. Create a Fragment Analyser names list file for each plate. Save to FragAlyser drive in networked drives.

8.2. Completing Samples in Clarity

a. Fill in batch reagent lot information in the Clarity interface.
b. Select printer BCode 10 or BCode 3.
c. Enter the Fragment Analyser QC data in the Clarity worksheet for each plate run.
d. Fill in adapter lot information in the worksheet for each plate and save.
e. Upload required worksheet, QC run data PDF report, and electropherogram to the Clarity interface.
f. ***Important:*** *Ensure the correct barcode printer is selected before completing Clarity steps.*
g. Click on the green colored NEXT STEPS
h. Click on the green colored FINISH button
i. A plate label will be printed for each plate on the selected printer.

j. Library plates will be pushed into the qPCR queue after this step.

8.3. Mark the plate as done in the Plate Availability/Schedule gDoc.

a. Highlight the plate in green (passing) or red (failed).

b. Fill out the Lib Create complete and GLS complete columns.

8.4. Tape Clarity barcode to the front of each library plate.

8.5. Transfer each plate to the plate request bin in the qPCR -20C freezer.

**Printable Setup Worksheet:**

| pa gDNA Lib plate prep 30min ligation * | | 2XSPRI date: | LC Date: | | SPRI cleanup date: |
|---|---|---|---|---|---|
| | | | | | |
| plate | Plate Barcode Label | #samples | Spike-in Barcode label | | Adapter bcode |
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |

| RI bead lot: | |
|---|---|
| pa Kit lot: | |

| | DAY 1 | 2X SPRI | | |
|---|---|---|---|---|
| SPRI beads 1rst addition | | | Dispense to all wells | |
| | 1 plate | 2 plates | 3 plates | 4 plates |
| | 43 | 75 | 107 | 138 |
| | DAY 1 | 2X SPRI | | |
| SPRI beads 2nd addition | | | Dispense to all wells | |
| | 1 plate | 2 plates | 3 plates | 4 plates |
| | 12 | 26 | 38 | 48 |
| | DAY 2 | Lib Creation | | |
| | SPRI beads: | Dispense to all wells | | |
| | 1 plate | 2 plates | 3 plates | 4 plates |
| | 32 | 54 | 76 | 98 |
| | DAY 3 | Adapter Cleanup | | |
| | SPRI beads: | Dispense to all wells | | |
| pdated for adding 5ul of 5uM adapter 3/4/24 | 1 plate | 2 plates | 3 plates | 4 plates |
| | 35 | 60 | 85 | 110 |

## Reagent Source Plate setup:

**Creation Reagent mix Preparation:**

End Repair/ A-Tail Mix

| | 1 plate | 2 plates | 3 plates | 4 plates |
|---|---|---|---|---|
| ER A-tail Buffer | 246 | 491 | 737 | 982 |
| ER A-tail Enzyme | 105 | 211 | 316 | 421 |
| | 351 | 702 | 1053 | 1403 |
| | 44 | 88 | 132 | 175 |

Ligation Mix

| | 1 plate | 2 plates | 3 plates | 4 plates |
|---|---|---|---|---|
| Ligation buffer | 972 | 1944 | 2916 | 3888 |
| Ligation Enzyme | 324 | 648 | 972 | 1296 |
| No H2O addition to compensate for increased adapter volume | | | | |
| | 1296 | 2592 | 3888 | 5184 |
| | 162 | 324 | 486 | 648 |

**ent Source plate setup** 486-324=162

STOR plate in Col 1,2 and 3 only

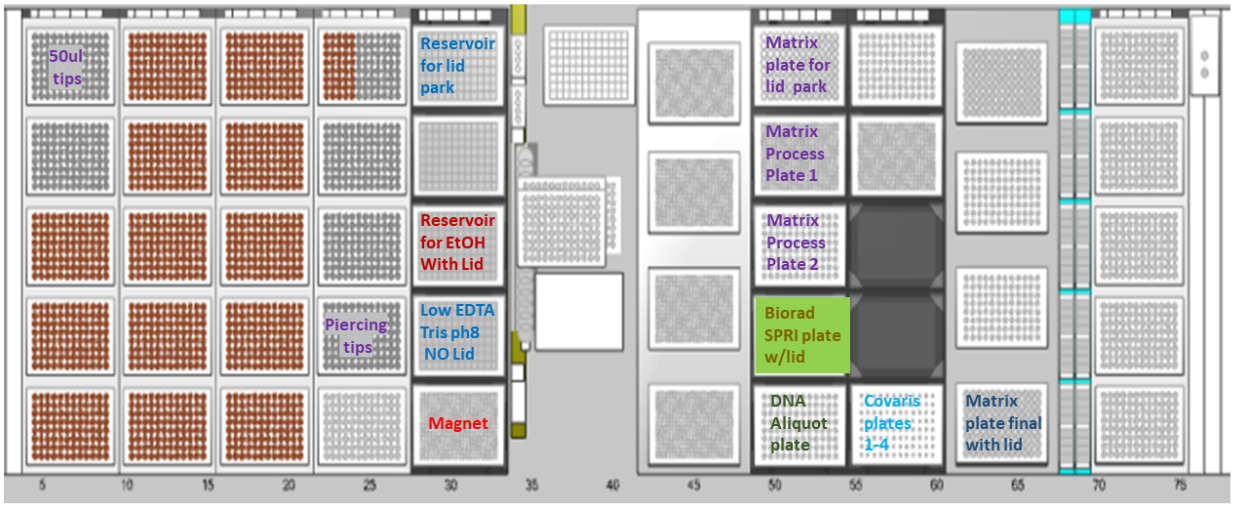
| | End repair/Atail | Ligase mix | Ligase mix |
|---|---|---|---|
| | Col 1 | Col 2 | Col 3 |
| plate | 42 | 158 | 0 |

| | End repair/Atail | Ligase mix | Ligase mix |
|---|---|---|---|
| | Col 1 | Col 2 | Col 3 |
| lates | 84 | 315 | 0 |

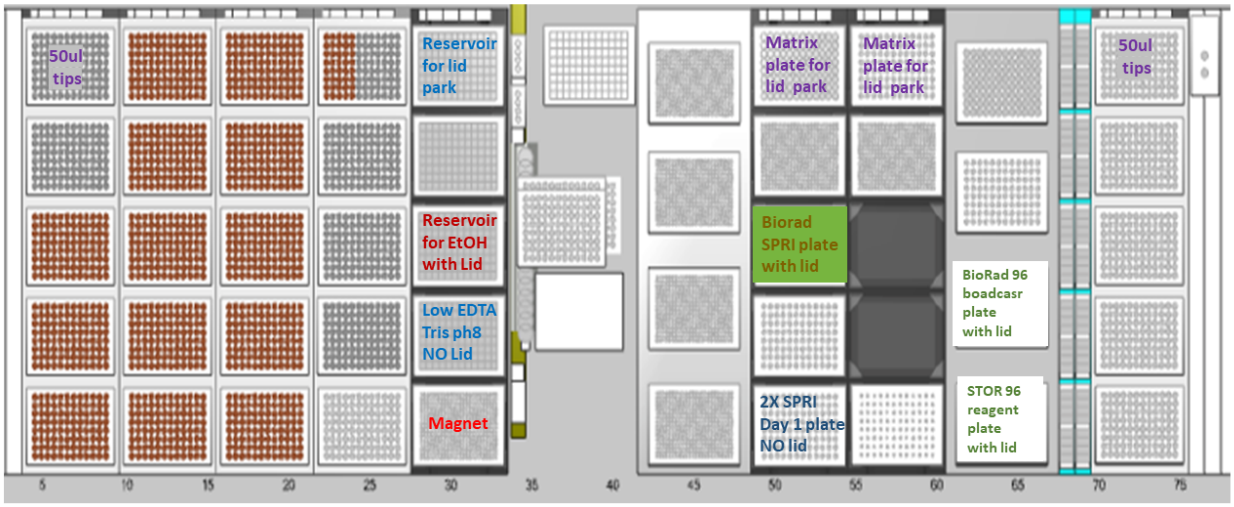
| | End repair/Atail | Ligase mix | Ligase mix |
|---|---|---|---|
| | Col 1 | Col 2 | Col 3 |
| lates | 126 | 315 | 158 |

| | End repair/Atail | Ligase mix | Ligase mix |
|---|---|---|---|
| | Col 1 | Col 2 | Col 3 |
| lates | 169 | 315 | 315 |

## Deck Set-up placards

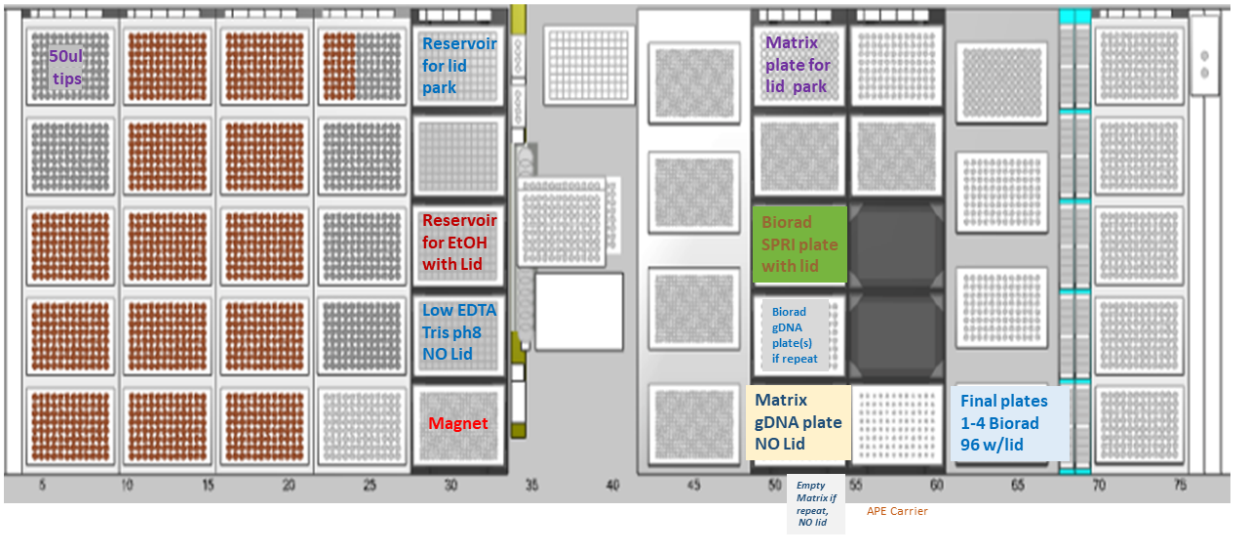



Wait to fill EtOH, H2O and SPRI plate until shearing is complete.




Wait to fill EtOH, H2O and SPRI plate until ligation is complete

Day 3: gDNA SPRI Adapter Cleanup in Vantage



**SPRI Aliquots**

1. Place the 1L SPRI bead bottle on a tilting mixer for 4 hours to mix and bring beads to room temperature. Tape bottle down on its side, and set speed and tilt to maximum settings. (VWR mixer in 459).
2. Pour beads into screw cap 50 ml tubes. Invert several times between pouring each aliquot to ensure consistent bead to buffer ratios.
3. Label each tube with the bead lot number, expiration date, aliquot date, and your initials.

**Perkin Adapter Plate stamp-out from Biorad HS 96 well into 384 plates**

**Note**: Do not vortex adapter plates.

1. Adapter stock plates (96 well) are provided by Sample Management.
2. Defrost stock plate at 4C.
3. Place on the plate shaker for 30 minutes.
4. Spin down stock plate.
5. Prepare a fresh trough of elution buffer to dilute adapters to 10uM.
6. Use the Sciclone to dilute and gently pipette mix the diluted adapter plate. Spin down.
7. Number the front left face of each adapter plate with color coded adapter plate type 1-8
8. Stamp out several 5ul single use 10uM adapter plates into BioRad 384 HS plates (for Vantage).
9. Program path: On Sciclone 3 > production methods > adapter creation > adapter creation adapter stamping 384 well. Choose Biorad plates
10. Spin down single use plates and check to make sure each has all 96 adapters in Q1.
11. Seal with foil seals.
12. Print barcode labels with adapter type, date, and lot info from Sample Management .
    a. Lot info: adapter type #, source plate date and aliquot number (ex: 02-091418-02).

    b. Format for printing of barcodes on printer 3 or 10 :
    line 1: **PE01-091418-01** (lot and plate type) **02/07/23 PE 5ul 10uM** (vol, conc)

c. Affix labels to the top of each adapter plate.

13. Store plates in -20C in adapter plate bins.

**Instrument Maintenance**

**LE220 Covaris Sonicator**

Change water every month
Sanitize instrument every month (*See maintenance printout on instrument*).
Replace filter every 6 months (done by service technician).

**Sanitize Covaris monthly (35 min)**

1. Turn degas off.
2. WCS off
3. Disconnect tubes from tray (tubes connecting water bath and WCS)
4. Click on "Service bath" to raise acoustic assembly.
5. Press open door and slide out bath tray, empty & re-install.
6. Turn on degas briefly to empty out lines.
7. Plug in left line from WCS (flows from WCS to bath) and turn on WCS briefly to empty WCS.
8. Disconnect WCS line from water bath.
9. Fill bath with milliQ water and re-install.
10. Dissolve (2) NaDCC 13.1 G tablets in the water tank.
11. Click load plate to lower assembly. Ensure water covers acoustic assembly and degas tubes.
12. Turn degas on and let degas run for 5 minutes.
13. Degas off > service bath > open door > empty tray > re-install > refill with Milli-Q water > load plate > run degas for 5 min to wash out bleach.
14. Repeat step 12 water washes 3 times to remove all sanitizer from lines.
15. Refill a 4th time to level 6 on the run side, turn on degas.
16. Reconnect tubes and turn on WCS.

**Instrument**

Hamilton and Covaris PM performed, and pipettes calibrated every 6 months.

## Troubleshooting

## SOP Approval

| DEPARTMENT | APPROVED BY | DATE |
|---|---|---|
| **Lab Supervisor** | Chris Daum | 4/22/2022 |

## Appendix

## Change History

- Implemented into production Vantage #3: 2/14/2022
- Updated for Vantage #1 use: 4/15/2023
- Update for 10uM Perkin Elmer adapter, and 15 min ligation 11/28/23
- Update for 5uM PE adapter using 5ul, with reduced master mix volume, with change to 30min ligation time. Also, updated covaris sanitizing method. 4/22/24