Plasma WGS Sciclone Library Preparation - KAPA

Purpose

To describe the KAPA Hyper Prep Kit Plasma Whole Genome Sequencing (pWGS) library preparation procedure using the Sciclone G3 liquid handler.

Scope

This Standard Operating Procedure (SOP) covers the entire procedure for creating plasma WGS libraries on the Sciclone G3 liquid handler using the KAPA Hyper Prep kit. It includes QC steps, and LIMS entry. The final result is a library that consists of DNA fragments with ligated adapters and indices which can be sequenced on an Illumina instrument.

This SOP applies to all staff who perform plasma WGS library preparation on the Sciclone G3 platform.

For document control and training purposes, the specific methods and reagents required to complete some steps of this assay are described in detail in separate TMs which are listed in this document and available on the Quality Management SharePoint. Only personnel with specific, up-to-date training are permitted to perform any validated assay.

Responsibilities

Laboratory Staff:
Follow the SOP, document all required metrics, and report any non-conformances that may occur

QA Manager:
Monitor quality output from this procedure

Management:
Review and update procedure, as required
Approve data at appropriate sign-off points
Communicate progress to the customer, as required

Reagents and Consumables

Note: Plastic consumables **cannot** be substituted for another product

Item Description	Vendor	Catalogue #
NA12878 DNA from LCL (gDNA positive control); GLCS_0002	Coriell	NA12878
Seraseq® Blood TMB Mix Score 7 (positive control) ; GLCS_0035	SeraCare	SER-0710-2087
KAPA Hyper Prep Kit	Roche	7962363001
NucleoMag® NGS Clean-up and Size Select beads or AMPure XP Beads	Macherey-Nagel Beckman	744970 A36881 (60 ml)
100% Ethanol	Sigma-Aldrich	E7023
1X Low TE Buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA	Life Technologies	12090-015
xGen Duplex Seq Adapter-Tech Access; 2nmol at 15uM, dual 3bp UMI; approximately 133.3ul (stock solution); -20°C	Integrated DNA Technologies	1080799
IDT Dual Index (IDT unique indexing primer pool 20uM)		custom with standard desalting
Hard-Shell® 96-Well PCR Plates	Bio-Rad Laboratories	HSP9601
150 µL - Barrier Sterile 96 Rack Tips	Perkin Elmer	111426
Polypropylene 12 column reservoir plate		6008700
StorPlate-96-well V-bottom plate, 450µL		6008290
Clear Lid		6005619
Polypropylene low-volume microplate, 384-well		6008890
96 microTUBE Plate	Covaris	520078
microTUBE-50 AFA Fiber Screw-cap		520166
High Sensitivity NGS Fragment Analysis Kit	Agilent	DNF-474
TapeStation High Sensitivity D1000 ScreenTape		5067-5584

TapeStation High Sensitivity D1000 Reagents		5067-5585
TapeStation High Sensitivity D1000 Ladder		5067-5587
Qubit dsDNA HS Assay	ThermoFisher	Q32854
Ambion Nuclease-free Water		AM9937

Equipment

Item Description	Vendor	Catalogue #
Sciclone G3	Perkin Elmer	SG3-11020-0100/B SG3-31020-0300/E
Centrifuge	Eppendorf	Various
QubitFlex Fluorometer	ThermoFisher	Q33327
TapeStation 4200	Agilent	G2991A
Fragment Analyzer Automated CE System 48/96		FSv2-CE10F
c1000 Thermocycler	Bio-Rad	1841100

Sample Requirements

1. 10-20ng of cfDNA sample is diluted in a total of 50µL of low TE buffer and used for library preparation.
   Minimum batch size is 14 samples, but less may be run at the discretion of a manager.
   Maximum batch size is 88 samples.
   Note: the validated input range is 10-50 ng. The default input is listed above, however, Management may specify a different input within this range as required.

Sample Transfer

1. Samples are received in a 96-well plate.
2. Wells G03, H03, G06, H06, G09, H09, G12, H12 are always empty. They are reserved for positive and no template controls.
3. Each occupied well has 50µL of cfDNA at a minimum of 0.2ng/µL.

Positive Control Requirements

1. Dilute 20 ng of Positive control (GLCS_0002 or GLCS_0035) in 10 mM Tris pH 8.0-8.5 to a final volume of 50 µl.
2. Transfer samples to Covaris microTUBE-50 AFA tube through septum (Spin down briefly; ensure there are no bubbles around fiber prior to shearing). Load the tube into the sonication chamber.
3. Run protocol: 50ul_shear_150bp (Peak Incident Power: 75; Duty factor: 10%; Cycles/Burst: 200; Treatment Time: 360 seconds; Temp 20°C).
4. Briefly spin microtube containing sheared DNA, twist off cap and transfer.

Batch Controls

At least one pair of no template (NTC) and positive DNA controls must be included for every quadrant. A plate has 4 quadrants: Column 1-3, 4-6, 7-9 and 10-12 (inclusive). DNA positive controls GLCS_0002 (NA12878) and GLCS_0035 (TMB Mix score 7) are purchased from the Coriell Institute and SeraCare respectively. Control libraries are not sequenced but are included in **each** library synthesis batch and recorded in a MISO batch within the respective MISO project(s). Control libraries must be synthesized at the same time as a production library batch.

Control Distribution
Maximum sample capacity of the plate is 88 samples. In a full plate, 8 wells must be allocated to controls. In a full plate of 88 samples, every quadrant must end with a pair of controls, 1 positive and 1 negative control, for a total of 4 pairs of controls (**Figure 1**).

For partial plates, a pair of controls must be allocated closest to the final sample in the plate, while ensuring one pair of controls within each quadrant (**Figure 2**).

Pass/Fail Guidelines

QC standards are defined in QM. Quality Control and Calibration Procedures**.**

Pass: If the controls within quadrant meet QC standards, that quadrant of samples are considered a ‘pass’

Fail: If the controls within a quadrant do not meet QC standards, that quadrant of samples are considered a ‘fail’

In the case of Figure 2, if the controls on column 3 meet QC standards, but the controls on column 4 don’t, then samples within the 1st quadrant pass while samples in the 2nd quadrant (column 4) fail.

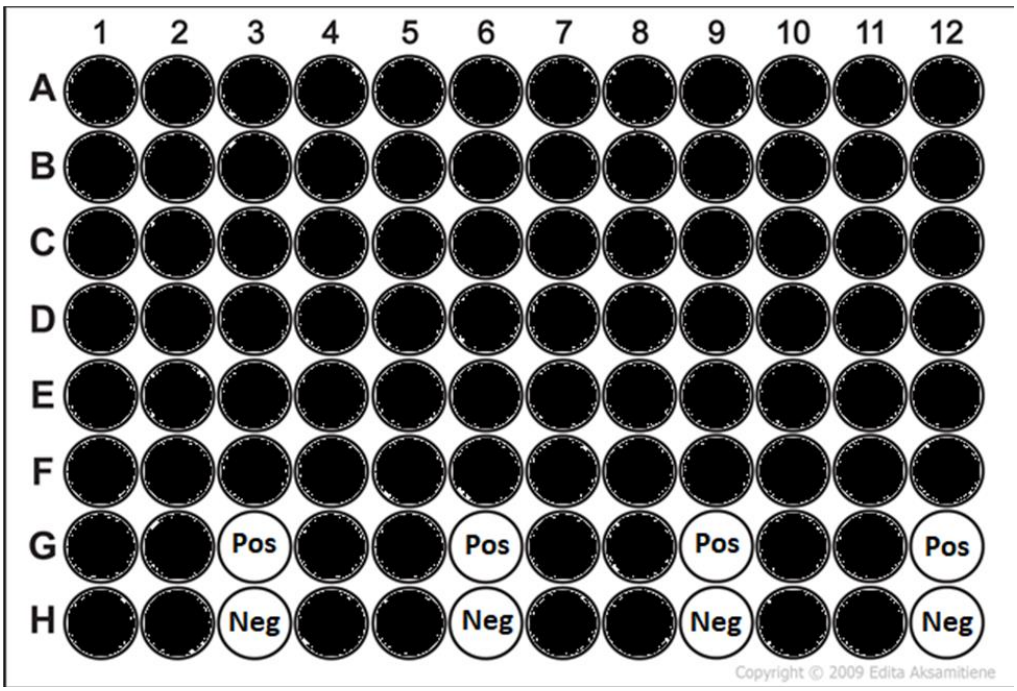


Figure 1: Full plate capacity with 88 samples and 4 pairs of controls

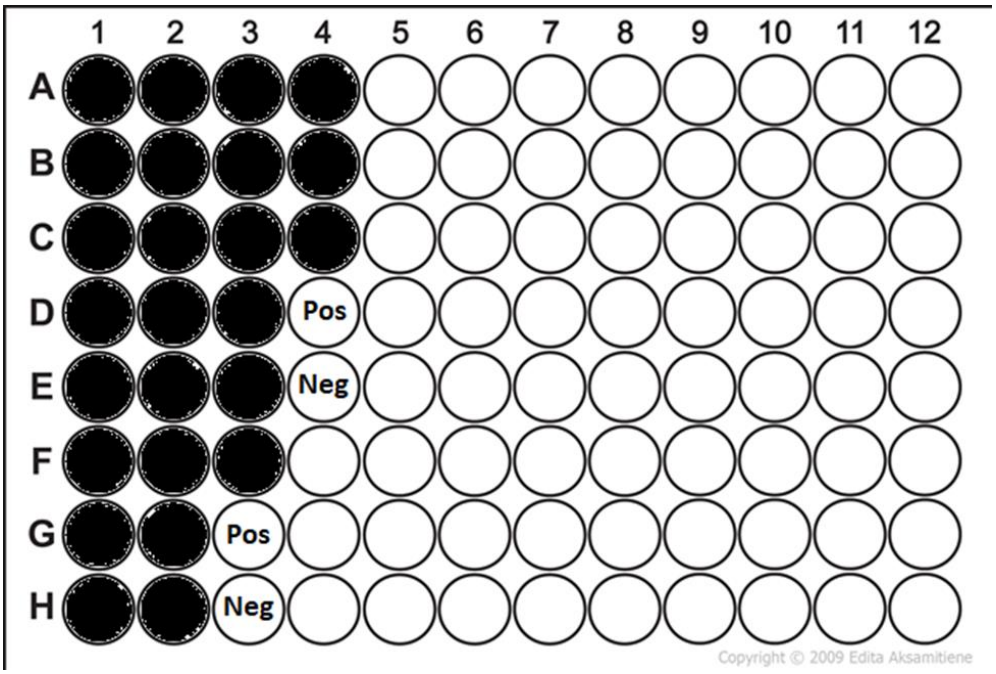


Figure 2: Example of partial plate with 25 samples and 2 pairs of controls

Variables and Observations to Record

The following library information is recorded and logged in MISO for **all** libraries.

- KAPA, IDT, index and other reagent lots
- Index used
- Quantity of input DNA (ng)
- Qubit quantification (ng/µl) of library
- % Adapter contamination
- Library size (bp) (Tapestation)

Important Considerations

Ensure that the following are used
- IDT xGen UMI Adapters
- IDT Dual Index (custom order, no cat#)
- Number of Library Amplification cycles (12).
- Use freshly-prepared 80% ethanol.

Reagent Lots and Expiration
- Prior to starting an assay, inspect reagents to ensure that they have not expired.
- Lot numbers for critical reagents must be recorded in MISO.

Recovering from Sciclone Errors

Errors listed below can be fixed and then the application can be continued. If the error is not listed below, follow the Emergency Response Procedure detailed in Sciclone G3 Operation SOP

1. **Initial lid movement error**
   If the first two lid movements fail to place the lid correctly, pause application, manually correct the lid placement, and resume the application.
2. **Util_UpdateConsumables error**
   This is an inconsistent and uncommon error that may randomly occur during a run. This occurs before any pipetting is done, so it is safe to click 'Retry action' to continue the application.

Procedure

Preparing Reagents Plates Ahead of Time

1. AMPure XP beads and low TE buffer used in the Sciclone library preparation can be aliquoted in plates ahead of time and stored at 4°C for at most 1 month.
- Volumes and which plates to use are specified in the Sciclone spreadsheet (C:\ProgramData\CaliperLS\Maestro\Workbooks\KAPA HyperPrep Plasma WG.xls).
- Thaw the following reagents:
  - AMPure XP Beads plate at room temperature.
  - 1X Low TE Buffer plate at room temperature.
  - IDT xGen UMI Adapters on ice.

- IDT Dual Index plate on ice.

Reagent Volumes

Volume used for one well. Extra volume may have been used to account for pipetting loss.

Library Prep

Reagent	**Volume (µL)**	**Comment**
End Repair + A-Tail Master mix	16	11.8 µL 10X KAPA End Repair/A-Tailing Buffer 5.0 µL KAPA End Repair/A-Tailing Enzyme
Ligation Master mix	51	35.5 µL KAPA Ligation Buffer 11.9 µL DNA Ligase 5.9 µL PCR Grade Water
KAPA HiFi HotStart ReadyMix	28	
Low TE Buffer	35	
AMPure XP Beads	110	
IDT xGen UMI Adapters	7	
IDT Dual Index	5	

Library Bead Cleanup

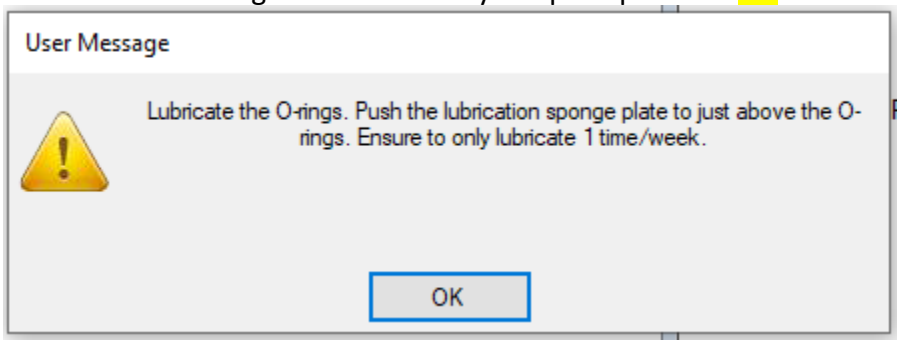
Reagent	Volume (µL)	Comment
AMPure XP Beads	65	
Low TE Buffer	35	

Library Preparation on Sciclone

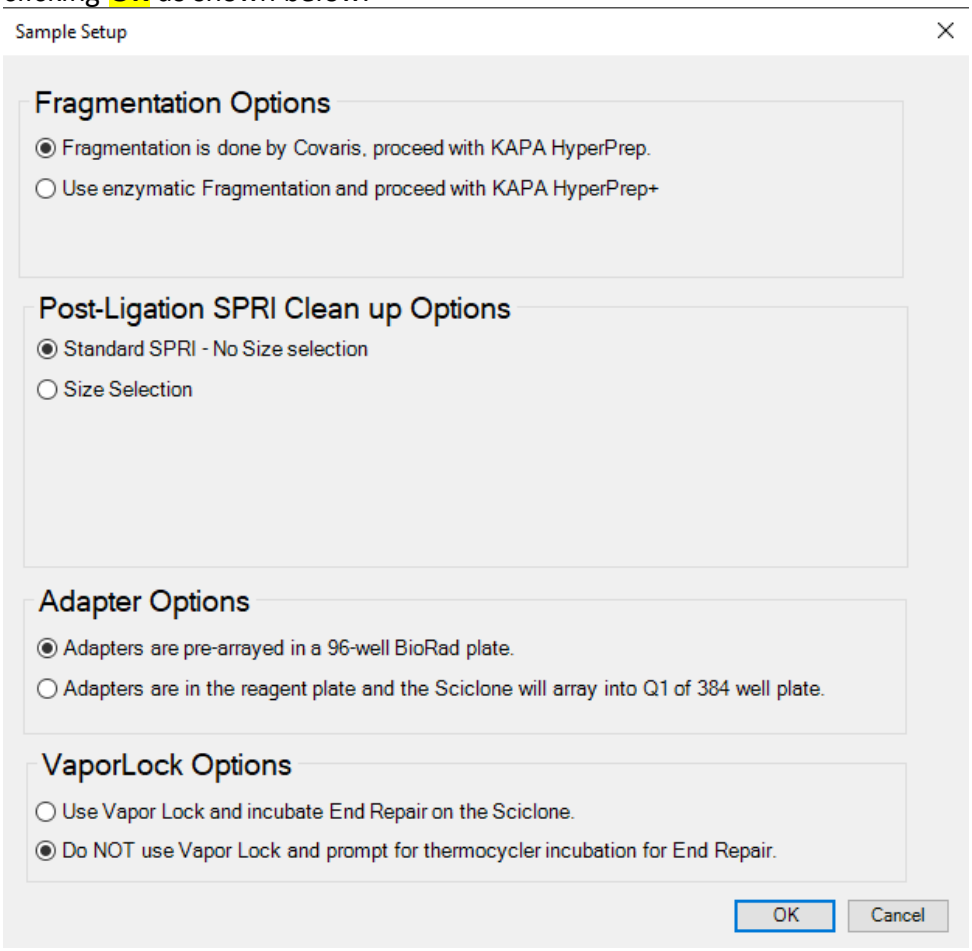
Sciclone startup is described in detail in the Sciclone G3 Operation SOP, located on the Genomics Quality SharePoint.

1. Wipe down the inside of the machine with 70% ethanol. Do not clean the gantry, gripper or main array.

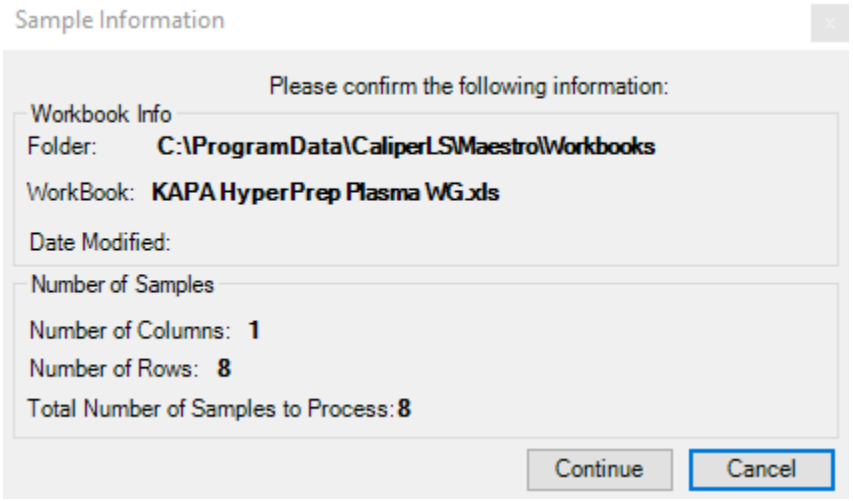
2. Turn on the Sciclone using the power switch on the left-hand side of the Sciclone. Ensure two green indicator lights on the upper left of the machine are on.
3. Turn on the INHECO Control Unit using the power switch on the back.
4. Open 'Maestro Workstation' on desktop.
5. Go to 'File' → 'Open Application'. All validated protocols will be in the PRODUCTION folder.
6. Open Application at **PRODUCTION\Kapa HyperPrep Plasma WG\1_Library_Prep_2022-08-25** and click the execution button ▶.
7. Lubricate the O-rings as instructed by the prompt. Press **OK** once completed.



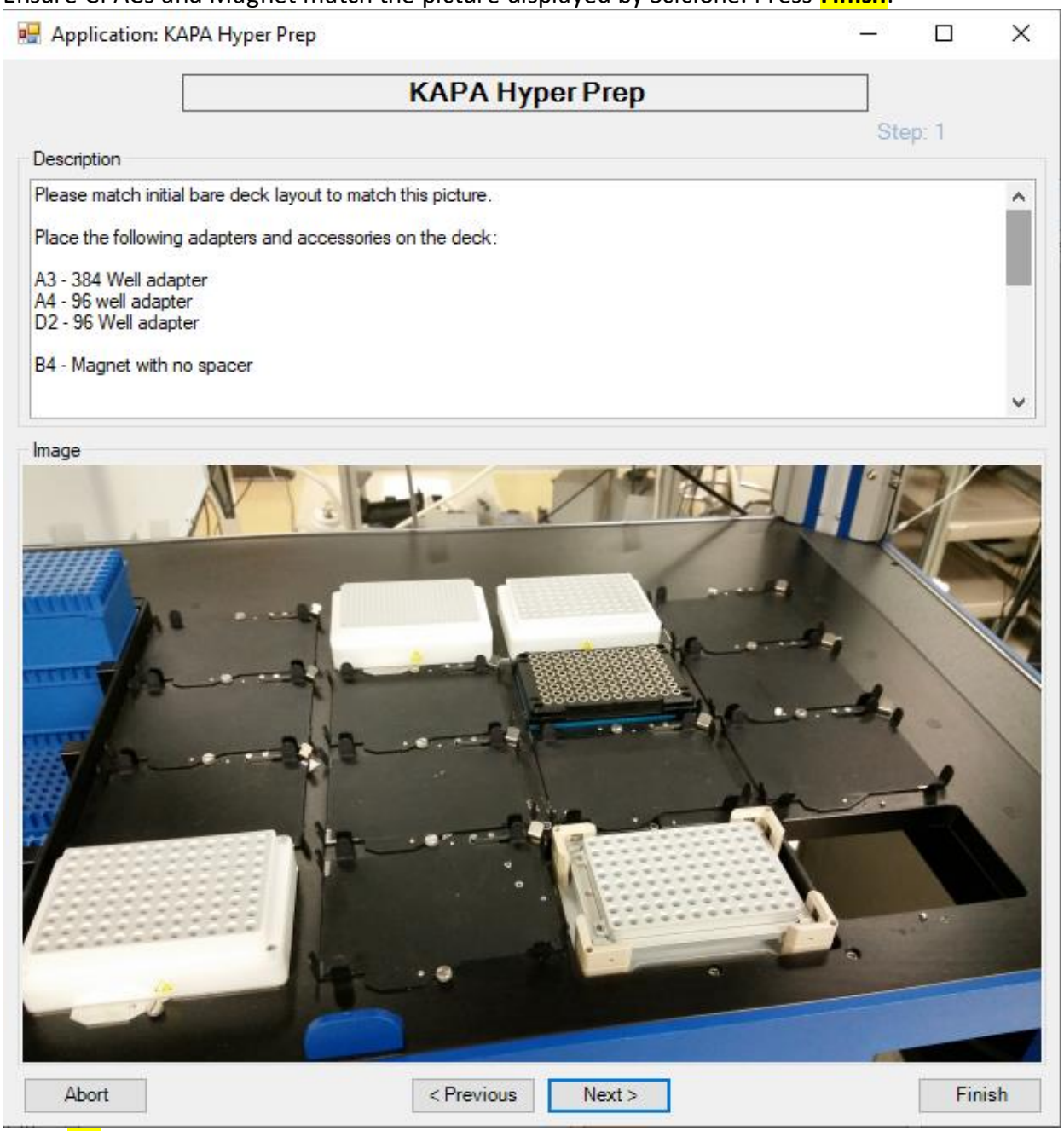
8. The application setup window will open. Ensure default options are selected before clicking **OK** as shown below:



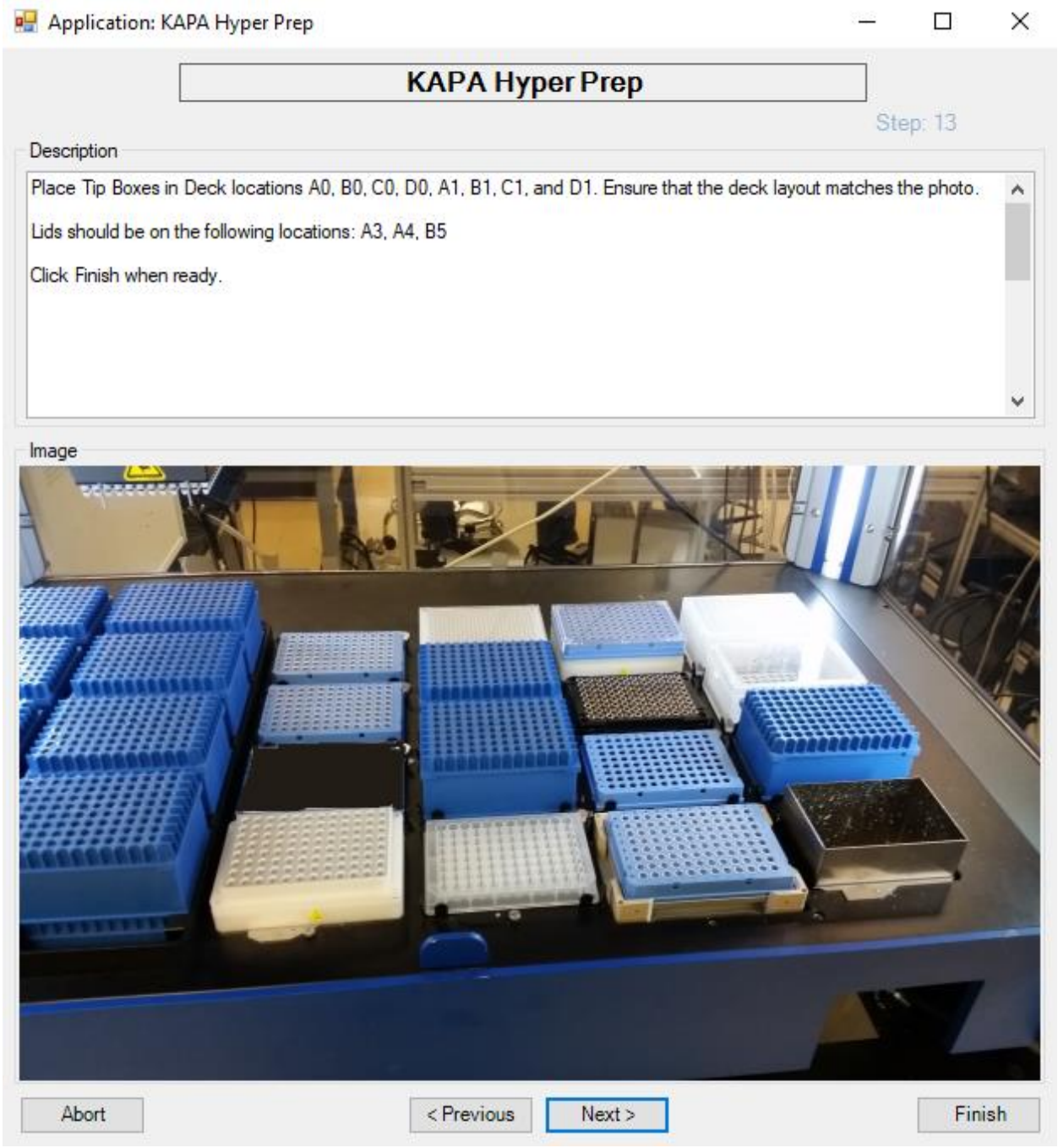
9. Wait for the Dialog box. Click **OK** after following instructions: "The Excel workbook has opened. Select the "***KapaHyperPrep Plasma WG***" sheet and enter the number of columns to process in the "*Number of Columns*" field. Save the Excel workbook. Click **OK** on this window."
    a. If Excel does not open, manually open the file at: C:\ProgramData\CaliperLS\Maestro\Workbooks\KAPA HyperPrep Plasma WG.xls
10. If reagent plates were prepared ahead of time, take out and thaw as described below. If not, take out reagents specified in Excel sheet and thaw as described in the 'Preparing Reagent Plates Ahead of Time' section. Aliquot reagents to plates specified in Excel sheet.
11. Press **Continue** after confirming '*Number of Columns*' is correct.



12. Ensure CPACs and Magnet match the picture displayed by Sciclone. Press **Finish**.

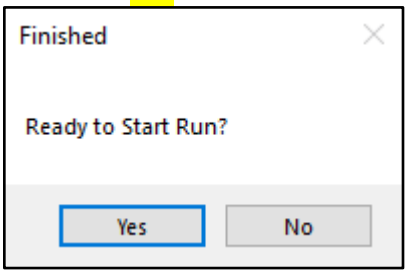


13. Press **OK** on the "Ready to Start Run?" dialog box.
14. Follow instructions for deck setup, using volumes specified in the open Excel sheet. Pull each plate to the top left (A1) position. Press the **Next >** button to proceed. Once the last picture is reached (see below), ensure the physical deck matches the displayed picture.



15. Ensure that lids are cleaned with 70% EtOH prior to placing them on plates.
16. Ensure that columns 0 and 1 contain full tip boxes (no lids).
17. Press the **Finish** button to continue the application.

18. Press the **Yes** button to continue. The Sciclone will start manipulating the deck.

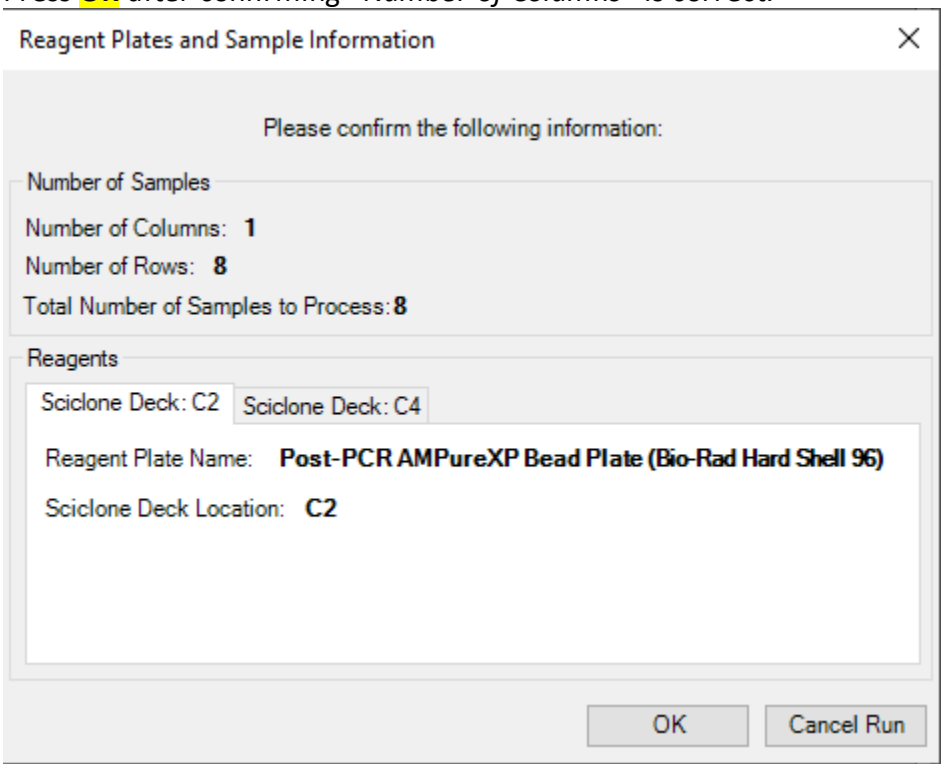


19. Follow the instructions of the Dialog Box when it opens: "1. Remove plate from location D4, seal carefully and place on thermocycler and run the CAP WG ER AT. 2. Click OK NOW to pre-broadcast Ligation Mix while the thermocycler runs."
    a. **Program Name**: CAP WG ER AT
    b. **Reaction Volume**: 60 µL
    c. **Cover Temperature**: 85 °C
20. A dialog Box will open with "Please wait for the End Repair A-Tail incubation to finish. When complete, spin down plate, remove seal and place plate on location B4. **NO LID!**" Follow instructions and click **OK**.
21. A dialog box will open with "Please place pre-arrayed UMI adapter plate at location D2 with LID". Follow instructions and click **OK**.
    Note: The Sciclone will proceed to perform the 2-hour ligation incubation at 20 °C on deck. On completion, it will immediately proceed to the post-ligation clean-up. During this clean-up the operator will be required to address the following prompts for additional reagent plates:
    a. A dialog box will open with "Please place pre-arrayed unique dual index primers at deck location D2 - WITHOUT LID". Follow instructions and click **OK**.
    b. A dialog box will open with "Remove and seal the PCR plate from D2. Centrifuge briefly and then run PCR enrichment program on thermocycler. Click **OK** to continue power down". Follow instructions and click **OK**.
22. Run the following program on the thermocycler:
    a. **Program Name**: CAP WG PCR Amplification
    b. **Reaction Volume**: 50 µL
    c. **Cover Temperature**: 105°C

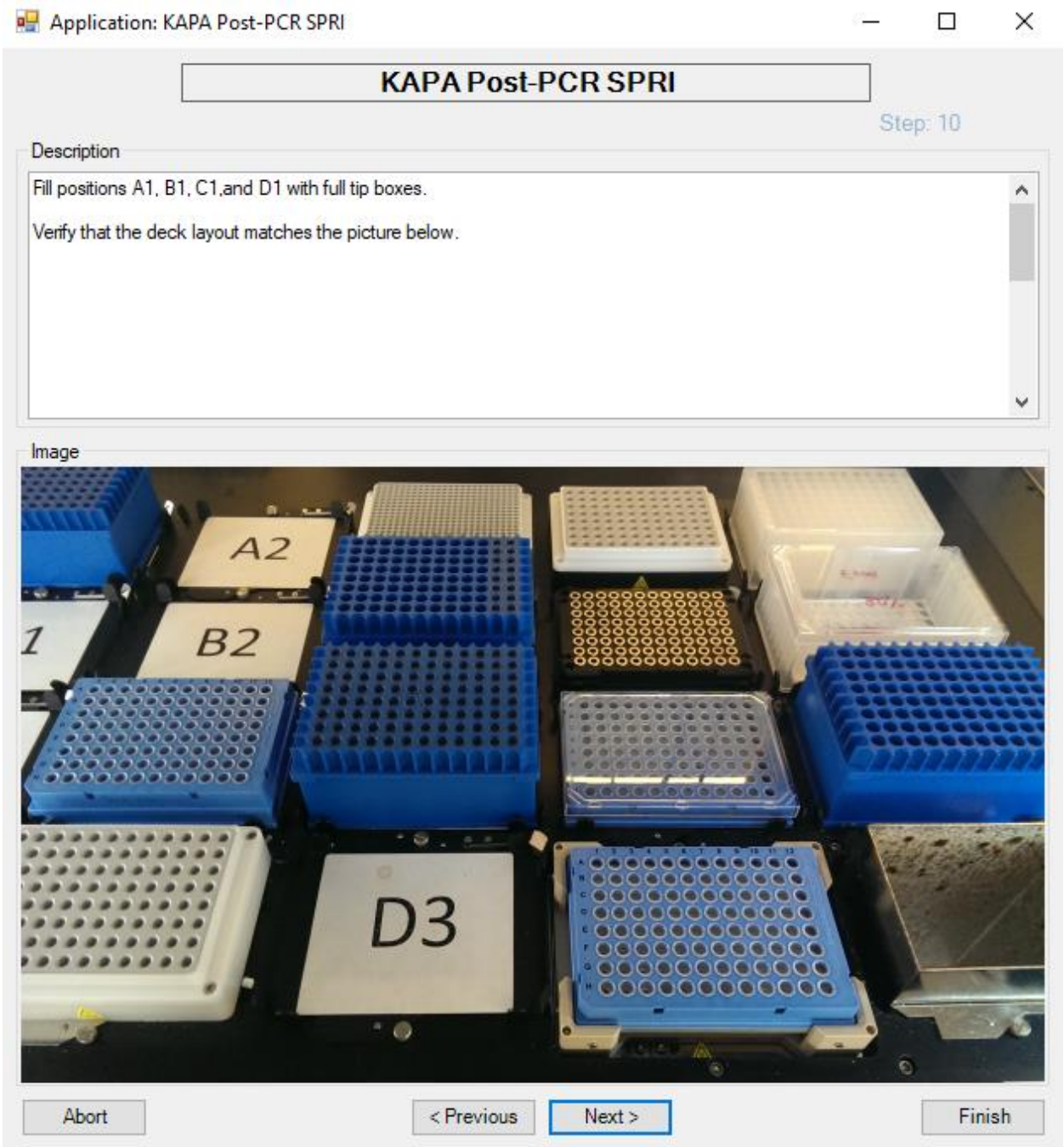
Step	Temperature (°C)	Time	Cycles
1	98	45 sec	1
2	98	15 sec	11
3	60	30 sec	
4	72	30 sec	
5	72	60 sec	1

6	4	Hold	1

23. Visually check the PCR reaction plate volumes and make note of any discrepancies.
24. The application has finished. While the PCR is running, start the application at **PRODUCTION\Kapa HyperPrep Plasma WG\2_Bead_Cleanup_2022-08-25** as described in the Sciclone Operation SOP.
25. Wait for the dialog box. Click **OK** after following instructions: "The Excel workbook has opened. Select the "***KAPA Post-PCR SPRI***" sheet and enter the number of columns to process in the "*Number of Columns*" field. Save the Excel workbook. Click OK on this window."
    a. If Excel does not open, manually open the file at: C:\ProgramData\CaliperLS\Maestro\Workbooks\KAPA HyperPrep Plasma WG.xls
26. Press **OK** after confirming "*Number of Columns*" is correct.

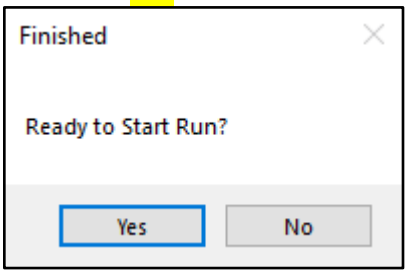


27. Follow instructions for deck setup, using volumes specified in the open Excel sheet. Press the **Next >** button to proceed. Once the last picture is reached (see below), ensure the physical deck matches the displayed picture.



28. Ensure that lids are cleaned with 70% EtOH prior to placing them on plates.
29. Ensure each plate is pulled to the top left (A1 position) and positioned correctly.
30. Ensure that columns 0 and 1 contain full tip boxes (no lids).
31. Press the **Finish** button to continue the application.

32. Press the **Yes** button to continue. The Sciclone will start manipulating the deck.



33. Once the bead clean-up is complete, the dialog box will show "Run complete. Remove the purified DNA sample plate from the shaker at D4 and store at -20°C." Store at -20°C or proceed to Library Quantity and Quality Assessment.
34. Press **OK** on the dialog box to finish up the application.
35. Go to 'Head Position' on the left control panel and move the gantry to deck location 'D5'.
36. Close 'Maestro Workstation'. Do not save any changes if prompted.
37. Turn off the Sciclone with the left side switch.
38. Turn off the INHECO Control Unit using the switch on the back.

    Note: Sciclone shutdown is described in detail in the Sciclone G3 Operation SOP, located on the Genomics Quality SharePoint.

Library Quantity and Quality Assessment

Plan Ahead: Thaw Qubit standards and Fragment Analyzer/TapeStation reagents to room temperature

1. Assess the library quantity by following TM. Genomics Qubit Fluorometric Quantification.
    a. Dilute libraries 1:5 before quantification as Qubit has an upper maximum of 120 ng/µL.
2. Assess library quality using the Fragment Analyzer or TapeStation
    a. Dilute libraries to 5 ng/µL using TM. Sciclone Aliquotting Procedure.
    b. Refer to the TM. Fragment Analyzer Assays SOP or TM. High Sensitivity TapeStation Assay, located on the Genomics Quality SharePoint.
3. Store plate at -20°C.

LIMS Entries

1. For general MISO instruction and training, see TM. LIMS Usage - MISO.
2. Propagate all MISO libraries from their gDNA Aliquots in the box. Check 'Create New Box' and provide all details, including the barcode using a scanner.
   Enter the following information for each Library:
    - Box Position: Set to 'Match parent positions'
    - Creation Date: Defaults to current date, but set to date of library creation
    - SOP: Set to "Plasma WGS Library Preparation- KAPA" (see version at the footer of SOP)
    - Thermal Cycler: Set to name of thermal cycler used

- Design: “PG”
- Platform: “Illumina”
- Type: “Paired End”
- Index Kit: “GRP TGL Dual Index UD Set 1 or 2”
- Index 1: Set to “index used for the library. Will autofill Index 2
- Has UMIs: “True”
- Kit: “KAPA Hyper Prep”
- Kit Lot: The lot number of the KAPA Hyper Prep Kit
- QC Passed: See Library Quality Assessment for criteria
- Size (bp): Determined during Library Quality Assessment
- Volume: Set to 28 µL, unless elution volume was less than expected
    - Elution volume is 30 µL, but 2 µL are used for QC
- Concentration Determined during Library Quality Assessment
- GroupID: Do not enter a groupID unless you are certain that it is required (e.g. for re-validation).

3. Propagate Library Aliquot. Prepare at least a 15 µL aliquot at 5 ng/µL of the stock library and propagate a matching MISO Library Aliquot from the MISO Library. Check “Create New Box” and provide all details, including the barcode using a scanner.
    - Box Position: Match parent positions here
    - QC Passed: “True”
    - Conc. 5 ng/µL
    - Volume: As prepared (µL)
    - Parent ng Used: Record the amount of stock library used to create the dilution
    - Parent Vol. Used: Record the volume (µL) of stock library used to create the dilution
    - GroupID: Do not enter a groupID unless you are certain it is required (e.g. for revalidation).
    - Creation Date: Defaults to current date, but set to date of dilution/aliquot creation
    - Prior to saving Libraries, check that all information is correct, and that the MISO entry matches the LIMs tracking sheet

4. Attach batch QC files to all stock libraries that were prepared during the same library synthesis batch:
    - Select all stock libraries prepared together in the same batch
    - Select Attach Files: Upload New Files
    - Choose files and pick the corresponding file “Category”:
        - Qubit csv file
        - Fragment Analyzer/TapeStation traces PDF
    - Only one file can be uploaded at a time
    - Repeat previous steps both QC files have been uploaded.

5. After all QC has been entered and the appropriate files have been uploaded Library aliquot plate can be passed to GRP as follows for sequencing:

a. Move the Library aliquot plate in the Freezer Tortuga, Shelf 2, Loose Storage 1 and update the locations in MISO.
b. Add the LDI for every sample passed on for sequencing into the Workset- GRP MiSeq QC Queue (For CAP-stream NovaSeq 2x151) in MISO.
c. Send an email to GenomicsLibrarySubmissions@oicr.on.ca with the Library Aliquot Plate ID to alert the sequencing team that there are libraries pending sequencing.