WGS Hamilton STAR Library Preparation - KAPA

Purpose

To describe the KAPA HyperPrep Kit Whole Genome Sequencing (WGS) library preparation procedure on the Hamilton Sequential Transfer Aliquoting Robot (STAR) liquid handler for up to 48 samples.

Scope

Following extraction and initial Quality Control (QC), samples in the WGS workflow must undergo library preparation. A library consists of DNA fragments with ligated adapters and indexes that are suitable for sequencing. This Standard Operating Procedure (SOP) outlines the procedure for the creation of WGS libraries on the Hamilton STAR from extracted gDNA using the KAPA HyperPrep kit and applies to all staff who perform WGS library preparation.

Responsibilities

Management:
Review and update procedure, as required.

QA Manager:
Monitor quality output from this procedure.

Laboratory Staff:
Follow this procedure and report any non-conformances that occur.

Reagents and Consumables

Note: All plastic consumables are dimension-specific and **cannot** be substituted.

Item Description	Vendor	Catalogue #
NA12878 DNA from LCL (gDNA positive control); GLCS_0002	Coriell	NA12878
KAPA HyperPrep Kit	Roche	7962363001
NucleoMag® NGS Clean-up and Size Select beads	Macherey-Nagel	744970
AMPure XP Beads	Beckman/Cedarlane	A36881

100% Ethanol	Sigma-Aldrich	E7023
1X Low TE Buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA	Life Technologies	12090-015
Ambion Nuclease-free Water	ThermoFisher	AM9937
IDT xGen Stubby Adapters 15 µM stock	Integrated DNA Technologies	10005924
IDT Dual Index (IDT unique indexing primer pool 20 µM)	Integrated DNA Technologies	custom with standard desalting
Hard-Shell® 96-Well PCR Plates	BIO-RAD	HSP9601
High Sensitivity NGS Fragment Analysis Kit	Agilent	DNF-474
DNA LoBind® Tubes	Eppendorf	022431021
50 µL Conductive Filter Tips	Hamilton Robotics	235948
300 µL Conductive Filter Tips	Hamilton Robotics	235903
1000 µL Conductive Filter Tips	Hamilton Robotics	235905
PCR ComfortLid	Hamilton Robotics	814300
96 Well 0.8 mL DeepWell Plates	ThermoFisher	AB0859
60-mL Reagent Reservoir	Hamilton Robotics	194051
Standard Sensitivity NGS Fragment Analysis Kit	Agilent	DNF-473
Covaris 96 microTube plate	Covaris (via D-Mark)	520078
Qubit dsDNA HS Assay Kit; RT, 4°C	ThermoFisher	Q32854
Qubit Assay Tubes	ThermoFisher	Q32856
LightSafe centrifuge Tubes	Sigma Aldrich	Z688320

Equipment

Item Description	Vendor	Catalogue #
Hamilton STAR	Hamilton Robotics	93774-03 (Microlab)
Centrifuge	Eppendorf	Various
Fragment Analyzer Automated CE System 48/96 Cap	Agilent	FSv2-CE10F
TapeStation 4200	Agilent	G2991AA
Covaris E220	Covaris (via D-Mark)	COV-500239
Qubit 4.0 Fluorometer	Thermofisher Scientific	Q33226
Qubit Flex Fluorometer	Thermofisher Scientific	Q33227

Variables and Observations to Record

The following library information is recorded and logged in MISO for **all** libraries including the no template control (NTC) and positive controls.

Record
Quantity of input DNA (ng)
Qubit quantification (ng/µL) (final library)
% Adapter contamination (final library)
Library size (bp) (TapeStation or Fragment Analyzer)
KAPA HyperPrep Kit lot number
IDT Dual indexes and other reagent lot numbers

Batch Controls

A plate has 4 quadrants: columns 1-3, 4-6, 7-9 and 10-12 (inclusive). At least one pair of NTCs and DNA positive controls must be included for every quadrant. DNA positive controls are purchased from the Coriell Institute (NA12878, MISO alias GLCS_0002). Control libraries must be synthesized at the same time as a production library batch.

Control Distribution

Maximum sample capacity of the plate is 48 samples. In a full plate of 48 samples, every quadrant must end with a pair of controls—1 positive and 1 NTC—for a total of 2 pairs of controls (Figure 1).

For partial plates, a pair of controls must be allocated closest to the final sample in the plate, while maintaining 1 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 the quadrant meet QC standards, then that quadrant of samples has passed.

Fail: If the controls within a quadrant do not meet QC standards, then that quadrant of samples has failed.

In the case of Figure 2, where the controls in column 3 meet QC standards and the controls in column 4 do not, the samples within the 1st quadrant pass, while samples in the 2nd quadrant (column 4) fail.

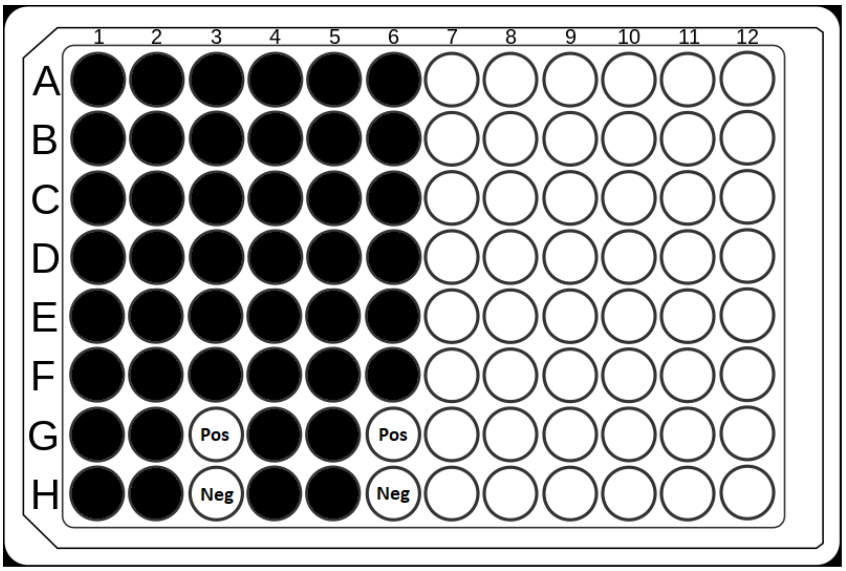

Figure 1: Full plate capacity with 44 samples and 2 pairs of controls.

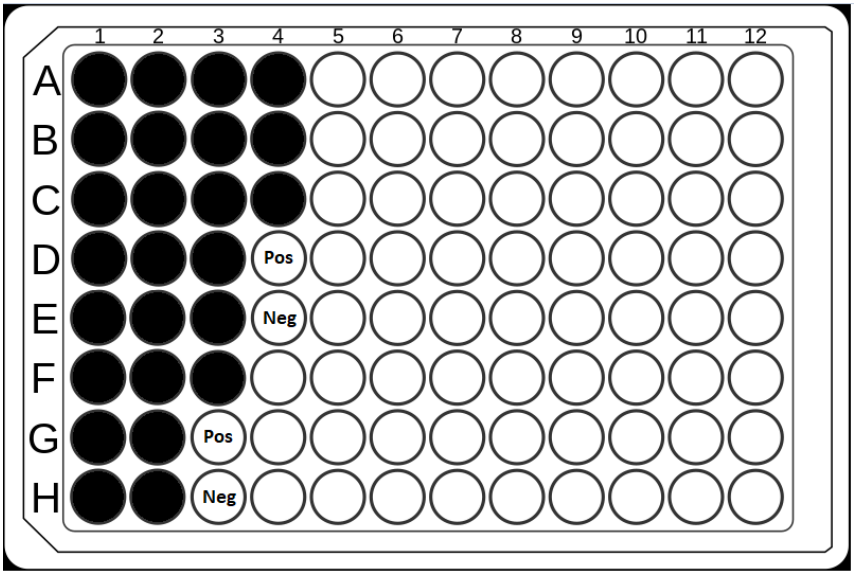

Figure 2: Example of a partial plate with 25 samples and 2 pairs of controls.

Important Considerations

<u>Ensure that the following are used:</u>

- IDT xGen Stubby Adapters
- IDT Dual Index
- Freshly prepared 80% ethanol

Reagent Lots and Expiration

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

Responding To Hamilton STAR Errors

Follow the Error Recovery procedure posted on the front of the machine.

Procedure

Sample Preparation

1. Samples are to be prepared or received in a 96-well Hard-Shell Plate (HSP), where wells G03, H03, G06 and H06 are always empty, as they are reserved for the positive and NTCs.
2. An input of 25 ng is used for fresh frozen (FF) tumour gDNA and 100 ng is used for buffy coat.
3. All input DNA is at a volume of 50 µL in low TE buffer.
4. If there are insufficient samples to fill a quadrant of the plate, add a positive control and a NTC to the last two available wells (Figure 2).

DNA Shearing

Shearing can be performed using either the Covaris M220 (individual samples) or E220 (larger batches) at the discretion of the operator and/or production manager. Brief instructions for each instrument are provided below, with detailed instructions in the TM. Covaris M220_E220 Use and Maintenance SOP.

Note: E220 degassing takes approximately 1-2 hours, so plan experiments accordingly.

Instructions for Covaris M220

1. Transfer DNA solution to a Covaris microTUBE-50 AFA Fiber Screw-Cap (PN520166).
    a. Pipette slowly and carefully to avoid creating bubbles in the tube.
2. Load microTUBE into the M220 insert, lower the tube lever and close the chamber door.
3. Use the following parameters:
    - M220 Shearing Parameters (WGS)
    - Shearing Program Name: **DNA_0550_bp_microtube-50_CAP**
    - Target Shearing Size: **~550bp**
    - Temperature Range:
      Min: 18°C / **Set Point:** 20.0°C / Max 22.0°C

Running Time	Peak Power	Duty Factor	Cyclers / Burst
40 seconds	75.0	10.0	200

4. After verifying parameters, select "**Run**".
5. Once the run is complete, remove the microTUBE and dry with Kimwipes.

SAFE STOP: The Covaris microTUBE-50 are **not** designed for long term storage. Samples must be transferred as soon as possible to a new PCR plate, which may be stored at -20°C for a maximum of 5 days.

Instructions for Covaris E220

1. Turn on the E220 and wait for 1-2 hours for it to degas prior to use.
2. Transfer the 50 µL from the prepared 96-well plate to a Covaris 96 microTube plate (no.520078).
3. Briefly centrifuge the microTube plate once all samples have been added.
4. Ensure that the following E220 shearing protocol is used:
   CAP - KAPA Hyper Prep WG Library Prep 60s
   Shearing parameters are as follows:
   - Peak Power: 140W
   - Duty Factor: 10
   - Cycles / burst: 200
   - Time: 60 seconds
   - Temperature Range: 4-7°C
5. Briefly centrifuge the microTube plate down once the E220 run has completed then transfer the sheared DNA to a Bio-Rad HSP.

SAFE STOP: The Covaris plate is **not** designed for long term storage. Samples must be transferred as soon as possible to a new Bio-Rad HSP, which may be stored at -20°C, for a maximum of 5 days.

Plan Ahead

Have these prepared for deck setup:

- Thaw IDT xGen Stubby Adapter (15 µM) on ice.
- Thaw KAPA HyperPrep Kit reagents (ERAT, Ligation and HotStart) on ice.
- Warm AMPure XP or Nucleomag beads to room temperature for at least 30 minutes.
- Thaw primer plate (20 µM) on ice.
- Thaw sample plate on ice.

Initialization

1. Detailed initialization is described in the Hamilton STAR Operation and Maintenance SOP, located on the Genomics Quality SharePoint.
2. Turn on the Hamilton STAR.
3. Turn on the Computer Programmable Automation Controller (CPAC).
4. Turn on the Hamilton Heater Shaker (HHS).
5. Turn on the On-Deck Thermocycler Controller (ODTC).

Log in to the computer and select "WGS KAPA Hamilton STAR Library Preparation" from MethodManager.exe.

Run Configuration

1. A window will appear to select a user.
    a. Select your name from the dropdown menu and press "Continue".
    b. If your name isn't in the dropdown menu, contact your manager.
2. A window will appear to select a start and end point.
    a. Ensure the following dropdowns are filled as listed:
        i. Start Point: "1. End Repair and A-Tailing"
        ii. End Point: "5. Post Amplification Cleanup"
    b. Press "Continue".
3. A window will appear to select a worklist.
    a. In the dropdown, select a number of samples that matches what you are. If the value of samples that you are working with does not match, round up to the nearest value.
    b. Press "Continue".

Deck Setup

1. A window will inform you that the front cover can be opened for deck loading.
    a. Press "OK".
2. A window will appear titled "Loading Plate Stacks and Tips".
    a. Place a PCR Comfort Lid in the PCR Lid position.
    b. Pull each of the plate and tip carriers from Track 1 to Track 24. Ensure that they are not pulled off the loading tray.
    c. For the plate carrier on track 1:
        i. Add one 0.8 mL 96 Well Plate to position 1.
        ii. Add one 0.8 mL 96 Well Plate to position 2.
        iii. Add two HSPs (stacked) to position 4.
    d. For tip carriers on Tracks 6 and 12:
        i. Add the required amount of 50 µL filter tips to any position on these racks.
    e. For tip carriers on Tracks 18 and 24:

i. Add the required amount of 300 µL filter tips to any position on Track 18 and positions 3, 4 and 5 on Track 24.
ii. Add the required amount of 1000 µL filter tips to position 1 and 2 of Track 24.

f. Add the required number of tips and ensure that they are in an appropriate position.

3. Push each of the plate and tip carriers in from Track 1 to Track 24. Ensure that they are pushed all the way back.
4. A window will appear instructing the user to store prepared reagents on ice and capped. The reagents are to be uncapped once instructed to put them on the deck.
    a. Click "OK".
5. A window will appear titled "End Repair & A-Tailing Mastermix".
    a. Create the End Repair - A-Tailing (ERAT) Mastermix in a 1.5 mL Eppendorf DNA LoBind tube using the two volumes specified in the window.
    b. Place the tube on ice.
    c. Click "OK".
6. A window will appear titled "Adapter Ligation Mastermix".
    a. Create the Adapter Ligation Master Mix in a 1.5 mL Eppendorf DNA LoBind tube using the two volumes specified in the window.
    b. Place the tube on ice.
    c. Click "OK".
7. A window will appear titled "Dilute 15 uM Stubby Adapters".
    a. Create the dilution in an Eppendorf DNA LoBind 1.5 mL tube using the two volumes specified in the window.
    b. Place the tube on ice.
    c. Click "OK".
8. A window will appear titled "Loading Reagents and Sample Plate".
    a. Prepare the remaining reagents required using the specified volumes listed in the diagram.
    b. Uncap all tubes before putting them on the deck.
    c. Place the following reagents according to the diagram on the screen (Figure 3):

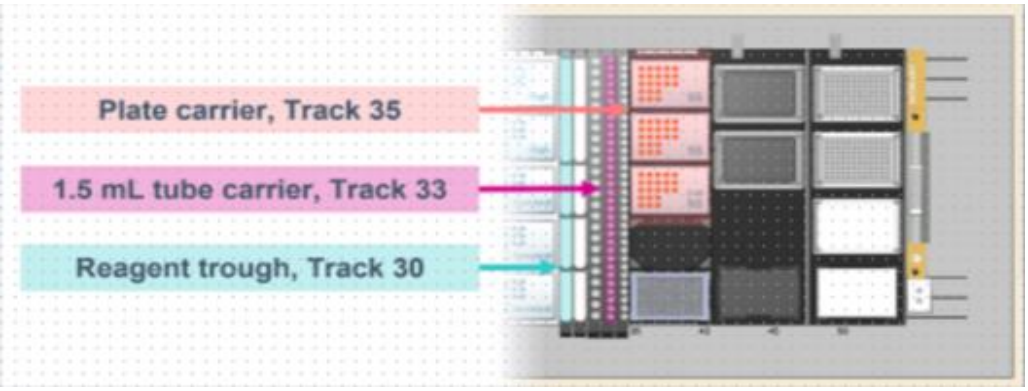

Figure 3: A visual representation of the Hamilton deck, and the key carriers for WGS Deck setup.

i. Low TE Buffer: trough on track 30, position 2.
ii. Ethanol (80%): trough on track 30, position 4 and, if required, position 5.
iii. UDI-Primer Plate (7 µL per well): carrier on track 35, position 1.
iv. Sample plate: carrier on track 35, position 2.
v. KAPA End Repair A-Tailing Mastermix: tube carrier on track 33, position 1.
vi. KAPA Ligation Mastermix: tube carrier on track 33, position 5.
vii. Stubby Adapter in an Eppendorf DNA LoBind 1.5 mL tube: tube carrier on track 33, position 7.
viii. KAPA HiFi Hotstart Readymix in a 1.5 mL tube: tube carrier on track 33, position 8.
ix. AMPure XP Beads in an Eppendorf DNA LoBind 1.5 mL Tube: tube carrier on track 33, position 19 through 26, inclusive.

d. Press "Continue".

9. A window will appear titled "Edit tip count".
    a. This is for the position of 50 µL tips
    b. Edit the window to have the virtual deck match the physical deck.
    c. Press "Continue".
10. A second window will appear titled "Edit tip count".
    a. This is for the position of 300 µL tips
    b. Edit the window to have the virtual deck match the physical deck.
    c. Press "Continue".
11. A third window will appear titled "Edit tip count".
    a. This is for the position of 1000 µL tips
    b. Edit the window to have the virtual deck match the physical deck.
    c. Press "Continue".
12. A window will appear titled "Setup Checklist".
    a. Check each item on the checklist and ensure that they have been carried out.
    b. Press "Continue".
13. A window will appear titled "Close Door Prompt".
    a. Ensure that the Hamilton STAR's front door has been closed.
    b. Select the checkbox once this has been completed.
    c. Press "OK".
14. A window will appear titled "Start of Run"; click "Ok" to start the run.
    a. Instrument will now move, the historical duration of methods is 5 hours (1 column) and 6 hours (3 columns).

Deck Cleanup

1. A window will appear titled "Deck Cleanup".
    a. Deck cleanup entails:

i. Remove the sample plate and either store the plate at -20°C or proceed to QC.
ii. Discard any consumables that are left within the Hamilton STAR.

2. At the end of the method, a window will appear titled “Cleanup Checklist”.
    a. Ensure each point has been successfully carried out.
    b. Press “Continue”.
3. A window will appear titled “End of Run”.
    a. Congratulations, your run has successfully completed.
    b. Press “OK” to exit the application.
4. Turn off the Hamilton Star and Accessory devices
    a. Turn off the HHS controller by flicking the green power button, located on the front of the device on the right-hand side.
    b. Turn off the CPAC by flicking the power button, which is located on the back of the device, left-hand side, near the top.
    c. Turn off the ODTC by flicking the power button on the back of the device, in the middle.
    d. Turn off the Hamilton STAR by flicking the green switch, located on the bottom-left corner of the machine.

Library Quality and Quantity Assessment

1. Assess the library quantity by running 1 µL of each library on Qubit dsDNA HS Assay.
    a. Refer to the TM. Genomics Qubit Fluorometric Quantitation SOP, located on the Genomics Quality SharePoint.
2. Library Quality may be assessed using either the TapeStation or Fragment Analyzer.
    a. Assess library quality using the TapeStation (High Sensitivity Tape).
        i. Refer to the TM. High Sensitivity TapeStation Assay SOP, located on the Genomics Quality SharePoint.
    b. Assess library quality using the Fragment Analyzer.
        i. Refer to the TM. Fragment Analyzer Assays SOP, located on the Genomics Quality SharePoint.
3. Store at -20°C.

LIMS Entries

1. For general MISO instruction and training, see TM. LIMS Usage - MISO.
2. Propagate one or more MISO Libraries from their respective gDNA Aliquots. If using the MISO bulk edit function, ensure that the order of samples has not been changed in MISO when propagating samples. Enter the following information for each Library:
    a. Matrix Barcode: Use barcode scanner while column is selected.
    b. Box Alias and Position: Use Box Search column to find correct Box Alias.
    c. Creation Date: Defaults to current date, but set to date of library creation.
    d. SOP: Set to WG Library Prep – KAPA Hyperprep v.X (see version at the footer of SOP).

e. Thermal Cycler
f. Design: Set to WG.
    i. Design tells the analysis pipeline to do alignment against a whole genome.
g. Platform: Set to Illumina.
h. Type: Set to Paired End.
i. Index Kit: Set to the Index set used for your batch.
j. Index 1: Set to the index used for the library. Will autofill Index 2.
k. Kit: Set to KAPA Hyper Prep.
l. Kit Lot: The lot number of the KAPA Hyper Prep Kit.
m. QC Passed: See Library Quality Assessment for criteria.
n. Size (bp): Determined during Library Quality Assessment.
o. Volume: Set to 31 µL, unless elution volume was less than expected.
    i. Elution volume is 33 µL, but 2 µL are used for QC.
p. Conc.: Determined during Library Quality Assessment.
q. Group ID: Do not enter a group ID unless you are certain that it is required (e.g. for re-validation). Group ID is inherited and has been set during sample accessioning.

3. Propagate Library Aliquot. Prepare a 25 µL aliquot at 5 ng/µl of the stock library and propagate a matching MISO Library Aliquot from the MISO Library:
    a. Matrix Barcode: Use barcode scanner while column is selected.
    b. Box Alias and Position: Use Box Search column to find correct Box Alias.
    c. QC Passed: Set to "True".
    d. Conc.: 5 ng/µL
    e. Volume: 25 µL
    f. Parent ng Used: Record the amount of stock library used to create the dilution.
    g. Parent Vol. Used: Record the volume (µL) of the stock library used to create the dilution.
    h. Group ID: Do not enter a group ID unless you are certain it is required (e.g. for re-validation). Group ID is inherited and has been set during sample accessioning.
    i. Creation Date: Defaults to current date, but set to date of dilution/aliquot creation.
    j. 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:
    a. Select all stock libraries prepared together in the same batch.
    b. Select "Attach Files": Upload New Files.
    c. Category:
        i. "LIMS tracking sheet": for scanned batch worksheet.
        ii. "Misc": for Qubit csv file.
        iii. "TapeStation": for run report.

d. Choose ”Files”: Attach the appropriate file corresponding to the category previously selected. Only one file can be uploaded at a time.
e. Press “Upload”
f. Repeat previous steps until all three Batch QC files have been uploaded.

5. After all QC has been entered and the appropriate files have been uploaded Library aliquots can be passed to GRP as follows for sequencing:
    a. Move the Library aliquot tubes into the GRP “CAP Miseq Inbox” BOX1580 and update the locations in MISO.
    b. Add the Library Dilution ID (LDI) for every sample passed on for sequencing into the following MISO workset: GRP MiSeq QC Queue (For NovaSeq 2x151).
    c. Send an email to GenomicsLibrarySubmissions@oicr.on.ca with the LDIs that were added to the Workset to alert the sequencing team that there are CAP libraries pending sequencing.