Sciclone Nextera XT DNA Library Creation SOP

Version Number: 1.2
Production Start Date: 02/17/2016
Version 1.2 Date: 6/23/2021

Summary

The purpose of this protocol is to generate DNA libraries from ultra-low input DNA samples with Caliper/Perkin Elmer Sciclone NGS robot and Illumina's Nextera XT DNA sample preparation reagents for sequencing on Illumina NextSeq and MiSeq analyzers. (Optional) If necessary, DNA libraries go through a size selection on Coastal Genomic's Ranger instrument.

Materials & Reagents

Material/Reagents/Equipment	**Vendor**	**Part Number**
Disposables		
BioRad 96 well HSP	BioRad	HSP 9631
Corning 384 well plate	Corning	3672
Seahorse Universal Lid	ISC Bioexpress	T-3181-11
Seahorse 96 Well 2ml Plate,	ISC Bioexpress	T-3180-24
Seahorse 12-Column Reagent Reservoir	ISC Bioexpress	B-0813-12
Barrier Sterile 96 Rack Tips, 150ul	Perkin Elmer	111426
Montage SEQ$_{96}$ Cleanup Kit (24 filter plates)	EMD Millipore	LSKS09624
StorPlate-96V, 450mL	Perkin Elmer	6008299
Pipet Tips, 300 ul, filtered	Hamilton	235903
Reagents		
Nextera XT DNA Sample Preparation Kit, includes: ATM (Amplicon Tagment Mix) TD (Tagment DNA Buffer) NT (Neutralize Tagment Buffer) NPM (Nextera PCR Master Mix)	Illumina	FC-131-1096
JGI unique 8bp dual-index (UDI) adapters,	IDT, custom design	06-######-##

plate 6		
(optional) Nextera XT Index Kit v2 Set A (96 indexes, 384 samples)	Illumina	FC-131-2001
(optional) Nextera XT Index Kit v2 Set B (96 indexes, 384 samples)	Illumina	FC-131-2002
(optional) Nextera XT Index Kit v2 Set C (96 indexes, 384 samples)	Illumina	FC-131-2003
(optional) Nextera XT Index Kit v2 Set D (96 indexes, 384 samples)	Illumina	FC-131-2004
Fragment Analyzer High Sensitivity Kit	Advanced Analytics	DNF-474-0500
1.5% Gel Cassette with 0.3+2Kb Marker	Coastal Genomics	CG-10600-01-150-12-24/96
Mag-Bind Total Pure NGS	Omega Bio-Tek	M1378-02
Alcohol, Ethyl, 200 proof, Pint	Fisher	111000200CSPP
Nuclease Free Water	Growcells	NUPW-1000
TE Buffer	Ambion	AM9849
Equipment		
Sciclone NGS	Perkin Elmer	
Ranger System	Coastal Genomics	
Fragment Analyzer	Advanced Analytics	
PCR Thermal Cycler	MJ Research	
Centrifuge		

EH&S

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

Procedure

NOTE: Defrost reagents ahead of time.

NOTE: Lubricate mandrils each time tip boxes are loaded.

NOTE: Starting DNA material amount is **1ng** of DNA.

1 Sciclone Start Up

1.1 Lubricate mandrils/head with black oil rack.

1.2 Open Maestro software on desktop.

Note: Pressing emergency stop or stop will make the software lose its place in the method.

Note: Open Sciclone door or press pause button in Maestro software to pause program.

2. Sample Controls and Reagent Plates Prep

2.1 Sample plates are listed in Plate Availability/Schedule gDoc under "Scheduled" tab.
   a. Enter your initials in the Library Creator cell to claim a plate.

2.2 Thaw sample plate on ice or in 4°C deli.

2.3 Samples should be 1ng in 5ul.

2.4 Centrifuge plate at 1,000rpm for 1 minute before peeling off the plate seal.

2.5 Set positive controls: Add 1ng of Lambda DNA to cell A1 and cell H12 and NF- free water to 5ul.

2.6 Set negative controls: Add 5ul NF- free water to cell A12 and cell H1.

2.7 Open Workbook 'Nextera XT Library Prep Workbook'
   a. Enter the number of columns to process in cell D2 and save.
   b. Follow excel instruction to prepare reagent plates.

2.8 Get all reagent plates ready before starting the Sciclone method.

3. Nextera Library Creation

3.1 Open Nextera XT library creation program in Maestra software.
   a. File > Open Application > production methods > (Nextera XT method)
   b. Click green execute arrow to start program.
   c. Follow Maestro instruction to setup deck.

3.2 Sciclone performs the following steps:
   a. Add 10ul of Tagment Buffer to each sample well.
   b. Add 5ul of Tagment Enzyme Mix to each well.
   c. Incubate at 55°C for 5 minutes, hold at 10°C.
   d. Add 5ul of Neutralize Tagment Buffer to each sample well.

e. Incubate at room temperature for 5 minutes.
f. Add 10ul of Index Primers (i5/i7) to each sample well.
g. Add 15ul of PCR Master Mix to each sample well.

3.3 Prompt user: Move plate to thermal cycler for amplification.

a. Remove sample plate from Sciclone and centrifuge at 1,000rpm for 1minute.
b. Put sample plate on thermal cycler and run program 'NEXTER > NEXTERA'
c. PCR conditions:
   - 72°C for 3 minutes
   - 95°C for 30 seconds
   - 12 cycles of:
     - 95°C for 10 seconds
     - 55°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 10°C

3.4 Once amplification complete, centrifuge PCR plate for 1 minute at 1,000rpm. Put plate back on Sciclone to finish up the remaining steps.

3.5 Safe stop point: Store sample plate in -20°C freezer until next step.

4. Library Size Selection on Ranger (optional)

Note: Perform this step when library samples need size selection

4.1 Prepare sample plate for DNA size selection on Ranger Instrument

a. Thaw the sample plate at room temperature. Quick spin plate for 1 minute at 1,000rpm, then put plate on ice.
b. Prepare a new Bio-Rad HSP96 plate. This will be the DNA Source plate. Add 3.5ul of 300bp&2kb DNA Marker to each well.
c. Transfer 25 ul of sample into above DNA Marker containing Bio-Rad HSP96 plate. Mix gently and spin the plate for 1 minute at 1,000rpm. Seal and store the remaining samples in -20°C freezer.

d. Keep sample plate on ice before loading it on Ranger instrument.

4.2 Setup deck

a. Cassette type: Use 1.5% agarose with 25 ul well capacity cassettes.
b. Place the DNA Source plate (BioRad 96 well HSP) on the deck.
c. Place an empty destination plate (StorPlate-96V, 450ml) on the deck.
d. Place sufficient cassettes on the electrophoresis pedestals.
a. Place cassette on pedestal, with the label facing forward
b. Insert electrodes, starting with the front electrodes

4.3 Start software

a. On Ranger computer: open program 'SSR'.
b. Define the agarose-type of each cassette on the deck: 1.5% agarose.
c. Define the samples in the source plate.
d. Select plate type ABGene 1000 (=Bio-Rad HSP96).
e. Select sample-type to run: 'Nextera-500'
f. Specify the loading volume for the samples: click on the 28.5ul button.

'Nextera-500' run parameters:

Marker Set:	300 bp+2000 bp
Cassette:	1.5% agarose
Voltage:	100VDC
Max Run Time(sec):	9000
Max Speed:	50%
Targeting:	**450 -600bp**
Raw output vol:	200ul

4.4 Close the instrument door.

4.5 Hit the green 'Start' button to begin the run

4.6 Clean-up when run complete

a. Place a tape-seal on the destination plate. Remove from the deck and spin down at 1,000rpm for 1 minute.

b. If not continue on step 5, store sample plate in -20°C freezer.

c. Shut down the software

d. Remove the electrodes from the cassettes. Rinse the electrodes and leave to dry for next run.

5. Library Concentration through 96-well filter plate (Optional)

Note: Perform this step on Ranger size selected DNA samples

5.1 On Ranger computer: open program 'Filter Plate Method'.

5.2 Press green arrow Start(F5) button to open Deck Layout window.

5.3 Follow up the deck layout instructions carefully to setup the deck.

5.4 Fill Elution Buffer Reservoir with TE or EB buffer.

5.5 Quick spin the sample plate to get rid of any bubbles in the samples before loading the sample plate on the deck.

5.6 Press 'OK' button to start the run.

5.7 Select sample volume 200ul from the pulldown list.

5.8 Prompt user to replace the sample plate with destination plate: Use Bio-Rad HSP96 as destination plate.

5.9 Turn on the vacuum when prompting user to do so.

5.10 Usually takes about 30 to 50 minutes to get all liquid through down the filter. Check frequently.

5.11 Once all liquid is gone, turn off the vacuum and wait for the suction on the filter plate to be released.

5.12 Continue program until it completes.

5.13 Remove the sample plate. Centrifuge at 1,000rpm for 1 minute. Keep it on ice.

6. QC Samples with Fragment Analyzer

6.1 Use High Sensitivity NGS Fragment Analysis Kit, 1bp-6,000bp.

6.2 Prepare sample QC plate according to manufacture user guide.

a. Mix 2ul Samples or Ladder with 22ul Diluent Marker.

b. Add 24ul Blank Solution to unused wells.

6.3 Save QC files in Fragment Analyzer folder.

7. Clarity Submission

7.1 Open Clarity (https://clarity-prd01.jgi-psf.org/clarity/login/auth).

7.2 Click on LAB VIEW icon to show the AVAILABLE WORK list.

7.3 Find the sample plate from LC Illumina Nextera with Ranger Size Selection,plate, or LC Illumina Nextera, plate, or LC Production RnD, plates, click on Step 1>> LC Library Creation.

7.4 Select the sample plate and click on Add Group to add into the ICE Bucket

7.5 Click on the green VIEW ICE BUCKET button.

7.6 Click on BEGIN WORK button.

7.7 In the new window, the samples were placed in a new plate. Check and ensure the pattern is correct.

7.8 Click on RECORD DETAILS button.

7.9 Fill in all the details. Select printer (BCode 10 is for plate samples).

7.10 Download, fill in and upload the worksheet.

7.11 Upload required files

7.12 Click on NEXT STEPS button.

7.13 Click on FINISH button

7.14 Label the library plate with barcode label.

8. qPCR Submission

8.1 Open GLS worksheet saved in Fragment Analyzer folder (from previous step).

8.2 Open “qPCR Sample Submission and Tracking” google doc.

a. Open the current plate request tab, ex: Plate Request Jan’17.

b. Find an empty section and fill in all of the highlighted cells (ex: plate name, sample size).

c. Erase any names for empty wells and ensure corners are marked as controls.

d. Open “MiSeq and pools qPCR request” tab and fill out one line.

8.3 Transfer the library plate to qPCR box in -20°C freezer.

a. Ensure the max volume/well no larger than 50ul.

9. Plate Availability/Schedule qDoc Update

9.1 Update tab Scheduled:

a. Remove “in progress” comment, and enter dates for “lab lib creation complete” and “GLS lib creation complete”.

b. Highlight row in green to signify it was successfully completed.

c. Highlight row pink if plate failed.

9.2 Update tab Nextera

Reagent/Stock Preparation

JGI UDI Adapter Plate 6: Single Use Plate 6 Preparation, 5 uM /10 ul per well

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91

D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

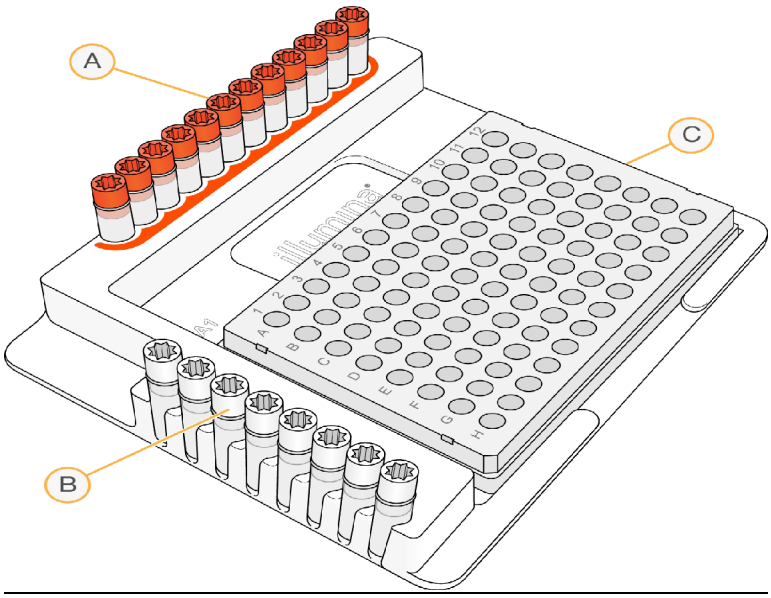
Note : Do not vortex adapter plates.

1. Adapter stock plates 6 (96 well) are provided by Sample Management.
2. Defrost stock plate at 4C.
3. Use Sciclone to gently pipette mix adapter stock plate. Spin down plate.
4. Use nuclease free water to dilute adapters to 5 uM.
5. Use Sciclone to gently pipette mix diluted adapter plate. Spin down.
6. Stamp out single use adapter plates with 5 uM and 10ul in BioRad HardShell 96 well plates.
7. Spin down single use plates and check to make sure each has all 96 adapters in plate.
8. Label plates with adapter type, concentration, volume, date, and lot info (ex: 06-042021-01).
9. Record adapter plate creation in the Plate Availability/Schedule gDoc in the operator notes tab.

(optional) Nextera XT Index kits: Single Use Index Primer Plate preparation

1. Arrange the index primers in the TruSeq Index Plate Fixture.
2. Arrange index 1 (i7) primers (orange caps) in order horizontally.
3. Arrange index 2 (i5) primers (white caps) in order vertically.
4. Using a multichannel pipette, add 5 µl index 1 primers (i7) to each row of the primer plate. ***Changing tips between rows is required to avoid cross-contamination.***

5. Using a multichannel pipette, add 5 µl index 2 primers (i5) (orange caps) to each column of the primer plate. ***Tips must be changed after each column to avoid index cross-contamination.***
6. To avoid index cross-contamination, discard the original caps and apply new caps provided in the kit.



Instrument Setup / Maintenance

Sciclone NGS:

1. Power cycle the inheco controllers each week.

2. Dye QC
   Note: Perform QC at start of each week.
   Note: Store dye at room temperature, and protect from light.

   a. In Maestro: File > open application > production methods > Dye QC.

Method adds 75ul water into costar plate, then adds 2ul dye.

b. Follow set-up instructions and pour just enough dye into a reservoir to cover the bottom.
c. After running the method, pour extra dye into a 50ml tube and protect from light. Discard dye after 2 weeks into SAA.
d. Seal costar plate with a foil seal, spin down, and place on plate shaker to allow dye to disperse for 1 minute.
e. Remove seal and read using Eosin Y Dye QC method on Victor.
f. Copy the well data and paste into the appropriate tab in Sciclone 2ul Eosin Y 370mgL MK vVictor file.
g. File located at: Octopus > prodseq > Dye QC tests > Eosin Y Dye QC > Sciclone 2ul Eosin Y 370mgL MK Victor.
h. Check that the average and CV both pass.
i. Notify Instrumentation group if a well fails two times in a row.

Ranger Gel Electrods Cleanup :

1. Every time when DNA size selection is completed, rinse all gel electrodes with warm tap water followed with Milli-Q water, leave dry in a clean container for next run.

Troubleshooting

SOP Approval

DEPARTMENT	APPROVED BY	DATE
Lab Supervisor		

Appendix

Change History

01/23/2017 – Changed from GLS date entry (v1.0) to Clarity data entry. Updated from v1.0 to v1.1.

06/23/2021 – Started using a new index plate, JGI UDI plate 6, for sample plates. Added the new UDI plate 6 in SOP and updated to v1.2. Still kept the Illumina Indexes in SOP as options.