FLUIDIGM®

PN 100-7135 I1

Using C1 to Generate Single-Cell Libraries for DNA Sequencing

PROTOCOL

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About this Guide

CAUTION ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For complete safety information, see the safety appendix on page 83.

For detailed instructions on instrument and software operation, refer to the C1 System User Guide (PN 100-4977).

Safety Alert Conventions

This guide uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.

Safety Alerts for Chemicals

Fluidigm follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) for communicating chemical hazard information. GHS provides a common means of classifying chemical hazards and a standardized approach to chemical label elements and safety data sheets (SDSs). Key elements include:

- Pictograms that consist of a symbol on a white background within a red diamond shaped frame. Refer to the individual SDS for the applicable pictograms and warnings pertaining to the chemicals being used.

- Signal words that alert the user to a potential hazard and indicate the severity level. The signal words used for chemical hazards under GHS:

  DANGER Indicates more severe hazards.

  WARNING Indicates less severe hazards.

Safety Alerts for Instruments

For hazards associated with instruments, this guide uses the following indicators:

- Pictograms that consist of a symbol on a white background within a black triangle shaped frame.

- Signal words that alert the user to a potential hazard and indicate the severity level. The signal words used for instrument hazards:

  DANGER Indicates an imminent hazard that will result in severe injury or death if not avoided.

  WARNING Indicates a potentially hazardous situation that could result in serious injury or death.

  CAUTION Indicates a potentially hazardous situation that could result in minor or moderate personal injury.

  IMPORTANT Indicates information necessary for proper use of products or successful outcome of experiments.

Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain SDSs for chemicals ordered from Fluidigm Corporation, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Revision History

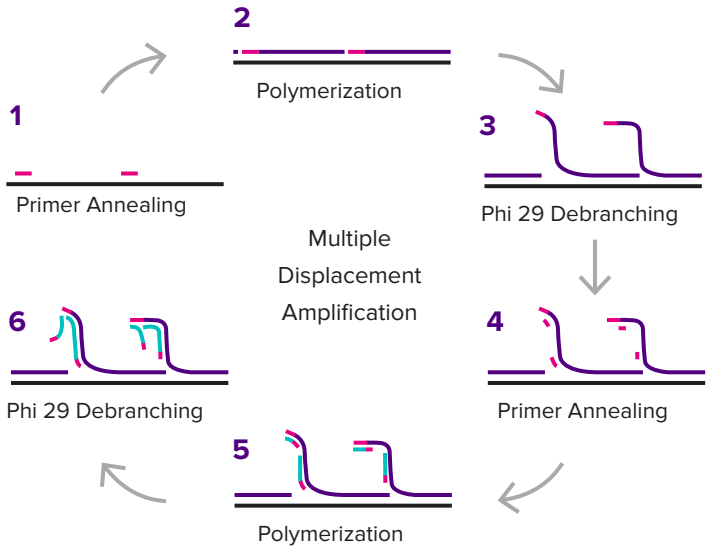
Revision	Date	Description of change
I1	07 June 2016	• Updated formatting. • Changed all instances of Life Technologies to Thermo Fisher Scientific (various pages throughout). • Updated the legal boilerplate (see page 2). • Updated the priming and loading/staining script times (see page 11, page 14, page 32, and page 37). • Updated the C1 reagent kit name (see page 16 and page 79). • Updated the cell dilution recommendation (see page 34). • Updated the input cell concentration range (see page 35). • Updated cell suspension ratio recommendation (see page 35). • Increased cell mix load volume to 6 µL (see page 36 and page 78).
H1	29 May 2015	Updated safety and technical support contact information.
G1	15 April 2015	Changed “C1 Suspension Reagent” to “Suspension Reagent.” (See Required Reagents on page 16.)
F1	10 February 2015	• Changed the volume of Stop Tagment Buffer from “an equal volume” to 3.75 µL. (See Prepare Genomic Samples: Perform Tagmentation, step 13.) • Changed “C1 DNA Suspension Reagent” to “C1 DNA Dilution Reagent.” (See Prepare Genomic Samples: Perform Tagmentation, step 2.) • Improved formatting in the protocol and added new capture site map. (See Harvest the Amplified DNA Products on page 44.)
E1	3 November 2014	Updated product names, illustrations, and legal boilerplate to new branding specifications.
D1	9 July 2014	• Updated content on preparing genomic samples, whole genome sequencing, and whole exome sequencing. • Included new instructions on use of 1X Access Array Hydration Reagent v2 to prepare libraries for targeted sequencing. (See Prime the Access Array 48.48 IFC on page 50 and Harvest the Access Array 48.48 IFC on page 55).
C1	8 April 2014	Added new protocols on whole genome and whole exome sequencing.
B1	4 April 2014	Added new content on thawing cells.
A1	4 December 2013	Released new document on use of the Fluidigm® C1 system with the C1 Reagent Kit for DNA Seq to perform targeted sequencing of genomic DNA from single cells.

Generate Single-Cell Libraries for DNA Sequencing

Introduction

This protocol describes how you perform DNA sequencing from single cells. First, whole genome amplification (WGA) is performed with the C1™ system and the C1 integrated fluidic circuit (IFC).

Whole genome amplification as described in this protocol is achieved through multiple displacement amplification with the illustra™ GenomiPhi™ V2 DNA Amplification Kit (GE Healthcare Life Sciences). After cells are lysed and the DNA denatured, random hexamers anneal to single-stranded DNA. Phi29 polymerase is used to amplify the randomly primed regions of the genome. The amplification process continues with the displacement of the DNA strand and annealing of random hexamers, generating a high single-cell genomic yield for analysis. Multiple displacement amplification is isothermal and has a low error rate due to the proof-reading capability of the Phi29 polymerase. The average product length with multiple displacement amplification is ~10kb, and the average yield using a C1 IFC is 12 ng/µL per cell in ~13 µL final volume:



After whole genome amplification, you choose targeted, whole genome sequencing,* or whole exome sequencing of your libraries. For targeted sequencing, you perform targeted enrichment with the Access Array™ system and Access Array IFC. For whole genome sequencing, you perform tagmentation of the harvested products from the C1 system followed by PCR amplification and library pooling. For whole exome sequencing, you follow the steps for whole genome sequencing, and then perform two hybridization steps, two enrichment steps, and PCR amplification.

Targeted sequencing, whole genome sequencing, and whole exome sequencing provide you unique and common benefits in genomic analysis:

DNA sequencing applications	Unique benefits	Common benefits
Targeted sequencing (See Targeted Sequencing on page 50.)	• Investigate selected genes or gene regions that are putatively associated with complex disease. • Perform targeted resequencing spanning all protein-coding regions of the genome. • Reduce analytical time and resources.	• Discover germline or somatic variants to characterize disease mechanisms and risk. • Identify the unique characteristics of cell subpopulations. • Sequence and structural variations (SNPs, small indels, translocations, and inversions). • Identify and authenticate induced pluripotent cells to ensure traceability to origin.
Whole genome sequencing (See Prepare Samples for Whole Genome and Whole Exome Sequencing on page 58.)	Discover variants in regulatory and functional regions of the genome in a hypothesis-free manner.	
Whole exome sequencing (See Whole Exome Sequencing on page 67.)	Run hypothesis-free analysis of functional, protein-coding regions of the genome.	

* The section, “Whole Genome Sequencing” (see Whole Genome Sequencing on page 64) is an Experimental Method and is provided “as is.” NO WARRANTIES ARE PROVIDED, EXPRESSED, OR IMPLIED. ALL WARRANTIES, INCLUDING THE IMPLIED WARRANTIES OF FITNESS FOR PURPOSE, MERCHANTABILITY, AND NON-INFRINGMENT, ARE EXPRESSLY DISCLAIMED. Every effort has been made to avoid errors in the text, diagrams, illustrations, figures, and screen captures. However, Fluidigm assumes no responsibility for any errors that may appear in this publication. It is Fluidigm policy to improve products as new techniques and components become available. Therefore, Fluidigm reserves the right to change specifications at any time. Information in “Whole Genome Sequencing” is subject to change without notice. Fluidigm assumes no responsibility for any errors or omissions. In no event shall Fluidigm be liable for any damages in connection with or arising from the use of this Experimental Method.

Workflows

Follow the appropriate workflow according to your DNA sequencing application:

- Cell capture and amplification: See Whole Genome Amplification and Targeted Sequencing of Single Cells by DNA Sequencing on page 11.
- Targeted sequencing: See Whole Genome Amplification and Targeted Sequencing of Single Cells by DNA Sequencing on page 11.
- Cell capture and preparation of genomic samples for whole genome sequencing and whole exome sequencing: See Prepare Genomic Samples, Whole Genome Sequencing (WGS), and Whole Exome Sequencing (WES) on page 14.
- Whole genome sequencing and whole exome sequencing: See Prepare Genomic Samples, Whole Genome Sequencing (WGS), and Whole Exome Sequencing (WES) on page 14.

Whole Genome Amplification and Targeted Sequencing of Single Cells by DNA Sequencing

Day 1: C1 IFC run

Day 2: Access Array IFC runs. Runs require two Access Array 48.48 IFCs to prepare 96 samples. Begin loading the second IFC 1 hour after thermal cycling begins on the first IFC.

Day 3: Barcoding and sequencing

Table 1. Whole genome amplification and targeted sequencing of single cells by DNA sequencing

	Reagent handling	Automated steps	Estimated time
Day 1: C1 IFC Run			
1	Thaw reagents.		5 min
2	Pipet priming solutions into IFC.		5 min
3		Prime IFC on the C1 system.	• Small- or large-cell IFC: 10 min • Medium-cell IFC: 12 min
4	Pipet cells into IFC.		5 min
5		Load cells on the C1 system.	Small-cell IFC: • 30 min if staining • 20 min if not staining Medium-cell IFC: • 65 min if staining • 35 min if not staining Large-cell IFC: • 60 min if staining • 30 min if not staining
6	Image cells with microscope.		15–30 min
7	Prepare reagent premixes.		15 min
8	Pipet lysis, stop buffer, reaction-enzyme mix, and harvest reagents into IFC.		5 min

Table 1. Whole genome amplification and targeted sequencing of single cells by DNA sequencing

	Reagent handling	Automated steps	Estimated time
9		Run DNA Seq script on the C1 system: Loading, thermal, and harvest protocol for single-cell lysis and whole genome amplification.	375 min for small-cell IFC 450 min for medium- or large-cell large IFC You can run the script overnight with a pause between DNA Seq and Harvest functions. You have up to 60 min after the script finishes to remove the harvest amplicons from the IFC.
10	Proceed to targeted sequencing (Access Array IFC run) or whole genome sequencing		

Table 1. Whole genome amplification and targeted sequencing of single cells by DNA sequencing

	Reagent handling	Automated steps	Estimated time
Day 2: Access Array IFC Runs			
Runs require two Access Array 48.48 IFCs to prepare 96 samples. Begin loading the second IFC 1 hour after thermal cycling begins on the first IFC.			
1	Harvest amplified DNA from IFC.		10 min. Store amplicons at 4 °C until use on a 48.48 Access Array (AA) IFC.
2	QC harvest (PicoGreen® suggested)		30 min
3	Pipet priming solutions into IFC.		5 min
4		Prime AA IFC on pre-PCR IFC Controller AX	20 min
5	Prepare reagent pre-mixes, samples, and assays.		15 min
6		Load on AA pre-PCR IFC Controller	60 min
7		Thermal cycle AA	2.5 hr
8	Pipet harvest reagents.		10 min
9		Harvest amplicons from IFC on AA post-PCR IFC Controller AX	60 min
10	Harvest amplicons from the Access Array IFC.		10 min
11	QC amplicons (Bioanalyzer® Assay)		30 min
	Reagent handling	Automated steps	Estimated time
Day 3: Barcoding and sequencing			
1	Prepare barcoding reaction (96-well plate)		30 min
2		Run barcoding reaction (stand-alone plate thermal cycler)	50 min
3	Pool and clean up products		60 min
4		QC on Bioanalyzer	30 min
5		Sequencing	
6		Sequence analysis	

Prepare Genomic Samples, Whole Genome Sequencing (WGS), and Whole Exome Sequencing (WES)

Day 1: C1 IFC run

Day 2: Prepare genomic samples

Day 3: WGS or WES

Table 2. Prepare genomic samples, whole genome sequencing (WGS), and whole exome sequencing (WES)

	Reagent handling	Automated steps	Estimated time
Day 1: C1 IFC Run			
1	Thaw reagents.		5 min
2	Pipet priming solutions into IFC.		5 min
3		Prime IFC on the C1 system.	• Small- or large-cell IFC: 10 min • Medium-cell IFC: 12 min
4	Pipet cells into IFC.		5 min
5		Load cells on the C1 system.	Small-cell IFC: • 30 min if staining • 20 min if not staining Medium-cell IFC: • 65 min if staining • 35 min if not staining Large-cell IFC: • 60 min if staining • 30 min if not staining
6	Image cells with microscope.		15–30 min
7	Prepare reagent pre-mixes.		15 min
8	Pipet lysis, stop buffer, reaction-enzyme mix, and harvest reagents into IFC.		5 min
9		Run DNA Seq script on the C1 system: Loading, thermal, and harvest protocol for single-cell lysis and whole genome amplification.	375 min for small-cell IFCS 450 min for medium- or large-cell large IFCs. You can run the script overnight with a pause between DNA Seq and Harvest functions. You have up to 60 min after the script finishes to remove the harvest amplicons from the IFC.

Table 2. Prepare genomic samples, whole genome sequencing (WGS), and whole exome sequencing (WES) (Continued)

	Reagent handling	Automated steps	Estimated time
Day 2: Prepare genomic samples			
1	Harvest amplified samples and measure concentrations by PicoGreen.		30–40 min
2	Dilute single-harvest DNA samples from C1 to 10 ng/µL.		30 min
3	Remeasure DNA concentration of the diluted samples by PicoGreen.		40 min
4	Further dilute the DNA to 5 ng/µL.		30 min
5	Tagment the DNA.		15 min
6	Clean up tagmented DNA.		40 min
7		PCR amplify the tagmented DNA.	30 min
	(Optional) Stopping point		
8	QC by Bioanalyzer		40 min
9	Choose a sequencing application: **WGS** or **WES**		
Day 3: WGS only			
1	Pool and clean up libraries		45 min
2	QC by Bioanalyzer		40 min
3		Sequence library pool on HiSeq® system.	
Day 3: WES only			
1	Pool and clean up libraries		10 min
2	Clean up the pool.		40 min
3	First hybridization.		130 min
4	First enrichment		190 min
	(Optional) Stopping point		
5	Second hybridization.		130 min
6	Second enrichment.		190 min
7	Clean up enriched library pool.		40 min
8		Amplify cleaned-up library pool.	30 min
9	Clean up amplified library pool.		40 min
10	QC by Bioanalyzer		40 min
11		Sequence library pool on either MiSeq™ or HiSeq System.	

Required Reagents

IMPORTANT

- Thaw reagents on ice unless directed to thaw them at room temperature. Store reagents as soon as they are received according to manufacturer's storage recommendations. Vortex and then centrifuge reagents as directed.
- The name of this reagent has changed:

Previous Reagent Name	New Reagent Name
C1 Suspension Reagent	Suspension Reagent

Use the reagent as before. Only use reagents provided in the required kit. Do not swap reagents between kits.

Required Reagents for Whole Genome Amplification

See a diagram of the C1 Single-Cell Auto Prep Reagent Kit for DNA Seq in Appendix B on page 79.

Product Name	Company	Part Number	Storage
C1 Single-Cell Auto Prep Reagent Kit for DNA Seq	Fluidigm	100-7357	Module 1: 4 °C Module 2: –20 °C Module 3: –20 °C

Product Name	Company	Part Number
illustra GenomiPhi V2 DNA Amplification Kit	GE Healthcare Life Sciences	25-6600-30* 25-6600-31†

* ~5 chips with tube controls or ~8 chips with no tube controls.

† ~22 chips with tube controls or ~30 chips without tube controls.

Required Reagents for Targeted Sequencing

Product Name	Company	Part Number	Storage
20X Access Array Loading Reagent	Fluidigm	100-7604	–20 °C
1X Access Array Harvest Solution*	Fluidigm	100-1031	–20 °C
1X Access Array Hydration Reagent v2	Fluidigm	100-7966	–20 °C
Access Array Barcode Library for Illumina Sequencers—384 (Single Direction)	Fluidigm	100-4876	–20 °C
Custom uniplex primers	Fluidigm	ASY-AA	–20 °C
Custom multiplex primers	Fluidigm	ASY-AAX	–20 °C

* 1X Access Array Harvest Solution (Fluidigm, PN 100-1031) is not packaged for individual sale. It can be purchased as a component in the Access Array 48.48 Loading Reagent Kit, PN 100-1032.

Product Name	Company	Part Number
FastStart™ High Fidelity PCR System, dNTPack	Roche	04 738 292 001 (500 U)
Agencourt® AMPure® XP	Beckman Coulter	A63880
Agilent DNA 1000 Kit	Agilent Technologies	5067-1504

For more information on equipment required for targeted sequencing, refer to the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).

Required Reagents for Preparing Genomic DNA

Product Name	Company	Part Number
Nextera Rapid Capture Kit	Illumina	FC-140-1003
Index adapters from the Nextera Rapid Capture Custom Enrichment Kit 288 Samples*	Illumina	15055366
Quant-IT™ PicoGreen dsDNA Assay Kit	Thermo Fisher Scientific	P11496
Agilent High Sensitivity DNA Kit	Agilent Technologies	5067-4626
UltraPure™ DNase/RNase-Free Distilled Water	Thermo Fisher Scientific	10977-015

* Required if processing >12 samples at one time.

Required Reagents for Whole Genomic Sequencing

Product Name	Company	Part Number
Nextera Rapid Capture Kit	Illumina	FC-140-1003

Required Reagents for Whole Exome Sequencing

Product Name	Company	Part Number
Nextera Rapid Capture Kit	Illumina	FC-140-1003
Index adapters from the Nextera Rapid Capture Custom Enrichment Kit 288 Samples	Illumina	15055366
Ethanol, absolute	Sigma-Aldrich	459844-500ML
Quant-IT PicoGreen dsDNA Assay Kit	Thermo Fisher Scientific	P11496
Agilent High Sensitivity DNA Kit	Agilent Technologies	5067-4626
UltraPure DNase/RNase-Free Distilled Water	Thermo Fisher Scientific	10977-015

Suggested Reagents

Suggested Reagents for Whole Genome Amplification

Product Name	Company	Part Number
LIVE/DEAD Viability/Cytotoxicity Kit	Thermo Fisher Scientific	L-3224
Quant-IT PicoGreen dsDNA Assay Kit	Thermo Fisher Scientific	P11496

Required Consumables

Required Consumables for Whole Genome Amplification

Product Name	Company	Part Number
Select the IFC needed: • C1 IFC for DNA Seq (5–10 µm) • C1 IFC for DNA Seq (10–17 µm) • C1 IFC for DNA Seq (17–25 µm)	Fluidigm	 • 100-5762 • 100-5763 • 100-5764*
VWR® Slick Disposable Microcentrifuge Tubes, Polypropylene, 1.5 mL	VWR	20170-666
TempPlate™ semi-skirted 96-well PCR plates	USA Scientific	1402-9700
MicroAmp® Clear Adhesive Film	Thermo Fisher Scientific	4306311
15 mL centrifuge tubes	Major laboratory supplier (MLS)	—
Lint-free cloth	MLS	—

* See Appendix D: IFC Types and Related Scripts on page 81.

Required Consumables for Targeted Sequencing

Product Name	Company	Part Number
Access Array 48.48 IFC (Two IFCs required to process 96 cells)	Fluidigm	AA-M-48.48
Agilent DNA 1000 Kit	Agilent Technologies	5067-1504
Eppendorf® 0.2 mL PCR Tubes	VWR	47730-598
VWR Slick Disposable Microcentrifuge Tubes, Polypropylene, 1.5 mL	VWR	20170-666
TempPlate semi-skirted 96-well PCR plates	USA Scientific	1402-9700
MicroAmp Clear Adhesive Film	Thermo Fisher Scientific	4306311

For more information on required consumables after library preparation, refer to the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).

Required Consumables for Preparing Genomic Samples

Product Name	Company	Part Number
VWR Slick Disposable Microcentrifuge Tubes, Polypropylene, 1.5 mL	VWR	20170-666
MicroAmp Clear Adhesive Film	Thermo Fisher Scientific	4306311
TempPlate semi-skirted 96-well PCR plates	USA Scientific	1402-9700
Corning® 384 Well Low Flange Black Flat Bottom Polystyrene Not Treated Microplate	Corning	3573
Agilent High Sensitivity DNA Kit	Agilent Technologies	5067-4626

Required Consumables for Whole Genome Sequencing

Product Name	Company	Part Number
VWR Slick Disposable Microcentrifuge Tubes, Polypropylene, 1.5 mL	VWR	20170-666
Agilent High Sensitivity DNA Kit	Agilent Technologies	5067-4626

Required Consumables for Whole Exome Sequencing

Product Name	Company	Part Number
Eppendorf 0.2 mL PCR Tubes	VWR	47730-598
VWR Slick Disposable Microcentrifuge Tubes, Polypropylene, 1.5 mL	VWR	20170-666
Agilent High Sensitivity DNA Kit	Agilent Technologies	5067-4626

Suggested Consumables

Suggested Consumables for Whole Genome Amplification

Product Name	Company	Part Number
INCYTO C-Chip™ Disposable Hemocytometer, Neubauer Improved	INCYTO	PN DHC-N01

Suggested Consumables for Targeted Sequencing

Product Name	Company	Part Number
Barrier Tape	Fluidigm	PN 100-5920

Required Equipment

Required Equipment for Whole Genome Amplification

Product Name	Company	Part Number
C1 System	Fluidigm	100-7000
Two centrifuges: one for microcentrifuge tubes and one for 96-well plates	MLS	—
Vortexer	MLS	—
Thermal cycler	MLS	—
Water bath (37 °C; to thaw frozen cells)	MLS	—
Low-lint cloth	Fluidigm	Supplied in 100-7357
Two biocontainment hoods*	MLS	—

* To prevent DNA contamination of lab and samples.

Required Equipment for Targeted Sequencing

Product Name	Company	Part Number
Access Array System	Fluidigm	—*
2100 Bioanalyzer	Agilent	G2940CA
Vortexer	MLS	—
Centrifuge for microcentrifuge tubes	MLS	—
Thermal cycler	MLS	—
Magnetic stand for 1.5 mL microcentrifuge tubes	MLS	—

* For more information on equipment required for targeted sequencing, refer to the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).

Required Equipment for Preparing Genomic Samples

Product Name	Company	Part Number
2100 Bioanalyzer	Agilent	G2940CA
Vortexer	MLS	—
Centrifuge for microcentrifuge tubes	MLS	—
Thermal cycler	MLS	—
Magnetic stand for 1.5 mL microcentrifuge tubes	MLS	—
Fluorometer (for PicoGreen assay)	MLS	—

Required Equipment for Whole Genome Sequencing

Product Name	Company	Part Number
2100 Bioanalyzer	Agilent	G2940CA
Vortexer	MLS	—
Centrifuge for microcentrifuge tubes	MLS	—
Magnetic stand for 1.5 mL microcentrifuge tubes	MLS	—

Required Equipment for Whole Exome Sequencing

Product Name	Company	Part Number
2100 Bioanalyzer	Agilent	G2940CA
Vortexer	MLS	—
Centrifuge for microcentrifuge tubes	MLS	—
Thermal cycler	MLS	—
Magnetic stand for 1.5 mL microcentrifuge tubes	MLS	—
Thermoshaker	MLS	—
Laboratory rotator	MLS	—

Suggested Equipment

Suggested Equipment for Whole Genome Amplification

Product Name	Company	Part Number
Imaging equipment compatible with C1 IFCs*	MLS	—

* Refer to the Minimum Specifications for Single-Cell Imaging Specification Sheet, PN 100-5004.

Suggested Equipment for Preparing Genomic Samples, Whole Genome Sequencing, and Whole Exome Sequencing

Product Name	Company	Part Number
NanoDrop™ spectrophotometer	Thermo Fisher Scientific	—
Qubit® Fluorometer	Thermo Fisher Scientific	—

Best Practices

- Use good laboratory practices to minimize contamination of samples. Use a new pipette tip for every new sample. Whenever possible, separate pre- and post-PCR activities. Dedicate laboratory materials to designated areas.
- Thaw reagents on ice unless directed to thaw them at room temperature.

Reagent Retrieval for Cell Capture and Amplification

Table 3. Cell and DNA sequencing preparation

	Required Reagents	Preparation	Kit Name
1 Prime the IFC	C1 Preloading Reagent	Remove from −20 °C and thaw to room temperature in a DNA-free hood	DNA Seq (Module 2) (Fluidigm)
	C1 Harvest Reagent	Remove from −20 °C and thaw to room temperature in a DNA-free hood	DNA Seq (Module 2) (Fluidigm)
	C1 Blocking Reagent	Remove from 4 °C and keep at room temperature in a DNA-free hood	DNA Seq (Module 1) (Fluidigm)
	C1 DNA Seq Cell Wash Buffer	Remove from −20 °C and thaw to room temperature in a DNA-free hood	DNA Seq (Module 2) (Fluidigm)
2 Prepare the (Optional) LIVE/DEAD Cell Staining	Ethidium homodimer-1	Remove from −20 °C and keep in the dark as much as possible	LIVE/DEAD Kit, Thermo Fisher Scientific
	Calcein AM	Remove from −20 °C and keep in the dark as much as possible	LIVE/DEAD Kit, Thermo Fisher Scientific
3 Prepare the Cell Mix	Suspension Reagent	Remove from 4 °C and keep at room temperature in a DNA-free hood	DNA Seq (Module 1) (Fluidigm)
4 Obtain the C1 DNA Stop Buffer	C1 DNA Stop Buffer*	Remove from −20 °C, thaw on ice, and keep on ice	DNA Seq (Module 3) (Fluidigm)
* Note that the C1 DNA Seq Stop Buffer in the kit has a blue cap but no “S” on the cap.			
5 Prepare the DTT Mix	PCR Water	Remove from −20 °C and keep at room temperature	DNA Seq (Module 3) (Fluidigm)
	GE Kit Sample Buffer	Remove from −20 °C, thaw on ice, and keep on ice	GE Healthcare Life Sciences, illustra GenomiPhi V2 DNA Amplification Kit
	GE Kit Reaction Buffer	Remove from −20 °C, thaw on ice, and keep on ice	GE Healthcare Life Sciences, illustra GenomiPhi V2 DNA Amplification Kit
	C1 DTT (1 M)	Remove only one tube from −20 °C and thaw on ice. Keep all other tubes frozen. Thaw a new tube only as needed.	DNA Seq (Module 3) (Fluidigm)

	Required Reagents	Preparation	Kit Name
6 Prepare the Lysis Mix	C1 DNA Seq Lysis Buffer	Remove from −20 °C and thaw to room temperature in a DNA-free hood	DNA Seq (Module 3) (Fluidigm)
7 Prepare the Reaction-Enzyme Mix	C1 DNA Seq Reaction Mix	Remove from −20 °C and thaw to room temperature in a DNA-free hood	DNA Seq (Module 3) (Fluidigm)
	GE Kit Enzyme Mix	Remove from −80 °C, vortex well, and keep on ice	GE Healthcare Life Sciences, illustra GenomiPhi V2 DNA Amplification Kit

Reagent Retrieval for DNA Sequencing

	Required Reagents	Preparation	Kit Name/Part Number
8 Perform Access Array for Targeted Sequencing	FastStart High Fidelity PCR System, dNTPack	Remove from −20 °C, thaw on ice, and keep on ice	Roche, PN 04-738-292-001
	20X Access Array Loading Reagent	Remove from −20 °C, thaw on ice, and keep on ice	Fluidigm, PN 100-7604
	1X Access Array Harvest Solution	Remove from −20 °C, thaw on ice, and keep on ice	Access Array 48.48 Loading Reagent Kit, Fluidigm, PN 100-1032
	1X Access Array Hydration Reagent v2	Remove from −20 °C, thaw on ice, and keep on ice	Fluidigm, PN 100-7966
	C1 $MgCl_2$	Remove from −20 °C, thaw on ice, and keep on ice	DNA Seq (Module 2) (Fluidigm)
	Access Array Barcode Library for Illumina Sequencers—384	Remove from −20 °C, thaw on ice, and keep on ice	Fluidigm, PN 100-4876
	C1 DNA Dilution Reagent	Remove from 4 °C, thaw on ice, and keep on ice	DNA Seq (Module 1) (Fluidigm)
	Custom uniplex or multiplex primers	Remove from −20 °C, thaw on ice, and keep on ice	Fluidigm, PN ASY-AAX
	20X primer solution preparation*	Remove from −20 °C, thaw on ice, and keep on ice	Primers customer-supplied
	PCR Water	Remove from −20 °C, and keep at room temperature	DNA Seq (Module 3) (Fluidigm)

* Refer to the Fluidigm Access Array Multiplex 20X Primer Solution Preparation Quick Reference (PN 100-3895)

		Required Reagents	Preparation	Kit Name/Part Number
9	Prepare Genomic DNA	Sample Purification Beads (SPB)	Remove from 4 °C and keep at room temperature	Illumina, Nextera Rapid Capture, Box 1
		Tagment DNA Enzyme (TDE1)	Remove from −20 °C and keep on ice	Illumina, Nextera Rapid Capture, Box 2
		Tagment DNA Buffer (TD)	Remove from −20 °C and thaw to room temperature	Illumina, Nextera Rapid Capture, Box 2
		Stop Tagment Buffer (ST)	Keep at room temperature	Illumina, Nextera Rapid Capture, Box 1
		Resuspension Buffer (RSB)	Remove from −20 °C and thaw to room temperature	Illumina, Nextera Rapid Capture, Box 2
		Index 1 primers (i7, N701–N712)*	Remove from −20 °C and thaw to room temperature	Illumina, Nextera Rapid Capture, Box 3
		Index 2 primers (i5, E502–E508 and E517)	Remove from −20 °C and thaw to room temperature	Illumina, Nextera Rapid Capture, Box 3
		Nextera Library Amplification Mix (NLM)	Remove from −20 °C and thaw to room temperature	Illumina, Nextera Rapid Capture, Box 2
		Ethanol, absolute	Keep at room temperature	Major laboratory suppliers
		Lambda DNA	Remove from −20 °C and thaw to room temperature	Quant-IT PicoGreen dsDNA Assay Kit, Thermo Fisher Scientific
		PicoGreen dsDNA quantitation assay	Remove from −20 °C and thaw to room temperature	Quant-IT PicoGreen dsDNA Assay Kit, Thermo Fisher Scientific
10	Perform Whole Genomic Sequencing	Resuspension Buffer (RSB)	Remove from −20 °C and thaw to room temperature	Illumina, Nextera Rapid Capture, Box 2

		Required Reagents	Preparation	Kit Name/Part Number
11	Perform Whole Exome Sequencing	Enrichment Hybridization Buffer (EHB)	Remove from −20 °C and keep on ice	Illumina, Nextera Rapid Capture, Box 2
		Coding Exome Oligos (CEX) or Expanded Exome Oligos (EEX)	Remove from −20 °C and keep on ice	Illumina, Nextera Rapid Capture, Box 3
		Elute Target Buffer 2 (ET2)	Remove from 4 °C, thaw on ice, and keep on ice	Illumina, Nextera Rapid Capture, Box 2
		2N NaOH (HP3)	Remove from −20 °C and thaw it to room temperature	Illumina, Nextera Rapid Capture, Box 2
		Enrichment Elution Buffer 1 (EE1)	Remove from −20 °C and thaw it to room temperature	Illumina, Nextera Rapid Capture, Box 2
		Enrichment Wash Solution (EWS)	Remove from −20 °C and keep on ice	Illumina, Nextera Rapid Capture, Box 2
		Streptavidin Magnetic Beads (SMB)	Remove from 4 °C and keep at room temperature	Illumina, Nextera Rapid Capture, Box 1
		PCR Primer Cocktail (PPC)	Remove from −20 °C, thaw it to room temperature, and then place on ice	Illumina, Nextera Rapid Capture, Box 2

* The Illumina Nextera Rapid Capture Kit contains 24 indices. For labeling with up to 96 indices, use the indices from the Illumina Nextera Rapid Capture Custom Enrichment Kit 288 Samples, PN 15055366.

Use the IFC Map Loading Plate

A black IFC map loading plate accessory can be used to assist IFC pipetting.

1 Obtain an IFC map loading plate:

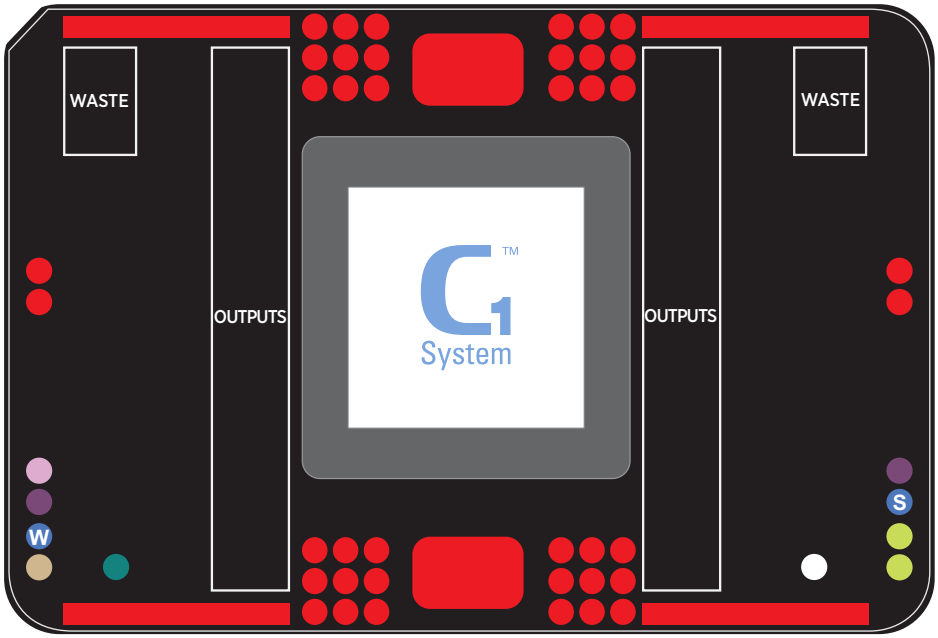


Figure 1. IFC map loading plate. Note that the C1 DNA Seq Stop Buffer in the kit has a blue cap but no “S” on the cap.

NOTE The IFC map loading plate is supplied with the C1 system.

2 Place the C1 IFC onto the IFC map loading plate. For details on IFC loading, see Appendix A: IFC Pipetting Map on page 78.

3 Prime the IFC. (See Prime the IFC on page 31.)

Prime the IFC

NOTE

- When pipetting into the C1 IFC, always stop at the first stop on the pipette to avoid creating bubbles in the inlets. If a bubble is introduced, ensure that it floats to the top of the well.
- Vortex and then centrifuge all reagents before pipetting into the IFC.

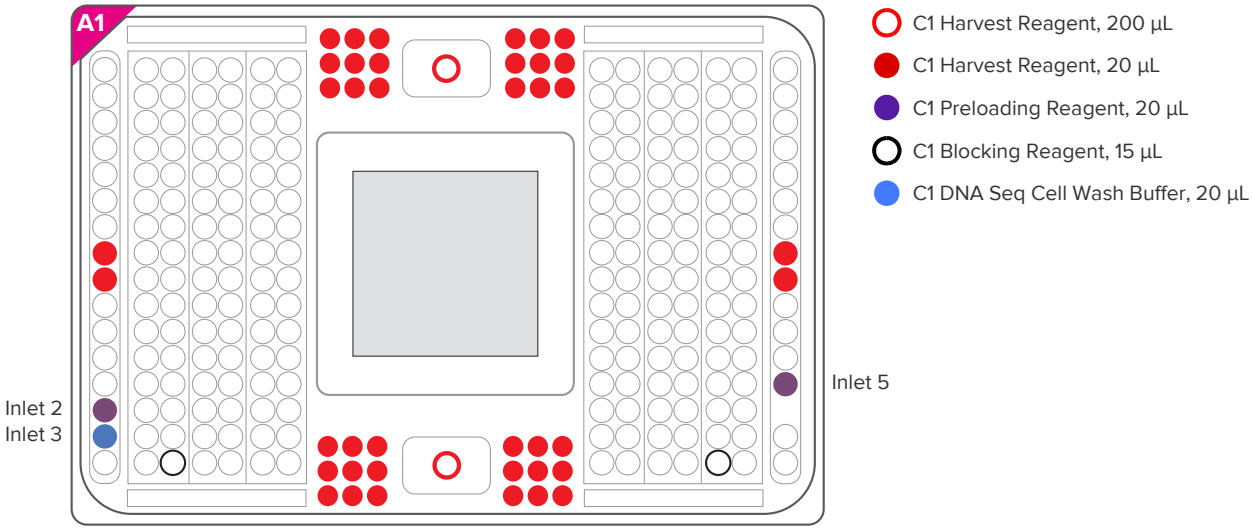


Figure 2. C1 IFC priming pipetting map.

IMPORTANT Allow C1 DNA Seq Cell Wash Buffer (Fluidigm) to equilibrate to room temperature prior to use.

1. Pipet 200 µL of C1 Harvest Reagent from 4 mL bottle into accumulators, marked with red outlined circles in Figure 2.
2. Pipet 20 µL of C1 Harvest Reagent into inlets, marked with solid red circles on each side of the accumulators (36 total).
3. Pipet 20 µL of C1 Harvest Reagent into the inlets marked with solid red circles in the middle of the outside columns of inlets on each side of the IFC. These wells are marked on the bottom of the IFC with a notch to ensure they are easily located.
4. Pipet 20 µL of C1 Preloading Reagent into inlet 2 and inlet 5, both marked with a purple dot.
5. Pipet 15 µL of C1 Blocking Reagent into the cell inlet and outlet, marked with white dots encircled by heavy black rule.
6. Pipet 20 µL of C1 DNA Seq Cell Wash Buffer, marked with a blue dot, into inlet 3.
7. Peel off white tape on bottom of IFC.

8 Place the IFC into the C1 system and then run the **DNA Seq: Prime (1791x/1792x/1793x)** script. Priming small- or large-cell IFCs takes 10 minutes, and priming medium-cell IFCs takes 12 minutes. When the prime script has finished, tap **Eject** to remove the primed IFC from the instrument.

NOTE After priming the IFC, you have up to 1 hour to load the cells on the IFC in the C1 system.

Prepare Cells

IMPORTANT
- Allow C1 DNA Seq Cell Wash Buffer (Fluidigm) to equilibrate to room temperature prior to use.
- Use the mixes no more than 30 minutes after preparation.

(Optional) Prepare LIVE/DEAD Cell Staining Solution

The optional live/dead cell staining step uses the LIVE/DEAD Viability/Cytotoxicity Kit, which tests the viability of a cell based on the integrity of the cell membrane. This test contains two chemical dyes. The first dye is green-fluorescent calcein AM, which stains live cells. This dye is cell-permeable and tests for active esterase activity in live cells. The second dye is red-fluorescent ethidium homodimer-1, which will stain nucleic acids only if the integrity of the cell membrane has been lost.

NOTE
- Keep the dye tubes closed and in the dark as much as possible, as they can hydrolyze over time. When not in use, store in dark, airtight bag with desiccant pack at −20 °C.
- Cell staining solution may be prepared up to two hours before loading onto the C1 IFC. Keep on ice and protected from light before pipetting into IFC.

IMPORTANT Approximate staining times: small cells (5–10 µm) takes 30 minutes, medium cells (10–17 µm) takes 65 minutes, and large cells (17–25 µm) cells takes 60 minutes. To easily visualize small cells, you may need to double the amount of stain used.

1 Vortex the dyes for 10 seconds and then centrifuge them before pipetting.

2 Prepare the LIVE/DEAD staining solution by combining reagents in this order:

Components	Volume (µL)
C1 DNA Seq Cell Wash Buffer (Fluidigm) (26 mL bottle)	1,250.0
Ethidium homodimer-1 (LIVE/DEAD kit, Thermo Fisher Scientific)	2.5
Calcein AM (LIVE/DEAD kit, Thermo Fisher Scientific)	0.625
Total	**1,253.125**

3 Vortex the LIVE/DEAD staining solution for 10 seconds before pipetting into the IFC.

Prepare the Cells

(Optional) Thaw the cells

If you do not need to thaw the cells, proceed to Wash the Cells on page 34

IMPORTANT The thawing procedure is stressful to frozen cells. Using good technique and working quickly ensures that a high proportion of the cells survive the procedure.

1 Warm 10 mL of the appropriate cell culture medium to 37 °C.

2 Thaw 1 mL of cells in a 37 °C water bath: For <1 minute, shake the tube of cells in the water bath to steadily increase the temperature of the cell suspension.

3 Transfer the entire thawed cell suspension to a 15 mL centrifuge tube.

4 Slowly add 9 mL the warmed cell culture media drop-wise.

5 Close the 15 mL centrifuge tube, and then gently invert the tube two to three times.

6 Centrifuge the cells at 300 x *g* for 5 min, and then remove and discard the supernatant.

7 Gently resuspend the cell pellet in 1 mL of C1 DNA Seq Cell Wash Buffer.

Wash the Cells

IMPORTANT The whole genome amplification protocol amplifies all DNA in the reaction. You must remove as much debris from the cells as possible. Washing the cells before loading them on the IFC is required.

1 Transfer 1 mL of the cell suspension to a new tube.

2 Centrifuge to pellet the cells at 300 x *g* for 5 min.

3 Remove 900 µL of supernatant.

4 Add 900 µL of C1 DNA Seq Cell Wash Buffer, and then gently pipet the cells up and down more than 5 times.

5 Centrifuge the cells at 300 x *g* for 5 min.

IMPORTANT For the last wash of thawed cells, centrifuge the cells at 100–200 x *g*.

6 Remove 900 µL of supernatant.

IMPORTANT For the last wash of thawed cells, save the supernatant should you need to recover additional cells. Recover more cells by centrifuging the saved supernatant at 500 x *g*.

7 Add 900 µL of C1 DNA Seq Cell Wash Buffer, and then gently pipet the cells up and down more than 5 times.

8 Repeat washing the cells:

- Fresh cells: Repeat steps 5–7 one time.
- Thawed cells from frozen sample: Repeat steps 5–7 three times.

9 Count the cells, and then dilute the cells with C1 DNA Seq Cell Wash Buffer to 66–333 cells/µL.

Prepare a Cell Suspension

NOTE Cells may be counted by any preferred method. If an established cell counting protocol does not exist, we suggest using the disposable hemocytometer C-Chip by INCYTO. See incyto.com for instructions for use.

Prepare a cell suspension at a concentration of 66–333 cells/µL in C1 DNA Seq Cell Wash Buffer prior to mixing with Suspension Reagent and loading onto the IFC. This will ensure a total cell count pipetted on the IFC of approximately 200–1000 cells. As few as 200 cells total, from 66,000 /mL in native medium, may be loaded on the IFC. Fewer cells loaded may yield fewer captured cells. A final volume of 0.5–1 mL is desirable so that there are enough cells for both the C1 IFC and the tube controls.

Prepare the Cell Mix

IMPORTANT Vortex the Suspension Reagent for 5 seconds before use. If Suspension Reagent contains particulates, ensure they are properly removed by vortexing. **Do not vortex** the cells.

1 Ensure that you have begun priming the IFC. (See Prime the IFC on page 31.)

2 Before mixing cells with Suspension Reagent and loading them into the IFC, prepare a cell suspension in native medium of 66,000-333,000 cells/mL. The recommended concentration range ensures that a total of 200-1000 cells are loaded into the IFC. You can prepare a cell suspension with a minimum concentration of 66,000 cells/mL, but fewer cells will be loaded and captured in the IFC. Preparing a cell suspension of >333,000 cells/mL may clog the fluidic channels. Suspend the cells in a final volume of 0.5–1 mL to ensure enough cells are available for the IFC and tube controls.

NOTE

- Cells may be counted by any preferred method. If an established cell counting protocol does not exist, we suggest using the disposable hemocytometer C-Chip by INCYTO. See incyto.com/product/product02_detail.php for instructions for use.
- Make sure to record your final cell concentration.

3 Prepare cell mix by combining cells with Suspension Reagent at a ratio optimized in advance for your cell type, to create a neutrally buoyant cell suspension. Many cell types use the standard ratio of 3:2 as shown below.

Components	Volume (µL)
66–333 cells/µL	60
Suspension Reagent (Fluidigm)	40
Total	**100**

NOTE

- For more information, see the Fluidigm Single-Cell Preparation Guide (PN 100-7697).
- The volume of cell mix may be scaled depending on volume of cells available. You will load 6 µL of the cell mix into the IFC (see Figure 3).

4 Set a P200 pipette to 60 µL, and then pipet the cell mix up and down 5–10 times to mix, depending on whether the cells tend to clump. **Do not vortex** the cell mix. Avoid bubbles when mixing.

Load Cells

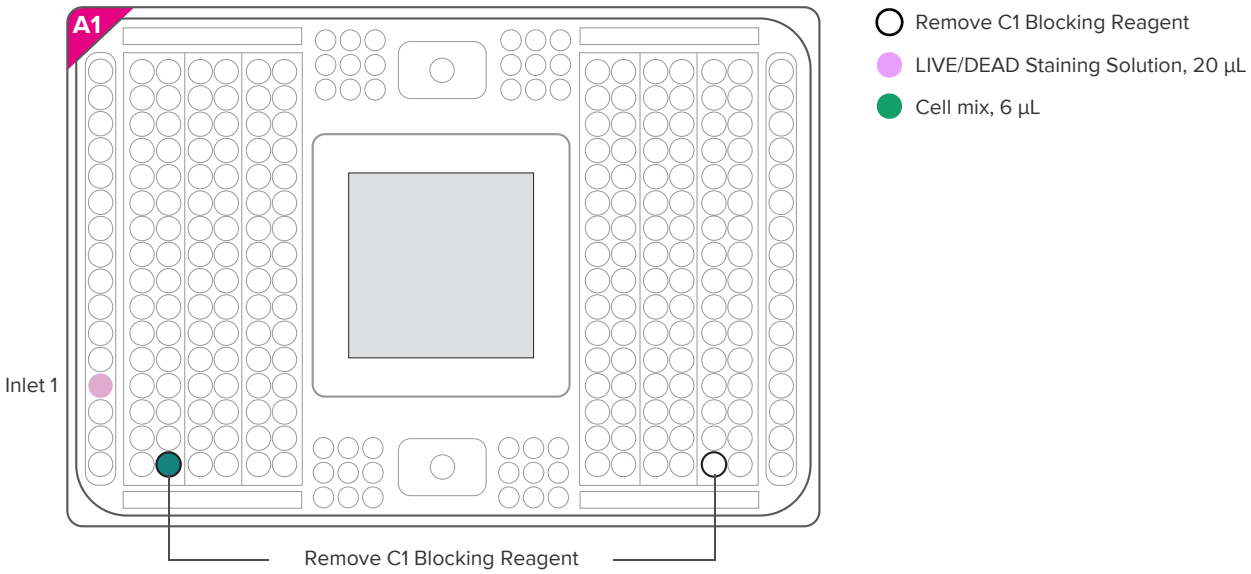


Figure 3. C1 IFC loading pipetting map

1 Use a pipette and tip to remove blocking solutions from cell inlet and outlet, marked with teal and white dots in Figure 3.

2 Set a P200 pipette to 60 µL, and then pipet the cell mix up and down 5–10 times to mix, depending on whether the cells tend to clump. **Do not vortex** the cell mix. Avoid bubbles when mixing.

3 Pipet 6 µL of cell mix into the cell inlet marked with the teal dot.

4 Perform one of these tasks:

- Staining cells: Vortex the LIVE/DEAD staining solution for 10 seconds, and then pipet 20 µL of the solution into inlet 1, marked with a pink dot.
- Not staining cells: Pipet 20 µL of C1 DNA Seq Cell Wash Buffer into inlet 1, marked with a pink dot.

5 Place the IFC into the C1 system. Run the **DNA Seq: Cell Load (1791x/1792x/1793x)** or **DNA Seq: Cell Load & Stain (1791x/1792x/1793x)** script.

NOTE Approximate staining times: small cells (5–10 µm) takes 30 minutes, medium cells (10–17 µm) takes 65 minutes, and large (17–25 µm) cells takes 60 minutes.

6 When the script has finished, tap **Eject** to remove the IFC from the C1 system.

Image Cells

Cells may be imaged on a microscope compatible with C1 IFCs. Guidelines for the selection of a microscope are outlined in Minimum Specifications for Single-Cell Imaging, PN 100-5004.

Prepare Reagent Mixes

- Obtain C1 DNA Seq Stop Buffer
- Prepare the DTT Mix on page 38
- Prepare the Lysis Mix on page 38
- Prepare the Reaction-Enzyme Mix on page 39

NOTE Vortex and then centrifuge all reagent mixes before pipetting into the IFC.

Obtain C1 DNA Seq Stop Buffer

C1 DNA Seq Stop Buffer is ready to use.

1 Vortex the solution for 5 seconds, and then centrifuge it to collect the buffer.

2 Keep on ice until use.

IMPORTANT Use the mixes no more than 30 minutes after preparation as described in the next sections.

Prepare the DTT Mix

IMPORTANT Remove only 1 tube of C1 DTT from −20 °C and thaw on ice. Keep all other tubes frozen. Thaw a new tube only as needed.

1 Label a new tube (suggestions: “DTT Mix” or “D”).

2 Mix the reagents in the labeled tube:

Components	Volume (µL)
PCR Water	193.1
GE Kit Sample Buffer	2.3
GE Kit Reaction Buffer	2.3
C1 DTT (1 M; Fluidigm)	2.3
Total	**200.0**

3 Vortex the solution for 3 seconds, and then centrifuge it to collect the mix. Keep on ice until use.

Prepare the Lysis Mix

1 Label a new tube (suggestions: “Lysis Mix” or “L”).

2 Mix the reagents in the new labeled tube:

Components	Volume for One C1 IFC (µL)	Volume Including Tube Controls (µL)*
C1 DNA Seq Lysis Buffer (Fluidigm)	13.5	19.8
C1 DTT (1 M; Fluidigm)	1.5	2.2
Total	**15.0**	**22.0**

* See Prepare the DNA Sequencing Reaction Tube Controls on page 43.

3 Vortex the solution for 5 seconds, and then centrifuge it to collect the mix. Keep on ice until use.

Prepare the Reaction-Enzyme Mix

IMPORTANT Use DTT Mix previously prepared (see Prepare the DTT Mix on page 38), not stock DTT solution. Use C1 DNA Seq Reaction Buffer, not GE Reaction Buffer.

1 Label a new tube (suggestions: “Reaction-Enzyme Mix” or “RE”).

2 Mix the reagents in the labeled tube:

Components	Volume for One C1 IFC (µL)	Volume Including Tube Controls (µL)*
C1 DNA Seq Reaction Mix	30.0	45.0
GE Kit Enzyme Mix	3.0	4.5
DTT Mix (See Prepare the DTT Mix on page 38.)	21.0	31.5
Total	**54.0**	**81.0**

* See Prepare the DNA Sequencing Reaction Tube Controls on page 43.

NOTE Discard the remaining DTT Mix. Do not reuse. Prepare fresh mix as needed.

3 Vortex the solution for 5 seconds, and then centrifuge it to collect the mix. Keep on ice until use.

Run Lysis and DNA Amplification on the C1 System

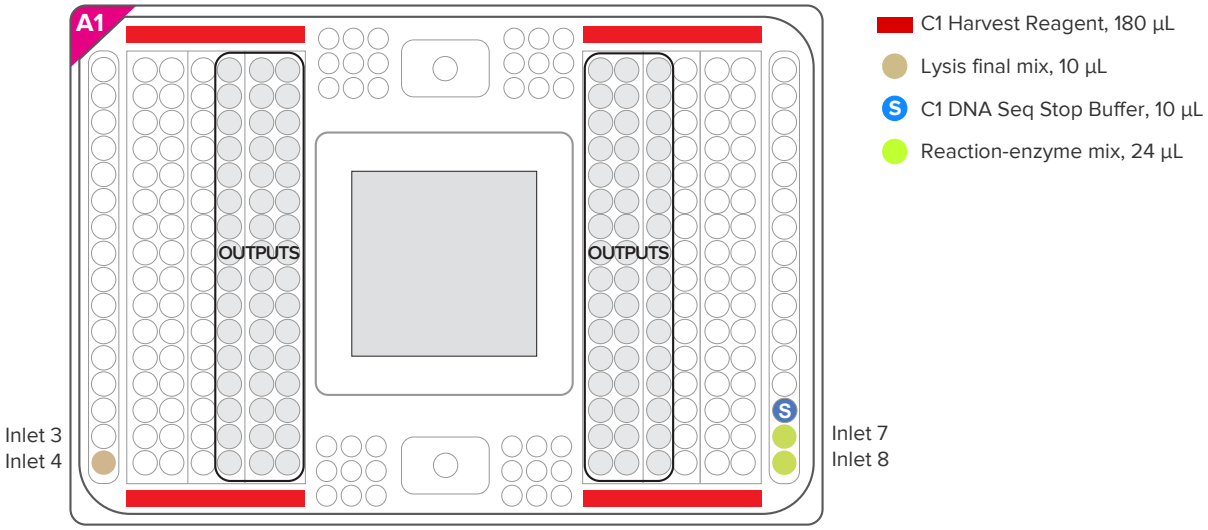


Figure 4. C1 IFC lysis, neutralization, and DNA sequencing pipetting map. Note that the C1 DNA Seq Stop Buffer in the kit has a blue cap but no "S" on the cap.

1. Pipet 180 µL of C1 Harvest Reagent into the four reservoirs marked with large solid red rectangles in Figure 4.
2. Pipet 10 µL of lysis mix in inlet 4, marked with a light tan dot.
3. Pipet 10 µL of C1 DNA Seq Stop Buffer in inlet 6, marked with a dark blue dot ("S").
4. Pipet 24 µL of Reaction-Enzyme Mix in inlets 7 and 8, marked with light green dots.
5. Place the IFC into the C1 system and run the **DNA Seq: Amplify (1791x/1792x/1793x)** script.

NOTE The **DNA Seq: Amplify (1791x/1792x/1793x)** script may be run overnight. Approximate run times are:

- Small-cell IFC: ~6.25 hours (~5.0 hours for lysis and amplification; 1.25 hours for harvest)
- Medium- and large-cell IFCs: ~7.5 hours (~5.0 hours for lysis and amplification; ~2.5 hours for harvest)

  This protocol can be programmed to harvest at a convenient time. Slide the orange box (end time) to the desired time. For example, the harvest function can be programmed so that the system harvests samples the morning after the run:

NOTE To abort the harvest, tap **ABORT**. The IFC will no longer be usable. Start a new experiment with a new IFC.

The **DNA Seq: Amplify (1791x/1792x/1793x)** script contains these thermal cycling protocols:

Lysis	
Temperature	**Time (min)**
4 °C	10
25 °C	1

DNA Sequencing	
Temperature	**Time (min)**
38 °C	120
70 °C	15

Stop lysis	
Temperature	**Time (min)**
23 °C	3

(Optional) Prepare the Tube Controls

The tube controls are used as positive and negative controls for the DNA Sequencing workflow. You prepare three tube controls: the lysis reaction, stop lysis reaction, and DNA seq tube controls. For each control, you prepare three solutions containing gDNA (tube 1), cells (tube 2), or PCR water (tube 3; no template control or NTC).

Prepare the Lysis Reaction Tube Controls

1 Add lysis mix (see Prepare the Lysis Mix on page 38) to gDNA (GE Healthcare Life Sciences), cells, and PCR water in new tubes:

Components	Tube 1: gDNA (µL)	Tube 2: Cells (µL)	Tube 3: NTC (µL)
gDNA, GE Kit Control DNA, 10 ng/µL	1.0	—	—
Cell Mix	—	1.0	—
PCR Water	—	—	1.0
Lysis Mix	2.0	2.0	2.0
Total	**3.0**	**3.0**	**3.0**

2 Vortex the solutions for 5 seconds, and then centrifuge to collect the mix.

3 Place the lysis reaction tube controls on ice for 10 minutes, and then immediately proceed to Prepare the Stop Lysis Reaction Tube Controls.

Prepare the Stop Lysis Reaction Tube Controls

1 Add C1 DNA Seq Stop Buffer (Fluidigm) to the same tubes containing the lysis reaction tube controls (see Prepare the Lysis Reaction Tube Controls):

Components	Tube 1: gDNA (µL)	Tube 2: Cells (µL)	Tube 3: NTC (µL)
Lysis Reaction	3.0	3.0	3.0
C1 DNA Seq Stop Buffer (Tube in the kit has a blue cap but no “S” on the cap)	4.0	4.0	4.0
Total	**7.0**	**7.0**	**7.0**

2 Vortex the solutions for 5 seconds, and then centrifuge to collect the mix.

3 Incubate the stop lysis reaction tube controls at room temperature for 3 minutes, and then keep the controls at room temperature.

Prepare the DNA Sequencing Reaction Tube Controls

1 Add reaction-enzyme mix (see Prepare the Reaction-Enzyme Mix on page 39) to aliquots of stop lysis reaction (see Prepare the Stop Lysis Reaction Tube Controls on page 42) in new tubes:

Components	Tube 1: gDNA (µL)	Tube 2: Cells (µL)	Tube 3: NTC (µL)
Stop Lysis Reaction	1.05	1.05	1.05
Reaction-Enzyme Mix	8.95	8.95	8.95
Total	**10.00**	**10.00**	**10.00**

2 Vortex the solutions for 5 seconds, and then centrifuge the tubes to collect the mix.

3 Place the tubes in a thermal cycler and run the following program:

Temperature	Time (min)
38 °C	120
70 °C	15
4 °C	Hold

4 Dilute the three amplified tube controls:

a Transfer prepared material to a post-PCR room.

b Vortex the solution for 5 seconds, and then centrifuge it to collect the mix.

c Combine:

Components	Volume (µL)
C1 DNA Dilution Reagent (Fluidigm)	9
PCR Products	1
Total	**10**

5 Quantify the tube controls with the PicoGreen dsDNA quantitation assay to perform quality control on the protocol. (See Quantify Harvest Amplicons on page 48.)

Harvest the Amplified DNA Products

1 When the **DNA Seq: Amplify** script has finished, tap **Eject** to remove the IFC from the instrument.

NOTE The IFC may remain in the C1 system for up to one hour after harvest before removing products from their inlets.

2 Transfer the C1 IFC to a post-PCR lab environment.

3 Label a new 96-well plate “Harvest Plate” and write the date.

4 Aliquot 10 µL of C1 DNA Dilution Reagent into each well of the Harvest Plate.

5 Carefully pull back the tape covering the harvesting inlets of the IFC using the plastic removal tool:

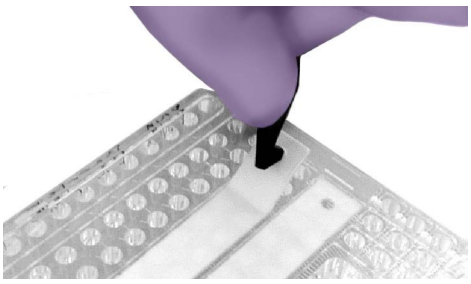


Figure 5. Tape removal.

6 Use an eight-channel pipette to pipet the harvested amplicons from the inlets according to Figure 6 and Table 4 and place in the harvest plate:

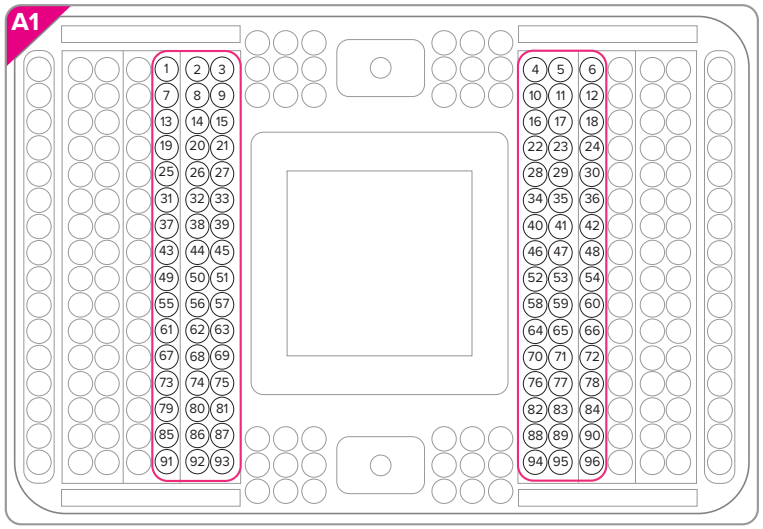


Figure 6. Pipette map of reaction products on the C1 IFC

NOTE Harvest volumes may vary. Set a pipette to 4.0 µL to ensure entire volume is extracted.

Table 4. Harvest amplicon dilution

Components	Volume (µL)
C1 DNA Dilution Reagent (Fluidigm)	10
C1 Harvest Amplicons	~3.5
Total	**~13.5**

NOTE For detailed instructions on pipetting the harvested aliquots to the "Harvest Plate," proceed to steps 7–10.

7 Pipet the entire volume of C1 harvest amplicons out of the left-side wells of the C1 IFC into the 10 µL of C1 DNA Dilution Reagent in each well of the harvest plate:

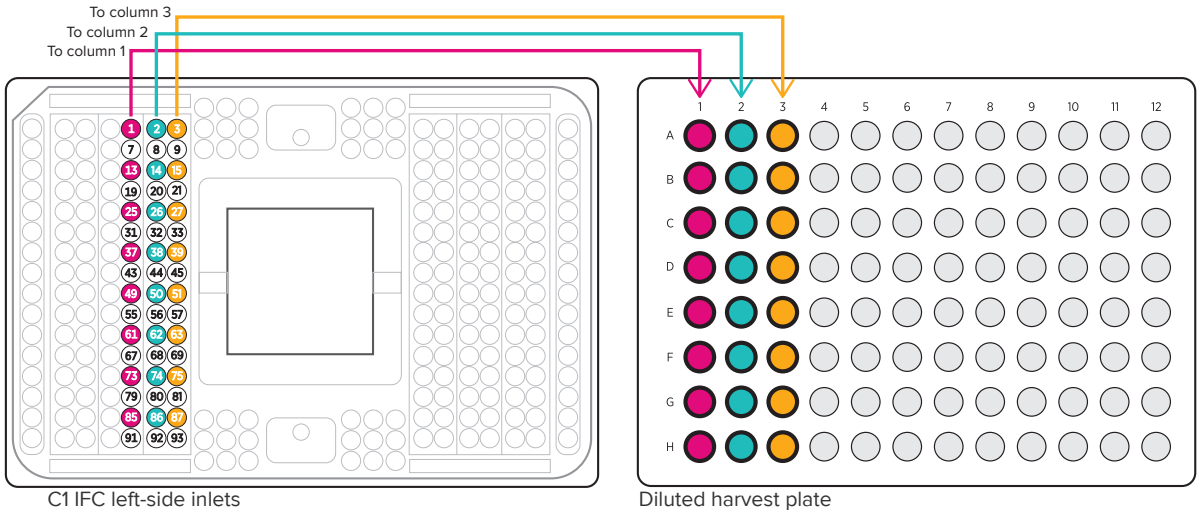


Figure 7. First three harvest product pipette steps

8 Pipet the entire volume of C1 harvest amplicons out of the right-side wells of the C1 IFC into the 10 µL of C1 DNA Dilution Reagent in each well of the harvest plate:

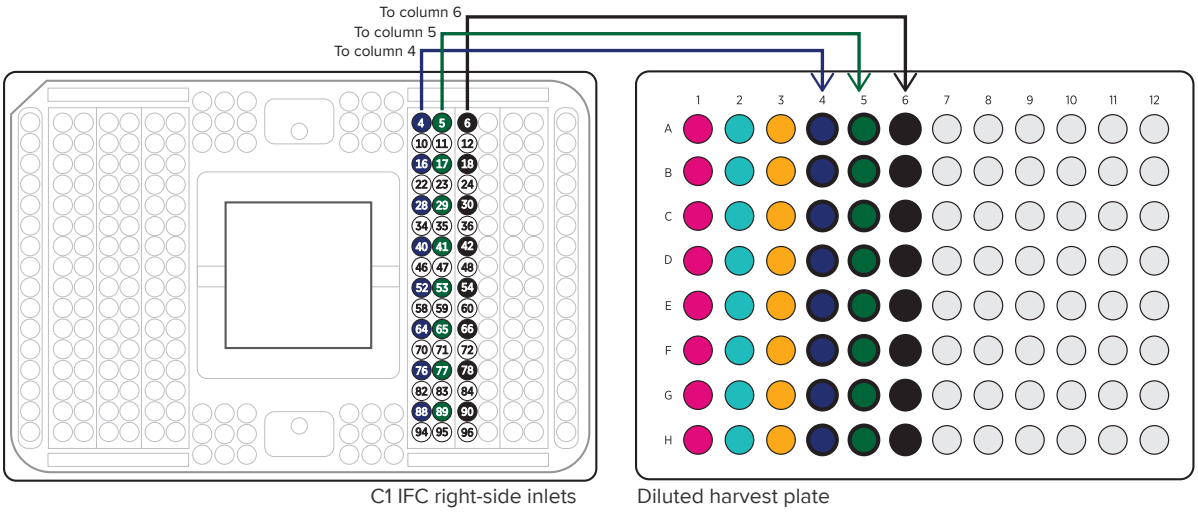


Figure 8. Fourth, fifth, and sixth pipetting steps

9 Pipet the entire volume of C1 harvest amplicons out of the left-side wells of the C1 IFC into the 10 µL of C1 DNA Dilution Reagent in each well of the harvest plate

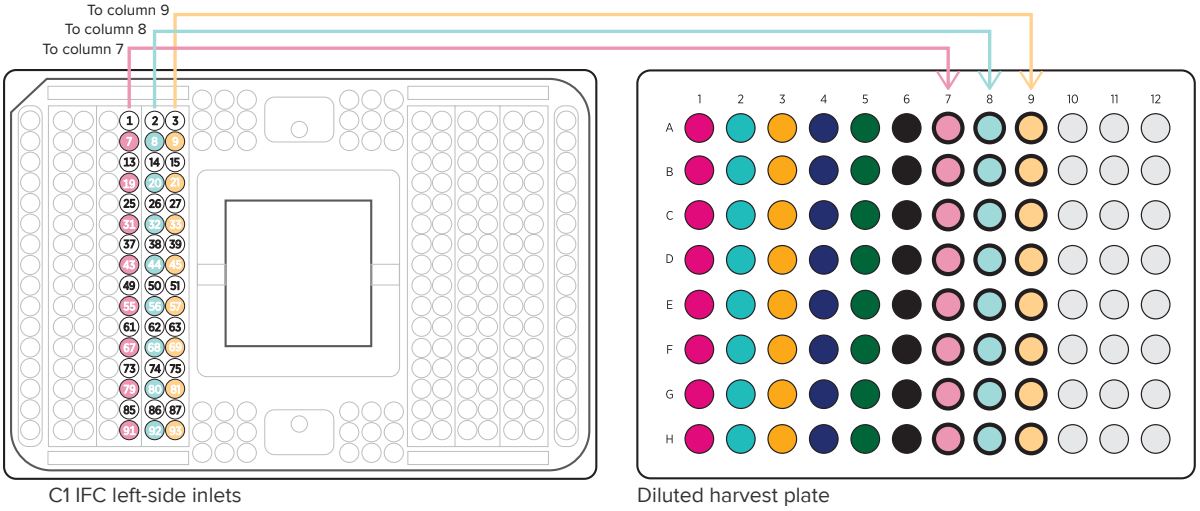


Figure 9. Seventh, eighth, and ninth pipetting steps

10 Pipet the entire volume of C1 harvest amplicons out of the right-side wells of the C1 IFC into the 10 µL of C1 DNA Dilution Reagent in each well of the harvest plate:

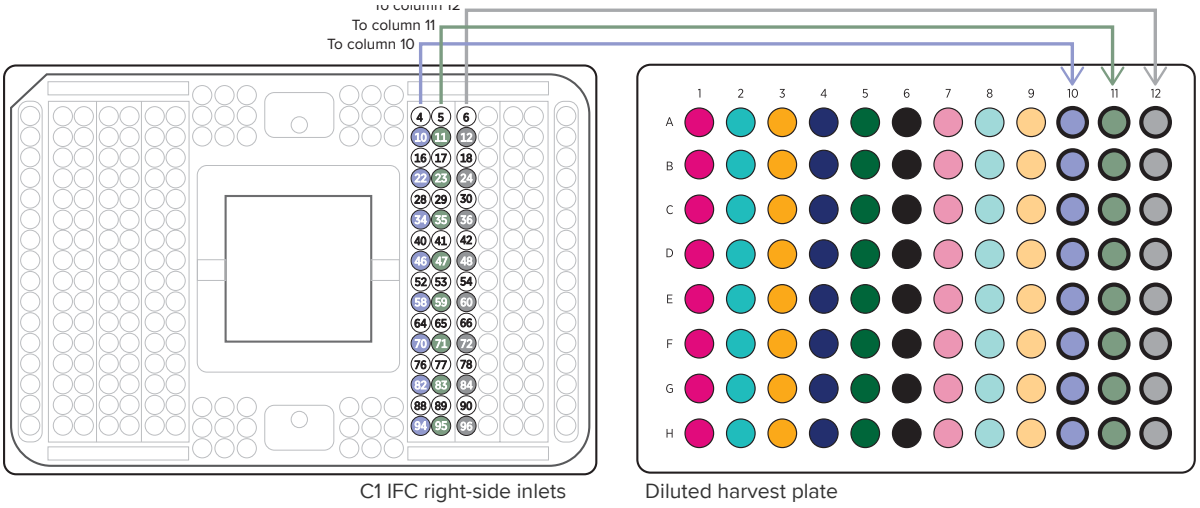
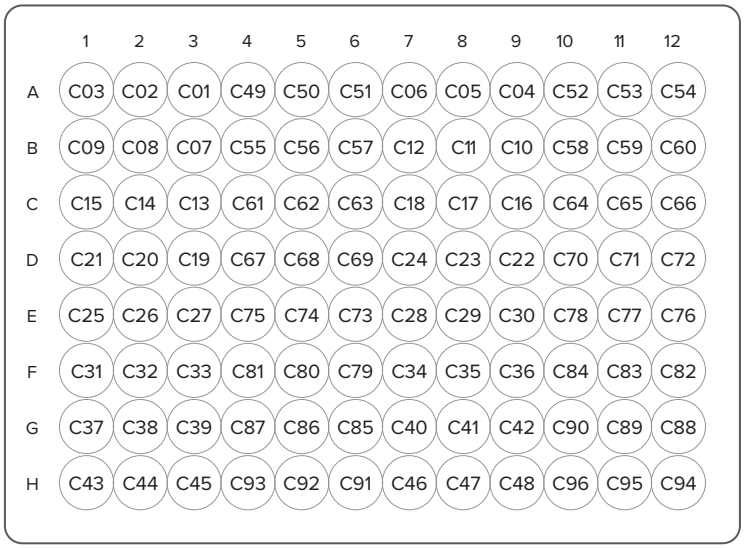


Figure 10. Tenth, eleventh, and twelfth pipetting steps

11 Seal, vortex the harvest plate for 10 seconds, and then centrifuge it to collect harvest products.

After harvesting, material from the capture sites is arranged on the harvest plate as follows:



NOTE These samples are now ready for library preparation for sequencing. Samples can be stored for up to 1 week at 4 ºC or at −20 ºC for long-term storage.

Quantify Harvest Amplicons

NOTE If you prepared tube controls, you can replace some harvest amplicons with tube controls to perform quality control on the protocol with the PicoGreen dsDNA quantitation assay. [See (Optional) Prepare the Tube Controls on page 42.]

Before proceeding to library preparation, quantify the harvest amplicons to verify DNA concentrations:

- We suggest using the PicoGreen dsDNA quantitation assay to determine the concentration of DNA samples; however, alternate methods can be used. The PicoGreen dsDNA quantitation assay is required to quantify DNA samples for whole exome sequencing.
- We suggest using the Microsoft Excel worksheet, Single-Cell Sequencing PicoGreen Template (Fluidigm, PN 100-6260), to quantify the library.
- The typical concentration of diluted product is 5–15 ng/µL. If you cannot dilute the product to 5-15 ng/µL, contact Fluidigm Technical Support.

Choose a Sequencing Application

After whole genome amplification and harvesting with the C1 DNA Seq Kit on the C1 system, choose the appropriate sequencing application of interest:

Sequencing Application	Coverage	Proceed to
Targeted DNA Sequencing	150–250 bp amplicons for targeted genomic loci	Targeted Sequencing on page 50
Whole Genome Sequencing	Intronic and exomic	Prepare Samples for Whole Genome and Whole Exome Sequencing on page 58
Whole Exome Sequencing	Exomic only	Whole Exome Sequencing on page 67

Targeted Sequencing

Follow these sections to perform targeted sequencing:

Targeted Sequencing: Workflow

Review the workflow for targeted sequencing. (See Whole Genome Amplification and Targeted Sequencing of Single Cells by DNA Sequencing on page 11.)

Targeted Sequencing: Required Materials

See Required Reagents for Targeted Sequencing on page 17 and Required Consumables for Targeted Sequencing on page 20.

Targeted Sequencing: Perform Access Array Amplification

Prime the Access Array 48.48 IFC

IMPORTANT

- Use the Access Array 48.48 IFC within 24 hours of opening the package.
- Due to the different accumulator volumes, use only 48.48 syringes with 300 µL of control line fluid (Fluidigm, PN 89000020).
- Control line fluid on the IFC or in the inlets makes the IFC unusable.
- Load the IFC into the pre-PCR IFC Controller AX in the pre-PCR lab within 60 minutes of priming.
- Be certain that the reagents 1X Access Array Harvest Solution and 1X Access Array Hydration Reagent v2 are thawed completely to room temperature and mixed thoroughly prior to use.

1 Inject control line fluid into each accumulator on the IFC. [Refer to the Fluidigm Control Line Fluid Loading Procedure Quick Reference (PN 68000132) and see Figure 11 on page 51.]

2 Add 500 µL of 1X Access Array Harvest Solution (Fluidigm, PN 100-1031) into the H1–H3 wells on the IFC. (See Figure 11 on page 51.)

3 Add 500 µL of 1X Access Array Hydration Reagent v2 (blue cap, Fluidigm, PN 100-7966) to the H4 well. (See Figure 11.)

IMPORTANT Hydration Reagent v2 ensures uniform harvest volumes.

4 Remove and discard the blue protective film from the bottom of the AA IFC.

5 Load the IFC into the pre-PCR IFC Controller AX located in the pre-PCR lab.

6 Tap **Eject** to move the tray out of the pre-PCR IFC Controller AX.

7 Place the IFC onto the tray by aligning the notched corner of the IFC to the A1 mark.

8 Tap **Load Chip** to register the barcode of the IFC and activate the script selection.

9 Select **Prime (151x)** and **Run Script** to prime the IFC.

10 Once the script is complete, tap **Eject** to remove the IFC.

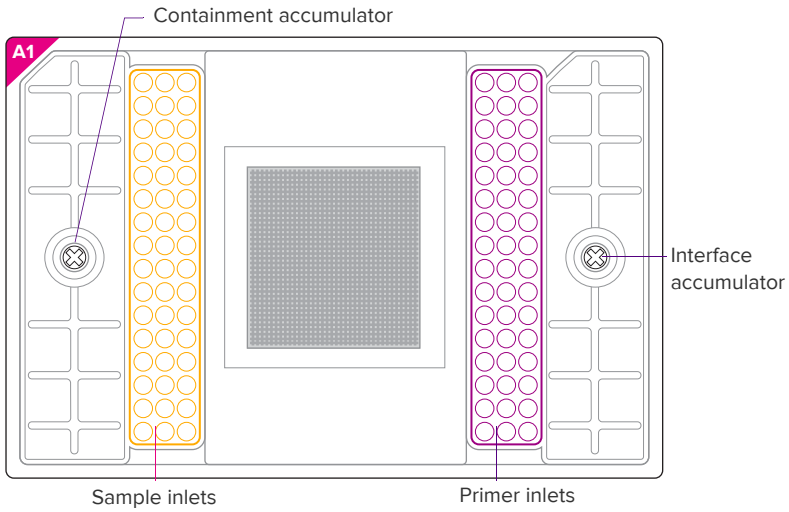


Figure 11. Access Array 48.48 IFC overview

Prepare the 20X Primer Solutions

IMPORTANT Warm the 20X Access Array Loading Reagent to room temperature before use.

Prepare 20X primer solutions according to your performing uniplex or multiplex PCR:

- **Multiplex PCR**: Refer to the Fluidigm Access Array Multiplex 20X Primer Solution Preparation Quick Reference (PN 100-3895).

NOTE The final Tagged TS forward and reverse concentrations for multiplex PCR are 1 µM per primer in the 20X Primer Solutions. The final TS forward and reverse primer concentrations in the Access Array reaction chamber are 50 nM per primer.

- **Uniplex PCR**: Refer to "Preparing the 20X Primers Solutions," in the Fluidigm Access Array System for Illumina Sequencing Systems User Guide (PN 100-3770).

  NOTE The final TS forward and reverse primer concentrations for uniplex PCR are 4 µM per primer in the 20X primer solution. The final TS forward and reverse primer concentrations in the Access Array IFC reaction chamber are 200 nM per primer.

Prepare Sample Master Mix Solutions

All DNA samples need to be added into the Sample PreMix individually, prior to loading the Sample Mix solutions into the sample inlets of an Access Array 48.48 IFC.

PREPARE THE SAMPLE PREMIX SOLUTIONS

1 Working in a DNA-free hood, combine the components:

Component	Volume per Reaction (µL)	Volume for 60 Reactions (µL) for One Access Array 48.48 IFC	Volume for 120 Reactions (µL) for Two Access Array 48.48 IFCs
10X FastStart High Fidelity Reaction Buffer without 18 mM $MgCl_2$ (Roche)	0.50	30.0	60.0
C1 $MgCl_2$ for AA (Fluidigm)*	0.02	1.4	2.7
DMSO (Roche)	0.25	15.0	30.0
10 mM PCR Grade Nucleotide Mix (Roche)	0.10	6.0	12.0
5 U/µL FastStart High Fidelity Enzyme Blend (Roche)	0.05	3.0	6.0
20X Access Array Loading Reagent (Fluidigm PN 100-7604)	0.25	15.0	30.0
PCR Water (Fluidigm)	0.08	4.6	9.3
Total	**1.25**	**75.0**	**150.0†**

* Use the $MgCl_2$ provided in the Fluidigm DNA Seq Kit, which is supplied at a higher concentration of $MgCl_2$ than is provided in the Roche kit

† This is enough reagent to load two Access Array 48.48 IFCs with 24 additional reactions to compensate for dead volume and pipetting error.

2 Vortex the Sample PreMix for a minimum of 20 seconds, and centrifuge for 30 seconds to collect all components.

PREPARE THE SAMPLE MIX SOLUTIONS

1 Combine the components listed in a 96-well plate to prepare 48 individual Sample Mix solutions:

Component	Volume per Reaction (µL)
Sample PreMix	1.25
DNA Sequencing Diluted Harvest	3.75
Total	**5.0**

2 Seal the plate with MicroAmp clear adhesive film, vortex the Sample Mix solutions for a minimum of 20 seconds, and centrifuge for 30 seconds to collect all components.

IMPORTANT It is essential to vortex all components to ensure complete mixing.

Load the Access Array 48.48 IFC

1 Pipet **4 µL** of 20X primer solution into each of the Primer Inlets.

2 Remove the adhesive seal, and then pipet 4 µL of Sample Mix solution into each of the sample inlets:

IMPORTANT While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into the inlets.

NOTE An eight-channel pipette is recommended to load the Sample Mix and 20X primer solutions. The recommended pipetting order is shown:

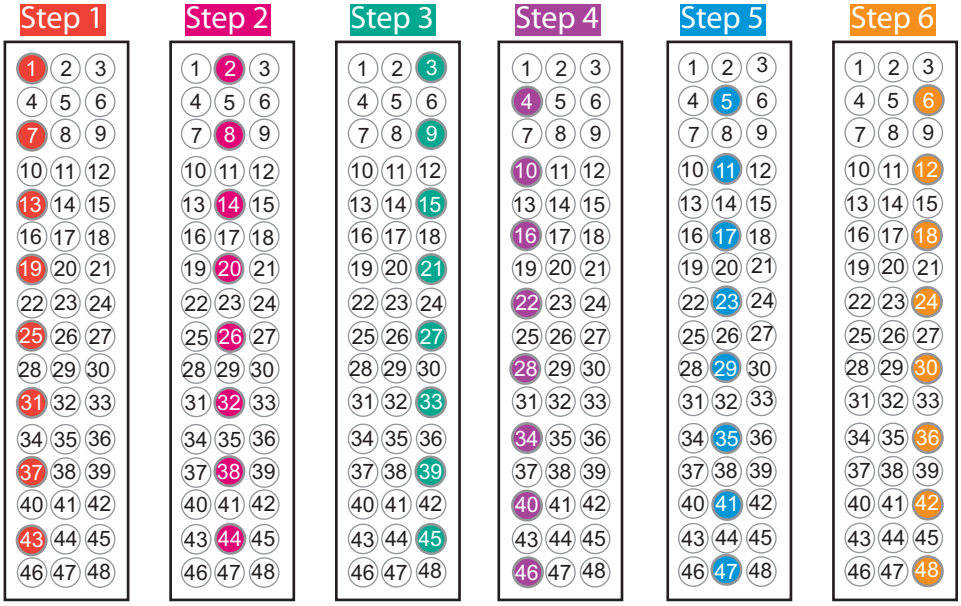


Figure 12. Pipetting order

3 Ensure that the IFC Controller AX is located in the pre-PCR lab.

4 Tap **Eject** to move the tray out of the IFC Controller AX.

5 Place the IFC onto the tray by aligning the notched corner of the IFC to the A1 mark.

6 Tap **Load Chip** to register the barcode of the IFC and activate the script selection.

7 Select **Load Mix (151x)** and **Run Script**.

8 After the script is complete, tap **Eject** to remove the IFC.

9 Place the IFC onto one of the following thermal cyclers and run the PCR according to the protocol specified:

- Fluidigm FC1 cycler: Select the **AA 48X48 Standard v1** protocol [refer to the Fluidigm FC1 Cycler Usage Quick Reference, (PN100-1250)].
- Fluidigm Stand-Alone Thermal Cycler: Select the **AA48v1** protocol [refer to the Fluidigm Stand-Alone Thermal Cycler Usage Quick Reference (PN 68000111)].

Harvest the Access Array 48.48 IFC

Fluidigm recommends harvesting the amplicons in a post-PCR lab.

1. After the PCR has finished, move the Access Array 48.48 IFC from the Biomark system or Biomark HD system into the post-PCR lab for harvesting.
2. Remove the remaining fluids from the H1-H4 wells.
3. Pipet **600 µL** of fresh 1X Access Array Harvest Solution into the H1–H4 wells. Do not use the hydration reagent here.
4. Pipet **2 µL** of 1X Access Array Harvest Solution into each of the sample inlets on the IFC.
5. Load the IFC in the post-PCR IFC Controller AX located in the post-PCR lab. If a post-PCR lab is not available, apply barrier tapes to the IFC. (See Appendix C: How to Use Barrier Tape on page 80.)
6. Tap **Eject** to move the tray out of the post-PCR IFC Controller AX.
7. Place the IFC onto the tray by aligning the notched corner of the IFC to the A1 mark.
8. Tap **Load Chip** to register the barcode of the IFC and activate the script selection.
9. Select **Harvest v5 (151x)** and **Run Script**.

   NOTE **Harvest v5 (151x)** is a script update from **Harvest (151x)** and is available on the Fluidigm website. For assistance, contact technical support.
10. Once the script is complete, tap **Eject** to remove the IFC.
11. Label a 96-well plate with the Access Array 48.48 IFC barcode. Carefully transfer 10 µL of harvested PCR products from each of the sample inlets into columns 1–6 of the 96-well PCR plate, using an eight-channel pipette.

Perform Quality Control (QC) on the Harvested PCR products

Perform QC on the harvested PCR products using the Agilent 2100 Bioanalyzer with DNA 1000 Chips.

1 Take 1 µL of each of the harvested PCR products in a random sampling of the products and run them on a Bioanalyzer DNA 1000 Chip following the manufacturer's instructions.

2 Ensure that amplicon sizes and distribution are within the expected range (±5% for amplicons of 200–400 bp including tags). The primer-dimer contamination in the PCR product pool (in the range of 50–130 bp) should be <25% based on the Bioanalyzer quantification:

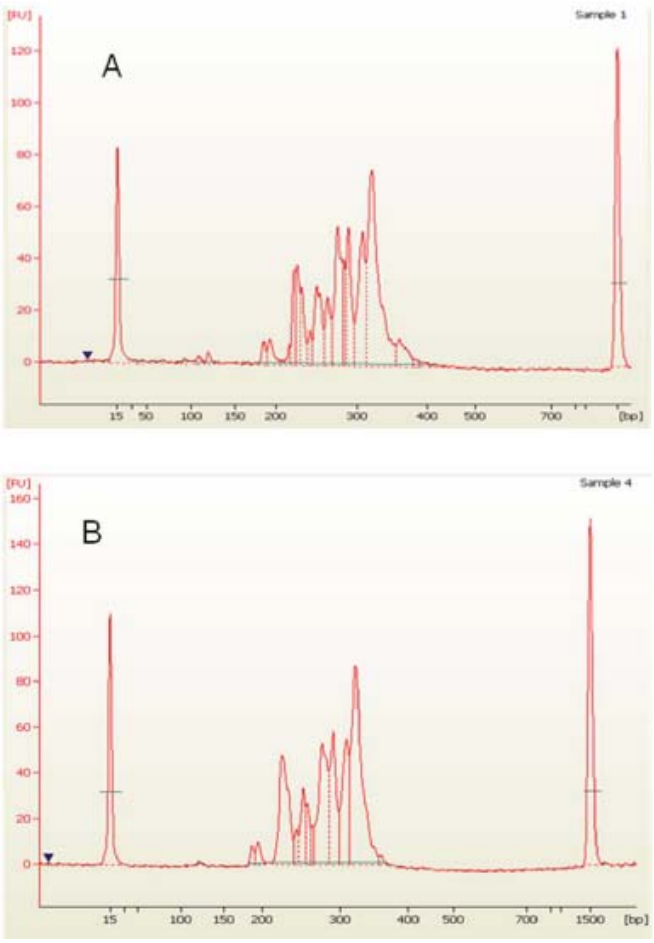


Figure 13. DNA 1000 Chip electropherogram of a pooled PCR product with 48 amplicons ranging between 180–350 bp. A. Harvested PCR product pool from a Access Array 48.48 IFC. B. The same PCR product pool after cleanup with magnetic beads.

Targeted Sequencing: Prepare Barcoded Libraries for Sequencing by the Illumina System

To prepare your library for Illumina sequencing:

- Attach Sequence Tags and Sample Barcodes to the Harvested PCR Products
- Pool, Purify, and Qualify the Barcoded Library
- Prepare Reagents for Sequencing on the Illumina Sequencing System

Attach Sequence Tags and Sample Barcodes to the Harvested PCR Products

1. Attach sequence tags and sample barcodes to the harvested PCR products. Refer to "Attaching Sequence Tags and Sample Barcodes" in the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).
2. Check the barcoded PCR products on the Agilent 2100 Bioanalyzer. Refer to "Checking PCR Products on the Agilent 2100 Bioanalyzer" in the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).

Pool, Purify, and Qualify the Barcoded Library

1. Pool the barcoded PCR products. Refer to "Pooling Products from Multiple Access Array IFCs" in the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).
2. Purify the barcoded library. Refer to "Purification of Harvested PCR Products" in the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).
3. Qualify the barcoded library. Refer to "Agilent 2100 Bioanalyzer Qualification" in the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).

Prepare Reagents for Sequencing on the Illumina Sequencing System

1. Follow the appropriate protocol to prepare reagents for the sequencing run according to your Illumina Sequencing System. Refer to "Preparing Reagents for Sequencing on the Illumina GAII and HiSeq Sequencing Systems" in the Access Array for Illumina Sequencing Systems User Guide (PN 100-3770).
2. Perform the sequencing run on the pooled, barcoded library. Refer to the appropriate Illumina sequencing guide before performing the sequencing run.

Prepare Samples for Whole Genome and Whole Exome Sequencing

IMPORTANT To prepare samples for whole exome sequencing, you must first prepare genomic samples. (See Prepare Genomic Samples.)

Prepare Genomic Samples

NOTE This section is adapted from the Illumina Nextera Rapid Capture Guide. Although we have adapted the Nextera chemistry to single-cell WGS and WES applications, we highly recommend that you carefully read the Illumina Nextera Rapid Capture Guide before proceeding.

Follow these sections to prepare genomic samples:

- Prepare Genomic Samples: Workflow
- Prepare Genomic Samples: Required Materials
- Prepare Genomic Samples: Perform Tagmentation on page 59
- Prepare Genomic Samples: Clean Up the Tagmented DNA on page 60
- Prepare Genomic Samples: PCR Amplify the Tagmented DNA on page 62
- Prepare Genomic Samples: Perform Quality Control (QC) on the Amplified Tagmented DNA on page 63

Prepare Genomic Samples: Workflow

Review the workflow to prepare genomic samples. See Prepare Genomic Samples, Whole Genome Sequencing (WGS), and Whole Exome Sequencing (WES) on page 14.

Prepare Genomic Samples: Required Materials

See Required Reagents for Preparing Genomic DNA on page 18, Required Consumables for Preparing Genomic Samples on page 21, and Required Consumables for Preparing Genomic Samples on page 21.

Prepare Genomic Samples: Perform Tagmentation

1 Label a 96-well PCR plate “Sample Tagment.”

2 From the PicoGreen dsDNA quantitation assay estimate, dilute the harvested DNA from single live cells with C1 DNA Dilution Reagent to 10 ng/µL. (See Quantify Harvest Amplicons on page 48.) If initial concentrations are between 5–10 ng/µL, dilute the samples to 5 ng/uL and proceed to step 5.

NOTE

- Select single-cell whole genome amplification samples to be diluted according to the cell capture image and PicoGreen dsDNA quantitation assay results. The qualified sample should be a sample from a single live cell and should meet the minimal DNA concentration required.
- Fluidigm highly recommends using unamplified gDNA from the same cell line as a control.

3 Remeasure concentrations of the diluted samples. [See the Single-Cell WTA PicoGreen Template (Fluidigm, PN 100-6260).]

NOTE Remeasure the sample concentrations for accuracy. Accurate concentrations are important to ensure that the tagmentation reaction generates libraries with the proper size distribution. The ratio of enzyme to DNA (in ng) determine insert size.

4 Dilute the samples to 5 ng/µL with Resuspension Buffer (RSB).

5 Prepare tagment mix, and then place the mix on ice:

Component	Volume per Reaction (µL)	Volume for 96 Reactions with Overage (µL)
Tagment DNA Buffer (TD)	6.25	684.0
Tagment DNA Enzyme (TDE1)	3.75	410.0
Total	**10.00**	**1,094.0**

6 Aliquot equal amounts of the tagment mix into each tube of a new eight-well strip.

7 From the eight-well strip, pipet 10 µL of the tagment mix into each well of the “sample tagment” plate.

8 Pipet 2 µL of the diluted DNA sample (5 ng/µL; see step 4) into each well of the plate.

9 Seal the plate with MicroAmp Clear Adhesive Film, vortex the plate at medium speed for 20 seconds, and then centrifuge it at 1,500 x *g* for 1 minute.

IMPORTANT Ensure that there are no bubbles in the wells of the plate. If necessary, centrifuge the plate at a higher centrifugal force and for a longer time to remove bubbles.

10 Place the plate in a thermal cycler and run the following program to perform the tagmentation:

Cycles	Temperature	Time
1	58 °C	10 min
1	10 °C	Hold

During the incubation, you can proceed to the next step.

11 Pipet 54 µL of Stop Tagment Buffer (ST) into each tube of a new eight-well strip. You will use the buffer in step 13.

12 When the temperature of the thermal cycler reaches 10 °C, remove the plate and place it on ice.

13 Remove the adhesive seal, and then pipet 3.75 µL of the Stop Tagment Buffer (ST) from step 11 into each well of the plate.

14 Seal the plate with MicroAmp Clear Adhesive Film, vortex the plate at medium speed for 20 seconds, and then centrifuge it at 1,500 x *g* for 1 minute.

15 Incubate the reactions at room temperature for 5 min.

Prepare Genomic Samples: Clean Up the Tagmented DNA

1 Allow the Sample Purification Beads (SPB) to reach room temperature.

2 Prepare 80% ethanol in a new 50 mL tube:

Component	Volume (mL)
UltraPure DNase/RNase-Free Distilled Water	10
Ethanol, absolute	Fill to 50

Invert the tube five times to mix. You use the 80% ethanol in step 10.

NOTE You can use freshly prepared 80% ethanol for up to 1 week. Store 80% ethanol at room temperature.

3 Vortex the Sample Purification Beads (SPB) at high speed for 1 minute.

4 Pipet 240 µL of Sample Purification Beads (SPB) into each tube of a new eight-well strip.

5 Remove the adhesive seal of the 96-well PCR plate containing the tagmented DNA, and then add 16 µL of the Sample Purification Beads (SPB) from step 4 to each well. The volume in each well of the eight-well strip is sufficient to pipet 16-µL aliquots across one row of the plate.

6 Seal the plate with MicroAmp Clear Adhesive Film, and then vortex the plate at medium speed for 30 seconds.

7 Incubate the tagmented DNA and beads at room temperature for 10 min.

8 Place the plate on a 96-well magnetic stand for 2 minutes.

9 Without disturbing the beads and keeping the plate on the magnetic stand, remove and discard the supernatant from each well.

10 Keeping the plate on the magnetic stand, pipet 180 µL of the 80% ethanol (see step 2) to each well of the plate, and then incubate the samples for 30 seconds.

11 Without disturbing the beads and keeping the plate on the magnetic stand, remove and discard the 80% ethanol from each well.

12 Repeat steps 10–11 once. Completely remove and discard all of the 80% ethanol.

13 Place the plate in a thermal cycler and incubate the tagmented DNA and beads at 37 °C for 2–5 minutes or at room temperature for 5–10 minutes with the lid open to air-dry the beads.

14 Pipet 100 µL of Resuspension Buffer (RSB) into each tube of a new eight-well strip.

15 From the eight-well strip, pipet 6 µL of the Resuspension Buffer (RSB) into each well of the plate. The volume in each well of the eight-well strip is sufficient to pipet 6-µL aliquots across one row of the plate.

16 Seal the plate with MicroAmp Clear Adhesive Film, and then vortex the plate at medium speed for 20 seconds to ensure that all beads are suspended.

17 Incubate the samples at room temperature for 2 minutes.

18 Centrifuge the plate at 1,500 x *g* for 2 minutes.

19 Place the plate onto a 96-well magnetic stand for 2 minutes. While the plate is on the stand, proceed to the next step.

20 Label a new 96-well PCR plate, “SC Lib Prep” and add the current date.

21 Keeping the original plate on the magnetic stand, carefully transfer 5 µL of each sample to the SC Lib Prep plate.

NOTE Minimize bead carryover by careful pipetting. A small amount of carryover will not affect subsequent PCR amplification.

Prepare Genomic Samples: PCR Amplify the Tagmented DNA

1 Pipet an equal volume of Nextera Library Amplification Mix (NLM) into each tube of a new eight-well strip.

2 Pipet 5 µL of the Nextera Library Amplification Mix (NLM) from step 1 into each well on the "SC Lib Prep" plate.

3 Pipet the index primers to the corresponding samples on the plate:

a Pipet 1.25 µL of Index 1 primers (N701–N712; orange caps) according to the index primer map in this step.

b Pipet 1.25 µL of Index 2 primers (E502–E508 and E517; white caps) according to the index primer map:

	N701	N702	N703	N704	N705	N706	N707	N708	N709	N710	N711	N712
	1	2	3	4	5	6	7	8	9	10	11	12
E517 A	E517 / N701	E517 / N702	E517 / N703	E517 / N704	E517 / N705	E517 / N706	E517 / N707	E517 / N708	E517 / N709	E517 / N710	E517 / N711	E517 / N712
E502 B	E502/ N701	E502/ N702	E502/ N703	E502/ N704	E502/ N705	E502/ N706	E502/ N707	E502/ N708	E502/ N709	E502/ N710	E502/ N711	E502/ N712
E503 C	E503/ N701	E503/ N702	E503/ N703	E503/ N704	E503/ N705	E503/ N706	E503/ N707	E503/ N708	E503/ N709	E503/ N710	E503/ N711	E503/ N712
E504 D	E504/ N701	E504/ N702	E504/ N703	E504/ N704	E504/ N705	E504/ N706	E504/ N707	E504/ N708	E504/ N709	E504/ N710	E504/ N711	E504/ N712
E505 E	E505/ N701	E505/ N702	E505/ N703	E505/ N704	E505/ N705	E505/ N706	E505/ N707	E505/ N708	E505/ N709	E505/ N710	E505/ N711	E505/ N712
E506 F	E506/ N701	E506/ N702	E506/ N703	E506/ N704	E506/ N705	E506/ N706	E506/ N707	E506/ N708	E506/ N709	E506/ N710	E506/ N711	E506/ N712
E507 G	E507/ N701	E507/ N702	E507/ N703	E507/ N704	E507/ N705	E507/ N706	E507/ N707	E507/ N708	E507/ N709	E507/ N710	E507/ N711	E507/ N712
E508 H	E508/ N701	E508/ N702	E508/ N703	E508/ N704	E508/ N705	E508/ N706	E508/ N707	E508/ N708	E508/ N709	E508/ N710	E508/ N711	E508/ N712

NOTE The number of indices used for the library prep is dictated by the number of indices available in the Illumina Nextera Rapid Capture Kit. Illumina provides 24 indices. For labeling with up to 96 indices, use the indices from the Illumina Nextera Rapid Capture Custom Enrichment Kit 288 Samples, PN 15055366.

4 Seal the plate with MicroAmp Clear Adhesive Film, vortex the plate at medium speed for 20 seconds and centrifuge it at 1,500 x *g* for 1 minute.

5 Place the plate in a thermal cycler and perform PCR amplification:

Cycles	Temperature	Time
1	72 °C	3 min
1	98 °C	30 sec
10	98 °C	10 sec
	60 °C	30 sec
	72 °C	30 sec
1	72 °C	5 min
1	10 °C	Hold

STOPPING POINT You can store the amplified, tagmented DNA at 2–8 °C for up to 72 hours.

Prepare Genomic Samples: Perform Quality Control (QC) on the Amplified Tagmented DNA

Perform QC on the amplified tagmented DNA using the Agilent 2100 Bioanalyzer with DNA High Sensitivity Chips.

1 Randomly choose 11 amplified libraries, and then dilute 1 µL of each library in 15 µL of Resuspension Buffer (RSB).

2 Run each library on a Bioanalyzer DNA High Sensitivity Chip following the manufacturer's instructions.

3 Ensure that the library passes QC:

- If the sizes of the amplified tagmented DNA are within normal range (150–2,000 bp and an average peak of 200–500 bp), proceed to Choose a Sequencing Application on page 64
- If the sizes of the library pool are outside normal range, and then troubleshoot the size variations. The tagment sizes vary with DNA input. Large tagment sizes indicate that the input concentration is too high. Small tagment sizes indicate that the DNA input is too low. If the libraries do not pass QC, adjust the DNA input concentration, and then repeat the protocol, Prepare Genomic Samples: Perform Tagmentation on page 59.

Choose a Sequencing Application

- To continue with whole genome sequencing, proceed to Whole Genome Sequencing.
- To perform whole exome sequencing, proceed to Whole Exome Sequencing on page 67.

Whole Genome Sequencing

Pool, clean up, and perform quality control on pooled libraries before whole genome sequencing.

Follow these sections for whole genome sequencing:

- Whole Genome Sequencing: Workflow
- Whole Genome Sequencing: Required Materials
- Whole Genome Sequencing: Pool and Clean Up the Libraries
- Whole Genome Sequencing: Perform Quality Control (QC) on the Cleaned-Up Library Pool on page 66

Whole Genome Sequencing: Workflow

Review the workflow to prepare to prepare for whole genome sequencing, Prepare Genomic Samples, Whole Genome Sequencing (WGS), and Whole Exome Sequencing (WES) on page 14.

Whole Genome Sequencing: Required Materials

See Required Reagents for Whole Genomic Sequencing on page 19, Required Consumables for Whole Genome Sequencing on page 22, and Required Consumables for Whole Genome Sequencing on page 22.

Whole Genome Sequencing: Pool and Clean Up the Libraries

1. Determine the number of libraries to be pooled based on the desired sequencing depth per sample.
2. Allow the Sample Purification Beads (SPB) to reach room temperature.

3 In a new 1.5 mL microcentrifuge tube, pipet 2 µL from each desired amplified sample to create a pooled library. (See Prepare Genomic Samples: PCR Amplify the Tagmented DNA on page 62.)

4 Determine the volume of Sample Purification Beads (SPB) required. Use an SPB volume that is 1.8x the library volume:

If the number of samples to pool is...	Then the volume of the library pool is... (µL)	And the volume of Sample Purification Beads (SPB) to use is... (µL)
6	12	22
12	24	43
24	48	86

5 Vortex the Sample Purification Beads (SPB) at maximum speed for 1 minute.

6 Add the required volume of the Sample Purification Beads (SPB) to the library pool. (See step 4.)

7 Vortex the library pool with beads at medium speed for 20 seconds, and then incubate the mixture at room temperature for 10 min.

8 Place the pool with beads on a magnetic stand for 2 min.

9 Without disturbing the beads and keeping the tube on the magnetic stand, remove and discard the supernatant from the tube.

10 Keeping the plate on the magnetic stand, pipet 200 µL of 80% ethanol to the beads (see preparation of 80% ethanol in Prepare Genomic Samples: Clean Up the Tagmented DNA on page 60), and then incubate the mixture for 1 minute.

11 Without disturbing the beads and keeping the tube on the magnetic stand, remove the 80% ethanol from the tube.

12 Repeat steps 10–11 once. Completely remove and discard all of the 80% ethanol.

13 Remove the tube from the magnetic stand, and then air-dry the beads at room temperature for 5–10 minutes or until the beads are completely dry.

14 To the air-dried beads, pipet the same volume of Resuspension Buffer (RSB) as the original volume of the library pool.

15 Vortex the suspension at medium speed for 20 seconds, and then incubate the beads at room temperature for 2 min.

16 Place the tube on a magnetic stand for 2 min. Keeping the tube on the stand, proceed to the next step.

17 Label a new 1.5 mL microcentrifuge tube with “WGS Lib Pool” and the date.

18 Keeping the tube on the magnetic stand, pipet the entire supernatant to the “WGS Lib Pool” tube.

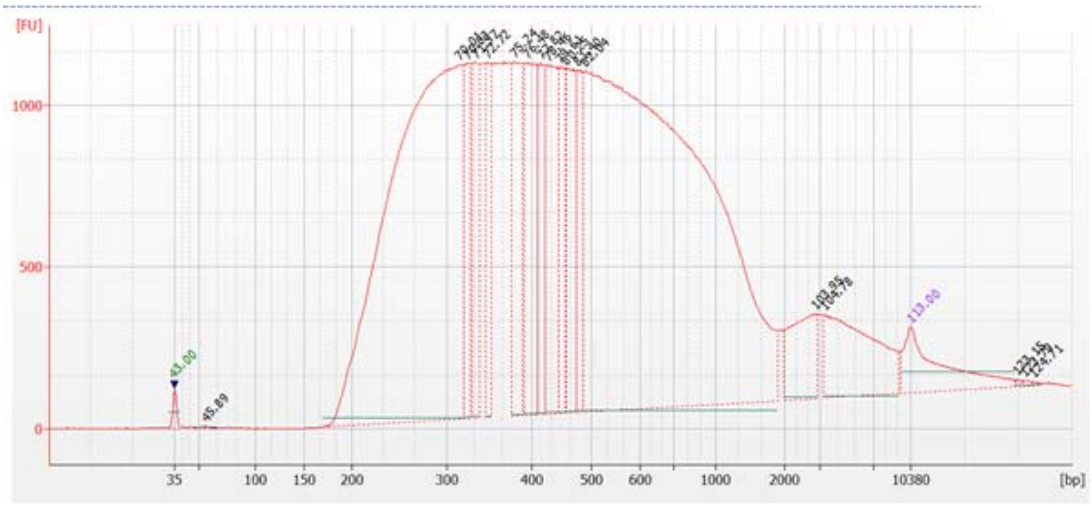
STOPPING POINT You can store the cleaned-up libraries at 2–8 °C for up to 72 hours or at –20 °C for long-term storage.

Whole Genome Sequencing: Perform Quality Control (QC) on the Cleaned-Up Library Pool

Perform QC on the cleaned-up library pool using the Agilent 2100 Bioanalyzer with DNA High Sensitivity Chips.

1 Dilute 1 µL of cleaned-up library pool in 9 µL of Resuspension Buffer (RSB), which should be at room temperature.

2 Run the diluted library pool on a Bioanalyzer DNA High Sensitivity Chip following the manufacturer’s instructions. For example:



3 Ensure that the library pool passes QC:

- If the sizes of the library pool are within acceptable range (150–2,000 bp and an average peak of 200–500 bp), perform whole genome sequencing on the pool with an appropriate Illumina sequencing system.
- If the sizes of the library pool are outside normal range, then troubleshoot the size variations. The sizes vary with DNA input. Large sizes indicate that the input concentration is too high. Small sizes indicate that the DNA input is too low. If the

library pool does not pass QC, adjust the DNA input concentration, then repeat the protocol, Prepare Genomic Samples: Perform Tagmentation on page 59.

- Before sequencing the library pool on the Illumina HiSeq system, you can sequence the library pool on the Illumina MiSeq system to examine library quality.

Whole Exome Sequencing

This protocol uses tagmented samples from the "Whole Genome Sequencing" protocol. (See Prepare Samples for Whole Genome and Whole Exome Sequencing on page 58.)

NOTE This section is adapted from the Illumina Nextera Rapid Capture Guide. Although we have adapted the Nextera chemistry to single-cell WGS and WES applications, we highly recommend that you carefully read the Illumina Nextera Rapid Capture Guide before proceeding.

Follow these sections to prepare for whole exome sequencing:

- Whole Exome Sequencing: Workflow
- Whole Exome Sequencing: Required Materials
- Whole Exome Sequencing: Pool and Clean Up the Libraries on page 68
- Whole Exome Sequencing: Perform the First Enrichment of the Library Pool on page 69
- Whole Exome Sequencing: Perform the Second Enrichment of the Library Pool on page 72
- Whole Exome Sequencing: Clean Up the Twice-Enriched Library Pool on page 74
- Whole Exome Sequencing: Amplify the Cleaned-Up Library Pool on page 75
- Whole Exome Sequencing: Clean Up the Amplified Library Pool on page 75
- Whole Exome Sequencing: Perform Quality Control (QC) on the Cleaned-Up Library Pool on page 76

Whole Exome Sequencing: Workflow

Review the workflow to prepare for exome sequencing, Prepare Genomic Samples, Whole Genome Sequencing (WGS), and Whole Exome Sequencing (WES) on page 14.

Whole Exome Sequencing: Required Materials

See Required Reagents for Whole Exome Sequencing on page 19, Suggested Reagents for Whole Genome Amplification on page 19, and Required Consumables for Whole Exome Sequencing on page 22.

Whole Exome Sequencing: Pool and Clean Up the Libraries

1 Determine the sequencing depth and number of samples to be sequenced.

NOTE The pool size is dictated by the number of indices provided in Illumina Nextera Rapid Capture Kit and the desired sequencing depth. The kit contains 24 indices. (For 96 indices, use the Illumina Nextera Rapid Capture Custom Enrichment Kit 288 Samples, PN 15055366.) Depending on the experimental design and desired sequencing depth for each exome, the pooled library can contain up to 96 samples.

2 Ensure that the amplified, tagmented DNA to be used for whole exome sequencing has passed quality control. (See Prepare Genomic Samples: Perform Quality Control (QC) on the Amplified Tagmented DNA on page 63.)

3 Allow the Sample Purification Beads (SPB) to reach room temperature.

4 In a new 1.5 mL microcentrifuge tube, pipet 2 µL from each desired amplified sample to create a pooled library. Use a new pipette tip after pipetting each sample.

5 Determine the volume of Sample Purification Beads (SPB) required:

If the number of samples to pool is...	Then the total volume of the library pool is... (µL)	And the volume of Sample Purification Beads (SPB) to use is...(µL)
24	48	48
48	96	96
72	144	144
96	192	192

6 Vortex the Sample Purification Beads (SPB) beads at maximum speed for 1 minute.

7 Add the required volume of the Sample Purification Beads (SPB) to the library pool. (See step 5.)

8 Vortex the library pool with beads at medium speed for 20 seconds, and then incubate the mixture at room temperature for 10 min.

9 Place the tube with the library pool and beads on a magnetic stand for 2 min.

10 Without disturbing the beads, remove and discard the supernatant from the tube.

11 Keeping the tube on the magnetic stand, pipet 500 µL of 80% ethanol to the beads (see the preparation of 80% ethanol in Prepare Genomic Samples: Clean Up the Tagmented DNA on page 60), and then incubate the mixture for 1 minute. Keep the tube on the magnetic stand.

12 Without disturbing the beads and keeping the tube on the magnetic stand, remove and discard the 80% ethanol from the tube.

13 Repeat steps 11–12 once. Completely remove and discard all of the 80% ethanol.

14 Remove the tube from the magnetic stand, and then air-dry the beads at room temperature for 5–10 minutes or until the beads are completely dry.

15 To the air-dried beads, pipet 45 µL of Resuspension Buffer (RSB).

16 Vortex the suspension at medium speed for 20 seconds, and then incubate the beads at room temperature for 2 min.

17 Place the tube on a magnetic stand for 2 min. While the tube is on the stand, proceed to the next step.

18 Label a new 1.5 mL microcentrifuge tube with “Exome Lib Pool” and the date.

19 After the two minutes on the magnetic stand, pipet the entire supernatant to the “Exome Lib Pool” tube.

20 Measure the concentration of the pool by one of these three methods:

- NanoDrop Spectrophotometer
- Quant-IT PicoGreen dsDNA Assay Kit
- Qubit 2.0 Fluorometer.

Whole Exome Sequencing: Perform the First Enrichment of the Library Pool

1 Set a thermoshaker to 40 °C.

2 Bring the Enrichment Hybridization Buffer (EHB) to room temperature, and then vortex the solution until it is clear.

3 Determine the volume of library pool to use (x). The volume used should be ≤40 µL and contain 500 ng–4 µg of the pooled library.

4 In a new 0.25 mL PCR tube, prepare the enrichment mix:

Component	Volume (µL)
Resuspension Buffer (RSB)	40–x
Library Pool	x
Enrichment Hybridization Buffer (EHB)	50
Coding Exome Oligos (CEX) or Expanded Exome Oligos (EEX)	10
Total	**100**

5 Vortex the enrichment mix at medium speed for 10 seconds and centrifuge at 1,500 x *g* for 1 minute.

6 Place the tube in a thermal cycler and run the following program:

Cycles	Temperature	Time
1	95 °C	1 min
1	58 °C	2 h

STOPPING POINT You can store the amplified, tagmented DNA at 2–8 °C overnight.

7 Briefly centrifuge the tube at 1,500 x *g*.

8 Pipet the entire mix to a new 1.5 mL microcentrifuge tube. You need to use the larger tube to hold the Streptavidin Magnetic Beads (SMB) and the enrichment mix.

9 Vortex Streptavidin Magnetic Beads (SMB) at maximum speed for 1 minute.

10 Pipet 250 µL of Streptavidin Magnetic Beads (SMB) to the enrichment mix.

11 Vortex the enrichment mix with beads at medium speed for 10 seconds.

12 Place the tube in a thermoshaker, and then incubate the enrichment mix with beads at 40 °C for 15 minutes and shaker speed of 1,500 rpm.

13 Place the tube on a laboratory rotator, and then rotate the tube at room temperature for 10 minutes.

14 While the tube is rotating, Increase the temperature of the thermoshaker to 50 °C.

15 Place the tube on a magnetic stand for 2 minutes.

16 Keeping the tube on the magnetic stand, carefully pipet the entire supernatant to a new 1.5 mL microcentrifuge tube, and then place the tube with the supernatant on ice. Retain the beads.

IMPORTANT Do not discard the supernatant until the experiment is complete. You may be able to recover the library by repeating the cleanup with Sample Purification Beads (SPB).

17 Pipet 200 µL of Enrichment Wash Solution (EWS) to the Streptavidin Magnetic Beads (SMB).

18 Vortex the suspension at medium speed for 20 seconds, and then incubate the suspension at 50 °C in the thermoshaker at 1,500 rpm for 15 minutes. After the 15 minute incubation, immediately proceed to the next step.

19 Place the tube on a magnetic stand for 2 minutes, and then carefully remove and discard the supernatant.

20 Repeat steps 17–19 once. Keep the sample at room temperature.

21 In a new 1.5 mL microcentrifuge tube, prepare elution solution:

Component	Volume (µL)
Enrichment Elution Buffer 1 (EE1)	28.5
2 N NaOH (HP3)	1.5
Total	**30.0**

22 Vortex the elution solution at medium speed for 20 seconds, and then briefly centrifuge the tube at 1,500 x *g*.

23 Pipet 23 µL of elution solution to the beads, and then vortex the suspension at medium speed for 20 seconds.

24 Incubate the suspension at room temperature for 2 minutes.

25 Place the tube on a magnetic stand for 1 minute.

26 Carefully pipet 21 µL of the supernatant to a new 0.2 mL PCR tube.

27 Pipet 4 µL of Elute Target Buffer 2 (ET2) to the elution to neutralize it.

28 Vortex the neutralized elution at medium speed for 20 seconds, and then centrifuge at 1,500 x *g* for 1 minute.

STOPPING POINT You can store the enriched library at 2–8 °C for up to 72 hours.

Whole Exome Sequencing: Perform the Second Enrichment of the Library Pool

1 Set a thermoshaker to 40 °C.

2 In a new 0.2 mL PCR tube, prepare the enrichment mix:

Component	Volume (µL)
Library pool from first enrichment	25
Resuspension Buffer (RSB)	15
Enrichment Hybridization Buffer (EHB)	50
Coding Exome Oligos (CEX) or Expanded Exome Oligos (EEX)	10
Total	**100**

3 Vortex the enrichment mix at medium speed for 10 seconds, and then centrifuge the mix at 1,500 x *g* for 1 minute.

4 Place the tube in a thermal cycler and run the following program:

Cycles	Temperature	Time
1	95 °C	1 min
1	58 °C	2 hr

5 Briefly centrifuge the tube at 1,500 x *g*.

6 Pipet the entire mix to a new 1.5 mL microcentrifuge tube. You need to use the larger tube to hold the Streptavidin Magnetic Beads (SMB) and the enrichment mix.

7 Vortex Streptavidin Magnetic Beads (SMB) at maximum speed for 1 minute.

8 Pipet 250 µL of Streptavidin Magnetic Beads (SMB) to the enrichment mix.

9 Vortex the enrichment mix with beads at medium speed for 10 seconds.

10 Place the tube in a thermoshaker, and then incubate the enrichment mix with beads at 40 °C for 15 minutes and shaker speed of 1,500 rpm.

11 Place the tube on a laboratory rotator, and then rotate the tube at room temperature for 10 minutes.

12 While the tube is rotating, Increase the temperature of the thermoshaker to 50 °C.

13 Place the tube on a magnetic stand for 2 min.

14 Keeping the tube on the magnetic stand, remove and discard the supernatant. Retain the beads.

15 Pipet 200 µL of Enrichment Wash Solution (EWS) to the beads.

16 Vortex the suspension at medium speed for 20 seconds, and then incubate the suspension at 50 °C in the thermoshaker at 1,500 rpm for 15 minutes. After the 15-minute incubation, immediately proceed to the next step.

17 Place the tube on a magnetic stand for 2 minutes, and then carefully remove and discard the supernatant.

18 Repeat steps 15–17 once.

19 Pipet 200 µL of Enrichment Wash Solution (EWS) to the beads.

20 Vortex the suspension at medium speed for 20 seconds.

21 Place the tube on a magnetic stand for 2 minutes, and then carefully remove and discard the supernatant.

22 In a new 1.5 mL microcentrifuge tube, prepare elution solution:

Component	Volume (µL)
Enrichment Elution Buffer 1 (EE1)	28.5
2 N NaOH (HP3)	1.5
Total	**30.0**

23 Vortex the elution solution at medium speed for 20 seconds, and then briefly centrifuge the tube at 1,500 x *g*.

24 Pipet 23 µL of elution solution to the beads, and then vortex the suspension at medium speed for 20 seconds.

25 Incubate the elution solution with beads at room temperature for 2 minutes.

26 Place the tube on a magnetic stand for 1 minute.

27 Carefully pipet 21 µL of the supernatant to a new 0.25 mL PCR tube.

28 Pipet 4 µL of Elute Target Buffer 2 (ET2) to the elution to neutralize it.

29 Vortex the neutralized elution at medium speed for 20 seconds, and then centrifuge at 1,500 x *g* for 1 minute.

STOPPING POINT You can store the enriched library at 2–8 °C for up to 72 hours.

Whole Exome Sequencing: Clean Up the Twice-Enriched Library Pool

1. Bring the Sample Purification Beads (SPB) to room temperature.
2. Pipet 75 µL of Resuspension Buffer (RSB) to the twice-enriched library pool. (See Whole Exome Sequencing: Perform the Second Enrichment of the Library Pool on page 72.)
3. Vortex the Sample Purification Beads (SPB) at maximum speed for 20 seconds, and then pipet 100 µL of the Sample Purification Beads (SPB) to the library pool.
4. Vortex the suspension at medium speed for 20 seconds, and then incubate the suspension at room temperature for 10 minutes.
5. Place the tube on a magnetic stand for 2 minutes, and then carefully remove and discard the supernatant without disturbing the beads.
6. Keeping the tube on the magnetic stand, pipet 200 µL of 80% ethanol (see the preparation of 80% ethanol in Prepare Genomic Samples: Clean Up the Tagmented DNA on page 60), and then incubate the suspension for 1 minute.
7. Without disturbing the beads and keeping the tube on the magnetic stand, remove and discard the 80% ethanol from the tube.
8. Repeat steps 6–7 once, and then carefully remove and discard the supernatant.
9. Place the tube at room temperature to air-dry the beads for 5–10 minutes or until the beads are completely dry.
10. Pipet 27.5 µL of Resuspension Buffer (RSB) to the air-dried beads.
11. Vortex the suspension at medium speed for 20 seconds, and then incubate the suspension at room temperature for 2 minutes.
12. Place the tube on a magnetic stand for 2 minutes. While the tube is on the stand, proceed to the next step.
13. Label a new 0.25 mL PCR tube “Enriched Sample.”
14. Keeping the tube on the magnetic stand, pipet 25 µL of the supernatant to the new 0.25 mL PCR tube labeled “Enriched Sample.”

Whole Exome Sequencing: Amplify the Cleaned-Up Library Pool

1 In a new 0.25 mL tube, prepare the PCR reaction:

Component	Volume (µL)
Cleaned-up library pool (See Whole Exome Sequencing: Clean Up the Twice-Enriched Library Pool.)	25.0
PCR Primer Cocktail (PPC)	5.0
Nextera Enrichment Amplification Mix (NEM)	20.0
Total	**50.0**

2 Vortex the enrichment mix at medium speed for 10 seconds, and then centrifuge the mix at 1,500 x *g* for 1 minute.

3 Place the tube in a thermal cycler and perform PCR amplification:

Cycles	Temperature	Time
1	98 °C	30 sec
10	98 °C	10 sec
	60 °C	30 sec
	72 °C	30 sec
1	72 °C	5 min
1	4 °C	Hold

Whole Exome Sequencing: Clean Up the Amplified Library Pool

1 Bring the Sample Purification Beads (SPB) to room temperature.

2 To the amplified library pool (~50 µL; see Whole Exome Sequencing: Amplify the Cleaned-Up Library Pool on page 75), pipet 50 µL of Resuspension Buffer (RSB).

3 Vortex the Sample Purification Beads (SPB) at maximum speed for 20 seconds.

4 Pipet 100 µL of the Sample Purification Beads (SPB) to the library pool with Resuspension Buffer (RSB).

5 Vortex the suspension at medium speed for 20 seconds, and then incubate the suspension at room temperature for 10 minutes.

6 Place the tube on a magnetic stand for 2 minutes, and then carefully remove and discard the supernatant without disturbing the beads. Keep the tube on the magnetic stand.

7 Keeping the tube on the magnetic stand, pipet 200 µL of 80% ethanol (see the preparation of 80% ethanol in Prepare Genomic Samples: Clean Up the Tagmented DNA on page 60), and then incubate the suspension for 1 minute.

8 Without disturbing the beads and keeping the tube on the magnetic stand, remove and discard the 80% ethanol from the tube.

9 Repeat steps 7–8 once, and then carefully remove and discard the supernatant.

10 Place the tube at room temperature to air-dry the beads for 5–10 minutes or until the beads are completely dry.

11 Pipet 40 µL of Resuspension Buffer (RSB) to the air-dried beads.

12 Vortex the Resuspension Buffer with beads at medium speed for 20 seconds, and then incubate the Resuspension Buffer with beads at room temperature for 2 minutes.

13 Place the tube on a magnetic stand for 2 minutes. While the tube is on the stand, proceed to the next step.

14 Label a new 0.25 mL PCR tube with “Enriched Lib” and the date.

15 Keeping the tube on the magnetic stand, pipet the entire supernatant to the 0.25 mL PCR tube labeled “Enriched Lib.”

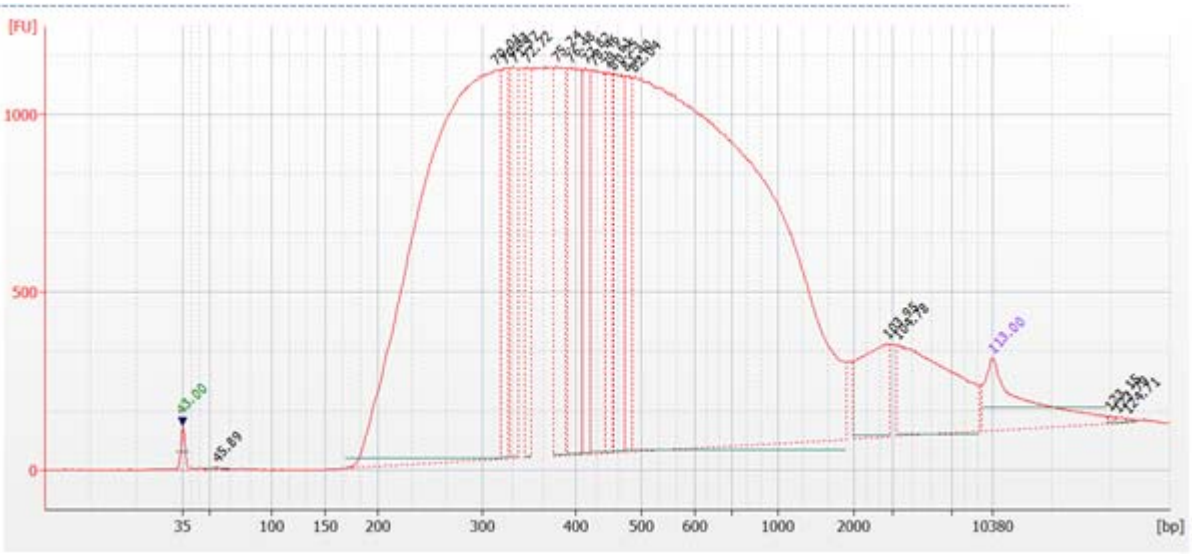
STOPPING POINT You can store the cleaned-up libraries at 2–8 °C for up to 72 hours or at −20 °C for long-term storage.

Whole Exome Sequencing: Perform Quality Control (QC) on the Cleaned-Up Library Pool

Perform QC on the cleaned-up library pool using the Agilent 2100 Bioanalyzer with DNA High Sensitivity Chips.

1 Dilute 1 µL of cleaned-up library pool in 4 µL of Resuspension Buffer (RSB), which should be at room temperature.

2 Run the diluted library pool on a Bioanalyzer DNA High Sensitivity Chip following the manufacturer's instructions. For example:



3 Ensure that the library pool passes QC:

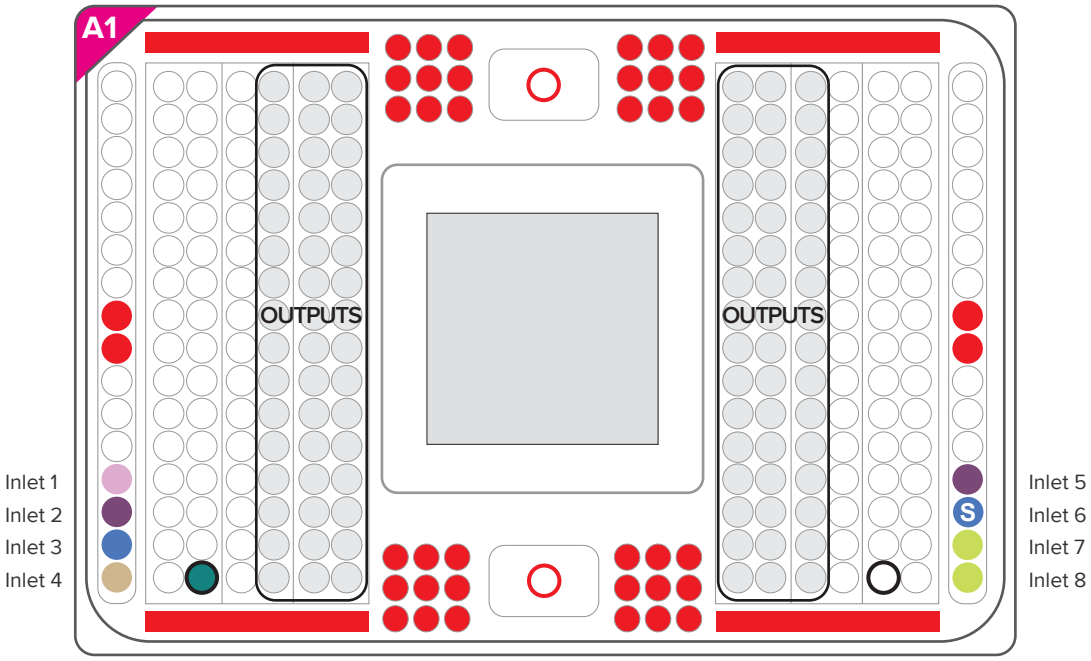
- If the sizes of the library pool are within acceptable range (150–2,000 bp and an average peak of 200–500 bp), perform whole genome exome sequencing on the pool with an appropriate Illumina sequencing system.
- If the sizes of the library pool are outside normal range, then troubleshoot the size variations. Ensure that all of the correct primers and probes were used to prepare the enriched library. If the library pool does not pass QC, make any necessary adjustments to the reagents, and repeat the protocol, Whole Exome Sequencing: Pool and Clean Up the Libraries on page 68.
- Before sequencing the library pool on the Illumina HiSeq system, you can sequence the library pool on the Illumina MiSeq system to examine library quality.
- The yield should be ~5% of the amount of starting material that was used in the first enrichment. (See Whole Exome Sequencing: Perform the First Enrichment of the Library Pool on page 69.)

Sequence Analysis

To analyze a library after targeted or whole exome sequencing, refer to the Singular Analysis Toolset User Guide (PN 100-5066).

Appendix A: IFC Pipetting Map

Overview of IFC Pipetting



Prime the IFC:

- C1 Harvest Reagent, 200 µL
- C1 Harvest Reagent, 20 µL
- C1 Preloading Reagent, 20 µL
- C1 Blocking Reagent, 15 µL
- C1 DNA Seq Cell Wash Buffer, 20 µL

Load the IFC:

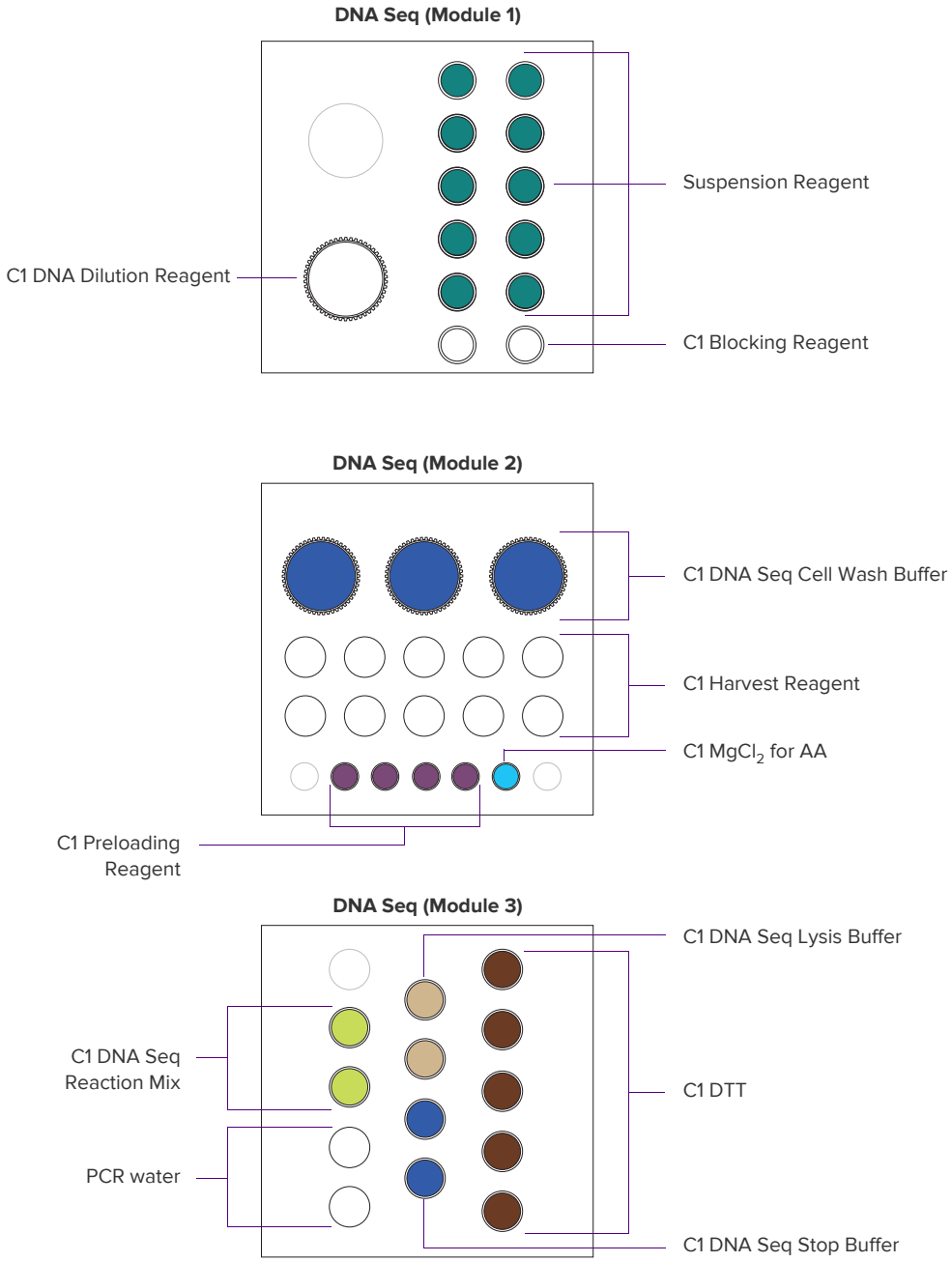
- Remove C1 Blocking Reagent
- LIVE/DEAD Staining Solution, 20 µL
- Cell mix, 6 µL

Lyse and amplify:

- C1 Harvest Reagent, 180 µL
- Lysis final mix, 10 µL
- DNA Reaction-enzyme mix, 24 µL
- C1 DNA Seq Stop Buffer, 10 µL

Appendix B: C1 Single-Cell Auto Prep Reagent Kit for DNA Seq, PN 100-7357

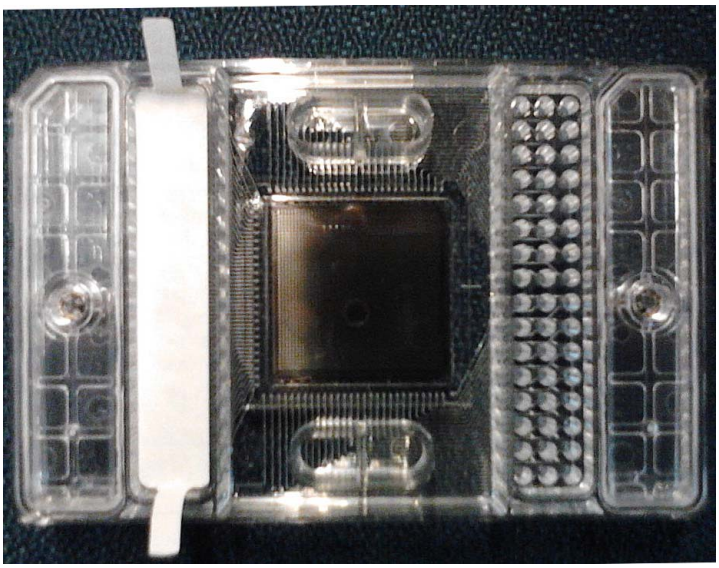
For storage conditions, see page 16.



Appendix C: How to Use Barrier Tape

If you cannot harvest the Access Array IFC in a post-PCR lab, apply barrier tapes (Fluidigm, PN 100-5920) to the IFC.

1. Separate the barrier tape from its base layer and discard the base layer.
2. Affix the tape onto the sample inlets before harvesting.
3. Ensure that the barrier tape covers all the sample Inlets:



Appendix D: IFC Types and Related Scripts

There are currently three C1 system-compatible IFCs for small, medium, and large single cells:

Table 1. IFC types and related scripts

Cell size/IFC Name and Part Numbers	Barcode (prefix)	Script Names	Description
Small (5–10 µm) C1 IFC for DNA Seq (5–10 µm) PN 100-5762	1791x	DNA Seq: Prime (1791x)	Priming the control line and cell capture channels of the 5–10 µm DNA Seq IFC (1791x)
		DNA Seq: Cell Load (1791x)	Cell loading and washing without staining for DNA sequencing of 5–10 µm diameter cells (1791x)
		DNA Seq: Cell Load & Stain (1791x)	Cell loading, staining, and washing for DNA sequencing of 5–10 µm diameter cells (1791x)
		DNA Seq: Amplify (1791x)	Loading, thermal, and harvest protocol for single-cell lysis and whole genome amplification of 5–10 µm diameter cells (1791x)
Medium (10–17 µm) C1 IFC for DNA Seq (10–17 µm) PN 100-5763	1792x	DNA Seq: Prime (1792x)	Priming the control line and cell capture channels of the 10–17 µm DNA Seq IFC (1792x)
		DNA Seq: Cell Load (1792x)	Cell loading and washing without staining for DNA sequencing of 10–17 µm diameter cells (1792x)
		DNA Seq: Cell Load & Stain (1792x)	Cell loading, staining, and washing for DNA sequencing of 10–17 µm diameter cells (1792x)
		DNA Seq: Amplify (1792x)	Loading, thermal, and harvest protocol for single-cell lysis and whole genome amplification of 10–17 µm diameter cells (1792x)
Large (17–25 µm) C1 IFC for DNA Seq (17–25 µm) PN 100-5764	1793x	DNA Seq: Prime (1793x)	Priming the control line and cell capture channels of the 17–25 µm DNA Seq IFC (1793x)
		DNA Seq: Cell Load (1793x)	Cell loading and washing without staining for DNA sequencing of 17–25 µm diameter cells (1793x)
		DNA Seq: Cell Load & Stain (1793x)	Cell loading, staining, and washing for DNA sequencing of 17–25 µm diameter cells (1793x)
		DNA Seq: Amplify (1793x)	Loading, thermal, and harvest protocol for single-cell lysis and whole genome amplification of 17–25 µm diameter cells (1793x)

Appendix E: Related Documentation

- C1 System User Guide (Fluidigm, PN 100-4977)
- C1 System Site Requirements Guide (Fluidigm, PN 100-5201)
- LIVE/DEAD® Viability/Cytotoxicity Kit, for mammalian cells (Thermo Fisher Scientific, PN L-3224)
- Minimum Specifications for Single-Cell Imaging (Fluidigm, PN 100-5004)
- INCYTO Disposable Hemocytometer, incyto.com/product/product02_detail.php
- Single-Cell WTA PicoGreen® Template (Fluidigm, PN 100-6260)
- Singular Analysis Toolset User Guide (Fluidigm, PN 100-5066)
- Agilent® Bioanalyzer User Guide
- Illumina® Nextera™ Rapid Capture Guide
- Voet, T. et al. “Single-cell paired-end genome sequencing reveals structural variation per cell cycle.” *Nucleic Acids Research.* 41 (2013): 6,119-38.
- Dean, F.B. et al. “Comprehensive human genome amplification using multiple displacement amplification.” *PNAS* 99 (2002): 5,261-66.

Appendix F: Safety

General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Use personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves.
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.
- Do not eat, drink, or smoke in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

Instrument Safety

WARNING Do not modify this device. Unauthorized modifications may create a safety hazard.

CAUTION HOT SURFACE. The C1 thermal cycler chuck gets hot and can burn your skin. Use caution when working near the chuck.

CAUTION PINCH HAZARD. The C1 door and shuttle can pinch your hand. Make sure your fingers, hand, shirt sleeve, etc., are clear of the door and shuttle when loading or ejecting a IFC.

WARNING BIOHAZARD. If you are putting biohazardous material on the instrument, use appropriate personal protective equipment and adhere to *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) from the Centers for Disease Control and Prevention and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines at: cdc.gov/biosafety/publications/index.htm.

For a full list of the symbols on the instrument, refer to the C1 System User Guide (PN 100-4977).

Chemical Safety

Read and comprehend all safety data sheets (SDSs) by chemical manufacturers before you use, store, or handle any chemicals or hazardous materials.

Wear personal protective equipment (gloves, safety glasses, fully enclosed shoes, lab coats) when handling chemicals.

Do not inhale fumes from chemicals. Use adequate ventilation, and return caps to bottles immediately after use.

Check regularly for chemical spills or leaks. Follow SDS recommendations for cleaning up spills or leaks.

Disposal of Products

Used IFCs should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.

Do not dispose of this product in unsorted municipal waste. This equipment may contain hazardous substances that could affect health and the environment. Use appropriate take-back systems when disposing of materials and equipment.

Learn more at fluidigm.com/compliance.