Total RNA-Seq Using C1 and SMART-Seq Stranded Kit

Introduction

In this technical note, we describe a method to generate Illumina®-compatible total RNA-seq libraries from single cells using C1™ IFCs (integrated fluidic circuits) and the SMART-Seq® Stranded Kit (Takara Bio USA, Inc.) on the C1 system.

Workflow

An estimated timeline is shown below, but your actual timeline may vary.

	Step	Time
Pre-C1	**Dilute indexing primers.**	30 min
C1	**Prepare reagent mixes.**	30 min
	Run Total RNA-Seq script on C1.	6 hr
	Harvest PCR1 products.*	5 min
Post-C1†	**A. Pool harvested PCR1 products.***	15 min
	B. Purify pooled products.	45 min
	C. Deplete ribosomal cDNA.	1 hr 20 min
	D. Perform PCR2.*	30 min
	E. Purify total RNA-seq library.	1 hr 30 min

* Potential stopping point

† The post-C1 workflow is modified from the SMART-Seq Stranded Kit User Manual (Takara Bio USA, Inc. PN 070518)

Materials

Required Script

Total RNA-Seq script and accompanying protocol (available for download from Script Hub™ at fluidigm.com/c1openapp/scripthub).

Required Reagents

IMPORTANT Store reagents as soon as they are received, according to manufacturer's storage recommendations.

- SMART-Seq Stranded Kit (Takara Bio USA, Inc. PN 634444, 96 rxns)
- Agencourt® AMPure XP (Beckman Coulter PN A63880, 5 mL)
- PCR-certified water
- Ethanol (200 proof, anhydrous)

Required Consumables

- C1 Open App™ IFC or C1 mRNA Seq IFC (Fluidigm, any available cell size range)
- 0.5 and 1.5 mL low-bind Eppendorf® microtubes
- 8-well PCR tube strips or individual PCR tubes
- 96-well PCR plates and sealing films

Required Equipment

- C1 system
- 2100 Bioanalyzer® with High Sensitivity DNA Kit (Agilent Technologies)
- Vortexer
- Three centrifuges: one for microtubes, one for 8-well PCR tube strips, and one for 96-well PCR plates
- Thermal cycler with heated lid and 100 µL sample volume capacity
- Calibrated pipettes (single- and multi-channel) and appropriate low-retention filter tips
- Two magnetic separation devices: one for 1.5 mL microtubes and one for 8-well PCR tube strips

Before You Begin

IMPORTANT Before using Fluidigm reagents, read and understand the detailed instructions and safety guidelines in this document. For complete safety information, see Appendix F.

- Vortex and centrifuge all reagents according to manufacturer's instructions.
- Use good laboratory practices to minimize contamination of samples.
- For detailed instructions on Fluidigm instrument and software operation, see the C1 System User Guide (PN 100-4977).

Pre-C1 Protocol

Dilute the Indexing Primers

1 Dilute the 3′ PCR primers (8 total, from 3′ 1 to 3′ 8) in separate 0.5 mL microtubes by combining the components in Table 1.

Table 1. Diluted 3′ PCR Primers. This example shows the volumes needed for one primer.

Component	Vol. per Primer (µL)*
Nuclease-Free Water (Takara Bio)	64.8
3′ PCR Primer (3′ 1 to 3′ 8) (12.5 µM each, Takara Bio)	12.0
Total	**76.8**

* Includes overage.

2 Dilute the 5′ PCR primers (12 total, from 5′ 1 to 5′ 12) in separate 0.5 mL microtubes by combining the components in Table 2.

Table 2. Diluted 5′ PCR Primers. This example shows the volumes needed for one primer.

Component	Vol. per Primer (µL)*
Nuclease-Free Water (Takara Bio)	40.5
5′ PCR Primer (5′ 1 to 5′ 12) (12.5 µM each, Takara Bio)	7.5
Total	**48.0**

* Includes overage.

3 Transfer 5 µL of each diluted primer to the appropriate well of a new 96-well PCR plate, according to the plate map in Figure 1.

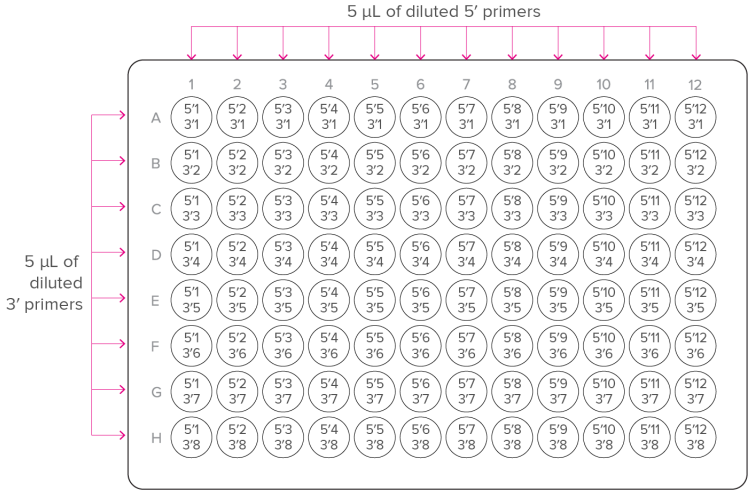


Figure 1. Diluted primer mix plate.

The plate now contains 96 wells of dual-indexing primers at the working dilution.

C1 Protocol

Prepare Reagent Mixes and Run the Total RNA-Seq Script on C1

1 Go to fluidigm.com/c1openapp/scripthub to download the **Total RNA-Seq** script and accompanying protocol from Script Hub.

2 Follow the protocol instructions to prepare the reagent mixes, load reagents and cells into the C1 IFC, and run the Total RNA-Seq script on the C1.

Harvest the PCR1 Products

1 When the Total RNA-Seq Sample Prep script has finished, tap EJECT to remove the IFC from the instrument.

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

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

3 Label a new 96-well plate "Harvest Plate."

4 Carefully pull back the tape covering the harvest outlets of the IFC using the plastic removal tool.

5 Using an 8-channel pipette set to 8.5 µL, pipet the harvested PCR1 amplicons from the IFC outlets according to Figure 2 and place into the 96-well Harvest Plate.

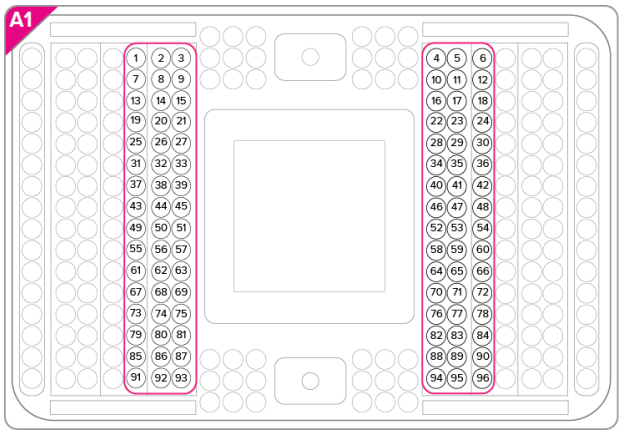


Figure 2. Pipette map of PCR1 products on the C1 IFC.

NOTE Harvest volumes may vary. Set a pipette to 8.5 µL to ensure entire volume is extracted. For detailed instructions on pipetting the harvest amplicons from the IFC to the 96-well harvest plate, see Appendix D: Detailed Harvest Pipetting Maps.

6 Seal the Harvest Plate and then centrifuge it to collect harvest products.

After harvesting, material from the capture sites is arranged on the Harvest Plate as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A	C03	C02	C01	C49	C50	C51	C06	C05	C04	C52	C53	C54
B	C09	C08	C07	C55	C56	C57	C12	C11	C10	C58	C59	C60
C	C15	C14	C13	C61	C62	C63	C18	C17	C16	C64	C65	C66
D	C21	C20	C19	C67	C68	C69	C24	C23	C22	C70	C71	C72
E	C25	C26	C27	C75	C74	C73	C28	C29	C30	C78	C77	C76
F	C31	C32	C33	C81	C80	C79	C34	C35	C36	C84	C83	C82
G	C37	C38	C39	C87	C86	C85	C40	C41	C42	C90	C89	C88
H	C43	C44	C45	C93	C92	C91	C46	C47	C48	C96	C95	C94

STOPPING POINT The harvested PCR1 products are now ready for the Post-C1 Protocol (see below). Harvest products can be stored at –20°C for up to two weeks before proceeding to the next step.

Post-C1 Protocol

Sections B–E are modified from pages 21–25 in the SMART-Seq Stranded Kit User Manual (Takara Bio USA, Inc. PN 070518).

Program the Thermal Cycler

For convenience, pre-program the following thermal protocols before proceeding to Section A. Pool the Harvested Products.

PreZap

Temperature	Time
72 °C	2 min
4 °C	∞

Zap

Temperature	Time
37 °C	60 min
72 °C	10 min
4 °C	∞

PCR2

Temperature	Time	Cycle	
94 °C	1 min	Hold	
98 °C	15 sec	Denaturation	12–14 cycles
55 °C	15 sec	Annealing	
68 °C	30 sec	Extension	
4 °C	∞	Hold	

NOTE Start a specific thermal protocol as indicated in the following sections in order to pre-heat the thermal cycler. Once the cycler has reached the target temperature for the first step, pause the protocol. After you place your samples in the cycler, resume the protocol and run it to completion.

A. Pool the Harvested Products

Transfer 4 µL from each of the 96 PCR1 products harvested from the C1 IFC into a single 1.5 mL microtube. The expected total pooled harvest volume is 384 µL.

NOTE We do not recommend pooling less than 2 µL of each harvest. You can pool a larger volume for cells with low RNA yield. If needed, you can pool the entire harvest volume.

STOPPING POINT Pooled harvest products can be stored at –20°C for one week.

B. Purify the Pooled Products

IMPORTANT DO NOT start this procedure if you do not have time to perform all steps up to Section D. Perform PCR2–RNA-Seq Library Amplification.

Before You Begin

You will need the following components, plus a magnetic separation device for microtubes.

- Remove Nuclease-Free Water from the SMART-Seq Stranded Kit at –20°C and thaw at room temperature. Do the same for ZapR Buffer, in preparation for Section C. Deplete Ribosomal cDNA.
- Warm AMPure XP beads up to room temperature (~30 min).
- Prepare ~2 mL of 80% EtOH by measuring each component separately and mixing immediately before use. **Do not** top off.

Component	Volume (mL)
PCR-certified water	0.4
EtOH (200 proof, anhydrous)	1.6

Purify the Products

1. Vortex the beads for 1 min immediately before use, and then pipet 269 µL beads to the pooled harvest sample of 384 µL (for a 0.7x ratio). Adjust the bead volume if a different pooled sample volume is used (see Section A. Pool the Harvested Products).

   NOTE The beads are viscous; pipet the entire volume up and down slowly. Accurate pipetting of beads is critical.

2. Mix by vortexing for 5 sec, and then incubate at room temperature for 8 min to allow the DNA to bind to the beads.
3. Briefly centrifuge the tube at ~2,000 x *g* to collect the liquid at the bottom. Place the tube on the magnetic separation device for 10 min or longer, until the solution is completely clear.
4. Keep the tube on the magnet and use a pipette to carefully remove and discard the **supernatant** without disturbing the beads.
5. Keeping the tube on the magnet and without disturbing the beads: carefully pipet 700 µL of freshly prepared 80% EtOH to the tube to wash away contaminants. Incubate for 30 sec and then use a pipette to carefully remove and discard the **supernatant**. cDNA will remain bound to the beads during the washing process.
6. Repeat Step B.5.

7 Briefly centrifuge the tube at ~2,000 x *g* to collect the remaining EtOH at the bottom of the tube. Place the tube on the magnet for 30 sec, and then use a pipette to carefully remove and discard any remaining EtOH without disturbing the beads.

8 Keep the tube open and at room temperature for ~5–10 min until the pellet appears dry.

NOTE You may see a tiny crack in the pellet when dry. Do not overdry.

9 Once the beads are dry, pipet 75 µl of Nuclease-Free Water to cover the beads. Remove the tube from the magnet and mix thoroughly by pipetting up and down until all the beads have been washed off the sides of the tube.

10 Incubate at room temperature for 5 min to rehydrate.

11 Briefly centrifuge the tube at ~2,000 x *g* to collect the liquid at the bottom. Place the tube on the magnet for 1 min or longer, until the solution is completely clear.

12 Transfer 73 µL of the **supernatant** to a new 1.5 mL microtube without disturbing the beads.

13 Vortex the AMPure XP beads for 1 min immediately before use, and then pipet 58 µL of beads to the new tube (for a 0.8x ratio).

14 Mix by vortexing for 5 sec, and then incubate the bead mixture at room temperature for 8 min to allow the DNA to bind to the beads. During the incubation time, proceed immediately to Section C. Deplete Ribosomal cDNA.

C. Deplete Ribosomal cDNA with scZapR and scR-Probes

Before You Begin

You will need the following components:

- Nuclease-Free Water, ZapR Buffer, and AMPure XP beads at room temperature, plus the freshly prepared 80% EtOH and magnetic separation device from previous section.
- Remove scZapR and scR-Probes from the SMART-Seq Stranded Kit at –20°C and –70°C, respectively. Keep scZapR on ice during use and return to –20°C immediately after use. Thaw scR-Probes to room temperature, and then immediately place on ice.
- Pre-chill an empty PCR tube by placing it on ice.
- Start the **PreZap** thermal protocol to preheat the thermal cycler (see Program the Thermal Cycler).

Deplete the Ribosomal cDNA

1 After the 8-min bead mixture incubation time in Step B.14 is complete, briefly centrifuge the tube at ~2,000 x *g* to collect the liquid at the bottom. Place the tube on the magnet for 5 min or longer, until the solution is completely clear.

2 During this 5-min bead mixture incubation time, pipet 1.5 µL scR-Probes per reaction into a pre-chilled PCR tube (see above). Keep this tube on ice and immediately return the unused scR-Probes to –70°C.

3 Incubate the microtube containing scR-Probes at 72°C in a thermal cycler pre-heated using the **PreZap** thermal protocol (see above).

4 Leave the scR-Probes tube in the thermal cycler at 4°C for at least 2 min, but for no more than 10–15 min, before using it in the scZapR Master Mix (see Step C.10).

5 Once the 5-min incubation on the magnet is complete and the solution is clear (see Step C.1), keep the tube on the magnet and use a pipette to remove and discard the **supernatant**.

6 Keeping the tube on the magnet and without disturbing the beads: carefully pipet 200 µL of freshly prepared 80% EtOH to the tube to wash away contaminants. Incubate for 30 sec and then use a pipette to carefully remove and discard the **supernatant**. cDNA will remain bound to the beads during the washing process.

7 Repeat Step C.6.

8 Briefly centrifuge the tube at ~2,000 x *g* to collect the remaining EtOH at the bottom of the tube. Place the tube on the magnet for 30 sec, and then use a pipette to carefully remove and discard any remaining EtOH without disturbing the beads.

9 Keep the tube open and at room temperature for ~1–2 min until the pellet appears dry.

NOTE Although 1–2 min may be sufficient, you can let the beads air-dry for up to 5 min while you prepare the scZapR Master Mix in Step C.10.

10 While the beads are drying, prepare the scZapR Master Mix by combining the components in Table 3 at room temperature and in the order shown. Make sure to add the preheated and chilled scR-Probes from Step C.4 last. Return scZapR to –20°C immediately after use. Briefly vortex and centrifuge at ~2,000 x *g* to mix.

Table 3. scZapR Master Mix. Combine components in the order shown.

	Component	Vol. per Rxn (µL)
1	Nuclease-Free Water (Takara Bio)	16.8
2	10X ZapR Buffer (Takara Bio)	2.2
3	scZapR (Takara Bio)	1.5
4	scr-Probes (Takara Bio)	1.5
Total		**22.0**

11 Once the beads are dry from Step C.9, pipet 22 µl of the scZapR Master Mix to cover the beads, and then close the tube. Remove the tube from the magnet and mix thoroughly by vortexing to resuspend the beads.

12 Incubate at room temperature for 5 min to rehydrate.

13 During this incubation time, start the **Zap** thermal protocol to preheat the thermal cycler (see Program the Thermal Cycler).

14 Briefly centrifuge the tube at ~2,000 x *g* to collect the liquid at the bottom. Place the tube on the magnet for 1 min or longer, until the solution is completely clear.

15 Keep the tube on the magnet and pipet 20 µl of the **supernatant** to a new PCR tube without disturbing the beads.

16 Incubate the tube in a thermal cycler pre-heated using the **Zap** thermal protocol (see above).

NOTE You can leave the tube in the thermal cycler at 4°C for up to 1 hr. However, we recommend proceeding immediately to Section D. Perform PCR2–RNA-Seq Library Amplification.

D. Perform PCR2–RNA-Seq Library Amplification

Before You Begin

You will need the following components:

- Nuclease-Free Water at room temperature, from previous section.
- Remove SeqAmp CB PCR Buffer (2X), PCR2 Primers and SeqAmp DNA Polymerase from the SMART-Seq Stranded Kit at –20°C and thaw on ice.
- Start the **PCR2** thermal protocol to preheat the thermal cycler (see Program the Thermal Cycler).

Perform PCR2

1 Prepare the PCR2 Master Mix by combining the components in Table 4 in the order shown. Briefly vortex and centrifuge at ~2,000 x *g* to mix.

Table 4. PCR2 Master Mix. Combine components in the order shown.

	Component	Vol. per Rxn (µL)
1	Nuclease-Free Water (Takara Bio)	26
2	SeqAmp CB PCR Buffer (Takara Bio)	50
3	PCR2 Primers (Takara Bio)	2
4	SeqAmp DNA Polymerase (Takara Bio)	2
Total		**80**

IMPORTANT DO NOT reduce the total PCR2 Master Mix volume as the final reaction volume of 100 µL in Step D.2 is crucial for adequate yield.

2 Pipet 80 µL of PCR2 Master Mix to the incubated tube from Step C.15, for a final reaction volume of 100 µL. Tap gently and briefly centrifuge at ~2,000 x *g* to mix.

IMPORTANT If your thermal cycler cannot accommodate 100 µL sample volumes, divide the reaction into two ~50 µL aliquots *after* adding the PCR Master Mix, but *before* thermal cycling in Step D.3.

3 Place the tube in the pre-heated thermal cycler and resume the **PCR2** thermal protocol (see above).

STOPPING POINT You can leave the PCR2 products in the thermal cycler at 4°C overnight to process the next day, or store them at –20°C for up to 2 weeks.

E. Purify the RNA Seq Library

Before You Begin

You will need the following components, plus a magnetic separation device for PCR tubes.

- Remove Nuclease-Free Water and Tris Buffer (5 mM) from the SMART-Seq Stranded Kit at –20°C and thaw at room temperature.
- Warm AMPure XP beads up to room temperature (~30 min).
- Prepare ~1 mL of 80% EtOH by measuring each component separately and mixing immediately before use. **Do not** top off.

Component	Volume (mL)
PCR-certified water	0.2
EtOH (200 proof, anhydrous)	0.8

Purify the PCR2 Products – First Cleanup

1 Vortex the AMPure XP beads for 1 min immediately before use, and then pipet 100 µL of beads to the tube containing PCR2 products (for a 1.0x ratio). Mix well by vortexing for 5 sec.

NOTE Accurate pipetting of AMPure XP beads with a well-calibrated pipette is critical.

2 Incubate at room temperature for 8 min to let the cDNA bind to the beads.

3 Briefly centrifuge the tube at ~2,000 x *g* to collect the liquid at the bottom. Place the tube on the magnetic separation device for 10 min or longer, until the solution is completely clear.

4 Keep the tube on the magnet and use a pipette to carefully remove and discard the **supernatant** without disturbing the beads.

5 Keeping the tube on the magnet and without disturbing the beads: carefully pipet 200 µL of freshly prepared 80% EtOH to the tube to wash away contaminants. Incubate for 30 sec and then use a pipette to carefully remove and discard the **supernatant**. cDNA will remain bound to the beads during the washing process.

6 Repeat Step E.5.

7 Briefly centrifuge the tube at ~2,000 x *g* to collect the remaining EtOH at the bottom of the tube. Place the tube on the magnet for 30 sec, and then use a pipette to carefully remove and discard any remaining EtOH without disturbing the beads.

8 Keep the tube open and at room temperature for 10 min until the pellet appears dry.

NOTE You may see a tiny crack in the pellet when dry. Do not overdry.

9 Once the beads are dry, pipet 52 µL of Nuclease-Free Water to cover the beads, and then close the tube. Remove the tube from the magnet and mix thoroughly by vortexing until all the beads have been washed off the sides of the tube.

10 Incubate at room temperature for 5 min to rehydrate.

11 Briefly centrifuge the tube at ~2,000 x *g* to collect the liquid at the bottom. Place the tube on the magnet for 5 min or longer, until the solution is completely clear.

12 Keep the tube on the magnet and pipet 50 µL of the **supernatant** to a new PCR tube without disturbing the beads.

Purify the PCR2 Products – Second Cleanup

13 Vortex the AMPure XP beads for 1 min immediately before use, and then pipet 50 µL to the tube containing supernatant (for a 1.0x ratio). Mix well by vortexing for 5 sec.

NOTE Accurate pipetting of AMPure XP beads with a well-calibrated pipet is critical.

14 Incubate at room temperature for 8 min to let the DNA bind to the beads.

15 Briefly centrifuge the tube at ~2,000 x *g* to collect the liquid at the bottom. Place the tube on the magnet for 5 min or longer, until the solution is completely clear.

16 Keep the tube on the magnet and use a pipette to carefully remove and discard the **supernatant** without disturbing the beads.

17 Keeping the tube on the magnet and without disturbing the beads: carefully pipet 200 µL of freshly prepared 80% EtOH to the tube to wash away contaminants. Incubate for 30 sec and then use a pipette to carefully remove and discard the **supernatant**. DNA will remain bound to the beads during the washing process.

18 Repeat Step E.17.

19 Briefly centrifuge the tube at ~2,000 x *g* to collect the remaining EtOH at the bottom of the tube. Place the tube on the magnet for 30 sec, and then use a pipette to carefully remove and discard any remaining EtOH without disturbing the beads.

20 Keep the tube open and at room temperature for 5 min until the pellet appears dry.

NOTE You may see a tiny crack in the pellet when dry. Do not overdry.

21 Once the beads are dry, pipet 22 µL of Tris Buffer to cover the beads, and then close the tube. Remove the tube from the magnet and mix thoroughly by vortexing until all the beads have been washed off the sides of the tube.

22 Incubate at room temperature for 5 min to rehydrate.

23 Briefly centrifuge the tube at ~2,000 x *g* to collect the liquid at the bottom. Place the tube on the magnet for 5 min or longer, until the solution is completely clear.

24 Keep the tube on the magnet and pipet 20 µL of the **supernatant** to a new low-bind microtube without disturbing the beads.

25 Proceed immediately to evaluate the RNA-seq library size distribution using the Agilent 2100 Bioanalyzer with the High Sensitivity DNA Kit, or store the purified library at –20°C.

Appendix A: Sequencing Guidelines

The following guidelines are modified from pages 30–31 in the SMART-Seq Stranded Kit User Manual (Takara Bio USA, Inc. PN 070518).

Sequencing Recommendation

Prepare a Sequencing Library

After library validation, prepare a 4 nM library for the sequencing run:

1. Dilute final library to 4 nM in nuclease-free water.
2. Depending on the Illumina sequencing library preparation protocol, use either a 5 µL or 10 µL aliquot of the diluted libraries. Follow the library denaturation protocol in the latest version of your Illumina sequencing instrument's user guide.

(Optional) Include a PhiX Control Library

For best quality sequencing data, include 1–5% PhiX control spike-in to the sequencing library.

Follow Illumina guidelines on how to denature, dilute, and combine a PhiX control library with your own pool of libraries. Make sure to use a fresh and reliable stock of the PhiX control library.

Library Loading Guidelines

Libraries generated with the SMART-Seq Stranded Kit cluster very efficiently, therefore make sure to avoid over-clustering. Use the library loading guidelines for various Illumina sequencing instruments in Table 5 as a starting point.

Table 5. Library loading guidelines for various Ilumina sequencing instruments.

Sequencing Instrument	Loading Conc. (pM)
MiSeq® – v2 chemistry	8
MiSeq – v3 chemistry	10
MiniSeq™	1.2
NextSeq® 500/550	1.1–1.2

Additional Recommendations for NextSeq and MiniSeq Instruments

Follow the additional recommendations for optimal performance of libraries sequenced on NextSeq and MiniSeq instruments from page 31 in the SMART-Seq Stranded Kit User Manual (Takara Bio USA, Inc. PN 070518).

Appendix B: Sequencing Analysis Considerations

IMPORTANT Follow the guidelines for sequence trimming and strand-of-origin information from page 13 in the SMART-Seq Stranded Kit User Manual (Takara Bio USA, Inc. PN 070518).

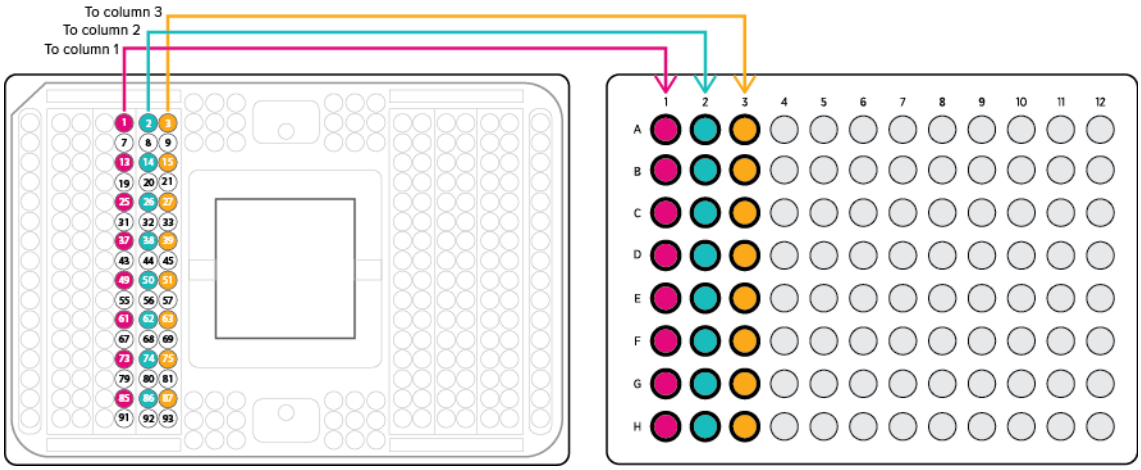
Appendix C: Indexing Primer Sets Adapter Sequences

i5 Index (tube label)	i5 Illumina Index Name	i5 Bases for Sample Sheet: MiSeq, NovaSeq™, HiSeq® 2000/2500	i5 Bases for Sample Sheet: MiniSeq, NextSeq, HiSeq 3000/4000	i7 Index (tube label)	i7 Illumina Index Name	i7 Bases for Sample Sheet
3′ 1	D501	TATAGCCT	AGGCTATA	5′ 1	D701	ATTACTCG
3′ 2	D502	ATAGAGGC	GCCTCTAT	5′ 2	D702	TCCGGAGA
3′ 3	D503	CCTATCCT	AGGATAGG	5′ 3	D703	CGCTCATT
3′ 4	D504	GGCTCTGA	TCAGAGCC	5′ 4	D704	GAGATTCC
3′ 5	D505	AGGCGAAG	CTTCGCCT	5′ 5	D705	ATTCAGAA
3′ 6	D506	TAATCTTA	TAAGATTA	5′ 6	D706	GAATTCGT
3′ 7	D507	CAGGACGT	ACGTCCTG	5′ 7	D707	CTGAAGCT
3′ 8	D508	GTACTGAC	GTCAGTAC	5′ 8	D708	TAATGCGC
				5′ 9	D709	CGGCTATG
				5′ 10	D710	TCCGCGAA
				5′ 11	D711	TCTCGCGC
				5′ 12	D712	AGCGATAG

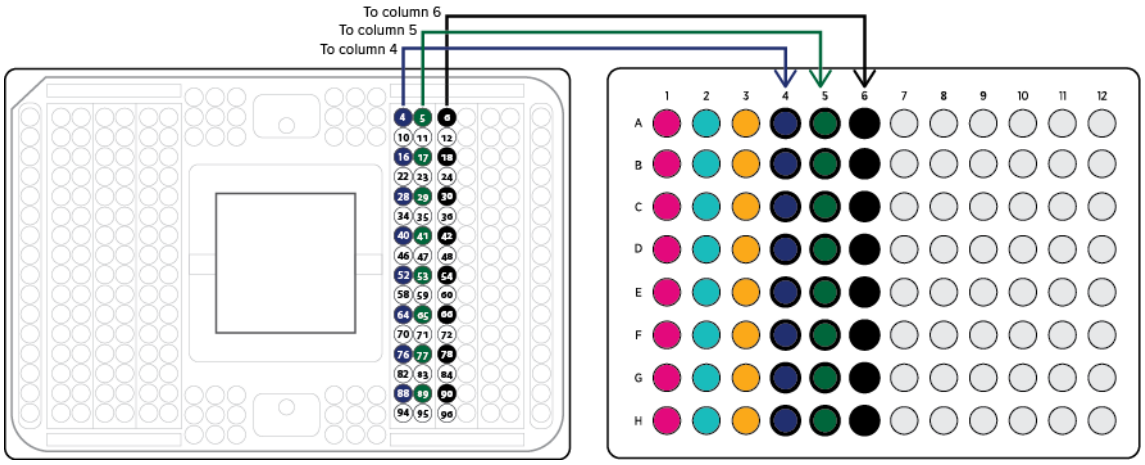
Appendix D: Detailed Harvest Pipetting Maps

The following are detailed instructions on pipetting the harvested PCR1 products from the C1 IFC to the 96-well Harvest Plate (see Harvest the PCR1 Products on page 3).

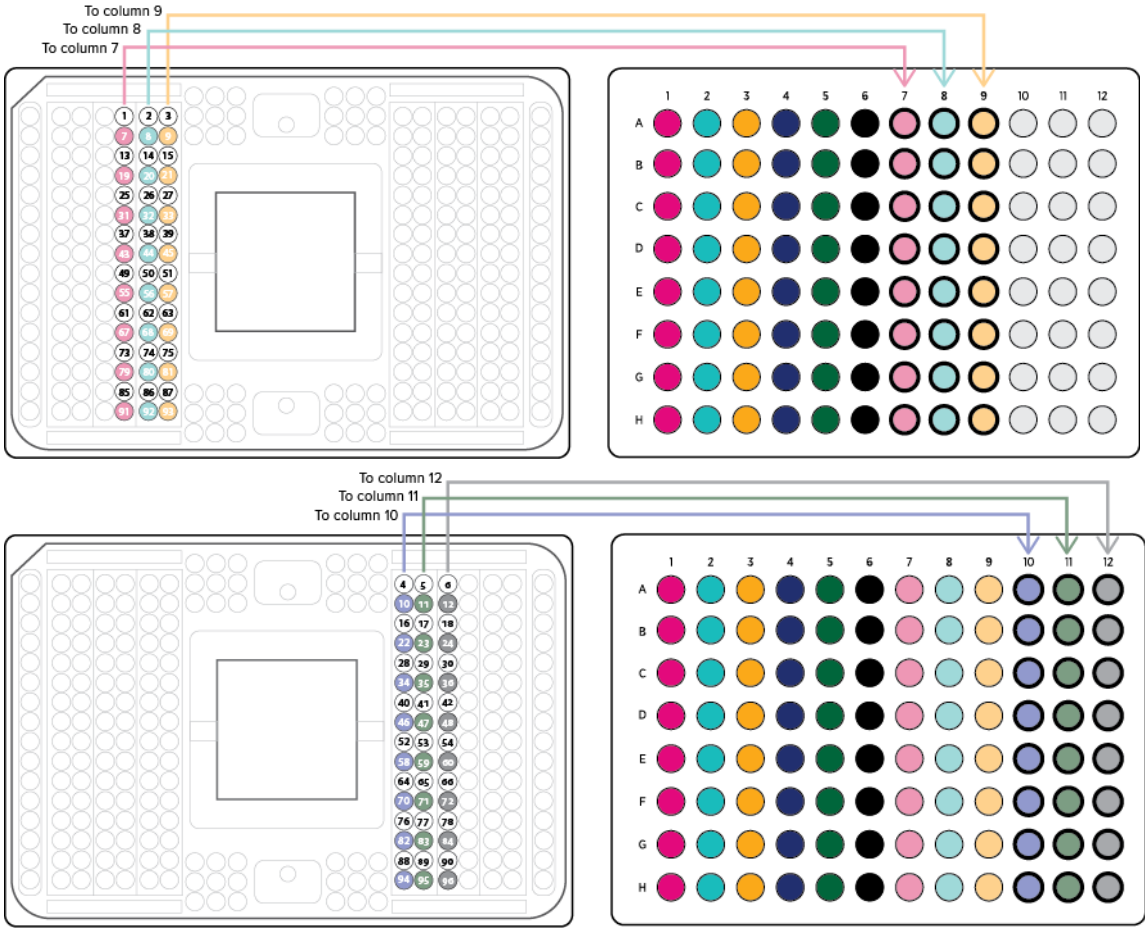
1 Pipet the entire volume of C1 harvest amplicons out of the first set of wells on the *left*-side of the C1 IFC into the *first* three columns of the harvest plate:



2 Pipet the entire volume of C1 harvest amplicons out of the first set of wells on the *right*-side of the C1 IFC into the *next* three columns of the harvest plate:



3 Repeat this process for the remaining left-side and right-side wells of the C1 IFC:



Appendix E: Related Documents

The post-C1 workflow is modified from the SMART-Seq Stranded Kit User Manual (Takara Bio USA, Inc. PN 070518). Go to fluidigm.com to download the following related Fluidigm documents.

Title	Part Number
C1 System User Guide	100-4977
Updating the C1 System Software Quick Reference	100-6217

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 the appropriate personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves, according to your laboratory safety practices.
- 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

For complete instrument safety information, including a full list of the symbols on the instrument, refer to the instrument user guide (see Appendix E).

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), a publication 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 online at cdc.gov/biosafety/publications/index.htm.

Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier.

Disposal of Products

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

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