Preparation of Single-Cell RNA-Seq Libraries for Next Generation Sequencing

John J. Trombetta,David Gennert,Diana Lu,Rahul Satija,Alex K. Shalek,Aviv Regev

Abstract

For the past several decades, due to technical limitations, the field of transcriptomics has focused on population-level measurements that can mask significant differences between individual cells. With the advent of single-cell RNA-Seq, it is now possible to profile the responses of individual cells at unprecedented depth and thereby uncover, transcriptome-wide, the heterogeneity that exists within these populations. This unit describes a method that merges several important technologies to produce, in high-throughput, single-cell RNA-Seq libraries. Complementary DNA (cDNA) is made from full-length mRNA transcripts using a reverse transcriptase that has terminal transferase activity. This, when combined with a second “template-switch” primer, allows for cDNAs to be constructed that have two universal priming sequences. Following preamplification from these common sequences, Nextera XT is used to prepare a pool of 96 uniquely indexed samples ready for Illumina sequencing. *Curr. Protoc. Mol. Biol*. 107:4.22.1-4.22.17. © 2014 by John Wiley & Sons, Inc.

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Guidelines

[Please see the full manuscript for additional information.]

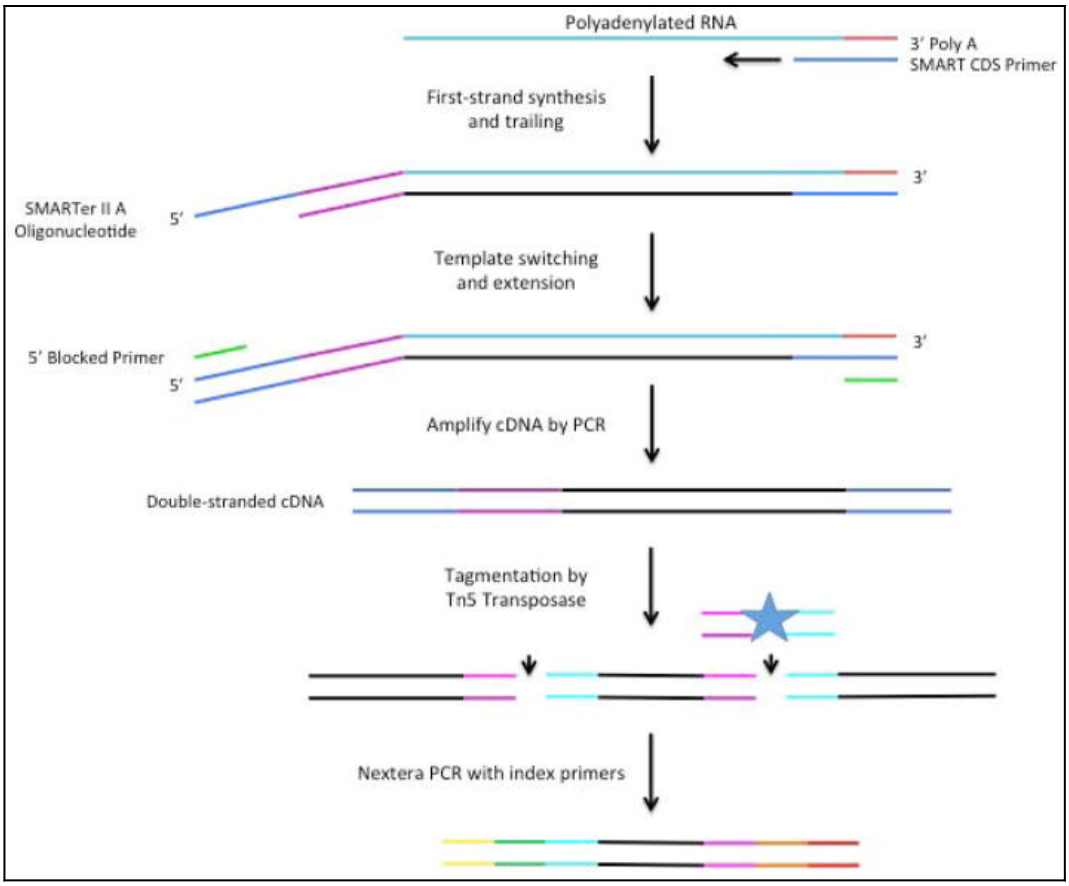

Figure 1. Single-cell RNA-Seq library construction. Illustrated are the steps required to convert mRNA to cDNA with sequencing adapters.

Oligonucleotide Primer Sequences

3′ SMART CDS Primer IIA: 5′ AAGCAGTGGTATCAACGCAGAGTACT(30)VN

SMARTer II A Oligonucleotide: 5′AAGCAGTGGTATCAACGCAGAGTACATrGrGrG

IS PCR Primer: 5′ AAGCAGTGGTATCAACGCAGAGT

TSO: 5′ AAGCAGTGGTATCAACGCAGAGTACATrGrG+G

The protocol workflow is as follows:

Stage I: Preparation of single-cell lysates

Note: In this protocol, we use Microseal F to seal for long-term storage and Microseal B when thermal cycling.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Note: These steps can be performed inside a biosafety cabinet or a RNA workstation (if available); otherwise, they can be carefully performed on a standard benchtop.

Stage III: Performing whole transcriptome amplification) and post-PCR cleanup

Stage IV: Nextera XT sequencing-library construction

Stage V: Pooling and DNA SPRI bead cleanup

Stage VI: Sequencing

Critical Parameters and Troubleshooting

Please see the full manuscript for extensive troubleshooting information.

Time Considerations

The SMARTer library construction and normalization take 2–3 days, and Nextera XT library construction and pooling takes 1 day.

Before start

TE Buffer, 1x

- 10 mM Tris-HCl, pH 8.0
- 1 mM EDTA

Materials

Microseal® 'F' Foil MSF-1001 by BioRad Sciences

Microseal® 'B' Adhesive Seals MSB-1001 by BioRad Sciences

✓ 2-mercaptoethanol, ≥ 99.0% by Contributed by users

Buffer TCL 1031576 by Qiagen

Eppendorf twin.tec® PCR 96-well plate, skirted 951020401 by Eppendorf

✓ Dry ice by Contributed by users

✓ RNeasy Micro Kit by Contributed by users

Agencourt RNAClean XP SPRI beads A63987 by Beckman Coulter

RNaseZap® RNAse Decontamination Solution AM9780 by Life Technologies

✓ RNase-free water by Contributed by users

✓ 200 proof ethanol by Contributed by users

SMARTer® Ultra™ Low Input RNA Kit for Illumina® Sequencing-HV, 96 reactions 634828 by Clontech

Advantage® 2 PCR Kit, 100 reactions 639206 by Clontech

Agencourt AMPure XP SPRI beads A63881 by Beckman Coulter

✓ TE Buffer, 1x (see recipe) by Contributed by users

Agilent High Sensitivity DNA Kit 5067-4626 by Agilent Technologies

Qubit® dsDNA HS assay kit, 100 reactions Q32851 by Life Technologies

Qubit® Assay Tubes Q32856 by Life Technologies

Taqman® Fast Advanced Master Mix, 100 reactions 4444557 by Life Technologies

✓ Taqman® Probe set (Choose housekeeping genes that are appropriate for your system) by Contributed by users

Nextera XT DNA Sample Preparation Kit, 96 samples FC-131-1096 by illumina

Nextera XT Index Kit, 96 indices, 384 samples FC-131-1002 by illumina

TruSeq Index Plate Fixture Kit FC-130-1005 by illumina

✓ Plate centrifuge by Contributed by users

✓ Vortex by Contributed by users

DynaMag™-96 side skirted magnet 12027 by Life Technologies

✓ RNAse and DNAse-free 1.5ml tubes by Contributed by users

✓ Thermal cycler by Contributed by users

Qubit® 2.0 Fluorometer Q32866 by Life

Technologies

2100 Electrophoresis Bioanalyzer Instrument G2939AA by Agilent Technologies

DynaMag™-2 Magnet 12321D by Life Technologies

Protocol

Stage I: Preparation of single-cell lysates

Step 1.

Prepare a solution of 1% 2-mercaptoethanol (by volume) in TCL buffer and distribute 5 µL of this solution into each well of a skirted-side 96-well PCR plate, and 350 µL into a 1.5 mL RNase-free centrifuge tube. Cover this plate with Microseal F and keep at room temperature until ready for single-cell isolation.

NOTES

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In this protocol, we use Microseal F to seal for long-term storage and Microseal B when thermal cycling.

Stage I: Preparation of single-cell lysates

Step 2.

Prepare a cell suspension in complete media and use a FACS machine to sort a single cell into each well of the abovementioned 96-well plate containing Buffer TCL, and an additional sample of ≥10,000 cells into the 1.5 mL centrifuge tube to use as a population control.

NOTES

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To improve yield, we recommend sorting single cells on both the presence of a positive viability indicator (e.g., Calcein AM, Life Technologies) and the absence of a cell death marker (e.g., the membrane-impermeant DNA stain EthD-1, Life Technologies).

Stage I: Preparation of single-cell lysates

Step 3.

Once sorting is completed seal plate with Microseal F and centrifuge (800g, 1 min).

Stage I: Preparation of single-cell lysates

Step 4.

Immediately freeze plate and population control on dry ice and keep at −80°C until ready for lysate cleanup.

TEMPERATURE

-80 °C Additional info:
Freezing

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 5.

Purify RNA from the population control sample using the RNEasy Plus Micro Kit (Qiagen) according to the manufacturers recommendations. Keep purified RNA at 4°C until section "Performing whole transcriptome amplification and post-PCR cleanup", step 28. (−80°C for long term storage).

TEMPERATURE

4 °C Additional info: Short
term storage

TEMPERATURE

-80 °C Additional info:
Long term storage

NOTES

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The following steps can be performed inside a biosafety cabinet or a RNA workstation (if available); otherwise, they can be carefully performed on a standard benchtop.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 6.

Bring RNA-SPRI beads (Agencourt RNAClean XP SPRI beads) to room temperature (allow 30 minutes) and use RNAseZap to clean workbench and all equipment used to process RNA.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 7.

Thaw lysate plate on ice, and then centrifuge (800g, 1 min).

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 8.

Optional: Add 10 µL RNase-free H_2O to lysate to reduce the difficulty of performing the following bead-cleanup steps.

AMOUNT

10 µl Additional info:
RNase-free H2O

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 9.

Thoroughly vortex RNA-SPRI beads to ensure a uniform suspension. Add 2.2 volumes RNA-SPRI beads to each well of lysate (2.2 × 5 µL cell lysate = 11 µL of beads are added, and 2.2 × 15 µL = 33 µL) and mix

well by pipetting 10 times. If each solution is not in the bottom of its respective well, centrifuge plate (300×g, 1 min).

NOTES

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The following steps are done at room temperature.

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Do not centrifuge plates containing SPRI beads at greater than 300 × g. Beads will adhere to the well sidewall and impair RNA recovery.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 10.

Incubate lysate and bead suspension for 10 minutes on bench.

NOTES

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If this procedure is performed on a standard benchtop, the plate should be kept covered (we use the lid from a fresh box of pipette tips, placed slightly ajar) to prevent dust and debris from falling into samples during this and subsequent incubation steps.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 11.

Place plate on 96-well plate magnet and incubate for 5 min.

NOTES

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The magnet described draws the beads to alternating sides of each column of the plate. It is therefore recommended to use an eight-channel pipette to remove the supernatant.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 12.

Remove supernatant from each column of the plate, being careful not to aspirate the beads collected on the side of each well.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 13.

Wash beads by adding 75 µL of 80% ethanol (prepared same day with nuclease-free water) to each well.

AMOUNT

75 µl Additional info: 80% ethanol

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 14.

Wait approximately 30 seconds.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 15.

Aspirate ethanol and repeat wash by adding 75 µL of 80% ethanol to each well and wait for 30 seconds. (2/3)

AMOUNT

75 µl Additional info: 80% ethanol

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 16.

Aspirate ethanol and repeat wash by adding 75 µL of 80% ethanol to each well and wait for 30 seconds. (3/3)

AMOUNT

75 µl Additional info: 80% ethanol

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 17.

Aspirate final ethanol wash.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 18.

Leave the plate on the magnet and allow beads to dry at room temperature for approximately 10 minutes.

NOTES

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Allowing beads to dry for too long will impair recovery of RNA. Immediately proceed with protocol when visible cracks appear in bead pellet.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 19.

Keep plate loosely covered with the lid from a fresh box of pipette tips, placed slightly ajar.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 20.

Once bead pellet has dried, remove plate from magnet and elute RNA from beads by resuspending dried beads in 4.5 µL of the following mix, using reagents from the SMARTer Ultra Low Input RNA Kit (amounts per sample):

- 2.375 µL Dilution Buffer
- 1 µL RNase-free H_2O

- 1 µL 24 µM 3′ SMART CDS Primer II A
- 0.125 µL RNase Inhibitor

AMOUNT

2.375 µl Additional info:
Dilution Buffer

AMOUNT

1 µl Additional info:
RNase-free H2O

AMOUNT

1 µl Additional info: 24 µM
3′ SMART CDS Primer II A

AMOUNT

0.125 µl Additional info:
RNase Inhibitor

NOTES

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Do not remove eluent from original plate!

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For population control, substitute 1 µL purified RNA in place of H_2O.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 21.
Seal and incubate for 1 minute at room temperature.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 22.
Centrifuge (800g, 1 min).

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 23.
Incubate for 3 min at 72°C to anneal 3′ SMART CDS Primer II A.

TEMPERATURE

72 °C Additional info:
Incubation

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 24.
Place plate on ice immediately following incubation.

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 25.

Remove seal and add the following as a mastermix (amounts per sample):

- 2 µL First Strand Buffer
- 0.25 µL 100 mM DTT
- 1 µL 10 mM dNTP Mix
- 1 µL 12 uM SMARTer II A Oligo
- 0.25 µL RNase Inhibitor
- 1 µL SMARTScribe Reverse Transcriptase

AMOUNT

2 µl Additional info: First Strand Buffer

AMOUNT

0.25 µl Additional info: 100 mM DTT

AMOUNT

1 µl Additional info: 10 mM dNTP Mix

AMOUNT

1 µl Additional info: 12 uM SMARTer II A Oligo

AMOUNT

0.25 µl Additional info: RNase Inhibitor

AMOUNT

1 µl Additional info: SMARTScribe Reverse Transcriptase

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 26.

Mix well by pipetting, reseal with a new Microseal B, and centrifuge (800g, 1 min).

Stage II: Lysate cleanup and reverse transcription of mRNA species

Step 27.

Carry out the following Reverse Transcription (RT) in a thermal cycler using the following conditions:

Initial step:	90 min	42°C
Inactivate:	10 min	72°C
Cool:	Hold	4°C

NOTES

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Following this step, the product can be stored at 4°C overnight.

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Residual SPRI beads remain in each well with the libraries until step 43.

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*The protocol provided with the SMARTer kit describes a SPRI cleanup at this stage of the protocol, which we omit here. It is important to avoid losing material before amplification and it has been found that the cleanup is not essential to generating a high-quality sequencing library (*Shalek et al., 2013; Picelli et al., 2013).

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 28.

Centrifuge the plate (800g, 1 min) and add the following as a mastermix (amounts per sample):
5 µL 10X Advantage 2 PCR Buffer
2 µL 10 mM dNTP mix
2 µL 12 uM IS PCR Primer (from the Clontech Ultra Low input RNA Kit)
2 µL 50X Advantage 2 Polymerase Mix
29 µL H_2O

AMOUNT

5 µl Additional info: 10X Advantage 2 PCR Buffer

AMOUNT

2 µl Additional info: 10 mM dNTP mix

AMOUNT

2 µl Additional info: 12 uM IS PCR Primer (from the Clontech Ultra Low input RNA Kit)

AMOUNT

2 µl Additional info: 50X Advantage 2 Polymerase Mix

AMOUNT

29 µl Additional info: H2O

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 29.

Mix well by pipetting, seal, and centrifuge (800g, 1 min).

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 30.

Carry out the whole transcriptome amplification (WTA) in a thermal cycler using the following conditions:

Initial step:	1 min	95°C (initial denaturation)
5 cycles:	20 sec 4 min 6 min	95°C (denaturation) 58°C (annealing) 68°C (extension)
9 cycles:	20 sec 30 sec 6 min	95°C (denaturation) 64°C (annealing) 68°C (extension)
7 cycles:	20 sec 30 sec 7 min	95°C (denaturation) 64°C (annealing) 68°C (extension)
1 cycle:	10 min	72°C (final extension)
Cool:	Hold	4°C

NOTES

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Following this step, the product can be stored at 4°C overnight.

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For this amplification, we have made a few modifications to the thermal cycling steps described in the original SMARTer protocol. We have found that these modifications improve annealing, denaturation, and extension for the diverse transcript sets with which we work.

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 31.

Bring DNA SPRI beads (Agencourt AMPure XP) to room temperature.

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 32.

Centrifuge WTA plate (800g, 1 min).

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 33.

Unseal and add 0.8 volumes of DNA SPRI beads to each well and mix well by pipetting.

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 34.

Cover with a clean lid from a pipette tip box and incubate bead suspension for 5 min on bench.

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 35.

Place plate on 96-well plate magnet and incubate for another 5 minutes.

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 36.

Wash beads by adding 100 µL 80% ethanol to each well and move the plate left and right on the magnet to move the beads from side to side in each well. Continue shifting plate on magnet for approximately 30 sec. (1/3)

AMOUNT

100 µl Additional info:
80% ethanol

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 37.

Aspirate ethanol. (1/3)

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 38.

Wash beads by adding 100 µL 80% ethanol to each well and move the plate left and right on the magnet to move the beads from side to side in each well. Continue shifting plate on magnet for approximately 30 sec. (2/3)

AMOUNT

100 µl Additional info:
80% ethanol

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 39.

Aspirate ethanol. (2/3)

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 40.

Wash beads by adding 100 µL 80% ethanol to each well and move the plate left and right on the magnet to move the beads from side to side in each well. Continue shifting plate on magnet for approximately 30 sec. (3/3)

AMOUNT

100 µl Additional info:
80% ethanol

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 41.

Aspirate final ethanol wash. Leave the plate on the magnet and allow beads to dry at room temperature for approximately 10 minutes. Keep plate covered with the lid of a pipette tip box (left slightly ajar) to prevent dust and debris from falling into samples.

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 42.

Once bead pellet has dried, remove plate from magnet and elute DNA from beads by resuspending dried beads with 20 µL TE buffer. Transfer eluent to a new 96-well plate.

AMOUNT

20 µl Additional info: TE
buffer

NOTES

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Following this step, the new product plate can be sealed and stored at 4°C overnight or at −20°C for months.

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 43.

Use 1 µL of the purified PCR product to measure the fragment size distribution using the Agilent HS DNA BioAnalyzer or similar instrumentation (as per the manufacturer's recommendations) and 1 µL to estimate the library concentration using the Qubit® dsDNA HS Assay kit with Qubit® assay tubes and the Qubit® fluorometer or similar instrumentation (as per the manufacturer's recommendations).

AMOUNT

2 µl Additional info:
Purified PCR product

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 44.

Use 1 µL of the purified PCR product to measure single-cell viability with the Taqman qPCR assay.

AMOUNT

1 µl Additional info:
Purified PCR product

NOTES

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*In most single-cell sample sets, a small subset of libraries will have low complexity and will lack expression of highly expressed housekeeping genes. Since there are, in most systems, a set of housekeeping genes that should be expressed in every cell (*Shalek et al., 2013*), we use qPCR of two housekeeping genes (usually ACTB and B2M) to identify viable samples. Samples with low or no expression of these genes can be removed from Nextera library construction to reduce cost.*

Stage III: Performing whole transcriptome amplification and post-PCR cleanup

Step 45.

For each sample, dilute the purified PCR product to a concentration of between 0.1 and 0.2 ng/µL with buffer TE in preparation for Nextera XT library construction.

Stage IV: Nextera XT sequencing-library construction

Step 46.
Before beginning library construction, thaw index primers and mix by brief vortexing followed by centrifugation. Arrange Nextera index tubes in the TruSeq Index Plate Fixture, such each slot in the fixture holds one index tube. The 12 index 1 (i7) primers, which have orange caps, should be arranged in order horizontally, such that each tube corresponds to a column (Figure 2). The column 1 slot should hold N701 and the column 12 slot should hold N712. Similarly, the index 2 (i5) primers should be arranged in order vertically, such that S501 is in the slot for row A and S508 is in the slot for row H.

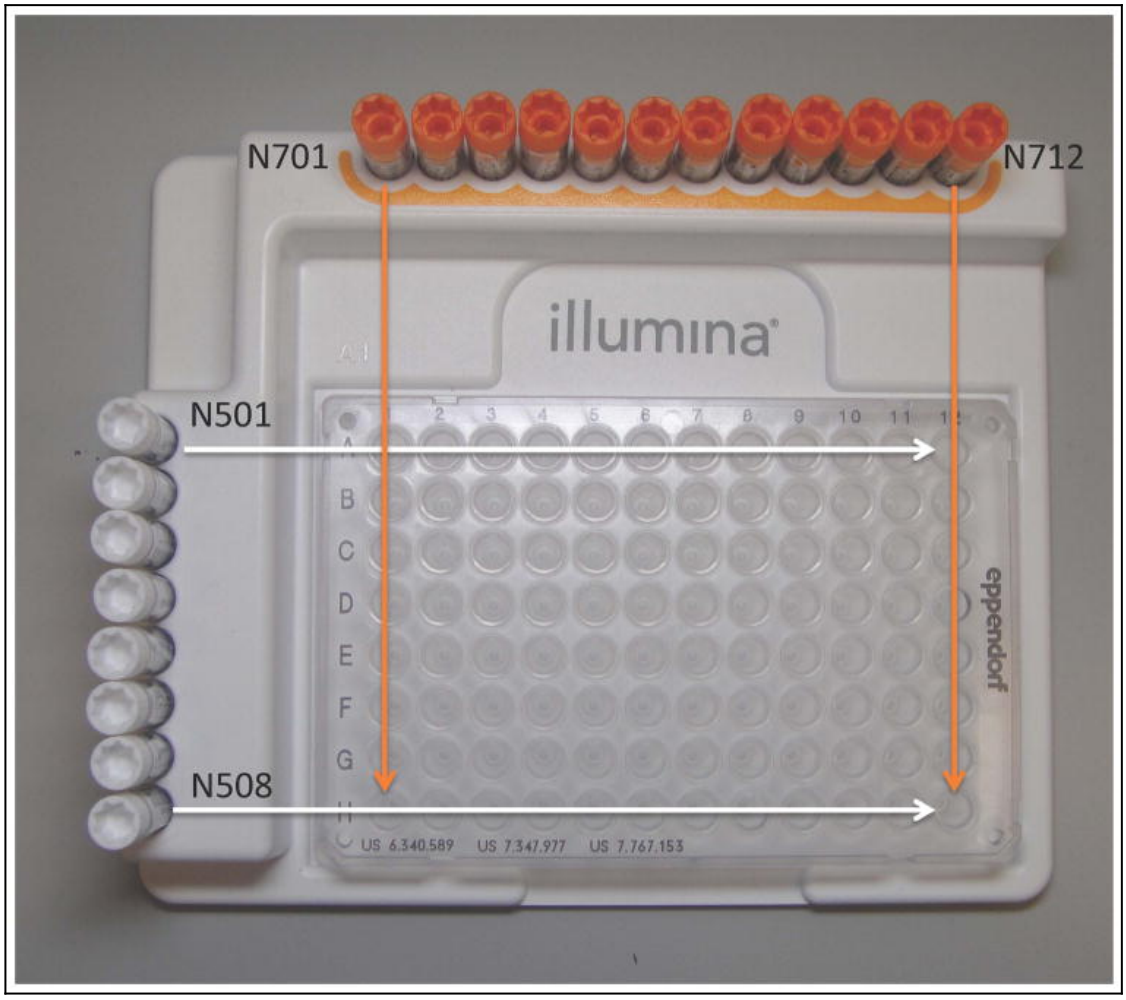

Figure 2
Layout to prepare the combined dual-index primer plate that is used in Nextera XT library construction.

Using a multichannel pipette, distribute 10 µL of each index into the corresponding row/column. (i7 primers will be distributed down rows, while i5 primers will be distributed across columns.)
Seal, vortex mildly to mix, and then centrifuge (800g, 1 min). These mixed index primers can be used immediately, or stored long-term at −20°C.

NOTES

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There is enough combined index primer solution in each well to generate 8 × 96 single-cell libraries.

Stage IV: Nextera XT sequencing-library construction

Step 47.

Using a new 96-well plate, add to each well:
2.5 µL TD Buffer
1.25 µL ATM
1.25 µL Diluted PCR product from SMART RT-PCR

Mix well by pipetting, seal, and centrifuge (800g, 1 min).

AMOUNT

2.5 µl Additional info: TD Buffer

AMOUNT

1.25 µl Additional info: ATM

AMOUNT

1.25 µl Additional info: Diluted PCR product from SMART RT-PCR

Stage IV: Nextera XT sequencing-library construction

Step 48.

Carry out tagmentation using the following conditions:

Initial step:	10 min	55°C
Cool:	Hold	10°C

Stage IV: Nextera XT sequencing-library construction

Step 49.

Unseal and immediately add 1.25 µL NT Buffer to each well and mix well by pipetting.

Stage IV: Nextera XT sequencing-library construction

Step 50.

Wait 5 minutes at room temperature.

Stage IV: Nextera XT sequencing-library construction

Step 51.

Add 3.75 µL NPM and 2.5 µL combined Index primer solution to each well. Mix well by pipetting, seal, and centrifuge (800g, 1 min).

Stage IV: Nextera XT sequencing-library construction

Step 52.

Carry out the amplification in a thermal cycler using the following conditions:

Initial step:	3 min	72°C (initial annealing)
1 cycle:	30 sec	95°C (denaturation)
12 cycles:	10 sec 30 sec 60 sec	95°C 50°C 72°C
1 cycle:	5 min	72°C
Cool:	Hold	4°C

NOTES

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Following this step, the product can be stored at 4°C overnight.

Stage V: Pooling and DNA SPRI bead cleanup

Step 53.

Bring DNA SPRI beads (Agencourt AMPure XP) to room temperature.

Stage V: Pooling and DNA SPRI bead cleanup

Step 54.

Spin down plate (800g, 1 min), unseal, and pool 2.5 µL from each well into a single 1.5 mL tube. Measure total volume of resulting pool and add 0.9 volumes SPRI beads to tube and mix well by pipetting.

Stage V: Pooling and DNA SPRI bead cleanup

Step 55.

Incubate bead suspension for 5 min on bench.

Stage V: Pooling and DNA SPRI bead cleanup

Step 56.

Place tube on Dynamag™-2 magnet, and incubate for 5 min.

Stage V: Pooling and DNA SPRI bead cleanup

Step 57.

Wash beads by adding 500 µL 80% ethanol to the tube and rotate it on the magnet to move the beads from front to back across the tube. (1/2)

AMOUNT

500 µl Additional info:
80% ethanol

Stage V: Pooling and DNA SPRI bead cleanup

Step 58.

Aspirate ethanol.

Stage V: Pooling and DNA SPRI bead cleanup

Step 59.

Wash beads by adding 500 µL 80% ethanol to the tube and rotate it on the magnet to move the beads from front to back across the tube. (2/2)

AMOUNT

500 µl Additional info:
80% ethanol

Stage V: Pooling and DNA SPRI bead cleanup

Step 60.

Aspirate final ethanol wash. Leave the tube on the magnet and allow beads to dry at room temperature for approximately 10 minutes. Keep the tube covered to prevent dust and debris from falling into your sample.

Stage V: Pooling and DNA SPRI bead cleanup

Step 61.

Once bead pellet has dried, remove the tube from the magnet and elute DNA from beads using 30 µL TE buffer (consider smaller volumes if working with fewer than 96 samples). Transfer eluent to a new 1.5 mL tube and repeat 0.9X SPRI bead cleanup once.

AMOUNT

30 µl Additional info: TE
buffer

GOTO

Repeating 0.9X SPRI bead cleanup -> go to step #53

Stage V: Pooling and DNA SPRI bead cleanup

Step 62.

Use 1 µL of the purified PCR product to measure the fragment size distribution using the Agilent HS DNA BioAnalyzer or similar instrumentation (as per the manufacturer's recommendations) and 1 µL to estimate the library concentration using the Qubit® dsDNA HS Assay kit with Qubit® assay tubes and the Qubit® fluorometer or similar instrumentation (as per the manufacturer's recommendations).

AMOUNT

2 µl Additional info:
Purified PCR product

Stage VI: Sequencing

Step 63.

Sequence libraries on an Illumina sequencer using paired-end reads.

NOTES

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We typical run 2x25bp for expression quantification.

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Expression estimates will saturate around ~ 1 million aligned reads, so one lane of HiSeq should be sufficient for 96 single cells.

Warnings

Please refer to the SDS (Saftey Data Sheet) for safety warning and hazard information.