Prepare, Count, and Assess Viability of Single-Cell Suspension

☐1 Dissociate cells for the cell or tissue type you are using .

☐2 If using adherent cells, neutralize the trypsin by adding 4x the volume .

☐3 Wash cells once in cold 1X PBS + 0.1% BSA at a volume sufficient to remove carryover components.

☐4 Centrifuge briefly and resuspend cells in appropriate volume of cold 1X PBS + 0.1% BSA .

☐5 Pipette cell suspension through the chilled cell filter and transfer filtered cells from the cell filter tube.

☐6 Use a microscope or automated cell counter to assess cell dissociation, viability, and concentration.

☐7 If cells are not dissociated to single-cell suspension, mix thoroughly with a P200 or P1000 pipette, as appropriate.

☐8 Dilute the stock cell preparation to target 2500 cells/µl in 1X PBS + 0.1% BSA solution.

Prepare Cell and Barcode Suspension Mixes

☐1 Combine the following components (red caps) in a 1.7 ml tube. Pipette 10–15 times while on ice, then briefly centrifuge.

Cell Enzyme Mix Component	Volume (µl) for 1 Cartridge (4 Samples)	Volume (µl) for 2 Cartridges (8 Samples)
Cell Suspend Buffer	60	120
DTT	8	16
RNA Stabilizer	6	12
RT Enzyme	13.2	26.4
Enhancer Enzyme	12	24
Total	99.2	198.4

☐2 Create the Cell Suspension Mix by combining the following components in a 1.7 ml tube on ice. Vortex the cells for 1 second, repeat.

Cell Suspension Mix Component	Volume (µl) per Sample	Volume (µl) for 1 Cartridge (4 Samples)	Volume (µl) for 2 Cartridges (8 Samples)
Cell Enzyme Mix	21.5	86	172
Filtered Cells (2500 cells/µl)	4.5	18	36

☐3 Create the Barcode Suspension Mix by combining the following components (blue caps) in a 1.7 ml tube on ice. Vortex for 1 second, repeat , then immediately add to the Barcode Buffer.

Barcode Suspension Mix Component	Volume (µl) for 1 Cartridge (4 Samples)	Volume (µl) for 2 Cartridges (8 Samples)
Barcode Buffer	60	120
3' Barcode Mix	60	120

Isolate Single Cells

- ☐1 Add 25 µl of ddSEQ Priming Solution to each well of an 8-tube strip.
- ☐2 Add 20 µl of ddSEQ Priming Solution to each well of the second row of the cartridge.
- ☐3 Allow the ddSEQ Priming Solution to remain in wells for 1 minute, then remove.
- ☐4 Vortex the Barcode Suspension Mix for 1 second, repeat.
- ☐5 Load 20 µl of the Barcode Suspension Mix into the bottom of the **B** ports (Blue).
- ☐6 Vortex the Cell Suspension Mix for 1 second, repeat .
- ☐7 Load 20 µl of Cell Suspension Mix into the bottom of the red ports, numbered 1–4.
- ☐8 Load 80 µl of Encapsulation Oil into each well of the bottom row of the cartridge labeled OIL.
- ☐9 Keep the loaded cartridge for single cell isolation on the ddSEQ Single-Cell Isolator.
- ☐10 Open the ddSEQ Single-Cell Isolator and place the cartridge holder into the instrument.
- ☐11 Close the instrument to initiate single-cell isolation.
- ☐12 Remove the cartridge holder from the ddSEQ Single-Cell Isolator.
- ☐13 Chill a 96-well plate on a chilled 96-well cooling block.
- ☐14 Gently aspirate all encapsulated sample from the output wells.
- ☐15 Dispense the encapsulated sample into the corresponding column of the plate.
- ☐16 Cover sample wells and keep on the 96-well cooling block.
- ☐17 If you are processing a second cartridge, proceed to *Prepare Cartridge* on page 1.
- ☐18 If you have finished processing cartridges, proceed to *Reverse Transcribe Samples* on page 2.

Reverse Transcribe Samples

- ☐1 Place the 96-well plate on the thermal cycler and run the RT program.
- ☐2 Bring Purification Beads (SPB) to room temperature.

Break Emulsion

- ☐1 Remove the 96-well plate from the thermal cycler.
- ☐2 Examine the samples ensuring they all have equal volumes.
- ☐3 Remove the 8-tube strip caps to avoid cross-sample contamination.
- ☐4 Add 20 µl of Droplet Disruptor above each sample.
- ☐5 Wait 30 seconds, then add 100 µl of water above each sample.

Clean Up First Strand Synthesis

- ☐1 Vortex Purification Beads (SPB) until well-dispersed.
- ☐2 Add 90 µl Purification Beads (SPB) to the samples by dispensing above the aqueous layer without mixing.
- ☐3 Pipette mix Purification Beads (SPB) *in the aqueous layer only* until the layer is evenly distributed.
- ☐4 Incubate at room temperature for 10 minutes.
- ☐5 Place on a magnetic peg stand.
- ☐6 Remove and discard all supernatant Use a fresh pipette tip to go into the well again to discard more supernatant.
- ☐7 Wash with 80% EtOH, incubate on the magnetic peg stand for 30 seconds, then remove all supernatant from each well. Repeat.
- ☐8 Seal the plate and centrifuge at 280 × g for 10 seconds.
- ☐9 Place on a magnetic peg stand and wait 30 seconds.
- ☐10 Remove residual 80% EtOH from each well.
- ☐11 Air-dry on the magnetic peg stand for 5 minutes.
- ☐12 Remove the sample plate from the magnetic peg stand.
- ☐13 35µl Resuspension Buffer (RSB) to each sample well. Pipette to mix, making sure all beads are resuspended.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Seal the plate and centrifuge at 280 × g for 10 seconds.
- ☐16 Place on a DynaMag 96 side magnet and wait 2 minutes.
- ☐17 Combine the 2 wells for each sample into a single well by transferring 34 µl of supernatant from each sample well to a new plate, as follows.
  - Sample 1, rows A—B to row A of the corresponding column in the new plate.
  - Sample 2, rows C—D to row B of the corresponding column in the new plate.
  - Sample 3, rows E—F to row C of the corresponding column in the new plate.
  - Sample 4, rows G—H to row D of the corresponding column in the new plate.

Synthesize Second Strand cDNA

☐1 Prepare Second Strand Synthesis Master Mix by adding the following to a 1.7 ml tube on ice. Pipette to mix.

Second Strand Synthesis Component	Volume (µl) for 1 Cartridge (4 Samples)	Volume (µl) for 2 Cartridges (8 Samples)
Second Strand Buffer (SSB)	36	72
Second Strand Enzyme (SSE)	18	36

☐2 Add 12 µl of Second Strand Master Mix to each sample well.

☐3 Pipette to thoroughly mix each sample well.

☐4 Centrifuge at 280 × g for 10 seconds.

☐5 Place on the thermal cycler and run the Second Strand Synthesis (SSS) program.

SAFE STOPPING POINT

If you are stopping, leave the plate on the thermal cycler at 4°C overnight or store at -25°C to -15°C for up to 2 days.

Clean Up cDNA

☐1 Centrifuge sample plate at 280 × g for 30 seconds.

☐2 Vortex Purification Beads (SPB) until well-dispersed.

☐3 Add 44 µl Purification Beads (SPB) to each sample well. Pipette mix until evenly distributed.

☐4 Incubate at room temperature for 5 minutes.

☐5 Place on a magnetic peg stand until the liquid is clear.

☐6 Remove and discard all supernatant from each well.

☐7 Wash 2 times with 200 µl 80% EtOH.

☐8 Air-dry on the magnetic peg stand for 5 minutes.

☐9 Remove residual 80% EtOH from each well.

☐10 Remove from the magnetic peg stand.

☐11 Add 11 µl Resuspension Buffer (RSB) to each sample well. Pipette to mix, making sure all beads are resuspended.

☐12 Incubate at room temperature for 2 minutes.

☐13 Seal the plate and centrifuge at 280 × g for 10 seconds.

☐14 Place on a DynaMag 96 side magnet and wait until the liquid is clear .

☐15 Transfer 10 µl of supernatant from each sample well to a new 96-well plate.

☐16 Run 1 µl of undiluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip.

☐17 Drag the blue regions to capture the 200-8000 bp range.

☐18 Record the cDNA library fragment size and cDNA yield.

Tagment cDNA

☐1 Prepare Tagmentation Mix in a 1.7 ml tube on ice as follows. Pipette to mix.

Tagmentation Mix Component	Volume (µl) for 1 Cartridge (4 Samples)	Volume (µl) for 2 Cartridges (8 Samples)
Tagment Buffer (TCB)	88	176
Tagment Enzyme (TCE)	44	88

☐2 Add 30 µl of Tagmentation Mix to each sample well. Mix .

☐3 Centrifuge at 280 × g for 10 seconds.

☐4 Place on the thermal cycler and run the TGM program.

☐5 Remove the plate from the thermal cycler as soon as the temperature reaches 4° C.

☐6 Remove the seal to avoid cross-sample contamination.

☐7 Add 10 µl of Tagment Stop Buffer to each well. Pipette to mix .

☐8 Centrifuge at 280 × g for 10 seconds.

☐9 Incubate at room temperature for 5 minutes.

Amplify Tagmented cDNA

- ☐1 Arrange the DNA Adapters in a tube rack. Use a different index for each sample well. Record.
- ☐2 Add 30 µl of Tagmentation PCR Mix (TPM) to each of the tagmented samples.
- ☐3 Add 10 µl of Tagment PCR Adapter (TPP1) to each of the tagmented samples.
- ☐4 Add 10 µl of each DNA Adapter to each tagmented sample.
- ☐5 Pipette to mix .
- ☐6 Seal the plate and centrifuge at 280 × g at 20°C for 30 seconds.
- ☐7 Place on the thermal cycler and run the LA program.

SAFE STOPPING POINT

If you are stopping, leave the plate on the thermal cycler at 4°C overnight or store at -25°C to -15°C for up to 2 days.

Clean Up Libraries

- ☐1 Centrifuge sample plate at 280 × g for 30 seconds.
- ☐2 Vortex Purification Beads (SPB) until well-dispersed.
- ☐3 Add 58 µl of Purification Beads (SPB) to each sample well. Pipette to mix, making sure that all beads are resuspended.
- ☐4 Incubate at room temperature for 5 minutes.
- ☐5 Place on a magnetic peg stand until the liquid is clear.
- ☐6 Remove and discard all supernatant from each well.
- ☐7 Wash 2 times with 200 µl 80% EtOH.
- ☐8 Remove residual 80% EtOH from each well.
- ☐9 Air-dry on the magnetic peg stand for 5 minutes.
- ☐10 Remove from the magnetic peg stand.
- ☐11 Add 51 µl of Resuspension Buffer (RSB) to each sample well. Pipette mix until beads are thoroughly resuspended.
- ☐12 Incubate at room temperature for 2 minutes.
- ☐13 Centrifuge at 280 × g for 10 seconds to bring entire solution to the bottom of the well.
- ☐14 Place on a DynaMag 96 side magnet until the liquid is clear.
- ☐15 Transfer 50 µl of supernatant from each sample well to a new sample 96-well plate.
- ☐16 Vortex Purification Beads (SPB) until well-dispersed.
- ☐17 Add 30 µl of Purification Beads (SPB) to each sample well. Pipette until evenly distributed .
- ☐18 Incubate at room temperature for 5 minutes.
- ☐19 Place on a magnetic peg stand until the liquid is clear .
- ☐20 Remove and discard all supernatant from each well.
- ☐21 Wash 2 times with 200 µl 80% EtOH.
- ☐22 Remove residual 80% EtOH from each well.
- ☐23 Air-dry on the magnetic peg stand for 5 minutes.
- ☐24 Remove the 96-well plate from the magnetic peg stand.
- ☐25 Add 22 µl Resuspension Buffer (RSB) to each sample well. Pipette mix until beads are resuspended.
- ☐26 Incubate at room temperature for 2 minutes.
- ☐27 Seal the plate and centrifuge at 280 × g for 10 seconds to bring entire solution to the bottom of the well.
- ☐28 Place on a DynaMag 96 side magnet until the liquid is clear .
- ☐29 Transfer 20 µl of supernatant from each sample well to a new 96-well plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Assess Libraries

- ☐1 Run 1 µl undiluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip.
- ☐2 Determine the concentration of the library using the Agilent Technology 2100 Bioanalyzer.
- ☐3 Select the **Region Analysis** tab.
- ☐4 Drag the blue region lines to capture the 200–8000 bp region. Record the final library fragment size and final library yield.

Acronyms

Acronym	Definition
BSA	Bovine Serum Albumin
PBS	Phosphate-Buffered Saline
RSB	Resuspension Buffer
RTE	Reverse Transcription Enzyme
SPB	(Sample) Purification Beads
SSB	Second Strand Buffer
SSE	Second Strand Enzyme
TCB	Tagment Buffer
TCE	Tagment Enzyme
TPM	Tagmentation PCR Mix
TPP1	Tagment PCR Adapter
TSB	Tagment Stop Buffer