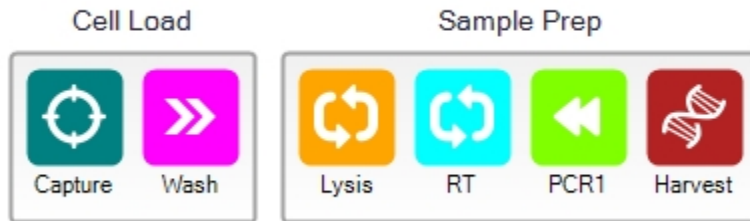




Name Total RNA-Seq  
 Revision B  
 Description Total RNA-Seq on Fluidigm C1  
 Authors Single-Cell Genomics R&D  
 Institution Fluidigm Corporation  
 Lab Single-Cell Genomics  
 Special Instructions



### Script Summary - Prime

#### Runtime Estimates

| Barcode                         | Estimate            |
|---------------------------------|---------------------|
| 1861x (5-10 um diameter cells)  | 0 hours, 11 minutes |
| 1862x (10-17 um diameter cells) | 0 hours, 13 minutes |
| 1863x (17-25 um diameter cells) | 0 hours, 12 minutes |
| 1771x (5-10 um diameter cells)  | 0 hours, 11 minutes |
| 1772x (10-17 um diameter cells) | 0 hours, 13 minutes |
| 1773x (17-25 um diameter cells) | 0 hours, 12 minutes |

### Script Summary - Cell Load

#### Runtime Estimates

| Barcode                         | Estimate            |
|---------------------------------|---------------------|
| 1861x (5-10 um diameter cells)  | 0 hours, 15 minutes |
| 1862x (10-17 um diameter cells) | 0 hours, 34 minutes |
| 1863x (17-25 um diameter cells) | 0 hours, 27 minutes |
| 1771x (5-10 um diameter cells)  | 0 hours, 15 minutes |
| 1772x (10-17 um diameter cells) | 0 hours, 34 minutes |
| 1773x (17-25 um diameter cells) | 0 hours, 27 minutes |

### Script Summary - Sample Prep

#### Runtime Estimates

| Barcode                         | Estimate            |
|---------------------------------|---------------------|
| 1861x (5-10 um diameter cells)  | 4 hours, 54 minutes |
| 1862x (10-17 um diameter cells) | 4 hours, 54 minutes |
| 1863x (17-25 um diameter cells) | 4 hours, 54 minutes |
| 1771x (5-10 um diameter cells)  | 4 hours, 54 minutes |
| 1772x (10-17 um diameter cells) | 4 hours, 54 minutes |



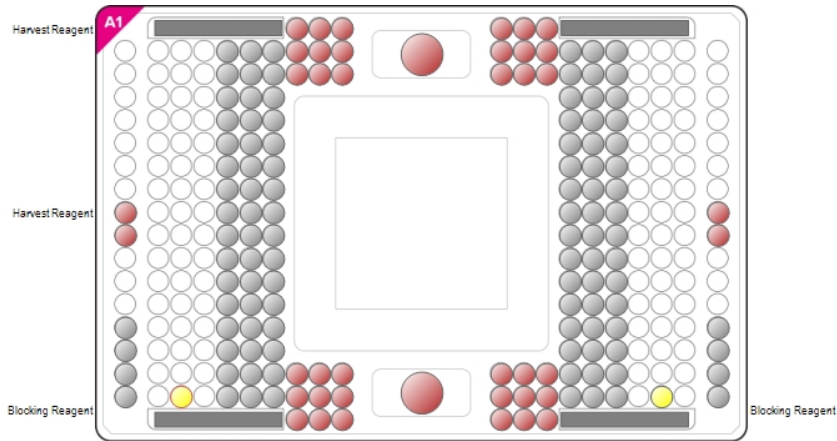
1773x (17-25 um diameter cells)      4 hours, 54 minutes

### Incubation Profile

| Script Step | Operation              |                   | Temperature (C) | Duration (s) |
|-------------|------------------------|-------------------|-----------------|--------------|
| Lysis       | Lysis                  | RNA Fragmentation | 85              | 360          |
|             |                        | Cool Down         | 10              | 120          |
| RT          | RT                     | RT                | 42              | 5400         |
|             |                        | RT Inactivation   | 70              | 600          |
|             |                        | Cool Down         | 10              | 60           |
| PCR1        | Hot Start              | Manual            | 94              | 60           |
| PCR1        | PCR Cycle (3 Step) x10 | Denaturation      | 98              | 15           |
|             |                        | Annealing         | 55              | 15           |
|             |                        | Extension         | 68              | 30           |
| PCR1        | Final Extension        | Extension         | 68              | 120          |



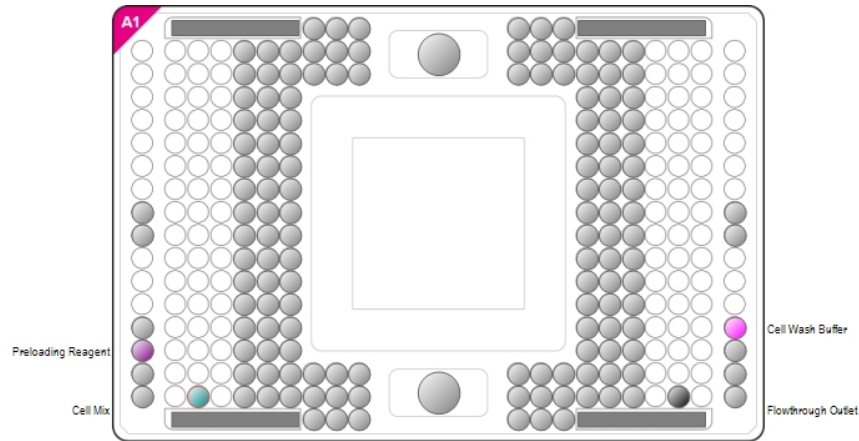
**Script Reagent Details - Prime**



| Reagent Loading               |               |            |               |
|-------------------------------|---------------|------------|---------------|
| Name                          | Volume (µl)   | IFC Inlet  | Notes         |
| ● Harvest Reagent             | 200 µl        | A1         |               |
| ● Harvest Reagent             | 200 µl        | A2         |               |
| ● Blocking Reagent            | 15 µl         | C1         |               |
| ● Blocking Reagent            | 15 µl         | C2         |               |
| ● Harvest Reagent             | 20 µl         | P1         |               |
| ● Harvest Reagent             | 20 µl         | P2         |               |
| Reagent Mix Recipe - Prime    |               |            |               |
| Blocking Reagent              |               |            |               |
| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
| C1 Blocking RGT (1X)          |               |            |               |
| Harvest Reagent               |               |            |               |
| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
| C1 Harvest RGT (1X)           |               |            |               |



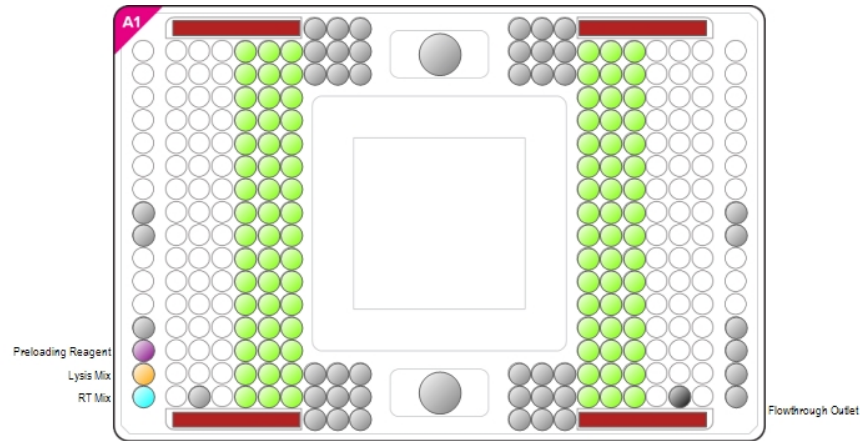
**Script Reagent Details - Cell Load**



| Inlet Reuse                    |               |  |               |
|--------------------------------|---------------|--|---------------|
| Name                           | IFC Inlet     | Instructions   |               |
| ● Cell Mix                     | C1            | Aspirate inlet prior to loading reagents                     |               |
| ● Flowthrough Outlet           | C2            | Aspirate inlet prior to loading reagents (1862x, 1863x only) |               |
| Reagent Loading                |               |  |               |
| Name                           | Volume (µl)   | IFC Inlet  | Notes         |
| ● Preloading Reagent           | 24            | 2  |               |
| ● Cell Wash Buffer             | 7             | 5  |               |
| ● Cell Mix                     | ↻ 6           | C1   |               |
| Reagent Mix Recipe - Cell Load |               |  |               |
| Preloading Reagent             |               |  |               |
| Reagent (Stock Concentration)  | Mix Prep (µl) | Prep Conc.   | Chamber Conc. |
| C1 Preloading RGT (1X)         |               |  |               |
| Cell Mix                       |               |  |               |
| Reagent (Stock Concentration)  | Mix Prep (µl) | Prep Conc.   | Chamber Conc. |
| Suspension RGT (2.5X)          | 40            | 1  | 1             |
| Cells 66-330 / µL              | 60            |  |               |
| 100 Total Prep Volume          |               |  |               |
| Cell Wash Buffer               |               |  |               |
| Reagent (Stock Concentration)  | Mix Prep (µl) | Prep Conc.   | Chamber Conc. |
| Cell Wash BUF (1X)             |               |  |               |



## Script Reagent Details - Sample Prep



| Inlet Reuse                      |               |  |               |
|----------------------------------|---------------|--|---------------|
| Name                             | IFC Inlet     | Instructions   |               |
| ● Flowthrough Outlet             | C2            | Aspirate inlet prior to loading reagents (1862x, 1863x only) |               |
| Reagent Loading                  |               |  |               |
| Name                             | Volume (μl)   | IFC Inlet  | Notes         |
| ● Preloading Reagent             | 0             | 2  |               |
| ● Lysis Mix                      | 7             | 3  |               |
| ● RT Mix                         | 8             | 4  |               |
| ● Harvest Reagent                | 180 μl each   | Harvest Inlets   |               |
| ● PCR1 Final Mix                 | 5 each        | Harvest Outlets  |               |
| Reagent Mix Recipe - Sample Prep |               |  |               |
| Preloading Reagent               |               |  |               |
| Reagent (Stock Concentration)    | Mix Prep (μl) | Prep Conc.   | Chamber Conc. |
| C1 Preloading RGT (1X)           |               |  |               |
| Harvest Reagent                  |               |  |               |
| Reagent (Stock Concentration)    | Mix Prep (μl) | Prep Conc.   | Chamber Conc. |
| C1 Harvest RGT (1X)              |               |  |               |
| 10X Lysis Mix (Secondary)        |               |  |               |
| Reagent (Stock Concentration)    | Mix Prep (μl) | Prep Conc.   | Chamber Conc. |
| 10X Lysis Buffer (10X)           | 19            | 9.5  |               |
| RNase Inhibitor                  | 1             |  |               |
| 20 Total Prep Volume             |               |  |               |
| Lysis Mix                        |               |  |               |
| Reagent (Stock Concentration)    | Mix Prep (μl) | Prep Conc.   | Chamber Conc. |

|                      |      |     |        |
|----------------------|------|-----|--------|
| SMART scN6           | 1.68 |     |        |
| scRT Buffer          | 2.88 |     |        |
| 10X Lysis Mix        | 1.2  |     |        |
| Nuclease-Free Water  | 3.12 |     |        |
| C1 Loading RGT (20X) | 0.72 | 1.5 | 1.0005 |

9.6 Total Prep Volume

| <b>RT Mix</b>                 |               |            |               |
|-------------------------------|---------------|------------|---------------|
| Reagent (Stock Concentration) | Mix Prep (μl) | Prep Conc. | Chamber Conc. |
| scRT Buffer                   | 2             |            |               |
| SMART scTSO Mix               | 3.95          |            |               |
| RNase Inhibitor (40 U/μl)     | 0.5           | 2          | 1.142         |
| SMARTScribe RT (100 U/μl)     | 1.75          | 17.5       | 9.9925        |
| Nuclease-Free Water           | 1.3           |            |               |
| C1 Loading RGT (20X)          | 0.5           | 1          | 0.571         |

10 Total Prep Volume

| <b>PCR1 Mix (Secondary)</b>    |               |            |               |
|--------------------------------|---------------|------------|---------------|
| Reagent (Stock Concentration)  | Mix Prep (μl) | Prep Conc. | Chamber Conc. |
| SeqAmp CB PCR Buffer (2X) (2X) | 420           | 1.5625     |               |
| SeqAmp DNA Polymerase          | 16.8          |            |               |
| C1 Loading RGT (20X)           | 37.8          | 1.4063     |               |
| Nuclease-Free Water            | 63            |            |               |

537.6 Total Prep Volume

| <b>Diluted Primer Mix (Secondary)</b>  |               |            |               |
|--|---------------|------------|---------------|
| Special Instructions:<br>-----<br>Please refer to the Technical Note (101-8496 A1) Total RNA Seq Using C1 and SMART-Seq Stranded Kit, under the section Pre-C1 Protocol for the instructions on diluting 3' and 5' primers, and the preparation of the Diluted Primer Mix plate. |               |            |               |
| Reagent (Stock Concentration)  | Mix Prep (μl) | Prep Conc. | Chamber Conc. |
| Diluted 3' Primer  | 5             |            |               |
| Diluted 5' Primer  | 5             |            |               |

10 Total Prep Volume

| <b>PCR1 Final Mix</b>   |  |  |  |
|---|--|--|--|
| Special Instructions:<br>-----<br>Aliquot 64 μL of PCR1 Mix into each well of a new 8-well PCR tube strip. Using a multi-channel pipette, distribute 5.0 μL of PCR1 Mix into each well of a new 96-well plate labeled PCR1 Final Mix. Using a multi-channel pipette, add 2.0 μL of Diluted Primer Mix from the Diluted Primer Mix plate to the corresponding wells in the PCR1 Final Mix plate. |  |  |  |
| When it is ready to run the Total RNA Seq Sample Prep script, carefully pull back the tape covering the harvest outlets of the IFC using the plastic removal tool. Using a multi-channel pipette, transfer 5 μL of each specific  |  |  |  |



PCR1 Final Mix from the PCR1 Final Mix plate into corresponding harvest outlets. Replace the tape to cover the harvest outlets.

| Reagent (Stock Concentration) | Mix Prep (μl) | Prep Conc. | Chamber Conc. |
|-------------------------------|---------------|------------|---------------|
| PCR1 Mix                      | 5             |            |               |
| Diluted Primer Mix            | 2             |            |               |

7 Total Prep Volume

**Protocol Reagent Shopping List**

| Reagent Name              | Vendor     | Part Number | Kit Part Number | Stock Concentration |
|---------------------------|------------|-------------|-----------------|---------------------|
| SMART scN6                | Takara Bio | ST1679      | 634444          |                     |
| scRT Buffer               | Takara Bio | ST1680      | 634444          |                     |
| 10X Lysis Buffer          | Takara Bio | ST1683      | 634444          | 10X                 |
| RNase Inhibitor           | Takara Bio | ST1682      | 634444          |                     |
| Nuclease-Free Water       | Takara Bio | ST1287      | 634444          |                     |
| SMART scTSO Mix           | Takara Bio | ST1676      | 634444          |                     |
| RNase Inhibitor           | Takara Bio | ST1682      | 634444          | 40 U/μl             |
| SMARTScribe RT            | Takara Bio | ST1681      | 634444          | 100 U/μl            |
| SeqAmp CB PCR Buffer (2X) | Takara Bio | ST1281      | 634444          | 2X                  |
| SeqAmp DNA Polymerase     | Takara Bio | ST1280      | 634444          |                     |
| Diluted 3' Primer         | Takara Bio |             | 634444          |                     |
| Diluted 5' Primer         | Takara Bio |             | 634444          |                     |

**Fluidigm Reagent Kits**

| Reagent Name      | Part Number | Stock Concentration | PN 100-8920                         | PN 100-6201                         | PN 100-5319                         | PN 100-7357                         | PN 100-8921                         |
|-------------------|-------------|---------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| C1 Blocking RGT   | 100-5316    | 1X                  | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |                                     |
| C1 Harvest RGT    | 100-6248    | 1X                  | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |                                     | <input checked="" type="checkbox"/> |                                     |
| C1 Preloading RGT | 100-5311    | 1X                  | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |                                     |
| Suspension RGT    | 100-5315    | 2.5X                | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |                                     |
| Cell Wash BUF     | 100-5314    | 1X                  | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |                                     |                                     |
| C1 Loading RGT    | 100-5170    | 20X                 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |                                     |                                     | <input checked="" type="checkbox"/> |