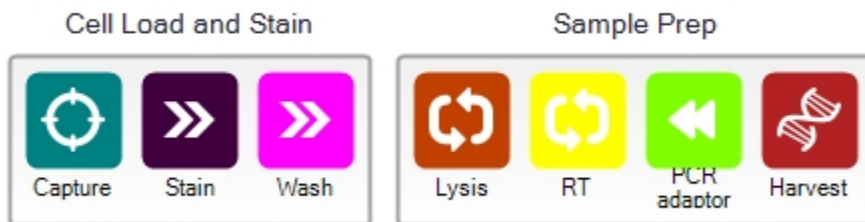


Name Single-cell mRNA Seq with Integrated Barcoding  
Revision A  
Description A cost effective 5' selective single cell transcriptome profiling approach.  
Authors Arguel et al.  
Institution CNRS - University of Cote d'Azur  
Lab Pascal Barbry - UCA Genomix - IPMC  
Special Instructions For library preparation after C1, cDNA were pooled and used with an adapted tagmentation protocol from Picelli et al. Refer to the documents "Off chip protocol for Ion Torrent sequencing" and "Off chip protocol for Illumina sequencing" for instructions on sample processing and library preparation. These files are included with the script hub download. Be sure to use the appropriate protocol for the sequencing platform you intend to use.



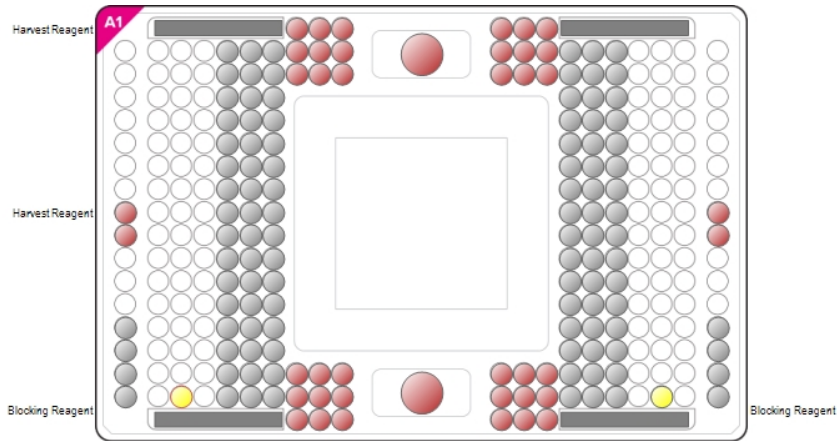
| Script Summary - Prime               |                     |        |                 |              |
|--------------------------------------|---------------------|--------|-----------------|--------------|
| Runtime Estimates                    |                     |        |                 |              |
| 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 |        |                 |              |
| Script Summary - Cell Load and Stain |                     |        |                 |              |
| Runtime Estimates                    |                     |        |                 |              |
| Barcode                              | Estimate            |        |                 |              |
| 1861x (5-10 um diameter cells)       | 0 hours, 21 minutes |        |                 |              |
| 1862x (10-17 um diameter cells)      | 0 hours, 50 minutes |        |                 |              |
| 1863x (17-25 um diameter cells)      | 0 hours, 41 minutes |        |                 |              |
| Script Summary - Sample Prep         |                     |        |                 |              |
| Runtime Estimates                    |                     |        |                 |              |
| Barcode                              | Estimate            |        |                 |              |
| 1861x (5-10 um diameter cells)       | 6 hours, 57 minutes |        |                 |              |
| 1862x (10-17 um diameter cells)      | 6 hours, 57 minutes |        |                 |              |
| 1863x (17-25 um diameter cells)      | 6 hours, 57 minutes |        |                 |              |
| Incubation Profile                   |                     |        |                 |              |
| Script Step                          | Operation           |        | Temperature (C) | Duration (s) |
| Lysis                                | Incubation          | S1     | 72              | 180          |
| Lysis                                | Cooling             | Manual | 10              | 180          |



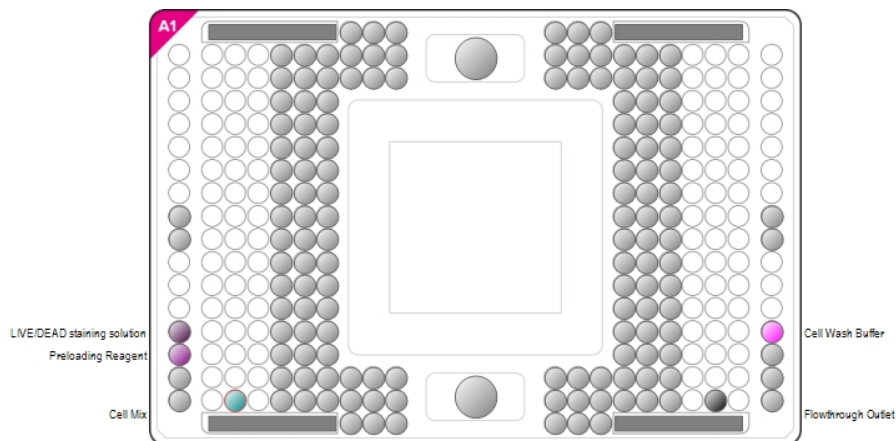
|             |                           |                                 |    |      |
|-------------|---------------------------|---------------------------------|----|------|
| RT          | Incubation                | RT and<br>Template<br>Switching | 42 | 5400 |
| RT          | Inactivation              | RT<br>inactivation              | 70 | 900  |
| PCR adaptor | Denature                  | Hot Start                       | 98 | 180  |
| PCR adaptor | PCR Cycle (3<br>Step) x18 | Denaturation                    | 98 | 20   |
|             |                           | Annealling                      | 64 | 15   |
|             |                           | Extension                       | 72 | 360  |
| PCR adaptor | End                       | Inactivation                    | 72 | 300  |
| PCR adaptor | Hold                      | Incubation                      | 10 | 300  |



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 and Stain**


| <b>Inlet Reuse</b>                              |                       |  |               |
|---|-----------------------|--|---------------|
| 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) |               |
| <b>Reagent Loading</b>                          |                       |  |               |
| Name  | Volume (μl)           | IFC Inlet  | Notes         |
| ● LIVE/DEAD staining solution                   | 7                     | 1  |               |
| ● Preloading Reagent                            | 24                    | 2  |               |
| ● Cell Wash Buffer                              | 7                     | 5  |               |
| ● Cell Mix                                      | 6                     | C1   |               |
| <b>Reagent Mix Recipe - Cell Load and Stain</b> |                       |  |               |
| <b>Preloading Reagent</b>                       |                       |  |               |
| Reagent (Stock Concentration)                   | Mix Prep (μl)         | Prep Conc.   | Chamber Conc. |
| C1 Preloading RGT (1X)                          |                       |  |               |
| <b>Cell Mix</b>                                 |                       |  |               |
| Reagent (Stock Concentration)                   | Mix Prep (μl)         | Prep Conc.   | Chamber Conc. |
| Suspension RGT (2.5X)                           | 40                    | 1  | 1             |
| Cells 250 / μL                                  | 60                    |  |               |
|   | 100 Total Prep Volume |  |               |
| <b>LIVE/DEAD staining solution</b>              |                       |  |               |
| Reagent (Stock Concentration)                   | Mix Prep (μl)         | Prep Conc.   | Chamber Conc. |
| Cell Wash BUF (1X)                              | 1250                  | 0.9975   | 0.9975        |
| Calcein AM (4 mM)                               | 0.625                 | 0.002  | 0.002         |
| Ethidium homodimer-1 (2 mM)                     | 2.5                   | 0.004  | 0.004         |

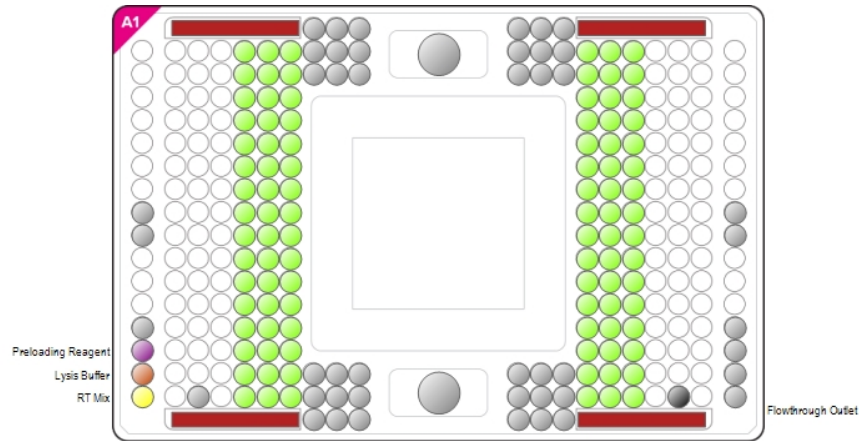


1253.125 Total Prep Volume

| <b>Cell Wash Buffer</b>              |                      |                   |                      |
|--------------------------------------|----------------------|-------------------|----------------------|
| <b>Reagent (Stock Concentration)</b> | <b>Mix Prep (μl)</b> | <b>Prep Conc.</b> | <b>Chamber Conc.</b> |
| Cell Wash BUF (1X)                   |                      |                   |                      |



## Script Reagent Details - Sample Prep



| Inlet Reuse  |               |  |               |
|--|---------------|--|---------------|
| Name   | IFC Inlet     | Instructions   |               |
| ● Preloading Reagent   | 2             | 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  |               |
| ● Lysis Buffer   | 7             | 3  |               |
| ● RT Mix   | 8             | 4  |               |
| ● Harvest Reagent  | 180 µl each   | Harvest Inlets   |               |
| ● PCR reaction 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  |               |  |               |
| Special Instructions:  |               |  |               |
| -----  |               |  |               |
| Refer to the documents "Off chip protocol for Ion Torrent sequencing" and "Off chip protocol for Illumina sequencing" for instructions on sample harvesting and cleanup. |               |  |               |
|  |               |  |               |
| Reagent (Stock Concentration)  | Mix Prep (µl) | Prep Conc.   | Chamber Conc. |
| C1 Harvest RGT (1X)  |               |  |               |



### Lysis Buffer

Special Instructions:

-----  
ERCC aliquots are conserved at -80°C. When needed, thaw on ice, make serial dilutions and do not reuse dilutions.  
For the reverse transcription primer sequence, refer to the files "Oligo Fequences for Ion Torrent Sequencing" or "Oligo Sequences for Illumina Sequencing" included with the script hub download.

| Reagent (Stock Concentration)          | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|--|---------------|------------|---------------|
| Tween 20 (0.4%)                        | 4.05          | 0.18       | 0.1201        |
| RNasin® Plus RNase Inhibitor (40 U/µl) | 0.25          | 1.1111     | 0.7411        |
| Set of dATP, dCTP, dGTP, dTTP (20 mM)  | 1.125         | 2.5        | 1.6675        |
| ERCC RNA Spike IN Mix1 (0.0204 µg/ml)  | 0.497         | 0.0011     | 0.0008        |
| reverse transcritption primer (10 µM)  | 1.8           | 2          | 1.334         |
| C1 Loading RGT (20X)                   | 0.45          | 1          | 0.667         |
| PCR Water                              | 0.828         |            |               |

9 Total Prep Volume

### RT Mix

Special Instructions:

-----  
MgCl2 at 25mM is added to complete MgCL2 from first-strand buffer  
For template switching oligonucleotide sequence, refer to the documents "Oligo Sequences for Ion Torrent Sequencing" or "Oligo Sequences for Illumina Sequencing" included with the script hub download.

| Reagent (Stock Concentration)                   | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---------------|------------|---------------|
| C1 Loading RGT (20X)                            | 0.9           | 1          | 0.571         |
| first-strand buffer (5X)                        | 6.3           | 1.75       | 0.9993        |
| DTT (100 mM)                                    | 1.58          | 8.7778     | 5.0121        |
| Betaine (5M)                                    | 6.3           | 1.75       | 0.9993        |
| RNasin® Plus RNase Inhibitor (40 U/µl)          | 0.25          | 0.5556     | 0.3172        |
| MgCl2 (25 mM)                                   | 0.76          | 1.0556     | 0.6027        |
| SuperScript II reverse transcriptase (200 U/µl) | 0.5           | 5.5556     | 3.1722        |
| template switching oligonucleotide (50 µM)      | 0.63          | 1.75       | 0.9993        |
| PCR Water                                       | 0.78          |            |               |

18 Total Prep Volume

**PCR reaction Mix**

## Special Instructions:

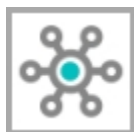
-----  
 For 1 IFC prepare a PreMix of 120X reactions without barcode primer. Dispatch 6.53ul in a 96 plate on ice with corresponding barcode primer before loading IFC wells.

For primer sequence, refer to the files "Oligo Fequences for Ion Torrent Sequencing" and "Oligo Sequences for Illumina Sequencing" included with the script hub download. Note that the barcode primers used for Ion Torrent and Illumina sequencers are different.

| Reagent (Stock Concentration)                | Mix Prep (μl) | Prep Conc. | Chamber Conc. |
|--|---------------|------------|---------------|
| C1 Loading RGT (20X)                         | 0.42          | 1.1923     | 1.0683        |
| Kapa HiFi Hot Start ReadyMix PCR Kit (2X)    | 4.69          | 1.3314     | 1.193         |
| barcode primer (0.25 μM)                     | 0.055         | 0.002      | 0.0017        |
| biotynylated PCR primer (10 μM)              | 0.94          | 1.3343     | 1.1955        |
| optional illumina reverse PCR primer (10 μM) | 0.94          | 1.3343     | 1.1955        |

7.045 Total Prep Volume





#### Protocol Reagent Shopping List

| Reagent Name                         | Vendor            | Part Number  | Kit Part Number | Stock Concentration |
|--------------------------------------|-------------------|--------------|-----------------|---------------------|
| Calcein AM                           | Life Technologies |              | L-3224          | 4 mM                |
| Ethidium homodimer-1                 | Life Technologies |              | L-3224          | 2 mM                |
| Tween 20                             | Sigma             | P9416-50ML   |                 | 0.4%                |
| RNasin® Plus RNase Inhibitor         | Promega           | N2611        |                 | 40 U/μl             |
| Set of dATP, dCTP, dGTP, dTTP        | Promega           | U1330        |                 | 20 mM               |
| ERCC RNA Spike IN Mix1               | ThermoFisher      | 4456740      |                 | 0.0204 μg/ml        |
| reverse transcription primer         | IDT               |              |                 | 10 μM               |
| first-strand buffer                  | ThermoFisher      |              | 18064071        | 5X                  |
| DTT                                  | ThermoFisher      |              | 18064071        | 100 mM              |
| Betaïne                              | Sigma             | B0300-1VL    |                 | 5M                  |
| MgCl <sub>2</sub>                    | Sigma             | M1028-10X1ML |                 | 25 mM               |
| SuperScript II reverse transcriptase | ThermoFisher      |              | 18064071        | 200 U/μl            |
| template switching oligonucleotide   | IDT               |              |                 | 50 μM               |
| Kapa HiFi Hot Start ReadyMix PCR Kit | Clinisciences     | KK2602       |                 | 2X                  |
| barcode primer                       | IDT               |              |                 | 0.25 μM             |
| biotynylated PCR primer              | IDT               |              |                 | 10 μM               |
| optional illumina reverse PCR primer | IDT               |              |                 | 10 μM               |

#### Fluidigm Reagent Kits

| Reagent Name      | Part Number | Stock Concentration | PN 100-8920                         | PN 100-6201                         | PN 100-7357                         | PN 100-5319                         | 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"/> |
| PCR Water         | 100-5941    |                     |                                     |                                     | <input checked="" type="checkbox"/> |                                     |                                     |