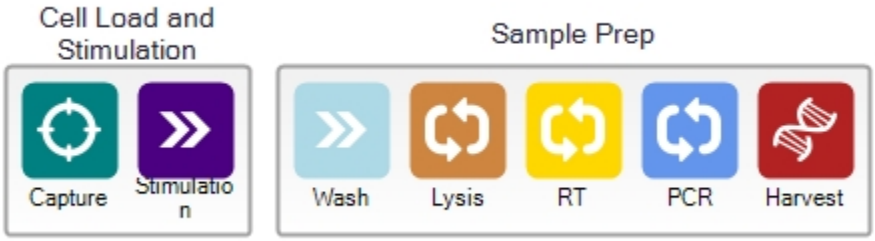
| | |
|---|---|
| Name | LPS Simulation |
| Revision | B |
| Description | script used in Shalek et al., 2014<br>Stimulation of cells with LPS |
| Authors | Shalek AK, Satija R, Shuga J, Trombetta JJ, Gennert D, et al. |
| Institution | Broad Institute, Harvard University, MIT, Hebrew University, Fluidigm Corporation |
| Lab | Regev, Park and May |
| 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 and Stimulation

### Runtime Estimates

| Barcode | Estimate |
|---|---|
| 1861x (5-10 um diameter cells) | 0 hours, 25 minutes |
| 1862x (10-17 um diameter cells) | 0 hours, 44 minutes |
| 1863x (17-25 um diameter cells) | 0 hours, 37 minutes |
| 1771x (5-10 um diameter cells) | 0 hours, 25 minutes |
| 1772x (10-17 um diameter cells) | 0 hours, 44 minutes |
| 1773x (17-25 um diameter cells) | 0 hours, 37 minutes |

### Incubation Profile

| Script Step | Operation | Temperature (C) | Duration (s) |
|---|---|---|---|
| Stimulation | Incubation | 37 | 600 |

## Script Summary - Sample Prep

### Runtime Estimates

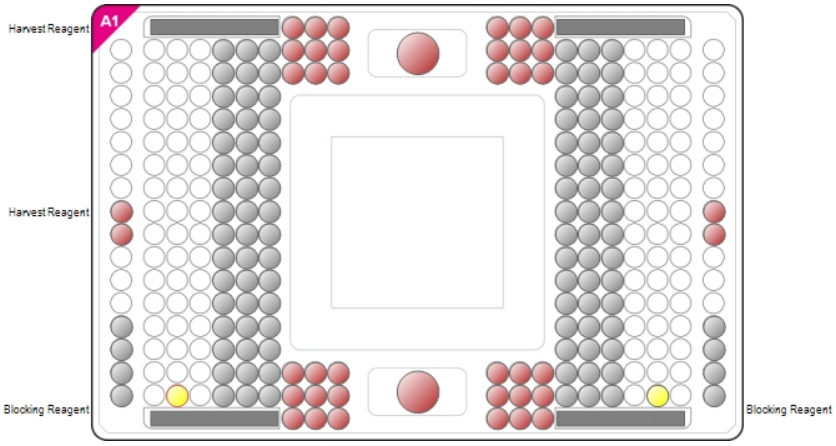
| Barcode | Estimate |
|---|---|
| 1861x (5-10 um diameter cells) | 8 hours, 6 minutes |

| | |
|---|---|
| 1862x (10-17 um diameter cells) | 8 hours, 16 minutes |
| 1863x (17-25 um diameter cells) | 8 hours, 14 minutes |
| 1771x (5-10 um diameter cells) | 8 hours, 6 minutes |
| 1772x (10-17 um diameter cells) | 8 hours, 16 minutes |
| 1773x (17-25 um diameter cells) | 8 hours, 14 minutes |

**Incubation Profile**

| Script Step | Operation | | Temperature (C) | Duration (s) |
|---|---|---|---|---|
| Lysis | Incubation | S1 | 72 | 180 |
| | | S2 | 4 | 600 |
| | | S3 | 25 | 60 |
| RT | Incubation | S1 | 42 | 5400 |
| | | S2 | 70 | 600 |
| PCR | Hot Start | 95C | 95 | 60 |
| PCR | PCR x5 | Denaturation | 95 | 20 |
| | | Annealling | 58 | 240 |
| | | Extension | 68 | 360 |
| PCR | PCR x9 | Denaturation | 95 | 20 |
| | | Annealling | 64 | 30 |
| | | Extension | 68 | 360 |
| PCR | PCR x7 | Denaturation | 95 | 30 |
| | | Annealling | 64 | 30 |
| | | Extension | 68 | 420 |
| PCR | Extension | 72C | 72 | 600 |

## 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**

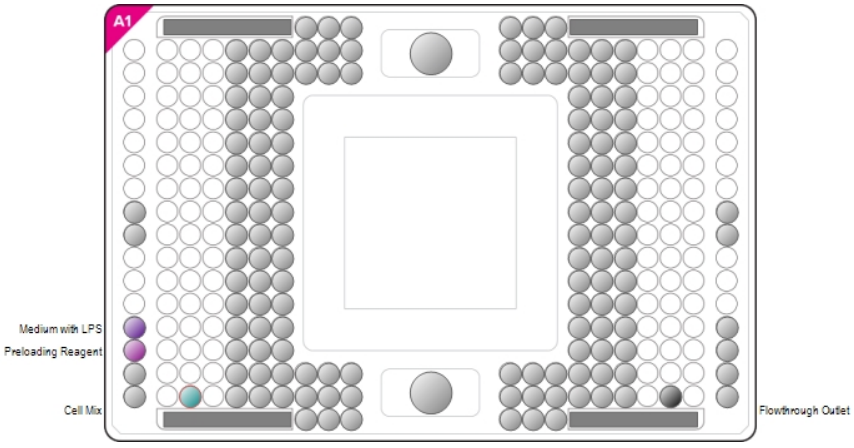
| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| C1 Blocking RGT (1X) | | | |

**Harvest Reagent**

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| C1 Harvest RGT (1X) | | | |

## Script Reagent Details - Cell Load and Stimulation

Cell load and LPS stimulation




| 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** |
| Medium with LPS | 7 | 1 | |
| Preloading Reagent | 20 | 2 | |
| Cell Mix | 6 | C1 | |

### Reagent Mix Recipe - Cell Load and Stimulation

**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. |
|---|---|---|---|
| C1 Suspension RGT (2.5X) | 40 | 1 | 1 |
| Cells 66 -330 / µL | 60 | | |
| | 100 Total Prep Volume | | |

**Medium with LPS**

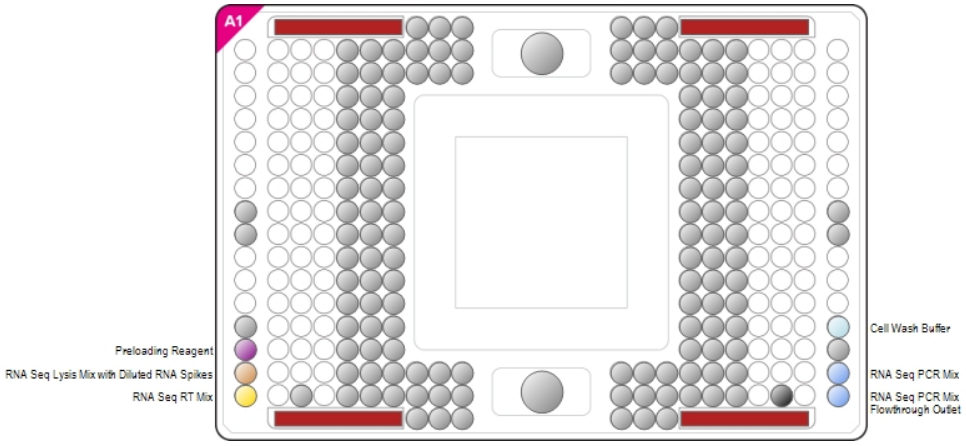
Special Instructions:
---
Incubate in tissue culture incubator with environment control for desired time
Note: any activators or inhibitors can also be used.

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| Cell culture medium (1X) | 9990 | 0.999 | 0.999 |

| | | | |
|---|---|---|---|
| Ultrapure LPS-EK (100 mg/ml) | 10 | 0.1 | 0.1 |

10000 Total Prep Volume

## 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 | 20 | 2 | |
| RNA Seq Lysis Mix with Diluted RNA Spikes | 7 | 3 | |
| RNA Seq RT Mix | 8 | 4 | |
| Cell Wash Buffer | 7 | 5 | |
| RNA Seq PCR Mix | 24 | 7 | |
| RNA Seq PCR Mix | 24 | 8 | |
| Harvest Reagent | 180 µl each | Harvest Inlets | |

## Reagent Mix Recipe - Sample Prep

### Preloading Reagent

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| C1 Preloading RGT (1X) | | | |

### ArrayControl RNA Spikes (Secondary: 1X)

Special Instructions:
---
After the ArrayControl RNA Spikes have thawed, remove spikes 1, 4, and 7 from the box.
Pipette the following in three tubes:

Tube A - 13.5 µl THE RNA Storage Solution, RNA Spikes #7 - 1.5 µl

Tube B - 12.5 µl THE RNA Storage Solution, RNA Spikes #4 - 1.5 µl

Tube C - 148.5 µl THE RNA Storage Solution, RNA Spikes #1 - 1.5 µl

Vortex briefly tube A and spin to collect contents. Pipette 1.5 µl from tube A into tube B. Discard tube A.

Vortex briefly tube B and spin to collect contents. Pipette 1.5 µl from tube B into tube C. Discard tube B.
Vortex briefly tube C and spin to collect contents.

Tube C is the concentrated RNA Standard that may be aliquoted and frozen for future use.
Aliquot in tubes containing 1 µl volumes and store at -80 ºC until use. One tube is necessary for each C1 chip run.

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| THE RNA Storage Solution | 174 | | |
| ArrayControl RNA Spikes #1 (100 ng/µl) | 1.5 | 0.8403 | |
| ArrayControl RNA Spikes #4 (100 ng/µl) | 1.5 | 0.8403 | |
| ArrayControl RNA Spikes #7 (100 ng/µl) | 1.5 | 0.8403 | |
| | 178.5 Total Prep Volume | | |

**RNA Seq Diluted RNA spikes (Secondary: 20X)**

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| C1 Loading RGT (20X) | 99 | 19.8 | |
| ArrayControl RNA Spikes (1X) | 1 | 0.01 | |
| | 100 Total Prep Volume | | |

**RNA Seq Lysis Mix with Diluted RNA Spikes**

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| RNA Seq Diluted RNA spikes (20X) | 1 | 1 | 0.667 |
| RNase Inhibitor (40 U/µl) | 0.5 | 1 | 0.667 |
| 3' SMART CDS Primer IIA (12 µM) | 7 | 4.2 | 2.8014 |
| Clontech Dilution Buffer (1X) | 11.5 | 0.575 | 0.3835 |
| | 20 Total Prep Volume | | |

**RNA Seq RT Mix**

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| C1 Loading RGT (20X) | 1.2 | 0.75 | 0.4283 |
| 5X First-Strand Buffer (Rnase-free) (5X) | 11.2 | 1.75 | 0.9992 |
| Dithiothreitol (100 mM) | 1.4 | 4.375 | 2.4981 |
| dNTP Mix (10 mM) | 5.6 | 1.75 | 0.9992 |
| SMARTer IIA Oligonucleotide (12 µM) | 5.6 | 2.1 | 1.1991 |
| RNase Inhibitor (40 U/µl) | 1.4 | 1.75 | 0.9992 |
| SMARTScribe Reverse Transcriptase (100 U/µl) | 5.6 | 17.5 | 9.9925 |
| | 32 Total Prep Volume | | |

**RNA Seq PCR Mix**

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|

| | | | |
|---|---|---|---|
| C1 Loading RGT (20X) | 4.5 | 1 | 0.811 |
| PCR-Grade Water | 63.5 | | |
| 10X Advantage 2 PCR Buffer (10X) | 10 | 1.1111 | 0.9011 |
| 50X dNTP Mix (10 mM) | 4 | 0.4444 | 0.3604 |
| IS PCR primer (12 µM) | 4 | 0.5333 | 0.4325 |
| 50X Advantage 2 Polymerase Mix (50X) | 4 | 2.2222 | 1.8022 |
| | 90 Total Prep Volume | | |

**Harvest Reagent**

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| C1 Harvest RGT (1X) | | | |

**Cell Wash Buffer**

Special Instructions:
---
Wash step: Wash cells after incubation and before mRNA preparation

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| C1 Cell Wash BUF (1X) | 20 | 1 | 1 |
| | 20 Total Prep Volume | | |

## Protocol Reagent Shopping List

| Reagent Name | Vendor | Part Number | Kit Part Number | Stock Concentration |
|---|---|---|---|---|
| Ultrapure LPS-EK | Invivogen | TLRL-PEKLPS | | 100 mg/ml |
| THE RNA Storage Solution | Life Technologies | AM7000 | | |
| ArrayControl RNA Spikes #1 | Life Technologies | | AM1780 | 100 ng/µl |
| ArrayControl RNA Spikes #4 | Life Technologies | | AM1780 | 100 ng/µl |
| ArrayControl RNA Spikes #7 | Life Technologies | | AM1780 | 100 ng/µl |
| RNase Inhibitor | Clontech | | 634833 | 40 U/µl |
| 3' SMART CDS Primer IIA | Clontech | | 634833 | 12 µM |
| Clontech Dilution Buffer | Clontech | | | 1X |
| 5X First-Strand Buffer (Rnase-free) | Clontech | | 634833 | 5X |
| Dithiothreitol | Clontech | | 634833 | 100 mM |
| dNTP Mix | Clontech | | 634833 | 10 mM |
| SMARTer IIA Oligonucleotide | Clontech | | 634833 | 12 µM |
| SMARTScribe Reverse Transcriptase | Clontech | | 634833 | 100 U/µl |
| PCR-Grade Water | Clontech | | 639207 | |
| 10X Advantage 2 PCR Buffer | Clontech | | 639207 | 10X |
| 50X dNTP Mix | Clontech | | 639207 | 10 mM |
| IS PCR primer | Clontech | | 634833 | 12 µM |
| 50X Advantage 2 Polymerase Mix | Clontech | | 639207 | 50X |

## 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 | ☑ | ☑ | ☑ | ☑ | |
| C1 Harvest RGT | 100-6248 | 1X | ☑ | ☑ | | ☑ | |
| C1 Preloading RGT | 100-5311 | 1X | ☑ | ☑ | ☑ | ☑ | |
| C1 Suspension RGT | 100-5315 | 2.5X | ☑ | ☑ | ☑ | ☑ | |
| C1 Loading RGT | 100-5170 | 20X | ☑ | ☑ | | | ☑ |
| C1 Cell Wash BUF | 100-5314 | 1X | ☑ | ☑ | ☑ | | |