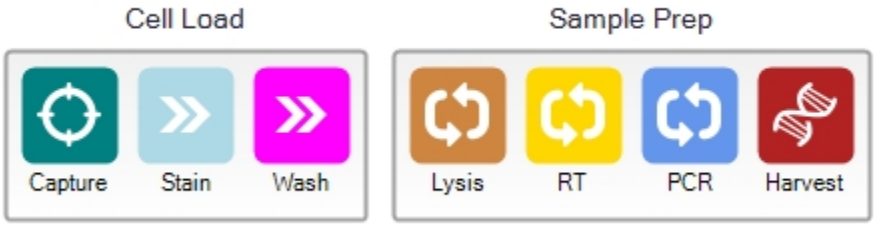
| | |
|---|---|
| Name | C1 CAGE |
| Revision | B |
| Description | C1 CAGE is a method for single-cell transcriptome analysis for molecular counting of RNA 5'-ends. Paired-end sequencing, random priming and unique molecular identifiers are used for single-molecule fragment assembly of mRNAs and long non-coding RNAs. |
| Authors | T.Kouno, S.Kato, M.Mendez, I.Abugessaisa, J.Shin and C.Plessy |
| Institution | RIKEN, JAPAN |
| Lab | Division of Genomics Technologies, Center for Life Sciences Technologies |
| 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 |

## Script Summary - Cell Load

### Runtime Estimates

| Barcode | Estimate |
|---|---|
| 1861x (5-10 um diameter cells) | 0 hours, 31 minutes |
| 1862x (10-17 um diameter cells) | 1 hours, 0 minutes |
| 1863x (17-25 um diameter cells) | 0 hours, 51 minutes |

### Incubation Profile

| Script Step | Operation | Temperature (C) | Duration (s) |
|---|---|---|---|
| Stain | Incubation | 25 | 600 |

## Script Summary - Sample Prep

### Runtime Estimates

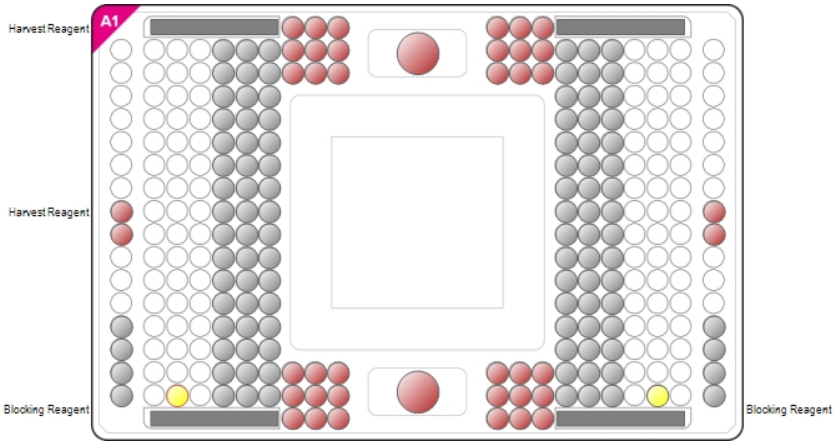
| Barcode | Estimate |
|---|---|
| 1861x (5-10 um diameter cells) | 8 hours, 52 minutes |
| 1862x (10-17 um diameter cells) | 8 hours, 52 minutes |
| 1863x (17-25 um diameter cells) | 8 hours, 52 minutes |

### Incubation Profile

| Script Step | Operation | | Temperature (C) | Duration (s) |
|---|---|---|---|---|
| Lysis | Incubation | S1 | 72 | 180 |

| | | | | |
|---|---|---|---|---|
| | | S2 | 4 | 600 |
| | | S3 | 25 | 60 |
| RT | Incubation | S1 | 22 | 600 |
| | | Reverse Transcription | 42 | 5400 |
| | | Enzyme inactivation | 75 | 900 |
| | | S4 | 25 | 10 |
| PCR | Hot Start | 95C | 95 | 60 |
| PCR | PCR x30 | Denaturation | 95 | 15 |
| | | Annealling | 65 | 30 |
| | | Extension | 68 | 360 |
| 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

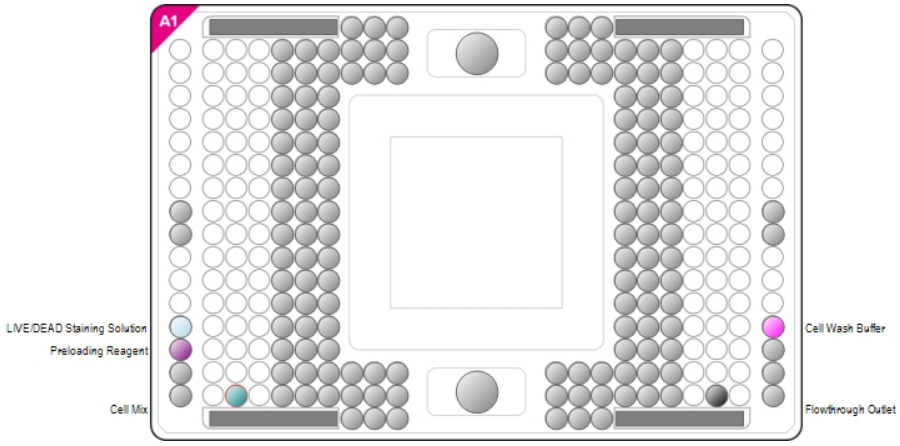
**Blocking Reagent**

| 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




### 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 |
|---|---|---|---|
| LIVE/DEAD Staining Solution | 7 | 1 | |
| 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 250 / µL | 60 | | |
| | 100 Total Prep Volume | | |

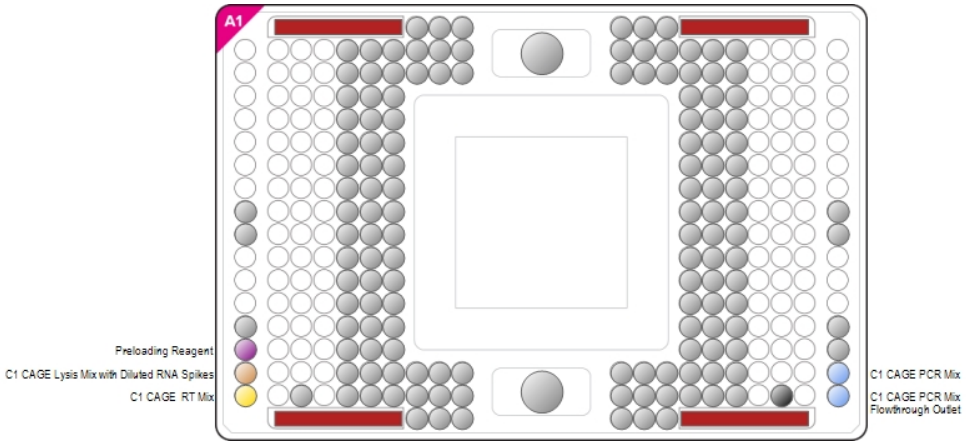
### LIVE/DEAD Staining Solution

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| Cell Wash BUF (1X) | 1250 | 0.9975 | 0.9975 |
| Ethidium homodimer-1 (2 mM) | 2.5 | 0.004 | 0.004 |
| Calcein AM (4 mM) | 0.625 | 0.002 | 0.002 |

1253.125 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 |
|---|---|---|
| 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 | |
| C1 CAGE Lysis Mix with Diluted RNA Spikes | 7 | 3 | |
| C1 CAGE RT Mix | 8 | 4 | |
| C1 CAGE PCR Mix | 24 | 7 | |
| C1 CAGE 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) | | | |

#### C1 CAGE Diluted RNA spikes (Secondary)

Special Instructions:
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Dilution of spike is performed by serial dilution.
Like 1 uL ERCC RNA Spike-In Mix I to 9 uL C1 Loading RGT, and put 1 uL into 132.2 uL C1 Loading RGT.

| Reagent (Stock Concentration) | Mix Prep (µl) | Prep Conc. | Chamber Conc. |
|---|---|---|---|
| C1 Loading RGT (20X) | 1332 | 19.985 | |
| ERCC RNA Spike-In Mix I | 1 | | |
| | 1333 Total Prep Volume | | |

| C1 CAGE Lysis Mix with Diluted RNA Spikes | | | |
|---|---|---|---|
| Special Instructions:<br>----------<br>RT primer sequence: TCGTCGGCAGCGTCAGATGTGNNNNNN | | | |
| **Reagent (Stock Concentration)** | **Mix Prep (µl)** | **Prep Conc.** | **Chamber Conc.** |
| Triton X-100 solution -10% in H2O (10%) | 0.67 | 0.2975 | 0.1984 |
| DNA suspension buffer | 18.65 | | |
| RNase inhibitor (40 U/µl) | 0.57 | 1.0124 | 0.6753 |
| RT primer (SupN6i) (50 µM) | 1.13 | 2.5089 | 1.6734 |
| C1 CAGE Diluted RNA spikes | 1.5 | | |
| | 22.52 Total Prep Volume | | |

| C1 CAGE RT Mix | | | |
|---|---|---|---|
| Special Instructions:<br>----------<br>TS oligo sequence: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNTATA(rG)(rG)(rG)<br>* r is RNA | | | |
| **Reagent (Stock Concentration)** | **Mix Prep (µl)** | **Prep Conc.** | **Chamber Conc.** |
| C1 Loading reagent | 1.05 | | |
| 5x First strand buffer (5X) | 9.8 | 1.6752 | 0.9565 |
| 0.1 M DTT (0.1M) | 4.9 | 0.0168 | 0.0096 |
| 10mM dNTP Mix (10 mM) | 3.08 | 1.053 | 0.6013 |
| Betaine (5M) | 3.08 | 0.5265 | 0.3006 |
| RNase inhibitor (40 U/µl) | 1.24 | 1.6957 | 0.9683 |
| TS oligo (NC2lg_Nbi) (500 µM) | 0.7 | 11.9658 | 6.8325 |
| Superscript III (200 U/µl) | 4.9 | 33.5043 | 19.1309 |
| Nuclease-Free Water | 0.5 | | |
| | 29.25 Total Prep Volume | | |

| C1 CAGE PCR Mix | | | |
|---|---|---|---|
| Special Instructions:<br>----------<br>PCR primer sequence: TCGTCGGCAGCGTCAGATGTG | | | |
| **Reagent (Stock Concentration)** | **Mix Prep (µl)** | **Prep Conc.** | **Chamber Conc.** |
| C1 Loading reagent | 3.75 | | |
| PCR-Grade water | 54.75 | | |
| 10x Advantage2 PCR buffer (not SA) | 7.5 | | |
| 50x dNTP Mix (10 mM) | 3 | 0.4 | 0.3244 |
| PCR primer (Supi) (12 µM) | 3 | 0.48 | 0.3893 |
| 50x Advantage2 Polymerase Mix | 3 | | |
| | 75 Total Prep Volume | | |

| Harvest Reagent |
|---|

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

## Protocol Reagent Shopping List

| Reagent Name | Vendor | Part Number | Kit Part Number | Stock Concentration |
|---|---|---|---|---|
| Ethidium homodimer-1 | Life Technologies | | L-3224 | 2 mM |
| Calcein AM | Life Technologies | | L-3224 | 4 mM |
| Triton X-100 solution -10% in H2O | SIGMA | 93443-100ML | | 10% |
| DNA suspension buffer | TEKnova | T0221 | | |
| RNase inhibitor | TaKaRa | 2313A | 6110A | 40 U/µl |
| RT primer (SupN6i) | Invitrogen | | | 50 µM |
| ERCC RNA Spike-In Mix I | Ambion | 4456740 | | |
| 5x First strand buffer | Life Technologies | Y02321 | 18080044 | 5X |
| 0.1 M DTT | Invitrogen | Y00147 | | 0.1M |
| 10mM dNTP Mix | TaKaRa | | 6110A | 10 mM |
| Betaine | Wako | 027-10865 | | 5M |
| TS oligo (NC2lg_Nbi) | IDT | | | 500 µM |
| Superscript III | Life Technologies | 56575 | 18080044 | 200 U/µl |
| Nuclease-Free Water | Ambion | AM9937 | | |
| PCR-Grade water | TaKaRa | S1785 | 639206 | |
| 10x Advantage2 PCR buffer (not SA) | TaKaRa | S1799 | 639206 | |
| 50x dNTP Mix | TaKaRa | S1800 | 639206 | 10 mM |
| PCR primer (Supi) | Invitrogen | | | 12 µM |
| 50x Advantage2 Polymerase Mix | TaKaRa | S1798 | 639206 | |

## 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 | ☑ | ☑ | ☑ | ☑ | |
| Suspension RGT | 100-5315 | 2.5X | ☑ | ☑ | ☑ | ☑ | |
| Cell Wash BUF | 100-5314 | 1X | ☑ | ☑ | ☑ | | |
| C1 Loading RGT | 100-5170 | 20X | ☑ | ☑ | | | ☑ |
| C1 Loading reagent | 100-5170 | | ☑ | ☑ | | | ☑ |