# Chromium
# Single Cell
# 3' Reagent Kits v3

oebiotech 欧易生物

# CATALOG

1.Kits And Instruments....3
1.1 Kits....3
1.2 Instruments....3
2. Protocol....4
2.1 GEM Generation & Barcoding....4
2.1.1 Prepare Master Mix....4
2.1.2 Transfer GEMs....5
2.2 Post GEM–RT Cleanup & cDNA Amplification....5
2.2.1 Post GEM-RT Cleanup – Dynabeads....5
2.2.2 cDNA Amplification....7
2.2.3 cDNA Cleanup – SPRIselect....7
2.3 3′ Gene Expression Library Construction....8
2.3.1 Fragmentation, End Repair & A-tailing....8
2.3.2 Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect....9
2.3.3 Adaptor Ligation....9
2.3.4 Post Ligation Cleanup – SPRIselect....10
2.3.5 Sample Index PCR....10
2.3.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect....11
2.4 Post Library Construction QC....12

# 1.Kits And Instruments

## 1.1 Kits

| Kits Name | Producers | Cat.No. |
|---|---|---|
| Chromium Single Cell 3' Library & Single Cell 3′ v3 Gel Beads | Chromium | PN-1000075 |
| Qubit dsDNA Assay Kit | Life Technologies | Q328520 |
| DynaBeads® MyOne™ Silane Beads* | Life Technologies | 37002D |
| Agilent High Sensitivity DNA Kit | Agilent | 5067-4626 |
| SPRIselect Reagent Kit | Life Technologies | B23318 |
| Buffer EB | | 19086 |

## 1.2 Instruments

| Instrument Name | Producers | Model |
|---|---|---|
| Chromium Controller | 10xGENOMICS | GCG-SR-1 |
| Centrifuge | Eppendorf | Centrifuge 5418R |
| PCR | Bio-rad | MyCycler |
| Quantitometer | Invitrogen | Qubit3.0 |
| Magnetic Grate | Chromium | 10×Magnetic H |
| Bioanalyzer | Agilent | 2100 |
| Eppendorf PCR Tubes, 0.2 mL | Eppendorf | 0030124.359 |
| Oscillator | | Votex-6 |

# 2. Protocol

## 2.1 GEM Generation & Barcoding

### 2.1.1 Prepare Master Mix

a. Prepare Master Mix on ice. Pipette mix 15x and centrifuge briefly.

| Master Mix<br>*Add reagents in the order listed* | PN | 1X (µl) | 4X + 10% (µl) | 8X + 10% (µl) |
|---|---|---|---|---|
| ● RT Reagent | 2000086 | 20.0 | 88.0 | 176.0 |
| ● Template Switch Oligo | 3000228 | 3.1 | 13.9 | 27.7 |
| ○ Reducing Agent B | 2000087 | 2.0 | 8.7 | 17.3 |
| ● RT Enzyme C | 2000085/<br>2000102 | 8.3 | 36.6 | 73.1 |
| Total | - | 33.4 | 147.1 | 294.2 |

b. Add 33.4 µl Master Mix into each tube of a PCR 8-tube strip on ice.

c. Assemble Chromium Chip B in a 10x Chip Holder.

d. Dispense 50% Glycerol Solution into Unused Chip Wells

i. 75 µl to unused wells in row labeled 1.

ii. 40 µl to unused wells in row labeled 2.

iii. 280 µl to unused wells in row labeled 3.

e. Prepare Master Mix + Cell Suspension

f. dispense 75 µl Master Mix + Cell Suspension into the bottom center of each well in row labeled 1 without introducing bubbles.


g. Prepare Gel Beads Snap the Gel Bead strip into a 10x Vortex Adapter. Vortex 30 sec.

h. Slowly aspirate 40 µl Gel Beads. Dispense into the wells in row labeled 2 without introducing bubbles.


i. Dispense 280 µl Partitioning Oil into the wells in row labeled 3


j. Attach 10x Gasket Run the Chromium Controller

2.1.2 Transfer GEMs

a. Place a tube strip on ice.
b. Slowly aspirate 100 µl GEMs from the lowest points of the Recovery Wells in the top row without creating a seal between the tips and the bottom of the wells.
c. If multiple chips are run back-to-back, cap/ cover the GEM-containing tube strip and place on ice for no more than 1 h.
d. GEM-RT Incubation

| Lid Temperature | Reaction Volume | Run Time |
|---|---|---|
| 53°C | 125 µl | ~55 min |
| **Step** | **Temperature** | **Time** |
| 1 | 53°C | 00:45:00 |
| 2 | 85°C | 00:05:00 |
| 3 | 4°C | Hold |

e. Store at 4°C for up to 72 h or at −20°C for up to a week, or proceed to the next step.

**2.2 Post GEM–RT Cleanup & cDNA Amplification**

2.2.1 Post GEM-RT Cleanup – Dynabeads

a. Add 125 µl Recovery Agent to each sample at room temperature. DO NOT pipette mix or vortex the biphasic mixture. Wait 60 sec.
b. Slowly remove 125 µl Recovery Agent/ Partitioning Oil (pink) from the bottom of the tube. DO NOT aspirate any aqueous sample.

c. Prepare Dynabeads Cleanup Mix.

| Dynabeads Cleanup Mix<br>*Add reagents in the order listed* | PN | 1X (µl) | 4X + 10% (µl) | 8X + 10% (µl) |
|---|---|---|---|---|
| Cleanup Buffer | 2000088 | 182 | 801 | 1602 |
| **Dynabeads MyOne SILANE**<br>Vortex thoroughly (≥30 sec) **immediately** before adding to the mix. If still clumpy, pipette mix to resuspend completely.<br>DO NOT centrifuge before use. | 2000048 | 8 | 35 | 70 |
| Reducing Agent B | 2000087 | 5 | 22 | 44 |
| Nuclease-free Water | | 5 | 22 | 44 |

d. Vortex and add 200 µl to each sample. Pipette mix 10x (pipette set to 200 µl).

e. Incubate 10 min at room temperature. Pipette mix again at ~5 min after start of incubation to resuspend settled beads.

f. Prepare Elution Solution I. Vortex and centrifuge briefly.

| Elution Solution I<br>*Add reagents in the order listed* | PN | 1X (µl) | 10X (µl) |
|---|---|---|---|
| Buffer EB | - | 98 | 980 |
| 10% Tween 20 | - | 1 | 10 |
| Reducing Agent B | 2000087 | 1 | 10 |
| Total | - | 100 | 1000 |

g. At the end of 10 min incubation, place on a 10x Magnetic Separator•High position (magnet•High) until the solution clears.
A white interface between the aqueous phase and Recovery Agent is normal.

h. Remove the supernatant.

i. Add 300 µl 80% ethanol to the pellet while on the magnet. Wait 30 sec.

j. Remove the ethanol.

k. Add 200 µl 80% ethanol to pellet. Wait 30 sec.

l. Remove the ethanol.

m. Centrifuge briefly. Place on the magnet•Low.

n. Remove remaining ethanol. Air dry for 1 min.

o. Remove from the magnet. Immediately add 35.5 µl Elution Solution I.

p. Pipette mix (pipette set to 30 µl) without introducing bubbles.

q. Incubate 2 min at room temperature.

r. Place on the magnet•Low until the solution clears.

s. Transfer 35 µl sample to a new tube strip.

2.2.2 cDNA Amplification

a. Prepare cDNA Amplification Mix on ice. Vortex and centrifuge briefly.

| cDNA Amplification Reaction Mix *Add reagents in the order listed* | PN | 1X (µl) | 4X + 10% (µl) | 8X + 10% (µl) |
|---|---|---|---|---|
| ○ Amp Mix | 2000047/ 2000103 | 50 | 220 | 440 |
| ● cDNA Primers | 2000089 | 15 | 66 | 132 |
| Total | - | 65 | 286 | 572 |

b. Add 65 µl cDNA Amplification Reaction Mix to 35 µl sample.
c. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.
d. Incubate in a thermal cycler with the following protocol.

| Lid Temperature | Reaction Volume | Run Time |
|---|---|---|
| 105°C | 100 µl | ~30-45 min |

| Step | Temperature | Time |
|---|---|---|
| 1 | 98°C | 00:03:00 |
| 2 | 98°C | 00:00:15 |
| 3 | 63°C<br>Version Specific<br>Updated Temperature | 00:00:20 |
| 4 | 72°C | 00:01:00 |
| 5 | Go to Step 2, see table below for total # of cycles | |
| 6 | 72°C | 00:01:00 |
| 7 | 4°C | Hold |

e. Store at 4°C for up to 72 h or proceed to the next step.

2.2.3 cDNA Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add 60 µl SPRIselect reagent (0.6X) to each sample and pipette mix 15x (pipette set to 150 µl).
b. Incubate 5 min at room temperature.
c. Place on the magnet•High until the solution clears.
d. Remove the supernatant.
e. Add 200 µl 80% ethanol to the pellet. Wait 30 sec.

f. Remove the ethanol.
g. Repeat steps e and f for a total of 2 washes.
h. Centrifuge briefly and place on the magnet•Low.
i. Remove any remaining ethanol. Air dry for 2 min. DO NOT exceed 2 min as this will decrease elution efficiency.
j. Remove from the magnet. Add 40.5 µl Buffer EB. Pipette mix 15x.
k. Incubate 2 min at room temperature.
l. Place the tube strip on the magnet•High until the solution clears.
m. Transfer 40 µl sample to a new tube strip.
n. Store at 4°C for up to 72 h or at −20°C for up to 4 weeks, or proceed to the next step.

**2.3 3′ Gene Expression Library Construction**

2.3.1 Fragmentation, End Repair & A-tailing

a. Prepare a thermal cycler with the following incubation protocol. .

| Lid Temperature | Reaction Volume | Run Time |
|---|---|---|
| 65°C | 50 µl | ~35 min |
| **Step** | **Temperature** | **Time** |
| Pre-cool block<br>Pre-cool block prior to preparing the Fragmentation Mix | 4°C | Hold |
| Fragmentation | 32°C | 00:05:00 |
| End Repair & A-tailing | 65°C | 00:30:00 |
| Hold | 4°C | Hold |

b. Vortex Fragmentation Buffer. Verify there is no precipitate.
c. Prepare Fragmentation Mix on ice. Pipette mix and centrifuge briefly.

| Fragmentation Mix<br>*Add reagents in the order listed* | PN | 1X (µl) | 4X + 10% (µl) | 8X + 10% (µl) |
|---|---|---|---|---|
| Fragmentation Buffer | 2000091/ 2000104 | 5 | 22 | 44 |
| Fragmentation Enzyme | 2000090 | 10 | 44 | 88 |
| Total | - | 15 | 66 | 132 |

d. Transfer ONLY 10 µl purified cDNA sample from cDNA Cleanup (step 2.3n) to a tube strip.
e. Add 25 µl Buffer EB to each sample.

f. Add 15 µl Fragmentation Mix to each sample.
g. Pipette mix 15x (pipette set to 35 µl) on ice. Centrifuge briefly.
h. Transfer into the pre-cooled thermal cycler (4°C) and press “SKIP” to initiate the protocol.

2.3.2 Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add 30 µl SPRIselect (0.6X) reagent to each sample. Pipette mix 15x (pipette set to 75 µl).
b. Incubate 5 min at room temperature.
c. Place on the magnet•High until the solution clears. DO NOT discard supernatant.
d. Transfer 75 µl supernatant to a new tube strip.
e. Vortex to resuspend SPRIselect reagent. Add 10 µl SPRIselect reagent (0.8X) to each sample. Pipette mix 15x (pipette set to 80 l).
f. Incubate 5 min at room temperature.
g. Place on the magnet•High until the solution clears.
h. Remove 80 µl supernatant. DO NOT discard any beads.
i. Add 125 µl 80% ethanol to the pellet. Wait 30 sec.
j. Remove the ethanol.
k. Repeat steps i and j for a total of 2 washes.
l. Centrifuge briefly. Place on the magnet•Low until the solution clears. Remove remaining ethanol. DO NOT over dry to ensure maximum elution efficiency.
m. Remove from the magnet. Add 50.5 µl Buffer EB to each sample. Pipette mix 15x.
n. Incubate 2 min at room temperature.
o. Place on the magnet•High until the solution clears.
p. Transfer 50 µl sample to a new tube strip.

2.3.3 Adaptor Ligation

a. Prepare Adaptor Ligation Mix. Pipette mix and centrifuge briefly.

| Adaptor Ligation Mix<br>*Add reagents in the order listed* | PN | 1X (µl) | 4X + 10% (µl) | 8X + 10% (µl) |
|---|---|---|---|---|
| Ligation Buffer | 2000092 | 20 | 88 | 176 |
| DNA Ligase | 220110/ 220131 | 10 | 44 | 88 |
| Adaptor Oligos | 2000094 | 20 | 88 | 176 |
| Total | - | 50 | 220 | 440 |

b. Add 50 µl Adaptor Ligation Mix to 50 µl sample. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.
c. Incubate in a thermal cycler with the following protocol.

| Lid Temperature | Reaction Volume | Run Time |
|---|---|---|
| 30°C | 100 µl | 15 min |

| Step | Temperature | Time |
|---|---|---|
| 1 | 20°C | 00:15:00 |
| 2 | 4°C | Hold |

2.3.4 Post Ligation Cleanup – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add 80 µl SPRIselect Reagent (0.8X) to each sample. Pipette mix 15x (pipette set to 150 µl).
b. Incubate 5 min at room temperature.
c. Place on the magnet•High until the solution clears.
d. Remove the supernatant.
e. Add 200 µl 80% ethanol to the pellet. Wait 30 sec.
f. Remove the ethanol.
g. Repeat steps e and f for a total of 2 washes.
h. Centrifuge briefly. Place on the magnet•Low.
i. Remove any remaining ethanol. Air dry for 2 min. DO NOT exceed 2 min as this will decrease elution efficiency.
j. Remove from the magnet. Add 30.5 µl Buffer EB. Pipette mix 15x.
k. Incubate 2 min at room temperature.
l. Place on the magnet•Low until the solution clears.
m. Transfer 30 µl sample to a new tube strip.

2.3.5 Sample Index PCR

a. Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run. Record the 10x Sample Index name (PN-220103 Chromium i7 Sample Index Plate well ID) used.
b. Prepare Sample Index PCR Mix.

| Sample Index PCR Mix<br>*Add reagents in the order listed* | PN | 1X (µl) | 4X + 10% (µl) | 8X + 10% (µl) |
|---|---|---|---|---|
| ○ Amp Mix | 2000047/ 2000103 | 50 | 220 | 440 |
| SI Primer | 2000095 | 10 | 44 | 88 |
| Total | - | 60 | 264 | 528 |

c. Add 60 µl Sample Index PCR Mix to 30 µl sample.

d. Add 10 µl of an individual Chromium i7 Sample Index to each well and record the well ID used. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.
e. Incubate in a thermal cycler with the following protocol.

| Lid Temperature | Reaction Volume | Run Time |
|---|---|---|
| 105°C | 100 µl | ~25-40 min |
| **Step** | **Temperature** | **Time** |
| 1 | 98°C | 00:00:45 |
| 2 | 98°C | 00:00:20 |
| 3 | 54°C | 00:00:30 |
| 4 | 72°C | 00:00:20 |
| 5 | Go to step 2, see below for # of cycles | |
| 6 | 72°C | 00:01:00 |
| 7 | 4°C | Hold |

2.3.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add 60 µl SPRIselect Reagent (0.6X) to each sample. Pipette mix 15x (pipette set to 150 µl).
b. Incubate 5 min at room temperature.
c. Place the magnet•High until the solution clears. DO NOT discard supernatant.
d. Transfer 150 µl supernatant to a new tube strip.
e. Vortex to resuspend the SPRIselect reagent. Add 20 µl SPRIselect Reagent (0.8X) to each sample. Pipette mix 15x (pipette set to 150 µl).
f. Incubate 5 min at room temperature.
g. Place the magnet•High until the solution clears.
h. Remove 165 µl supernatant. DO NOT discard any beads.
i. With the tube still in the magnet, add 200 µl 80% ethanol to the pellet. Wait 30 sec.
j. Remove the ethanol.
k. Repeat steps i and j for a total of 2 washes.
l. Centrifuge briefly. Place on the magnet•Low. Remove remaining ethanol.
m. Remove from the magnet. Add 35.5 µl Buffer EB. Pipette mix 15x.
n. Incubate 2 min at room temperature.
o. Place on the magnet•Low until the solution clears.
p. Transfer 35 µl to a new tube strip.
q. Store at 4°C for up to 72 h or at −20°C for long-term storage.

**2.4 Post Library Construction QC**

a. Load 1 μL sample on an Agilent 2100 Bioanalyzer.
b. Check the size and purity of the library.