Chromium™

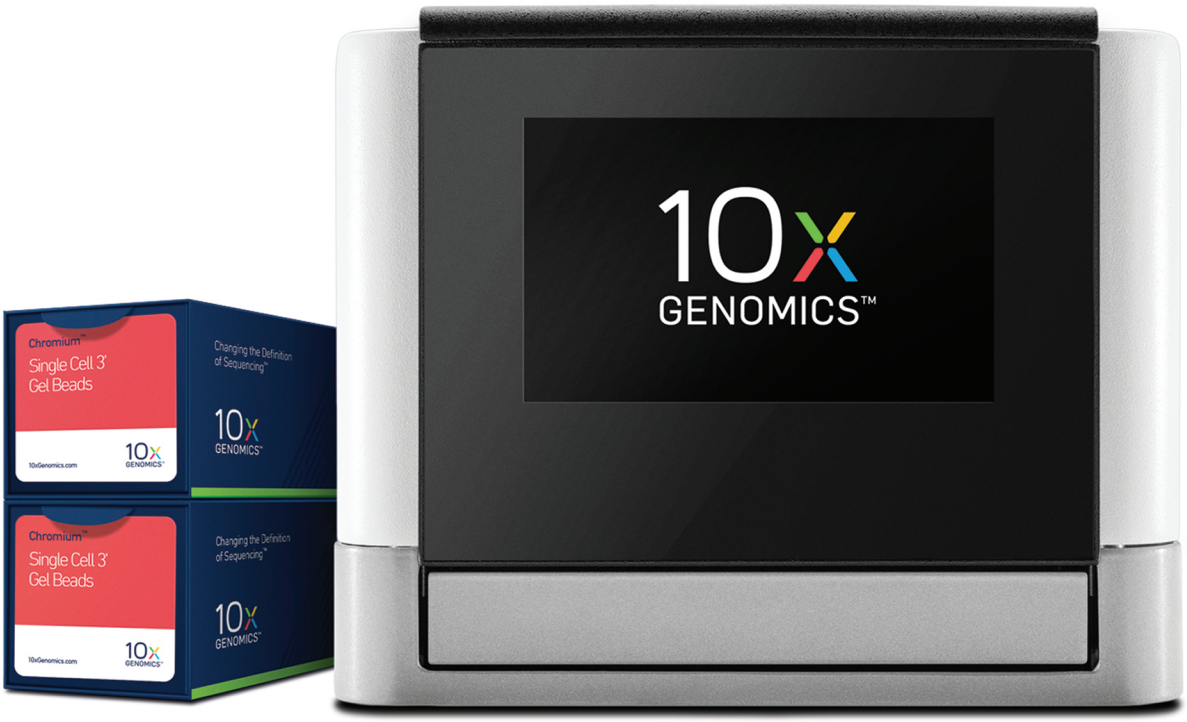
Single Cell 3' Reagent Kits

Quick Reference Cards

FOR USE WITH

Chromium™ Single Cell 3' Library, Gel Bead & Multiplex Kit, 16 rxns PN-120233

Chromium™ Single Cell 3' Chip Kit PN-120232



Chromium™ Single Cell 3'

1. Cell Preparation

1.1 Cell Harvesting - Suspension Cell Lines

– Determine the cell concentration. Ideally, 3 x 10^5 – 1 x 10^6 /ml with >90% alive

Cell concentration > 6 x 10^5 / ml	Cell concentration < 6 x 10^5 / ml
1.5 ml into 2 ml tube	1.5 ml into two 2 ml tubes (total 3 ml)

1.2 Cell Harvesting - Adherent Cell Lines

– Remove and discard culture medium

– Rinse cell layer: 1.5 ml of 0.25% Trypsin-EDTA solution. Discard spent trypsin solution

– Surface dissociation: 5 - 6 ml of 0.25% Trypsin-EDTA solution at 37°C for 5 - 15 min

– Add 10 ml medium to stop digestion, transfer cell suspension to 50 ml conical tube

– Rinse flask with 2 ml medium, combine wash into 50 ml conical tube

– Centrifuge cells at 250 rcf for 5 min, remove supernatant

– Resuspend pelleted cells in medium and determine cell concentration

Cell concentration > 6 x 10^5 / ml	Cell concentration < 6 x 10^5 / ml
1.5 ml into 2 ml tube	1.5 ml into two 2 ml tubes (total 3 ml)

1.3 Cell Washing

– Centrifuge cells in a 2 ml Eppendorf tube at 150 rcf for 3 min, remove supernatant

– Add 1 ml 1X PBS (0.04% BSA) to each tube, pipette mix 5x and invert tubes to resuspend cells. Pool tubes if necessary

– Centrifuge cells at 150 rcf for 3 min, remove supernatant

– Add 1 ml of 1X PBS (0.04% BSA) to the tube, pipette mix 5x and invert tubes to resuspend cells

– Spin cells at 150 rcf for 3 min, remove supernatant

– Add 500 µl 1X PBS (0.04% BSA) or volume for cell concentration >1 x 10^6 /ml. Pipette mix until complete suspension

– Determine the cell concentration. Adjust concentration to 1 x 10^6 /ml (1000/µl)

– Place on ice and continue to next step

Chromium™ Single Cell 3'

2. GEM Generation & Barcoding

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Getting Started!

Equilibrate to room temperature before use:

- RT Reagent Mix
- RT Primer (See User Guide for resuspension)
- Additive A
- Surrogate Fluid (if <8 samples)
- Heat block from plate sealer
- Single Cell 3' Gel Beads. Vortex Gel Bead strip in a 10x™ Vortex Adapter and then flick sharply downwards to maximize Gel Bead recovery
- *Ensure no precipitate in liquid reagents before use*

Place on ice:

- RT Enzyme
- RNase Inhibitor
- Chilled metal block

Obtain:

- Partitioning Oil
- Single Cell 3' Chip(s)
- 10x Chip Holder
- 10x Gasket(s)

2.1 Single Cell Suspension Volume Table

- The table below outlines the volume of single cell suspension (µl) and the volume of Nuclease-Free Water (µl) to be added to a single GEM generation reaction for a range of cell stock concentrations and targeted cell recovery

Cell Stock Concentration (Cells / µl)	Targeted Cell Recovery					
	1200 cells	2000 cells	3000 cells	4000 cells	5000 cells	6000 cells
100	25.6 | 11.8	n/a	n/a	n/a	n/a	n/a
200	12.8 | 24.6	21.3 | 16.1	32.0 | 5.4	n/a	n/a	n/a
300	8.5 | 28.9	14.2 | 23.2	21.3 | 16.1	28.4 | 9.0	35.6 | 1.8	n/a
400	6.4 | 31.0	10.7 | 26.7	16.0 | 21.4	21.3 | 16.1	26.7 | 10.7	32.0 | 5.4
500	5.1 | 32.3	8.5 | 28.9	12.8 | 24.6	17.1 | 20.3	21.3 | 16.1	25.6 | 11.8
600	4.3 | 33.1	7.1 | 30.3	10.7 | 26.7	14.2 | 23.2	17.8 | 19.6	21.3 | 16.1
700	3.7 | 33.7	6.1 |31.3	9.1 | 28.3	12.2 | 25.2	15.2 | 22.2	18.3 | 19.1
800	3.2 | 34.2	5.3 | 32.1	8.0 | 29.4	10.7 | 26.7	13.3 | 24.1	16.0 | 21.4
900	2.8 | 34.6	4.7 | 32.7	7.1 | 30.3	9.5 | 27.9	11.9 | 25.5	14.2 | 23.2
1000	2.6 | 34.8	4.3 | 33.1	6.4 | 31.0	8.5 | 28.9	10.7 | 26.7	12.8 | 24.6

Cell Stock Concentration (Cells / µl)	Targeted Cell Recovery					
	1200 cells	2000 cells	3000 cells	4000 cells	5000 cells	6000 cells
1100	2.3 | 35.1	3.9 | 33.5	5.8 | 31.6	7.8 | 29.6	9.7 | 27.7	11.6 | 25.8
1200	2.1 | 35.3	3.6 | 33.8	5.3 | 32.1	7.1 | 30.3	8.9 | 28.5	10.7 | 26.7
1300	2.0 | 35.4	3.3 | 34.1	4.9 | 32.5	6.6 | 30.8	8.2 | 29.2	9.8 | 27.6
1400	1.8 | 35.6	3.0 | 34.4	4.6 | 32.8	6.1 | 31.3	7.6 | 29.8	9.1 | 28.3
1500	1.7 | 35.7	2.8 | 34.6	4.3 | 33.1	5.7 | 31.7	7.1 | 30.3	8.5 | 28.9
1600	1.6 | 35.8	2.7 | 34.7	4.0 | 33.4	5.3 | 32.1	6.7 | 30.7	8.0 | 29.4
1700	1.5 | 35.9	2.5 | 34.9	3.8 | 33.6	5.0 | 32.4	6.3 | 31.1	7.5 | 29.9
1800	1.4 | 36.0	2.4 | 35.0	3.6 | 33.8	4.7 | 32.7	5.9 | 31.5	7.1 | 30.3
1900	1.3 | 36.1	2.2 | 35.2	3.4 | 34.0	4.5 | 32.9	5.6 | 31.8	6.7 | 30.7
2000	1.3 | 36.1	2.1 | 35.3	3.2 | 34.2	4.3 | 33.1	5.3 | 32.1	6.4 | 31.0

- *Grey boxes: Volumes that would exceed the allowable water volume in each reaction*
- *Yellow boxes: A low transfer volume that may result in higher cell load variability*

2.2 Preparing Single Cell Master Mix

- Make and maintain Master Mix on ice
- Add reagents in order shown
- **Do not add single cell suspension at this point**
- Pipette mix 15x, centrifuge briefly
- Calculate the total **volume of Master Mix** in each reaction for the **volume of Nuclease-Free Water** determined from the table above

Master Mix	1X (µl)	8.8X (µl)
Nuclease-Free Water	See Table	Calculate
RT Reagent Mix	50.0	440.0
RT Primer	4.0	35.2
RNase Inhibitor	1.5	13.2
Additive A	2.5	22.0
RT Enzyme	4.6	40.5
Total	**Calculate**	Calculate

Chromium™ Single Cell 3'

2. GEM Generation & Barcoding

2.3 Loading the Chromium™ Single Cell 3' Chip

- If <8 samples, dispense **Surrogate Fluid** to each unused well: 90 µl - row labeled 1, 40 µl - row labeled 2, 270 µl - row labeled 3. Do not add Surrogate Fluid to Recovery Wells (row labeled ◀)
- Dispense **calculated volume of Master Mix** into each well of a tube strip on ice
- Pipette mix cell suspension, add appropriate **volume of single cell suspension** (from table) into each tube strip well
- Pipette mix 5x on ice (pipette set at 90 µl)
- Dispense **90 µl** Master Mix + Cells into bottom of row labeled **1**. Place the tips into the bottom center of the wells and raise the tips slightly above the bottom before slowly dispensing
- Wait **30 sec**
- Slowly dispense **40 µl** Single Cell 3' Gel Beads into bottom of row labeled **2**
- Dispense **2 x 135 µl** (total 270 µl) Partitioning Oil into row labeled **3**
- Attach 10x™ Gasket - notched cut top left, holes aligned with the wells

2.4 Running the Chromium Controller

- Press the touchscreen button to eject the tray
- Place assembled Chip, 10x Chip Holder and 10x Gasket on the tray
- Press the touchscreen button again to retract the tray
- Confirm Single Cell 3' program on screen, press play button to start the run
- At run completion (~6 min), Chromium Controller will chime

2.5 Transferring GEMs

- Maintain an Eppendorf twin.tec® 96-well PCR plate on ice
- Remove and discard the 10x Gasket
- Open the 10x Chip Holder and fold the lid all the way back until it clicks
- Slowly aspirate **105 µl** GEMs from the lowest points of the Recovery Wells
- Dispense GEMs into the PCR plate on ice with the pipette tips **against the sidewalls of the wells**
- *If running >1 Chip, keep PCR plate on ice, seal wells with Strip Caps*

2.6 GEM-RT Incubation

- Seal PCR plate with foil heat seal at 185°C for 6 sec, promptly remove
- Place PCR plate in the thermal cycler and run the incubation program (right)
- Store in PCR plate at 4°C ≤72 h before next step STOP

Lid Temperature	Reaction Volume	Run Time
55°C	125 µl	~2 h 10 min
Step	**Temperature**	**Time**
1	55°C	2:00:00
2	85°C	5:00
3	4°C	Hold

Chromium™ Single Cell 3'

3. Post GEM-RT Cleanup & cDNA Amplification

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Getting Started!

Equilibrate to room temperature before use:

- Additive A
  - DynaBeads® MyOne™ Silane beads
  - Beckman Coulter SPRIselect Reagent
- Amplification Master Mix
- cDNA Primer Mix
- cDNA Additive
  - Agilent Bioanalyzer High Sensitivity Kit

- *Ensure no precipitate in liquid reagents before use*

Thaw at 65°C:

- Buffer for Sample Cleanup 1 at 65°C for 10 min at max speed on a thermomixer. Cool to room temperature

Obtain:

- Recovery Agent
  - Qiagen Buffer EB
  - Bio-Rad 10% Tween 20

Prepare:

- 80% Ethanol (15 ml for 8 samples)

For Silane DynaBeads & SPRIselect Reagent Cleanups:

- *Vortex to fully resuspend Dynabeads and SPRIselect Reagent before adding to each sample in the tube strip*
- *Incubations are at room temperature*
- *Mix 15x = Pipette mix 15x*
- *Magnet H or L = Place in a 10x™ Magnetic Separator in the High or Low position*
- *Supernatant and ethanol wash should be discarded except where noted*

3.1 Post GEM-RT Cleanup - Silane DynaBeads

- Remove foil seal
- Add **125 µl** Recovery Agent
- Wait **60 sec**. Transfer entire volume to tube strip
- Remove **125 µl** Recovery Agent/Partitioning Oil, discard
- Add **200 µl** DynaBeads Cleanup Mix
- Pipette mix 5x. Incubate **10 min** (mix 5x after 5 min)
- Magnet **H**. Remove supernatant
- Add **2 x 150 µl** (total 300 µl) 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- Add **200 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol, air dry **2 min**
- Remove from magnet
- Add **50.5 µl Elution Solution I**. Mix ≥15x. Incubate **1 min**
- Magnet **H**
- Transfer **50 µl** sample to new tube strip

DynaBeads Cleanup Mix	1X (µl)	8.8X (µl)
Buffer for Sample Cleanup 1	182	1602
DynaBeads MyOne Silane	14	123
Additive A	4	35
Total	**200**	**1760**

Elution Solution I	1 rxn (µl)	10 rxns (µl)
Buffer EB	98	980
10% Tween 20	1	10
Additive A	1	10
Total	**100**	**1000**

3.2 Post GEM-RT Cleanup - SPRIselect

- Add **30 µl** SPRIselect Reagent. Mix 15x. Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **125 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol, air dry **1 min**
- Remove from magnet
- Add **35.5 µl Elution Solution II**. Mix 15x. Incubate **2 min**
- Magnet **L**
- Transfer **35 µl** sample to new tube strip, cap wells

Elution Solution II	1 rxn (µl)	10 rxns (µl)
Buffer EB	99	990
Additive A	1	10
Total	**100**	**1000**

3.3 cDNA Amplification Reaction

cDNA Amplification Rxn Mix	1X (µl)	8.8X (µl)
Nuclease-Free Water	8	70
Amplification Master Mix	50	440
cDNA Additive	5	44
cDNA Primer Mix	2	18
Total	**65**	**572**

- Add **65 µl** cDNA Amplification Reaction Mix to each tube containing 35 µl purified GEM-RT product
- Pipette mix 15x (pipette setting 80 µl) and centrifuge briefly
- Cap strip, place in the thermal cycler, and run the program (right)
- Store at 4°C ≤72 h or proceed to next step

STOP

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~45 min
Step	**Temperature**	**Time**
1	98°C	3:00
2	98°C	0:15
3	67°C	0:20
4	72°C	1:00
5	Go to Step 2, (see below for total cycles)	
6	72°C	1:00
7	4°C	Hold

- *Targeted cell recovery <2000 - 14 cycles total*
- *Targeted cell recovery 2000-6000 - 12 cycles total*
- *Targeted cell recovery >6000 - 10 cycles total*

3.4 Post cDNA Amplification Reaction Cleanup - SPRIselect

- Add **60 µl** SPRIselect Reagent. Mix 15x. Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **200 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol, air dry **2 min**
- Remove from magnet
- Add **55.5 µl Buffer EB**. Mix 15x. Incubate **2 min**
- Magnet **H**
- Transfer **55 µl** sample to new tube strip, cap wells
- Store at 4°C ≤72 h, –20°C ≤1 week, or proceed to next step

STOP

3.5 Post cDNA Amplication Reaction QC

- Run 1 µl of sample at 1:5 dilution on the Agilent Bioanalyzer High Sensitivity chip for qualitative analysis

Chromium™ Single Cell 3'

4. Library Construction

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Getting Started!

Equilibrate to room temperature before use:

- End Repair and A-tailing Buffer
- SI-PCR Primer
- Ligation Buffer
- R1 Adaptor Mix
- Single Cell 3' Sample Index Plate
- Beckman Coulter SPRIselect Reagent
- Agilent Bioanalyzer High Sensitivity Kit, or
- Agilent TapeStation High Sensitivity D1000 ScreenTape & Reagents
- Kapa DNA Quantification Kit for Illumina Platforms

Place on ice:

- End Repair and A-tailing Enzyme
- DNA Ligase
- Amplification Master Mix

Obtain:

- Qiagen Buffer EB

Prepare:

- 80% Ethanol (20 ml for 8 samples)

- *Ensure no precipitate in liquid reagents before use*

4.1 Shearing

- Shear **51 µl** sample from Post cDNA Amplification Reaction Cleanup according to Covaris's recommended settings for the instrument and tube - target peak size of 200 bp, standard DNA sample
- Centrifuge tubes briefly and transfer **50 µl** sheared sample to a new tube strip

4.2 Post Shearing Size Selection - SPRIselect

- Add **30 µl** SPRIselect Reagent. Mix 15x. Incubate **5 min**
- Magnet **H**
- **DO NOT discard supernatant**
- **Transfer 75 µl supernatant to new tube strip**, discard old tube strip
- Add **10 µl** SPRIselect Reagent. Mix 15x
- Incubate **5 min**
- Magnet **H**. Remove supernatant. **DO NOT remove any beads**
- Add **125 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **50.5 µl Buffer EB**. Mix 15x. Incubate **2 min**
- Magnet **H**
- Transfer **50 µl** sample to new tube strip, cap wells

For SPRIselect Reagent Size Selection & Cleanups:

- *Vortex to fully resuspend SPRIselect Reagent before adding to each sample in the tube strip*
- *Incubations are at room temperature*
- *Mix 15x = Pipette mix 15x*
- *Magnet H or L = Place in a 10x™ Magnetic Separator in the High or Low position*
- *Supernatant and ethanol wash should be discarded except where noted*

Chromium™ Single Cell 3'

4. Library Construction

4.3 Library Construction: End Repair & A-tailing

End Repair and A-tailing Mix	1X (µl)	8.8X (µl)
End Repair & A-tailing Buffer	7	62
End Repair & A-tailing Enzyme	3	26
Total	**10**	**88**

Lid Temperature	Reaction Volume	Run Time
85°C	60 µl	~ 1h 5 min
Step	**Temperature**	**Time**
End Repair	20°C	30:00
A-tailing	65°C	30:00
Hold	4°C	Hold

- Add **10 µl** End Repair and A-tailing Mix to each tube containing 50 µl sample from Post Shearing Size Selection
- Pipette mix 15x and centrifuge briefly
- Place tube strip in the thermal cycler and run the End Repair and A-tailing program (above right)

4.4 Library Construction: Adaptor Ligation

Adaptor Ligation Mix	1X (µl)	8.8X (µl)
Nuclease-Free Water	7.5	66
Ligation Buffer	30	264
DNA Ligase	10	88
R1 Adaptor Mix	2.5	22
Total	**50**	**440**

Lid Temperature	Reaction Volume	Run Time
30°C	110 µl	-
Step	**Temperature**	**Time**
1	20°C	15:00

- Add **50 µl** Adaptor Ligation Mix to each tube containing 60 µl sample from the End Repair and A-tailing step
- Pipette mix 15x and centrifuge briefly
- Place tube strip in the thermal cycler and run the Ligation program (above right)

4.5 Post Ligation Cleanup 1 & 2 - SPRIselect

- **Cleanup 1**
- Add **88 µl** SPRIselect Reagent
- Mix 15x. Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **200 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol, air dry **2 min**
- Remove from magnet
- Add **50.5 µl Buffer EB.** Mix 15x. Incubate **2 min**
- Magnet **H**
- Transfer **50 µl** sample to new tube strip
- Proceed to Cleanup 2

- **Cleanup 2**
- Add **50 µl** SPRIselect Reagent
- Mix 15x. Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **125 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol, air dry **2 min**
- Remove from magnet
- Add **30.5 µl Buffer EB.** Mix 15x. Incubate **2 min**
- Magnet **L**
- Transfer **30 µl** sample to new tube strip
- Proceed to next step

Chromium™ Single Cell 3'

4. Library Construction

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4.6 Sample Index PCR

Sample Index PCR Mix	1X (µl)	8.8X (µl)
Nuclease-Free Water	8	70
Amplification Master Mix	50	440
SI-PCR Primer	2	18
Total	**60**	**528**

- Add **60 µl** Sample Index PCR Mix into each tube containing 30 µl purified Post Ligation sample
- Add **10 µl** of an individual Single Cell 3' Sample Index to each well and record assignment
- Pipette mix 15x and centrifuge briefly
- Place tube strip in the thermal cycler and run the program (right)

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~25 min

Step	Temperature	Time
1	98°C	0:45
2	98°C	0:20
3	60°C	0:30
4	72°C	0:20
5	Go to Step 2, 9X (for 10 cycles total)	
6	72°C	1:00
7	4°C	Hold

- Store at 4°C ≤72 h or proceed to next step

STOP

4.7 Post Sample Index PCR Cleanup 1 & 2 - SPRIselect

- **Cleanup 1**
- Add **100 µl** SPRIselect Reagent
- Mix 15x. Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **200 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol, air dry **2 min**
- Remove from magnet
- Add **50.5 µl Buffer EB.** Mix 15x. Incubate **2 min**
- Magnet **H**
- Transfer **50 µl** sample to new tube strip
- Proceed to Cleanup 2

- **Cleanup 2**
- Add **50 µl** SPRIselect Reagent
- Mix 15x. Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **125 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol, air dry **2 min**
- Remove from magnet
- Add **35.5 µl Buffer EB.** Mix 15x. Incubate **2 min**
- Magnet **L**
- Transfer **35 µl** sample to new tube strip
- Proceed to next step
- Store at 4°C ≤72 h or –20°C for long-term storage

STOP

4.8 Post Library Construction QC

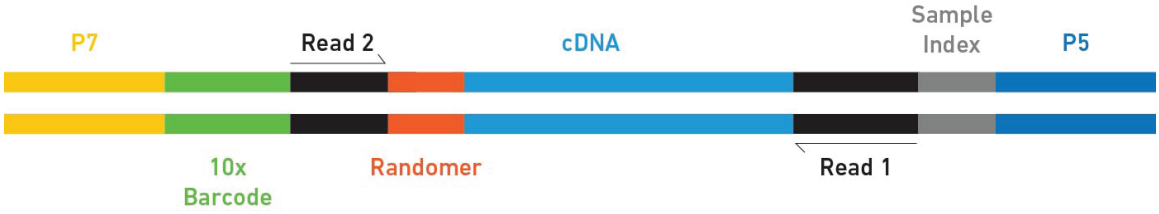
- Either run 1 µl of sample at 1:10 dilution on the Agilent Bioanalyzer High Sensitivity chip or 1 µl of sample at 1:10 dilution on the Agilent TapeStation High Sensitivity D1000 ScreenTape for qualitative analysis

4.9 Post Library Construction Quantification

- Quantify libraries using Kapa DNA Quantification Kit for Illumina platforms following manufacturer's recommendations

5. Sequencing

– The Single Cell 3' Solution produces Illumina-ready sequencing libraries. See the Chromium™ Single Cell 3' Reagent Kit User Guide (Document CG00026, Section 5) for further sequencing run parameters and loading libraries



– Single Cell libraries have been validated for sequencing on the Illumina NextSeq 500 (V2 chemistry, 150 cycle kit) and the HiSeq 2500 in Rapid Run mode (V1 and V2 chemistry, 200 cycle kits)

– Single Cell libraries must be run using paired-end sequencing with dual indexing. Recommended read lengths:
  – Read 1: 98 nt (shorter reads may result in reduced alignment rates)
  – i7 Index: 14 nt (any other length will result in a failed run)
  – i5 Index: 8 nt (any other length will result in a failed run)
  – Read 2: 10 nt (any other length will result in a failed run)

Changing the Definition of Sequencing™

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