User-Demonstrated Protocol: BD™ Single-Cell Multiplexing Kit—Human

For use with the 10x Chromium™ Single Cell 3' Reagent Kit v2

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Contents

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Overview

The BD™ Single-Cell Multiplexing Kit—Human uses an innovative antibody-oligo technology to increase sample throughput of single cell 3' RNA-seq assays. Every antibody-oligo in the kit, called a Sample Tag, has a unique sample barcode conjugated to a human universal antibody. (See Figure 1.) Adjacent to the Sample Tag barcode are a universal PCR handle and poly(A) tail, which allow each Sample Tag to be captured and amplified by 3' single cell RNA-seq methods. The BD Single-Cell Multiplexing Kit—Human contains 12 Sample Tags and two Sample Tag-specific primers for Sample Tag library generation.

Cells are labelled with Sample Tags through a simple antibody staining procedure before being pooled into a single lane on the 10x Chromium™ system. The system is used with the 10x Chromium™ Single Cell 3' Reagent Kit v2. By pooling multiple samples into a single lane, the BD Single-Cell Multiplexing Kit—Human:

- Increases sample throughput to reduce library preparation costs.
- Reduces sample-to-sample variability due to technical errors.
- Detects inter-Sample Tag multiplets.

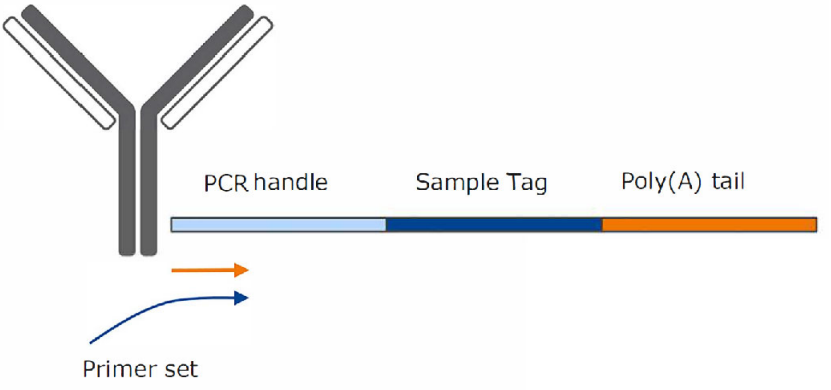


Figure 1 The Sample Tag design ensures compatibility with the 10x Chromium Single Cell 3′ assay.

Workflow

Sample Tags from the BD Single-Cell Multiplexing Kit—Human are captured with transcripts of single cells during the 10x Chromium workflow, and cDNA is generated. (See Figure 2.) During cDNA amplification, Sample Tags are amplified together with mRNA content. After Sample Tags are separated from the cDNA products by size selection purification, they are amplified in a Sample Tag library amplification step. As a result, two sequencing libraries are created: an mRNA library and a Sample Tag library. Since Sample Tags are more abundant than typical mRNA transcripts, this parallel approach allows for optimization of the percentage of reads allocated to Sample Tags in a sequencing run.

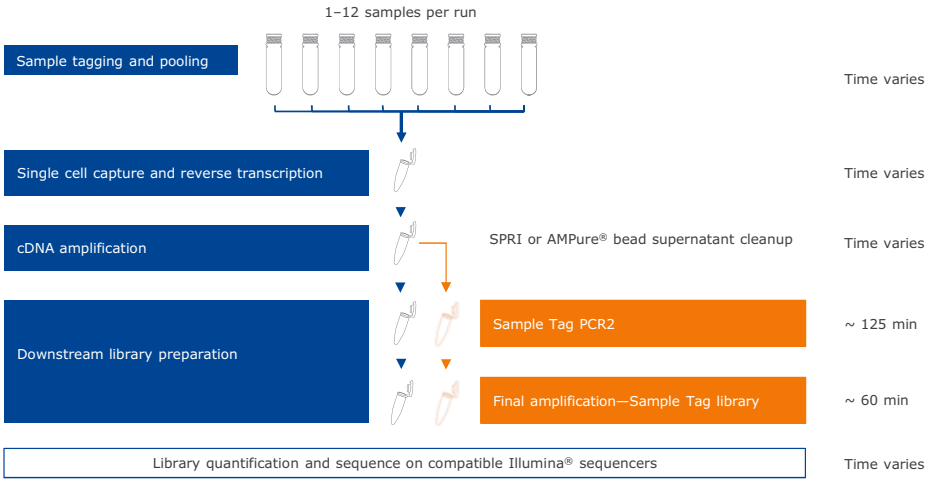


Figure 2 Workflow for generating parallel libraries with Sample Tags.

Reference for library construction

For a detailed protocol for library construction other than with Sample Tags, see the *10x Chromium™ Single Cell 3' Reagent Kits v2 User Guide* (PN CG00052).

Required and recommended materials

Required kits

- Store the kit boxes at the specified storage temperatures.

  Sample Tags should not be frozen.

- Each reagent is stable until the expiration date shown on the label when stored as directed.

- Keep the reagents on ice during the procedure unless instructed otherwise.

Kit	Components	Quantity	Volume per unit	Storage
BD Single-Cell Multiplexing Kit—Human Sample Tag (12) Component[a]	Sample Tag 1—Human	1 vial	20 µL	4°C
	Sample Tag 2—Human	1 vial	20 µL	
	Sample Tag 3—Human	1 vial	20 µL	
	Sample Tag 4—Human	1 vial	20 µL	
	Sample Tag 5—Human	1 vial	20 µL	
	Sample Tag 6—Human	1 vial	20 µL	
	Sample Tag 7—Human	1 vial	20 µL	
	Sample Tag 8—Human	1 vial	20 µL	
	Sample Tag 9—Human	1 vial	20 µL	
	Sample Tag 10—Human	1 vial	20 µL	
	Sample Tag 11—Human	1 vial	20 µL	
	Sample Tag 12—Human	1 vial	20 µL	

a. Required for multiplexing samples. Purchased by ordering PN 633780, which includes the BD Single-Cell Multiplexing Kit—Human Sample Tag (12) Component and the BD™ Single-Cell Multiplexing Kit Library Amplification Component.

Kit	Components	Quantity	Volume per unit	Storage
BD Single-Cell Multiplexing Kit Library Amplification Component (PN 633782)[a]	PCR MasterMix	1 vial	300 µL	–20°C
	Elution Buffer	1 vial	500 µL	
	Universal Oligo	1 vial	20 µL	
	Library Forward Primer	1 vial	20 µL	
	Sample Tag PCR1 Primer[b]	1 vial	20 µL	
	Sample Tag PCR2 Primer	1 vial	20 µL	

a. Required for multiplexing samples. This kit can be purchased as a standalone kit.
b. Not required for use with the 10x Chromium system.

Kit	Supplier	Catalog no.
Chromium™ i7 Sample Index Plate well ID	10x Genomics®	220103
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851

Required reagents

Material	Supplier	Catalog no.
BD™ Stain Buffer (FBS)[a]	BD Biosciences	554656
Beads for purification:		
• SPRIselect® Reagent, 5 mL	Beckman Coulter	B23317
OR		
• Agencourt® AMPure® XP magnetic beads	Beckman Coulter Life Sciences	A63880
Ethyl alcohol, Pure (200 proof, molecular biology grade)	Sigma-Aldrich	E7023-500ML
70% isopropyl alcohol	Major supplier	—
Nuclease-free water	Major supplier	—

a. Or alternative staining buffer. For example, you can use 1X PBS with 1% FBS or 0.1% BSA.

Required consumables and equipment

Material	Supplier	Catalog no.
Falcon® Tube with Cell Strainer Cap	Thermo Fisher Scientific	352235
DNA LoBind Tubes, 1.5 mL[a]	Eppendorf	0030108051
DNA LoBind Tubes, 1.5 mL[a]	Eppendorf	0030108051
Qubit™ 3.0 Fluorometer	Thermo Fisher Scientific	Q33216
2100 Bioanalyzer	Agilent Technologies	G2940CA
0.2 mL PCR 12-strip tubes[a]	Major supplier	—
Low retention filtered pipette tips[a]	Major supplier	—
Laminar flow hood	Major supplier	—
Digital timer[a]	Major supplier	—
Multi-channel pipette, 2–20 µL and 20–200 µL	Major supplier	—
Pipettes (P10, P20, P200, P1000)[a]	Major supplier	—
Microcentrifuge for 1.5–2.0 mL tubes[a]	Major supplier	—
Microcentrifuge for 0.2 mL tubes[a]	Major supplier	—
Centrifuge and rotor for 15 mL tubes	Major supplier	—
Vortexer[a]	Major supplier	—
Pipet-Aid	Major supplier	—

a. Provide material in both pre- and post-amplification workspaces.

Preparing cDNA from pooled Sample Tag-labelled single cells

Unless specified, perform the procedure in a pre-amplification workspace.

Some cell dissociation reagents, such as trypsin, may damage cell surface markers and decrease Sample Tag sensitivity. Use cell dissociation reagents suitable for cell surface antibody labelling.

Cells may be lost during the wash steps (25–50%). For low-abundance samples (<100,000 cells), account for cell loss when preparing single cell samples.

1 Resuspend 12,000–2 x 10^6 cells in 200.0 µL of BD Stain Buffer (FBS).

2 Briefly centrifuge the Sample Tag tubes to collect the contents at the bottom.

3 For each sample, transfer 180.0 µL of the cell suspension to a Sample Tag tube, and mix by pipette only.

Caution. Aqueous buffered solution (Sample Tag) contains BSA and ≤0.1% sodium azide. Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

4 Incubate the cell suspension at room temperature for 20 minutes.

5 Add 200.0 µL of BD Stain Buffer (FBS) to the cell suspension, and mix by pipette only.

6 Centrifuge the cells at 300 x *g* for 5 minutes.

7 Remove the supernatant without disturbing the pellet, and resuspend the pellet in 500.0 µL of BD Stain Buffer (FBS).

For low-abundance samples, leave ~50 µL of supernatant before resuspending the pellet in 500.0 µL of BD Stain Buffer (FBS).

8 Centrifuge the cells at 300 x *g* for 5 minutes.

9 Remove the supernatant without disturbing the pellet, and resuspend the cells in 500.0 µL of buffer recommended by the *10x Chromium™ Single Cell 3' Reagent Kits v2 User Guide*.

For low-abundance samples, leave ~50 µL of supernatant. Resuspend the cells in appropriate buffer so that the total volume is 100.0 µL. Do not filter the sample. Proceed to step 11.

10 Filter the cell suspension through a Falcon Tube with Cell Strainer Cap (Thermo Fisher Scientific Cat. No. 352235). Place the cell suspension on ice.

11 Count each sample according to the *10x Chromium™ Single Cell 3' Reagent Kits v2 User Guide*, and pool cells according to the desired relative amounts of cells and the volumes calculated.

Minimize the time between cell pooling and single cell capture.

12 Proceed with single cell capture, reverse transcription, and cDNA amplification according to the *10x Chromium™ Single Cell 3' Reagent Kits v2 User Guide*.

Separating Sample Tags from cDNA products using SPRIselect Reagent or Agencourt AMPure XP magnetic beads

Retain both the beads and the supernatant that contain cDNA and Sample Tags, respectively.

Follow the *10x Chromium™ Single Cell 3' Reagent Kits v2 User Guide* for DNA purification and downstream library preparation for mRNA libraries.

Bring the SPRIselect Reagent or Agencourt AMPure XP magnetic beads to room temperature.

1. Vortex the SPRIselect Reagent or Agencourt AMPure XP magnetic beads at high speed until the beads are fully suspended.

2. Pipet 60 µL (0.6X) of SPRIselect Reagent or Agencourt AMPure XP magnetic beads to each sample in the tube strip containing cDNA amplification products, and pipet the suspensions up and down 15 times to mix.

3. Incubate the suspensions at room temperature for 5 minutes, and then place the tube strip in a 10x™ Magnetic Separator in the **High** position until the solution is clear.

4. Label a new 0.2 mL PCR tube strip **ST**.

5. **Without disturbing the beads and while leaving the tube strip in a magnetic separator, carefully pipet the supernatant, which has the Sample Tags, into the tube strip labelled ST.**

   Do not dispose of the beads, which have the cDNA products.

6. Set the ST tube strip aside until completion of cDNA purification and elution. The cDNA products and Sample Tags go through separate workflows at this point.

7 For cDNA that is bound to the SPRIselect Reagent or Agencourt AMPure XP magnetic beads, follow the *10x Chromium™ Single Cell 3' Reagent Kits v2 User Guide* for cDNA purification and downstream library preparation. Proceed with Sample Tag library generation in step 8.

8 To the **ST** tube strip, pipet 40.0 µL (1X) of SPRIselect Reagent or Agencourt AMPure XP magnetic beads into each supernatant, and then pipet the mix up and down 15 times.

9 Briefly vortex the suspensions at high speed, and then briefly centrifuge the tube strip (<1 second).

10 Incubate the suspensions at room temperature for 5 minutes, and then place the tube strip in a 10x Magnetic Separator in the **High** position until the solution is clear.

11 Carefully remove and discard only the supernatant without disturbing the beads and while leaving the tube in a magnetic separator.

12 Keeping the tube strip in a magnetic separator, gently pipet 200.0 µL of fresh 80% ethyl alcohol to the side of the tube strip opposite each pellet. Leave the tube open.

13 Incubate the samples for 30 seconds on the magnetic separator.

14 Carefully remove and discard only the supernatant without disturbing the beads and while leaving the tube in a magnetic separator.

15 Repeat steps 12–14 once for a total of two washes.

16 Keeping the tube strip on the magnetic separator, use a small-volume pipette to remove any residual supernatant from each tube.

17 Leave the tube strip open on the magnetic separator to dry the beads at room temperature for 2 minutes. Do not exceed 2 minutes.

18 Remove the tube strip from the magnetic separator, and then pipet 30.0 µL of Buffer EB into each tube, close the tubes, and then pulse vortex to completely resuspend the beads.

19 Briefly centrifuge the tube strip to collect the contents at the bottom, and then incubate the samples at room temperature for 2 minutes.

20 Place the tube strip on the magnetic separator until the solution is clear, usually ≤30 seconds.

21 Pipet the entire eluate (~30.0 µL) from each tube into a labelled new 1.5 mL LoBind Tube. These are the purified Sample Tags.

Performing amplification of Sample Tags

1 Add these components in the following order on ice to prepare the Sample Tag reaction mix in a new 1.5 mL LoBind Tube:

Sample Tag reaction mix

Component	1 lane (µL)	1 lane + 10% overage (µL)
Nuclease-Free Water (PN 650000076)	15.0	16.5
PCR Master Mix (PN 650000073)	25.0	27.5
Universal Oligo (PN 91-1054)	2.0	2.2
Sample Tag PCR2 Primer (PN 91-1061)	3.0	3.3
Total	**45.0**	**49.5**

2 Gently vortex and centrifuge the mixes, and place them back on ice until use.

3 Bring the Sample Tag reaction mix to the post-amplification workspace.

4 In a new 0.2 mL PCR tube strip, pipet 5.0 µL of the purified tagged samples to 45.0 µL of the Sample Tag reaction mix, and mix by pipette for a total of 50.0 µL.

5 Run the mix in the thermal cycler:

Step	Cycles	Temperature	Time
Hot start	1	95°C	3 min
Denaturation		95°C	30 s
Annealing	15[a]	60°C	3 min
Extension		72°C	1 min
Final extension	1	72°C	5 min
Hold	1	4°C	∞

a. Cycle number depends on cell type and cell number and may require optimization.

Stopping point: The PCR can run overnight.

Purifying the amplified Sample Tags with SPRIselect Reagent or Agencourt AMPure XP beads

Bring the SPRIselect Reagent or Agencourt AMPure XP magnetic beads to room temperature.

1. Vortex the SPRIselect Reagent or Agencourt AMPure XP magnetic beads at high speed until the beads are fully suspended.

2. Pipet 30.0 µL (0.6X) of SPRIselect Reagent or Agencourt AMPure XP magnetic beads into each PCR product, and then pipet the suspensions up and down 15 times.

3. Incubate the suspensions at room temperature for 5 minutes, and then place the tube strip in a 10x Magnetic Separator in the **Low** position until the solution is clear.

4. Carefully transfer each supernatant to a new tube in a tube strip without disturbing the beads and while leaving the tube in a magnetic separator.

5. Pipet 20.0 µL (1X) of SPRIselect Reagent or Agencourt AMPure XP magnetic beads into each supernatant, and then pipet the suspensions up and down 15 times.

6. Incubate the suspensions at room temperature for 5 minutes, and then place the tube strip in a 10x Magnetic Separator in the **Low** position until the solution is clear.

7. Carefully remove and discard only the supernatant without disturbing the beads and while leaving the tube in a magnetic separator.

8. Keeping the tube strip in a magnetic separator, gently pipet 200.0 µL of fresh 80% ethyl alcohol to the side of the tube strip opposite each pellet. Leave the tube open.

9. Incubate the samples for 30 seconds on the magnetic separator.

10 Carefully remove and discard only the supernatant without disturbing the beads and while leaving the tube in a magnetic separator.

11 Repeat steps 8–10 once for a total of two washes.

12 Keeping the tube strip on the magnetic separator, use a small-volume pipette to remove any residual supernatant from each tube.

13 Leave the tube strip open on the magnetic separator to dry the beads at room temperature for 2 minutes. Do not exceed 2 minutes.

14 Remove the tube strip from the magnetic separator, and then pipet 30.0 µL of Buffer EB into each tube, close the tubes, and then pulse vortex to completely resuspend the beads.

15 Briefly centrifuge the tube strip to collect the contents at the bottom, and then incubate the samples at room temperature for 2 minutes.

16 Place the tube strip on the magnetic separator until the solution is clear, usually ≤30 seconds.

17 Pipet the entire eluate (~30 µL) from each tube in a labelled new 1.5 mL LoBind Tube. These are the purified and amplified Sample Tags.

18 Quantify the amplified Sample Tags with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay.

19 Dilute an aliquot of amplified Sample Tags to ≤10 ng/µL.

Performing Sample Index PCR of the amplified Sample Tags

Choose the appropriate sample index sets [Chromium™ i7 Sample Index Plate well ID (PN-220103)] to ensure that no sample indices overlap in the same sequencing run. The same sample index set used for Sample Tags and the cDNA library is preferred but not required.

1 In the pre-amplification workspace, add these components in the following order on ice to prepare the mix in a new 1.5 mL LoBind Tube:

Sample Tag Sample Index PCR reaction mix

Component	1 lane (µL)	1 lane + 10% overage (µL)
Nuclease-Free Water (PN 650000076)	15.0	16.5
PCR MasterMix (PN 91-1052)	25.0	27.5
SI-PCR Primer	2.0	2.2
Total	**42.0**	**46.2**

2 To each Sample Tag library, pipet 5.0 µL of an individual Chromium i7 Sample Index to 42.0 µL of the Sample Tag Sample Index PCR reaction mix.

3 Gently vortex and centrifuge the mixes, and place them back on ice until use.

4 Bring the Sample Tag Sample Index PCR reaction mix to the post-amplification workspace.

5 In a new 0.2 mL PCR tube strip, pipet 3.0 µL of ≤10 ng/µL of the amplified Sample Tags to 47.0 µL of the Sample Tag Sample Index PCR reaction mix for a total of 50 µL. Mix by pipette only, and briefly centrifuge the tube.

6 Run the mix in the thermal cycler:

Step	Cycles	Temperature	Time
Hot start	1	95°C	3 min
Denaturation		95°C	30 s
Annealing	8[a]	60°C	3 min
Extension		72°C	1 min
Final extension	1	72°C	5 min
Hold	1	4°C	∞

a. Cycle number depends on cell type and cell number and may require optimization.

Stopping point: The PCR can run overnight.

Purifying the final library for Sample Tags

Bring the SPRIselect Reagent or Agencourt AMPure XP magnetic beads to room temperature.

1. Vortex the SPRIselect Reagent or Agencourt AMPure XP magnetic beads at high speed until the beads are fully suspended.
2. Pipet 30.0 µL (0.6X) of SPRIselect Reagent or Agencourt AMPure XP magnetic beads into each PCR product, and then pipet the suspensions up and down 15 times.
3. Incubate the suspensions at room temperature for 5 minutes, and then place the tube strip in a 10x Magnetic Separator in the **Low** position until the solution is clear.
4. Carefully transfer each supernatant to a new tube in a tube strip without disturbing the beads and while leaving the tube in a magnetic separator.
5. Pipet 20.0 µL (1X) of SPRIselect Reagent or Agencourt AMPure XP magnetic beads into each supernatant, and then pipet the suspensions up and down 15 times.
6. Incubate the suspensions at room temperature for 5 minutes, and then place the tube strip in a 10x Magnetic Separator in the **Low** position until the solution is clear.
7. Carefully remove and discard only the supernatant without disturbing the beads and while leaving the tube in a magnetic separator.
8. Keeping the tube strip in a magnetic separator, gently pipet 200.0 µL of fresh 80% ethyl alcohol to the side of the tube strip opposite each pellet. Leave the tube open.
9. Incubate the samples for 30 seconds in the magnetic separator.
10. Carefully remove and discard only the supernatant without disturbing the beads and while leaving the tube in a magnetic separator.
11. Repeat steps 8–10 once for a total of two washes.

12 Keeping the tube strip on the magnetic separator, use a small-volume pipette to remove any residual supernatant from each tube.

13 Leave the tube strip open on the magnetic separator to dry the beads at room temperature for 2 minutes. Do not exceed 2 minutes.

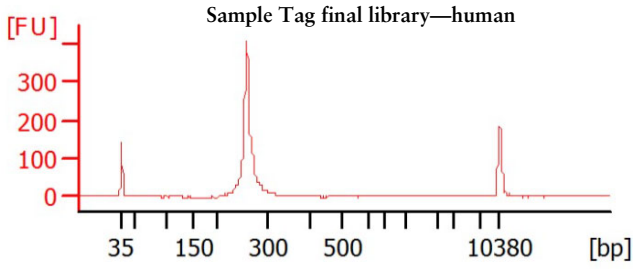
14 Remove the tube strip from the magnetic separator, and then pipet 30.0 µL of Buffer EB into each tube, close the tubes, and then pulse vortex to completely resuspend the beads.

15 Briefly centrifuge the tube strip to collect the contents at the bottom, and then incubate the samples at room temperature for 2 minutes.

16 Place the tube strip on the magnetic separator until the solution is clear, usually ≤30 seconds.

17 Pipet the entire eluate (~30 µL) from each tube in a labelled new 1.5 mL LoBind Tube. These are the purified final libraries for Sample Tags.

18 Perform quality control and sequence the final libraries. The Sample Tag final library shows a fragment distribution of 200–350 bp. For example, library quality control performed on the Agilent 2100 Bioanalyzer:



Sequencing

Sequencing requirements

Follow the *10x Chromium™ Single Cell 3' Reagent Kits v2 User Guide* for sequencing setup.

Sequencing recommendations

- BD recommends pooling the cDNA library and the corresponding Sample Tag library in the same sequencing run. The Sample Tag library should make up ≤5% of the combined reads.
- cDNA library: Follow the 10x Genomics sequencing recommendations for the cDNA library.
- Sample Tag library:
  - Pooling samples of the same cell type: ≥120 reads/cell. For example: combining different donor peripheral blood mononuclear cells.
  - Pooling different sample cell types: ≥600 reads/cell. For example: combining Jurkat cells with peripheral blood mononuclear cells.
  - Sample Tag library sequenced alone: Use 50% of PhiX and a loading concentration of 1.0 pM.

NOTE To determine the ratio of 10x Genomics cDNA library to Sample Tag library to pool for sequencing, a Sample Tag sequencing calculator is available. Contact BD Biosciences technical support at researchapplications@bd.com.

Sequencing analysis

Workflow

NOTE See workflow examples and open source pipeline code for Sample Tag analysis at bitbucket.org/CRSwDev/multiplexing_tools.

1. Generate the Sample Tag reference files.
2. Combine the Sample Tag reference with your desired reference genome.
3. Run the 10x single-cell RNASeq pipeline to generate the read alignment and cell-gene count table.
4. Analyze the Sample Tag reads, and assign cells to samples.

Sample Tag sequences

Each Sample Tag is a human universal antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends and the Sample Tag sequence:

GTTGTCAAGATGCTACCGTTCAGAG[**Sample Tag sequence**]AAAAAAAAAAAAAAAAAAAAAAAA

Sample Tag	Sample Tag sequence
Sample Tag 1—Human	ATTCAAGGGCAGCCGCGTCACGATTGGATACGACTGTTGGACCGG
Sample Tag 2—Human	TGGATGGGATAAGTGCGTGATGGACCGAAGGGACCTCGTGGCCGG
Sample Tag 3—Human	CGGCTCGTGCTGCGTCGTCTCAAGTCCAGAAACTCCGTGTATCCT
Sample Tag 4—Human	ATTGGGAGGCTTTCGTACCGCTGCCGCCACCAGGTGATACCCGCT
Sample Tag 5—Human	CTCCCTGGTGTTCAATACCCGATGTGGTGGGCAGAATGTGGCTGG
Sample Tag 6—Human	TTACCCGCAGGAAGACGTATACCCCTCGTGCCAGGCGACCAATGC
Sample Tag 7—Human	TGTCTACGTCGGACCGCAAGAAGTGAGTCAGAGGCTGCACGCTGT
Sample Tag 8—Human	CCCCACCAGGTTGCTTTGTCGGACGAGCCCGCACAGCGCTAGGAT
Sample Tag 9—Human	GTGATCCGCGCAGGCACACATACCGACTCAGATGGGTTGTCCAGG
Sample Tag 10—Human	GCAGCCGGCGTCGTACGAGGCACAGCGGAGACTAGATGAGGCCCC
Sample Tag 11—Human	CGCGTCCAATTTCCGAAGCCCCGCCCTAGGAGTTCCCCTGCGTGC
Sample Tag 12—Human	GCCCATTCATTGCACCCGCCAGTGATCGACCCTAGTGGAGCTAAG

Troubleshooting

Cell preparation troubleshooting

Observation	Possible causes	Recommended solutions
No pellet after centrifuging cells or very few cells	Rare or dilute sample	After each centrifugation step, leave 50 µL of supernatant.
Do not have the recommended buffer for labelling with Sample Tags	Various	Label with Sample Tags in BD Stain Buffer (FBS) (PN 554656) or 1X PBS with 1% FBS or 0.1% BSA.

Library preparation troubleshooting

Observation	Possible causes	Recommended solutions
Yield of Sample Tag Library is too low (<1 ng/µL)	Various	• Ensure that the cells were stained correctly during sample preparation. • During library preparation, Sample Tag PCR1 and PCR2 Primers may have been swapped. Ensure that the correct primers are used for each step. • Ensure that the correct ratio of SPRIselect or AMPure beads to sample is used at the appropriate steps.
Low sensitivity of Sample Tags (<95%)	Insufficient sequencing of the Sample Tag library	• Pooled samples of the same cell type: 120 reads/cell. • Pooled samples of different cell types: 600 reads/cell.

Sequencing analysis troubleshooting

Observation	Possible causes	Recommended solutions
Warning status for Sample Tags in sequence analysis output	Over-amplification of Sample Tag library	Reduce the number of PCR cycles for Sample Tag amplification.

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Regulatory information

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

History

Revision	Date	Change made
Doc ID: 179682 Rev. 1.0	01/2018	Initial release

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