C10024 (Rev 0) MultiPro® Human Discovery Panel with HashMax™-5 Cell Hashing Antibodies Staining Protocol for GEM-X Flex Cat Nos. G900150 and G900081

Protocol Description

This protocol provides instructions for cell staining with HashMax-5 Cell Hashing Antibodies (Cat No. G900081) followed by cell staining with the MultiPro Human Discovery Panel (Cat No. G900150). For convenience, this protocol contains steps taken from relevant 10x Genomics protocols. Please read the entire protocol before starting.

This protocol results in the generation of three sequencing libraries: Gene Expression (GEX), and two Protein Expression libraries, Panel and Cell Hashing Antibody libraries.

Product Description

The Human Discovery Panel (HDP) contains 347 DNA-barcoded antibodies separated into a Cell Surface (purple cap) and Intracellular (white cap) cocktails. Each reaction of the Human Discovery Panel contains enough material to stain up to 2M cells.

HashMax-5 Cell Hashing Antibodies are available in 8 Hashcodes (A-H). Each Hashcode is a cocktail of anti-CD29 (TS2/16) and anti-B2M (1H3F4) antibodies.

Oligonucleotide Structure

Human Discovery Panel antibodies are conjugated to **5CFLX** oligonucleotides with the following structure:

CGGAGATGTGTATAAGAGACAG-[15N Barcode]-CCCATATAAGA*A*A

HashMax-5 Cell Hashing Antibodies are conjugated to **TCFLX** oligonucleotides with the following structure:

GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT—[15N Barcode]-CCCATATAAGA*A*A

N represents randomly chosen A, T, C, or G. * Phosphorothioate bonds

Barcode sequences are available in each product's **Feature Reference File**, which can be downloaded from the HDP product page and the HashMax-5 product page.

Workflow and Associated Protocols

1. Prepare single-cell suspension
2. Stain cells with HashMax-5 Cell Hashing Antibodies
3. Stain cells with the Human Discovery Panel
4. Final cell fixation
5. 10x Genomics Flex
    a. Probe Hybridization
    b. Optional storage*
    c. Single-cell partitioning
    d. Sequencing library prep
    e. Sequencing
    f. Data analysis

This document will guide you through steps 2-4 and 5c. For your convenience we've included protocol steps and buffer formulations from the appropriate 10x Genomics protocols below.

* If needed, Proteintech Genomics recommends storing antibody-stained cells post-probe hybridization.

Staining with the Human Discovery Panel

10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for GEM-X Flex Gene Expression CG000781 Rev A

10x Genomics Flex Fixation

10x Genomics Fixation of Cells & Nuclei for GEM-X Flex Gene Expression (CG000782 Rev B)

Next Steps

The following documents are required for completing the Flex workflow:

GEM-X Flex Gene Expression Reagent Kit for Multiplex samples with Feature Barcode technology for Protein Expression CG000789 Rev A

Required Reagents and Consumables

Description	Manufacturer	Part Number
HashMax™-5 Cell Hashing Antibodies	Proteintech Genomics	G900081
HashMax™ Primer T [2 µM] 5'-GTGACTGGAGTTCAGACGTGTGCTC-3' HPLC purified	Proteintech Genomics	G900149
MultiPro® Human Discovery Panel	Proteintech Genomics	G900150
MultiPro® Enhanced Blocking Reagent	Proteintech Genomics	G900005-65UL
1.5 mL Protein LoBind tubes	Eppendorf	022431081
Dual Index Kit TT Set A	10x Genomics	1000215
Fixed RNA Feature Barcode Multiplexing Kit, 64 rxns	10x Genomics	1000628
Additive C	10x Genomics	2001332
Conc. Fix & Perm Buffer B	10x Genomics	2001301
Conc. Quench Buffer B	10x Genomics	2001300
Protector RNase inhibitor	Roche	3335399001
Tween-20, 10%	Thermo Scientific	28320
NP-40, 10%	Thermo Scientific	85124
Dextran Sulfate Sodium Salt, 8 kDa	MilliporeSigma	RES2029D-A7
Bovine Serum Albumin, Nuclease-free	MilliporeSigma	126609100GM
Paraformaldehyde, 32%	Electron Microscopy Sciences	15714
Human TruStain FcX™	BioLegend	422302
PBS, Ultrapure (10X), pH 7.4	Invitrogen	AM9625
Nuclease-free Water	-	-

Required Equipment

- Centrifuge capable of 14,000 x g, with a rotor suitable for 1.5 mL microcentrifuge tubes.
- Swinging bucket centrifuge with adapter capable of holding 1.5 mL microcentrifuge tubes.
- Tabletop vortex mixer.

Before You Start

HashMax-5 Antibodies can be leveraged in a wide range of experimental contexts, including but not limited to:

- Enhancing target cell recovery
- Reducing undetected multiplet rates
- Facilitating workflows involving limited cell numbers

This protocol assumes a cell input of 2×10^6 cells per sample. However, since experimental needs may require that you work with a wide range of cell inputs - from as few as 50,000 to several million cells per sample, the following general best practices are recommended. Maintain consistency in staining volume and antibody concentration as outlined above. Scaling volumes beyond 2×10^6 cells may negatively impact staining performance.

- Avoid vacuum aspiration during wash steps to minimize the risk of complete cell loss.
- Use P1000 pipette tips for all aspiration steps. Be careful not to disturb the cell pellet or touch it with the pipette tip. Leaving ~20 µL of residual liquid in each tube at each step is acceptable.
- Handle cell suspensions gently. Avoid vigorous pipetting or mixing that could compromise cell viability or integrity.

Time required - Depending on the number of samples being stained, this protocol is expected to take ~3 to 4 hours

Sample preparation - A high-quality sample is critical for assay performance. The ideal cell suspension has high viability (> 80%) and minimal cell clumps and debris. We strongly recommend straining cells through a 30 µm filter such as Pre-Separation Filters (Miltenyi PN: 130-041-407) to remove cell aggregates. During resuspension and mixing steps, pipette gently and avoid introducing bubbles.

RNase awareness - To prevent RNA degradation, use nuclease-free reagents and consumables listed in this protocol. Before starting, decontaminate your work surfaces and pipettes using RNase Zap (Invitrogen PN: AM9780).

Centrifuge selection - All steps requiring centrifugation of a cell suspension should be performed using a swinging bucket rotor centrifuge. Use of a fixed angle rotor can result in smearing of the cell pellet, which may negatively impact cell recovery.

Buffer Preparation

20% BSA Stock Solution

Dissolve 4 g of nuclease-free BSA powder in 20 mL of ultrapure water. Filter-sterilize using a 0.2 µm filter and store at 4 °C for up to one week.

1% Dextran Sulfate Stock Solution

Dissolve 0.050 g of dextran sulfate, 8 kDa in 5 mL of nuclease-free water. Store at room temperature for up to 1 month.

Cell Surface (CS) Buffer – Chill on ice prior to using

Component	[Final]	1 sample +10%	4 samples +10%
BSA, 20%	1%	374	1496
10X PBS	1x	748	2992
Nuclease-free water	-	6358	25432
Total	**-**	**7480 µL**	**29920 µL**

Blocking Buffer

Component	[Final]	1 sample +10%	4 samples +10%
CS Buffer	-	47.5	190
Human TruStain FcX	-	7.5	30
Total	**-**	**55 µL**	**220 µL**

Intracellular (IC) Buffer

Component	[Final]	1 sample + 10%	4 samples + 10%
RNase inhibitor (200X)	1X	1.1	4.4
Nuclease-free BSA, 20%	7.55%	41.53	166.1
Tween-20, 10%	0.2%	2.2	8.8
NP-40 or Nonidet P40 Substitute, 10%	0.5%	5.5	22
Dextran Sulfate, 8 kDa, 1%	0.025%	2.75	11
Enhanced Blocking Reagent	-	15.95	63.8
Human TruStain FcX™	-	5.5	22
PBS, Ultrapure (10X)	1x	11	44
Nuclease-free Water	-	24.47	97.9
Total		**110 µL**	**440 µL**

Intracellular Wash Buffer

Component	[Final]	1 sample +10%	4 samples +10%
RNase Inhibitor 40 U/µL	0.2	11	44
Tween-20 10%	0.1%	22	88
Nuclease-free BSA 20%*	1%	110	440
10X PBS	1X	220	880
Nuclease-free water		1837	7348
Total		**2200 µL**	**8800 µL**

*The original 10x Genomics recipe calls for 30% BSA; we've adjusted this to 20% BSA for convenience.

Fixation Buffer B

Component	[Final]	1 sample + 10%	4 samples + 10%
Conc. Fix & Perm Buffer B Thaw at room temperature. Vortex, check for precipitation, and centrifuge briefly.	1X	110	440
Paraformaldehyde, 32%	4%	137.5	550
Nuclease-free water		852.5	3410
Total		**1100 µL**	**4400 µL**

Quenching Buffer B

Component	[Final]	1 sample + 10%	4 samples + 10%
Conc. Quench Buffer B Thaw at room temperature. Vortex and centrifuge briefly.	1X	275	1100
Nuclease-free water		1925	7700
Total		**2200 µL**	**8800 µL**

Reconstitution of the Human Discovery Panel

1. Remove the desired number of panel reactions from 4 °C storage.

Reaction (RXN) of HDP

Critical: All tubes in one reaction pouch are required to stain up to 2 million cells.

2. While keeping the tubes in their pouch, incubate at room temperature for 10 minutes.

3. Remove the cocktail tubes from the pouch and place each tube, with its cap still on, into a 2.0 mL Eppendorf tube.

4. Place the stacked tubes into a centrifuge with a fixed angle rotor.

5. Centrifuge at 1500 *x g* for 30 seconds at room temperature.

6. Carefully remove the cocktail tubes from the Eppendorf tubes and place them into a tube rack.

   Note: Reconstitution of both Cell Surface and Intracellular cocktails can be performed simultaneously.

7. Add 55 µL of **CS Buffer** to the Cell Surface cocktail tube (purple cap)

   Note: Make sure the volume is added BELOW the internal threads of the cocktail tube.

8. Add 55 µL of **IC Buffer** to the Intracellular cocktail tube (white cap)
   Critical: If your pouch contains TWO tubes with white caps add 27.5 µL of **IC Buffer** to each.
   Note: Make sure the volume is added BELOW the internal threads of the cocktail tube.

9. Securely cap the tubes and vortex for 10 seconds.

10. Place each cocktail tube into an Eppendorf tube.

11. Incubate the cocktail at room temperature for 5 minutes.

12. Place the stacked tubes into a centrifuge with a fixed angle rotor.

13. Centrifuge at 10,000 *x g* for 30 seconds at room temperature.

14. Remove the stacked tubes carefully from the centrifuge and place them into a tube rack.

15. Set the temperature of the centrifuge to 4 ˚C.

16. Using a P200 pipette with an **extended length pipette tip**, carefully transfer the contents of each cocktail tube to a new 1.5 mL Eppendorf tube.

    Critical: For processing multiple samples, can pool CS (purple) or IC (white) tubes into individual CS or IC tubes for convenience

17. Place each Eppendorf tube containing the reconstituted **CS** and **IC** pools into a centrifuge with a fixed angle rotor that has been chilled to 4 ˚C.

18. Centrifuge at 14,000 *x g* for 10 minutes at 4 ˚C.

19. Label two new 1.5 mL Eppendorf tube **CS** and **IC**.

20. Without touching the bottom of the Eppendorf tubes, transfer 52 µL of the contents of the **CS** and **IC** tubes to the newly labeled Eppendorf tubes.

    Critical: This step removes protein aggregates that may be present.

21. Keep **CS** and **IC** tubes on ice until use.

Prepare HashMax-5 Staining Solution (HSS)

Critical: Steps 22-29 are described for a single HashMax-5 hashcode, but should be carried out, in parallel, for all HashMax-5 hashcodes to be used for staining.

22. Clearly label 2 x 1.5 mL Protein LoBind tubes with a unique identifier corresponding to the HashMax-5 hashcode, e.g., A-H, or sample ID.

23. Retrieve HashMax-5 Antibody stock solutions from -20°C storage. Briefly vortex, then centrifuge at 1,500 × g for 30 seconds at room temperature to ensure all contents are collected at the bottom of the tube

    Critical: HashMax-5 antibodies are supplied in 1.4 mL Micronic vials. A 2 mL microcentrifuge tube can be used to hold the vial during centrifugation.

24. Create **Hashmax-5 Staining Solution** (**HSS**) by adding 2 µL of the HashMax-5 antibody stock solution to 53 µL of **CS buffer** in one of the pre-labeled 1.5 mL Protein LoBind tubes. Return the HashMax-5 Antibody stock vials to -20°C storage.

25. Cap the tube, vortex for 15 seconds

26. Centrifuge tubes containing HSS at 14,000 x *g* for 10 minutes at 4°C.

    Critical: This step pellets any protein aggregates that may be present in the cell hashing solution.

27. Being careful NOT to touch the bottom of the tube with your pipette tip, carefully transfer 52 µL of the HSS to the other pre-labeled 1.5 mL Protein LoBind tube.

    Critical: Avoid touching the bottom of the tube and do not transfer the entire volume of HSS, as doing so could result in the transfer of aggregates to the final staining solution.

28. Keep the final **HSS** on ice until ready to use.

Stain Cells with HashMax-5 Staining Solution (HSS)

29. Prepare up to 2 x 10^6 cells in 1 mL of **CS Buffer** in a 1.5 mL Protein LoBind tube.

Fc Block

30. Centrifuge cells at 400 x *g* for 5 minutes at 4°C.

31. Using a 1 mL pipette tip, carefully remove supernatant.

    Critical: Centrifugation at low-speed results in loose pellets that can be easily disturbed and aspirated during washing steps. Avoid disruption of the cell pellet as much as possible.

32. Resuspend cells in 50 µL of **Blocking Buffer** and gently pipette mix.

33. Incubate on ice for 10 minutes.

Stain with HSS

34. Without removing blocking solution, add 50 µL of **HSS** to the Fc Blocked cells and gently pipette mix 5-10 times.

35. Incubate on ice for 30 minutes

Cell Hashing Wash

36. Add 1.4 mL of **CS Buffer** and gently pipette mixing 5-10 times

37. Centrifuge cells at 400 x *g* for 5 minutes at 4°C.

38. Remove supernatant.

    Critical: Centrifugation at low-speed results in loose pellets that can be easily disturbed and aspirated during washing steps. Avoid disruption of the cell pellet as much as possible.

39. Add 1 mL of CS Buffer and gently pipette mix 5 - 10 times.

40. Centrifuge cells at 400 x *g* for 5 minutes at 4°C.

41. Remove supernatant

42. Resuspend the cells in 150 µL of **CS Buffer** for cell counting.

Count cells and create a sample pool.

43. Prepare 1: 10 dilutions of each sample in **CS Buffer** and count. Combine all samples equally or as desired into a single pool

    Example: 8 samples were stained with 8 unique hashcodes and combined into a single pool for staining with the Human Discovery Panel. We combine all 8 samples equally for a total of 2 x 10^6 cells.

Sample	Hashed Cells/mL	Volume (µL)	Dilution	Cells each	Volume Required (µL)
1	1.30 x 10^6	150	10	2.5 x 10^5	19.2
2	1.22 x 10^6	150	10	2.5 x 10^5	20.4
3	9.55 x 10^5	150	10	2.5 x 10^5	26.2
4	1.12 x 10^6	150	10	2.5 x 10^5	22.4
5	4.55 x 10^5	150	10	2.5 x 10^5	55
6	7.96 x 10^5	150	10	2.5 x 10^5	31.4
7	1.01 x 10^6	150	10	2.5 x 10^5	24.8
8	1.17 x 10^6	150	10	2.5 x 10^5	21.4

44. Centrifuge the tube of pooled cells at 400 *x g* for 5 minutes at 4 °C.

45. Remove the supernatant without disturbing the cell pellet.

    Critical: It is acceptable to leave some buffer to avoid aspirating the cell pellet. We recommend leaving less than 20µL.

46. Proceed to Cell Surface Staining.

Stain Cells with Human Discovery Panel (HDP)

Cell Surface Stain

47. Resuspend the cell pellet in 50 µL of reconstituted **Cell Surface Cocktail** (**CS**)

    Critical: If using single antibodies instead of a lyophilized cocktail, add **CS buffer** for a total volume of 50 µL, including the volume of antibodies to be used.

48. Add 50 µL chilled **CS Buffer** to the cells to bring the total volume to 100 µL, gently pipette mix.

49. Incubate for 30 minutes at 4°C.

Wash

50. Add 1.4 mL chilled **CS Buffer** to the labeled cells. Gently pipette mix.
    - Add 700 µL of **CS Buffer** to each sample and gently mix the cells by pipette 5X.
    - Add an additional 700 µL of **CS Buffer** and gently mix the cells by pipette 5X.

51. Centrifuge cells at 400 *x g* for 5 minutes at 4 °C.

52. Remove the supernatant without touching the bottom of the tube to avoid dislodging the pellet. Up to 30 µL supernatant may be left behind if working with < 3 x 10^5 cells.

53. Resuspend the cell pellet in 1.0 mL chilled **CS Buffer** and place on ice.

54. Centrifuge cells at 400 *x g* for 5 minutes at 4°C.

55. Remove the supernatant

Critical: Centrifugation at low-speed results in loose pellets that can be easily disturbed and aspirated during washing steps. Avoid disruption of the cell pellet as much as possible. Ensure no more than 20µL of volume is left behind. If you are working with <500k cells, 30µL of volume can be left behind.

Cell Fixation

56. Add 0.5 mL room temperature **Fixation Buffer B** to the and pipette mix 5x.

57. Incubate for 20 minutes at room temperature (20 °C).

Critical: DO NOT agitate or mix the sample during incubation.

58. Add 0.5 mL room temperature **Additive C** to the sample in Fixation Buffer B and pipette mix 5x.

59. Centrifuge at 2,000 *x g* for 5 minutes at room temperature.

60. Remove the supernatant without disturbing the pellet.

Critical: Centrifugation at low-speed results in loose pellets that can be easily disturbed and aspirated during washing steps. Avoid disruption of the cell pellet as much as possible. Ensure no more than 20µL of volume is left behind. If you are working with <500k cells, 30µL of volume can be left behind.

61. Add 1 mL chilled Quenching Buffer B to the sample pellet and pipette mix 5x and keep on ice.

Intracellular Block

62. Centrifuge at 2,000 *x g* for 5 minutes at 4°C. Remove the supernatant without disturbing the pellet.

63. Resuspend pellet in 50 µL **IC Buffer**.

64. Incubate for 10 mins at 4°C.

Intracellular Staining

65. Without removing blocking solution, add 50 µL reconstituted **Intracellular (IC) Cocktail** to the cells and gently pipette mix 10x.

    Critical: If using single antibodies instead of a lyophilized cocktail, add **IC buffer** for total volume of 50 µL, including the volume of antibodies to be used.

66. Incubate for 30 minutes at 4 °C.

Intracellular Wash

67. Add 1 mL chilled Intracellular **Wash Buffer** to the labeled cells. Gently pipette mix.

68. Centrifuge cells at 2,000 *x g* for 5 minutes at 4°C.

69. Remove the supernatant without touching the bottom of the tube to avoid dislodging the pellet. Up to 30 µL supernatant may be left behind if working with $<3 \times 10^5$ cells.

70. Repeat three steps above (67-69) one time for a total of 2 washes.

Post Intracellular Stain Fixation

71. Add 0.5 mL room temperature **Fixation Buffer B** to the sample pellet and pipette mix 5x.

72. Incubate for 1 hour at room temperature (20 °C) or for 16 to 24 hours at 4 °C. If planning to store fixed samples, a 16-to-24-hour fixation at 4 °C is recommended.

    Critical: DO NOT agitate or mix the sample during incubation.

73. Add 0.5 mL room temperature **Additive C** to the sample in Fixation Buffer B and pipette mix 5x.

74. Centrifuge at 2000 *x g* for 5 minutes at room temperature.

75. Remove the supernatant without disturbing the pellet. Up to 30 µL supernatant may be left behind if working with < 3 x 10^5 cells.

76. Add 1 mL* chilled **Quenching Buffer B** to the sample pellet and pipette mix 5x and keep on ice.

    *If cell numbers are expected to be < 2 x 10^5, resuspend sample pellet in 200 µL **Quenching Buffer B**. After counting, add an additional 800 µL **Quenching Buffer B** before proceeding to GEM-X Flex Gene Expression protocols or storage.

77. Determine cell concentration of the fixed sample using an automated cell counter or hemocytometer.

78. Proceed immediately to the appropriate GEM-X Flex Gene Expression protocols or store the sample as directed in the protocols below:

    GEM-X Flex Gene Expression Reagent Kit for Multiplex samples with Feature Barcode technology for Protein Expression CG000789 Rev A

Optional Storage

Fixed cell or nuclei suspensions can be stored at 4 °C for up to 1 week or at -80 °C for up to 12 months after resuspending in appropriate reagents.

Short-term Storage at 4 °C

a. Thaw Enhancer (10x Genomics PN: 2000482) for 10 min at 65 °C. Vortex and centrifuge briefly. Keep warm and verify no precipitate before use.

   DO NOT keep the thawed reagent on ice, or the solution will precipitate. Once thawed, Enhancer can be kept at 42 °C for up to 10 min. If not used within 10 min and precipitation
   is observed, reheat at 65 °C to ensure enhancer is fully dissolved again before use.

b. Add 0.1 volume pre-warmed Enhancer to fixed sample in **Quenching Buffer B**.
   For example, add 100 µl Enhancer to 1,000 µl fixed sample in **Quenching Buffer B**. Pipette mix.

   Alternatively, to conserve the Enhancer volume, centrifuge cells at 850 rcf, remove 500 µl Quenching Buffer B, and add 50 µl Enhancer to the sample.

c. Store sample at 4 °C for up to 1 week.

Long-term Storage at −80 °C

a. Thaw Enhancer (10x Genomics PN: 2000482) for 10 min at 65 °C. Vortex and centrifuge briefly. Keep warm and verify no precipitate before use.

   DO NOT keep the thawed reagent on ice, or the solution will precipitate. Once thawed, Enhancer can be kept at 42 °C for up to 10 min. If not used within 10 min and precipitation
   is observed, reheat at 65 °C to ensure enhancer is fully dissolved again before use.

b. Add 0.1 volume pre-warmed Enhancer to fixed sample in **Quenching Buffer B**.

   For example, add 100 µl Enhancer to 1,000 µl fixed sample in Quenching Buffer Pipette mix.

   Alternatively, to conserve the Enhancer volume, centrifuge cells at 850 rcf, remove 500 µl Quenching Buffer B, and add 50 µl Enhancer to the sample.

c. Add 50% glycerol for a final concentration of 10%.

   For example: add 275 µl 50% **glycerol** to 1,100 µl fixed sample in **Quenching Buffer B** and Enhancer. Pipette mix.

d. Store at −80 °C for up to 12 months.

   If planning to store the fixed samples, it is strongly recommended to perform a 16-24 hr fixation at 4 °C during the fixation step and store the fixed samples at -80°C for best results.

HashMax-5 Library Pre-Amplification

1. In the "Pre-Amplification PCR" step of the 10x Genomics Flex Library Construction protocol, spike-in the HashMax Primer T in addition to Pre-Amp Primers C as described below, for the appropriate chemistry.

Component	10x Genomics PN	1 sample (µL)
Amp Mix C (GEM-X)	2001311	25
Pre-Amp Primers C	2000953	10
HashMax-5_suppl primer (2 µM)	-	2
Total		**37 µL**

2. Add 37 µL Preamplification Mix to aqueous sample recovered from the Post-GEM Incubation – Recovery step, cap firmly, invert 8 times, **centrifuge** briefly and proceed with Pre-Amplification PCR.

HashMax-5 Sequencing Library Generation

3. In parallel with Sample Index PCR of protein library, transfer ONLY 20 µL of **post cleanup pre-amplified DNA** to a new well of a PCR strip

   Add 50 µL Amp Mix (10x Genomics PNs: 2000047/2000103) and 10 µL Buffer EB to the 20 µL of **post cleanup pre-amplified DNA** sample transferred in the previous step.

4. Add 20 µL of an individual Dual Index TT Set A well to each sample and. pipette mix 5x, centrifuge briefly.

   Critical: Make note of used wells and avoid their re-use.

5. Proceed to Sample Indexing PCR with the following cycle numbers:

Target Cells	Total Cycles
< 4,000	20
4,000-7,000	19-20
7,000-12,000	18-19
12,000-25,000	17-18
25,000-50,000	16-17
50,000-128,000	15-16

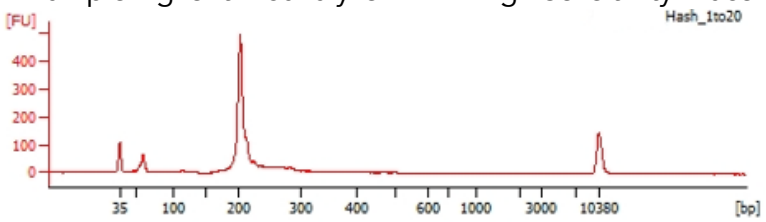
HashMax-5 Library Cleanup

6. HashMax-5 libraries have a similar length as protein libraries and cleanup of both libraries can be performed in parallel using the same parameters. For isolating cell hashing library, perform 1.0X SPRIselect clean up the same way as protein library cleanup described in the Protein library - Post Sample index PCR Size Selection - SPRIselect section of the GEM-X Flex 10x Genomics User Guide.

HashMax-5 Library Traces

1. To check the quality of the HashMax-5 sequencing library, perform a 1:10 to 1:20 dilution with TE buffer and run fragment size analysis following manufacturer recommendations.

Example Agilent Bioanalyzer DNA High Sensitivity Trace



Sequencing

Sequencing depth guidelines

Cell Hashing – Add 500 paired-end reads per cell (targeted cell recovery)

HDP library – Add a minimum of 10k paired-end reads per cell (targeted cell recovery)

Run configuration

Read 1:	28 cycles
Index 1:	10 cycles
Index 2:	10 cycles
Read 2:	90 cycles

Feature Reference File

Download from the HDP product page and HashMax-5 product page

Contact Us

Questions, concerns, or suggestions? Please contact us at genomics.support@ptglab.com