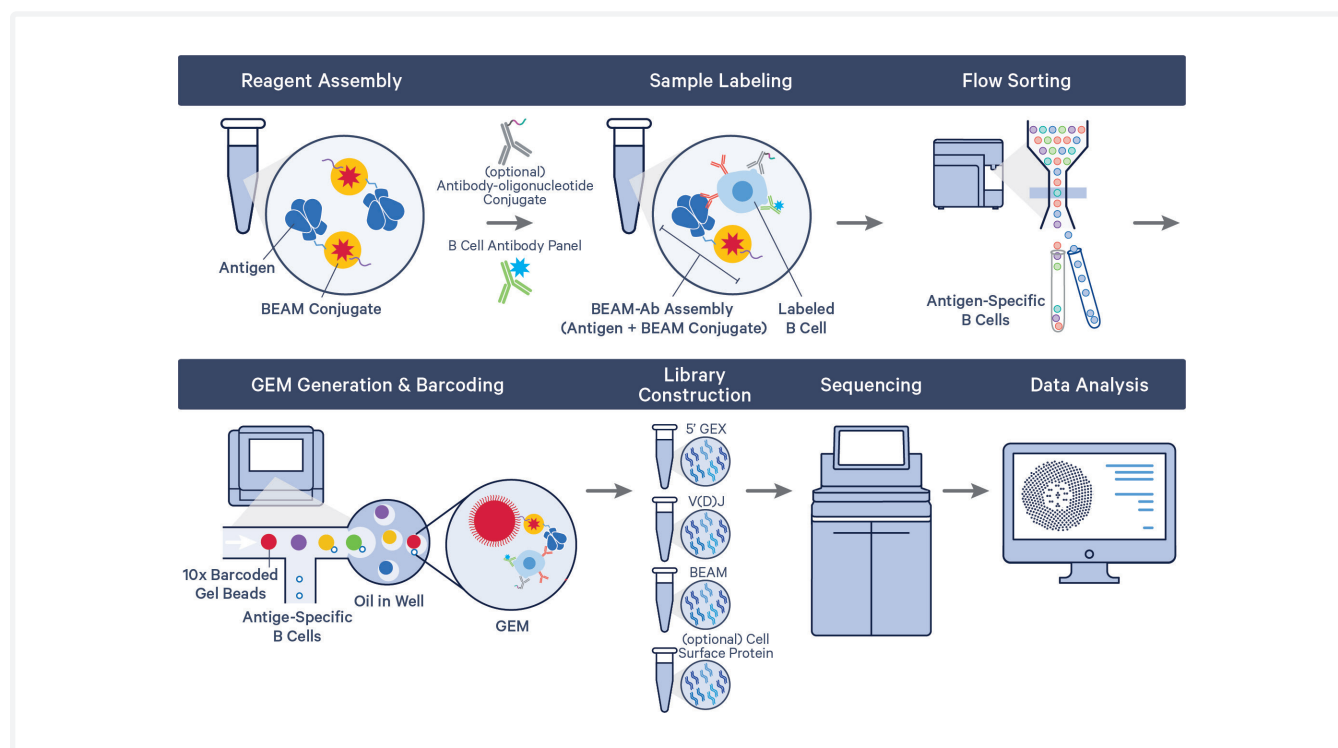


Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM) – Protocol Planner

Introduction

Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM) enables multiplexed screening of antigen targets to match unique antigens with their corresponding B-cell receptors (BCRs) and T-cell receptors (TCRs), allowing rapid discovery of antigen-specific BCR (BEAM-Ab) and TCR (BEAM-T), respectively. This is done by assembling antigens/peptides of interest with uniquely barcoded 10x Genomics BEAM Conjugates and by labeling samples with the assembled reagents (BEAM-Ab or BEAM-T Assembly), followed by flow sorting of antigen-specific cells. The cells can then be used with Chromium Single Cell 5' Reagent Kits with Feature Barcode technology for BEAM to generate BEAM, 5' Gene Expression, and V(D)J libraries. This document provides workflow overview and additional equipment, kits, and reagents for the Chromium Single Cell 5' BEAM protocols. The 10x Genomics Chromium Single Cell 5' Reagent Kits are not listed here. An overview of the Chromium Single Cell 5' BEAM data analysis is also included in this document.

Workflow Overview – BEAM-Ab



Workflow Overview – BEAM-T

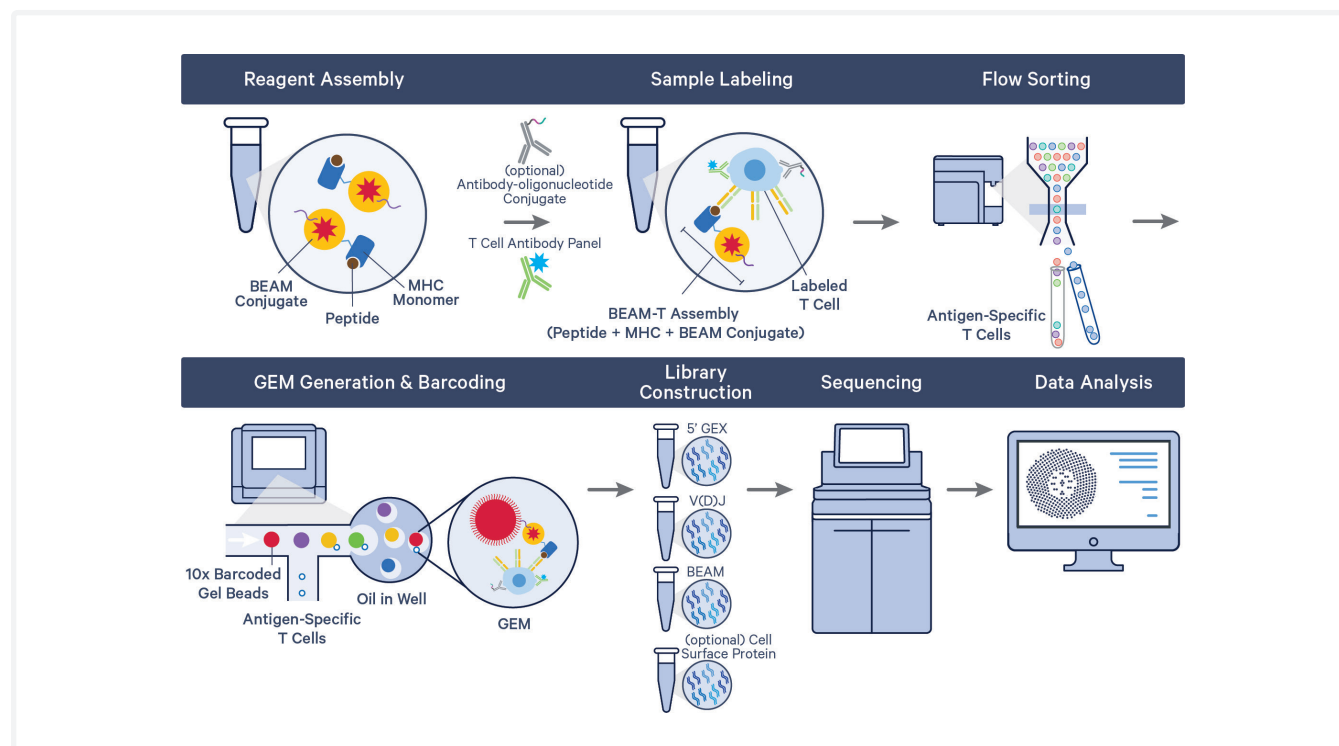


Figure 1. BEAM Workflow Overviews. Each BEAM Conjugate is composed of a streptavidin, a fluorophore molecule (Phycoerythrin, PE), and a Feature Barcode oligonucleotide.

Document Resources – Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM)

1 Experimental Design & Planning

Before starting the experiment, refer to these documents for protocol planning and preparation.

Protocol Planner

Workflow overview and additional reagents for BEAM workflow

Planner CG000590

Experimental Planning Guide

Key considerations before starting with BEAM workflow

Experimental Planning Guide CG000596

2 Sample Preparation

Perform BEAM reagent assembly, labeling, and flow sorting.

BEAM-Ab Calculator Workbook

BEAM-Ab Assemblies Calculation

Technical Note CG000597

BEAM-T Calculator Workbook

BEAM-T Assemblies Calculation

Technical Note CG000615

Reagent Assembly, Labeling & Flow Sorting

Prepare BEAM-Ab or BEAM-T Assemblies, label cells & sort antigen-specific B or T cells

Sample Prep User Guide CG000595

Flow Cytometry Guidelines

Key flow cytometry & sorting considerations

Technical Note CG000598

3 Library Construction

Perform library construction.

Library Construction

Construct 5' GEX, V(D)J, and BEAM Libraries

User Guide CG000591

Library Construction

Construct 5' HT GEX, V(D)J, and BEAM Libraries

User Guide CG000593

Library Construction

Construct 5' GEX, V(D)J, BEAM, and Cell Surface Protein Libraries

User Guide CG000592

Library Construction

Construct 5' HT GEX, V(D)J, BEAM & Cell Surface Protein Libraries

User Guide CG000594

Kits, Reagents & Equipment – Assembly, Labeling & Flow Sorting for Chromium Single Cell 5' BEAM protocols (CG000595)

The items in the tables below have been tested by 10x Genomics and perform optimally with the Chromium Single Cell 5' BEAM protocols. Substituting materials may adversely affect system performance. This list may not include standard laboratory equipment such as water baths, centrifuges, pH meters, freezers etc.

Item	Description		Supplier	Part Number (US)
Plastics				
0.2 ml PCR 8-tube strips	PCR Tubes 0.2 ml 8-tube strips	Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.	Eppendorf	951010022
	TempAssure PCR 8-tube strip		USA Scientific	1402-4700
	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8 -Cap Strip, clear		Thermo Fisher Scientific Thermo Fisher Scientific	N8010580 N8010535
1.5-ml tubes	DNA LoBind Tubes 1.5 ml	Choose either tubes for reagent & buffer preparation.	Eppendorf	22431005
2-ml tubes	DNA LoBind Tubes 2.0 ml		Eppendorf	22431048
15-ml tubes	Corning 15 ml centrifuge tubes		Corning	CLS430791
50-ml tubes	Corning 50 ml centrifuge tubes		Corning	CLS430829
Kits & Reagents				
Sterile 1X PBS	Phosphate-Buffered Saline without Calcium & Magnesium <i>Or any equivalent sterile PBS</i>		Corning	21-040-CM
Nuclease-free water	Molecular Grade Nuclease-free Water		Thermo Fisher Scientific	AM9937
FBS	Fetal Bovine Serum, qualified, heat inactivated		Thermo Fisher Scientific	16140071
	Avantor Seradigm Premium Grade Fetal Bovine Serum		VWR	97068-085
FcX	Human TruLabel FcX (Fc Receptor Blocking Solution) TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody		Biolegend Biolegend	422301 156603
Total-Seq C	TotalSeq™ Antibody Oligonucleotide Conjugates <i>Optional</i>		Biolegend	-
Antibodies	See Example Antibody Panels		-	-
Viability dye	Invitrogen eBioscience 7-AAD Viability Staining Solution		Invitrogen	00699350
Biotinylated HSA	Biotinylated human serum albumin <i>Optional</i>		Acro Biosystems	HSA-H82E3
Biotinylated MSA	Biotinylated mouse serum albumin <i>Optional</i>		Acro Biosystems	MSA-M82E4

Cell Counting				
Label	Trypan Blue Label (0.4%)	Choose counter based on availability & preference.	Thermo Fisher Scientific	T10282
Cell counter & slides	Countess II FL Automated Cell Counter		Thermo Fisher Scientific	AMQAF1000
	Countess 3 FL Automated Cell Counter		Thermo Fisher Scientific	A49866
	Countess Cell Counting Chamber Slides		Thermo Fisher Scientific	C10228
Equipment				
Vortex	Vortex Mixer		VWR	10153-838
Centrifuge	Refrigerated Eppendorf Centrifuge <i>Or any equivalent centrifuge</i>		Millipore-Sigma	5427R or 5424R
Sorter	MA900 Multi-Application Cell Sorter <i>Or any equivalent cell sorter</i>		Sony	MA900

For some items, a number of vendor options are listed. Choose item based on availability and preference. Refer to the manufacturer's website for regional part numbers.

Kits, Reagents & Equipment – BEAM Library Construction (CG000591 & CG000592)

The items in the tables below have been tested by 10x Genomics and perform optimally with the Chromium Single Cell protocols. Substituting materials may adversely affect system performance. This list may not include standard laboratory equipment such as water baths, centrifuges, pH meters, freezers etc.

Item	Description		Supplier	Part Number (US)
Plastics				
1.5-ml tubes	DNA LoBind Tubes, 1.5 ml		Eppendorf	022431021
2.0-ml tubes	DNA LoBind Tubes, 2.0 ml		Eppendorf	022431048
0.2 ml PCR 8-tube strips	PCR Tubes 0.2 ml 8-tube strips	Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.	Eppendorf	951010022
	TempAssure PCR 8-tube strip		USA Scientific	1402-4700
	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8 -Cap Strip, clear		Thermo Fisher Scientific Thermo Fisher Scientific	N8010580 N8010535
Kits & reagents				
Nuclease-free Water	Nuclease-free Water		Thermo Fisher Scientific	AM9937
Ethanol	Ethanol, Pure (200 Proof, anhydrous)		Millipore Sigma	E7023-500ML
SPRIselect	SPRIselect Reagent Kit		Beckman Coulter	B23318
Tween 20	10% Tween 20		Bio-Rad	1662404
50% Glycerol	Glycerin (glycerol), 50% (v/v) Aqueous Solution		Ricca Chemical Company	3290-32
Buffer EB	Qiagen Buffer EB		Qiagen	19086
Equipment				
Vortex mixer	Vortex Mixer		VWR	10153-838
Reagent reservoirs	Divided Polystyrene Reservoirs		VWR	41428-958
Vortex mixer	Mini Centrifuge <i>Or any equivalent mini centrifuge</i>		VWR	76269-064
Thermomixer	Eppendorf ThermoMixer C		Eppendorf	5382000023
Heat block	Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 reaction vessel <i>Or any equivalent temperature controlled heat block</i>		Eppendorf	5360000038

Quantification & Quality Control			
Bioanalyzer & associated reagents	2100 Bioanalyzer Laptop Bundle (discontinued) (Replacement 2100 Bioanalyzer Instrument/ 2100 Expert Laptop Bundle)	Agilent	G2943CA G2939BA/ G2953CA
	High Sensitivity DNA Kit	Agilent	5067-4626
TapeStation & associated reagents	4200 TapeStation	Agilent	G2991AA
	High Sensitivity D1000: ScreenTape/ Reagents	Agilent	5067-5592/ 5067-5593
	High Sensitivity D5000: ScreenTape/ Reagents	Agilent	5067-5584/ 5067-5585
LabChip & associated reagents	LabChip GX Touch HT Nucleic Acid Analyzer	PerkinElmer	CLS137031
	DNA High Sensitivity Reagent Kit	PerkinElmer	CLS760672
Library quantification kit	KAPA Library Quantification Kit for Illumina Platforms	KAPA Biosystems	KK4824

For some items, a number of vendor options are listed. Choose item based on availability and preference. Refer to the manufacturer's website for regional part numbers.

Recommended Thermal Cyclers

The table below lists the thermal cyclers that have been validated by 10x Genomics for all currently available Chromium Single Cell protocols.

Supplier	Description	Part Number (US)
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Analytik Jena [†]	Biometra TAdvanced 96 SG	846-x-070-241 (x = 2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)
Eppendorf [‡]	Mastercycler X50s	6311000010
	Mastercycler Pro (discontinued)	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler (discontinued)	4375786

For select instruments, ramp rates should be adjusted for all steps as described below:

[†]Analytik Jena Biometra TAdvanced 96 SG: 2°C/sec for both heating and cooling

[‡]Eppendorf Mastercycler X50s: 3°C/sec heating and 2°C/sec cooling

Antibody Panels for Flow Sorting

BEAM-Ab Antibody Panels

The following tables provide example panels for flow sorting antigen-specific human and mouse B cells. The purpose is to sort lymphocytes > single cells > live cells (7-AAD⁻) > lineage⁻ > CD19⁺ > dual CD19⁺PE⁺ cells.

Example Human B Cell Antibody Panel for Flow Sorting

Marker	Target Cell type	Antibody	Vendor	Part Number (US)
CD19 (+)	B Cells	PE/Cyanine7 anti-human CD19 Antibody	Biolegend	302215
CD56 (-)	Natural killer cells	Brilliant Violet 421 anti-human CD56 (NCAM) Antibody	Biolegend	318327
CD3 (-)	T cells	Brilliant Violet 421 anti-human CD3 Antibody	Biolegend	317344
CD14 (-)	Monocytes	Brilliant Violet 421 anti-human CD14 Antibody	Biolegend	367143
7-AAD (-)	Live Dead marker	Invitrogen eBioscience 7-AAD Viability Staining Solution	Invitrogen	00699350

Example Mouse B Cell Antibody Panel for Flow Sorting

Marker	Target Cell Type	Antibody	Vendor	Part Number (US)
CD19 (+)	B Cells	PE/Cyanine7 anti-mouse CD19 Antibody	Biolegend	115519
Ter119 (-)	Erythroid cells	Brilliant Violet 421 anti-mouse TER-119/Erythroid Cells Antibody	Biolegend	116233
Ly6g (-)	Myeloid cells	Brilliant Violet 421 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody	Biolegend	108433
CD3 (-)	T cells	Brilliant Violet 421 anti-mouse CD3 Antibody	Biolegend	100227
CD14 (-)	Dendritic cells	Brilliant Violet 421 anti-mouse CD14 Antibody	Biolegend	123329
7-AAD (-)	Live dead marker	Invitrogen eBioscience 7-AAD Viability Staining Solution	Invitrogen	00699350

Antibody Panels for Flow Sorting

BEAM-T Antibody Panels

The following tables provide example panels for flow sorting peptide-specific human and mouse CD8⁺ T cells. The purpose is to sort lymphocytes > single cells > live cells (7-AAD⁻) > lineage⁻ > CD8⁺ > dual CD8⁺PE⁺ cells.

Example Human T Cell Antibody Panel for Flow Sorting

Marker	Target Cell Type	Antibody	Vendor	Part Number (US)
CD3 (+)	Total T cells	Brilliant Violet 510 anti-human CD3 Antibody	Biolegend	317331
CD8 (+)	Cytotoxic T cells	Alexa Fluor 488 anti-human CD8 Antibody	Biolegend	344716
CD56 (-)	Natural killer cells	Brilliant Violet 421 anti-human CD56 (NCAM) Antibody	Biolegend	318327
7-AAD (-)	Live dead marker	Invitrogen eBioscience 7-AAD Viability Staining Solution	Invitrogen	00699350

Example Mouse T Cell Antibody Panel for Flow Sorting

Marker	Target Cell Type	Antibody	Vendor	Part Number (US)
CD3 (+)	Total T cells	Brilliant Violet 510 anti-mouse CD3 Antibody	Biolegend	100233
CD8 (+)	Cytotoxic T cells	FITC anti-Mouse CD8a Antibody	BD Biosciences	553030
Ter119 (-)	Erythroid cells	Brilliant Violet 421 anti-mouse TER-119/Erythroid Cells Antibody	Biolegend	116233
Ly6g (-)	Myeloid cells	Brilliant Violet 421 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody	Biolegend	108433
CD19 (-)	B cells	Brilliant Violet 421 anti-mouse CD19 Antibody	Biolegend	115537
CD14 (-)	Dendritic cells	Brilliant Violet 421 anti-mouse CD14 Antibody	Biolegend	123329
7-AAD (-)	Live dead marker	Invitrogen eBioscience 7-AAD Viability Staining Solution	Invitrogen	00699350

Analysis of Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM) Data

For detailed information on analysis of Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM) data, refer to 10x Genomics [Software Support](#) site.

Data Analysis Overview – Chromium Single Cell 5' Barcode Enabled Antigen Mapping

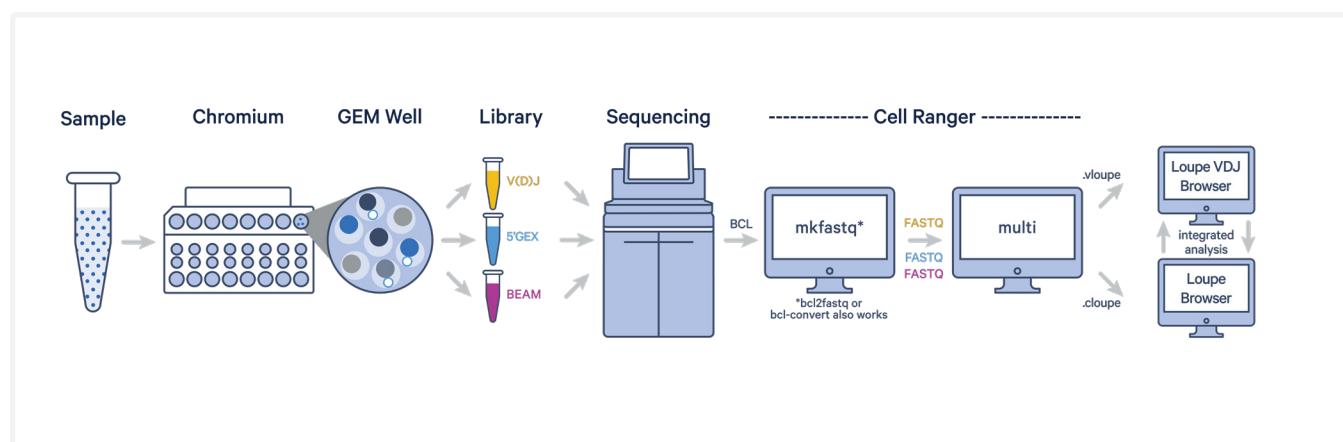


Figure 2. BEAM Data Analysis Overview.

Key information that should be collected and provided to the data analyst/bioinformatician to ensure that parameters are set appropriately for Cell Ranger to analyze the data:

- Libraries created (e.g., 5' Gene Expression, BEAM-Ab, BCR/TCR, Cell Surface Protein)
- Sample and Chromium Single Cell 5' library name to be appended to the FASTQ file name and the corresponding library type
- Cell types loaded and their proportions
- Expected number of cells per sample
- Negative Control Peptides and MHC Monomer used for BEAM-T experiments
- The sample indices used for each Chromium Single Cell 5' library generated

Document Revision Summary

Document Number	CG000590
Title	Barcode Enabled Antigen Mapping (BEAM) – Protocol Planner
Revision	Rev B
Revision Date	April 2023
General Changes	Updated for general minor consistency of language and terms throughout
Specific Changes	Added Biotinylated Human Serum Albumin and Mouse Serum Albumin to Kits, Reagents & Equipment – Assembly, Labeling & Flow Sorting for Chromium Single Cell 5' BEAM protocols (CG000595) (page 4) Updated Document Resources section (page 3)

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