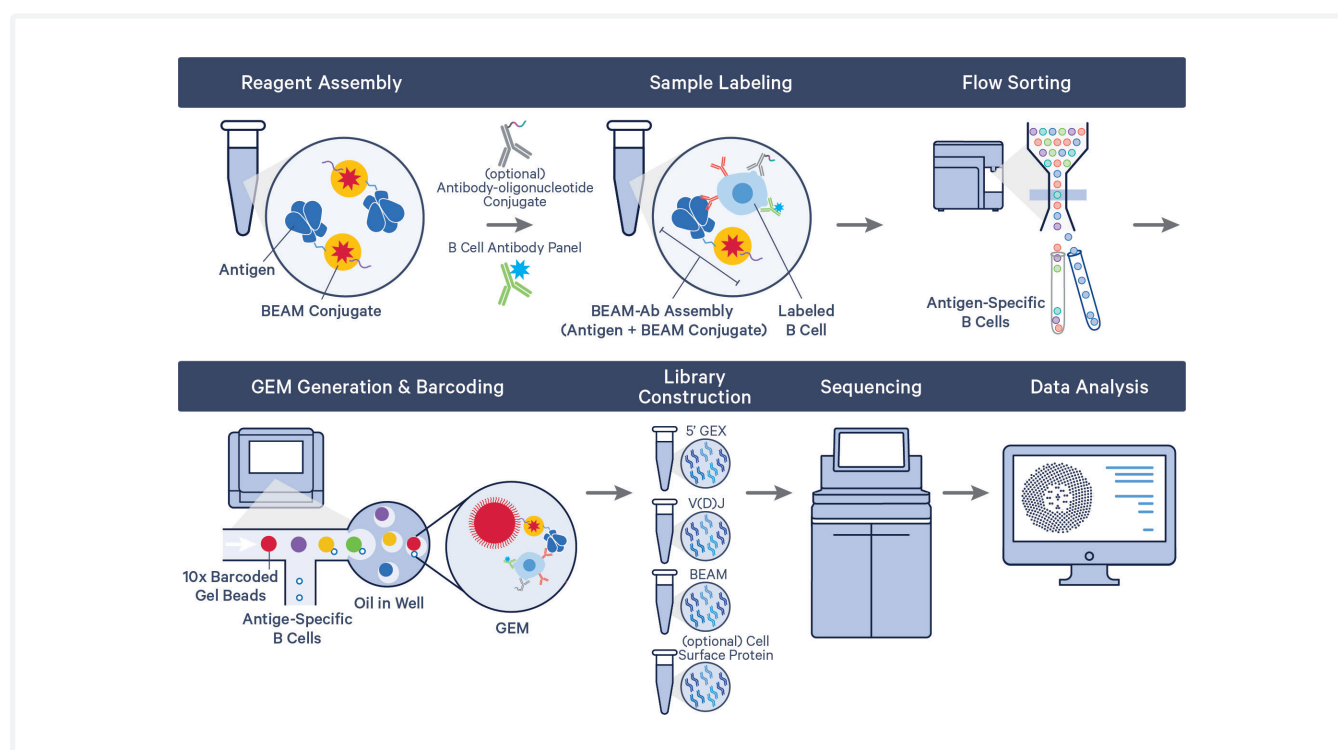


Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM) – Protocol Planner

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

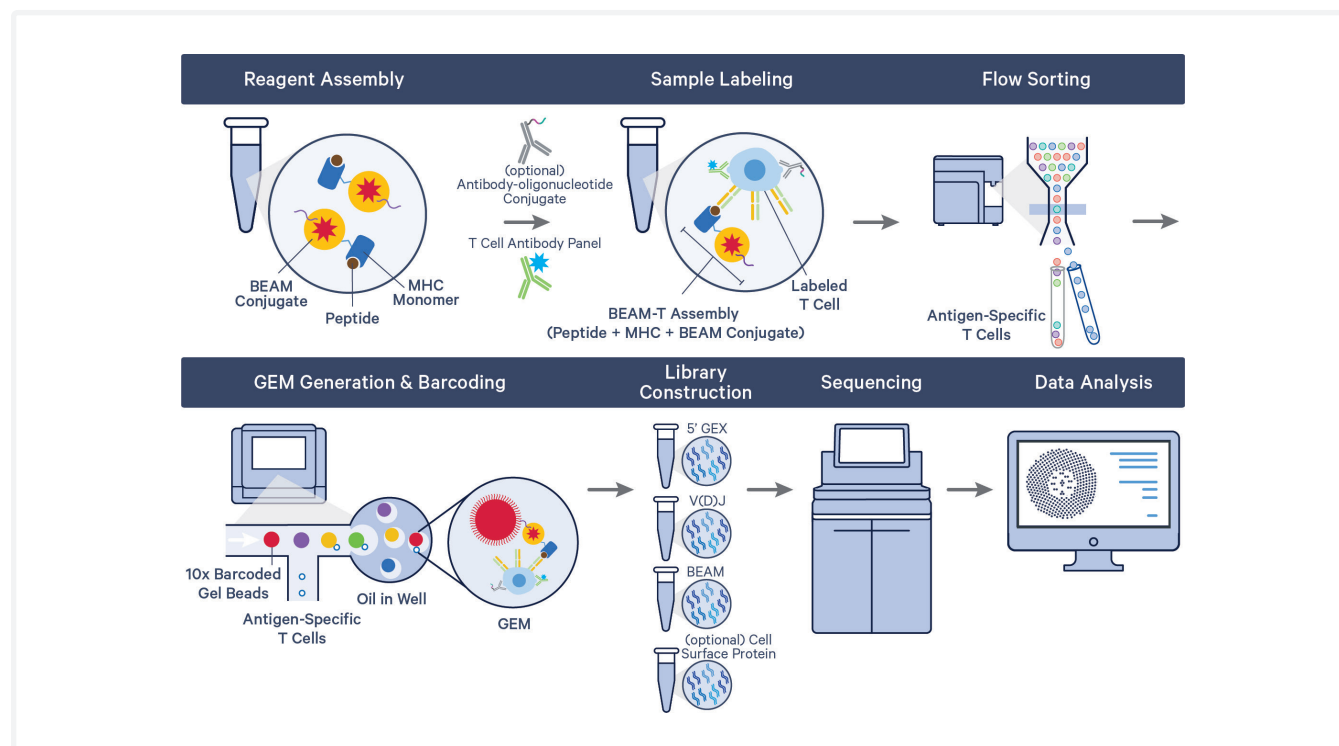


Figure 1. BEAM Workflow Overviews. Each BEAM Conjugate is composed of a streptavidin, a fluorophore molecule (Phycoerythrin, PE), and a Feature Barcode oligonucleotide.

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

User Guide CG000592

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 | | Eppendorf | 22431005 |
| 2-ml tubes | DNA LoBind Tubes 2.0 ml | Choose either tubes for reagent & buffer preparation. | 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 |
| 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 (7AAD⁻) > 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 |
| 7AAD (-) | 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 |
| 7AAD (-) | 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 (7AAD⁻) > 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 |
| 7AAD (-) | 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 |
| 7AAD (-) | 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

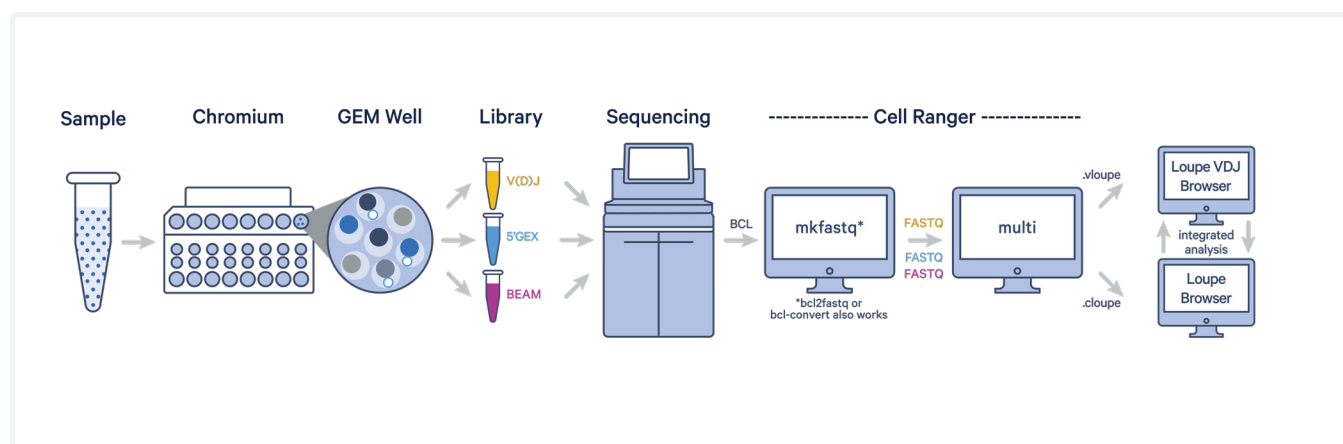


Figure 2. BEAM Data Analysis Overview.

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 A |
| Revision Date | November 2022 |

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