Barcode Enabled Antigen Mapping (BEAM) Experimental Planning Guide

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 accomplished by assembling antigens or peptides of interest with uniquely barcoded 10x Genomics BEAM Conjugates (16 unique 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 Barcode Enabled Antigen Mapping (BEAM) to generate BEAM, 5' Gene Expression, and V(D)J libraries. This document provides an overview of the key considerations for the various steps of the workflow when planning a BEAM experiment.

	Design			Execution			
BEAM-Ab	**Source Sample** • Human/ Mouse	**Establish Flow Sorting** • Appropriate flow sorter access	**Procure Antigen** • Biotinylated Antigen • 10 kDa to 200 kDa	**Order BEAM Reagent Kits** • Chromium 5' BEAM Core Kit	**Assemble Reagents, Label Samples & Flow Sort Cells** **Pre-screen Antigens** • Prepare BEAM-Ab Assemblies • Label samples • Flow analyze	**Test Multiple Antigens** • Prepare BEAM-Ab Assemblies • Pool assemblies • Label samples • Flow sort antigen-specific B cells*	**GEM Generation & Library Prep** • Generate GEMs • Cleanup and amplify cDNA • Prepare libraries
	~ 1-8 weeks	~ 2 weeks	5-7 weeks	~ 1 week	~ 1 day	~ 1 day	~ 1-2 days
BEAM-T	**Source Sample** • Human/ Mouse • Determine MHC type	**Establish Flow Sorting** • Appropriate flow sorter access	**Procure Peptide** • 9-12 amino acid long peptide	**Order BEAM Reagent Kits** • Chromium 5' BEAM Core Kit • MHC Monomer Kit	**Assemble Reagents, Label Samples & Flow Sort Cells** **Pre-screen Peptides** • Prepare BEAM-T Assemblies • Label samples • Flow analyze	**Test Multiple Peptides** • Prepare BEAM-T Assemblies • Pool assemblies • Label samples • Flow sort antigen-specific $CD8^+$ T cells*	**GEM Generation & Library Prep** • Generate GEMs • Cleanup and amplify cDNA • Prepare libraries
	~ 1-8 weeks	~ 2 weeks	~ 3-5 weeks	~ 1 week	~1 day	~ 1 day	~ 1-2 days

**Immediately proceed to chip loading after sorting.*

Figure 1. Overview of the design and execution steps for BEAM-Ab and BEAM-T workflows. All design steps can be performed in parallel.

Sample Sourcing

Before starting with the BEAM workflow, appropriate samples must be obtained.

Sample Types

BEAM workflow supports human and mouse samples. Other species are not supported. This workflow has been demonstrated on human peripheral blood mononuclear cells (PBMCs), enriched T and B cells from human PBMCs, mouse splenocytes, and mouse lymphocytes. Additional optimization may be needed if working with new sample types.

Mouse Immunization

One of the applications of the BEAM-Ab workflow is the high-throughput BCR discovery from immunized animals or convalescent patients. If interested in generating antibodies, a 6-8 weeks mice immunization plan using the antigen of interest will need to be followed. The harvested cells (splenocytes, lymphocytes, and/or bone marrow cells) can then be prepared and screened using the biotinylated antigen for specific antibodies (B cell receptors).

HLA/MHC Typing of Donor for BEAM-T

For the BEAM-T workflow, determine the human leukocyte antigen (HLA) type of the donors in order to choose the right 10x Genomics Monomer kit(s) to use. Many clinical research organizations provide HLA typing information on their donors. Chromium Single Cell 5' v2 Reagent Kits for BEAM include four Human MHC Class I Monomer Kits and one Mouse MHC Class I Monomer Kit. For the BEAM-T workflow in mouse samples, the MHC allele available (mouse H2Kb) is expressed in several common laboratory strains, including 129/- and C57BL/6. See this Mouse Haplotype Table for reference.

Screening of Donor for Peptide Reactivity for BEAM-T

There are various ways to pre-screen donors for reactivity to a particular antigen, including 1) performing enzyme-linked immunosorbent spot (ELISpot) assays, which are quantitative and measure key cellular functions of the immune cells, 2) performing antigen-specific expansion of the donor cells and then testing by peptide-loaded MHC monomer screening, such as BEAM-T.

Pre-enrichment

If the percentage of T or B cells is low in the starting sample, it is recommended to enrich the sample for the cell type of interest (B or T cells) to maximize the use of BEAM Conjugate and to decrease the flow sorting time. A negative selection kit should be used for enrichment to avoid interference with BEAM reagents.

Sample Viability

High viability samples give best results. Use samples with at least 70% viability for BEAM workflow. After sorting, the viability should be more than 90%.

Sample Size

Significant cell loss can occur at various stages of sample preparation. It is therefore recommended to start with a higher number of cells. The recommended minimum number of cells for flow sorting is $1x10^6$ cells depending on the expected frequency of antigen-positive cells.

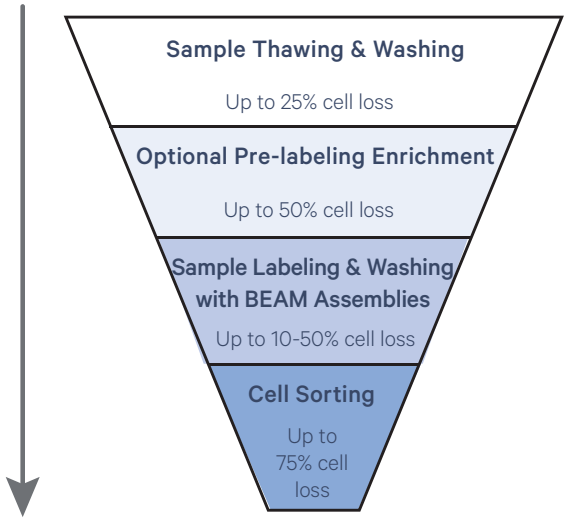


Figure 2. Percent loss of cells during various steps of sample preparation.

Establishing Flow Sorting Pipeline

Before starting the BEAM workflow, it is recommended to establish a flow sorting pipeline by getting access to an appropriate cell sorter and designing antibody panels.

Cell Sorter Key Considerations

Lasers

The cell sorter should include the following lasers:

- **Blue (488 nm)**
  To detect forward scatter, side scatter, and fluorophores such as Fluorescein Isothiocyanate (FITC). This laser is also used to detect live/dead dyes such as 7-AAD.
- **Yellow/Green (561 nm)**
  To excite the Phycoerythrin (PE) fluorophore on the BEAM Conjugate.
- **Violet (405 nm) or red (647 nm)**
  To ensure that other cell type-specific markers can be gated for/against.

Nozzle

Cell sorter nozzles should have milder pressure to preserve the viability and the integrity of cells.

Antibody Panel Designs

Before starting the BEAM workflow, antibody panels should be designed for flow sorting and appropriate flow antibodies should be ordered.

Antibody Panels

Each panel should consist of:

- **Markers for Desired Cells**
  Antibodies specifically labeling $CD8^+$ T cells (BEAM-T) or B cells (BEAM-Ab) should be included.
- **Markers for Undesired Cell Lineages**
  Markers for cells that are not desired should also be included so that these cell populations can be excluded during sorting.
- **Live/Dead Dyes**
  These dyes selectively dye dead cells, allowing the sorter to distinguish between live and dead cell populations.

Consult Technical Note BEAM-Ab & BEAM-T Flow Cytometry Guidelines (Document CG000598) for general guidance on cell sorting, as well as specific tips and best practices for optimizing sample enrichment for the BEAM workflow.

Procure Antigens – BEAM-Ab

Antigen

- Any antigen of interest should be of high purity and quality.
- Antigen should be properly biotinylated. If not experienced at antigen biotinylation, it is recommended to use commercial vendors or other researchers that are able to provide high-quality biotinylation services and advice.
- Mono-biotinylated antigens (His-Avi-tagged) are a recommended option, although poly-biotinylated antigens can also be used.
- It is reasonable to expect a turn-around time of 5-7 weeks for the generation of biotinylated antigen, though some vendors have popular or common antigens of interest in various biotinylated forms.

Optimal/Supported Number of Antigens

The Chromium Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns PN-1000539 contains a set of 16 BEAM Conjugates and allows users to test up to 15 antigens per sample in one experiment. Each experiment should also include a Negative Control Assembly (BEAM Conjugate + PBS). Refer to the section Assemble Reagents, Label Samples & Flow Sort Cells – BEAM-Ab for details.

Antigen Molecular Weight

BEAM workflow has been demonstrated on antigens with molecular weight ranging from 10 kDa to 200 kDa. It is important to know the exact molecular weight of the antigen as the volume of the antigen needed to prepare each BEAM-Ab Assembly is dependent on the molecular weight of the antigen. Consult the Sample Prep User Guide Reagent Assembly, Sample Labeling & Flow Sorting for BEAM (Document CG000595) for additional details.

Antigen QC/Pre-screening

Before starting with the BEAM-Ab workflow, each antigen must be individually pre-screened by assembling with the BEAM Conjugate, labeling non-experimental samples, and performing flow cytometric analysis. See Antigen Pre-screening for details.

Procure Peptides – BEAM-T

Peptide

- Peptide should be of high quality and purity (>90%).
- Associated QC steps such as Fast Protein Liquid Chromatography (FPLC) or mass spectrometry can be requested from the vendors to ensure the peptide purity as well to confirm the sequence.
- Peptides should have good solubility. Peptide solution should be properly checked to see any indication of poor solubility such as precipitation or cloudiness.
- Use appropriate MHC monomer-matched peptides. Prediction engines such as NetMHCpan can be used to predict peptides with potential for binding the HLA Class I alleles.
- Peptide should be 9-12 amino acids long.
- The lead time to procure peptides could be 3-5 weeks, although more common peptides (various virus peptides for EBV, Flu, or CMV) for the most common MHC types are sometimes in stock with certain vendors.

Peptide QC

Each peptide must be individually pre-screened by assembling with the BEAM Conjugates, labeling non-experimental samples, and performing flow cytometric analysis. See Peptide Pre-screening for details.

Optimal/Supported Number of Peptides

The Chromium Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns PN-1000539 contains a set of 16 BEAM Conjugates and allows users to test up to 15 peptides per sample in one experiment. Each experiment should also include a Negative Control Peptide Assembly, which includes BEAM Conjugate, MHC monomer, and Negative Control Peptide (part of 10x Genomics MHC kits). Refer to the section Assemble Reagents, Label Samples & Flow Sort Cells – BEAM-T for details.

HLA/MHC Compatibility of the Peptide

Choose a peptide that is compatible with the MHC/HLA type of the donor and choose the right 10x Genomics Monomer kit(s) to use.

Order BEAM Reagents

To perform BEAM workflow, the following kits must be purchased.

BEAM-Ab Reagents
Chromium Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns PN-1000539
BEAM-T Reagents
Chromium, Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns, PN-1000539
Chromium Human MHC Class I A0201 Monomer Kit, 32 rxns PN-1000542
Chromium Human MHC Class I A1101 Monomer Kit, 32 rxns PN-1000543
Chromium Human MHC Class I B0702 Monomer Kit, 32 rxns PN-1000544
Chromium Human MHC Class I A2402 Monomer Kit, 32 rxns PN-1000545
Chromium Mouse MHC Class I H2Kb Monomer Kit, 32 rxns PN-1000546

The Chromium Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns PN-1000539 contains a set of 16 BEAM Conjugates, each composed of a streptavidin, a fluorophore molecule (Phycoerythrin, PE), and a Feature Barcode oligonucleotide. Chromium Human MHC Class I kits (PN-1000542, 1000543, 1000544, and 1000545) and Mouse MHC Class I Kit (1000546) include empty, biotinylated monomers that can be loaded simply by mixing with MHC monomer-matched peptides and BEAM Conjugates.

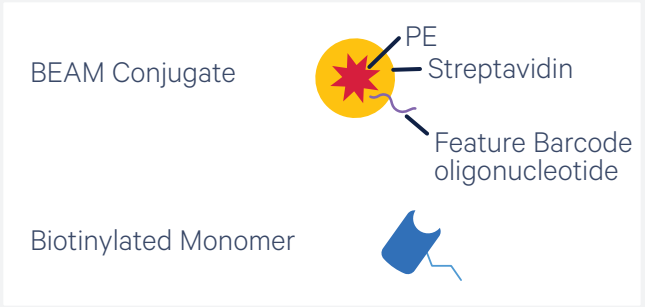


Figure 3. BEAM Conjugate and biotinylated MHC monomer.

Assemble Reagents, Label Samples & Flow Sort Cells – BEAM-Ab

Antigen Pre-screening

The first step of BEAM-Ab sample preparation workflow is to pre-screen each antigen to assess the non-specific binding of that antigen, which may be the result of lower purity or improper biotinylation.

During pre-screening, biotinylated antigens are combined with BEAM Conjugates to prepare BEAM-Ab Assemblies (Figure 4A). These assemblies are not quenched. The unquenched assemblies are used to label samples along with the B cell antibody panel for flow cytometry. Flow cytometric analysis is then performed to profile the loaded antigen. Depending on the model/cells being labeled, an antigen will have no or only a small amount of positivity (an exception could be in the case of a target in an engineered model that is known to have high positivity). If there is a large number of PE^+ events, that antigen should not be used in a multiplex BEAM experiment.

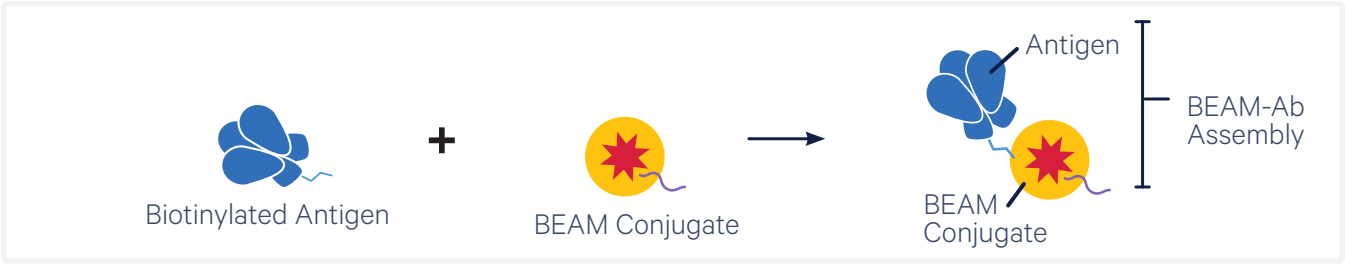
Testing Multiple Antigens

For each experiment, one Negative Control Assembly and one ore more (up to 15) Target Antigen Assemblies are prepared by combining BEAM Conjugates with the biotinylated antigen. For the Negative Control Assembly, PBS is used instead of the antigen. Negative controls are required for calculating the Antigen Specificity Score. All the assemblies undergo a quenching step and are then pooled together. Samples are then labeled with the quenched and pooled assemblies. During labeling, the B cell antibody panels for flow sorting are also added. The assembly and labeling protocols take 2-3 hours.

Flow Sorting

The labeled samples are then sorted on an appropriate sorter and PE positive B cells are collected. For this step, bead-based enrichment is not recommended. For guidance on pre-screening, assembly preparation, labeling, and sorting, consult Demonstrated Protocol Reagent Assembly, Sample Labeling & Flow Sorting for BEAM (Document CG000595).

A. BEAM-Ab Assembly



B. BEAM-Ab – Sample Labeling & Flow Sorting

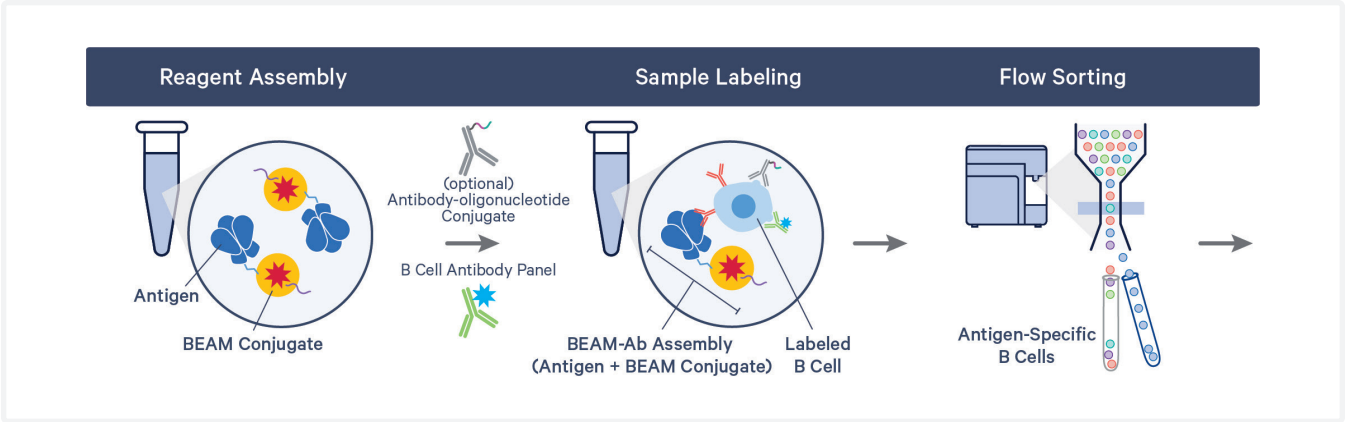


Figure 4. BEAM-Ab – Reagent assembly, sample labeling & flow sorting. A. BEAM-Ab Assembly (biotinylated antigen + BEAM Conjugate) **B.** Sample labeling with BEAM-Ab Assembly and flow sorting of antigen-specific (PE^+) B cells.

Assemble Reagents, Label Samples & Flow Sort Cells – BEAM-T

Peptide Pre-screening

The first step of BEAM-T sample preparation workflow is to pre-screen each peptide to determine the peptide quality as well as proper loading of the MHC monomer.

During pre-screening, peptides are combined with BEAM Conjugates and appropriate MHC monomers to prepare BEAM-T Assemblies. These assemblies are not quenched. The unquenched assemblies are then used to label samples along with the T cell antibody panel for flow cytometry. Flow cytometric analysis is then performed to profile the loaded peptide. Depending on the cells being labeled, a peptide will have no or only a small amount of positivity (an exception could be in the case of a target in an engineered model that is known to have high positivity). If there is a large number of PE^+ events, that peptide should not be used in a multiplex BEAM experiment.

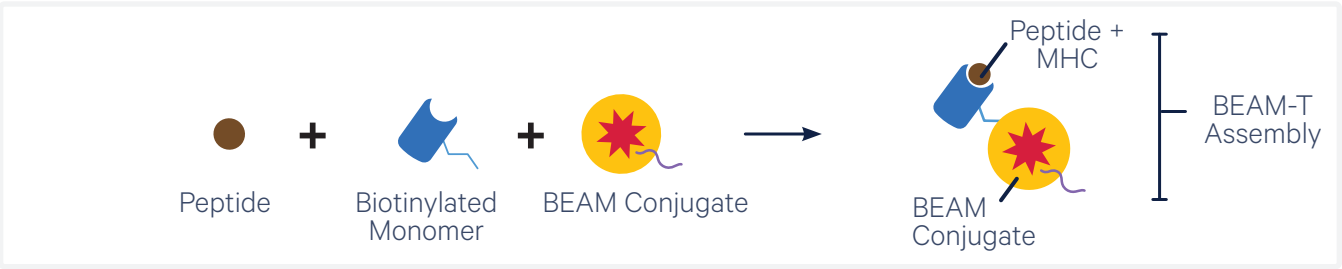
Testing Multiple Peptides

For each experiment, one Negative Control Assembly and one or more (up to 15) Target Peptide Assemblies are prepared by combining BEAM Conjugates with the MHC monomer and peptide. For the Negative Control Assembly, 10x Genomics provided non-targeting Negative Control Peptides are used. Negative controls are required for calculating the Antigen Specificity Score. All the assemblies undergo a quenching step and are then pooled together. Samples are then labeled with the quenched and pooled assemblies. During labeling, the T cell antibody panels for flow sorting are also added. The assembly and labeling protocols take 2-3 hours.

Flow Sorting

The labeled samples are sorted on an appropriate sorter to collect antigen positive (PE^+) T cells. For this step, bead-based enrichment is not recommended. For guidance on pre-screening, assembly preparation, labeling, and sorting, consult Demonstrated Protocol Reagent Assembly, Sample Labeling & Flow Sorting for BEAM (Document CG000595).

A. BEAM-T Assembly



B. BEAM-T – Sample Labeling & Flow Sorting

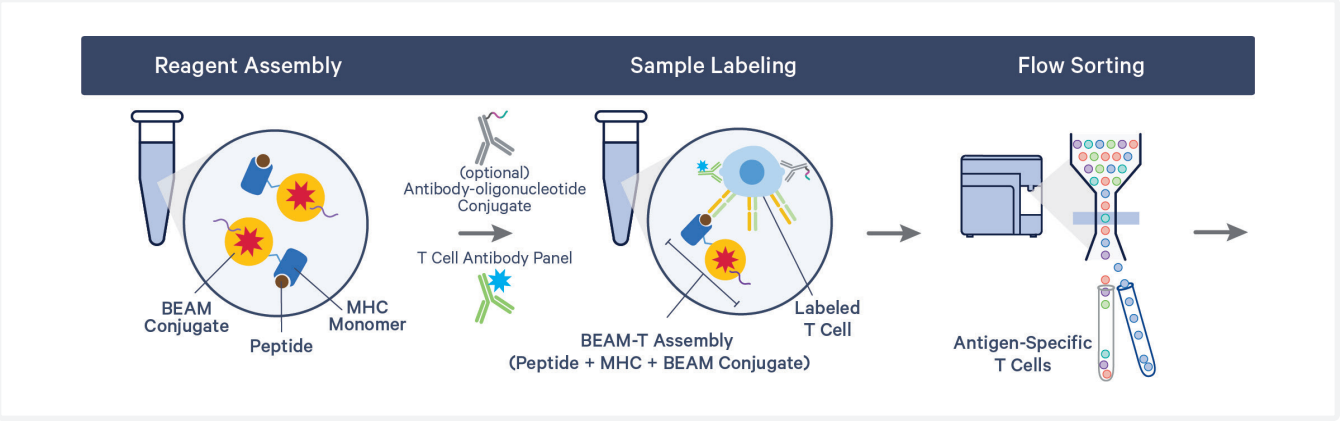
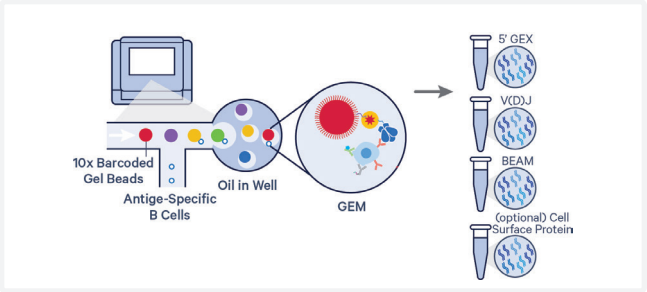


Figure 5. BEAM-T – Reagent assembly, sample labeling & flow sorting. A. BEAM-T Assembly (Peptide + Biotinylated monomer + BEAM Conjugate) **B.** Sample labeling with BEAM-T Assembly and flow sorting of antigen-specific (PE^+) $CD8^+$ T cells.

GEM Generation & Library Prep

Antigen-specific B or T cells are loaded into the 10x Genomics chip and Gel Beads-in-emulsion (GEMs) are generated. Libraries are prepared and sequenced from the DNA molecules and 10x Barcodes are used to associate the individual reads back to the individual partitions. Single Cell 5' Gene Expression, V(D)J, and BEAM libraries can be generated alone or in combination with Cell Surface Protein libraries using this workflow.

A. BEAM-Ab GEM Generation & Library Preparation



B. BEAM-T GEM Generation & Library Preparation

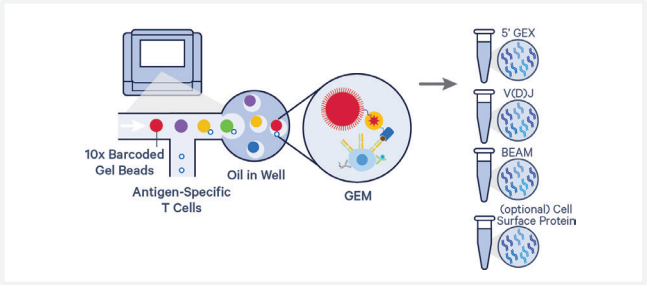


Figure 6. GEM generation & library preparation.

References

1. Reagent Assembly, Sample Labeling & Flow Sorting for Barcode Enabled Antigen Mapping (BEAM) Sample Prep User Guide (CG000595).
2. Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM) Protocol Planner (CG000590).
3. BEAM-Ab & BEAM-T Flow Cytometry Guidelines (CG000598).
4. BEAM-Ab Assembly Workbook (CG000597).
5. BEAM-T Assembly Workbook (CG000615).
6. Chromium Next GEM Single Cell 5' Reagent Kits with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) & Cell Surface Protein User Guide (CG000592).
7. Chromium Next GEM Single Cell 5' Reagent Kits with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) User Guide (CG000591).

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