Multiplexed antigen screening for rapid B- and T-cell clonotype discovery

Single Cell Immune Profiling with Barcode Enabled Antigen Mapping (BEAM)

Despite the huge therapeutic potential of the functional adaptive immune repertoire, it remains difficult to match antigen targets with specific B- and T-cell receptors (BCRs/TCRs) using conventional methods. BEAM empowers rapid discovery of antigen-specific B-cell (BEAM-Ab) and T-cell (BEAM-T) clonotypes with unparalleled cellular resolution. Built on the proven Chromium Single Cell Immune Profiling workflow, BEAM enables screening of BCRs/TCRs against putative antigens in conjunction with gene expression, V(D)J sequencing, and cell surface protein expression. Leverage multiplexed antigen screening to generate tens to hundreds of high-quality hits from the same sample, augmenting the diversity and magnitude of clonotypes/receptors for downstream validation in therapeutic applications.

Highlights

- Generate tens to hundreds of high-quality antigen-specific hits from the same sample

- Discover antigen-specific clonotypes with full-length, paired V(D)J sequences, gene expression, and surface protein expression from the same single cell

- Go from sample to antigen-specific immune repertoire sequencing data in a week

- Explore and interpret antigen–clonotype relationships with easy-to-use analysis and visualization software
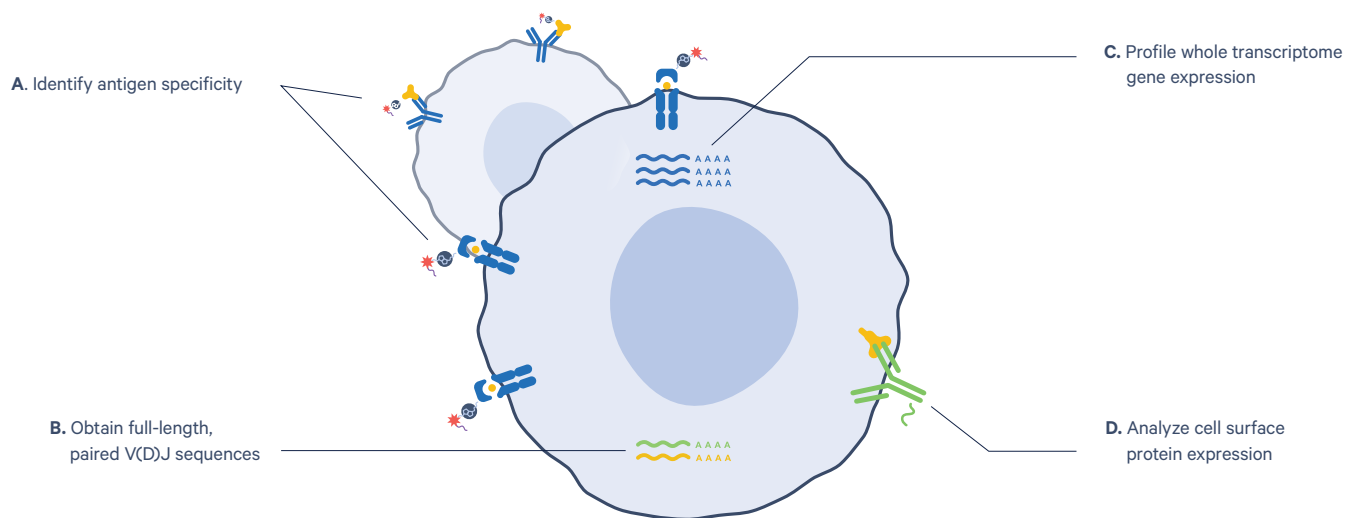

A. Identify antigen specificity

B. Obtain full-length, paired V(D)J sequences

C. Profile whole transcriptome gene expression

D. Analyze cell surface protein expression

Figure 1. Comprehensive profiling of the immune repertoire with antigen-specific clonotype characterization. A. Feature Barcode technology with unique BEAM-Ab and BEAM-T reagent assemblies allows antigen-specificity determination of target B and T cells, respectively. **B.** Obtain full-length, paired V(D)J sequences of antigen-specific B or T cells alongside whole transcriptome gene expression (**C**) and cell surface proteins (**D**) at the single cell level.

10x GENOMICS

Product features

BEAM-Ab

- Utilize a seamlessly integrated, end-to-end work-flow to go from sample to high-quality antibody hits in under a week

- Screen as many as 15 antigens in parallel to generate tens to hundreds of hits per sample and increase your funnel size for antibody candidates

- Leverage intuitive software solutions to identify expanded antigen-specific clonotypes for efficient selection of the ideal antibody candidates for downstream validation

BEAM-T

- Leverage kitted, custom loadable MHC monomers to easily prepare peptide–MHC complexes, offering flexibility to design and source peptides from any vendor

- Detect rare and/or therapeutically relevant TCR clonotypes with gene expression at single cell resolution for a comprehensive understanding of the immune response

- Explore antigen–clonotype relationships with easy-to-use software solutions to access the full diversity of antigen-reactive T cells from a single sample
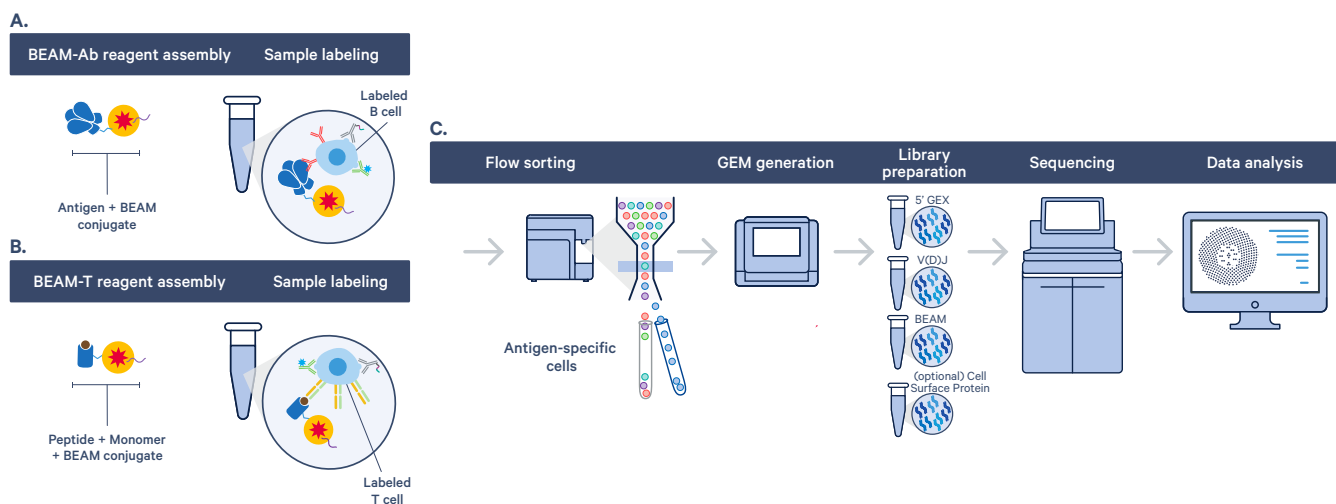

Figure 2. Fast and robust workflow for antigen-specific BCR and TCR discovery. The BEAM-Ab and BEAM-T workflows begin with user-supplied antigens, which are first assembled and uniquely barcoded using 10x Genomics BEAM reagents. **A.** BEAM-Ab reagent assemblies couple biotinylated antigen to a barcoded BEAM Conjugate complex that contains PE fluorescent marker. **B.** BEAM-T reagent assemblies couple antigenic peptide to empty loadable MHC, which are themselves coupled to BEAM conjugate. BEAM-T offers flexibility to design peptides of interest and choose from five different MHC Class I alleles provided through 10x Genomics Chromium Human/Mouse MHC Class I Monomer Kits. **C.** The barcoded assemblies are then used to stain B or T cells prior to flow sorting for enrichment (flow sorting is recommended). Normal library preparation steps are then carried out on the sample, generating multiple library types, including antigen, V(D)J, gene expression, and, optionally, cell surface protein. After sequencing, raw FASTQ files are processed for downstream analysis with Cell Ranger and Loupe V(D)J Browser, our fully integrated and easy-to-use analysis and visualization software tools. (Note: BEAM-Ab and BEAM-T are performed in separate workflows with unique reagents).
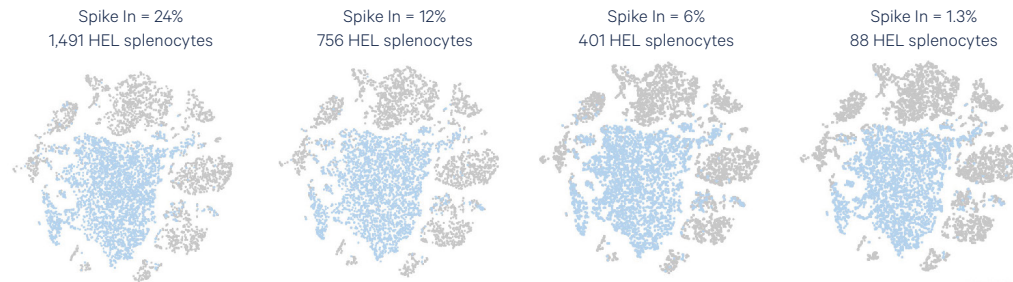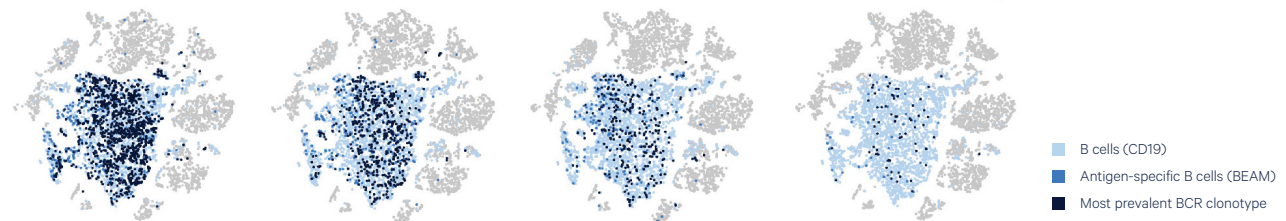
A. Gene expression t-SNE

Spike In = 24%
1,491 HEL splenocytes

Spike In = 12%
756 HEL splenocytes

Spike In = 6%
401 HEL splenocytes

Spike In = 1.3%
88 HEL splenocytes

B. Gene expression t-SNE overlaid with antigen-specific clonotypes

- B cells (CD19)
- Antigen-specific B cells (BEAM)
- Most prevalent BCR clonotype

Figure 3. BEAM-Ab provides a precise view of antigen-specific, expanded clonotypes. Transgenic mouse splenocytes that recognize the foreign antigen hen egg lysozyme (HEL) were labeled with a 2-plex BEAM-Ab reagent assembly of HEL and a negative control. These cells were flow sorted to purity and then spiked into a background of mouse splenocytes at the following percentages: 24%, 12%, 6%, and 1.3%. **A.** Aggregated t-SNE projections show splenocyte cells clustered by gene expression. Light blue dots indicate a cell expressing CD19 (a marker for B cells), while gray dots indicate non-B-cell populations clustered via 5' gene expression profiles. **B.** Antigen-specific B cells, identified by the presence of a BEAM Conjugate, were overlaid with gene expression and cells from the most prevalent BCR clonotypes in the B-cell cluster. As shown, the most prevalent BCR clonotypes were found to be specific for the HEL antigen.
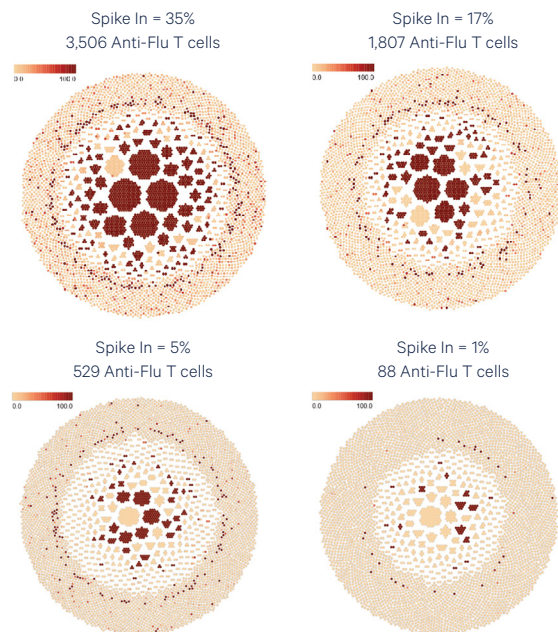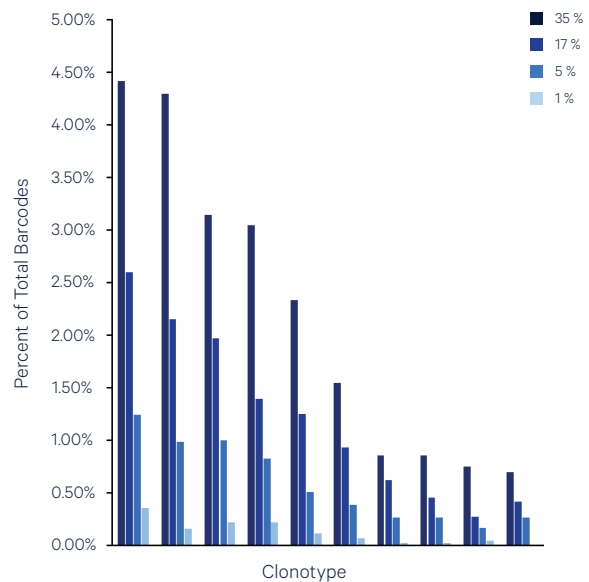
A. Clonotype distribution

Spike In = 35%
3,506 Anti-Flu T cells

Spike In = 17%
1,807 Anti-Flu T cells

Spike In = 5%
529 Anti-Flu T cells

Spike In = 1%
88 Anti-Flu T cells

B. Top ten clonotype distribution

Figure 4. Highly sensitive, consistent clonotype–antigen assignments with BEAM-T. HLA-A*02:01 anti-flu expanded human T cells were labeled with a 2-plex BEAM-T reagent assembly of FLU and the HLA-A*02:01 negative control. These cells were flow sorted to purity and then spiked into a background of HLA-A*02:01 peripheral blood mononuclear cells (PBMCs) at the following percentages: 35%, 17%, 5%, and 1%. **A.** Visualization of antigen-specific T cells identified via BEAM-T. Cells are represented as individual points and grouped by clonotype assignment. Cells are colored according to their antigen specificity, where dark red denotes higher antigen specificity. **B.** Top ten TCR clonotype frequency distribution demonstrates consistent clonotype detection and abundance consistent with the spike-in percentage.

Product specifications

- Multiplex up to 15 antigens per sample plus a negative control
- Diverse sample type compatibility, including PBMCs, splenocytes, lymph node aspirates, and enriched B or T cells
- Antigen specificity score computed for screened antigens against controls
- Low doublet rates of under 0.8% per 1,000 cells
- High cell capture rates of up to 65%

Gene expression and / or immune repertoire profiling	Product code
Chromium Next GEM Single Cell 5' Kit v2, 16 rxns	1000263
Chromium Next GEM Single Cell 5' Kit v2, 4 rxns	1000265
Chromium Next GEM Chip K Single Cell Kit, 48 rxns	1000286
Chromium Next GEM Chip K Single Cell Kit, 16 rxns	1000287
Library Construction Kit, 16 rxns	1000190
Dual Index Kit TT Set A, 96-rxns	1000215
Dual Index Kit TS Set A, 96-rxns	1000251

V(D)J amplification kits		Product code
Human T cell	Chromium Single Cell Human TCR Amplification Kit, 16 rxns	1000252
Human B cell	Chromium Single Cell Human BCR Amplification Kit, 16 rxns	1000253
Mouse T cell	Chromium Single Cell Mouse TCR Amplification Kit, 16 rxns	1000254
Mouse B cell	Chromium Single Cell Mouse BCR Amplification Kit, 16 rxns	1000255

Feature barcode technology products	Product code
5' Feature Barcode Kit, 16 rxns	1000541
Dual Index Kit TN Set A, 96-rxns	1000250

BEAM-Ab reagents	Product code
Chromium Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns	1000539

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

Instrument compatibility	Product code
Chromium iX & Accessory Kit, 12 Mo. Warranty	1000328
Chromium iX & Accessory Kit, 24 Mo. Warranty	1000329
Chromium X & Accessory Kit, 12 Mo. Warranty	1000331
Chromium X & Accessory Kit, 24 Mo. Warranty	1000332
Chromium X Upgrade Package	1000330
Chromium Controller & Next GEM Accessory Kit, 12 Mo. Warranty	1000202
Chromium Controller & Next GEM Accessory Kit, 24 Mo. Warranty	1000204

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