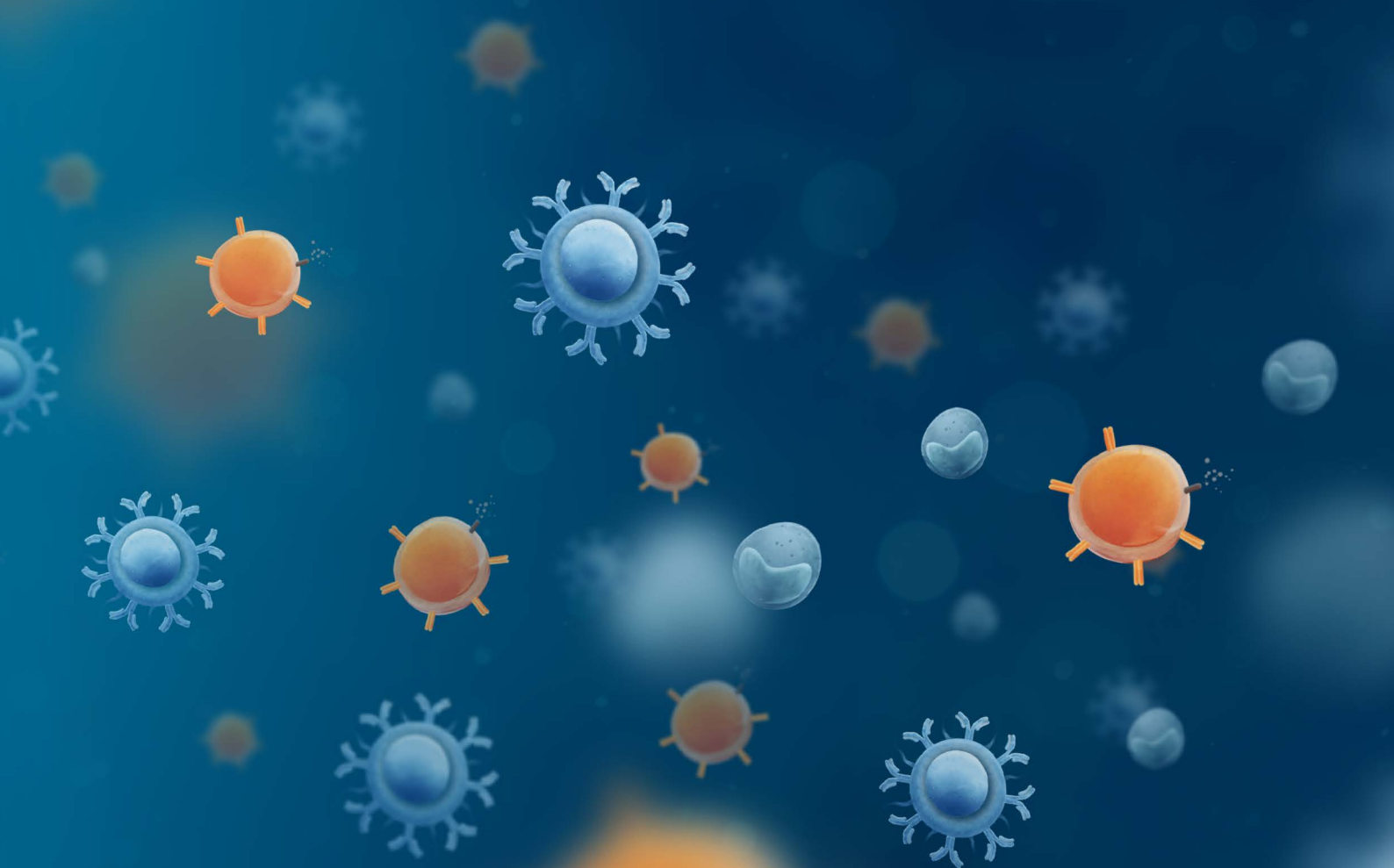


dCODE Dextramer<sup>®</sup> Compatible with 10x  
Genomics Single Cell Immune Profiling  
with Feature Barcode Technology

# Getting Started Guide

**Designing Experiments with dCODE Dextramer<sup>®</sup> Reagents**



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# 1. Single-Cell Immune Profiling and Multi-Omics

## Linking highly multiplexed peptide antigens to T-cell receptors using 10x Genomics Single Cell Immune Profiling with dCODE Dextramer<sup>®</sup>

### Single-Cell Profiling of Antigen-Specific T Cells

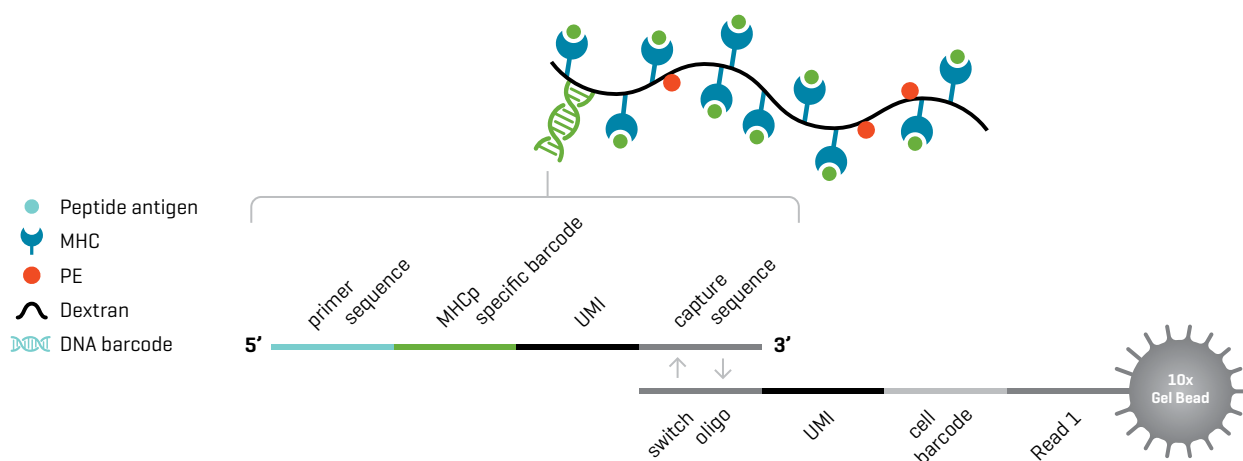
Activated by the interaction with antigenic peptides, T cells represent key players in the adaptive immune response. Understanding T-cell interactions may provide insights to disease development as well as the establishment of new treatments like vaccines or cell-based therapies [Ref. 1]. To completely understand the mounted immune response, the antigen-specific T cells should be characterized. dCODE Dextramer<sup>®</sup> reagents combine the sensitivity of the classical MHC Dextramer<sup>®</sup> technology and multi-omics by the introduction of DNA barcodes allowing identification of multiple antigen specificities in the same sample.

dCODE Dextramer<sup>®</sup> reagents consist of a dextran backbone carrying an optimized number of MHC molecules, fluorochromes, and the DNA barcode (Fig. 1). dCODE Dextramer<sup>®</sup> [10x] reagents are compatible with 10x Genomics Single Cell Immune Profiling and Feature Barcode technology, enabling gene expression profiling and paired TCR clonotypes at single-cell resolution in just one experimental workflow.

### Highlights

Together with single-cell immune profiling, dCODE Dextramer<sup>®</sup> reagents enable you to:

- Uncover data at single-cell resolution for TCR-pMHC binding specificity, gene and cell surface protein expression, and paired  $\alpha\beta$  T-cell receptor (TCR) sequences
- Analyze samples at large scale by designing panels of distinct dCODE Dextramer<sup>®</sup> reagents
- Assess the specificity of T cells across patient cohorts with coverage of over 100 human, murine, and primate MHC I alleles and 7 human MHC II alleles
- Explore the potential to develop novel experimental and analytical approaches



**Figure 1: Structure of dCODE Dextramer<sup>®</sup> Reagent and DNA Barcode**

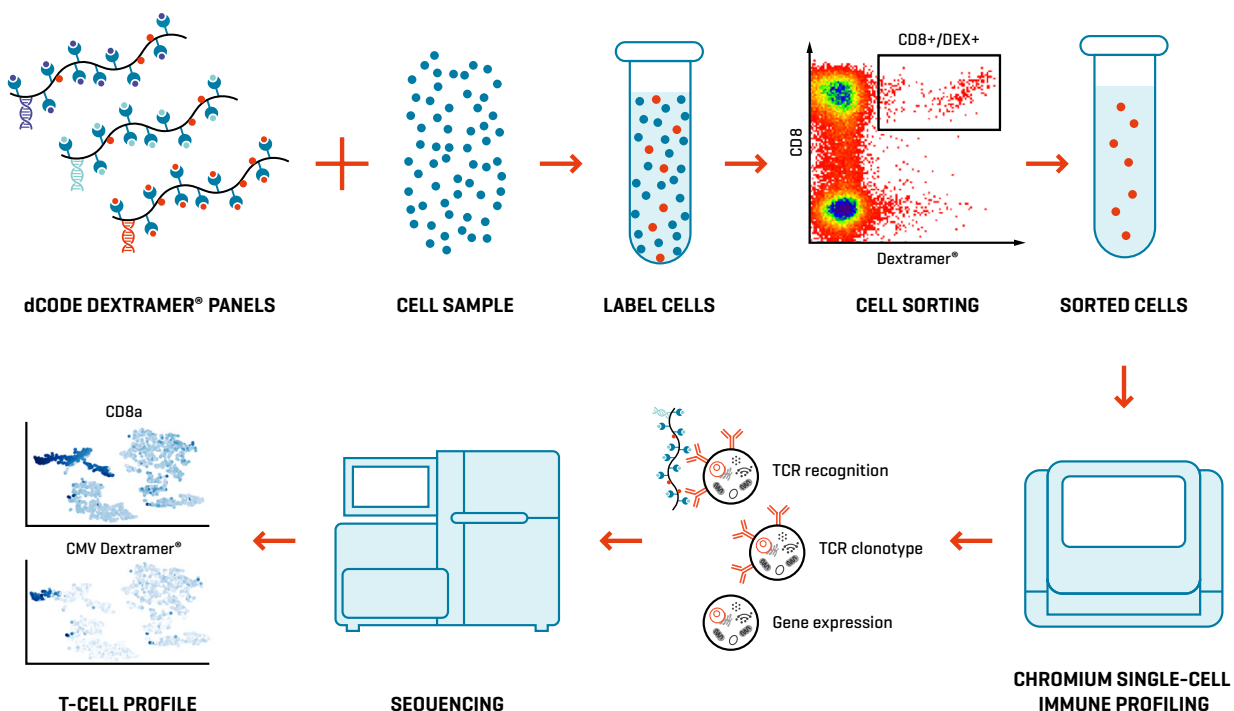
Ref. 1: Bentzen AK, Hadrup SR. Evolution of MHC-based technologies used for detection of antigen-responsive T cells. *Cancer Immunol Immunother.* 2017;66(5):657-666. doi:10.1007/s00262-017-1971-5

## 2. Workflow when Performing an Experiment with dCODE Dextramer® Reagents

Profiling antigen specificity with dCODE Dextramer® reagents and the 10x Genomics Single Cell Immune Profiling assay includes six crucial steps (Fig. 2):

1. Sample preparation and cell staining. Cells should be labeled with dCODE Dextramer® reagents then TotalSeq™-C antibody-oligonucleotide conjugates. dCODE Dextramer® reagent-only analyses are not supported currently by 10x Genomics
2. Enrichment of dCODE Dextramer® positive cells by FACS
3. Partitioning of dCODE Dextramer® positive cells into Gel Beads-in-emulsions (GEMs)
4. Library construction
5. Sequencing
6. Data analysis, including visualization and interpretation

There are experimental considerations and critical steps throughout the workflow that can impact results and determine a study's success. A well-planned and executed experiment is essential to ensure accurate data and draw insightful conclusions.



**Figure 2: Experimental Approach for Generating TCR Antigen-Binding Data at Scale.**

To identify antigen-specific T cells, cells are stained with dCODE Dextramer® and TotalSeq™-C antibodies. dCODE Dextramer® positive CD8+ T cells are then used as input for the 10x Genomics Single Cell Immune Profiling solution with Feature Barcode technology. Libraries are then prepared to gather TCR recognition, TCR clonotype, and gene expression data through sequencing. The acquired sequencing data provides you with a comprehensive characterization of your antigen-specific T cells of interest.

## 3. Designing Your Panel of dCODE Dextramer<sup>®</sup> Reagents

### Designing your dCODE Dextramer<sup>®</sup> Panel for Single-Cell Immune Profiling

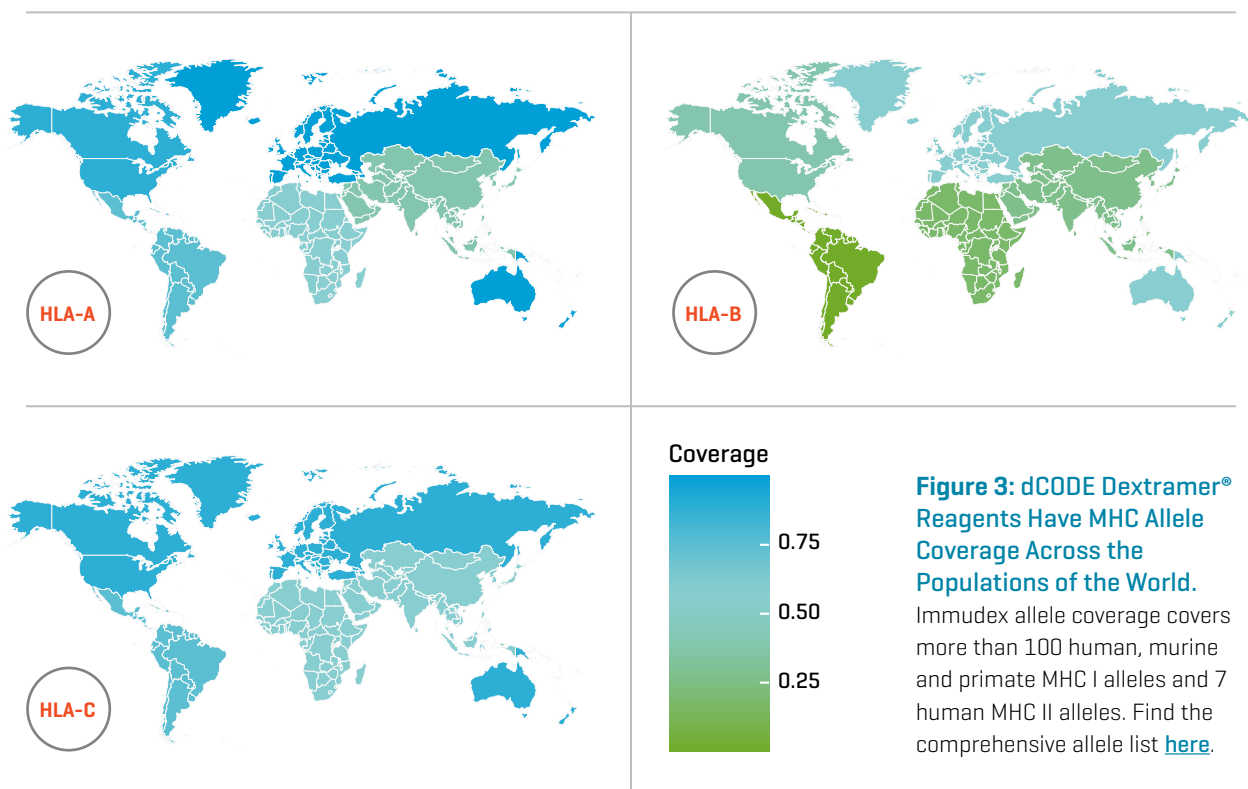
When starting an experiment with dCODE Dextramer<sup>®</sup> [10x] reagents for 10x Genomics Single Cell Immune Profiling, it is crucial to define the antigen peptide and consider its affinity towards the MHC allele.

#### Antigen Peptide Affinity

The antigen specificities that you wish to investigate ultimately determines which dCODE Dextramer<sup>®</sup> reagents and peptides to choose. Therefore, it is important to define the target antigen and peptide interaction, identify the epitope sequence, and evaluate if there are strain- or allele-specific sequence variations of the antigen included in your peptide or epitope library. To guarantee the stability of dCODE Dextramer<sup>®</sup> reagents, the antigen peptide should have high affinity towards the MHC allele. You may predict the peptide affinity using online tools such as [IEDB](#) or [NetMHC](#).

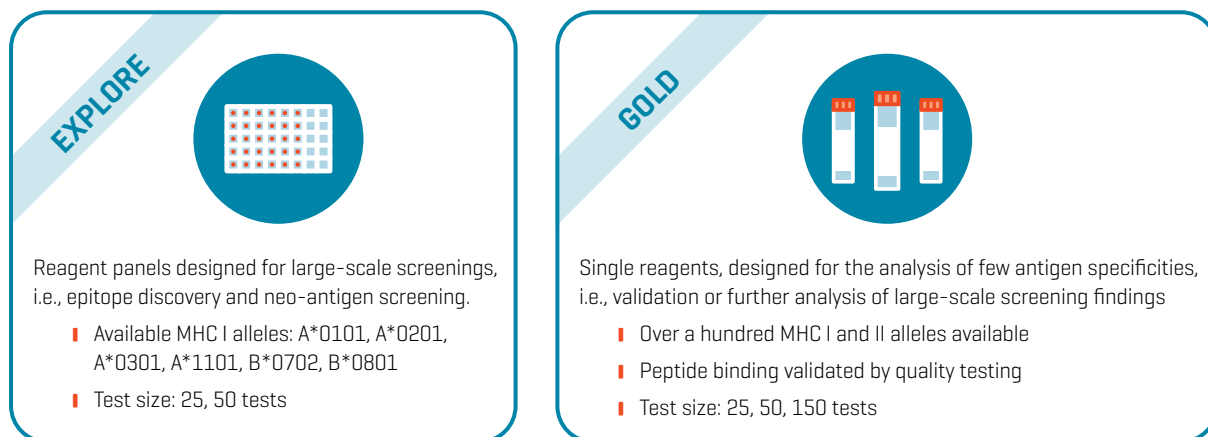
#### MHC Allele Compatibility

The MHC alleles displayed on the reagent and the investigated cells should be the same. Immudex offers over 100 human, murine and primate MHC I alleles and 7 human MHC II alleles (**Fig. 3**). Find the full allele list at [www.immudex.com/resources/mhc-alleles-list/](http://www.immudex.com/resources/mhc-alleles-list/). If a non-standard MHC allele is required, a customized dCODE Dextramer<sup>®</sup> reagent can be requested by contacting [customer@immudex.com](mailto:customer@immudex.com).



## dCODE Dextramer® Product Grades

Depending on the experimental needs, dCODE Dextramer® reagents are available in two different product grades: Explore for large-scale screenings and Gold for the analysis of few antigens (Fig. 4). The Explore grade is offered in limited alleles and test sizes, whereas the Gold grade is offered for more alleles and test sizes.



**Figure 4: dCODE Dextramer® Product Grades.**

dCODE Dextramer® reagents are available in two different product grades: Explore and Gold.

## Selection of dCODE Dextramer® Negative Controls

Once the alleles, peptide affinity, and grade of the dCODE Dextramer® reagents are chosen, it is important to consider which negative controls to use. Using one or more negative controls increases the chance of unambiguous results and maximizes the signal-to-noise ratio. Negative controls help discriminate real events from artifacts, allowing the selection of a background threshold to isolate the true signal. For the negative control, choose a matched or a mismatched allele.

Immudex offers two negative controls carrying non-sense peptide sequences for Human MHC I but also customizable negative controls. A customized negative control should include an MHC allele present in the dCODE Dextramer® panel but carry peptides, for which no signal is expected to be found. You can see the available dCODE Dextramer® negative controls in **Table 1**.

Negative controls	Allele	Cat. No.
Standard dCODE Dextramer® negative control 1	HLA-A*0201	WB02666
Standard dCODE Dextramer® negative control 2	HLA-B*0801	WI03233
Custom dCODE Dextramer® negative control reagent	Customizable and specific for your experimental setup and for the dCODE Dextramer® panel used.	N/A

**Table 1: dCODE Dextramer® Negative Controls**

## Summary

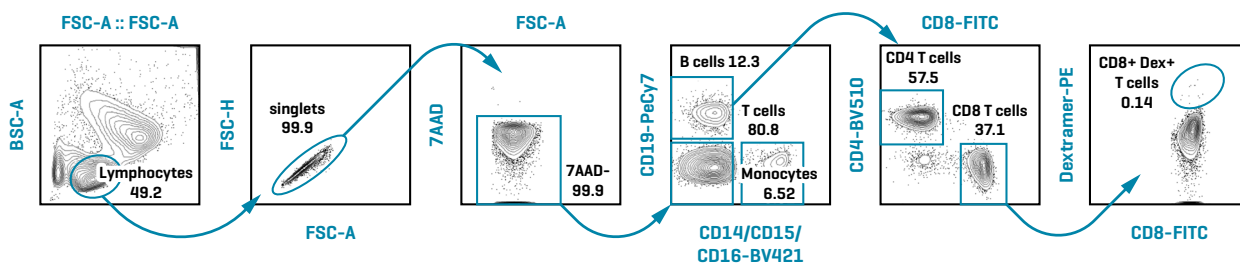
After the dCODE Dextramer® reagents and negative controls have been selected, researchers can proceed with confidence to cell staining, enrichment, and sequencing. If you would like to discuss dCODE Dextramer® reagent selection, panel setup or how dCODE Dextramer® reagents can be integrated with your research, please contact Immudex at [customer@immudex.com](mailto:customer@immudex.com).

## 4. Single-Cell Enrichment, Library Preparation and Sequencing

The next critical steps in the dCODE Dextramer<sup>®</sup> (10x) workflow include cell enrichment, single-cell partitioning, library preparation, and finally, sequencing.

### Cell Enrichment

Antigen-specific T cells are rare events and can therefore be challenging to detect in heterogeneous cell samples. To maximize the frequency of dCODE Dextramer<sup>®</sup> positive cells, these cells are enriched before performing single-cell partitioning with the 10x Genomics Chromium platform. The PE fluorophore on the dCODE Dextramer<sup>®</sup> allows flow cytometry-based cell sorting of antigen-specific T cells bound to dCODE Dextramer<sup>®</sup> reagents. Please see **Fig. 5** for an example of a gating strategy used to detect low-frequency, antigen-specific CD8+ T cells.



**Figure 5: Representative Enrichment Strategy for dCODE Dextramer<sup>®</sup> Positive CD8+ T Cells.**

Antigen-specific T cells are gated in the live, non monocyte/B cells as CD8+ dCODE Dextramer<sup>®</sup> positive T cells.

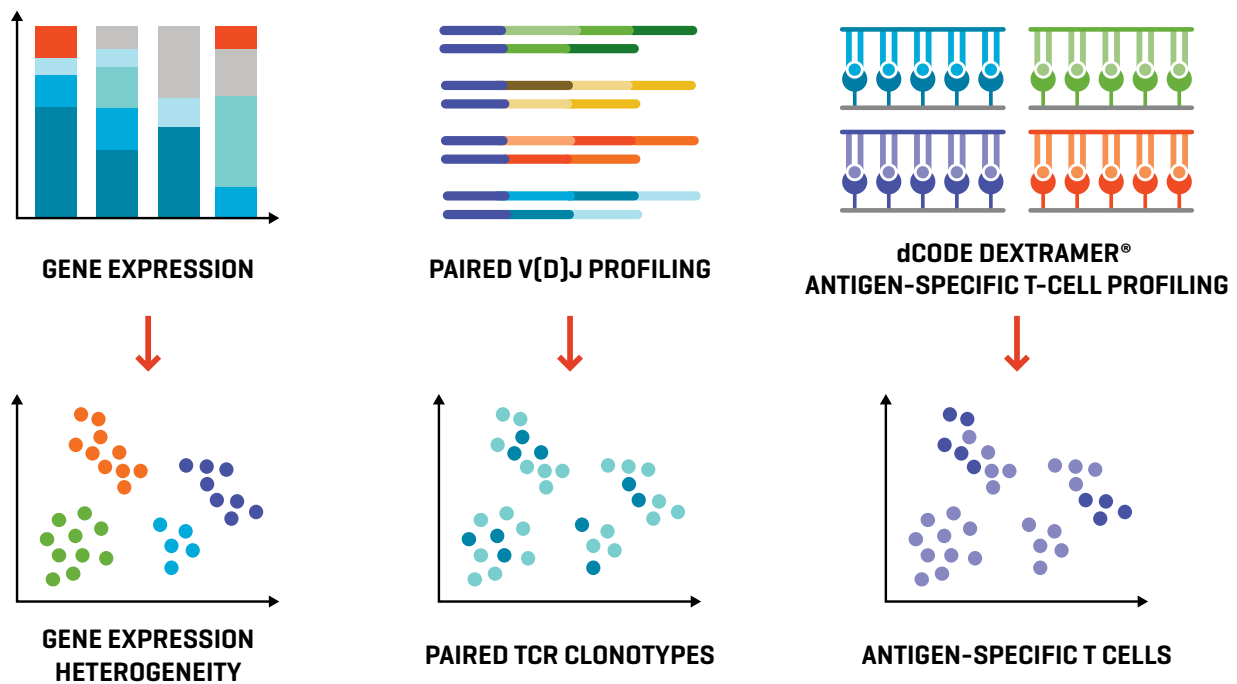
### Single-Cell Partitioning

Before sequencing, enriched dCODE Dextramer<sup>®</sup> positive cells are partitioned into GEMs and processed using the Chromium Single Cell V(D)J Reagent Kits on the Chromium Controller. The capture sequence within the dCODE Dextramer<sup>®</sup> DNA barcode allows for capture of antigen specificity information that can then be computationally associated with the same unique cell contained in the reaction vesicle.

The Chromium Controller platform uses microfluidics to process up to 10,000 single cells per channel, with up to 80,000 single cells per chip. It is crucial to ensure that cells are loaded at a limited dilution to provide a low doublet rate while maintaining a high cell recovery rate of up to ~65%. Within each GEM reaction vesicle, the single cell is lysed, the Gel Bead is dissolved to free the identically barcoded RT oligonucleotides into solution, and reverse transcription of polyadenylated mRNA occurs. As a result, all cDNAs from a single cell will have the same barcode, allowing the sequencing reads to be mapped back to their original single cells of origin.

## Library Preparation

The libraries for sequencing from the barcoded cDNAs are independently prepared due to different reads, primers, or sample indexes. A gene expression library is required when analyzing cell surface protein data with Cell Ranger. Using the appropriate library preparation protocols, the cells are processed and analyzed to simultaneously characterize gene expression, paired TCR sequences, and TCR antigen specificity in each single cell [Fig. 6]. After high-quality cDNA libraries have been prepared, researchers can proceed with confidence to sequencing.



**Figure 6: Library Preparation.**

The cells are processed and analyzed to simultaneously characterize gene expression, paired TCR sequences, and TCR antigen specificity in each single cell. The library constructs for gene expression, V(D)J, and dCODE Dextramer® are used to gain information on gene expression heterogeneity, paired TCR clonotypes, and the antigen-specificity. Modified from Application Note: Redefining Cellular Phenotyping: Comprehensive Characterization and Resolution of the Antigen-Specific T Cell Response, Document number LIT000038, 10x Genomics, [2019].



## Sequencing

Depending on the chemistry version of the 10x Genomics Single Cell Profiling kit, different sequencing configurations may be required. General recommendations are to pool all library types together prior to sequencing. *All libraries can be sequenced in the below configurations.*

Read	Read 1	I7	I5*	Read 2
Length**	26	8	0	91

**Table 2: 5' v1/v1.1 Library Sequencing Read Lengths**

Read	Read 1	I7	I5	Read 2
Length**	26	10	10	90

**Table 3: 5' v2 Library Sequencing Read Lengths**

\* Single Cell Immune Profiling (5') v1 and v1.1 libraries are single-indexed. We do not recommend sequencing these libraries with a dual-index configuration.

\*\* Shorter reads than indicated above can lead to decreased application performance. In particular, Read 2 length is critical for spanning the V-J junctions. Sample index reads must not be shorter than indicated. Any read can be longer than recommended.

Read depth recommendations are based on the library type. When performing an experiment using dCODE Dextramer<sup>®</sup> (10x) reagents, the 10x Genomics Single Cell Immune Profiling with Feature Barcode Technology read recommendation should be used:

- Gene Expression Library: 20,000 read pairs per cell
- V(D)J Amplified Library: 5,000 read pairs per cell
- Feature Barcode Library: 5,000 read pairs per cell

## Sequencing Platform

The following platforms are supported for dCODE Dextramer<sup>®</sup> (10x) and 10x Genomics libraries:

- Illumina<sup>®</sup> NovaSeq
- Illumina<sup>®</sup> HiSeq 3000/4000
- Illumina<sup>®</sup> HiSeq 2500 Rapid Run
- Illumina<sup>®</sup> NextSeq 500/550
- Illumina<sup>®</sup> MiSeq

## Considerations for Sequencing

More details on sequencing guidelines can be found [here](#).

## Summary

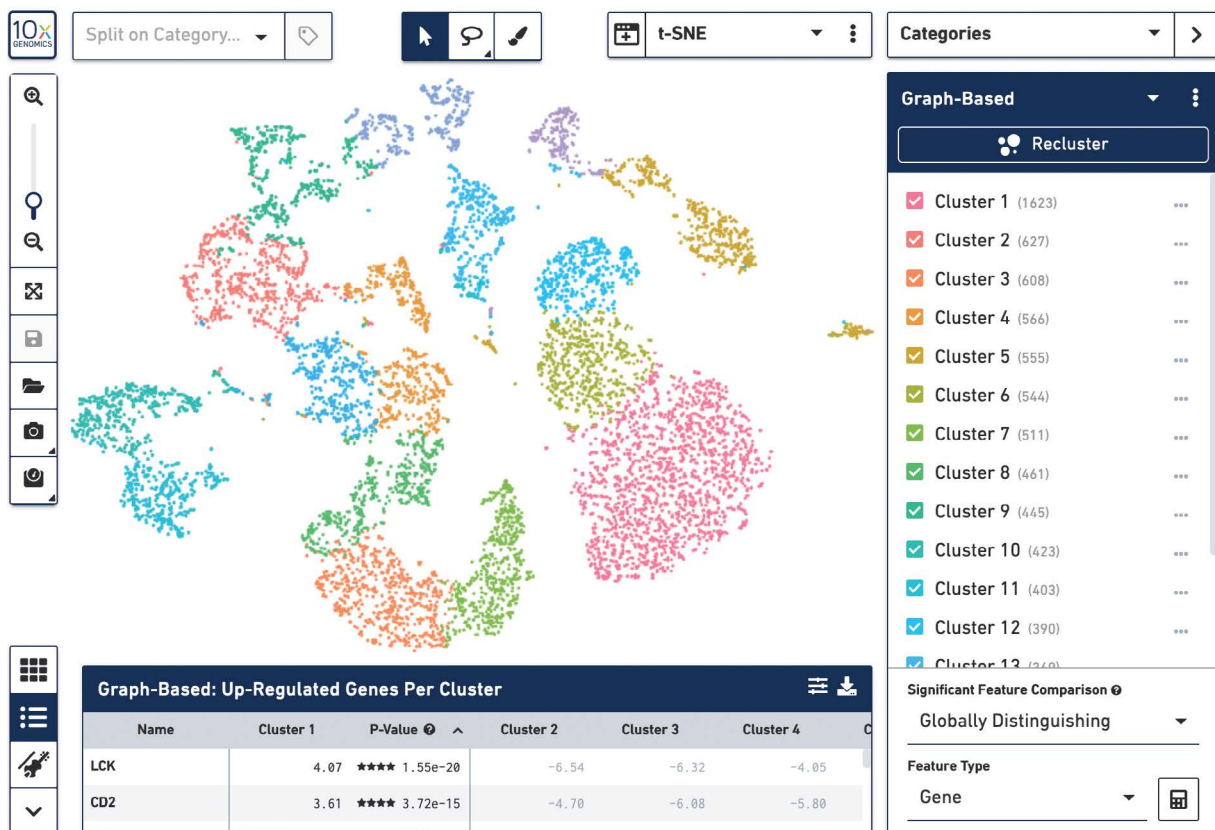
The steps leading up to sequencing are critical in the single-cell sequencing workflow and the specific protocol should be selected and optimized based on the experimental question. After high-quality cDNA libraries have been prepared, researchers can proceed with confidence to sequencing. If you would like to discuss library preparation or sequencing method, and how they can be integrated with your research, please contact 10x Genomics at [support@10xgenomics.com](mailto:support@10xgenomics.com) for more information.

## 5. Data Analysis

After sequencing of dCODE Dextramer® specific T cells is completed, downstream analysis can be performed. Generally, the study of single-cell sequencing data involves crude data analysis, including file conversion, initial data cleanup, and setting the thresholds for downstream analysis followed by visualization and interpretation. dCODE Dextramer® reagent-only analyses are not supported currently by 10x Genomics. For enhanced cell calling, dCODE Dextramer® staining should be combined with a small panel of TotalSeq™-C antibodies. *See below for some general considerations for analyzing dCODE Dextramer® sequencing data.*

### Data Visualization with Loupe Browser and Loupe V(D)J Browser Platforms

For visualization and data interpretation, the [Loupe Browser](#) and Loupe [V\(D\)J Browser](#) platforms are available. The research objective, single-cell isolation platform, and general workflow considerations will largely determine the analysis strategy. For further information on data analysis, see the [Application Note: Redefining Cellular Phenotyping: Comprehensive Characterization and Resolution of the Antigen-Specific T Cell Response](#).



**Figure 7: Analysis of Sequencing Data in 10x Genomics Loupe Browser.**

Loupe Browser is a desktop application that provides interactive visualization functionality to analyze data from different 10x Genomics solutions. Loupe Browser allows you to easily interrogate different views of your 10x Genomics data to quickly gain insights into the underlying biology.

## Selecting the UMI Count Threshold

Selecting a threshold for antigen binding will focus your analysis on the cells with the strongest evidence of specific binding.

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## Comparison to a Negative Control

Antigens that meet the minimum UMI threshold should be compared to the threshold set against the negative control dCODE Dextramer<sup>®</sup> reagents on a cell-by-cell basis to assess specificity.

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## Binding Specificity

Some cells may appear to show specific binding to more than one antigen even after filtering on UMI and negative control threshold criteria. This can represent a true biological event as each TCR may bind to different pMHCs but may also represent an artifact. For example, simultaneous binding to very similar pMHCs is very likely to be a genuine result.

If expanded clonotypes (groups of cells with the same TCR sequences) are observed, it should be considered whether the cells within that clonotype exhibit concordant dCODE Dextramer<sup>®</sup> specificities. Cells with the same TCR can be expected to exhibit the same binding specificities, and so analysis of the number of cells within a clonotype that bind each dCODE Dextramer<sup>®</sup> can be valuable.

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## Summary

There is no one correct way to carry out an analysis of single-cell sequencing experiments, and therefore, the data analysis strategy should be designed for each individual experiment. If you'd like to discuss data analysis strategy or experience difficulties with data analysis when using dCODE Dextramer<sup>®</sup> reagents, please contact 10x Genomics at [support@10xgenomics.com](mailto:support@10xgenomics.com) or Immudex at [customer@immudex.com](mailto:customer@immudex.com) for more information.

## 6. Applications of dCODE Dextramer®

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### Application of dCODE Dextramer®

The dCODE Dextramer® has a multitude of applications ranging from epitope discovery to monitoring immune status, detection of antigen-specific T cells down to a single-cell level, and more.

See below for some examples of applications using the dCODE Dextramer® reagents.

If you'd like to discuss your experiment further, you can always contact [customer@immudex.com](mailto:customer@immudex.com).

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#### Application 1:

### Simultaneous Single-Cell Analysis of Multiple Analytes Resolves T-Cell Populations at High Resolution

A panel of 12 dCODE Dextramer® reagents covering four different HLA types displaying peptides from CMV, EBV, or Flu was used to identify T-cell antigen specificities within a CMV seropositive and seronegative blood sample. Please see [poster](#) or [Application Note: Redefining Cellular Phenotyping: Comprehensive Characterization and Resolution of the Antigen-Specific T Cell Response](#) for more information.

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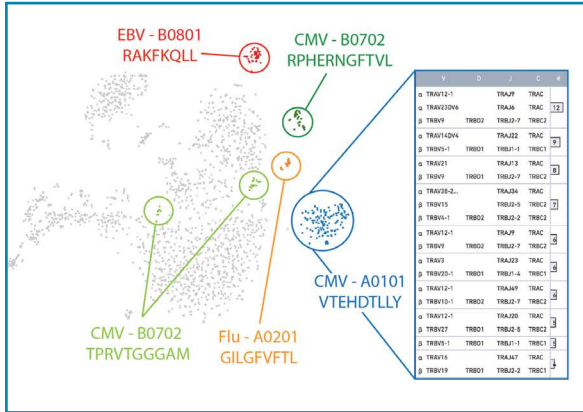
#### Data Analysis and Results

Following staining with dCODE Dextramer® reagents, cells were stained with TotalSeq™-C antibodies from BioLegend to enable analysis of surface protein expression. Cells were partitioned into single-cell reactions using the 10x Genomics Chromium platform, then sequenced.

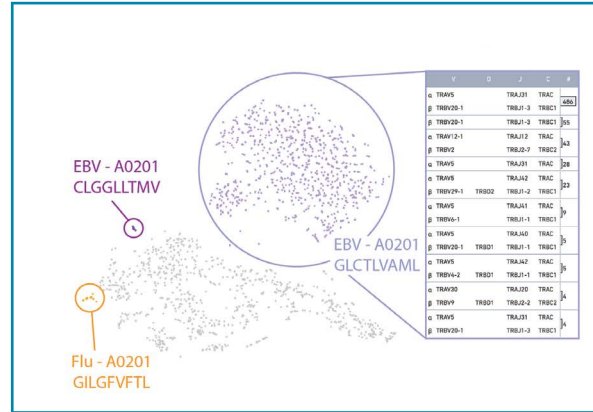
CD4-/CD8a+/dCODE Dextramer®+ positive cells were aggregated, and t-SNE projections were generated by Cell Ranger and visualized in Loupe Browser. Cells were clustered on gene expression data with graph-based clustering to provide deeper phenotyping of the identified antigen-specific populations at the single-cell level. Each identified T-cell subtype was associated with its TCR clonotype by V[D]J sequencing.

CMV-specific T-cell subpopulations, not present in the CMV seronegative donor, were identified in the CMV seropositive sample. EBV or Flu-specific T-cell populations were identified within both donors (**Fig. 8**).

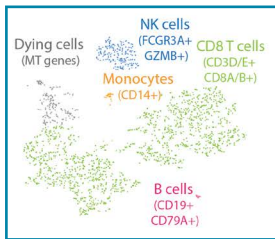
**CMV Seropositive Donor**  
Antigen Specificity and TCR clonotypes



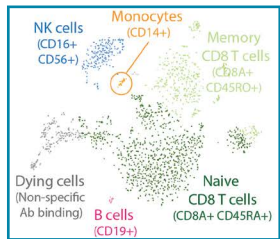
**CMV Seronegative Donor**  
Antigen Specificity and TCR clonotypes



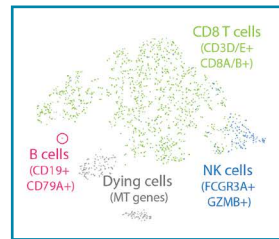
Gene Expression



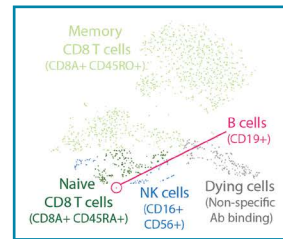
Surface Protein Expression



Gene Expression



Surface Protein Expression



**Figure 8: Sequencing Data, Including Clonal Distribution, Allowed Phenotyping of Each T-Cell Population.** Combining gene expression and cell surface protein expression using labelled barcoded antibodies provides increased resolution of cell-type characterization. Visual analysis was performed using Loupe Browser and Loupe V(D)J Browser. Each dot represents a single cell.

## Application 2:

# Single-Cell Immune Phenotyping of Antigen-Specific CD4+ T Cells using MHC II dCODE Dextramer®

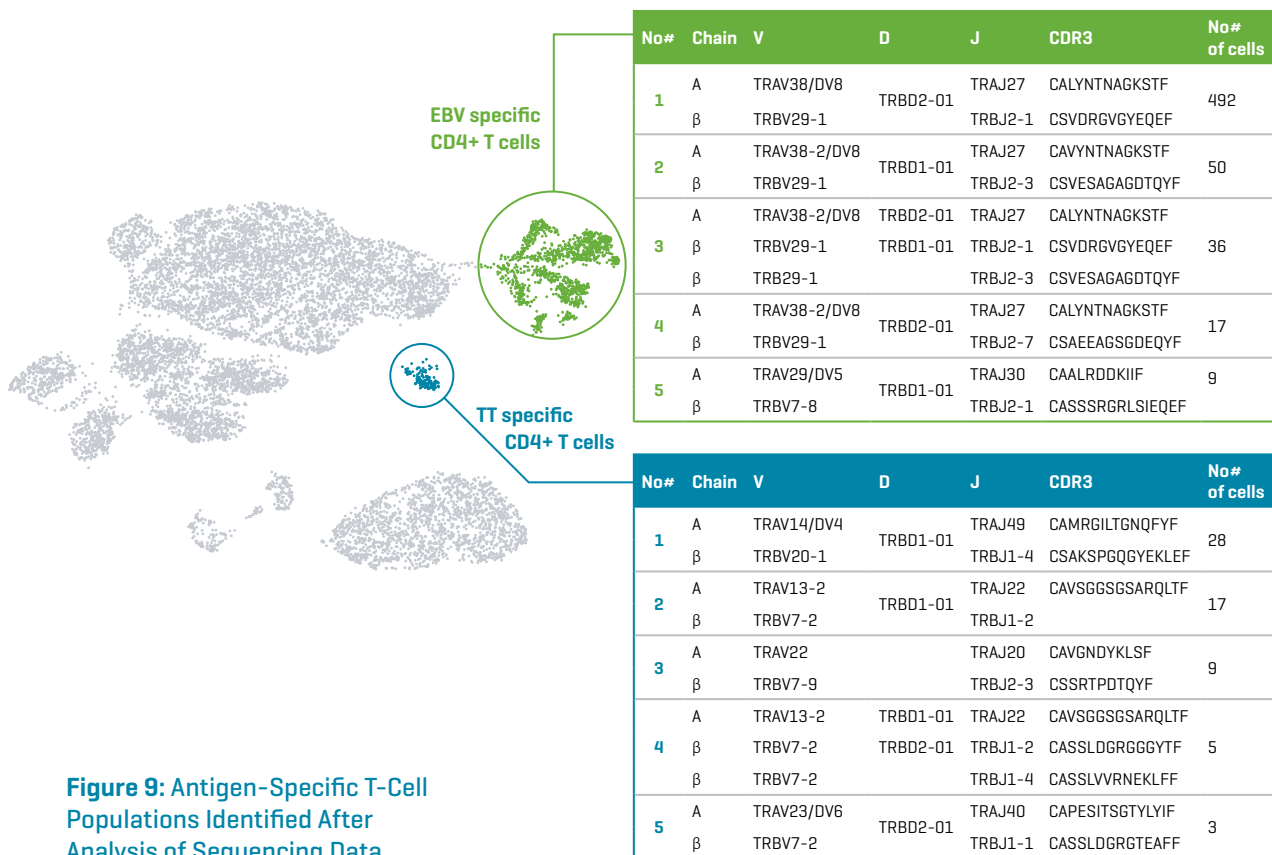
A panel of three different dCODE Dextramer® reagents was used to stain a sample of CD4+ T cells to identify EBV and Tetanus Toxoid (TT) antigen-specificities within the same sample. Please see case study available at [www.immudex.com](http://www.immudex.com) for more information.

## Results

The CD4+ T cells were expanded in the presence of EBV and TT peptides and subjected to the 10x Genomics Single Cell Immune Profiling workflow followed by sequencing without going through an enrichment step.

Sequencing data were analyzed using Cell Ranger analysis, and results were visualized by Loupe Browser. CD4+ T cells were clustered and classified based on gene and dCODE Dextramer® expression. The analysis of dCODE Dextramer® positive binding identified EBV- and TT-specific CD4+ T cells.

The TCR clonotypes for the dCODE Dextramer® positive EBV- and TT-specific populations were compared to known TCR sequences using the V(D)J database. None of the V(D)J sequences were identified in V(D)J database, indicating that dCODE Dextramer® can be used to uncover novel TCR sequences specific for MHC II/EBV and MHC II/TT, for which there was no available information before.



**Figure 9: Antigen-Specific T-Cell Populations Identified After Analysis of Sequencing Data.**

## 7. Frequently Asked Questions

### 1. Can I label my sample with antibodies and dCODE Dextramer® reagents?

Yes, cell staining with dCODE Dextramer® is compatible with staining with fluorescence- or DNA-barcoded antibodies. dCODE Dextramer® and TotalSeq™-C antibodies can be used in the same experiment, as long as the DNA barcodes of each reagent do not overlap. However, because binding of staining antibodies to surface receptors may result in steric hindrance for the dCODE Dextramer® binding to their cognate TCRs, it is crucial to stain with dCODE Dextramer® reagent before staining with antibodies. If combining dCODE Dextramer® and TotalSeq™-C antibodies with fluorescently labeled antibodies for FACS, it is important to select distinct clones for the included antibodies.

### 2. Are there any recommendations for staining with panels above 50 dCODE Dextramer® reagents?

Yes, each added dCODE Dextramer® will dilute the reaction, which should be considered when using large panels of dCODE Dextramer® (>50 reagents). The dilution effect is caused by an increase in the final staining volume resulting in a weaker signal of each dCODE Dextramer®. To avoid signal dilution when using large panels, we recommend:

- Using the lowest possible volume of cell suspension, for example, by resuspending the cell pellet directly in the dCODE Dextramer® mix
- Increasing incubation times to enhance the signal of each dCODE Dextramer®

### 3. Can dCODE Dextramer® technology also be used for B-cell research?

Yes, dCODE Dextramer® technology can also be used for applications beyond T-cell research due to the dCODE® Klickmer technology. dCODE® Klickmer is a customizable version of dCODE Dextramer® reagents. dCODE® Klickmer allows attachment of antigen-specific molecules directly onto the dextran backbone allowing enabling customized targeting. For example, detecting antigen-specific B-cell populations at a single-cell level is possible by attaching B-cell-specific antigens to dCODE® Klickmer.

### 4. Can I enrich my T-cell population using magnetic beads instead of flow sorting?

Enriching via magnetic beads is not recommended for use in conjunction with dCODE Dextramer® reagents due to the low frequency of the target population. At this time, only enriching via flow sorting has been validated for dCODE Dextramer® experiments.

### 5. Can two cells be found in the same GEM?

Yes, it is possible that two cells are encapsulated in one gel bead. To limit the chance of multiplets, a low number of cells is used [10,000 cells], while gel beads are added in excess. The possibility of two cells being in one gel bead, and therefore in one GEM is less than 10%. The presence of dCODE Dextramer® does not increase the possibility of having cell doublets. It has been observed that the number of doublets is constant with different amounts of dCODE Dextramer® and is comparable to the cells not stained with dCODE Dextramers®.

### 6. How many cells can be loaded on a 10x Genomics Chromium chip? What is the minimum number that can be loaded?

Each 10x Genomics Chromium chip has 8 channels, and it is recommended to recover between 500-10,000 cells per channel on the Chromium chip. The minimum number that should be recovered is 500 cells. Loading more cells is possible, but it increases the chances of cell doublets [2 cells landing in the same GEM].



## 8. Resources

We are dedicated to helping you get the most out of your Single Cell Immune Profiling with dCODE Dextramer® reagents by offering multiple helpful resources and support.

### Resources from Immudex

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**dCODE Dextramer®**

[www.immudex.com/  
products/basic-research/  
dcode-dextramer-ngsmulti-omics/](http://www.immudex.com/products/basic-research/dcode-dextramer-ngsmulti-omics/)

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**MHC allele list**

[www.immudex.com/resources/  
mhc-alleles-list/](http://www.immudex.com/resources/mhc-alleles-list/)

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**Webinars**

[www.immudex.com/resources/webinars/](http://www.immudex.com/resources/webinars/)

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**Protocol**

[www.immudex.com/resources/](http://www.immudex.com/resources/)

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**Case Studies**

[www.immudex.com/resources/  
Case-studies](http://www.immudex.com/resources/Case-studies)

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**Homepage**

[www.immudex.com](http://www.immudex.com)

### Resources from 10x Genomics

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**Chromium Single Cell Immune Profiling**

[www.10xgenomics.com/products/  
single-cell-immune-profiling](http://www.10xgenomics.com/products/single-cell-immune-profiling)

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**10x Genomics Support Site**

[support.10xgenomics.com](http://support.10xgenomics.com)

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**Loupe Browser**

[www.10xgenomics.com/products/  
loupe-browser](http://www.10xgenomics.com/products/loupe-browser)

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**Datasets**

[www.support.10xgenomics.com/  
single-cell-vdj/datasets](http://www.support.10xgenomics.com/single-cell-vdj/datasets)

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**Application Notes**

[www.10xgenomics.com/resources/  
document-library/Application-notes](http://www.10xgenomics.com/resources/document-library/Application-notes)

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**Homepage**

[www.10xgenomics.com/](http://www.10xgenomics.com/)

### Contact us

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Let us know if you experience difficulties or have questions. Together we will help you get the most out of your Single Cell Immune Profiling with dCODE Dextramer® products.

**Immudex ApS**

[customer@immudex.com](mailto:customer@immudex.com)

**10x Genomics**

[support@10xgenomics.com](mailto:support@10xgenomics.com)