IMMUDEX®
PRECISION IMMUNE MONITORING

Multiplex dCODE Dextramer® Profiling of Antigen-Specific T Cells

Single-Cell Evaluation Using a Panel of dCODE Dextramer® Reagents and the 10x Genomics Feature Barcode Protocol

Adapted from scientific poster by Jacobsen *et al.* presented at SITC 2019.

Identifying disease-specific T-cell epitopes is key to developing novel immunotherapies and vaccines, while profiling them during a cellular immune response (e.g., tumor development or destruction) informs personalized immunotherapy. Combining dCODE Dextramer® technology with the 10x Genomics Feature Barcode protocol allows coupling the T-cell receptor (TCR) specificity and sequence of antigen-specific T cells (ASTC) to analyses of their transcriptional profile. Here we show data from the analysis of a blood sample with a panel of 50 dCODE Dextramer® specificities spanning 8 alleles and include 6 negative controls.

Designing a Panel of dCODE Dextramer® Reagents

dCODE Dextramer® (10x) reagents have been designed for compatibility with the 10x Genomics Feature Barcode protocol for Single Cell Immune Profiling. Each unique DNA barcode is specific for the MHC-peptide displayed on the dCODE Dextramer® reagent, which also carries a PE-label for sorting and enrichment of rare cell populations.

dCODE Dextramer® reagents enable the multiplexed identification of many T-cell specificities in the same sample. Customized dCODE Dextramer® libraries can be used to profile donor T cells and gain detailed understanding of its T-cell immunity.

Highlights

We performed in-depth phenotyping of immune relevant cells:

- Identified four antigen-specific T-cell populations in samples of CD8-sorted hPBMCs
- Obtained and quantified paired clonal TCR sequence and TCR specificity by overlaying single-cell V(D)J expression onto dCODE Dextramer®-positive cell clusters
- Further discriminated HLA-DR and CCR7 subpopulations within each antigen-specific T-cell cluster using TotalSeq™ antibody data

The panel used in this study consisted of 50 dCODE Dextramer® reagents comprising different viral and cancer epitopes, and 6 negative control dCODE Dextramer® reagents (**Table 1**).

Cat. no	Alelle	Peptide	Antigen	Cat.no	Alelle	Peptide	Antigen
WA2131-PfBC	A*0101	VTEHDTLLY	CMV/Viral	WB2177-PfBC	A*0201	RMFPNAPYL	WT-1/cancer
WB2660-PfBC	A*0201	KTWGQYWQV	gp100/Cancer	WB2191-PfBC	A*0201	YLNDHLEPWI	BCL-X/Cancer
WB2162-PfBC	A*0201	ELAGIGILTV	MART-1/Cancer	WB2652-PfBC	A*0201	MLDLQPETT	HPV/Viral
WB3697-PfBC	A*0201	CLLWSFQTSA	Tyrosinase/Cancer	WC2197-PfBC	A*0301	KLGGALQAK	IE-1/CMV
WB2158-PfBC	A*0201	IMDQVPFSV	gp100/Cancer	WC2656-PfBC	A*0301	RLRAEAQVK	EBV/Viral
WB3247-PfBC	A*0201	SLLMWITQV	NY-ESO-1/Cancer	WC2632-PfBC	A*0301	RIAAWMATY	BCL-2L1/ Cancer/
WB3497-PfBC	A*0201	KVAELVHFL	MAGE A3/Cancer	WD2175-PfBC	A*1101	IVTDFSVIK	EBV/Viral
WB3474-PfBC	A*0201	KVLEYVIKV	MAGE-A1/Cancer	WD2149-PfBC	A*1101	AVFDRKSDAK	EBV/Viral
WB5066-PfBC	A*0201	CLLGTYTQDV	Kana. B dioxyg.	WK2138-PfBC	B*3501	IPSINVHHY	CMV/Viral
WB2143-PfBC	A*0201	LLDFVRFMGV	EBV/Viral	WF2196-PfBC	A*2402	AYAQKIFKI	CMV/Viral
WB3307-PfBC	A*0201	LLMGTLGIVC	HPV/Viral	WF2133-PfBC	A*2402	QYDPVAALF	CMV/Viral
WB2144-PfBC	A*0201	CLGGLLTMV	EBV/Viral	WH2165-PfBC	B*0702	QPRAPIRPI	EBV/Viral
WB3531-PfBC	A*0201	YLLEMLWRL	EBV/Viral	WH2136-PfBC	B*0702	TPRVTGGGAM	CMV/Viral
WB3529-PfBC	A*0201	FLYALALLL	EBV/Viral	WH2166-PfBC	B*0702	RPPIFIRRL	EBV/Viral
WB2161-PfBC	A*0201	GILGFVFTL	Flu/Viral	WH2135-PfBC	B*0702	RPHERNGFTVL	CMV/Viral
WB2130-PfBC	A*0201	GLCTLVAML	EBV/Viral	WI2148-PfBC	B*0801	RAKFKQLL	EBV/Viral
WB2132-PfBC	A*0201	NLVPMVATV	CMV/Viral	WI2137-PfBC	B*0801	ELRRKMMYM	CMV/Viral
WB2139-PfBC	A*0201	ILKEPVHGV	HIV/Viral	WI2147-PfBC	B*0801	FLRGRAYGL	EBV/Viral
WB5335-PfBC	A*0201	FLASKIGRLV	P-lipase				
WF2639-PfBC	A*2402	CYTWNQMNL	WT1/Cancer	**Negative controls**			
WB2646-PfBC	A*0201	RTLNAWVKV	HIV/Viral	WA3580-PfBC	A*0101	SLEGGGLGY	NC
WB2157-PfBC	A*0201	KLQCVDLHV	PSA/Cancer	WA3579-PfBC	A*0101	STEGGGLAY	NC
WB2141-PfBC	A*0201	LLFGYPVYV	HTLV-1/Viral	WB2666-PfBC	A*0201	ALIAPVHAV	NC
WB3338-PfBC	A*0201	SLFNTVATL	HIV/Viral	WF3231-PfBC	A*2402	AYSSAGASI	NC
WB3339-PfBC	A*0201	SLYNTVATLY	HIV/Viral	WH3397-PfBC	B*0702	GPAESAAGL	NC
WB3340-PfBC	A*0201	SLFNTVATLY	HIV/Viral	NI3233-PfBC	NR	AAKGRGAAL	NC

Table 1. dCODE Dextramer® panel

Materials and Methods

- Panel of dCODE Dextramer® reagents compatible with 10X Genomics [**Table 1**]
- TotalSeq™-C antibodies [BioLegend]
- Single-cell sorter [FACS]
- 10x Chromium controller
- Chromium single cell V[D]J reagent Kit

Workflow

- CD8-enriched hPBMCs from a healthy donor [HLA-A*201, A*1102, B*3501] were stained with the prepared panel of 50 dCODE Dextramer® reagents
- The stained cells were sorted to isolate the CD8+ dCODE Dextramer®+ cells, which were then loaded onto the 10x Chromium controller
- Three libraries were generated using the Chromium Single Cell V[D]J Reagent Kit with feature barcode technology – feature barcode, V[D]J and RNA expression libraries.
- Next-generation sequencing was performed on a Illumina® sequencing platform.

NGS Data Analysis

Sequencing data were analyzed by dimensional reduction, clustering and t-SNE calculation using Cell Ranger. Loupe Cell Browser and Loupe V[D]J Browser from the 10x Genomics software package were used for visual analysis.

The following stringent rules were set up for the analysis:

- Background threshold was defined by the negative controls. Specifically, thresholds 1–4 [threshold = log2[reads+1]] were tested on the negative controls and a threshold of 4 were used to analyze each dCODE Dextramer® reagent specificity. The same approach was used for the antibody staining, based on the isotype control antibodies.
- dCODE Dextramer®+ antigen-specific T cells were defined by a signal over the threshold
- Only MHC matching the alleles of the donor were considered positive antigen-specific T cells
- To be included in the analysis, ASCTs must be CD8+ and bind only one dCODE Dextramer® specificity

Results

Data for each of the 44 dCODE Dextramer® reagents were analyzed and visualized by t-SNE plot [**Fig. 1A**]. Four populations of antigen specific T cells were detectable: Influenza [Flu], two Epstein-Barr virus [EBV], and MART1.

WB2161 A*0201/GILGFVFTL [Flu MP, Influenza]
WD2175 A*1101/IVTDFSVIK [EBNA 3B, EBV]
WD2149 A*1101/AVFDRKSDAK [EBNA 3B, EBV]
WB2162 A*0201/ELAGIGILTV [MART-1]

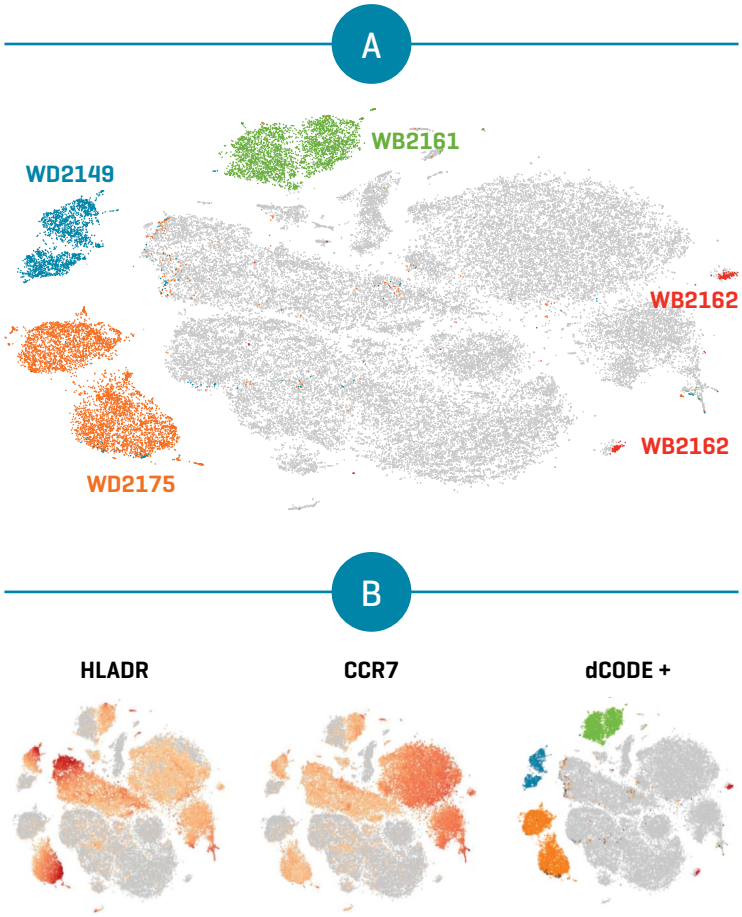

Fig. 1: [A] t-SNE plot of the dCODE Dextramer®+ T cells, clustered and colored according to specificities. [B] HLA-DR and CCR7 staining corresponds to the bisection of the clusters.

Each identified cluster was visibly bisected, indicating different phenotypes within a population. Using TotalSeq™ C Antibody data, the two markers HLA-DR and CCR7 were shown to stain one half of each bisected antigen-specific T cell cluster. Furthermore, labeling occurred on the same half of each identified population and thus, the segregation is likely driven by these two activation markers [**Fig. 1B**].

Based on V(D)J sequences, the distribution of unique TCR clones was evaluated for each of the four positive antigens (**Fig. 2**) with the following findings:

- A high number of different TCR clones was detected for the influenza epitope (Flu).
- Fewer but larger TCR clonotypes were detected for the two EBV epitopes.
- A low number of TCR clones with a wide distribution was detected for the MART1 epitope. In fact, only one clonal TCR with more than one cell was observed. The rest were single clonotypes.

Discussion

High-plex staining of T cells with dCODE Dextramer® reagents and subsequently TotalSeq™-C antibodies followed by single-cell multi-omic analysis using the 10x Chromium Feature Barcoding workflow is a powerful tool for deep phenotyping of immune relevant cells.

Overlaying the TotalSeq™-C antibody data for the antigen-specific T cells identified reveals a phenotypic difference in the two activation markers HLA-DR and CCR7 expression, which drive the bisection of each of the antigen-specific populations seen in the t-SNE plots. Indicating that the antigen-specific T cells populations consist of at least two phenotypic different antigen-specific T cells. We also easily paired clonal TCR sequence and TCR specificity by overlaying each cell's V(D)J expression onto the dCODE Dextramer®+ cell clusters (data not shown).

The four identified subsets of antigen-specific T-cell populations included; one influenza (Flu), two EBV, and one MART1 specific population. The influenza T-cell population exhibited a high number of TCR clones, potentially reflecting that influenza is a recurring infection, where some TCR clones fall away in between infective periods. Still, new clones are generated at each infection cycle, resulting in a highly diverse profile of expanded influenza-specific T cell clones. The EBV-specific T cells were fewer but exhibited large TCR clonotypes, possibly due to EBV being a persistent infection with a continuous selection of TCR clones. As a result, clonal distribution narrows, and the number of cells of the same clones increases. The MART1 TCR clones were detected with low frequency, and wide distribution as MART1 is an endogenous cancer antigen that remains unexpanded in healthy donors.

Specificity (pMHC)	FLU	EBV1	EBV2	MART1
Number of positive cells	2594	4472	1846	187
Number of specific clones	732	389	166	180
No. cell in highest freq. clone	110	2373	1241	8
Frequency of highest clone	4%	53%	67%	4%
Clones of > 1 cell	251	44	19	1
Clones of 1 cell	481	345	147	179
Freq. of Clones with 1 cell	66%	89%	89%	99%
Frequency of Clones >1 cell	34%	11%	11%	1%

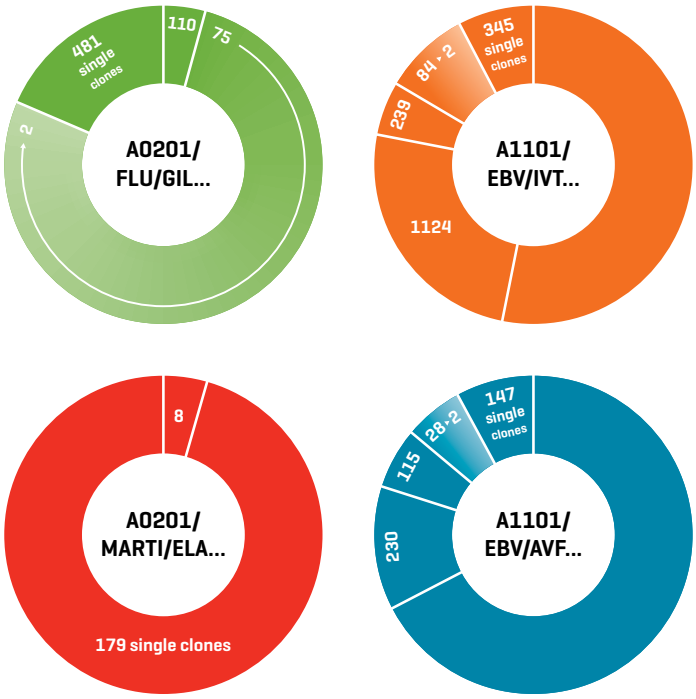

Fig. 2: Clonal distribution of antigen-specific T cells. (A) Parameters measured for each of the four positive antigens. (B) Distribution and frequency of clones for each positive antigen. While the EBV epitopes detected few but large clones, the influenza and MART1 epitopes detected high numbers of TCR clones, the first of variable sizes and the latter almost exclusively single clonotypes.

This proof-of-concept study confirmed that dCODE Dextramer reagents enable detection of antigen-specific T cells with additional information on phenotypic traits and V(D)J sequencing on the 10x Chromium platform. The dataset obtained contains essential unlimited information. Filtering relevant biological insights requires both unbiased bioinformatics and deep phenotyping analyses. However, for cells that do not cluster well in t-SNE space, such as low-frequency cells or dCODE Dextramer®+ cells with very diverse TCRs, the t-SNE cluster analysis was restrained - an issue overcome in later studies.

Conclusions

The combination of optimal dCODE Dextramer® reagents with the 10x Chromium Feature Barcoding workflow streamlines the characterization of antigen-specific T-cell populations in samples. Multiplexed staining and directly connected transcriptional information reveal detailed phenotypes and facilitate T-cell clonal analysis. The information obtained at the level of single cells can be instrumental in the development of novel therapies or decision-making in personalized immunotherapy.

Resources from Immudex

We are dedicated to helping you get the most out of your dCODE Dextramer ® reagents by offering multiple helpful resources and support:

dCODE Dextramer®

Access the dCODE Dextramer® site where you can find everything from how to order to the latest news on dCODE Dextramer® products.

Read more

Technical Support

Let us know if you experience difficulties or have questions. Immudex will help you get the most out of your dCODE Dextramer® products.

customer@immudex.com

Resources

Easy access to our complete library of customer publications, posters and webinars and protocols, and many other useful resources.

Read more

Customer Product Development

To advance and support promising research, Immudex provide customized solutions with the best quality. Visit the customer-defined product site to learn more of the service.

Read more

Case Studies and Application Notes

Immerse yourself in comprehensive case studies containing information on the applications of dCODE® reagents.

Read more

Contact us

Immudex ApS
Bredevej 2A
2830 Virum
Denmark

Email: info@immudex.com
Tel.: +45 29 13 42 24