Poster# XXXX

Advancements in single cell multiomic profiling of antigen-specific T cells with dCODE Dextramer® (RiO) and BD® AbSeq Reagents on the BD Rhapsody™ Single-Cell Analysis System

Cynthia Sakofsky,[1] Kivin Jacobsen,[2] Vadir Lopez-Salmeron,[4] Liselotte Brix,[2] Aruna Ayer[1].

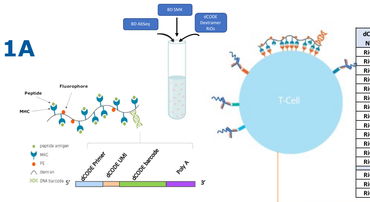
[1]BD Biosciences, 2350 Qume Drive, San Jose, CA 95131, [2]Immudex, Fruebjergvej 3 DK-2100 Copenhagen, Denmark, [3]BD Biosciences, Tullastrasse 8-12 69126 Heidelberg, Germany

Abstract

The advancement of immunotherapies and drug development relies on the characterization of antigen-specific T cells. However, due to their low frequency among T cells and their inherent weak affinity to bind known MHC-peptide complexes, detection of these cells has been difficult. Furthermore, pairing this information with the corresponding variable (V), diversity (D), and joining (J) [V(D)J] sequences of the antigen-specific T cell receptors has also been challenging due to hurdles in sequencing these large regions, particularly at the single-cell level. Here, we have expanded our previous work of combining two powerful technologies, Immudex® dCODE Dextramer® (RiO) Reagents and the BD Rhapsody™ Single-Cell Analysis System, to detect and characterize low-frequency antigen-specific T cells, including the full sequences of the V(D)J gene segments of the T cell receptors, as well as profile transcriptome and protein expression. Specifically, thousands of sorted PBMCs were multiplexed to provide high-throughput detection of individual antigen-specific CD8⁺ T cells in combination with the corresponding full V(D)J sequences of the T cell receptors. In addition, we simultaneously obtained gene expression data for over 350 immune-related mRNAs as well as cell phenotypes using a panel of 15 cell surface BD® AbSeq Protein Markers. Together these data can be used to define T cell phenotypes associated with T cell activation states, alongside antigen specificity of enriched CD8⁺ dCODE Dextramer® positive antigen-specific T cells from PBMC populations. This study showcases the importance of multiomic analysis for high-resolution T cell profiling that has broader implications and utility in immuno-oncology, infectious diseases and autoimmunity.

Methods

Seamless integration of dCODE Dextramer®(RiO) technology into the BD® Rhapsody AbSeq + SMK + TCR Full Length Workflow

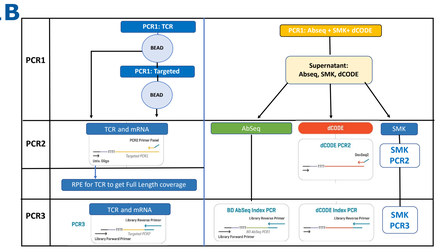


dCODE® RiO Panels

dCODE ID Number	Specificity	MHC allele/peptide	Sample
RiO_5024	Neg.Ctrl	A0101_SLEGGGLGY	1
RiO_5025	Neg.Ctrl	A0201_ALIAPVHAV	1
RiO_5026	Neg.Ctrl	A2402_AYSSAGASI	1
RiO_5027	Neg.Ctrl	B0702_GPAESAAGL	1
RiO_5028	Neg.Ctrl	General NC_NC	1
RiO_5029	FLU	A0201_GILGFVFTL	1
RiO_5030	EBV	A0201_GLCTLVAML	1
RiO_5031	EBV	A0201_FLYALALLL	1
RiO_5032	CMV	A0201_NLVPMVATV	1
RiO_5035	CMV	A0201_VLEETSVML	1
RiO_5036	EBV	B3501_EPLPQGQLTAY	1
RiO_5037	EBV	B3501_HPVGEADYFEY	1
RiO_5038	CMV	B3501_IPSINVHHY	1
RiO_5021	EBV	DRB1*0101_TSLYNLRRGTALA	2
RiO_5022	TT	DRB1*0101_KYSFYPSVISKV	2
RiO_5023	Neg.Ctrl	DRB1*0101_PVSKMRMATPLLMQA	2

BD® AbSeq Panel

BD Abseq specificity	Clone	Product Number
CD11b	M1/70	940008
CD45RA	HI100	940011
CD69	FN50	940019
CD279	EH12.1	940015
TCRgd	B1	940057
TIM-3	7D3	940066
LAG-3	T47-530	940080
CD56	NCAM16.2	940007
CD19	525C1	940004
CD4	SK3	940001
CD3	SK7	940000
HLA-DR	G46-6	940010
CD45RO	UCHL1	940022
CD62L	DREG-56	940041
TCRab	IP26	940074



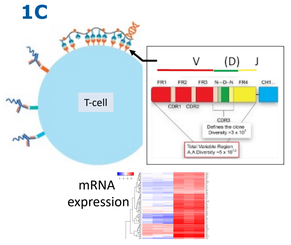


Figure 1. Integration of Immudex® dCODE Dextramer®(RiO) profiling into the TCR full length, Targeted mRNA, BD® AbSeq and Sample Tag workflow. dCODE (RiO) reagents contain a PE fluorophore that can be used for cell sorting, as well as a dCODE-specific barcode associated with a specific antigen epitope that can be amplified and sequenced simultaneously alongside BD abseq, sample tag, targeted mRNA and TCR full length libraries in the BD Rhapsody™ system. **(A)** In this study, two cell samples were first stained with a panel of dCODE (RiO) reagents followed by co-staining of BD® AbSeqs and Sample Tag. Cell sample 1 were resting hPBMCs that were sorted for dCODE⁺ and live CD8⁺ cells using a BD FACSAria™ flow sorter by additional staining with anit-CD8 APC-H7 antibody and 7-AAD dye for live/dead cell detection. Cell sample 2 were hPBMCs stimulated with two peptide antigens, EBV and Tetanus toxoid (TT) then enriched for CD4⁺ cells using magnetic separation. Both cell samples were multiplexed into a single BD Rhapsody cartridge system, as well as individually loaded into separate cartridges. Using the BD Rhapsody™ System, single cells were partitioned into microwells followed by the addition of beads prior to cell lysis. The beads enabled capturing of polyadenylated transcripts and oligonucleotide tags from single cells after cell lysis whereby, cDNA and library preparations were completed (as shown in **1B**). Next generation sequencing (NGS) and data analysis followed. **(C)** Using dCODE (RiO) reagents alongside our BD Rhapsody Full Length TCR assay we can identify the full length TCR sequence that can recognize a specific antigen epitope in the HLA complex, while also profiling protein and gene expression of single antigen-specific T cells.

Results

dCODE Dextramer® reagents were used alongside BD® AbSeqs to sensitively identify antigen specific T cells and profile cell surface proteins

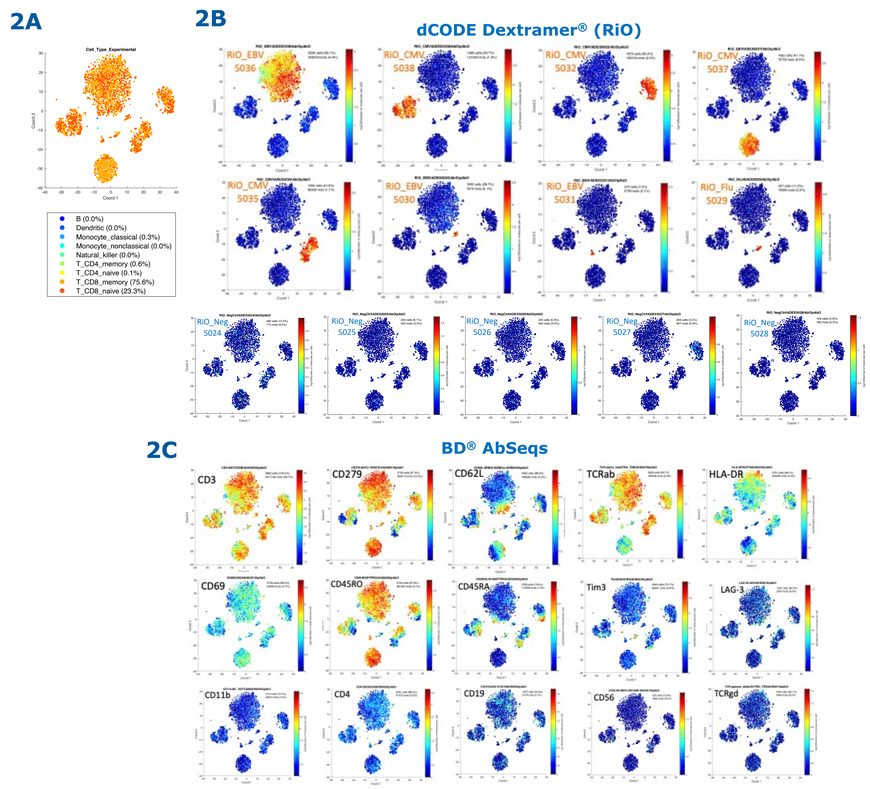
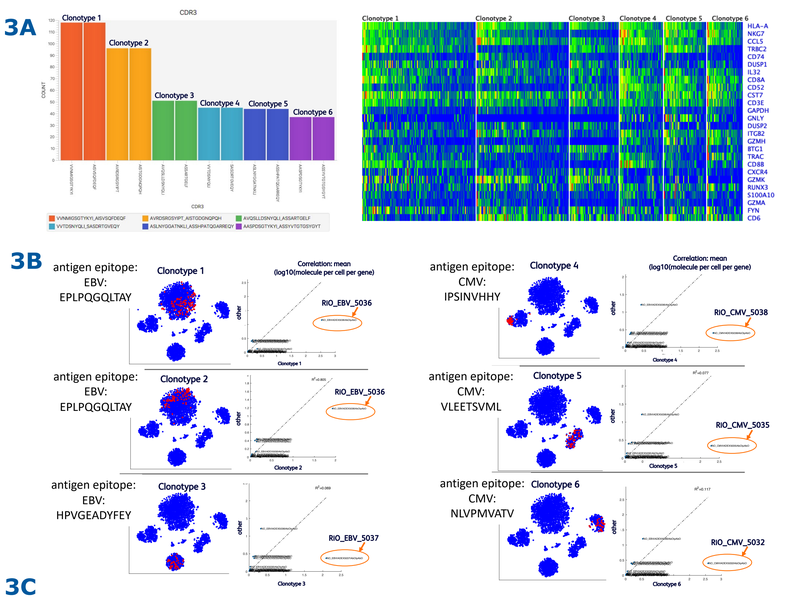


Figure 2. dCODE (RiO)® reagents and BD® AbSeqs were used simultaneously to identify antigen-specific T cells in hPBMCs and profile cell surface proteins. (A) tSNE plot from an experiment using ~ 5K sorted dCODE positive CD8⁺ cells with cell-type distribution shown. **(A)** tSNE plots highlighting the expression of a 13-plex dCODE Dextramer panel including 8 dCODE reagents with epitopes and 5 negative controls. **(B)** tSNE plots showing the expression of a 15-plex BD AbSeq panel that range in expression level.

Full length TCR sequences were identified in antigen specific CD8⁺ T cells and were linked to specific antigen epitopes using dCODE® (RiO) reagents



Clonotype	TCR chain	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
Clone 1	Alpha	RKEVEQDPGPFNVPEGATVAFNCTYS	NSASQS	FFWYRQDCRKEPKLLMS	VYSSGN	EDGRFTAQLNRASQYISLLIRDSKLSDSATYLC	VVNMIGSGTYKYI	FGTGTRLKVLA
	Beta	DAGITQSPRHKVTETGTPVTLRCHQT	ENHRY	MYWYRQDPGHGLRLIHY	SYGVKD	TDKGEVSDGYSVSRSKTEDFLLTLESATSSQTSVYFC	AISVSQFDEQF	FGPGTRLTVL
Clone 2	Alpha	GQNIDQPTEMTATEGAIVQINCTYQ	TSGFNG	LFWVQQHAGEAPTFLSY	NVLDGL	EEKGRFSSFLSRSKGYSYLLLKELQMKDSASYLC	AVRDSRGSYIPT	FGRGTSLIVHP
	Beta	DAGITQSPRHKVTETGTPVTLRCHQT	ENHRY	MYWYRQDPGHGLRLIHY	SYGVKD	TDKGEVSDGYSVSRSKTEDFLLTLESATSSQTSVYFC	AISTGDGNQPQH	FGDGTRLSIL
Clone 3	Alpha	EDQVTQSPEALRLQEGESSSLNCSYT	VSGLRG	LFWYRQDPGKGPEFLFT	LYSAGEE	KEKERLKATLTKKESFLHITAPKPEDSATYLC	AVQSLLDSNYQLI	WGAGTKLIIKP
	Beta	DSGVTQTPKHLITATGQRVTLRCSPR	SGDLS	VFWYQQSLDQGLQFLIQ	YYNGEE	RAKGNILERFSAQQFPDLHSELNLSSLELGDSALYFC	ASSARTGELF	FGEGSRLTVL
Clone 4	Alpha	RKEVEQDPGPFNVPEGATVAFNCTYS	NSASQS	FFWYRQDCRKEPKLLMS	VYSSGN	EDGRFTAQLNRASQYISLLIRDSKLSDSATYLC	VVTDSNYQLI	WGAGTKLIIKP
	Beta	GAVVSQHPSRVICKSGTSVKIECRSL	DFQATT	MFWYRQFPKKSLMLMAT	SNEGSKA	TYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYIC	SASDRTGVEQY	FGPGTRLTVT
Clone 5	Alpha	ILNVEQSPQSLIHVQEGDSTNFTCSFP	SSNFYA	LHWYRWETAKSPEALFV	MTLNGDE	KKKGRISATLNTKEGYSYLYIKGSQPEDSATYLC	ASLNYGGATNKLI	FGTGTLLAVQP
	Beta	EAEVAQSPRYKITEKSQAVAFWCDPI	SGHAT	LYWYRQILGQGPELLVQ	FQDESV	VDDSQLPKDRFSAERLKGVDSTLKIQPAELGDSAMYLC	ASSHPATQGARREQY	FGPGTRLTVT
Clone 6	Alpha	QQQVKQSPQSLIVQKGGIPIINCAYE	NTAFDY	FPWYQQFPGKGPALLIA	IRPDVSE	KKEGRFTISFNKSAKQFSLHIMDSQPGDSATYFC	AASPDSGTYKYI	FGTGTRLKVLA
Clone 6	Beta	NAGVTQTPKFQVLKTGQSMTLQCAQD	MNHEY	MSWYRQDPGMGLRLIHY	SVGAGI	TDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFC	ASSYVTGTGSYGYT	FGSGTRLTVV

Figure 3. Specific antigen epitopes linked to full length TCR sequences. (A) The six most frequent TCR CDR3 clonotypes were identified among CD8⁺ dCODE Dextramer positive antigen-specific T cells along with their mRNA gene expression profiles of 26 high expressing genes from the BD Rhapsody™ Immune Response panel (397 genes). **(B)** Differential gene expression plots of the 13 dCODE Dextarmer panel for each of the top six clonotypes show a high association to a single dCODE Dextramer. **(C)** Full length translated TCR sequence of the top six clonotypes.

BD Rhapsody sample multiplexing is compatible with dCODE Dextarmer® (RiO) reagents and provides a solution for high throughput detection of antigen-specific T cells

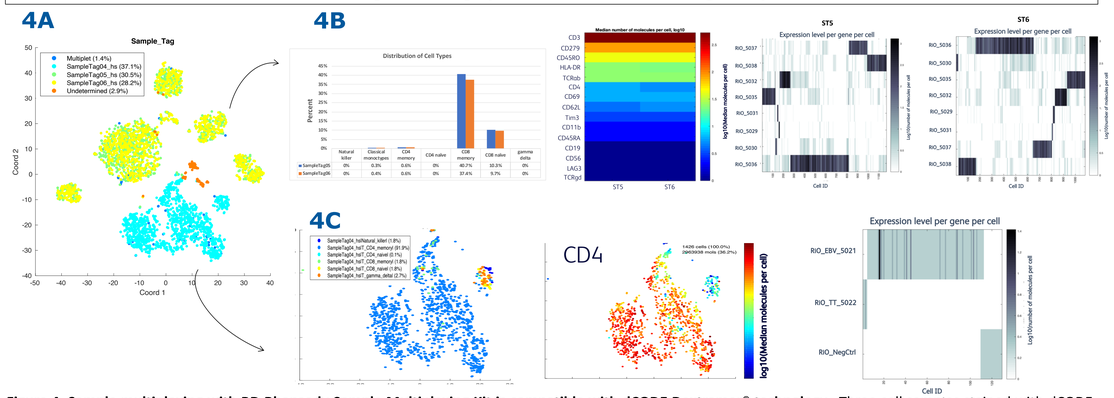


Figure 4. Sample multiplexing with BD Rhapsody Sample Multiplexing Kit is compatible with dCODE Dextramer® technology. Three cell samples stained with dCODE (RiO) reagents and BD AbSeq were multiplexed with BD SMK and loaded into a single BD Rhpasody cartridge. Two of the samples were CD8⁺ dCODE⁺ sorted cells derived from the same stock but independently stained with a 15-plex Abseq and 13-plex dCODE (RiO) panel to evaluate reproducibility of AbSeq and dCODE performance as shown in **(4B)** Note only antigen dCODEs are shown, no negative controls. The third cell sample was an enriched CD4⁺ cell population that was stimulated with EBV and tetnus toxoid (TT) peptides and stained with a 15-plex AbSeq and 3-plex Dextramer panel. AbSeq CD4 expression and antigen-specific TCR expression detected by dCODE (RiO) reagents are shown in **(4C).**

Conclusions

* dCODE Dextramer® (RiO) reagents can be seamlessly integrated into the BD Abseq, Sample Tag, Targeted mRNA and TCR Full Length workflow to profile hPBMCs.

* Antigen-specific T cells along with their corresponding full length TCR sequences were identified by viral specific HLA-peptide complexes displayed on dCODE Dextramer® (RiO) reagents used in combination with BD TCR Full Length assays.

* Sample multiplexing of 3 different samples showed reproducibility of AbSeq and dCODE (RiO) performance as well as sensitive detection of dCODE (RiO) reagents from different cell types (CD4⁺ and CD8⁺ cells).