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PRECISION IMMUNE MONITORING

Accurate Antibody Discovery with dCODE® Klickmer

dCODE® Klickmer Reagents Identify Antigen-specific B cells at Single-Cell Level for Antibody Discovery

Data provided by Symphogen, a Servier Company. Written by Immudex.

Antibodies against foreign antigens are critical components of the overall immune response and can facilitate pathogen clearance during primary infection. Sensitive detection and characterization of antigen-specific B cells are crucial for therapeutic antibody discovery. Nevertheless, these types of analyses are currently hampered by the lack of technologies allowing comprehensive multiplexed detection of antigen-specific B cells. This study describes the use of dCODE® Klickmer for customized, sensitive, and multiplexed detection of antigen-specific B cells in antibody discovery.

dCODE® Klickmer - Breaking the Limits of Antibody Discovery

Monoclonal antibodies represent the most crucial class of therapeutic proteins, delivering clinical benefits to millions of patients worldwide1. Analysis of B-cell specificities at the single-cell level provides essential information on B-cell responses during infection, antigen presentation, or vaccination2. Emerging technologies in single B-cell screening and cloning have made antibody discovery quicker and more efficient3. With the introduction of next-generation sequencing (NGS), it became easier to identify novel antigen-specific antibodies. NGS combined with single B-cell screening enables a more focused approach for antibody discovery compared to classical screens like ELISpot and flow cytometry3. Nevertheless, methods for detecting and analyzing antigen-specific B cells on a single cell level remain limited.

For multiplexed detection of antigen-specific immune cells, Immudex has developed dCODE® Klickmer. dCODE® Klickmer is a versatile reagent that combines the multiplexing power of DNA barcodes with the possibility to attach any biotinylated molecule with applications within vaccine development, the discovery of antibodies, studies of protein-protein interactions, and much more.

Highlights

We demonstrate the power of dCODE® Klickmer in mapping the specific B cell response to:

- Assess the specificities of 2000 murine lymph node derived B cells using a next-generation sequencing and custom-produced Protein-X dCODE® Klickmer in just one workflow
- Develop novel experimental and analytical approaches that ensure fast and reliable identification of antigen-specific B cells
- Uncover BCR sequences at single-cell resolution for identification of antibodies with Protein-X binding potential
- Help facilitate a greater understanding of the antigen-specific B-cell response and its applications in the discovery of therapeutic antibodies

Through the attachment of biotinylated B-cell antigens, dCODE® Klickmer becomes suitable for large-scale screening of B-cell specificities (**Fig. 1**). Due to the stabilizing dextran backbone and optimized number of acceptor sites, dCODE® Klickmer ensures sensitive detection even at low affinity. Additionally, the unique DNA barcode of dCODE® Klickmer enables efficient identification of B-cell specificity by sequencing.

This study describes the use of dCODE® Klickmer for customized, sensitive, and multiplexed detection of antigen-specific B cells as part of an antibody discovery workflow. The data presented was produced and provided by Symphogen, a Seriver Company.

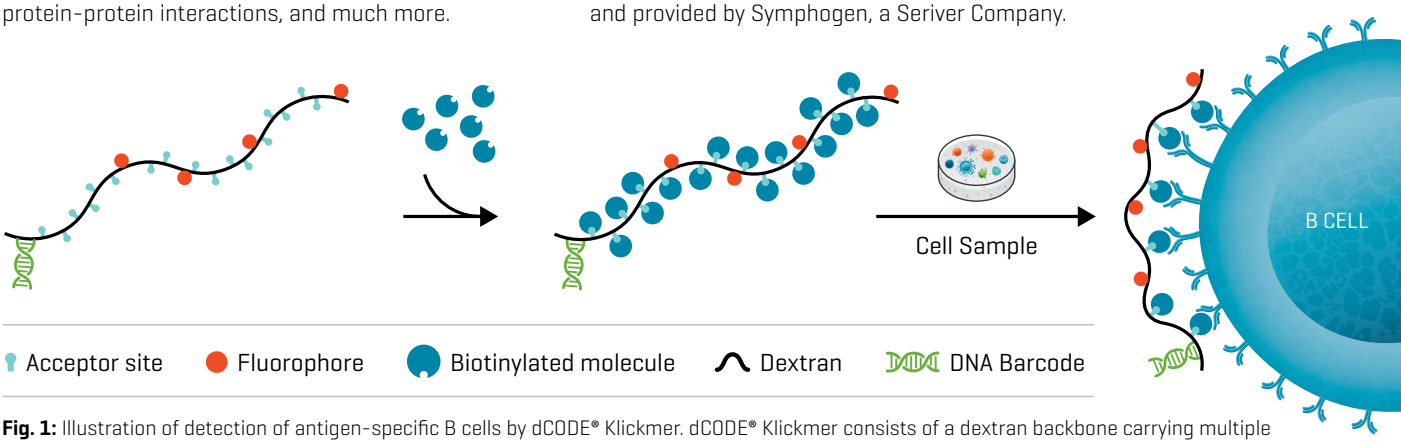

Fig. 1: Illustration of detection of antigen-specific B cells by dCODE® Klickmer. dCODE® Klickmer consists of a dextran backbone carrying multiple acceptor sites for the binding of biotinylated molecules. Loading different B-cell ligands to different dCODE® Klickmer allows investigation of multiple B-cell specificities in the same sample.

Materials and Methods

- Klickmer™ and dCODE® Klickmer reagents for the production of Protein-X specific multimers and negative controls were provided by Immudex ApS
- TotalSeq™ antibodies were purchased from BioLegend for quantification of surface protein expression
- Chromium cassette for single-cell reaction partitioning was purchased from 10x Genomics

Staining and cell enrichment

- For detection of Protein-X specific B cells, cryopreserved murine lymph-node cells resulting from immunizations with Protein-X were used.
- Cells were stained with dCODE® Klickmer for 10 minutes at room temperature, followed by staining with TotalSeq™ antibodies for 20 minutes at 4-8°C. Cells were washed three times and the supernatant decanted prior to enrichment.
- dCODE® Klickmer positive cells were enriched within the B-cell gate by FACS targeting a yield of 9000 cells per well.

Library preparation and sequencing

Enriched cells were directly loaded onto a Chromium chip. Libraries for gene expression, V(D)J, and dCODE® Klickmer were generated and quantified, normalized, and sequenced on an Illumina NovaSeq 6000 S2 according to manufacturers' instructions.

Sequencing depth

Libraries were targeted for sequencing depths of at least 10,000 read pairs per cell.

Data analysis

Sequencing data combined with the description of the sequences identifying dCODE® Klickmer reagents were analyzed with the Cell Ranger analysis pipeline and 10x Loupe software.

Protein-X-specific antibody-producing B cells were identified by clustering based on antigen specificity followed by B-cell markers and identification of cells exhibiting gene expression related to antibody production.

dCODE® Klickmer and 5´Prime Feature Barcode Protocol

To incorporate dCODE® Klickmer into the 5´Prime Feature Barcode Protocol enabling single-cell analysis on the 10x Chromium Gene expression system, the barcode on dCODE® Klickmer holds a capture sequence. The capture sequence then binds the capture beads in the wells of the Chromium chip and allows for single-cell partitioning. Thereby, single-cell analysis is enabled but also allows for the combination of different analyses, like combining information on antigen-specificity with gene expression, clonotype, and surface protein expression. This additional information gives a deeper characterization and understanding of the antigen-specific B-cells response in just one workflow. The full workflow for dCODE® Klickmer and antibody discovery is listed below.

Workflow

- For detection of Protein-X-specific B cells, murine lymph node cells were stained with dCODE® Klickmer, incubated for 10 minutes at room temperature, and stained with TotalSeq™ antibodies (BioLegend)
- After incubation and washing steps, cells were enriched for Protein-X specific B cells by FACS
- Sorted cells were partitioned into single-cell reactions in the Chromium chip on the 10x Chromium Gene Expression System
- Then libraries for dCODE® Klickmer, TotalSeq™ antibodies, and gene expression were prepared and sequenced
- Sequencing data were analyzed in Loupe browser (10x). Antibody-producing, Protein-X-specific B cells were identified using dCODE® Klickmer
- Based on the identified antigen-specific B cells, antibody sequences were chosen, isolated, and validated for protein-X binding

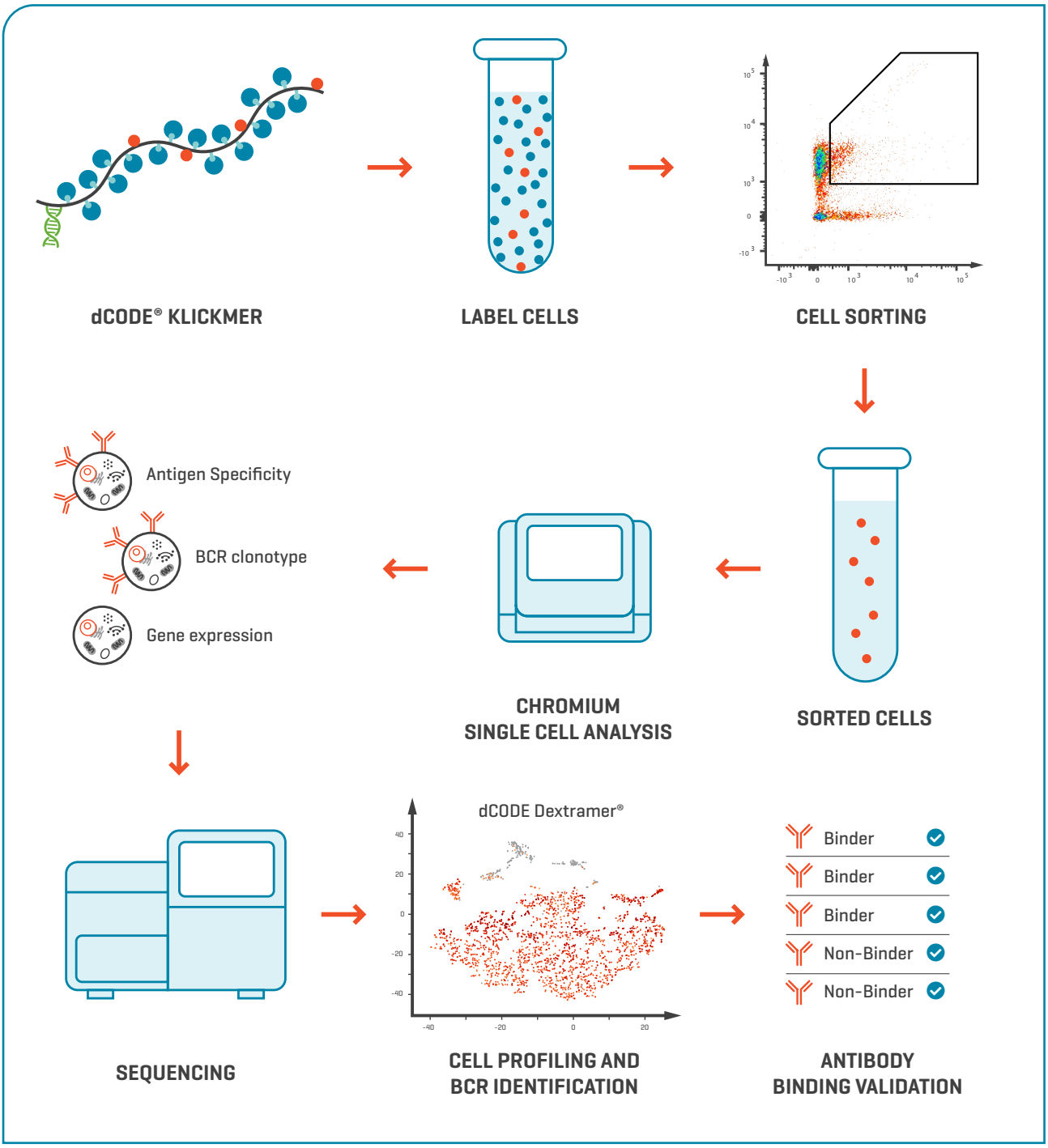

Fig. 2: Antibody Discovery with dCODE® Klickmer workflow. Cells derived from rat lymph nodes were stained with the Protein-X loaded dCODE® Klickmer. Stained cells were enriched for dCODE® Klickmer-bound B cells. The enriched cells were partitioned into single cells on the 10x Chromium system. After processing, three distinct DNA libraries for antigen-specificity, gene expression, and B-cell receptor clonotyping were generated and individually sequenced. Protein-X-specific antibodies were identified based on BCR clonotype and gene expression and validated using surface plasmon resonance (SPR).

dCODE® Klickmer Production and Cell Sorting

Biotinylated protein-X antigens were attached to the acceptor sites on the dCODE® Klickmer reagent to ensure sensitive detection of Protein-X antigen-specific B cells. The resulting Protein-X dCODE® Klickmer was then used to stain cryopreserved murine Protein-X immunized lymph node cells. Cells were thawed, washed, and stained with Protein-X dCODE® Klickmer and TotalSeq™ antibodies. Hereafter, cells were enriched for Protein-X-specific B cells by FACS. Protein-X dCODE® Klickmer positive cells were gated in the non-plasma antibody-producing B-cell population based on the PE-fluorophore attached to the dCODE® Klickmer (**Fig. 3**).

Single-Cell Partitioning and Sequencing

Sorted cells were partitioned into single cells on the Chromium assay for RNA-seq library preparation. Three DNA libraries were prepared, including detection of antigen specificity, identify BCR clonotypes, and gene expression.

Libraries were sequenced using an Illumina BCL sequencer, and the resulting data was interrogated for antigen specificity, surface marker, and gene expression using 10x Loupe software.

The dCODE® Klickmer (10x compatible) DNA barcode holds a capture sequence compatible with the 10x Genomics capture bead used to partition the cells in the Chromium chip on the 10x Genomics Chromium Single Cell Gene Expression platform. The DNA barcode also holds a unique molecular identifier and a unique DNA barcode sequence. Furthermore, these factors also enable differentiation of the T- cell specificities present in the sample during data analysis independent of how many and which dCODE® Klickmer a given cell has bound is also enabled.

Identification of Protein-X Antibody-Producing B cells

NGS analysis of the generated DNA from the procedure matched the antigen specificity measured by applying dCODE® Klickmer with the suitable antibodies. 1934 B cells expressing BCR complex genes were identified (**Fig. 4A**). 436 of the BCR complex expressing B cells were confirmed to be antibody-producing by estimating IgG expression (**Fig. 4B**). A large portion of the antibody-producing pool of B cells was further confirmed to bind Protein-X dCODE® Klickmer (**Fig. 4C**). Based on V(D)J sequencing, BCR clonotypes were identified for Protein-X specific B cells. The identified BCRs were validated through Protein-X binding studies.

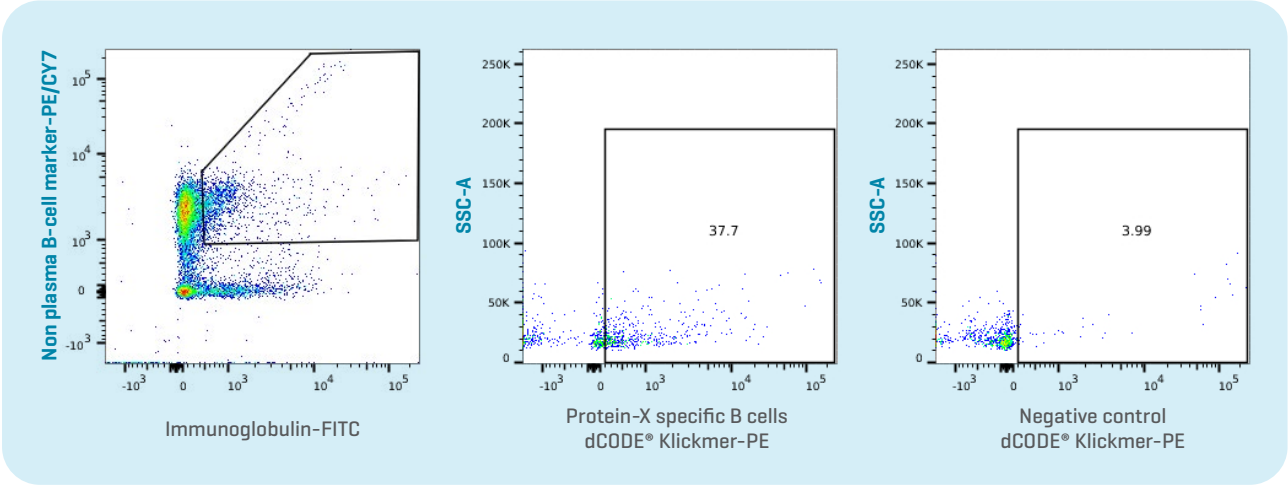

Fig. 3: FACS sorting of Protein-X dCODE® Klickmer binding B cells. Cells were gated into single cells, and Protein-X dCODE® Klickmer B cells were enriched in the non-plasma B-cell antibody (Immunoglobulin) producing population.

Antibody-Binding Study and Validation

40 monoclonal antibodies were selected from the identified BCR clonotypes for binding affinity validation studies. The selected antibodies were expressed, purified, immobilized, and exposed to single-cycle kinetics. To enable validation of the monovalent binding affinity of the selected antibodies by Surface Plasmon Resonance analysis monovalent antigen was added to the solution. 21 of the 40 selected antibodies (55%) with an estimated binding affinity towards Protein-X were confirmed to have binding affinity ranging from sub nM to 400 nM by Surface Plasmon Resonance measurements (**Fig. 4D**).

Discussion

This experiment, the first of its kind, demonstrates that dCODE® Klickmer loaded with Protein-X antigens enables the generation of highly multiplexed binding data of B-cell specificities in a single workflow. The dataset also illustrates the complexity inherent in performing and analyzing these experiments.

Even though the experimental design and the analyses presented here are not fully, the data will be useful in extracting detailed biological insights and developing future experimental and analytical approaches. Imminent workflow optimizations may allow an even higher percentage of binders after antibody sequence expression.

Conclusions

- dCODE® Klickmer allowed identification of 40 Protein-X specific antibodies. Of the identified antibodies, 55% were validated as true Protein-X binders with binding affinity ranging from sub-nanomolar to approximately 400 nM.
- For the first time, dCODE® Klickmer in combination with 10x 5´Feature Barcoding Technology was applied to antibody discovery. dCODE® Klickmer provides a faster and cost-effective alternative to classical methods used today for antibody discovery.

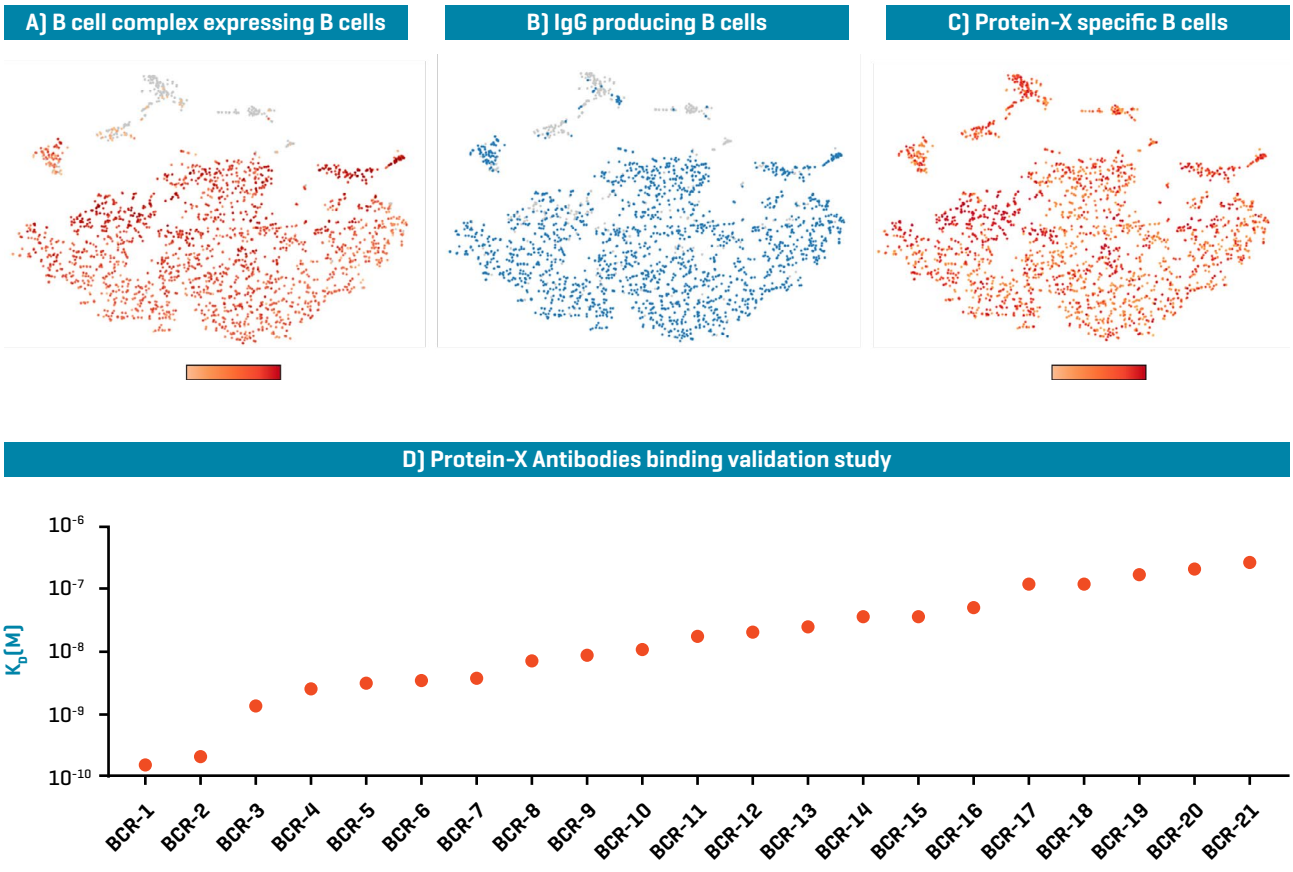

Fig. 4: Analysis of Protein-X sequencing data and antibody validation. A) t-SNE plots showing expression of surface markers related to memory B cells, B) Immunoglobulin expression, C) Protein-X dCODE® Klickmer binding cells. Color range bar shows the binding intensity of memory B-cell surface marker or Protein-X dCODE® Klickmer, D) Validation of antibody binding. Protein-X specific antibody binding was validated by surface plasmon resonance (SPR) analysis in affinity studies.

References

1. Liu, IJ. *et al.* "Development of therapeutic antibodies for the treatment of diseases". *J Biomed Sci* 27, 1 (2020).
2. Jahnmatz, P. *et al.* Multiplex analysis of antigen-specific memory B cells in humans using reversed B-cell FluoroSpot. *J Immunol Methods* 478: 11271 (2020)
3. Boonyaratanakornkit, J. & Taylor, J. J. Techniques to Study Antigen-Specific B Cell Responses. *Front Immunol* 10: 1694 (2019).

Resources from Immudex

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

dCODE® Klickmer

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

www.immudex.com/products/basic-research/dcode-dextramer-ngsmulti-omics/dcode-klickmer/

Technical Support

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

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Resources

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