Longitudinal single-cell V(D)J and transcriptomic profiling reveal dynamic B cell clonotype evolution during recurrent UTI and progression to ESKD

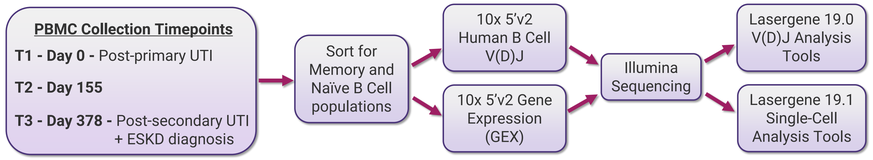
Sarah Quinn[1], Erik Edlund[1], Constantin Tivis[1], Thomas Leary[1], David Frisch[2], Charles Landry[2], Tim Durfee[1], and Frederick Blattner[1,2]

Author affiliations: [1]DNASTAR, Inc. Madison, WI, [2]Scarab Genomics LLC, Madison, WI

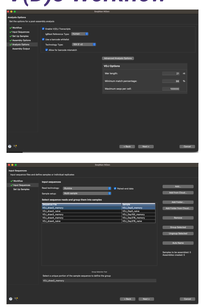
DNASTAR
SOFTWARE FOR LIFE SCIENTISTS

Chronic infection and disease progression: a case study

Project Design



V(D)J Workflow



GEX Workflow

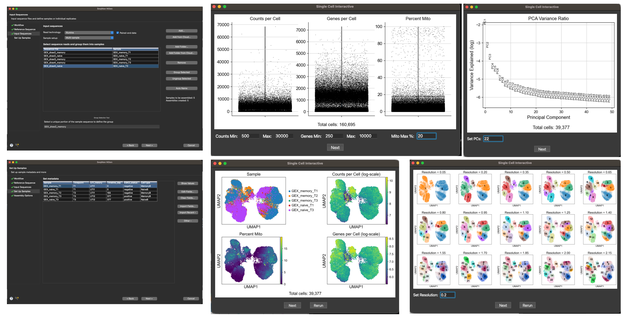


Figure 1. Project design (top) and snapshots of V(D)J (bottom left) and GEX (bottom right) data analysis steps in Lasergene.

This study aims to examine changes to the B cell repertoire during recurrent urinary tract infection (UTI) and progression to end-stage kidney disease (ESKD). To this end, peripheral blood samples were collected from an individual at three timepoints spanning persistent/recurrent UTI, treatment, and eventual ESKD diagnosis. Memory and naïve B cells were isolated and profiled using 10x Genomics 5' v2 single-cell V(D)J and gene expression sequencing. V(D)J data were analyzed using Lasergene 19.0 to characterize clonotype abundance and repertoire diversity, while Lasergene 19.1 Single-cell Analysis Tools were used to align gene expression data, perform quality control and clustering, and identify transcriptional features associated with cell populations and expanded clonotypes. Together, these analyses provide a longitudinal view of both clonal dynamics and the cellular states associated with those responses.

Frequency analysis reveals changing clonotype diversity throughout disease progression

The distribution of clonotype frequencies revealed substantial differences in repertoire structure across timepoints, particularly within memory B cells. Naïve B cell populations showed relatively few highly expanded clonotypes, while all memory populations contained multiple clonotypes with high read counts, consistent with a history of antigen-driven expansion. Notably, the second timepoint showed a broader group of higher-frequency clonotypes compared with the other timepoints. This sample was collected after UTI treatment and prior to ESKD diagnosis. As a result, it is expected to be the least dysregulated immune state with regard to this disease course. However, distinguishing recovery-associated changes from effects of prolonged disease would require additional longitudinal and control samples.

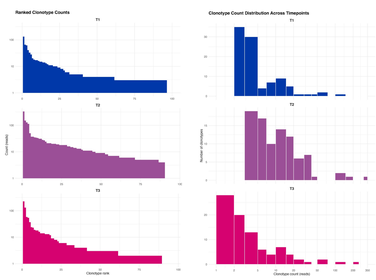


Figure 2. Distribution of counts for top 100 clonotypes of Memory B cell V(D)J samples, ranked by number of counts (left) and bucketed by counts (right).

Clonotype consistency and expansion persist throughout disease progression

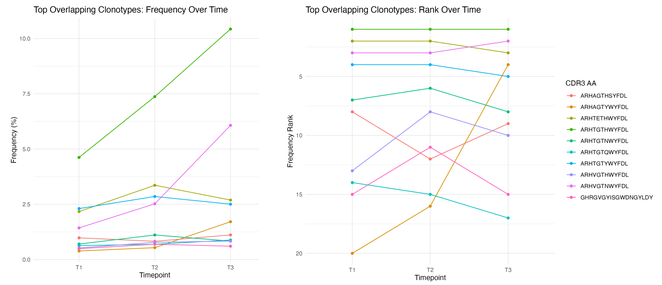


Figure 3. Frequency (left) and rank (right) of top overlapping Memory B Cell clonotypes across timepoints.

Tracking the most abundant clonotypes across timepoints revealed a persistent component of the memory B cell response. Several of the most abundant clonotypes were shared across timepoints, with the top four remaining among the most frequent clonotypes throughout the study. Most notably, the top clonotype was detected at all three timepoints and progressively increased in relative frequency, including after UTI treatment. Its greatest expansion occurred at the final timepoint, following recurrent UTI and ESKD diagnosis. This persistence and expansion suggest that a subset of B cell responses may be maintained over extended periods despite changes in clinical status, although the antigen specificity of these clonotypes remains unknown.

Aligned analysis reveals inter- and intra-cellular diversity of sequences

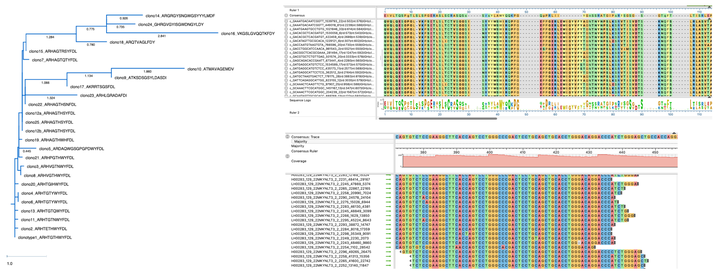


Figure 4. Phylogenic tree of top clonotypes (left). Representative snapshot of sequence diversity within cells of the same clonotype (top right) and an individual cell (bottom right).

Examining the sequence relationships among expanded clonotypes provided additional resolution beyond clonotype frequency alone. Phylogenetic analysis of CDR3 sequences revealed related sequence patterns among several highly abundant clonotypes, suggesting that clonotypes classified separately may potentially represent related B cell lineages or antigen-selection histories. We also observed substantial sequence diversity among cells assigned to the same clonotype, highlighting variation that is not apparent from clonotype abundance alone. Together, these findings suggest that longitudinal repertoire analysis may benefit from considering both clonotype-level expansion and the underlying sequence diversity within and between clonotypes.

Cell subset and expression shifts elucidate immune dynamics of infection

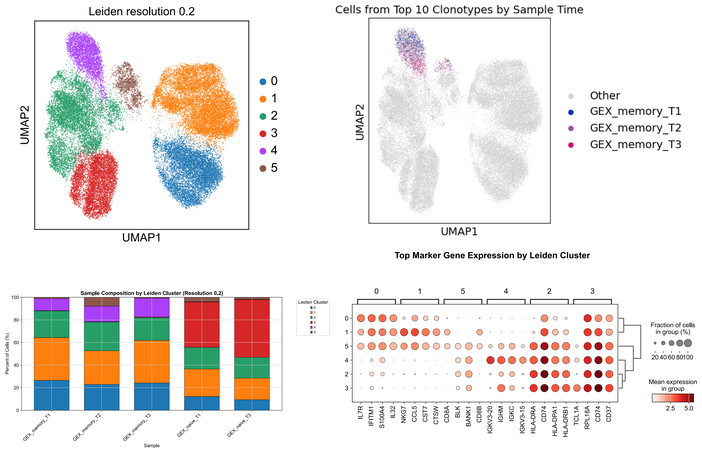


Figure 5. UMAP colored by Leiden cluster (res = 0.2) and sample (top). Cluster distribution by sample (middle left) and top positively expressed genes by cluster (bottom right).

Integration of V(D)J and gene expression data enables analysis of the cellular state of expanded clonotypes. Joint analysis of memory and naïve B cells identified six expression clusters and revealed differences in cluster composition across timepoints. Notably, cells belonging to the top overlapping clonotypes were concentrated within an IGK-dominated cluster, linking the longitudinally persistent clones to a distinct transcriptional state. This uniform transcriptional state across time points may indicate shared cellular function agnostic of disease state.

Conclusions

Longitudinal profiling revealed substantial remodeling of the B cell repertoire across recurrent UTI and progression to ESKD, including persistent and progressively expanding memory B cell clonotypes. The continued expansion of the most abundant clonotype across clinical timepoints raises the possibility of a sustained antigen-driven response, although its specificity is yet to be established. Sequence-level analysis further revealed diversity both among related clonotypes and within cells assigned to the same clonotype, suggesting additional complexity in the evolving repertoire that is not captured by clonotype frequency alone. Finally, integration of V(D)J and gene expression data linked the most persistent, expanded clonotypes to a distinct transcriptional state, providing a potential connection between clonal expansion and functional cell state. Future work will focus on determining the antigen specificity of these expanded clones and whether their persistence reflects responses to recurrent UTI-associated antigens, other chronic stimuli, or disease-associated immune changes.

Visit DNASTAR at booth 628 to learn more about Lasergene, integrated software workflows for single-cell analysis, guided project setup and downstream visualization.