Development and Proof-of-Concept for a Free Full-Stack Web-Based Epitope Finder Platform

UC Irvine

Lin Wang[1], Manxuan Zhang[2], Rodin Hooshiyar[3], Robert A Bray[4]

1. Dept. of Pathology and Laboratory Medicine, UC Irvine, CA | 2. University High School, Irvine, CA 3. Los Altos High School, Los Altos, CA | 4. Dept. of Pathology and Laboratory Medicine, Emory University, GA

Introduction

The Challenge: Solid-phase single antigen bead (SAB) assays offer high sensitivity for HLA antibody detection, but false-positive signals and non-specific reactivity often complicate clinical interpretation.

The Need: Definitive antibody assignment requires systematic epitope pattern recognition, exclusion of autoantibodies against recipient self-HLA, and alignment with high-resolution donor typing for DSA determination.

The Bottleneck: Major references—such as the **HLA Epitope Registry** and the **One Lambda Terasaki Epitope Bank**—are fragmented across separate databases and lack comprehensive integration into primary commercial testing platforms. Manual curation across disparate registries is time-consuming and susceptible to inter-operator error.

Our Solution: We developed **Epitope Finder**, an intuitive, open-access web platform that automates epitope pattern recognition, filters self-reactivity, and resolves donor-specific antibodies (DSAs) for rapid, standardized histocompatibility assessment.

Method

Back-End Infrastructure & Data Pipeline

Data Acquisition & Structuring: A custom Python-based ingestion tool automatically fetches data from the HLA Epitope Registry. The extracted data is structured, organized, and loaded into Google BigQuery to establish a scalable relational database validated via custom SQL filtering scripts.To ensure low latency operations, scalability, and universal compute, Implemented a backend system using Google Cloud Functions to connect the frontend to BigQuery to execute user-based queries and offload the compute intensive operations.

Serverless Architecture & Compute Offloading: To ensure low-latency operations and scalability, Google Cloud Functions serve as the core backend middleware, bridging the user interface and BigQuery to execute user-driven queries and offload resource-intensive computations.

Query Processing Workflow: Each analysis request from the client-side initiates a parameter-driven BigQuery SQL query via the backend server. Processed data is subsequently returned as a structured JSON response for visualization.

Deployment & Containerization: The Flutter-based front-end application is containerized using Docker and deployed via Google Cloud Run, ensuring a universally accessible, highly maintainable, and easily scalable deployment environment.

Front-End Architecture & UI Optimization

Custom High-Performance Rendering Engine: To mitigate performance degradation and UI "jank" when rendering thousands of data points, a custom-rendered paint engine (HeatmapRowPainter) is utilized instead of standard nested widgets. Each row is drawn directly onto the pixel canvas using canvas.drawRect to instantly color cells based on match status. This painter identifies **Green cells** as **positive matches** where a user's input allele exists within an epitope, and **Red cells** as **negative mismatches** where a required Luminex allele is missing from the patient's profile.

Clinical Indicator Overlay: Specific textual markers **"S" for self, indicating an allele identical to the patient's own typing** and **"D" for DSA to flag dangerous donor-specific antibodies)** are dynamically overlaid within the cells, providing immediate clinical insight without sacrificing scrolling performance.

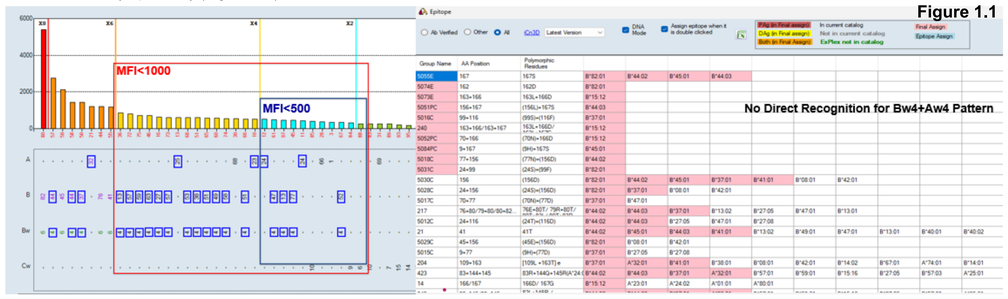
Layout Virtualization & Memory Management: The layout incorporates a dual-axis scrolling mechanism utilizing ListView.builder with fixed item extents, ensuring that only visible rows are rendered in memory at any given time.

Dynamic Scaling & Global Zoom: A responsive zoom system—controlled via sliders or keyboard shortcuts—dynamically scales base cell widths and font sizes, facilitating a seamless transition from a macro "bird's-eye view" of the entire antibody profile to granular inspection of specific mismatched alleles.

Case 1 Weak Bw4+Aw4 with DSAs

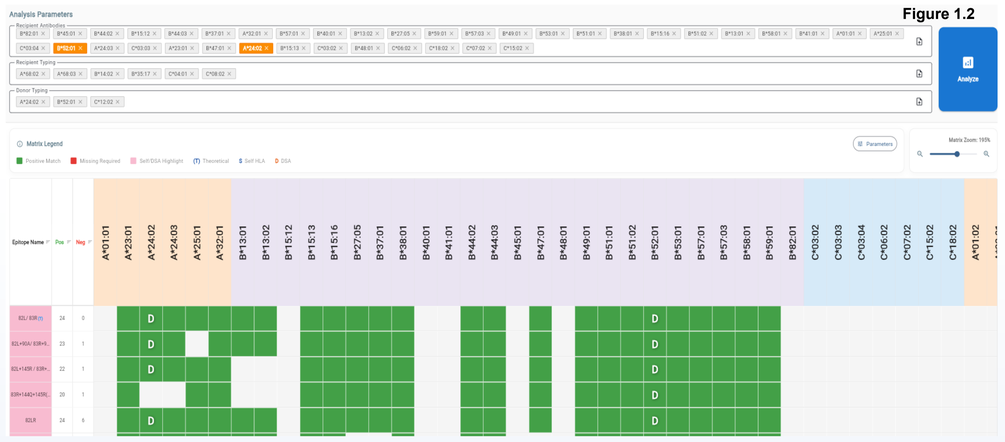
A 66-year-old female on hemodialysis for diabetic ESRD, with a history of hypertension and hypothyroidism, **presents for kidney transplant evaluation**. The HLA Class I single-antigen bead assay shows weak, low-level reactivity across multiple Bw4-associated beads—with the vast majority falling below 1,000 and 500 MFI. Consequently, One Lambda own epitope analysis shows no definitive direct recognition pattern for a Bw4/Aw4 antibody specificity.(Figure 1.1)

Figure 1.1



From the raw data table in One Lambda Fusion, sort beads from highest to lowest MFI, then copy and paste all alleles with reactive beads into the "Recipient Antibodies" field on the Epitope Finder interface. After entering the recipient's HLA typing and potential donor typing, click the blue **Analyze** button on the right. Unrestricted by fixed 500 or 1,000 MFI cutoffs, Epitope Finder identifies the public eplet **82L/83R** (the classic Bw4/Aw4 motif) as the top shared configuration, highlighting **potential donor-specific antibodies (DSA)** against donor antigens **A*24:02** and **B*52:01 (highlighted in orange).** Candidate epitopes are ranked in descending order by the number of reactive positive alleles with zero negative/missing alleles. Eplets labeled with a **blue (T)** denote entries derived from **the One Lambda Epitope Bank**, whereas unmarked eplets are derived from **the HLA Epitope Registry** (Figure 1.2).

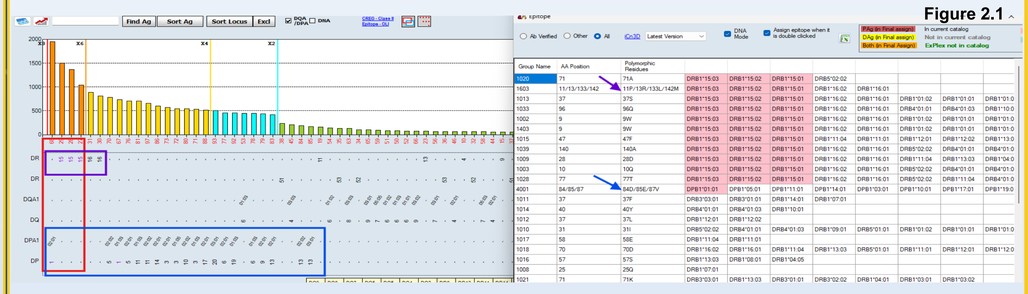
Figure 1.2



Case 2 Weak DP Pattern

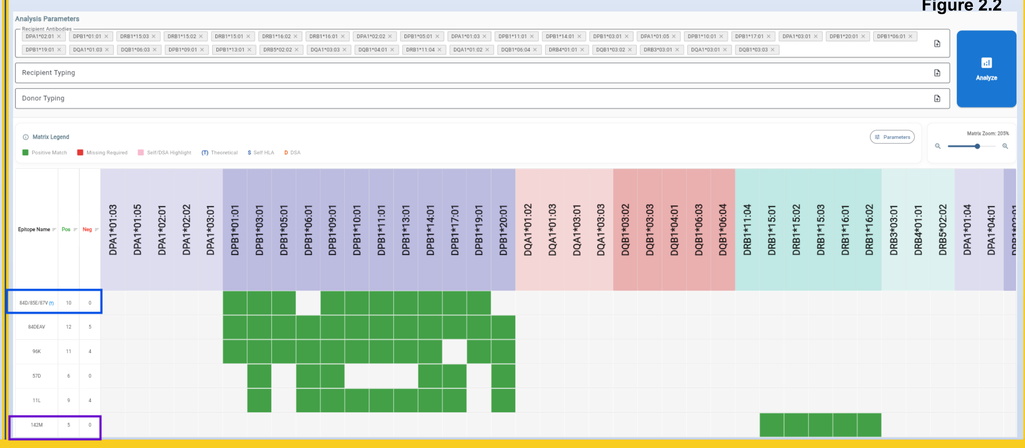
A 65-year-old female with CKD secondary to a congenital atrophic left kidney in the setting of diabetes mellitus presents for **kidney transplant evaluation**. In the HLA Class II single-antigen bead panel, low-level reactivity under 1,000 MFI fails to trigger direct epitope assignment on initial One Lambda Fusion analysis, missing DRB1*15 and DRB1*16 alleles **associated epitope** (**11P/13R/133L/142M**, boxed in purple) and a broader **DP pattern** (**84D/85E/87V**, boxed in blue) (Figure2.1).

Figure 2.1



Through a streamlined copy-and-paste workflow with one-click execution, Epitope Finder resolves sub-threshold reactivity by identifying both the One Lambda-defined DPB1 motif **84D/85E/87V (DEV)** and the HLA Epitope Registry Eplet **142M** (shared across DRB1*15 and *16 alleles), presenting a comprehensive visualization without the constraints of rigid MFI thresholds (Figure2.2).

Figure 2.2

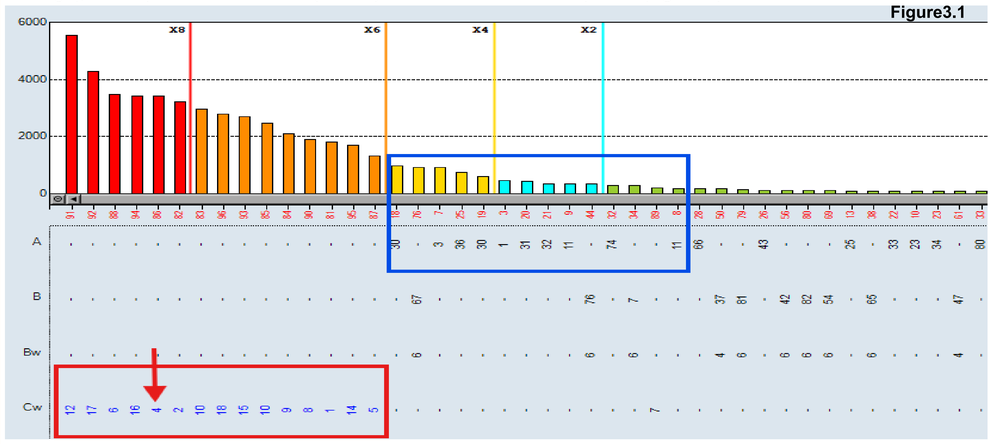


Case 3 PAN-C pattern with Self Ab

A 34-year-old Hispanic male with ESRD due to presumed diabetic nephropathy, **presents for kidney transplant evaluation**.

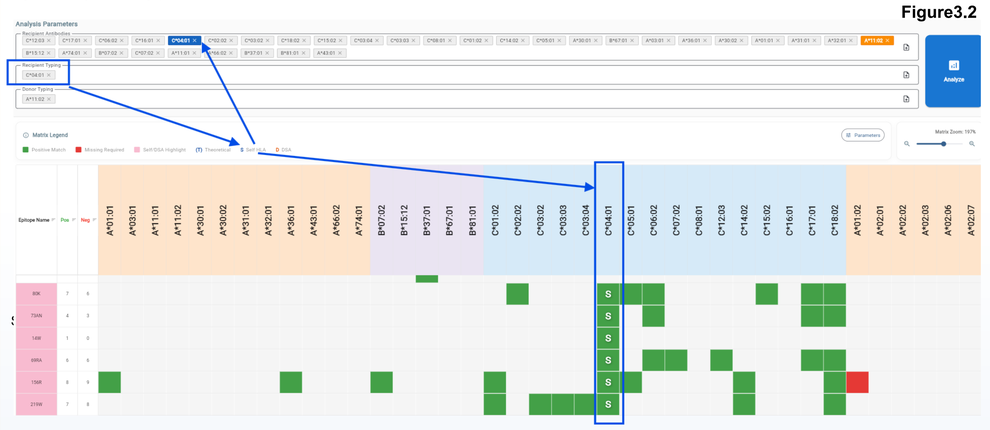
Pan-C Reactivity Profile with Self Ab: The assay demonstrates a broad, pan-reactive pattern across almost all classical HLA-C loci (in the red rectangle), with the patient's own **self-allele (C*04:01)** identified within the reactive spectrum (**Figure3.1).**

Figure3.1



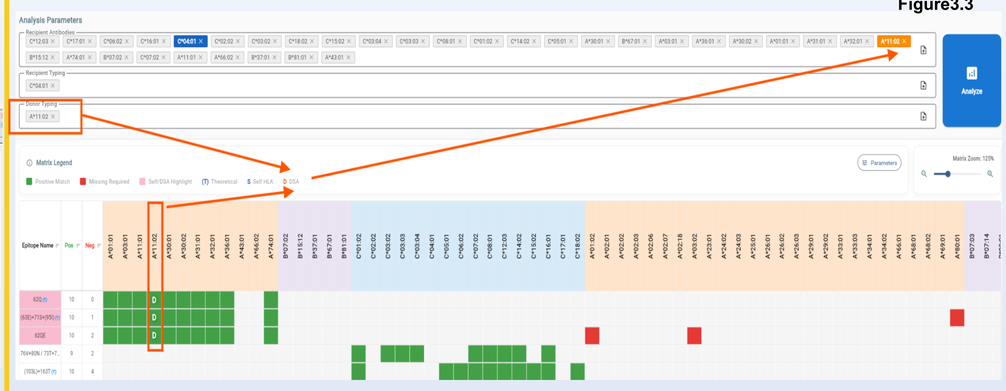
Within Epitope Finder, corresponding self-alleles are clearly delineated and highlighted with an "S" marker inside green match cells (**Figure3.2).** This feature allows professionals to instantly differentiate and rule out autoreactive shared-epitope patterns from true alloimmunization during high-density antibody profile interpretation.

Figure3.2



Donor-Specific Antibody (DSA) Identification: Because standard software does not provide instant suggestions for low-MFI profiles (<1000 MFI), One Lambda Fusion raw data is sorted by bead MFI, and all reactive alleles are transferred into the recipient antibodies. Analysis reveals a prominent 62Q epitope cluster (including A1, A3, A11, A30, A31, A32, A36 and A74) where most patterns register under 1000 MFI. When evaluating potential donor antigens (such as A*11:02 and A*74:01), Epitope Finder successfully flags the DSA against the donor mismatch, highlighting it in orange within the recipient antibodies field and marking it with a distinctive "D" overlay on the green match cells in the structural matrix (**Figure3.3).**

Figure3.3



Results and Future Directions

Results: The platform successfully synthesized SAB data and HLA typing into a consolidated Epitope Analysis Matrix. Across the three validation cases, rapid data integration mapped complex patterns, the ranking algorithm flagged weak antibodies overlooked in routine review, and automatic filtering of self-HLA noise revealed true sensitization.

Conclusion: Epitope Finder successfully transitions HLA antibody analysis from manual interpretation to an automated, epitope-driven matrix. By consolidating databases, filtering self-antigens, and utilizing weighted ranking, it significantly enhances detection sensitivity for low-MFI patterns. This standardized tool minimizes human error and markedly improves the efficiency and accuracy of pre-transplant risk assessments.

Future Directions: To further enhance our software and provide richer analytical insights, our next steps focus on two key areas. First, we will refine our database by annotating epitopes as either 'confirmed' or 'unconfirmed'. While we currently distinguish data sources between One Lambda and Epitope Finder, this new categorization will allow users to assess the credibility and likelihood of each epitope during analysis. Second, we plan to stratify the epitopes according to their structural location. Epitopes in more exposed, easily accessible positions for antibody anchoring will be categorized into Tier 1, while less accessible locations will fall into Tier 2. This stratification will offer deeper context and more precise information for researchers.