Accelerating Immunoassay Development

with Antibody Biophysical Characterization

High Content Predictive Characterization delivers rapid lead discovery for sensitive immunoassay development.

Introduction

In this technology note, we apply our custom biophysical workflow to evaluate key attributes of 130 of our catalog and non-catalog IL-10 antibodies within more than 50,000 unique immunoassay leads.

Our process efficiently uncovers a diverse and highly curated set of leads that were screened for high-sensitivity performance on Simple Plex™ assays running on the Ella™ open access 48-Digoxigenin platform.

Method

Cytokines are core biomarkers for:

- Characterizing immune function
- Understanding and predicting disease
- Monitoring effects of treatment on the functional status of the body

Due to their critical roles, ultrasensitive (sub pg/ml) detection methods continue to attract considerable attention.

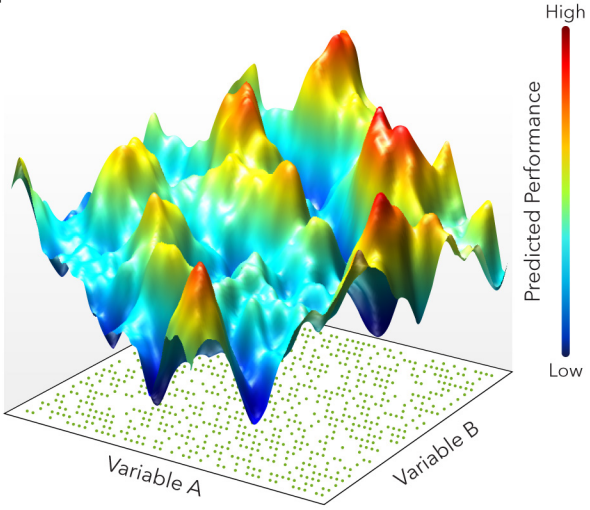
Here, we apply our custom, high throughput antibody characterization and analysis workflow to develop sensitive IL-10 cytokine biomarker assays for automated Ella ELISA system–a precision, benchtop immunoassay platform.

Our workflow was applied to screen a large panel of antibodies to identify matched pair leads. A multi-level analysis, including Carterra-LSA based evaluations, enabled 50,000 ELISA sandwiching pairs to be assessed.

We identify a curated set of 20 antibody leads with advantageous immunoassay properties. These 20 antibody leads were subsequently validated for deployment on the Ella platform.

Graphical Overview

Our custom assay development workflow combines our extensive library of biomarker-specific antibodies with high-content biophysical characterization to enhance and accelerate the identification of top-ranked leads and their deployment onto the Ella platform.



The 130 IL-10 Antibodies

Our Workflow Leverages Our Extensive Antibody Collection for Immunoassay Development.

The 130 IL-10 antibodies that were processed through our workflow derive from a variety of immunization methods, designed immunogens, and host species, and represent monoclonal hybridoma, recombinant monoclonal, and polyclonal antibodies (Figure 1).

Evaluating large collections of antibodies significantly increases the success rate of identifying high-performing leads for immunoassay applications. However, to fully leverage this antibody content, next-generation methodologies are required that afford an optimal balance of depth of analysis and speed to thoroughly and efficiently survey an expansive combinatorial landscape of lead possibilities.

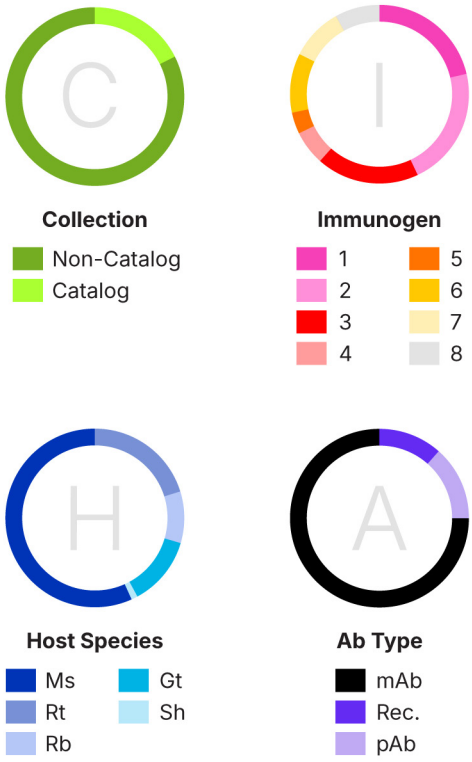


FIGURE // 01

Antibodies Utilized

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BTc-129				
BTc-130				

FIGURE 1. A diverse library of 130 IL-10 antibodies from our Catalog and Non-Catalog collections were entered into our immunoassay development workflow.

Matched Pair Reagents

Antibody Biophysics and Biomarker Interaction Establish the Lead Landscape.

The high-performance specifications that the Ella platform achieves derives from a variety of engineered optimizations unique to this platform. Significant among these is the custom development and precision selection of matched-pairs of capture and detection antibody reagents. These matched pair reagents require key attributes that are tuned for the specifications of a particular immunoassay.

Our high-content workflow initially serves to surround our library of antibodies with an expansive structure-activity dataset (Figure 2). Once obtained, this dataset is processed and analyzed to populate an information-rich landscape comprising all possible permutations of lead parameters, such as antibody pairing, titer, sample type, and incubation time.

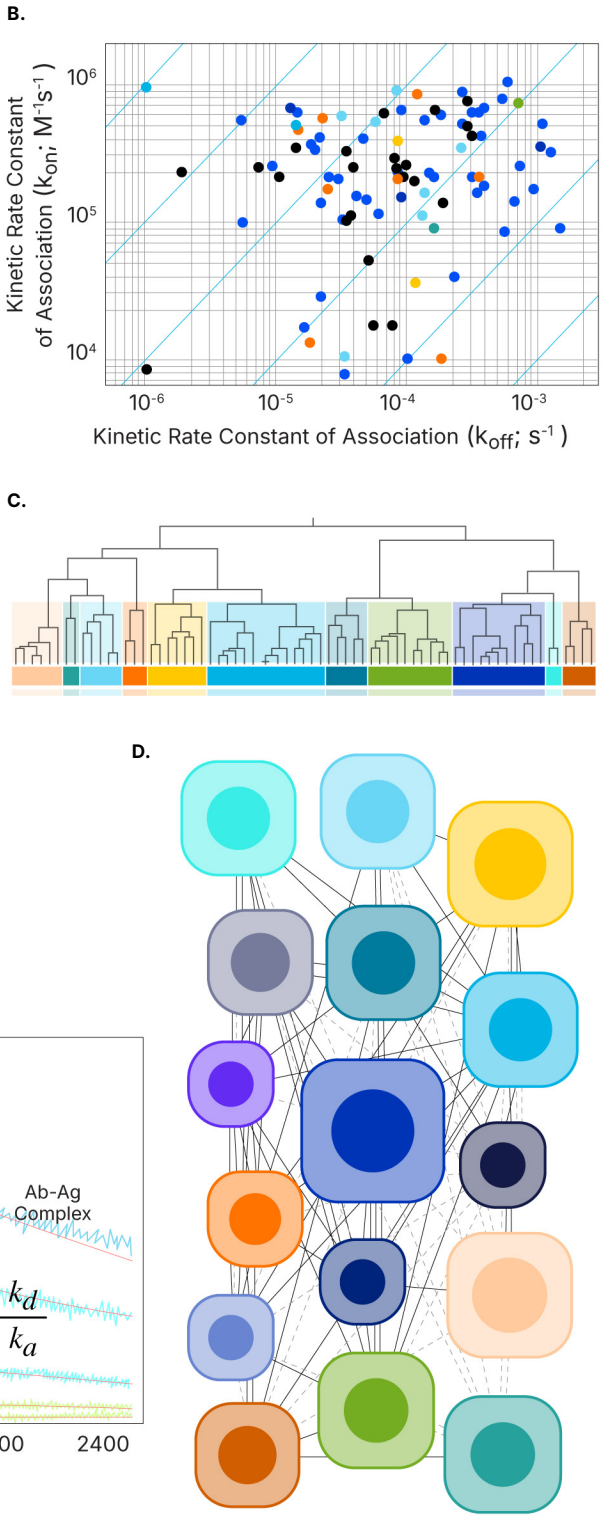


FIGURE // 02

Structural, biophysical, and kinetic examination

FIGURE 2. Structural, biophysical, and kinetic examination is performed on our library of IL-10 antibodies. Here, we enhance our workflow with foundational data regarding the critical attributes of matched pair immunoassay performance. These include (A) specificity and potential for cross-reactivity and (B) sensitivity,here graphically represented as binding response profiles; (C) structural epitope, represented as a dendrogram of diversity; and (D) the capacity for pairwise, matched-pair multiplexing, represented as a network map.

Performance Scoring

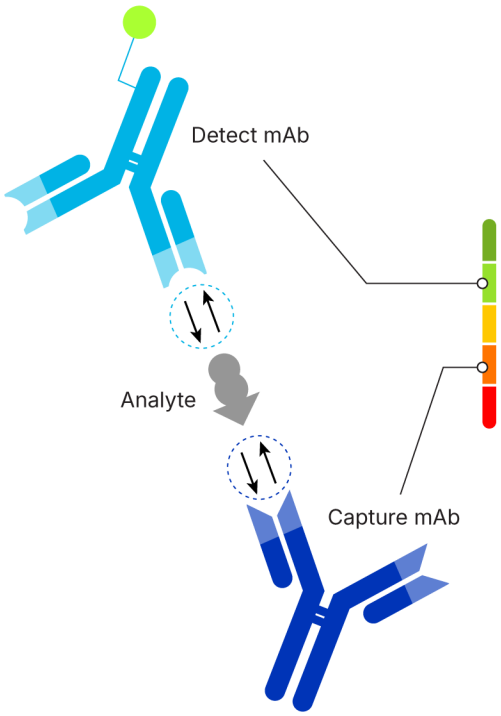
Our Algorithmic Process Scores and Filters the Landscape to Identify Top Leads.

Information regarding the degree to which each antibody and each composite lead possess the requisite performance qualities is contained with our generated landscape. Through the application of custom models and performance-scoring equations, the lead landscape is efficiently parsed according to, in this case study, predicted IL-10 Ella-specific performance (Figure 3).

Integration of our (1) extensive collection of target-specific antibodies, (2) high-content biophysical data collection methodologies, and (3) model-guided performance predictors enable rapid identification of the top 20 immunoassay leads—out of 50,000 enumerated and evaluated candidates—that were immediately directed downstream onto the Ella platform for performance validation.

FIGURE // 03

Ella Specific Performance



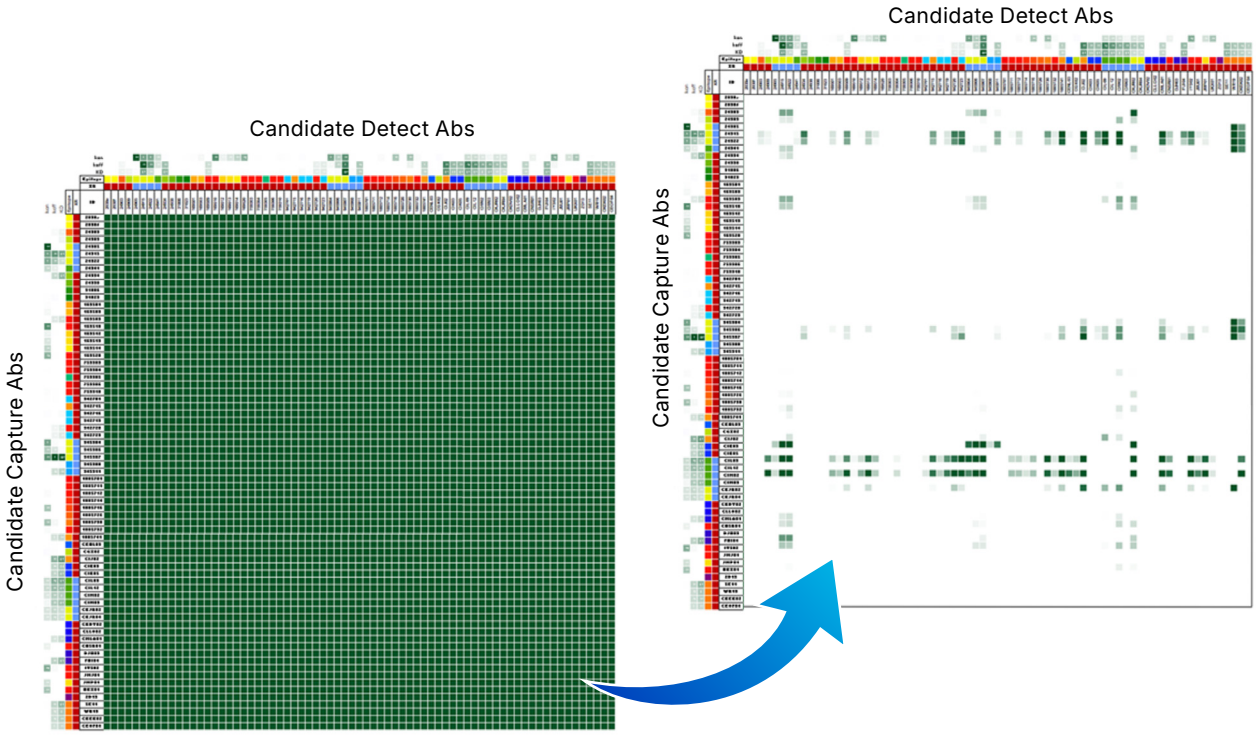


FIGURE 3. High-content data integration and algorithmic scoring filters and rank-orders the combinatorial landscape to empower the identification of a focused, custom-curated set of maximally diverse lead leads for downstream immunoassay development.

Assay Development Testing

Our Workflow Accelerates Development and Validation of High-Sensitivity Immunoassays.

We evaluated the set of 20 curated leads according to the assay-development best practices specified by the Ella platform.

This assay development process involves a sequence of validations, including core performance criteria such as specific detection of the biomarker calibrator protein; quantification of the detectability dose-response profile; assessment of the quantitative limits of detection; and percent detectability of endogenous levels of natural samples (e.g., serum, plasma, and cell culture supernates).

First, the 20 leads were evaluated for specific detection of the IL-10 calibrator (Figure 4A). Of the initial set of 20 leads, 18 moved to the next validation step. Second, calibration curves were produced to assess IL-10 biomarker dose response and non-specific binding (Figure 4B). The top six leads moved to the next validation step. Third, upper and lower limits of quantitation were determined (Figure 4C). All six leads moved to the next validation step. Finally, detectability of endogenous levels of IL-10 were examined (Figure 4D). Two leads (LF2 and LF11) with 100% endogenous level detectability were identified.

FIGURE // 04

Assay Development Tests on Ella

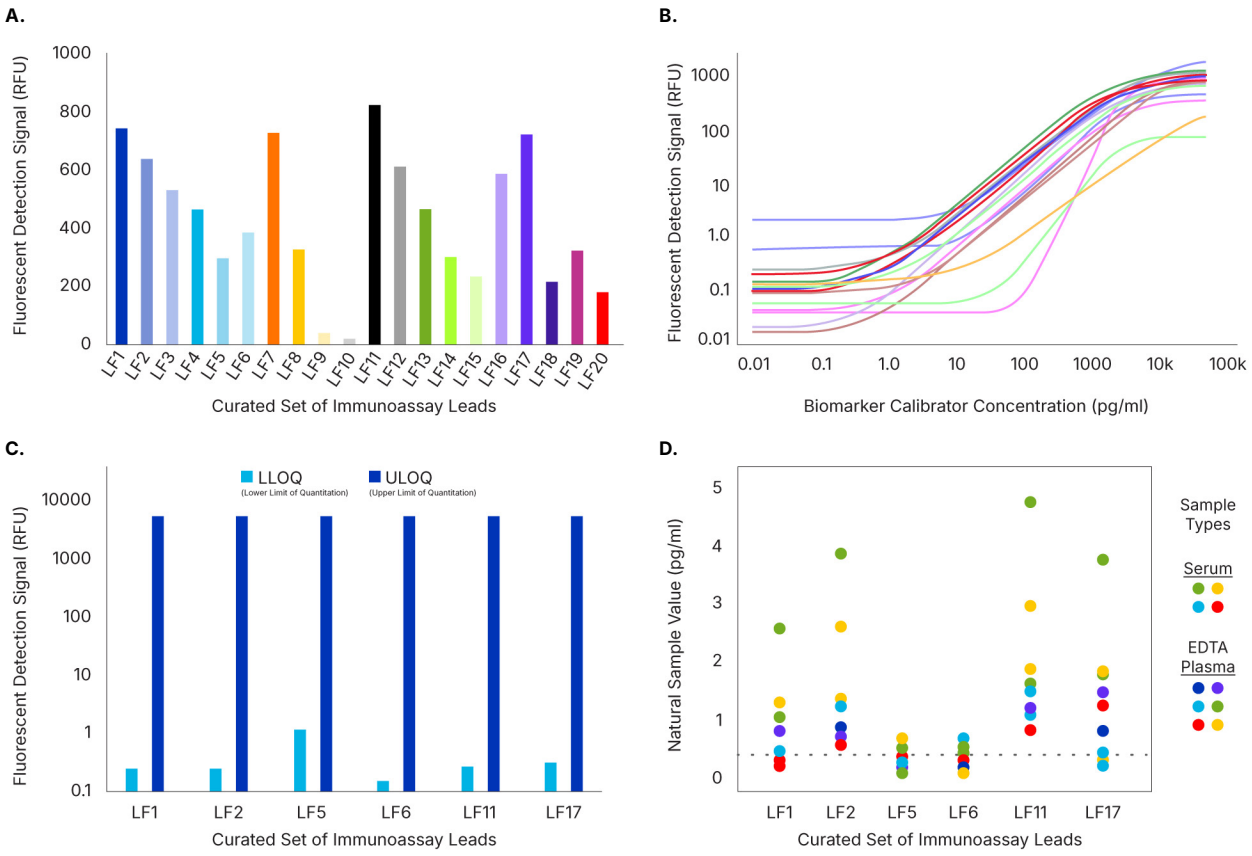


FIGURE 4. The identified leads were subjected to successive assay development tests on the Simple Plex Ella open access platform. We evaluated (A) the specific detection of the IL-10 calibrator, (B) the IL-10 calibration curves, (C) the limits of quantitation, (D) the endogenous level detectability of natural samples, and (E, F) the statistical precision of the selected leads.

Methodology Matters

Our Custom Workflow-Enabled Development Process Out-Performs the Conventional Approach.

To benchmark the performance of our workflow-enabled process, we compared our methodology to a conventional approach. To maximize the chances for a successful conventional development approach, 2700 leads were manually screened. Thus, the conventional approach requires considerable cost in operator time and reagents. Moreover, despite the large number of manually-screened leads, this sparsely sampled set covers only 5% of the total landscape of 50,000 leads. Further, because of the absence prior of performance predictors, once entered into the development process the conventional leads suffer severe step-to-step attrition, with the top two ultimately failing to meet the assay's requirements for precision and reproducibility.

In contrast, our custom workflow was capable of efficiently applying performance predictors to the entire combinatorial landscape candidates. This resulted in a specifically-curated set of 20 candidates to be entered into the immunoassay development process. Moreover, a significantly enhanced step-to-step success rate was observed that culminated in the rapid identification of two lead candidates that met all requisite standards of performance, precision, and reproducibility.

FIGURE // 05

Comparison of Methodologies

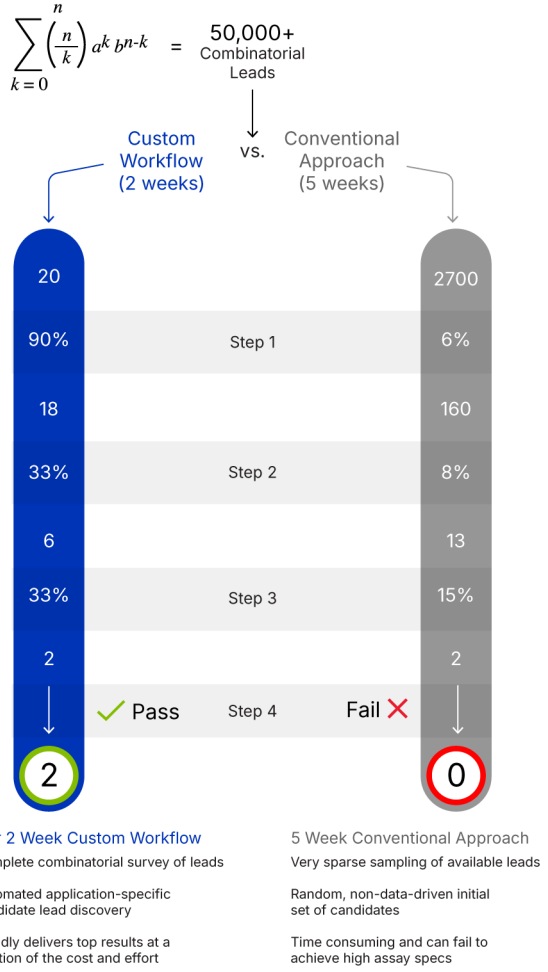


FIGURE 5. The performance of our workflow-enabled process (colored blue) was benchmarked against a conventional approach for large-scale screening (colored grey).

Conclusion

Bio-Techne's extensive collection of non-catalog antibodies and biophysical assessment capabilities enable us to offer customized antibody screening services tailored to the specifications and performance priorities of the intended application. Here, we apply a tailored screening process to evaluate key immunoassay performance characteristics of 130 of our catalog and non-catalog IL-10 antibodies, ultimately assessing more than 50,000 unique immunoassay antibody combinations. Our process efficiently uncovered a curated set of diverse leads that were then screened using Simple Plex assays running on the Ella open access 48-Digoxigenin platform for immunoassay development.