

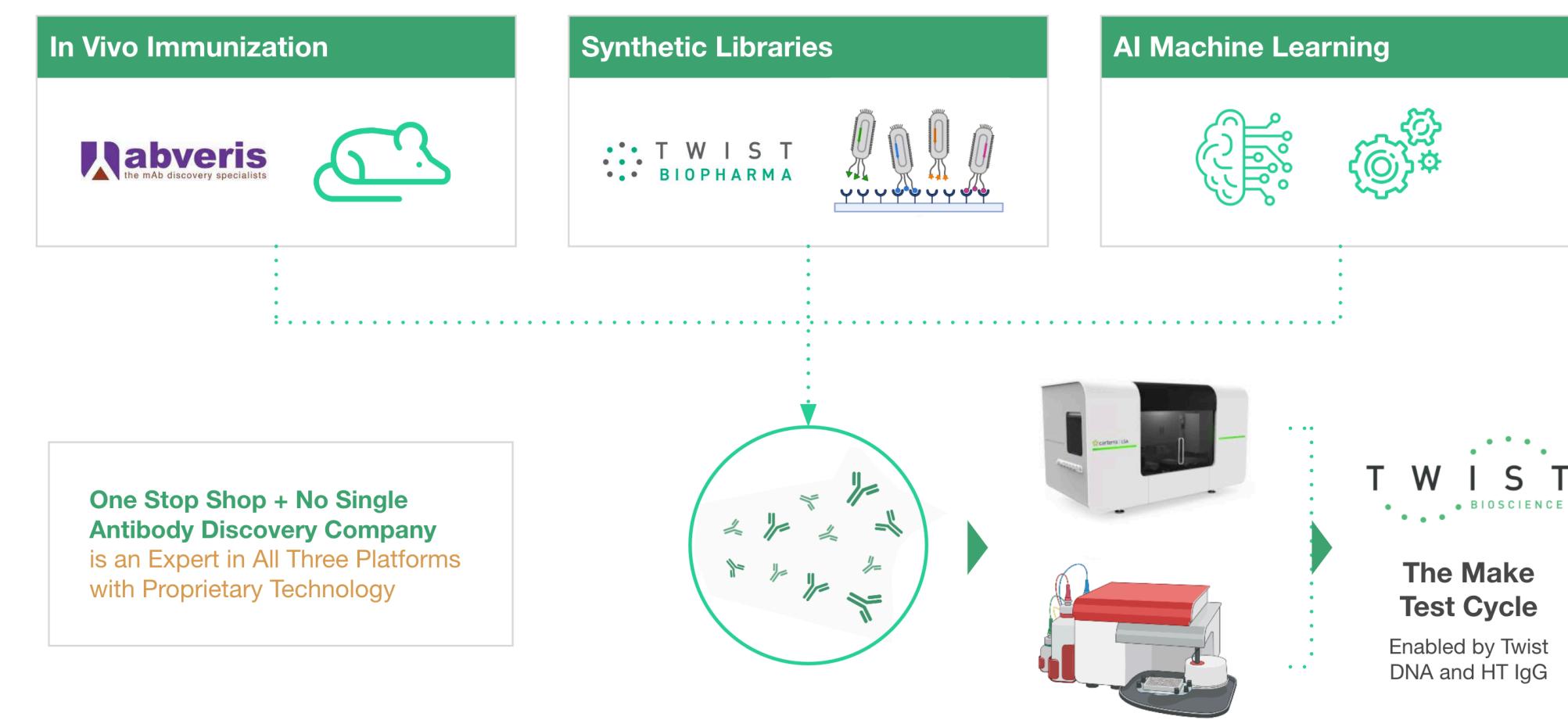
Antibody Therapeutic Development with An Integrated Platform Combines Immunized Libraries, Synthetic Libraries, AI/ML Guided Leads Optimization, and HT IgG Production at Twist Bioscience

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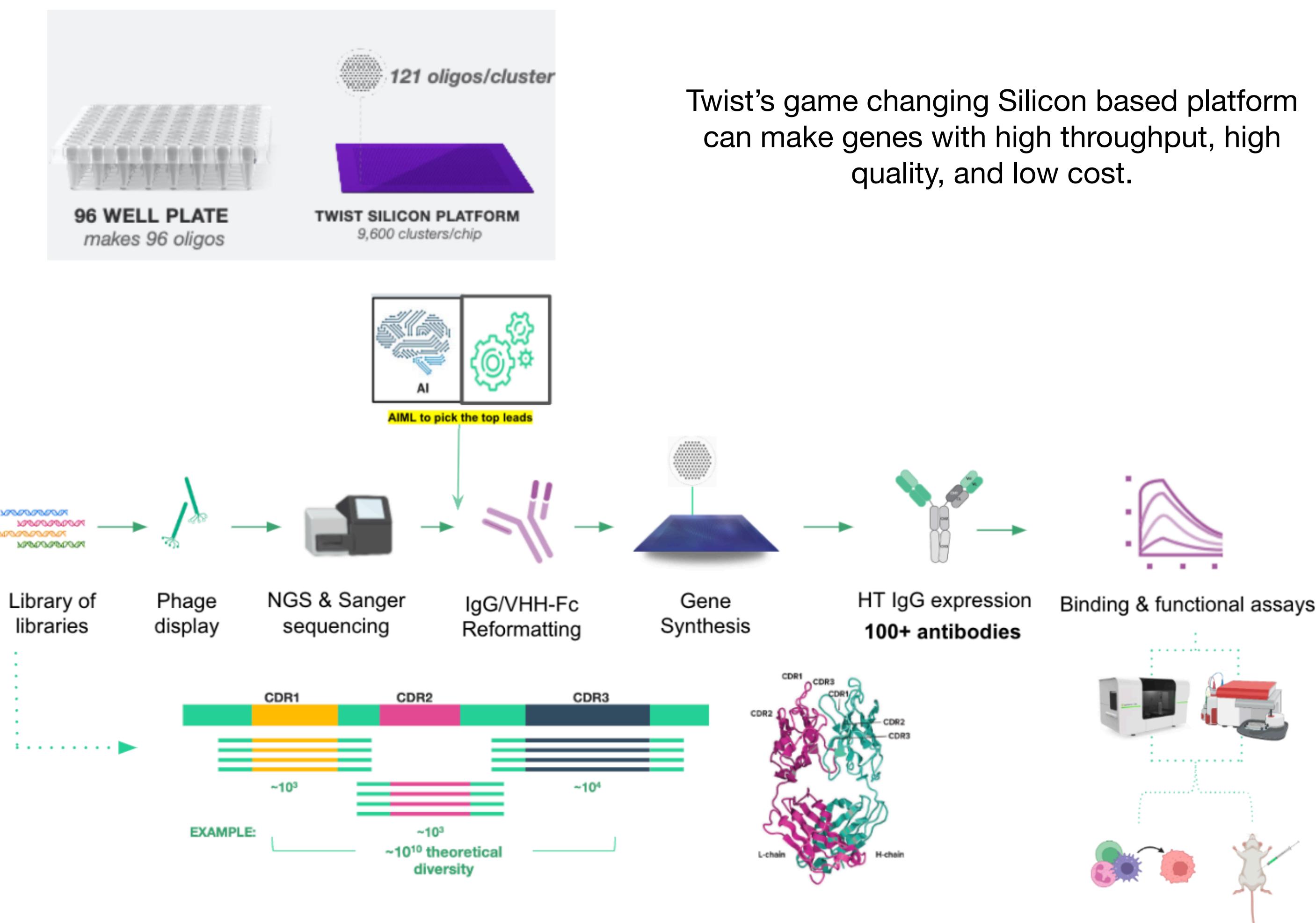
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ABSTRACT

Antibody drugs are a promising and rapidly growing component of the therapeutic landscape. The success of antibody therapeutics is largely driven by its specificity and selectivity in contrast to the off-targets liabilities of small molecule drugs. To advance antibody drug discovery, Twist integrated multiple techniques and platforms including synthetic and immunized libraries, artificial intelligence and machine learning (AI/ML), and High Throughput IgG Production (HT IgG) to discover, optimize, produce, and characterize antibody drug candidates against a wide range of inflammatory and immune-oncology targets to assist in generating leads for downstream pre-clinical and clinical campaigns. Wnt signaling pathway has been known to play important regulatory roles in tumorigenesis. Dickkopf-1 (DKK1) is an endogenous protein known to over-express in tumor micro-environments and can block Wnt/β-catenin signaling by interrupting Wnt-FZD-LRP interactions. Previous studies have shown the anti-tumor effect of anti-DKK1 antibodies in multiple different cancers including esophagogastric cancer, lung cancer, prostate cancer, etc. Here, we would like to show how Twist uses our integrated platform to identify functional anti-DKK1 antibody drug candidates through screening of hundreds of hits enriched from phage display and AI/ML affinity maturation. This platform can also be easily expanded to any other “difficult to target” drug targets.

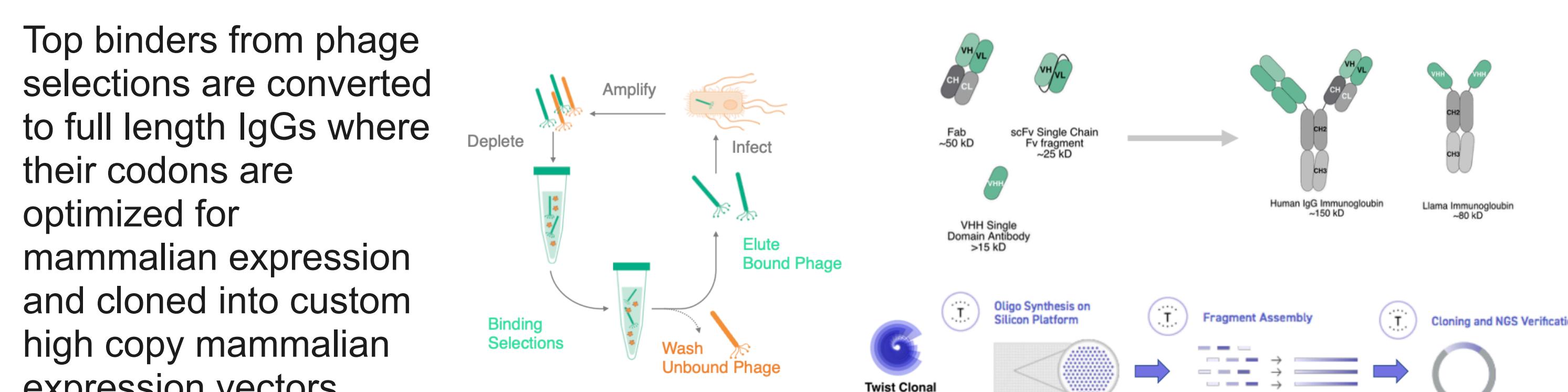


ANTIBODY DISCOVERY OVERVIEW

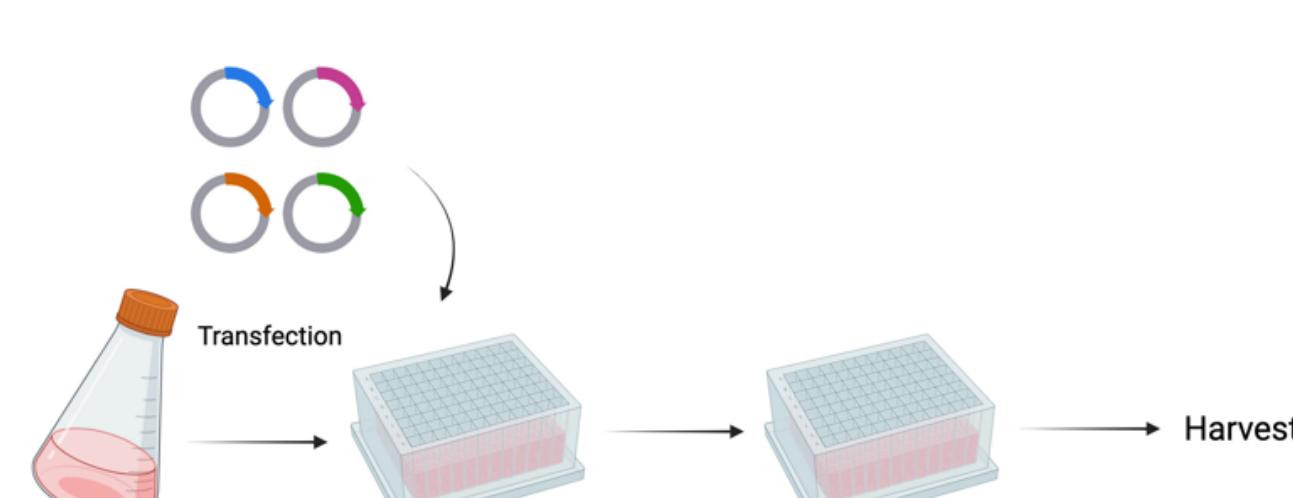


Twist's oligo synthesis technology can synthesize discrete oligo pools that are optimized during the design phase to maximize antibody library quality. Oligo pool's sequences are written directly from natural human diversity. Despite the natural sequences from human diversity, some may have isomerization, cleavage sites, deamination, and glycosylation sites but these liabilities can be removed upfront. Twist also developed a machine learning model to further enrich our libraries and to improve the specificity of binders. In these discovery campaigns, we were able to identify many binders against the DKK1 from the libraries.

A. Selection & High-Throughput IgG Conversion



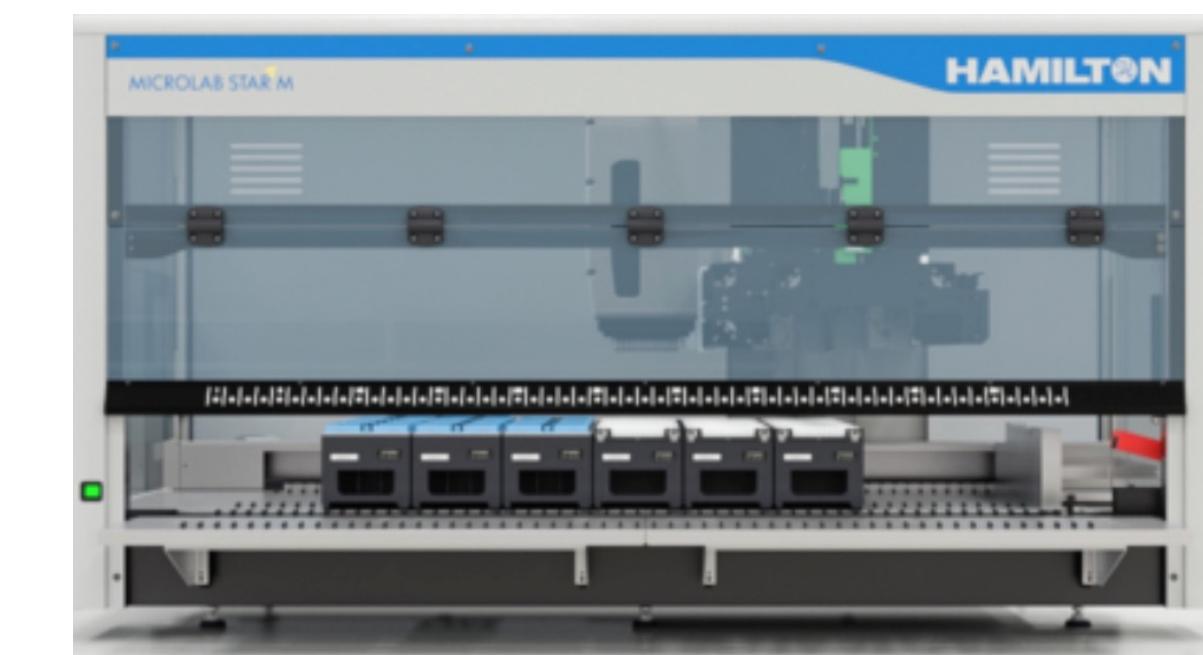
B. HT Antibody Production



The converted IgG DNA are transiently transfected into HEK293 cells in a 96 well format to produce antibodies. The 1mL transfected cultures will be developed for 4-5 days and harvested when culture's cell viability drop below 75%.

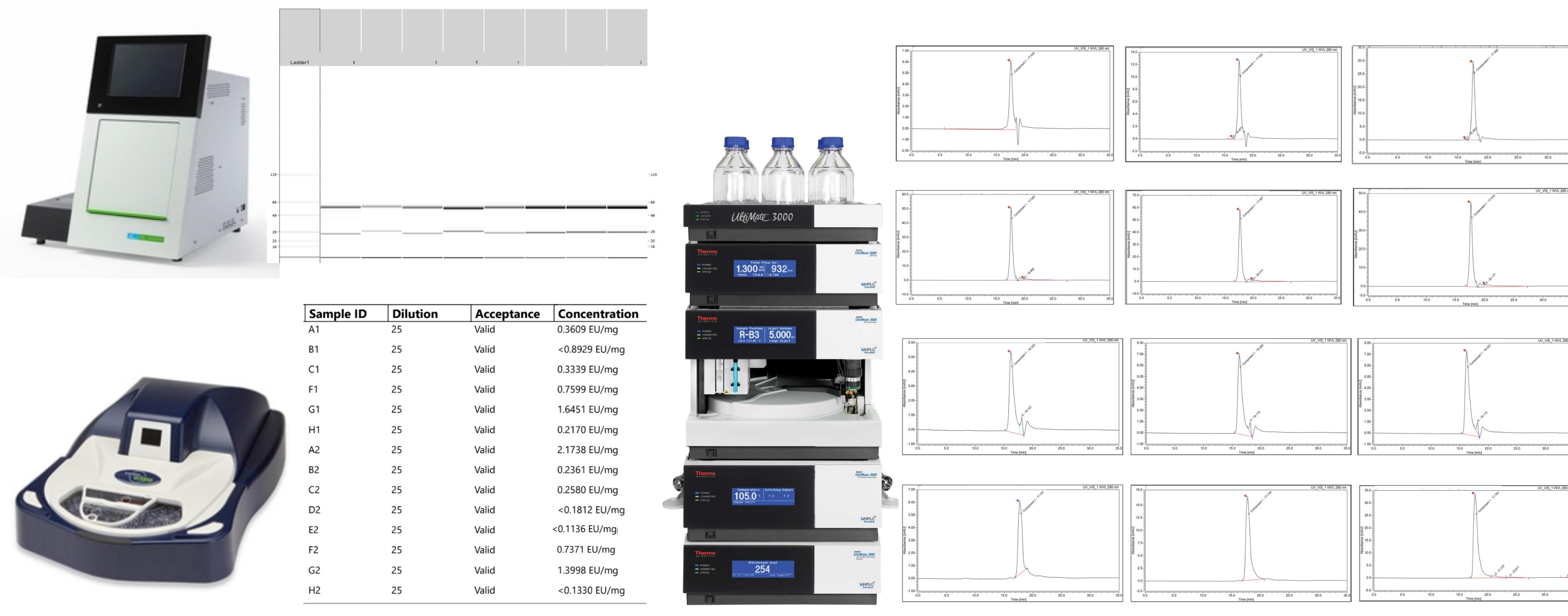
C. HT Antibody Purification via Hamilton Liquid Handler

The harvested cells are pelleted and the antibody-containing supernatants are transferred to purification plates where they'll then be subjected to automatic protein A purification with the Hamilton Star, an automated liquid handling system.

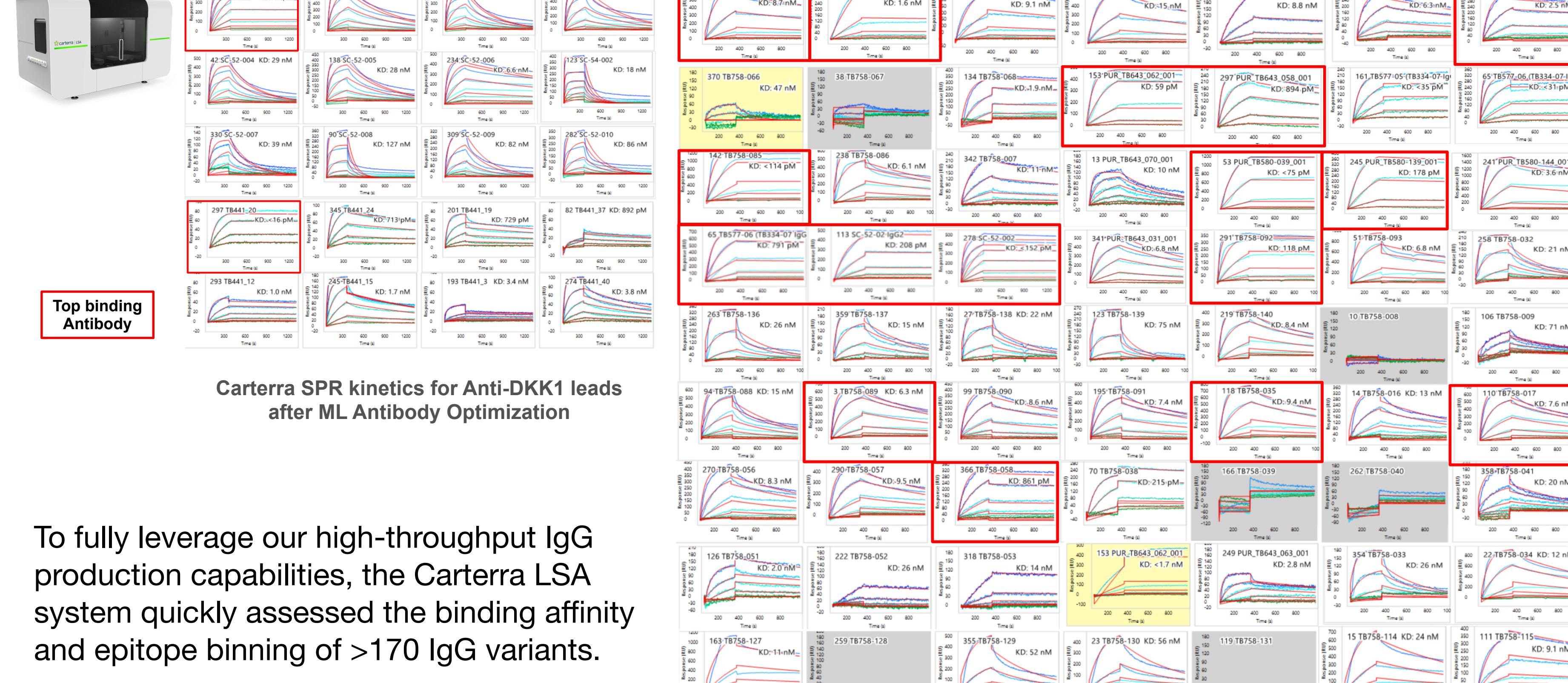


D. Biophysical Characterization of Purified IgGs

Purified antibodies are tested for its purity on Perkin Elmer Labchip, Sievers Eclipse and HPLC Analyses



BINDING ASSAY



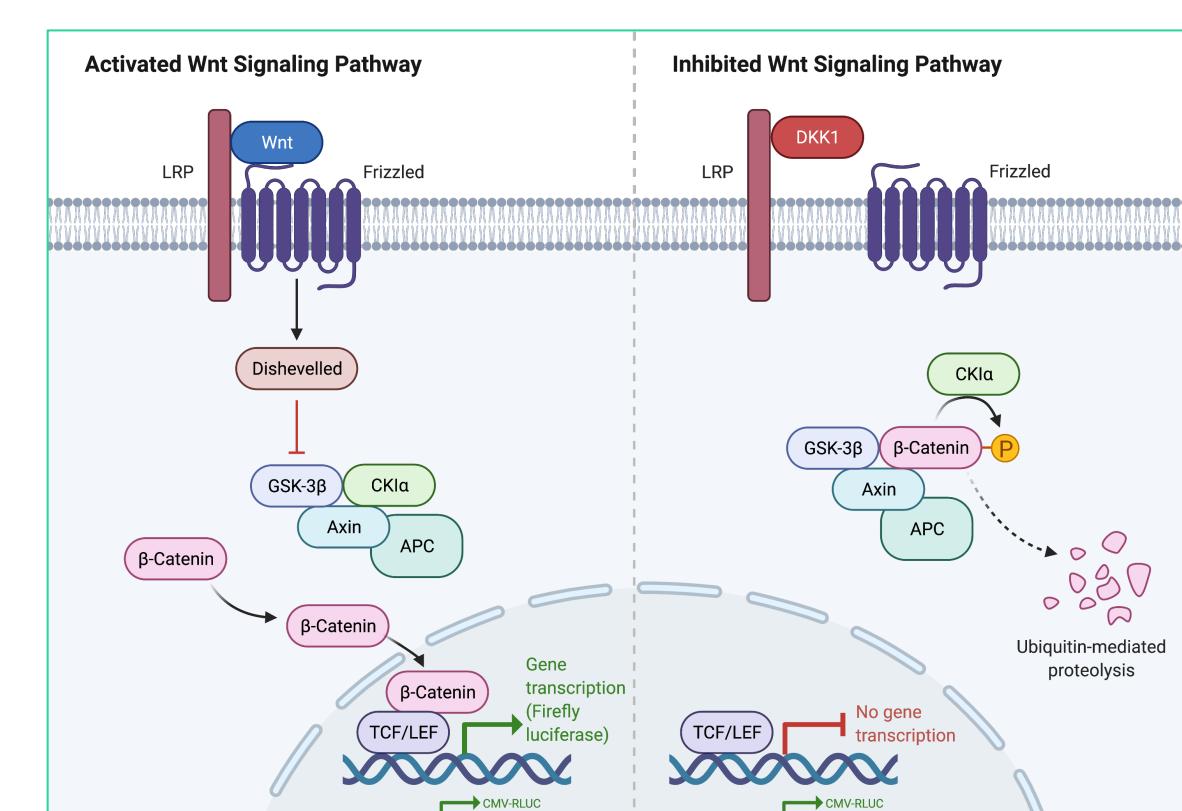
Carterra SPR kinetics for Anti-DKK1 leads after ML Antibody Optimization

To fully leverage our high-throughput IgG production capabilities, the Carterra LSA system quickly assessed the binding affinity and epitope binning of >170 IgG variants.

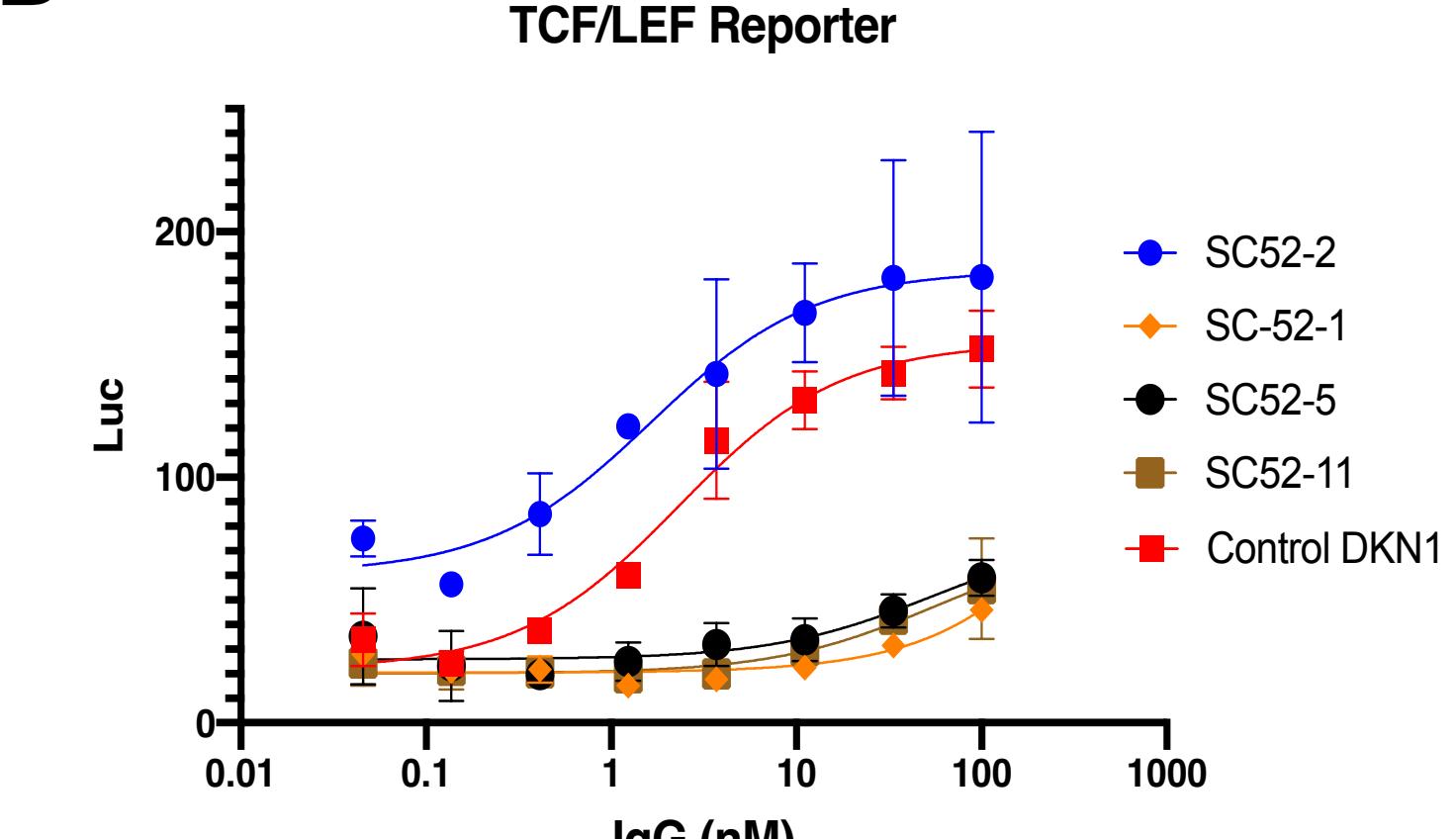
FUNCTIONAL ASSAY

1. TCF/LEF Reporter (Wnt Signaling) Assay

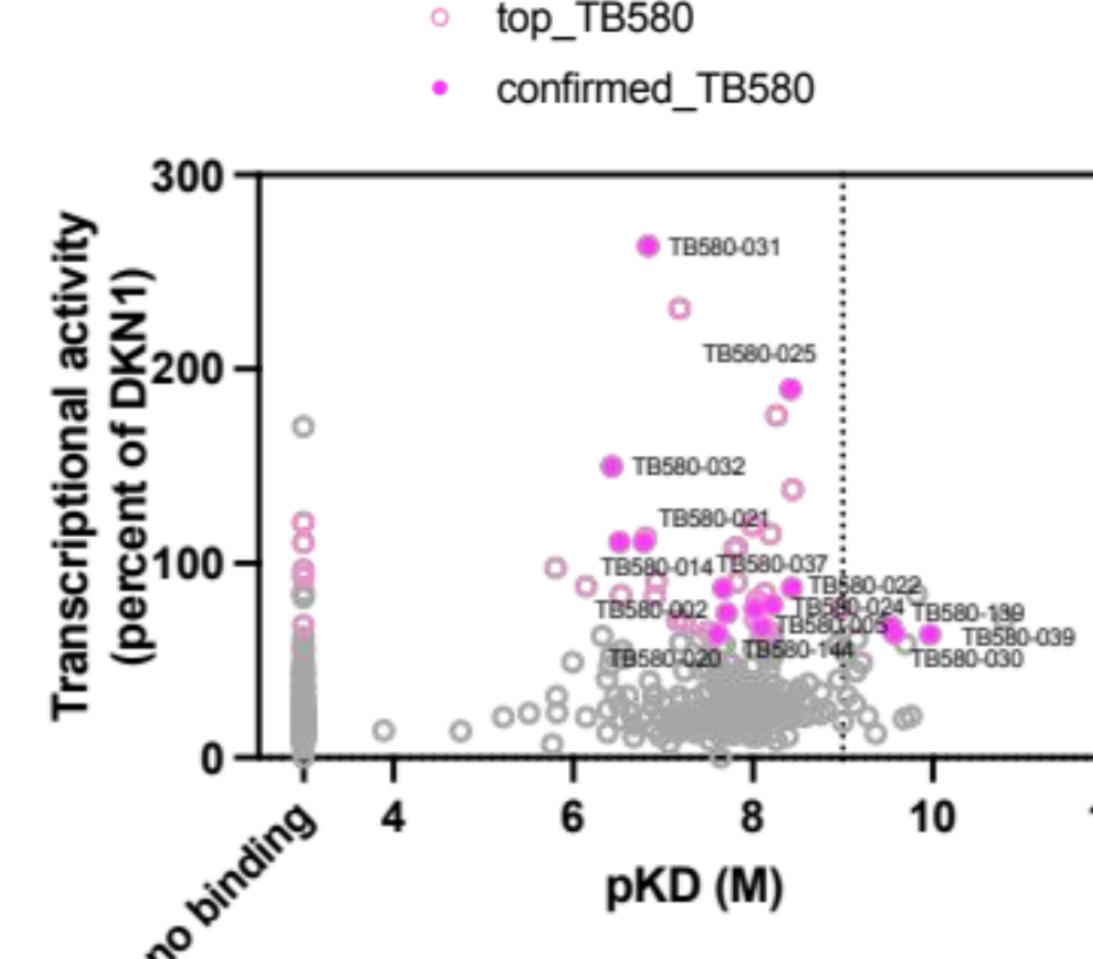
A



B



C



D

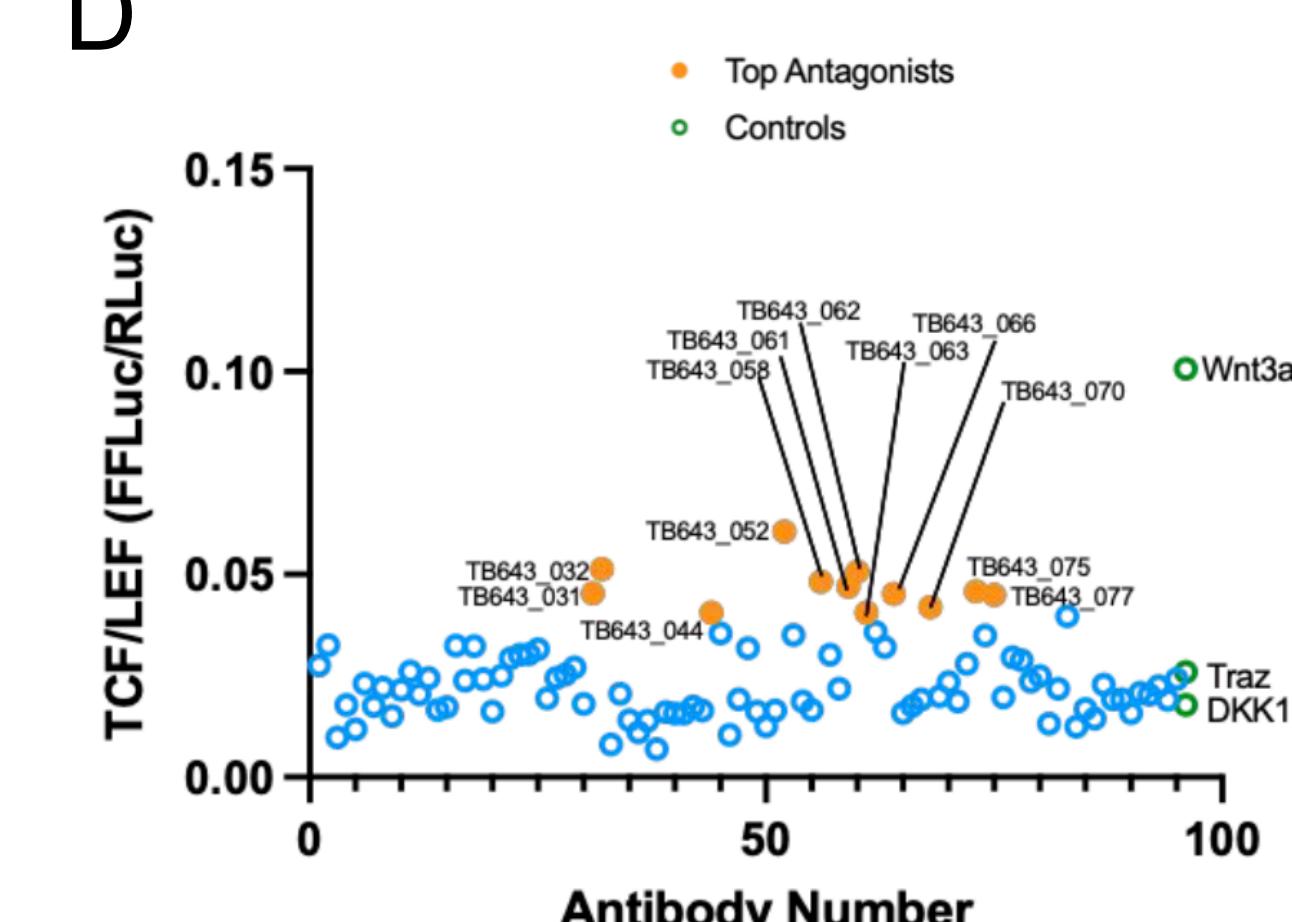


FIG. 1. Wnt TCF/LEF reporter assay screening. (A) Wnt TCF/LEF signaling is blocked by DKK1 binding to LRP5/6 receptor. (B) DKK1 VHH library screening, (C) and (D) ML Synthetic Library

SUMMARY

Our high-throughput antibody discovery platform utilizes synthetic libraries and immunized libraries, AI/ML, and HT IgG Production to support high throughput develop-ability studies of a panel of antibodies. Here, we have demonstrated how Twist uses the Integrated platform to identify, optimize, and characterize anti-DKK1 antibodies with high affinity to target and functional activity.