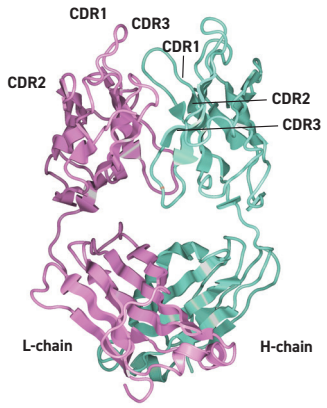
Twist Antibody Optimization Platform

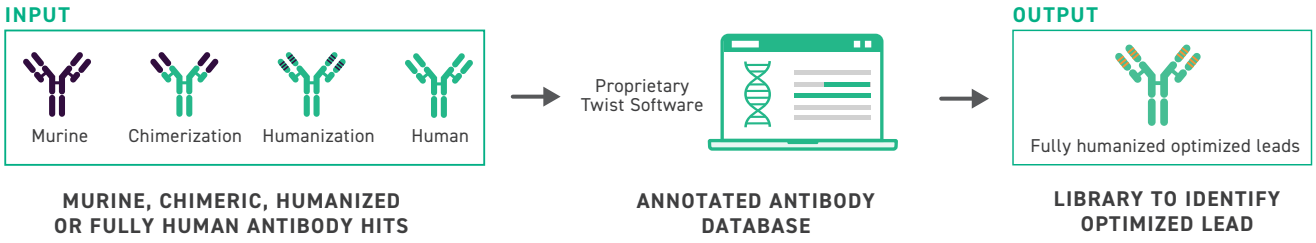
Quickly generate high-diversity, high-quality molecules inspired by human and non-human repertoires

Why generate custom libraries with TAO?

- For human antibody optimization, Twist uses a wide sequence space of tens of millions of natural human antibody sequences to crease an optimized library that precisely matches the human repertoire
- Liabilities that prevent optimal antibody expression and function are removed e.g. isomerization, cleavage, deamidation, glycosylation sites, liability dipeptide motifs
- Rational sampling from desired sequence space
- Workflows available for non-human species (e.g. felis or canis)
- Accurate sequence representation as motif sequences are explicitly encoded in the DNA oligos
- With our silicon-based DNA writing platform, have confidence that accurate, clone-perfect sequences are made when producing antibodies for screening

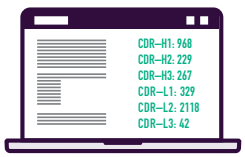


How it works: canonical humanization workflow

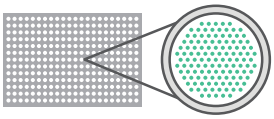


IN AS LITTLE AS 4 MONTHS

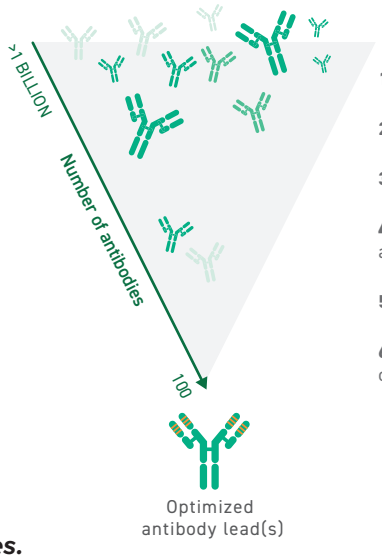
STEP 1
***In Silico* sequence assessment**



STEP 2
Order oligo pool sequences for library generation



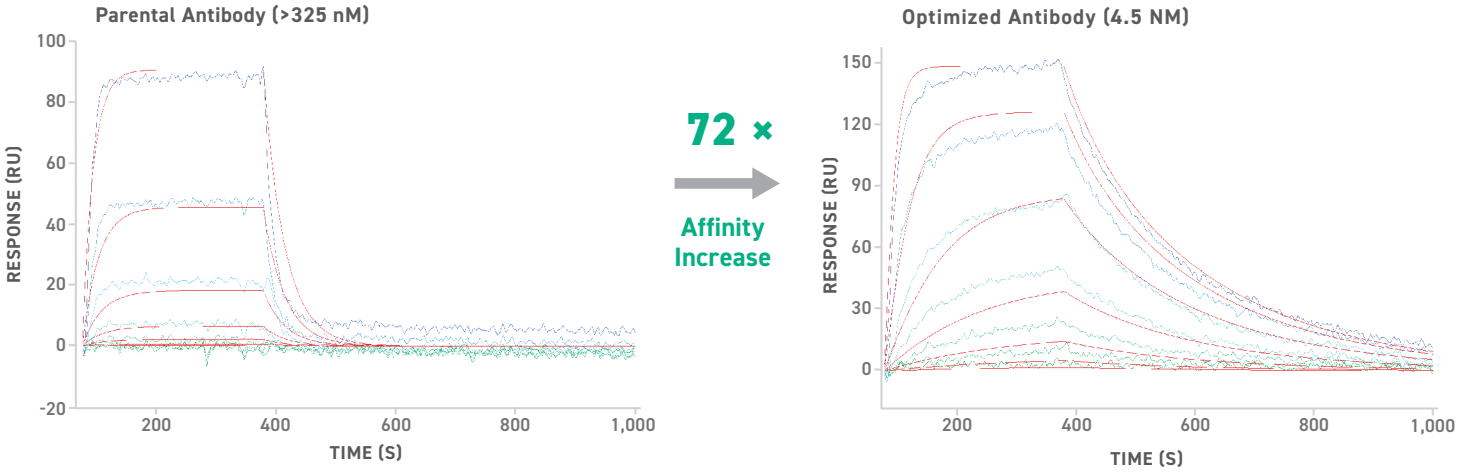
STEP 3
Twist Biopharma creates library



1. Construct phage or yeast library
2. Cell- or bead-based selection
3. NGS and/or clonal screening
4. Reformatting to IgG, DNA scale-up, and expression
5. High-throughput antibody purification
6. High-throughput analytical characterization and developability

For Research Use Only. Not for use in diagnostic procedures.

Using Twist Antibody Optimization, PD-1 inhibitors have higher affinity and potency

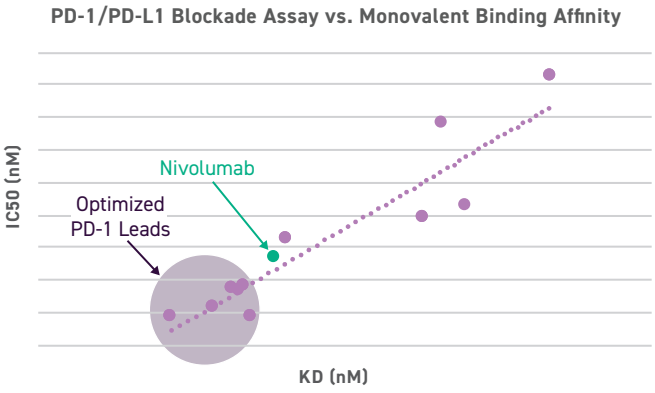


Multiple optimized leads block the PD-1/PD-L1 interaction

CLONE	SPR KD (nM)	IC50 (nM)
PD1_TA01	4.5	0.434
PD1_TA015	7.3	0.562
PD1_TA091	9.2	0.868
PD1_TA02	9.8	0.848
PD1_TA07	10.5	0.896
PD1_TA075	11.2	0.418
NIVOLUMAB	**14.5**	**1.345**
PD1_TA060	16.5	1.614
PD1_TA08	78.1	1.968
PD1_TA058	96.7	3,384
PD1_TA080	125	2.129
Parental	325	4.122

AFTER TWIST ANTIBODY OPTIMIZATION:

- Binding affinity went up 72×
- Function increased by 9.5×
- **Six antibodies identified with higher binding affinity and function than nivolumab**



Addition of anti-PD1 antibody blocks the PD-1/PD-L1 interaction, releases inhibitory signal and results in TCR activation and NFAT-RE-mediated luminescence (RU).

Affinity maturation of a functionally active 4-1BB antibody using Yeast Display

To improve antibody affinity, Twist uses both magnetic sorting and fluorescent based sorting methods coupled with our synthetic library design capabilities in a yeast display platform to reliably mature antibodies. A large population of enriched binders is seen after two rounds of FACS-based sorting. With careful control of antigen concentration we can carefully select for clones with improved binding to the target antigen. Individual clone performance can then be assessed by both clonal yeast cytometry and/or NGS.

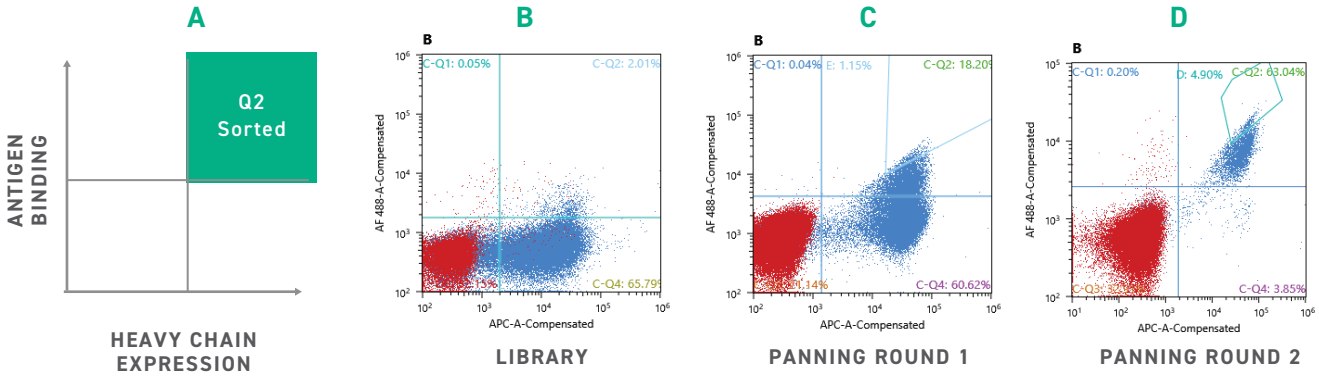
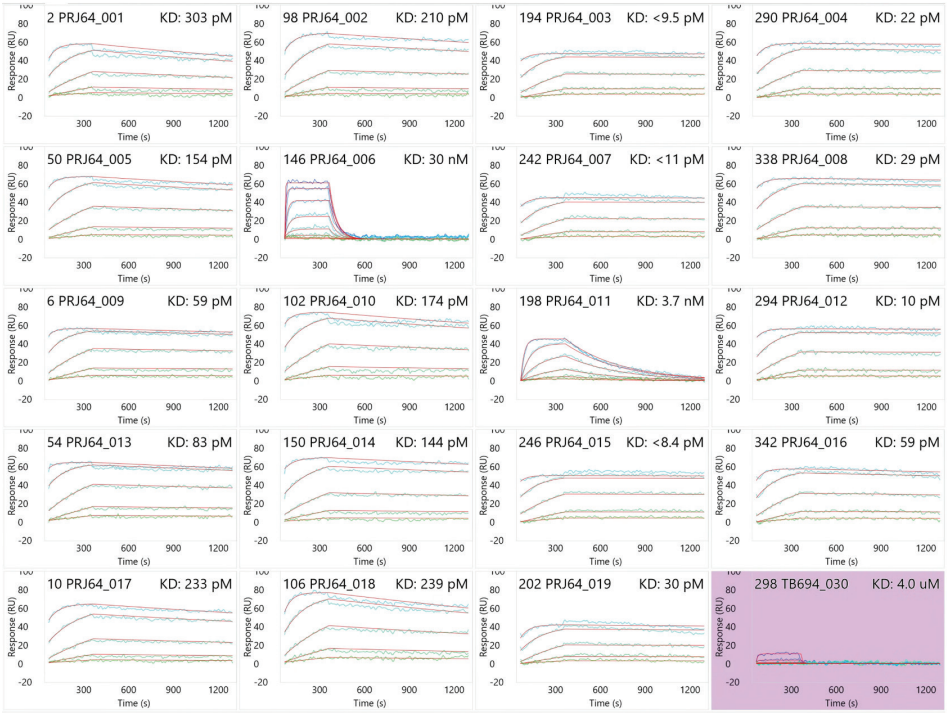


Figure Description: (A) Antigen binding is assessed by fluorescence shift along the y-axis and antibody fragment display along the x-axis. **(B)** Scatter plot of yeast sorting over multiple rounds of selection. The red population is the uninduced yeast population and the blue is the induced population. Aggressive gating schemes can be used to select for clones with ideal properties.



AFTER TWIST ANTIBODY OPTIMIZATION USING YEAST DISPLAY:

- Library design introduces combinatorial diversity for affinity maturation
- 19 unique clones were identified from Sanger sequencing
- **>10,000-fold affinity improvement to 41BB compared to starting antibody**

PARTNER WITH US.

Get in touch at **twistbioscience.com/antibody-discovery/twist-antibody-optimization** or contact us at **biopharmasales@twistbioscience.com**