

Machine Learning Enabled Humanization for More Efficient Antibody Engineering

Promising therapeutic antibodies from other species typically go through the process of humanization before they reach human patients. Humanization involves altering its amino acid sequence such that it more closely resembles a human antibody. While this is a common method used to minimize immunogenicity risk, humanization remains a substantial technical barrier. Twist Bioscience has developed a humanization platform that combines highly precise DNA synthesis with machine learning (ML) to improve the process to enable faster, more efficient, and ultimately more productive antibody engineering.

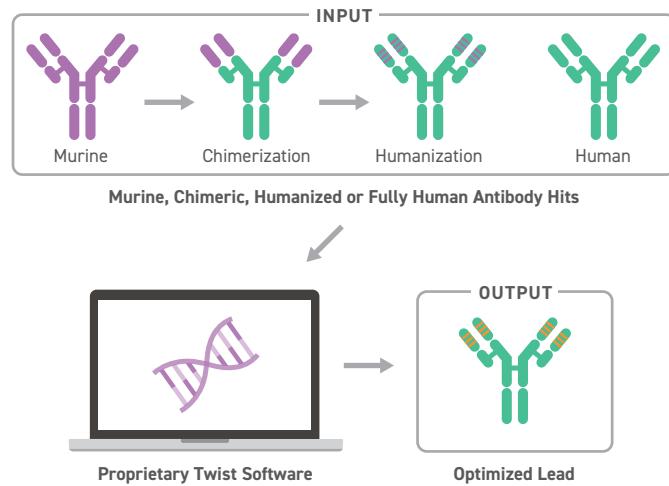
THE CHALLENGE OF HUMANIZATION

The human immune system is remarkably adept at recognizing foreign antigens. As such, biologics discovered in non-human animals have a substantial risk of triggering a patient's immune system, potentially leading to anti-drug antibody (ADA) formation which may impact pharmacokinetics, efficacy, or safety. It is therefore necessary to engineer them to appear more human. This process is known as humanization.

The process of humanization can be far from trivial. Every amino-acid substitution risks altering the antibody's behavior, from its binding affinity to its developability. Maintaining desirable properties during the humanization process can thus be tricky and often involves multiple cycles of engineering which can take a long time.

Researchers need the ability to predict the impact of amino-acid substitutions, both alone and in combination. The task is further complicated by a need to avoid amino acid liability motifs that, when present, may increase the antibody's likelihood to degrade, lose activity, aggregate or become immunogenic. This is where Twist can help.

HOW IT WORKS: CANONICAL HUMANIZATION WORKFLOW SHOWN

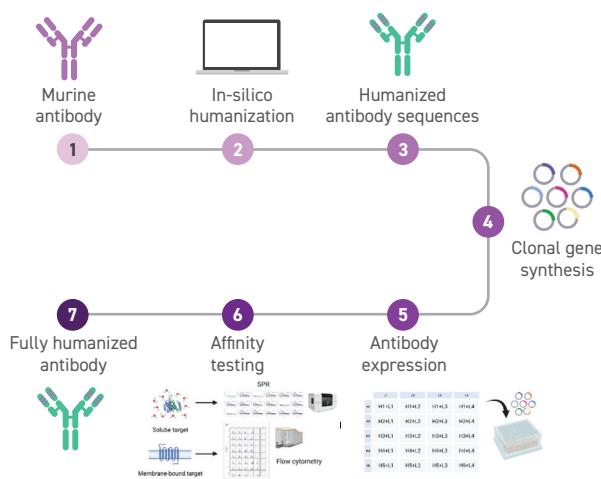


TWIST ML-ASSISTED HUMANIZATION

Twist has developed a humanization platform that leverages machine learning, Twist's industry-leading DNA synthesis capabilities, and Twist Biopharma's deep expertise in antibody engineering to rapidly generate humanized antibody candidates.

Importantly, the use of a powerful machine learning algorithm—one trained on millions of human antibody sequences—enables Twist to go beyond guesswork and instead design humanized antibody sequences that are highly likely to retain both functionality and developability. **Critically, this workflow bypasses the need to build and mine an antibody library, cutting down the time to delivery of a humanized antibody by more than half.**

With Twist's expertise, humanized candidates can be readily designed, synthesized and expressed for screening within 6–8 weeks.



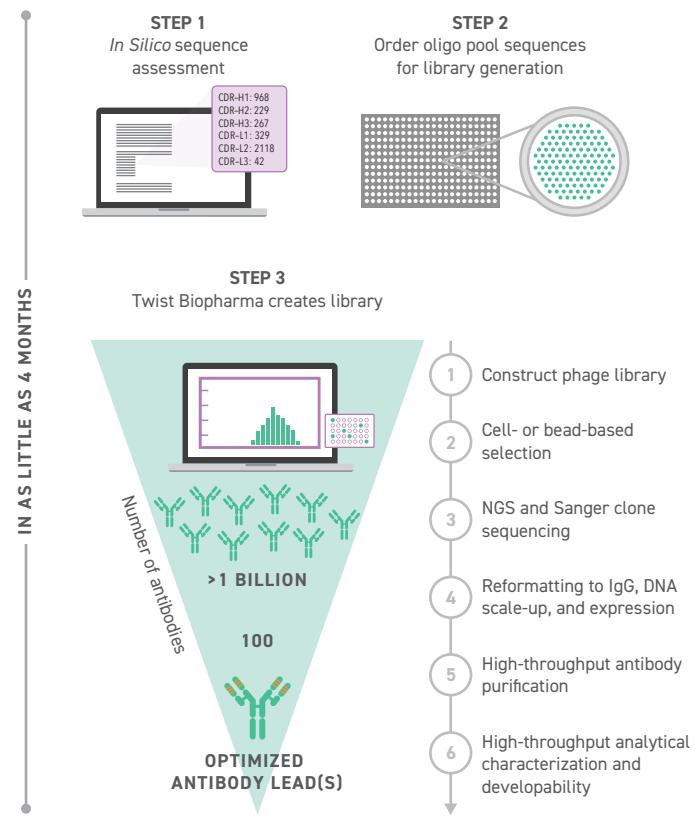
TWIST ANTIBODY OPTIMIZATION (TAO)

Following humanization, antibody candidates often need further optimization to improve binding affinity, selectivity, and pharmacokinetics. This multi-parameter optimization problem is particularly challenging as modifications that improve one feature may detract from another. Therefore, the optimization process requires designing and testing many different combinations of antibody variants to narrow in on a developable, functional, optimal candidate.

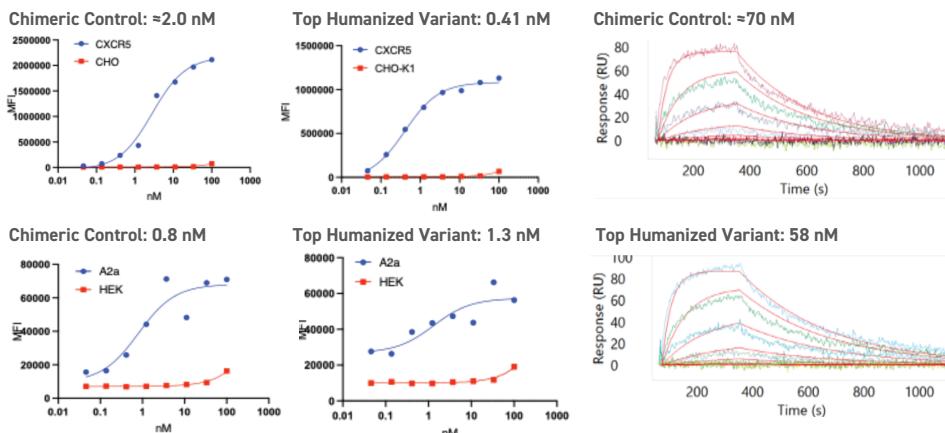
When such optimization is needed, researchers can leverage Twist's Antibody Optimization (TAO) platform. TAO leverages a database of tens of millions of natural human antibody sequences to design focused phage libraries to select the ideal candidate. TAO rationally samples from the natural sequence space while removing motifs that are prone to isomerization, cleavage, deamidation, and other liabilities.

Because libraries are created with Twist's industry-leading DNA synthesis platform with large oligo-pools, the resulting antibody library is highly uniform and accurate to design specifications. This means comprehensive screening can be achieved with minimal oversampling.

Together, Twist's humanization and TAO platforms enable rapid and efficient antibody engineering, ultimately helping researchers improve the productivity of antibody development pipelines.



FULLY-HUMANIZED AFFINITY ASSESSMENT



MURINE mAb	MURINE PARENTAL %HUMAN	CHIMERIC AFFINITY	HUMANIZED VARIANT %HUMAN	HUMANIZED TOP VARIANT AFFINITY
X	70% HC, 76% LC	$K_D \approx 70 \text{ nM}$	74% HC, 85% LC	$K_D \approx 58 \text{ nM}$
Y	63% HC, 70% LC	$EC_{50} \approx 0.8 \text{ nM}$	69% HC, 84% LC	$EC_{50} \approx 1.3 \text{ nM}$
Z	79% HC, 84% LC	$EC_{50} \approx 2.0 \text{ nM}$	88% HC, 94% LC	$EC_{50} \approx 0.41 \text{ nM}$

Using Twist's rapid humanization pipeline all three murine antibodies were successfully humanized. For 2/3 candidates, affinity of the top humanized variant was even modestly improved compared to its chimeric control.

TALK TO US ABOUT YOUR NEXT PROJECT.

Contact the Twist Bioscience team at support@twistbioscience.com or visit twistbioscience.com