TWIST BIOPHARMA

Library of Libraries

Twist Biopharma has leveraged Twist Bioscience's precise and massively parallel DNA synthesis technology to create the *Library of Libraries*, an unprecedented collection of synthetic antibody libraries that harnesses innovative structural and developability features to cover a wide range of antibody drug targets. Where discovery companies typically offer a single library, our experienced antibody discovery and engineering team has designed and constructed over 15 synthetic libraries to enable discovery of high-affinity drug-like antibodies, often without the need for affinity maturation. Each library contains up to 10^{10} antibodies in proven and highly developable human antibody frameworks across Fab, scFv, and VHH scaffolds. The *Library of Libraries* is rapidly expanding and offers highly diverse library choices, such as our VHH Library Series and Hyperimmune Library Series, as well as libraries specifically targeting hard-to-drug target classes like GPCRs, ion channels, and carbohydrates.

WRITING THE FUTURE OF BIOLOGICS

Please contact us to learn more about customizing your campaign at **biopharma@twistbioscience.com**

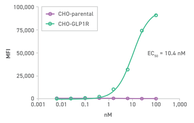
GPCR LIBRARY SERIES

GPCR 2.0 scFv ($1x10^{10}$ diversity)
GPCR 2.0 scFv is a fully human antibody library that leverages over 150,000 GPCR-binding motifs to direct antibodies to GPCR targets. This high variation library incorporates rules of the human repertoire.

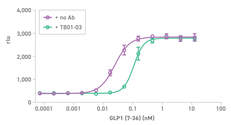
GPCR 3.0 scFv ($1x10^{10}$ diversity)
The GPCR 3.0 scFv library is modeled on 61 GPCR antibody sequences that target 22 different GPCR proteins. This library incorporates 2 heavy chain frameworks and 2 light chain frameworks.

VHH hShuffle GPCR ($1x10^{10}$ diversity)
The VHH hShuffle GPCR library shuffles GPCR-binding motifs in CDR3 from the GPCR 2.0 scFv library with sequences from a naïve llama repertoire (CDR1 and CDR2 regions) in the context of a partially humanized VHH framework.

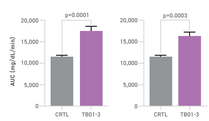
Twist's GLP-1R Antagonist Antibody: TB01-3
Twist has identified a high-affinity, potent GLP-1R antagonist, TB01-3, from our expertly designed and proprietary GPCR library. TB01-3 is a fully human IgG2 antibody and its function, pharmacokinetics, and *in vivo* efficacy have been characterized. TB01-3 acts as a dose-dependent competitive antagonist of GLP-1 and stabilizes higher blood glucose concentrations *in vivo*, making it an attractive candidate for further pre-clinical development. Twist has also characterized seven additional GLP-1R antagonists with EC_{50}s in the low nM range that are ready for additional testing.



I. Binding Assays and Flow Cytometry
TB01-3 binds with nanomolar affinity to specific GPCR target-expressing CHO cells and does not bind to CHO-parental cells.



II. cAMP Assay
The functional effect of IgG binding is assessed in a cAMP assay with GPCR target-expressing cells. The presence of TB01-3 results in a negative allosteric effect.



III. *In Vivo* Study
TB01-3 stabilizes higher blood glucose levels in an insulin tolerance test using a 6 hour (left) and 19+2 hour (right) dosing regimen.

VHH LIBRARY SERIES

VHH Ratio ($1x10^{10}$ diversity)
The VHH Ratio library models the natural VHH repertoire with 2,391 synthetic CDR sequences analyzed for position-specific variation. The library introduces controlled CDR diversity to produce amino acid ratios randomized at different positions.

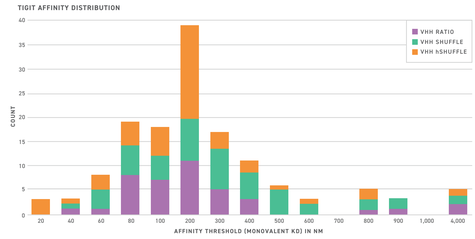
VHH Shuffle ($3.2x10^{9}$ diversity)
The VHH Shuffle library shuffles thousands of natural, individually sequenced llama CDR sequences within the context of a llama consensus framework.

VHH hShuffle ($3.2x10^{9}$ diversity)
The VHH hShuffle library shuffles thousands of natural llama CDR sequences within the context of a partially humanized VHH framework that incorporates 1,600 unique CDR3s. This framework confers lowered immunogenicity for therapeutic development.

VHH hShuffle Hyperimmune ($1x10^{10}$ diversity)
VHH hShuffle Hyperimmune is a hybrid library that shuffles llama CDR1 and CDR2 sequences with human CDR3 sequences. Building on the VHH hShuffle library, this library increases CDR3 diversity with over 2.5 million unique human CDR3s.

VHH hShuffle GPCR ($1x10^{10}$ diversity)
See GPCR Library Series.

Binding Affinities
An SPR array screen demonstrated tight anti-TIGIT antibody binding affinities. Of the 141 antibodies measured, all had binding affinities < 200 nM. A third had binding affinities < 100 nM.



HYPERIMMUNE LIBRARY SERIES

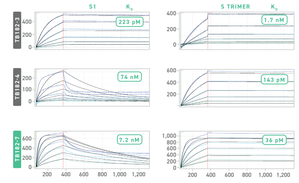
Hyperimmune Fab ($1x10^{10}$ diversity)
The Hyperimmune Fab library offers diversity in both heavy and light chains.

Hyperimmune scFv ($1x10^{10}$ diversity)
The Hyperimmune scFv library offers single-chain binders that are smaller than their Fab counterparts with the same diversity.

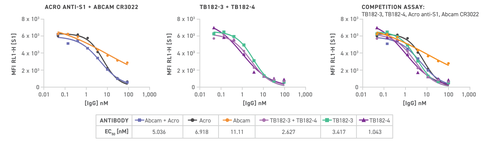
Hyperimmune Common Light Chain Fab ($1x10^{10}$ diversity)
The Hyperimmune Common Light Chain Fab library combines the heavy chain diversity from the Hyperimmune Fab library with a fixed trastuzumab light chain, making it useful for generating bispecifics.

VHH hShuffle Hyperimmune ($1x10^{10}$ diversity)
See VHH Library Series.

Anti-S1 mAbs Kinetics, SARS-CoV-2: Direct Coupled Antibodies
Twist's Hyperimmune Library has been effective at uncovering SARS-CoV-2 S1 Protein virus leads.



S1 RBD: VERO E6 Inhibition by FACS
TB182-3 and TB-182-4 Show Potent Inhibition of S1 Binding to ACE2-expressing VERO E6 cells.



scFv LIBRARY SERIES

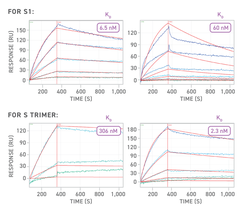
ANCESTRAL

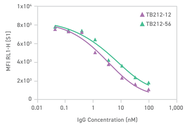
Ancestral scFv ($1x10^{9}$ diversity)
The Ancestral scFv Library is a synthetic antibody library developed using trends observed in a curated, yet broad, set of 22,426 therapeutic and diagnostic antibodies. By capturing the diversity observed in examined antibody sequences and mimicking the human antibody repertoire, this library offers higher quality sequences than naïve libraries to help you identify better hits against any target.

Proof of Concept Data
The Twist Ancestral scFv Library was successfully panned against SARS-CoV-2 Spike Protein S1. A large number of unique clones with diverse binding affinities were identified.

Uncover Anti-S1 Antibodies with High Binding Affinity
Kinetics with directly coupled anti-S1 antibodies via surface plasmon resonance identifies antibodies like TB212-12 and TB212-56 with high binding affinity for S1 and S trimer.

Potent Inhibition of VERO E6 Cells by FACS
Flow titration demonstrates that TB212-12 and TB212-56 show inhibition of S1 binding to ACE2-expressing VERO E6 cells.





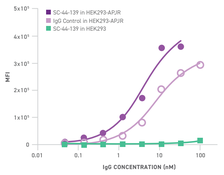
ANTIBODY NAME	EC_{50} (nM)
TB212-12	3.07
TB212-56	6.22

ION CHANNEL

Ion Channel scFv ($1x10^{9}$ diversity)
The Ion Channel scFv library integrates loop sequences from natural peptide toxins that target ion channels. This allows the library to target these classically difficult-to-drug proteins without cytotoxicity concerns. This library is available in two formats: one with paired cysteines (Cys+ Library) and one without paired cysteines (Cys- Library).

Proof of Concept Data
The Twist Ion Channel scFv Library was successfully panned against APJR, a multi-pass transmembrane receptor, to identify multiple unique clones that bind this cardiovascular target. Flow titration demonstrates that antibodies like SC-44-139 bind APJR+ positive cells with high affinity.

	SC-44-139 (Human IgG)	IgG Control (Mouse IgG)
EC_{50} (nM)	4.4	8.3

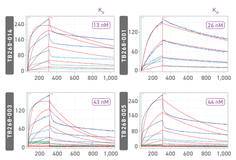


AI HYPERMUTATED

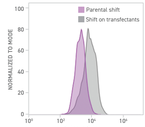
AI Hypermutated scFv ($1x10^{9}$ diversity)
The AI Hypermutated scFv Library unleashes the power of artificial intelligence to augment the design of a synthetic antibody library. A neural network mimics B cell receptor recombination and hypermutation and produces antibodies with developability in mind.

Proof of Concept Data
The Twist AI Hypermutated scFv Library was successfully panned against SARS-CoV-2 Spike Protein S1, a key protein on the surface of the coronavirus, to identify unique clones with desirable properties. Affinity was determined via surface plasmon resonance and activity was demonstrated in competition assays.

Kinetics with Directly Coupled Anti-S1 Antibodies
The AI Hypermutated scFv Library effectively uncovers SARS-CoV-2 S1 antibody leads with high binding affinities.



Potent Inhibition of VERO E6 Cells by FACS
A panel of anti-S1 antibodies from the AI Hypermutated scFv library shows inhibition of S1 binding to ACE2-expressing VERO E6 cells. A flow cytometry plot for representative clone TB268-14 illustrates a shift in the transfectant population compared to the parental population.



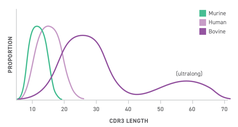
SAMPLE	MEDIAN RL1-H OF SINGLETS	COMPETITION FACTOR
TB268-014	1907.5	3.61
TB268-005	3790	1.82
TB268-001	4898	1.41
TB268-003	7807	0.88

MINOTAUR

Minotaur scFv (>10^{9} diversity)
This scFv library inserts ultralong bovine HCDR3s into a human antibody framework. The unique bovine HCDR3s provide access to hard-to-target epitopes, such as those found in pores and channels. This library includes two sublibraries: Sublibrary 1 with cysteines in HCDR3 only and Sublibrary 2 with cysteines in HCDR3 and other regions (HCDR2 and framework).

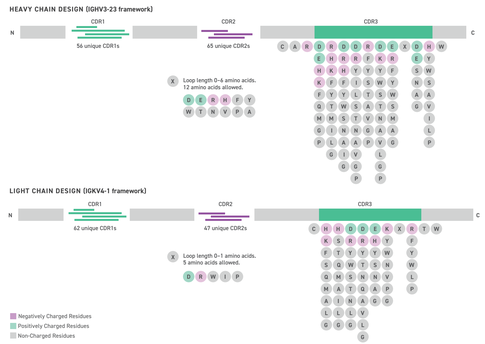
Library Specifications
The Twist Minotaur scFv Library incorporates ultralong bovine HCDR3s into the VH3-23/VK1-39 human antibody framework. The ultralong HCDR3 loops are up to 60 amino acids in length, making them three- to fourfold longer than the average human CDR3 loop.

The Twist Minotaur scFv Library includes a set of two sublibraries, each of which incorporates diversity from the Twist Hyperimmune Original Fab Library (HCDR1, HCDR2, LCDR1, LCDR2, and LCDR3) and a proprietary bovine antibody database (HCDR3). Both sublibraries are provided as a part of the Minotaur scFv library, and can be panned in parallel against your target of interest.



CARBOHYDRATE

Carbohydrate scFv ($2x10^{9}$ diversity)
To address the difficult-to-drug nature of carbohydrates, this library shuffles unique CDRs from 130 existing carbohydrate antibodies across the CDR1 and CDR2 regions. The CDR3 regions derive their diversity from 52 structures of antibodies in complex with carbohydrate antigens and are biased towards incorporating residues that make up the carbohydrate-antigen interface.



STRUCTURAL

Structural scFv ($4x10^{10}$ diversity)
This general-use scFv library incorporates CDR sequences from 3,700 antibodies with known crystal structures. By starting with structurally resolved antibodies, this library generates leads that are "well behaved" and therefore have more potential to be developable as therapeutics.

Proof of Concept Data
The Twist Structural scFv Library was successfully panned against CD3, an important cell surface target in immunology, to identify unique clones, such as TB138-6, with desirable properties.

Cross-reactive with cynomolgus CD3
Titration ELISA shows the cross-reactivity of TB138-6 with human and cynomolgus monkey CD3.

Binds cell surface CD3 on human CD8+ T-cells
Flow titration demonstrates that TB138-6 binds CD3+ cells (CD8+ T-cell) and not CD3- cells (CHO-GLP1R).