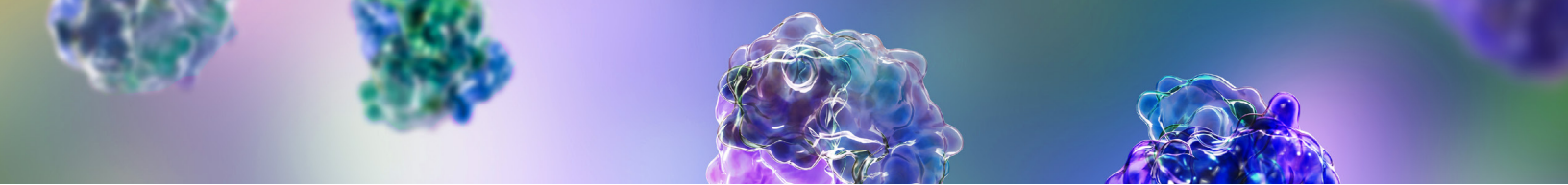


A large, detailed 3D visualization of an antibody molecule, rendered in a translucent, multi-colored (purple, blue, green) surface representation. The molecule is Y-shaped and occupies the right half of the slide. In the top left corner, there is a smaller, partially visible similar structure.

# Introduction to Antibody Libraries for Display-Based Antibody Discovery



**LakePharma**  
*The Biologics Company*



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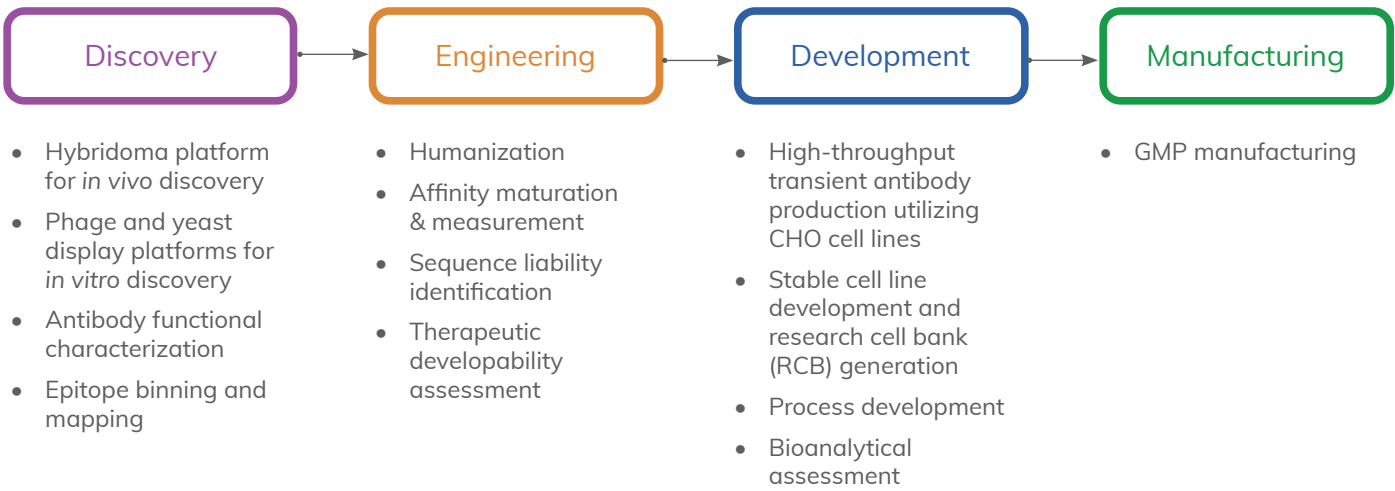
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# About the Author

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LakePharma is a leading US-based contract research, development, and manufacturing organization (CRDMO). LakePharma offers comprehensive, end-to-end integrated solutions for antibody discovery, development, and manufacturing.



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## Surface Display Technologies in a Nutshell

Protein engineering has greatly benefited by the advent of *in vitro* display platforms, where individual or large libraries of peptides or proteins are displayed on the surface of mammalian, yeast, or bacterial cells or even bacteriophage capsids. Here are the main principles of phage and yeast surface display platforms:

### Phage Display

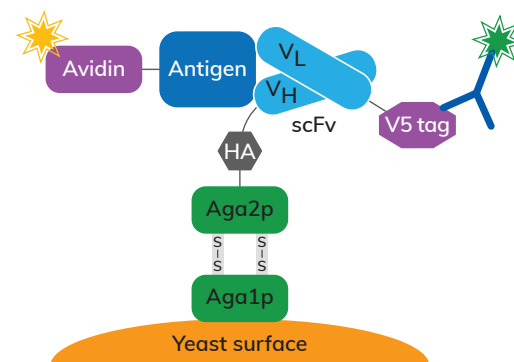
- Phage display is based on isolation of large pools of antibody genes cloned into different scaffolds such as antigen-binding fragments (Fab), single-chain variable fragments (scFv), or VHH (nanobody).
- The genes encoding these scaffolds are then fused into phage genome or phagemids and packaged into displaying bacteriophage, resulting in the generation of large and diverse antibody library repertoires.



Structure of a displaying bacteriophage is shown here.

### Yeast Display

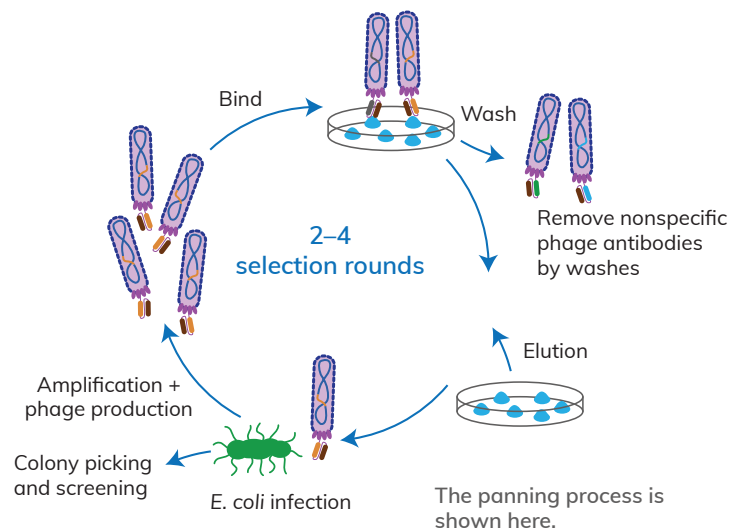
- Yeast display involves the insertion of a large spectrum of antibody sequences into yeast surface display vectors.
- Fusion of Fab, scFv, VHH antibody fragments, or full-size IgGs to the yeast Aga2 protein coupled with inducible expression of yeast Aga1 cell wall protein ensures successful display on the yeast cell surface.
- Yeast cells are then sorted by flow cytometry (FACS). Since large numbers of antibody fragments (up to  $10^5$ ) can be displayed on a single cell, quantitative selection by FACS can be employed to simultaneously monitor surface expression and antigen binding.



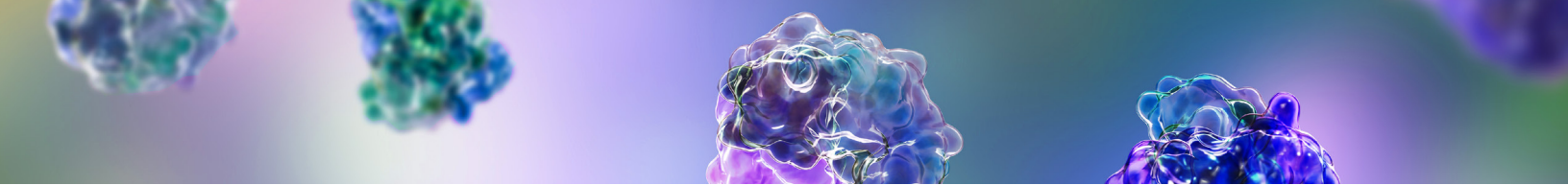
Structure of the yeast cell surface with display proteins are shown here.

## Antibody Phage Panning

Antibody panning is an affinity-driven process to select binding partners from phage display libraries. Following sequential rounds of incubation, washes, and amplification, selected binders are enriched over a larger population of non-specific antibody-displaying phage.







## Phage vs Yeast Display


Some of the most obvious advantages of yeast surface display is proper folding and/or improved solubility of complex proteins, both which is attributed to mammalian-like post-translational modifications (e.g. glycosylation). On the other hand, the major advantage of phage display is superior *E. coli* transformation efficiency, resulting in larger phagemid libraries<sup>1,2</sup>. These two platforms can be combined, starting with a couple of rounds of phage display selections involving a very large number of antibody fragments and followed by more stringent or focused selections of target-specific antibodies via yeast surface display<sup>3</sup>.

	Phage Display	Yeast Display
System	Prokaryotic Expression	Eukaryotic Expression
Library Size	Very Large: Up to 10 <sup>12</sup>	Medium to Large: Up to 10 <sup>9</sup>
Display	1~5 displayed copies/phage particle	Up to 10 <sup>5</sup> displayed protein copies/yeast cell
Selection & Screening	<ul style="list-style-type: none"><li>• Various panning formats<ul style="list-style-type: none"><li>• Immobilized</li><li>• Soluble</li><li>• Bead-based</li><li>• Cell-based</li></ul></li><li>• Screening via ELISA or FACS</li></ul>	<ul style="list-style-type: none"><li>• FACS enables antibody binding with more engineering options</li><li>• During FACS screening, selection parameters (e.g. real-time kinetic observations, number of collected cells) can be monitored, modified, and fixed</li><li>• Additional applications (affinity maturation, species cross-reactivity, epitope mapping)</li></ul>
Additional Features	Faster turnaround for conventional targets	More suitable for complex targets

<sup>1</sup> Ledsgaard L. et al. 2018. Basics of Antibody Phage Display Technology. *Toxins* (Basel). Jun 9;10(6)

<sup>2</sup> Sheenan J and Marasco WA . 2015. Phage and Yeast Display. *Microbiol Spectr.* Feb;3(1)

<sup>3</sup> Ferrara F. et al. 2019. Recombinant Antibody Selections by Combining Phage and Yeast Display. *Methods Mol Biol.* 2019;1904:339-352



# Overview of Different Display Library Types

Naïve Antibody Libraries

Immune Antibody Libraries

Synthetic Antibody Libraries

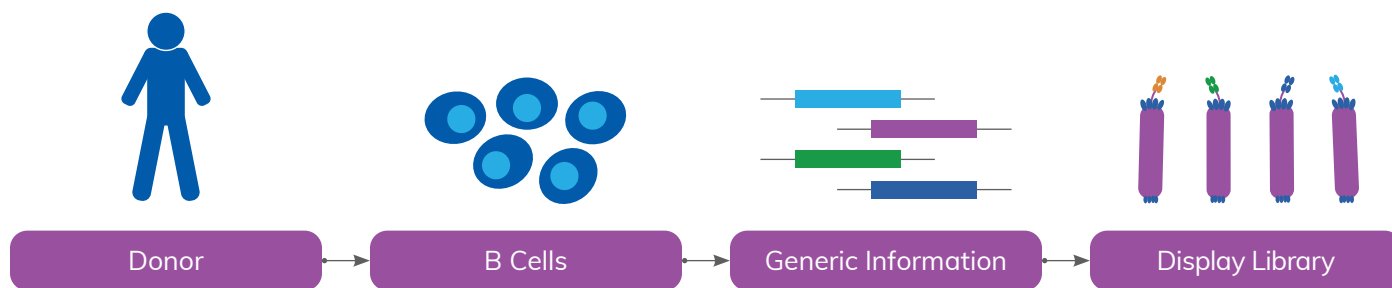
## Naïve Phage Libraries

### Overview

Naive phage libraries are comprised of large repertoires of antibody sequences generated from circulatory B cells that can be harnessed from bone marrow, tonsils, spleens, or peripheral blood mononuclear cells (PBMCs) of non-immunized human donors. B cells from other species such as rodents, chickens, rabbits, or llamas can also be utilized. Antibodies can then be generated against a large number of different types of antigens, such as toxins and peptides. These can be targets related to different indications, including solid or hematological malignancies, infectious or metabolic diseases, and many other conditions<sup>1,4</sup>.

### Highlights

- No need for immunization, therefore avoiding ethical considerations and shortening timelines
- Target a diverse set of epitopes with broad range of affinities
- No prior antigen exposure is required for targeting a large number of antigens



Circulatory B cells from donors are isolated. Antibody sequences are then obtained and cloned into a large number of phagemids, resulting in the formation of the display library.

### XOMA Human Naïve Phage Libraries at LakePharma

LakePharma has licensed XOMA's diverse naïve scFv and Fab human phage display libraries for antibody discovery. Key features of these libraries include:

- Large diversity (>10<sup>11</sup>)
- Fully human and natural repertoire (originate from 30 donors)
- Multiple antibodies generated from XOMA libraries are in clinical trials

<sup>4</sup> Lim CC. et al. 2019. Cognizance of Molecular Methods for the Generation of Mutagenic Phage Display Antibody Libraries for Affinity Maturation. *Int J Mol Sci.* Apr 15;20(8).

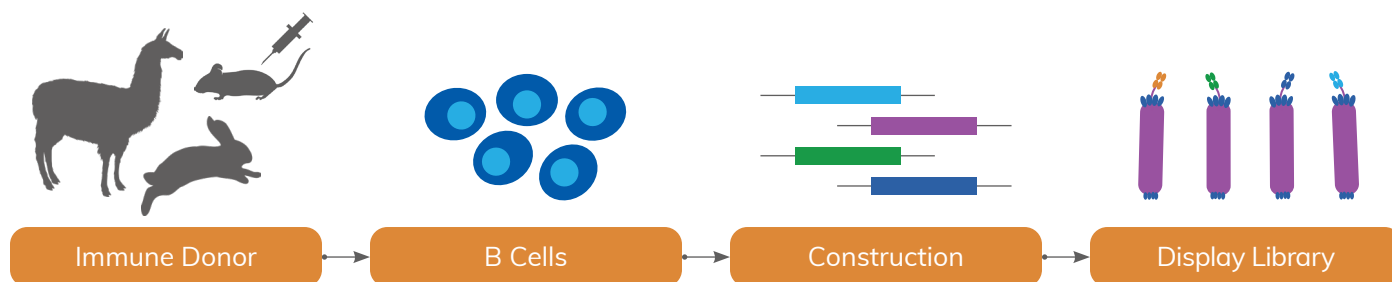
## Immune Phage or Yeast Display Libraries

### Overview

Immune phage or yeast display libraries are constructed using antibody sequences that are amplified from the immunoglobulin mRNA of immunized human donors or other species, such as rodents, chicken, rabbits, or llamas. Immune libraries are distinguished from their naïve counterparts since IgG genes are extracted from B cells that have been antigen-activated and subsequently undergone the *in vivo* affinity maturation process<sup>1,4</sup>.

### Highlights

- Usually antibodies derived from immune libraries have higher affinities against the specific immunogen, obviating the need for affinity maturation<sup>1</sup>
- The immune library size does not need to be as large, since it is comprised of more antigen-specific and high-affinity antibodies
- Co-immunization of animals with antigen orthologs from different species can result in the discovery of species-cross reactive antibody candidates which can then be utilized for animal *in vivo* studies
- Immunizations can be performed on transgenic or wild-type animal species
- Not appropriate for discovery of antibodies against self-antigens



Donors are immunized with the desired immunogen to elicit a proper immune response. B cells are then collected from these donors, and antibody sequences are obtained and cloned into phagemids to establish the immune library.

### LakePharma's Custom Immune Libraries for Antibody Discovery

LakePharma's custom immunization-based libraries are very versatile as they can be applicable to multiple species, antibody formats, and transgenic models. Key features of these libraries include:

- Utilizing patients' or animals' natural immune response coupled with phage/yeast display platforms enables discovery of high affinity and specificity antibodies
- Affinity maturation is often not needed when utilizing these libraries
- LakePharma has completed 100+ antibody discovery projects using llama (VHH), rabbit or murine (Fab or scFv) immune libraries and delivered various antibody lead candidates for therapeutic and diagnostic development



# Synthetic Phage Libraries

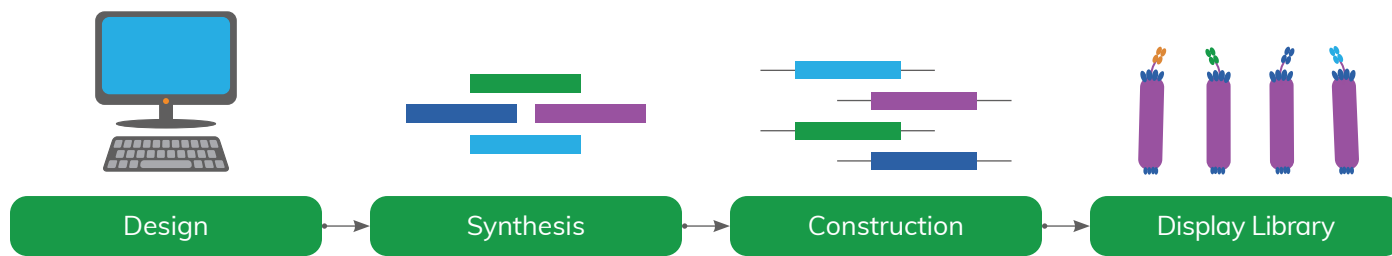
## Overview

Synthetic antibody libraries are constructed to allow for the artificial diversification of complementarity-determining region (CDR) positions that are responsible for antigen recognition while at the same time maintaining the use of mostly optimized framework sequences<sup>4,5</sup>. Different synthetic libraries were designed via randomization of VH CDR3 or additional CDRs, following the understanding that the binding contribution of amino acid residues from multiple CDRs is very significant.<sup>6</sup>

Synthetic antibody libraries can also be rationally designed towards the discovery of antibodies against specialized antigen targets, such as membrane protein receptors.

## Highlights

- An important advantage of synthetic (or semi-synthetic) libraries is that specific framework sequences can be designed which can be very helpful for subsequent antibody selections
- Synthetic antibody libraries can be extremely diverse and extensive, reaching a size of up to  $\sim 10^{13}$  and lacking any biases or redundancies, allowing the recognition of a very large number of antigens that includes toxins and self antigens
- Particular attention must be directed towards the proper design of synthetic library framework regions in order to improve the antibody thermostability properties and avoid potential downstream developability or manufacturability issues



Antibody sequences are synthetically designed and cloned into phagemids for expression.

## Twist Biopharma Synthetic Libraries at LakePharma

LakePharma has partnered with Twist Biopharma, a division of Twist Bioscience, to offer various synthetic libraries including the ones that identify antibodies against a difficult and long-sought class of drug targets: GPCRs. Key features of these libraries include:

- Rationally designed and empirically screened: >100,000 GPCR binding motifs
- Diversity up to  $10^{10}$
- HCDR3 sequences are included with an average length of 45 amino acids

<sup>5</sup> Nelson B. and Sidhu. SS. 2012. Synthetic Antibody Libraries. *Methods Mol Biol.* 899:27-41

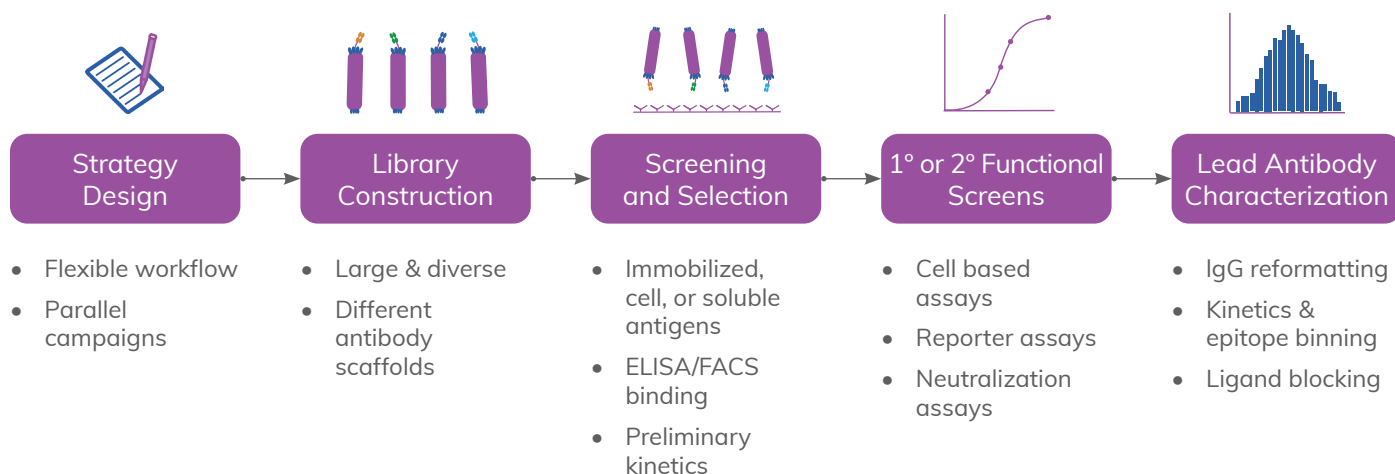
<sup>6</sup> Burkovitz A. and Ofra Y. 2016. Understanding Differences between synthetic and natural antibodies can help improve antibody engineering. *MAbs.* 8(2):278-87



# Power Your Discovery

With over 80 years of combined experience, LakePharma's Antibody Engineering team is ready to partner with you on your next antibody discovery and engineering project.

## LakePharma's Phage Display Antibody Discovery Services



## Downstream Antibody Engineering Services Include:

- One-step antibody humanization and maturation (HuMAT™ method)
- Epitope binning and mapping
- Affinity Measurement
- Sequence liability identification
- Antibody FcRγ and FcRn binding assays
- Therapeutic developability assessment

Learn More at [lakepharma.com/surface-display](https://lakepharma.com/surface-display)



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