Rethinking Lead Optimization: Adopting New Synthetic Biology Tools to Accelerate Development Timelines

Introduction

Since the FDA approval of the first monoclonal therapeutic antibody Muromonab-CD3 in 1986, antibody therapeutics have come to dominate the biologics market. Currently, more than 120 antibody therapeutics have been granted use approvals in the US or EU,[1] with an estimated market size valued at well over a hundred billion dollars.

Despite their preeminence in the biologics market however, the successful commercialization of a therapeutic antibody candidate is not a foregone certainty. After emerging from discovery workflows, which are facing their own challenges screening for rare hits against complex membrane protein families such as GPCRs and ion channels, lead antibody candidates must also be engineered during optimization workflows to meet therapeutic and safety performance requirements.

The magnitude of these challenges is perhaps underscored by the fact that over the past 15 years, the therapeutics landscape has seen a marked decrease in success rates for antibody drug discovery with greater than 90% of all molecules failing to advance to commercialization.[2]

Given the primacy that first-to-market drugs enjoy in the marketplace, there is now more pressure than ever before for biologics developers to rapidly generate, build and screen large numbers of candidate antibodies to continue meeting project milestone metrics. This situation has forced antibody therapeutic development companies to adapt. Enhanced methodologies, higher screening efficiencies, and emerging technologies are all required to meet demanding drug development timelines.

In this white paper we will discuss the key challenges and innovative synthetic biology tools available for researchers to streamline therapeutic antibody optimization.

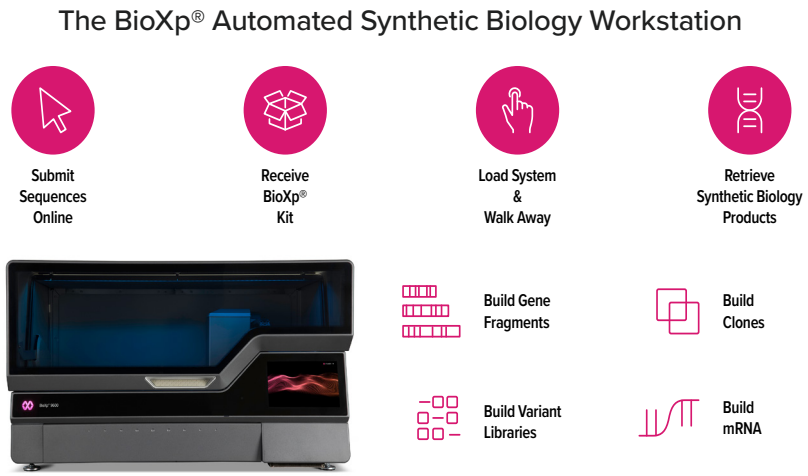

Figure 1: The automated BioXp platform can help streamline antibody development workflows. With simple ordering and push-button instrument setup, the BioXp delivers rapid synthesis of several key synthetic DNA constructs in less than 24 hours.

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Need for engineering

A critical lesson learned from almost 40 years of therapeutic antibody advancement has been the near ubiquitous need to improve desirable biological and physical properties of lead candidates via antibody engineering during development cycles.

These properties include:

- Manipulation of variable regions for affinity-maturation of antibodies
- Humanization of antibody sequences derived from animal models
- Design of complex antibody formats: multi-specifics and antibody-drug conjugates
- Fc engineering
- Isotype switching
- Glycoengineering

Foremost of all these is the manipulation of lead candidate variable regions for affinity-maturation. Monoclonal antibodies require affinities of 1 nM or less to their target antigen to be considered for clinical development, yet this value lies at the theoretical upper limit of what is biologically possible to achieve in-vivo. This B cell affinity ceiling means that one or several cycles of affinity maturation via targeted mutagenesis have become a standard requirement of therapeutic antibody development.

Overcoming challenges

Antibody Engineering leveraging in-vitro display technologies

Antibody engineering techniques together with in-vitro display technologies have become essential for optimizing high-affinity antibodies against difficult targets. Display libraries are produced from natural antibody sequences whose diversity is then increased through mutating one or more complementarity determining regions using synthetic variant library synthesis. As CDRH3 and CDRL3 variable domains have the greatest influence on antibody specificity and affinity, variant library designs invariably target these regions for mutagenesis, sometimes along with other CDRs.

Phage display technology remains the most prominent selection technology for panning antibody candidate sequences with 32% of researchers listing the platform as the best method for obtaining therapeutic antibodies, compared to 8% for yeast display3. However, regardless of the display method employed, driving efficiency by leveraging synthetic biology tools is essential.

Fortunately, critical technological and automation advancements in synthetic biology have emerged which are synergistically reshaping the molecular biology toolkit available to researchers for generating variable-domain variant libraries by replacing tedious manual methods such as error-prone PCR.

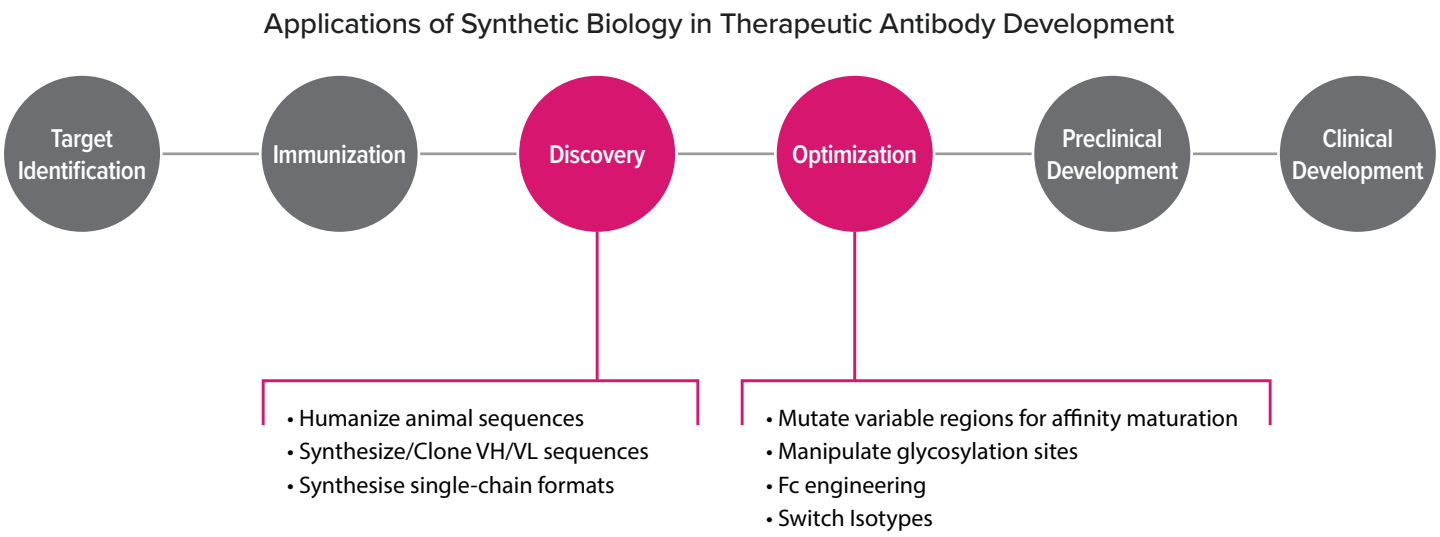

Figure 2: Automated synthetic biology solutions offer rapid, hi-fidelity synthesis of the DNA constructs required for antibody discovery and optimization tasks listed under the magenta brackets.

Accelerating development timelines with comprehensive workflow solutions for antibody discovery & engineering

Automation solutions are now available to researchers that can help streamline synthetic biology construct generation in both the discovery and optimization phases of therapeutic antibody development cycles. These solutions offer distinct advantages to researchers:

- **Overnight synthesis:** With instrument run times less than 24 hours, researchers can realize significant efficiency gains by freeing themselves of long turnaround times for delivery of synthetic biology products from service providers
- **Broad workflow utility:** Synthetic biology workstations offer centralized production of several commonly utilized biomolecules including gene fragments, clones, variant libraries, and mRNA that are critical through the lead discovery and optimization process
- **Chain of custody for proprietary reagents:** Since all synthesis is performed by the instrument on site, researchers never have to send proprietary expression vectors or other materials to 3rd party vendors. This completely abrogates any chance of key resources being sent outside the company.

Telesis Bio workflow solutions

Telesis Bio's comprehensive synthetic biology solutions enable researchers to build DNA constructs across a variety of common workflows in both antibody discovery and optimization. The BioXp® platform and BioXp kits for gene synthesis, cloning, and variant libraries can be leveraged to deliver overnight synthesis of constructs for all activities in the bulleted lists (Fig. 2).

The BioXp® platform

At Telesis Bio, our diverse team of scientists, engineers, and thinkers are invested in making synthetic biology efficient and broadly accessible to the scientific community. We believe that workflow optimization leveraging our technology can help address the challenges facing antibody therapeutic development.

Through the power of Gibson Assembly® and BioXp technology, Telesis Bio enables push-button, automated building of synthetic DNA. In a single run, researchers can construct variant DNA libraries, genes, or large clones – drastically reducing build time and improving productivity. Researchers no longer have to choose between long delivery times from synthetic biology service providers or labor-intensive manual benchtop protocols.

The BioXp platform is a suite of fully automated benchtop instruments that enables numerous synthetic biology workflows by providing a turnkey, end-to-end solution for generating synthetic DNA starting from DNA sequence. The platform is available in 2 models designed to meet the throughput demands of any laboratory:

BioXp 3250 system

- Synthesize up to 32 individual constructs per instrument run. A mid-throughput instrument.

BioXp 9600 system

- Synthesize up to 96 individual constructs per instrument run. This instrument is ideal for high-throughput antibody therapeutic development.

BioXp® variant library solutions

BioXp error-corrected libraries kits are built using proprietary technology that leverages *de novo* DNA synthesis, error correction, and amplification steps. The final product is purified library gene fragments ready for downstream applications such as cloning, screening, or selection.

BioXp error-corrected libraries are assembled in a single instrument run of less than 16 hours –enabling researchers to significantly reduce synthesis bottlenecks during Design-Build-Test cycles of lead optimization.

Accelerate your path to discovery

- Library synthesis time is independent of design complexity. With the BioXp system, any library, regardless of complexity, can be built in a single instrument run.

Confidence in your results

- DNA with the diversity you need & the fidelity you expect. The BioXp system delivers synthesis of both scanning and combinatorial libraries with both the mutational diversity required and excellent sequence fidelity at an average 1: 5,000 bp error-rate.

Solve complex challenges

- Escape from the design limitations of service providers. Build the library you need, not the one they can build.

Additional synthetic DNA solutions

In addition to variant libraries, researchers can also use the BioXp platform to construct gene fragments as well as clones, in a single instrument run – drastically reducing build time and improving productivity.

Whether you need to build antibody variable regions from digital sequence, interchange formats between full IgG and single-chain derivatives, or humanize antibody candidates from animal models, Telesis Bio has a suite of BioXp kits that can help you turn your sequence designs into DNA overnight.

BioXp® gene synthesis kits

BioXp™ gene synthesis kits contain all of the Gibson Assembly® reagents necessary to make error-corrected, *de novo* synthetic gene fragments of up to 1.8 kb in length.

BioXp® DNA cloning kits

BioXp DNA cloning kits contains all of the Gibson Assembly® reagents necessary to make error-corrected, *de novo* synthetic genes of up to 7.2 kb in length. Genes are then scarlessly ligated into standard made-to-stock vectors or your specified vectors, giving you the piece of mind knowing that proprietary reagents never leave your custody.

Find out how automated synthetic biology solutions can accelerate your lead optimization workflow

Visit: telesisbio.com/workflows/synthetic-dna/

Concluding remarks

Therapeutic antibodies are a key, dominant technology in the biopharmaceutical industry. The ability to engineer antibodies into many formats make them versatile biologic drugs that can be tailored to fit highly specialized treatments. However, as researchers strive to continue meeting the growing demand for antibody therapeutics, there is a strong need to streamline existing workflow steps in order to drive greater efficiencies.

Emerging new automated synthetic biology tools can readily assist in these efforts by delivering:

- **Improved productivity:** Eliminate hands-on molecular biology benchwork
- **Consolidated workflows:** Centralize production of critical construct designs while maintaining chain of custody of proprietary reagents
- **Time savings:** On-demand overnight synthesis instead of long turnaround times from services partners

Telesis Bio's comprehensive workflow solutions allow antibody therapeutic developers to build a variety of DNA constructs required for not only lead optimization, but also lead discovery, at a throughput and scale that keeps pace with the demands of development timelines.

References

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3. State of the industry report 2021. Antibody engineering & therapeutics; https://informa.turtl.co/!VwBLvN/