ChemPartner
Dedicated to LifeScience

TECHNICAL NOTE:

Rapid Antibody Discovery Through B-cell Cloning on the Beacon Platform

Single B-cell cloning approach, and its advancement for antibody discovery

Several methodologies have been developed for generating monoclonal antibodies. Hybridoma technology is the most widely used method for antibody generation. However, there are major disadvantages to this process, such as being time consuming, laborious and of low-efficiency. Phage display, another common method used for antibody discovery, can often provide limited and biased repertoires due to non-cognate VH/VL pairing. Both of these methods have notable limitations, despite being important and reliable antibody discovery engines.

Table 1. Comparison of antibody generation methods

METHODS	ADVANTAGES	DISADVANTAGES
Hybridoma	• Well-established processes • Natural VH/VL pairing • No need for special equipment • Low R&D costs	• Low fusion efficacy; high cell number required • Loss of clones due to chromosomal instability • Loss of functional light chains due to genetic rearrangements • Difficult to select and express low frequency positive clones from pools • Long duration (> 4 months) • Requires humanization of the Ab sequences • Labor intensive to screen clones for desired binding and functional characteristics
Phage display	• Provides a direct linkage between phenotype and genotype of the antibody • Avoids loss of sequence information in target-specific clones, unlike hybridoma and B cells • Cell-free based approach allows for flexible and specific screening approaches	• Limited B cell repertoire depending upon library size • Expression bias; folding errors by unnatural pairings of VH/VL • Restrictive post-translational modification due to prokaryotic expression • Limited quantitative assays during panning process • Labor intensive to screen clones for desired binding and functional characteristics
Single B cell cloning	• High probability of selecting low frequency target-specific antibodies • High sensitivity for indentifing low antibody secreted clones from pool • Avoids B cell immortalization and library construction which are essential in hybridoma and phage display methods • Allows de novo antibody discovery from various species • Natural VH/VL pairing • High specificity, high affinity, rich genetic diversity by using antigen-specific B cells or antigen-secreting plasma B cells • Short duration • High-throughput and automation (Beacon platform) • Allows screening of clones in multiplexed format with high specificity, crossreactivity and other functional characteristics (Beacon platform) • Require low quantity of reagents and cell counts (Beacon platform)	• High screening efficiency can be achieved with fresh samples • Strict operating regimen • Reagents require fluorescent labelling while retaining functionality • Requires humanization of the Ab sequences

+86 21 5132 0088 | contact@chempartner.com
1F & 3F, Block A, 2829 JinKe Road, Zhangjiang Hi-Tech Park, PuDong New Area, Shanghai, China, 201203

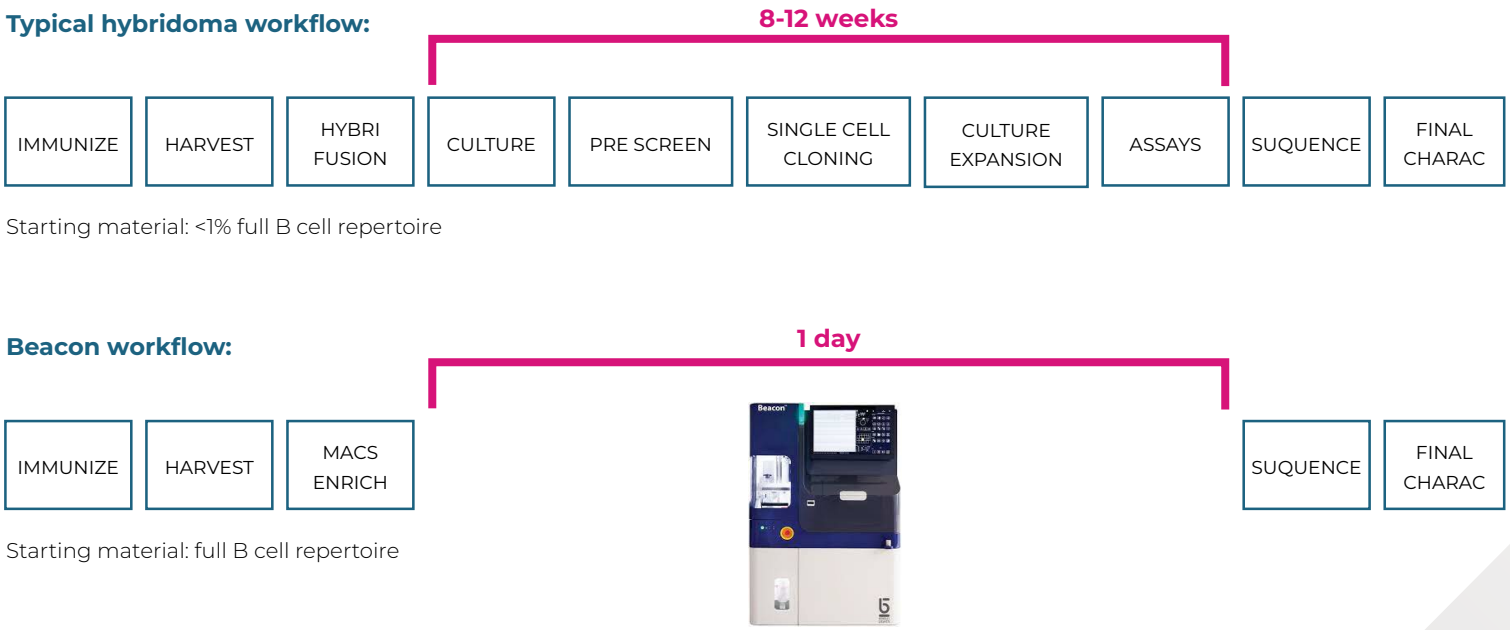
Single B cell cloning technology is an emerging microsystem-based screening method that is increasingly being adopted throughout the field of antibody discovery. Originally, this was achieved by isolating and screening antigen-specific memory B cells by flow-cytometric single-cell sorting or limiting dilution. Genes coding for VH/VL fragments from individual B cell were identified by isolating mRNA and then performing RT-PCR, cDNA amplification, and gene sequencing. This strategy of single B cell cloning by either cell sorting or limiting dilution dramatically overcame many limitations of conventional methods in the field of antibody discovery. Several microscale bioprocess technologies, such as microfluidic chambers, microencapsulation devices, among others, have been established that improved on the processing of single B-cell cloning. However, each technology is inflexibly designed for a very specific process, which therefore limits an integrated and streamlined approach.

Beacon® platform for single B-cell technology for antibody discovery

Recently, a nanofluidic optoelectronic antibody screening technique, the Berkeley Lights, Inc. Beacon® platform, was introduced that integrates individual cell trafficking, imaging and culturing on a chip that is smaller than a business card. This screening platform is designed with the concept of putting “function first”. The distinguishing feature of the discovery workflow is that it enables the user to directly screen single antibody-secreting plasma cells against multiple assays within hours of isolation from an immunized animal (or person). Standard assays include screening for antigen specificity, cross-reactivity, and functional activity. By using the Beacon® for antibody discovery, the probability of identifying higher numbers of quality antibody leads against difficult targets is significantly improved.

Through our partnership with Berkeley Lights, ChemPartner offers services using the innovative Optofluidic based Beacon® platform at our South San Francisco research facility. The nanochip technology of the Beacon® platform enables us to analyze binding and functional characteristics of antibodies secreted from tens of thousands of single plasma B cells. Our Beacon® antibody service offers an accelerated antibody discovery workflow for obtaining high affinity and diverse antibodies compared to standard methods such as hybridoma technology and phage display.

Figure 1 An accelerated antibody discovery workflow by the Beacon® platform



+86 21 5132 0088 | contact@chempartner.com
1F & 3F, Block A, 2829 JinKe Road, Zhangjiang Hi-Tech Park, PuDong New Area, Shanghai, China, 201203

Although a number of single-cell cloning approaches based on different technologies have been utilized for antibody discovery, the Beacon® offers many advantages over other platforms.

Table 2. Advantages of single B-cell cloning by Beacon platform over other single-cell cloning platforms

- **Fast:** Identify functional leads in a single day by multi-parameter phenotyping with high-throuput features
- **Function First:** Workflow enables streamlined selection of lead candidates through on-chip assays for antigen specificity, cross-reactivity, and functionality
- **Diversity:** Direct screening of plasma B cells representing a greater proportion of the immune repertoire
- **Automation:** Fully automated workflow including cell loading, single-cell penning, cell and/or protein-based assays, and exporting for sequencing
- **Low risk:** Reduced loss of positive clones for antibody sequencing
- **Superior quality:** Antibody leads with superior diversity, affinity and specificity

Why work with ChemPartner for antibody discovery by Beacon®?

ChemParner, a global research CRO/CDMO, has a well-established and integrated infrastructure for antibody discovery and production. Additionally, we offer full service in vitro and in vivo pharmacology and DMPK capabilities, as well.

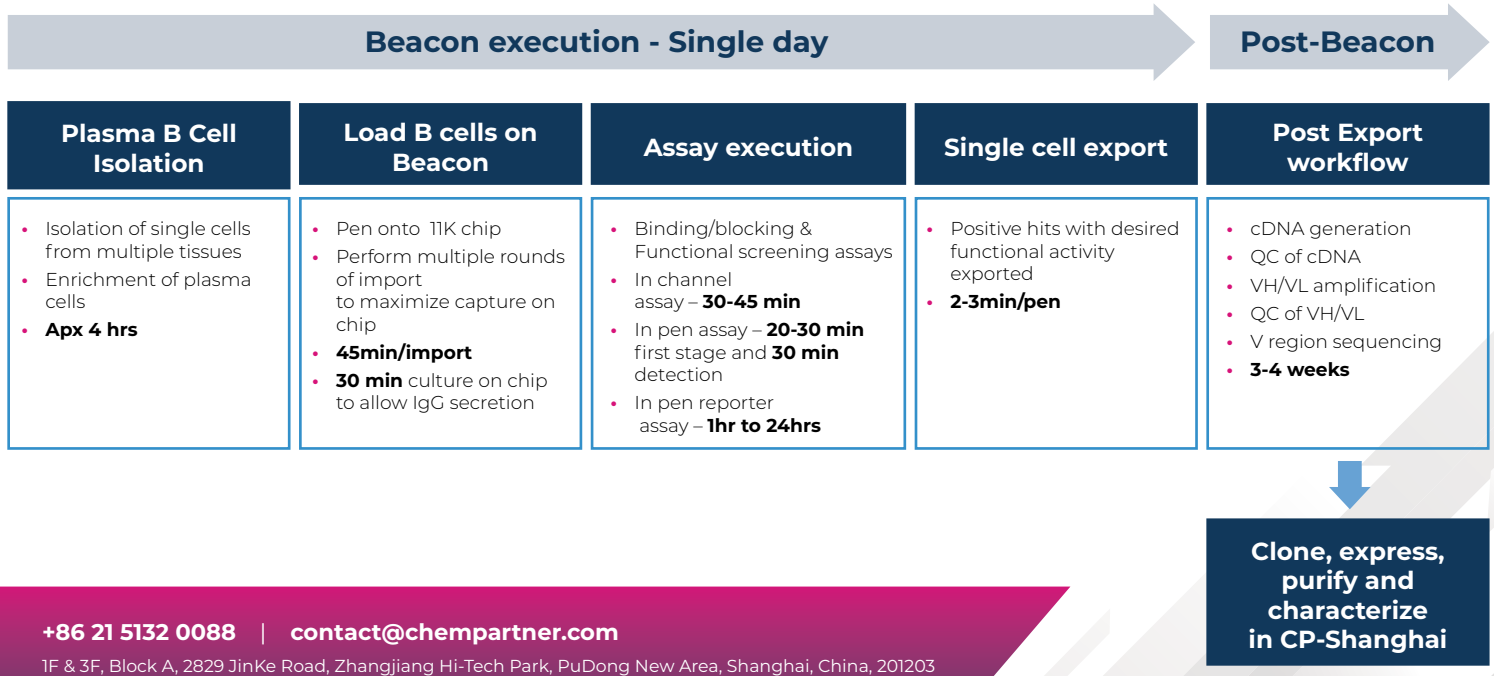
Table 3. Advantages of choosing Chempartner for your antibody discovery needs

- Extensive experience with Beacon screening strategy design
- Strong support for Biologics discovery and development from CRO/CDMO teams
- Collaborative Bay Area partnership with Berkeley Lights (Beacon platform) and our South San Francisco research team
- Established workflows that enable antibody generation from multiple animal species
- Exploring single-cell cloning approach in various applications and therapeutic areas

Established Beacon® Workflow and Project Timeline for Antibody Discovery in ChemPartner

By working with us on the Beacon® platform, positive functional hits of your antibody candidates can be identified in a single day. Antibody sequencing and production can also be completed with the integrated and streamlined services offered at ChemPartner.

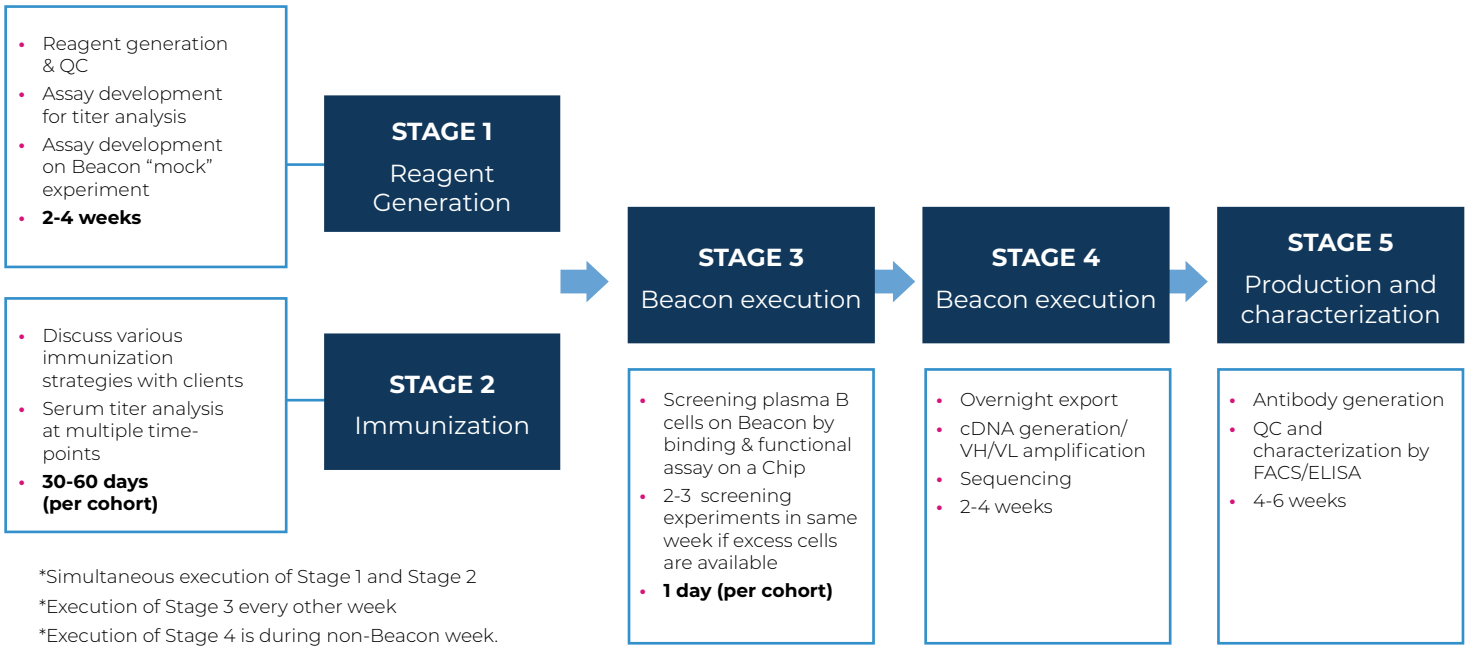
Figure 2 The workflow of plasma cell Screening by Beacon



+86 21 5132 0088 | contact@chempartner.com
1F & 3F, Block A, 2829 JinKe Road, Zhangjiang Hi-Tech Park, PuDong New Area, Shanghai, China, 201203

Each discovery project on the Beacon® platform covers five different stages. The entire process of antibody development can be completed in as little as 3 months.

Figure 3 Overview of Different Phases of Beacon Projects for Antibody Discovery



*Simultaneous execution of Stage 1 and Stage 2
*Execution of Stage 3 every other week
*Execution of Stage 4 is during non-Beacon week.

Single B-cell approach for antibody generation and more

In addition to our established capabilities for screening mouse and human antibodies, ChemPartner's Beacon® plasma B-cell workflow will be soon available for antibody discovery services for additional species including alpaca/llama, rabbit and chicken. Recently, Beacon® technology has also been used for single cell phenotypic and genotypic characterization, identification of CRISPR-Cas9 edited primary T cells and analysis of T-cell receptor repertoires. With a growing demand for T-cell therapeutics, ChemPartner will soon be offering our T-cell cloning services to enable clients to screen desired individual T cell phenotypes within heterogeneous T cell clones.

Table 4. Beacon applications for more

A. Antibody discovery: Various species available for plasma B cell workflow

SPECIES	AVAILABILITY	ASSAYS FOR
Mouse	Now	Specificity, cross-reactivity, blocking, functional activity
Rat		
Human		
Alpaca/llama	est. July, 2021	
Rabbit	est. July, 2021	
Chicken	est. Sept, 2021	

B. T cell therapy: Characterize single T-cell functionality

SPECIES	AVAILABILITY	ASSAYS FOR
Human	est. Sept, 2021	Cytokine secretion, proliferation, phenotyping

For more information: https://www.chempartner.com/2019/01/31/antibody-discovery-through-b-cell-cloning/

answers4beacon@chempartner.us | chchcheng@chempartner.us