BERKELEY LIGHTS BEACON® VS HYBRIDOMA PLATFORM
FOR RAPID ANTIBODY DISCOVERY AGAINST DIFFICULT TARGETS – THREE CASE STUDIES

DNA immunization in combination with our improved hybridoma platform has been the gold standard for many years. This approach has served very well for a variety of targets, including challenging multi-transmembrane spanning targets. In our first example, named **GPCR 1**, we were able to generate **13 conformational epitope-binding antibodies** using our highly efficient hybridoma method. **GPCR 1** is a 7-transmembrane spanning GPCR with a very small extracellular region and low sequence homology between the human and murine orthologs, which exhibits a relatively good expression level (depicted orange in the histogram, Fig. 1).

LIMITATIONS OF THE HYBRIDOMA TECHNOLOGY

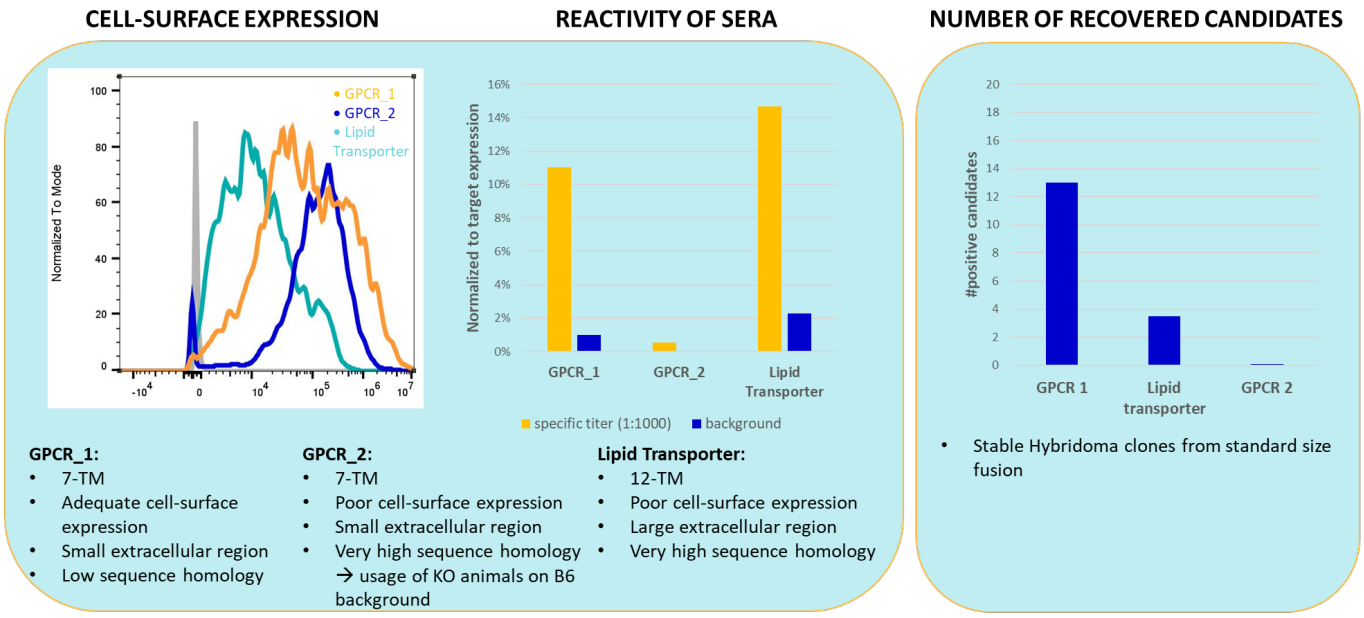

Figure 1 – Limitations of the Hybridoma Technology

In another case, the target was a 12-transmembrane-spanning **lipid transporter**, that exhibited a large extracellular region, but displayed very high sequence homology to the murine ortholog and also proved very challenging to express in transient transfection. Nevertheless, we were able to elicit an immune response and our hybridoma platform was able to deliver **three stable binders**.

In a third example, the target was a different 7-TM GPCR, here named **GPCR 2**, with a very small extracellular N-terminus and extremely high sequence homology. In this case, our client chose to use knockout animals, which existed only on black6 background, introducing a new challenge for this project. Hence, despite relatively good transient expression levels, we obtained only minimal titer and consequently **fusions did not yield a single antibody**, pointing out the limits of antibody discovery by hybridoma generation.

While hybridoma technology has limitations (low immunogenic targets), we continue to optimize and advance our processes.

GPCR 1:

- Class A (rhodopsin-like) GTP-binding protein-coupled receptor, belonging to subfamily A2
- 32 aa (small) extracellular N-terminus
- 7 transmembrane domains (7TM)
- Sequence identity between human and mouse: FL 74%, N-terminus 40%

GPCR 2:

- Belongs to the lysophospholipid receptor (LPL-R) group
- 50 aa (small) extracellular N-terminus
- 7 transmembrane domains (7TM)
- Sequence identity between human and mouse: FL 97%, N-terminus 92%

LIPID TRANSPORTER:

- 450 aa (large) extracellular domain
- 12 transmembrane domains (12TM)
- Sequence identity between human and mouse: FL 90%

In the light of our client's growing demands for antibody discovery campaigns against the most difficult targets, we have expanded our services with a B cell platform, that enables direct screening of primary B cells, as an alternative to the less efficient hybridoma technology. Berkeley Lights' Beacon® technology facilitates rapid screening of thousands of single plasma cells at extremely high monoclonality for their specificity in one day. The Beacon allows a more thorough characterization of antibodies, by performing a series of sequential assays, helping to identify the antibody of interest and limit downstream molecular biology work. Combined with cell-based assays performed in a sequential manner it enables an efficient sampling of the antibody repertoire of the immunized animal and preservation of the lead candidates. For example, cross-reactivity studies can be carried out on the Beacon by sequentially testing on different target orthologues thereby getting to the right antibody faster.

BERKLEY LIGHTS BEACON® FOR EXPEDITED ANTIBODY DISCOVERY AGAINST DIFFICULT TARGETS

Figure 1 – Berkeley Lights Beacon®

- Two side-by-side Beacons enable screening of up to 80.000 primary plasma cells in one day
- Sequential assays enable cross-reactivity studies
- On-chip characterization (e.g. cross-reactivity studies, blocking assays, internalization assays) to limit downstream molecular biology work
- Native pairing of heavy and light chains
- Upgraded AbD2.0 workflow for better screening efficiency and cDNA recovery
- AbD2.0 11k OptoSelect chip reduces loss of precious samples
- Cell-based assays to test on conformational target protein

The Genovac advantage has always been the discovery of antibodies binding conformational epitopes, made possible by optimized genetic immunization protocols combined with screening on target expressing cells. Along this line we have established cell-based assays on our Beacon.

With respect to our on-chip cell-based assays, we have optimized our in-house cell-based screening system, which includes multiple cell line options and can support cell lines provided by clients. We also offer our clients customized assay development.

Screening of primary B cell on Berkeley Lights Beacon® accelerates the antibody discovery process by weeks compared to standard hybridoma. Target-binding candidates are identified on the day of organ-harvest, and sequencing of variable chains as well as generation of recombinant antibodies is carried out in 2--4 weeks.

CONTACT US

info@genovac.com
www.genovac.com

EUROPE

Genovac GmbH
Waltershofener Str. 17
79111 Freiburg im Breisgau, Germany
Tel: +49-(0)761-45636-0

USA

Genovac Antibody Discovery LLC
1810 NDSU Research Circle N, Suite B
Fargo, ND 58102, USA
Tel: +1 (701)-551-2770

In addition to time, one of the Beacon's strongest advantages is its ability to "find the needle in the haystack".

The number of stable hybridoma clones from a standard size fusion was compared to a two-chip 20.000 plasma cell Beacon screening. Optimized cell-based assays for low detection threshold, paired with screening of primary plasma cells has allowed us to overcome the limitations observed with hybridoma. In a comparative campaign between hybridoma and Beacon for the three targets introduced previously, we were able increase yields by a factor of ten (Fig. 3). The more difficult the target, the more distinct the gain in antibodies obtained by the Beacon compared to hybridoma. This increased number of antibodies combined with an increased sequence variety distinctive for plasma cells allows us to increase the chances to find the antibody exhibiting the desired characteristics.

RECOVERY OF ANTIBODIES AGAINST DIFFICULT TARGETS USING THE BEACON® INSTRUMENT

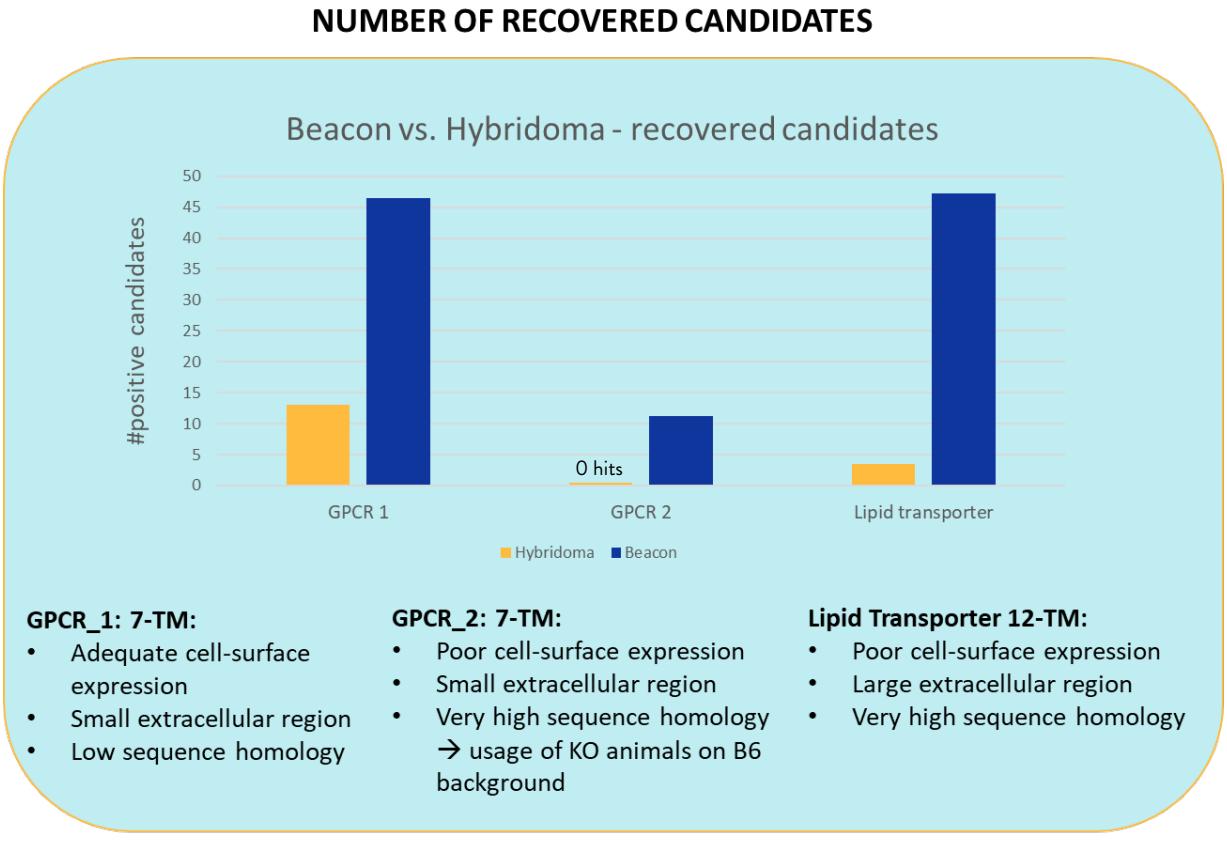

Figure 2 – Recovery of Antibodies Against Difficult Targets Using the Hybridoma Platform vs. Beacon® Screening

CONTACT US

info@genovac.com
www.genovac.com

EUROPE

Genovac GmbH
Waltershofener Str. 17
79111 Freiburg im Breisgau, Germany
Tel: +49-(0)761-45636-0

USA

Genovac Antibody Discovery LLC
1810 NDSU Research Circle N, Suite B
Fargo, ND 58102, USA
Tel: +1 (701)-551-2770

Here a summary of the comparison of our Hybridoma Platform vs. Beacon Screening regarding the yield of antibodies against difficult targets:

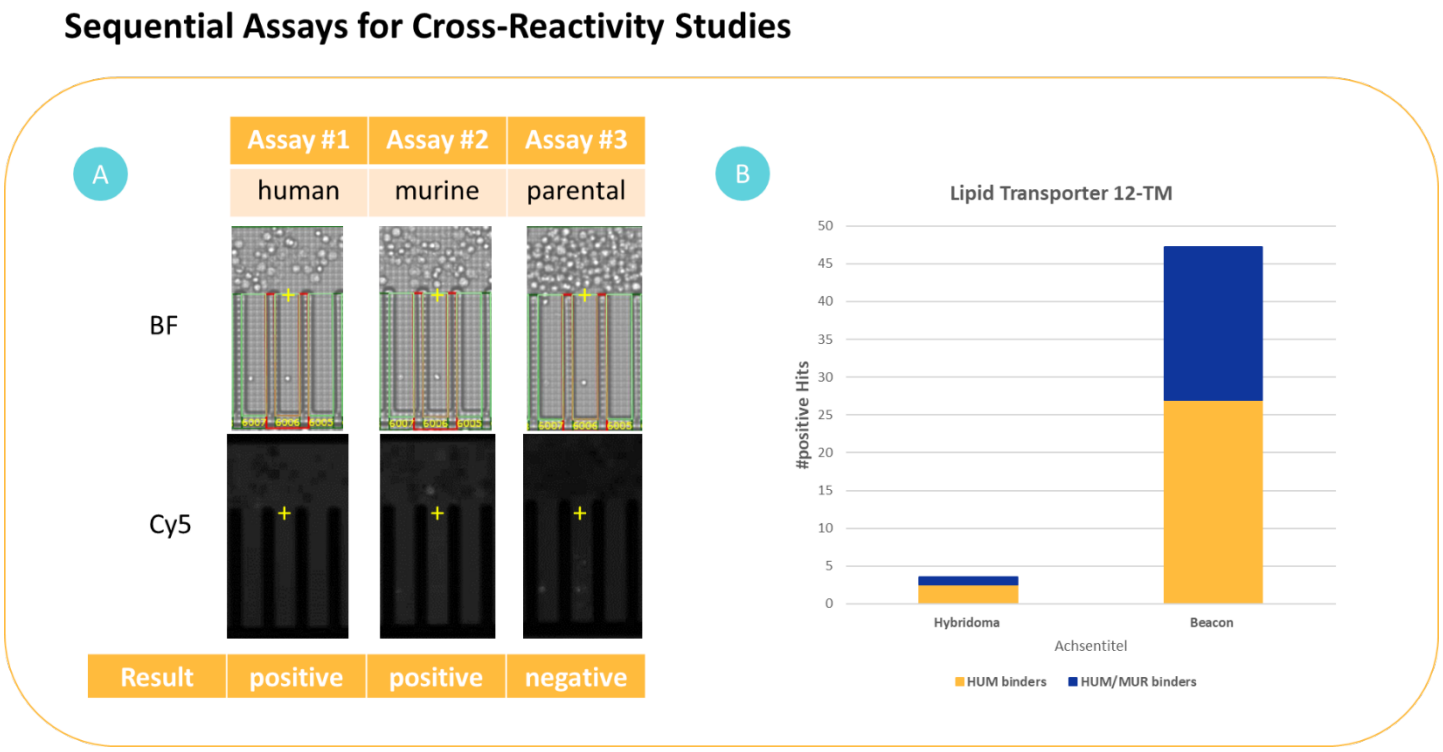

Figure 3 – Cross-Reactivity Studies Using the Hybridoma Platform vs. Beacon® Screening

Until recently, antibody discovery on the Beacon® was restricted to wildtype and transgenic mice. Based on client demand, we expanded our capabilities to include wildtype and transgenic rats, including Ligand Omnirats for the discovery of fully human antibodies without the need for complex humanization processes. In Q1 of 2021 we should also be able to use Rabbit-derived ASC on the Beacon.

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EUROPE

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