Case Study

# Rapid mAb Discovery against Cell Surface Receptor Based on B cell Screening on Adherent Cell Lines

# CASE STUDY: CELL SURFACE RECEPTOR ANTIBODY DISCOVERY

Abverris
A Division of Twist Bioscience

Advanced high-resolution single B cell screening for rapid antibody discovery - Beacon workflow

IMMUNIZATION & TITER TEST → TISSUE HARVEST & IMPORT OF PLASMA B CELLS → BEACON WORKFLOW (FUNCTIONAL SCREEN) → SEQUENCING OF B CELLS → RECOMBINANT ANTIBODY EXPRESSION & VALIDATION

**The Target:**

- A cell surface receptor, Target X (80% murine/human homology)

**The Goal:**

- Deliver a diverse panel of IgG and scFv anti-Target X binders

**The Challenges:**

- Tight timeline – partner required rapid antibody discovery
- Cell-based screening required adherent cell lines which is a big challenge with Beacon's default loading protocol
- Partner had previously attempted in vivo discovery and suspected the immunogen to be poorly immunogenic

**29 DAYS**

from immunization
to sequence for
a cell surface target

# CASE STUDY: CELL SURFACE RECEPTOR ANTIBODY DISCOVERY

An accelerated immunization protocol was employed to give harvest-ready titer in 3 weeks

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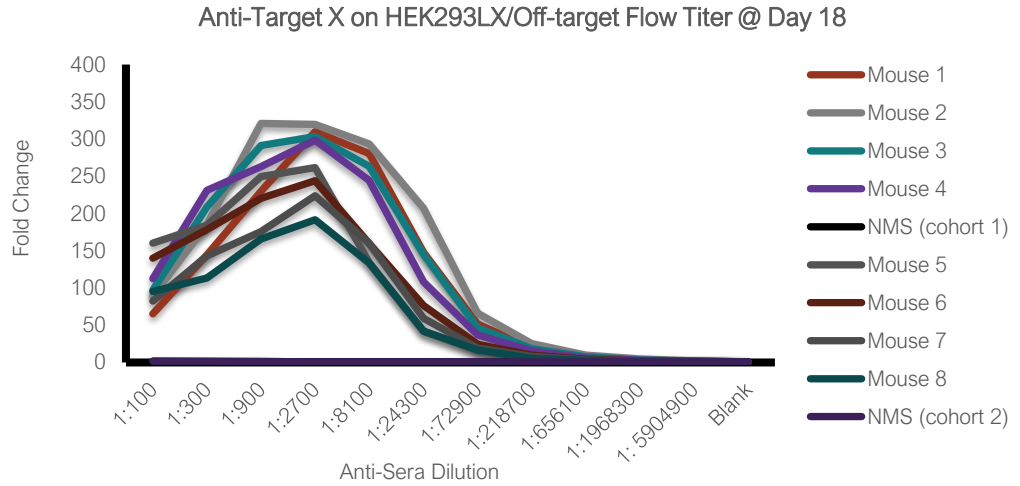
TIMELINE — **21 DAYS** — 1 DAY — 7 DAYS — 4 WEEKS

| Cohort | Immunogen | Immunization Protocol | Comment |
|---|---|---|---|
| 1 (Mouse 1 – 4) | huTarget X-His | Accelerated | Abveris proprietary adjuvant |
| 2 (Mouse 5 – 8) | huTarget X-huFc | Accelerated | Abveris proprietary adjuvant |

- Harvest-ready titer observed @ Day 18



# CASE STUDY: CELL SURFACE RECEPTOR ANTIBODY DISCOVERY

B cells from lymph and spleen tissues were isolated and enriched for loading into the Beacon platform

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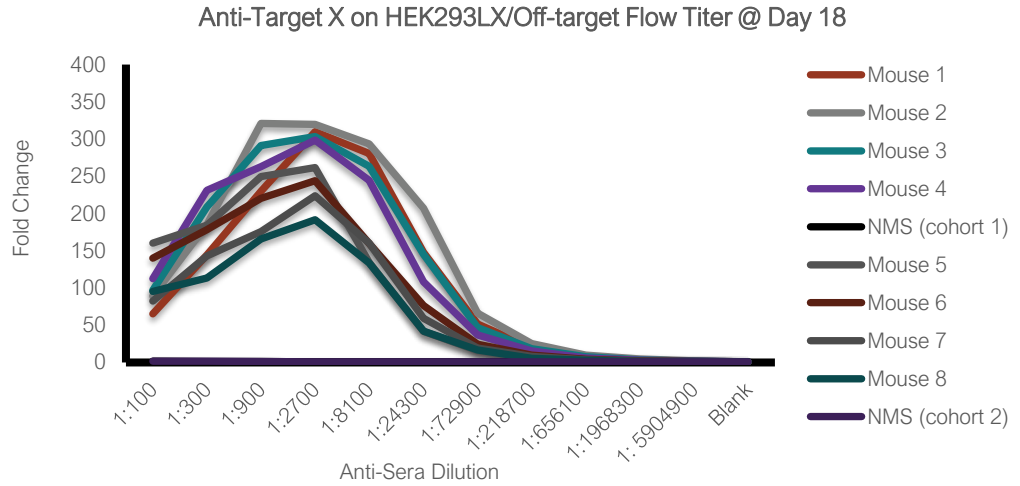
- Mouse 1 selected for tissue harvest @ Day 21
- Proprietary protocol to load >10K viable plasma B cell per chip



# BEACON ON-CHIP SCREENING USING ADHERENT CELL LINES

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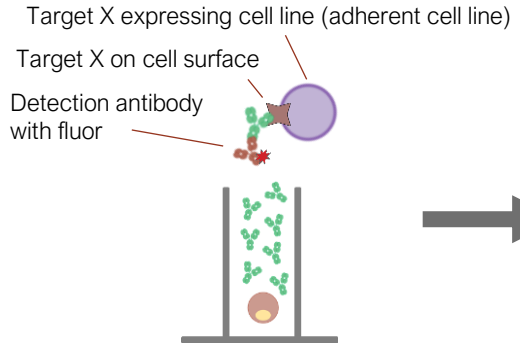
On-chip cell-based specificity screening identifies specific binding to the target-expressing cell line over the off-target cell line

IMMUNIZATION & TITER TEST | TISSUE HARVEST & IMPORT OF PLASMA B CELLS | **BEACON WORKFLOW (FUNCTIONAL SCREEN)** | SEQUENCING OF B CELLS | RECOMBINANT ANTIBODY EXPRESSION & VALIDATION

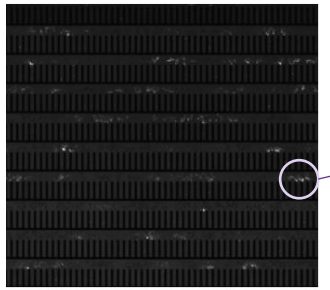
TIMELINE — **21 DAYS** — **1 DAY** — 7 DAYS — 4 WEEKS

## Assay #1

(+) screen with Target X-expressing cell line




Overview of chip

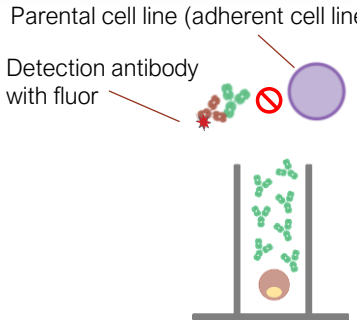


Representative results

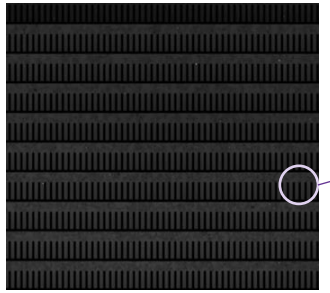
*"Bloom" signifies on-cell binding*

## Assay #2

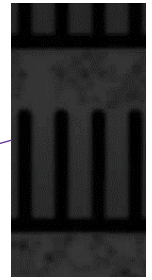
(-) screen with parental cell line




Overview of chip



Representative results



*No bloom*

# BEACON ON-CHIP SCREENING USING ADHERENT CELL LINES

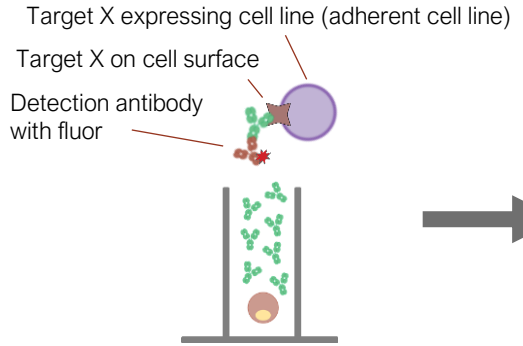
Clones with specific binding profile were exported for sequencing and small-scale expression

IMMUNIZATION & TITER TEST | TISSUE HARVEST & IMPORT OF PLASMA B CELLS | BEACON WORKFLOW (FUNCTIONAL SCREEN) | **SEQUENCING OF B CELLS** | RECOMBINANT ANTIBODY EXPRESSION & VALIDATION

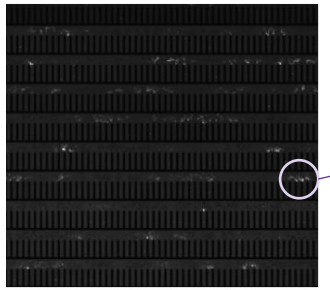
TIMELINE — 21 DAYS — 1 DAY — 7 DAYS — 4 WEEKS

## Assay #1

(+) screen with Target X-expressing cell line




Overview of chip



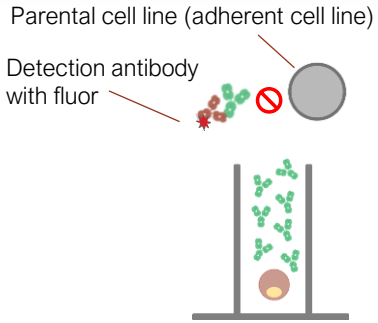
Representative results

*"Bloom" signifies on-cell binding*

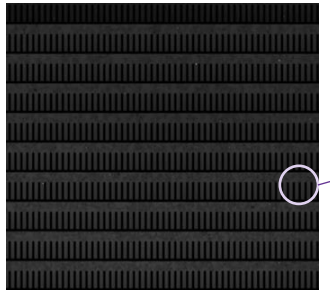
Top 192 clones exported for sequencing

## Assay #2

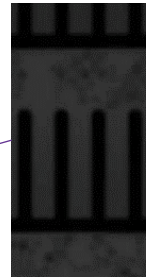
(-) screen with parental cell line




Overview of chip



Representative results



*No bloom*

# DOWNSTREAM VALIDATION OF HITS WITH HTP FLOW CYTOMETRY

Majority of antibodies recombinantly expressed as IgG and reformatted into scFv retained on-cell binding activity

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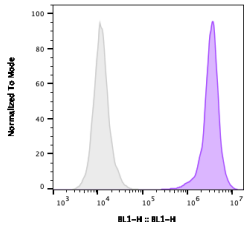
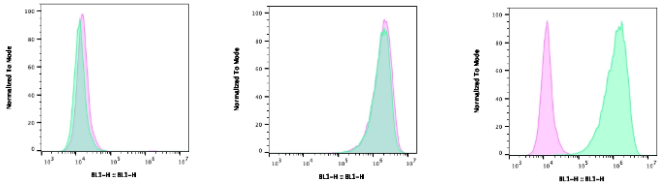
TIMELINE — **21 DAYS** — **1 DAY** — **7 DAYS** — **4 WEEKS**

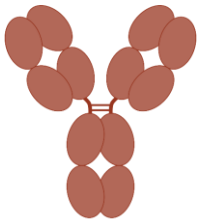
Target-X (+) Control Binding by Flow Cytometry



IgG vs. scFv Binding Validation on huTarget X Cells IgG scFv



Neither | Both | IgG only



| | | |
|---|---|---|
| Antibodies recombinantly expressed | 24 | 24 |
| Antibodies with validated binding after expression | 20 | 18 |
| Total binding recovery | 83% | 78% |

# From Immunization to Antibody Sequences in 29 Days

Successful delivery of 95 unique sequences to a challenging cell-surface receptor in 29 days using the DiversimAb™ technology

## SUMMARY

- Successful antibody discovery campaign against a known poorly-immunogenic cell surface target
- Effective cell-based screening using adherent cell lines on Beacon
- Conversion of IgG to scFv for a small subset of identified sequences
- Next step: reformat into CAR construct and test a larger panel of sequences

## Throughput & Results of B Cell Screening