



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

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

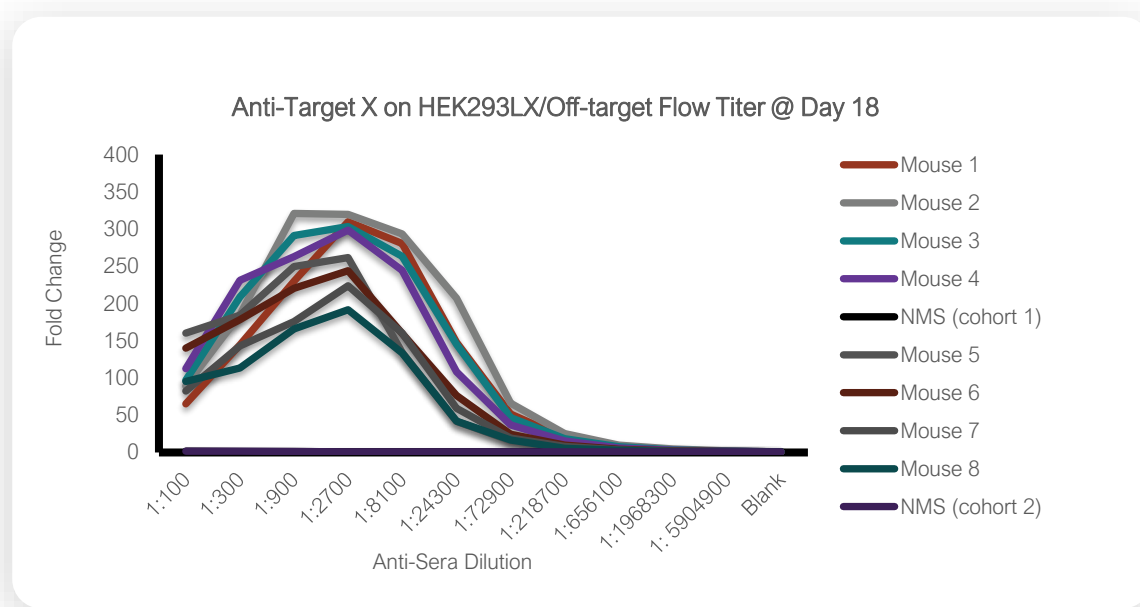
CASE STUDY: CELL SURFACE RECEPTOR ANTIBODY DISCOVERY

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



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



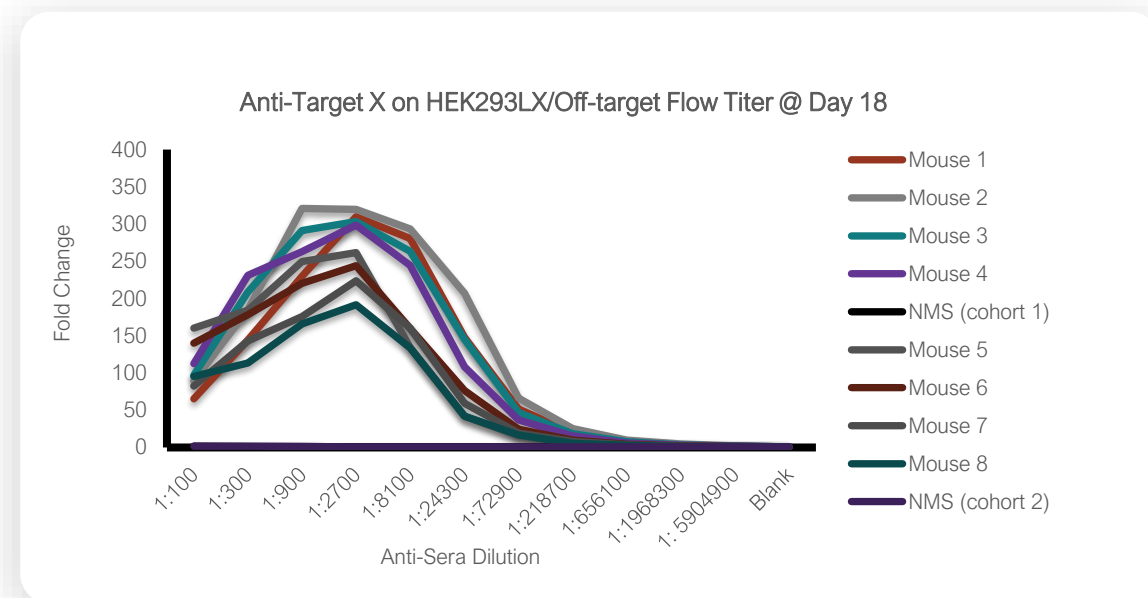
CASE STUDY: CELL SURFACE RECEPTOR ANTIBODY DISCOVERY

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



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

- 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

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

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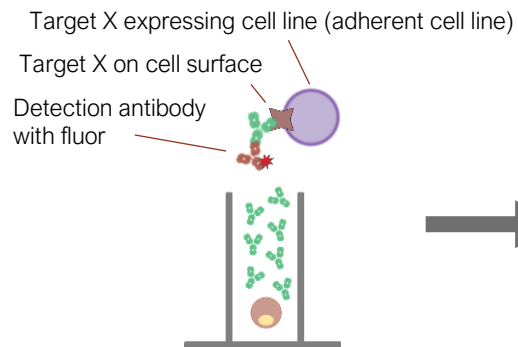
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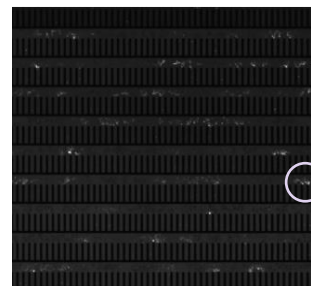
TIMELINE 21 DAYS 1 DAY 7 DAYS 4 WEEKS

Assay #1

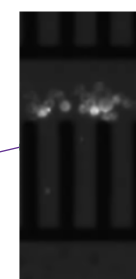
(+) screen with Target X-expressing cell line



Overview of chip



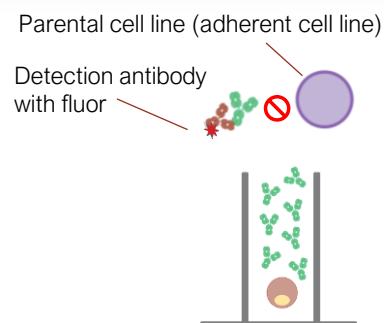
Representative results



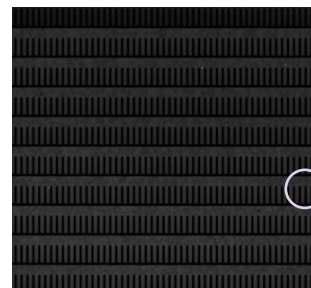
"Bloom" signifies on-cell binding

Assay #2

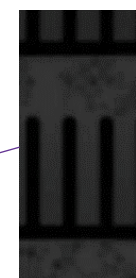
(-) screen with parental cell line



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
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BEACON WORKFLOW
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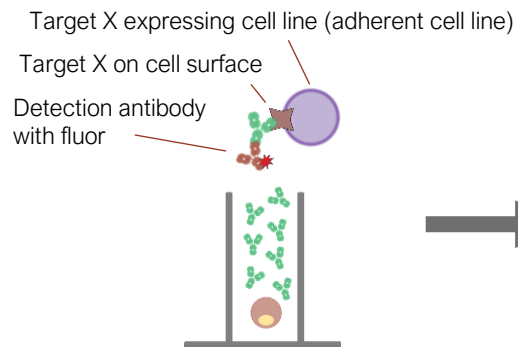
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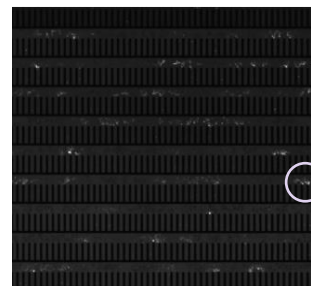
TIMELINE 21 DAYS 1 DAY 7 DAYS 4 WEEKS

Assay #1

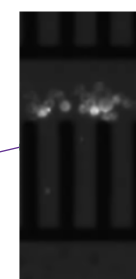
(+) screen with Target X-
expressing cell line



Overview of chip



Representative results

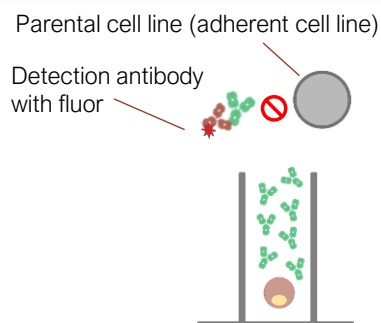


*"Bloom" signifies
on-cell binding*

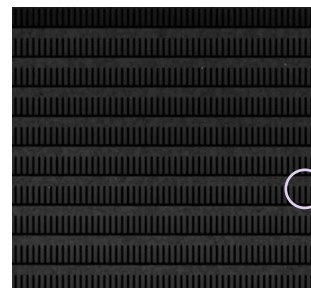
Top 192 clones exported
for sequencing

Assay #2

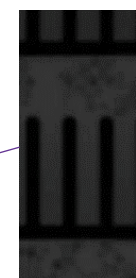
(-) screen with
parental cell line



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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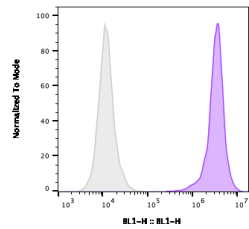
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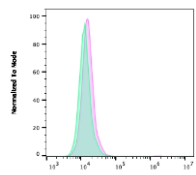
RECOMBINANT ANTIBODY
EXPRESSION & VALIDATION

TIMELINE •--- 21 DAYS •--- 1 DAY •--- 7 DAYS •--- 4 WEEKS •---

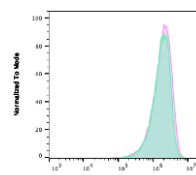
Target-X (+) Control Binding by Flow Cytometry



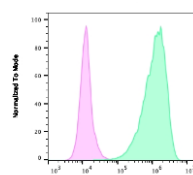
IgG vs. scFv Binding Validation on huTarget X Cells **IgG** **scFv**



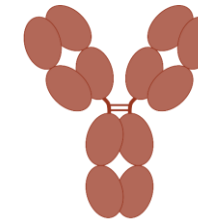
Neither



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

