Generation of Recombinant Tool Antibodies to Support Cell and Gene Therapy Development

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Overview

Background

- Cell and Gene Therapies (CGT) must comply to strict analytical testing requirements to meet regulatory agencies' standards.
- Development of bioanalytical tools is a critical step for the success of CGT.
- Critical reagents used in such assays include antibodies.

Solution

- Antibody phage display combined with SpyTag technology can generate highly-specific antibodies in a variety of formats for immediate use in relevant applications

Overview

How to deliver high-quality, critical reagents?

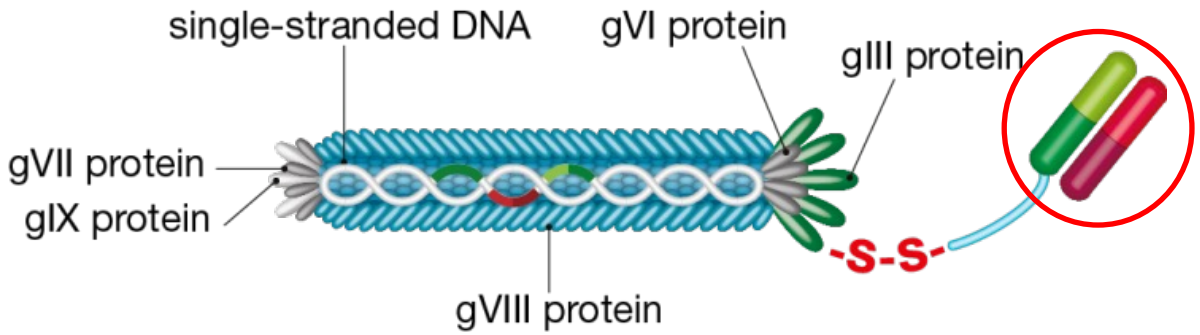
- Phage display technology applied to CAR T
- SpyTag technology: format switch
- Stable and reliable antibody supply

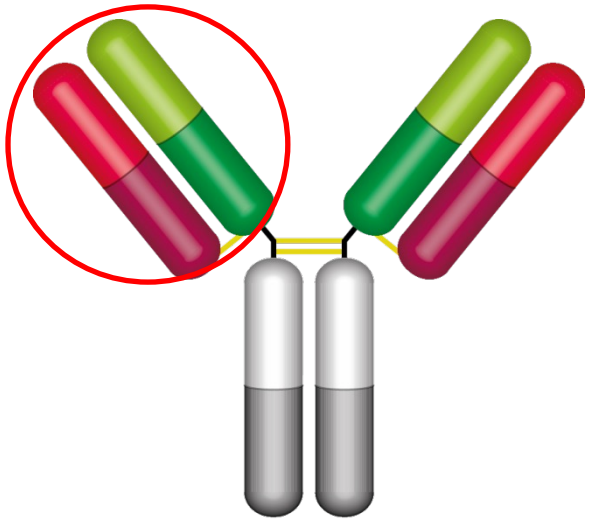
Real case application:

- Development of antibodies for use in flow QC of CAR T-products

Antibody Phage Display

- HuCAL PLATINUM®
- Fully synthetic, fully human, Fab library
- High diversity: theoretical library size of 45 billion antibodies
- Fast: library screening in as little as 8 weeks
- Nonanimal-derived antibodies, reducing the use of animals in science

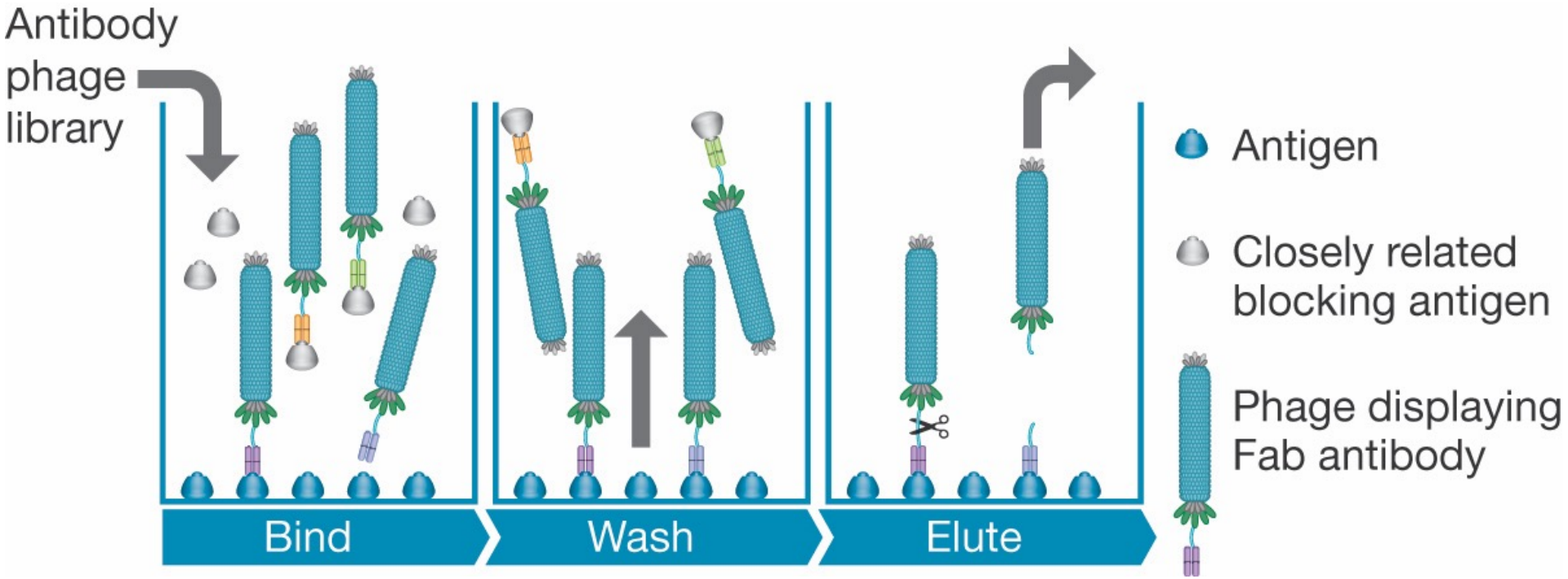




Guided Selection: Epitope Specificity

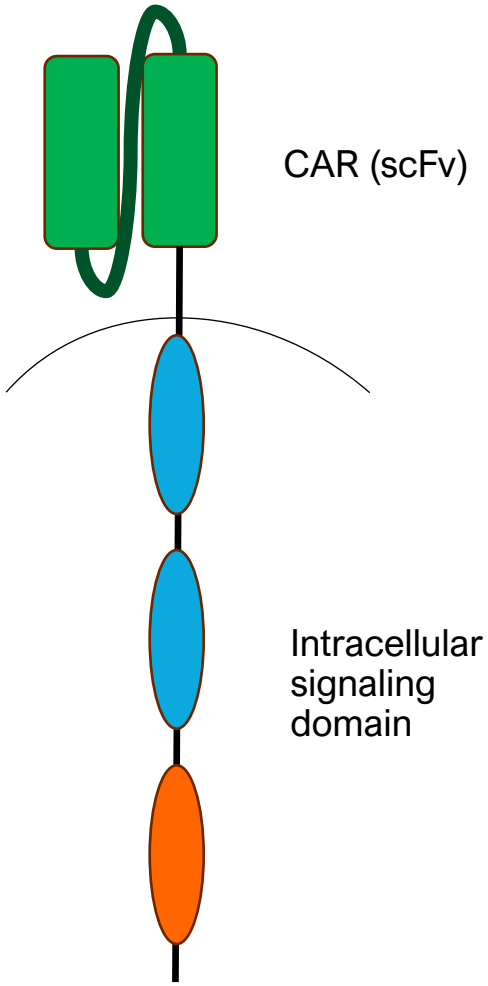
Requirements: Fab antibodies recognize antigen but not a closely related antigen (CRA)

Strategy: Selection on antigen and library subtraction using CRA

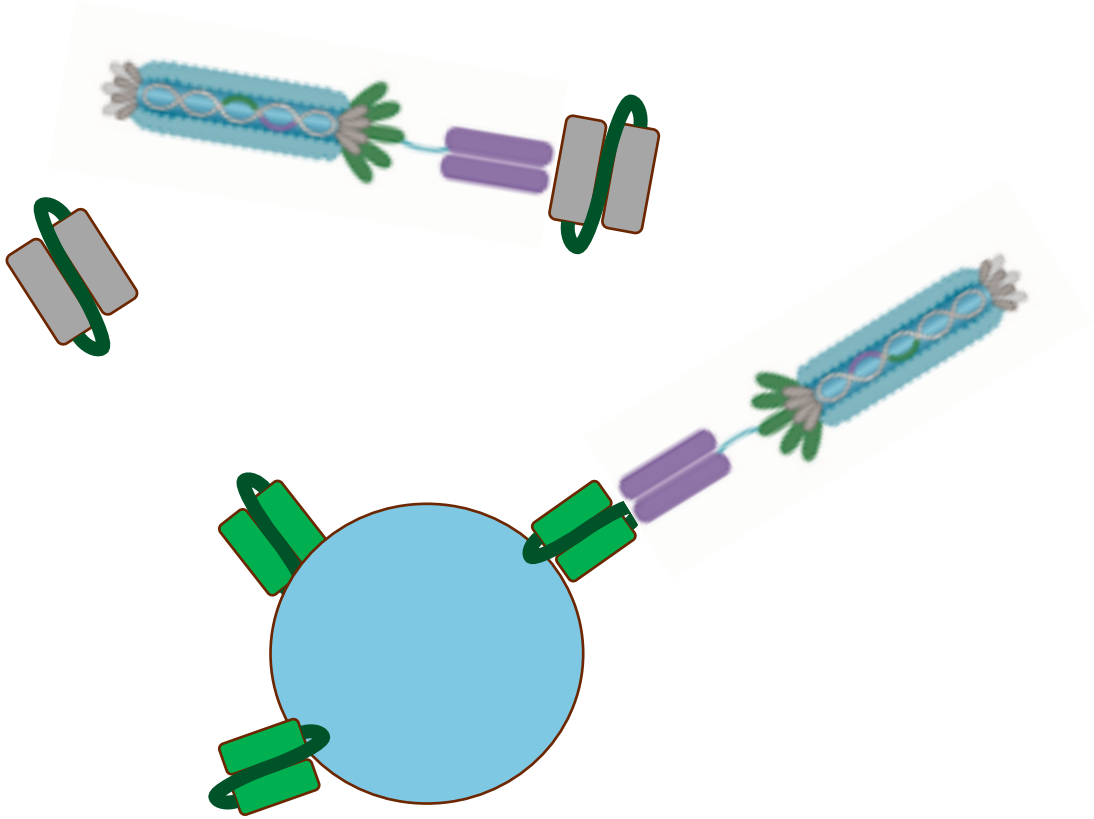


Guided Selection: Epitope Specificity

CAR-T receptor structure



Target antigen: recombinant scFv portion
CRA: control scFv



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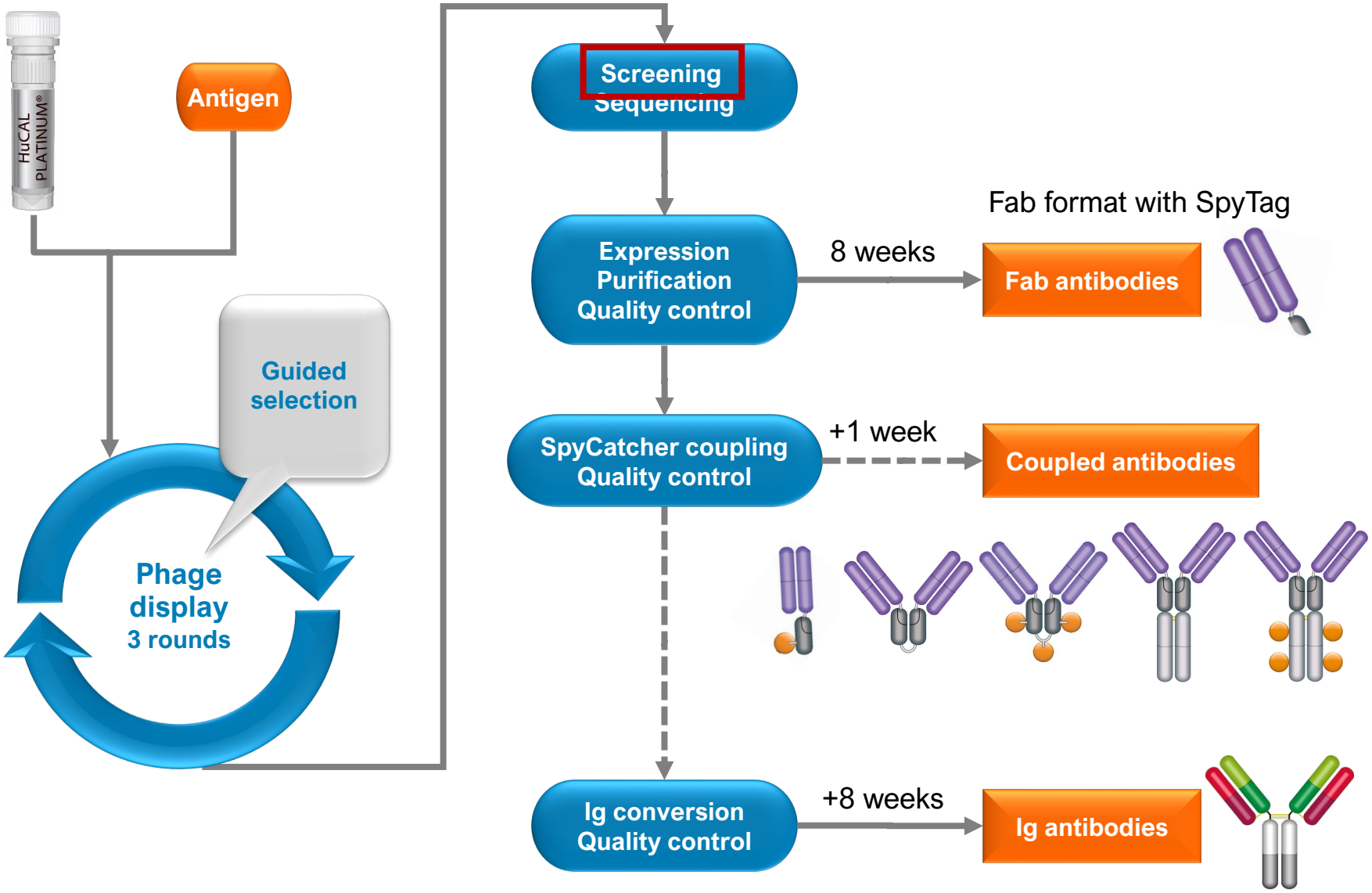
Antibody Generation Process



TrailBlazer

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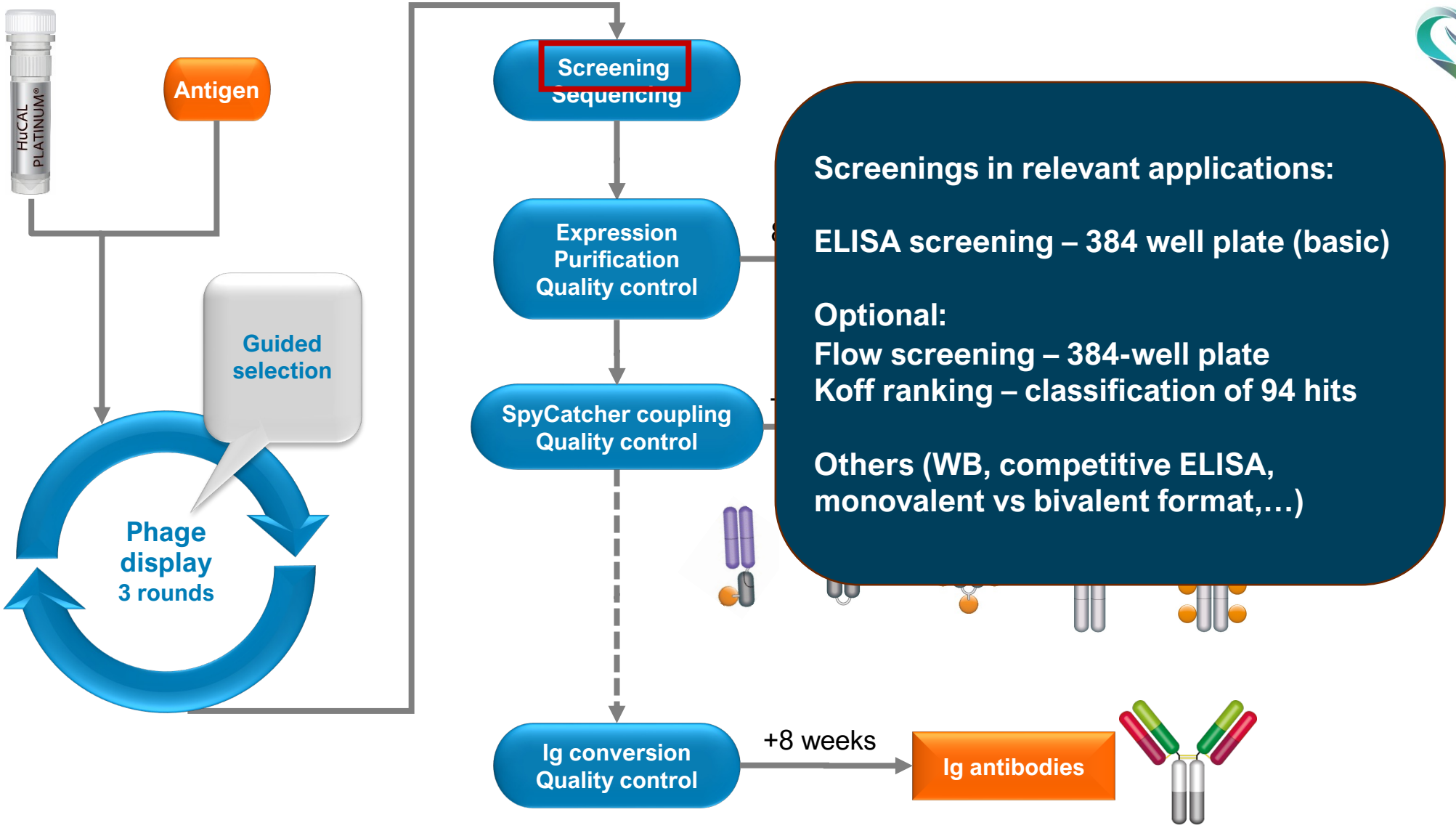
Antibody Generation Process



TrailBlazer

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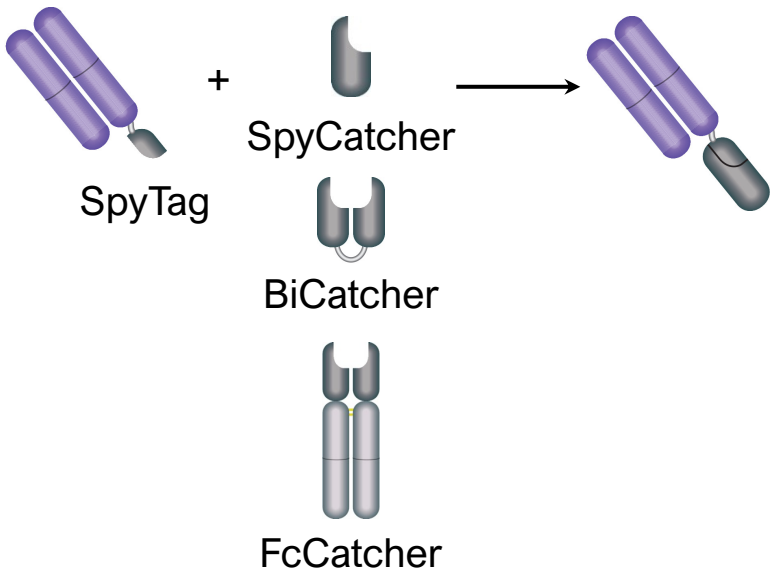
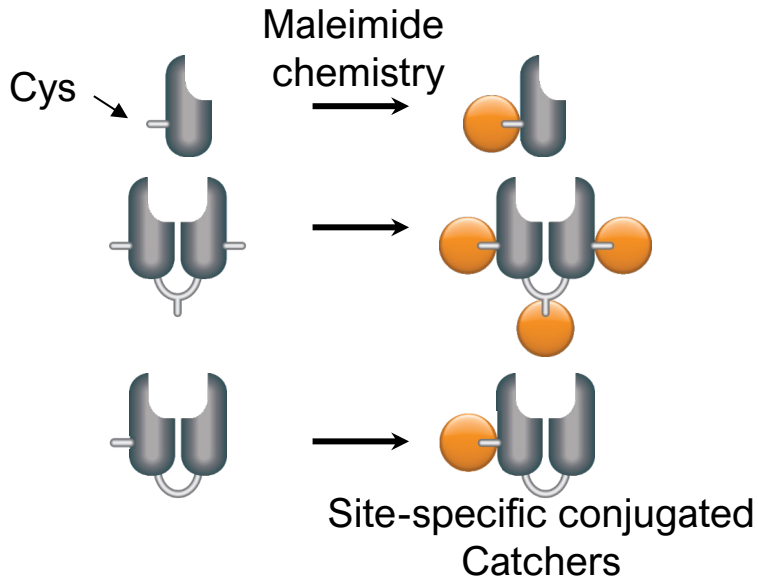
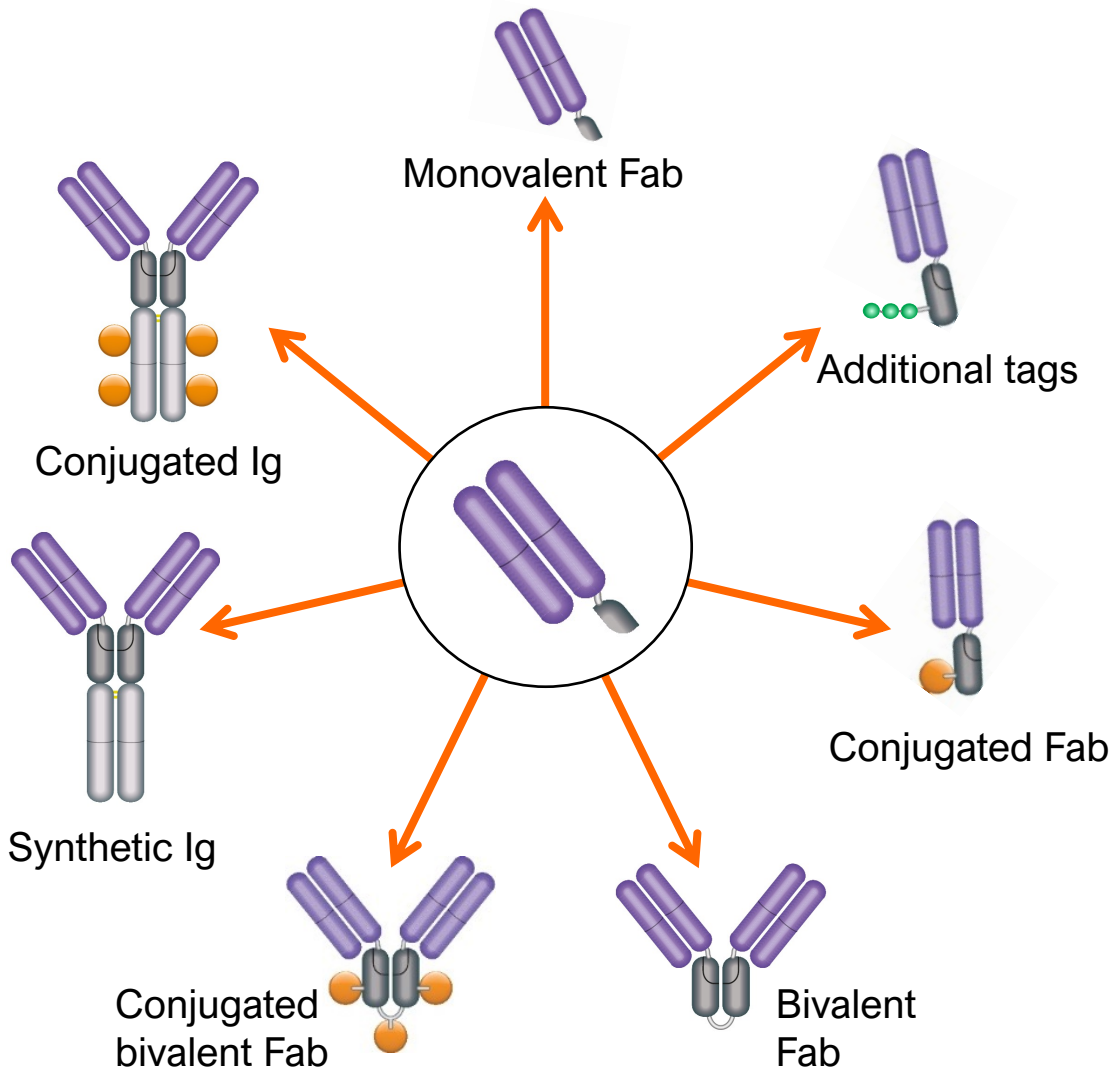
Modular Antibodies: One Antibody, Multiple Formats in an Instant







Hentrich C et al. (2021). Periplasmic expression of SpyTagged antibody fragments enables rapid modular antibody assembly. Cell Chem Biol 28, 1–12.

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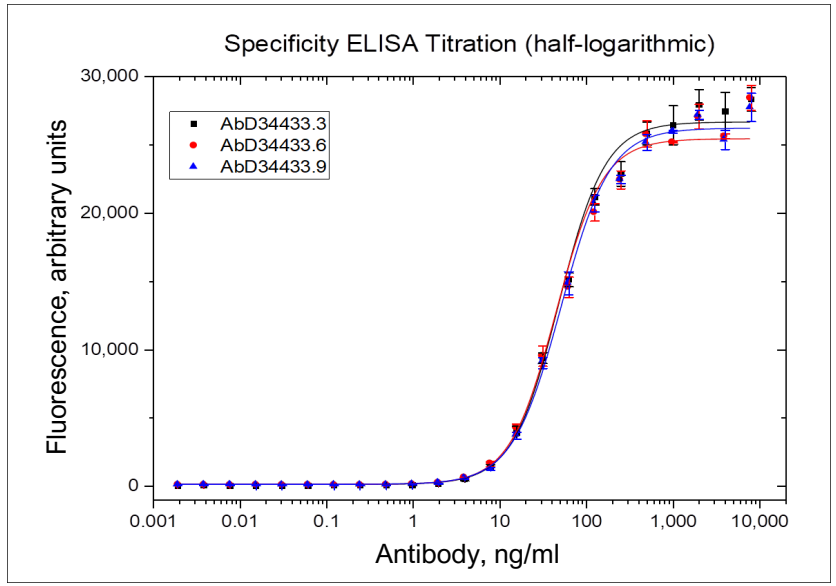
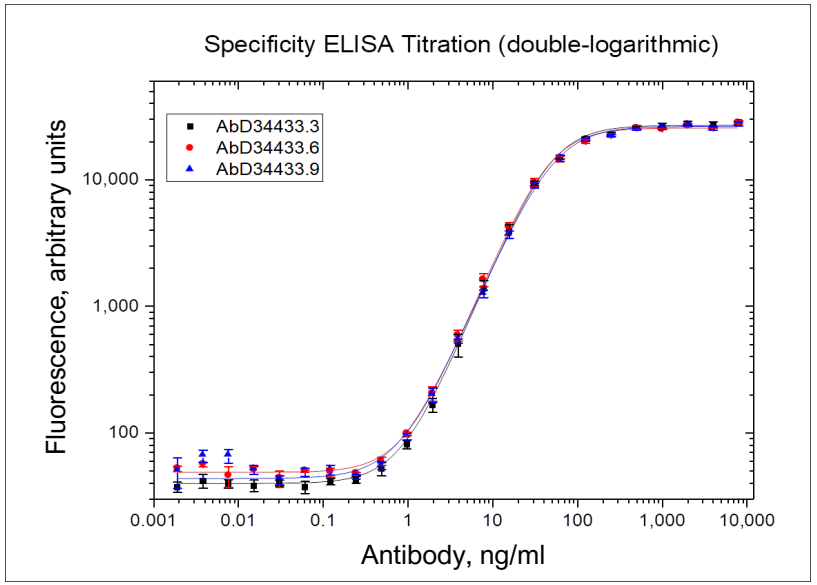
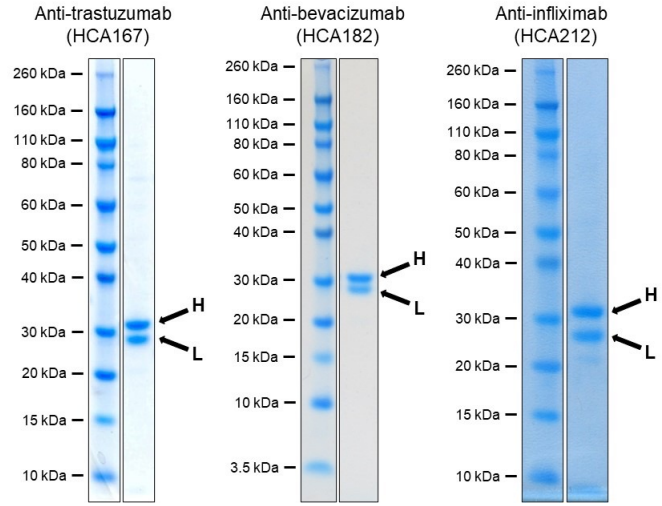
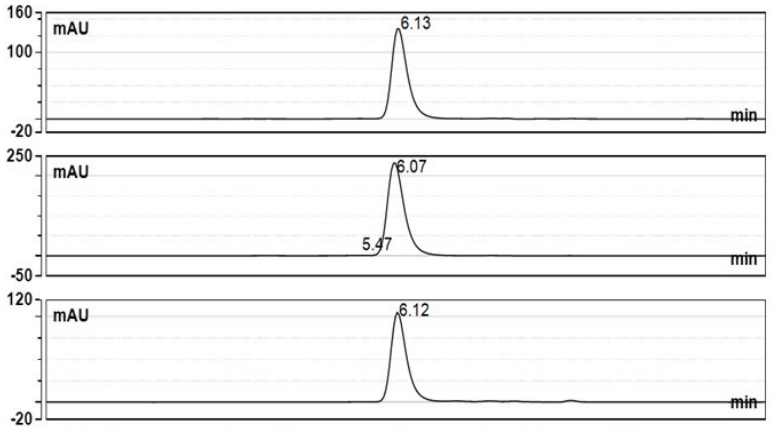
Recombinant Production is Highly Reproducible Between Lots

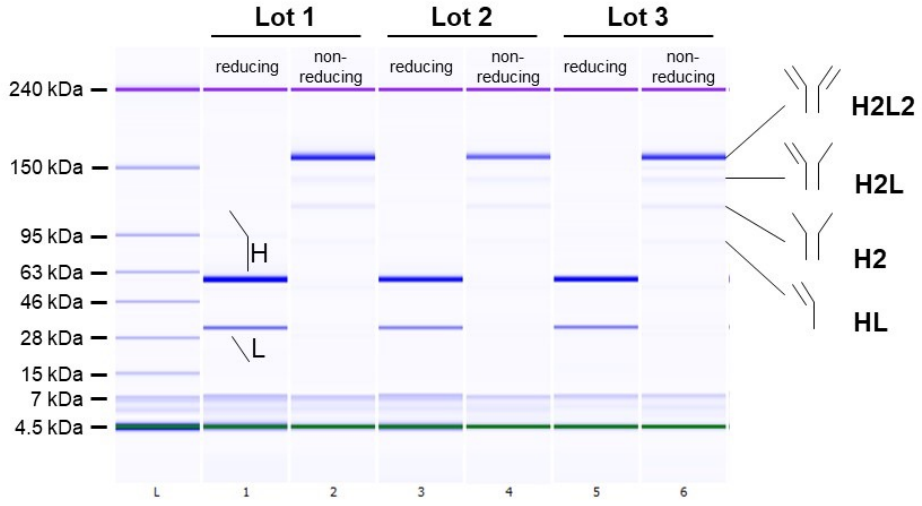








SEC analysis of three different batches of human anti-palivizumab hIgG (HCA262, Lot 1-3)



Generation of antibodies targeting a chimeric antigen receptor (CAR)

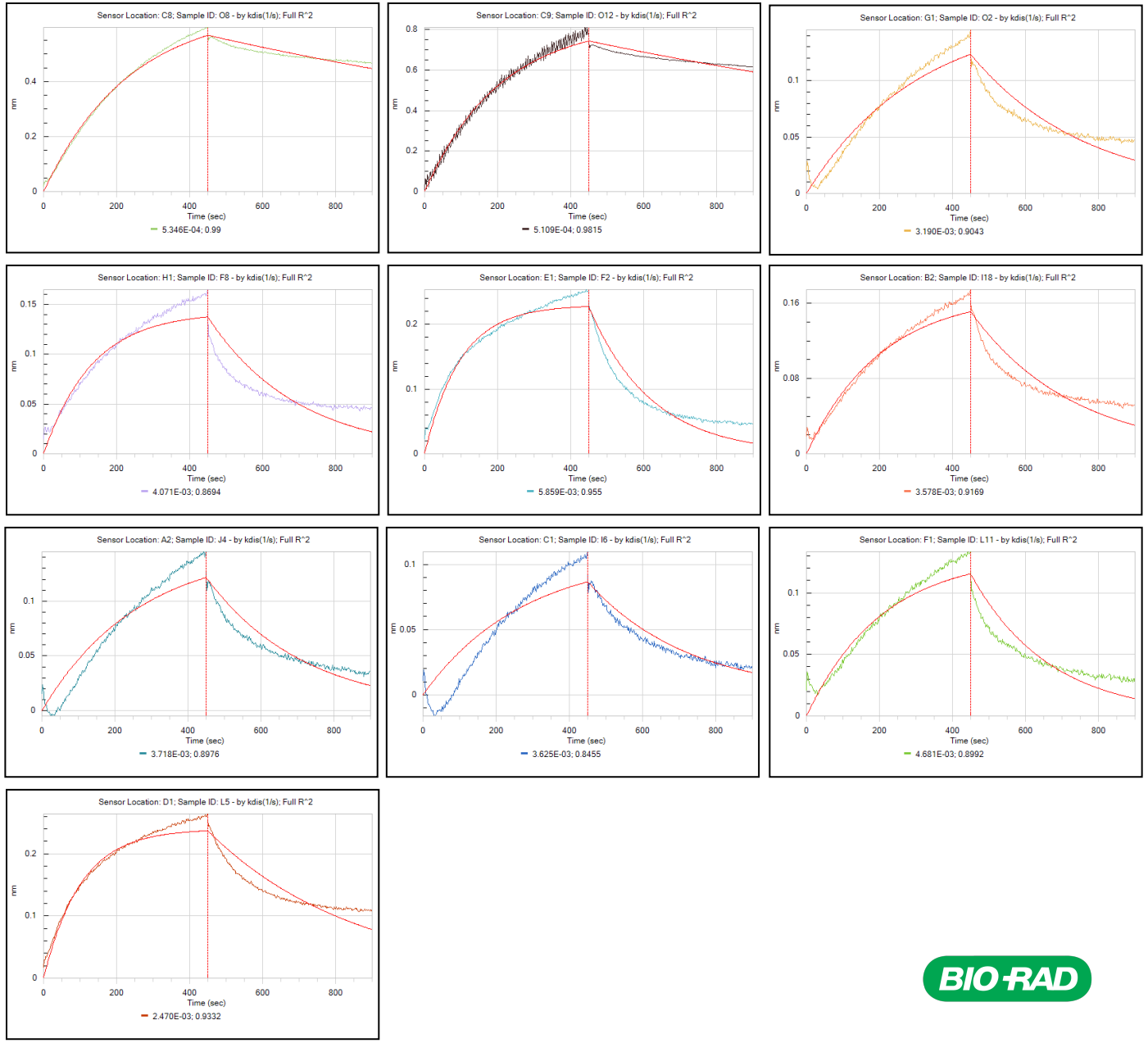
- Antigens: single chain fragment variable (scFv) and biotinylated scFv
- Screenings:
  - Primary: ELISA on scFv, biotinylated scFv, and unrelated scFv, identified **196 hits**
  - Secondary : flow cytometry on scFv expressing cells, resulted in **75 hits**
  - Tertiary : off-rate (k_{off}) ranking of positive hits using biolayer interferometry
- Sequencing of the 20 best clones identified **11 unique antibodies**
- Antibodies expressed and purified: 3 different formats provided thanks to TrailBlazer™ technology
- Quality control (QC) by ELISA and flow cytometry

Off-Rate Ranking Results of Unique Clones

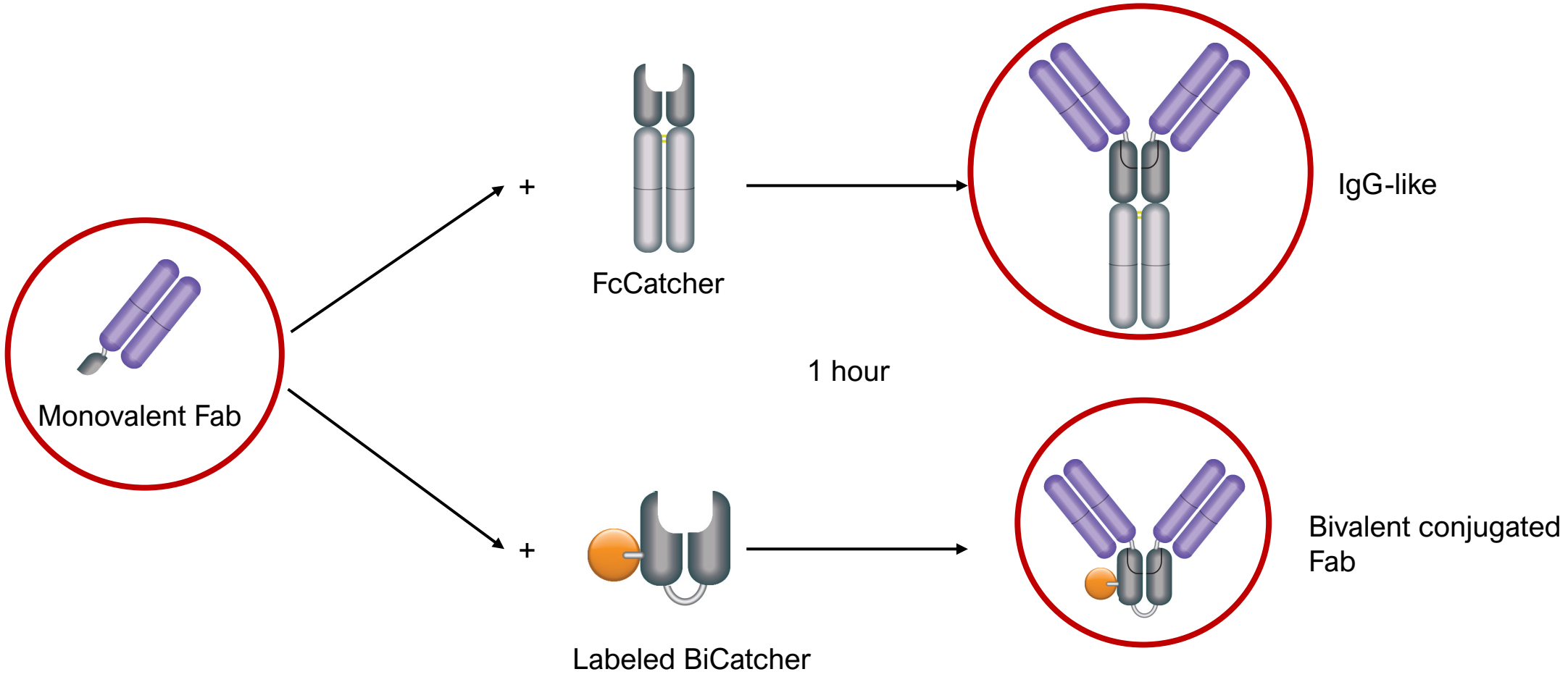
k_{off} ranking data for unique clones.

Measurement on the monovalent Fab

Sensor Location	Antibody Clone	Antigen	Response, nm	k_{off}, 1/s
C8	AbD99991ad-1	scFv-bio	0.59	5.35×10^{-4}
C9	AbD99992ad-1	scFv-bio	0.79	5.11×10^{-4}
G1	AbD99993ad-1	scFv-bio	0.14	3.19×10^{-3}
H1	AbD99994ad-1	scFv-bio	0.16	4.07×10^{-3}
E1	AbD99995ad-1	scFv-bio	0.25	5.86×10^{-3}
B2	AbD99996ad-1	scFv-bio	0.17	3.58×10^{-3}
A2	AbD99997ad-1	scFv-bio	0.14	3.72×10^{-3}
C1	AbD99998ad-1	scFv-bio	0.11	3.63×10^{-3}
F1	AbD99999ad-1	scFv-bio	0.13	4.68×10^{-3}
D1	AbD99990ad-1	scFv-bio	0.26	2.47×10^{-3}

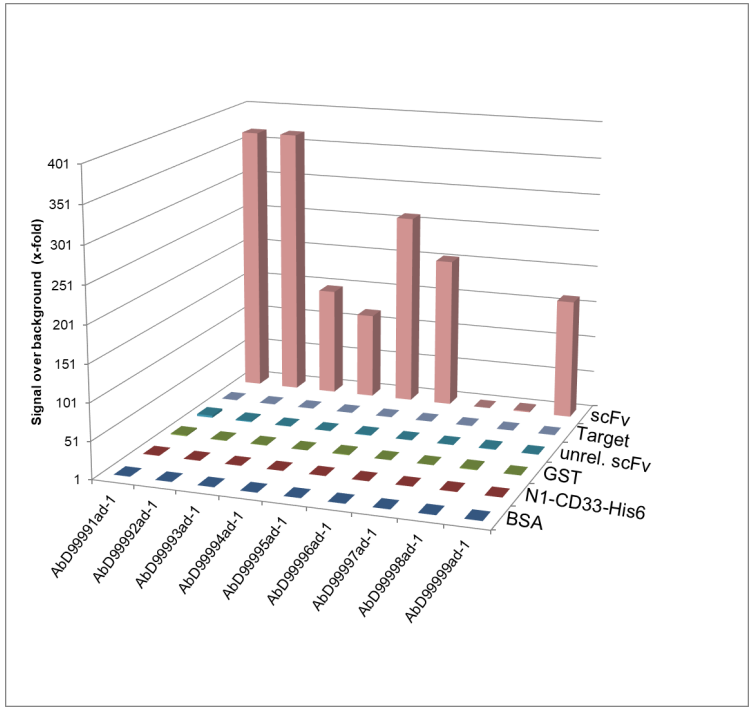


One Antibody Production Leads to Three Formats

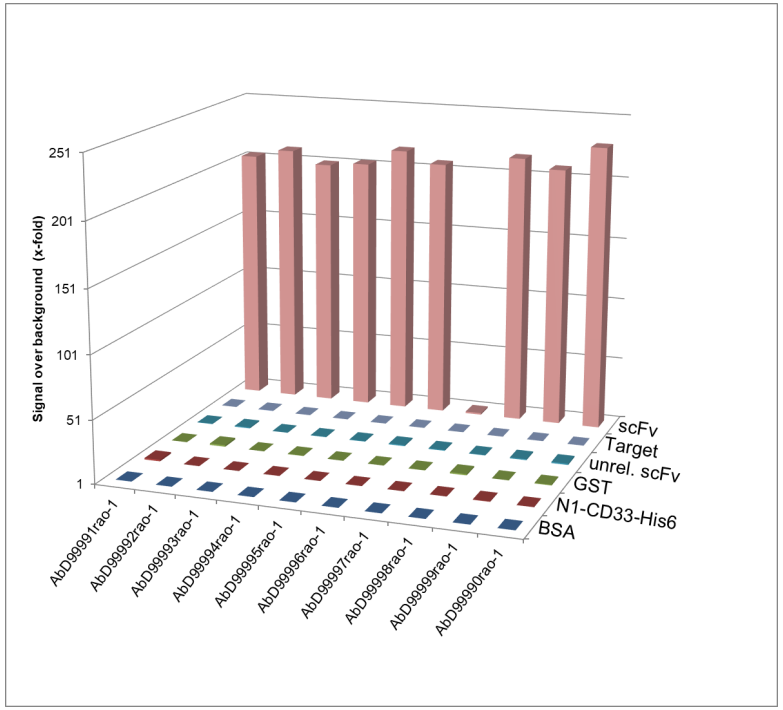


Quality Control ELISA

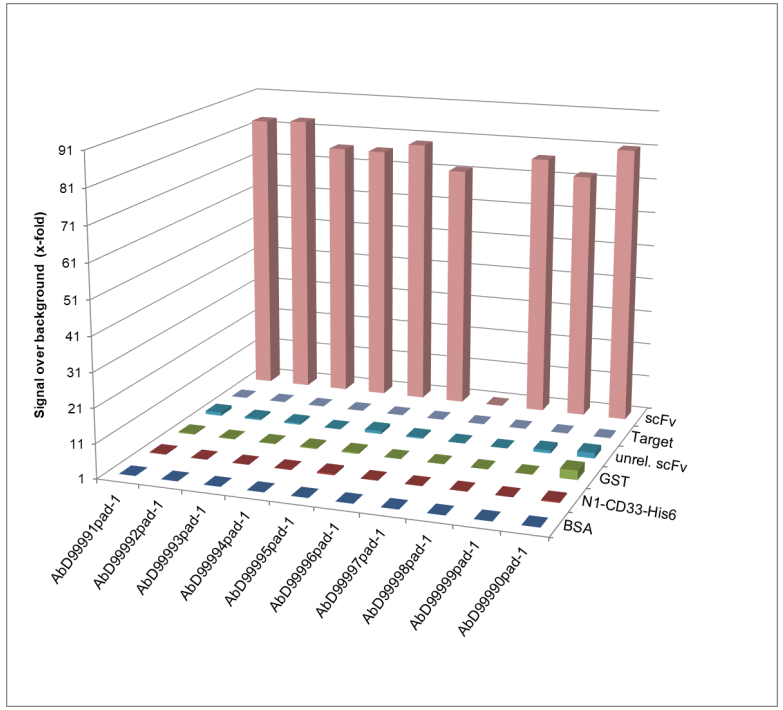
Fab



hIgG-like



Bivalent Fab-PE



- ELISA with the monovalent Fab format can be used to rank the antibodies according to their binding strength
- Bivalent formats (Fab or Ig-like) can be used in applications that benefit from the avidity effect to offer increased sensitivity

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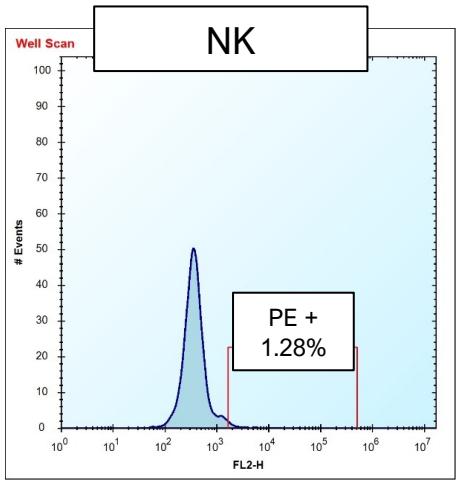
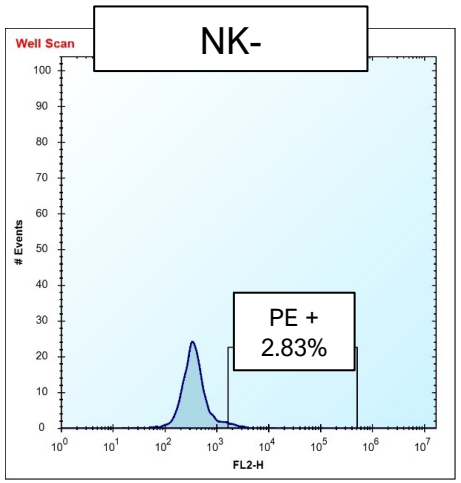
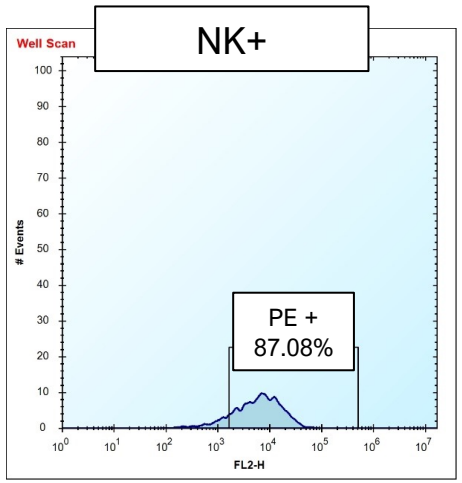
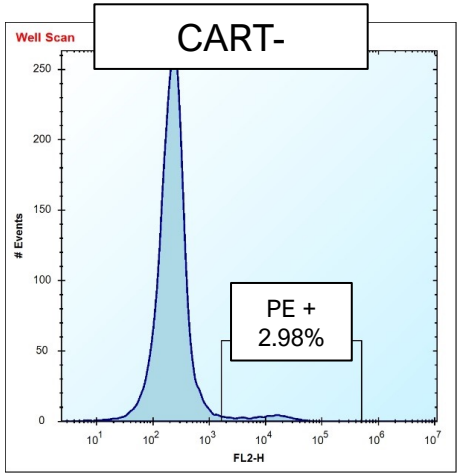
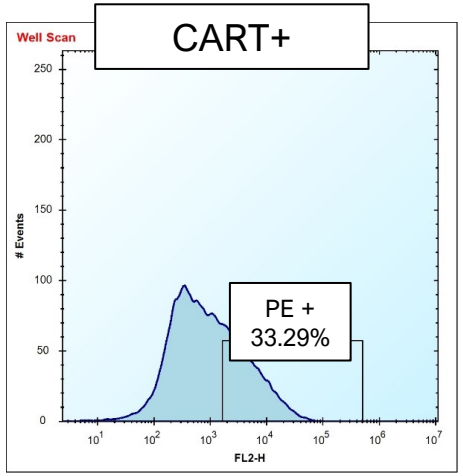
Flow Cytometry with Anti-scFv Antibody in Ig-Like Format











NK	Natural killer (NK) cell line
NK-	NK cell line transfected with unrelated scFv
NK+	NK cell line transfected with target scFv
CART-	CAR T-cells transfected with unrelated scFv
CART+	CAR T-cells transfected with target scFv

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Summary

- Fast, well-established platform for the generation of customized antibodies
- In vitro strategies for meeting challenging specificity requirements
- Screening and testing in relevant applications
- Modular antibody platform with SpyTag incorporated
- Reliable production, lot-to-lot consistency

Visit bio-rad-antibodies.com/TrailBlazer for more information.