Case Study 1

Hybridoma-based Anti-idiotypic mAb Discovery against a Bispecific Therapeutics for PK/ADA Assays Development

CASE STUDY ON ANTI-IDIOTYPIC ANTIBODY DISCOVERY

A campaign for the discovery for panels of anti-idiotypic mAb's for a bispecific molecule

Target and Applications:

- Therapeutic bispecific target (IgG-scFv) with two antigen-binding arms (Figure 1)
- Anti-Fab (Cohort 1) and anti-scFv (Cohort 2) antibodies required for PK/ADA assay development
- Unique antibody sequences and antibodies delivered to partner

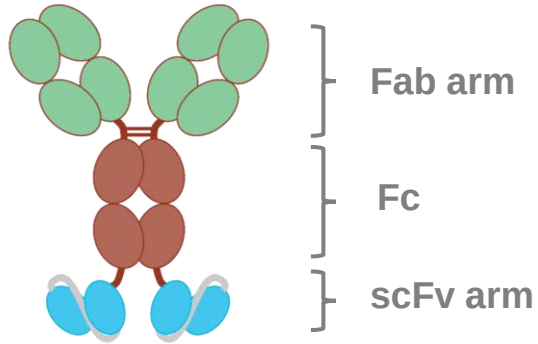


Figure 1. Bispecific Target (IgG-scFv)

Antibody characteristics desired
High specificity to the respective antigen-binding arm
Antibodies that works as pairs for bridging assay development (Figure 2)
A range of affinities for assay sensitivity (from sub-nanomolar to double-digit nanomolar affinities)

Optional: Identify neutralizers and non-neutralizers

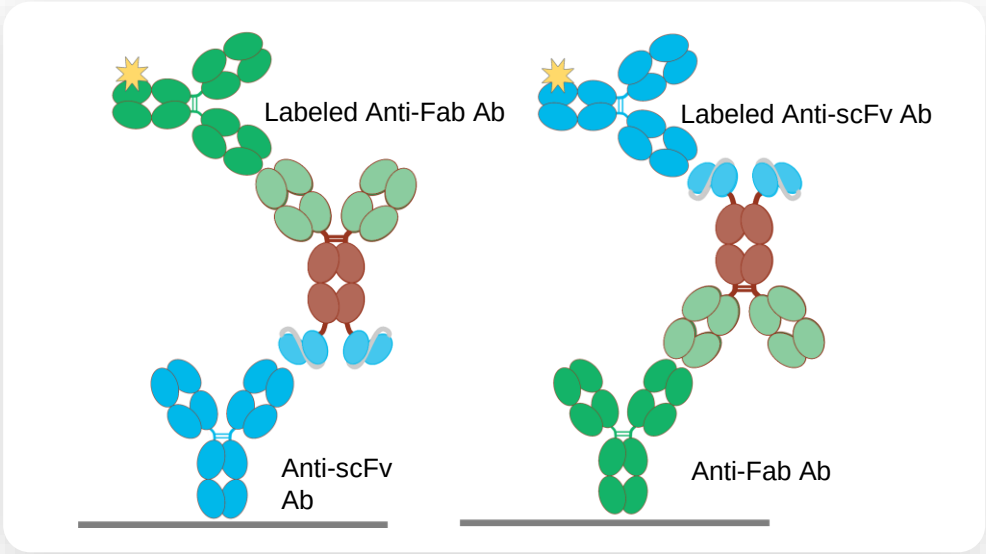


Figure 2. Illustration of the two formats of bridging assay with anti-Fab and anti-scFv as detection or capture antibodies

ANTI-IDIOTYPIC ANTIBODY DISCOVERY – IMMUNIZATION TO SEQUENCES

Abveris
A Division of Twist Bioscience

Workflow for two antigen-binding arms using two cohorts of mice: Cohort 1 (anti-Fab); Cohort 2 (anti-scFv)

01 Immunization & Hybridoma Generation	• Generation of antibodies by immunization of DiversimAb Mice • Rapid immunization schedule with proprietary Abveris adjuvant • Harvest of lymph tissues and generation of hybridoma by electrofusion	2 mice for each antigen-binding arm	3 WEEKS
02 ELISA Specificity Screens (screening in the presence of human serum)	• Primary ELISA screening for rapid identification of initial positive hits • Custom ELISA secondary specificity screen cascade o Screen for target binding o Screen in the presence of human serum to test for matrix effect o Counter screen against polyclonal human IgG for promiscuous binding	1760 hybridoma to 182 clones per cohort	3 WEEKS
03 Advanced Screen by Octet BLI – Affinity screen	• Direct biosensor-based kinetics on supernatant of hybridoma suspensions • Affinity ranking by off-rate for clone selection	96 clones per cohort	1 WEEKS
04 Advanced Screen by Octet BLI – Pairing and Neutralization	• Pairing study with a custom loading sequence • Neutralizing antibodies against ligand of each antigen-binding arm	24 clones per cohort	2 WEEKS
05 Sequencing and Recombinant Expression	• Cryopreservation of parental clones • Delivery of panel of anti-idiotypic antibodies by HTP sequencing of 24 parental clones for each cohort to obtain monoclonal antibody sequences • Expression and production of antibody	16 &17 unique sequences for each cohort	4 WEEKS

TWIST BIOSCIENCE

GENERATION OF ANTIBODIES FOR SPECIFICITY SCREENING

Robust immune from DiversimAb mouse allows for generation of hybridoma for screening

Immunization & Hybridoma Generation

ELISA Specificity Screens

Titer check of immunization

Figure 3A. Cohort 1 Anti-Fab ELISA Titer

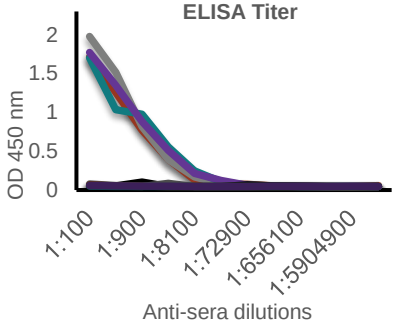
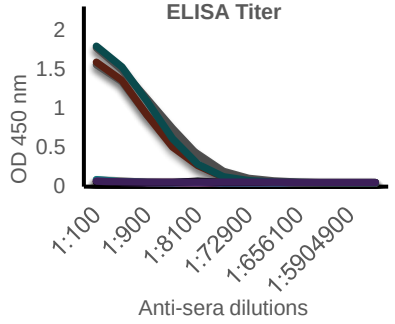


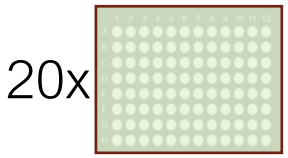
Figure 3B Cohort 2 Anti-scFv ELISA Titer



Cohort 1: Fab immunized for anti-Fab antibodies (Figure 3A)
Cohort 2: scFv immunized for anti-scFv antibodies (Figure 3B)

- Rapid immunization in 18 days
- Good immune response for both cohorts
- 2 mice from each cohort were selected for fusion based on ELISA titer responses
- Lymph tissues were harvested and fused with myeloma cells by electrofusion

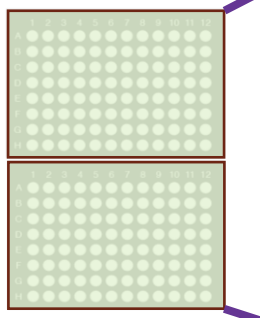
Primary screen



1760 wells for each cohort

Cohort 1 & Cohort 2
Bispecific target (+)
Polyclonal human IgG (-)

Secondary screen



182 clones for each cohort

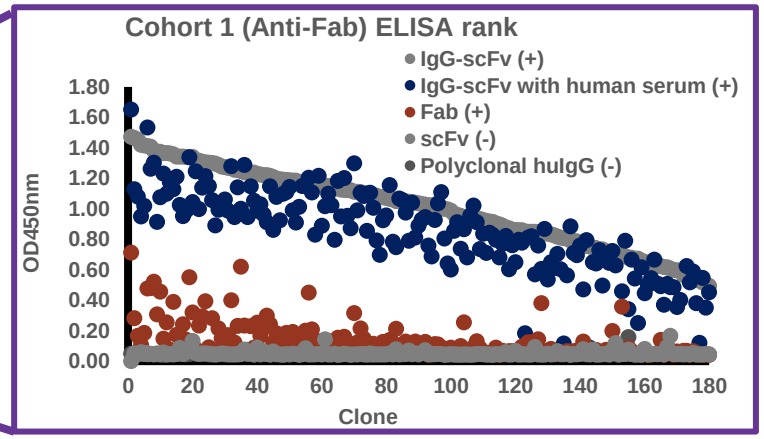


Figure 4. Example secondary ELISA specificity screening data

	Cohort 1 Anti-Fab	Cohort 2 Anti-scFv
Bispecific target	(+)	(+)
Bispecific target with 20% human serum	(+)	(+)
Fab	(+)	(-)
scFv	(-)	(+)
Polyclonal huIgG	(-)	(-)

ELISA SECONDARY SPECIFICITY SCREEN RESULTS

Interrogation of the 182 positive hits by a custom screen cascade allows for the selection of top 96 hits

Cohort 1 (Fab immunized)

Clone	Bispecific target (IgG-scFv) (+)	Bispecific target with 20% human serum (+)	Fab (+)	scFv (-)	Polyclonal hulgG (-)
1-1	1.474	1.653	0.713	0.048	0.049
1-2	1.466	1.129	0.282	0.052	0.051
1-3	1.414	1.536	0.478	0.053	0.044
1-4	1.408	1.264	0.479	0.046	0.046
1-5	1.384	1.304	0.523	0.044	0.044
1-6	1.378	0.915	0.308	0.052	0.045
1-7	1.375	1.077	0.456	0.046	0.043
1-8	1.366	1.101	0.256	0.045	0.043
1-9	1.359	1.129	0.389	0.045	0.044
1-10	1.343	0.953	0.243	0.047	0.046
1-11	1.341	1.339	0.551	0.076	0.047
1-12	1.341	1.046	0.320	0.134	0.076
1-13	1.317	0.999	0.235	0.046	0.051
1-14	1.311	1.139	0.296	0.051	0.05
1-15	1.305	1.215	0.393	0.052	0.049

Table 1A. 15 representative clones from secondary screen cascade ranked by bispecific target (+) readout

Cohort 2 (scFv immunized)

Clone	Bispecific target (IgG-scFv) (+)	Bispecific target with 20% human serum (+)	Fab (-)	scFv (+)	Polyclonal hulgG (-)
2-1	1.411	0.265	0.043	1.346	0.049
2-2	1.408	0.802	0.05	1.487	0.051
2-3	1.382	0.543	0.042	1.409	0.05
2-4	1.346	0.273	0.049	1.749	0.183
2-5	1.325	0.161	0.043	1.183	0.052
2-6	1.316	0.462	0.042	1.292	0.044
2-7	1.286	0.659	0.042	1.207	0.047
2-8	1.285	0.501	0.042	1.22	0.046
2-9	1.273	0.423	0.055	1.337	0.051
2-10	1.265	0.375	0.042	1.221	0.045
2-11	1.244	0.095	0.048	1.099	0.053
2-12	1.224	0.871	0.045	0.99	0.053
2-13	1.223	0.577	0.042	1.185	0.049
2-14	1.211	0.326	0.061	1.25	0.056
2-15	1.206	1.026	0.049	1.291	0.06

Table 1B. 15 representative clones from secondary screen cascade ranked by bispecific target (+) readout

- Ranking of 182 clones screen by readout of anti-bispecific target ELISA in secondary screen
- Screen cascade allows for the selection of binders with stringent specificity requirements
- 96 hits were selected for affinity ranking for each cohort

ELISA SECONDARY SPECIFICITY SCREEN RESULTS

Screening for the retainment of binding in the presence of human serum is critical

Several antibodies appeared specific to the bispecific and the ScFv then showed reduced binding capacity in the presence of human serum.

Screening in the presence of human serum early enables downselection of mAbs that are not suitable for clinical assay use

Cohort 2 (scFv immunized)

Clone	Bispecific target (IgG-scFv) (+)	Bispecific target with 20% human serum (+)	Fab (-)	scFv (+)	Polyclonal hulgG (-)
2-1	1.411	0.265	0.043	1.346	0.049
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2-14	1.211	0.326	0.061	1.25	0.056
2-15	1.206	1.026	0.049	1.291	0.06

Quality of anti-ID for Cohort 2 (anti-scFv) is significantly improved by screening out low readouts in the presence of human serum

OCTET BLI AFFINITY MEASUREMENT RESULTS

Affinity ranked by off-rate by direct measurement on crude supernatant of the hybridoma suspensions

Figure 5A Cohort 1 (anti-Fab) Binding Kinetics Representative Examples

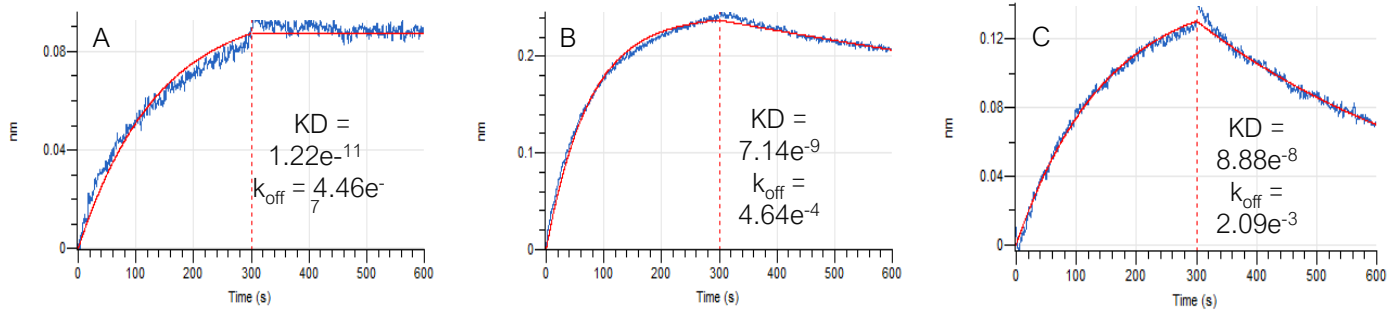
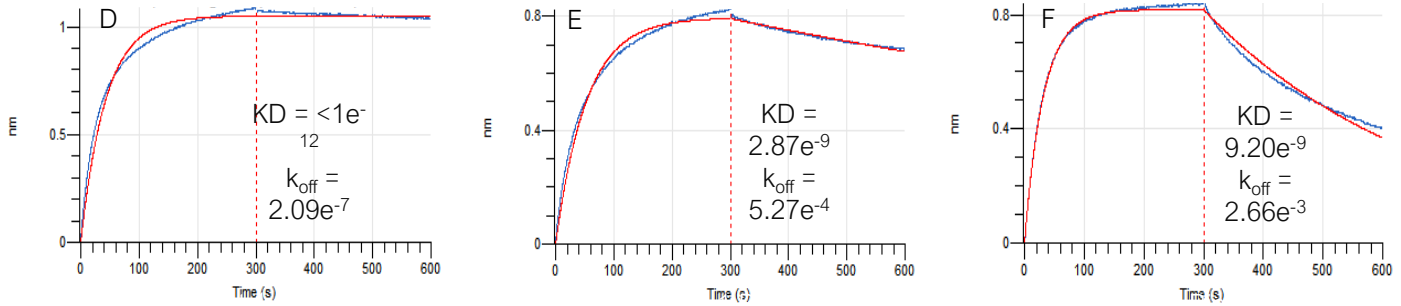
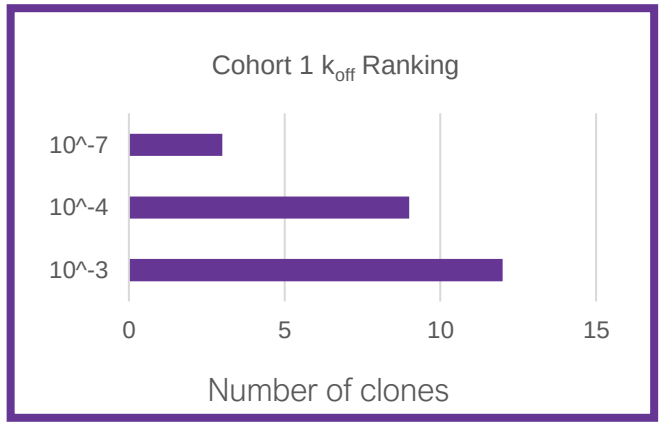
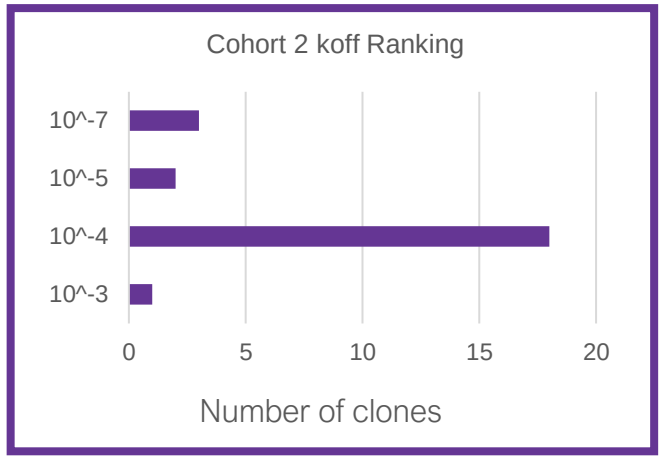


Figure 5B. Cohort 2 (anti-scFv) Binding Kinetics Representative Examples





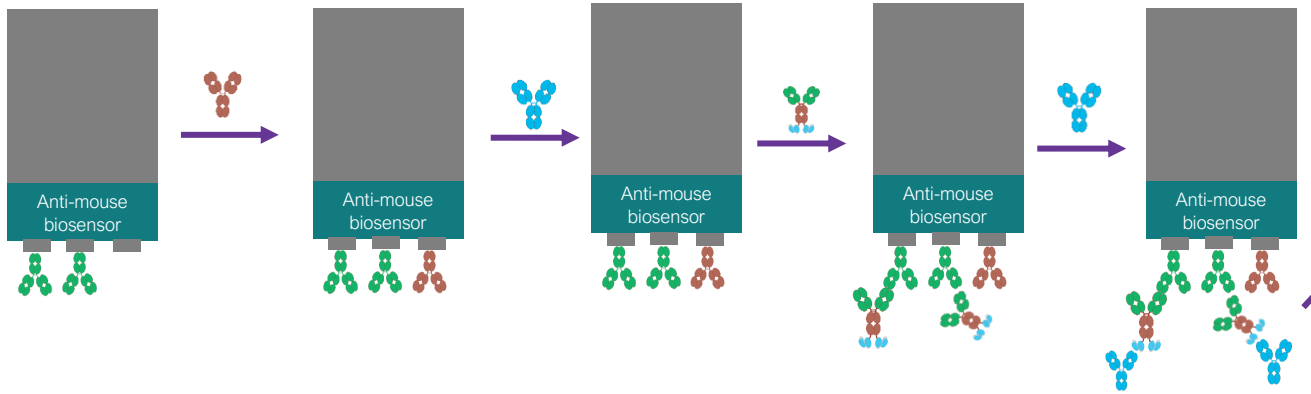


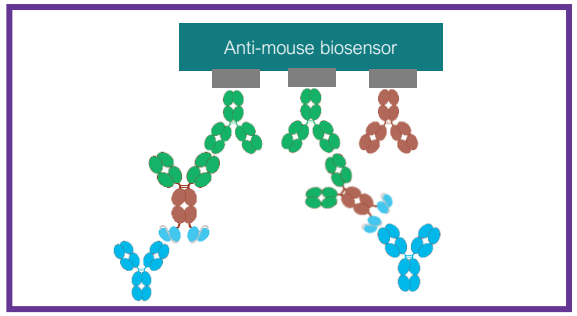
Conclusion

Two panels of clones with good ranges of affinity were ranked by off-rate
24 antibodies from each were selected to move on for pairing analysis

IDENTIFYING ANTIBODY PAIRS THAT BIND TO THE IgG-scFv

A loading sequence is designed to detect anti-Fab and anti-scFv that will bind to the antibodies





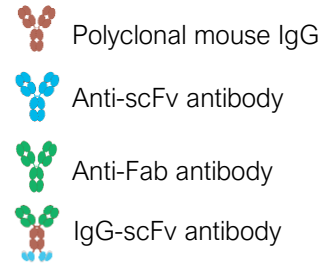
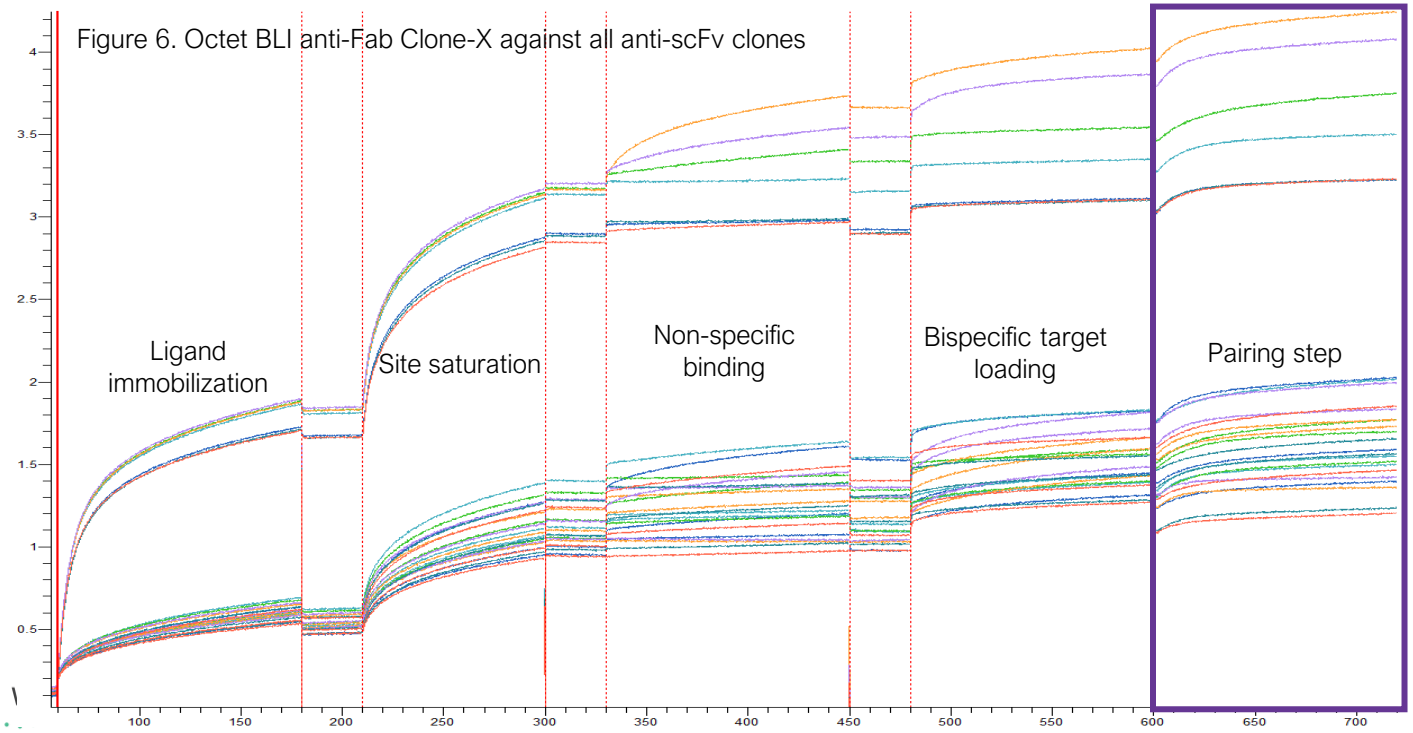
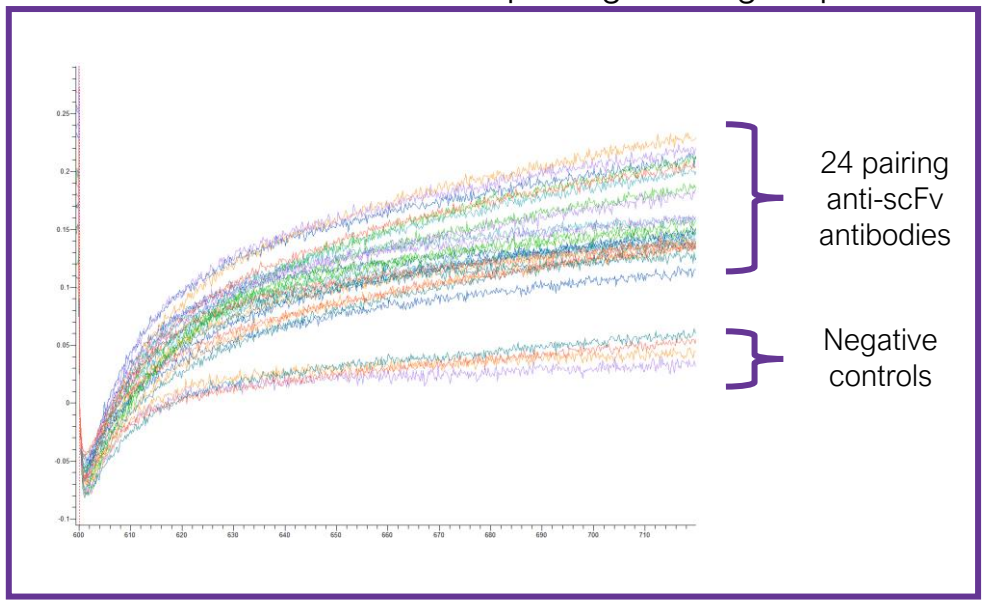


Figure 6. Octet BLI anti-Fab Clone-X against all anti-scFv clones



Normalized view of the pairing binding step

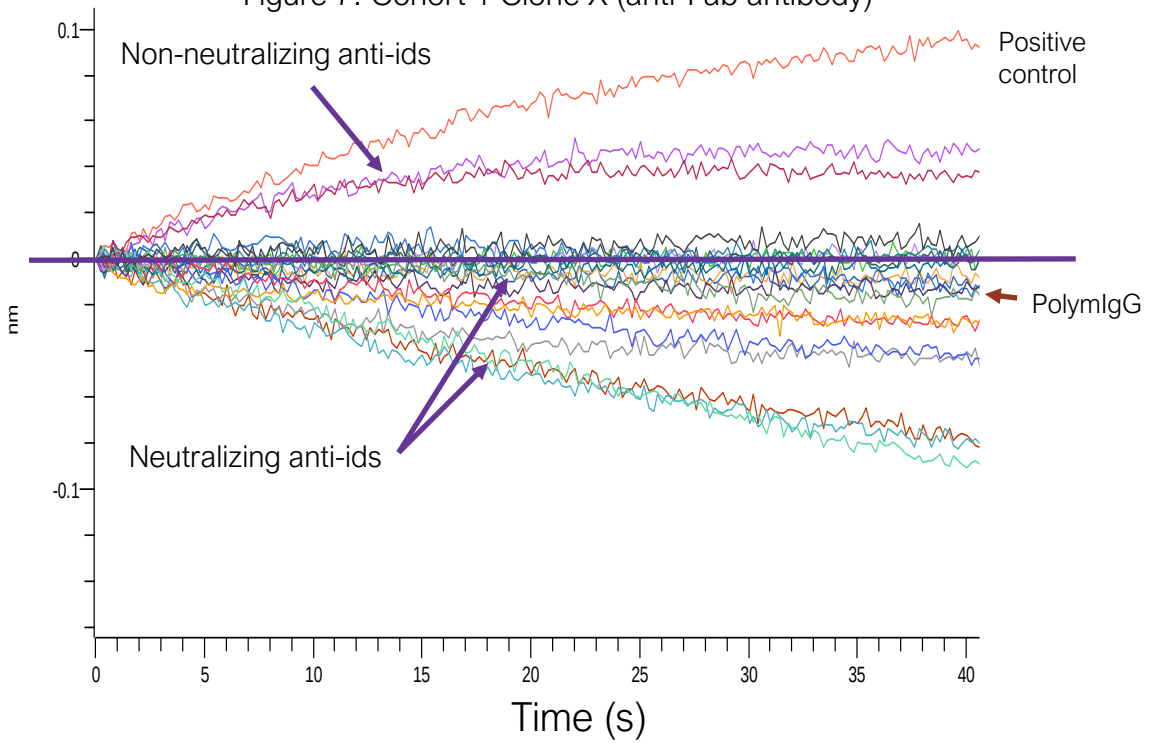


IDENTIFICATION OF NEUTRALIZERS/NON-NEUTRALIZERS

Successful identification of non-neutralizer for anti-Fab antibodies

2 non-neutralizers were identified in Cohort 1 (anti-Fab)

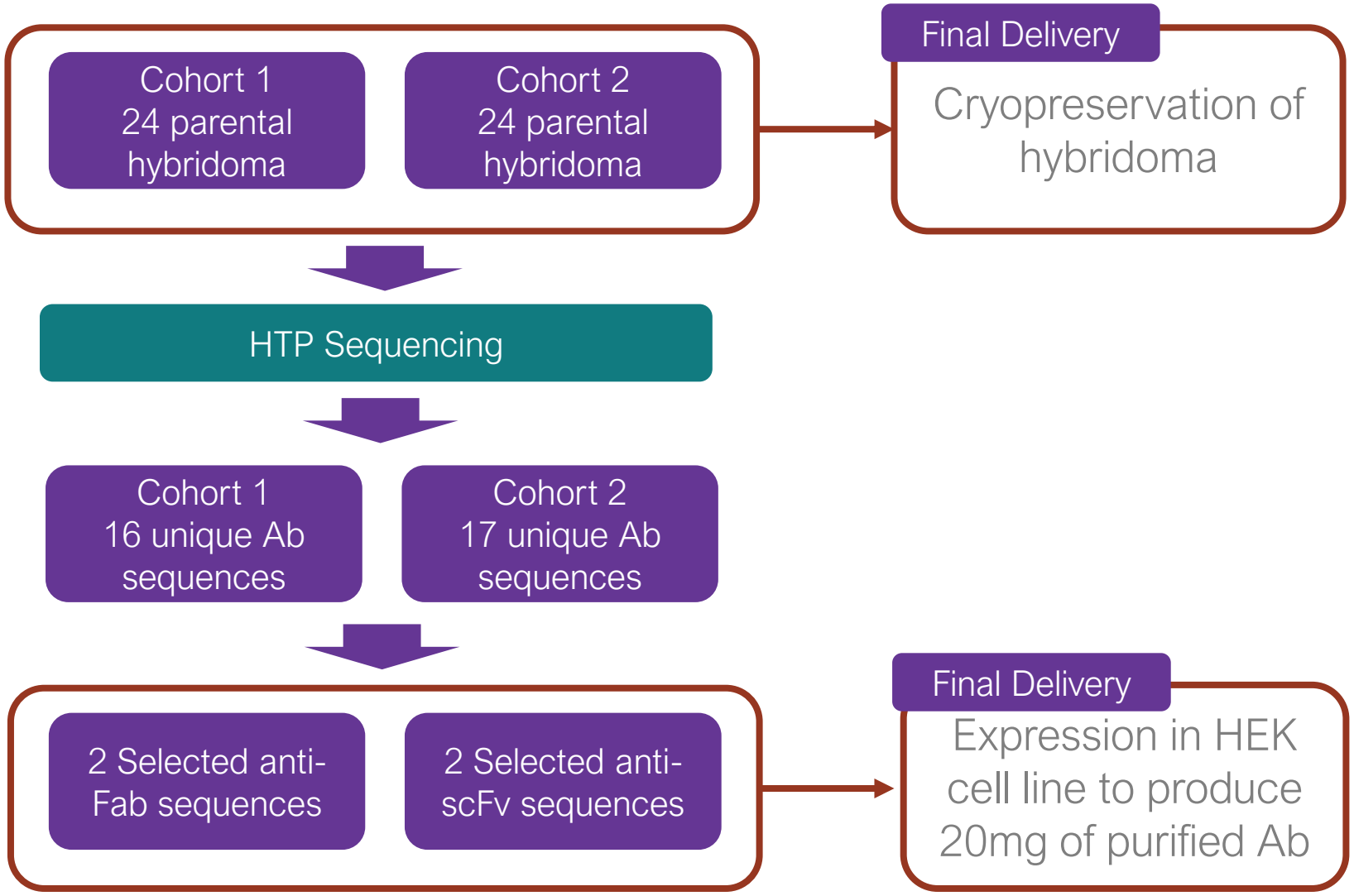
Figure 7. Cohort 1 Clone X (anti-Fab antibody)



*All Cohort 2 anti-svFc antibodies are neutralizers

DELIVERY PROJECTS AS SEQUENES AND PURIFIED ANTIBODIES

Direct HTP sequencing on parental hybridoma clones for efficient recovery of antibody HC/LC pairing



A SUCCESSFUL ANTI-ID mAb CAMPAIGN AGAINST A COMPLEX bsmAb THERAPEUTICS

Conclusions

- Rapidly generated mAbs against the ScFv and Fab arms of a bi-specific with the desired characteristics:
  - Highly specific, including in the presence of human serum
  - mAbs that work well as pairs
  - Blocking and non-blocking profiles identified
  - Diverse affinities
- Delivered:
  - HC/LC sequences
  - Purified and validated mAb
  - pcDNA3.4 vector
- Delivered final product 1 week ahead of pre-determined schedule