OmniAb®

High Throughput Antibody Characterization to Validate Novel Transgenic Animal Platforms

Bill Harriman, PhD

SVP Antibody Discovery, OmniAb Inc

Carterra Symposium, San Francisco, May 9, 2023

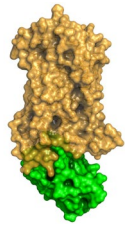
The OmniAb Technology Suite

OmniAb®

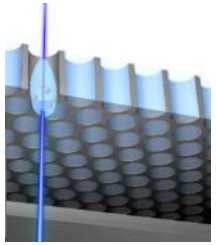
OmniRat | OmniMouse | OmniChicken | OmniTaur™

OmniFlic | OmniClic®

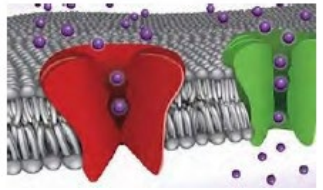
Antigen Design



xPloration®



ICAGEN®
ION CHANNEL TECHNOLOGY

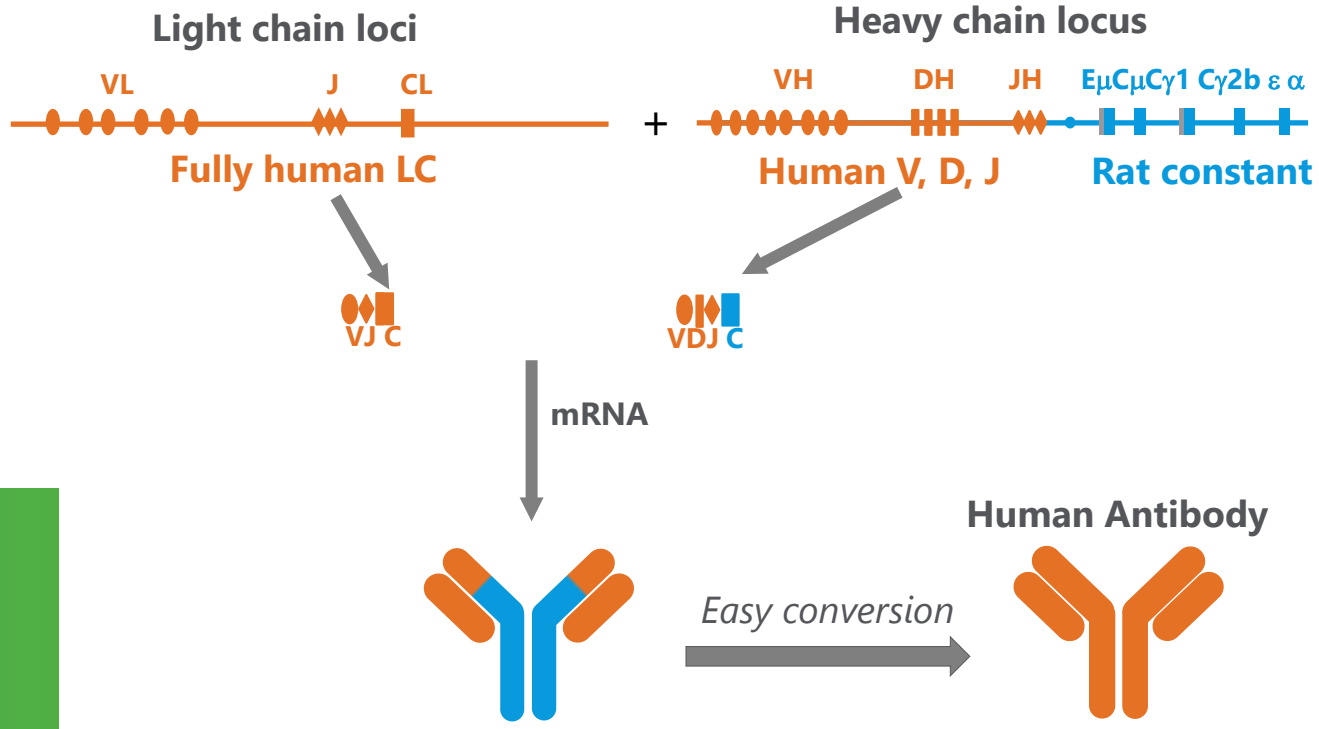


- The only platform leveraging **four species**
- Robust solutions for **bispecific antibodies**
- Human frameworks with **ultralong CDR-H3s**
- Industry-leading **broadest offering**
- **Proven success**

Rodent Platforms

OmniRat OmniMouse

- Endogenous Ig genes inactivated
- Expression of full human V gene diversity
- Streamlined conversion into fully human molecule



Well-validated transgene design utilizes rodent constant regions for robust immune responses from the B-cell repertoire

Geurts et al. Science 2009
Ménoret et al. Eur J Immunol 2010
Osborn et al. J. Immunol. 2013

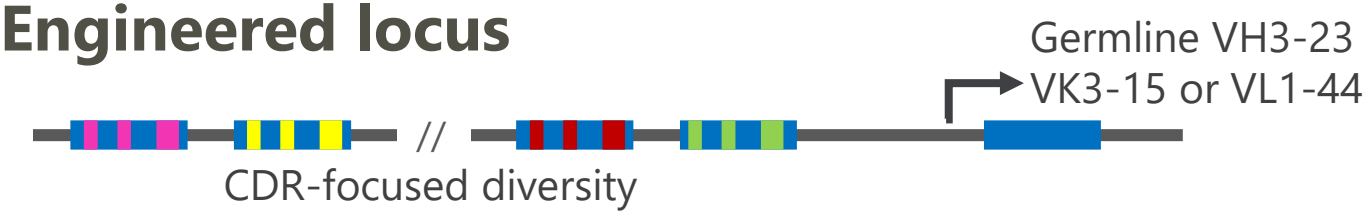
Engineering of Chicken Ig Loci

ADAPTATION TO CHICKEN GENE CONVERSION PROCESS

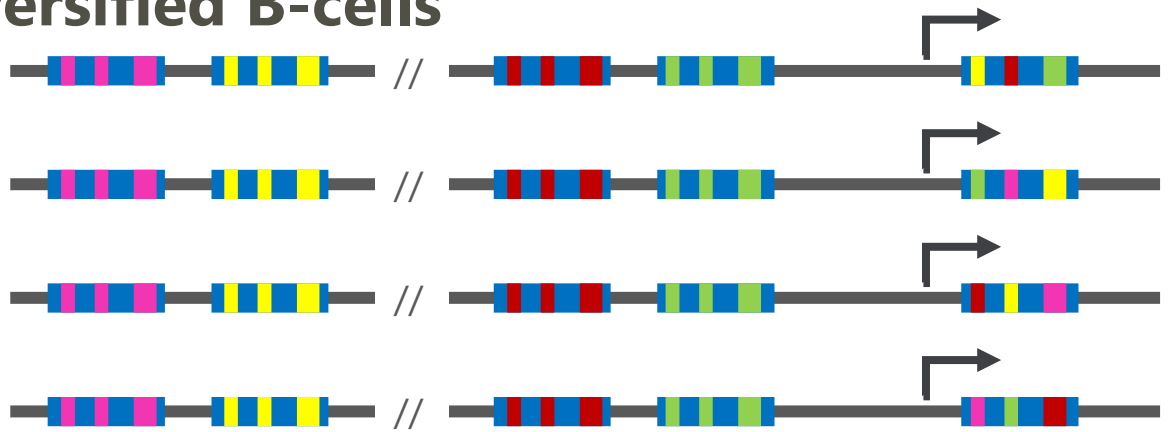
OmniChicken

Gene conversion

Engineered locus



Diversified B-cells



Human V's selected for:

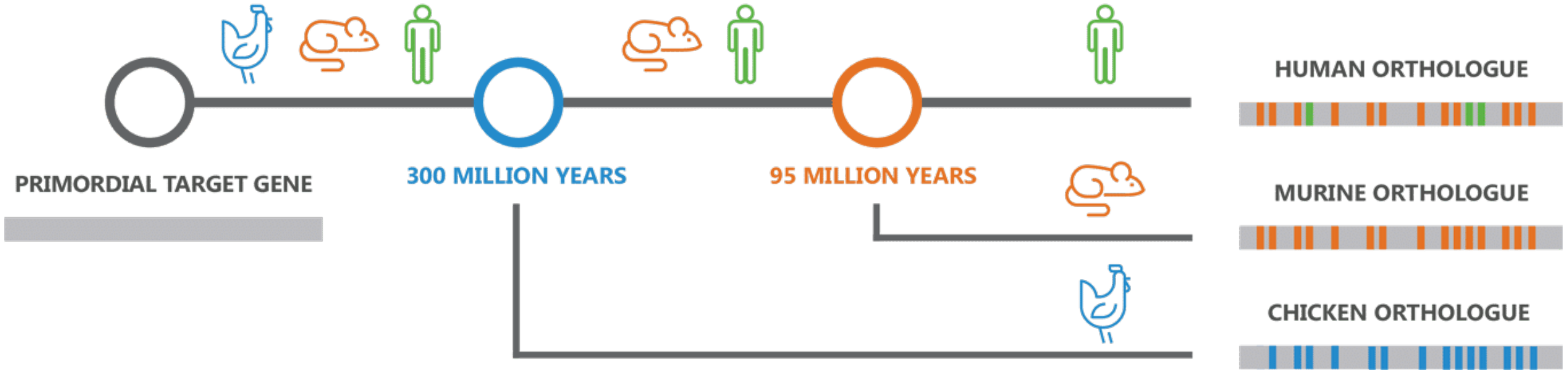
- High expression level, stability, ubiquity
- High sequence diversity in CDRs
- Low sequence diversity in FWs

OmniChicken®

POWERED BY EVOLUTION

OmniChicken

GREATER EVOLUTIONARY DISTANCE YIELDS
GREATER IMMUNOGENICITY AND MORE ANTIBODY DIVERSITY



Historical Success Stories

CHICKEN ANTIBODIES RAISED AGAINST CONSERVED MAMMALIAN TARGETS

Antigen	Reference
RNA polymerase II - bovine	(Carroll and Stollar 1983)
β2-microglobulin - human	(Horton, Holden et al. 1985)
Kallikrein - human	(Burger, Ramus et al. 1985)
IGF1-R & Insulin-R - human	(Stuart, Pietrzyk et al. 1988)
PCNA - bovine	(Gassmann, Thommes et al. 1990)
Activin A - human	(Murata, Saito et al. 1996)
Prion protein (PrP) peptide - bovine	(Matsushita, Horiuchi et al. 1998)
Mannose-6-P/IGFII-R - human	(Lemamy, Roger et al. 1999)
Hypoxia Inducible Factor-1α - human	(Camenisch, Tini et al. 1999)
Melatonin receptor - human	(Williams, Drew et al. 2001)
Cystatin C - human	(Hansson, Flodin et al. 2008)

Epitope Recognition in Wild-Type Animals

Report

Assessing kinetic and epitopic diversity across orthogonal monoclonal antibody generation platforms

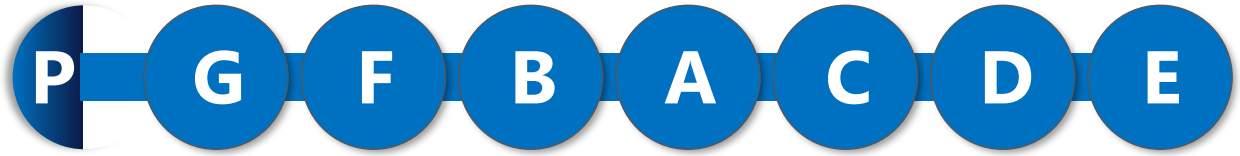
Yasmina Noubia Abdiche, Rian Harriman, Xiaodi Deng, Yik Andy Yeung, Adam Miles, Winse Morishige, Leila Boustany, Lei Zhu, Shelley Mettler Izquierdo & William Harriman

...show less

Pages 264-277 | Received 07 Oct 2015, Accepted 05 Nov 2015, Accepted author version posted online: 14 Dec 2015, Published online: 08 Feb 2016

Download citation https://doi.org/10.1080/19420862.2015.1118596 Check for updates

Model Antigen: Human Progranulin



OmniChicken Recapitulates WT Antigen Recognition

SIMILAR PROFILES SEEN FROM CHICKENS PRODUCING HUMAN SEQUENCE ANTIBODIES

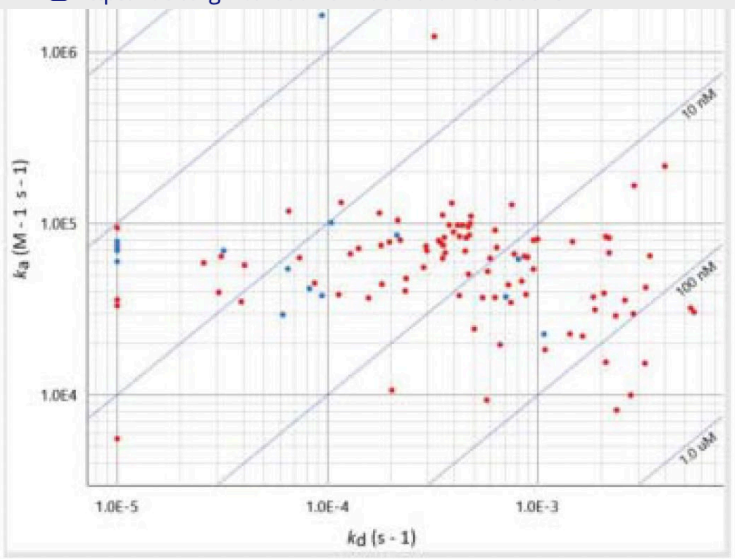
Report

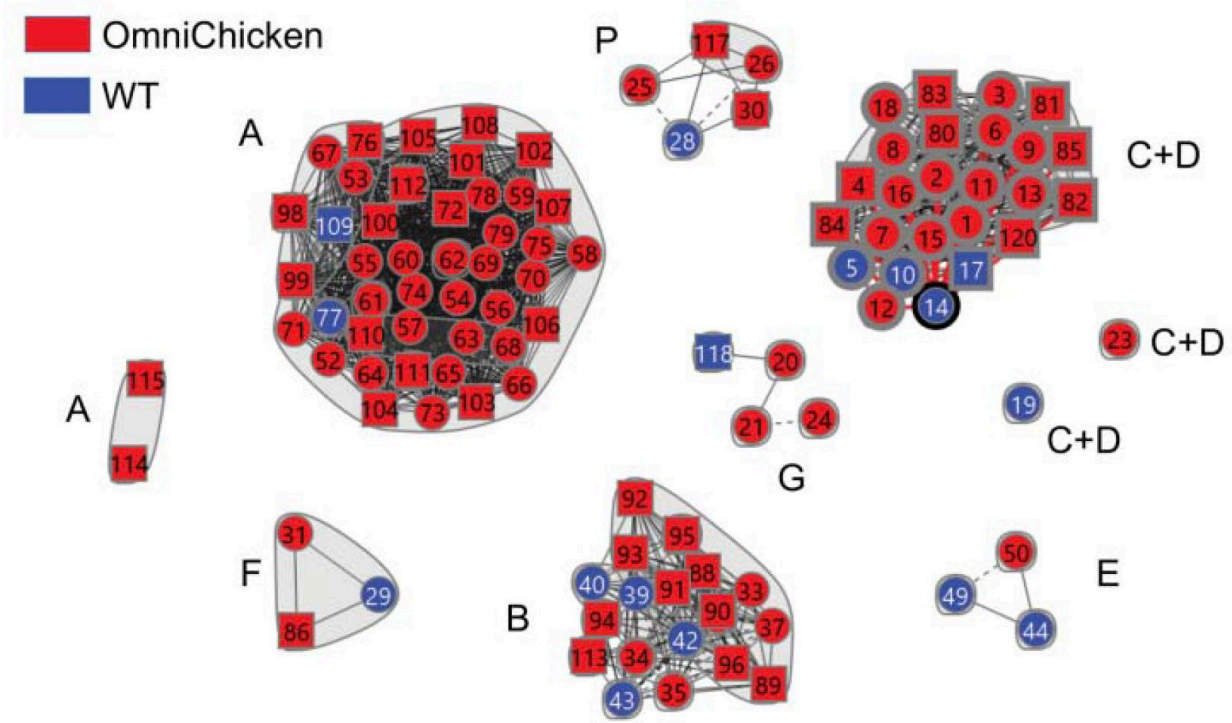
Chickens with humanized immunoglobulin genes generate antibodies with high affinity and broad epitope coverage to conserved targets

Kathryn H. Ching, Ellen J. Collarini, Yasmina N. Abdiche, Daniel Bedinger, Darlene Pedersen, Shelley Izquierdo, Rian Harriman, Lei Zhu, Robert J. Etches, Marie-Cecile van de Lavoir, William D. Harriman & **Philip A. Leighton** ...show less

Pages 71-80 | Received 07 Aug 2017, Accepted 26 Sep 2017, Accepted author version posted online: 16 Oct 2017, Published online: 02 Nov 2017

Download citation https://doi.org/10.1080/19420862.2017.1386825 Check for updates

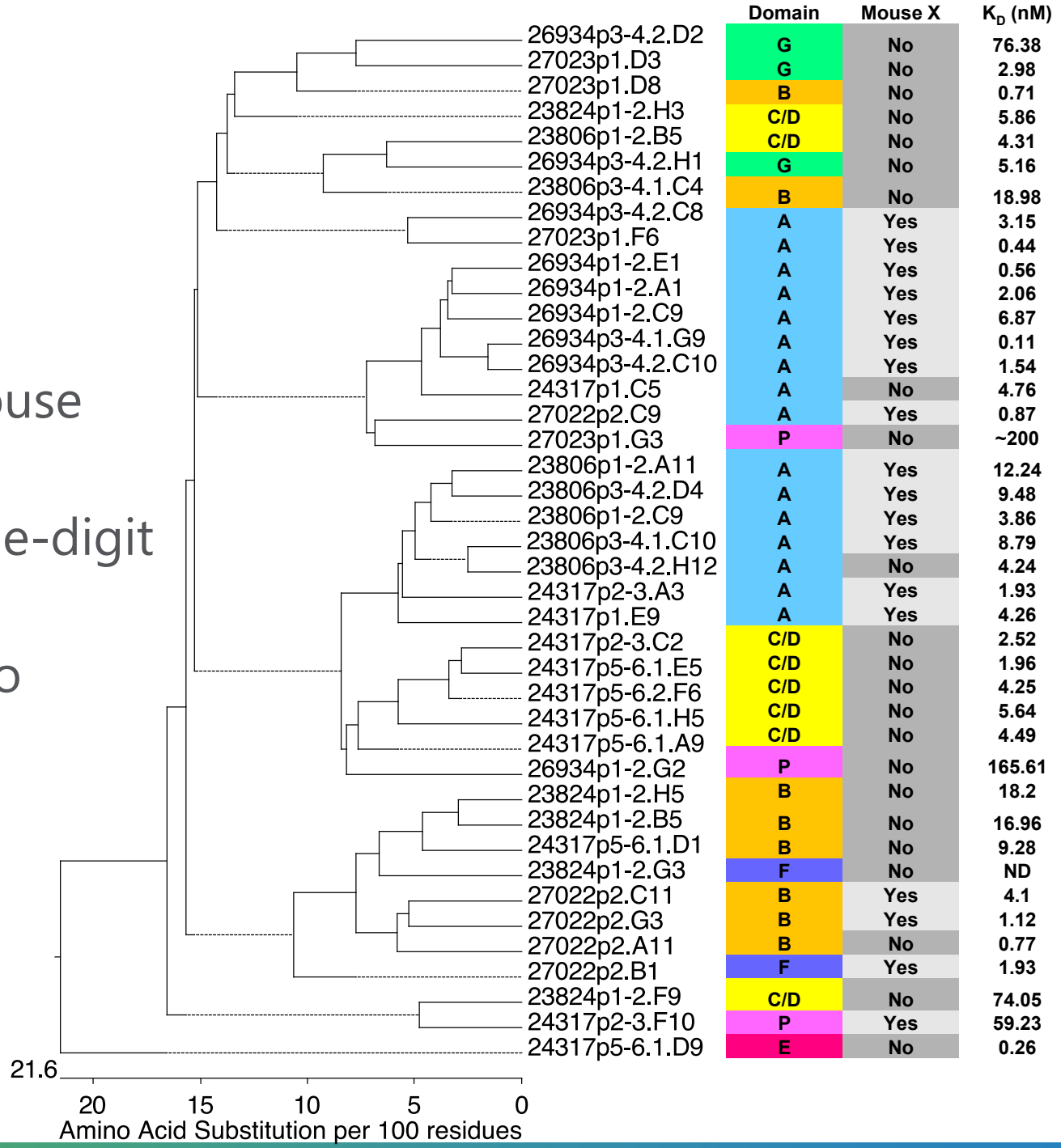




OmniAb

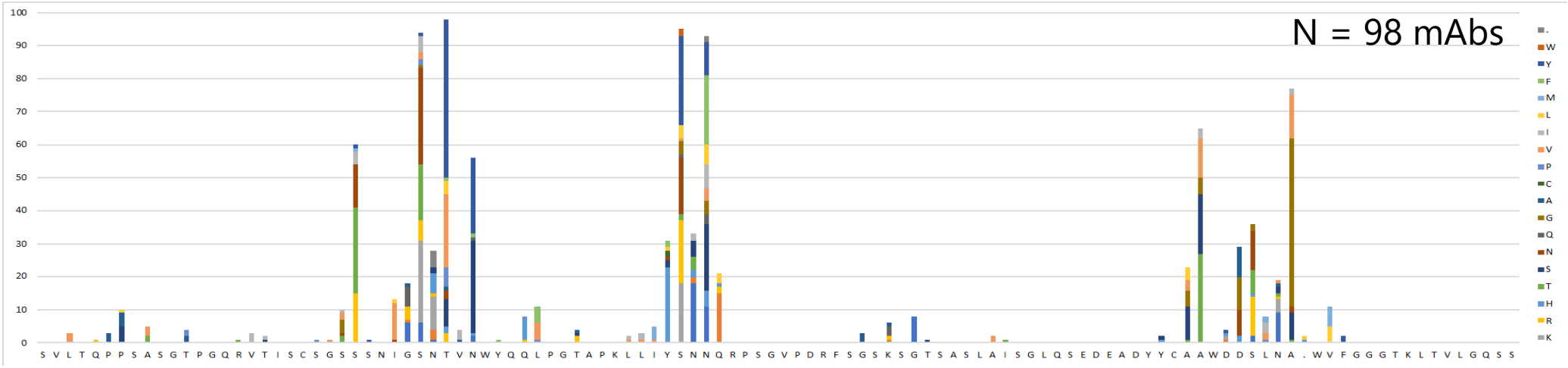
OmniChicken/PGRN

- Clones to all subdomains identified
- Over half of clones are mouse cross-reactive
- Affinities generally in single-digit nM or better range
- Phenotypic clusters map to sequence clusters

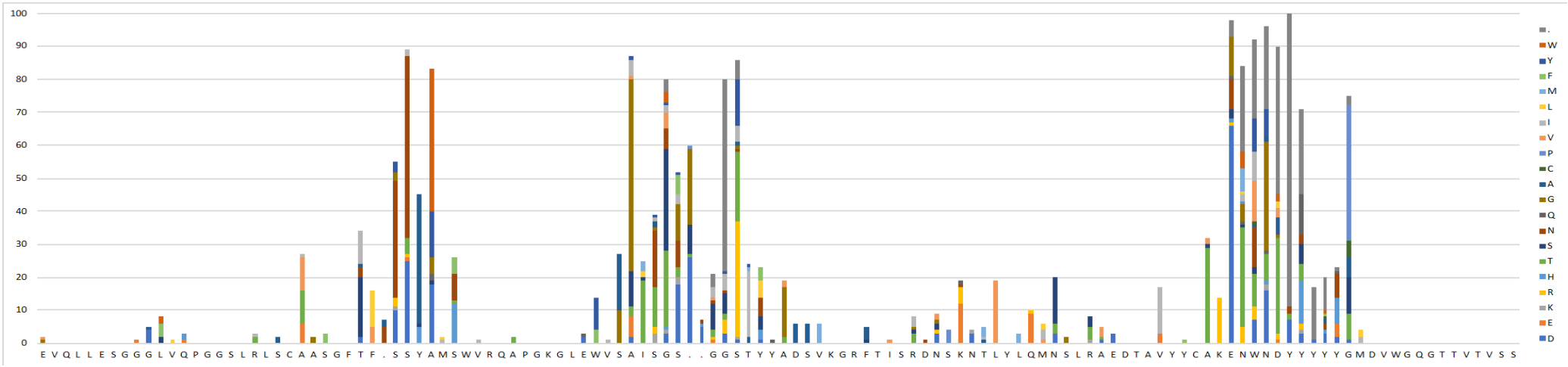


Sequence Diversity of PGRN mAbs from OmniChicken

VL



VH



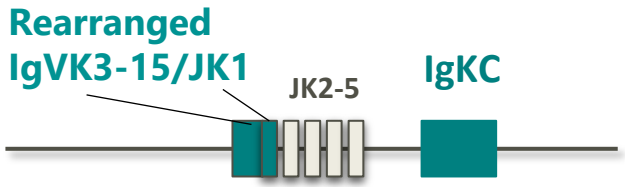
Diversity is principally focused in the CDR regions of human VH and VL;
a result of both transgene design and cellular selection

OmniAb®

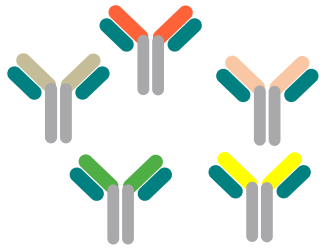
Common Light Chain Platforms

STANDARD IGG FORMAT TO DE-RISK DOWNSTREAM DEVELOPMENT[1] OF BISPECIFIC MABS

OmniFlic

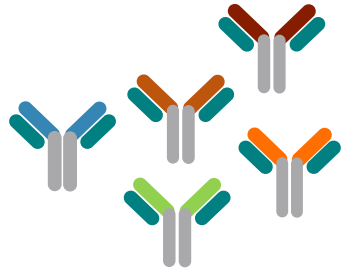


Rearranged human VK3-15 light chain combined with diversifying heavy chain



OmniClic®

"Germlining" human VK3-15 light chain combined with diversifying heavy chain



Monospecific IgG

Simple reformatting from monospecific into bispecific for efficient production

Bispecific IgG

Common light chain for OmniFlic® and OmniClic® allows interchangeability between the platforms

†The Evolution of Bispecifc Antibodies, Nimish Gera
https://doi.org/10.1080/14712598.2022.2040987

OmniAb®

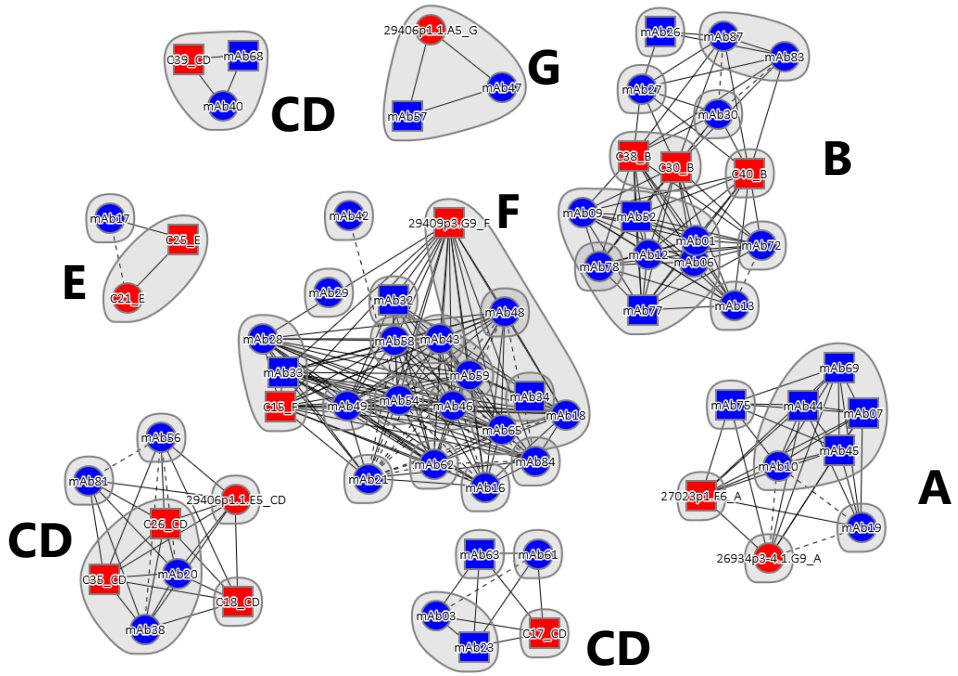
Broad Epitope Coverage Maintained in OmniClic

Report

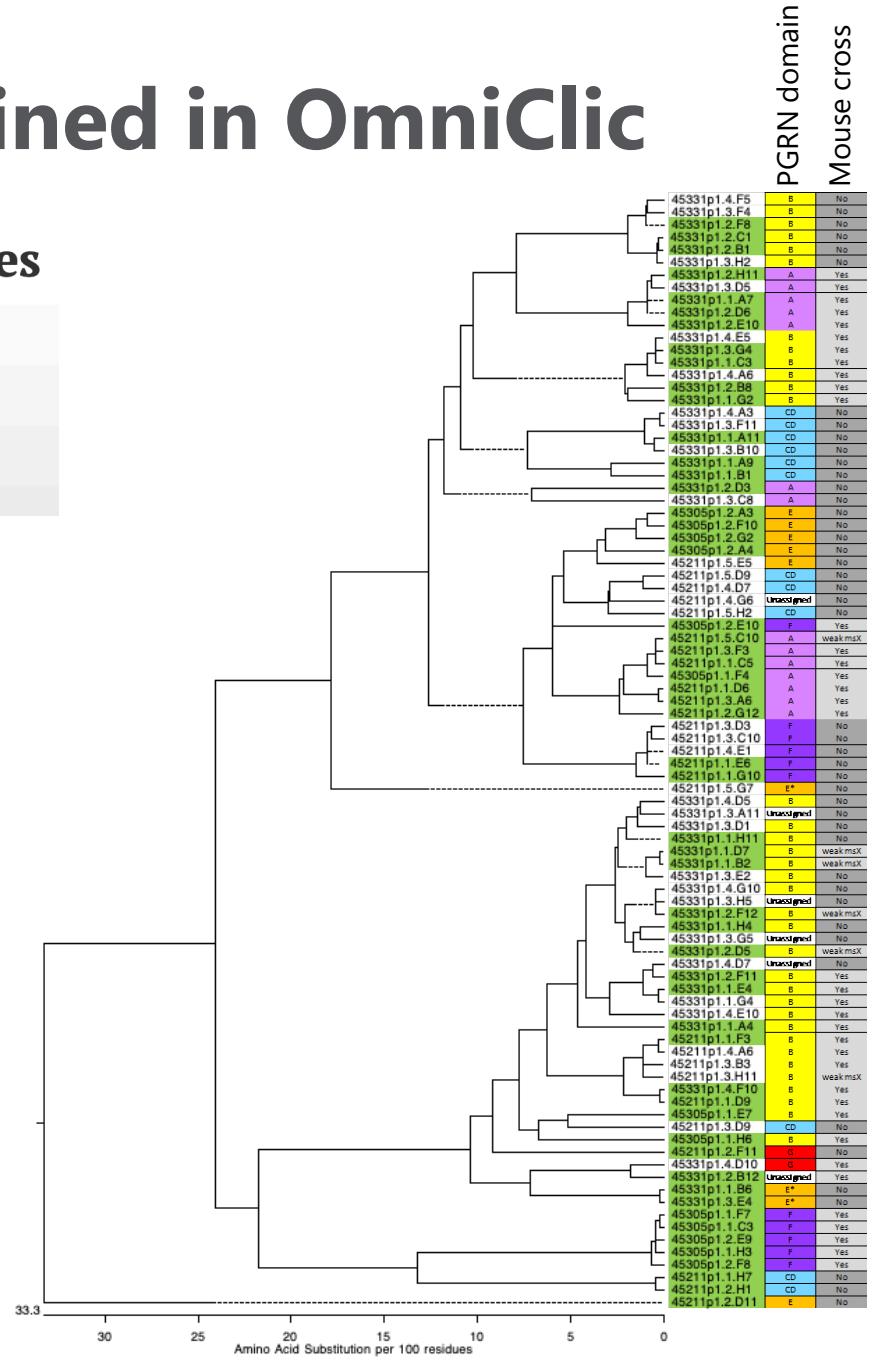
Common light chain chickens produce human antibodies of high affinity and broad epitope coverage for the engineering of bispecifics

Kathryn H. Ching, Kimberley Berg, Kevin Reynolds, Darlene Pedersen, Alba Macias, Yasmina N. Abdiche, William D. Harriman & Philip A. Leighton ...show less

Article: 1862451 | Received 04 Sep 2020, Accepted 07 Dec 2020, Published online: 25 Jan 2021



53 OmniClic mAbs
16 mAb standards

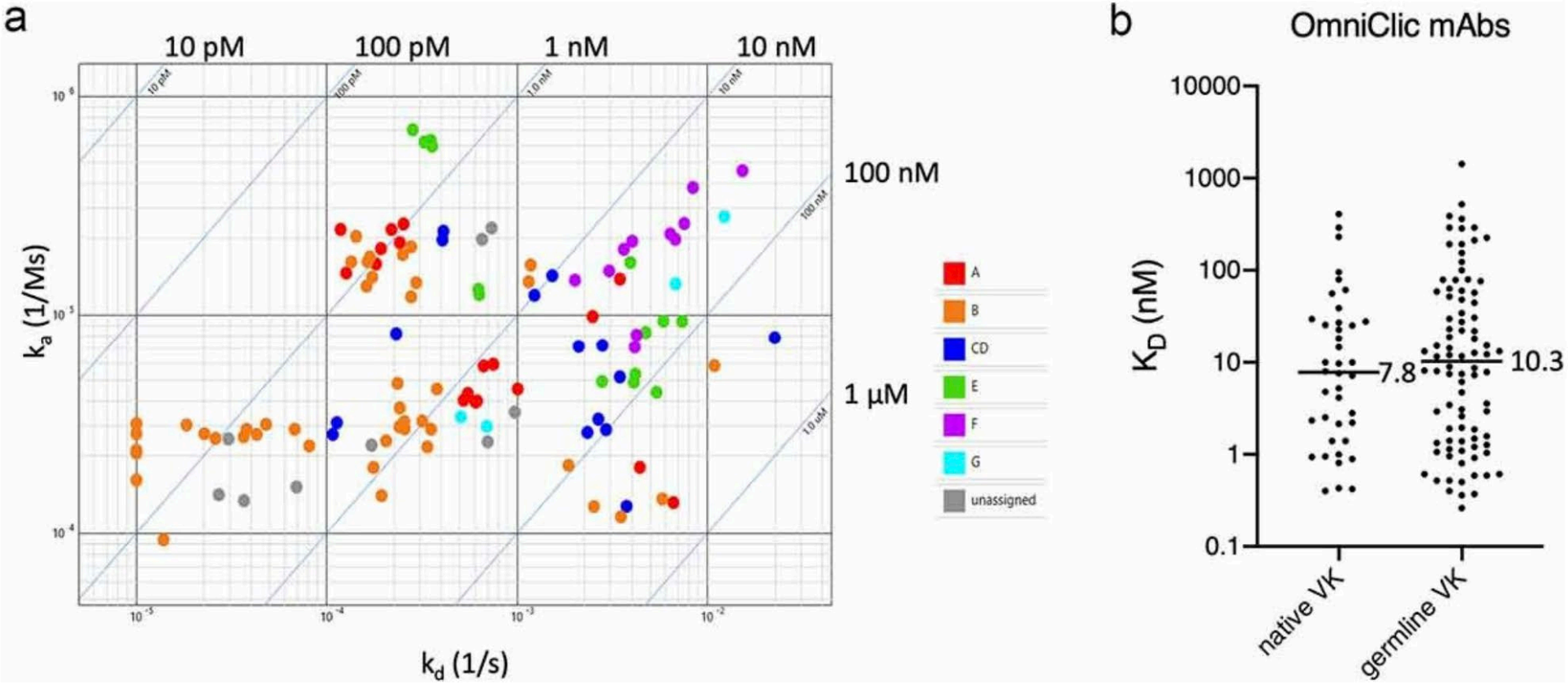


=germline light chain

OmniAb

Broad Kinetic Diversity in OmniClic

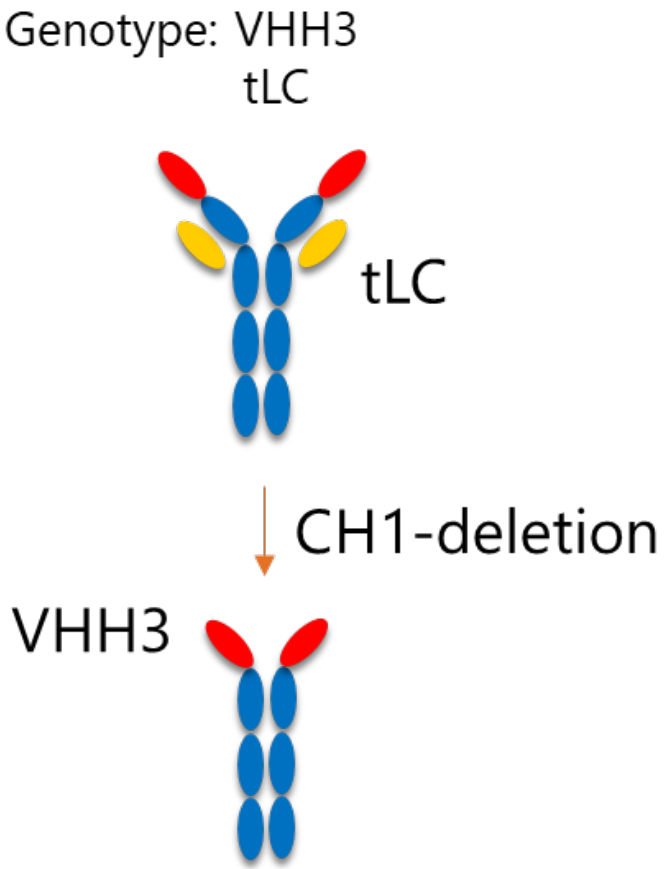
SUB-NANOMOLAR AFFINITIES ACHIEVABLE



Omni*dAb*™: Tg Chickens Expressing Human VHH

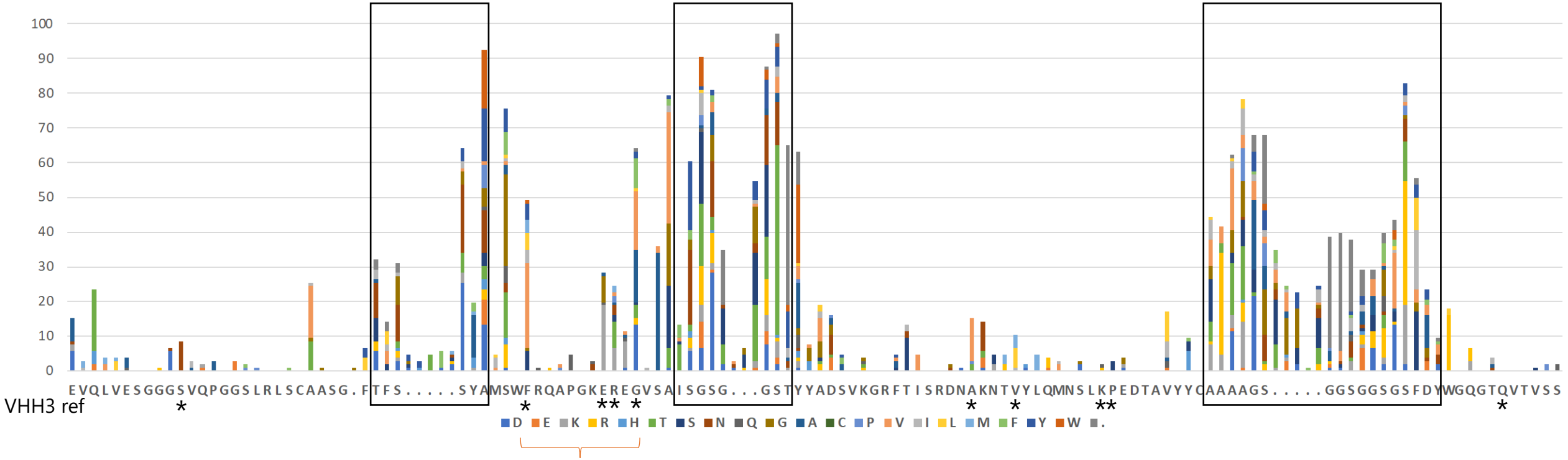
HUMAN VH3-23 WITH 10 CAMELIZING MUTATIONS IN THE FRAMEWORKS

- Birds express VHH3 but no VL
- Spliced to genetically WT heavy chain constant region
- Spontaneous CH1 deletion



Mutational Levels in Cloned dAbs

DATA FROM CONFIRMED PGRN, TIGIT AND NKP46 CLONES. N = 107



More change of FR2 camelizing mutations than others, in particular V37F and W47G

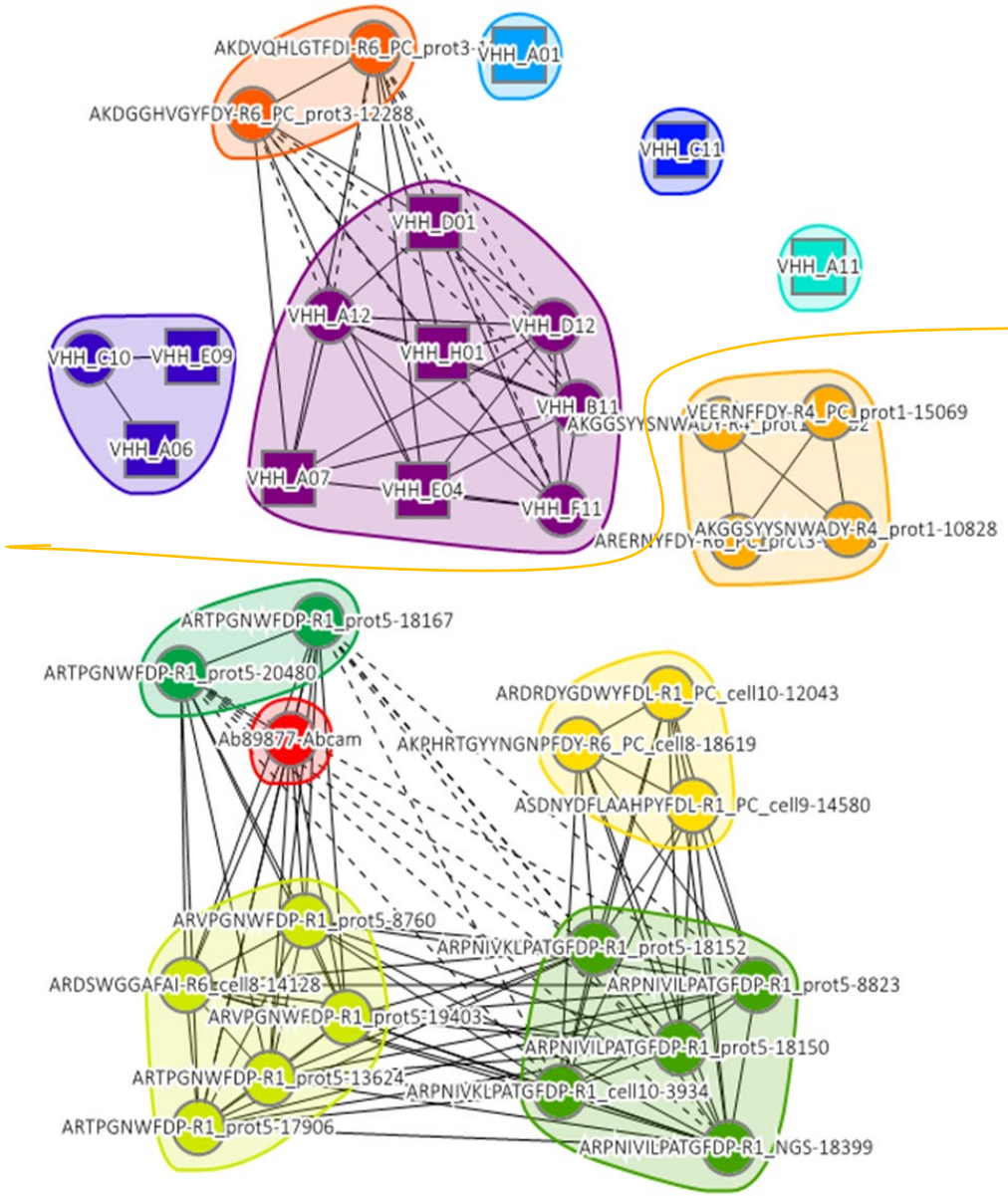
* Camelizing mutations

OmniAb

Epitope Binning – NKp46

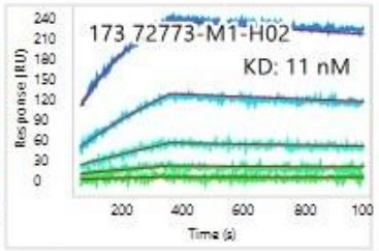
Omni***dAb*** VHH and OmniFlic binning on Carterra LSA

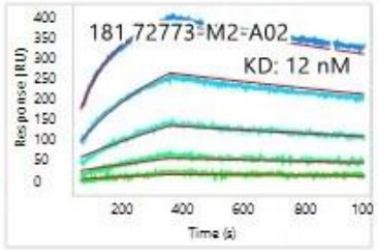
VHH bins are independent from those found in OmniFlic rat

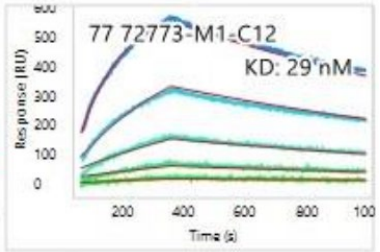


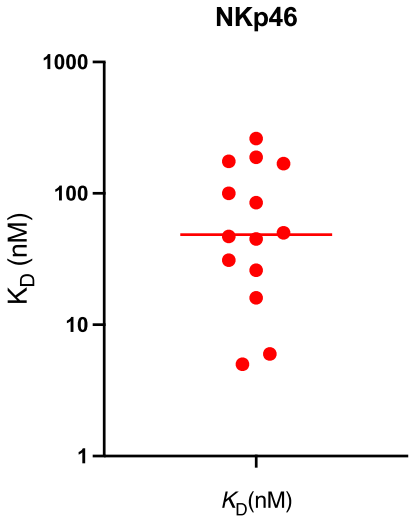
Omni*dAb* Kinetics

RANGES OBSERVED ON TWO TARGETS

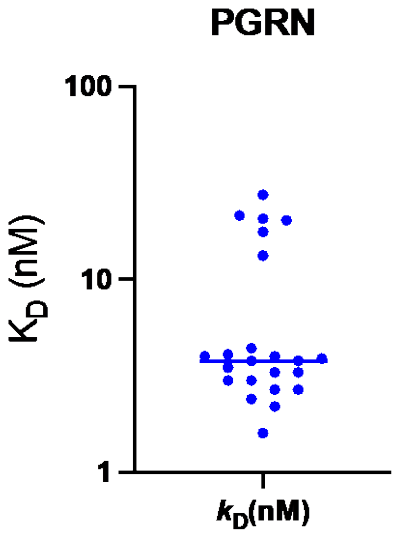




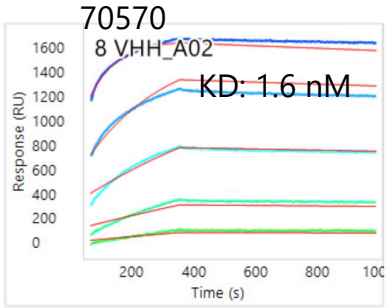


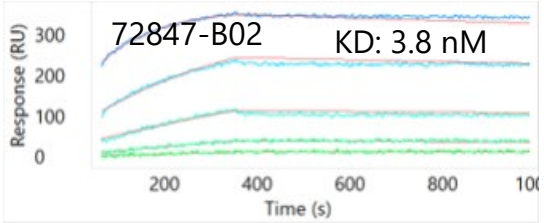


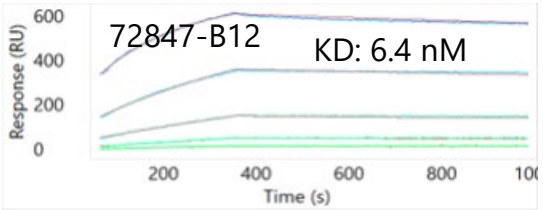
Number of clones	14
Minimum	5 nM
Maximum	261 nM
Mean	86 nM



Number of clones	23
Minimum	1.6 nM
Maximum	28 nM
Mean	8.7 nM







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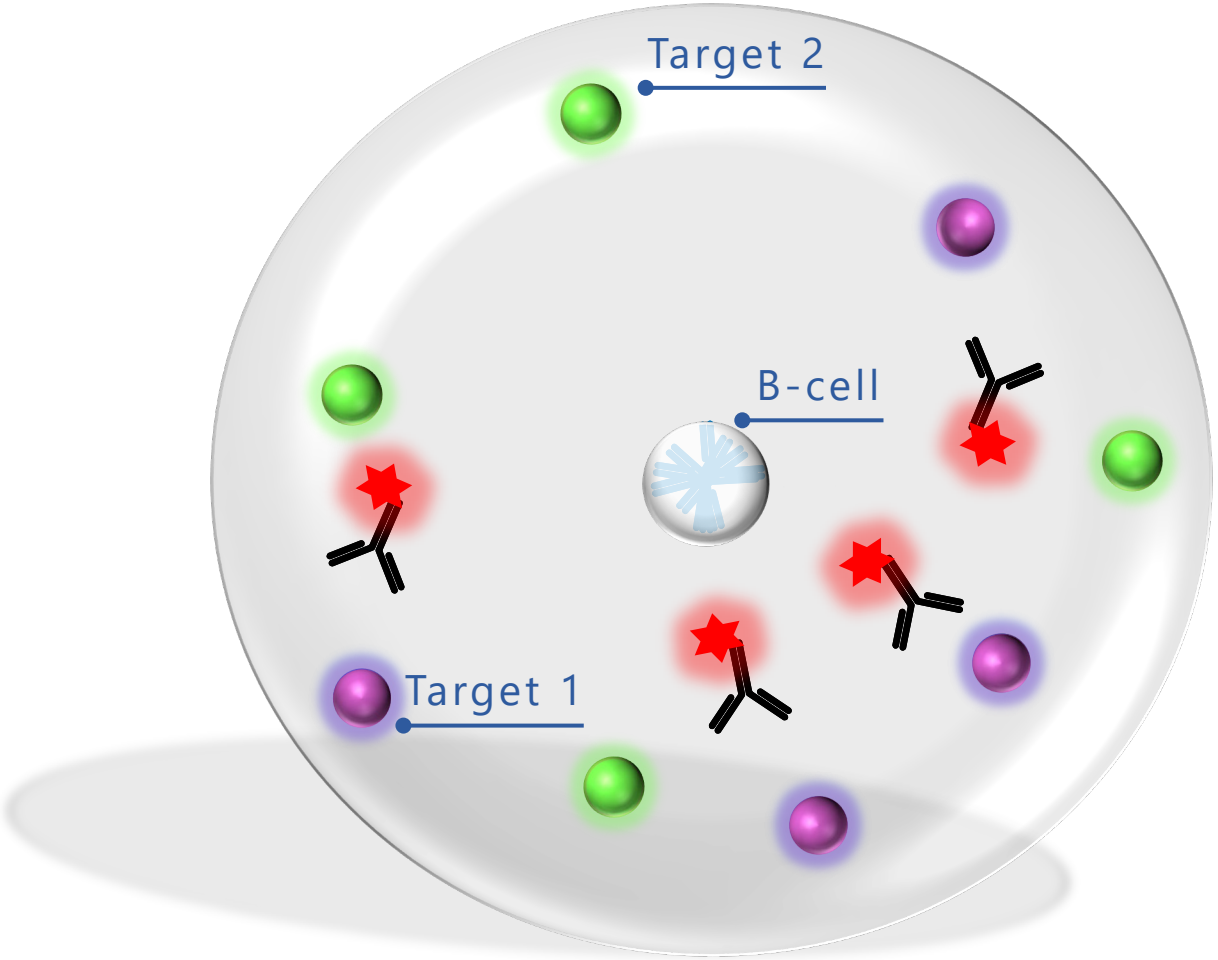
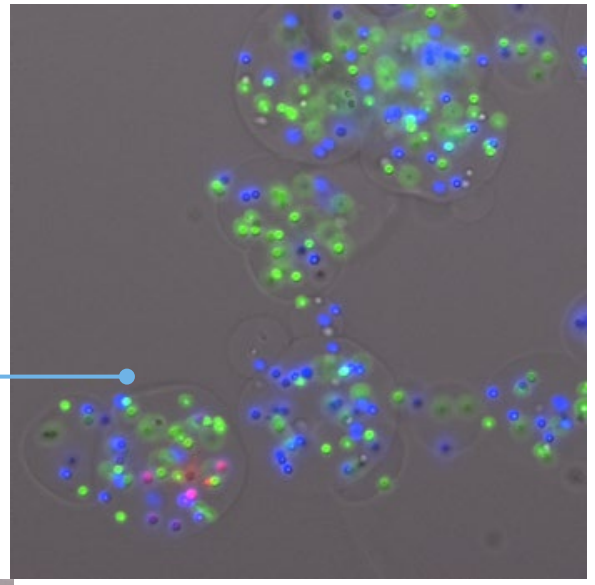
Screening and Workflows

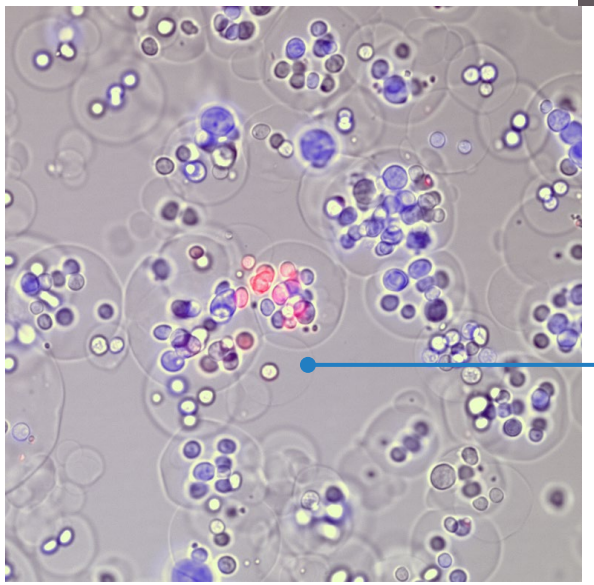
Screening Technology: GEM Assay

GEL ENCAPSULATED MICROENVIRONMENT



Dual bead GEM





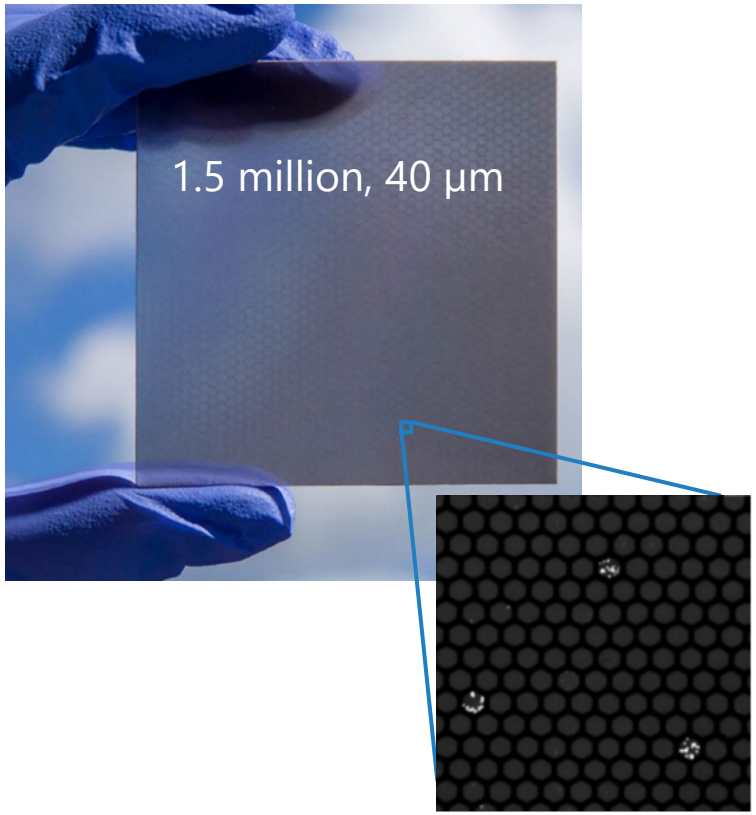
Cell-based GEM

Izquierdo et al. Microscopy, 2016, 1-12

OmniAb

Screening Technology: xPloration®

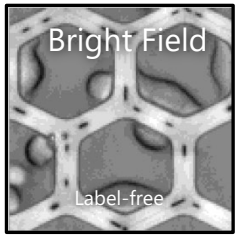
1 | Loading

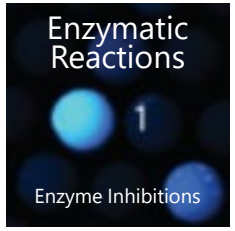


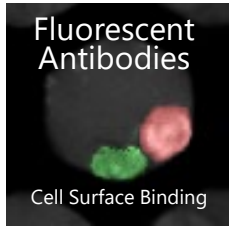
Unique through-hole format

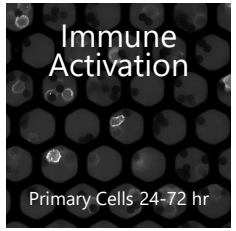
Workflows for OmniAb B-cells

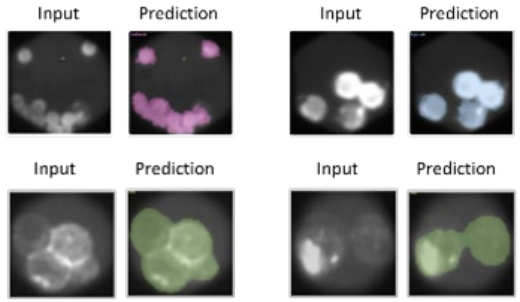
2 | Assay + Machine Vision





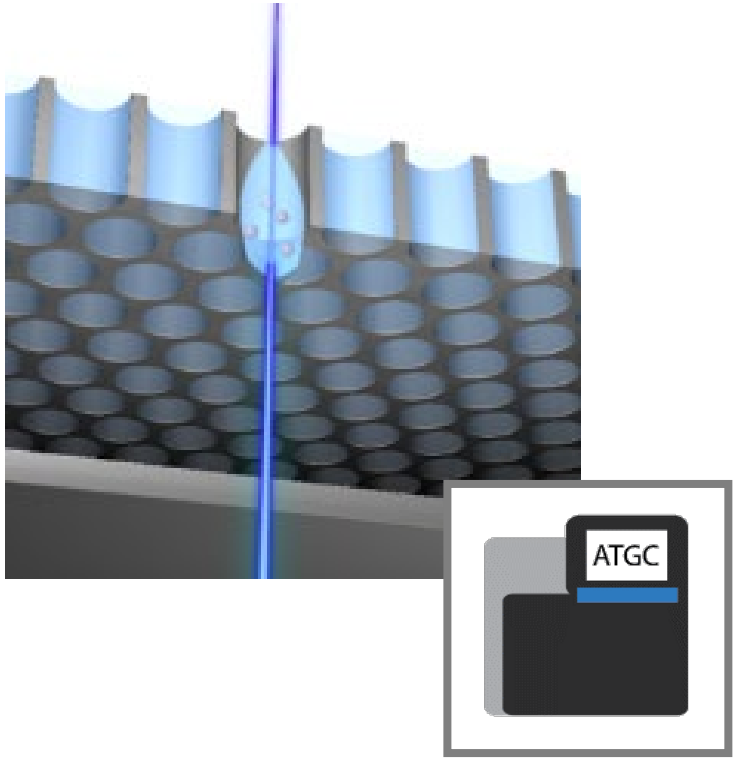






AI-driven hit detection

3 | Recovery & Single-Cell NGS



Precise laser-based recovery

1 cell/sec (single-cell mode), single-cell barcoding

scFv-Fc Cloning

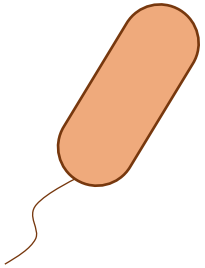
POSITIVE HITS FROM B CELL SCREENING ARE CLONED INTO SCFV-FC

Sorted positive binding cells

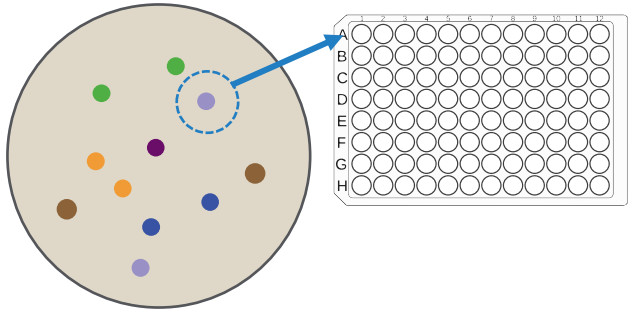
RT and PCR to assemble scFv-Fc

Native pairing
is retained

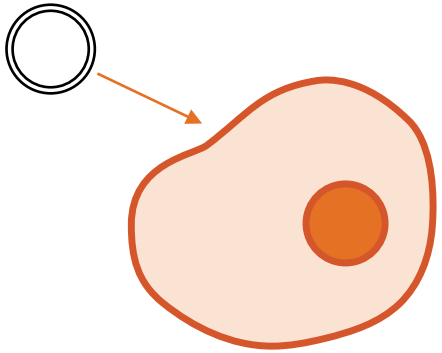
Pooled transformation



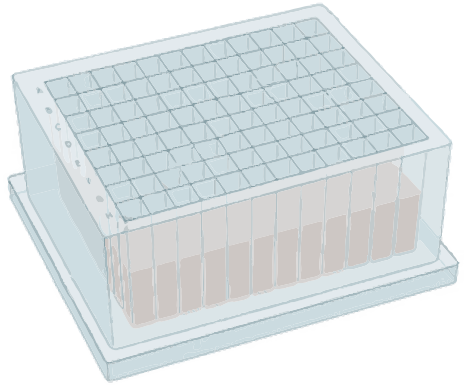
Colony picking



HT mini-prep and transfection



HT expression



OmniAb

Single-cell Processing Using Barcoding

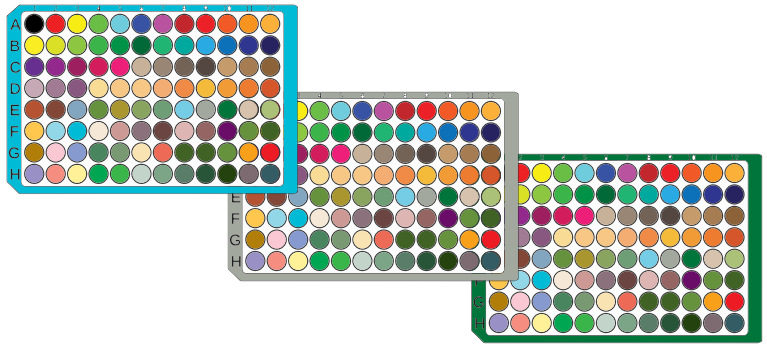
RT

Well barcoding

96 barcodes

Plate barcoding

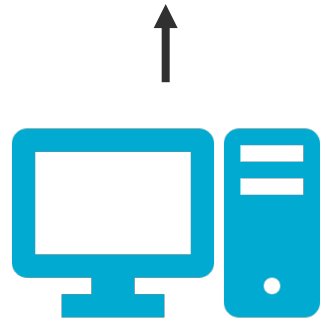
96 plate indexes



2 x 300bp Illumina MiSeq

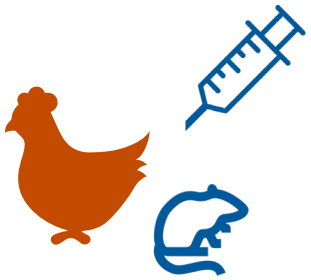
Paired heavy & light chain sequences

Target: >70% of paired sequences

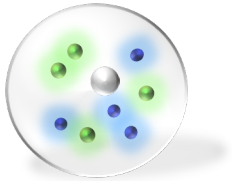


OmniAb

OmniAb Antibody Discovery Workflow



Immunization

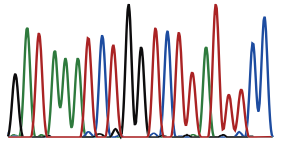


GEM

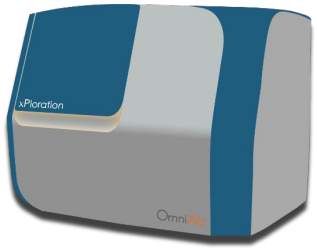
Cloning

Expression

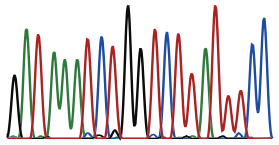
Assay Validation



Sequencing



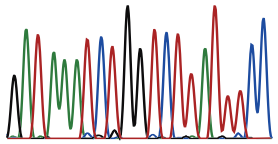
xPloration



Sequencing

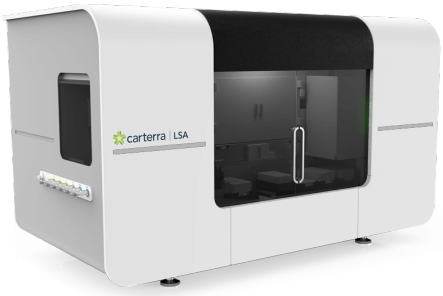
Expression

NGS Hit Expansion



Sequencing

Expression



Advanced Characterization

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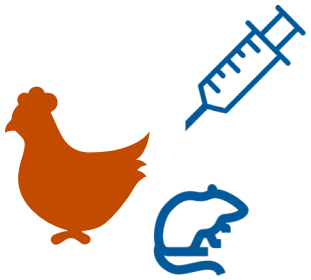
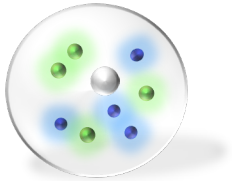
OmniAb Antibody Discovery Workflow

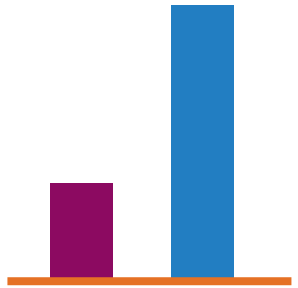


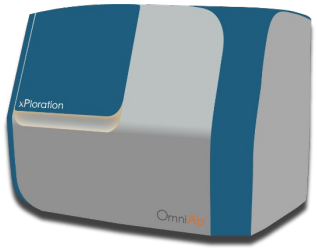
Immunization



GEM

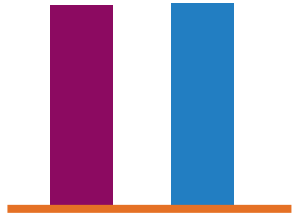
- Well validated, confirmed scFv-Fc binders
- ~100 natively paired sequences per program





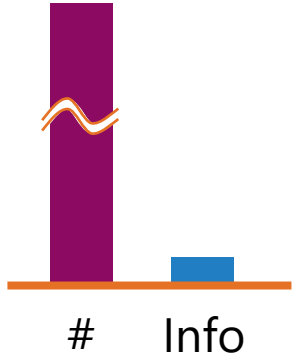
xPloration

- ~1000 natively paired sequences per program that passed functional screening
- Optional: ~100 confirmed binders



NGS Hit Expansion

- >10^6 VH only sequences from immunized animals
- Background sequences + relevant binding sequences



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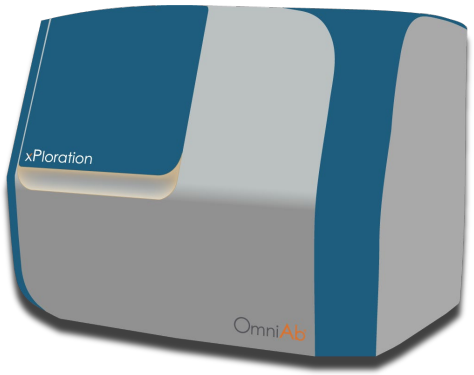
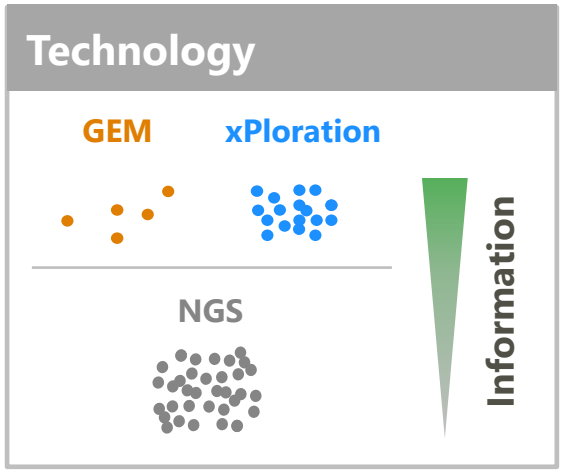
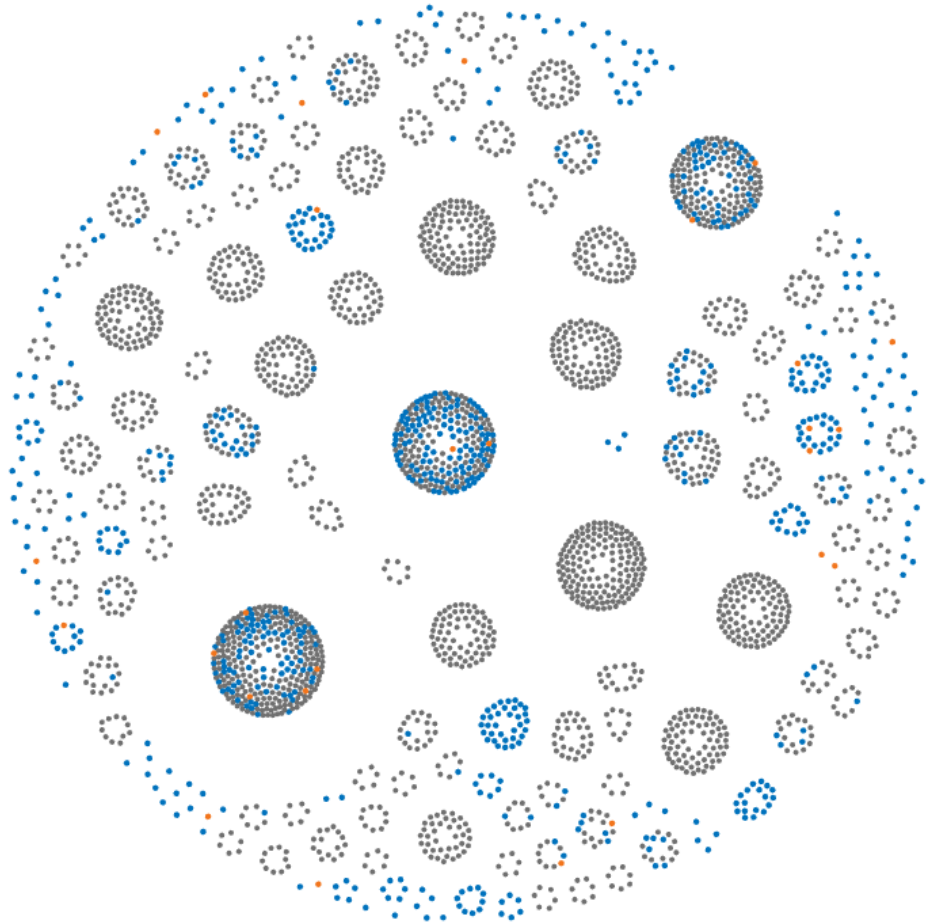
Deeper Sequence Analysis Identifies New Families





Each dot represents an antibody



- Multiple new clonotype families identified when overlaying data from various sources
- Next-generation sequencing (NGS) adds support to new clusters and reveals even more diversity
- Bioinformatics approach includes liability assessment, homology modeling, structure-based optimization

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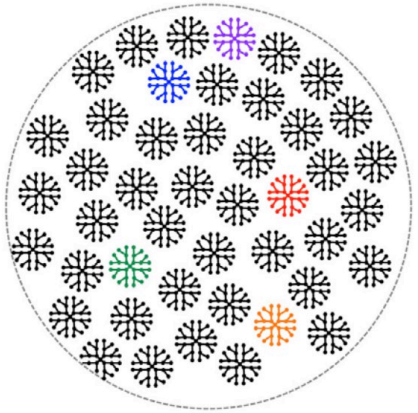
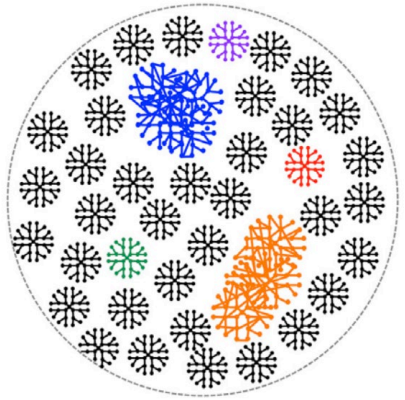
Platforms to Generate Custom Antibody Repertoires

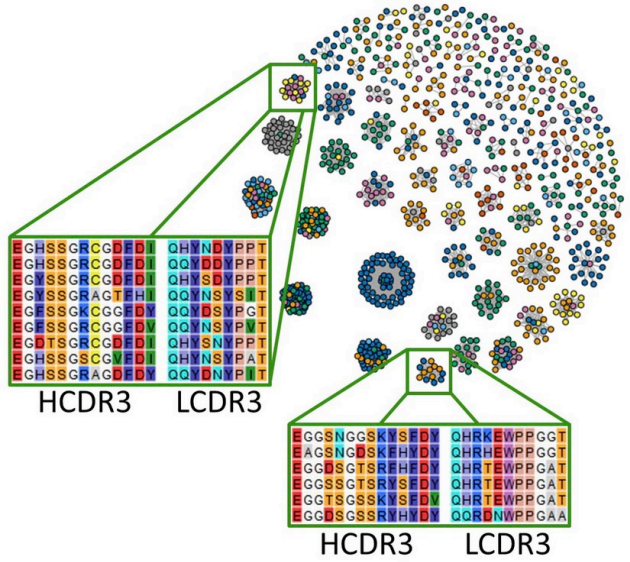
BIOLOGICAL INTELLIGENCE™: INTERPLAY BETWEEN RATIONAL GENETIC DESIGN AND POWERFUL IN VIVO PROCESSES

Naïve repertoire



Immune repertoire





Building the Animal System
V gene building blocks
Structural attributes
Diversification architecture
Transgene design
Immunological robustness

Repertoire Shaping
Antigen design
Host immune recognition
Immunization protocols
Campaign strategy
Immune response monitoring

Repertoire Mining
Phenotypic screening
Clonal sampling/sequencing
Antibody characterization
Clone & Repertoire ranking
Selective "Deep Dives"
NGS hit expansion

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Acknowledgements

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Gerry Rivera
Gonzalo Reyes
Devendra Srivastava

Confidential