OptiMAL®: Mammalian Display for Antibody Discovery

Olivier Touzelet, Lorna Stewart, Georgia Pentland, Conor Breen, Sean Tierney, Nicola Hamilton, Brendan Clarke, Chris King, Chris Murray, Richard Ma, Simon Larsen, Adrian Kinkaid and Richard Buick[1].

[1.]*Fusion Antibodies plc., Belfast, Northern Ireland.* richard.buick@fusionantibodies.com www.fusionantibodies.com

Introduction

OptiMAL® is a novel mammalian display platform that Fusion Antibodies plc has developed for the enrichment, sorting and selection of full-length human antibodies from a proprietary synthetic sequence library.

Benefits of OptiMAL®

- Library design is based on the **natural human antibody repertoire** with CDR and FR somatic hypermutations.
- **Full-length IgG** eliminates the need to convert antibody fragments into IgG.
- Mammalian display selects for **developable antibodies** (Fig.1).
- **Large functional library size** enabled by very high viral transduction efficiency.
- Multiple benefits over non-mammalian display platforms[1,2].
- **Negative depletion** removes polyreactive antibodies.
- Screening in the final IgG format increases **downstream success rate**.

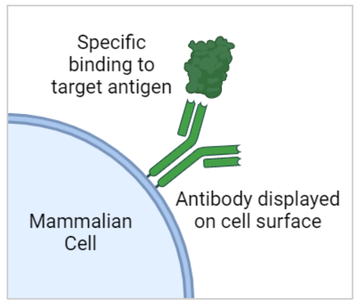


Fig 1. Mammalian cell displaying a full-length IgG that is binding specifically to a target antigen

Current findings

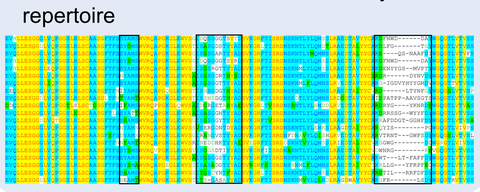
Fusion Antibodies have used the OptiMAL® mammalian display process successfully to enrich for antigen-specific antibodies with high affinity (**single-digit nanomolar**) from populations comprising up to **1x10⁹ cells**.

OptiMAL® Library Design*

Human Germline Frameworks with natural SHM

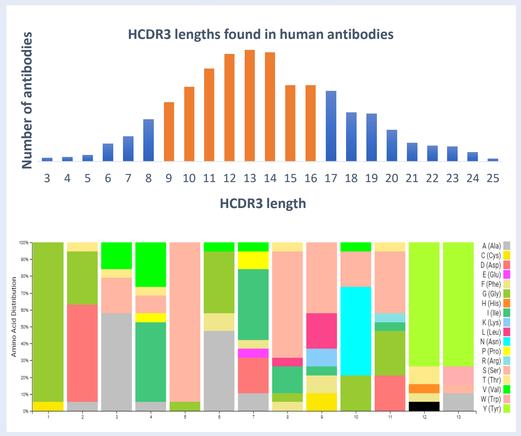
- **Step 1**: V-gene frameworks selected
  - Heavy Genes **IGHV3-23** and **IGHV1-46**
  - Light Genes **IGKV1-39** and **IGKV3-20**
- **Step 2**: Apply natural somatic hypermutation throughout V-gene (Framework Regions and CDRs)
- **Step 3**: Design CDR3 based on natural frequency distribution of each amino acid at each position within the CDR3.
- **Step 4**: Synthesise and clone the complete heavy chains and light chains

- **Result**: A library of antibodies that closely resembles the natural human antibody repertoire



Heavy Chain CDR3 Design

Our libraries contain multiple heavy CDR3 lengths



Each position within every HCDR3 length is designed according to the most frequently observed amino acids in human antibodies

Fig 2. Design of OptiMAL® mammalian display library
Diversity of 3.4x10⁹ plasmids with unique combinations of H+L antibody sequences

Mammalian Display

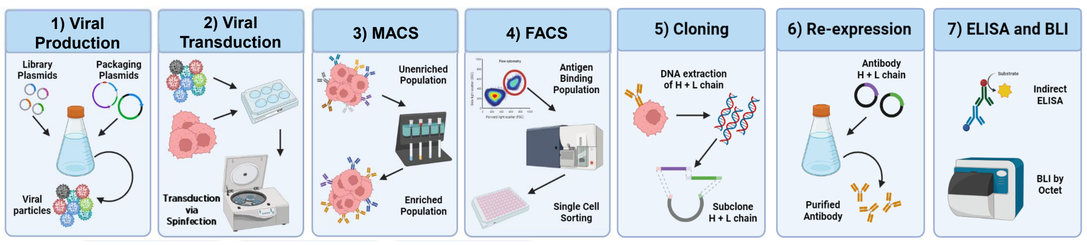
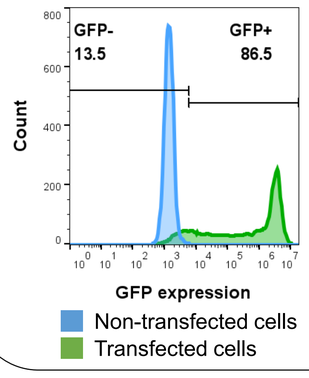


Fig 3. Schematic of OptiMAL® mammalian display platform

Viral Production



Viral Transduction (MOI=3)

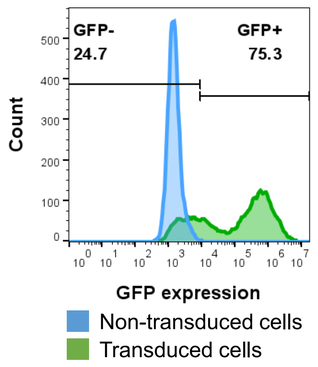


Fig 4. Viral Production HEK293T cells were transfected to produce lentiviral particles containing DNA sequences for different antibody sequences. Successfully transfected cells expressed GFP (48 hpt).

Viral transduction Cells were infected with lentivirus (**MOI=3**) to produce antibodies on the cell surface. Successfully transduced cells expressed GFP (24 hpi).

*Library design and method is protected under patent US12378696

Results

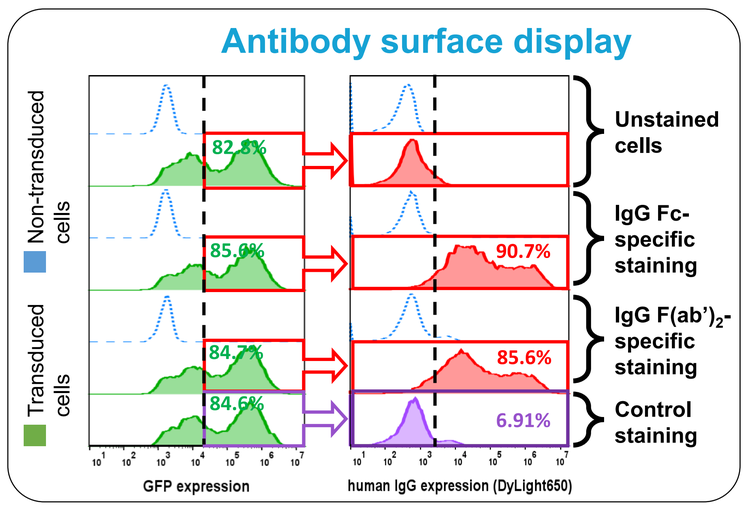


Fig 4. Antibody surface display. Transduced cells were stained with anti-human IgG antibodies (either Fc- or $F(ab')_2$-specific) conjugated to Dyelight650 fluorescent marker. A large proportion of cells (~90%) stained positively for surface display of human IgG Fc or $F(ab')_2$ fragments, as indicated by a high DyeLight650 signal in the GFP+ cell population (transduced cells expressing GFP) compared to non-transduced cells (blue population), unstained cells or cells stained with a negative antibody control (anti-Mouse IgG Fc-specific).

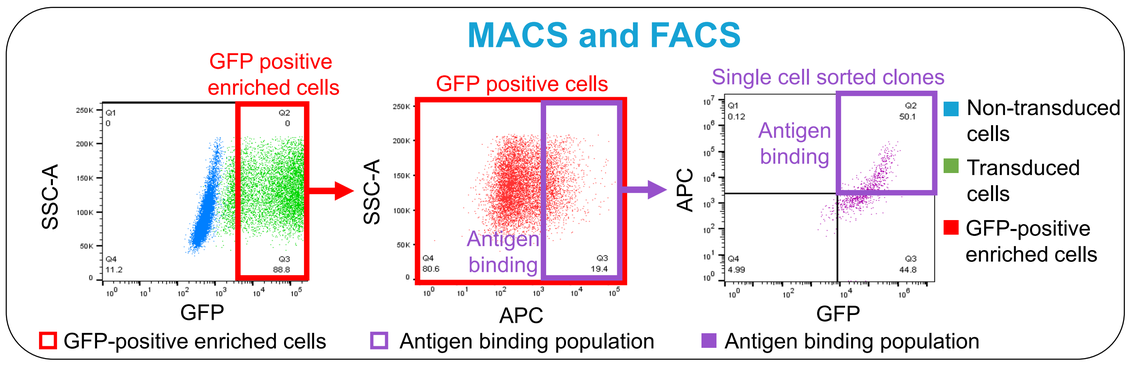


Fig 5. Enriching and selecting for antigen-specific clones. After MACS, an enriched population of GFP positive cells (red gating) was detected by flow cytometry. **GFP positive cells** that displayed binding to target antigen (purple gating) were selected and single-cell sorted by **FACS**. **Single-cell sorted clones** were expanded and validated for antigen binding by flow cytometry (FC). Clones were found to be GFP positive and antigen binding, indicating that the antibody (Ab) displayed on the cell surface was binding to target antigen (Ag).

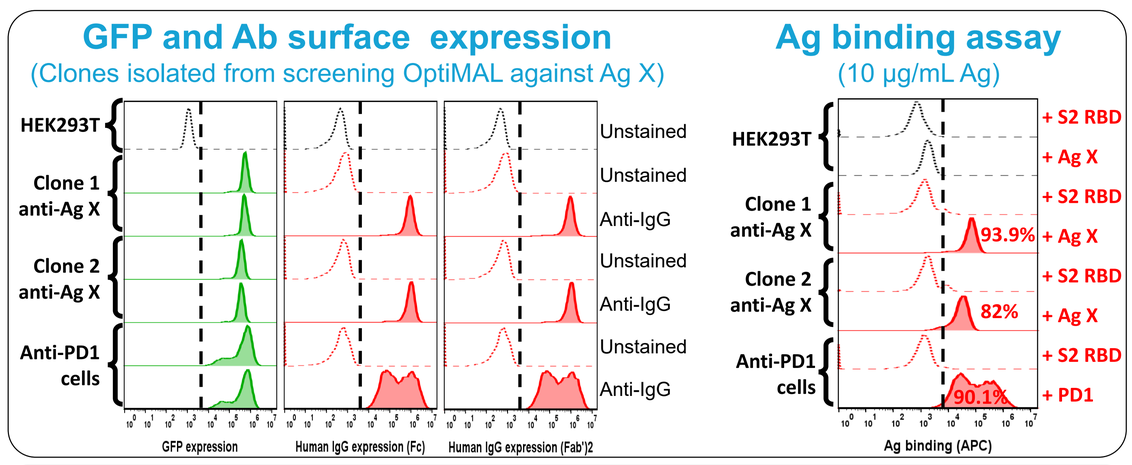
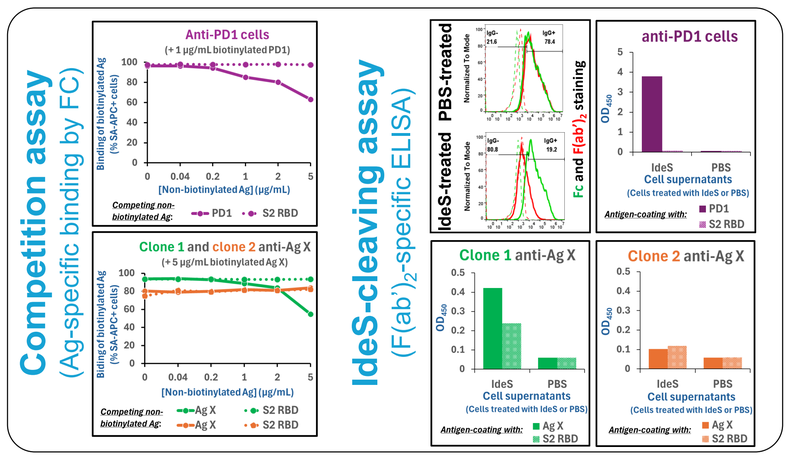


Fig 6. Validation of Ag-specific clones identified through OptiMAL screening. Single cell clones 1 and 2 isolated from screening OptiMAL against antigen X are further validated for **GFP and Ab surface expression,** while their antigen specificity are assessed with an **Ag binding assay.** Clones 1 and 2 are first incubated with biotinylated target (Ag X) or negative (S2-RBD) antigens (10 µg/mL), followed by APC-conjugated streptavidin (SA-APC). HEK293T cells expressing Pembrolizumab (anti-PD1 cells) were used as controls, with PD1 and S2 RBD as target and negative Ag, respectively.



BLI
(binding affinity to Ag)

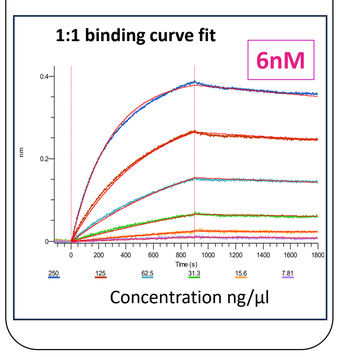


Fig 7. Assessing antibody binding specificity and affinity to target antigen. **Competition assay** Selected cell clones are co-incubated with non-biotinylated (target or negative Ag), and biotinylated target Ag. APC-conjugated streptavidin (SA-APC) positively stains Ag-binding cells, which are detected by flow cytometry (FC). **IdeS-cleaving assay** Clones are treated with IdeS (immunoglobulin-degrading enzyme, Genovis) to cleave $F(ab')_2$ fragments off the cell surface and $F(ab')_2$-specific binding to target Ag is measured by performing an ELISA on cell supernatants, using mouse pAb sera anti-IgG light chain (LC) as secondary detection. PBS-treated cells are used as control and IdeS-induced $F(ab')_2$ cleaving is confirmed by FC with a reduction in $F(ab')_2$-specific fluorescent staining (red) compared to Fc-specific staining (green).

Fig 8. BLI After cloning and sequencing the H+L antibody sequences from the FACS-selected clones, the antibody was expressed by transient gene expression and purified. Purified antibody was tested for binding affinity to target antigen by BLI.
Binding affinity of Ab from the OptiMAL® platform to target Ag was shown to be 6nM.

Conclusions and future work

- The OptiMAL® mammalian display platform has successfully identified binders to a target antigen through primary screening of a synthetic human antibody library.
- The OptiMAL® platform is well positioned for the next stage of this study: screening AI/ML-derived antibody libraries.

Acknowledgements Thank you to the entire Fusion Antibodies team who have been a part of the development of the OptiMAL® mammalian display platform to date. Thank you to Prof. Christopher Scott and the Fusion Antibodies Scientific Advisory Panel for their advice. Figures were created using Biorender.com.

References (1) Valldorf, Bernhard, Hinz, Steffen C., Russo, Giulio, Pekar, Lukas, Mohr, Laura, Klemm, Janina, Doerner, Achim, Krah, Simon, Hust, Michael and Zielonka, Stefan. "Antibody display technologies: selecting the cream of the crop" *Biological Chemistry*, 2022 vol. 403, no. 5-6,, pp. 455-477. (2) Dyson MR, Masters E, Pazeraitis D, Perera RL, Syrjanen JL, Surade S, Thorsteinson N, Parthiban K, Jones PC, Sattar M, Wozniak-Knopp G, Rueker F, Leah R, McCafferty J. Beyond affinity: selection of antibody variants with optimal biophysical properties and reduced immunogenicity from mammalian display libraries. *MAbs*. 2020 Jan-Dec;12(1):1829335