MORAFAB: A PHAGE DISPLAY HUMAN FAB LIBRARY

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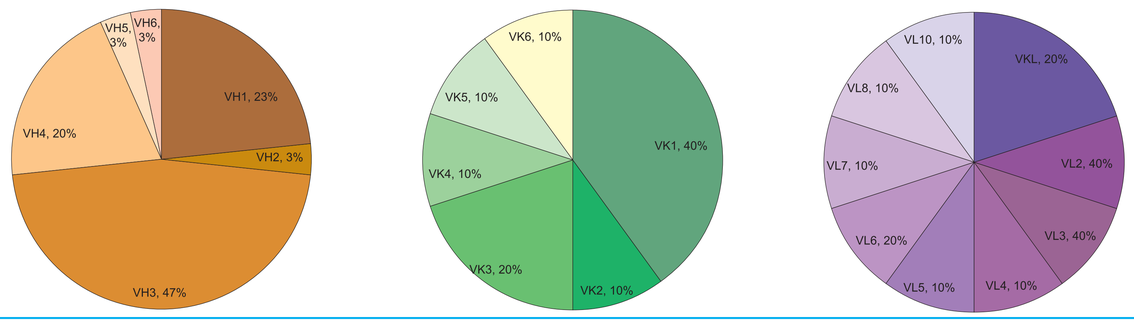
ABSTRACT

Phage display is a powerful tool to screen and select for high affinity binders from a large library. This technique has been successfully applied in the discovery and development of therapeutic antibodies and diagnostic tools. With the intention to deliver a large panel of fully human monoclonal antibodies to our customers, Moradec has designed and constructed a high quality phage-displayed human Fab library (MORAFAB), which comprises approximately 10^9 to 10^{10} unique antibodies. The MORAFAB library is a representation of 30 fully human heavy chain frame \works and 20 light chain (10 kappa and 10 lambda) frameworks, which were pre-selected for their good expression level and high stabilities. The library diversity is further enhanced with the carefully designed heavy and light chain CDR3s, which enables diverse epitope coverage.

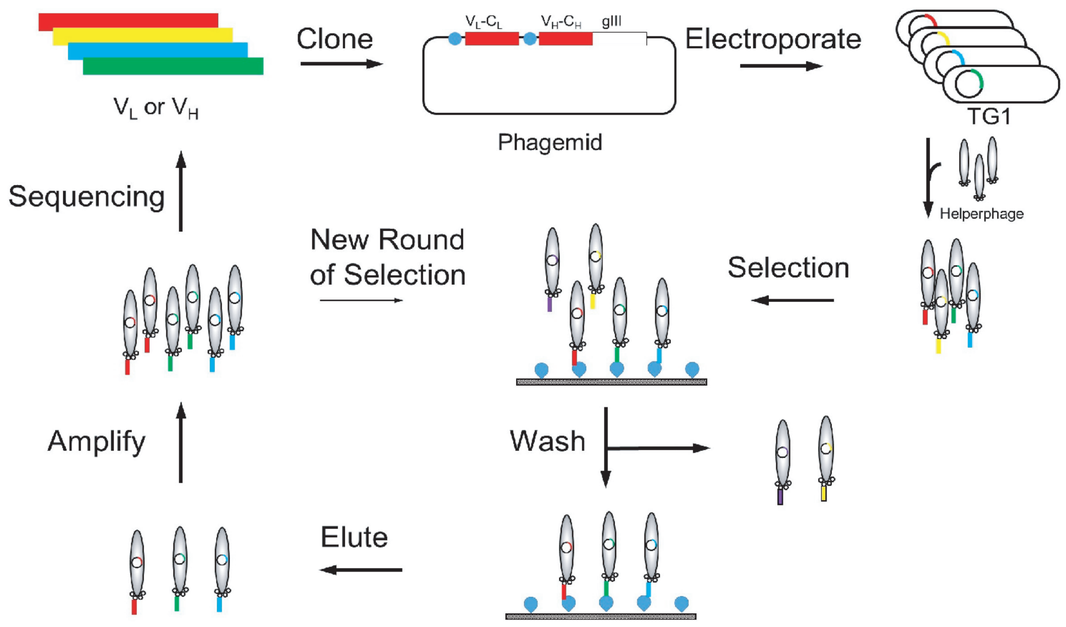
What's MORAFAB?

- High quality phage-display human Fab Library
- Representation of 30 fully human HC frameworks, 20 LC frame works
  - --30 HC frameworks from 6 different HC sub-families
  - --10 Kappa LC frameworks from 6 different LC sub-families
  - --10 Lambda LC frameworks from 9 different LC sub-families
- Diversity resides at heavy and light chain CDR3s--10^9 to 10^{10} unique antibodies
- Easy conversion to IgG without losing binding affinity

Framework Families Used in Constructing the Library



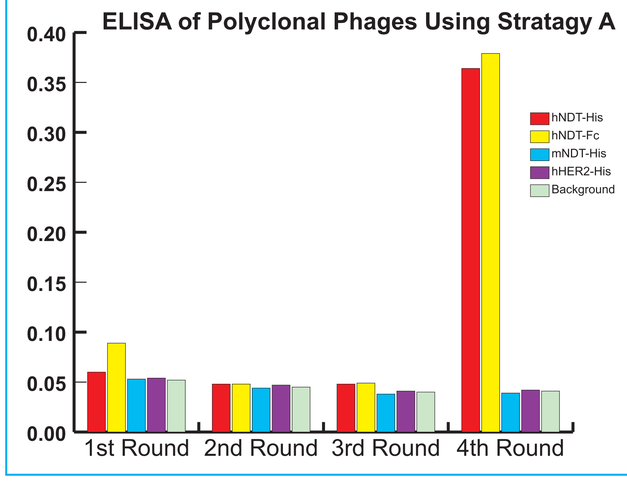
Schematics of Phage Display Selection

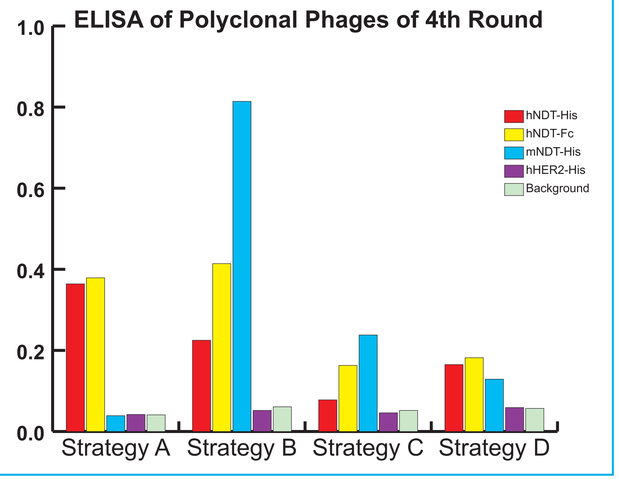


Example of a Selection Campaign

The Non-Disclosed Target (NDT) is a single-pass transmembrane receptor of the immunoglobulin superfamily. Overexpression of NDT, and the interaction between NDT and its ligands can lead to pro-inflammatory gene activation. Inhibition of NDT has been targeted for therapeutic efforts in several inflammatory diseases such as diabetic, psoriasis, Alzheimer's disease, and others. We set out to discover antibody leads against both human and mouse NDT using the MORAFAB phage display library. The human and mouse NDTs share approximately 80% homology. Our goal is to identify antibodies that (1) bind to only human NDT, (2) bind to only mouse NDT, (3) bind to both human and mouse NDTs. We have designed the four selection strategies by panning on different antigens for each round. After four rounds of selection, we observed target specific binding and enrichment on ELISA with the polyclonal phages.

Selection Round	Stratagy A	Stratagy B	Stratagy C	Stratagy D
1st Round	human NDT	mouse NDT	mouse NDT	human NDT
2nd Round	human NDT	mouse NDT	mouse NDT	human NDT
3rd Round	human NDT	mouse NDT	mouse NDT	mouse NDT
4th Round	human NDT	mouse NDT	human NDT	human NDT





After four rounds of phage panning using four different selection strategies, individual bacteria colonies were picked into 96-well blocks to express monoclonal phage. The 96-well block phages were tested for specific binding of either human NDT, or mouse NDT.

Strategy A, Phage ELISA on hNDT

0.03	0.57	0.03	0.53	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03
0.04	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.53	0.03
0.03	0.03	0.55	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.04
0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.03
0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04
0.03	0.03	0.04	0.03	0.03	0.04	0.04	0.03	0.03	0.03	0.17	0.04
0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.04
0.15	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03

Strategy B, Phage ELISA on mNDT

0.03	0.04	0.04	0.29	0.04	0.16	0.03	0.04	0.04	0.03	0.04	0.25
0.04	0.03	0.03	0.38	0.28	0.22	0.34	0.04	0.04	0.26	0.27	0.23
0.04	0.03	0.03	0.03	0.03	0.23	0.04	0.04	0.03	0.03	0.10	0.04
0.03	0.25	0.14	0.26	0.03	0.03	0.03	0.03	0.23	0.03	0.03	0.04
0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.18	0.26	0.04	0.03	0.03
0.04	0.21	0.24	0.22	0.25	0.32	0.22	0.03	0.03	0.31	0.03	0.04
0.24	0.03	0.03	0.03	0.04	0.04	0.30	0.04	0.23	0.18	0.03	0.18
0.11	0.04	0.03	0.15	0.03	0.03	0.07	0.03	0.19	0.15	0.03	0.03

Strategy A, Phage ELISA on mNDT

0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03
0.04	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.04	0.03
0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.04
0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.03
0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04
0.03	0.03	0.04	0.03	0.03	0.04	0.04	0.03	0.03	0.03	0.11	0.04
0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.04
0.10	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03

Strategy B, Phage ELISA on hNDT

0.03	0.03	0.04	0.20	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.04
0.03	0.04	0.04	0.38	0.04	0.04	0.20	0.04	0.04	0.03	0.04	0.04
0.04	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.04
0.03	0.04	0.03	0.04	0.03	0.03	0.04	0.03	0.04	0.03	0.04	0.04
0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.03
0.03	0.06	0.03	0.04	0.04	0.16	0.04	0.04	0.03	0.16	0.03	0.04
0.26	0.03	0.04	0.03	0.03	0.04	0.17	0.03	0.03	0.03	0.03	0.03
0.03	0.04	0.03	0.03	0.04	0.03	0.03	0.03	0.04	0.04	0.04	0.04

Strategy C, Phage ELISA on hNDT

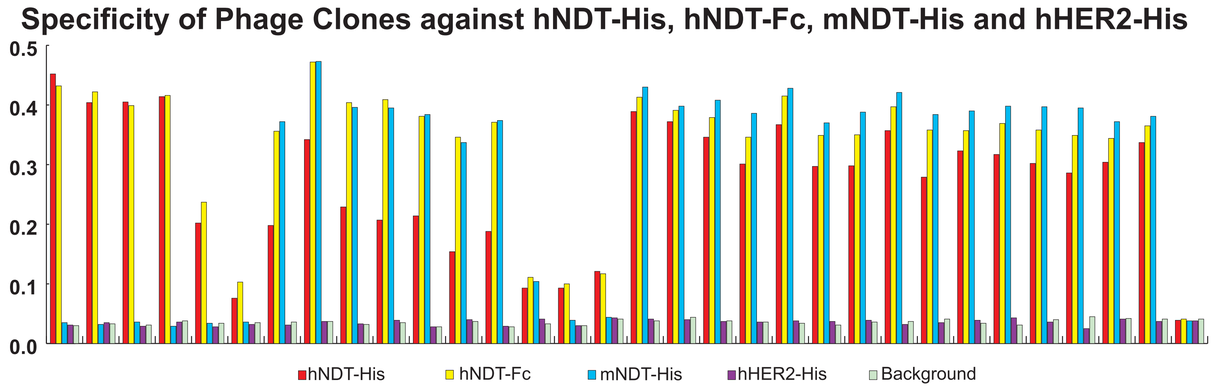
0.03	0.37	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.35
0.40	0.34	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.02	0.04	0.04
0.03	0.03	0.03	0.36	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.03
0.35	0.34	0.03	0.03	0.04	0.03	0.04	0.35	0.31	0.03	0.03	0.03
0.04	0.38	0.03	0.04	0.03	0.03	0.03	0.41	0.03	0.02	0.03	0.02
0.03	0.04	0.04	0.03	0.03	0.38	0.02	0.03	0.03	0.03	0.04	0.03
0.03	0.03	0.03	0.03	0.36	0.04	0.03	0.03	0.03	0.02	0.02	0.03
0.03	0.04	0.03	0.04	0.35	0.03	0.04	0.04	0.05	0.03	0.03	0.45

Strategy D, Phage ELISA on hNDT

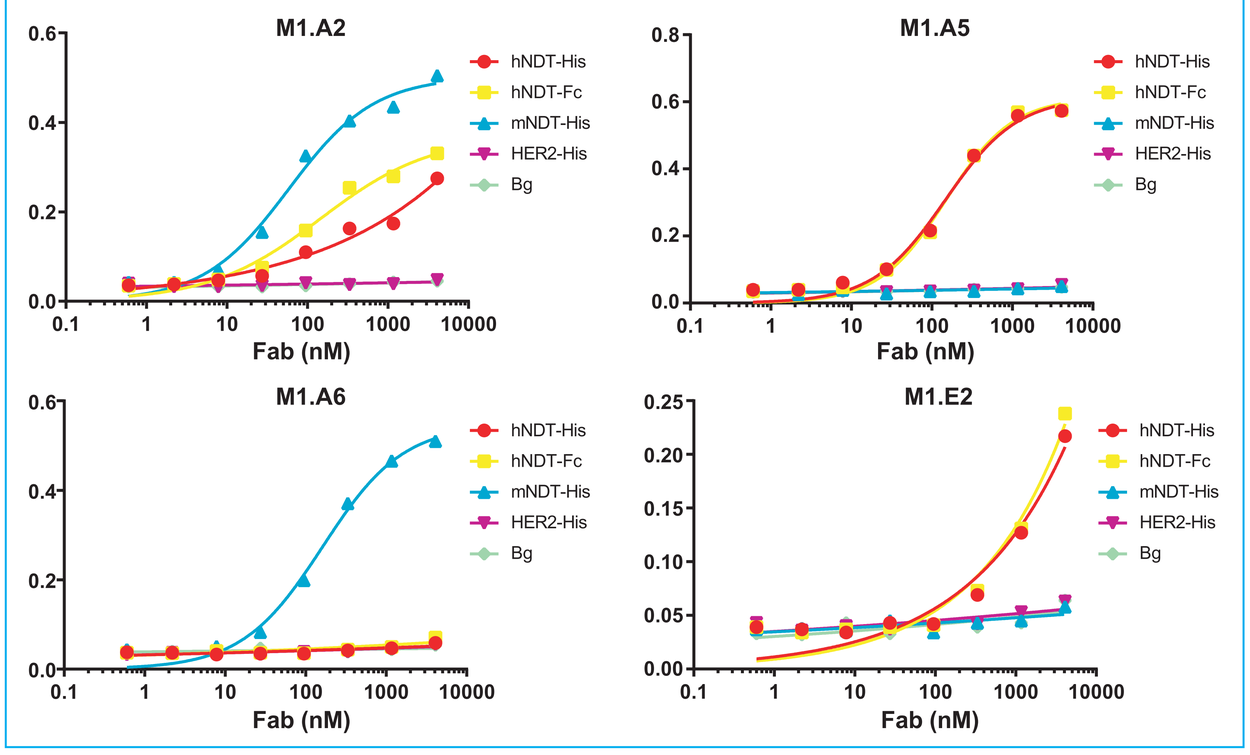
0.03	0.04	0.04	0.14	0.04	0.07	0.04	0.04	0.03	0.04	0.04	0.03
0.04	0.04	0.03	0.04	0.04	0.03	0.11	0.04	0.04	0.03	0.04	0.04
0.04	0.13	0.03	0.04	0.04	0.03	0.04	0.04	0.04	0.04	0.03	0.03
0.04	0.03	0.04	0.03	0.04	0.11	0.08	0.04	0.04	0.09	0.11	0.04
0.04	0.04	0.04	0.03	0.04	0.03	0.04	0.13	0.04	0.13	0.04	0.04
0.08	0.12	0.04	0.11	0.04	0.09	0.04	0.04	0.04	0.04	0.03	0.12
0.20	0.05	0.03	0.08	0.03	0.04	0.04	0.04	0.04	0.04	0.03	0.07
0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.04	0.04	0.04

Using Strategy A, we were able to identify clones that are human NDT specific, and a few clones also weakly cross-reactive to mouse NDT. The same weak cross-reactive clones from Strategy A were also confirmed using Strategy D. In contrast, using Strategy B, we not only found strong mouse NDT binders but also some strong cross-reactive binders to human NDT. The same cross-reactive clones from Strategy B were also selected using Strategy C. However, the mouse and human cross-reactive binders identified from Strategies A & D were different from those found with Strategies B & C. Henece selection strategies can help in identifying multiple different binding epitopes.

Specificity of Phage Clones against hNDT-His, hNDT-Fc, mNDT-His and hHER2-His



Specificity of Purified Fabs against hNDT-His, hNDT-Fc, mNDT-His and hHER2-His



Phage Display Services

Moradec offers the following services using the MORAFAB library:
1. Antibody discovery program (turnaround time of 4-6 weeks from antigen to lead identification).
2. Cross-species reactivity selection (turnaround time of 4-8 weeks from antigen to lead identification).
3. Affinity maturation (based upon the leads identified from the library).

Leads Out-License or Collaboration

Moradec has antibodies leads discovered using the MORAFAB library for the following immune checkpoint targets. We welcome potential opportunities for out-license or collaboration with these antibody leads.
OX40 (CD134; TNFRSF4), B7H5 (VISTA), TIM3 (HAVCR2), LAG3 (CD223), B7H3 (CD276), B7H4 (B7S1), and CD47 (IAP).