Yeast surface display antibody screening using MagDots

Yeast Surface Display (YSD)

Yeast Surface Display is used for the expression and subsequent screening of desired proteins and peptides in the field of antibody discovery and development.[i] Coupled with cell sorting techniques, YSD has allowed researchers to isolate antigen-specific antibodies (or antibody fragments) from a library of clones, with high affinity and specificity.[ii]

Current techniques involved in separation are tedious and require multiple enrichment steps as well as individual labeling for each separation step to achieve the desired protein with sufficient purity. Here, we detail a protocol that uses anti-biotin MagDots for rapid and effective enrichment of antigen-binding clones in a 2T open magnetic system.

MagDots in YSD

MagDot is a unique nanoparticle that combines quantum dots and superparamagnetic iron oxide particles within the same nanoparticle. (Figure 1) Owing to its dual nature, it is possible to perform cell separation on MagDot labeled cells via Magnet Activated Cell Sorting (MACS) and immediately transfer the cells to Fluorescence Activated Cell Sorting (FACS) for further purification without any additional fluorescent labeling procedure (Figure 3).

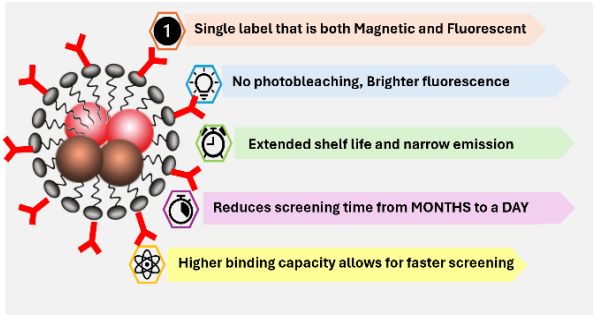

Figure 1. Schematic of MagDot and its advantages

Core Mag2T designed with a 2 Tesla magnet enables rapid and effective separation of MagDot labeled cell suspensions within minutes.

Figure 2. Core Mag2T

The labeled cell population may be visualized as they get separated in the tube with CQT's open magnetic separator and unlabeled cells may be aspirated out without disturbing the labeled population.

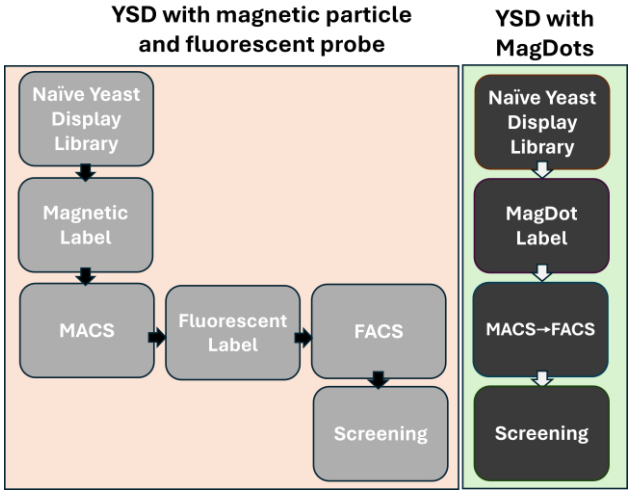

Figure 3. Standard multi-step protein screening from YSD vs single step screening with MagDot

Screening Yeast Display Nanobody Library (NbLib) using MagDots and CoreMag2T

Nanobody libraries with high expression diversity can be screened for affinity antibodies with enhanced efficiency using MagDot in a 2T open magnet. An experiment was performed to screen for engineered yeast strain using anti-biotin conjugated MagDots with an emission maximum of 610 nm. MagDots were used to label a biotinylated and ALFA-tagged fluorescent protein (mEos3.1)[iii] that was bound to yeast cells displaying anti-ALFA-tag nanobody (Figure 4a), and subsequently isolated from yeast cells expressing nanobodies that did not bind to ALFA-tagged protein (Figure 4b).

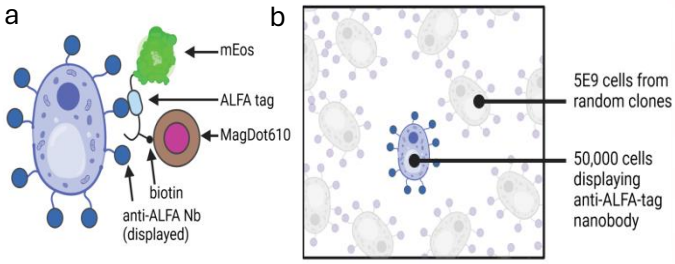

Figure 4 a. MagDots with anti-biotin bind to biotinylated ALFA-tags b. Yeast strain expressing anti-ALFA-tag nanobody (positive control) mixed with yeast cells from random clones.

CORE QUANTUM TECHNOLOGIES

Materials

- Yeast Display Nanobody Library
- Anti-Biotin MagDot 610
- Biotinylated Antigens
- Anti-HA Alexa Fluor 647
- CoreMag2T
- Selection Buffer (20 mM HEPES, pH 7.5; 150 mM sodium chloride, 0.1% (w/v) bovine serum albumin, 5 mM maltose)
- Propidium Iodide (PI)

Methods

a. Suspend yeast cells in 5 mL selection buffer (1E+09/mL) with appropriate diversity of nanobody expression.

b. Add 250 µl of **anti-biotin MagDot** to the cell suspension and incubate for 30 minutes at 4ºC.

Note: Antigen is not added to the solution in this step. Cells are incubated with MagDot to remove clones that non-specifically bind to the beads.

c. Centrifuge cells for 5 minutes at 4000 x rpm at 4ºC, discard supernatant.

d. Resuspend the cells in a 5 mL selection buffer and place it in CoreMag2T for 15 minutes.

e. With the tube still on the magnet, remove the supernatant and transfer the supernatant to a fresh tube.

f. Centrifuge cells for 5 minutes at 4000 x rpm at 4ºC and discard supernatant.

g. Resuspend the cells in a 5 mL selection buffer.

h. Add biotinylated antigen (*here biotinylated and ALFA-tagged fluorescent protein (mEos3.1)*) to the cell suspension and incubate with mixing for 1 hour at 4ºC.

i. Centrifuge cells for 5 minutes at 4000 x rpm at 4ºC and discard supernatant to remove unbound antigens.

j. Resuspend cells in 5 mL selection buffer, centrifuge as before, and discard supernatant.

k. Resuspend in 5 mL selection buffer, add 250 µL **anti-biotin MagDot** and incubate for 30 minutes at 4ºC.

Note: If additional epitopes are to be visualized on a cytometer, add fluorescent antibodies simultaneously with MagDots. For the library utilized in this protocol, anti-HA-Alexa Fluor 647 antibodies were added to stain the displayed nanobodies.

l. Centrifuge cells for 5 minutes at 4000 x rpm at 4ºC, discard supernatant.

m. Resuspend in 5 mL selection buffer and place in the CoreMag2T for 15 minutes.

n. Remove supernatant, resuspend immobilized cells with 5 mL selection buffer and repeat the magnet separation in CoreMag2T for another 15 minutes.

o. Remove supernatant and resuspend immobilized cells in 400 µL selection buffer containing 1 µg/mL PI for analysis.

p. Sort cells with FACS and recover into media

q. Perform Next Generation Sequencing (NGS) to screen for the clones of interest.

Result and Discussion

Performance of anti-biotin MagDot was first assessed by magnetically separating ALFA-Nb displaying cells added at 1% to yeast cells displaying random clones in Core 2T Magnet and analyzed in BD FACSymphony S6 Cell sorter. (These were not screened in NGS). An enrichment of 70 % was achieved in just a single magnetic separation step (Figure 5)

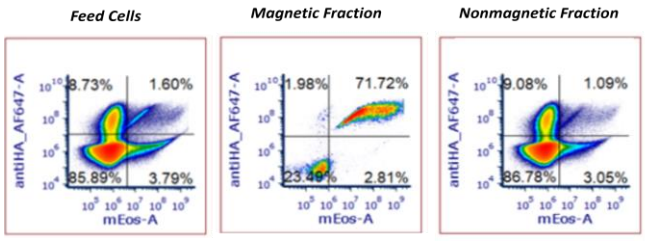

Figure 5. Flow cytometry analysis of 1% doped ALFA-Nb displaying cells. Feed cells, Magnetic Fraction, Non-Magnetic Fraction are shown.

To further test for the highest degree of purity that can be attained with the MagDots, the engineered yeast strain was doped as a positive control (50000 cells) into a background of negatives (5 billion cells) at 0.001%. The positive control cells were enriched using anti-biotin MagDots in Core2 T Magnet. The enriched cells were then sorted immediately using FACS for live cells and recovered for Next Generation Sequencing (NGS). NGS revealed doped in strain as

the top hit and was read three times more than the next abundant clone.

Figure 6. NGS analysis confirming the clone of interest

Eliminating multiple reagents and using MagDots in this proof-of-concept study with engineered yeast strain, therefore, simplified the workflow of antibody screening and consequently reduced the screening time from 5 weeks to a couple of days.

The high antigen-binding capacity of nanobodies combined with the enhanced isolating abilities of MagDots therefore allows for an excellent high throughput platform for antibody selection with high specificity.

Acknowledgment

William Bret Redwine, Head- Custom Protein Resources, Stowers Institute for Medical Research

[i] Eric T. Boder and K. Dane Wittrup, "Yeast Surface Display for Screening Combinatorial Polypeptide Libraries," *Nature Biotechnology* 15, no. 6 (June 1997): 553–57, https://doi.org/10.1038/nbt0697-553.

[ii] Conor McMahon et al., "Yeast Surface Display Platform for Rapid Discovery of Conformationally Selective Nanobodies," *Nature Structural & Molecular Biology* 25, no. 3 (March 2018): 289–96, https://doi.org/10.1038/s41594-018-0028-6.

[iii] Hansjörg Götzke et al., "The ALFA-Tag Is a Highly Versatile Tool for Nanobody-Based Bioscience Applications," *Nature Communications* 10, no. 1 (September 27, 2019): 4403, https://doi.org/10.1038/s41467-019-12301-7.