Mammalian display screening for increased antibody developability

Linnea Korhonen[1], Ph.D. Antti Kulmala[2], M.Sc. (tech.) Saara Östman[1,2]

[1]Department of Life Technologies, University of Turku
[2]Protein and Antibody Engineering, Orion Corporation, Turku

BIOTECHNOLOGY (tech.)

Introduction

Mammalian display is a technique where single antibodies or antibody libraries are expressed on the surface of mammalian cells (fig 1), and it is often used as a tool in therapeutic antibody discovery and development. It allows the selection of desired antibody characteristics from a large number of library variants.

By inserting the protein DNA sequence under the same promoter in every cell it is possible to normalize the transcription levels to make comparison possible[1]. Mammalian cells have been shown to act as a filter by reabsorbing and breaking up proteins that have poor biophysical attributes, leading to lower protein display level[2].

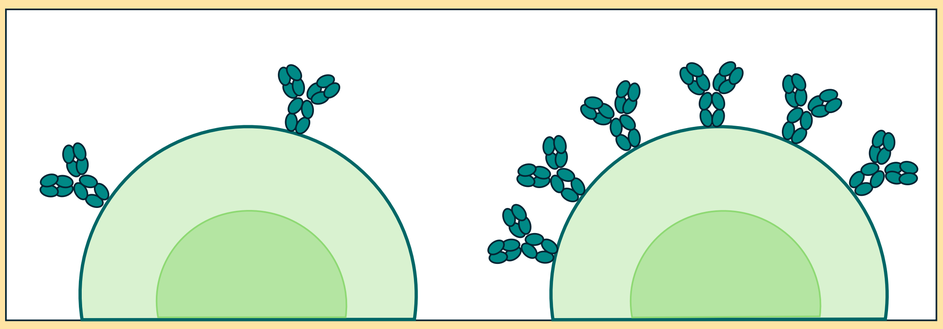

Figure 1. Antibodies displayed on the surface of a cell. On the left side lower display level and on the right higher display level are demonstrated. Only one antibody variant is expressed per cell due to the use of recombinase-mediated cassette exchange[1].

To increase the library size that is possible to achieve, nocodazole can be used to treat the cells. Nocodazole stops the cell division at the same point in every cell[3]. This makes it possible to have cell cycle synchronized before transfection, enhancing the transfection efficiency.

In addition to target binding properties of a therapeutic antibody, it is important that its biophysical properties are optimal to increase the likelihood of success in drug development process. These developability aspects should be considered in early stages of antibody discovery to avoid wasted time, resources, and money[4]. It could be beneficial to have a screening step using a deselection method for antibodies with poor biophysical properties.

In this project the developability of antibody libraries created by mutating three commercial antibodies were analyzed. This was done using a group of deselection reagents that bind to antibodies due to certain problematic biophysical properties.

Methods

Error-prone PCR was used to create variants from three different commercial antibodies. Variable light and heavy domains were mutated separately so that there were two different libraries created from each antibody. Before library constructions, nocodazole was tested by transfecting green fluorescent protein (GFP) into Chinese hamster ovary (CHO) cells.

After producing the library plasmids in Escherichia coli (E. coli) -cells, they were transfected by electroporation into CHO cells. The cells were treated with nocodazole a day before transfection to synchronize the cell cycle. The cells that expressed antibodies were analyzed with flow cytometry to see their display levels, antigen binding ability and which, if any, deselection reagents they bound. Interesting variants were collected by fluorescence-activated cell sorting for further analysis with next-generation sequencing to see what types of mutations they had. The workflow is presented in figure 2.

[1]Huhtinen et al. (2023) Selection of biophysically favorable antibody variants using a modified Flp-In CHO mammalian display platform. *Front Bioeng Biotechnol* **11**:1170081.
[2]Dyson et al. (2020) Beyond affinity: selection of antibody variants with optimal biophysical properties and reduced immunogenicity from mammalian display libraries. *MAbs* **12(1)**:1829335.
[3]Chen et al. (2023) High-fidelity large-diversity monoclonal mammalian cell libraries by cell cycle arrested recombinase-mediated cassette exchange. *Nucleic Acids Res* **51(22)**:e113.
[4]Jain et al. (2017) Biophysical properties of the clinical-stage antibody landscape. *Proc Natl Acad Sci USA* **114(5)**:944-949.

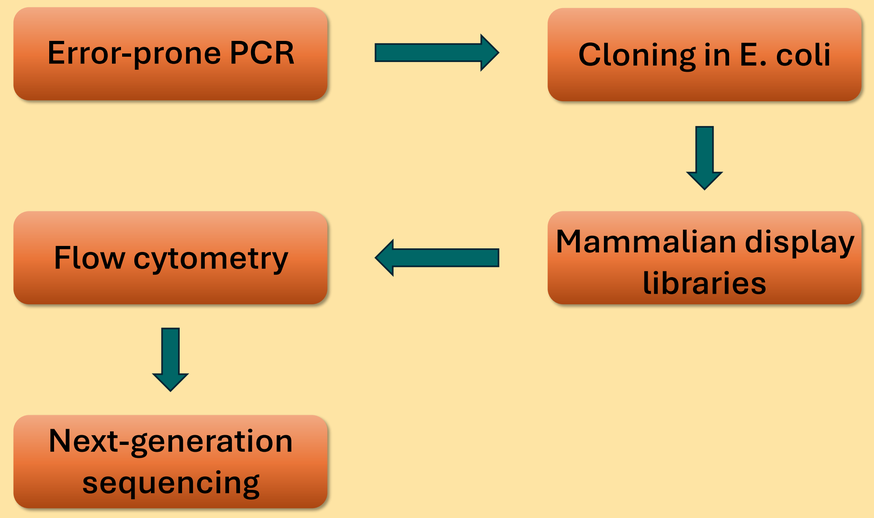

Figure 2. The workflow of the project. Error-prone PCR mutated DNA was cloned in E. coli cells and transfected into CHO cells to form antibody libraries. The deselection reagents were labeled with a fluorescent reagent to measure their binding in flow cytometry. The display levels and antigen binding were measured separately with flow cytometry by using a fluorescent labels. Interesting variants were sequenced with next-generation sequencing.

Results

Nocodazole was found to increase the amount of GFP positive cells by increasing the transfection efficiency. The percentage of 650 nM nocodazole treated GFP positive cells was 14% compared to 3.6% positive cells without nocodazole treatment. This shows that the intake of DNA in the nocodazole treated cells was greater. More results will be gathered once the display libraries are ready to be analyzed.

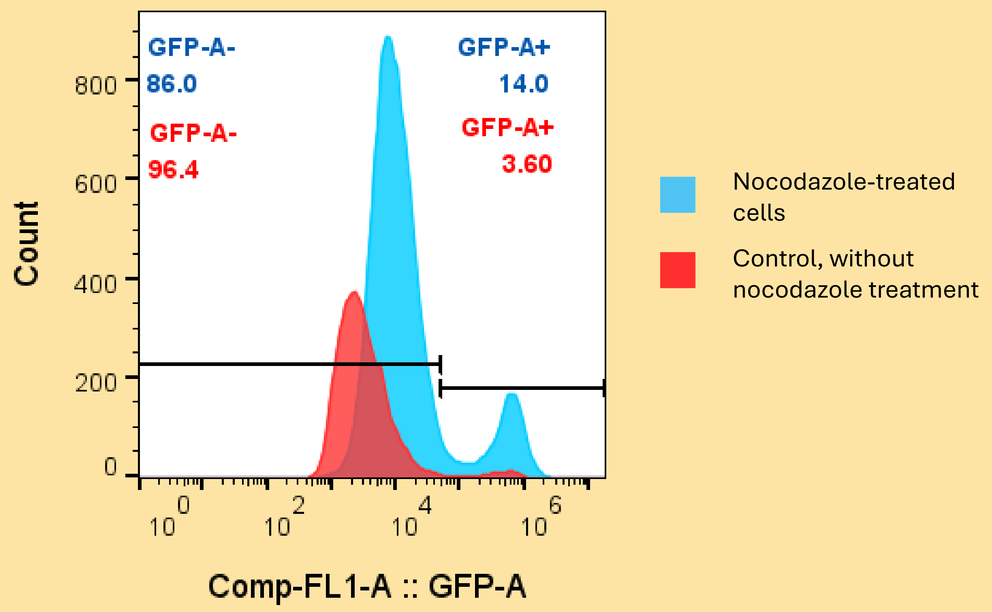

Figure 3. GFP-signal from the nocodazole-treated cells is marked with blue, control sample without nocodazole treatment in red. On the right-hand side of the gating (shown in black lines) are the GFP-positive cell signals. The percentages of negative and positive cells are indicated with blue and red texts that correspond to the colors of the samples.

Discussion

With nocodazole the transfection efficiency is better which is shown to increase library sizes.

Once the display libraries are analyzed, the expectation is to see variants with worsened and improved qualities. The display level, antigen binding and the deselection reagent binding or the lack thereof should reflect these changes in the antibody variants compared to the parental ones. Any changes in one direction or the other will be examined with next-generation sequencing. The data will be analyzed to pinpoint the changes in the antibody DNA sequence that led to the change in its properties.