new/era/mabs

WHITE PAPER

SELECTION OF ANTIBODIES USING SELMA TECHNOLOGY

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SELECTION OF ANTIBODIES

The ability to generate monoclonal antibodies (mAbs) for research, diagnostic and therapeutic purposes has opened a new century of predicting, preventing and curing many diseases.

Following the human immune system, where antibodies are generated after antigen contact, scientists were able to develop methods such as hybridoma technology or phage display that enabled a routine workflow for mAb generation (Fig. 1). These methods were a fundamental breakthrough for all antibody-related applications known today. This field is emerging strongly, now in combination with AI, bringing in new aspects to create even better tools for diagnosis and therapy.

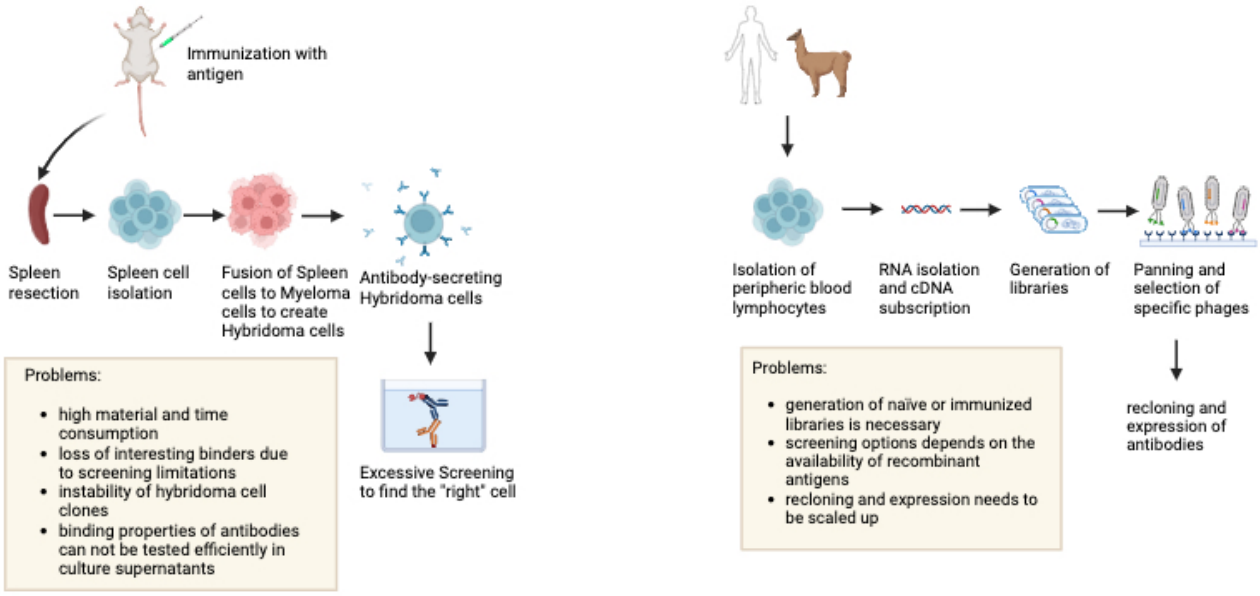

Figure 1: Schematic illustration of hybridoma technology (left) and phage display (right). Created in BioRender.com

In general, antibody candidates have been discovered by either hybridoma screening or phage display panning rounds, in which the antigen is coated on a solid phase and

cell culture supernatant or phage are selected according to their specific binding constants. Both methods are very time-consuming, and even though the processes have been automated in the last 20 years, it is still a low-probability endeavor to find the optimal binder among the many possible ones.

Several attempts have been made to circumvent the current problems. Some of these are related to the development of complex and fragile mammalian cell display systems that aim at rapid selection of the desired antibody-producing cells in a high-throughput manner, while others opt for parallelization of equipment, disposables and biological material.

An example of such a display approach is the Beacon system, in which B lymphocytes from patients are encapsulated in microdroplets followed by an indirect ELISA performed inside these microdroplets to determine the specificity of the released antibody[1]. The system is based on proprietary, highly sophisticated miniaturized electronic and optical equipment. It goes without saying that complexity is usually error-prone and leads to increased failure rates as well as time and cost increases.

Another complex display system developed particularly for the selection of recombinant IgG immunoglobulins uses antibodies anchored to the cell surface by an engineered transmembrane domain[2]. Displayed IgG antibodies can be detected using a fluorescently labeled antigen and the corresponding cells are selected by flow cytometry. However, the selected antibodies are structurally modified by fusion to the transmembrane anchor domain. This modification forces them to be in close proximity to the cell membrane and other proteins on the cell surface, which can affect their binding properties.

For both methods and most others, once the desired production cell has been identified, their antibody genes must be recloned for further development and production.

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[1] Mocciaro A, Roth TL, Bennett HM, Soumillon M, Shah A, Hiatt J, Chapman K, Marson A, Lavieu G. Light-activated cell identification and sorting (LACIS) for selection of edited clones on a nanofluidic device. Commun Biol. 2018 May 3;1:41. doi: 10.1038/s42003-018-0034-6.

[2] Kothai Parthiban, Rajika L. Perera, Maheen Sattar, Yanchao Huang, Sophie Mayle, Edward Masters, Daniel Griffiths, Sachin Surade, Rachael Leah, Michael R. Dyson & John McCafferty (2019) A comprehensive search of functional sequence space using large mammalian display libraries created by gene editing, mAbs, 11:5, 884-898, DOI: 10.1080/19420862.2019.1618673.

With ***selma***, we have developed an advanced continuous cell display system that enables the selection of antibody-producing cells in combination with early characterization and manufacturing without the need for antibody fragment recloning.

DESCRIPTION AND BENEFITS OF SELMA

selma was developed to overcome the current problems in selecting antibody-producing cells in high-throughput screening. Especially for hybridoma-related monoclonal antibodies, the gap between product and producing cell – the lack of a direct phenotype-genotype-linkage – leads to time-consuming and inefficient sorting procedures. Not just for hybridomas, ***selma*** is universally applicable to any type of antibody-releasing cell. Capturing the released antibody in its natural form and retaining it on the cell surface without external or structural modifications allows for complete and early characterization of the antibody and its direct subsequent production without the need for recloning.

The system was originally developed at the University of Potsdam and transferred to our academic spinoff company new/era/mabs that has improved and tuned the platform, owns the IP and offers a customized monoclonal and recombinant antibody production.

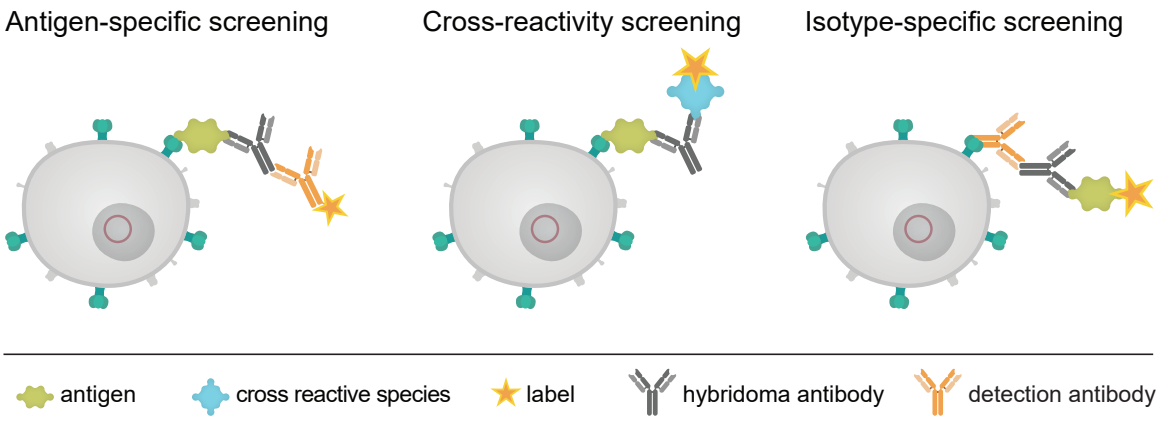


Figure 2: Schematic presentation of different selection options using NEM´s ***selma*** platform

selma is based on transgenic cell lines expressing an artificial cell surface marker[3]. This marker is used to bind an antibody capture matrix that captures the released antibody and displays it on the cell surface (Fig. 2). With the help of a fluorescence-labeled antigen, the antibodies can now be characterized with regard to their binding properties and the desired cells can be transferred directly into production after selection. The system is highly flexible so that different selection options can be chosen to search for appropriate antibody isotypes or cross-reactivities.

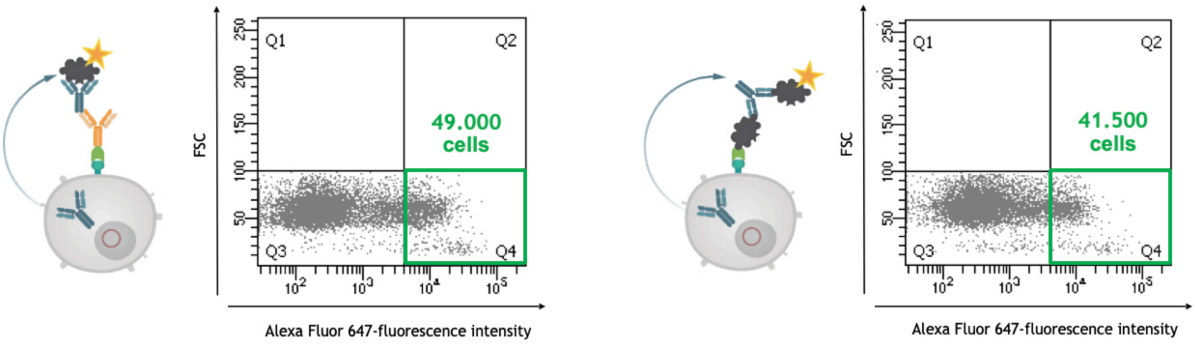


Figure 3: Exemplary sorting results for isotype-specific and cross-reactivity screening.

The easy-to-use and flow cytometry-based protocol allows selection of target cells from 1x10^6 hybridomas per screening with a single cell plating two weeks after splenocyte fusion (Fig. 3). When using cell lines expressing a recombinant antibody, the same principle can be applied to HEK293 cells to select high production cells or target cells from a mammalian cell library.

Table 1: Comparison of standard cell lines vs. ***selma*** cell lines to show the benefits of the ***selma*** system

options	standard cell line	*selma* cell line
selection via limited dilution	yes	yes
selection via flow cytometry	no	yes
antigen specific sorting	no	yes
isotype specific sorting	no	yes
combination of all sortings	no	yes
improved maintenance of high producers	no	yes

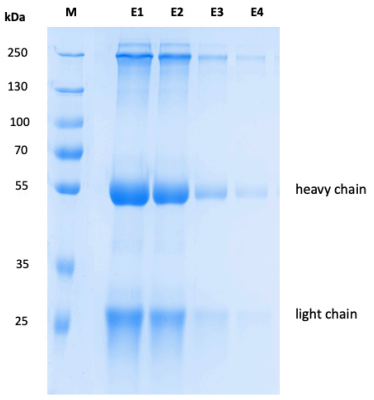
[3] Listek M, Hönow A, Gossen M, Hanack K. A novel selection strategy for antibody producing hybridoma cells based on a new transgenic fusion cell line. Sci Rep. 2020 Feb 3;10(1):1664. doi: 10.1038/s41598-020-58571-w.

USE CASE #1

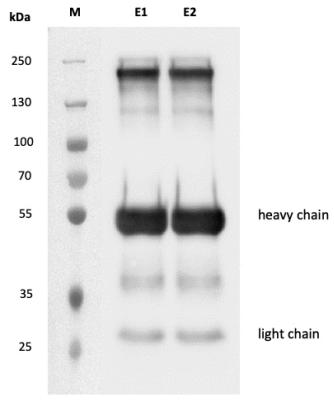
GENERATION OF HUMAN ANTIBODIES USING IN VITRO IMMUNIZATION AND SELMA

In combination with new/era/mabs' *in vitro* immunization approach, ***selma*** is a highly efficient and a short workflow to generate antibody candidates.

In this use case, peripheral B lymphocytes were isolated from a human donor and activated *in vitro* with the antigen of choice - a cancer-related membrane receptor. The B cells were activated in a 12-day protocol, fused with transgenic cell lines and selected with ***selma***. Antigen-specificity was demonstrated with the ***selma*** workflow using recombinant protein and transgenic cell lines expressing the receptor of interest. The purified antibodies were of IgG4 isotype and could be produced in large quantities (Fig. 4).



SDS-PAGE under reducing conditions (10%)
M - Marker (PageRuler)
E1 - elution fraction 1
E2 - elution fraction 2
E3 - elution fraction 3
E4 - elution fraction 4



Western Blot
5 µg purified antibody per lane
HRP-conjugated goat anti-human IgG
M - Marker (PageRuler)
E1 - elution fraction 1
E2 - elution fraction 2

clone	elution fraction	concentration [µg/ml]	yield [mg]
1 A1	1	957	1.628
	2	564	
	3	93	
	4	14	

Figure 4: Characterization of purified human IgG4 mAbs by SDS PAGE and Western Blot

The following steps in this project include advanced agonist/antagonist assays, affinity measurements, primary cell assays, and recombinant antibody production.

USE CASE #2

GENERATION OF ANTIBODIES FOR THE DIAGNOSIS OF OXIDATIVE DNA DAMAGE

Use case #2 demonstrates the feasibility of the ***selma*** system for isolating binders of oxidized DNA.

The detection of oxidative DNA damage in biological samples is of high clinical relevance and is significantly associated with various types of cancer as well as degenerative diseases. The new/era/mabs ***selma*** system enabled the selection of a highly specific binder for 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is useful for the visualization of oxidative DNA damage and the formation of foci – accumulations of oxidative modifications in highly condensed DNA (Fig. 5).

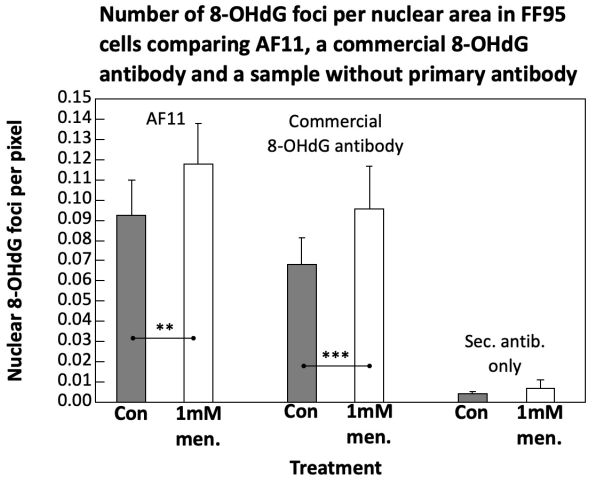


Figure 5: Result slide for antibody clone AF11 compared to a commercial mAb detecting nuclear foci in FF95 cells.

Compared with a commercial antibody, the newly ***selma***-selected antibody clone AF11 was able to react in the same trend as the commercial antibody, providing a viable tool for detecting 8-OHdG foci in nuclear regions of cells.[4]

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[4] Jung T, Findik N, Hartmann B, Hanack K, Grossmann K, Roggenbuck D, Wegmann M, Mantke R, Deckert M, Grune T. Automated determination of 8-OHdG in cells and tissue via immunofluorescence using a specially created antibody. Biotechnology Reports 2024. https://doi.org/10.1016/j.btre.2024.e00833.

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01 in unsurpassed short time

02 with maximum avoidance of animal use

03 with unmatched yields of specific clones

04 against otherwise inaccessible epitopes

The company offers strategic partners the transfer and use of its proprietary technologies including its ***selma*** discovery platform under license.

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