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Display of a Full Length IgG Antibody on the Surface of *Escherichia coli*: Towards the Screening of an Antibody Library

Carina Dilkaute[1,*], Wilhelmine Weckenbrock[1] and Joachim Jose[1]

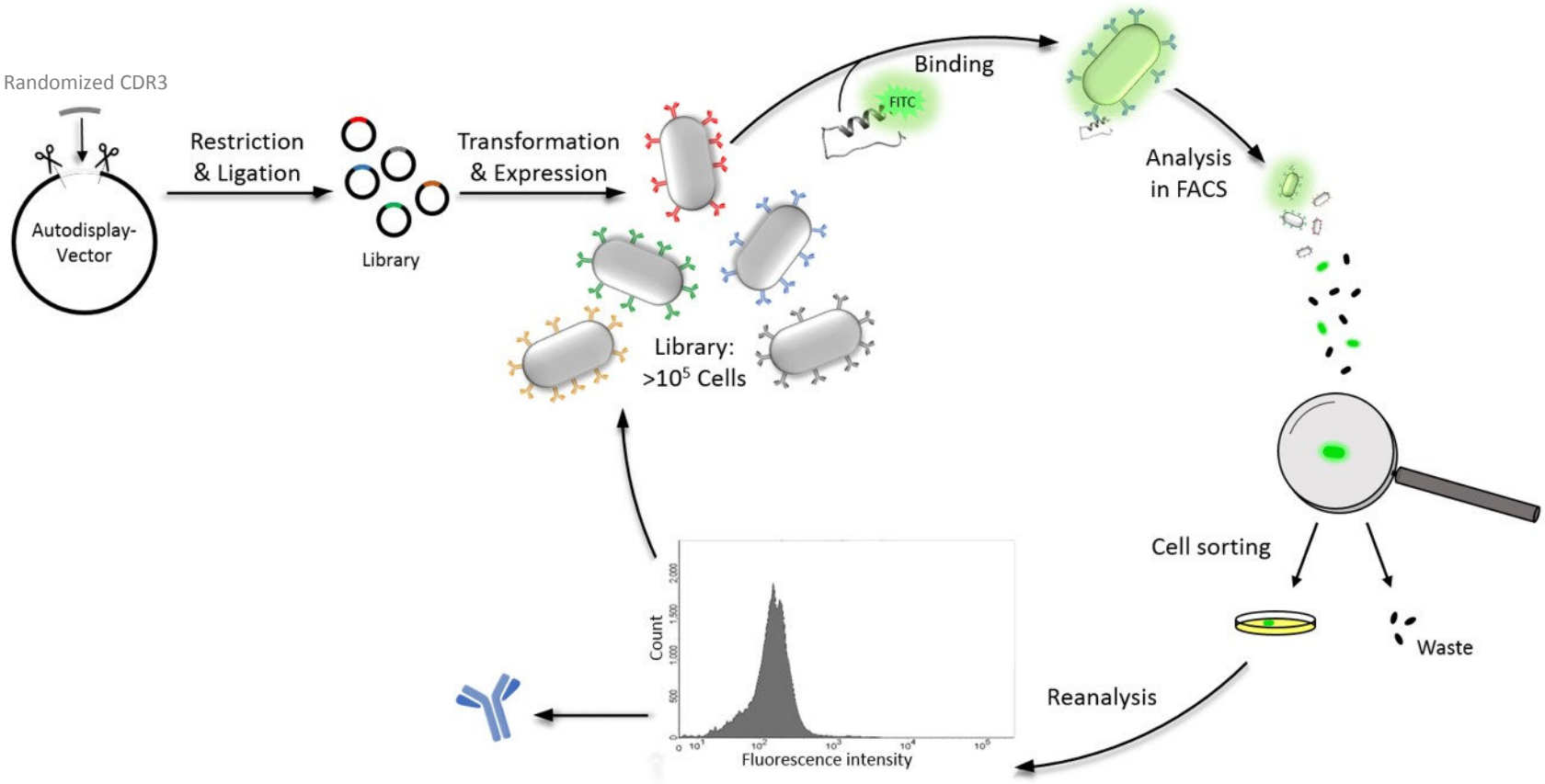
[1] Institute of Pharmaceutical and Medicinal Chemistry, PharmaCampus, Westfälische Wilhelms-Universität, Corrensstraße 48, 48151 Münster

***** Corresponding author: carina.dilkaute@uni-muenster.de

WWU PharmaCampus Institut für Pharmazeutische und Medizinische Chemie

GRAPHICAL ABSTRACT

Display of a Full Length IgG Antibody on the Surface of *Escherichia coli*: Towards the Screening of an Antibody Library



ABSTRACT

Phage display is an often used technique to identify new antibody variants. Nevertheless, it is associated with some drawbacks as the possible discrimination of the most potent binders during biopanning, the incompatibility with flow cytometry or the size limitation of the protein displayed on the surface [1]. To circumvent these disadvantages, we presented a full-length antibody on the surface of *Escherichia coli* using the autodisplay technique [2,3]. As a proof of principle, the display of antibody T84.66, which is directed against carcinoembryonic antigen (CEA), was investigated. Based on this antibody a library was generated using a ligation-restriction strategy. The resulting library consists of up to 10^5 clones which could be analyzed via flow cytometry after incubation with a fluorescently labelled target protein. To examine the optimal conditions for the screening, two different autotransporters in combination with two promoters were investigated: the AIDA-1 autotransporter [2] under control of a T7 promoter and the EhaA-autotransporter [3] controlled by an araBAD promoter. Experiments with the T84.66 antibody revealed that the EhaA-araBAD combination suited better with regard to surface presentation and cell survival after sorting. These results indicate that it is possible to generate a full-length antibody library on the surface of *E. coli.* This library can be screened with the advantageous high-throughput screening system of flow cytometry.

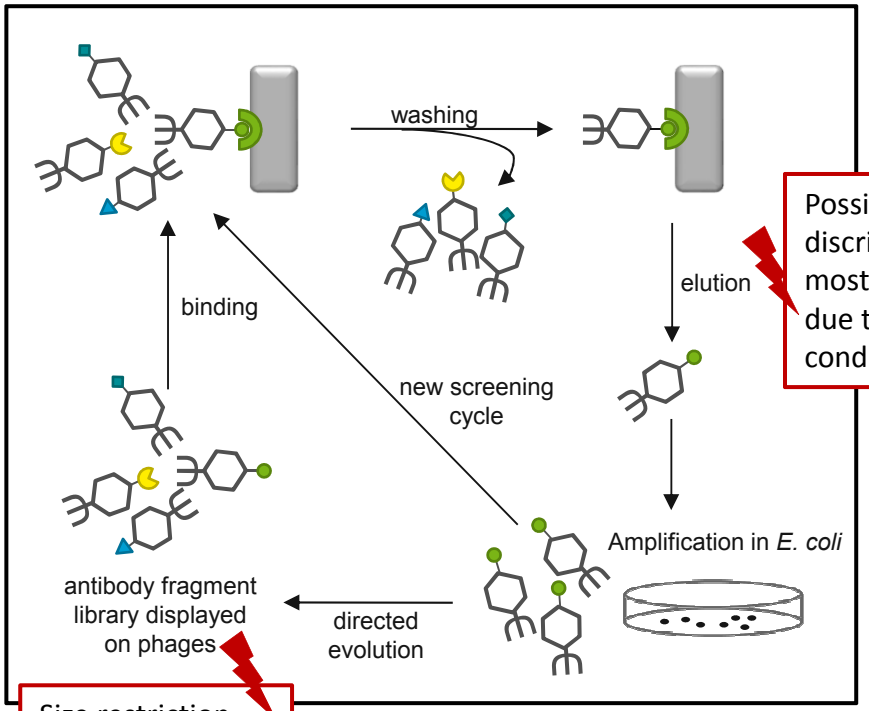
Keywords: antibody library; autodisplay; full-length antibody; surface display

References:
[1] Levin A. M., Weiss G.A.: *Mol. BioSyst* **2006**, 2: 49-57
[2] Jose J., Meyer T.F.: *Microbiol. Mol. Biol. Rev.,* **2007,** 71(4): 600-619
[3] Sichwart S. et al.: *Food Technol. Biotechnol*. **2015**, 53(3): 251-260

Discovery of new monoclonal antibody variants

Development

- Animal immunization in combination with hybridoma technology
- **Phage display** (State of the art)
- Ribosome display
- Yeast display
- …



PROBLEM

Possible discrimination of the most potent binders due to mild elution conditions

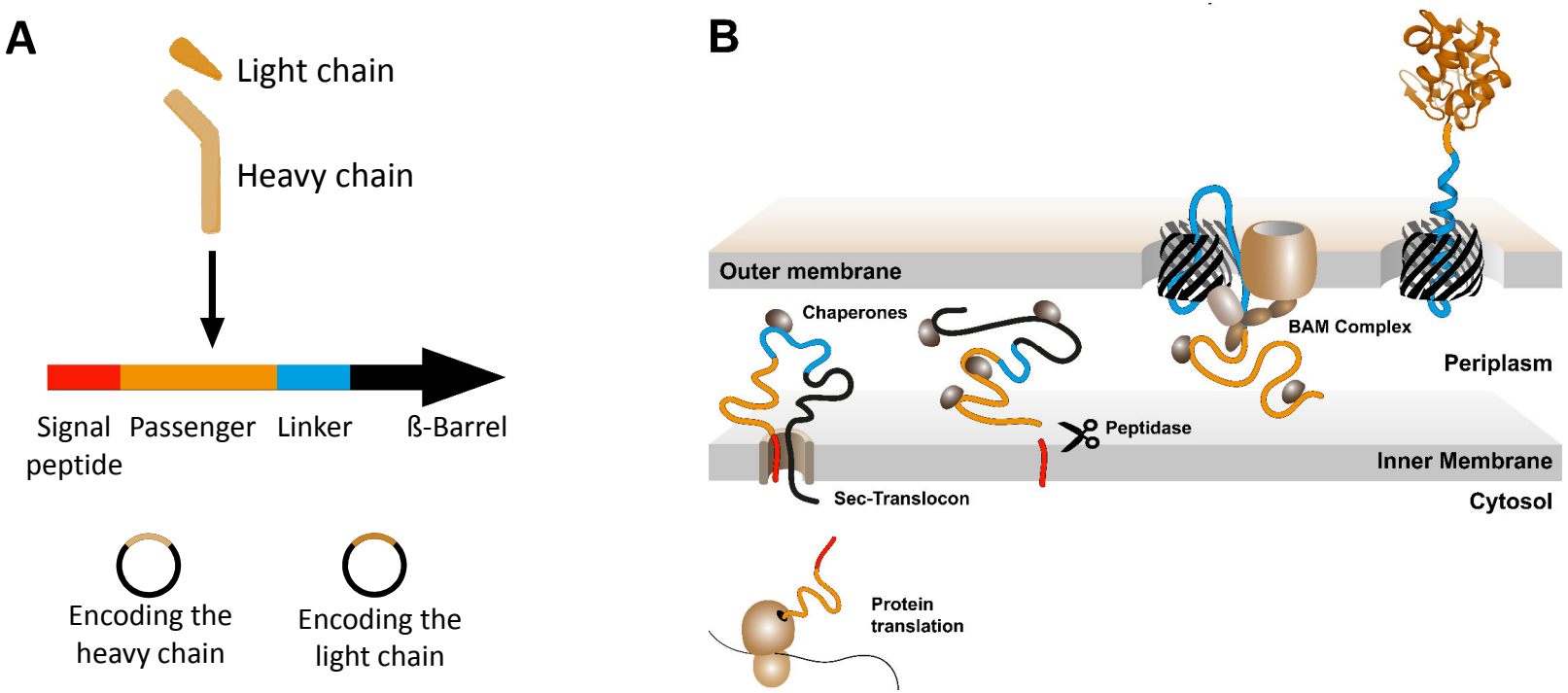
PROBLEM

Size restriction for the peptide displayed on the surface

SOLUTION

Surface presentation on *E. coli*

Presentation of antibodies on the surface of *E. coli* via autodisplay

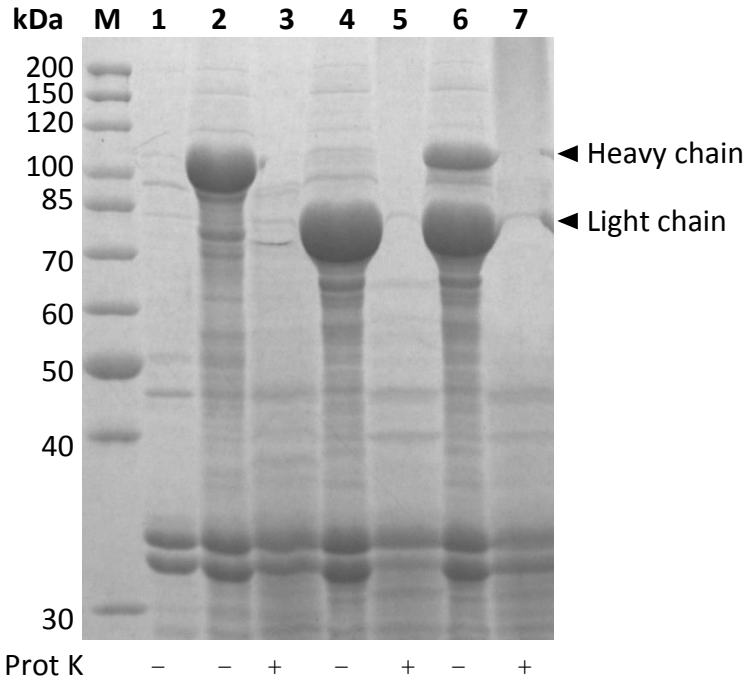


A: Structure of the autotransporter fusion precursor protein. One plasmid includes the gene for the fusion protein with the antibody's heavy chain as passenger, another the light chain**.**

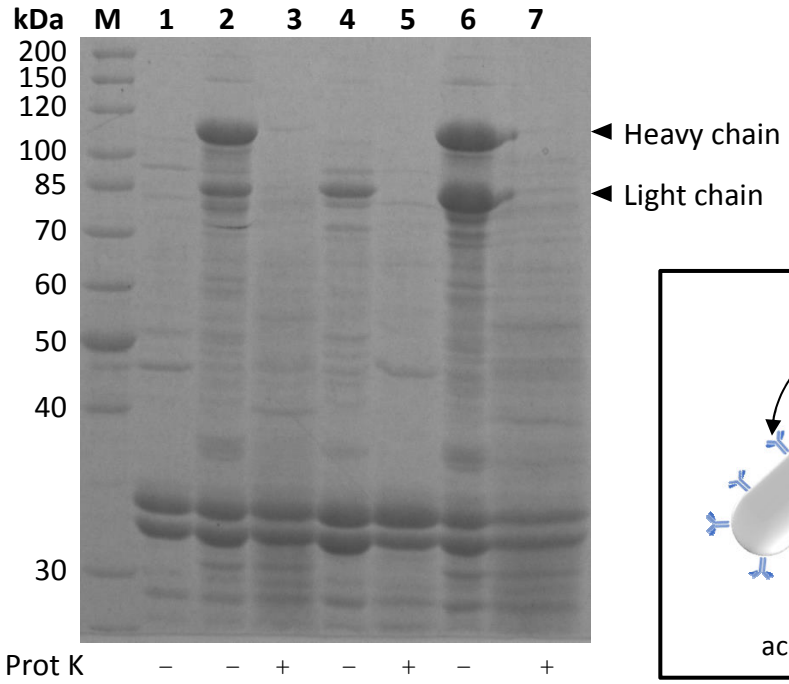
B: Mechanism of translocation. Due to the mobility of the ß-barrel in the outer membrane, the heavy and the light chain are able to find each other, when co-expressed in one cell.

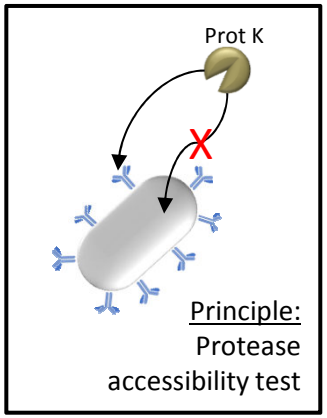
Proof of surface display by outer membrane preparation

AIDA-1 autotransporter, T7 promoter



EhaA autotransporter, araBAD promoter

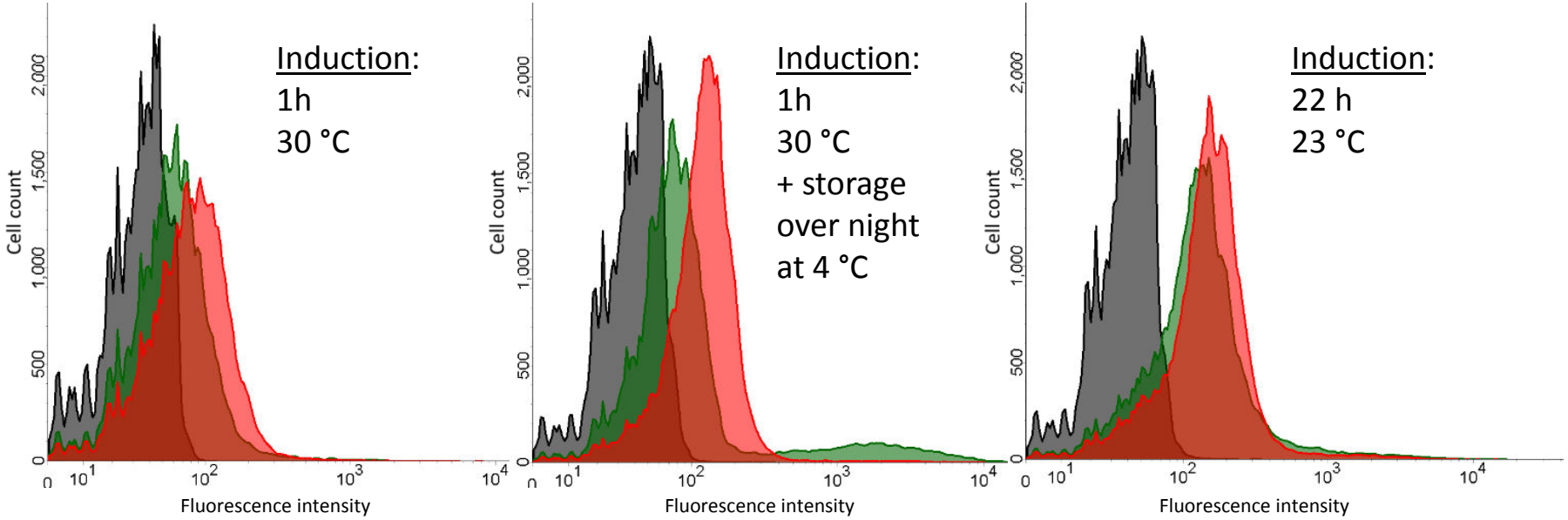




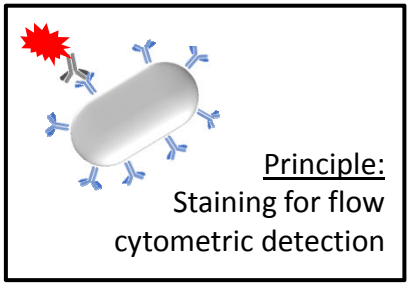
SDS-PAGE analysis of outer membrane fractions from *E. coli* UT5600(DE3) cells without plasmid (1), from cells displaying the heavy chain (2,3), the light chain (4,5) or both chains (6,7) of the anti-CEA antibody. The exposure at the surface was confirmed by digestion with proteinase K, which cannot enter the cell (3,5,7).

Proof of surface display via flow cytometry

Comparison of different induction conditions



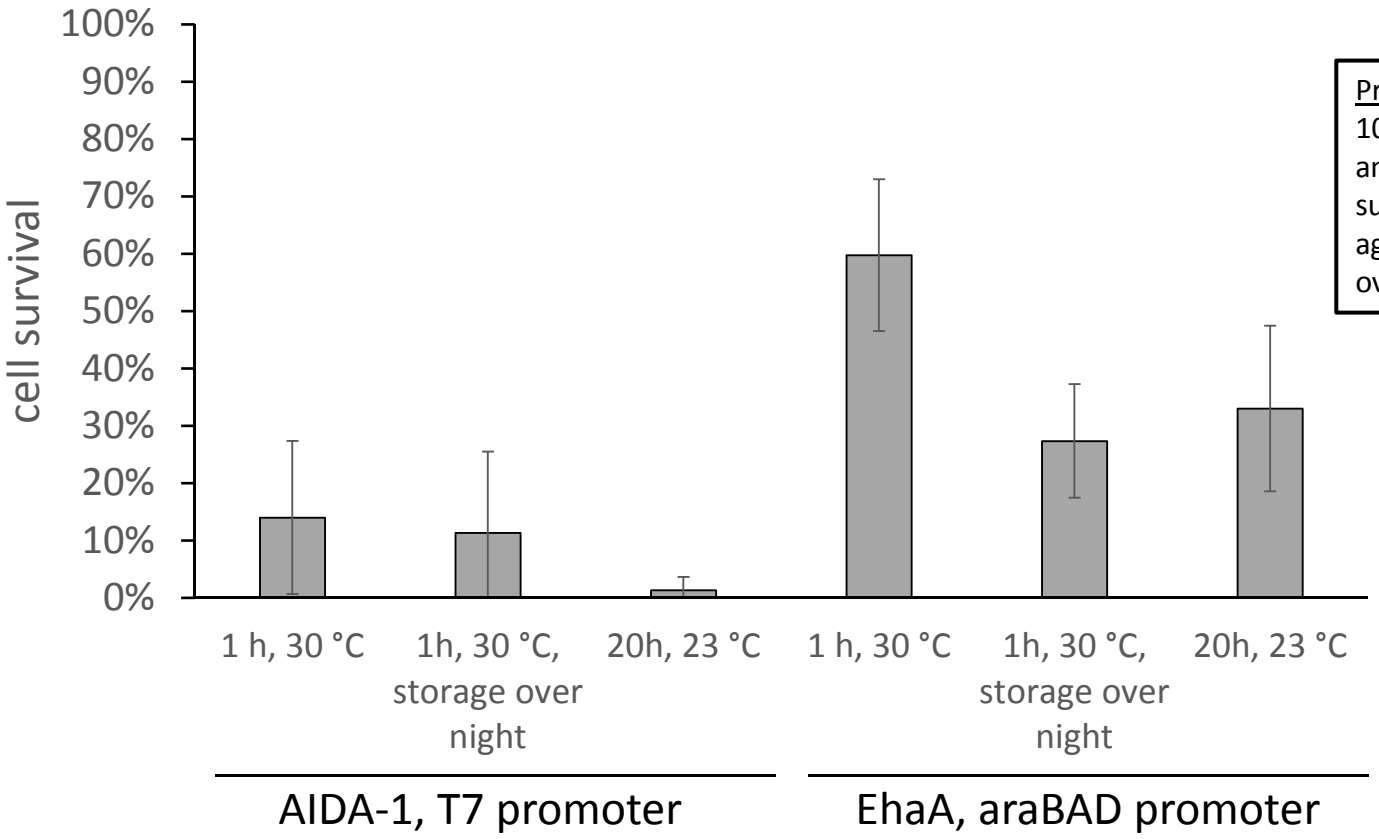
- Cells presenting an unrelated protein
- Cells presenting anti-CEA antibody, AIDA-1, T7 promoter
- Cells presenting anti-CEA antibody, EhaA, araBAD promoter



RESULTS AND DISCUSSION:
1. ANTI-CEA ANTIBODY AS PROOF OF PRINCIPLE

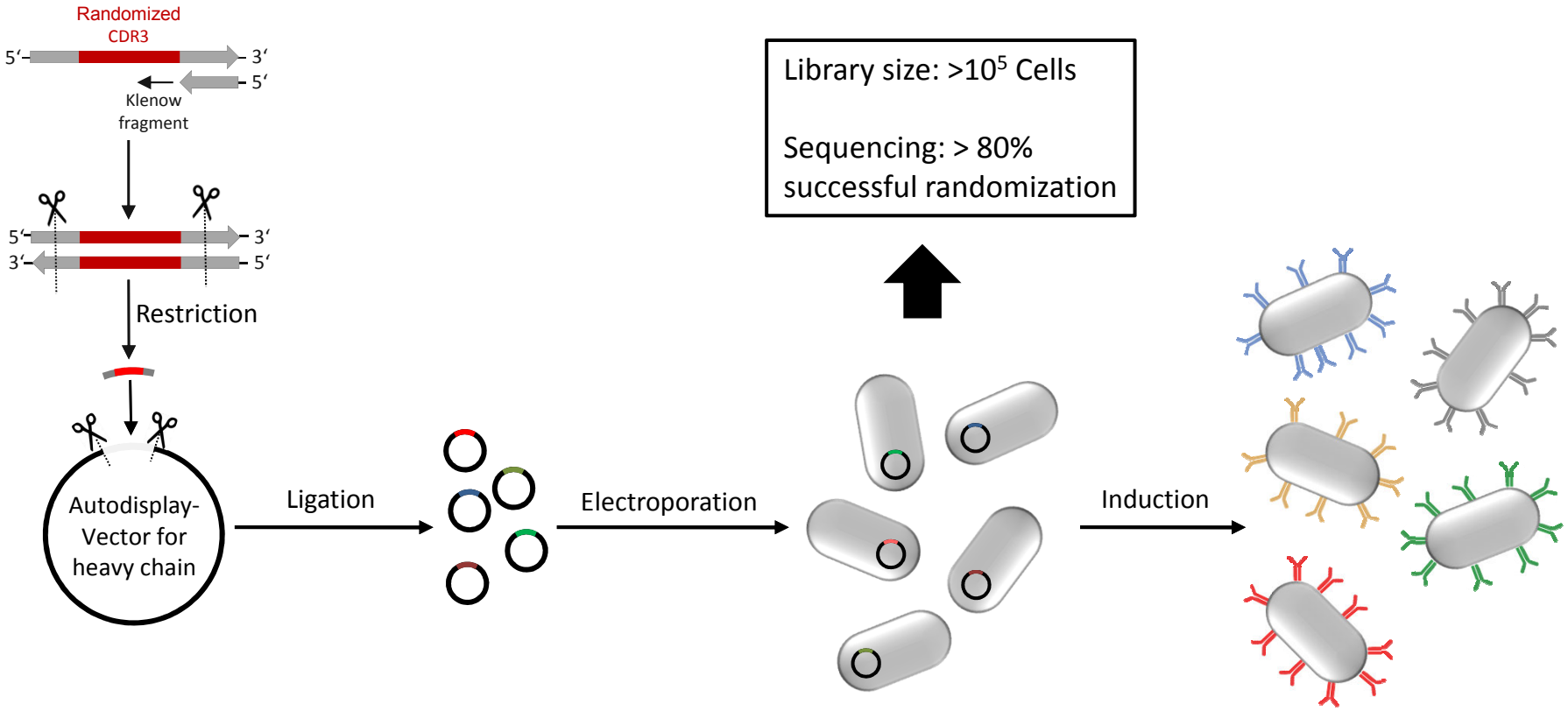
Cell survival after sorting by flow cytometry

Comparison of different induction conditions

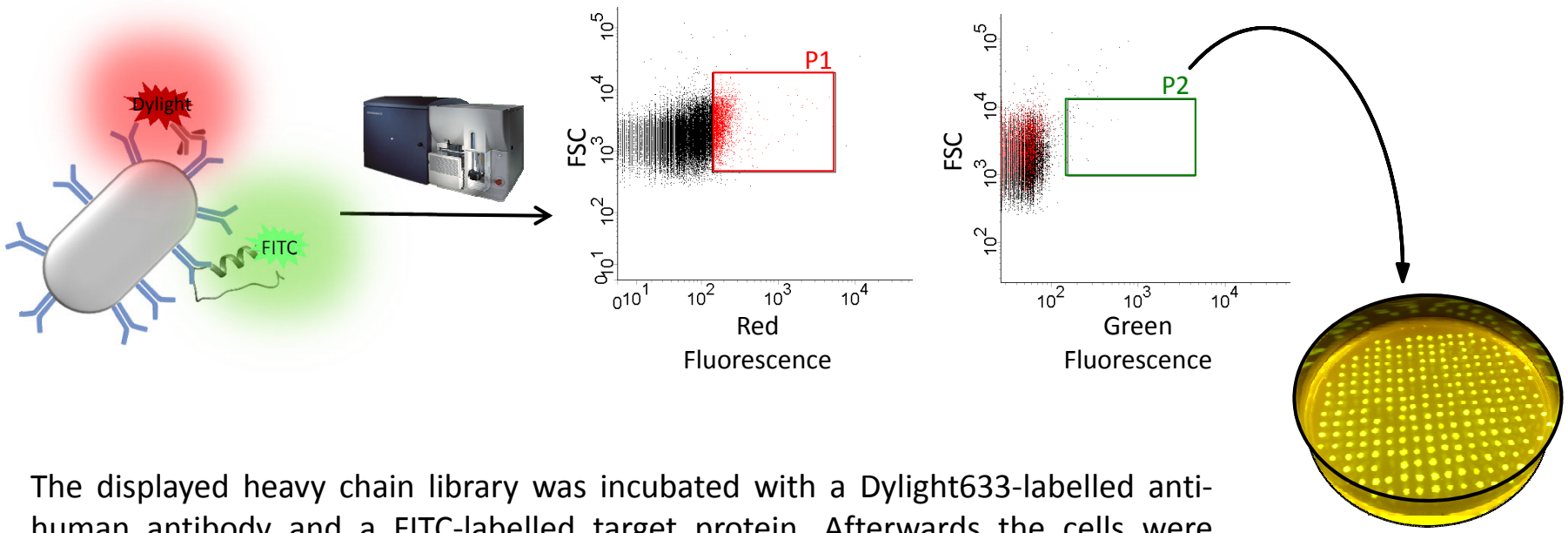


Principle:
100 clones presenting the anti-CEA antibody on its surface were sorted on an agar plate and incubated over night at 37 °C.

Generation of an heavy chain antibody library



Library screening via flow cytometry



The displayed heavy chain library was incubated with a Dylight633-labelled anti-human antibody and a FITC-labelled target protein. Afterwards the cells were analyzed by flow cytometry. Events with an increased fluorescence intensity for both dyes (Gate P1 and P2) should carry a target-binding antibody at their surface and were therefore sorted on an agar plate.

CONCLUSION

A full-length antibody was displayed on the surface of *E. coli.*

The combination of araBAD promoter and EhaA autotransporter was identified to be superior to an AIDA-1 autotransporter under control of a T7 promoter.

To achieve the best combination of surface presentation and cell survival, an induction time of 22 hours at 23 °C was chosen.

In further experiments, sorted variants from the library should be reanalyzed in order to identify new binding heavy chain variants. Furthermore, libraries consisting of heavy and light chain should be further investigated.

ACKNOWLEDGMENTS

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