Monoclonal expression and affinity characterization of anti-gelsolin Fab library produced in Expi293 suspension cells

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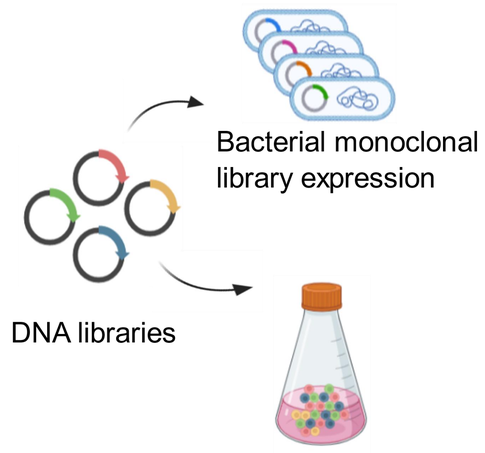
INTRODUCTION

- Direct transfection of DNA libraries into mammalian cells result in polyclonal phenotype → unsuitable to produce soluble antibodies for single clone screening.
- However, mammalian cells are superior in protein folding and posttranslational modifications for producing complex molecules, such as recombinant antibodies.

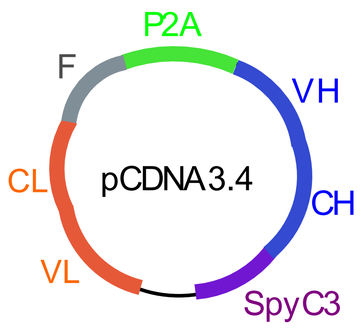


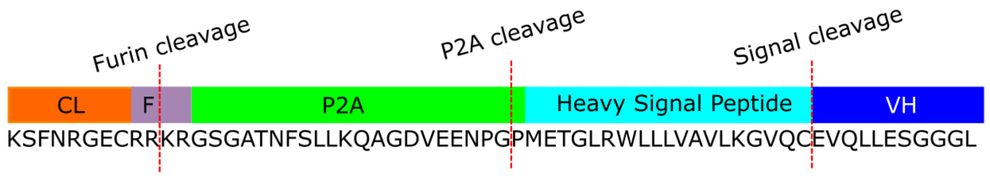
AIMS

Obtain monoclonal anti-gelsolin Fab libraries in mammalian cells as fusion protein with SpyCatcher3, that can be screened on bead array in multiplexed manner.

KEY METHODS

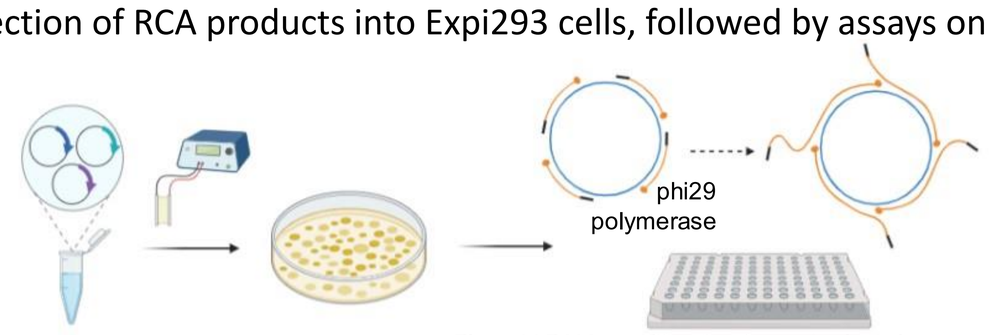
Single plasmid encoding complete Fab-SpyCatcher3 (pCDNA3.4 Fab skipper)





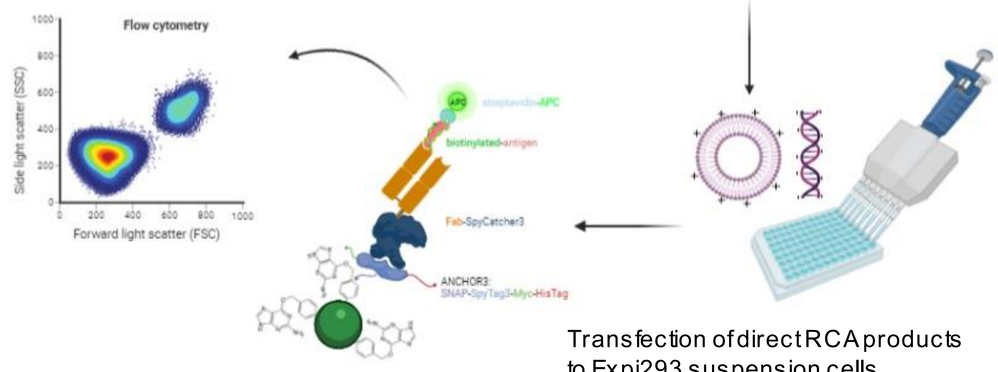
Furin recognition site sequences and P2A self-skipping peptide sequences allow multiple gene expression in single gene casette.

Transfection of RCA products into Expi293 cells, followed by assays on beads.



Transformation of DNA library encoding Fab to XL 1 E.coli

Plasmid DNA amplification from single colony using Rolling Circle Amplification (RCA)



Screening of monoclonal Fab library on beads and simultaneous analysis using flow cytometry

Transfection of direct RCA products to Expi293 suspension cells in 96-well format

RESULTS

Single Plasmid Construct Testing

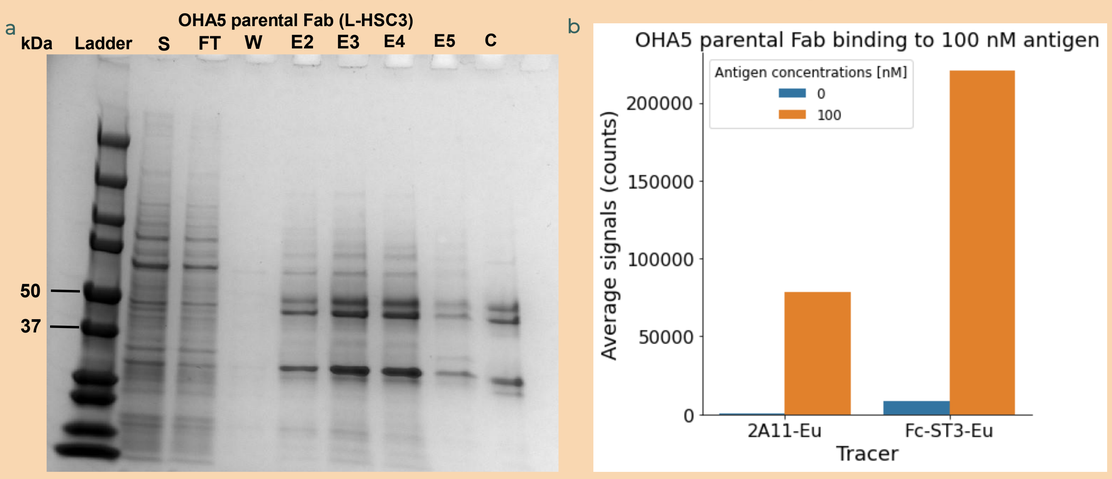


Figure 1. (a) SDS-PAGE of Ni-NTA purified OHA5 parental Fab from pCDNA3.4 Fab skipper transfection in Expi293 cells. (b) TRF-immunoassay for binding detection against 8 kDa gelsolin antigen on 96-well microtiter plate.

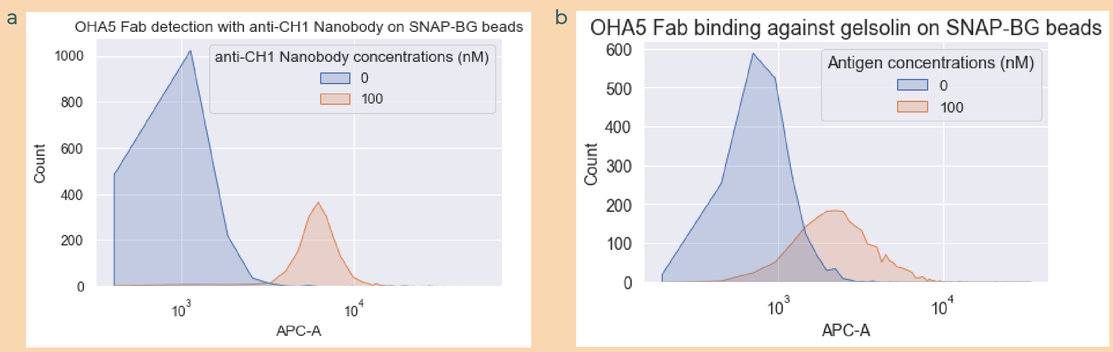


Figure 2. Testing of OHA5 parental Fab on color-coded beads analyzed in flow cytometry. (a) Fab detection assay using anti-CH1 Nanobody. (b) Antigen (8 kDa gelsolin) binding detection.

RCA optimization

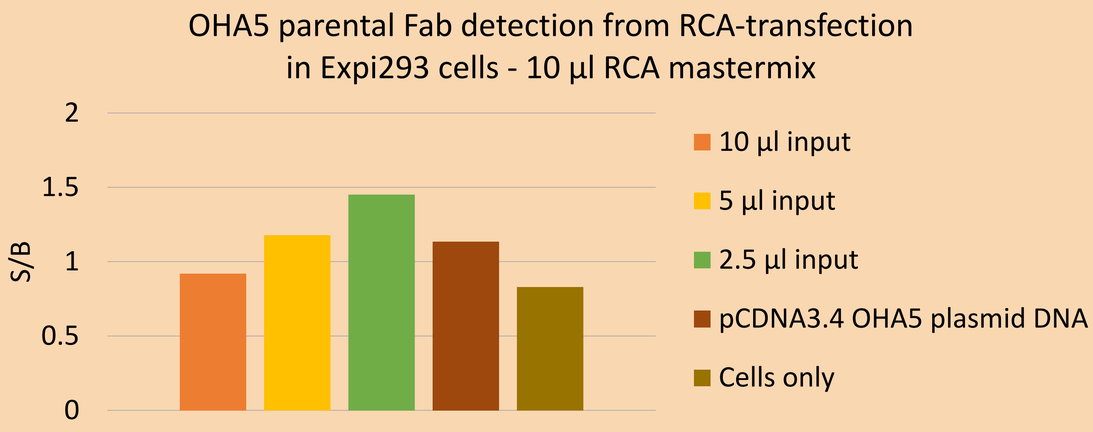


Figure 3. Fab detecting immunoassay of RCA-transfection in Expi293 cells. Different volume input of RCA products were tested in the transfection.

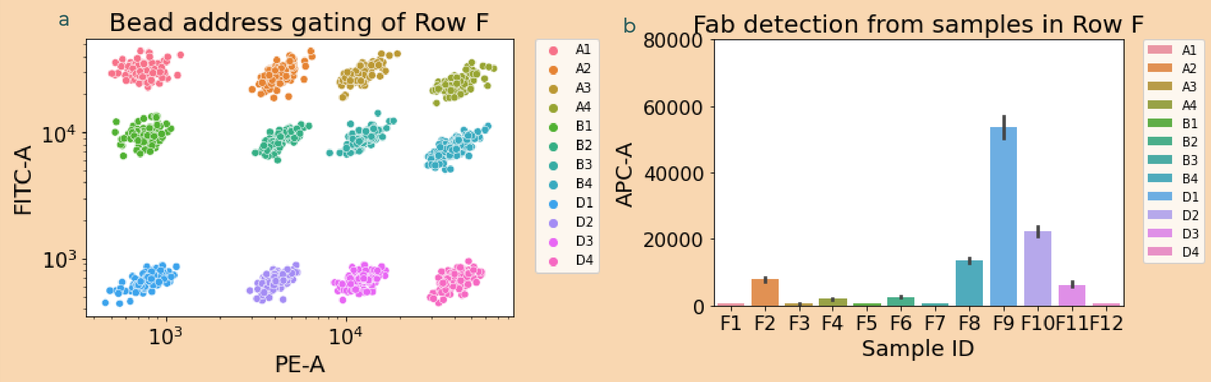


Figure 4. 12-plex assay demonstration of Fab produced from RCA-transfection in Expi293 cells. (a) Bead address gating based on FITC and RITC using DBScan algorithm. Each bead represents individual Fab samples. (b) Fab detection assay, sample 1-8: RCA of anti-gelsolin libraries, 9: plasmid DNA control, 10: RCA of parental Fab, 11: Cells control, 12: RCA NTC.

CONCLUSION

- Transfection of 2.5 µl RCA products from bacteria colony harboring single plasmid into mammalian cells successfully produced antibody and can be applied to obtain monoclonal antibody libraries expression for screening purposes.
- Uneven expression levels from RCA-transfection requires further optimization.