The Development of Bispecific Binders for Fast Isolation and Screening of High-producing Mammalian Cell Clones

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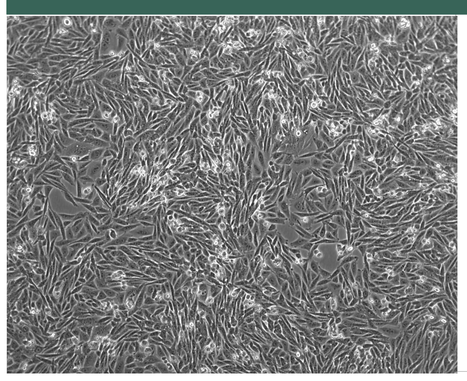
INTRODUCTION

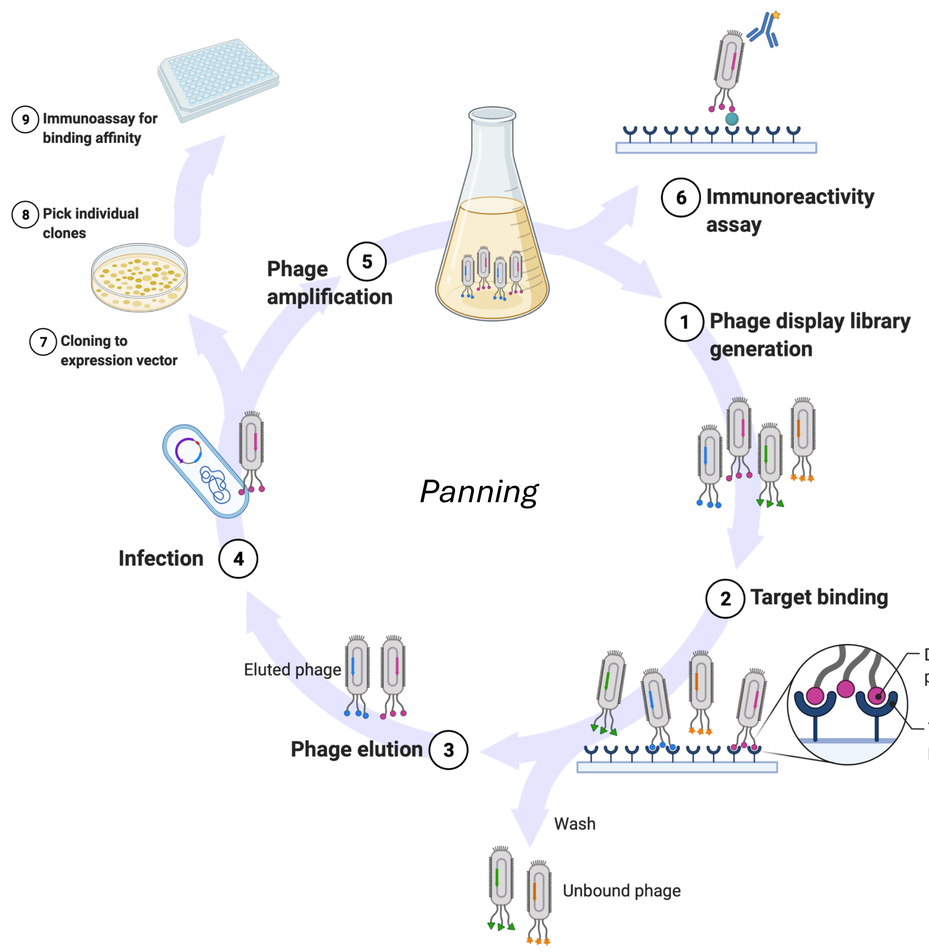


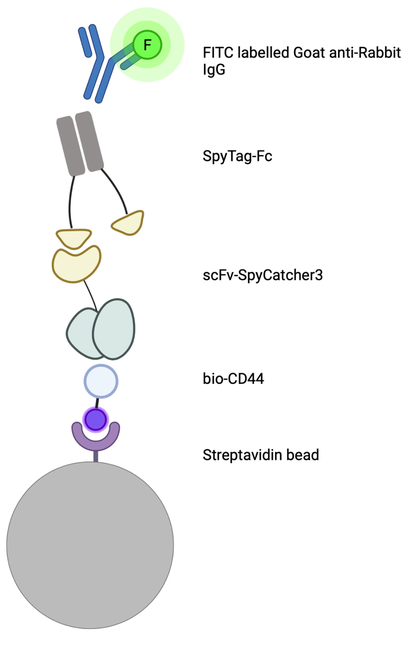
Chinese hamster ovary (CHO) cells are one of the most well-established and widely used systems for the production of recombinant therapeutic proteins. In contrast to other expression host systems, CHO cells enable complex posttranslational modifications and protein folding. However, cell productivity is often a limiting factor in large-scale manufacturing. Thus, stable mammalian cell lines with high expression yield are required for the industrial manufacture of recombinant proteins, including antibodies. This time-consuming and laborious method involves the extensive screening of a large number of cell clones from heterogeneous populations.

AIMS

- Discover and enrich single-chain variable fragments (scFv) against whole ExpiCHO cells and recombinant CD44 surface receptor by biopanning
- Produce scFv fragments as fusions with SpyCatcher
- Develop an efficient screening approach for the isolation of high-producing mammalian cell clones from those with a low level of protein production

MATERIALS AND METHODS





1. Selecting scFv fragments from synthetic antibody phage display libraries by biopanning
2. Screening of selected clones
3. Cloning selected single clones and polyclonal scFv gene libraries into the pHBSC3 vector to create scFv-SpyCatcher fusion proteins
4. Expressing fusion proteins in *E. coli* and purification
5. Coupling of scFv-SpyCatcher and SpyTag-Fc
6. Characterizing the performance of scFv-SpyCatcher/ SpyTag-Fc binders by flow cytometry

RESULTS

Panning

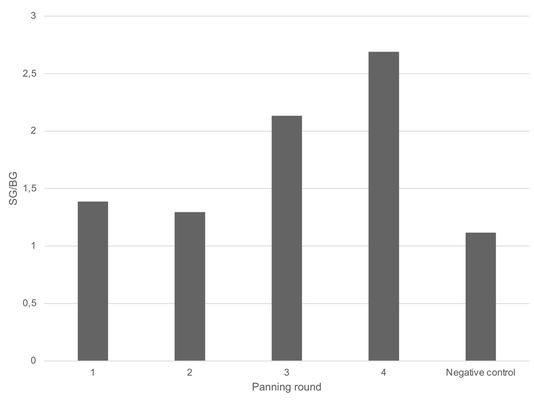


Figure 1. Immunoreactivity of phages. Phage stocks obtained from panning on ExpiCHO cells rounds 1-4 against ExpiCHO.

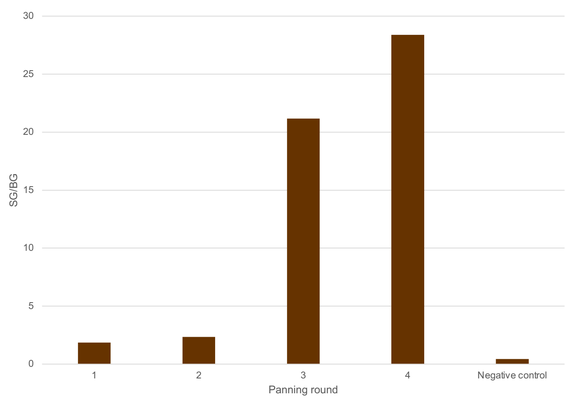


Figure 2. Immunoreactivity of phages. Phage stocks obtained from panning on bio-CD44 rounds 1-4 against bio-CD44.

Screening

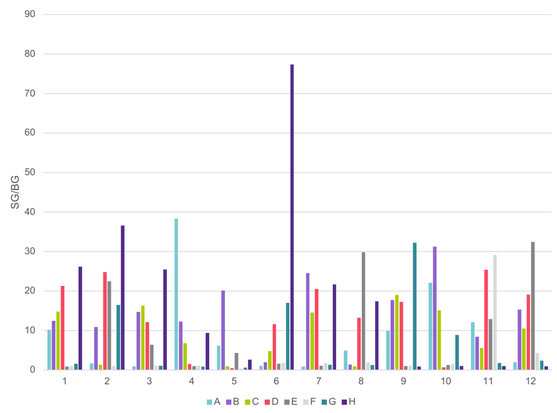
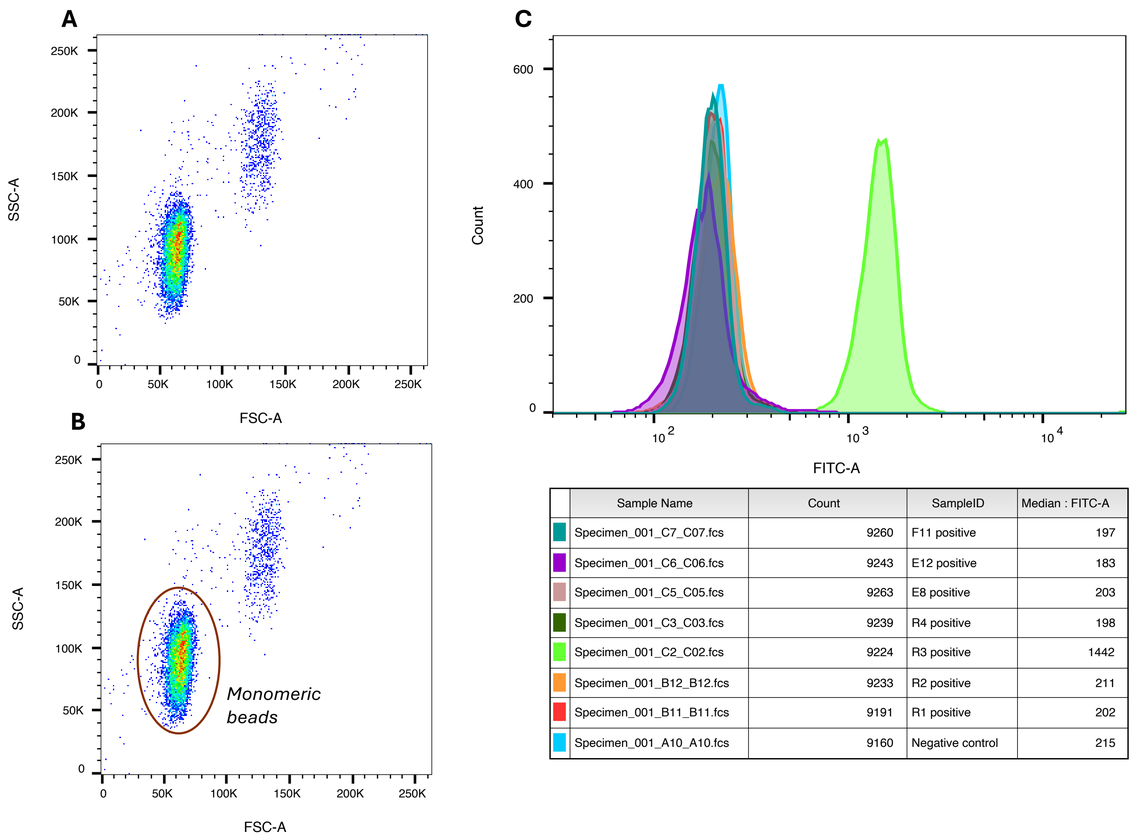


Figure 3. Screening of individual clones against bio-CD44.

S/B	1	2	3	4	5	6	7	8	9	10	11	12
A	10,2	1,6	0,9	38,3	6,1	1,1	0,9	4,9	9,9	22,1	12,1	2,0
B	12,4	10,9	14,7	12,2	20,1	2,0	24,5	1,4	17,7	31,3	8,4	15,3
C	14,8	1,4	16,3	6,8	0,9	4,8	14,5	0,9	19,0	15,1	5,5	10,5
D	21,3	24,8	12,1	1,5	0,5	11,6	20,5	13,2	17,3	0,7	25,4	19,1
E	0,9	22,5	6,4	1,0	4,3	1,6	1,0	29,8	1,0	1,3	12,9	32,4
F	1,1	1,1	1,1	1,2	0,3	1,8	1,8	1,9	1,2	1,5	29,1	4,3
G	1,6	16,5	1,1	0,9	0,6	17,0	1,3	1,3	32,2	8,9	1,8	2,4
H	26,2	36,6	25,5	9,4	2,6	77,4	21,7	17,4	0,8	1,0	1,0	1,0

Figure 4. Screening of individual clones. Signal to background ratio of scFv binders against bio-CD44.

Flow cytometry



	Sample Name	Count	SampleID	Median : FITC-A
	Specimen_001_C7_C07.fcs	9260	F11 positive	197
	Specimen_001_C6_C06.fcs	9243	E12 positive	183
	Specimen_001_C5_C05.fcs	9263	E8 positive	203
	Specimen_001_C3_C03.fcs	9239	R4 positive	198
	Specimen_001_C2_C02.fcs	9224	R3 positive	1442
	Specimen_001_B12_B12.fcs	9233	R2 positive	211
	Specimen_001_B11_B11.fcs	9191	R1 positive	202
	Specimen_001_A10_A10.fcs	9160	Negative control	215

Figure 5. Characterizing the performance of scFv-SpyCatcher003/ SpyTag003-Fc binders by flow cytometry. A) All events, B) Gated monomeric streptavidin beads and C) Histograms of bead assay where scFv-SpyCatcher003/ SpyTag003-Fc complexes were bound to beads via bio-CD44 and labelled with FITC labelled anti-Rabbit IgG.

CONCLUSIONS

- ScFv fragments against whole ExpiCHO cells and recombinant CD44 protein were successfully discovered and enriched from the synthetic antibody phage display libraries
- ScFv fragments were produced as fusions with SpyCatcher003 and were able to couple with SpyTag-Fc
- Flow cytometry results showed that only binders from 3rd panning round were able to recognize and bind to the bio-CD44
- Further optimization is required before moving on to cell-based detection