Predictable High-Yield CHO Cell Line Development via Site-Specific Integration

Authors: Wanjun Lan, Longzhi Lin, Michael Chen | DOI: 10.1186/s13036-025-00610-z

Abstract

Site-specific integration (SSI) accelerates CHO cell line development by directing genes of interest (GOIs) into genomic loci supporting stable, high expression. Using Bxb1 integrase–mediated SSI with AI-driven screening and robotic workflows, the authors established host lines achieving titers exceeding 15 g/L.

Uniformity of SSI-derived lines allows monoclonal performance prediction from minipools. Different types of proteins have exhibited high titer on the CHO-SSI platform, including monoclonal antibodies, bispecific antibodies, difficult-to-express proteins.

Methods

- Build CHO host cell lines with genomic landing pad.
- Bxb1 integrase for marker/GOI exchange (RMCE).
- AI + automation for high-throughput selection.
- Assess titer, stability, and quality in fed-batch.

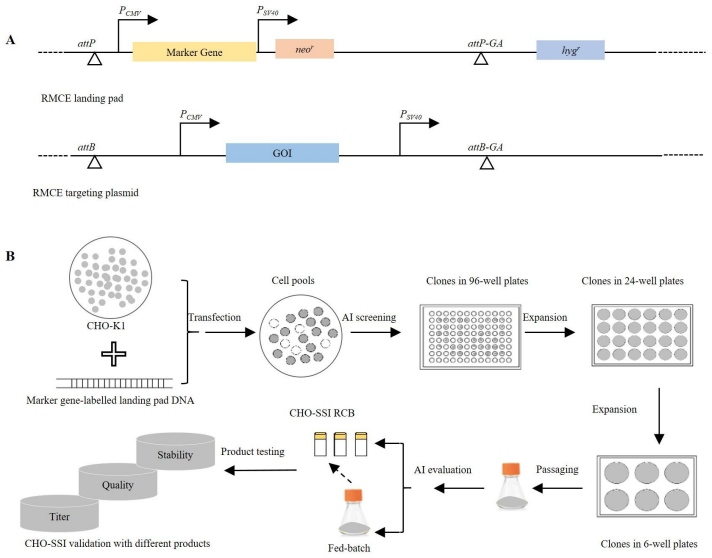


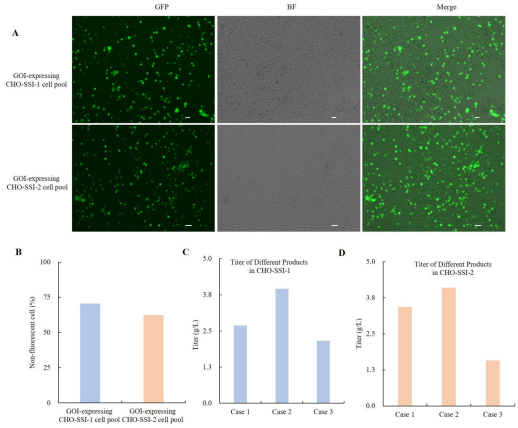
Fig. 1 | SSI platform workflow.

(A) Bxb1-mediated recombinase-mediated cassette exchange (RMCE). Maps of representative landing pad elements and target vector for RMCE.

(B) CHO-SSI screening workflow. Transfect CHO-K1 cells with linearized pLanding1.1 plasmid and EGFP-expressing cell pools are generated.

Results

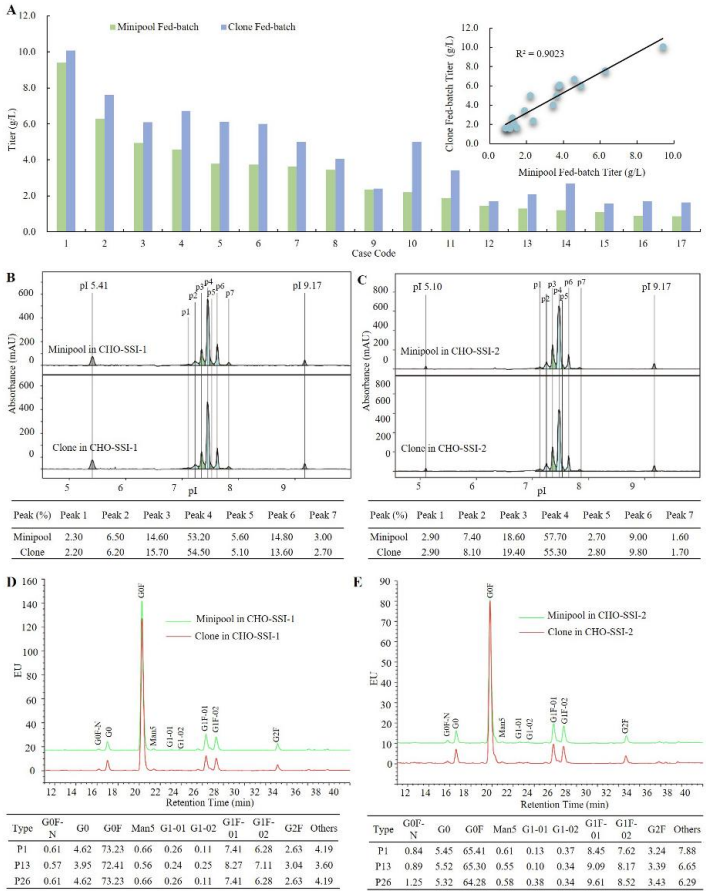
1 CHO-SSI host cell lines can be applied to the expression of different proteins.



（A）Images of minipools after transfecting different GOIs into CHO-SSI host cell lines.
（B）Proportion of non-fluorescent cells in minipools.
（C-D）Titer of monoclonal cells from 3 different cases in both CHO-SSI-1 (C) and CHO-SSI-2 (D) at day 14 of fed-batch culture.

After transfecting, the resultant cell pools contained both fluorescent and non-fluorescent cells, the proportion of non-fluorescent cells was generally greater than 60% after enrichment. Two different monoclonal antibodies (mAbs, case 1 and case 2) and a bispecific antibody (bsAb, case 3) were expressed in CHO-SSI-1 and CHO-SSI-2 respectively.

2 The Predictability and Consistency in CHO-SSI Host Cell lines



Peak (%)	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
Minipool	2.30	6.50	14.60	53.20	5.60	14.80	3.00
Clone	2.20	6.20	15.70	54.50	5.10	13.60	2.70

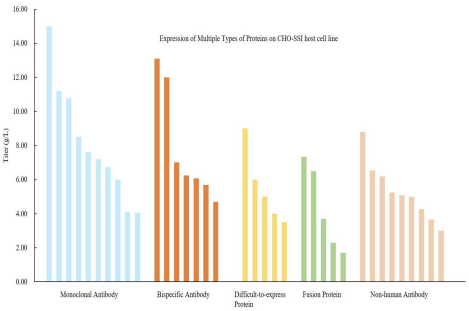
Peak (%)	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
Minipool	2.90	7.40	18.60	57.70	2.70	9.00	1.60
Clone	2.90	8.10	19.40	55.30	2.80	9.80	1.70

Type	G0F-N	G0	G0F	Man5	G1-01	G1-02	G1F-01	G1F-02	G2F	Others
P1	0.61	4.62	73.23	0.66	0.26	0.11	7.41	6.28	2.63	4.19
P13	0.57	3.95	72.41	0.56	0.24	0.25	8.27	7.11	3.04	3.60
P26	0.61	4.62	73.23	0.66	0.26	0.11	7.41	6.28	2.63	4.19

Type	G0F-N	G0	G0F	Man5	G1-01	G1-02	G1F-01	G1F-02	G2F	Others
P1	0.84	5.45	65.41	0.61	0.13	0.37	8.45	7.62	3.24	7.88
P13	0.89	5.52	65.30	0.55	0.10	0.34	9.09	8.17	3.39	6.65
P26	1.25	5.32	64.28	0.58	0.38	0.34	9.61	8.52	3.43	6.29

(A) Correlation between minipool titer and monoclonal cell titer in 14-day fed-batch culture.**(B-C)** Charge variation of minipool and monoclonal cell expressing the same product in CHO-SSI-1 (B) and CHO-SSI-2 (C); p refers to peak.**(D-E)** Glycan profile of minipool and monoclonal cell expressing the same product in CHO-SSI-1 (D) and CHO-SSI-2 (E).

3 CHO-SSI Host Cell Line Demonstrated Universality



The titer of multiple monoclonal antibodies exceeded 10 g/L (some even hit 15 g/L); several bispecific antibodies presented with a titer of more than 6 g/L (some even exceeded 12 g/L); for difficult-to-express proteins, fusion proteins and non-human antibodies, there were also cases with titer over 5 g/L.

Discussion

The platform demonstrates that SSI is superior to random integration for speed and predictability. By fixing the genomic location, researchers can focus on GOI optimization rather than screening thousands of clones for "lucky" integration events.

• Bxb1-mediated SSI enables predictable development. • Minipools anticipate monoclonal performance. • Platform demonstrates universality.