Utilization of *ProSpeed*™ Expression System To Empower Single B Cell based Antibody Discovery Platform

Derek Chen, Yu Liang, Lindi Wang, Wenwan Fang, Jie Ma

ABSTRACT

In the last four years, as a newly emerged technology featuring shorter timeline, better diversity and better developability, single B cell approach contributed to the discovery of almost 20% of FDA approved Ab drugs. However, this new technology platform also faces some limitations, including difficulty in functional screening, limited assay sensitivity and poor cost-effectiveness when the hit rate is low. To address these limitations, here we integrated a ***ProSpeed™*** linear expression system to the Beacon Single B cell platform, allowing a fast and cost-effective discovery of functional Ab leads.

CHALLENGES & SOLUTIONS

Due to the extremely low cell density, antibody concentrations in Beacon on-chip screening tend to be low and fluctuate substantially, which may lead to a number of limitations.

- **Difficulty in on-chip functional Assays** (RGA/ Enzymatic/ internalization/epitope binning/affinity ranking, etc.)
- **Reduced success rate for multi-round (>3) of on-chip screening**
- **Limited assay sensitivity leading to false negative/positive**
- **Poor cost-effectiveness when hit rate is low**

Fig 1. Limitations in Beacon-based single B cell screening

To confirm the binding & function of Ab sequences derived from exported single B cells, in the workflow of conventional Beacon screening (left panel), recombinant expression based on costly and time-consuming gene syntheses/plasmid preparation is used. In contrast, in a ProSpeed™ Expression workflow (right panel), a PCR based linear expression cassette is first constructed and used for transient expression, to allow the recombinant production of mAbs derived from the exported single B cells in the supernatant, and their functional characterization. Since not all exported B cells but only those secreting confirmed binding Abs with desirable function profile will be sequenced and pursued, the turnaround time needed from exported B cell to sequence of confirmed hits is greatly reduced, from 5-6 wks to 2-3 weeks, and with a substantial reduction of cost as well.

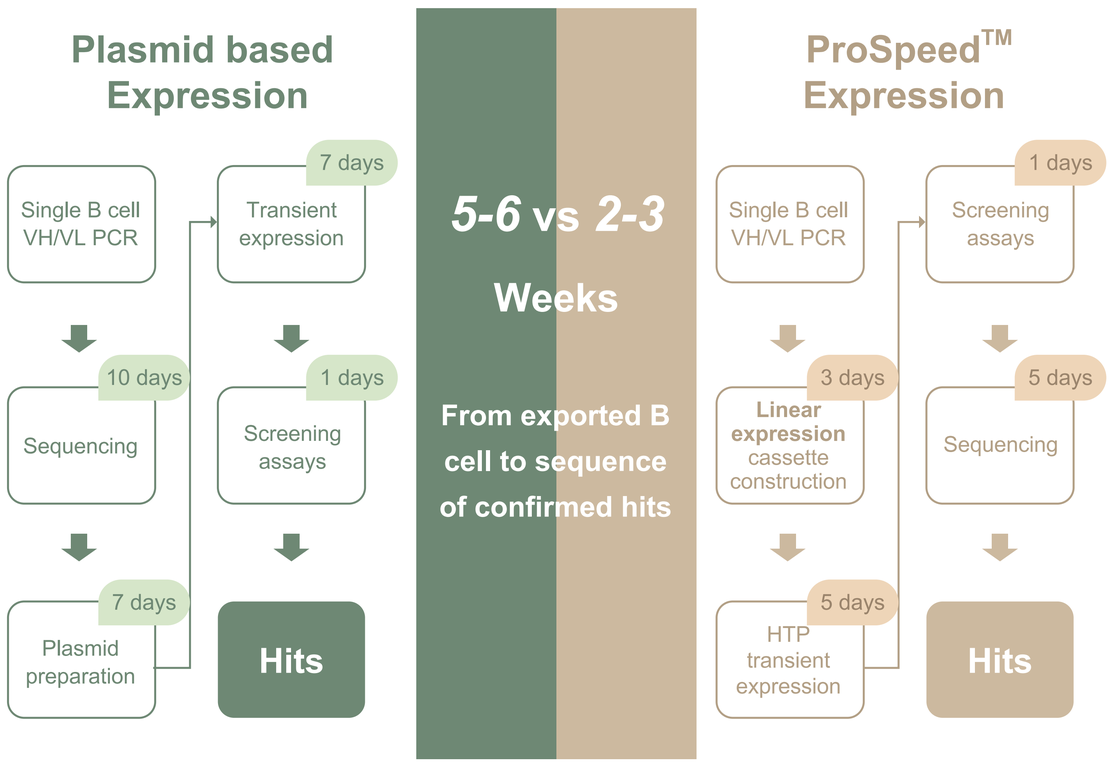
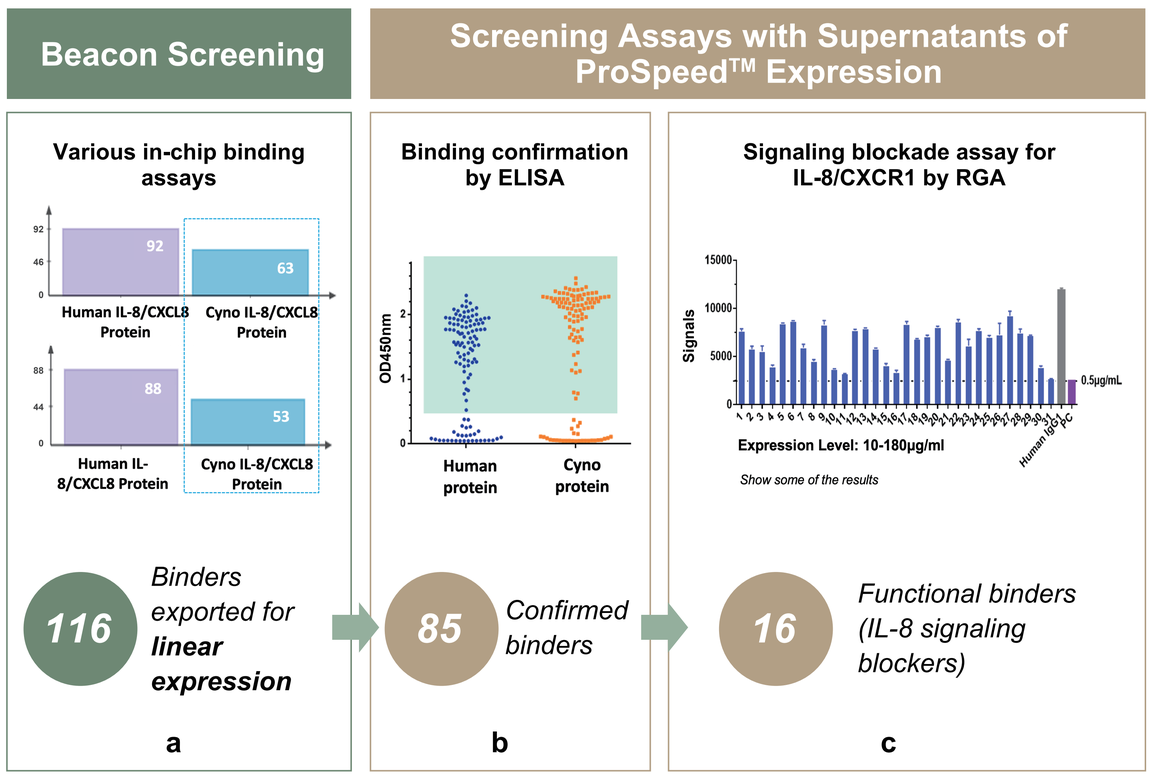


Fig 2. Workflow of plasmid-based and ProSpeed™ expression

APPLICATION SCENARIOS

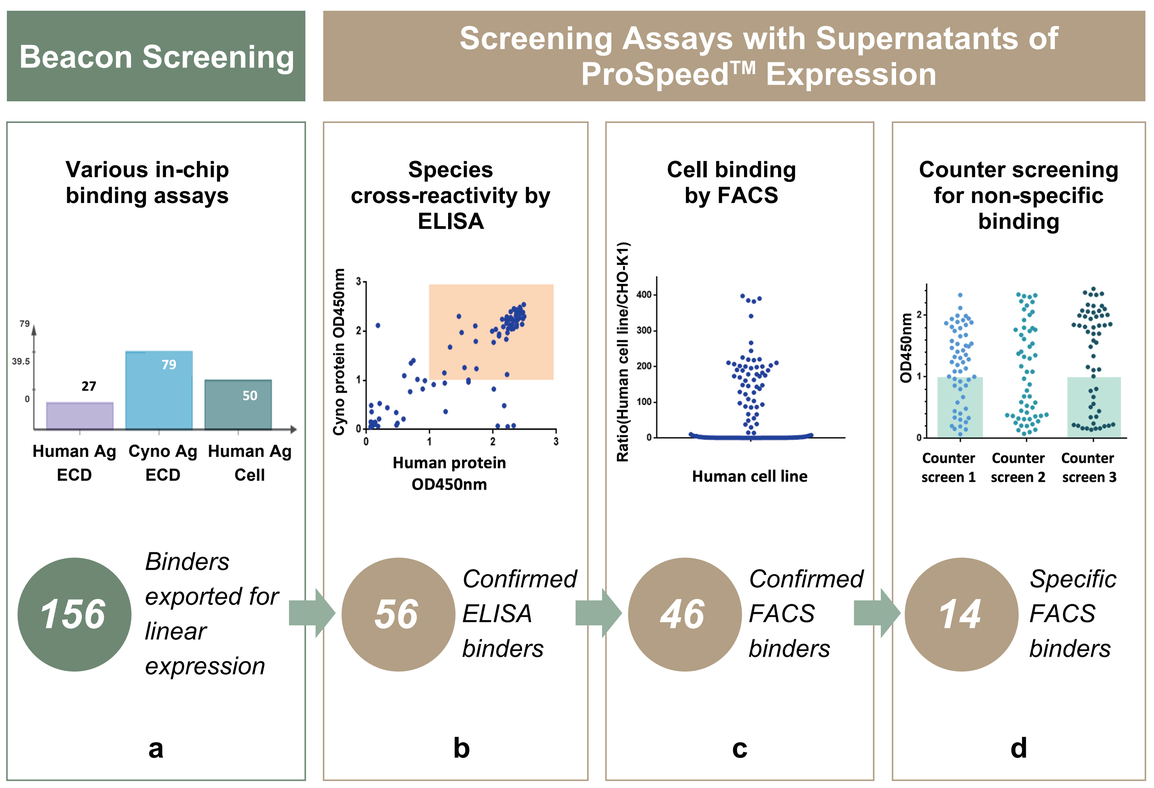
1. Functional Screening with Recombinant Supernatants

Fig 3. Functional screening assays were applied using ProSpeed™ expression supernatants to identify IL-8 mAbs that block the IL-8/CXCR1 signaling. (a) 116 positive B cells were identified by Beacon on-chip screening with human and cyno IL-8 proteins, followed by ProSpeed™ linear expression. The supernatant of ProSpeed™ expression were tested by ELISA for binding to human and cyno IL-8, confirming 85 hits were dual binders (b), among which 16 candidates were shown to block IL-8/CXCR1 signaling by reporter gene assay (c)



2. Multiple Rounds of Screening Beyond Beacon's Limits

Fig 4. Identification of specific binders against a matrix of positive and negative antigens by multiple rounds of screening. (a) 156 positive B cells were identified by Beacon on-chip screening with binding to human, cyno ECD proteins and human antigen cell line, followed by ProSpeed™ linear expression. Using this ProSpeed™ expression supernatant, 56 candidates were confirmed to be binders to hu and cyno Ag proteins by ELISA (b), and 46 candidates to be specific binder to human Ag cell line by FACS (c), among which only 14 showed low nonspecific binding to 3 undesired Ags (with high homology to the target Ag) in additional counter-screen assays (d)



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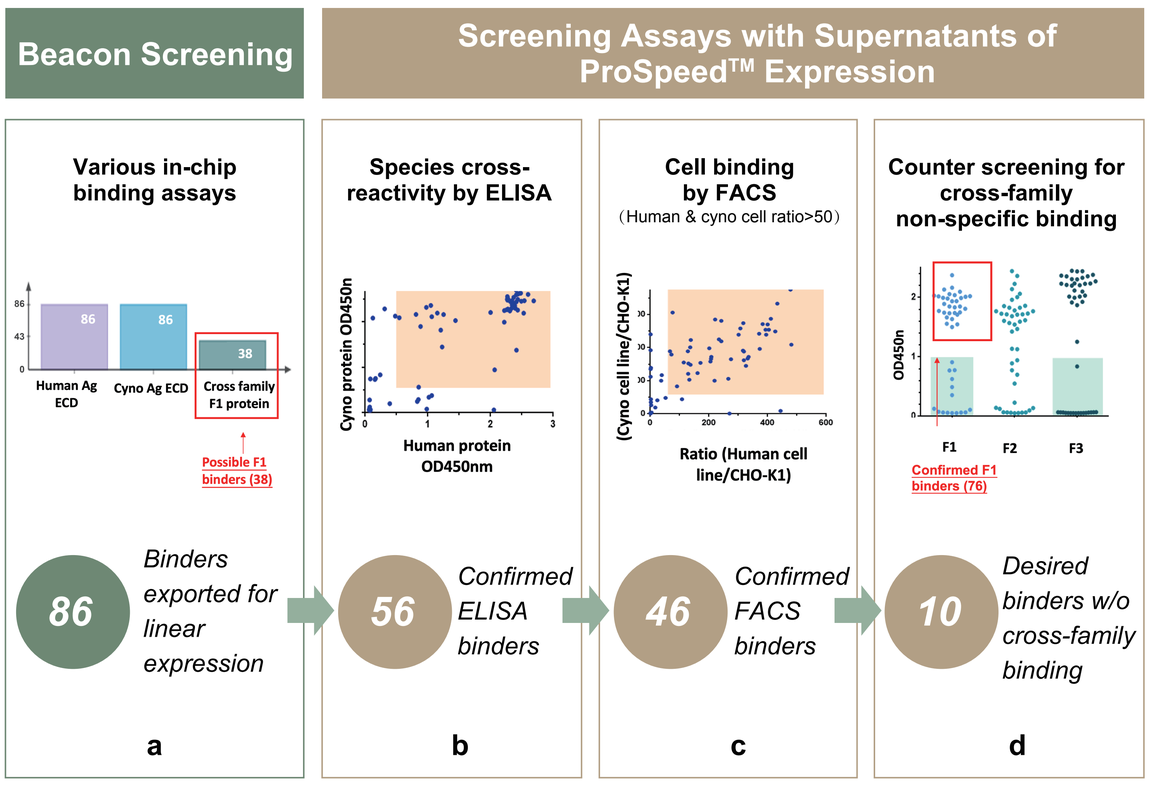
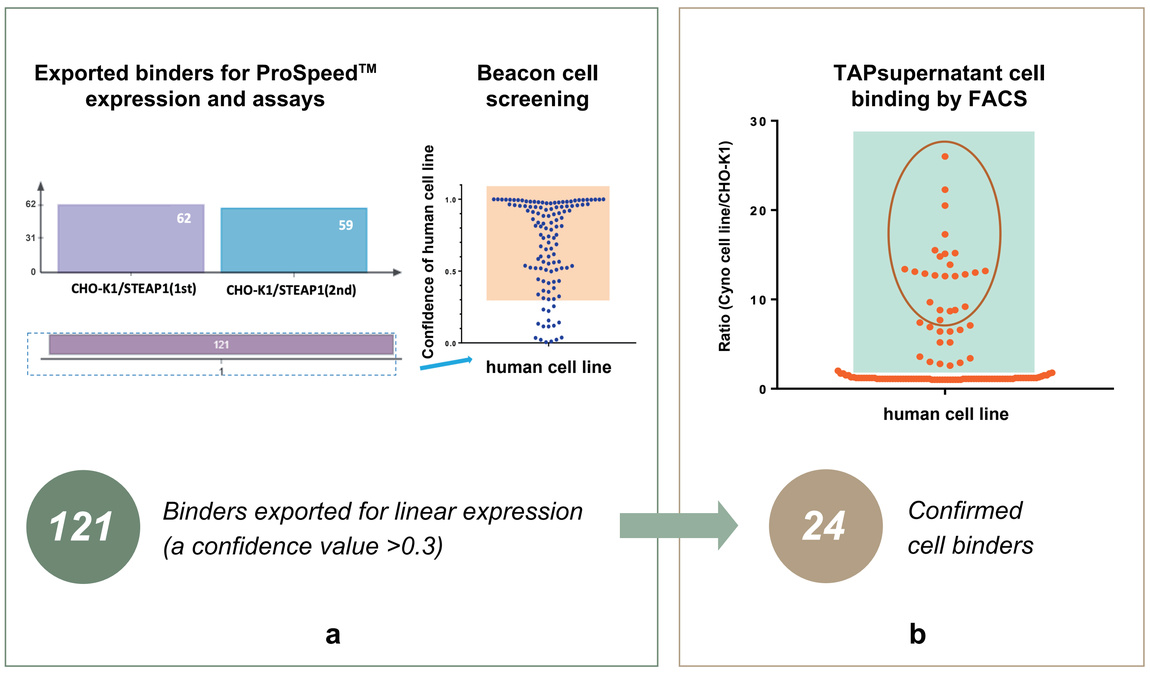
3. Improved Assay Sensitivity to Reduce False Negatives

Fig 5. (a) The initial on-chip Beacon screening identified 86 positive B cells binding to human and cyno antigen proteins, among which only 38 hits shown undesirable binding to cross-famility Ag F1. However, in the follow-up screening using supernatant of ProSpeed™ expression with improved assay sensitivity, 76 of them were shown to non-specifically bind to cross family Ag F1, and only 10 hits were confirmed to be the desirable low binders to all 3 cross-famility Ags F1, F2 and F3.



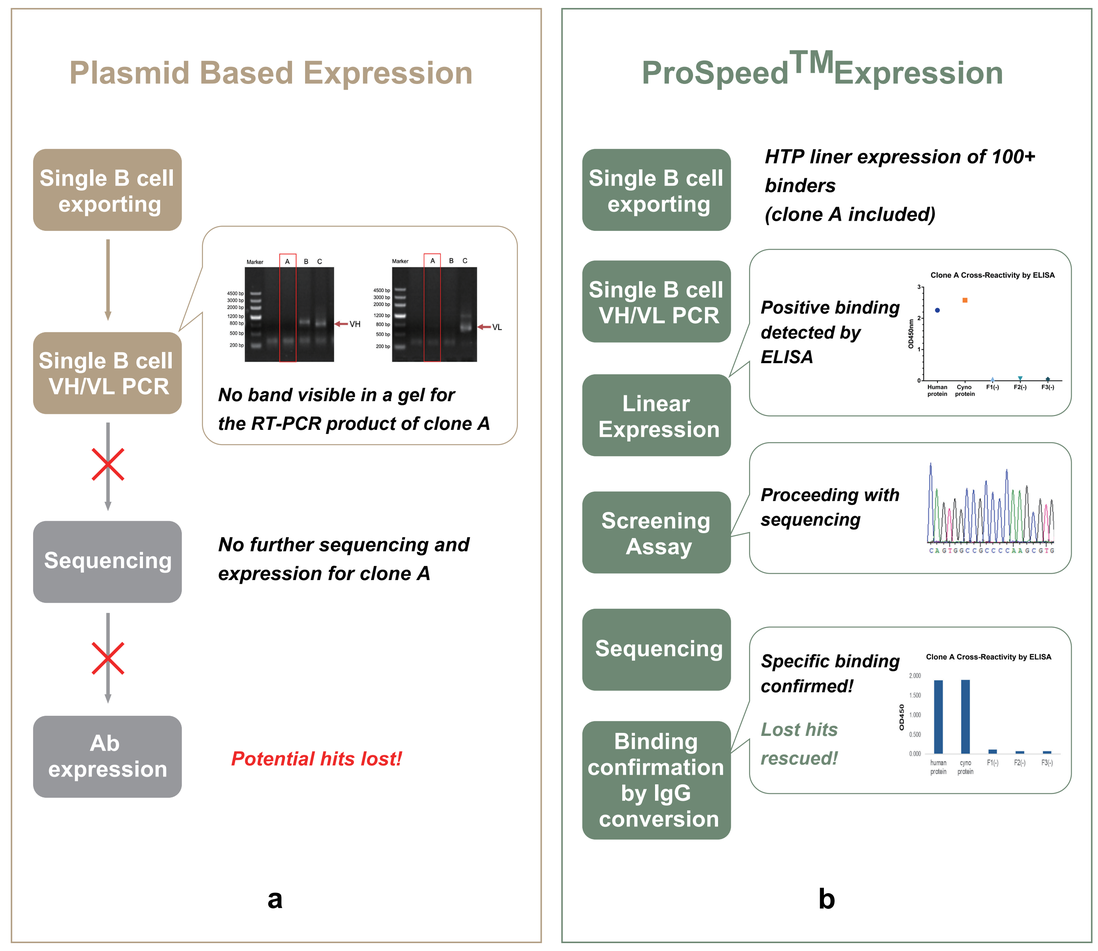
4. Improved Assay Sensitivity to Identify True Cell Binders

Fig 6. Due to the low and fluctuating Ab concentration in a Beacon on-chip screening, FACS binding data may not be conclusive especially when the cell surface Ag level is low. (a) initially 121 hits were identified by Beacon on-chip screening to be potential binders to Hu Ag cell. However, in a follow-up FACS assay using ProSpeed™ expression supernatant with improved assay sensitivity, only 24 of them were confirmed to be true cell binders (b).



5. "Rescuing" Missed Hits Due to Unsuccessful RT-PCR

Fig 7. (a) In the plasmid-based approach, the overall outcome heavily relies on the success of RT-PCR. If this is not successful, no further sequencing and expression of a certain B cell clone will be pursued and such a potential hit will be lost. (b) In a ProSpeed expression workflow, a nested-PCR with improved sensitivity was employed. Thus even trace amount of cDNA below the detection limit of a conventional PCR may still be effectively amplified and incorporated into the linear expression system, allowing further characterization and sequencing of such hits that may be missed in a conventional Beacon workflow.



CONCLUSION

In summary, the ProSpeed™ workflow addressed the bottlenecks of current single B cell platform based on Beacon, by the integration of a linear expression technology to Beacon platform. This upgrade of the Beacon platform allows high thruput functional screenings based on recombinant supernatant without the costly gene synthesis/plasmid preparation, makes it feasible for multiple rounds of screening beyond Beacon's limits, increases the assay sensitivity to reduce false negative/positive results, improves the cost-effectiveness of single B cell screening especially when the hit rate is low, and as a bonus may potentially "rescue" missed hits due to unsuccessful gene amplification by RT-PCR. Overall, with these inviting new features, the ProSpeed™ Single B Cell Ab Discovery Platform, coupled with our substantial experience of over 100 projects, may offer a favorable solution for antibody discovery campaigns with expedited timeline, good Ab sequence diversity and better cost-effectiveness.

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