Site-directed integration platform supports industry-relevant titers while reducing CLD time and effort

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Abstract

Traditional random integration (RI) cell line development (CLD) workflows are limited by the time and effort required, as well as by the risk of not isolating high-expressing stable clones. Until recently, attempts to address these limitations by using alternative workflows such as single-site integration have been thwarted primarily by subpar titers in a space where high titer is paramount. Improvements in expression vectors and integration site screens have enabled single-site integration platforms that support industry-relevant titers. Herein we share data generated in our new site-directed integration (SDI) platform that utilizes a novel integration site in the GOCHO™ host. This platform not only supports multi-gram/liter clones from a single integration site, but also saves ~ 4 wk as compared to RI while also substantially reducing the throughput required to identify high-producing clones. In addition, we will show the built-in long-term expression stability of the chosen integration site, which reduces project risk by allowing for process development to occur in parallel to clone expression stability confirmation, potentially saving an additional ~ 9 wk. Thus, the SDI CLD workflow has the potential to save ~ 13 wk over RI from transfection to initiation of process development. Finally, we will compare how changes in the SDI workflow impact clone titers.

(A) RI timeline

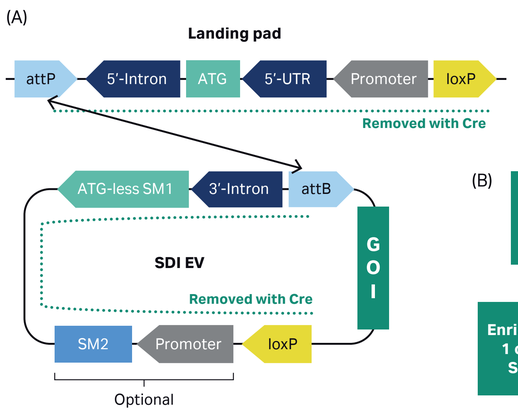
(B) SDI timeline

Fig 1. Timeline comparison between GOCHO CLD workflows. (A) RI timeline. From transfection to clone research cell bank (RCB) is ~ 15 wk. Expression stability is done prior to initiating process development (PD) since this information is necessary to define the top clone (= total ~ 24 wk from transfection to initiation of PD). (B) SDI timeline. From transfection to clone RCB is ~ 11 wk. In addition, due to successful screening for expression stability in the integration site, PD can occur in parallel to expression stability assessment, saving an additional ~ 9 wk as compared to RI (= total ~ 11 wk from transfection to initiation of PD).

Materials and methods

There are two key elements of SDI: the landing pad (LP)-modified host and the matched expression vector (SDI EV; Fig 2) to be integrated at the LP via site-specific recombination (herein using phiC31 integrase). Initial LP screening identified a locus that was compatible with high stable expression (Fig 3). Subsequent modifications of the LP were enabled by phiC31-directed integration of elements via the SDI EV. Clone data was generated from a Gen3 LP host. The original design and workflow includes two selection markers (SMs) and allows for elimination of cells containing off-site integration events. A second version of the SDI EV was generated with only 1 SM to assess a more streamlined workflow (Fig 2B).

(A)



(B)

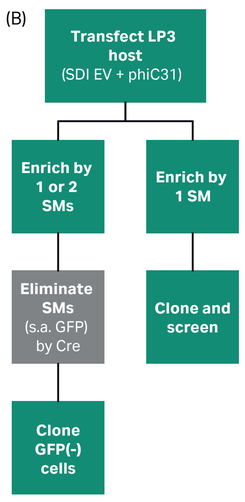


Fig 2. SDI overview. (A) SDI elements. An LP containing an attP phiC31 recognition site, promoter elements, and a loxP site was integrated into GOCHO cell line. The SDI EV contains an attB site as well as 1-2 selection markers (SM1 and SM2), a 2nd loxP site and gene(s) of interest (GOI) expression cassettes. Dotted lines: what Cre could remove post-integration: only SMs integrated at the LP will be removed by Cre. (B) 2- vs 1-SM workflows.

Results

Screening candidate LP loci by titer and expression stability

GOCHO cell line was transfected with a vector containing Gen1 LP elements plus an artificial mAb comprised of LC plus an HC-GFP fusion protein. With the demonstrated correlation between titers and GFP expression (Fig 3A), GFP was used to enrich for high expression cells (Fig 3B) before single-cell cloning. Clones identified as having a single copy of LP by PCR were assessed by titer (Fig 3C) and GFP (Fig 3D) to identify loci supporting high stable expression over time.

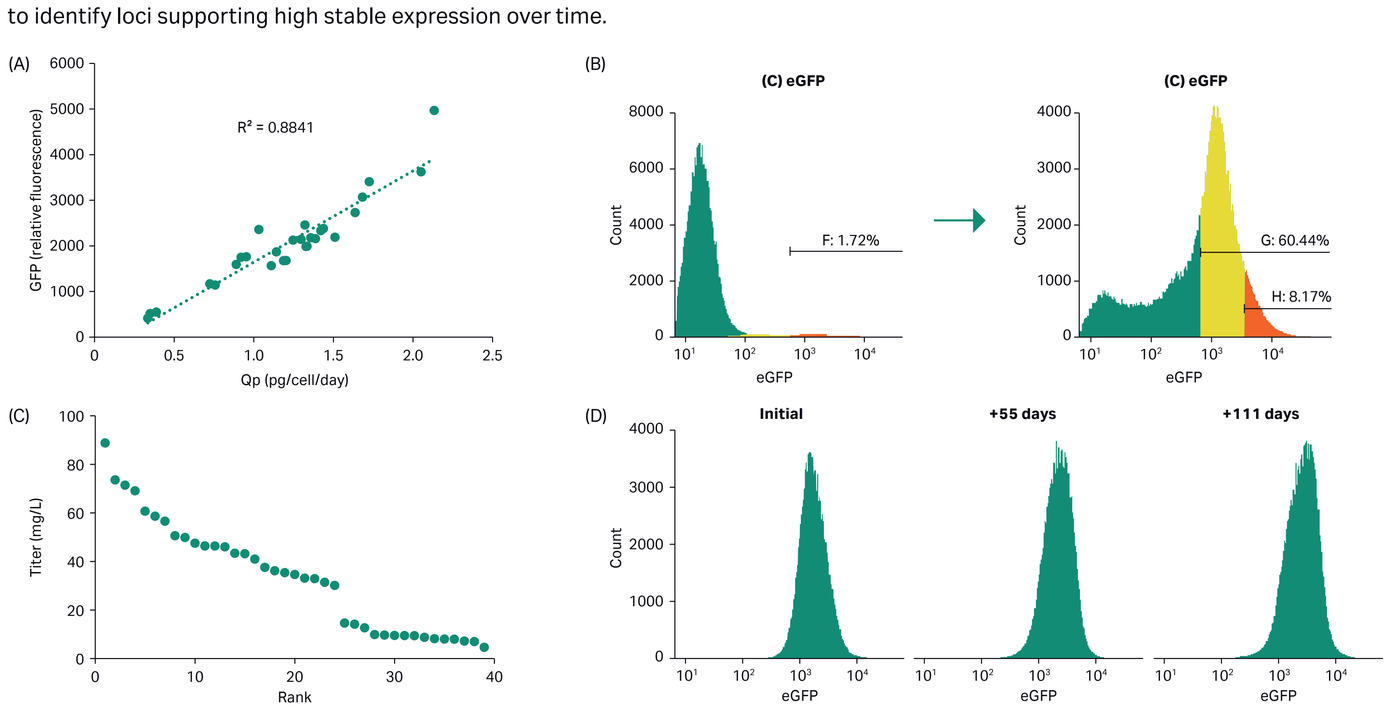


Fig 3. LP site screening via titer and expression stability. (A) Correlation of specific productivity with GFP expression in cells expressing LC + HC-GFP. (B) GFP enrichment of LP integration events. Clones were isolated from regions G and H of the right histogram. (C) Titer in clones identified by PCR to have integrated a single copy of the LP. (D) Example of GFP expression stability over time in a single-copy LP clone candidate.

SDI workflow clones versus RI

Clones from 3 CLD workflows were compared for titer and expression stability with the same model mAb. While all RI clones expressed higher than SDI clones, only one RI clone (RI-1) was clearly stable through 60 generations, and this required G418 to be included in the train. All six SDI clones were defined as stable for > 60 generations, and without selection pressure. We have yet to isolate an SDI clone that does not maintain titers > 70% of initial values (DNS). Work to optimize titers in SDI is ongoing.

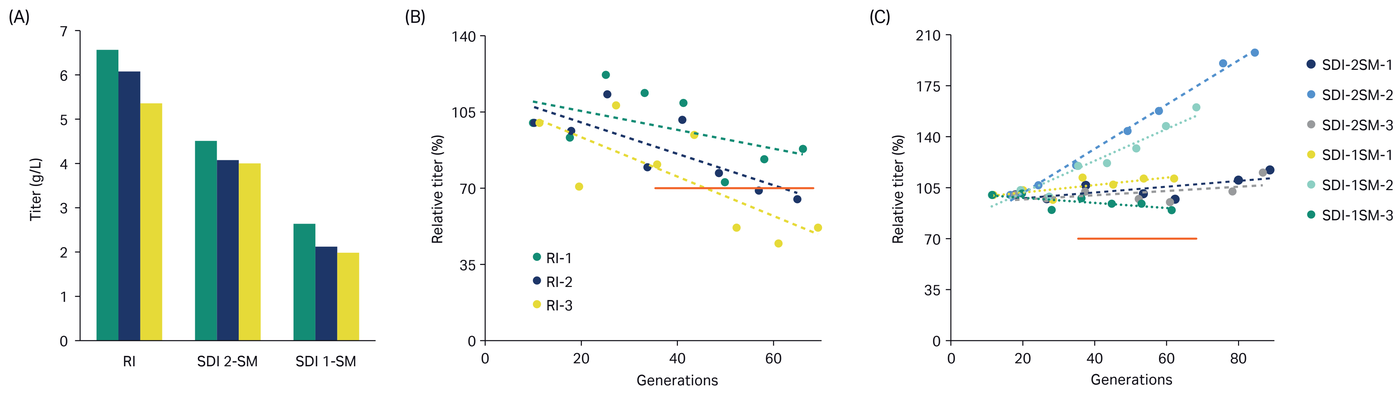


Fig 4. Final clone titers and expression stability. Clones expressing the same model mAb were isolated from the 3 different workflows and compared in ActiPro™ medium + Cell Boost™ 7a/7b fed-batch (A), as well as in expression stability over 60+ generations (B-RI; C-SDI). Orange line: expression stability limit (70%).

RI versus SDI workflow timelines

A single model mAb was taken through 3 workflows: RI, 2-SM SDI, and 1-SM SDI. Actual time needed for each phase is shown.

Table 1. RI vs SDI timelines. Workflow comparison between RI, 2-selection marker SDI (SDI, 2-SM), and 1-selection marker SDI (SDI, 1-SM). Note: Regarding FTE, RI requires high-throughput screening twice (1st in selection, 2nd in cloning), while SDI only requires high throughput screening of clones and at half the scale used for RI.

Workflow	Phase 1	Phase 2	Phase 3	Total time
RI	Transfect/select (~ 7 wk)	Clone + screen (~ 8 wk)	Stability (~ 9 wk*)	24 wk to identify top clone (*requires stability assessment)
SDI, 2-SM	Transfect/GFP-enrich/Cre SM removal (~ 4 wk)			12 wk *stability not required to advance top clone to PD
SDI, 1-SM	Transfect/select (~ 3 wk)			11 wk *stability not required to advance top clone to PD

Acknowledgments

The primary SDI designs (LP, SDI EV), modifications, and screens were a team effort led by Daniel and Ann (Sweden). The RI and 1-SM workflow modification + testing, as well as final titer stabilities, were led by Michelle (USA). The two teams thank the many Cytiva associates not listed for their input and support.

Conclusions

Clones expressing > 4 g/L were readily isolated from RI and the 2-SM SDI workflow. Reasonably high titer clones (> 2.5 g/L) were isolated using a simpler 1-SM SDI workflow, thus giving CLD flexibility to prioritize time/effort/cost as needed per project. Both SDI workflows provide the distinct advantages over RI of shorter timelines, less labor, and built-in expression stability. With an additional week of work, Cre removal of the SMs from the SDI LP provides a clear titer advantage.

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