GINKGO BIOWORKS

Chemical Transfection of Mammalian Cells using Ginkgo's Reconfigurable Automation Cart (RAC) Platform

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93%

mean chemical transfection efficiency

< 2%

CV in chemical transfection efficiency

80%

hands-on time reduction with fully automated (RACs) execution vs. manual execution

Chemical Transfection of Mammalian Cells using Ginkgo's Reconfigurable Automation Cart (RAC) Platform

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INTRODUCTION

Chemical transfection is a common laboratory technique used to introduce foreign nucleic acids into mammalian cells, via nucleic acid-encapsulating positively charged chemicals, such as lipofectamine, which facilitate fusion with a negatively charged cell membrane.[1] The method has a wide range of applications, including protein production and precision genome editing.[2,3] Fully automated chemical transfection is foundational to mammalian cell high-throughput screening, but requires special attention to accurate, precise, timely and sterile execution, to enable robust hit identification.

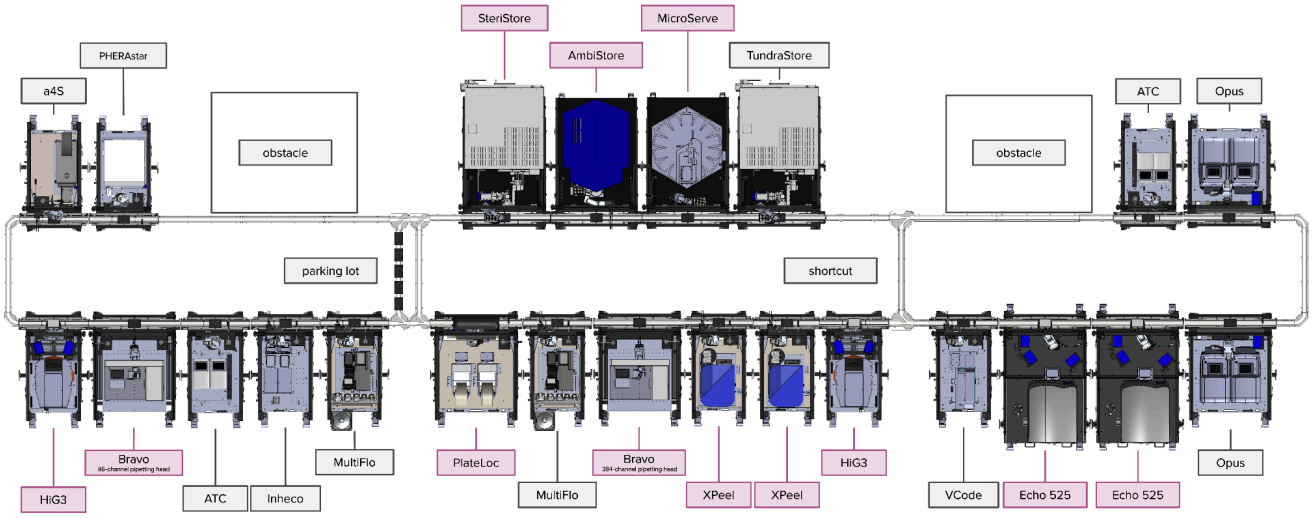

Figure 1. Internal Ginkgo RAC system used to execute fully automated chemical transfection. RACs encompassing devices relevant to the process are highlighted in pink (ambient temperature storage - AmbiStore, HighRes Biosolutions; cold storage - SteriStore, HighRes Biosolutions; tip box storage - MicroServe, HighRes Biosolutions; centrifugation - HiG3, BioNex; hit picking via acoustic dispensing - Echo 525, Beckman Coulter; plate seal peeling - XPeel, Azenta; plate sealing - PlateLoc, Agilent; plate stamping - 2x Bravo with 96-channel and 384-channel pipetting head, Agilent); RACs are connected via the MagneMover LITE (Rockwell Automation) transport system.

Here, we showcase a fully automated chemical transfection using Ginkgo's Reconfigurable Automation Carts (RACs), used to transiently express green fluorescent protein (GFP) in Expi293F human cells (Fig 1). **The fully automated protocol unlocked a significant (80%) hands-on time reduction, while generating high-quality results, on par with the previous manual protocol. A total of 384 independent chemical transfections were executed across four 96-well plates in less than 1.5 hours, without any contamination issues observed. The mean chemical transfection efficiency was 93%, with < 2 %CV - overall demonstrating the RAC platform's utility in demanding high-throughput mammalian biology work.**

Table 1. Chemical transfection - experimental conditions

Protocol Execution	Variables		Number of Replicates
	Transfection Reagent	Plasmid DNA	
RAC	+	+	192
	+	-	192
Manual	+	+	8
	+	-	8
	-	-	8

Table 2. Chemical transfection protocol on Ginkgo's RAC system

Steps #	Step Description*	Instruments / RACs Utilized
Step 1	Mix pDNA in its 384-well source plate; quickly centrifuge it; un-seal 384-well source plate	BioShake orbital shaker, HiG centrifuge, XPeel automated plate seal remover
Step 2	Cherry pick pDNA or water from the 384-well source plate into an empty 384-well destination plate	Echo acoustic liquid handler
Step 3	Dilute pDNA by stamping in and mixing in Opti-MEM medium from a 384-well source plate to the 384-well destination plate from step 2	Bravo liquid handling platform (384 channel pipetting head)
Step 4	Mix ExpiFectamine 293 reagent in its 384-well source plate; quick centrifuge it; and un-seal the 384-well source plate	BioShake orbital shaker, HiG centrifuge, XPeel automated plate seal remover
Step 5	Hit pick ExpiFectamine 293 reagent from its 384-well source plate into an empty 384-well destination plate	Echo acoustic liquid handler
Step 6	Dilute ExpiFectamine 293 reagent by stamping in and mixing in Opti-MEM medium from a 96-well source plate to the 384-well destination plate from step 5 (four stamps into quardrants 1 through 4)	Bravo liquid handling platform (96 channel pipetting head)
Step 7	Incubate at room temperature for 5 minutes	Bravo liquid handling platform deck
Step 8	Stamp the diluted pDNA or water from the 384-well plate in step 3 into the diluted ExpiFectamine 293 reagents in the 384-well plate in step 6; mix well (quardrant 1 stamp to quardrant 1, 2 to 2, 3 to 3, 4 to 4)	Bravo liquid handling platform (96 channel pipetting head)
Step 9	Incubate at room temperature for 18 minutes for complexation	Bravo liquid handling platform deck
Step 10	Stamp pDNA / water and ExpiFectamine 293 reagent complex from the 384-well destination plate in step 8 to cells in four 96-well deep-well plates; mix well (quardrant 1 stamps to deep-well plates 1, 2 to 2, 3 to 3, 4 to 4)	Bravo liquid handling platform (96 channel pipetting head)

**Transport of plates and tip boxes on the MagneMover LITE transport track occurs throughout step execution; De-lidding and re-lidding of plates are handled by the Bravo liquid handler gripper. Plate sealing is done using Agilent PlateLoc plate sealer. Transport, lid handling, and plate sealing steps are omitted in this process table.*

RESULTS

The fully automated protocol achieved high chemical transfection efficiency and transient protein expression at levels equivalent to manual chemical transfection.

The fully automated protocol (Tables 1 and 2) took less than 1.5 hrs to complete and did not require any in-person monitoring (Fig 2). The chemically transfected mammalian cell cultures were incubated offline at 37°C with shaking, CO_2 and humidity control for 3 days. Transient expression of GFP was measured via flow cytometry, which enabled relative GFP fluorescence quantification at the single-cell level. GFP positive (GFP+) population was established by gating for single cells exhibiting GFP fluorescence intensity beyond the cutoff, set by untransfected cells, not expressing GFP (Supplementary Fig 1).

Chemical transfection efficiency for each sample was quantified by dividing the number of GFP+ cells by the total number of cells. **For 192 biological replicate transfections with plasmid DNA (pDNA), executed by Ginkgo's RAC system, the mean transfection efficiency was 93.3%, and was comparable to the manual transfection efficiency (Fig 3a).** Relative transient GFP expression was determined using the GFP mean fluorescence intensity (MFI) values. The average MFI of cells transfected with pDNA and processed by Ginkgo's RAC system was over 2 orders of magnitude higher compared to the average MFI of cells transfected with water only. These results are comparable to the results obtained from the manual protocol (Fig 3b, c).

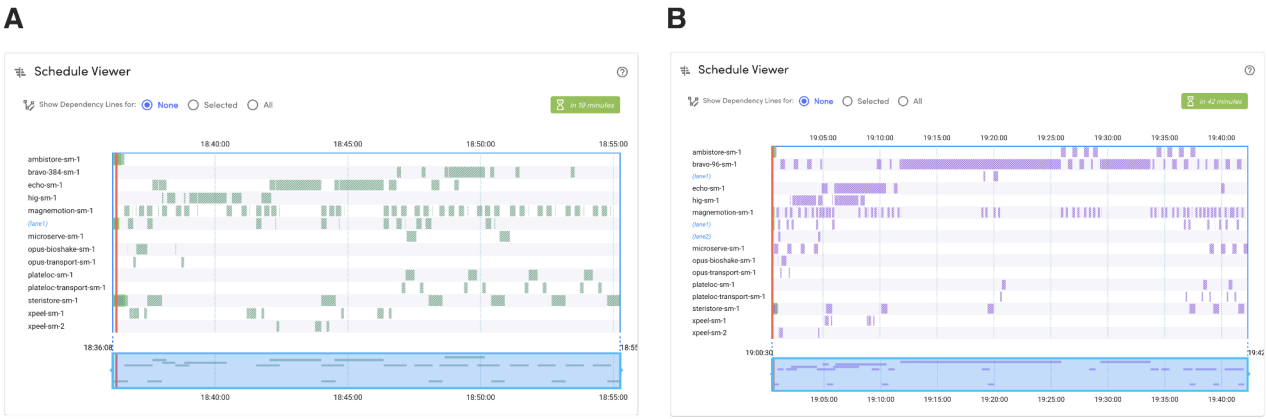

Figure 2. Catalyst ACS schedule viewer to monitor chemical transfection protocol execution. The protocol was separated into two sequential sub-processes: (A) plasmid DNA preparation followed by (B) complexation of transfection reagent and plasmid DNA. The fully automated protocol successfully completed 384 independent chemical transfections in less than 1.5 hours.

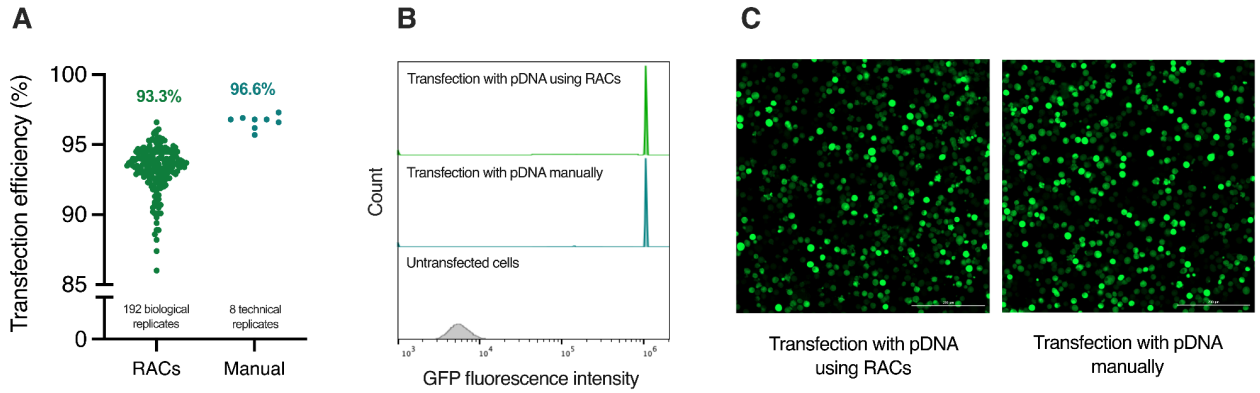

Figure 3. The fully automated protocol achieved high chemical transfection efficiency and transient protein expression at levels equivalent to manual chemical transfection. (A) For 192 biological replicate chemical transfections executed on the Ginkgo's RAC system, the mean efficiency was 93.3%, only 3.3% less than the mean efficiency of 8 biological replicate manual chemical transfections. Out of 192 biological replicate chemical transfections on the RAC system, 184 of them (95.8%) resulted in > 90% transfection efficiency. (B) The fluorescence intensity of GFP in transfected cells processed on the Ginkgo's RAC system was equivalent to that in cells transfected manually. (C) Representative fluorescence images of GFP expression in transfected cells processed on the Ginkgo's RAC system and in cells transfected manually.

Chemical transfection using RACs demonstrated low intra-plate and inter-plate biological replicate variability.

The intra-plate variability was determined using the coefficient of variation (CV) of samples within each plate, whereas the inter-plate variability was determined using CV of samples in all plates. Among biological replicate chemical transfections with pDNA in each 96-well plate, CV of chemical transfection efficiency ranged between 1.5% and 1.9%, CV of MFI ranged between 5.5% and 12.1%, and CVs of cell viability (measured with a dead cell stain during flow cytometry) were all under 1.0% (Fig 4a, b, c). Across biological replicate transfections with pDNA in all the plates, CV of chemical transfection efficiency was 1.8%, CV of MFI was 9.9%, and CV of cell viability was 0.3%. **These results thus showed low variability between biological replicates within the same plate and across different plates.**

No sample cross-contamination was detected during fully automated protocol execution.

Chances of sample cross-contamination could rise with increasing throughput, plate density or reduced direct scientist oversight. Therefore, we intentionally created a plate layout allowing us to detect any sample cross-contamination events. Each plate harbored an alternating pattern of chemical transfections with pDNA and with water only (no pDNA) (Fig 5a). **After examining GFP expression among cells chemically transfected with water only, we confirmed absence of detectable sample**

cross-contamination, by comparing the %GFP+ values to the flow cytometry gating control established using untransfected cells (Fig 5b-d).

The RAC platform helps flexibly increase protocol scale and throughput, while ensuring high walk-away time and attention to pre-defined time constraints.

The fully automated chemical transfection enabled scientists to load samples and confidently walk away, while the Ginkgo Automation Support Team remotely monitored protocol execution (as part of Ginkgo's Catalyst Flow Managed Automation Solution offering). Throughout protocol execution, the RAC Automation Control Software (Catalyst ACS) continuously optimized the sequence of protocol steps being executed, taking into account user-defined time constraints (particularly during complexation of chemical transfection reagents with plasmid DNA), therefore both maximizing throughput and ensuring high protocol execution quality (Fig 2). **In this study, the fully automated protocol successfully completed 384 independent chemical transfections in less than 1.5 hours, utilizing 9 RACs in total (Fig 1), resulting in 80% hands-on time reduction.** It would cost an experienced scientist > 1 hour to manually perform the same chemical transfection using the same set of devices. The rate-limiting protocol step was liquid handling with a 96-channel pipetting head, performed on a single Agilent Bravo RAC. In the future, this throughput bottleneck could be addressed by a simple and quick addition of another copy of that RAC.

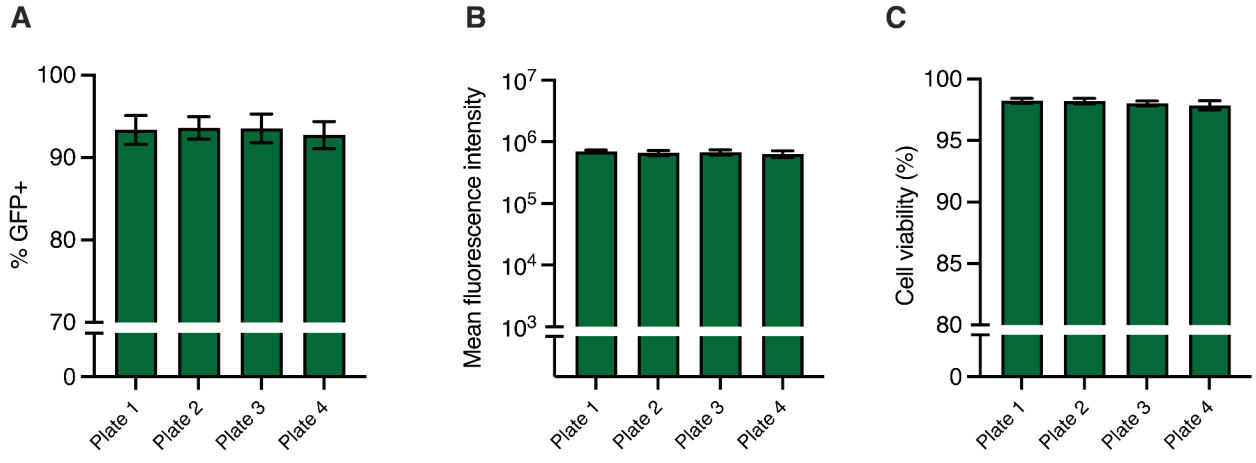

Figure 4. **Chemical transfection using RACs demonstrated low intra-plate and inter-plate biological replicate variability**. Among replicate transfections with pDNA in each 96-well plate, (A) the mean chemical transfection efficiency was between 92.7% and 93.6%, with CV between 1.47% and 1.87%, (B) the average MFI of GFP was between 6.4e5 and 7.0e5, with CV between 5.5% and 12.1%, (C) the mean cell viability was between 97.0% to 98.2%, with CV all under 1.0%.

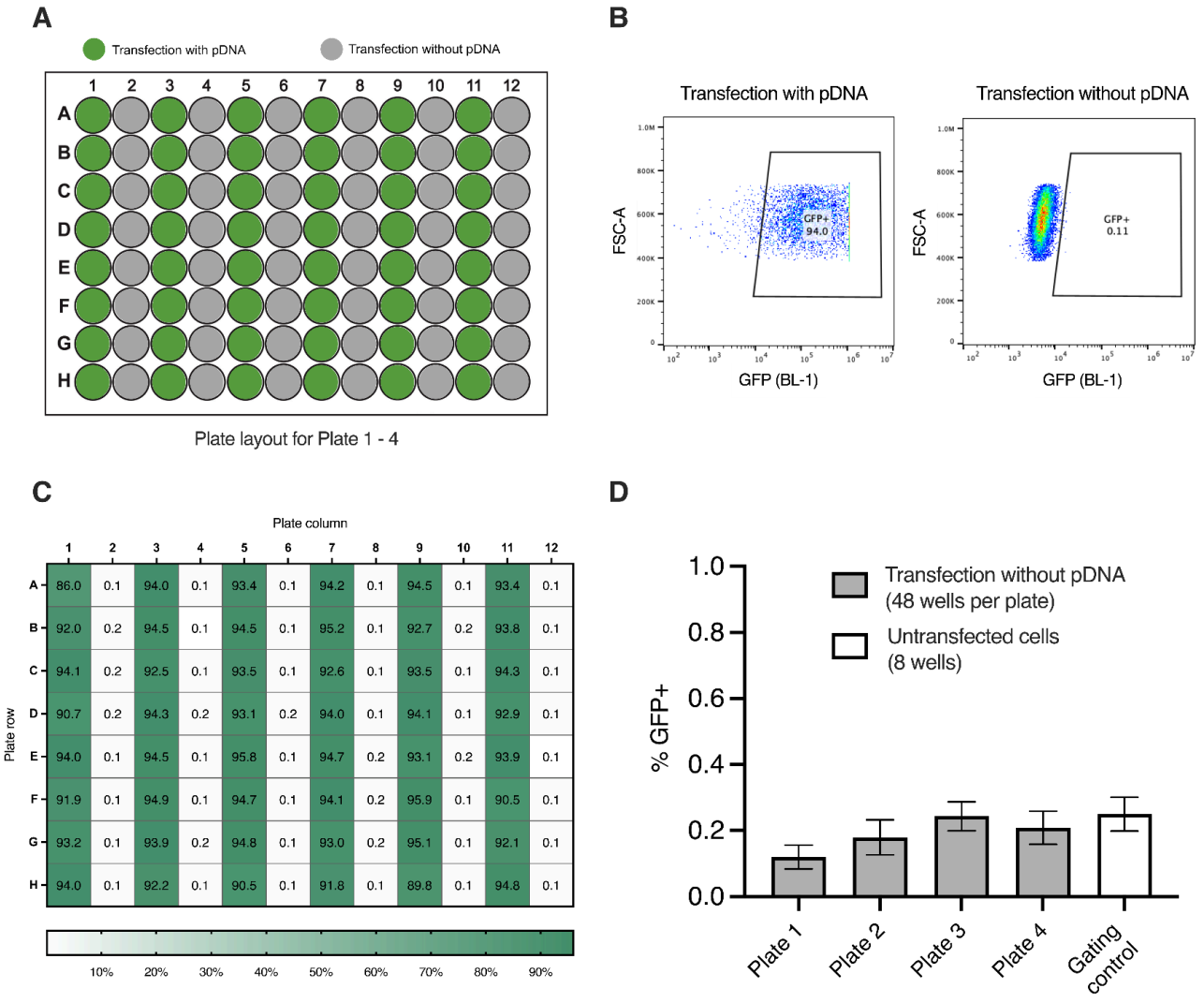

Figure 5. No sample cross contamination was detected during fully automated protocol execution. (A) Plate layout with alternating pattern of chemical transfection with and without plasmid DNA for detection of sample cross contamination. (B) Dot plots showing GFP expression in single cells transfected with and without GFP by the RAC system. Transfection with plasmid DNA resulted in expression of GFP, detectable via flow cytometry. Transfection without plasmid DNA in the absence of contamination showed lack of meaningful GFP signal. (C) Heat map of %GFP+ cells in Plate 1 executed by RAC system. (D) None of the chemical transfections without addition of plasmid DNA executed by Ginkgo's RAC system resulted in %GFP+ above 0.4%, similar or lower than that of the flow cytometry gating control established using untransfected cells.

CONCLUSIONS

In this study, we showcased successful chemical transfection for transient protein expression in human cells using RACs. The high quality of the fully automated protocol execution was demonstrated by high chemical transfection efficiency and protein expression, low variability between biological replicates, and absence of sample cross-contamination. The RAC platform hardware, software and complementary services overall ensured robust, time-efficient, issue-free and walk-away protocol execution, with options to expand throughput and functionality (e.g. additional upstream or downstream protocol execution) via straightforward relevant RAC additions.

More broadly, the chemical transfection protocol described is core to a broad range of research areas, including antibody drug development, functional genomics studies, gene therapy, and drug discovery. Therefore, its successful full automation, using Ginkgo's RAC system, demonstrates the RAC platform's potential in executing demanding, time-sensitive mammalian biology work.

GLOSSARY

Catalyst Automation Control Software (Catalyst ACS): a modern, web-based software for controlling our RAC systems. It allows dynamic scheduling and running of multiple workflows for multiple users, at the same time, through constraints-based protocol interleaving, ensuring maximum instrument device utilization and uptime.

Catalyst Flow: ticketless automation support service. It enables our customers to access our automation team as an extension of their own. We continuously monitor (5 days/week, 15 hours/day) our customers' RAC systems, resolving 80-90% of errors in real time, remotely - without the end user needing to alert us of the problem.

Reconfigurable Automation Cart (RAC): a standardized enclosure for integrating scientific instrumentation.

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MATERIALS AND METHODS

Cell culture

Expi293F cells, culture media, and transfection reagents were purchased as part of the Expi293 Expression System Kit (Thermo Fisher, A14635). Cells were thawed, subcultured and scaled up for transfection in shake flasks following the Expi293 Expression System user guide (Pub. No. MAN0007814 C.0) from the manufacturer.

Transfection

The transfection workflow was adapted from the manufacturer's user guide (Pub. No. MAN0007814 C.0) to transfect 0.5 µg of GFP-expressing plasmid DNA (pDNA) (Lonza Biosciences) into 1.5e6 cells in 500 µL per well in a 96-well round bottom 1.1 mL polypropylene deep well plate (Corning, P-DW-11-C-S) using 2.7 µL of ExpiFectamine 293 reagent. Expi293F cells were seeded to 3.0e6 viable cells/mL in one shake flask and grown to a density between 5.0-5.5e6 cells/mL (20-24 hours). Immediately before transfection, cells were diluted to 3.0e6 viable cells/mL in Expi293 Expression Medium (Thermo Fisher, A14635) supplemented with Antibiotic-Antimycotic (Thermo Fisher, 15240062) and aliquoted into deep well plates. After transfection, cells were cultured in 96-well round bottom plates with shaking at 37°C, 80% humidity, 8% CO_2, 850 rpm. Transfection enhancers were not added to transfected cells.

Definition of experimental conditions

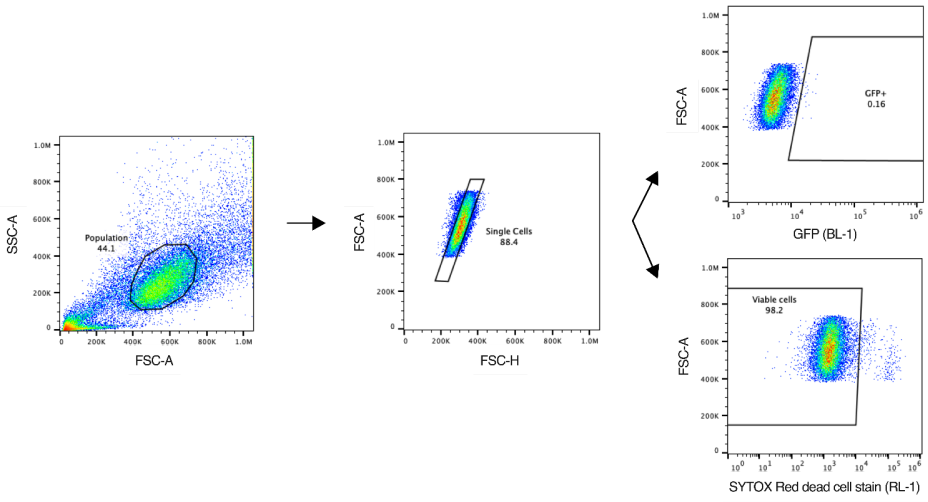
Experimental conditions were defined based on transfection condition, addition of pDNA, and execution (Table 1). A total of 384 samples across four 96-well plates were treated as biological replicates and transfected independently by RACs. Among them, 192 wells were transfected with pDNA and 192 wells were transfected with equal volume of water (Thermo Fisher, 10977015). Transfection efficiency, transient GFP expression, and cell viability were evaluated for all 192 transfection with pDNA, as well as for each set of 48 transfection with pDNA in each plate. The plate layout was specifically designed to evaluate cross-contamination of samples during experiment execution by RACs: pDNA was added to wells in odd number columns (column 1,3,5…) and water was added to wells in even number columns (column 2,4,6…).

In addition, 16 samples in a separate 96-well plate were transfected by an experienced scientist manually using the equivalent

transfection protocol, serving as positive control for transfections executed by RACs. Among them, pDNA was added to 8 samples while water was added to the other 8 samples. Another 8 samples with untransfected cells (no pDNA) were established as flow cytometry gating controls in the same plate. Equal volume of media was added to untransfected cells in place of pDNA and transfection reagent complex, so the final viable cell density remains the same as that in transfected cells.

Flow cytometry

Cell viability and transient GFP expression in transfected cells were measured using Attune NxT Flow Cytometer after 3 days of incubation post transfection. Approximately 2.5e5 cells were sampled from each well, washed once with Dulbecco's phosphate-buffered saline (Thermo Fisher, 14190144), stained with 150 µL of 5 nM SYTOX Red Dead Cell Stain diluted in Cell Staining Buffer (Thermo Fisher, S34859; BioLegend, 420201), and incubated for 15 minutes in darkness following manufacturer's manual in preparation for flow cytometry. GFP was excited with the 488 nm laser and emission was measured in the BL-1 channel. SYTOX Red was excited with the 638 nm laser and emission was measured in the RL-1 channel. No compensation was needed to correct for any fluorescence spillover. Flow cytometry data was analyzed using FlowJo (BD Biosciences). Gates were drawn using gating controls containing untransfected cells without pDNA as reference, then applied to samples in all experimental groups (Supplementary Fig 1). Minimum of 7400 single cell events were present in all flow cytometry samples.



Supplementary Figure 1. Flow cytometry gating strategy. Green fluorescence protein positive (GFP+) population was established by gating for single cells exhibiting GFP fluorescence intensity beyond the cutoff, set by untransfected cells. Viable cell population was established by gating for single cells not exhibiting SYTOX Red dead cell stain signal.