GINKGO BIOWORKS

Accelerating Drug Discovery with Miniaturized Cell-based Assays on Ginkgo's Flexible Reconfigurable Automation Cart (RAC) System

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7x

Throughput increase over manual process

88%

Reduction in hands-on time

100x

Improvement in lead compound series potency

Accelerating Drug Discovery with Miniaturized Cell-based Assays on Ginkgo's Flexible Reconfigurable Automation Cart (RAC) System

Lizvette Ayala-Valdez, Jeff Tang Ph.D., Joshua Paul Ph.D., Jennifer Fredlund Ph.D.

INTRODUCTION

Octant is a platform drug discovery company focused on building small molecule correctors. These are drugs that address the large (and underserved) class of diseases resulting from protein misfolding and mistrafficking by *correcting* the underlying dysfunction, thereby restoring proper folding and trafficking pathways. Because the underlying biology plays out in a complex cellular context, traditional drug discovery approaches that consider a protein in isolation – either biochemically or computationally – are not well suited to building correctors. Octant's platform uses synthetic biology to build reporters for these mechanisms in human cells. These cellular assays are coupled with high throughput nanoscale chemical syntheses, enabling an iterative, scaled, and empirical exploration of chemical space to identify and evolve drug candidates.

In practice, producing quantitative data reliably and consistently from cell-based assays, especially at scale, is incredibly challenging. Moreover, each drug program develops a diverse set of such assays (varying in cell types and mechanisms) and uses chemical inputs specific to the underlying mechanism of the disease. Octant executes this heterogeneous and dynamic set of assay workflows using a flexible Reconfigurable Automation Cart (RAC) system from Ginkgo Bioworks. This application note details how Ginkgo's RAC technology enables Octant to produce consistent, high-quality data while also providing the scale required to drive rapid iteration and progress across several drug programs with a remarkably lean team.

THE RAC SYSTEM

Octant's system includes 11 RACs tailored to run mammalian cell-based assays from assay ready plate preparation to plate reader based results. This system has capabilities for nanoliter acoustic liquid dispensing, microliter stamping, centrifugal plate washing, microliter bulk filling, multimodal plate reading, and

mammalian cell incubation, in addition to centrifugation, plate sealing and peeling, delidding, and ambient plate and tip storage (see **FIG. 1**). This configuration allows for execution of production screening campaigns as well as direct assay development on the RAC system. The RAC system is controlled by the Automation Control Software (ACS) software suite and is actively and remotely monitored by the Ginkgo team as part of our Managed Automation Solution (MAS) to maximize researcher walkaway time.

CASE STUDY: A CELL-BASED ENZYMATIC ACTIVITY ASSAY FOR FABRY DISEASE

Fabry disease is a lysosomal storage disorder in which the enzyme α-galactosidase A (α-galA) is mutated to a less- or non-functional form, leading to the toxic buildup of glycolipids in various tissues. In pursuit of a novel therapy, Octant has developed cell-based reporter assays that enable large-scale, quantitative evaluation of chemical compounds targeting this disease. This case study highlights one

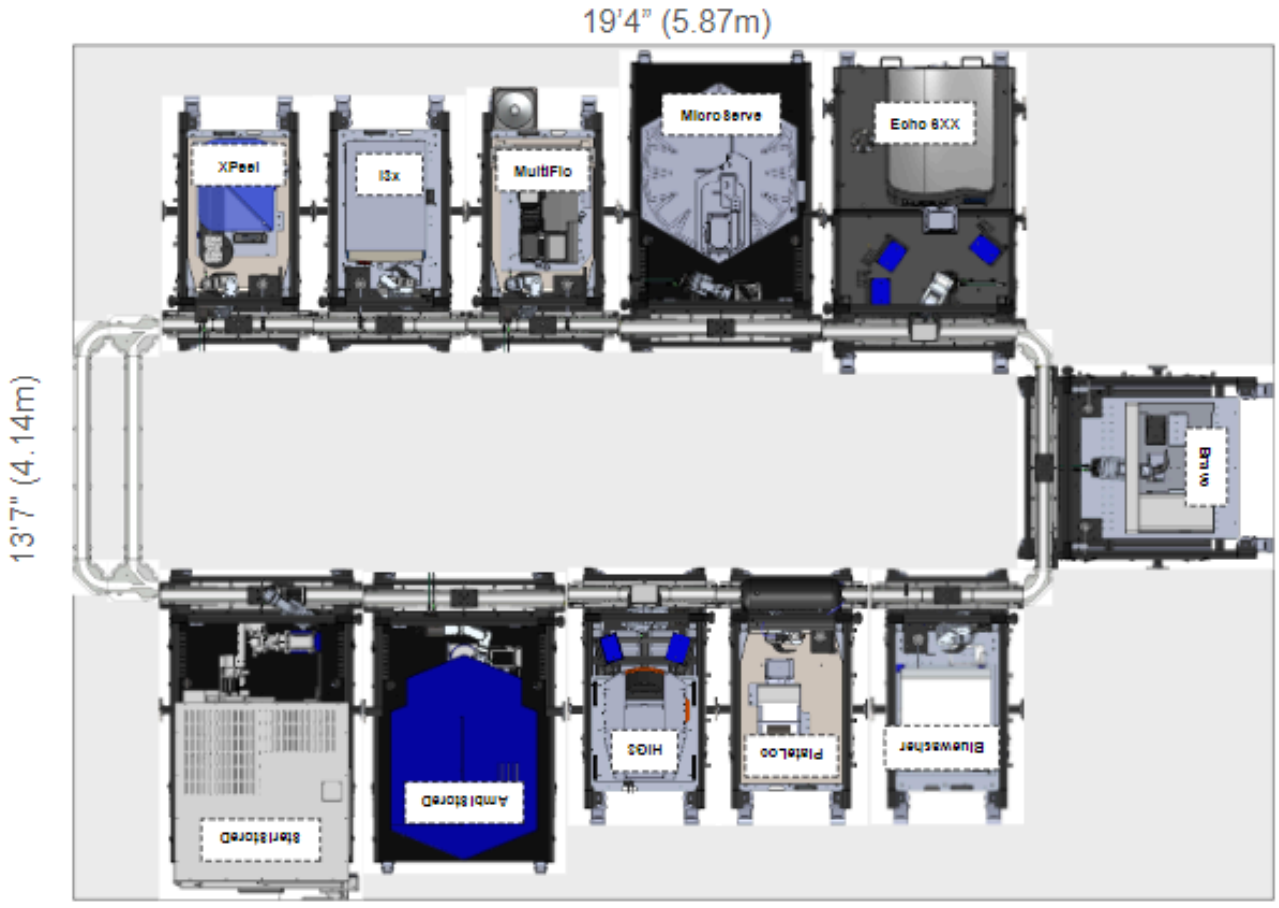

FIGURE 1. RAC System Octant's RAC system, called Hypatia, contains 11 instruments and is actively and remotely monitored by the Ginkgo team.

such assay, its integration into Octant's RAC system, and the resulting benefits and impact.

RAC implementation of a cell-based assay

In one of the screening assays for this program, a fluorescence-based, cellular assay was implemented to measure the ability of small molecule compounds to correct the misfolding and rescue the function of mutant variants of α-galA[1]. A human cell line was engineered to express the mutant enzyme in its native cellular environment with the assay measuring the activity of the protein upon treatment with our compounds.

The assay begins with the preparation of Assay Ready Plates (ARPs), where nanoliter volumes of test compounds are dispensed into tissue culture-compatible microtiter plates. Human cells are then added directly to the compounds in a BSC and subsequently incubated in the RAC Steristore for 48 hours. The humidity control of this instrument prevents appreciable evaporation and enables assays to be robustly run in 1536-well plates.

After 48 hours, a user initiates a series of protocols by submitting a csv file of plate barcodes, generated by Octant's custom experimental design software, to the ACS launch form. The cells are centrifugally washed with phosphate-buffered saline (PBS) using a BlueCatBio BlueWasher before moving to the BioTek MultiFlo for lysis buffer addition. The lysis buffer contains a fluorogenic substrate, which specifically interacts with the target enzyme α-galA. The enzyme catalyzes a reaction with the substrate, producing a fluorescent signal that is proportional to α-galA's activity. Following an incubation period of 60 min, the plates are returned to the MultiFlo for the addition of stop buffer, which halts the reaction and stabilizes the fluorescent signal. The plates are centrifuged and immediately read on a Molecular Devices SpectraMax i3x plate reader. Plates are returned to room temperature storage in the AmbiStore to be disposed of later (see **FIG. 2**).

As the protocol progresses, ACS publishes event notifications to particular channels on the

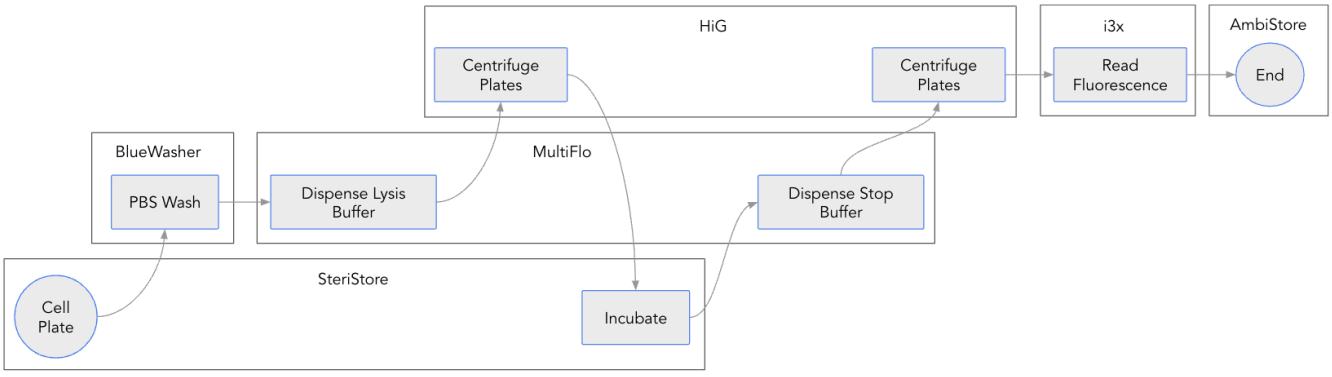

FIGURE 2. Fabry Screening Assay Plate Flow on the RAC System

stream-processing platform, Kafka. A separate process monitors these channels for incoming messages, one of which signals that data is available from the i3x. That monitoring process uses the information in the message to retrieve the data and move it to a separate data store, in this case AWS S3, for archiving and downstream processing.

RACs minimize hands-on time during assay execution

For the Fabry assay described in the previous section, the average user set up time is 10 minutes to load plates and reagents and 5 minutes to launch the protocol in ACS. The average clean up time is similarly 10 minutes. Ginkgo's MAS team monitors the system during the entirety of the run and quickly recovers from any errors that occur, without researchers needing to file a ticket. The average time per plate to complete the assay (after the 48 hour incubation) is 95 minutes and thanks to tight interleaving of individual plates by the ACS scheduler, the average time for a typical screening experiment (10 x 1536-well plates) is 5 hours. **Researchers running this protocol spend only 25 minutes of hands-on time on the system.** The "bench" version of the protocol similarly requires 10 min of setup and cleanup but also requires 3.5 hours of hands-on time, with 1 hour of walkaway time during the incubation. For 10 plates, the bench protocol takes 4.5 hours. Running on RACs saves researchers 3 hours per screening experiment compared to executing on the bench. This available time can then be spent on designing experiments and has enabled Octant to both optimize assays and move more assays onto the RAC system.

In addition to minimizing hands-on time, the RACs provide high quality assay data. The Fabry assay detailed above generates consistent, high quality data in 1536-well plates, as evidenced by the consistent Z' score seen in **FIG. 3**. This consistency minimizes rework and allows scientists to make decisions with confidence.

RACs enable iteration and scale to efficiently drive potency improvements

For the Fabry program, a single researcher tested more than 400,000 novel compounds over a 12 month period using the RAC system. Each week of screening represents an entire design-build-test-learn (DBTL) loop and iteratively tests newly synthesized compounds in the 1536-well cell-based assay. Results from the previous screen inform the next set of chemical designs, and each week a new ~10k member analog compound library is synthesized and then tested using the RACs. This tight DBTL loop, enabled by RACs, resulted in potency increases of Octant's lead compound series by >2 orders of magnitude (from micromolar to single-digit nanomolar EC_{50}'s). The RAC system was instrumental in enabling the efficient and rapid progress for that program's hit-to-lead efforts (see **FIG. 4**).

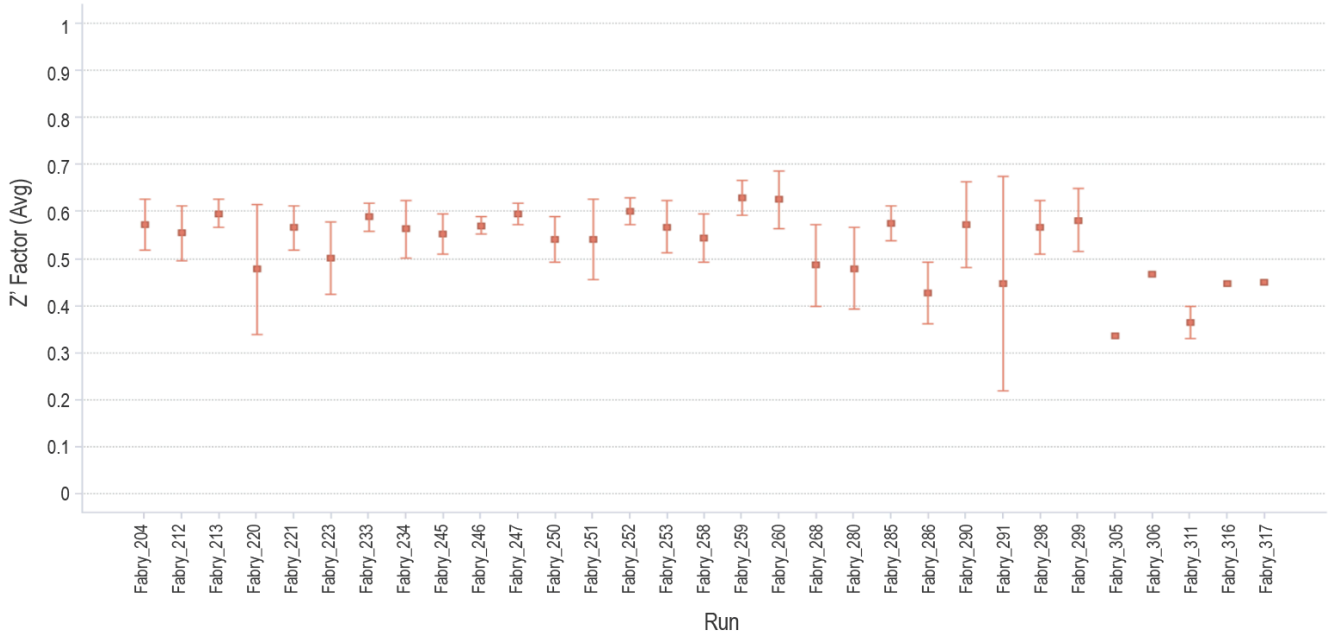

FIGURE 3. Fabry Screening Assay Runs Each point is a single day's Fabry screening assay run and indicates the average Z' score across plates for that day with error bars showing the standard deviation.

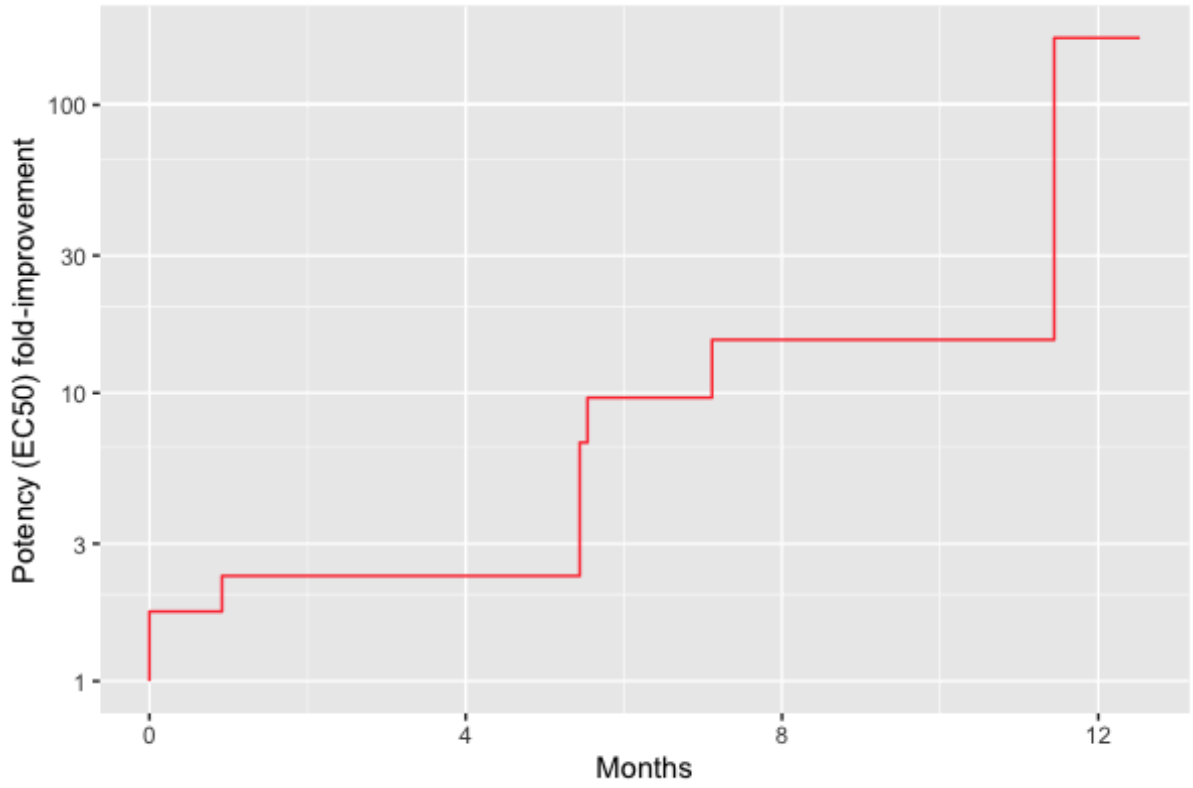

FIGURE 4. Improving Potency For the lead compound series of Octant's Fabry program, screening on the RAC system was critical for driving potency improvements by two orders of magnitude.

SCALING ASSAYS AND PROGRAMS

The case study above illustrates how the RAC system benefits a single cell-based assay within one drug program. However, this is just one of a dynamic and heterogeneous collection of assays Octant currently runs on the RAC system (see **FIG. 5**). The platform has enabled Octant to scale operations across multiple programs without expanding the team, with RACs now serving as the primary engine of scientific discovery, reliably producing high-quality data. Ginkgo and Octant work together to ensure onboarding assays from the bench to the RAC system is straightforward and users can parameterize or lock down assay inputs as they see fit. The MAS program also enables Octant to run late into the night without having onsite staff. This has been instrumental in speeding up the DBTL cycle across programs and driving progress with a rapid experimental cadence.

CONCLUSIONS

Octant's platform strategy relies on the ability to run a heterogeneous and dynamic set of mammalian cell-based assays at scale, iteratively generating measurements for tens of thousands of novel compounds every week. Ginkgo's RAC system is a critical component of this strategy, providing the flexibility to rapidly onboard and automate new assays and subsequently execute them at scale. In the past twelve months, Octant has onboarded many assays across four different drug targets, and produced data for millions of wells.

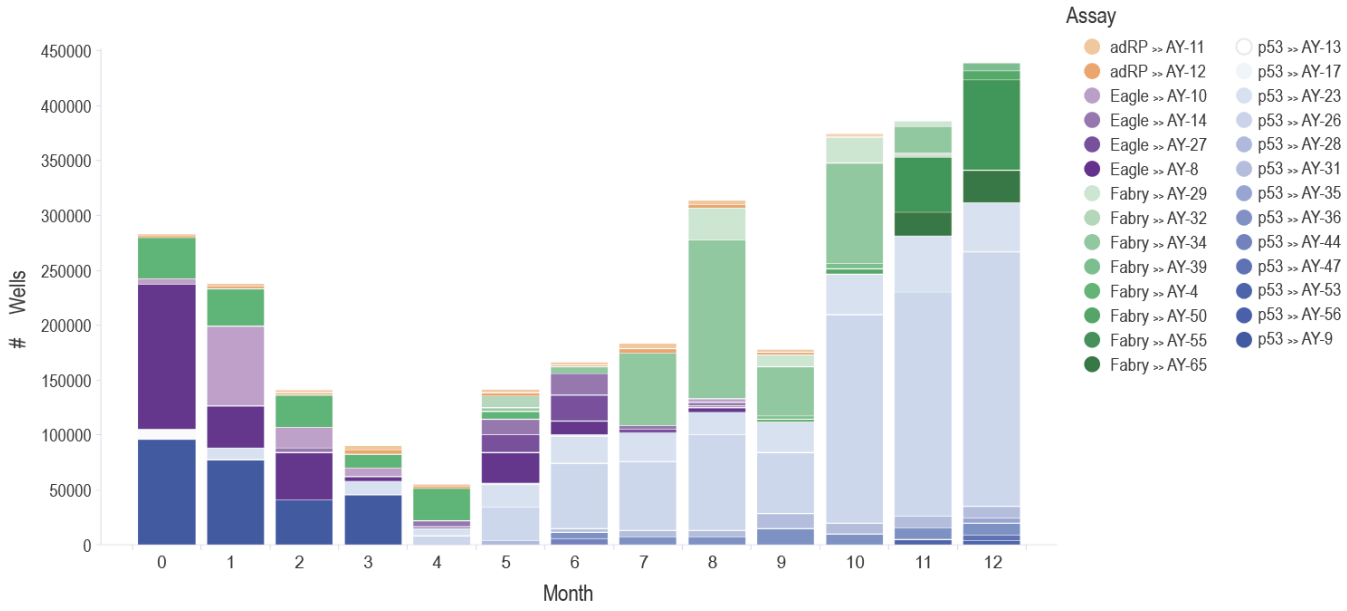

FIGURE 5. High-Throughput Operations Multiple assays can be run on the system at the same time, resulting in high-throughput operations and time savings across many programs. Over the 12 months shown, Octant ran four programs and supported 10s of different assays on their RAC system with recent throughput over 400,000 wells per month.

Octant's usage of the RAC system is reinforced through Ginkgo's Managed Automation Solutions (MAS), which is responsible for both streamlining the onboarding of new assay workflows onto the system and real-time operational support. In most cases, error conditions on the RACs – and the instruments contained therein – are handled remotely and without intervention, benefiting Octant scientists with increased walkaway time and the ability to confidently run automated workflows across extended time frames in a way that most other workcells could not.

Together, Ginkgo's RAC system and MAS have enabled scaled and rapid DBTL cycles at Octant while maintaining a small and agile internal operations team. This has, in turn, resulted in substantial improvements in compound potency and ADMET properties across several drug programs in the misfolded or mistrafficked protein space, accelerating Octant's mission of developing effective therapies for a challenging and underserved class of disease.

REFERENCES

1. Wu, X. et al. A pharmacogenetic approach to identify mutant forms of α-galactosidase A that respond to a pharmacological chaperone for Fabry Disease. Hum Mutat 32(8): 965-977 (2011).