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Semi-Automated Cell Line Development with Rapid Timelines, 80% Less Manual Handling and End-to-End Digital Traceability

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Abstract: **Traditional single-cell colony isolation remains a significant bottleneck in drug discovery due to intensive manual labor and susceptibility to pipetting errors, which limit throughput and scalability. EnquBio, a San Diego-based CRO, has developed an optimized, semi-automated workflow that integrates high-content imaging (HCI) with precision liquid handling. The workflow utilizes the Revvity Operetta CLS to identify and map single-cell colonies within 384-well microplates, generating a digital coordinate map for downstream processing. The CERTUS FLEX liquid dispenser then utilizes this map to precisely dispense dissociation reagents. Finally, the Opentrons Flex™ platform reformats these colonies into 96-well plates for expansion. This high-throughput screening (HTS) compatible workflow was validated by generating human GLP-1 receptor (GLP-1R) clones. We successfully isolated and characterized four functional colonies within three weeks, identifying clones with distinct high and low surface receptor expression profiles. Pharmacological validation was performed using the full agonist GLP-1 and the non-peptide partial agonist Orforglipron (LY3502970). This semi-automated approach accelerates the identification of high-quality screening materials while ensuring robust data traceability consistent with GxP standards.**

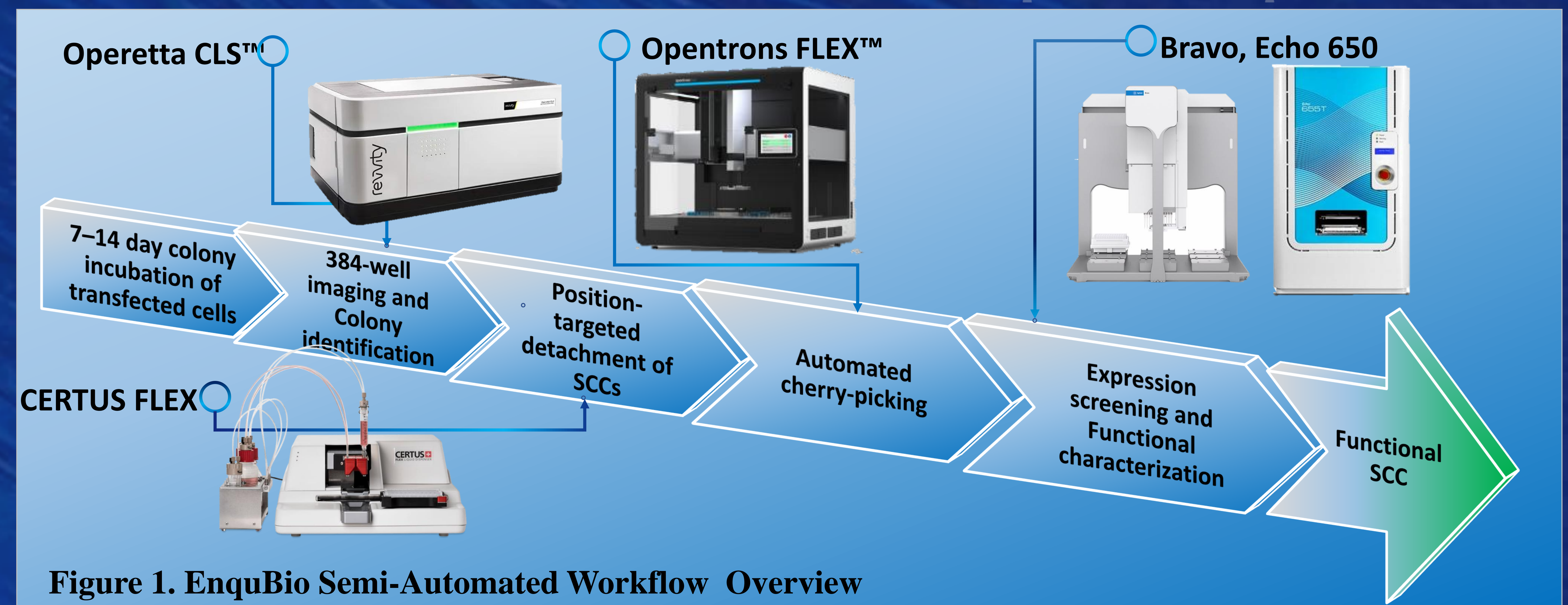

Figure 1. EnquBio Semi-Automated Workflow Overview

Introduction:

Single-cell cloning is foundational to biological research; however, conventional methods suffer from high manual intervention and inter-operator variability. Furthermore, manual workflows lack the rigorous data traceability. EnquBio has modernized this process by mobilizing key steps through an automated lab system (Figure 1). By digitizing the "command-and-record" cycle, we have significantly increased throughput, reduced human error, and standardized methods.

Methods:

1. Cell Seeding: CHO cells transfected with human GLP-1R were seeded into 384-well plates at 1–2 cells/well. After a 7-10-day incubation, plates were screened for optimal colony size.

2. High Content Imaging and Spatial Mapping: Colonies were scanned using the Revvity Operetta CLS (4x objective). To isolate colonies from background, we employed Sliding Parabola and Texture SER filters, refined by a Linear Classifier based on SER features (Figure 2A, B). These 384-well images were digitized into heatmaps representing colony distribution and density (Figure 2C), generating CSV coordinate maps for downstream automation.

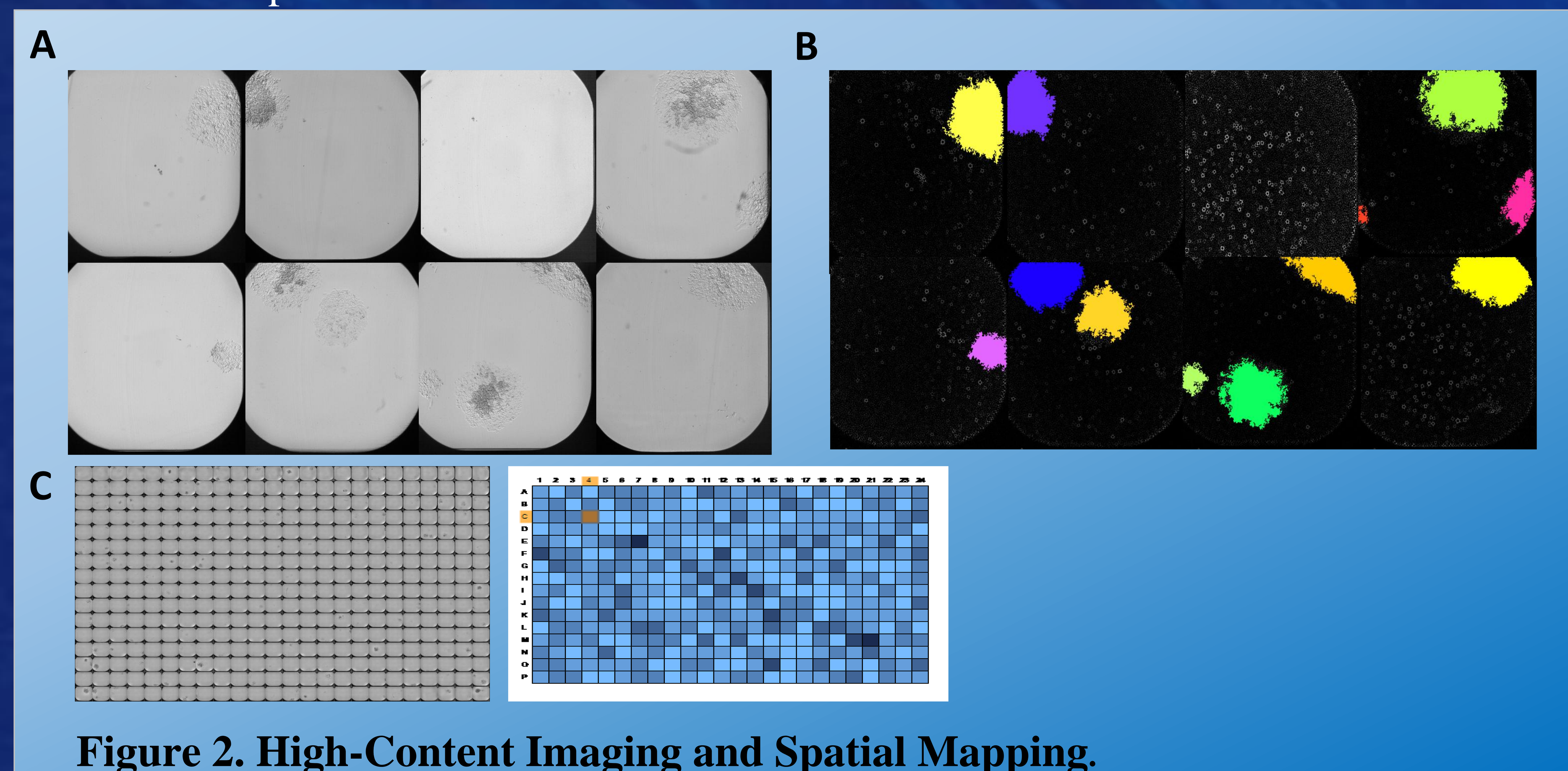

Figure 2. High-Content Imaging and Spatial Mapping.

3. Automated Digestion and Reformatting: We integrated the CERTUS FLEX dispenser and BlueCatBio Blue®Washer for media exchange. Using the HCI-derived map, 20uL of TrypLE was targeted only to colony-containing wells (Figure 3A). This precision dispensing, followed by a 5-minute incubation and media addition, achieved a homogenous cell suspension from previously intact colonies (Figure 3B). Finally, the Opentrons Flex executed a point-to-point cherry-picking protocol, transferring 50uL of suspension to a destination 96-well plate based on automated script generation (Figure 3C, D).

4. Functional and Expression Validation: Upon reaching 90% confluence, clones were reformatted into 384-well assay plates via Agilent Bravo 96LT. Plates were pre-stamped with GLP-1 or DMSO using a Beckman Coulter Echo® 650. Intracellular cAMP levels were quantified on an EnVision® reader (HTRF protocol) (Figure 4A), and surface expression was characterized via Attune™ flow cytometry.

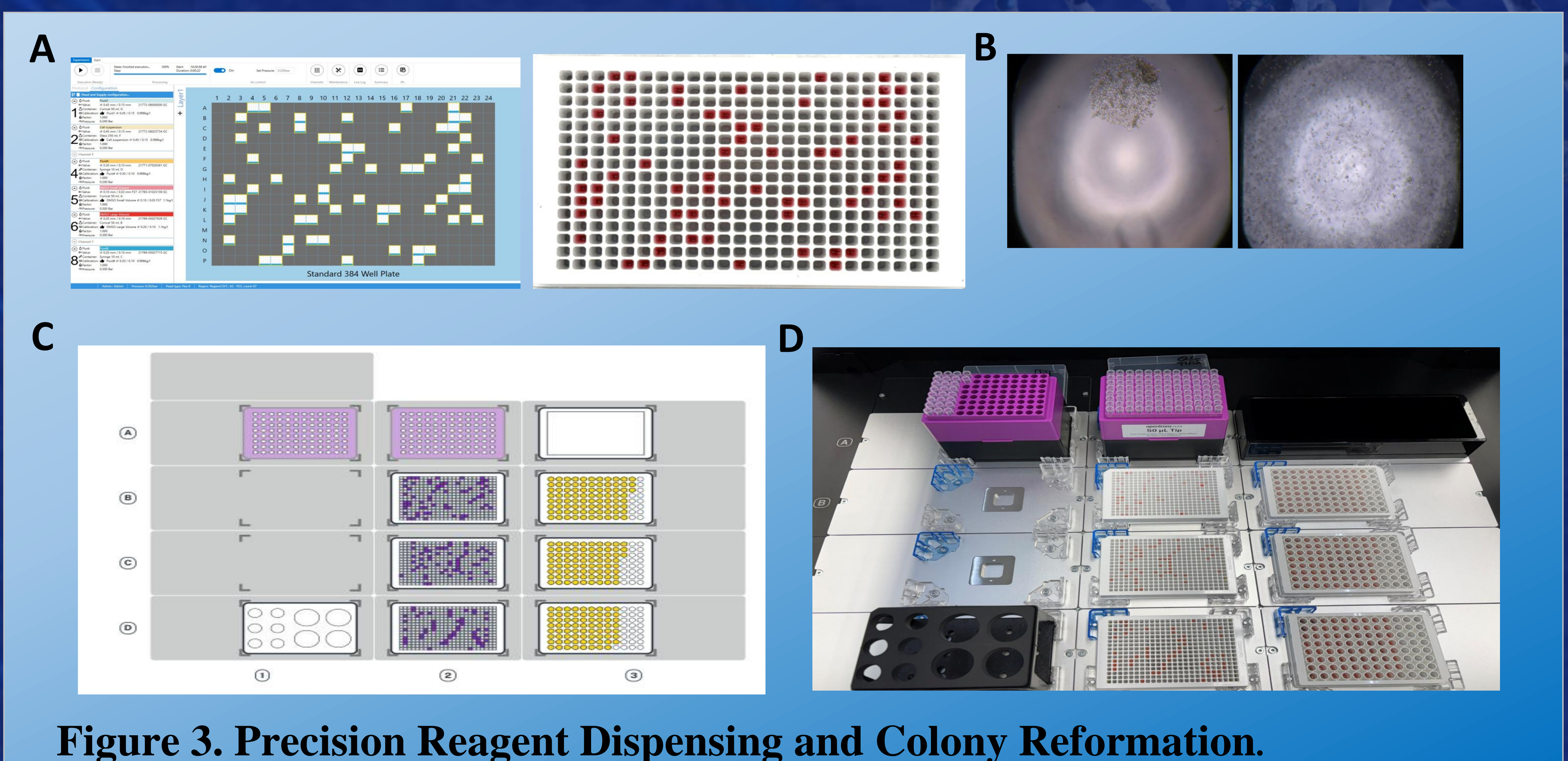

Figure 3. Precision Reagent Dispensing and Colony Reformation.

Result:

The automated workflow isolated 229 single-cell colonies from 1,152 wells across three plates, achieving a 90% hit rate with zero false positives, demonstrating superior precision over manual operators. Ten top candidates were identified via cAMP functional assays and GLP-1R surface expression were confirmed by flow cytometry (Figure 4B). cAMP signaling assays distinguished the pharmacology of the full agonist GLP-1 from the G-biased partial agonist Orforglipron (Figure 4C). This semi-automated process reduced the timeline to three weeks, yielding an 80% reduction in manual labor. Furthermore, the generation of digital "cell footprints" provided 100% data traceability, ensuring a robust audit trail for ELN integration.

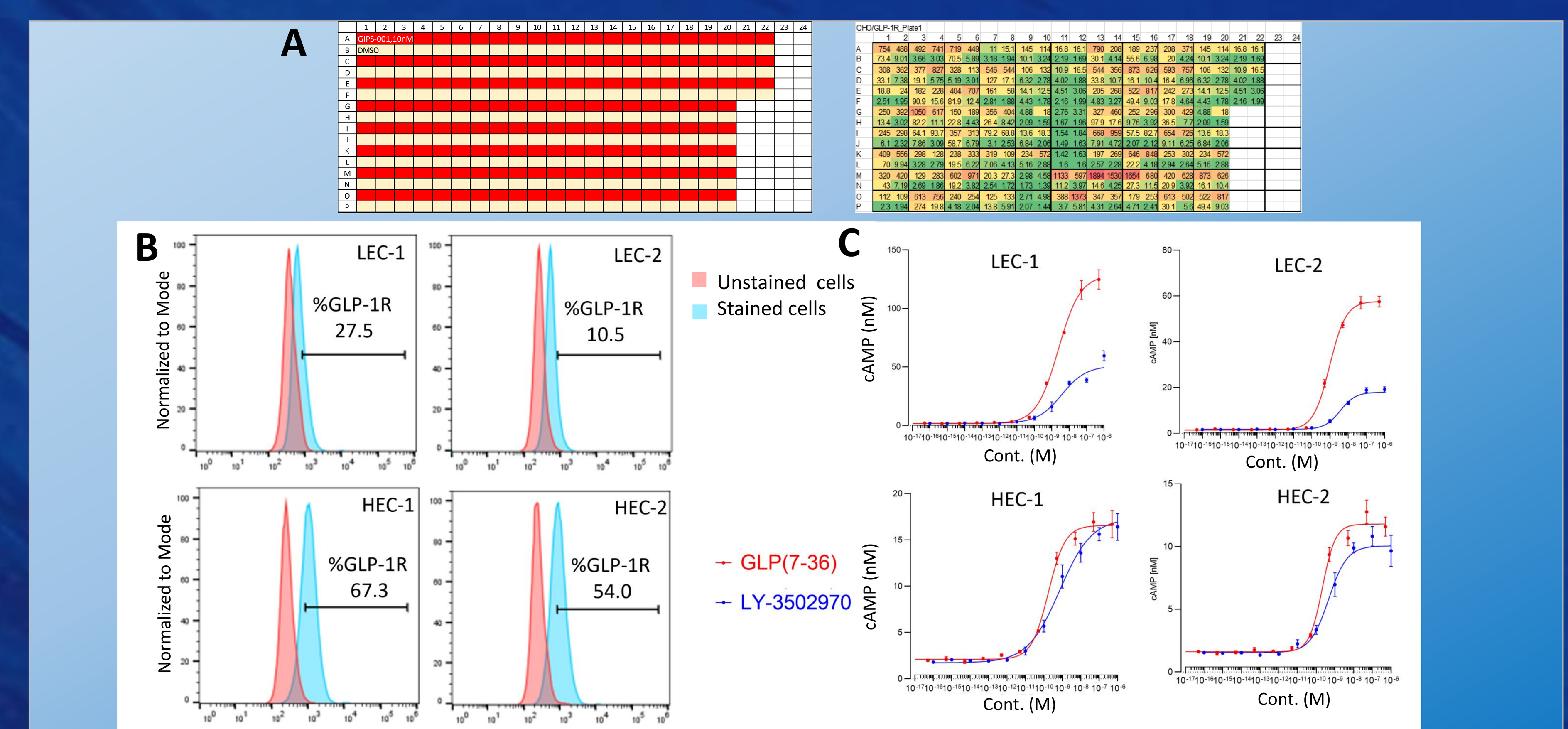

Figure 4. Expression Profiling and Functional Validation of High Expression Clones (HEC) and Low Expression Clones (LEC).

Conclusions:

The EnquBio Semi-Automated Workflow overcomes the limitations of manual cell line development by integrating high-content imaging with flexible liquid handling. This approach maximizes throughput, reduces human error, and enables HTS-scale capabilities with robust data integrity.