Plant Physiology, 2026, 200, 1–9
https://doi.org/10.1093/plphys/kiag066
Published: 19 February 2026
Research report

Plant Physiology® OXFORD

BOTany methods: accessible automation for plant synthetic biology

Moni Qiande [1,2], Abigail Lin [1,2], Lianna Larson [1,2], and Cătălin Voiniciuc [1,2]*

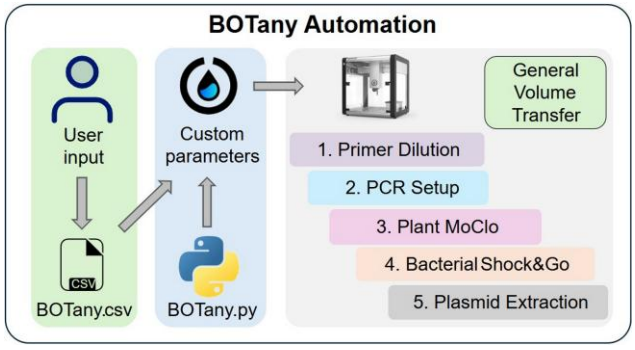
[1]Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, United States
[2]Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, United States

*Corresponding author: Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, United States. cvoiniciuc@ufl.edu
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plphys/pages/General-Instructions) is: Cătălin Voiniciuc (cvoiniciuc@ufl.edu).

Abstract

Most members of the synthetic biology community, particularly plant scientists, lack access to liquid handling robots to scale up experiments, enhance reproducibility, and accelerate the Design, Build, Test, Learn cycle. Biofoundries enable high-throughput data acquisition to train AI models and to develop bioproducts, but they are capital-intensive to set up and not widely distributed. Entry-level, 3D-printed robots offer more affordable alternatives, but suffer from a shortage of validated protocols that can be modified without prior coding experience. To enhance access to biological automation, we developed a collection of modular BOTany Methods using Opentrons OT-2 robots to streamline the most common methods for molecular biology research and education. Our comprehensive workflow offers automation for a variety of procedures, ranging from simple but repetitive tasks (such as primer dilution and PCR setup) to more complex operations, including Plant Modular Cloning (MoClo), bacterial transformation, and plasmid extraction. Our BOTany Methods enable users across different training levels (from undergraduate students to senior scientists) to run designer experiments using table-based inputs, without editing the custom Python scripts. This pipeline enables end-to-end molecular cloning with minimal user intervention, enhancing throughput and traceability for synthetic biology applications.

Graphical Abstract



Introduction

Despite the rise of generative artificial intelligence (AI), and integration of robots in both industrial and domestic settings (IFR, 2024), most students and bench scientists still carry out molecular biology techniques in an artisanal manner using manual pipettes. To clone a gene, assemble a multi-part construct, and/or transform an organism, success often depends on an individual's prior experience and

Received: August 21, 2025. **Accepted:** December 22, 2025

Table 1 Summary of customizable parameters in BOTany protocols and Opentrons App. Protocol features: user-defined (✓), fixed in Python code (•); not available (–).

			BOTany1-Primers	BOTany2-PCR BOTany3-MoClo	BOTany4-Shock&Go	Botany5-MagBead	BOTany6-Universal
BOTany Methods and CSV input	**Labware**	Module	•	•	•	•	✓
		Labware	•	•	•	•	✓
	Reagents	Location	•	✓	•	•	✓
		Volume	✓	✓	✓	✓	✓
	Liquid transfers	Source	•	✓	✓	•	✓
		Destination	•	✓	✓	•	✓
		Transfer volume	✓	✓	✓	•	✓
		Tip use	•	✓	✓	•	✓
Opentrons App parameters	**Pipettes**	Type	P300	P20	P20 and P300	8-channel P300	P20, P300, P1000
		Mount	✓	✓	✓	✓	✓
	Starting tip location		✓	✓	✓	✓	✓
	Thermocycler		—	✓	—	—	—
	Sample number		✓	—	—	✓	—

attention to detail. Pipetting liquids at the microliter (μL) scale is subject to technical errors, challenging to scale up, and can lead to repetitive stress injuries. Biofoundries can address these issues by offering a dedicated infrastructure for academic and industry stakeholders to automate common procedures in biology and bioengineering (Tellechea-Luzardo *et al.* 2022). A select group of institutions have set up a global alliance of biofoundries (Hillson *et al.* 2019) to speed up the design-build-test-learn cycle in synthetic biology. Biofoundries enable high-throughput experiments necessary to train AI models, accelerate scientific research, and empower product development, but are not available to most research-intensive universities in the United States and most other countries due to the high startup and maintenance costs.

High-end laboratory robots (in excess of $100,000 per unit) typically require dedicated personnel, proprietary software with limited flexibility, expensive consumables, and/or mandatory service contracts (Torres-Acosta *et al.* 2022; Stephenson *et al.* 2023). To save costs, some labs have built do-it-yourself (DIY) systems that integrate entry-level robotic arms and 3D-printed components (Gome *et al.* 2019; Rupp *et al.* 2022). Because engineering and programming skills required to build and operate DIY robots pose barriers to biologists, modular benchtop liquid handlers such as the Opentrons OT-2 have become the go-to option for entry-level lab automation. The OT-2 robot consists of a modular chassis (resembling a biosafety cabinet) and 11 plug-and-play deck slots for standard labware plus add-on modules for sample cooling, shaking, magnet-based purification and thermocycling. As an example of their utility at scale, numerous OT-2 robots were used in research and clinical settings to automate SARS-CoV-2 diagnostic workflows and to test thousands of patient samples per day (Lázaro-Perona *et al.* 2021; Villanueva-Cañas *et al.* 2021). In the bioengineering community, several research groups have showcased the suitability and affordability of OT-2 robots for high-throughput DNA assembly (Storch *et al.* 2020; Bryant *et al.* 2023), *Agrobacterium* transformation (Annese *et al.* 2025), RNA extraction (Moufarrej and Quake 2023), and single-cell proteomics (Montes *et al.* 2024), but comprehensive user-friendly solutions are still lacking for plant biologists.

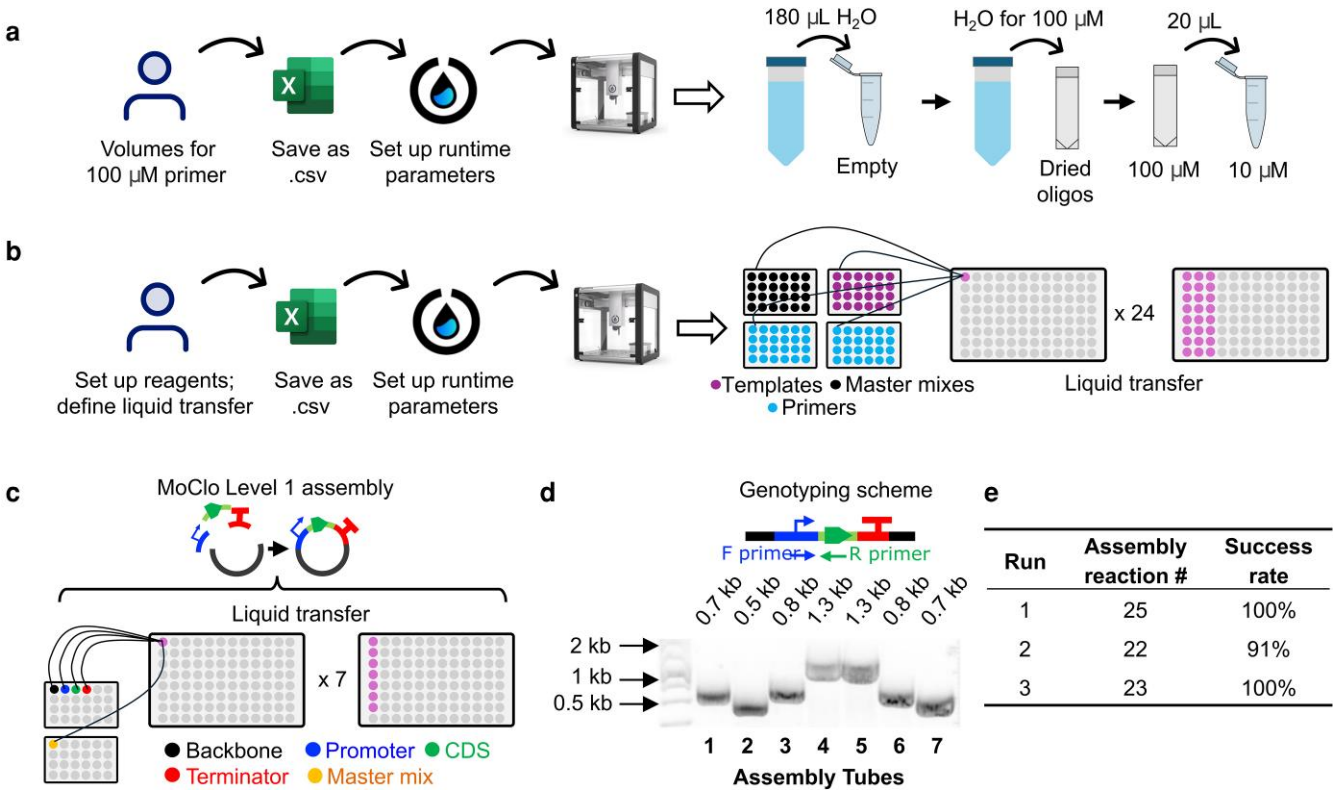
While the open-source Opentrons application programming interface (API) supports Python-based protocol development and sharing, it still poses barriers to biologists and engineers without programming skills. Protocol Designer, an Opentrons web-based drag-and-drop interface, offers simple workflows for first-time or casual users, but it quickly becomes cumbersome to design complex liquid transfer schemes which require multiple changes in parameters and labware between experimental runs. From our hands-on experience and from personal communication with other synthetic biologists working with plants and microbes, many of the OT-2 protocols shared online (eg Opentrons Protocol Library) have not been experimentally validated and are not sufficiently accessible or versatile.

To address this limitation, we developed a collection of OT-2 protocols that enable biologists, including learners and researchers interested in plant molecular biology, to automate several essential techniques by modifying spreadsheets. Instead of diving into the Python code or generating new scripts, users simply fill spreadsheets with reagents, volumes, and transfer steps, and export the Comma Separated Values (CSV) to run in the Opentrons App. Our Python scripts parse the table and execute each instruction on the OT-2 robot, preserving full control over liquid handling without any user modifications to the code. By shifting protocol design into a familiar spreadsheet format, we offer a user-friendly interface that still delivers the flexibility and precision of custom scripting. The BOTany Methods automate primer dilution, PCR, DNA assembly, *Escherichia coli* transformation, plasmid extraction, and more, thereby reducing hands-on time and increasing throughput for essential molecular biology tasks.

Results

CSV-based OT-2 protocols with runtime parameters

Our BOTany Methods can be applied to 1 or more robots, with separate units offering operational efficiency without changing the hardware between protocols (Figure S1). We initially built and/or modified custom OT-2 protocols in Python, but the manual editing of the code proved inefficient between experiments and challenging for lab members without training in computer science. To address this, protocol commands such as well locations and reagent definitions were migrated into CSV-editable files. Our early implementation relied on manual copy-and-paste of CSV instructions into Python scripts, leaving room for errors. Since the release of API v2.20 and App v7.3.0, Opentrons added the possibility to upload user-defined CSV files containing custom runtime parameters for Python scripts (Table 1).



e

Run	Assembly reaction #	Success rate
1	25	100%
2	22	91%
3	23	100%

Figure 1 Workflow of molecular cloning. **(a)** Automated primer dilution using BOTany1-Primers. **(b)** DNA amplification using BOTany2-PCR methods. **(c)** Plant modular cloning (MoClo) using BOTany3-MoClo methods, with MoClo Level 1 assemblies as an example. **(d)** Agarose gel of PCR amplicons from 7 different plant MoClo level 1 DNA assembly reactions shown in panel **(c)** using genotyping primers prepared in **(a)**. **(e)** Summary of 3 independent Golden Gate assembly batches, each containing more than 20 assembly reactions, and their success rates.

This shift to CSV-driven workflows enabled us to create more flexible protocols that require no coding knowledge or modification of the scripts, greatly shortening the learning curve to start automating molecular biology research and education. These protocols are equally valuable for students, technicians, senior researchers, and teaching laboratories. Our spreadsheet templates can be edited with multiple software, including the web version of Excel (free, no installation required), LibreOffice Calc and Google Sheets. We verified that all of these options export CSV files compatible with the BOTany protocols and the Opentrons App. Additional text editors or scripting languages could be used to modify the tables, but we recommend spreadsheet editing in Excel or similar tools to benefit from the user-friendly colorful formatting and drop-down menus for different columns.

Automation of vector construction

From automated primer dilution to PCR

Synthetic DNA oligonucleotides (oligos or primers) serve as starting points for numerous molecular biology workflows including DNA synthesis, target sequence amplification for cloning or genotyping plants/microbes, and for Sanger sequencing. Since most labs order primers in a shelf-stable, lyophilized format that requires manual resuspension, we developed a fully automated protocol for the preparation of primer stocks (Fig. 1a). We use the BOTany1-Primers method as a practical solution that introduces new users to biological automation. First, the CSV file instructs the robot to add variable volumes of nuclease-free water to the lyophilized primer tubes whose final concentrations should be 100 μM. After pipette mixing the concentrated solutions, the robot also transfers aliquots to prepare 10 μM primer stocks in 1.5 mL tubes (Method S1). We have successfully applied this method to process more than 200 primers for multiple users in our laboratory.

Next, we developed BOTany2-PCR protocols (Fig. 1b) that support up to 120 reagent tubes in a 1.5 mL format (primers, templates, master mixes, etc.) to prepare reactions in 8-well PCR strips or in a 96-well plate. To accommodate different laboratory setups, there are 2 versions for the protocol to support the Opentrons thermocycler module in running on-deck reactions (version A) after all the liquid transfers are completed (Method S2); version B supports off-deck thermocyclers from most other molecular biology companies. In both cases, a CSV file defines the pipetting instructions, including the liquids that will be loaded in the OT-2 and the desired transfer steps (eg source, destination, volume, tip, and pipette choices). Sensitive reagents such as enzymes are chilled on-deck for the duration of the experiment using the on-deck temperature module. Although the OT-2 temperature module can cool samples to 4 °C, we have found it practical to prechill its aluminum blocks in the fridge and/or set the cooling temperature in the scripts to 14 °C to strike a balance between time required to reach the set point (< 2 min in a room at 23 °C) and the need to protect the reagents.

Automated golden gate assembly

Golden Gate assembly enables seamless, scarless assembly of multiple DNA fragments in a single reaction, making it ideal for constructing

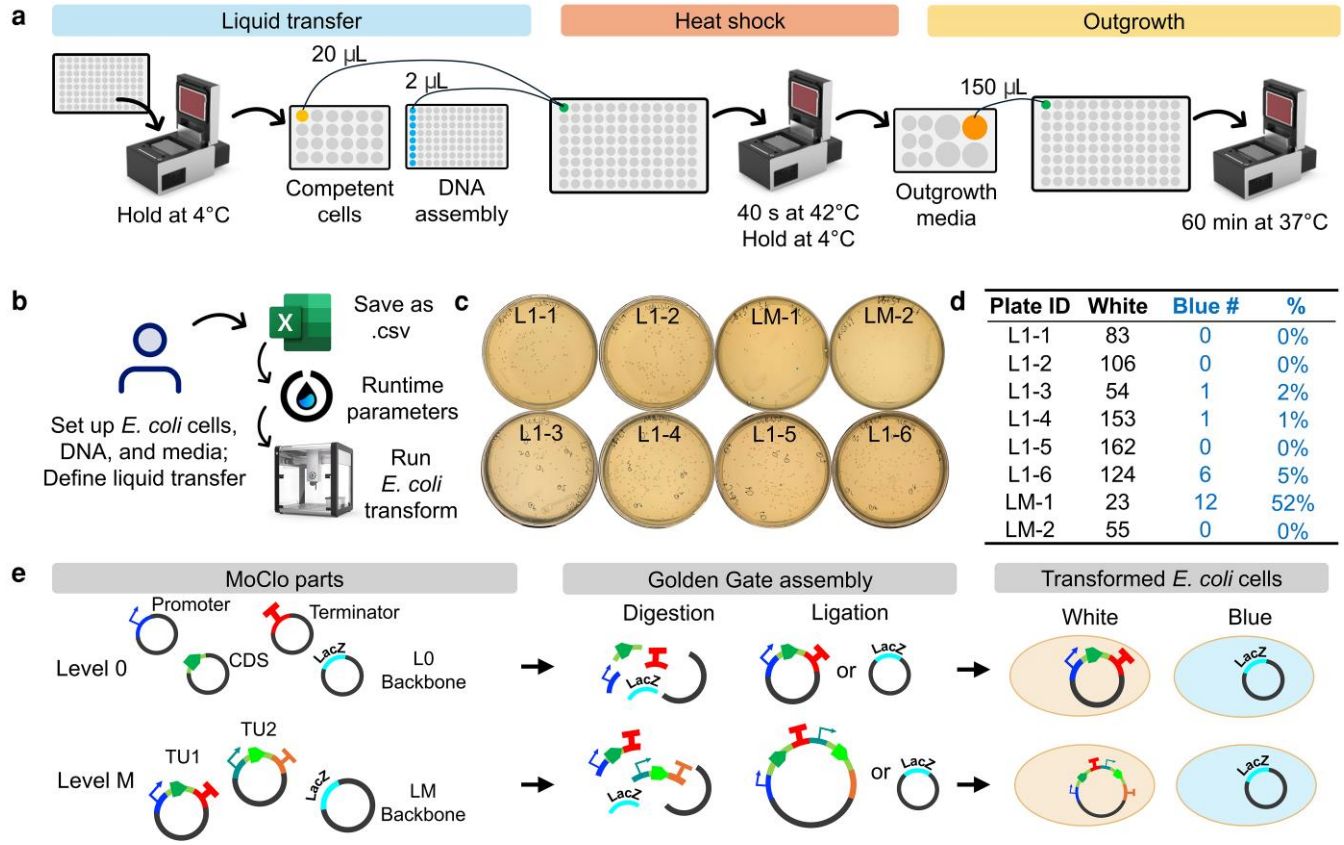


Figure 2 Automated *E. coli* transformation using BOTany4-Shock&Go. **(a)** OT-2 workflow of the bacterial transformation protocol. **(b)** Setup steps for the protocol. **(c)** Representative colony growth from 50 μL aliquots of transformed cells using different DNA assemblies prepared via BOTany3 method. The L1 plates contain MoClo level 1 assemblies, while LM indicates MoClo level M assemblies that contain larger, multimeric constructs. **(d)** Colony numbers after overnight growth and AQ-white screening. Blue colonies contain re-ligated destination vectors, without the gene of interest. **(e)** Illustrations of MoClo L1 and LM assembly outcomes from digestion-ligation reaction and *E. coli* transformation.

complex genetic constructs quickly and with high fidelity (Engler *et al.* 2008). Golden Gate standards have been established in the plant synthetic biology community (Patron *et al.* 2015), and collections such as the plant modular cloning (MoClo) toolkit provide reusable coding sequences and regulatory elements for plants (Engler *et al.* 2014) and other hosts (Bird *et al.* 2022), including yeast (Lee *et al.* 2015; Püllmann *et al.* 2021; Shaw *et al.* 2023; Grieß-Osowski *et al.* 2025). Plant MoClo automation improves throughput and accuracy for users at all levels of expertise, facilitating parallel assembly of multiple constructs, or the generation of DNA libraries. Like the PCR protocols (Fig. 1b), we created fully automated BOTany3-MoClo versions for on-deck and off-deck thermocycling (Fig. 1c). In version A, users can specify the desired ligation time, temperature, and the number of digestion-ligation cycles for efficient Gate assembly (Method S3). Seven Plant MoClo Level 1 vectors, consisting of a desired promoter, various coding sequences (cDNA), and a transcriptional terminator (Fig. 1c), were correctly assembled by the robot on the first try (Fig. 1d and e).

Automated bacterial transformation

Automating the transformation of assembled plasmids into bacterial hosts such as *Escherichia coli* would improve reproducibility and throughput across cloning workflows, providing more consistent results regardless of user experience level. The BOTany4-Shock&Go protocol uses the Opentrons HEPA and Thermocycler modules (Fig. 2a) to perform up to 96 parallel *E. coli* transformations (Method S4). First, the OT-2 robot aliquots competent cells (prepared in house) in the sterile PCR tubes, then adds newly assembled plasmid mixtures (Fig. 2b). The on-deck thermocycler heat shocks the cells, cools them, and finally aids their recovery by adding a nutrient-rich medium. The tubes are manually capped and then incubated on-deck for an hour before manual plating. To validate this protocol, we transformed 8 Plant MoClo plasmid assemblies, ranging from 4 to 9 kb, and obtained around 30 to 160 colonies per plate using an aliquot of the recovered cells (Fig. 2c). Using blue-white colony screening, we found that fewer than 5% of colonies on each plate showed the *lacZ* activity of empty vectors (Fig. 2d and 2e), except for 1 multimeric assembly with a low colony count. This protocol minimized the need for manual interventions (eg moving tubes between a water bath, an ice bucket, and a shaking incubator) and miniaturized the transformation volumes found in standard *E. coli* protocols. Our Shock&Go approach worked consistently and yielded more colonies than the commercial *Mix&Go!* kit (Zymo Research), which requires proprietary reagents for competent cell preparation. In our hands, competent cells prepared with *Mix&Go!* reagents yielded insufficient *E. coli* colonies for Level 0 assemblies without heat shock (Method S4). Cells from the same preparation

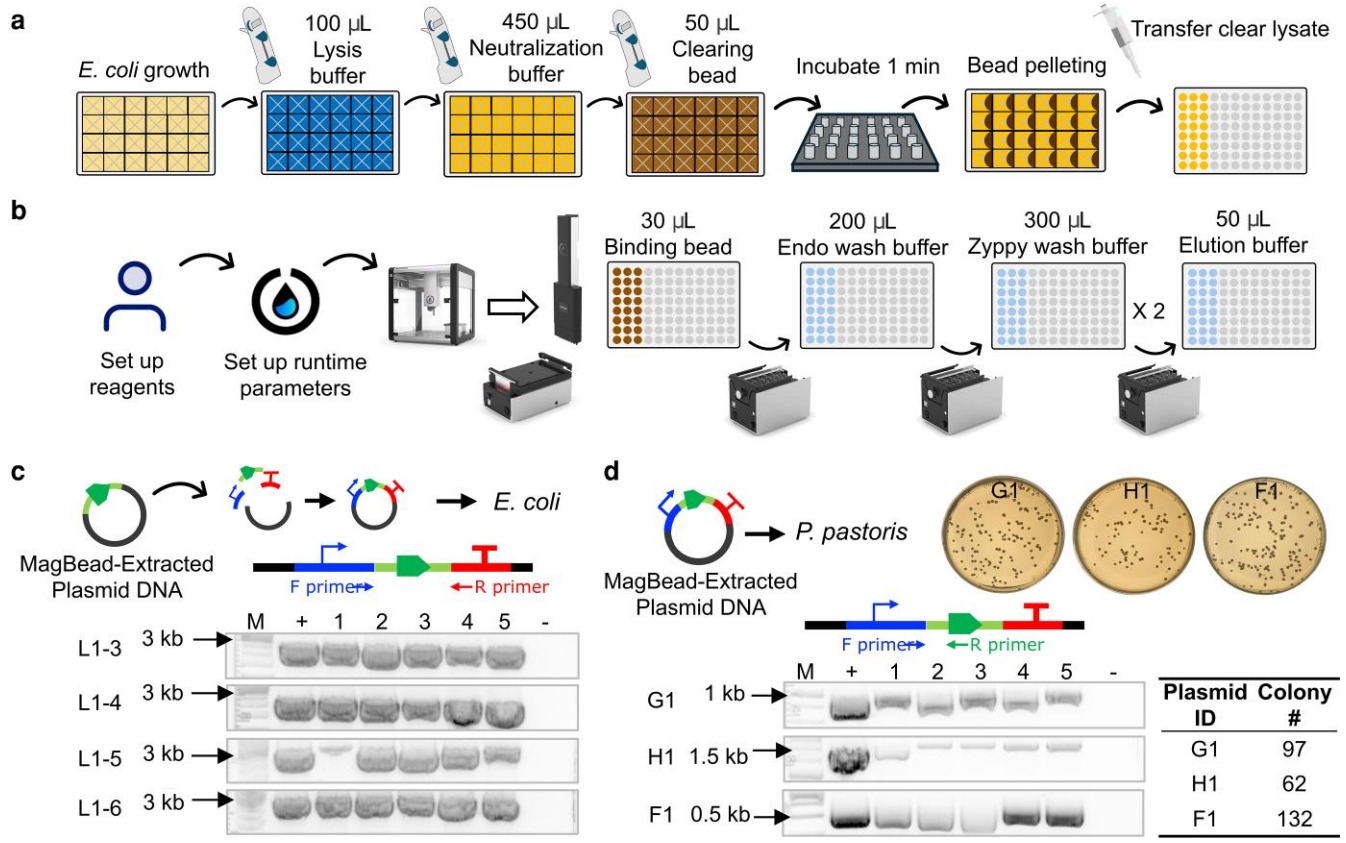


Figure 3 BOTany5-MagBead automation of plasmid extraction. **(a)** Manual steps to obtain clear bacterial cell lysate. **(b)** Workflow for automated binding, washing, and elution of plasmid. **(c)** Agarose gel of colony PCR amplicons after *E. coli* transformation with Golden Gate assemblies using MagBead-extracted plasmids. **(d)** Agarose gel of colony PCR amplicons after *Pichia* electroporation using MagBead-extracted plasmids. Five colonies per transformation were genotyped in **(c)** and **(d)**. "M" indicates DNA markers, "-" indicates negative control that includes all PCR components excluding DNA templates and is run under the same PCR conditions as other samples.

yielded substantially higher transformation efficiency with our automated Shock&Go method, matching the colony numbers obtained using manual DNA assembly and transformation workflows (Method S4).

Semi-automated MagBead plasmid extraction

Most laboratories extract plasmid DNA using commercial miniprep kits containing spin columns, which require extensive pipetting and multiple centrifugation steps (plus capping and uncapping tubes), or the complete removal of solvent washes using a vacuum manifold. Our semi-automated protocol can process up to 96 samples using the Zyppy-96 Plasmid MagBead Miniprep kit, which offers cost parity on a per sample basis ($1.6 to $2.0 each) with traditional spin column kits. The kit uses 2 types of magnetic beads (MagBeads) to purify *E. coli* plasmid DNA and was initially selected based on the availability of a "compatible" script in the Opentrons Protocol Library. However, neither the OT-2 scripts available online nor the standard kit instructions yielded sufficient DNA in our laboratory, so we had to develop a novel manual workflow and a Python script in consultation with Opentrons and Zymo. Neither the OT-2 Magnetic Module, nor the ZR-96 MagStand (recommended by Zymo) were strong enough to fully pellet the clearing MagBeads in 96-well plates. To address this, we pivoted to growing cells in autoclavable 24 deep-well plates that yield ample growth in standard laboratory shakers (eg 250 rpm at 1.9 cm orbit), are compatible with the Zymo MagStand, and provide extra space for initial pipetting steps that were difficult to automate (Fig. 3a). The BOTany5-MagBead method performs the remaining steps, including plasmid binding, washing, and elution using an 8-channel pipette (Fig. 3b). The Magnetic Module and the Heater Shaker are used to pull down the DNA during multiple wash and incubation steps (Method S5), respectively. Although the MagBead-extracted plasmid concentration reached only 5 to 10% of our typical column-purified miniprep yield (Table S1 and Figure S2), both methods displayed comparable restriction enzyme digestion patterns (Figure S3). The MagBead samples also showed high-molecular weight chromosomal DNA, but this did not interfere with the standard applications. We used MagBead-extracted plasmids (corresponding to coding sequences in Plant MoClo Level 0) for automated Golden Gate assembly and *E. coli* transformation using the aforementioned BOTany methods. PCR genotyping showed that all tested colonies contained the desired genes (Fig. 3c). Finally, we demonstrated that MagBead-extracted episomal plasmids (MoClo Level 1) were suitable for *Pichia* yeast cell electroporation (Lin-Cereghino *et al.* 2005), which requires more stringent DNA concentration and purity than bacterial transformation (Fig. 3d). Although the spin column method yielded 3 to 10 times more colonies, both methods consistently produced sufficient transformants for genotyping (Figure S4).

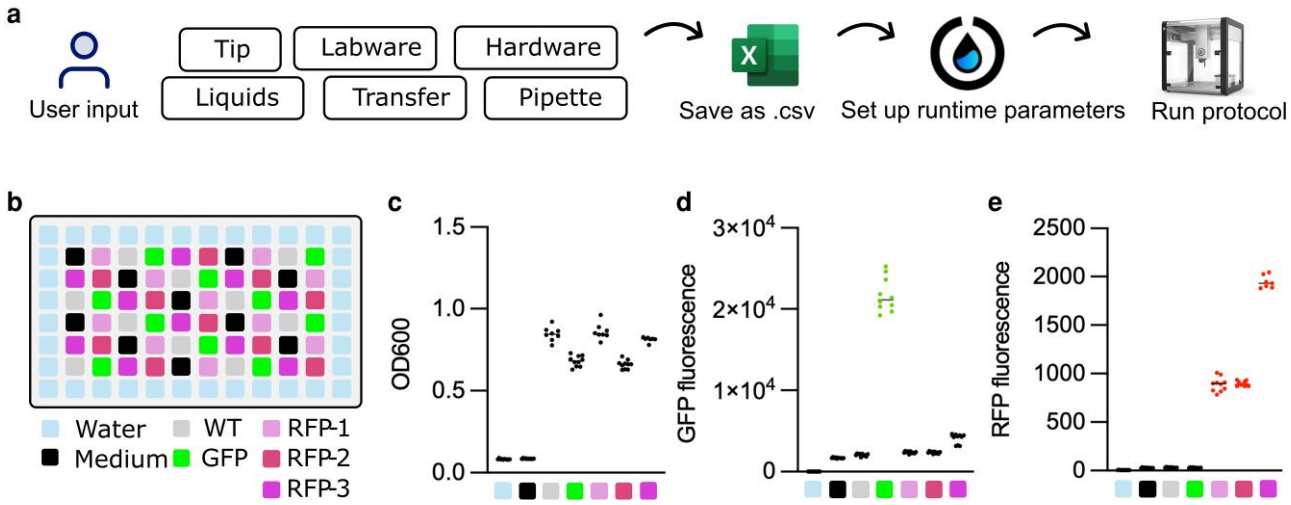


Figure 4 BOTany6-universal liquid transfer. **(a)** Workflow of a general liquid transfer protocol. **(b)** Inoculation of a 96 deep-well plate using different *Pichia* fluorescent cell strains and liquids in a mosaic pattern. After 72 h incubation at 800 rpm and 30 °C, **(c)** OD600, **(d)** GFP fluorescence, and **(e)** RFP fluorescence were measured using 1/10 diluted aliquots of yeast cell cultures.

A versatile BOTany protocol

In addition to the common procedures described above, we created a BOTany6-Universal pipetting protocol (Fig. 4a) that offers full flexibility in terms of hardware, labware, pipettes, tips, and liquid transfers (Method S6). Our spreadsheet has drop-down selections with defined parts from the Opentrons API (eg tip rack, labware, and hardware modules) to simplify user input and avoid typing errors. This protocol was used to fill a 96 deep-well plate with 500 μL of media and to inoculate wells with fluorescent *Pichia* yeast strains in a mosaic pattern that would be difficult to replicate by hand (Fig. 4b). After 3 d of cultivation, green and red fluorescent cells were only detected for the expected wells (Fig. 4d and e). We did not detect any cross-contamination or growth in nutrient-rich wells that were not inoculated with yeast cells, highlighting the efficiency of the HEPA module and accuracy of the pipetting steps (Fig. 4c).

Discussion

Recently, the iBioFAB platform was programed to automate tedious steps in plant transformation (Dong *et al.* 2025), such as protoplast preparation. However, high-end biofoundries remain inaccessible to most researchers because they are prohibitively expensive to replicate. Here, we present an affordable hardware setup and custom BOTany Methods that automate several time-consuming steps in plant synthetic biology and can enable other labs to integrate liquid handlers in their research and teaching activities. The BOTany collection provides a streamlined approach that overcomes technical challenges in adopting automated plant molecular biology methods, thereby accelerating research as well as education across a wide range of laboratory settings. The Opentrons OT-2 system is currently one of the most affordable commercial solutions for liquid handling. The robot is sufficiently compact and robustly built to be occasionally transported, and we have successfully moved it on rolling carts for classroom demonstrations in neighboring labs or buildings. With considerable interest from industry, academic, and community science labs, full-time researchers as well as DIY biologists have promoted a protocol-sharing culture for the OT-2. Despite the convenience of the Opentrons Protocols Library (https://protocols.opentrons.com/), we caution that shared protocols should not be taken for granted since they may lack experimental validation or rigorous testing. For example, the Zymo MagBead protocol originally published by Opentrons failed for several reasons, such as inadequate Magnetic Module parameters. Peer-reviewed pipelines such as AssemblyTron (Bryant *et al.* 2023) are more robust, but require users to be proficient with multiple software and to modify scripts, which can deter plant biologists and other users.

Our implementation of customizable tables in CSV format overcomes programming hurdles and leverages software that is familiar to most users in biological sciences. The recently released OT-Mation also employs CSV files to generate Python protocols for routine OT-2 liquid transfer tasks that do not require advanced module control (Laverick *et al.* 2025). While this resembles the BOTany approach, OT-Mation did not provide detailed, biologically relevant examples. Meanwhile, our protocols offer a complete workflow from dry primers to the purification of newly assembled constructs. In essence, our platform enables the modular assembly of plant constructs (eg for single or multi-step enzymatic reactions), which can be quickly prototyped in heterologous hosts such as yeast before more time consuming experiments in model plants or crops (Grieß-Osowski *et al.* 2025). Users are only required to modify spreadsheets, since the Python code development and experimental validation was completed by our team. These supplied tables can be edited using any software that supports CSV file export; however, we recommend using a desktop or web-based spreadsheet programs (eg Excel) to preserves user-friendly layout and avoid disrupting the column organization, which could disrupt protocol execution. As a result, no additional programming knowledge or code modifications are needed: users simply load or drag our BOTany protocols and updated CSV files onto Opentrons App to execute the procedures, thereby providing a direct route to laboratory automation. We have rigorously validated each protocol step using concrete example workflows, demonstrating that each protocol works reliably under standard laboratory conditions. We also supply step-by-step instructions for each BOTany method,

annotated screenshots and troubleshooting tips, so that researchers unfamiliar with the OT-2 interface can install and execute our protocols successfully from the first day.

In addition to OT-2 protocols dedicated to common procedures in synthetic biology, we also present a universal transfer protocol to serve as the foundation for additional tasks, such as RNA normalization and cDNA synthesis prior to real-time PCR. Our protocols and the OT-2 platform support a wide range of consumables, which provides experimental flexibility and does not restrict users to 1 vendor or proprietary supplies. Pipette tips from other manufacturers (eg universal 20 and 200 μL tips from Sarstedt) can be integrated seamlessly on the OT-2, and the system can accommodate custom labware files for plates or reservoirs. Although pipette tips and other modules must be calibrated during deck setup, at least during first-time use for a given protocol and slot, the OT-2 system bypasses the service contract required for more expensive robots, including the new Opentrons Flex. One benefit for more expensive robots such as the Flex is the option of a gripper to move labware between deck slots and modules. This could further automate our MagBead-based plasmid extraction method, which already yielded sufficient plasmids for DNA assembly and yeast transformation.

In conclusion, the BOTany Methods combine affordable robots with modular, CSV-driven protocols to complete routine as well as advanced molecular biology tasks. Our detailed workflows are feasible to implement at other institutions and improve experimental throughput and reproducibility while remaining accessible to researchers with diverse backgrounds and without any programming expertise. Due to the modular nature of OT-2 robots and BOTany methods, our open-access protocols are easily modifiable by the community and can be integrated with other recent methods for plant bioengineering (Annese *et al.* 2025). This combination of modular hardware and scriptable workflows, in turn, supports shared use: a single OT-2 robot can be flexibly used for different projects within 1 laboratory or integrated as a core instrument for several research groups within a department or other institutional units. Shared access to robots further democratizes laboratory automation, reduces equipment redundancy, and enhances the return on investment for taxpayer dollars within publicly funded organizations. By lowering the barriers to start automating biology, our workflows for end-to-end molecular cloning will accelerate research and product development in plant sciences, microbiology, and beyond.

Materials and methods

BOTany method setup

The BOTany protocols were developed using Python framework of the Opentrons OT-2 API. Custom labware were measured using a digital caliper, and the .json files were created via link (https://labware.opentrons.com/#/create). Method S1 features a general guide for using the BOTany Methods and step-by-step instructions for the BOTany1-Primers protocol. Tables S2 and S3 summarize the files and physical plasticware used in BOTany Methods. All the Python protocols, customizable tables for the CSV runtime parameters, and third-party labware files developed in this study can be found on our public GitHub repository (https://github.com/cvoiniciuc/BOTany).

Primer dilution and PCR setup

Synthetic oligos were synthesized by Eurofins Genomics (USA) in dry, salt-free, 25 nM scale, which typically require 100 to 300 μL water to prepare the 100 mM stocks (Method S1). Primers used to validate successful DNA assemblies in Fig. 1d, and colony genotyping in Fig. 3c and d, are listed in Method S2.

The BOTany2-PCR-Table.xlsx template on GitHub illustrates 10 μL genotyping reactions containing 5 μL Red Taq 2X Master Mix (VWR International; 76620-474) and 2 μL water (together referred to as Master mix1), along with 1 μL of each 10 mM primer and 1 μL of DNA template. In this setup, Master mix1 is aliquoted first, followed by single transfers of primers and DNA templates. To save tips and time when genotyping many plant or microbial samples, primers could be premixed with the master mix, leaving the DNA template as the only variable. This PCR setup can be scaled up to a 20 μL reaction volume and works with Phusion DNA Polymerase (Thermo Fisher Scientific) or other high-fidelity polymerases for cloning.

Golden gate assembly

DNA parts provided in the MoClo Toolkit (Addgene kit # 1000000044) and the GoldenPiCS Kit (Addgene kit #1000000133), were gifts from Sylvestre Marillonnet (Weber *et al.* 2011; Werner *et al.* 2012) and Gasser/Mattanovich/Sauer Group (Prielhofer *et al.* 2017), respectively. Golden Gate reactions used FastDigest Eco31I (BsaI), and BpiI (BbsI) type IIS restriction enzymes (Thermo Fisher Scientific), but the protocols are compatible with alternative suppliers (New England Biolabs) or enzymes. Efficient ligation was obtained with T4 DNA ligases from Thermo Fisher Scientific (MA, USA; 5 U/μL; EL0012) at 22 °C, and New England Biolabs (MA, USA; M0202) at 16 °C. Both suppliers provided efficient assembly in our methods. All the Golden Gate assemblies constructed in this study are summarized in Method S3.

In BOTany3-MoClo-Table.xlsx, the example illustrates 10 μL Golden Gate reactions consisting of 1 μL each of the MoClo parts (backbone, promoter, CDS, and terminator), combined with 6 μL of Master Mix containing 1 μL 10 mM ATP (Thermo Fisher Scientific, MA, USA; R0441), 1 μL 10× FastDigest Buffer, 0.5 μL FastDigest type IIS restriction enzyme, 0.5 μL T4 DNA ligase (either brand mentioned above), and 3 μL water.

E. coli Methods

Escherichia coli calcium competent cells were prepared based on an online protocol (Krantz 2013). The transformation efficiency of these cells was approximately 10^6 colonies per μg of pUC19 plasmid DNA. After outgrowth on OT-2, 50 μL *E. coli* cell cultures were plated on 60 mm plates containing low salt LB agar supplemented with proper antibiotics (50 μg/mL carbenicillin and spectinomycin for MoClo L1 and LM constructs, respectively). For blue-white screening, 10 μL of X-gal stock (20 mg/mL in dimethyl sulfoxide) and 30 μL of IPTG stock (Isopropyl β-D-1-thiogalactopyranoside; 100 mM in water) were spread onto each 60-mm LB agar plate together with the transformed *E. coli* culture. Plates were incubated overnight at 37 °C to allow colony formation.

The *Mix & Go! E. coli* Transformation Kit and Buffer Set (Zymo research; T3002) was used according to the supplied manual with TOP10F′ strain. For no-shock transformation, 100 μL of *Mix & Go! E. coli* competent cells were mixed with 2 μL of MoClo DNA assembly, incubated on ice for 30 min, recovered for 1 h in 400 μL of SOC media, and plated (50 μL aliquots) on solid media.

Plasmid DNA extraction

For DNA extraction, positively genotyped *E. coli* colonies were inoculated in 1.6 mL low-salt LB medium with antibiotics in 24 deep-well

plate sealed with an air-permeable cover (Sigma Aldrich; Cat# AXYPDW10ML24C). The cultures were incubated overnight at 250 rpm at 37 °C in an Innova 42 shaker. For each culture, 800 μL was mixed with 200 μL of 75% glycerol to prepare glycerol stocks, which were stored in 1.6-mL CryoPure tubes (Sarstedt), and the rest of the culture was used for Zyppy-96 Plasmid MagBead Miniprep kit (Zymo Research, Cat# D4102) in Method S5. After the automated steps, beads may be present in the elution plate but did not interfere with plasmid use, including the DNA quantification using a NanoDrop spectrophotometer. For manual plasmid extraction, we used universal miniprep spin columns (Syd Labs, MA, USA; MB082-PC200) and lab-made buffers as described in Method S5.

Yeast growth for universal method validation

Pichia experiments were conducted as previously described (Grieß-Osowski *et al.* 2025), using strains expressing sfGFP and mScarlet fluorescent reporters under the methanol-inducible *pAOX1* promoter. Transformed GFP, RFP-1, RFP-2, RFP-3 lines along with X-33 strain were grown in 500 μL YP media supplemented with 1.5% (v/v) methanol, 0.5% (v/v) glycerol, and 150 μg/mL hygromycin in a NEST 96-well 2 mL plate using a Multitron shaker (Infors HT, Switzerland) at 30 °C, 800 rpm, 80% humidity for 72 h. Optical density and fluorescent signals were quantified on a BioTek Synergy H1 Microplate Reader (Grieß-Osowski *et al.* 2025).

Acknowledgments

We dedicate this manuscript to Andrew D. Hanson who championed advances in plant synthetic biology and the establishment of a biofoundry at the University of Florida (UF). We also acknowledge the Opentrons Education Initiative for helping us bring automation to the classroom through the donation of a teaching robot and support with the MagBead Python script to enhance the plant molecular biology curriculum. We thank Syd Labs for sharing protocols for spin columns.

Author contributions

M.Q. led the implementation of the protocols, the manuscript drafting, the data acquisition and analysis. A.L. programed the Python scripts and runtime parameters, and L.L. performed biological experiments using the robots. C.V. conceived the project, supervised and supported the method development, analyzed data, and revised the manuscript.

Supplementary material

Supplementary material is available at *Plant Physiology* online.

Funding

The BOTany setup was funded by a UF Institute of Food and Agricultural Sciences (UF/IFAS) Equipment Grant, and personnel funding from the Horticultural Sciences Department. The work of C.V. was also supported by the USDA National Institute of Food and Agriculture, Research Capacity Fund (Hatch) project 7004470. A.L. and L.L. were funded in part by the UF AI Scholars and the UF/IFAS Summer Research Internships programs, respectively. The CSL constructs used to validate the workflow were obtained through the Joint Genome Institute Proposal No. CSP-510074. The work conducted by the U.S. Department of Energy Joint Genome Institute (https://ror.org/04xm1d337), a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy operated under Contract No. DE-AC02-05CH11231.

Conflict of interest

None declared.

Data availability

Data is provided in the supplementary section, with additional Python scripts, tables and custom labware files publicly available on GitHub (https://github.com/cvoiniciuc/BOTany). Additional information is available upon request from the corresponding author.

References

Annese D *et al.* 2025. Semi-automated workflow for high-throughput Agrobacterium-mediated plant transformation. *Plant J Cell Mol Biol.* 122:e70118. https://doi.org/10.1111/tpj.70118.

Bird JE, Marles-Wright J, Giachino A. 2022. A user's guide to golden gate cloning methods and standards. *ACS Synth Biol.* 11:3551–3563. https://doi.org/10.1021/acssynbio.2c00355.

Bryant JA Jr, Kellinger M, Longmire C, Miller R, Wright RC. 2023. AssemblyTron: flexible automation of DNA assembly with Opentrons OT-2 lab robots. *Synth Biol.* 8:ysac032. https://doi.org/10.1093/synbio/ysac032.

Dong J *et al.* 2025. Enhancing lipid production in plant cells through automated high-throughput genome engineering and phenotyping. *Plant Cell.* 37:koaf026. https://doi.org/10.1093/plcell/koaf026.

Engler C *et al.* 2014. A golden gate modular cloning toolbox for plants. *ACS Synth Biol.* 3:839–843. https://doi.org/10.1021/sb4001504.

Engler C, Kandzia R, Marillonnet S. 2008. A one pot, one step, precision cloning method with high throughput capability. *PLoS One.* 3:e3647. https://doi.org/10.1371/journal.pone.0003647.

Gome G, Waksberg J, Grishko A, Wald IY, Zuckerman O. 2019. OpenLH: open liquid-handling system for creative experimentation with biology. Proc. Thirteen. Int. Conf. Tangible Embed. Embodied Interact. New York, NY, USA, Association for Computing Machinery, pp 55–64

Grieß-Osowski A, Robert M, Qiande M, Clauss S, Voiniciuc C. 2025. Illuminating glucomannan synthases to explore cell wall synthesis bottlenecks. *ACS Synth Biol.* 14:2472–2479. https://doi.org/10.1021/acssynbio.5c00181.

Hillson N *et al.* 2019. Building a global alliance of biofoundries. *Nat Commun.* 10:2040. https://doi.org/10.1038/s41467-019-10079-2.

IFR. 2024. Top 5 Robot Trends 2024. IFR Int. Fed. Robot. https://ifr.org/ifr-press-releases/news/top-5-robot-trends-2024. Accessed 18 Apr. 2025.

Krantz B. 2013. Making Calcium Competent Cells. Krantz Lab Website. https://mcb.berkeley.edu/labs/krantz/protocols/calcium_comp_cells.pdf. Accessed 22 Oct. 2025.

Laverick A *et al.* 2025. OT-Mation: an open-source code for parsing CSV files into python scripts for control of OT-2 liquid-handling robotics. *Synth Biol.* 10:ysaf009. https://doi.org/10.1093/synbio/ysaf009.

Lázaro-Perona F *et al.*. 2021. Evaluation of two automated low-cost RNA extraction protocols for SARS-CoV-2 detection. *PLoS One.* 16:e0246302 https://doi.org/10.1371/journal.pone.0246302.

Lee ME, DeLoache WC, Cervantes B, Dueber JE. 2015. A highly characterized yeast toolkit for modular, multipart assembly. *ACS Synth Biol.* 4:975–986. https://doi.org/10.1021/sb500366v.

Lin-Cereghino J *et al.* 2005. Condensed protocol for competent cell preparation and transformation of the methylotrophic yeast Pichia pastoris. *BioTechniques.* 38:44–48. https://doi.org/10.2144/05381BM04.

Montes C, Zhang J, Nolan TM, Walley JW. 2024. Single-cell proteomics differentiates Arabidopsis root cell types. *New Phytol*. 244:1750–1759. https://doi.org/10.1111/nph.19923.

Moufarrej MN, Quake SR. 2023. An inexpensive semi-automated sample processing pipeline for cell-free RNA extraction. *Nat Protoc*. 18:2772–2793. https://doi.org/10.1038/s41596-023-00855-2.

Patron NJ *et al.* 2015. Standards for plant synthetic biology: a common syntax for exchange of DNA parts. *New Phytol*. 208:13–19. https://doi.org/10.1111/nph.13532.

Prielhofer R *et al.* 2017. GoldenPiCS: a golden gate-derived modular cloning system for applied synthetic biology in the yeast Pichia pastoris. *BMC Syst Biol*. 11:123. https://doi.org/10.1186/s12918-017-0492-3.

Püllmann P *et al.* 2021. A modular two yeast species secretion system for the production and preparative application of unspecific peroxygenases. *Commun Biol*. 4:1–20. https://doi.org/10.1038/s42003-021-02076-3.

Rupp N, Peschke K, Köppl M, Drissner D, Zuchner T. 2022. Establishment of low-cost laboratory automation processes using AutoIt and 4-axis robots. *SLAS Technol*. 27:312–318. https://doi.org/10.1016/j.slast.2022.07.001.

Shaw WM, Khalil AS, Ellis T. 2023. A multiplex MoClo toolkit for extensive and flexible engineering of saccharomyces cerevisiae. *ACS Synth Biol*. 12:3393–3405. https://doi.org/10.1021/acssynbio.3c00423.

Stephenson A *et al.* 2023. Physical laboratory automation in synthetic biology. *ACS Synth Biol*. 12:3156–3169. https://doi.org/10.1021/acssynbio.3c00345.

Storch M, Haines MC, Baldwin GS. 2020. DNA-BOT: a low-cost, automated DNA assembly platform for synthetic biology. *Synth Biol*. 5:ysaa010. https://doi.org/10.1093/synbio/ysaa010.

Tellechea-Luzardo J, Otero-Muras I, Goñi-Moreno A, Carbonell P. 2022. Fast biofoundries: coping with the challenges of biomanufacturing. *Trends Biotechnol*. 40:831–842. https://doi.org/10.1016/j.tibtech.2021.12.006.

Torres-Acosta MA, Lye GJ, Dikicioglu D. 2022. Automated liquid-handling operations for robust, resilient, and efficient bio-based laboratory practices. *Biochem Eng J*. 188:108713. https://doi.org/10.1016/j.bej.2022.108713.

Villanueva-Cañas JL *et al.* 2021. Implementation of an open-source robotic platform for SARS-CoV-2 testing by real-time RT-PCR. *PLoS One*. 16:e0252509. https://doi.org/10.1371/journal.pone.0252509.

Weber E, Engler C, Gruetzner R, Werner S, Marillonnet S. 2011. A modular cloning system for standardized assembly of multigene constructs. *PLoS One*. 6:e16765. https://doi.org/10.1371/journal.pone.0016765.

Werner S, Engler C, Weber E, Gruetzner R, Marillonnet S. 2012. Fast track assembly of multigene constructs using golden gate cloning and the MoClo system. *Bioengineered*. 3:38–43. https://doi.org/10.4161/bbug.3.1.18223.