

Enabling Automated Microplate Replication Using the Agilent Bravo BenchCel Workstation

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Introduction

Microplate replication is one of the most common workflows used in compound management research facilities. Because of the large quantity of microplates that must be processed daily, it is a labor-intensive task ideally suited for automation. In addition to increasing throughput, automation itself provides added consistency and reliability.

The Agilent Bravo Automated Liquid Handling Platform is equipped to handle a wide range of automated workflows, including microplate replication. It has nine standard deck positions that can hold any ANSI-standard microplate, and be customized with a variety of accessories to enable functionalities such as heating, cooling, shaking, barcode reading, and tip washing. The Agilent Bravo Pipetting Head can enable volume transfers by column or row, from 300 nL to 200 μ L. The integration of the Agilent BenchCel Microplate Handler with the Bravo provides an efficient way of storing and dispensing microplates and pipette tip boxes, permitting users to process multiple batches of microplates with more walk-away time. This Application Note describes how these two integrated instruments, combined as an Agilent Bravo BenchCel Workstation, can provide a complete workflow solution for automating microplate replication. Instrumentation is driven by the Agilent VWorks Automation Control Software using a simplified interface that is easy to use and fully enabled for this application.

Experimental

Equipment

The microplate replication workflow was carried out using an Agilent Bravo BenchCel Workstation System consisting of:

- Agilent BenchCel 4R Microplate Handler with 660 mm Front Loading Racks
- Agilent Bravo Automation Liquid Handling Platform with Gripper
- Agilent 96LT Disposable Pipette Tip Head
- Agilent 96LT 250 μL Disposable Pipette Tips
- 96 well polystyrene, clear flat bottom microplates (Greiner 655101)
- Nunc 96-deep well polypropylene, U bottom plates (ThermoFisher 260251)

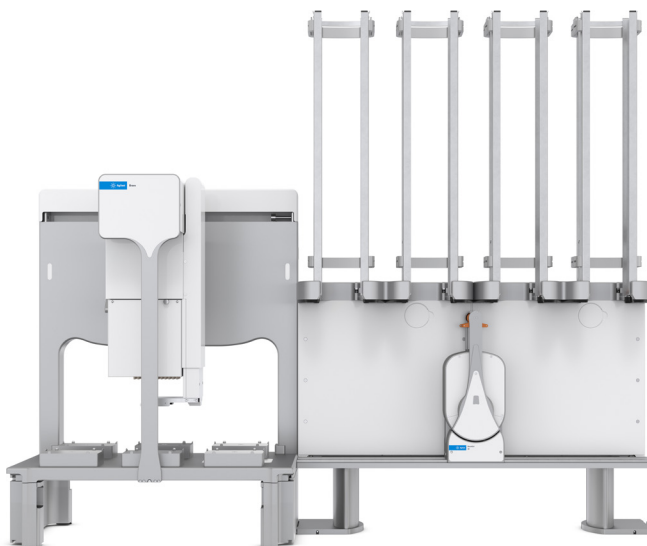


Figure 1. Agilent Bravo BenchCel Workstation System for Automated Microplate Replication.

Agilent VWorks Automation Control Software

To simplify startup for new users, preloaded form-based protocols for automated microplate replication are provided with each Bravo BenchCel Workstation. Protocol variations for each BenchCel Configuration are included.

Microplate Replication Protocol Instrument setup

1. Open VWorks Automation Control Software and load the Plate Replication System Form (Figure 2).
2. Load Source microplates into BenchCel Stacker 3.
3. Stack Destination plates onto Bravo deck position 4.
4. Load pipette tip boxes into BenchCel Stacker 1.

 A screenshot of the Agilent VWorks Protocol Form for Automated Plate Replication Systems. The form is titled "Plate Replication System" and includes various setup sections:

- Stacker Setup:** Shows four stackers (Stacker 1-4) with tip boxes and source plates.
- Bravo Deck Setup:** Shows a 6-well deck with positions 1-6. Position 4 is labeled "Downstack Location - Destination", position 8 is "Upstack Location - Destination", and others are "Empty".
- Pipette Settings:** Includes fields for Aspirate Volume (0-251 μL), Pre-Aspirate Volume (0-251 μL), Post-Aspirate Volume (0-251 μL), Dispense Volume (0-251 μL), Blowout Volume (0-251 μL), Distance from Well Bottom (0-100 mm), and Dynamic Tip Retraction (0-20 mm/ μL).
- Run Setup:** Includes Number of Plates to Process, Number of Plate Replicates, Labware Type (Source and Destination), and dropdown menus for Source (96 Nunc Deep Well 1 mL) and Destination (96 Greiner 655101 PS Clr Rnd Well Flat Btm).
- Control Panel:** Includes buttons for Start Run, Pause, Initialize all devices, Full Screen on/off, and Reset All Values to Default.

Figure 2. Agilent VWorks Protocol Form for Automated Plate Replication Systems.

- In the **Pipette Settings** submenu, enter the desired pipetting parameters.
- In the **Run Setup** submenu, enter the desired number of Source microplates under **Plates to Process** and Destination microplates under **Plate Replicates**. Select the labware type used for Source and Destination microplates.
- Select **Start Run** to begin automated workflow.

Instrument workflow

- Transfer pipette boxes from BenchCel Stacker 1 to Bravo deck position 2.
- Transfer source microplates from BenchCel Stacker 3 to Bravo deck position 6.
- Transfer destination microplates from Bravo deck position 4 to Bravo deck position 5.
- Transfer solutions from source microplates to destination microplates using set dispensing and replicating parameters. Pipette tips are replaced for each source microplate used in the protocol.
- Upon completion, source microplate is transferred back to BenchCel Stacker 4.
- Destination microplates are stacked vertically on Bravo deck position 8.
- Used pipette tips are re-racked into their tip boxes and transferred back to BenchCel Stacker 2.

Results and Discussion

Microplate replication is a straightforward process in which defined quantities of a solution are transferred from a single source microplate into multiple destination microplates (Figure 3). These destination plates are then used for multiple downstream applications such as small molecule screening in research or toxicity studies. Having an automated workflow for this routine task enables greater consistency, while increasing walk-away time.

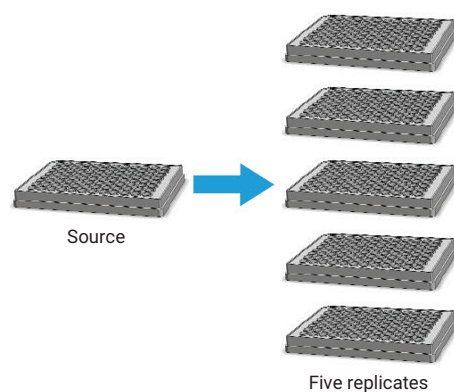


Figure 3. Microplate Replication Workflow Example.

To demonstrate this workflow, an automation microplate replication workstation was assembled using the Bravo Liquid Handling Platform with the 96LT Channel Pipette Head and the BenchCel 4R Microplate Handler. The Bravo Liquid Handler included a gripper attachment to move microplates between various deck positions. The 96-channel pipetting tip head was used to handle Agilent 250- μ L disposable pipette tips. Transfer and storage of microplates or pipette tip boxes was performed by the BenchCel 4R.

While a stand-alone Bravo Liquid Handling Platform is capable of performing microplate replication, there are limitations for high-throughput workflows. Integration with the BenchCel Microplate Handler significantly increases the microplate capacity of this protocol by simultaneous and independent transfer of microplates and pipette tip boxes at Bravo deck position 3.

The Bravo BenchCel Workstation control was handled by Agilent VWorks Automation Control software using a form-based interface designed for automating microplate replication. Source microplates can be replicated in a variety of ratios into new Destination plates using the adjustable fields found in the **Pipette Settings** and **Run Setup** submenus. To ensure reproducibility and minimize contamination, pipette tips are designed to be changed after each set of destination microplates is produced.

Conclusion

The Agilent Bravo BenchCel Workstation provides a complete liquid handling and microplate management solution for enabling automated microplate replication workflows. The Agilent BenchCel Microplate Handler facilitates simultaneous delivery of Source microplates, Destination microplates, and pipette tips to the Agilent Bravo Liquid Handling Platform. Inclusion of a protocol form in the Agilent VWorks Automation Control Software enables quick start-up time and the ability make simple protocol adjustments to achieve customizable source-to-destination microplate ratios.

Depending on the number of plates to process, and the pipetting parameters used, it takes approximately 7 minutes generate five replicate plates from one Source plate and one pipette tip box on a Bravo BenchCel Workstation. Additionally, these forms can be used as a template to build more customized workflows, when the workstation is integrated with additional devices onto the BenchCel Microplate Handler. Overall, the Bravo BenchCel Workstation provides a simplified method for automating microplate replication to meet the needs of high-throughput screening research facilities.

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