

A complete microbial workflow from plating and picking through growth curve measurement and plasmid DNA quantitation

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Introduction

Microbial strains are central to biotechnology, healthcare, and environmental science, yet manual workflows introduce variability, contamination risks, and throughput bottlenecks. This study presents a microbial processing workflow from plating and colony picking through growth analysis and, ultimately, plasmid DNA purification and quantification.

The QPix® FLEX Integrated Work Cell automated the process of plating/streaking, colony selection, colony picking, and data management within a sterile framework to eliminate manual variability and boost reproducibility. The SpectraMax® iD3s Multi-Mode Microplate Reader with dual injectors enabled real-time monitoring of microbial growth and the delivery of nutrient or antibiotic to designated wells for the assessment of their effects on growth as measured by OD₆₀₀. The sensitive fluorescence detection mode of the reader was used to accurately measure the amount of DNA purified from the small-volume cultures.

Methods

Automated colony plating & picking

E. coli colonies were plated, imaged, and automatically picked based on blue/white phenotypes. Mapped colonies were transferred into a 96-well plate, and overnight growth reproduced the expected color outputs, confirming accurate plating, picking, and data generation.

- E. coli* strain JM109 transformed with pGemT vector + (control) insert was streaked onto LB agar plates using the QPix FLEX Microbial Colony Picker and screened with colorimetric markers (blue/white) combined with morphology criteria
- Colonies ranging from 0.5 to 3 mm were transferred into 96-well plates containing liquid media and incubated overnight verified via OD₆₀₀ readings
- A single resulting colony of interest from the overnight culture was selected and subsequently inoculated (manually) into 5 mL of LB broth and grown in a 15-mL culture tube to an OD₆₀₀ of 0.9 with shaking for 14 to 16 hours at 37°C. This was then diluted with fresh LB broth to an OD₆₀₀ of about 0.1 OD
- 190 µL of this *E. coli* culture was then added to wells B2 to G11 of a clear 96-well microplate, and to wells in columns 4 and 5, glucose was added to a final concentration of 30 mM
- The microplate was transferred to the microplate reader for subsequent experiments

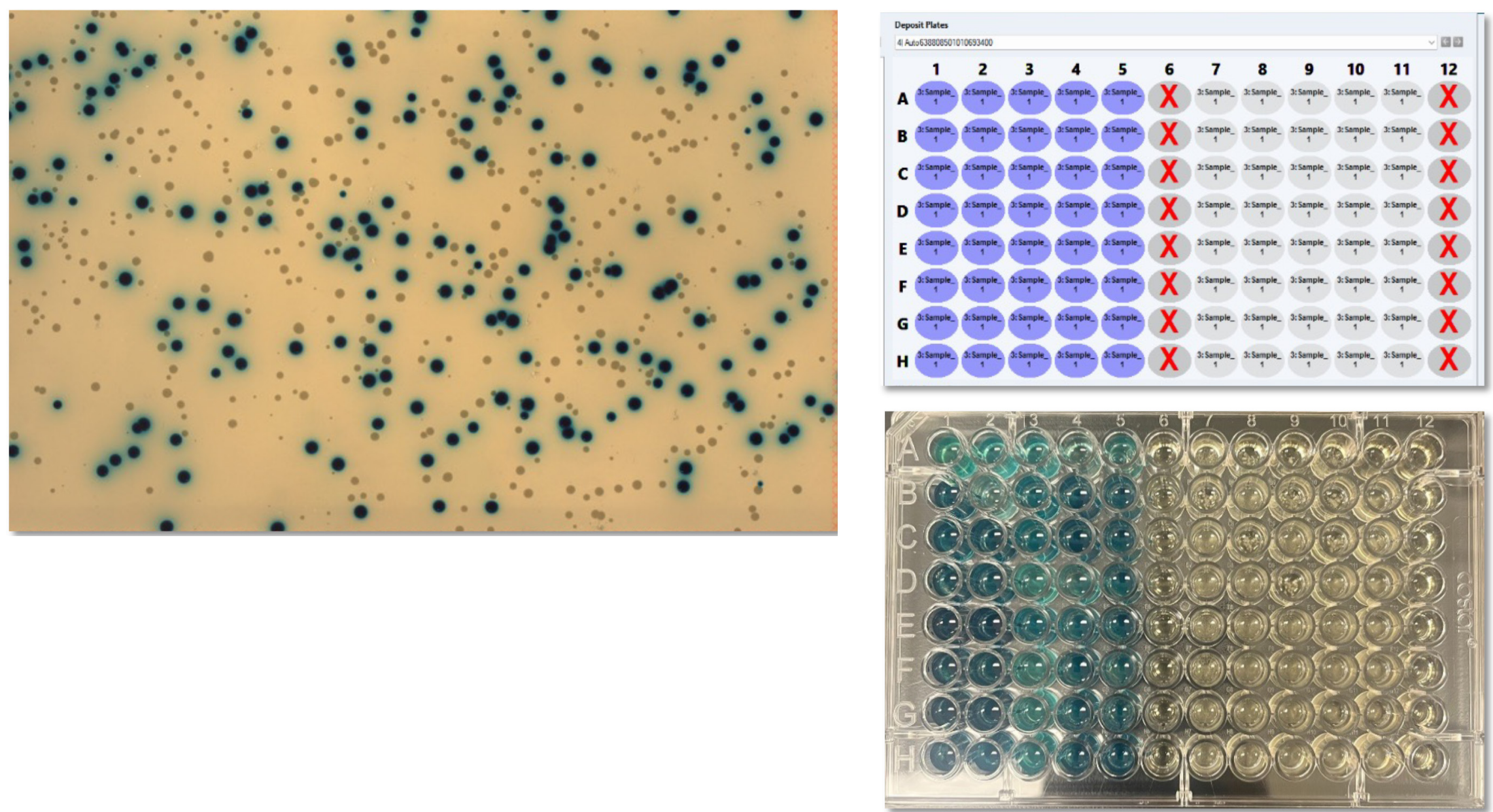


Figure 1. Image (left) shows a true color image of a Nunc™ OmniTray™ plated with *E. coli* colonies, where distinct blue (+lacZ) and white (ΔlacZ) phenotypes were observed. Image (top right) presents the user-defined destination plate map created to guide colony transfer into specific wells. Image (bottom right) displays the corresponding destination plate after inoculation and incubation overnight at 37°C.



Figure 2. QPix FLEX Microbial Colony Picker. Features automated plating, streaking, colony picking, liquid handling, and high-resolution color imaging.

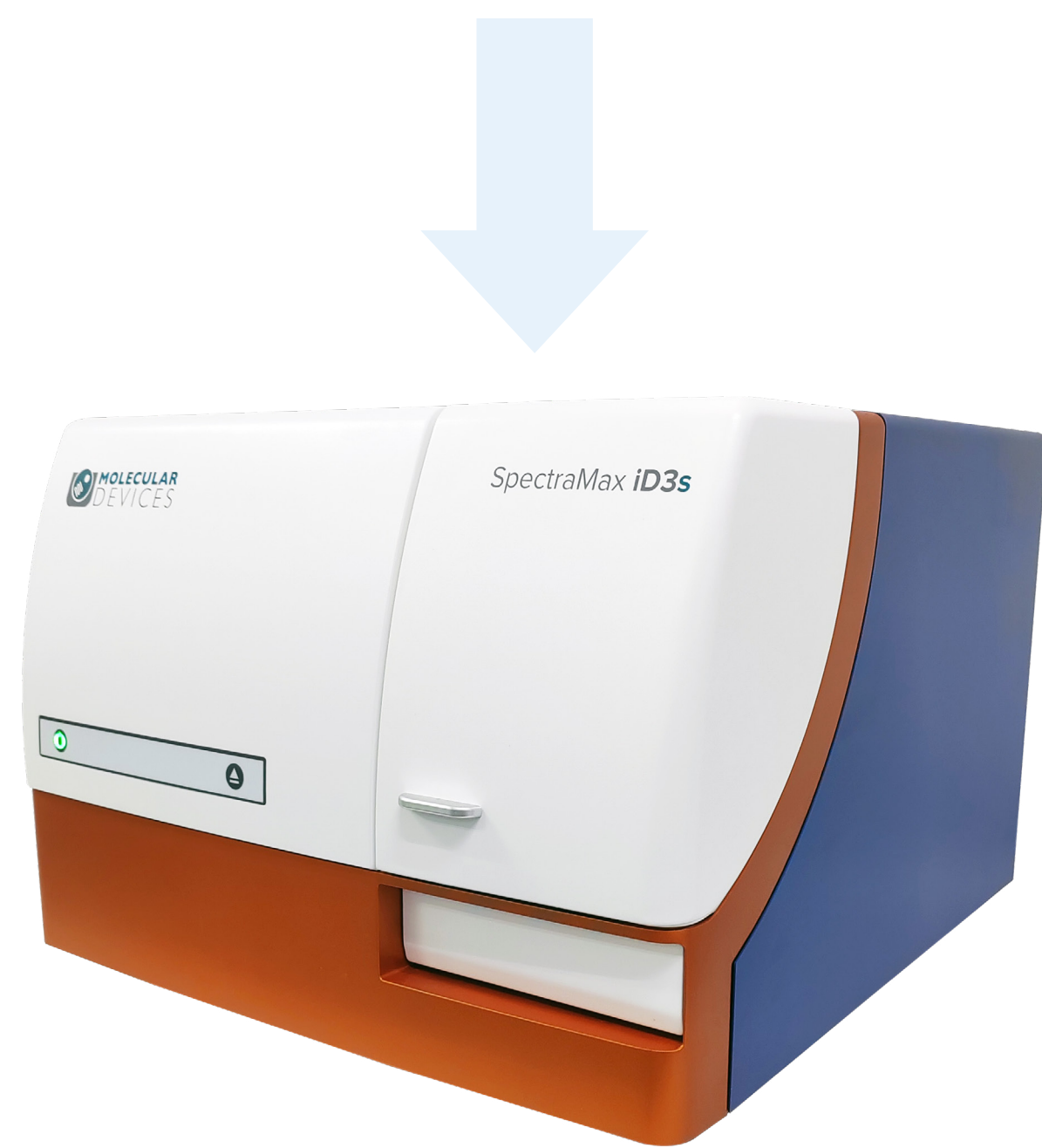


Figure 3. SpectraMax iD3s Multi-Mode Microplate Reader. Features absorbance, fluorescence, and luminescence detection, with optional Advanced Shaking and dual injectors.

Microbial growth & response monitoring

The microplate was placed in the SpectraMax iD3s reader, and a kinetic read was initiated using a protocol with workflow set up in SoftMax® Pro software as described below.



Figure 4. Workflow Editor in SoftMax Pro software was used to set up a three-part workflow: (1) 14 cycles of OD₆₀₀ reads followed by Advanced Shaking configured to match settings of a typical bacterial shaker (orbital mode, diameter 2.5 mm and speed 500 rpm); (2) injections of glucose or chloramphenicol into specified wells; (3) continuation of OD₆₀₀ reads with shaking. Total time: 14 hours.

Plasmid purification & quantitation

- Following incubation, plasmid DNA extraction and purification was performed on 200-µL samples of bacterial cultures using the Mag-Bind UltraPure Plasmid DNA kit
- Purified plasmid DNA was quantitated using the Quant-iT PicoGreen dsDNA Kit, with results detected and analyzed using the SpectraMax iD3s reader and SoftMax Pro software

Results

Kinetic growth monitoring

Raw data on bacterial growth were generated using SoftMax Pro software and could be viewed as continuous kinetic traces at any time during the workflow (Figure 3). Further analysis of growth response to the addition of glucose or chloramphenicol are shown in Figure 4.

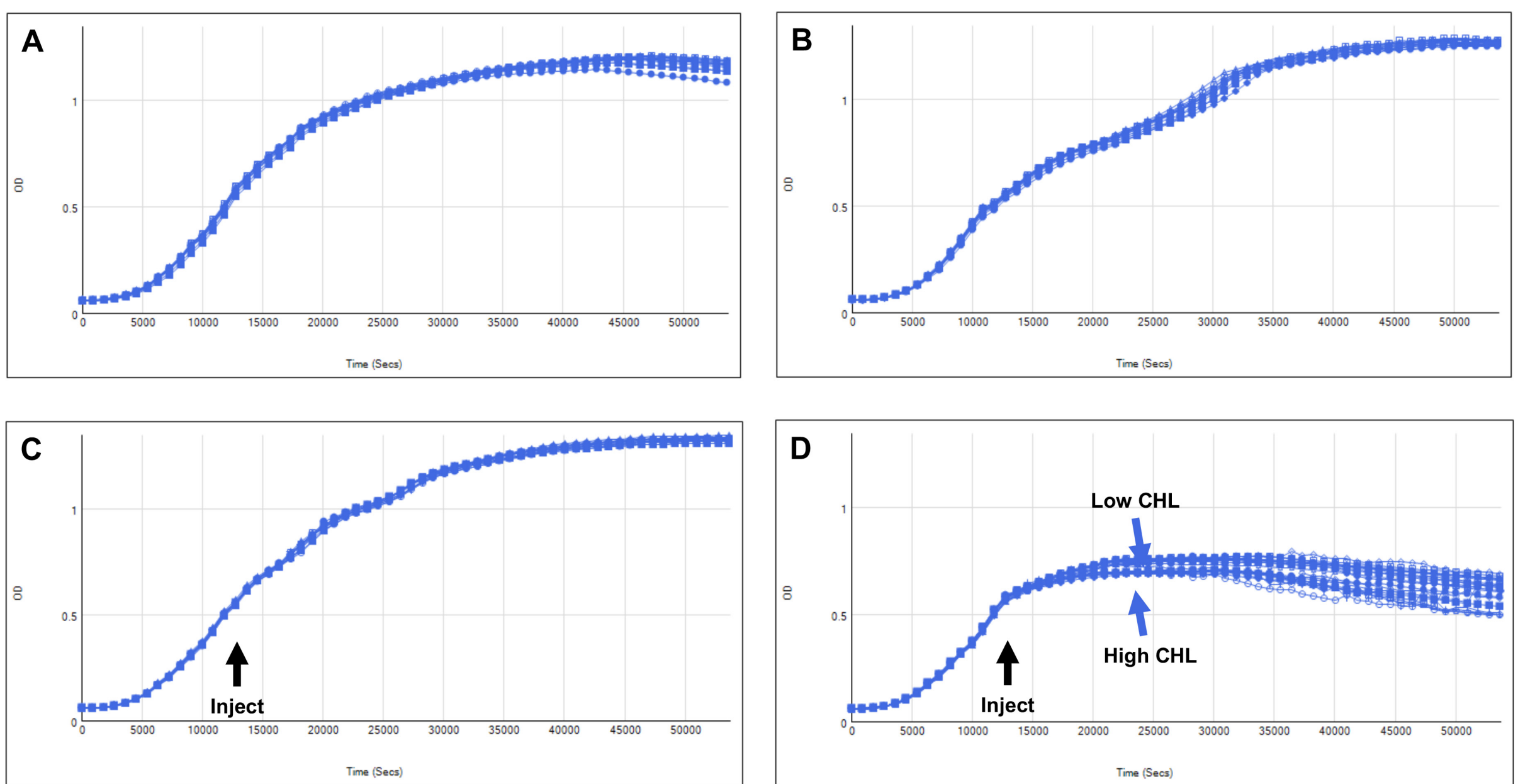


Figure 5. Kinetic traces of replicate wells. A, control; B, glucose added at time zero; C, glucose added by injector at mid-log phase; D, high or low concentration of chloramphenicol (CHL) added by injector at mid-log phase of growth.

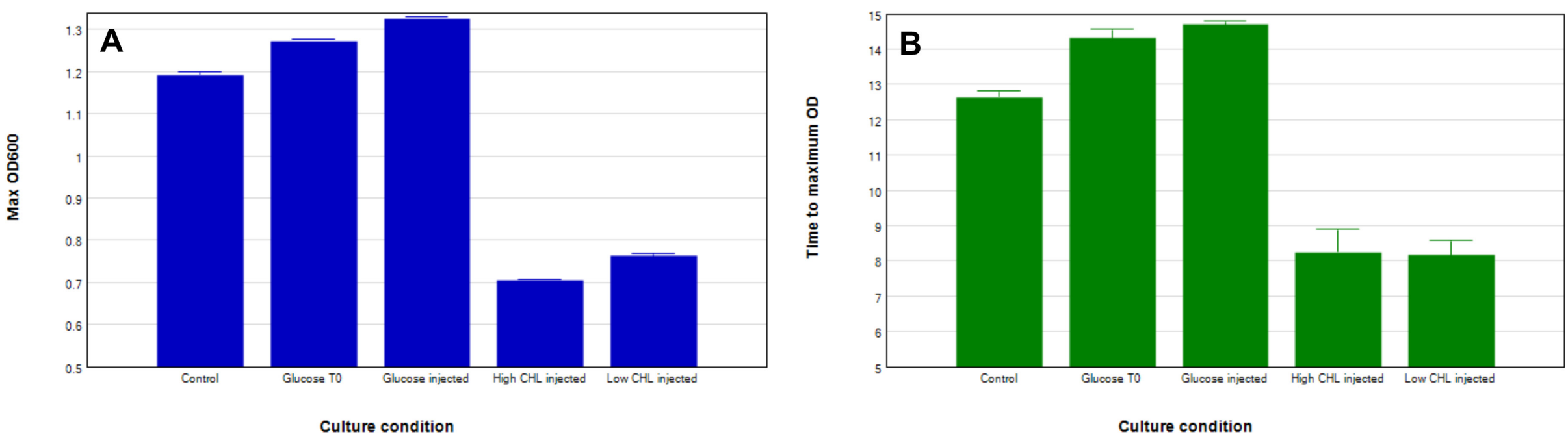


Figure 6. Raw kinetic growth curve data with calculations applied using the data reduction function in SoftMax Pro software. Results for different culture conditions were plotted: A, maximum OD₆₀₀ reached during the kinetic read; B, time required (hours) to reach maximum OD₆₀₀

Fluorescent DNA quantitation

The sensitivity of the assay in combination with the SpectraMax iD3s reader enabled accurate quantitation of plasmid preps from small-volume cultures grown in a microplate.

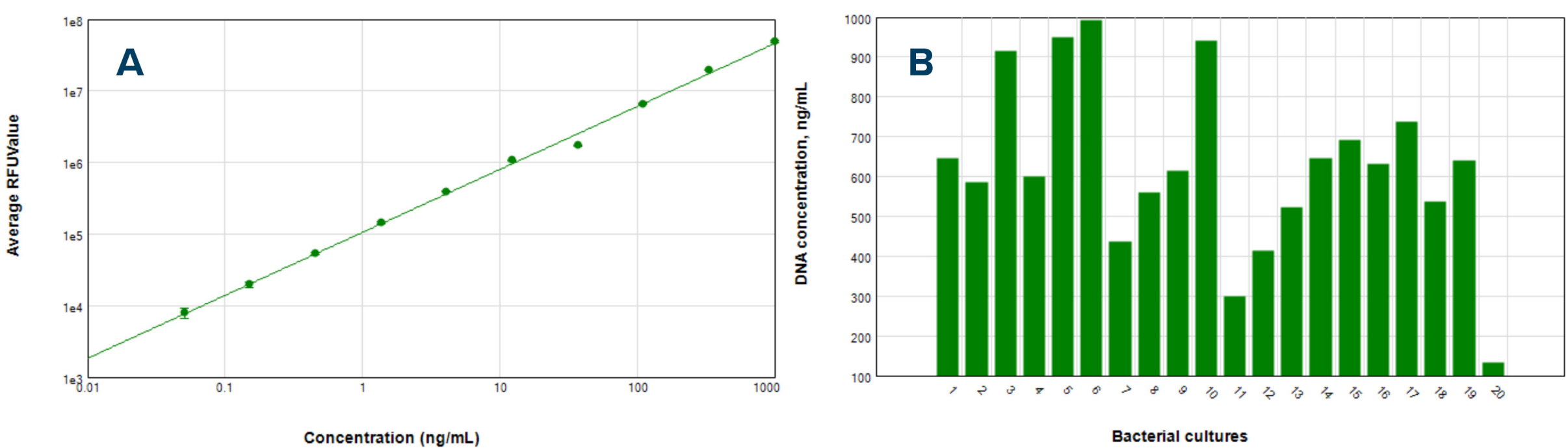


Figure 7. A, DNA standard curve, with standard concentrations ranging from 50 pg/mL to 1000 ng/mL ($r^2 = 0.998$). B, plasmid prep DNA concentrations interpolated from the standard curve.

Conclusion

- Automating steps of a complex workflow streamlines the process for higher throughput with faster results.
- Uniting plating, streaking, colony picking, liquid handling, and high-resolution color imaging into one benchtop system, the QPix FLEX system replaces multiple instruments and eliminates tedious manual steps.
- The SpectraMax iD3 reader's Workflow Editor and onboard injectors enable the execution of more complex experiments with timed additions of reagents while growth data are generated. The Advanced Shake feature allows selection of shaking conditions for optimal microbial growth.