

# Automated cell handling and lipofection-based transient transfection design of experiments (DOE) on the new Biomek i3 benchtop liquid handler

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## Introduction

Transient transfection of target cell lines allow for the study of gene expression, protein function, expression of therapeutic targets, activation of cell functionality and a variety of other biological processes. During the initial stages of experimental design, scanning the gamut of DNA concentrations and transfection reagents is important because it helps to identify the appropriate transfection efficiency ranges at which specific experiments need to be carried out. Use of an automated liquid handler capable of performing reagent prep, cell handling and time-dependent addition of complexes can deliver precision and reproducibility in this process. Beckman Coulter Life Sciences presents our newest lab companion, the Biomek i3 benchtop liquid handler. In this poster we showcase cell handling, passaging and transfection of HEK 293 cells on the new Biomek automated workstation for up to 96 samples. Our method demonstrates ease of use for the new automated liquid handler, highlights method writing and optimization steps, and shows the integrated data handling capability built into the system.

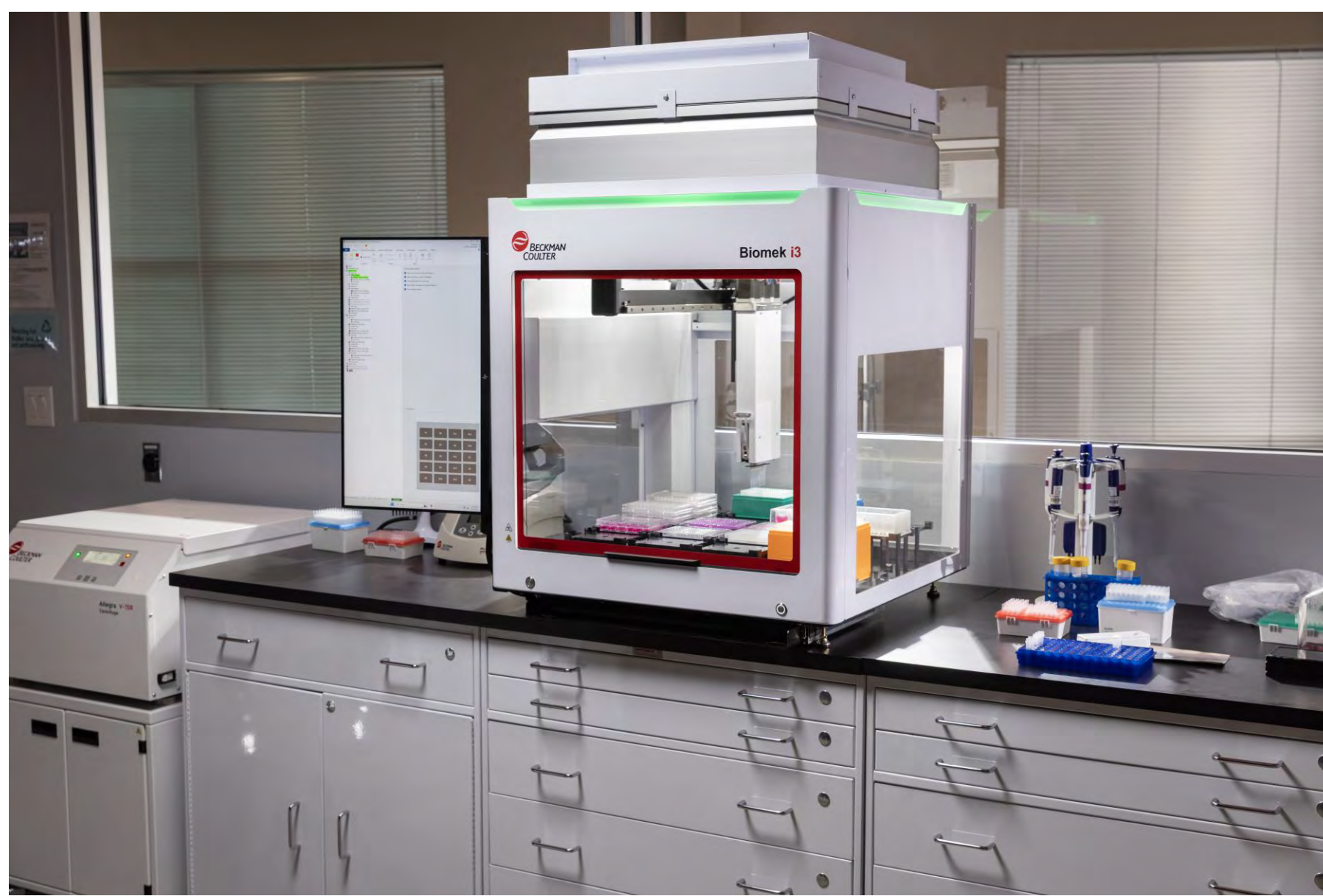


Figure 1. A Biomek i3 benchtop liquid handler easily fits into busy labs

## The Biomek i3 benchtop liquid handler – Key Features

### Hardware

- ❖ Small form factor with 15 fully pipettable positions + 5 flex positions
- ❖ Fully enclosed with available positive or negative HEPA filtration
- ❖ Onboard interlocked UV-C with 15-minute auto timer or manual on/off
- ❖ Top-mounted cameras with 30-second buffered automatic error logging
- ❖ Integrate for additional functionality – heating, cooling, shaking, tilting, and thermal cycling
- ❖ Easily selectable 1-8 tips with 1-1000 µL pipetting range
- ❖ Sturdy keyboard and mouse tray doubles as material staging space

### Software

- ❖ Touchscreen-enabled Biomek 5.5 software on Windows 11
- ❖ Fully functional real-time or warped 3D simulator – program at your desk, not in the lab
- ❖ Guided Labware Setup for functional, informative deck setup instructions
- ❖ Method Options Selector tool to easily build HTML user interfaces and variable inputs
- ❖ Biomek Accounts and Permissions for administrative control and 21 CFR Part 11 compliance
- ❖ Access to MyBeckmanLearning for downloadable methods, tools, and coaching resources

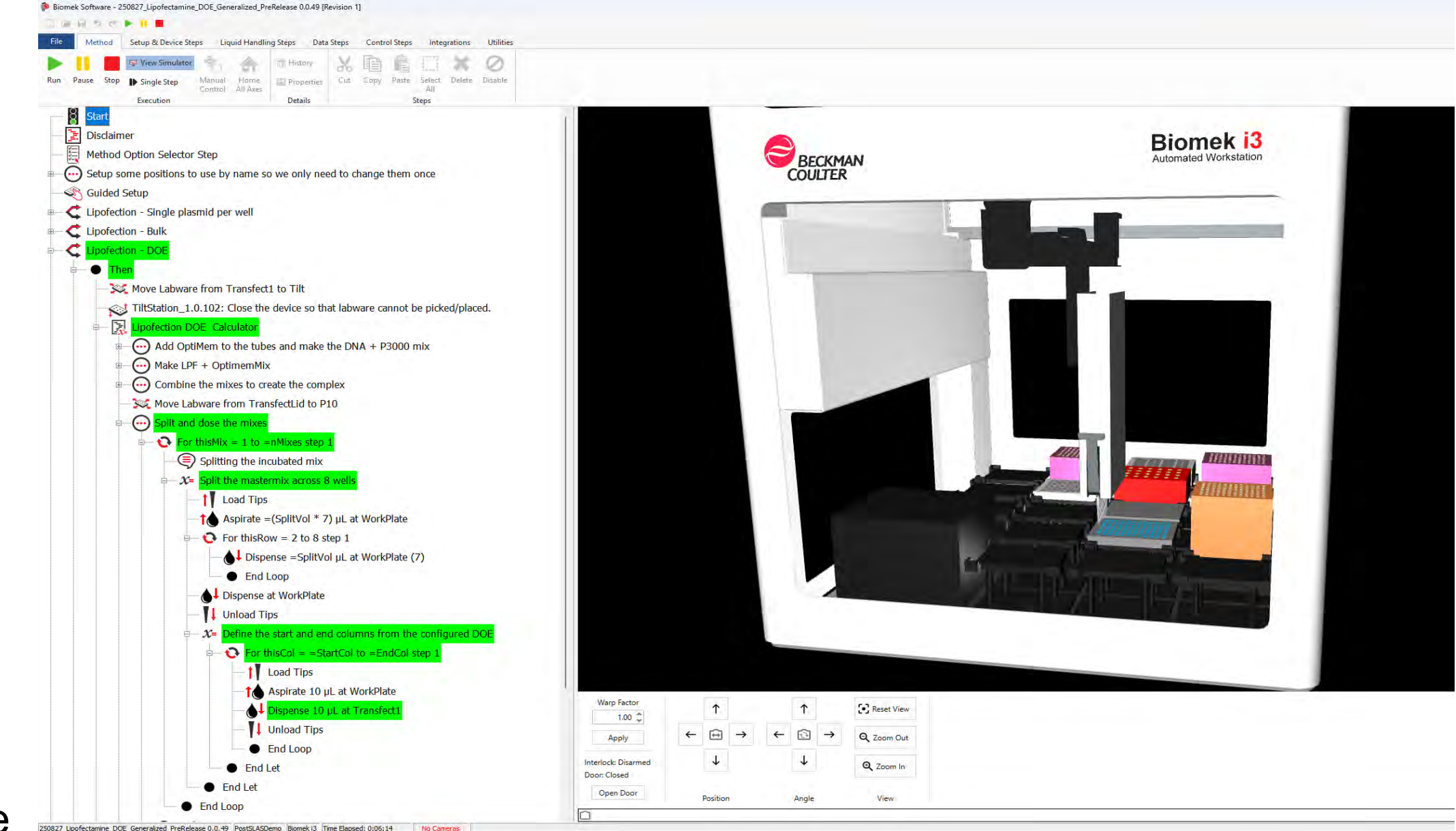


Figure 2. Biomek 5.5 software running in simulation enables desk development and frees up valuable machine time

## Cell seeding

On day -1 a freshly passaged suspension of human embryonic kidney 293 (HEK293) cells were transferred to a reservoir and counted utilizing a Vi-CELL BLU analyzer (Beckman Coulter Life Sciences, Indiana USA). Two dilutions were made to target concentrations of 25,000 and 50,000 cells per 100 µL. Diluted cell suspensions were then seeded across a 96-well flat, clear-bottom black-walled plate alternating by column using 100 µL at alternating densities (Fig. 1). The seeded cells were allowed to settle, and seeding was confirmed using an i3X MiniMax imager (Molecular Devices, California USA). This plate was then returned to the incubator at 37° C & 5% CO<sub>2</sub>.

## Lipofectamine™ 3000 Transfection

On day 0, the Biomek i3 Liquid Handler deck was loaded according to the on-screen directions with labware and tips. Lipofectamine 3000 kit tubes (Thermo Fisher Scientific, Massachusetts USA), a tube containing 1.5 mL OptiMEM (Gibco, New York USA), and a tube containing Monster Green phmGFP vector (Promega, Wisconsin USA) were loaded directly into a chilled reagent block. The seeded cell plate was loaded onto a warm position and held at 37° C. At method run time, experimental values were entered into the on-screen dialog on a µL/well basis. The experimental design reflected the manufacturer's recommended initial trial conditions for 96-well transfection using 0.15 µL or 0.3 µL Lipofectamine 3000 / well and fixed P3000 reagent ratio of 2 µL/µg DNA. DNA loading was chosen to reflect a .5X and 2X multiple of the recommended loading of 200 ng.

	DNA (µL / well)	P3000 Reagent (µL / well)	Lipofectamine 3000 (µL / well)	wells / column	# of columns
Mastermix 1	0.2	0.2	0.15	8	2
Mastermix 2	0.2	0.2	0.3	8	2
Mastermix 3	0.8	0.8	0.15	8	2
Mastermix 4	0.8	0.8	0.3	8	2

Table 1: Values provided to Biomek i3 DOE calculator. Global coverage margin was set to 15%.

The system then provided user feedback to confirm sufficient volumes were loaded to complete the experiment as designed. Following confirmation, the Biomek system then performed all mastermix creation, broadcasting, and dosing. For each mastermix, incubation of the DNA + P3000 and Lipofectamine 3000 reagent was automatically staggered by the system to result in a uniform incubation time of 12 minutes from mixing to payload dosing. Transfection payloads were dosed using a combination of gentle pipette mixing and tilting on the Biomek i3 liquid handler. When prompted, the transfection plate was returned to the incubator at 37° C & 5% CO<sub>2</sub>.

96-well plate	Cells seeded per well											
	25K		50K		25K		50K		25K		50K	
	1	2	3	4	5	6	7	8	9	10	11	12
A	No transfection											
B	Mastermix 1											
C	Mastermix 2											
D	Mastermix 3											
E	Mastermix 4											
F	No transfection											
G	No transfection											
H	No transfection											

Figure 3: Biomek i3 design-of-experiments plate layout

## TrypLE Cell harvesting

On day 4, approximately 60 hours post transfection, all wells were imaged using i3X MiniMax imager and a subset using a fluorescent microscope, cell media was removed, cells were washed with DPBS, and 50 µL TrypLE™ (Gibco) was added to all wells. The plate was then incubated at 37° C for 5m followed by 30 sec of orbital shaking at 1600 RPM. Cell detachment was verified with a brightfield microscope, and the plate was returned to the Biomek liquid handler. 150 µL DMEM was then added, and suspensions were triturated to ensure single-cell suspensions.

## Discussion

Here we demonstrate the use of the Biomek i3 benchtop liquid handler as a robust tool for lipofection-based transient transfection workflows. In our design, we intentionally used a DNA concentration we suspected to be in excess and may result in cytotoxic effects. The data supports this assumption, as all test conditions at 400 ng exhibited reduced transfection efficiency both in fluorescent imaging and in flow cytometry data. When considering the dosing of lipofectamine, we did not observe a significant difference between 0.15 µL and 0.3 µL additives in downstream transfection efficiency, except in the case of a 50k cell seeding density and high DNA input. This conforms to expectations that larger cell populations may be less susceptible to the cytotoxic effects of over-transfection. Further, we conclude that future experiments may benefit from reduced cost-per-data-point by proceeding with the lower lipofectamine concentration and reducing consumption of costly reagents. Further studies on the Biomek i3 instrument could include end point protein production and viability analysis to further investigate and optimize the seeding density and growth times. The method infrastructure and inputs are also amenable to other lipid complex-based transfection systems such as FuGENE (Promega) or ExpiCHO (Thermo Fisher Scientific). The Biomek i3 liquid handler is a capable test bed for the development and execution of automated multi-variate experimental designs that reduce hands-on time, increase experimental robustness, and provide high-confidence data suitable for driving meaningful workflow decisions.

## Fluorescent imaging

Prior to trypLE harvesting, wells were imaged using an EVOS fl inverted digital fluorescent microscope (AMG, Washington USA) using overlaid brightfield and 470nm / 525nm GFP settings. All intensity, gain, and contrast settings were held consistent between wells and only focal adjustments were made as needed. Wells were imaged as near to center as practicable.

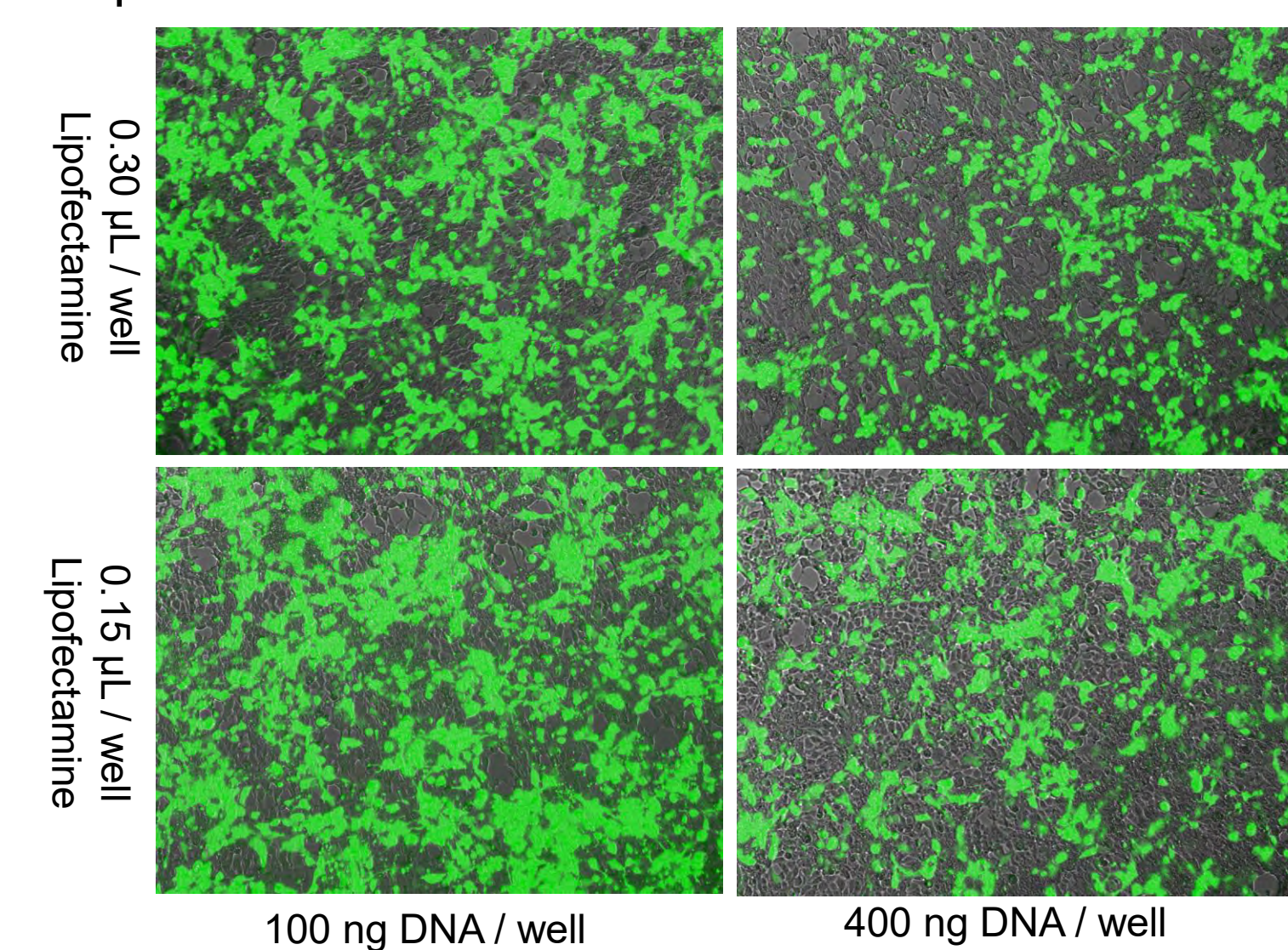


Figure 4: Representative fluorescent microscopy of the 4 mastermix conditions on 25K / well cell seeding density.

## Flow cytometry analysis

Daily QC flow beads (Beckman Coulter Life Sciences, PN C65719) were run with passing results. The Cytotflex cytometer was configured with an excitation/detection spectrum of 488/500-520 nm. A 510/520 OD bandpass filter was used with gain settings of FSC=36; SSC=95; and GFP=20. A target of 10,000 events were collected and gated first for cells, then singlet cells, then fluorescent singlets as shown in figure 5. "No transfection" well A12 was used to establish the fluorescent gate. Gain and gating settings were applied to all wells uniformly.

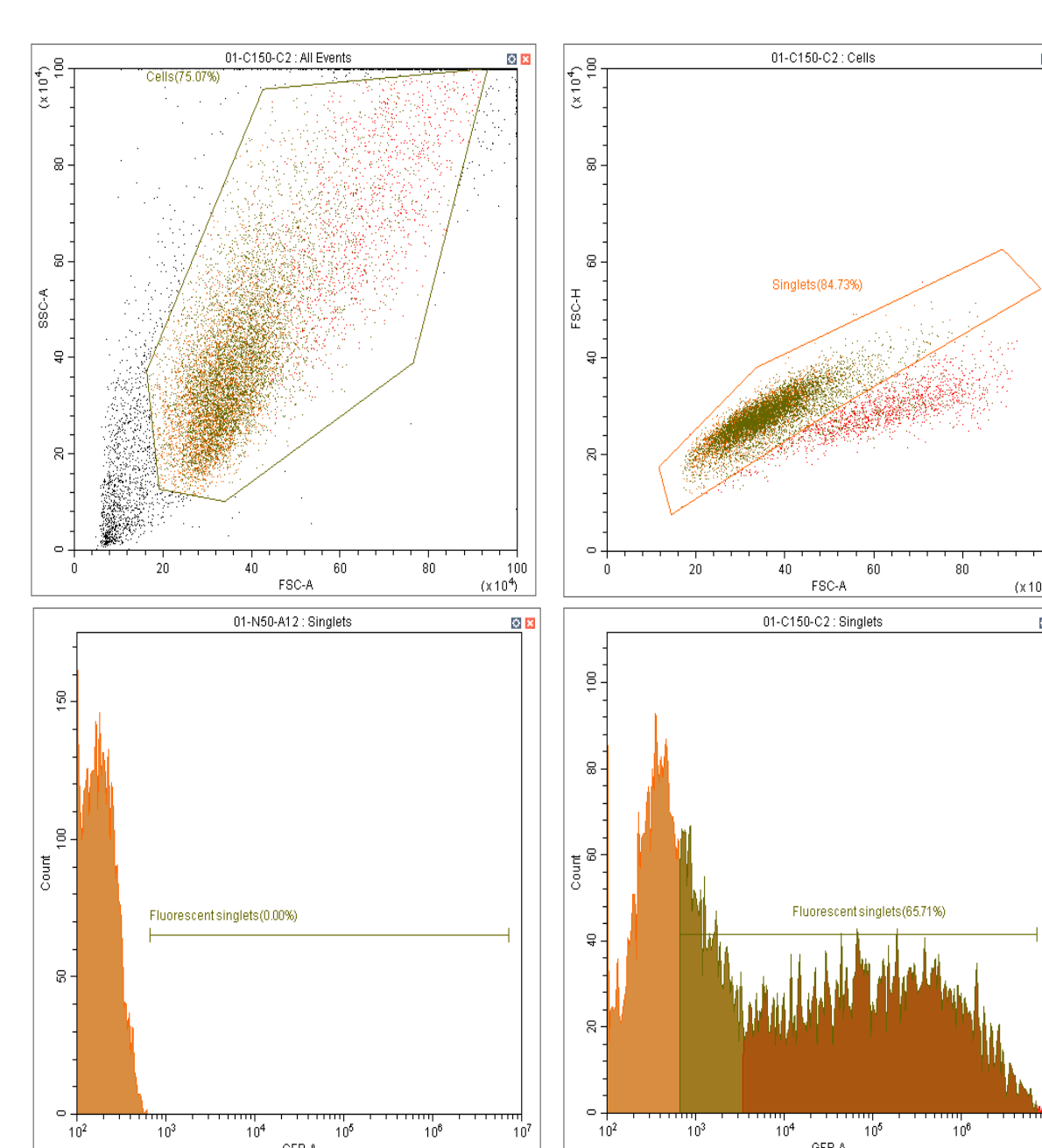


Figure 5: Flow cytometry gating strategy

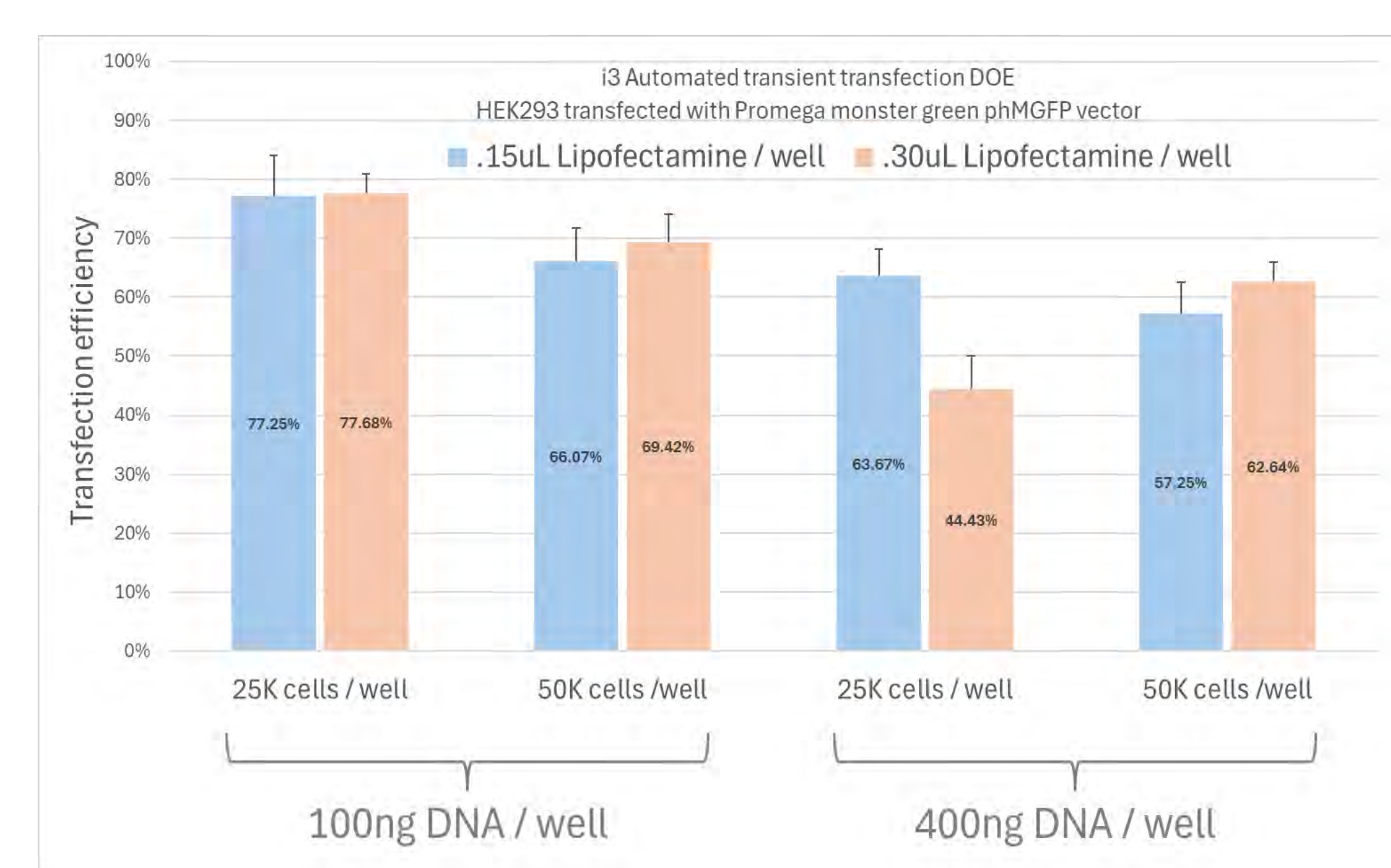


Figure 6: Singlet transfection efficiency. Error bars represent +1 SD

## Automated workflow and timings

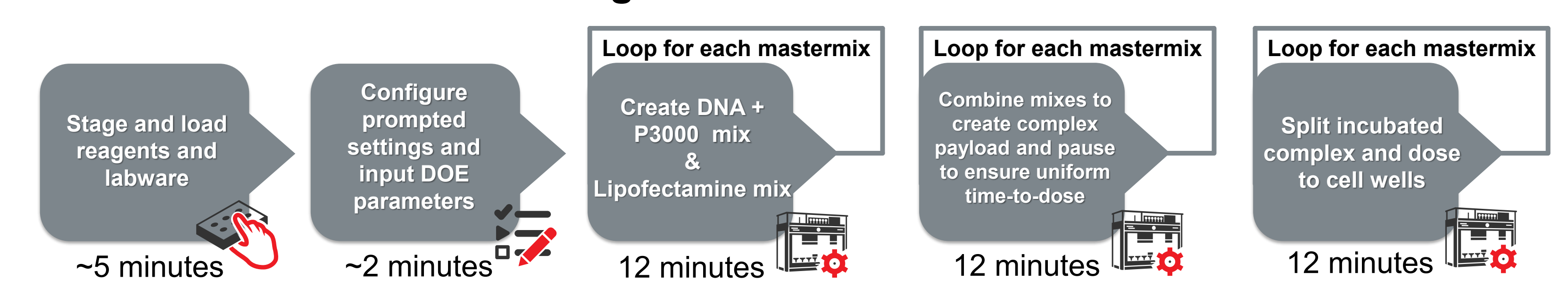


Figure 7: Approximate run times for an 8 condition by 8 well design-of-experiments

Product in Development. Performance characteristics have not been validated. Biomek Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

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