EXECUTING SERIAL DILUTIONS ON THE OT-2

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ABSTRACT

Serial dilutions are an important part of various biological experiments. For example, generating standard curves for DNA, protein or cell culture quantification are common use cases that require diluting samples with a constant dilution factor. Here we present an alternative to carrying out these repetitive steps with manual pipetting. The Opentrons OT-2 robot can serially dilute eight samples for 10 dilutions in about 2 minutes with high reliability across replicates. The precision represented by the coefficient of variance per column is comparable to much more expensive liquid handling platforms. We encourage users to adapt the accompanying parametric protocol for their specific scientific applications.

INTRODUCTION

Serial dilutions are both independent experiments and building blocks of more complex experimental pipelines. Applications can be as simple as generating a standard curve or more complex phenotype characterization across cell culture concentrations. In any application, precise pipetting is required to generate reliable results.

In general, manual lab work can introduce random errors at any pipetting step in a protocol. These pipetting inaccuracies can incorporate the incorrect dilution factor across samples. Pipette mixing speed and tip height within samples can affect precision, but are challenging to keep constant during manual pipetting. Additionally, repetitive pipetting can lead to stress injuries over time. The OT-2 is an affordable option for ensuring pipetting reliability and ultimately data quality of serial dilution experiments.

MATERIALS

- Opentrons OT-2 Liquid Handling Robot
- Opentrons OT-2 Software Version 3.1.2
- Opentrons p300 multi-channel pipette
- Opentrons 300 µL Tip Rack
- USA Scientific 12-channel reservoir for automation (1061-8150)
- Corning 96-well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene (3368)
- Corning 500 mL Molecular Biology Grade Water (46-000-CV)
- McCormick Assorted Food Color and Egg Dye - Blue
- Tecan Infinite F200 Pro

PROTOCOL

1. Prepare the blue dye solution with 50 µL of blue food dye and 10 mL of molecular biology grade water.

2. Add 200 µL of the blue dye solution to the wells A1-H1 of the 96-well flat bottom plate.

3. Add 20 mL of diluent to A1 well of the trough reservoir.
   Note: The column after the last dilution is the default location of the blanks. The last well of the trough/reservoir is the default liquid trash.

4. Arrange labware in slots 1-3 according to the deck layout in **Figure 1a**.

   - Slot 1 = Opentrons 300 µL tip rack
   - Slot 2 = 12-channel reservoir
   - Slot 3 = 96-well flat bottom plate

5. Change the parameters in the protocol to the following:
   - Dilution factor = 1.5
   - Final Volume = 200
   - Number of dilutions = 10

6. Proceed to the OT-2 run app to upload your protocol and calibrate the labware.

7. Seal the plate and quantify the absorbance at 450 nm with 25 flashes per well on the plate reader.

Figure 1a
Suggested deck layout for serial dilution.
Deck layout rendering from the OT-2 run app.

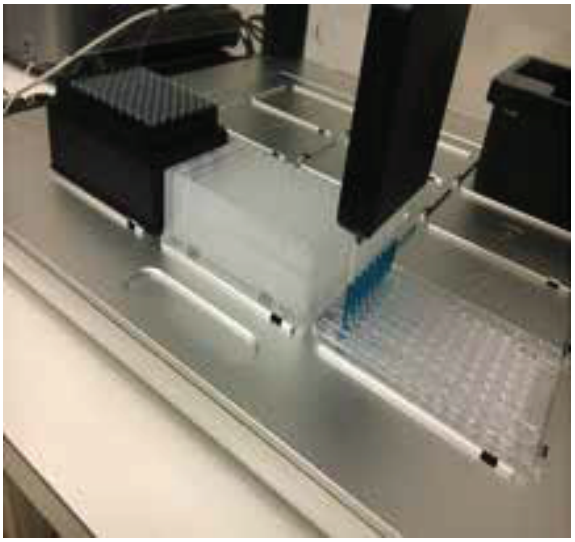

Figure 1b

OT-2 aspirating 100µL from eight blue dye samples in column 1 of a 96-well flat bottom plate.

RESULTS

The average raw absorbance values in the first six columns of Plate 1 are shown in **Figure 2**. Each column shows a 1.5-fold dilution from the previous column. The coefficient of variance (CV%) illustrates the high reliability across replicates. Table 1 shows the column-wise CV% for each plate of dilutions. The average precision of each column is about 2%, comparable to the values for a similar experiment carried out with a much more expensive liquid handler than the OT-2[1]. Each serial dilution plate was completed on the OT-2 in about 2 minutes.

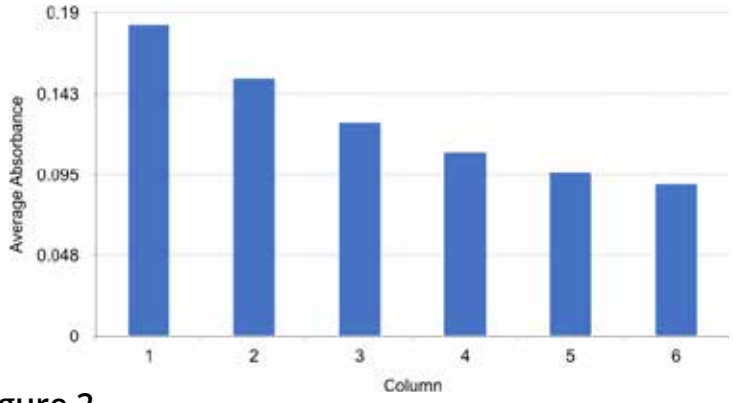

Figure 2
Raw average absorbance values for Plate 1 (Columns 1 - 6) for 1.5-fold dilutions of eight samples of blue dye.

CONCLUSIONS

Here we present a simple serial dilution experiment that illustrates the high precision pipetting of the OT-2 liquid handling robot. In addition to precision, the OT-2 is capable of highly customizable pipetting techniques. Users can optimize aspirate/dispense speed, mix speed, air-gaps and many other options for more accurate and efficient liquid handling. Automating serial dilutions will provide scientists with reliable pipetting along with valuable walk away time from tedious, error-prone manual work.

Table 1.
Comparable column-wise variance as Agilent Bravo. Average coefficient of variance (%) per column is shown in each table.

OT2

	Average CV/column (%)								Average precision
	1	2	3	4	5	6	7	8	
Plate 1	1.0	1.3	1.0	1.6	1.4	2.5	1.2	1.2	**1.8**
Plate 2	0.9	0.8	1.2	0.7	0.8	0.9	1.4	1.2	**2.1**
Plate 3	6.4	0.7	0.7	0.9	1.1	1.1	1.0	1.3	**1.9**

Agilent Bravo

	Average CV/column (%)								Average precision
	1	2	3	4	5	6	7	8	
Plate 1	1.3	1.0	2.3	1.4	2.9	2.8	3.4	6.9	**2.8**
Plate 2	1.4	1.7	1.8	1.6	1.5	2.5	4.5	4.4	**2.4**
Plate 3	0.6	1.4	1.8	3.3	2.2	3.1	5.2	8.6	**3.3**

Reference
1. Gomez and Whitman. Agilent Technologies
'Enabling Automated Serial Dilutions Using the Agilent Bravo BenchCel Workstation'