Feb 10, 2025 Version 2

Absorbance assay from an M2P flower plate V.2

Forked from a private protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

Although synthetic biology can produce valuable chemicals in a renewable manner, its progress is still hindered by a lack of predictive capabilities. Media optimization is a critical, and often overlooked, process which is essential to obtain the titers, rates and yields needed for commercial viability. Here, we present a molecule- and host-agnostic active learning process for media optimization that is enabled by a fast and highly repeatable semi-automated pipeline. Its application yielded 60% and 70% increases in titer, and 350% increase in process yield in three different campaigns for flaviolin production in Pseudomonas putida KT2440. Explainable Artificial Intelligence techniques pinpointed that, surprisingly, common salt (NaCl) is the most important component influencing production. The optimal salt concentration is very high, comparable to seawater and close to the limits that P. putida can tolerate. The availability of fast Design-Build-Test-Learn (DBTL) cycles allowed us to show that performance improvements for active learning are rarely monotonous. This work illustrates how machine learning and automation can change the paradigm of current synthetic biology research to make it more effective and informative, and suggests a cost-effective and underexploited strategy to facilitate the high titers, rates and yields essential for commercial viability.

This protocol explains the steps for preparing samples and taking OD600 and OD340 measurements from a BioLector plate.

Safety warnings

Wear proper PPE.

Before start

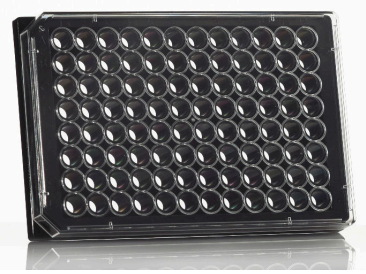
Make sure you booked the equipment in the calendar:

- 978-4-BIOMEK-NX-S8_ SPECTRAMAX (4148) EQ (1) (or any other "plate-reader" capable of measuring OD340 and OD600)
- 978-4-BIOMEK-NXP (4148) EQ (1)

Make sure you received proper training before operating on the Biomeks.

Required equipment/labware

1 **Destination plate:**



Flat-black clear-bottom Tecan plate x2

Water plate:

deep reservoir (available at Robotics lab)

Supernatant plate:

96-deep-well plate x2

2 Liquid handler:

Equipment	
new equipment	NAME
Beckman Coulter	BRAND
Biomek NXp	SKU

Pipette tips needed (available at Robotics lab):
- tips s200 (green box)
- tips p1000 (yellow box)

Spectrophotometer/plate-reader:
- Molecular Devices SpectraMax M2
- or any other spectrophotometer capable of measuring OD340 and OD600

3 Additional components:
- H2O
- m2p plate with cultures

Centrifuge options:
- Avanti-15R-centrifuge
- Allegra-25R
- Eppendorf 5810R
- (any swinging-bucket centrifuge capable of spinning a 96-deepwell plate)
- NOT the Eppendorf 5430

Biomek setup

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Note

If you wish to set up a method in advance of a run, you may access the Biomek remotely.

- Follow the instruction provided in **this file**.
- Request a password for a Windows Active Directory (AD) account from Arthur Panganiban (ahpanganiban@lbl.gov)
- Find your Biomek, use password **Robotp@ss978**

We will use two Biomek methods for this assay.

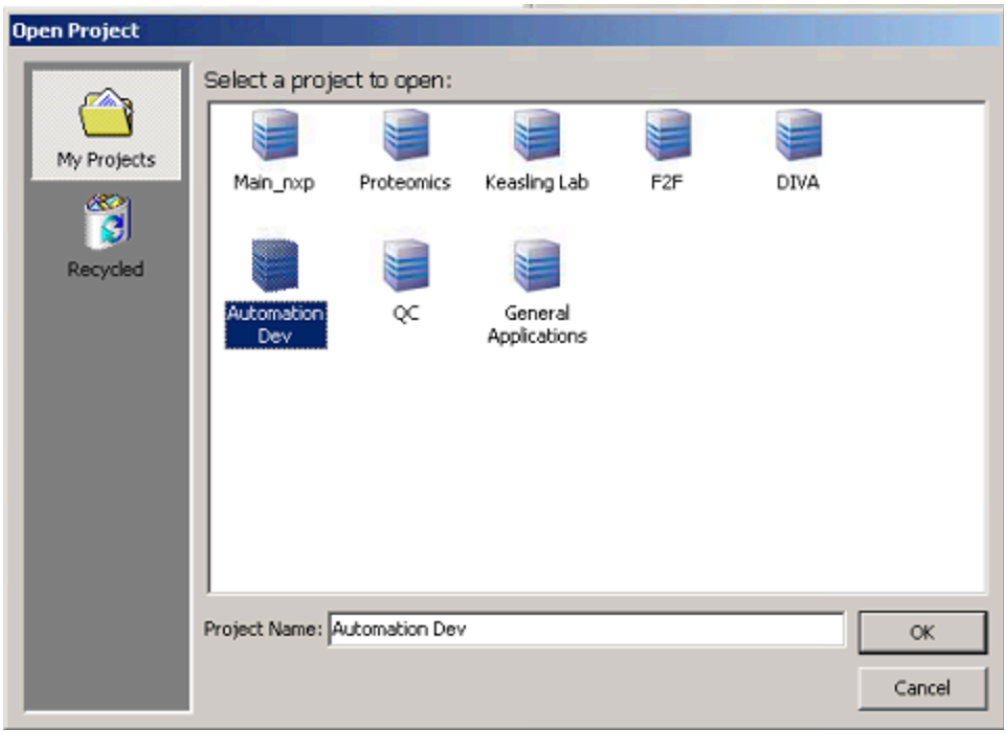
The **first** method will prepare:

- a Tecan plate for OD600 measurements, with 10x dilution of the samples
- two 96-deepwell plates as an intermediate step for preparing a Tecan plate for OD340 measurements - one with 1 mL of samples (supernatant plate) and the other with 1 mL of water for balance in the centrifuge

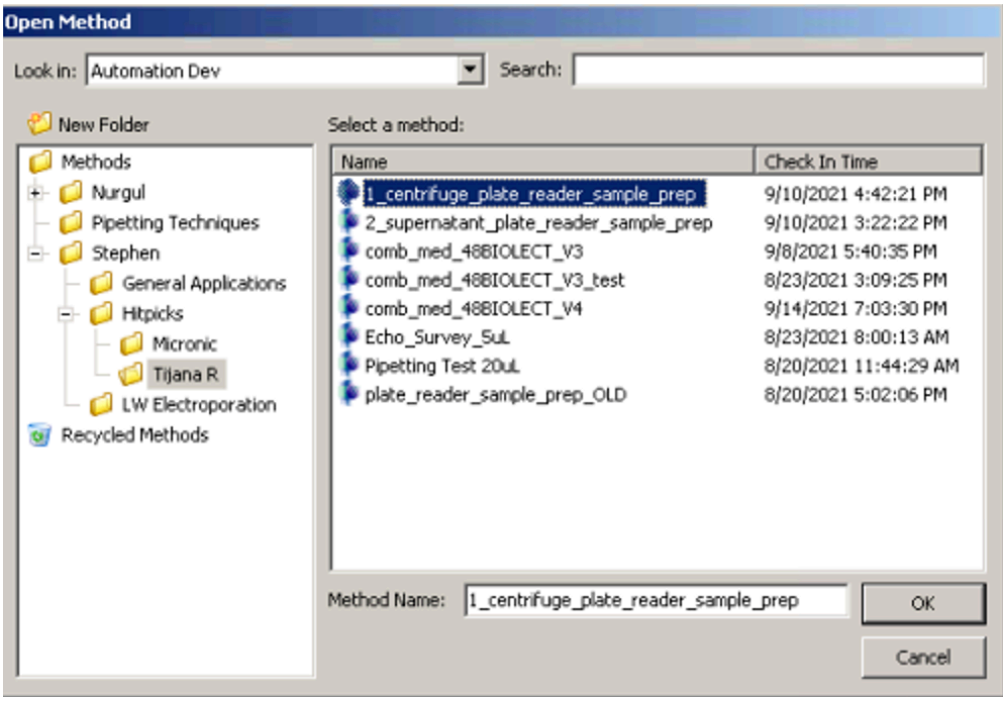
The **second** method will prepare:

- a Tecan plate for OD340 measurements (aliquoting cell supernatants from the 96-deepwell plate)

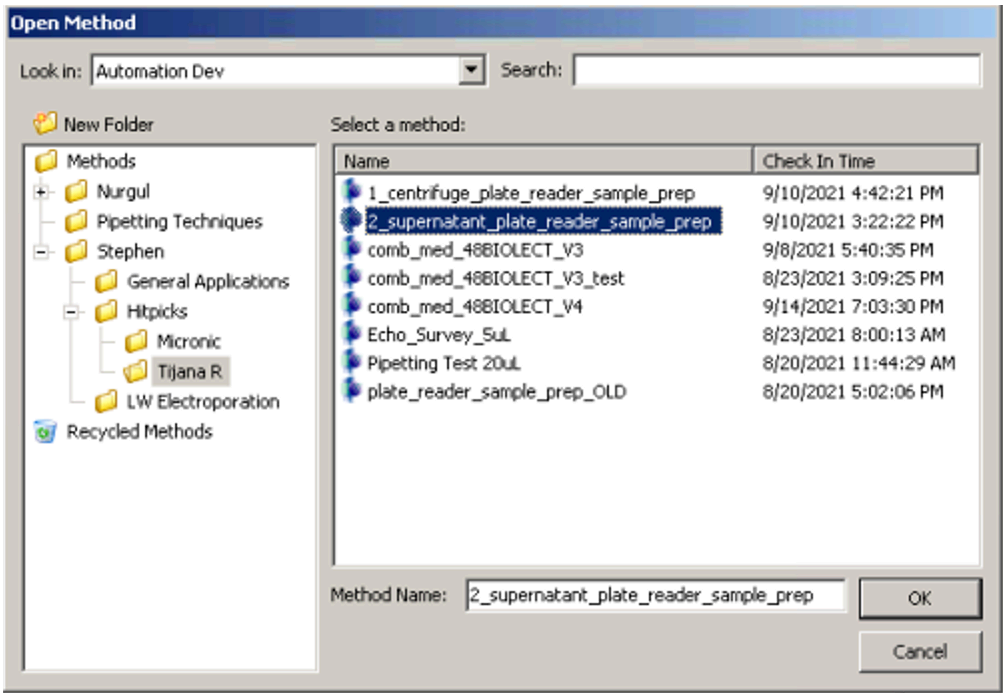
4.1 Open the Biomek Software application on the Biomek. At the top toolbar, select Project > Open Project to open the "Automation Dev" project.



4.2 At the top toolbar of the Biomek Software, select File > Open to open the first method.



To open the second choose:



Biomek run

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> Note
>
> Do not start a Biomek run remotely if it is not in the Simulation mode.

Hit the play button (green arrow) to run the method.

5.1 10m

- The first method takes around 00:05:00 before the pause and 00:05:00 after the pause
- When the method pauses, remove the two 96-deepwell plates and continue the Biomek run

5.2 5m

Centrifuge run:

- Place the two 96-deepwell plates sealed in the centrifuge and spin at max speed for 00:05:00

5.3 5m

- Carefully remove the supernatant plate from the centrifuge, remove the seal and place it back in the Biomek
- Start the second Biomek method to prepare a Tecan plate for OD340 measurements (takes around 00:05:00)

SpectraMax run

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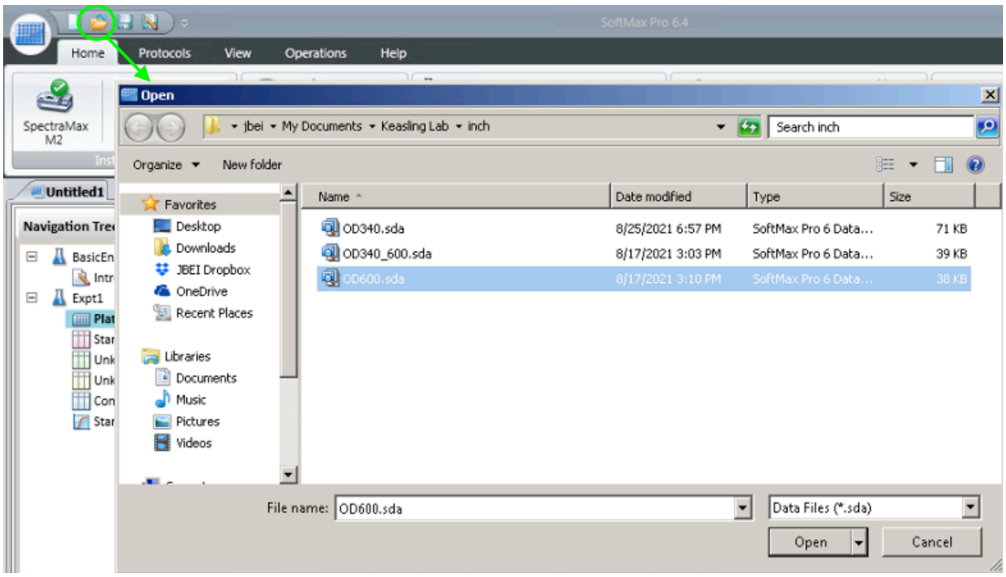
- Start the Spectramax software "SoftMax"
- Click "Open a data file" in the top left of the SoftMax window

- Open the method for measuring OD600 named **OD600.sda** in the "My_Documents/Keasling_Lab/inch" subfolder on the Spectramax (NX-S8) computer



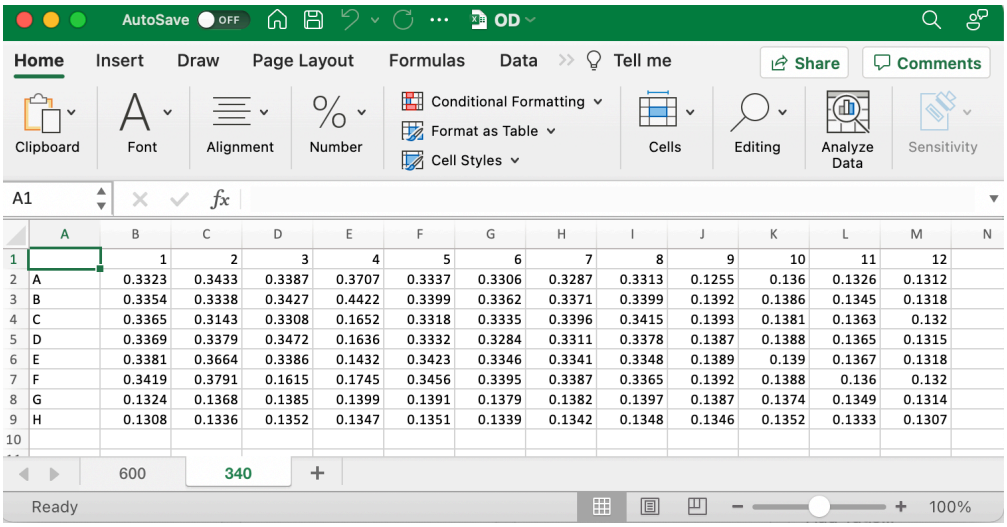
- Open tray and place plate
- Measure OD600 from the first prepared 96-well Tecan plate
- Save the data (see step 7)

- Click "Open a data file"
- Open a method for measuring OD340 named **OD340.sda** in "My_Documents/Keasling_Lab/inch" subfolder on the Spectramax (NX-S8) computer
- Measure OD340 from the second prepared 96-well Tecan plate
- Save the data (see step 7)

Saving data

7

- Copy the values for OD600 from the SoftMax window into an empty Excel file and rename the spreadsheet to "600"
- Copy the values for OD340 to another spreadsheet of the same file and rename to "340"
- Save the Excel file as "OD.xlsx"



	A	B	C	D	E	F	G	H	I	J	K	L	M
1		1	2	3	4	5	6	7	8	9	10	11	12
2	A	0.3323	0.3433	0.3387	0.3707	0.3337	0.3306	0.3287	0.3313	0.1255	0.136	0.1326	0.1312
3	B	0.3354	0.3338	0.3427	0.4422	0.3399	0.3362	0.3371	0.3399	0.1392	0.1386	0.1345	0.1318
4	C	0.3365	0.3143	0.3308	0.1652	0.3318	0.3335	0.3396	0.3415	0.1393	0.1381	0.1363	0.132
5	D	0.3369	0.3379	0.3472	0.1636	0.3332	0.3284	0.3311	0.3378	0.1387	0.1388	0.1365	0.1315
6	E	0.3381	0.3664	0.3386	0.1432	0.3423	0.3346	0.3341	0.3348	0.1389	0.139	0.1367	0.1318
7	F	0.3419	0.3791	0.1615	0.1745	0.3456	0.3395	0.3387	0.3365	0.1392	0.1388	0.136	0.132
8	G	0.1324	0.1368	0.1385	0.1399	0.1391	0.1379	0.1382	0.1397	0.1387	0.1374	0.1349	0.1314
9	H	0.1308	0.1336	0.1352	0.1347	0.1351	0.1339	0.1342	0.1348	0.1346	0.1352	0.1333	0.1307
10													