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Absorbance assay from an M2P flower plate

Forked from Absorbance assay from an m2p flower plate

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We use this protocol and it's working

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Abstract

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

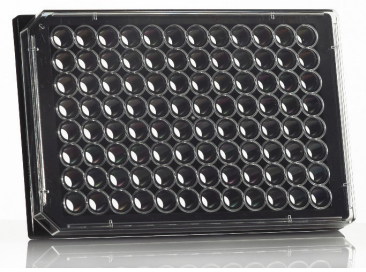
Make sure you booked the equipment in the calendar:

- 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

4

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.

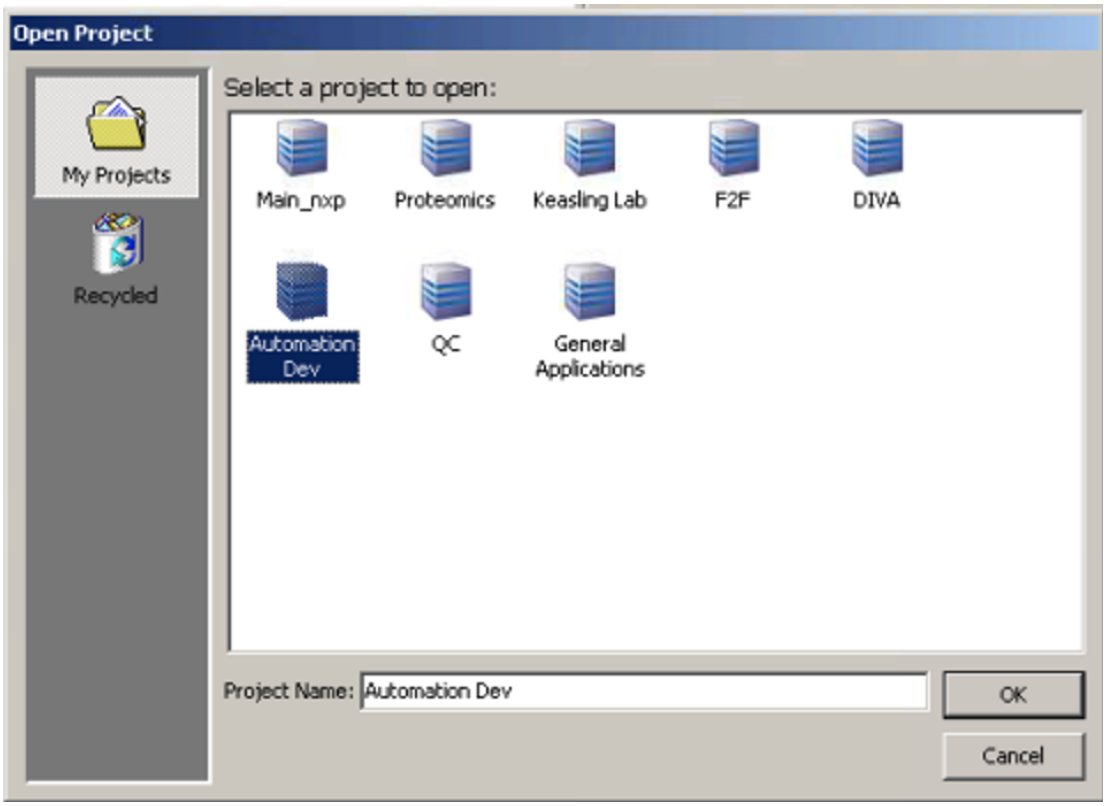
The **first** method will prepare:

- 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

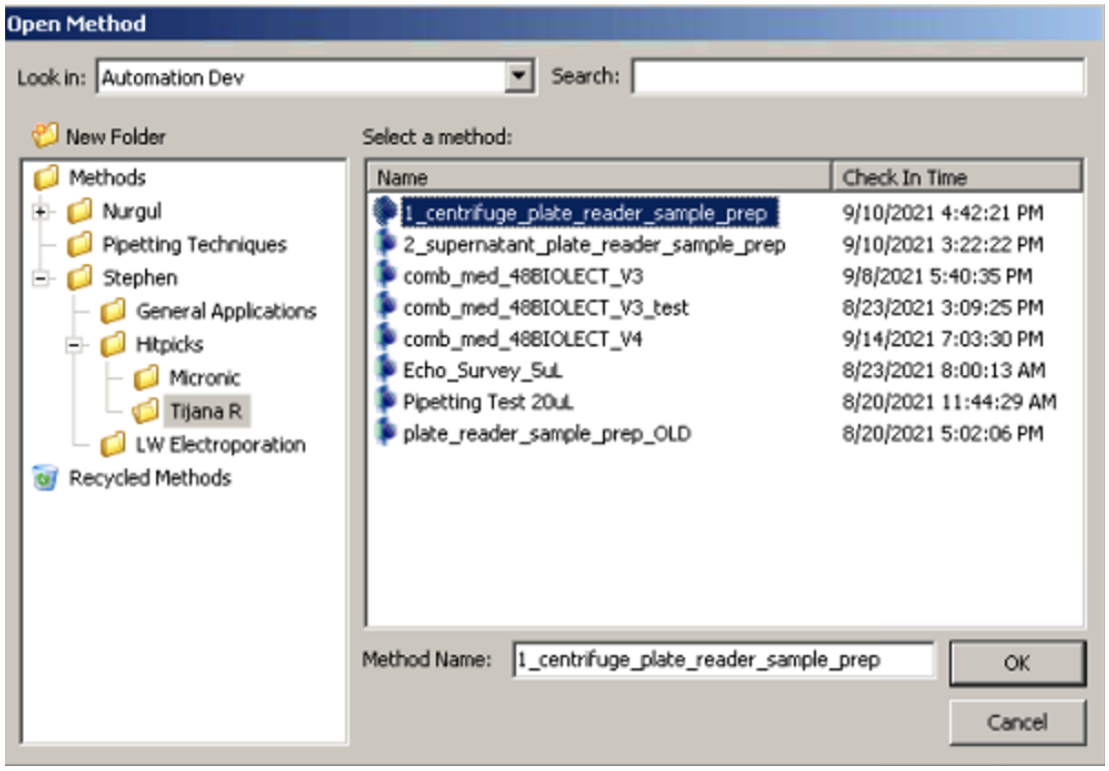
The **second** method will prepare:

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

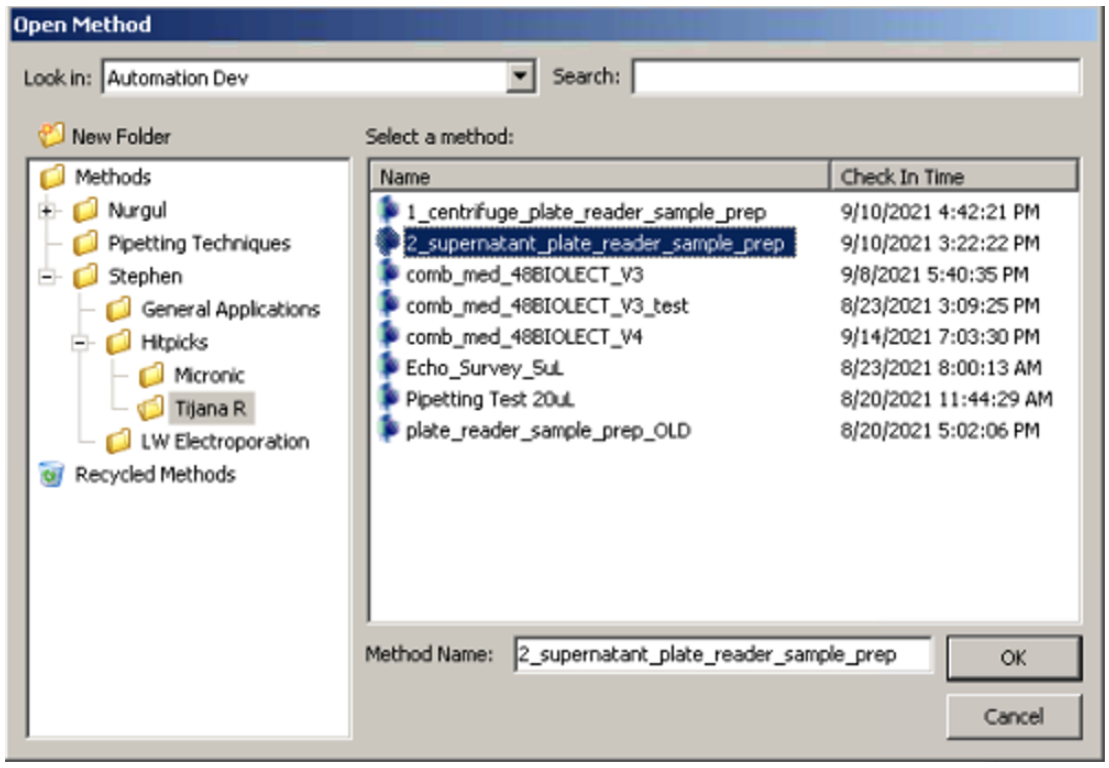
4.1 Open the Biomek Software application on the Biomek. At the top toolbar, select Project > Open Project to open the "Automation Dev" project.



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



To open the second choose:

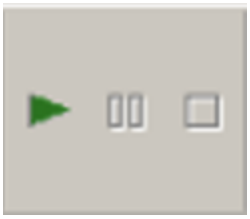


Biomek run

5

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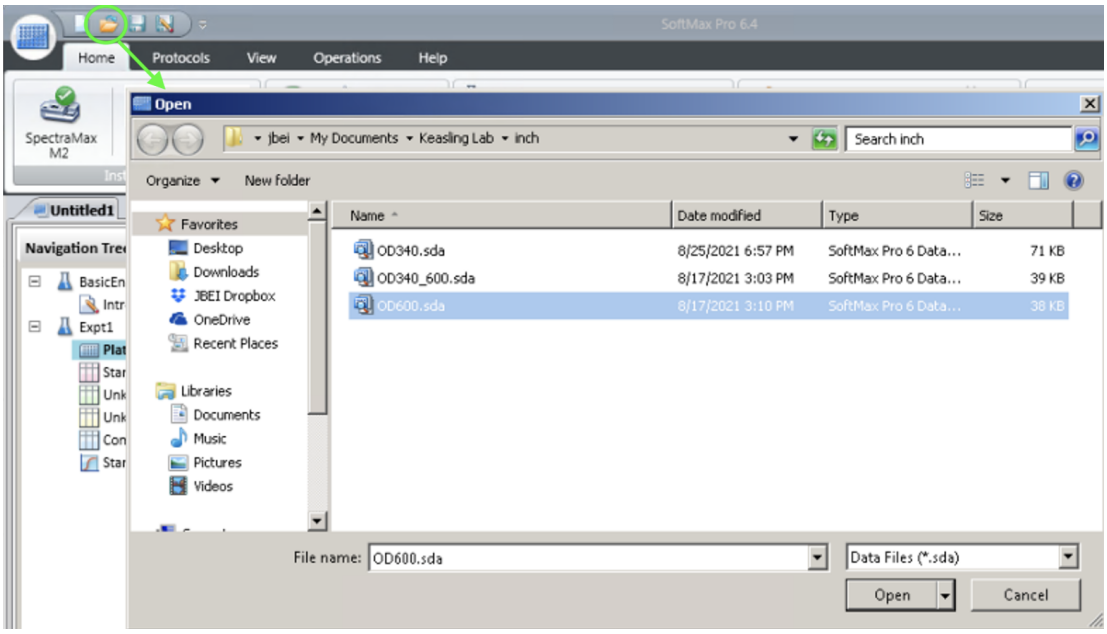
- Start the Spectramax software "SoftMax"
- Click "Open a data file" in the top left of the SoftMax window

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



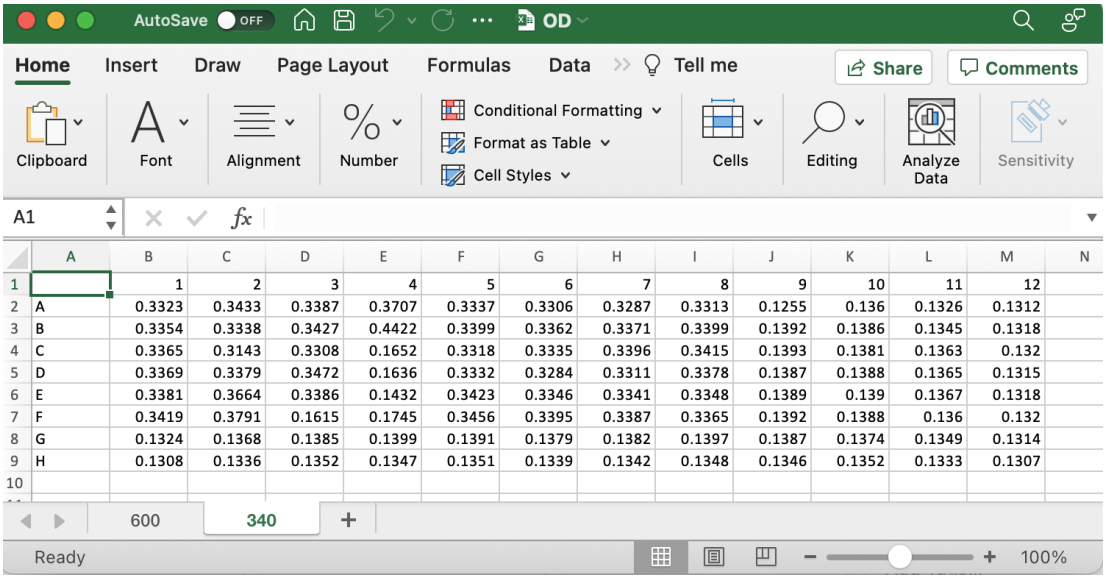
- Open tray and place plate
- Measure OD600 from the first prepared 96-well Tecan plate
- Save the data (see step 7)

- 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													