Automated MetClo Assembly Plan

OT2 Set-Up Instructions

Welcome to the MetcloOT2, plan specific to your assemblies!

This documentation is a userguide that instructs the setup of the OT2 robot for multiplexed MetClo based DNA assembly. Reading Lin & O'Callaghan (2018 & 2020) is highly recomended.

MATERIALS

> Software:

- OpenTrons OT-2 App
- Python 3

> FILES within metclo_plan_files:

- assembly_data.csv
- part_data.csv
- position_data.csv
- reagents_data.csv

> Hardware:

- OpenTron OT-2
- OpenTrons P20 Single Channel Electronic Pipette
- Opentrons Thermocycler
- Opentrons 96 Tip Rack 20ul

> Consumables and reagents

- 30fmol of all part containing insert plasmids and assembly vectors.
- T4 ligase buffer
- T4 DNA ligase
- BsaI-HFv2
- ddH20

PROTOCOL

1. Within the terminal, simulate the OpenTrons OT-2 protocol: ot2metclo.py. Review and confirm the output of the simulation. https://support.opentrons.com/s/article/Simulating-OT-2-protocols-on-your-computer?)

2. Follow the OpenTrons OT-2 get-started guidelines to prepare the Opentron OT2. https://support.opentrons.com/s/ot2-get-started).

3. Prepare the insert plasmids and assembly vectors as described in Lin & O' Callaghan (2020).

4. As mentioned in the README.md, dilute the plasmids and vectors where necessary.

5. Set up the OpenTrons OT-2 deck as depicted in the "OT2 LAYOUT" section. Additionally attach the P20 Single Channel Electronic Pipette to the left mount.

6. The "REAGENT PLATE LAYOUT (ul)" depicts the positioning and volume of the reagents and the parts needed for the assembly. Manually pipette the volume of reagents and parts to their respective well. Example [A1 ('ligase_buffer.1',7.2)] means that 7.2ul of T4 ligase buffer needs to be allocated to A1.

7. Assure that the metclo_plan_files produced from metclo_plan.py and the ot2metclo.py are within the same file. Transfer this file to the computer which is connected to the OT2 robot and has the downloaded OpenTrons OT-2 App.

8. Run the ot2metclo.py protocol on the OpenTrons OT-2 App. For assistance refer to OT - 2 : Getting Started. https://support.opentrons.com/s/ot2-get-started).

9. The final assemblies will be stored within the thermocycler module at 4 degrees Celsius. The positions of the assemblies are depicted in "THERMOCYCLER PLATE WITH ASSEMBLIES". These assemblies can be used for further applications.

NOTES

- The "Assemblies" section provides a summary of the assemblies' size, recommended transformation method based on size, parts and reagent volumes.

- The "Parts" section provides a summary of the parts' size, concentration (ng/ul), number of assemblies in which the part was used, the volume containing 30fmol, and the total volume of the part needed for the protocol.

REFERENCE

Lin, D. & O'Callaghan, C.A. (2018) MetClo: methylase-assisted hierarchical DNA assembly using a single type IIS restriction enzyme. Nucleic Acids Research. 46 (19), e113. doi:10.1093/nar/gky596.

Lin, D. & O'Callaghan, C.A. (2020) Hierarchical Modular DNA Assembly Using MetClo. In: S. Chandran & K.W. George (eds.). DNA Cloning and Assembly. Methods in Molecular Biology. New York, NY, Springer US. pp. 143-159. doi:10.1007/978-1-0716-0908-8_9.

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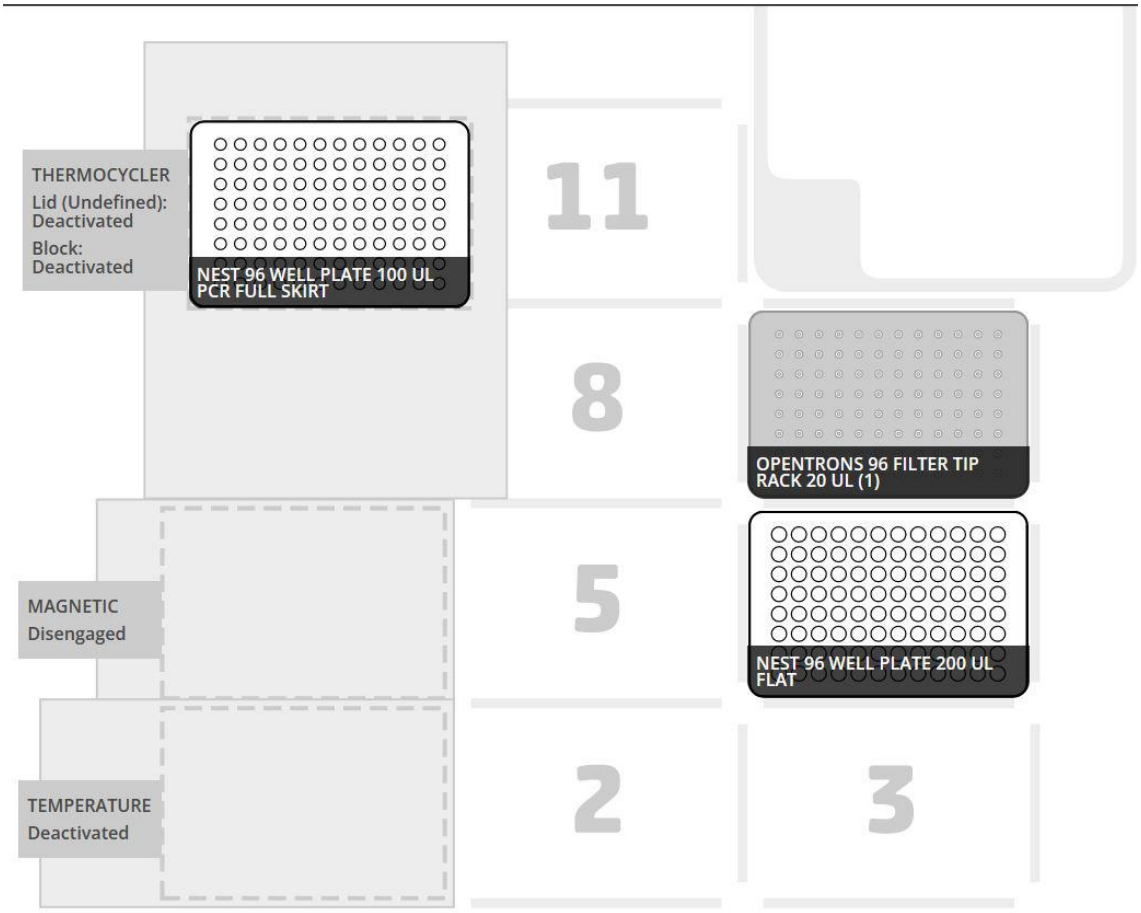
OT2 Layout



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Reagent Plate Layout (ul)

A1 ('ligase_buffer', 7.2)	B1 ('ligase', 1.8)	C1 ('bsai', 2.4)	D1 ('water', 1.807)
A2 ('bq', 7.163)	B2 ('bc', 5.147)	C2 ('cq', 12.569)	D2
A3	B3	C3	D3
A4	B4	C4	D4
A5	B5	C5	D5
A6	B6	C6	D6
A7	B7	C7	D7
A8	B8	C8	D8
A9	B9	C9	D9
A10	B10	C10	D10
A11	B11	C11	D11
A12	B12	C12	D12

E1 ('MpMXBP_pFa', 13.453)	F1 ('pa', 23.537)	G1 ('aq', 6.263)	H1 ('ab', 18.984)
E2	F2	G2	H2
E3	F3	G3	H3
E4	F4	G4	H4
E5	F5	G5	H5
E6	F6	G6	H6
E7	F7	G7	H7
E8	F8	G8	H8
E9	F9	G9	H9
E10	F10	G10	H10
E11	F11	G11	H11
E12	F12	G12	H12

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Thermocycler Plate with Assemblies

A1 2frag	B1 3frag	C1 4frag	D1
A2	B2	C2	D2
A3	B3	C3	D3
A4	B4	C4	D4
A5	B5	C5	D5
A6	B6	C6	D6
A7	B7	C7	D7
A8	B8	C8	D8
A9	B9	C9	D9
A10	B10	C10	D10
A11	B11	C11	D11
A12	B12	C12	D12

E1	F1	G1	H1
E2	F2	G2	H2
E3	F3	G3	H3
E4	F4	G4	H4
E5	F5	G5	H5
E6	F6	G6	H6
E7	F7	G7	H7
E8	F8	G8	H8
E9	F9	G9	H9
E10	F10	G10	H10
E11	F11	G11	H11
E12	F12	G12	H12

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3 Assemblies

Assembly Name: 2frag

Assembly Size: 21,949 (Recomend: Electroporation)

3 Parts

MpMXBP_pFa	pa	aq

Reagents (ul)

ligase_buffer	ligase	bsai	water
2	0.5	0.5	1.506

Assembly Name: 3frag

Assembly Size: 29,494 (Recomend: Electroporation)

4 Parts

MpMXBP_pFa	pa	ab	bq

Reagents (ul)

ligase_buffer	ligase	bsai	water
2	0.5	0.5	0

Assembly Name: 4frag

Assembly Size: 37,286 (Recomend: Electroporation)

5 Parts

MpMXBP_pFa	pa	ab	bc
cq			

Reagents (ul)

ligase_buffer	ligase	bsai	water
2	0.5	1.0	0

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7 Parts

Part Name	Size	Conc.	Times Used	30fmol (ul)	Total Volume
MpMXBP_pFa	8067	40	3	3.737	13.453
pa	14112	40	3	6.538	23.537
aq	14083	50	1	5.219	6.263
ab	14087	33	2	7.91	18.984
bq	12884	40	1	5.969	7.163
bc	13888	60	1	4.289	5.147
cq	14131	25	1	10.474	12.569

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Total Reagents Volumes Required (ul) *1.2

ligase_buffer	ligase	bsai	water
7.2	1.8	2.4	1.807