# Instructions for MoClo_Transformation

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## About

This document is intended to guide users through OT2 Modular Cloning (MoClo) and Transformation in *E. coli workflow*.
This workflow enables end-to-end molecular cloning using the OT2 liquid handling robot by OpenTrons. This includes:

- Performing Modular Cloning (Golden Gate) reactions.
- Transforming plasmids into *E. coli* via hear shock.
- Plating cell transformations onto Rectangular agar plates.

## Materials

Below we list the materials previously used to implement OT2 Modular Cloning (MoClo) and Transformation in *E. coli* workflow. We recommend starting with these consumables, however certain standard labwares may be altered. Labware needs to be defined carefully when working with OpenTrons OT-2 liquid handling robot and changes to labware requires the user to update the template scripts and the labwares used during the execution of OT2 MoClo and Transformation runs. Here is a general OpenTrons' Guideline for utilizing labware for users new to OT2, and additional details regarding labware can be found on OpenTrons Labware Library.

Software:

- OpenTrons OT-2 App (Version 3.10.3 or later)
- Python 3
- OT2 Modular Cloning and Transformation GitHub repository

Hardware:

- OpenTrons OT-2
- OpenTrons P10 Single-Channel Electronic Pipette
- OpenTrons P300 Multi-Channel Electronic Pipette
- OpenTrons Temperature Module
- Opentrons 96 Tip Rack 10 µL
- Opentrons 96 Tip Rack 300 µL
- Bio-Rad 96 Well Plate 200 µL PCR
- USA Scientific 12 Well Reservoir 22 mL
- Thermo Scientific™ Nunc™ OmniTray™ Single-Well Plate, Non-reated

Consumables & Reagents:

| **Item** | URL |
|---|---|
| **OpenTrons 10 µL Tips** | https://shop.opentrons.com/collections/opentrons-tips/products/opentrons-10ul-tips?_ga=2.54859875.995557980.1585316723-1185431017.1585316723 |
| **OpenTrons 300 µL Tips** | https://shop.opentrons.com/collections/opentrons-tips/products/opentrons-300ul-tips?_ga=2.54859875.995557980.1585316723-1185431017.1585316723 |
| **Bio-Rad 96-well PCR Plate** | https://www.bio-rad.com/en-us/sku/hsp9601-hard-shell-96-well-pcr-plates-low-profile-thin-wall-skirted-white-clear?ID=hsp9601 |
| **USA Scientific 12-well Reservoir** | https://www.usascientific.com/12-channel-automation-reservoir/p/1061-8150 |
| **Thermo Scientific OmniTray** | https://www.fishersci.com/shop/products/nunc-omnitray/12565296?searchHijack=true&searchTerm=12565296&searchType=RAPID&matchedCatNo=12565296 |
| **Thermo Scientific Adhesive PCR Plate Foils** | https://www.fishersci.com/shop/products/adhesive-pcr-plate-seals/ab0558#?keyword=AB-0558 |
| **NEB BsaI** | https://www.neb.com/products/r0535-bsai#Product%20Information |

| Item | URL |
|---|---|
| **NEB T4 DNA Ligase** | https://www.neb.com/products/m0202-t4-dna-ligase#Product%20Information |
| **NEB 5-alpha Competent E. coli (High Efficiency)** | https://www.neb.com/products/c2987-neb-5-alpha-competent-e-coli-high-efficiency#Product%20Information |

Additional Reagents:

- dd$H_2O$
- SOC Media
- LB-Agar with appropriate antibiotics

# Protocol

## OT-2 Preparation

Follow the Opentrons guidelines for setting up the OT-2 before executing any protocols.

## Construct Supplemental CSV Files

Using any web browser, navigate to the following GitHub directory: https://github.com/DAMPLAB/OT2-MoClo-Transformation-Ecoli, and follow the Step 1 and 2 of the Initial Setup for Modular Cloning, Cell Transformation, and Cell Plating to generate necessary supplemental CSV files, input DNA plate map and combination list. Examples of input DNA plate map and combination list are provided in the examples folder of the GitHub directory.

## Prepare Plasmid DNA and Reagents for Assembly [2 Days]

1. [Day 1] Using a sterile inoculation loop, and working near an open flame, inoculate streak out bacterial glycerol stocks with 2 mL of LB broth supplemented with appropriate antibiotics. Do this for all necessary DNA parts, be sure to sterilize the loop between every sample. Incubate cultures overnight at 37 °C while shaking at 300 RPM.
   **NOTE:** Frozen bacterial glycerol stocks should be kept on ice as much as possible. Repeated freeze-thaw cycles lower the viability of the stock and should be avoided.
2. [Day 2] Purify plasmid DNA from the bacterial cultures using any commercially available mini-prep plasmid purification kit.
3. Dilute each sample of plasmid DNA to a concentration of 10,20 and 40 nM in water or TE buffer and transfer appropriate volume of each diluted DNA part into the corresponding well on a Bio-Rad 96-well PCR plate according to the input-dna-map.csv file provided in the examples folder. Seal the plate with an adhesive seal and store at -20°C. [30 Minutes]

4. On ice, transfer calculated amount of buffer, restriction enzyme, and ligase into appropriate well of a Bio-Rad 96-well PCR plate according to this ratio: for each 20 µL of reaction add 2 µL of 10x T4 DNA ligase buffer, 1 µL of BsaI restriction enzyme, and 0.5 µL of T4 DNA Ligase (HC) as illustrated in Plate A of Figure 1B. Store at -20°C. [10 Minutes]

## Execute MoClo and Transformation Protocols on OT-2

1. Using any web browser, navigate to the following GitHub directory: https://github.com/CIDARLAB/OT2-MoClo, and follow the instructions provided in the README.md for the general installation.
2. Open up a new command line interpreter at *moclo_transformation* folder, and type in *python3 moclo_transform_generator.py*.
   **NOTE:** MoClo and Transformation protocol can be executing as a single protocol or separate protocols by using individual *protocol_generator.py*, which are provided in the *moclo_transformation* folder.
3. Select the two CSV files, input DNA plate map and combinations list, and an output folder for the protocol when prompted.
4. A protocol named *moclo_transform_protocol.py* and a CSV file named agar_plate should be saved in the output folder.
5. Open up OT2 APP, and upload the generate *moclo_transform_protocol.py* under the Protocol Tab. [3 Minutes / Variable]
6. Once the protocol is uploaded, following the calibration instructions provided by the OT2 APP, place the Temperature Module and an empty Bio-Rad 96-well PCR plate (Reaction Plate) on the top of the Module, the Input_DNA_Plate, the Reagents Plate, the Reagent Trough, the Agar Plate, and one Opentrons 10 µL and two 300 µL Tip Racks onto the deck of the liquid handler (Figure 1A and 1B). [8.5 Minutes / Variable]
   **NOTE:** Pipette replacement might be necessary, please follow the instructions provided by OT2 App.
7. Once the calibration process is completed, proceed to running the protocol. [90 Minutes / Variable]
   **NOTE:** Always allow the robotic liquid handler to complete the execution of a script before trying to access the deck space.
8. The robotic liquid handler would automatically pause when the modular cloning protocol is completed, and indication light of the Temperature Module should be off. Seal the Reaction Plate with adhesive film at the beginning of the thermocycling process, and execute the Reaction Plate with the following thermocycling settings on a benchtop Thermocycler (35 cycles of 37°C for 1.5 minutes and 16°C for 3 minutes, and 1 cycle of 50°C for 5 minutes and 80°C for 10 minutes). Remove the Input_DNA_Plate from the Deck Space. Remaining DNA may be saved by sealing the Input_DNA_Plate with adhesive film and stored at -20°C. [172.5 Minutes]
9. Prior to the start of the *E. coli* Transformation Protocol, load 10 µL of chemically competent cells into each well (well 1 through well 72) of an empty Bio-Rad 96-well PCR plate. Place the new Bio-Rad 96-well PCR plate containing competent cells on the top of the Temperature Module, and place the Reaction Plate on deck slot 7. (**Note:** At the beginning of the execution, slot 7 is labeled with Post-MoClo Reaction Plate, but should be empty until this step). Load appropriate amount of SOC outgrow media into the fourth well of the Reagent Trough. Be sure to remove the adhesive film before proceeding to robotic execution. [15 Minutes]

10. Continue to the Cell Transformation protocol by unpausing the execution for the robotic liquid handler and proceed to run. [50 Minutes / Variable]
    **NOTE**: Always allow the robotic liquid handler to complete the execution of a script before trying to access the deck space.
11. Once 2 µL of each MoClo assembled DNA plasmids are transferred and mixed with 10 µL of the component cells the robotic liquid handler would automatically pause the pipette movements to enter heat shocking and recovering stage. The conditions are 4°C for 30 minutes, heat shock at 42°C for 1 minutes, recovering at 4°C for 5 minutes. [36 Minutes]
12. After cells have recovered, robot liquid handler would transfer 150 µL of SOC outgrowth medium to each well containing reactions and any cells to recover and reproduce in SOC outgrowth medium for 1 hour at 37°C. [65 Minutes / Variable]
    **NOTE:** Apply adhesive film to the new Reagent Plate to avoid evaporation.
13. Robotic liquid handler would automatically pause when the Cell Transformation is completed before proceeding to the Cell Plating protocol. Robotic liquid handler first dilution the recovered transformation reactions to the 3% of the original concentration for the 2-part DNA assembly and 30% of the original concentration for the 5-part DNA assembly, then plate 10 µL of each recovered transformation reactions on the rectangular agar plate with appropriate antibiotic. [43 Minutes / Variable]
    **NOTE:** Remove the adhesive film before proceeding to run. Mark well location **A1** on the side of the agar plate as a location indicator for the preceding protocol.
14. Remove the agar plate from the deck of the robotic liquid handler and incubate at 37 °C overnight.
15. We used this workflow to assemble 72 plasmids using modular cloning.

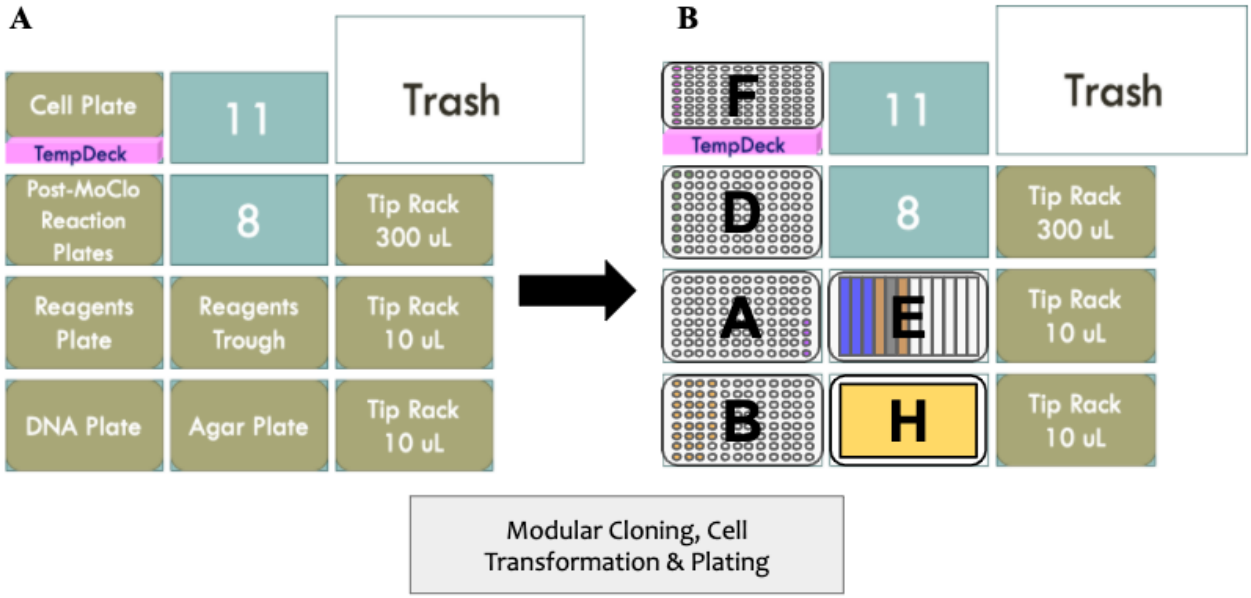


**Figure 1**: End-to-end cloning pipeline. (A) Representative OT-2 deck layout with labwares and pipette tip boxes placement locations. (B) Representative OT-2 deck layout for plate setup instructions.