Instructions for OT2-SARS-CoV2

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About

This document is intended to guide users through OT2 SARS CoV2 detection workflow based on TaqPath™ COVID-19 Combo Kit.

This workflow enables end-to-end RT-qPCR using the OT2 liquid handling robot by OpenTrons. This includes:

- Executing magnetic beads-based RNA extraction protocol.
- Performing qPCR assay preparation protocol in 96-well plate format.

Materials

Below we list the materials previously used to implement OT2 SARS CoV2 detection 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 protocol scripts and the labwares used during the execution of OT2 SARS CoV2 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 SARS CoV2 GitHub repository

Hardware:

- OpenTrons OT-2
- OpenTrons P10 Multi-Channel Electronic Pipette
- OpenTrons P300 Multi-Channel Electronic Pipette
- OpenTrons Temperature Module
- OpenTrons Magnetic Module
- Opentrons 20 µL Filter Tips
- Opentrons 200 µL Filter Tips
- Bio-Rad 96 Well Plate 200 µL PCR
- USA Scientific 96 Deep Well Plate 2.4 mL
- Agilent 1 Well Reservoir 290 mL
- USA Scientific 12 Well Reservoir 22 mL

Consumables & Reagents:

Item	URL
OpenTrons 20 µL Filter Tips	https://shop.opentrons.com/collections/opentrons-tips/products/opentrons-20ul-filter-tips
OpenTrons 200 µL Filter Tips	https://shop.opentrons.com/collections/opentrons-tips/products/opentrons-200ul-filter-tips
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 96 Deep Well Plate 2.4 mL	https://www.usascientific.com/plateone-96-deep-well-2ml/p/PlateOne-96-Deep-Well-2mL

Agilent 1 Well Reservoir 290 mL	https://www.agilent.com/store/en_US/Prod-201252-100/201252-100
USA Scientific 12-well Reservoir	https://www.usascientific.com/12-channel-automation-reservoir/p/1061-8150
MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	https://www.thermofisher.com/order/catalog/product/A48383#/A48383
TaqPath™ COVID-19 Combo Kit	https://www.thermofisher.com/order/catalog/product/A47814#/A47814
Thermo Scientific Adhesive PCR Plate Foils	https://www.fishersci.com/shop/products/adhesive-pcr-plate-seals/ab0558#?keyword=AB-0558

Additional Reagents:

- Nuclease-free Water (not DEPC-Treated)
- Ethanol, Absolute, Molecular Biology Grade

Protocol

OT-2 Preparation

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

General Installation

Using any web browser, navigate to the following GitHub directory: https://github.com/DAMPLAB/OT2-SARS-CoV2, and follow the instructions provided in the README.md for the General Installation to install the necessary software setup.

Prepare Fresh 80% Ethanol

Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, plus 10% overage.

Execute RNA Extraction Protocols on OT-2

1. Open up OT2 APP, and upload the *rna_extraction_magmax.py* under the Protocol the folder **rna_extraction_magmax**. [3 Minutes / Variable]
2. 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 plate (Output Plate), the Magnetic Module and a USA Scientific 96 deep well plate containing the samples (Reaction Plate), the Reagents Plate, the Reagent Trough, the Waste Reservoir, two Opentrons 20 µL Filter Tips, and four Opentrons 200 µL Filter Tips Racks onto the deck of the liquid handler (Figure 1A and 1B). [5 Minutes / Variable]
   NOTE: Pipette replacement might be necessary, please follow the instructions provided by OT2 App.
3. Once the calibration process is completed, proceed to running the protocol. [2 Hours / Variable]
   NOTE: Always allow the robotic liquid handler to complete the execution of a script before trying to access the deck space.
4. The robotic liquid handler would automatically pause when the RNA extraction protocol is completed, and indication light of the Temperature Module should be off. Aliquot 10 µL of the purified RNA extract to an empty Bio-Rad 96 well plate for the next workflow, and seal the Reaction Plate with adhesive film.

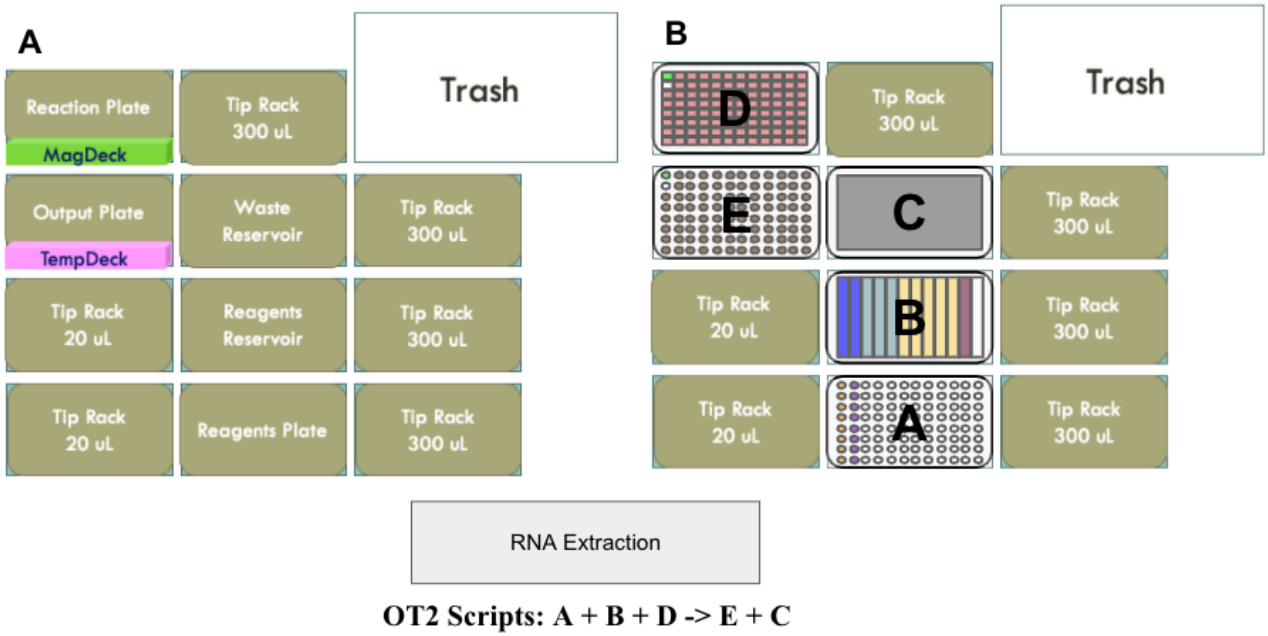

Figure 1: Workflow for executing RNA extraction protocols on OT-2. (A) Representative OT-2 deck layout with labwares and pipette tip boxes placement locations. (B) Representative OT-2 deck layout for plate setup instructions

Execute qPCR Assay Preparation Protocols on OT-2

1. Open up OT2 APP, and upload the *qPCR_taqpath_multiplex.py* under the Protocol the folder **qPCR_taqpath_multiplex**. [3 Minutes / Variable]
2. Once the protocol is uploaded, following the calibration instructions provided by the OT2 APP, place the Temperature Modules 1 and a Bio-Rad 96 well plate containing purified RNA extraction form the previous workflow (Reaction Plate), place the Temperature Modules 2 and a Bio-Rad 96 well plate containing TaqPath™ master mixes (Reagents Plate), and one Opentrons 200 µL Filter Tips Racks onto the deck of the liquid handler (Figure 2A and 2B). [2 Minutes / Variable]
   NOTE: Pipette replacement might be necessary, please follow the instructions provided by OT2 App.
3. Once the calibration process is completed, proceed to running the protocol. [5 Minutes/ Variable]
   NOTE: Always allow the robotic liquid handler to complete the execution of a script before trying to access the deck space.
4. The robotic liquid handler would automatically pause when the qPCR assay preparation protocol is completed, and indication light of the Temperature Module should be off. Seal the Reaction Plate with adhesive film before beginning the qPCR assay.

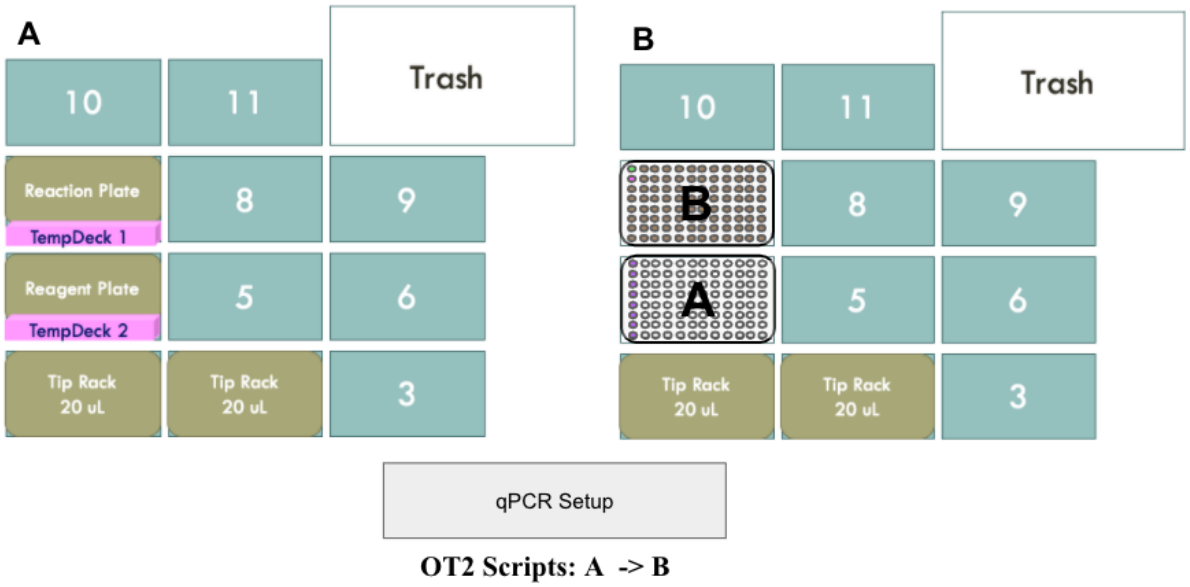

Figure 2: Workflow for executing qPCR assay preparation protocols on OT-2. (A) Representative OT-2 deck layout with labwares and pipette tip boxes placement locations. (B) Representative OT-2 deck layout for plate setup instructions