Station C: ThermoFisher TaqPath RT-PCR COVID-19 Setup guide

Code parameters:
- Change the sample number on line 14 (default is 32, maximum is 94)
- Change the sample volume on line 15 (default is 5µl)
- Change whether or not if the mastermix is created on the robot or manually on line 16 (default is True)
- Tip rack tracking can be changed from False to True on line 17 (default is False)

Pipettes:
- P20 multichannel on the right mount
- P300 single channel on the left mount

Deck Layout:
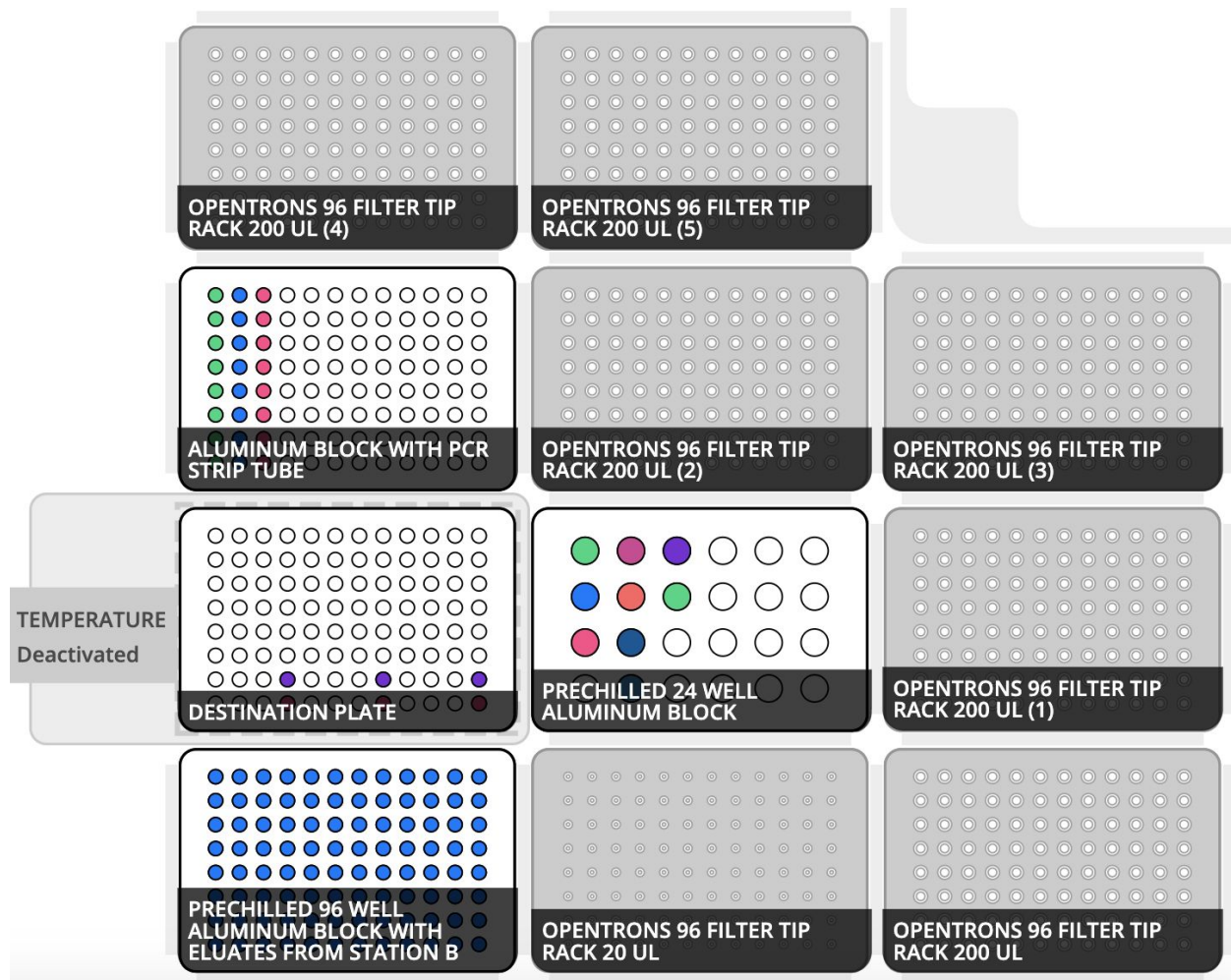
Labware and module requirements:
- 1 x Temperature Module
- 6 x 20µl tipracks
- 1 x 200µl tiprack

- 3 x 96 well aluminum blocks (1 x **prechilled*** in slot 1, 1 x on top of the Temperature Module in slot 4, 1 x in slot 7)
- 1 x 24 well **prechilled*** aluminum block **[holds 1.5 - 2mL tubes with master components]**
- 9 x 2mL tubes (if you select **False** for mastermix creation on deck, then there are 3 x 1.5-2mL tubes)
- 1 x 200 µl PCR strip tubes on top of the 96 well aluminum block in slot 7
- 1 x 96 well plate **[Input - holds eluates/extractions from Station B)**
- 1 x RT-PCR Plate (can be 96 well plate or PCR strip tubes) **[Output]**

Prechilled means the aluminum block has been chilled in the -20C before beginning the protocol

Volume requirements:
***Note**: the below volumes account for a 10% overage - the dead volume can be adjusted depending on the calibration of the pipette to the labware, but it's recommended to have an overage of about 10%*

Mastermix components	Volume per sample (µl)	Volume for 8 samples (µl)	Volume for 16 samples (µl)	Volume for 32 samples (µl)
TaqPathTM 1-Step RT-qPCR Master Mix	6.25	75	325	625
2019-nCoV assay or RNaseP	1.25	15	65	125
Nuclease-free Water	11.25	90	390	750

Slot 5, 24 well prechilled aluminum block setup - Mastermix components tube in A1, B1, and C1 loaded empty. Load mastermix components in remaining tubes

Type	Opentrons 24 Well Aluminum E	
Nickname	prechilled 24 well alu...	✏

- 🟢 RDRP MASTERMIX ⌄
- 🔵 GENE S MASTERMIX ⌄
- 🔴 GENE N MASTERMIX ⌄
- 🟣 TAQPATHTM 1-STEP RT-QPCR MASTER MIX ⌄
- 🔴 S GENE ⌄
- 🔵 N GENE ⌄
- 🔵 RNASEP ⌄
- 🟣 ORF1AB ⌄
- 🟢 WATER ⌄

Slot 7 96 well aluminum block setup
Load 3 **empty** strip tube (these will be used for the mastermix components mixture during the run)

Slot 4 Destination Plate setup

For 32 sample throughput: Add 5 µl for positive control in G4, G8, and G12 and negative control is H4, H8, and H12
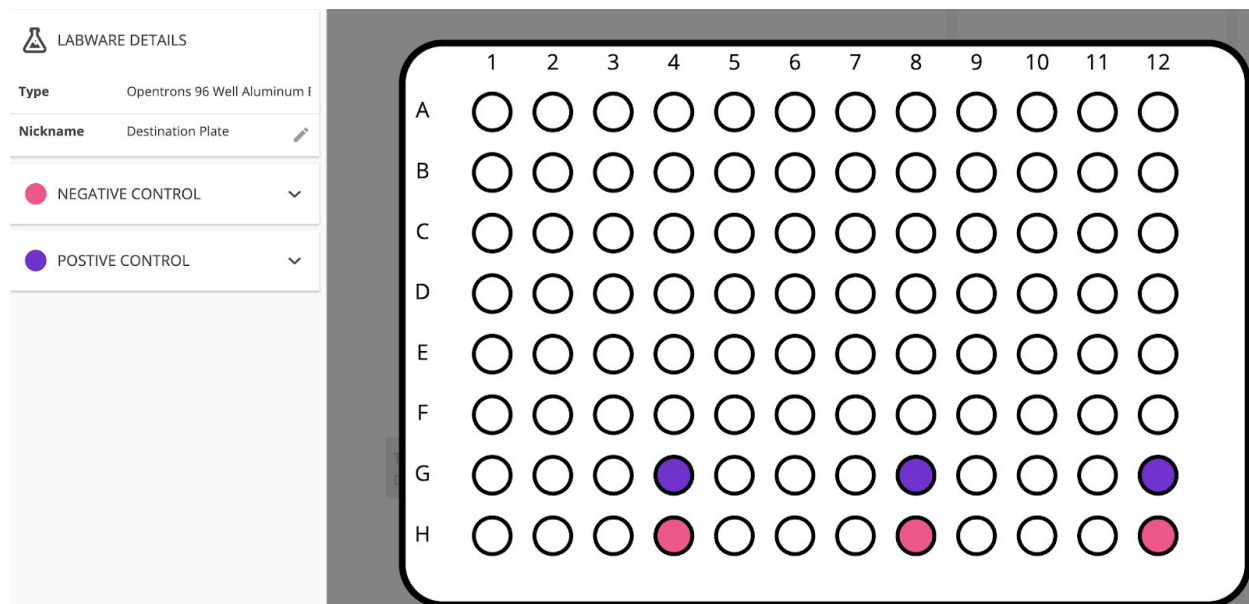
Before you begin:
1. Pre-cool the Temperature Module in the Opentrons App to 4°C
2. Eluates (extractions) from Station B are loaded onto a **Prechilled** 96 well aluminum block on slot 1.
3. Add the Master mixture tube (loaded empty if choosing true for mastermix creation) and mastermix component tubes to the **Prechilled** 24 well aluminum block in slot 5.
4. Add the empty strip tube to the 96 well aluminum block in slot 7
5. Add the **5 µl of control** to each corresponding tube in the destination plate
6. Check again to make sure each component is added and the Temperature Module is pre-cooled to 4°C.

The final destination RT-PCR plate will be in Slot 4 on top of the Temperature Module. Once the protocol is complete, the plate will be ready to be sealed, spun down, and loaded onto an RT-PCR machine.

Plate setup:
Note: if you are running 8 samples, columns 1, 5, and 9 will be used. If you are running 16 samples, columnes 1,2 5,6 and 9,10 will be used, etc.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	1	9	17	25	1	9	17	25
B	2	10	18	26	2	10	18	26	2	10	18	26
C	3	11	19	27	3	11	19	27	3	11	19	27
D	4	12	20	28	4	12	20	28	4	12	20	28
E	5	13	21	29	5	13	21	29	5	13	21	29
F	6	14	22	30	6	14	22	30	6	14	22	30
G	7	15	23	positive control	7	15	23	positive control	7	15	23	positive control
H	8	16	24	negative control	8	16	24	negative control	8	16	24	negative control