Station C: ThermoFisher TaqPath RT-PCR COVID-19

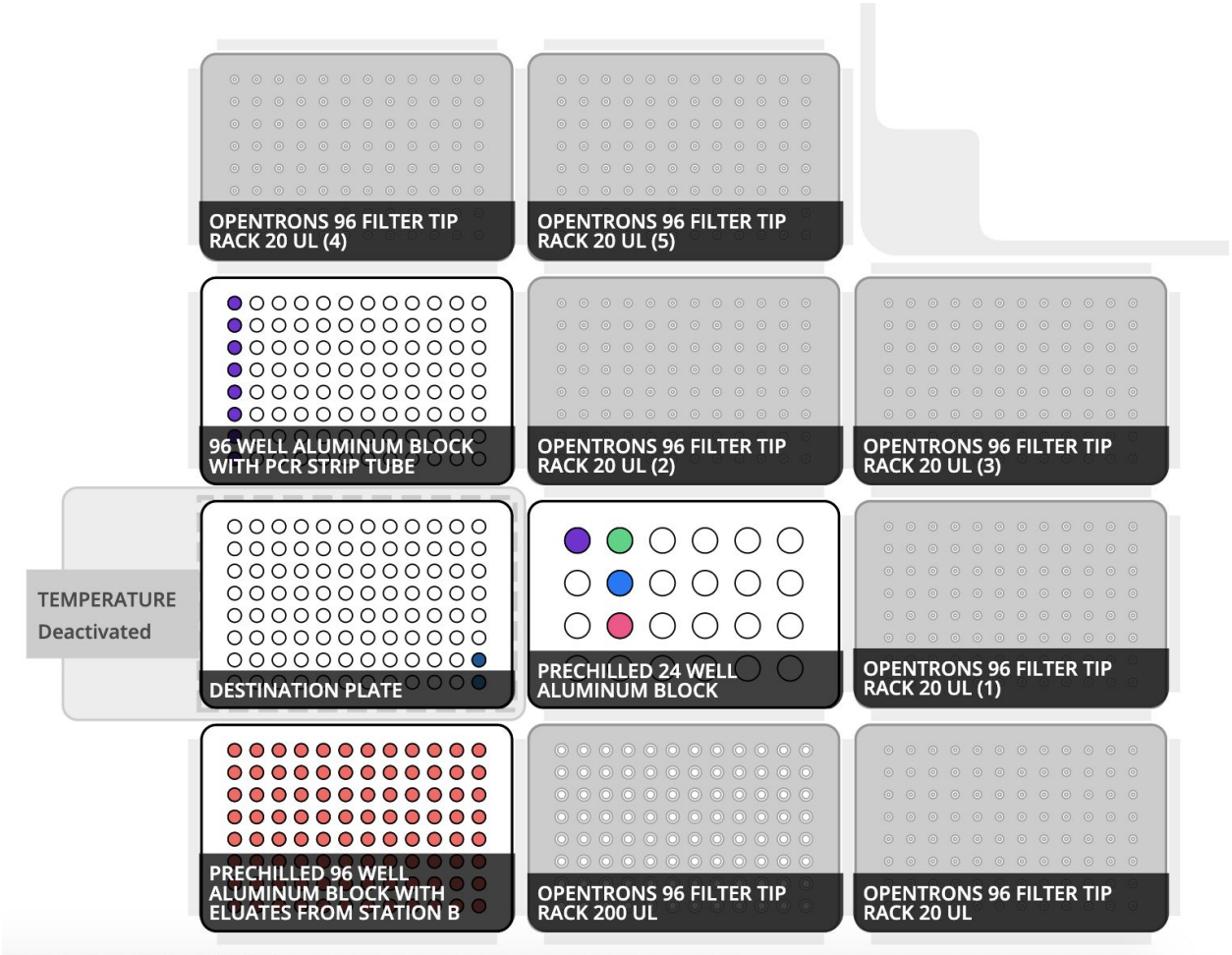
Code parameters:

- Change the sample number on line 14 (default is 8, maximum is 94)
- Change the sample volume on line 15 (default is 10µl)
- 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]**
- 4 x 1.5-2mL tubes (if you select **False** for mastermix creation on deck, then there are 1 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]**

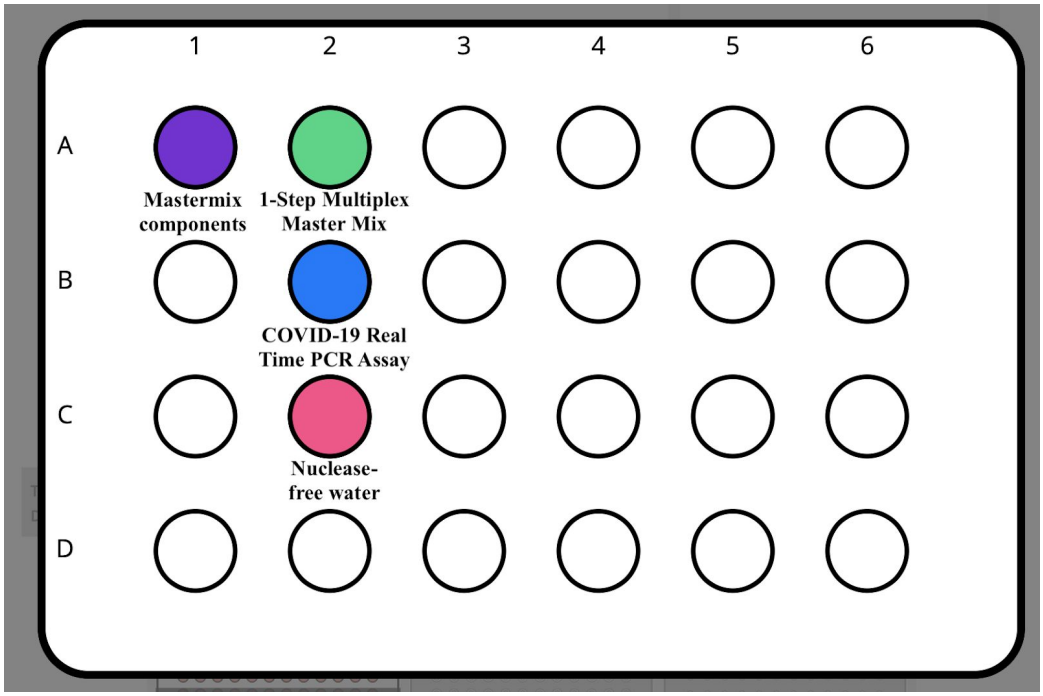
**Prechilled means the aluminum block has been chilled in the -20C before beginning the protocol*

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%*

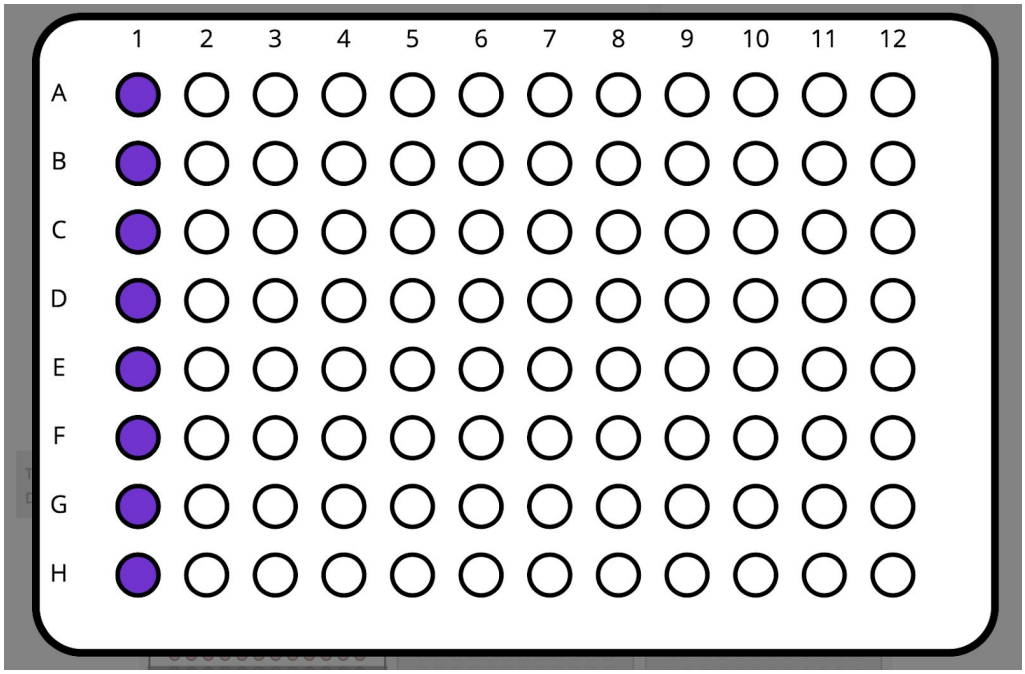
Mastermix components	Volume per sample (µl)	Volume for 8 samples (µl)	Volume for 48 samples (µl)	Volume for 96 samples (µl)
TaqPath™ 1-Step Multiplex Master Mix	6.25	75	325	625
COVID-19 Real Time PCR Assay Multiplex	1.25	15	65	125
Nuclease-free Water	7.5	90	390	750

Slot 5, 24 well prechilled aluminum block setup - Mastermix components tube in A1 loaded empty. Load mastermix components in A2 - C2



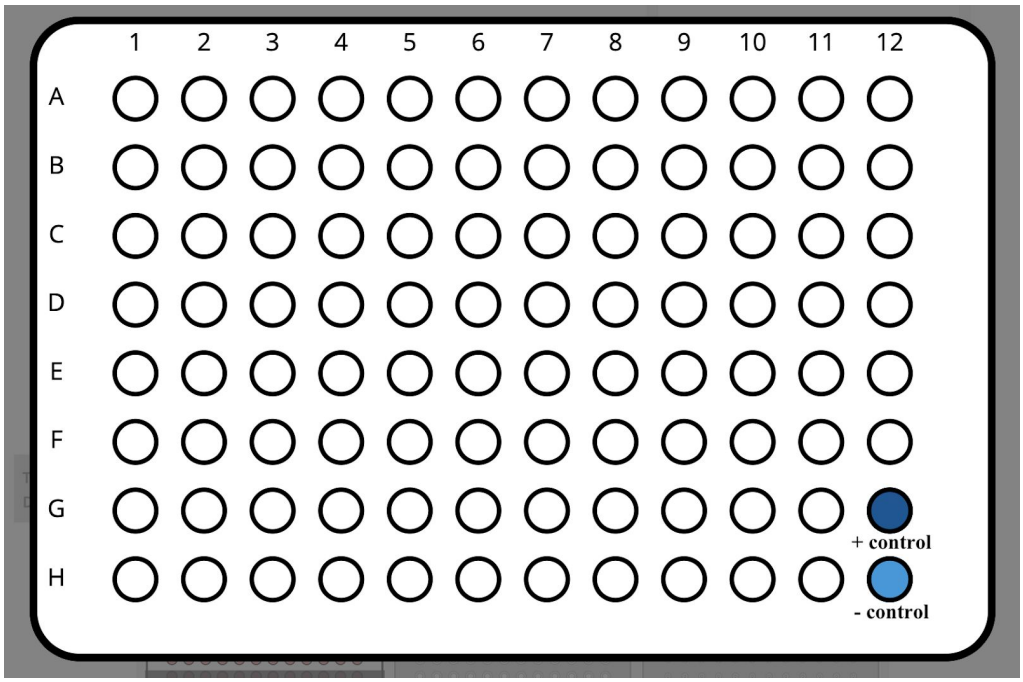
Slot 7 96 well aluminum block setup

Load 1 **empty** strip tube (these will be used for the mastermix components mixture during the run)



Slot 4 Destination Plate setup

For 94 sample throughput: Add 10 µl for positive control in G12 and negative control is 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 **10 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.

8 sample plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	**+ control**										
B	2	**- Control**										
C	3											
D	4											
E	5											
F	6											
G	7											
H	8											

94 sample plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	**+ control**
H	8	16	24	32	40	48	56	64	72	80	88	**- Control**