

Station C: ABM GenomeCoV19 Detection kit

Please familiarize yourself with the manual instructions before proceeding.

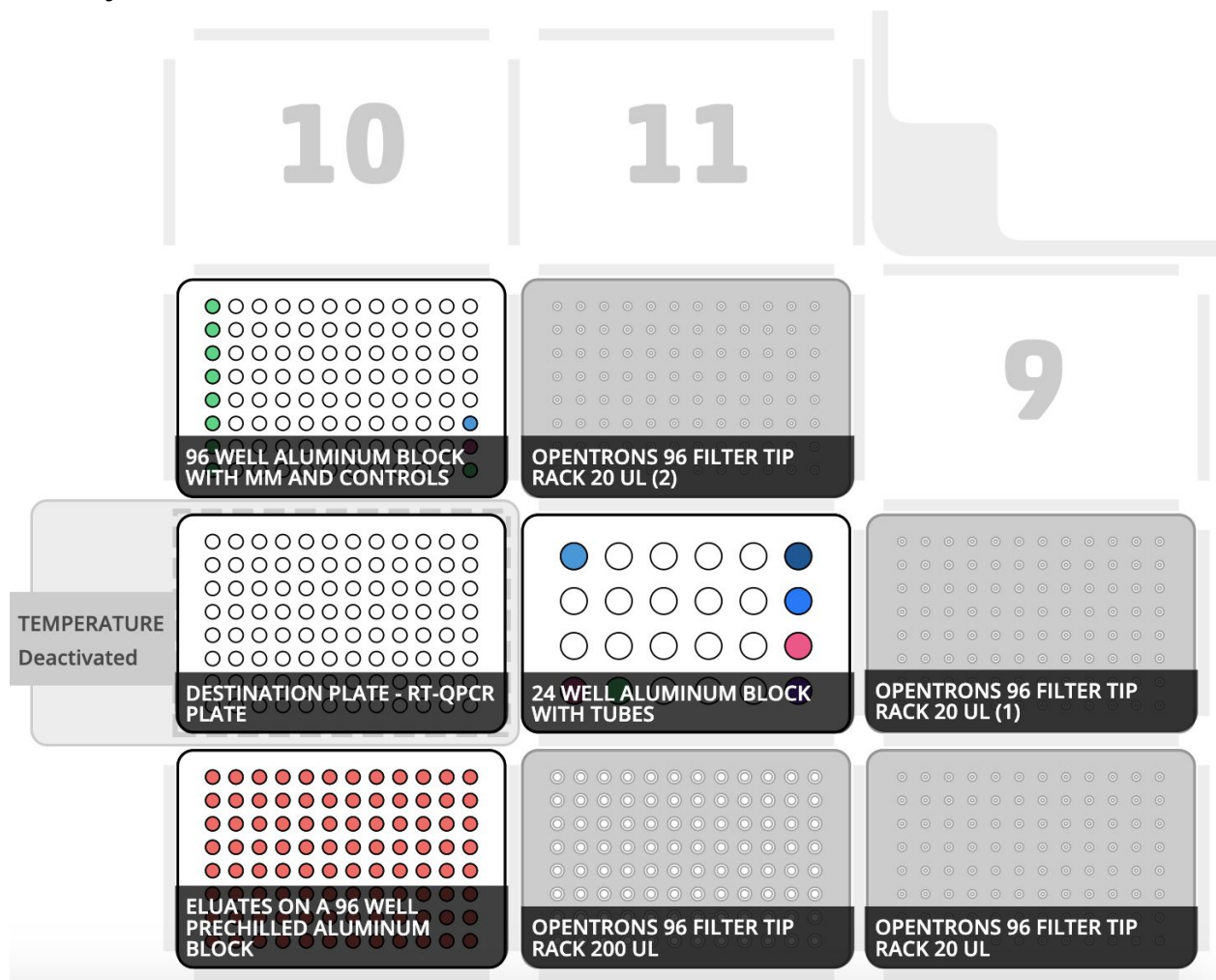
Code parameters:

- Change the sample number on line 14 (default is 8, max is 94)
- Change whether or not if the mastermix is created on the robot or manually on line 16 (default is True)
- Change if the the robot adds the controls or manually on line 17 (default is True)
- Tip rack tracking can be changed from False to True on line 16 (default is False)
- Change the sample volume on line 15 (default is 5µl)

Pipettes:

- P20 multi channel on the right mount
- P300 single channel on the left mount

Deck layout:



Labware and module requirements:

- 1 x Temperature Module

- 3 x 20µl tipracks (up to 6 x 20uL tipracks if you select **True** for tiprack tracking)
- 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 2mL tubes with mastermix mastermix components**]
- 7 x 2mL tubes (if you select **False** for mastermix creation on deck and **True** for controls added on deck, then there are 3 x 2mL tubes, if you select **True** for mastermix creation on deck and **False** for controls added on deck, then there are five 2 x 2mL tubes, if you select **False** for both, there is 1 x 2mL tubes)
- 2 x strip tubes on top of the 96 well aluminum block in slot 7
- 1 x 96 well plate or strip tubes [**Input - holds eluates/extractions from Station B**]
- 1 x RT-PCR Plate (can be 96 well plate or PCR 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 dead volume - the dead volume can be adjusted depending on the calibration of the pipette to the labware, but it's best to have a dead volume of at least 10%

Mastermix Reagents	Volume per sample (µl)	Volume for 8 samples (µl)	Volume for 48 samples (µl)	Volume for 96 samples (µl)
COVID-19 Primers/Probes	2	24	104	200
2x RT-qPCR Mastermix	10	120	520	1,000
RT-qPCR Enzyme Mix	0.4	4.8	20.8	40
Nuclease-free H2O	2.6	31.2	135.2	260

Slot 5 24 well prechilled aluminum block

Note: mastermix tube in D2 is loaded empty unless mastermix is created manually

LABWARE DETAILS

Type

Opentrons 24 Well Aluminum f

Nickname

24 well aluminum bloc...

MASTERMIX

▼

2X RT-QPCR MASTERMIX

▼

RT-QPCR ENZYME MIX

▼

NUCLEASE-FREE H2O

▼

COVID-19 PRIMERS/PROBES

▼

POSITIVE CONTROL

▼


NEGATIVE CONTROL

▼


	1	2	3	4	5	6
A						
B						
C						
D						

Slot 7 96 well aluminum block setup

Load 2 **empty** strip tubes (these will be used for mastermix and controls during the run)

LABWARE DETAILS

TypeOpentrons 96 Well Aluminum i

Nickname96 Well Aluminum Blo...

MASTERMIX

▼

POSITIVE CONTROL

































































































▼

NEGATIVE CONTROL

▼

NO TEMPLATE CONTROL

▼

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

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 Mastermix tube (loaded empty if choosing true for mastermix creation) and mastermix component tubes and control tubes to the **Prechilled** 24 well aluminum block in slot 5.
4. Add empty strip tubes to the 96 well aluminum block in slot 7
5. Check again to make sure each component is added and the Temperature Module had pre-cooled.

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:

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

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	+ control
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	NTC

