# Station C: Allplex 2019-nCov Detection Assay - Seegene

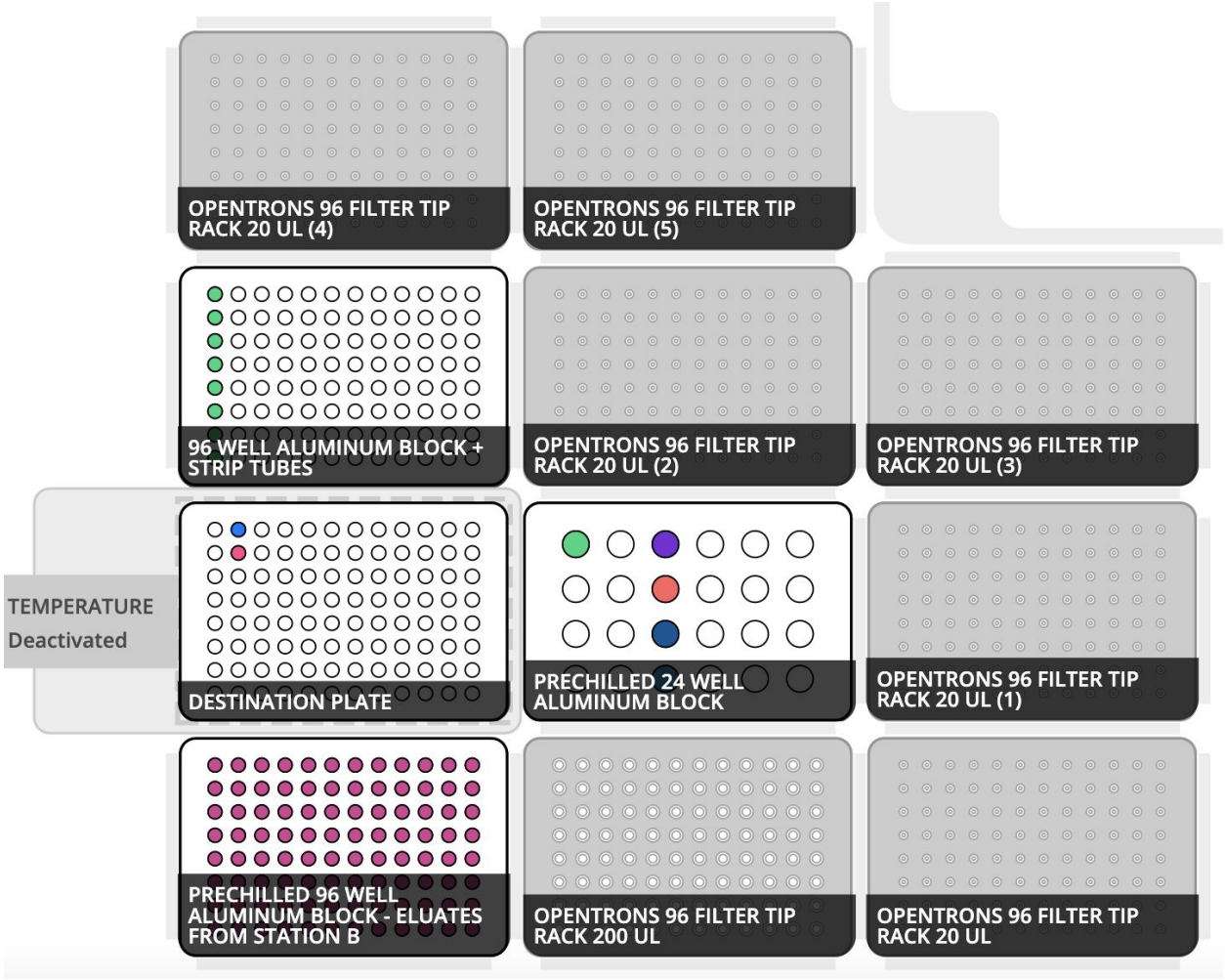
**Code parameters:**

- Change the sample number on line 14 (default is 94, max is 94)
- Change whether or not if the mastermix is created on the robot or manually on line 15 (default is True)
- Tip rack tracking can be changed from False to True on line 16 (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 mastermix mastermix components]**
- 5 x 1.5-2mL tubes (if you select **False** for mastermix creation on deck, then there are 1 x 1.5-2mL 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*

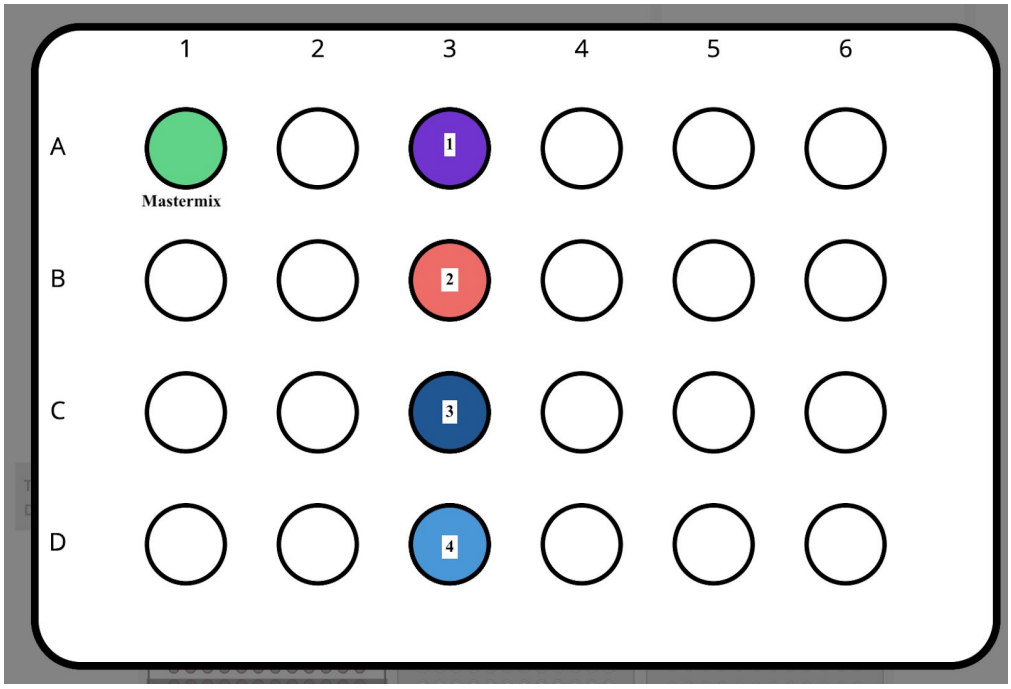
**Volume components**

**Note**: the belows 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 Component** | **What the component is** | **Volume for 1 sample (µl)** | **Volume for 8 samples (µl)** | **Volume for 48 samples (µl)** | **Volume for 96 samples (µl)** |
|---|---|---|---|---|---|
| 1 | nCov MOM probe | 5 | 60 | 260 | 500 |
| 2 | H20 | 5 | 60 | 260 | 500 |
| 3 | 5x Real time one-step buffer | 5 | 60 | 260 | 500 |
| 4 | Real time one-step enzyme | 2 | 24 | 105 | 200 |

**Slot 5 - 24 well aluminum block tube layout**

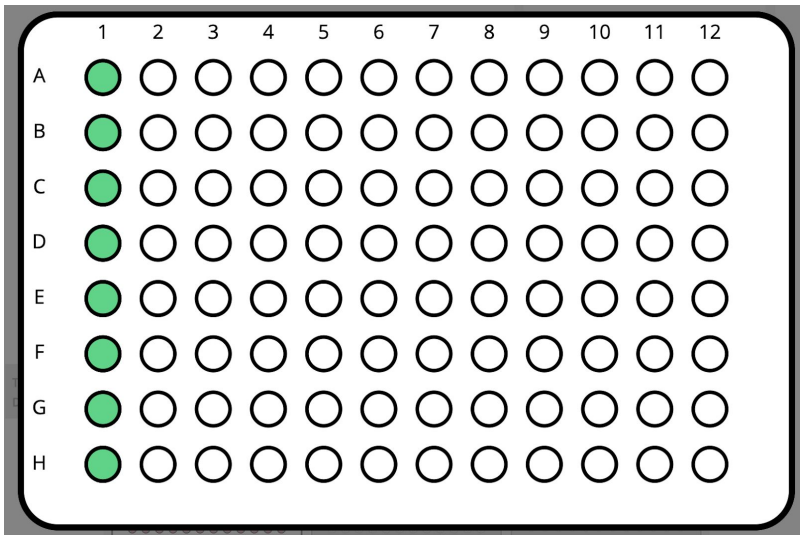
*Note: mastermix tube in A1 is loaded empty unless mastermix is created manually*




**Slot 7 96 well aluminum block setup**

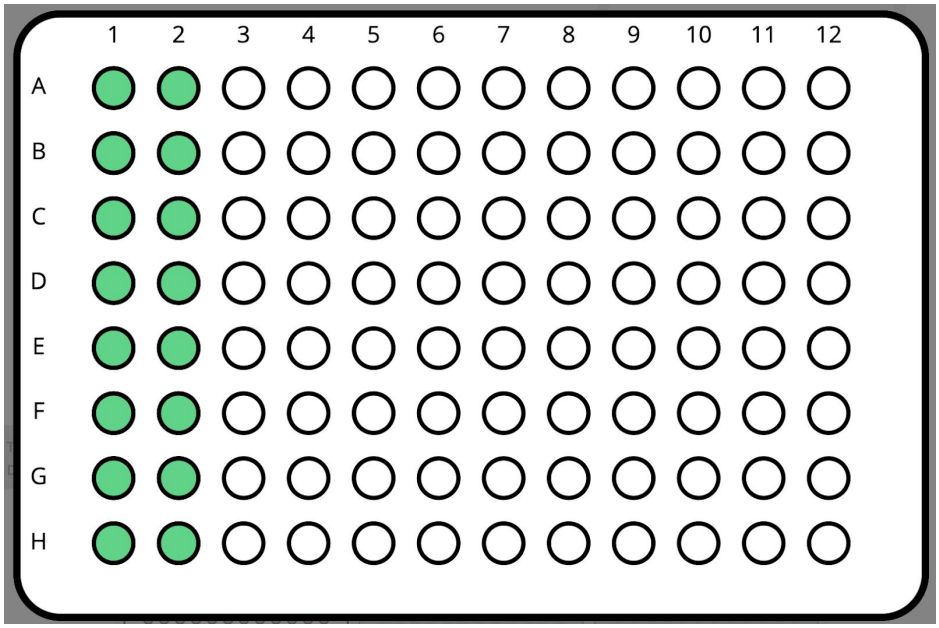
**For less than 72 sample throughput:**

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



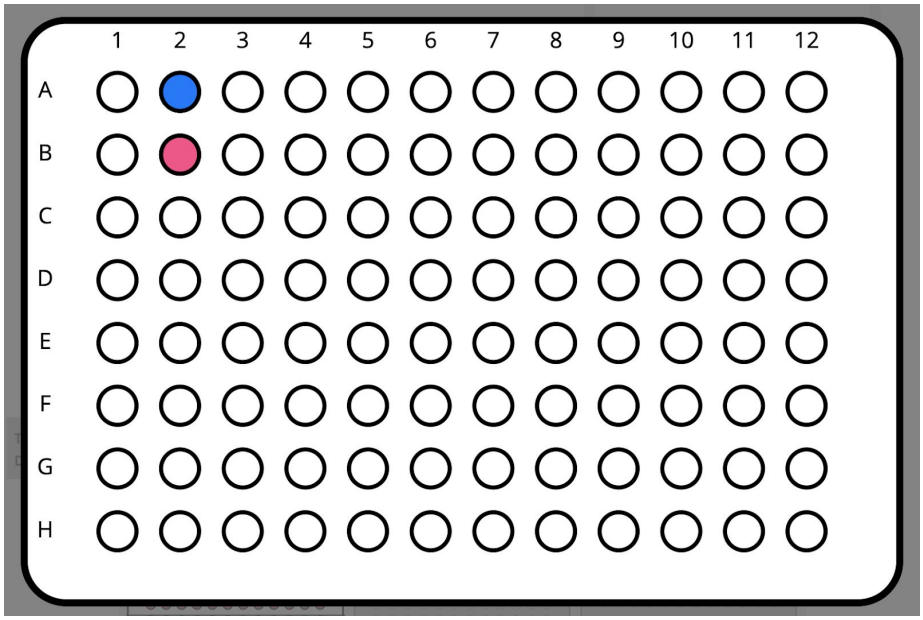
**For 72 or greater sample throughput:**

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

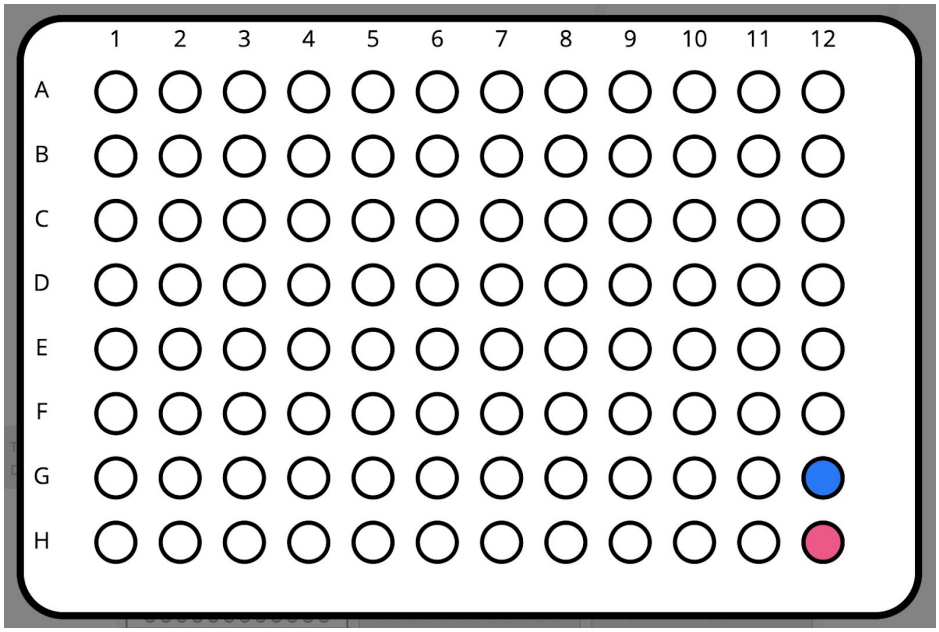



**Slot 4 Destination Plate setup**

For 8 sample throughput: Add 8 µl of each control



For 94 sample throughput: Add 8 µl of each control




**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 to the **Prechilled** 24 well aluminum block in slot 5.
4. Add empty strip tube(s) to the 96 well aluminum block in slot 7
5. Add the **8 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 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:**

| **Seegene setup** | 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** |