Last Update: April 29, 2020

Station C: Diatheva Detection Kit

The python code can be found here

Please familiarize yourself with the manual instructions before proceeding.

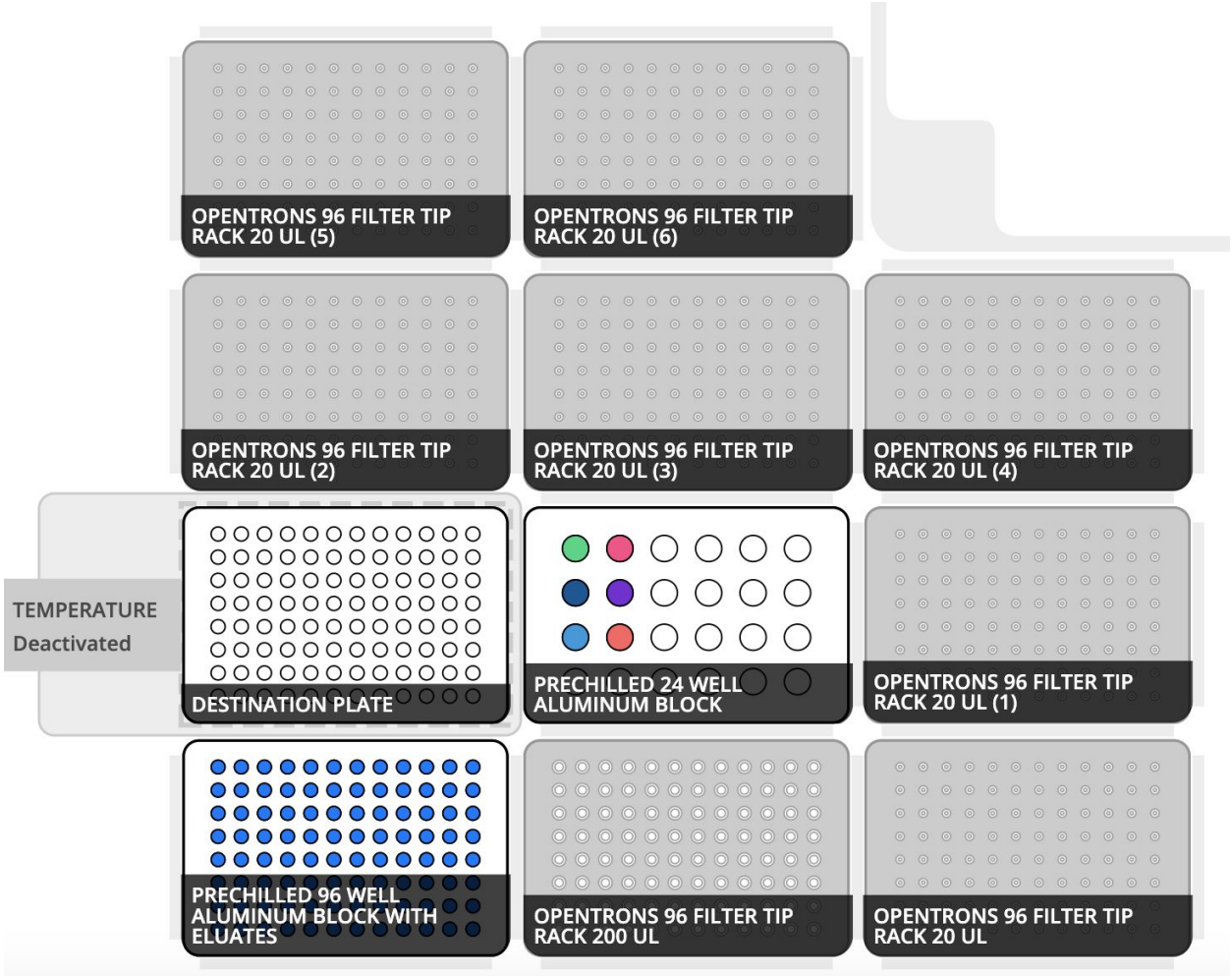
Code parameters:

- Change the sample number on line 13 (default is 8, max is 94)
- Change whether or not if the mastermix is created on the robot or manually on line 14 (default is True)
- Tip rack tracking can be changed from False to True on line 15 (default is False)

Pipettes:

- P20 single 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 (if you select **True** for tip rack tracking, then there are 7 x 20µl tipracks)
- 1 x 200µl tiprack

- 2 x 96 well aluminum blocks (1 x **prechilled*** in slot 1, 1 x on top of the Temperature Module in slot 4)
- 1 x 24 well **prechilled*** aluminum block **[holds 1.5 - 2mL tubes with mastermix, controls, and mastermix components]**
- 6 x 1.5-2mL tubes (if you select **False** for mastermix creation on deck, then there are 3 x 1.5-2mL tubes)
- 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*

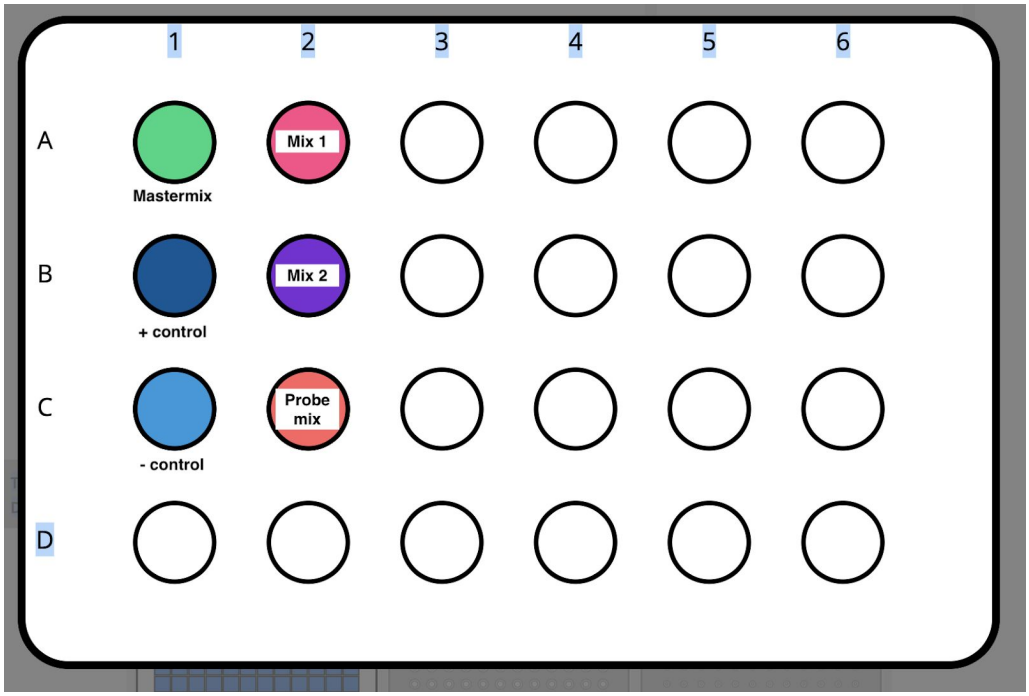
Volume requirements:

***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)**
Mix 1	5	60	260	500
Mix 2	0.625	7.5	32.5	62.5
Mix Primer/Probe	9.375	112.5	482.5	937.5

Slot 5 24 well prechilled aluminum block

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



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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), positive and negative control tubes, and mastermix component tubes to the **Prechilled** 24 well aluminum block in slot 5.

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:

	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**