Station C: ViaSure Real Time PCR Detection Kits: CerTest BioTek

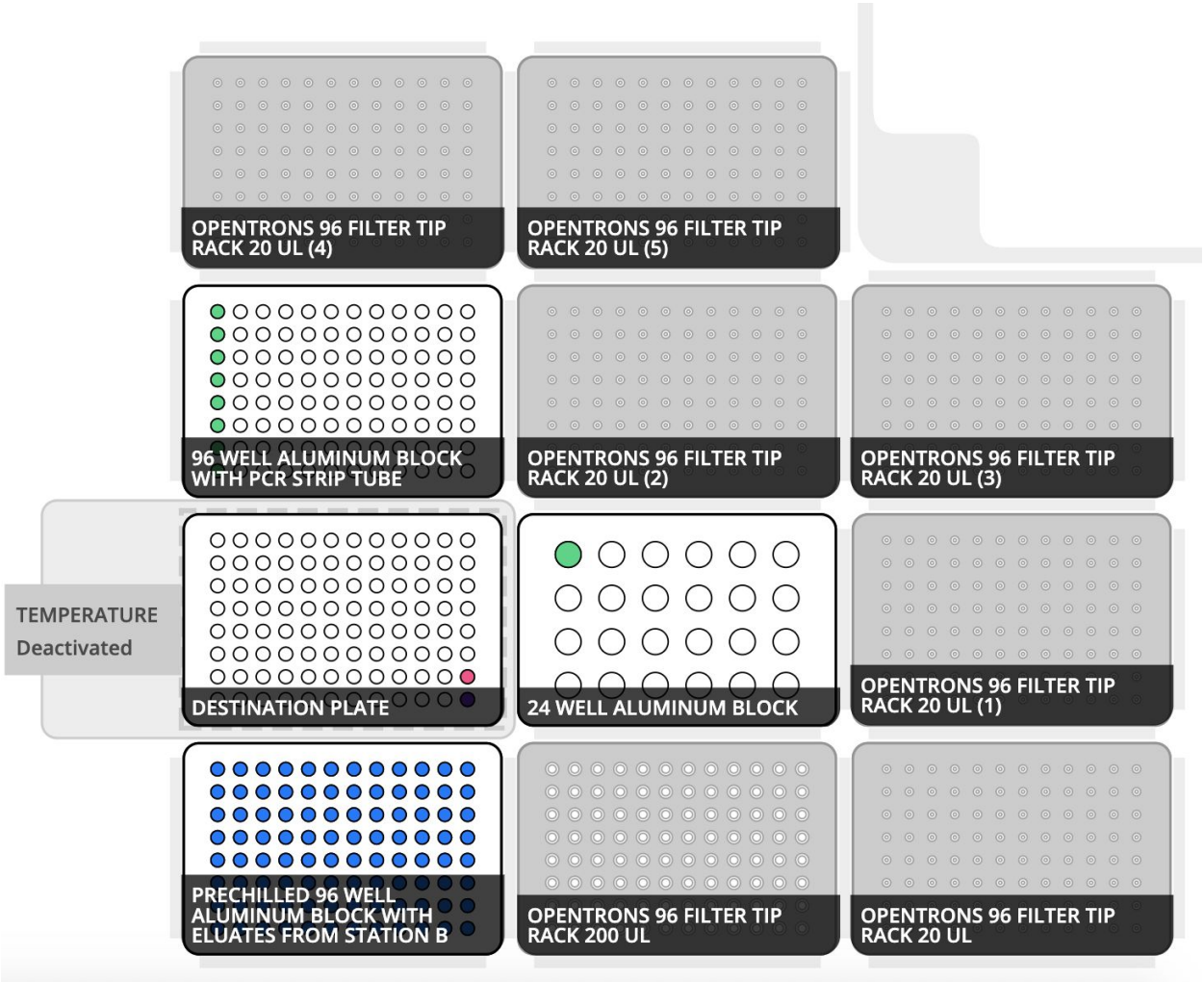
Code parameters:

- Change the sample number on line 14 (default is 94, maximum is 94)
- Change the sample volume on line 15 (default is 5µl)
- 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 aluminum block **[holds 2mL tube for rehydration buffer]**

- 1 x 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]**

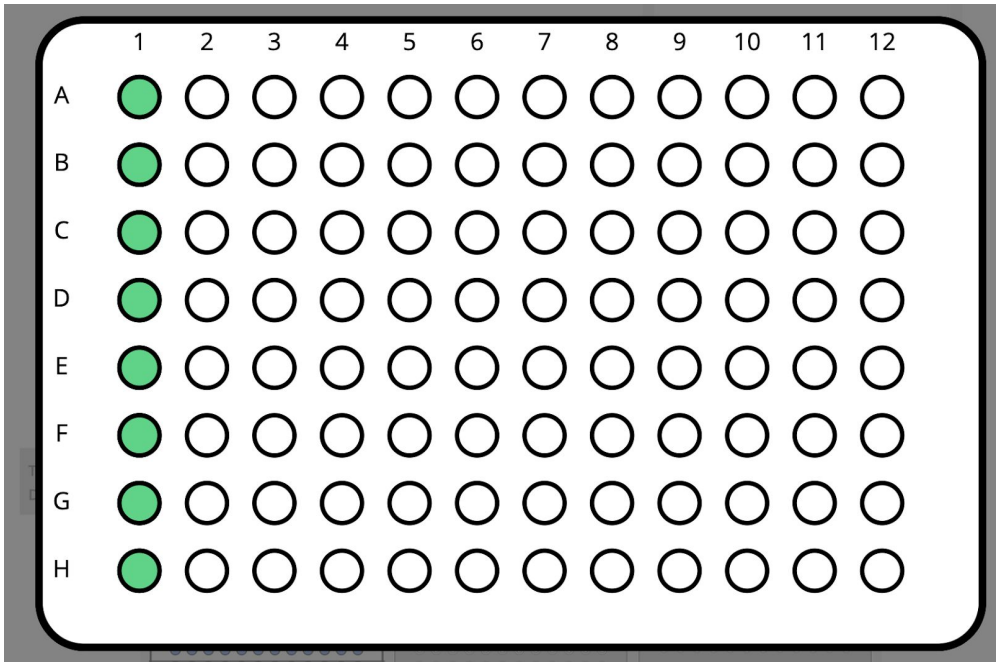
**Prechilled means the aluminum block has been chilled in the -20C before beginning the protocol*

Volume requirements:

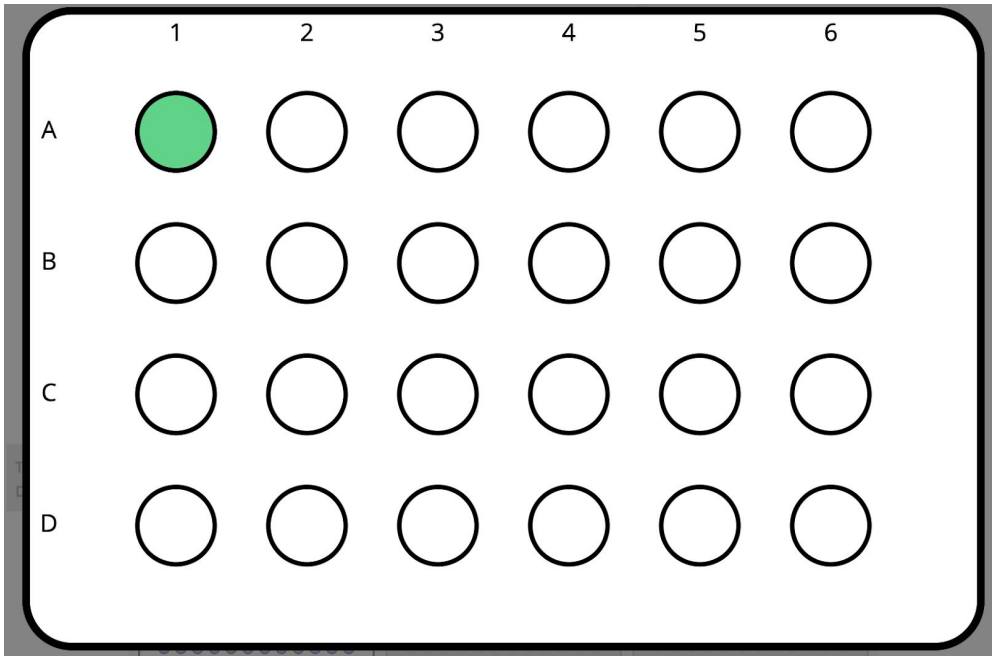
***Note**: the below volumes account for a 10% overage - the dead volume can be adjusted depending on the calibration of the pipette to the labware, but it's recommended to have an overage of about 10%*

	Volume per sample (µl)	**Volume for 8 samples (µl)**	**Volume for 48 samples (µl)**	**Volume for 96 samples (µl)**
Rehydration Buffer	15	60	780	1,500

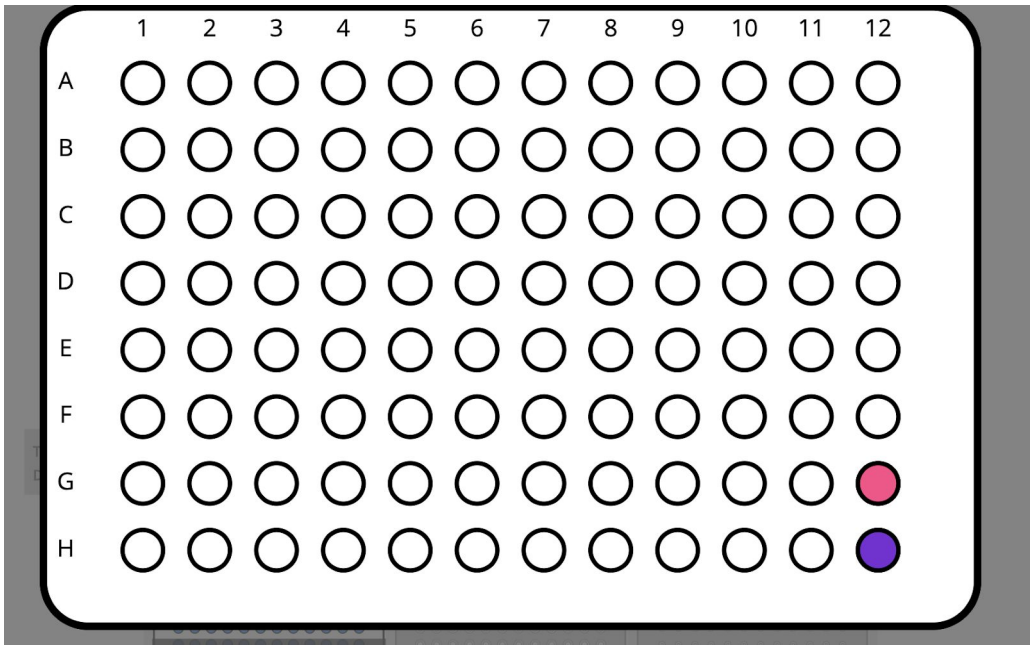
Slot 7 - load an **empty** PCR strip tube in column 1



Slot 5 - load a 2mL tube with rehydration buffer in well A1



Slot 4 - destination plate - load 5µl of positive and negative control into wells G12 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 Rehydration buffer tube to the 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.

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**