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BSCI:414--Lab 4 Plate Setup for COVID RT-qPCR

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ABSTRACT

The purpose of this experiment is to show where saliva samples and positive and negative controls will be placed on a 96-well plate by an OpenTrons robot.

PROTOCOL CITATION

Harley King 2020. BSCI:414--Lab 4 Plate Setup for COVID RT-qPCR. **protocols.io**
<https://protocols.io/view/bsci-414-lab-4-plate-setup-for-covid-rt-qpcr-bmqyk5xw>



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42488

Discuss Sample Types

1

Review SalivaDirect for sample processing protocol: <https://www.protocols.io/view/salivadirect-rna-extraction-free-sars-cov-2-diagno-bkjgkuju?step=11>

Inset Well Plate in Benchling Lab Notebook

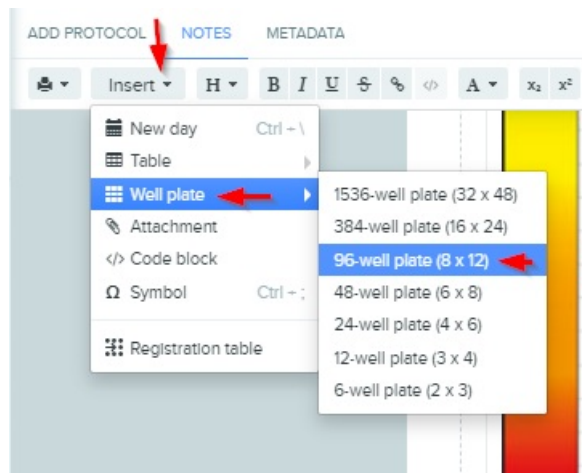
2

Open your Benchling Lab Notebook and insert "Lab 4" with heading "H1". Add pertinent links to protocols like the SalivaDirect protocol.

3

We need to set up the format for our experiment in a 96-well plate.

Add a 96-well well plate by selecting "Insert>Well plate>96-well plate".



Add a 96-w "Well plate" in Benchling.

- 4 Add saliva samples 1 to 4, the no-template control and positive control using plasmid "pEX-A128-nCoV_CD". Make sure to "@-mention" the plasmid "pEX-A128-nCoV_CD" located in the BSCI:414 directory. Do not copy this to a new folder.

HK_F20_BSCI:414_96-well Plate for SARS-CoV2 Testing													
	1	2	3	4	5	6	7	8	9	10	11	12	
A	Saliva sample 1												
B	Saliva sample 2												
C	Saliva sample 3												
D	Saliva sample 4												
E	NTC (water)												
F	pEX-A128-nCoV_CD												
G	pEX-A128-nCoV_CD												
H													

96-well plate outline in Benchling.

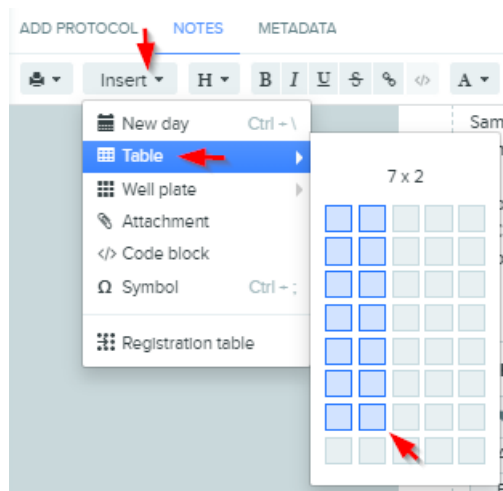
Add qPCR Schedule to Lab Notebook

- 5 Add a 2x7 table using "Insert>Table".

Copy the qPCR schedule from Step 11 in SalivaDirect into the table . Use the values from the "NEB Luna" kit.

Add a "Saliva Sample" row containing 5ul.

Add a "TOTAL" row at the bottom and ensure reagents total 20ul.



Add a 2x7 table.

qPCR Schedule for Each Sample using NEB Luna Reagents			
	A	B	
1	Component	NEB Luna	
2	Master mix	10 µL	
3	RT	1 µL	
4	Primer-probe-water mix (see above)	4 µL	
5	Nuclease-free water	-	
6	Saliva Sample	5 µL	
7	TOTAL	20 ul	

qPCR Schedule in Benchling.