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# BSCI:414 Lab 6 and Lab 7--Analyze RT-qPCR Results from COVID-19 Test

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1 Works for me This protocol is published without a DOI.

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## PROTOCOL CITATION

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<https://protocols.io/view/bsci-414-lab-6-and-lab-7-analyze-rt-qpcr-results-f-bm8ck9sw>

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## CREATED

Oct 09, 2020

## LAST MODIFIED

Oct 16, 2020

## PROTOCOL INTEGER ID

42980

## Review Experiment

- 1 Watch and discuss experimental video.

<https://youtu.be/9s5sACRqkZ8>

## Review Results

- 2 Download screenshots and "Human.sample.chart.results.JPG" in our class folder, "Files>Lab Results>Lab 6 qPCR Data".

7300 System SDS Software - [SARS-CoV2\_human\_samples.sds (Standard Curve)]

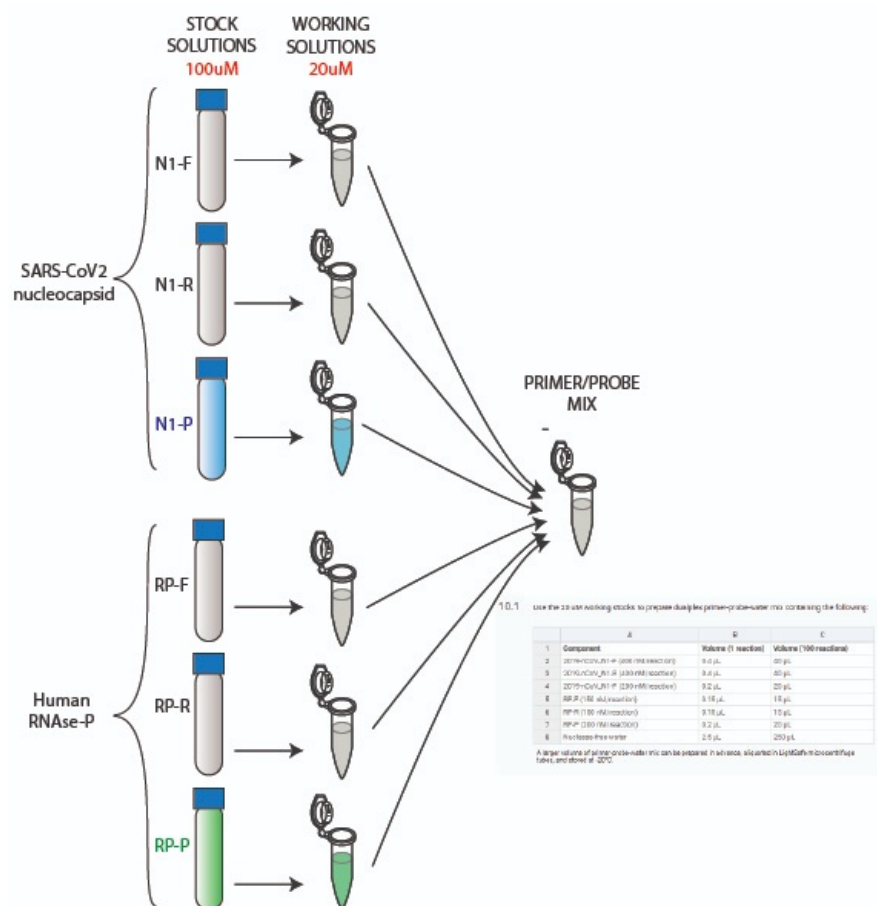
File View Tools Instrument Analysis Window Help

Setup Instrument Results

Plate Spectra Component Amplification Plot Standard Curve Dissociation Report

Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
A1	Saliva_Sample_1	RNAse-P	Unknown	25.6109	0.581					
A1	Saliva_Sample_1	NP	Unknown	Undet.						
A2	Saliva_Sample_1	RNAse-P	Unknown	24.7895	0.581					
A2	Saliva_Sample_1	NP	Unknown	Undet.						
B1	Saliva_Samples_2	RNAse-P	Unknown	26.8639	0.102					
B1	Saliva_Samples_2	NP	Unknown	Undet.						
B2	Saliva_Samples_2	RNAse-P	Unknown	27.0082	0.102					
B2	Saliva_Samples_2	NP	Unknown	Undet.						
C1	Saliva_Sample_3	RNAse-P	Unknown	25.5291	0.438					
C1	Saliva_Sample_3	NP	Unknown	26.7596	0.411					
C2	Saliva_Sample_3	RNAse-P	Unknown	24.9103	0.438					
C2	Saliva_Sample_3	NP	Unknown	26.1787	0.411					
D1	blank	RNAse-P	Unknown	39.0896	0.819					
D1	blank	NP	Unknown	Undet.						
D2	blank	RNAse-P	Unknown	37.9307	0.819					
D2	blank	NP	Unknown	38.949						
E1	std_Tube4	RNAse-P	Unknown	23.2151						
E1	std_Tube4	NP	Unknown	19.659						
F1	std_Tube3	RNAse-P	Unknown	20.2816						
F1	std_Tube3	NP	Unknown	16.8728						
G1	std_Tube2	RNAse-P	Unknown	17.3628						
G1	std_Tube2	NP	Unknown	14.5282						
H1	std_Tube1	RNAse-P	Unknown	17.0393						
H1	std_Tube1	NP	Unknown	14.0592						

qPCR threshold results for human saliva samples.



Mastermix concentrations of probes and primers. Chart in bottom right corner is to [SalivaDirect protocol, step 10.1](#).

### 3 Calculate the concentration of plasmid DNA used to generate the standards.

7 plasmid standards were created.

Tube	Conc	Notes
1	?	Diluted 1ug plasmid DNA in 100ul H2O
2	?	Diluted 10ul from tube 1 into 990ul H2O of tube 2
3	?	Diluted 100ul from tube 2 into 900ul H2O
4	?	Diluted 100ul of tube 3 into 900ul H2O
5	?	Diluted 100ul of tube 4 into 900ul H2O
6	?	Diluted 100ul of tube 5 into 900ul H2O
7	?	Diluted 100ul of tube 6 into 900ul H2O

Use the "Notes" column to calculate the concentration of control plasmid DNA in each of the tubes.

Our assay used the "[nCoV-CDC-Control Plasmid](#)" from Operon. The plasmid has been [registered](#) in Benchling.

**nCoV-CDC-Control Plasmid**

The nCoV-CDC-Control Plasmid includes **all genes of 2019-nCoV**, recommended from the CDC.

- N1 region
- N2 region
- N3 region
- RNase P Gen

1µg of the control plasmid is delivered lyophilised individual tubes. Shipped at ambient temperature.

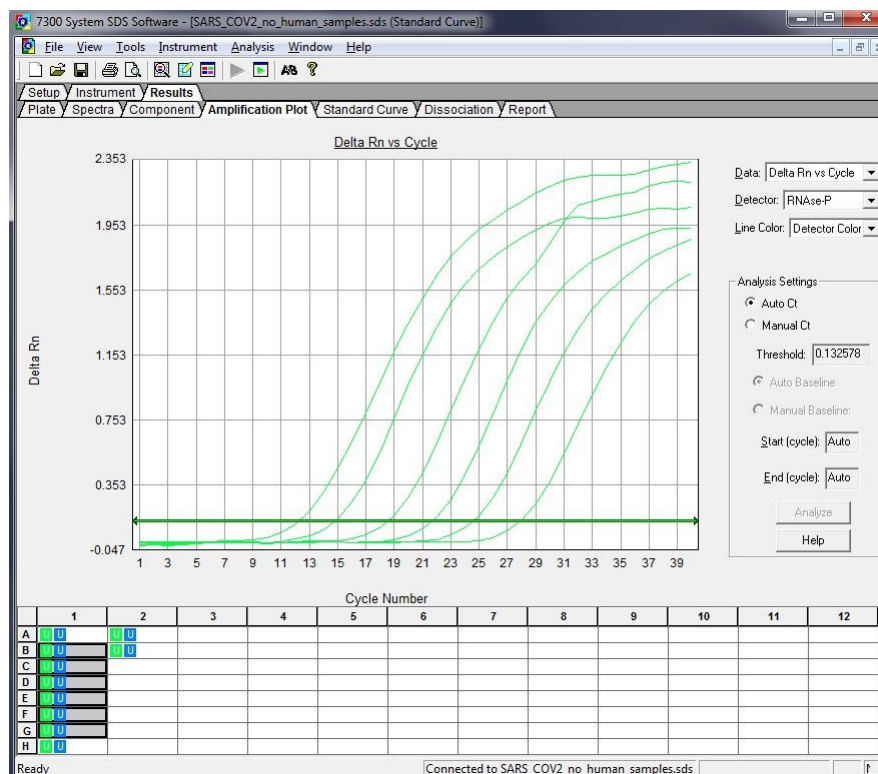
[Sequence incl vector >>](#)

Quantity

Price **\$43.40**

**ADD TO CART**

nCoV-CDC-Control Plasmid from Operon



Serially diluted standards produce staggered inflections.