Jan 19, 2021

Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE_Hudson Alpha

Forked from Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE updated

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1 Works for me dx.doi.org/10.17504/protocols.io.brixm4fn

ashwinrc

ABSTRACT

The Stanford University and Purigen Biosystems team's SARS-CoV-2 assay leverages on-chip microfluidics to eliminate laborious and time consuming steps associated with standard molecular diagnostics such as solid phase spin-column extraction and PCR amplification. Purification of nucleic acids from a variety of biological sources is achieved in a one-step, automated fashion using on-chip isotachophoresis (ITP). The purified nucleic acids are then amplified using reverse transcription (RT) loop-mediated isothermal amplification (LAMP) in 30 minutes, less than half of the time associated with standard qPCR. We then use CRISPR-Cas12 fluorescent detection to identify amplicons associated with the SARS-CoV-2 genome for enhanced specificity.

DOI

dx.doi.org/10.17504/protocols.io.brixm4fn

PROTOCOL CITATION

jnesvet 2021. Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE_Hudson Alpha. **protocols.io** https://dx.doi.org/10.17504/protocols.io.brixm4fn

FORK NOTE

FORK FROM

Forked from Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE updated, ashwinrc

KEYWORDS

CRISPR-diagnostics, microfluidics, SARS-CoV-2, RNA, Nucleic acid test, Isotachophoresis, Stanford, Purigen, RT-LAMP, COVID-19, CRISPR-Cas12

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MATERIALS TEXT

Materials for Purigen Ionic Install:

Required Materials

Table2: Contents provided in the Virtual Installation and Training Kit

Item Name	Description
Ionic System Cleaning Chip	1 plastic cleaning chip
Leveling Kit	1 Leveling tool and charging cable
Web Camera	1 Adjustable web camera on stand
USB Flash Drive	1 64 G USB flash drive

Table 3: Contents provided in the Ionic System Installation and Training Kit

Item Name	Description
System Validation Kit	2 fluidic chips, 2 reservoirs, and reagents
Reference Samples	16 cell or FFPE tissue reference samples
Reagent Reservoirs	10 reagent reservoirs
Training Purification Kit	3 fluidic chips and reagents for the following protocols: • Viral RNA - PBS • Viral RNA - Saliva • Viral RNA - Water 3 fluidic chips for pipetting practice only 50, 1.5 mL Eppendorf LoBind DNA Tubes

Equipment provided by Purigen

Citation: jnesvet (01/19/2021). Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE_Hudson Alpha. https://dx.doi.org/10.17504/protocols.io.brixm4fn

Table 4: Equipment Provided by the user

User-Supplied Equipment	Installation	Training
Scissors	✓	
Box Cutter	✓	
P200 Multichannel Pipette	✓	✓
P200 Single Channel Pipette	✓	✓
P1000 Single Channel Pipette	✓	✓
P20 Single Channel Pipette	✓	✓
Pipette Tips	✓	✓
Microcentrifuge	✓	✓
Programmable Thermomixer	✓	✓
Vortex Mixer (adjustable speed)	✓	✓
15 mL centrifuge tube		✓

User Provided Laptop

Laptop or PC for RingCentral Webconference

1. Operating System
   - Windows 10
2. Connecting Port Type
   - USB2.0, or 3.0 High Speed
3. Network
   - Internet connection required
4. Audio
   - Must have functioning speakers and microphone. Use of a headset is not recommended but can be used if it provides enough flexibility to manipulate the instrument and perform basic laboratory tasks.
5. RingCentral App
   - Install RingCentral app: https://app.ringcentral.com/unified-login

⚠ Please **test the compatibility of your laptop or PC** with RingCentral prior to your scheduled installation and training

Equipment provided by the user for the Purigen Install

Materials for Purigen Ionic Extraction:

Citation: jnesvet (01/19/2021). Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE_Hudson Alpha. https://dx.doi.org/10.17504/protocols.io.brixm4fn

Kit Contents

Table1: Contents provided in the Viral RNA Kit

Container	Item Name	Description	Volume	Quantity per Kit
-20ºC Reagents Box	Lysis 1	Lysis Buffer 1	1.4 mL	6
-20ºC Reagents Box	Proteinase K	Proteinase K Reagent	1.0 mL	1
RT Reagents Box	Lysis 2	Lysis Buffer 2	4.0 mL	1
RT Reagents Box	1 - Extraction Buffer	Extraction Buffer	12.0 mL	1
RT Reagents Box	2 - Anodic Buffer	Anodic Buffer	12.0 mL	1
RT Reagents Box	3 - Separation Buffer	Separation Buffer	2.5 mL	6
RT Reagents Box	4 - Neutralization Buffer	Neutralization Buffer	12.0 mL	1
RT Reagents Box	5 - Cathodic Buffer	Cathodic Buffer	12.0 mL	1
Fluidic Chip Set Box	Purigen Ionic Fluidic Chip	Fluidic Chips	N/A	6

Table 2: Reagents Provided by the User

User-Supplied Reagents	For Lysate Preparation	For Purification
Viral Samples in PBS, Saliva or Water	✓	

Table 3: Equipment Provided by the User

User-Supplied Equipment	For Lysate Preparation	For Purification
12-column Reservoir (Agilent 204365-100)		✓
P200 Multichannel Pipette		✓
P200 Single Channel Pipette	✓	
P1000 Single Channel Pipette	✓	✓
P20 Single Channel Pipette		✓
Microcentrifuge	✓	
Programmable ThermoMixer	✓	
Vortex Mixer (adjustable speed)	✓	✓
15 mL centrifuge tube (or larger if preparing for more than 6 chips)	✓	

Table 4: Labware Provided by the User

User-Supplied Equipment	For Lysate Preparation	For Extract Collection
DNA LoBind Tube, 1.5 mL (Eppendorf 22431021)	✓	✓
Optional DNA LoBind Tube, 2.0 mL (Eppendorf 22431048)	✓	
Optional DNA LoBind Plate, 96 well (Eppendorf 951032000)		✓

FOR RESEARCH USE ONLY AND NOT FOR USE IN DIAGNOSTIC PROCEDURES 5

Kit contents and Equipment provided by the user for the Purigen Extraction

Materials list for LAMP and CRISPR reaction steps:

Citation: jnesvet (01/19/2021). Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE_Hudson Alpha. https://dx.doi.org/10.17504/protocols.io.brixm4fn

A	B	C
Reagent or Consumable	**Supplier**	**Catalog #**
10x isothermal amplification buffer	New England Biolabs	B0537S
25x primers	Custom	N/A
WarmStart Bst2.0 DNA Polymerase	New England Biolabs	M0538L
WarmStart RTx Reverse Transcriptase	New England Biolabs	M0380L
CRISPR RNP mix	Custom	N/A
CRISPR Reporters	Custom	N/A
LAMP Positive control (PC)	Custom	N/A
Nuclease free water	Thermo Fisher	10977015
MgSO4 Solution	New England Biolabs	B1003S
dNTP Solution Mix	New England Biolabs	N0447L

Equipment and consumables:

- Pipette (P10, P20 and P200)
- Pipette tips (10 µL, 20 µL and 200 µL)
- 8-tube strips or 96 well plates
- 1.5 mL Eppendorf tubes
- Thermal cycler or Plate reader (any company's product capable of fluorescence readout)

Primers and guide RNA sequences used in this assay:

A	B
LAMP primer	**Sequence (5'-3')**
E-gene F3	CCG ACG ACG ACT ACT AGC
E-gene B3	AGA GTA AAC GTA AAA AGA AGG TT
E-gene FIP	ACC TGT CTC TTC CGA AAC GAA TTT GTA AGC ACA AGC TGA TG
E-gene BIP	CTA GCC ATC CTT ACT GCG CTA CTC ACG TTA ACA ATA TTG CA
E-gene LF	TCG ATT GTG TGC GTA CTG C
E-gene LB	TGA GTA CAT AAG TTC GTA C
Guide RNA and reporter sequences	Sequence (5'-3')
E gene guide RNA	UAA UUU CUA CUA AGU GUA GAU GUG GUA UUC UUG CUA GUU AC
ssDNA reporter	/56-FAM/TTATT/3IABkFQ/

Note that the LAMP primers and reporters are already part of the "**Materials list for LAMP and CRISPR reaction steps**", and the sequences here are provided only for reference

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BEFORE STARTING

Wear appropriate PPE including gloves, lab coat, goggles.
Raw NP swab samples must be handled according to BSL-2 safety level or higher.

Preparation for Purigen Ionic Instrument

1 ⚠

Prepare your laboratory for the receipt of the Purigen Ionic Instrument.

Ionic™ Purification System Site Prep Guide - Version 3.0.pdf

NOTE: When handling nucleic acids and amplicon, it is critical to reduce contamination. Therefore, 3 separate preparation areas are required for this protocol: 1) a "patient sample preparation area" for viral RNA extraction with the Purigen Ionic Instrument and dispensing sample into LAMP reaction 8-strip tubes, 96, or 384 well plates, 2) a "LAMP reagent preparation area" for preparing the LAMP mastermix and dispensing it into 8-strip tubes, 96, or 384 well plates prior to viral RNA addition, and 3) a "CRISPR/amplicon preparation area" for preparing the CRISPR reaction mix and performing the CRISPR reactions.

To aid in reducing contamination, spray surfaces, vortex, microcentrifuge, pipettes, and all reagent tubes with RNase Away, or 10% bleach and RNase Zap prior to use. Each of the three preparation areas should have dedicated pipettes, tips, etc. to reduce cross-contamination. Change gloves often and when necessary.

2 Complete Purigen Ionic installation and training with Purigen's Field Application Support.

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Ionic System Virtual Installation and Training Guide 12202020.pdf

During the training run with the Field Application Support, you will process positive and negative control samples. The eluates can be stored at -80 °C for future downstream processing with LAMP and CRISPR, or stored on ice for same day downstream LAMP and CRISPR processing to ensure the positive and negative controls are validated.

Viral RNA Extraction from Patient Samples

3 Purify viral RNA using the Purigen Ionic Viral RNA kit.

Ionic Viral RNA Kit User Guide_20210117.pdf

This is a safe stopping point. If you are not proceeding directly to LAMP/CRISPR, store the resulting viral RNA samples on ice for same-day use, at -80 °C for long-terms storage.

Program thermal cycler for LAMP 30m

4 30m

LAMP can be performed on any thermal cycler. For the QuantStudio instruments, follow below instructions.

Open a new template. Select "Presence/Absence" then "Presence Absence Post."

Program the thermal cycler to hold 62 °C for 00:30:00 . Save the protocol as "Stanford Purigen LAMP protocol" for future use.

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4.1 If applicable, set the lid heater to a value greater than **62 °C** . We recommend **105 °C** .

4.2 No fluorescence readouts are required for the LAMP amplification step. Adjust the program accordingly, if necessary.

Preparation of LAMP Mastermix

5 ⚠

NOTE: To reduce cross-contamination, prepare the LAMP mastermix and dispense in 8-tube strips, 96, or 384 well plates in an area separate from the patient sample preparation area and the CRISPR/amplicon preparation area.

Decontaminate LAMP reagent preparation area with RNase Away or 10% bleach plus RNase Zap spray, including all surfaces and pipettes. Thaw all reagents on ice.

6 After thawing, briefly vortex 10x isothermal amplification buffer, 25x primers, dNTPs, and MgSO4 while inverting Bst2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase to mix. Collect reagents at the bottom of the tubes with a brief centrifuge on benchtop microcentrifuge. Keep all reagents on ice, decontaminate all reagent tubes prior to use.

7 Determine number of reactions (*N*) to prepare for each batch of LAMP amplifications. Each patient sample should have duplicate LAMP reactions performed. Additionally, be sure to include one LAMP Positive Control (PC) and one No Template Control (NTC) in each batch. Mastermix should be prepared in slight excess to account for pipette error, therefore the total number of reactions (*N*) = (2.2 x [# patient samples]) + 1 PC + 1 NTC. So for example, if you were to amplify all 8 patient samples from a single chip, *N* = (2.2 x 8) + 1PC + 1 NTC = 19.6

7.1 Calculate the volume of each reagent required for N total reactions according to the table below.

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https://dx.doi.org/10.17504/protocols.io.brixm4fn

Reagent	Mastermix Reagent Volume
10x Isothermal Amplification Buffer	N x 2.5 uL
25x primers	N x 1.0 uL
WarmStart Bst2.0 DNA Polymerase	N x 1.0 uL
WarmStart RTx Reverse Transcriptase	N x 0.5 uL
dNTP mix	N x 3.5 uL
MgSO4	N x 1.5 uL
Total Mastermix Volume	N x 10 uL

8 Organize the 8-tube strips, 96, or 384 well plate for the given *N* number of reactions. As an example, Patient Samples 1-8 (P1-P8) duplicates plus a PC and NTC are shown below. Please scale accordingly; note for 384 well plates use every other row and column to reduce cross-contamination between samples and isolate NTC and PC wells. Label the strips with the corresponding patient sample number.

A	B	C	D	E	F	G	H	I	J	K	L	M
	1	2	3	4	5	6	7	8	9	10	11	12
A	P1 A		P1 B									NTC
B												
C	P2 A		P2 B									
D												
E	P3 A		P3 B									
F												
G	P4 A		P4 B									
H												
I	P5 A		P5 B									
J												
K	P6 A		P6 B									
L												
M	P7 A		P7 B									
N												
O	P8 A		P8 B									
P												PC

*Note that because no fluorescent data is collected in this step, the top of the tube can be labeled if using 8-tube strips.

9 Dispense mastermix reagents in a clean 1.5 mL microcentrifuge tube. Vortex to thoroughly mix mastermix. Briefly centrifuge to collect reagents at bottom of tube.

10 Set up 8-tube strips, 96, or 384 well plate over ice or cold rack.

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10.1 Dispense **10 µl** of mastermix into each tube or well.

11 Prepare NTC reactions in reagent preparation area. Decontaminate Nuclease Free Water with RNase Away or RNase Zap, then pipette **15 µl** into appropriately labeled tubes and securely cap them, if applicable. Additionally, dispense **13 µl** of Nuclease Free Water into the Positive Control well. Now, you can move the strips loaded with the mastermix to the patient sample preparation area.

Patient Sample Template Addition

12 If sample extracts are stored in individual Lo-Bind microcentrifuge tubes, vortex prior to dispensing extract into LAMP reaction. If extracts were stored in 8-tube strips or 96-well plates, mix by gently pipetting up and down prior to dispensing extract into LAMP reaction. Centrifuge to collect contents at bottom of the tube. Keep samples in cold rack or on ice.

13 Carefully dispense **15 µl** of patient sample eluates into the corresponding wells containing the **10 µl** of mastermix . Pipette the sample up and down 5 times to mix, *DO NOT VORTEX.* Change tips after each addition to reduce contamination

14 Carefully dispense **2 µl** of the LAMP positive control (LAMP Pos Ctrl) to appropriate tube or well.

15 Securely cap or seal wells after addition, briefly centrifuge to collect samples at bottom.

Perform 30 Minute LAMP Reaction on Thermal Cycler 30m

16 Load "Stanford Purigen LAMP protocol" on thermal cycler. Add sealed tubes or well plate to thermal cycler and perform LAMP for **00:30:00** . 30m

17 After LAMP reaction is complete, briefly centrifuge tubes or well plate and keep on ice in CRISPR/amplicon handling area.

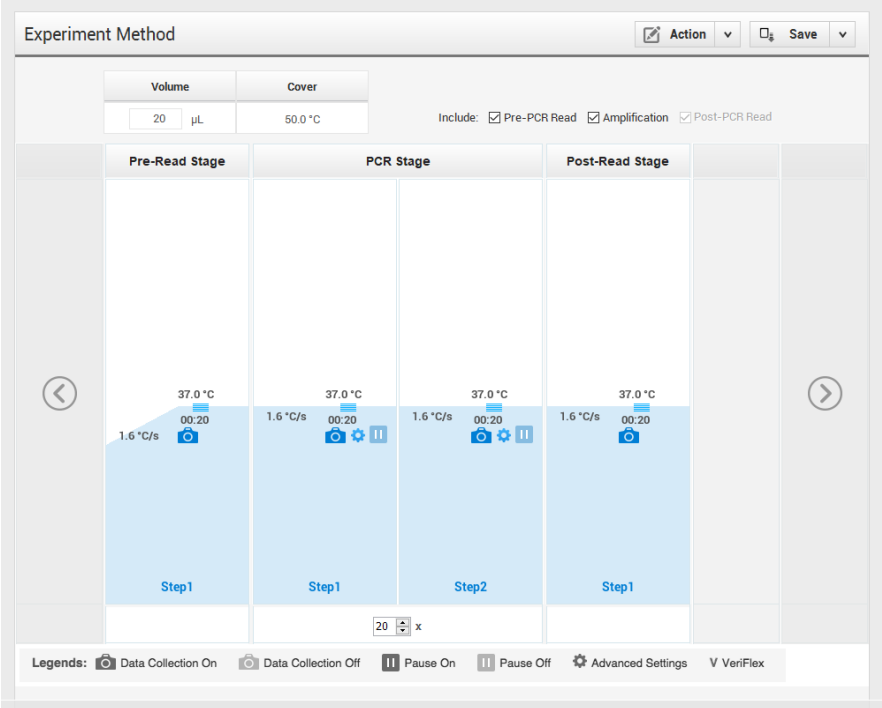
Program thermal cycler for CRISPR Reaction 30m

18 Program the QuantStudio thermal cycler for the CRISPR reaction. Open a new template and select "Presence/Absence"

Citation: jnesvet (01/19/2021). Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE_Hudson Alpha. https://dx.doi.org/10.17504/protocols.io.brixm4fn

Ensure Pre-PCR Read and Post-PCR Read are selected.

Set cover to **50 °C** . Save experiment method as "Stanford Purigen CRISPR protocol."



Note the reporters are FAM labeled, so ensure the reading is either in the FAM or SYBR green fluorescent channel.

Citation: jnesvet (01/19/2021). Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE_Hudson Alpha. https://dx.doi.org/10.17504/protocols.io.brixm4fn

Preparation of CRISPR Reaction Mix

19 Using the same number of *N* determined for LAMP reactions in Step 7, prepare the CRISPR reaction mix

Note that because tubes containing amplicon will be handled, all steps in this section should be performed in an area dedicated for amplicon handling, separate from both the reagent preparation and patient sample preparation area. Decontaminate surfaces and pipettes with RNase Away or 10% bleach RNase Zap.

Note that the CRISPR reaction proceeds immediately after LAMP amplicons are added. Therefore, this reaction is very time sensitive and the order of reagent addition is extremely important. Because of the time sensitive nature, each laboratory personnel should perform no more than 48 CRISPR reactions (6 columns) per run.

20 Calculate the volume of each reagent required for N total reactions according to the table below.

Reagent	CRISPR Reaction Mix Volume
RNP mix	N x 17 uL
reporters	N x 1 uL
Total volume	N x 18 uL

21 Organize clean 8-tube strips, 96, or 384 well plate for the given *N* number of reactions in the same format as in Step 8 for the CRISPR reaction.

Load this plate format on the QuantStudio touch screen. The patient samples should be classified as "Unknown" under the sample type, while the NTC and LAMP Positive Control are "Negative Control" and "Positive Control," respectively.

A	B	C	D	E	F	G	H	I	J	K	L	M
	1	2	3	4	5	6	7	8	9	10	11	12
A	P1 A		P1 B									NTC
B												
C	P2 A		P2 B									
D												
E	P3 A		P3 B									
F												
G	P4 A		P4 B									
H												
I	P5 A		P5 B									
J												
K	P6 A		P6 B									
L												
M	P7 A		P7 B									
N												
O	P8 A		P8 B									
P												PC

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Note that fluorescent measurements will be taken during CRISPR reaction, so do not label the top of the tubes or wells.

22 Dispense reagents in a clean 1.5 mL microcentrifuge tube to prepare CRISPR reaction mix. Vortex to thoroughly mix. Briefly centrifuge to collect reagents at bottom of tube, then keep on ice.

23 ⚠

Add **2 µl** from LAMP reaction tube into the new corresponding tube or 96 well plate. Store the remaining solution in the freezer.

Note that the amplicons must be added ***BEFORE*** the CRISPR reaction mix to ensure speedy pipetting in the following step.

If using a sealed well plate, ensure to carefully remove the seal to avoid sample cross-contamination.

24 ⚠

Quickly add **18 µl** of the CRISPR reaction mix to each tube, changing pipette tips after each addition. It is important to add the CRISPR reaction mix to the PC and NTC tubes prior to the patient samples to ensure the timing is accurate.

Note: do not pipette-mix or vortex for this step. At most, 48 CRISPR reactions should be performed at a time, and it should take approximately 3 minutes to fully dispense the CRISPR reaction mix into the 48 individual tubes.

25 Load the "Stanford Purigen CRISPR protocol" on the thermal cycler. Add tube strips or well plate and run CRISPR reaction for 15 minutes.

CRISPR Data Analysis - Threshold Calibration

26 Data analysis of the CRISPR data will be a simple presence/absence call. However, it is necessary to determine the fluorescence threshold for presence/absence on each unique QuantStudio instrument. Therefore, calibration of the fluorescence threshold will be necessary during the first run on each QuantStudio instrument, which will occur during the Purigen instrument training using known samples. Calibration of each instrument is necessary prior to processing patient samples.

If the QuantStudio has already been calibrated and the fluorescence threshold established, continue onto step 30. If the

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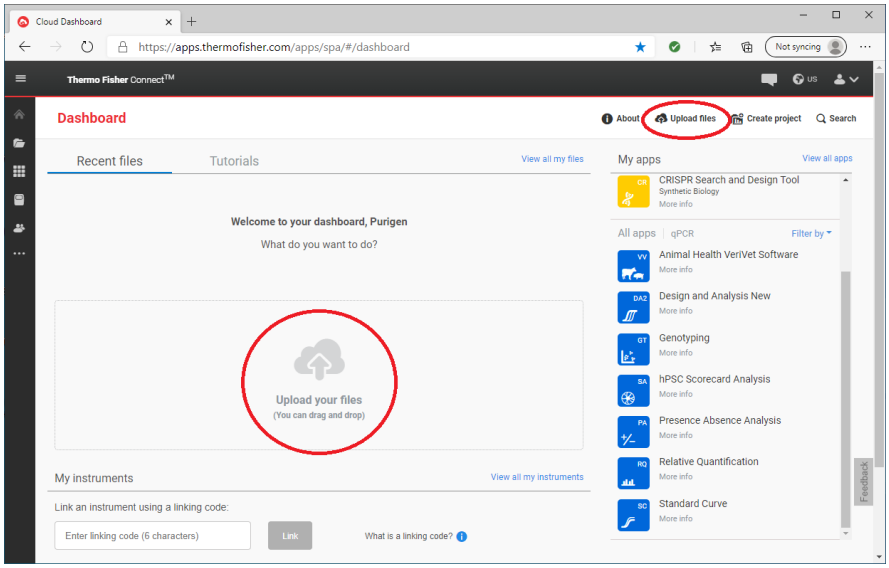
QuantStudio has not been calibrated, continue onto the next step.

27 The CRISPR fluorescent data can be analyzed on the cloud-based platform Thermo Fisher Connect.

Register for an account here: https://www.thermofisher.com/account-center/cloud-signin-identifier.html

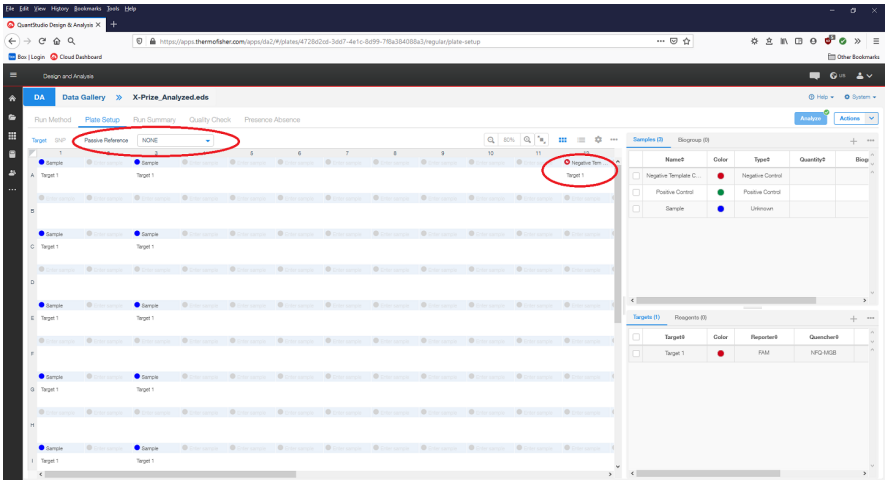
The Thermo Fisher Connect dashboard can be found here: https://apps.thermofisher.com/apps/spa/#/dashboard

Upload the .eds file from the Purigen training run to the Thermo Fisher Connect platform and open the file.



27.1

Go to the "Plate Setup" menu and change the Passive Reference to "None." Additionally, ensure that wells are correctly labeled as "Unknown," "Negative Control," or "Positive Control" according to the plate format.



27.2

Go to the "Quality Check" menu. The Amplification Plot should be displayed, as shown below.

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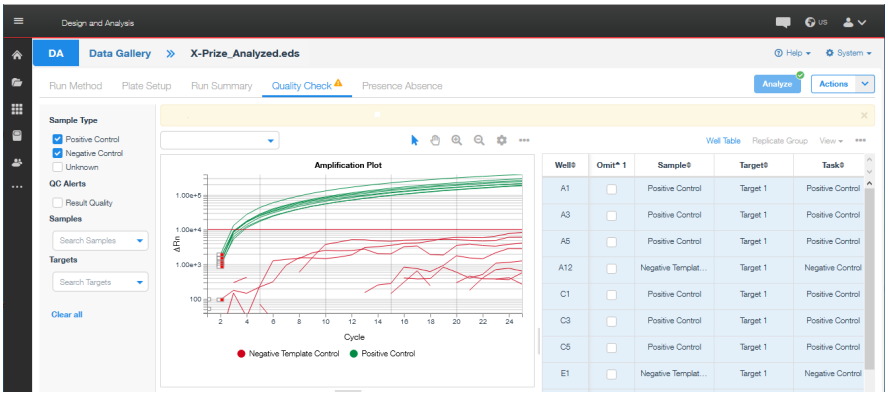
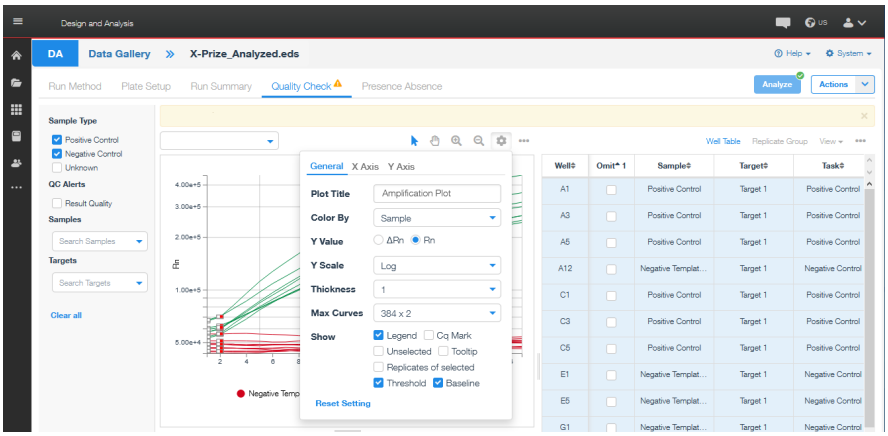
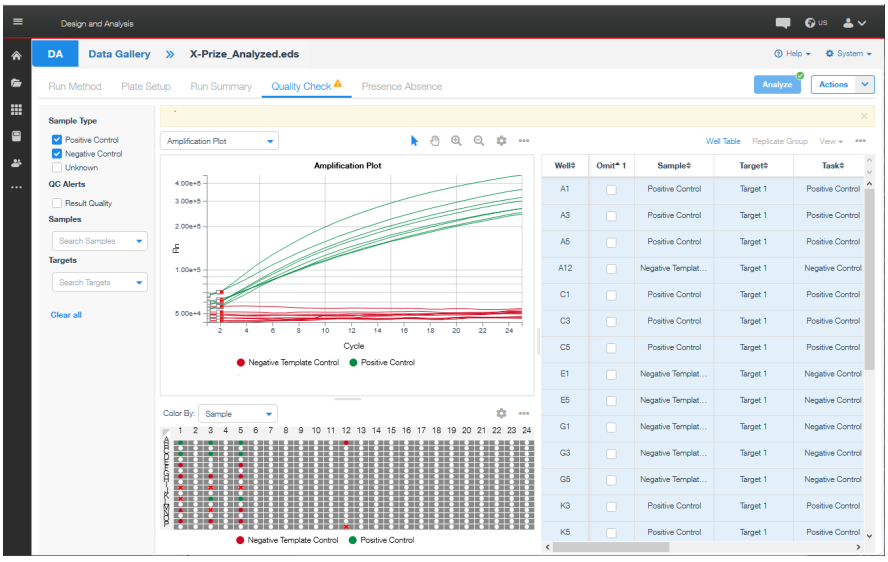


27.3 Go to the Settings menu, and change Y value from ΔRn to Rn.

Ensure the CRISPR fluorescence data qualitatively passes quality control, the Purigen Negative Controls should show no CRISPR fluorescent signal, and while the Purigen Positive Controls should show an increase in CRISPR fluorescent signal, as shown below. Note any negative controls that show an increase in CRISPR fluorescence signal and exclude them in analysis for calculating threshold.





Quality Check of the CRISPR amplification. The positive controls (green) should qualitatively show amplification, whereas the negative controls (red) should not.

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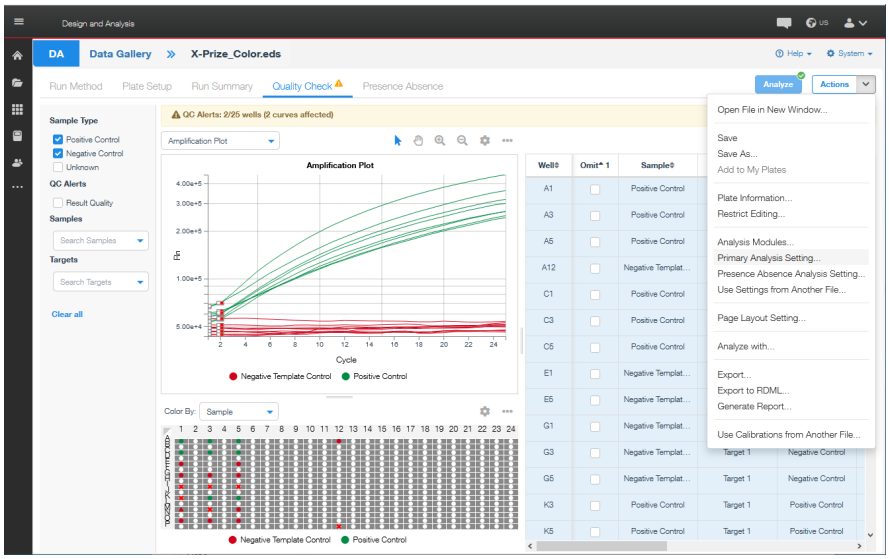
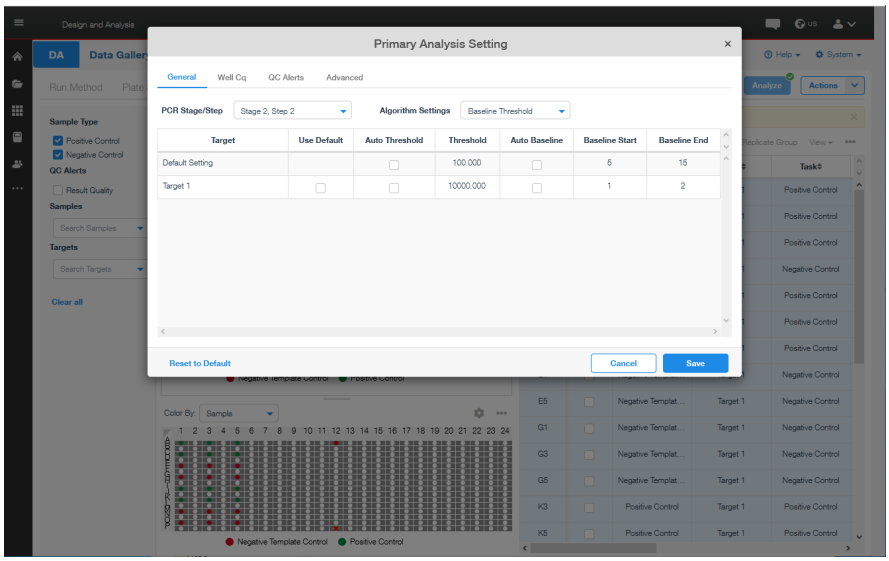
28 Select the "Actions" dropdown menu in the top right of the screen and select "Primary Analysis Setting." Set "Algorithm Settings" to "Baseline Threshold." Change the Baseline Start to 1 and the Baseline End to 2. Click "Save."





29 To calculate the fluorescence threshold, go to the "Presence Absence" menu. Click "..." and choose Export. This will create and download a csv file, which should contain the ΔRn for each well.

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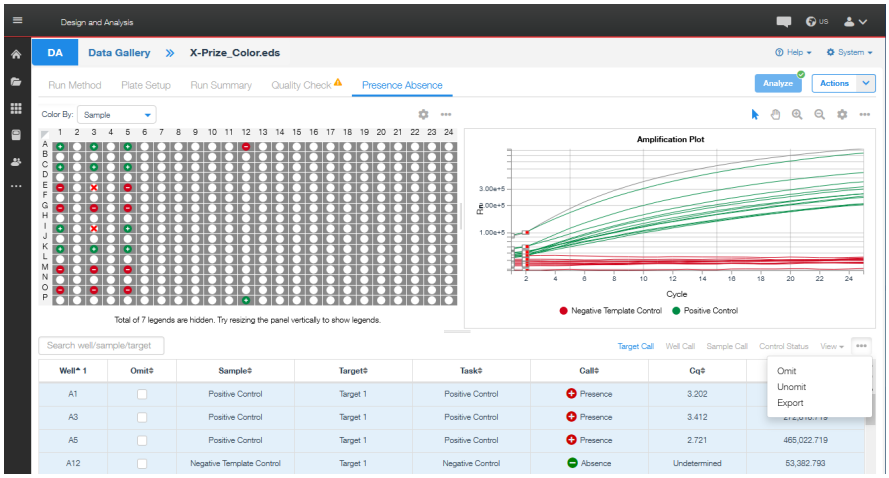
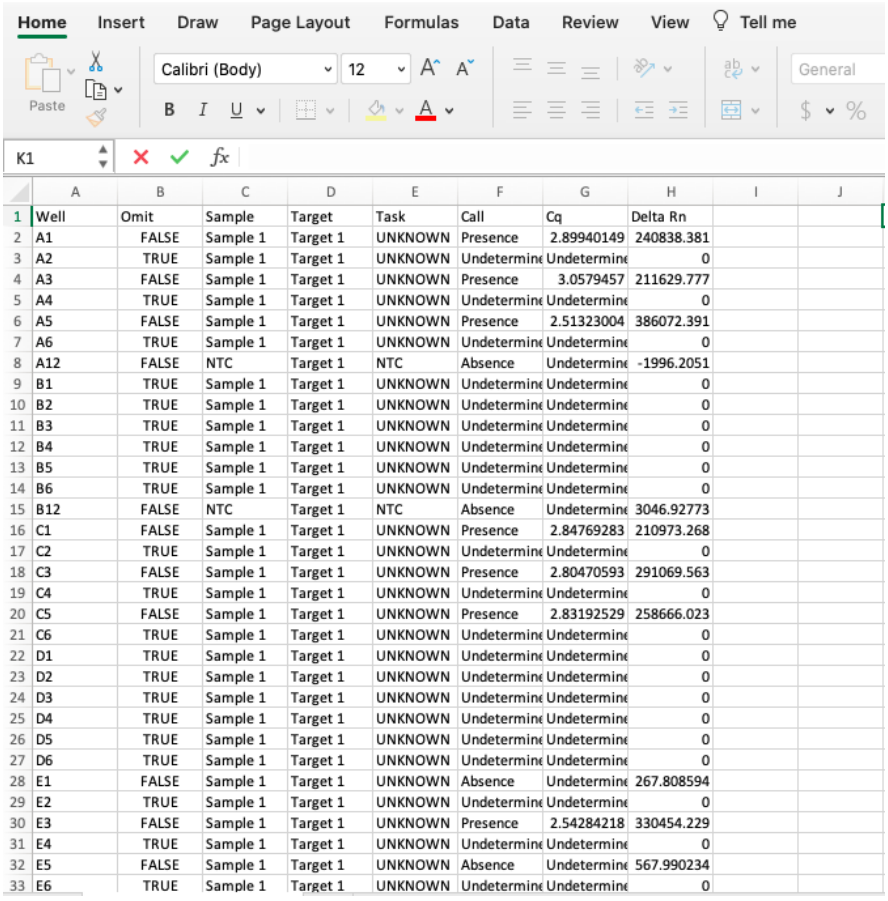


29.1

Identify the ΔRn for each Purigen Negative Control (8 samples) sample and the No Template Control (1 sample). Calculate the average ΔRn of these 9 samples and the standard deviation (σ). The threshold is equal to:

$$Threshold = avg\Delta Rn + 2\sigma$$



	A	B	C	D	E	F	G	H	I	J
1	Well	Omit	Sample	Target	Task	Call	Cq	Delta Rn		
2	A1	FALSE	Sample 1	Target 1	UNKNOWN	Presence	2.89940149	240838.381		
3	A2	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
4	A3	FALSE	Sample 1	Target 1	UNKNOWN	Presence	3.0579457	211629.777		
5	A4	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
6	A5	FALSE	Sample 1	Target 1	UNKNOWN	Presence	2.51323004	386072.391		
7	A6	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
8	A12	FALSE	NTC	Target 1	NTC	Absence	Undetermine	-1996.2051		
9	B1	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
10	B2	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
11	B3	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
12	B4	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
13	B5	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
14	B6	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
15	B12	FALSE	NTC	Target 1	NTC	Absence	Undetermine	3046.92773		
16	C1	FALSE	Sample 1	Target 1	UNKNOWN	Presence	2.84769283	210973.268		
17	C2	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
18	C3	FALSE	Sample 1	Target 1	UNKNOWN	Presence	2.80470593	291069.563		
19	C4	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
20	C5	FALSE	Sample 1	Target 1	UNKNOWN	Presence	2.83192529	258666.023		
21	C6	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
22	D1	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
23	D2	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
24	D3	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
25	D4	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
26	D5	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
27	D6	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
28	E1	FALSE	Sample 1	Target 1	UNKNOWN	Absence	Undetermine	267.808594		
29	E2	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
30	E3	FALSE	Sample 1	Target 1	UNKNOWN	Presence	2.54284218	330454.229		
31	E4	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		
32	E5	FALSE	Sample 1	Target 1	UNKNOWN	Absence	Undetermine	567.990234		
33	E6	TRUE	Sample 1	Target 1	UNKNOWN	Undetermine	Undetermine	0		

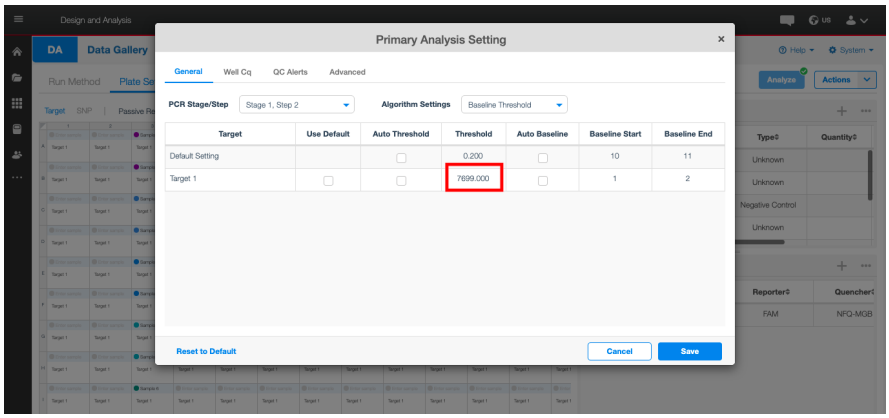
Citation: jnesvet (01/19/2021). Stanford and Purigen Biosystems Microfluidics Team protocol XPRIZE_Hudson Alpha. https://dx.doi.org/10.17504/protocols.io.brixm4fn

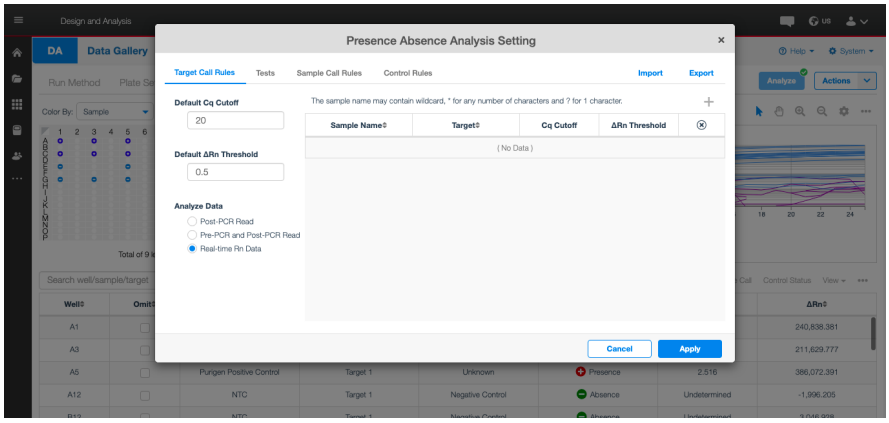
Record the value of the threshold, as it will be the same for all subsequent runs on this QuantStudio instrument.

CRISPR Data Analysis

30 Return to the "Primary Analysis Setting" from the "Actions" dropdown menu and enter the threshold unique to the QuantStudio, then click "Save."

In the "Actions" dropdown menu, go to the "Presence Absence Analysis Setting," set the Default Cq Cutoff to 20 and select Real-time Rn Data, then click "Apply."





31 Click "Analyze." The sample wells should now show a call of either presence or absence.

Export the data as a csv, which will contain the well, presence/absence call, and ΔRn. Results can be analyzed on the exported csv, or they can be analyzed on the cloud platform.

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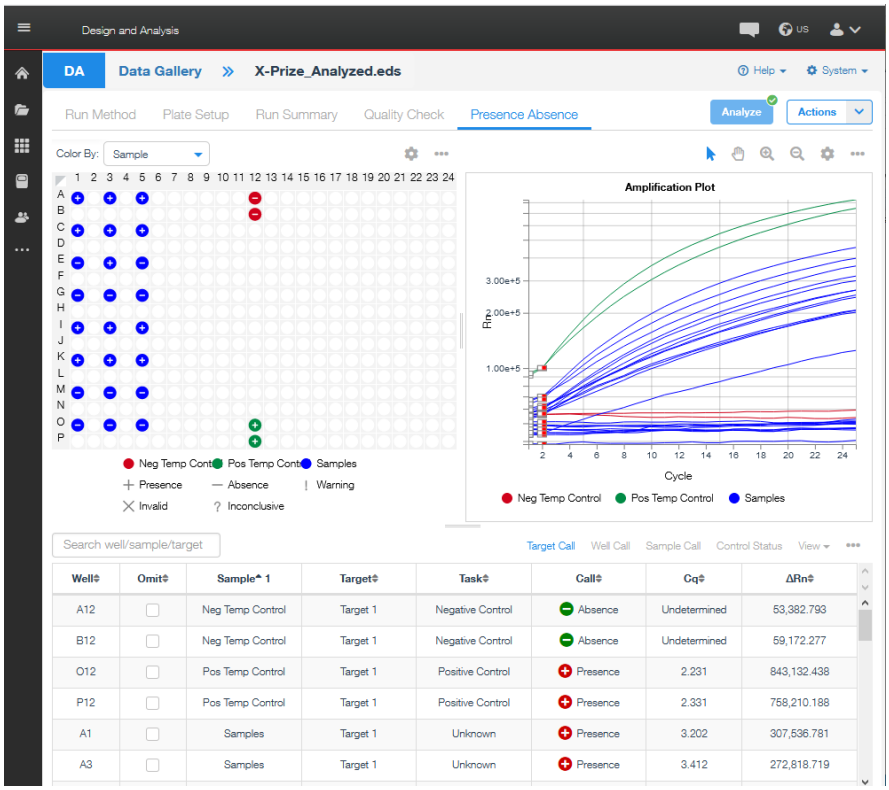

Well	Omit	Sample 1	Target	Task	Call	Cq	ΔRn
A12		Neg Temp Control	Target 1	Negative Control	Absence	Undetermined	53,382.793
B12		Neg Temp Control	Target 1	Negative Control	Absence	Undetermined	59,172.277
O12		Pos Temp Control	Target 1	Positive Control	Presence	2.231	843,132.438
P12		Pos Temp Control	Target 1	Positive Control	Presence	2.331	758,210.188
A1		Samples	Target 1	Unknown	Presence	3.202	307,536.781
A3		Samples	Target 1	Unknown	Presence	3.412	272,818.719

Note: During the Purigen training run, ensure that the threshold produces the correct presence/absence assignments to the respective positive and negative samples.

Interpretation of Results

32 Ensure that the NTC sample is classified as "absence" for every run. If the NTC sample does appear positive, this is likely due to contamination from the LAMP reaction setup and the results are invalid. If possible, repeat the protocol from the sample extraction on remaining raw sample using the Purigen system (Step 3).

Ensure that the LAMP Positive Control sample is classified as "presence" for every run. If the sample does not appear positive, this could be due to the time sensitive nature of CRISPR, and it may have taken too long to add the CRISPR reaction mix to all 48 samples. To troubleshoot, try running another CRISPR reaction on just the LAMP PC. If it now appears positive, it likely took too long to load the CRISPR reaction mix into the 48 samples. Redo the CRISPR reaction starting from Step 23, instead doing 16 samples at a time. If this troubleshooting did not solve the issue, repeat the protocol from the sample extraction on remaining raw sample using the Purigen system (Step 3).

33 Once the results have been validated by the controls, the patient samples can be interpreted as follows:

If either 1 of 2 or 2 of 2 of the patient sample replicates is classified as "presence" this can be interpreted as positive for SARS-CoV-2.

If both patient sample replicates are classified as "absence" this can be interpreted as negative for SARS-CoV-2.

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