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GenSwab Assay (SARS-CoV-2)

Forked from GenSwab Assay (SARS-CoV-2)

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1 Works for me dx.doi.org/10.17504/protocols.io.bp8jmrun

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ABSTRACT

The GenapSys SARS-CoV-2 GenSwab Assay is a reverse transcription sequencing (RT-Seq) test. The assay uses primers and probes that were first designed by OctantBio to detect RNA from SARS-CoV-2 in respiratory and saliva specimens from patients. This is the workflow for purified RNA samples, and starts with purified samples. Our protocol will accept RNA purified from any kit.

ATTACHMENTS

QRC GenSwab Purified RNA.pdf

DOI

dx.doi.org/10.17504/protocols.io.bp8jmrun

PROTOCOL CITATION

huiyi.chen , Sid Roy 2020. GenSwab Assay (SARS-CoV-2). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp8jmrun

FORK NOTE

Purified RNA samples are now supported.

FORK FROM

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MATERIALS TEXT

MATERIALS

Tris-EDTA, pH 8.0 **Ambion Catalog #AM9849**

Agencourt AMPure XP SPRI beads **Beckman Coulter Catalog #A63881**

Qubit dsDNA HS (High sensitivity) Assays **Thermo Fisher Scientific Catalog #Q32851**

DNA LoBind Tubes, 1.5 mL **Eppendorf Catalog #0030108051**

200 Proof Ethanol pure **Sigma Aldrich Catalog #E7023**

Tween-20 **Sigma Aldrich Catalog #P9416**

Nuclease-Free Water (not DEPC-Treated) **Thermo Fisher Catalog #AM9937**

TaqPath™ 1-Step RT-qPCR Master Mix, CG **Thermo Fisher Catalog #A15299**

GenapSys will provide S2 spike-in RNA, and 10X Indexed Primers.
User should make the following stock solutions:
- 1% Tween-20 in TE (for diluting spike-in RNA)

Equipment:

Qubit 2.0 Fluorometer instrument Q33226
with Qubit RNA HS Assays

- Vortexer
- Plate mixer
- Plate centrifuge
- Thermocycler
- Set of pipettors

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The GenapSys SARS-CoV-2 GenSwab Assay is a reverse transcription sequencing (RT-Seq) test. The assay uses primers and probes that were first designed by OctantBio to detect RNA from SARS-CoV-2 in respiratory and saliva specimens from patients. This is the workflow for purified RNA samples, and starts with purified samples. Our protocol will accept RNA purified from any kit.

BEFORE STARTING

This is the protocol for purified RNA samples.

Pre-PCR: S2 Spike-in Dilution

1 Start with a stock solution of 1% Tween-20 in TE. Dilute 10x in TE to obtain 0.1% Tween-20 in TE.

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2 Retrieve S2 spike-in RNA from -80C freezer.
S2 spike-in RNA is provided at 1e10 copies/ul. Perform three 100x dilutions, followed by a 10x dilution to obtain 1000 copies/ul. Dilutions should be done in Lo-Bind DNA tubes in 0.1% Tween-20 in TE.

2.1 Add 198ul to 0.1% Tween-20 in TE to the tube containing the S2 spike-in RNA. This is the Dilution 1.

2.2 Remove 2ul of Dilution 1 to 198ul of 0.1% Tween-20 in TE. This is Dilution 2.

2.3 Remove 2ul of Dilution 2 to 198ul of 0.1% Tween-20 in TE. This is Dilution 3.

2.4 Make a final 10x dilution by adding 10ul of Dilution 3 to 90ul of 0.1% Tween-20 in TE. Keep on ice until use.

Pre-PCR: Setting up RT-PCR

3

Component	Final Conc	1x reaction	____ x reactions
TaqPath 1-Step RT-qPCR Master Mix, CG (4x)	1x	5 ul	
S2 Spike-In RNA (1000/ul)	500 copies	0.5 ul	
Water		7.5 ul	
10x Indexed Primer Mix	1x	2 ul	
Sample		5 ul	
Total Volume		20 ul	

Each batch of reactions should be accompanied by 1 positive control and 1 no-template control, with a minimum of 1 set of controls per 96-well plate.

4 To the 2 ul of 10x Indexed Primer Mix in each well, add 5 ul of the sample (purified RNA).

5 Incubate the primer and sample mix at **65 °C** for 5 min, then place on ice for at least 2 min.

6 While the primer and sample mix incubates on ice, prepare the mastermix. The mastermix comprises the reagents in blue. Combine the TaqPath Master Mix, diluted S2 spike-in RNA and water in the necessary quantities, and mix well by pipetting.

7 Dispense 13 ul of MasterMix into each well.

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8 Seal the plate. Spin down the plate at 1000xg for 1 min to collect the contents, then mix using a plate mixer at 2000 rpm for 2 min (96-well plate). Load into thermocycler.

9 Run the following PCR program:

# of cycles	Temperature	Duration
1	25C	2 min
1	50C	15 min
1	95C	2 min
40	95C	10 sec
	60C	30 sec
1	12C	hold

Post-PCR: Bead Clean Up

10 Pool the samples.
Remove 5ul from each reaction to be combined to a fresh tube or sterile reservoir (if using a multi-channel pipettor).

11 Transfer the reaction mix from the reservoir to a tube of appropriate size.
Vortex to mix.
Transfer 100ul to a fresh Lo-Bind 1.5ml tube.

12 Clean up using a dual-sided purification.
Add **50 µl** of AmpureXP beads. Vortex to mix, and incubate at room temperature for **00:05:00** .

13 Use a magnetic rack to collect the beads for at least 1 minute.

14 Transfer supernatant to a new tube.
Add **130 µl** of AmpureXP beads.
Vortex and incubate at room temperature for **00:05:00** .

15 Use magnetic rack to collect the beads for at least 1 minute.

16 Remove and discard supernatant.

17 Wash the beads twice with **500 µl** of freshly made 80% ethanol.

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18 After the final wash, leave the lid open for 3 minutes to let the residual ethanol evaporate.

19 Elute in **40 µl** of TE buffer. Vortex to mix and incubate for 2 minutes. Spin down briefly to collect.

20 Use magnetic rack to collect the beads for at least 1 minute. Collect 35ul of the eluate.

Post-PCR: Quantification of library

21 Dilute the eluted library 1:10 in TE buffer: add 5ul of library to 45ul TE buffer.

22 Quantify the 1:10 dilution using the High Sensitivity DNA Qubit kit.

23 Convert the Qubit measurement to molarity assuming a 120bp amplicon.
For convenience, an online tool like NEB's NEBioCalculator can provide the conversion.

23.1 Optional: qPCR measurement of the library would provide a more accurate concentration, and is recommended for initial tests. Subsequent tests can extrapolate from the conversion between Qubit and qPCR measurements.

24 Dilute the eluted library with TE to 33pM in Lo-bind tubes.

25 Proceed to clonal amplification. The suggested starting point for clonal amplification is 4ul of the 33pM library.

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