Implementation of the Promega GoTaq® Enviro Wastewater SARS-CoV-2 Assay on the Analytik Jena qTOWER³

Abstract

Detection of molecular targets from wastewater is challenging due to the high dilution of the target(s) and the potential presence of PCR inhibitors. It requires nucleic acid enrichment (e.g., by filtration), and magnetic bead-based nucleic acid purification to remove inhibitors. This measuring protocol verifies the general functionality of the Promega AM2100 kit on Analytik Jena's qTOWER³ RT-qPCR devices and presents the main qPCR parameters required for successful operation.

At a Glance

Implementation and technical proof of functionality of the Promega GoTaq® Enviro Wastewater SARS-CoV-2 Assay (AM2100) on the Analytik Jena qTOWER³.

This functional verification is also applicable for AM2110, AM2120, and AM2130.

Introduction

Wastewater based epidemiology (WBE) has been increasingly considered as a useful tool for monitoring local disease outbreaks. The most prominent example is the monitoring of SARS-CoV-2 concentrations within wastewater. As the virus is cleared from an infected individual, viral particles are excreted through solid fecal matter that is deposited in untreated wastewater. These noninfectious viral particles contain both intact and partially disintegrated RNA from the SARS-CoV-2 virus. Utilizing traditional qPCR molecular techniques, that RNA can be quantified and tracked overtime. As the amount of viral RNA increases, the number of infections within a population has increased. The same is true for the decrease of viral RNA detected. Tracking the concentration of viral RNA in wastewater thus can be used to monitor the status of outbreaks and how a certain pathogen is moving through a population.

The Promega GoTaq® Enviro Wastewater SARS-CoV-2 assay is a real-time PCR kit designed to detect the quantity of viral RNA in wastewater samples and is designed specifically to address this public health need. The three channel multiplex assay measures one of three SARS-CoV-2 targets (N1, N2 or E), an internal amplification control (IAC) and Pepper Mild Mottle Virus (PMMoV) used as a process control. To showcase the capability of the assay, it was run using the Analytik Jena qTOWER^3 real-time PCR system. Three separate assay mixed were created (N1, N2 & E) assay groups to showcase all three assays in a single run. Two wastewater samples were collected and run in all three assays to showcase robust detection in all targets.

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Materials and Methods

Materials required

- GoTaq® Enviro Wastewater SARS-CoV-2 kit w/included technical manual (Part# AM2100, Promega)
- qTOWER³ G real-time PCR instrument configured with color modules 1, 2, 4 and 5 (Part# 844-00554-2, Analytik Jena)
- 96-well full-skirted PCR plate, volume 0.2 mL, low profile, white (Part# 844-70038-S, Analytik Jena)
- Optical sealing foil for qPCR applications (Part# 844-70045-0, Analytik Jena)

A color compensation profile was measured in order to ensure fluorescent crosstalk is properly controlled for. This compensation data file (compensation.cmp) needs to be installed in qPCRsoft prior to running this assay. This compensation is available upon request from Analytik Jena along with instructions for installation.

Protocol

1. Thaw RNA standards according to the Promega technical manual.
2. SARS-CoV-2 (4 x 10^6 copies/µL) and PMMoV (4 x 10^6 copies/µL) RNA standards were combined and diluted 100-fold for a final concentration of 4 x 10^4 copies/µL. 10-fold serial dilutions were subsequently performed to create the standard concentrations below. Detailed instructions are provided in the Promega technical manual (TM661).

Table 1. Overview of standard concentrations for the generation of standard curves. Table reproduced from the assay technical manual.

Standard	RNA (copies/µL)	Copies/Well (5 µL sample/20 µL reaction)
A	4 x 10^4	2 x 10^5
B	4 x 10^3	2 x 10^4
C	4 x 10^2	2 x 10^3
D	40	2 x 10^2
E	4	20

Three separate master mixes were prepared (N1, N2 & E) and plated onto a single 96-well PCR plate. The plate layout prepared is summarized below in figure 1. All samples were run in triplicate.

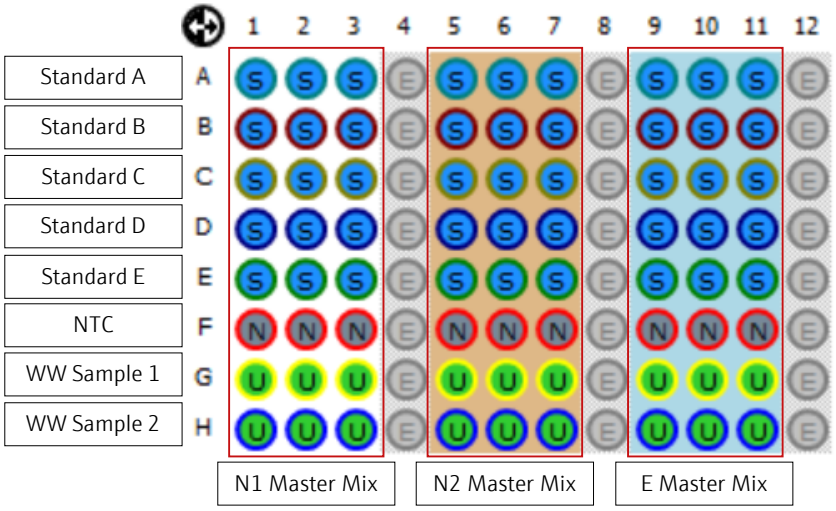

Figure 1. PCR plate map combining all three available assays and with two WW samples. All reactions were performed in triplicate.

3. After reagent preparation, 5µL of each sample was added to 15µL of master mix in an Analytik Jena white PCR plate. The plate was sealed using Analytik Jena optical sealing film then spun down at 2000 rpm for 1 min using a plate centrifuge.

4. The prepared PCR plate was placed on the qTOWER^3 and run with the following instrument settings. A template was created to store instrument settings for future runs.

Lid temp. °C: 100 Preheat lid Device: qTOWER³G

4 steps		scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
40x[	1		45.0	15:00	--	---	--.-	---	8.0
	2		95.0	02:00	--	---	--.-	---	8.0
	3		95.0	00:15	--	---	--.-	---	6.0
	4	◆	62.0	01:00	3	39	--.-	---	6.0

Figure 2a. PCR cycling conditions used on the qTOWER^{3}G

Pos.	Channel	Dye	Gain	Measurement	Pass. Ref.
1	Blue	FAM	3	◆	
2	Green	JOE	3	◆	
3	Yellow	TAMRA	5		
4	Orange	ROX	5	◆	✔
5	Red	Cy5	5	◆	
6	NIR1	Cy5.5	5		

Meas. repeats: 3 Color compensation: GoTaq Enviro Wast

Figure 2b. Optical filter sets selected to collect fluorescent data. The qTOWER³ allows for up to six fluorophores to be scanned simultaneously. Passive reference was selected for this run; however, it is not required on the qTOWER³G.

Results & Analysis

Analysis of standard curves and subsequent wastewater samples:

1) SARS-CoV-2, PMMoV & IAC Quality Control

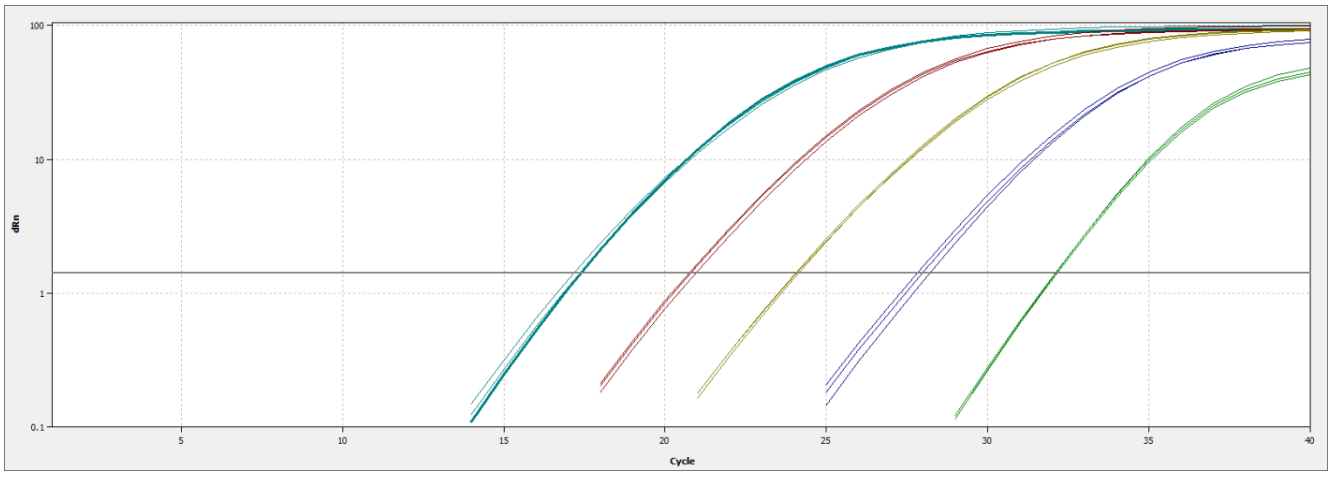

Figure 3. SARS-CoV-2 N1 assay (FAM) standard curves ordered from left to right, standard A-E. Only the N1 assay in FAM is shown above.

Table 2. Summary of N1, N2 and E reaction mixes qPCR quality control metrics. qPCR metrics were calculated using qPCRsoft and is highly recommended when analyzing run data. A run with metrics outside of the acceptable ranges decrease the integrity of the data and should not be relied upon. All QC metrics are recorded in detail in the assay technical manual.

Target	R^2	Slope	Efficiency	QC Pass
SARS-CoV-2 N1 (FAM)	0.99802	-3.68	0.87	Yes
SARS-CoV-2 N2 (FAM)	0.99754	-3.69	0.87	Yes
SARS-CoV-2 E (FAM)	0.99976	-3.45	0.95	Yes
PMMoV N1 Mix (Cy5)	0.99887	-3.66	0.88	Yes
PMMoV N2 Mix (Cy5)	0.99687	-3.62	0.89	Yes
PMMoV E Mix (Cy5)	0.99211	-3.64	0.88	Yes

Metric	Acceptance
R^2	>0.970
Slope	-3.0 to -3.7
Efficiency	86% < E < 115%

2) IAC quality control and clustering assessment

Table 3. Summary of IAC's measured in the green (JOE) channel. IAC analysis is covered in detail in section 6.B of the assay technical manual. Each sample group should be clustered together across all three multiplex assays. For brevity the Ct values of all replicates across multiplexes were averaged and displayed with a standard deviation. All IACs for each sample did amplify successfully and fit within the published Ct range and within the acceptable clustering range. A Ct value that is greater than 2 Ct values from the NTC indicates potential inhibition for that sample. For this run, all samples passed IAC QC and does not show signs of inhibition.

Sample (all assays)	Mean Ct	Standard Deviation Ct	QC Pass
NTC (N1, N2, E) all replicates	26.35	0.44	Yes
SARS-CoV-2 & PMMoV Standards	26.69	0.53	Yes
WW Sample 1	26.9	0.42	Yes
WW Sample 2	27.49	0.55	Yes

Metric	Acceptance (Ct)
Ct range for NTC's for all assays	20-30
Acceptable Ct distance from NTC	2

3) Wastewater Sample Analysis

Table 4. Wastewater sample result summary for all three SARS-CoV-2 targets (FAM). Concentration calculations are based on the average Ct of each replicate for each sample.

Well	Sample Name	Ct Value	Standard Deviation (Ct)	Concentration (copies/well)	Standard Deviation (copies/well)
G1	WW Sample 1 N1	30.05	0.46	44	13
G2	WW Sample 1 N1	30.63			
G3	WW Sample 1 N1	30.96			
G5	WW Sample 1 N2	30.55	0.15	57	5.3
G6	WW Sample 1 N2	30.25			
G7	WW Sample 1 N2	30.33			
G9	WW Sample 1 E	30.37	0.17	45	5.0
G10	WW Sample 1 E	30.03			
G11	WW Sample 1 E	30.22			
H1	WW Sample 2 N1	32.58	0.58	16	5.7
H2	WW Sample 2 N1	31.52			
H3	WW Sample 2 N1	32.45			
H5	WW Sample 2 N2	32.70	0.26	13	2.1
H6	WW Sample 2 N2	32.61			
H7	WW Sample 2 N2	33.11			
H9	WW Sample 2 E	33.05	0.59	10	4.1
H10	WW Sample 2 E	32.32			
H11	WW Sample 2 E	31.87			

Discussion

The GoTaq® Enviro Wastewater SARS-CoV-2 provided by Promega has been successfully validated to work the Analytik Jena qTOWER^3/G Realtime PCR instrument and software. The run data above fits within the QC metrics provided by Promega in the technical manual for the assay. Users are encouraged to contact either Promega or Analytik Jena for support in adopting this assay and instrument for WBE applications. The experimental design presented here is to showcase all three multiplex assays being successfully used in a single run. Individual experimental designs may vary. Promega does not require all three assays to be run in order to obtain a valid result, each lab may choose their own target(s) for detecting SARS-CoV-2. The average concentration calculation for both WW sample 1 and WW sample 2 are highly similar to each other showing that reliable results may be generated with any of the three assays.

References

[1] Application Note - Complete Wastewater-based Epidemiology (WBE) Workflow for detection of SARS-CoV-2 via RT-qPCR.
Link: https://www.analytik-jena.com/fileadmin/import/assets/12576279_AppNote_Extraction_0005v2_en_SARS_wastewater.pdf

[2] GoTaq® Enviro Wastewater SARS-CoV-2 Systems Technical Manual
Link to website: https://www.promega.de/en/products/pcr/qpcr-and-rt-qpcr/sars-cov-2-rt-qpcr-kit-for-wastewater/?catNum=AM2100

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