Promega

Methods for Wastewater Disease Surveillance: A Reagent Manufacturer's Perspective

Presented by:
Subhanjan Mondal, Ph.D.
Sr. Research Scientist

Promega

Core Technologies in:

- **Nucleic Acid Extraction and Amplification**
- **Protein Expression, Purification and Analysis**
- **Cell Health, Viability and Energy Metabolism**
- **Cellular, Genetic and Protein Reporters**

Our “Wastewater Surveillance Journey”

SARS-CoV-2 detected in wastewater

Interlaboratory Study Participation ->

Direct Capture Method developed

Kits available in catalogue

Automation Kits including Ceres Nanotrap® A

March 2020 → April 2020 → Spring 2020 → July 2020 → October 2020 → Q2 2021 → Nov 2021 → Q1 2022 → Ongoing

Adoption in >50 countries

KWR

Global Wastewater Surveillance Summit

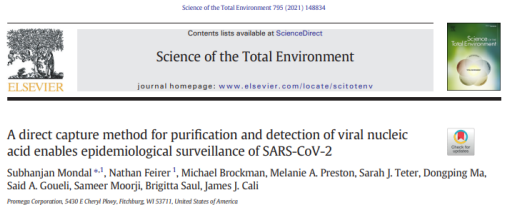
THE Water Research FOUNDATION®

World Health Organization

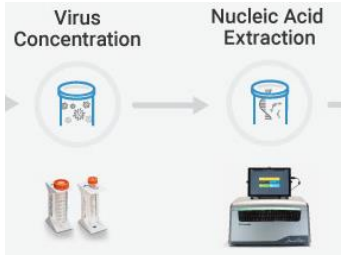
Public Health Agency of Canada / Agence de la santé publique du Canada

RT-qPCR Detection kit for wastewater including quant **standards** and **controls**

Method validation paper in STOTEN



Additional methods and qPCR Kits under development



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…. and the journey continues…

Challenges of Wastewater Samples for Molecular Biology

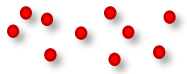
- Sample composition greatly **varies** between locations/sewersheds
- Analytes are present in **low abundance**
- Wastewater contains compounds that **inhibit PCR**
- **“Solids”** vs “**Liquid**” phase – which one to use?
- Simple, scalable and consistent workflow is needed for surveillance.

Madison Metropolitan Sewerage District

The Wastewater : A simplistic view

Dietary Fibers
(cellulosic)

Tissue paper
(cellulosic)



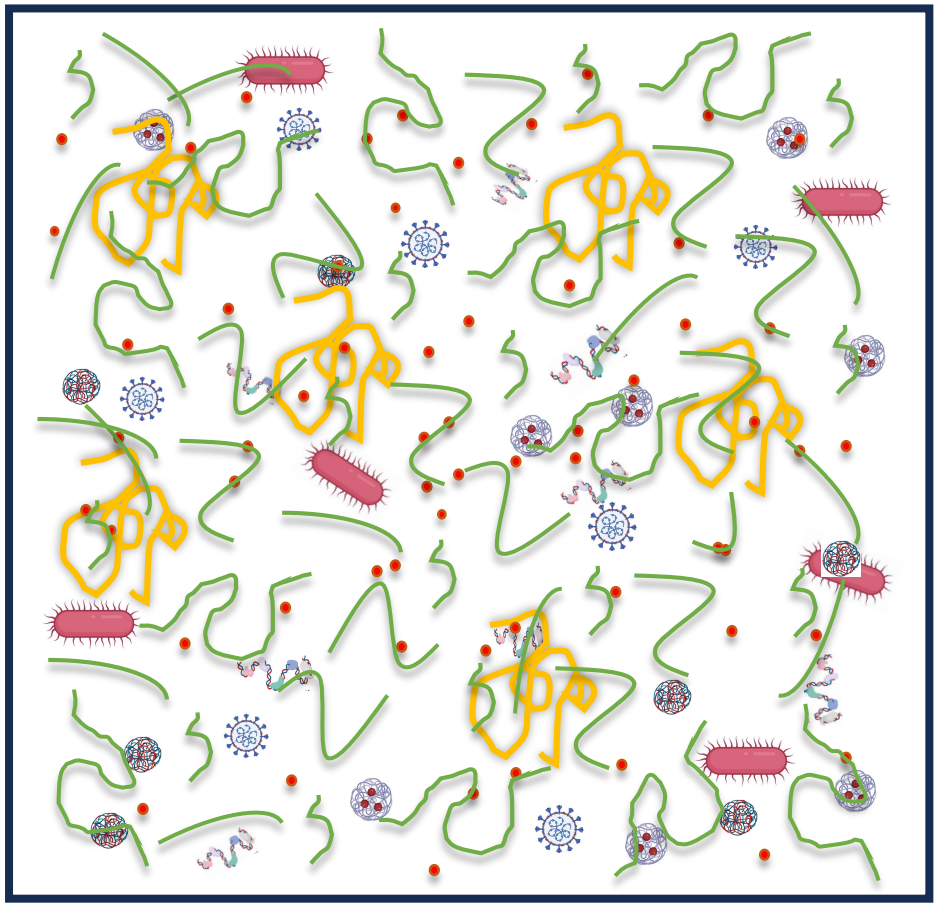
Particulates
(silica)

<u>Chemicals</u>

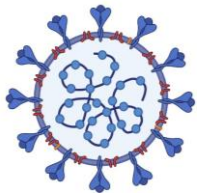
- Urea
- Detergents
- Salts

<u>PCR inhibitors</u>

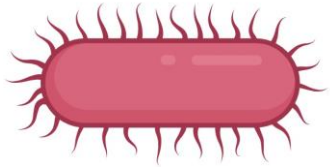
- Bilirubin
- Humic acid
- Fulvic acid



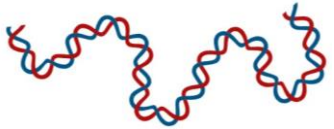
Virus



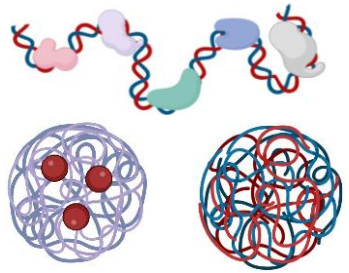
Bacteria



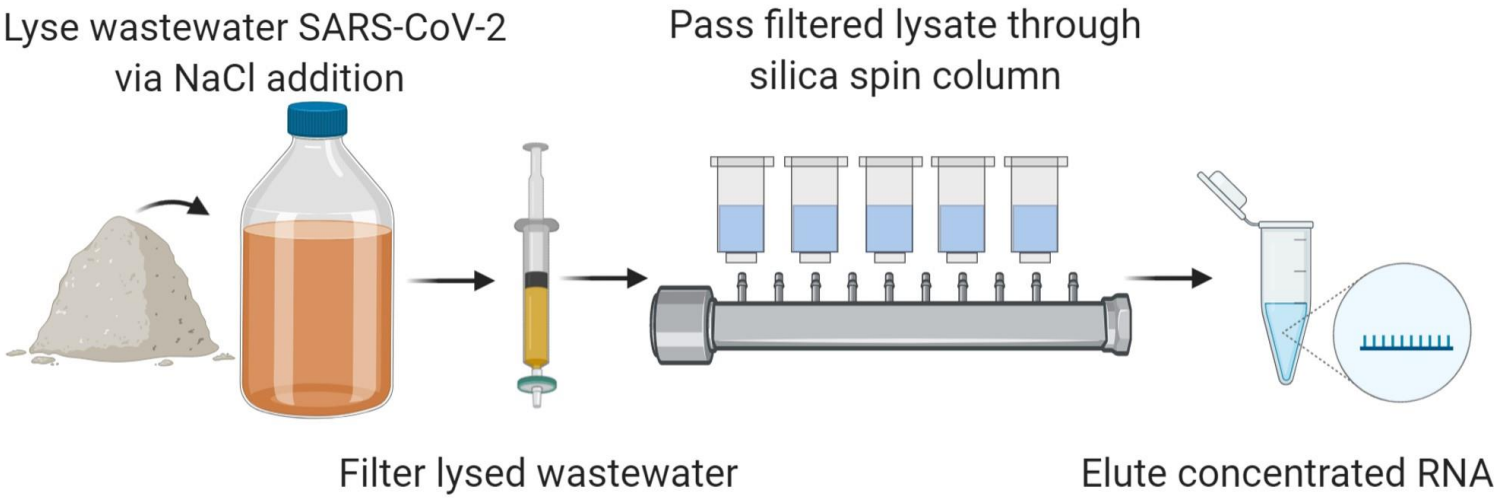
Nucleic acid
naked



Nucleic acid
protected



The Inspiration: Sewage, Salt, Silica and SARS-CoV2 (4S) method



Sewage, Salt, Silica, and SARS-CoV-2 (4S): An Economical Kit-Free Method for Direct Capture of SARS-CoV-2 RNA from Wastewater

Oscar N. Whitney, Lauren C. Kennedy, Vinson B. Fan, Adrian Hinkle, Rose Kantor, Hannah Greenwald, Alexander Crits-Christoph, Basem Al-Shayeb, Mira Chaplin, Anna C. Maurer, Robert Tjian, and Kara L. Nelson*

Cite This: *Environ. Sci. Technol.* 2021, 55, 4880–4888 Read Online

Sample Concentration

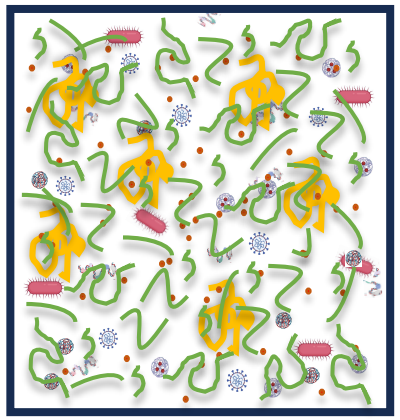
Release TNA from solid fraction **30’ Protease**

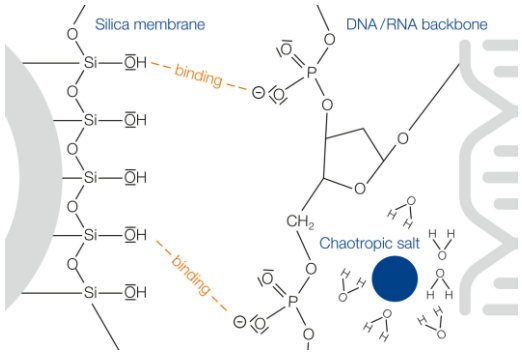
Prepare TNA for capture on silica: Chaotropic agents + alcohol

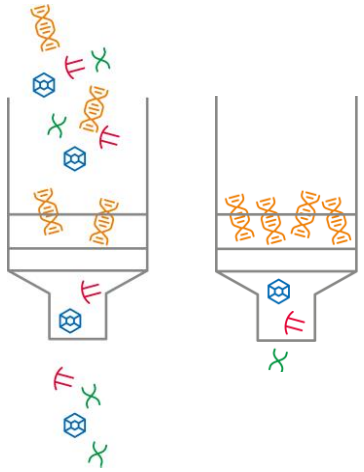
Bind TNA to Midi-column on Vacuum Manifold **Wash & Inhibitor Removal**

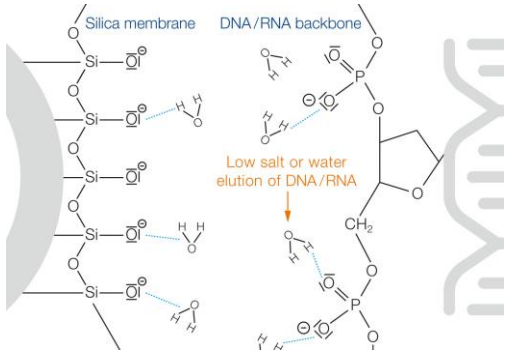
Elute TNA 1ml Eluate

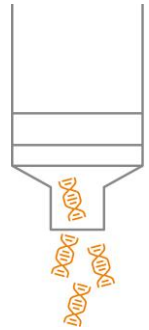
0.5-1 ml TNA





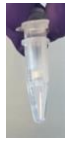






Wizard® Enviro TNA Kit

Direct Capture & Spin column



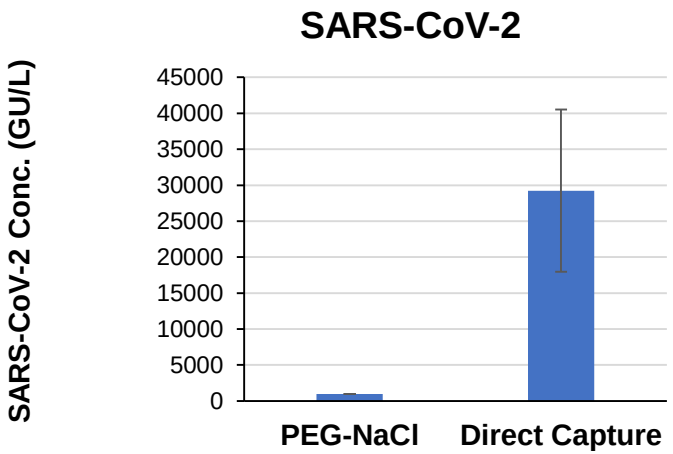
Maxwell RSC® Enviro TNA Kit

Direct Capture & Maxwell® RSC Cartridges

Advantages of Direct TNA Capture

Total Nucleic Acid concentration

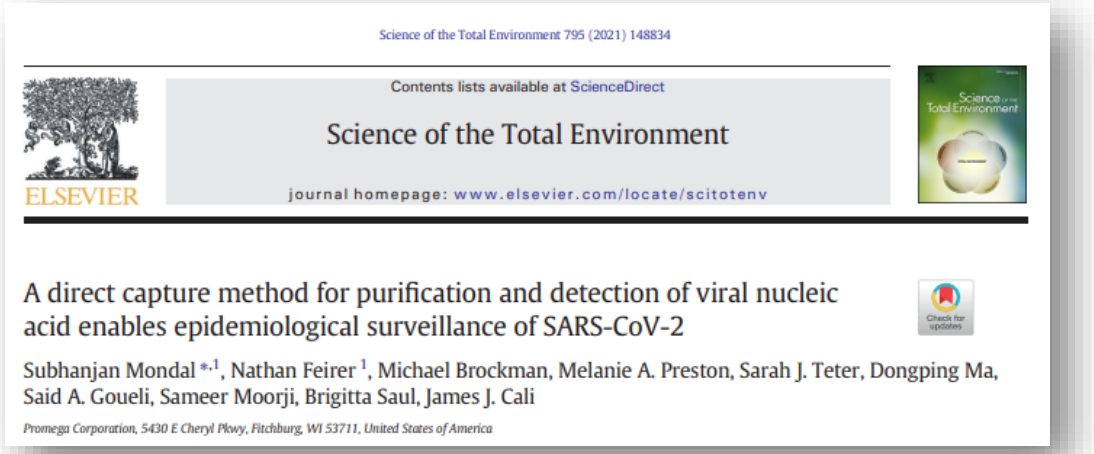
- 40 ml input provides higher yield than PEG/NaCl Method
- Consistent Recovery is ideal for surveillance



Recovery of SARS-CoV-2 genetic material was more than 20-times more than the PEG/NaCl method. Mondal et al, 2021

% Recovery of Direct Capture Method	
Spike virus*	**% Recovery**
MS2 phage	39.67 ± 10.66
OC43 coronavirus	63.13 ± 4.16
229E coronavirus	40.09 ± 10.36

- **Consistent yield**
- **Good Recovery**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journal homepage: www.elsevier.com/locate/scitotenv

ELSEVIER

A direct capture method for purification and detection of viral nucleic acid enables epidemiological surveillance of SARS-CoV-2

Subhanjan Mondal *,[1], Nathan Feirer [1], Michael Brockman, Melanie A. Preston, Sarah J. Teter, Dongping Ma, Said A. Goueli, Sameer Moorji, Brigitta Saul, James J. Cali

Promega Corporation, 5430 E Cheryl Pkwy, Fitchburg, WI 53711, United States of America

High Yield and Quality for Sequencing

Case Report

Evaluation of viral concentration and extraction methods for SARS-CoV-2 recovery from wastewater using droplet digital and quantitative RT-PCR

Lampros Dimitrakopoulos [1], Aikaterini Kontou [1], Areti Strati, Aikaterini Galani, Marios Kostakis, Vasileios Kapes, Evrikleia Lianidou, Nikolaos Thomaidis [**], Athina Markou [*]

Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, University Campus, Zografou, 15771, Athens, Greece

Evaluation of two different concentration methods for surveillance of human viruses in sewage and their effects on SARS-CoV-2 sequencing

Inés Girón-Guzmán [a,1], Azahara Díaz-Reolid [a,1], Enric Cuevas-Ferrando [a], Irene Falcó [a], Pablo Cano-Jiménez [b,c], Iñaki Comas [b,c], Alba Pérez-Cataluña [a,*], Gloria Sánchez [a]

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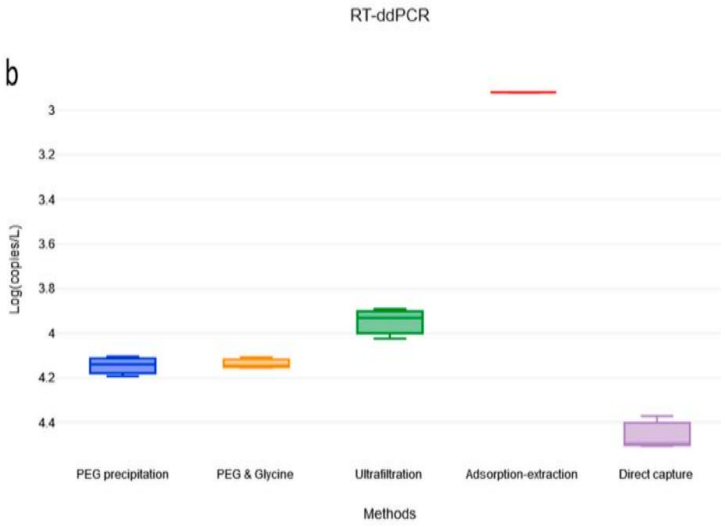
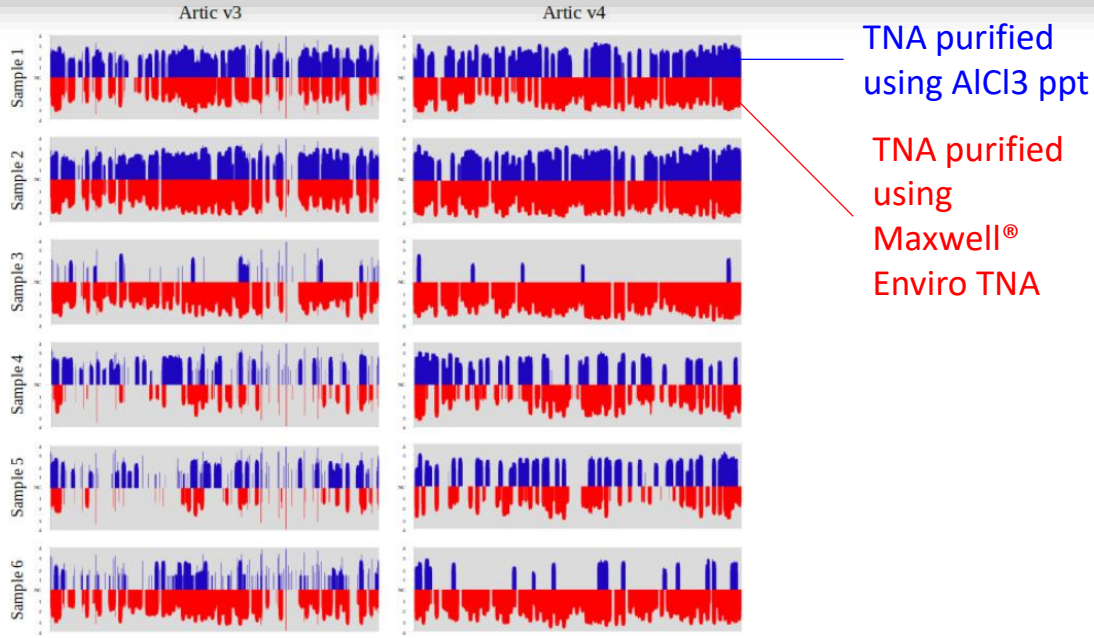
[b] *Instituto de Biomedicina de Valencia (IBV-CSIC), C/ Jaume Roig, 11, Valencia 46010, Spain*
[c] *CIBER in Epidemiology and Public Health (CIBERESP), Valencia, Spain*



Recoveries (%) of the SARS-CoV-2 spiked viral standard (standard deviation values (SD)) on three different days with all five different concentration methods and all four different extraction kits based on RT-qPCR.

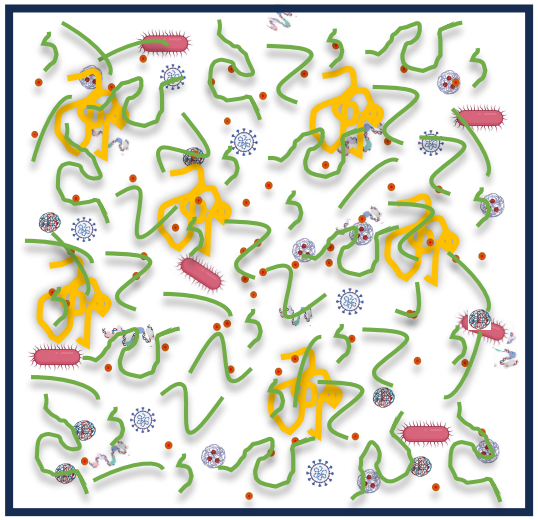
Concentration method	DAY 1 Recovery (SD)	DAY 2 Recovery (SD)	DAY 3 Recovery (SD)	AVERAGE
PEG precipitation	0.1289 (0.0165)	0.1067 (0.0123)	0.0864 (0.0142)	0.1073
PEG & Glycine	0.1373 (0.0082)	0.2703 (0.0217)	0.3031 (0.0042)	0.2369
Ultrafiltration	0.0104 (–)	0.0018 (0.0000)	0.0115 (0.0065)	0.0079
Adsorption-extraction	0.0202 (0.0044)	0.0042 (0.0028)	N/A	0.0122
Direct capture	1.82 (0.06)	1.63 (0.01)	2.73 (0.02)	2.06
Extraction method	DAY 1 Recovery (SD)	DAY 2 Recovery (SD)	DAY 3 Recovery (SD)	AVERAGE
IDEXX	6.31 (0.0012)	7.07 (0.0039)	12.7 (0.0054)	8.7
QIAGEN Viral	22.0 (0.0035)	14.8 (0.013)	25.6 (0.0122)	20.8
QIAGEN Water	1.02 (0.0016)	0.90 (0.0013)	4.41 (0.0028)	2.11
Promega	39.3 (0.0071)	36.0 (0.0239)	37.6 (0.0258)	37.6



Enviro TNA kits yield more nucleic acid from a smaller sample volume than other methods

Enviro TNA purified nucleic acid provides high quality sequencing data

New Purification Methods: Going after the “solids”



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Vol. 31, No. 3
Printed in U.S.A.

Demonstration of Solids-Associated Virus in Wastewater and Sludge

FLORA MAE WELLINGS,* ARTHUR L. LEWIS, AND CARROL W. MOUNTAIN
Epidemiology Research Center, State of Florida, Department of Health and Rehabilitative Services, Tampa, Florida 33614

Contents lists available at ScienceDirect
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journal homepage: www.elsevier.com/locate/scitotenv

Comparative analysis of Adsorption-Extraction (AE) and Nanotrap® Magnetic Virus Particles (NMVP) workflows for the recovery of endogenous enveloped and non-enveloped viruses in wastewater

Warish Ahmed [a,*], Aaron Bivins [b], Asja Korajkic [c], Suzanne Metcalfe [a], Wendy J.M. Smith [a], Stuart L. Simpson [d]
LA 70803, USA

Adsorption of Respiratory Syncytial Virus, Rhinovirus, SARS-CoV-2, and F+ Bacteriophage MS2 RNA onto Wastewater Solids from Raw Wastewater

Laura Roldan-Hernandez and Alexandria B. Boehm*

Cite This: *Environ. Sci. Technol.* 2023, 57, 13346−13355
Read Online

- Nucleic acid concentrations are 1-3 orders of magnitude higher in the solid fraction than the liquid fraction
- Require smaller volumes: simple and scalable
- Eliminate any purification bias
- Steps to effectively remove PCR inhibitors

Detection and Quantification

Sample concentration

- Direct TNA capture

Nucleic Acid Extraction

- Manual Spin column
- Automated Extraction

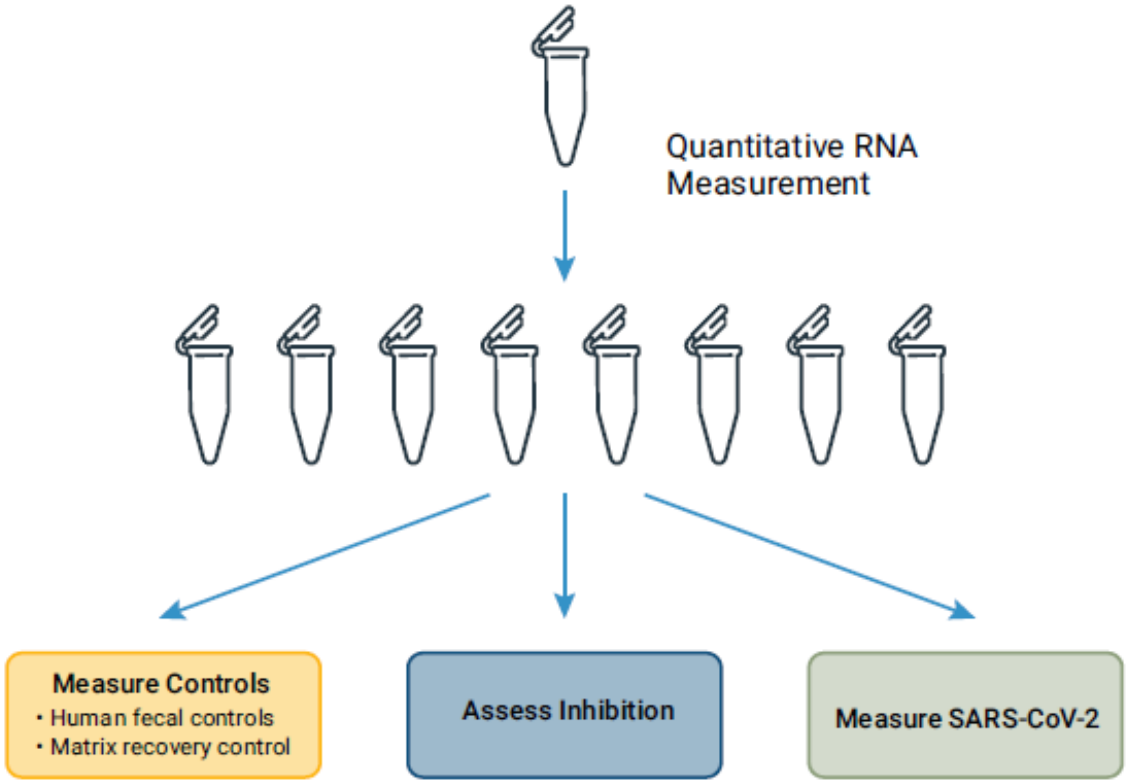
Detection and Quantification

- PCR
- Sequencing

GoTaq® Enviro qPCR kits
made for **wastewater surveillance**:

- PCR inhibitor tolerant qPCR and RT-qPCR Master-Mix
- Multiplexed to include process control (PMMoV or CrAssphage)
- **Quantitation standard RNA/DNA** included

Design of the Wastewater SARS-CoV-2 RT-qPCR Detection Kit

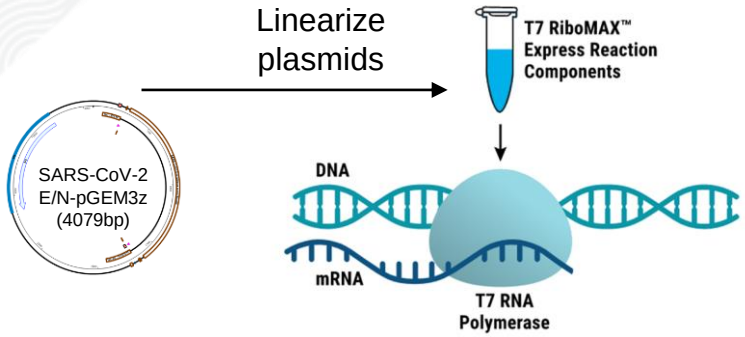


	Detection Channel in Multiplex			
Cat.#	FAM	HEX	ROX	Cy5
AM2150	N1	N2	IAC	PMMoV
AM2160	E	N2	IAC	PMMoV
AM2100-2130	N1, N2, or E	IAC		PMMoV
CS317431	Flu A	Flu B	SC2	PMMoV

Quantitation Standard RNAs are available for: SARS-CoV-2 (N+E), SC2, PMMoV, FluA, FluB

Assays with Proper QA/QC controls

Quantitation Standards

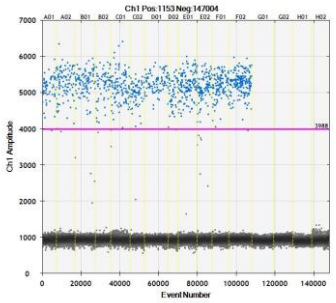


In vitro transcription to generate RNA

DNase treatment to remove plasmid DNA

Quantify RNA using QuantiFluor RNA System and droplet digital RT-PCR.

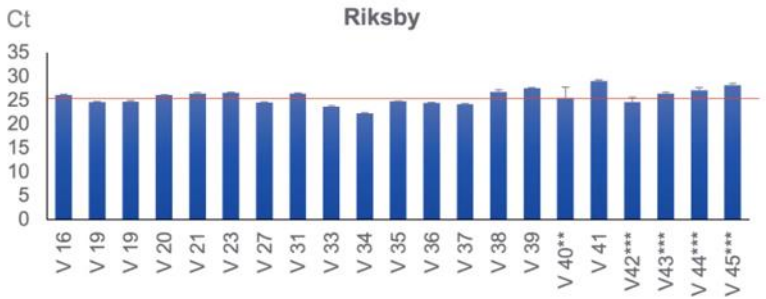
Dilute the RNA at $4x10^6$ copies/ul in TE-Buffer + protectant



Human Fecal Control

Pepper mild mottle virus

- An **RNA** plant virus that is commonly found in human feces (diet)
- It is present in wastewater globally and serves as **human fecal indicator**



PMMoV levels in wastewater from one sampling point over multiple days provided by Zeynep Cetecioglu, KTH, Stockholm

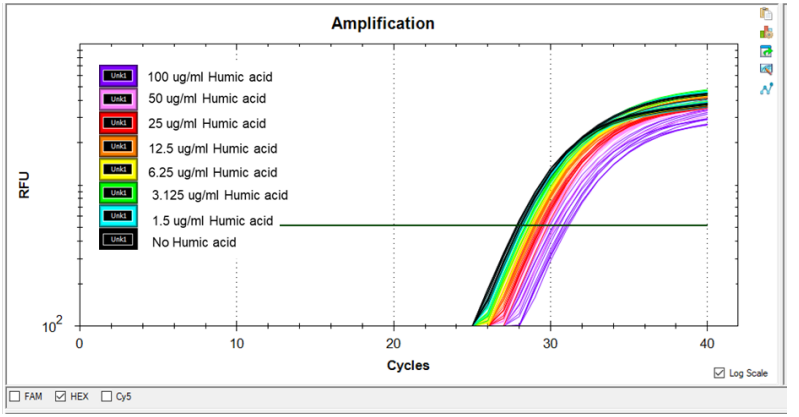
Internal Amplification Control

- An exogenous RNA that is in every qPCR assay serving as an amplification control
- Template for the Internal control used is Firefly Luciferase RNA

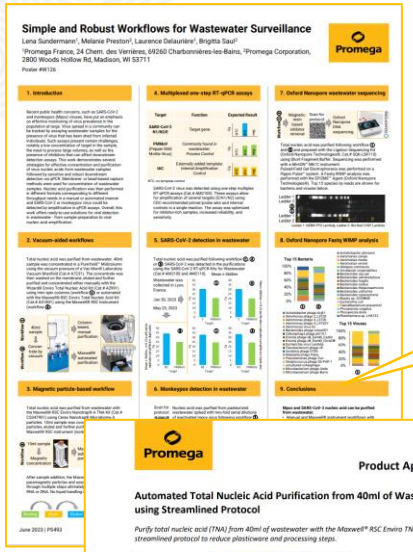
A shift in Cq value from the no template control (NTC) reflects the level of RT-qPCR inhibition in a sample.

$\Delta Cq = Cq_{[Sample]} - Cq_{[NTC]}$

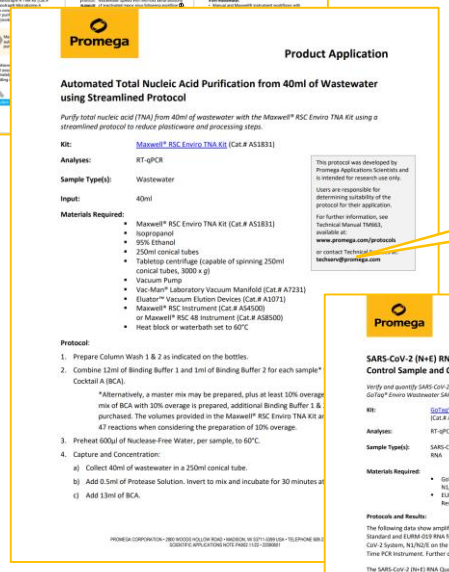
$\Delta Cq > 2$ represents significant inhibition of the reaction



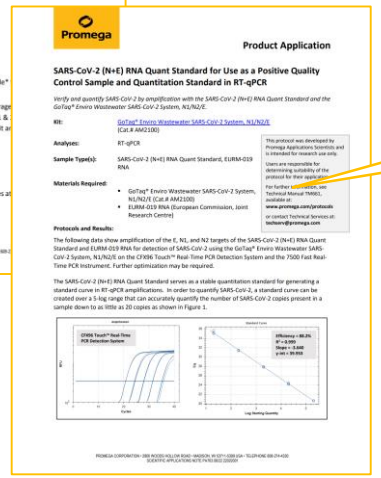
Application scientists develop additional applications:



Promega purification used for qPCR and for **Oxford Nanopore Sequencing**

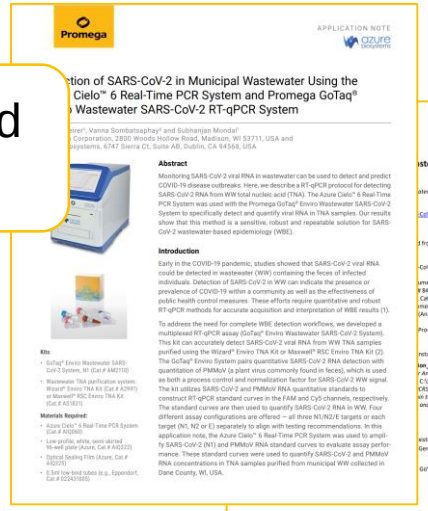


Reduce hands-on time by using larger containers



Compare Standard RNAs

Automation for Concentration and purification



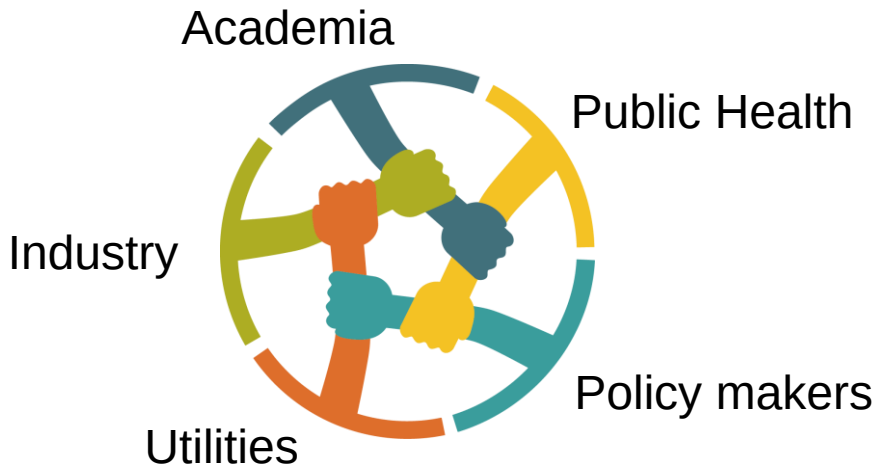
Using additional **qPCR instruments**

...and more

Where Next?

Now that the COVID-19 pandemic is behind us, how do we sustain the surveillance system that was set up during the pandemic

- Targets?
- What sample-type to use thats best for the targets
- What throughput



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Reach us at:
applied@promega.com

Thank you