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RT-PCR amplification for the discrimination between wild type and mutation alleles in SARS-CoV-2 genomes from wastewater

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We use this protocol and it's working

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Abstract

This protocol describes the procedure of RT-PCR for the allelic discrimination between wild type and mutation alleles in specific positions of the SARS-CoV-2 genome. Starting with isolated RNA from SARS-CoV-2 positive wastewater samples, this workflow combines Applied Biosystems TaqMan SNP Genotyping Assays with a one-step RT-PCR reaction to detect whether there are known SARS-CoV-2 mutations present in the wastewater samples.

Sequence-specific forward and reverse primers amplify the target sequence region in each mutation assay. The reverse primer also primes reverse transcription of the SARS-CoV-2 genomic RNA sequences. Each mutation assay includes two TaqMan minor groove binder probes with nonfluorescent quenchers: one VIC dye labelled probe to detect the reference sequence and one FAM dye labelled probe to detect the mutation sequence.

Materials

- TaqPath 1-Step RT-qPCR Master Mix CG, ThermoFisher Scientific, Catalog A15299
- AcroMterix SARS-CoV-2 Control, ThermoFisher Scientific, Catalog 954519
- Mutation Assay: S.G339D.GGT.GAT, ThermoFisher Scientific, Catalog CV47VRX
- Mutation Assay: S.Q493R.CAA.CGA, ThermoFisher Scientific, Catalog CVH49P2
- Mutation Assay: S.L452R.CTG.CGG, ThermoFisher Scientific, Catalog CVAAAAD
- Mutation Assay: S.T547K.ACA.AAA, ThermoFisher Scientific, Catalog CVYMJGA
- Mutation Assay: M.D3N.GAT.AAT, ThermoFisher Scientific, Catalog CVAAAAK
- Mutation Assay: ORF7b.L11F.TTG.TTT, ThermoFisher Scientifc, Catalog CVCE3VH

Troubleshooting

Before start

Wipe bench surfaces with RNAse Away and keep working environment clean. Maintain the RNA samples on ice, and prepare the RT-PCR mixture on ice.

RT-PCR amplification

1 Prepare the amplification mixture as follows:

	Reagent	Concentration in the reaction
	TaqPath 1-Step RT-qPCR Master Mix CG (4X)	1X
	Mutation assay (40X)	2X
	DNAse/RNAse free water	up to 15 µL

To the 15 µl of reaction mix, add 5 µl of wastewater RNA sample, or no-template control (DNAse/RNAse free water), or a wild-type AcroMetrix Coronavirus 2019 (COVID-19) RNA control, to complete 20 µl of reaction volume.

2 Thermocycling conditions

	Step	Temperature (ºC)	Time	No. Cycles
	Reverse transcription	45	15'	
	Taq polymerase activation	95	2'	
	Denaturation	95	15''	45
	Annealing	58	45''	
	Post-read	60	30'	

Set the detection module for FAM and VIC labelled probes.

Data analysis

3 The allelic discrimination results can be graphed on a scatter plot contrasting reporter dye florescence (*i.e.*, allele X versus allele Y), and analyzed with the QuantStudio Design and Analysis Software, using the genotyping analysis module with real-time data.