Application of qPCR-Based SARS-CoV-2 Variant Detection to Compliment Next Generation Sequencing (NGS)

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Introduction

- The coronavirus (SARS-CoV-2) is one of the deadliest infectious disease outbreaks in recent history.
- Several variants of SARS-CoV-2 have emerged and some variants have caused second and third waves of the deadly infection.
- Some variants may be resistant to available vaccines and therapies.
- World Health Organization (WHO) has declared it as Variants of Concern (VOC); Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529)
- There continues to be a need for swift and cost-effective SARS CoV-2 variant detection and monitoring.
- Next Generation Sequence (NGS) has been instrumental in discovering new mutations but the technique is expensive and time-consuming for mass deployment.
- Real-time PCR is widely used for the diagnosis of SARS-CoV-2, and the technology can be deployed for mass surveillance of known mutations and variants.

Objective

- Variant analysis of SARS-Cov-2 from the East Texas region using Next Generation Sequencing (NGS) and qPCR.
- Comparative application of qPCR in the detection of SARS-CoV-2 variants

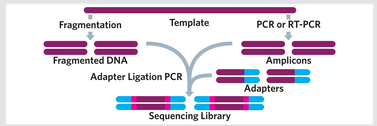
Materials & Methods

Study Design

- This investigation performed Whole Genome Sequencing of 97 de-identified COVID positive (Ct<30) specimens and classified them into WHO label and Pango lineages.
- We also examined the COVID variant of 76 samples with two commercially available qPCR assays and analogized the results with those identified by NGS at Advanta Genetics in Tyler, Texas.

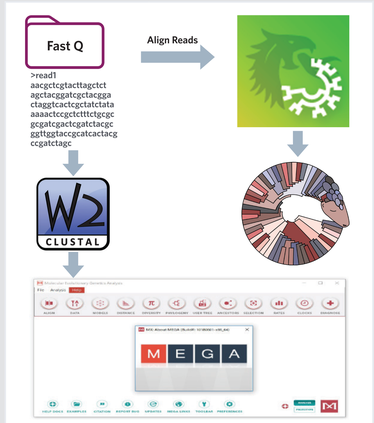
Step 1: Extraction

Step 2: Library Preparation



Step 3: Sequencing

Step 4: Analysis



Sequencing of SARS-CoV-2

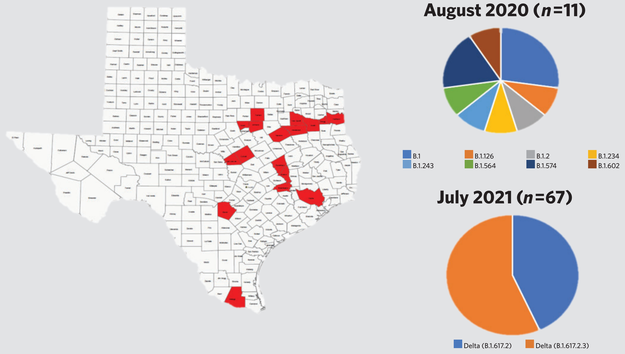
- We prepared the sequencing libraries using Illumina COVIDSeq protocol (Illumina Inc).
- The dual indexed libraries were sequenced using the Illumina MiniSeq® instrument to yield paired-end reads of 75bp length.
- A single consensus FASTA file was assembled using DRAGEN COVID Lineage (Version: 3.5.4) application.
- Consensus FASTA was further analysed for lineage designation using PANGOLIN software (https://pangolin.cog-uk.io).
- Clustal omega server was used for further phylogenetic analysis and the Mega X tool to construct the phylogenetic tree.

COVID-19 Lineage Assignment Using qPCR

Mutation	α	β	γ	δ	O
del69-70	+	–	–	–	–
N501Y	+	+	+	–	+
E484K	–	+	+	–	–
K417N	–	+	–	–	+
K417T	–	–	+	–	–
T478K	–	–	–	+	+
L452R	–	–	–	+	–
P681R	–	–	–	+	–
N679K	–	–	–	–	+
Q954H	–	–	–	–	+

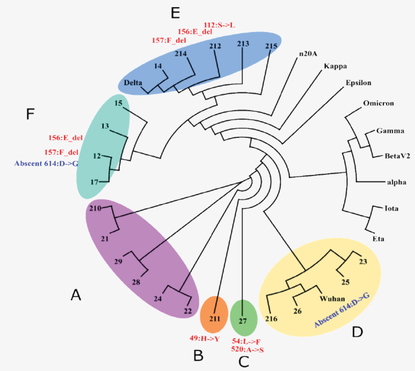
Results

Evolution of the SARS COV-2 Variant in East Texas Over One Year (Aug 2020 – July 2021)



- We detected 8 different lineages from the 11 samples collected during August 2020.
- All 67 samples collected in July 2021 were harboring with Variant of Concern (VOC) of Delta (B.1.617.2) lineage, which was superseded by Omicron by the end of January 2022.

Phylogenetic Analysis of SARS-Cov-2 Samples from East Texas



Minimum spanning tree of the SARS COV-2 samples (n=20) collected August 2020 and July 2021

Comparative Analysis of COVID-19 Lineage Assignment Using qPCR and NGS

Evaluation of qPCR Based SARS-CoV-2 Variant Detection Solutions					Delta Variants (n=67)		Non-VOC (n=11)	
					PCR	NGS	PCR	NGS
Manufacturer	Catalog #	Rxn	Targets	Variants (WHO label) harboring this Mutation				
GT MOLECULAR	100180	1	N501Y	Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1)	0	0	0	0
			Del69-70	Alpha (UK, B.1.1.7)	0	0	0	0
		2	N501Y	Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1)	0	0	0	0
			E484K	Beta (B.1.351), Gamma (P.1)	1	1	0	0
			K417N	Beta (B.1.351), Delta plus	0	0	0	0
			K417T	Gamma (P.1)	0	0	0	0
		3	L452R	Epsilon (B.1.427/B.1.429), Delta (B.1.617.2)	67	67	0	0
			T478K	Delta (India, B.1.617.2)	62	64	0	0
ThermoFisher SCIENTIFIC	A51819	1	L452R	B.1.617, B.1.617.1, Delta (India, B.1.617.2), B.1.617.3, B.1.429	63	67	0	0
	A51822	2	P681R	B.1.617.1, B.1.617.2, B.1.617.3	55	64	0	0

- qPCR-based assays determined the L452R mutation with 100% (67/67; GT Molecular) and 94% (63/67; Thermo Fisher) accuracy compared to NGS.
- 100% concordance between NGS and qPCR assays for detection of Non-VOC.

Conclusion

- NGS remains the gold standard for discovering new variants as they emerge.
- qPCR-based detection of the known COVID variant can be implemented as an alternative to sequencing for clinical and epidemiological interests.
- Findings provide support for clinical laboratories like Advanta to validate COVID variant testing using qPCR as a proxy for NGS development and application.
- Such testing capabilities are likely to be clinically useful in cases of SARS-COV-2 mutagenic progression.

Acknowledgment: GT Molecular for providing the Omicron-specific PCR kits