The Utility of Twist Respiratory Viral Controls for SARS-CoV-2 and Multiplexed Pathogen Detection

INTRODUCTION

Emerging viral infectious diseases require rapid responses to develop assays for detection and measurements. This is especially true with the COVID-19 pandemic. Researchers responded by developing rapid detection tests including quantitative PCR (qPCR) and Next Generation Sequencing (NGS) for detecting the SARS-CoV-2 viral genome.

The symptoms for SARS-CoV-2 are similar to other diverse viral pathogens resulting in respiratory illnesses, thus there is a push to develop assays capable of detecting more than one pathogen in a single test, which would allow for rapid pathogen identification without lengthy systematic testing to rule out individual pathogens.

With a multiplexed assay comes the need for an increased set of diverse viral controls. In this technote we demonstrate the compatibility of the diverse Twist Respiratory Viral controls as positive controls for the Twist Respiratory Virus Research Panel. We also demonstrate that these same viral controls serve as useful negative controls for routine SARS-CoV-2 testing.

RESULTS

To determine the compatibility of the Twist Respiratory Viral controls as positive controls for the Twist Respiratory Virus Research Panel (PN 103066, 103067 & 103068) we created libraries for NGS target enrichment. Libraries were made with the indicated viral control at 1,000,000 copies spiked into 50 ng of human reference RNA. Captures were performed using a 16 hour hybridization with the Twist Standard hybridization kit (PN 101279, 101025, 101026).

Capture of the viral controls at 1 million copies showed excellent coverage, enrichment and uniformity. Every viral template was enriched at more than 2500-fold above the input fraction and had more than 99.9% of bases covered with at least 1x coverage (Figure 1). After capture, coverage was relatively even across the viral genomes, even for segmented viruses such as influenza. This uniform coverage is due to capture efficiency but also reflects the synthesis and manufacturing excellence of these viral controls. In Figure 2 we show 99.9% of bases that were captured with at least 20x coverage represented with at least 95% of reads showing the expected reference allele.

For the viral controls, hybrid captures were very specific leading to little or no contamination or cross-alignment. To measure cross-contamination or cross-alignment we aligned capture results against each Twist Respiratory Viral control. For every captured control, at least 99.3% of reads mapping to the capture space of the panel mapped to the target genome (Figure 3).

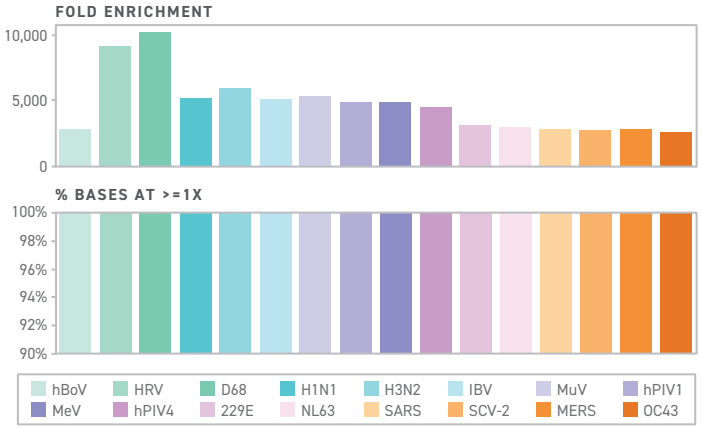

Figure 1: Fold-enrichment and percent of genome covered at 1x for viral controls after target enrichment. Full names for abbreviated viruses are present in Table 1.

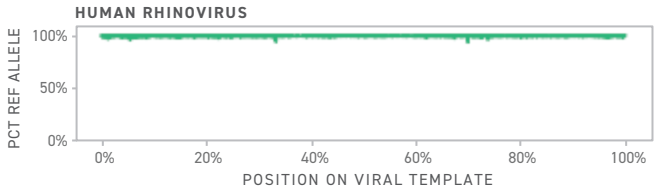

Figure 2: Fraction of reads with the reference allele at all bases across the human rhinovirus synthetic template.

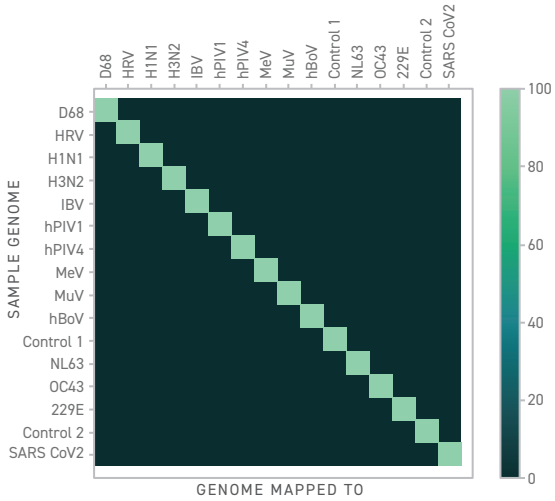

Figure 3: Percent of reads from each enrichment sample (rows) mapping to each genome covered by the enrichment panel (columns). Entries on the diagonal line represent percentages of on-target reads, while entries off the diagonal line represent percentages of off-target reads. Full names for abbreviated viruses are present in Table 1.

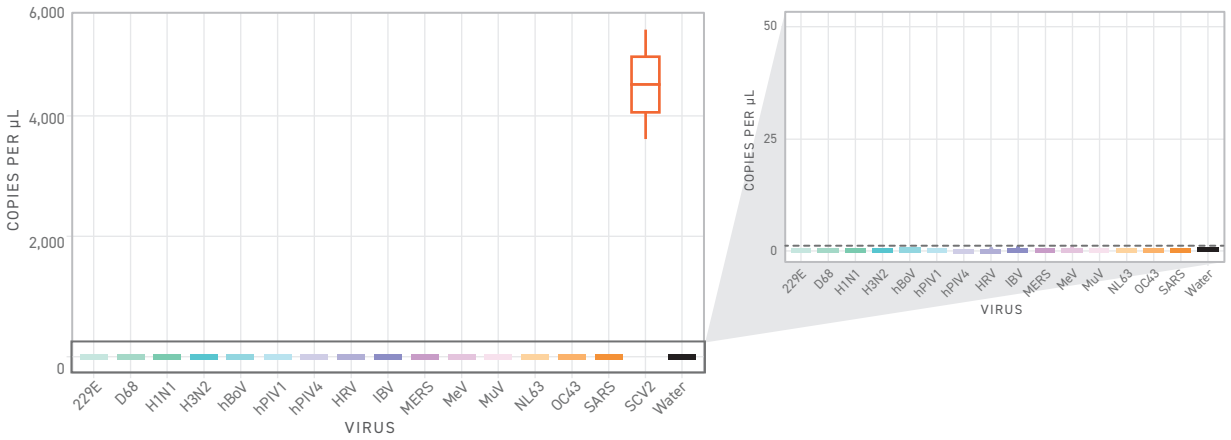

Figure 4: Boxplot showing qPCR data using the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel for each of the Twist Respiratory Virus Controls. The Twist SARS-CoV-2 RNA control 2 was added at 5,000 copies as a positive control. All of the viruses show similar behavior to the no-template water control, with fewer than 1 copy per µl of SARS-CoV-2. Full names for abbreviated viruses are present in Table 1. The zoomed-in boxplot shows qPCR data from the above RNA controls between 0 and 50 copies per µl.

We also put these Twist Respiratory Viral Controls through a FDA-EUA approved qPCR diagnostic assay for SARS-CoV-2 to determine if the Twist Respiratory Viral controls could potentially serve as negative controls in diagnostic assays. Using the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel with TaqPath 1-Step RT-qPCR Master Mix, we performed a series of assays with each of the Twist Respiratory Viral Controls at approximately 10^6 copies per µl as templates. In addition, we used the Twist SARS-CoV-2 RNA Control 2 (PN 102024) at 5,000 copies as a positive control against the ATCC Quantitative Synthetic SARS-CoV-2 RNA as a quantitative reference standard. As expected, we found that SARS-CoV-2 was practically undetectable in 6 technical replicates for each virus, with SARS-CoV-2 genome copies at fewer than 1 copy per µl. The Twist Respiratory Viral control essentially performs as well as the no-template water control. The results below indicate these viral controls serve as valuable negative controls in routine SARS-CoV-2 detection assays.

SUMMARY

In this document we highlight the utility of 15 Twist Respiratory Viral Controls as positive controls for Multiplexed Pathogen Detection via target enrichment and negative controls for qPCR assays detecting SARS-CoV-2. The 15 Twist Respiratory Viral controls resulted in 99.9% sequence coverage with a minimum of 2500 fold enrichment after capture with the Twist Respiratory Panel. When used in a FDA-EUA approved qPCR SARS-CoV-2 detection assay, we found the Twist Respiratory controls gave similar background levels as the no-template water controls, indicating these controls serve as useful negative controls in SARS-CoV-2 assays.

PART NUMBER	TWIST SYNTHETIC CONTROL NAME	ABBREVIATED NAME	NUCLEIC ACID SPECIES	STORAGE
102024	SARS-CoV-2 RNA Control 2 (MN908947.3)	SCV-2	ssRNA	–90 to –70°C
103001	Influenza H1N1 (2009) RNA control	H1N1	ssRNA	–90 to –70°C
103002	Influenza H3N2 RNA control	H3N2	ssRNA	–90 to –70°C
103003	Influenza B RNA control	IBV	ssRNA	–90 to –70°C
103004	Human bocavirus 1 DNA control	hBoV	ssDNA	–90 to –70°C
103005	Human enterovirus 68 RNA control	D68	ssRNA	–90 to –70°C
103006	Human rhinovirus 89 RNA control	HRV	ssRNA	–90 to –70°C
103007	Mumps virus RNA control	MuV	ssRNA	–90 to –70°C
103008	Human parainfluenza virus 1 RNA control	hPIV1	ssRNA	–90 to –70°C
103009	Measles virus RNA control	MeV	ssRNA	–90 to –70°C
103010	Human parainfluenza virus 4 RNA control	hPIV4	ssRNA	–90 to –70°C
103011	Human coronavirus 229E RNA control	229E	ssRNA	–90 to –70°C
103012	Human coronavirus NL63 RNA control	NL63	ssRNA	–90 to –70°C
103013	Human coronavirus OC43 RNA control	OC43	ssRNA	–90 to –70°C

Table 1: Twist Respiratory Synthetic Controls.