Viral Detection and Characterization

Using the Twist Fast Hybridization Target Enrichment Protocol

INTRODUCTION

Twist's Fast Hybridization Target Enrichment system, an alternative to the standard 16 hour hybridization workflow, is designed with the flexibility to both save time and enhance the performance of targeted sequencing efforts. With this system, both time and process stringency can be tuned to fit the needs of the investigator.

We previously illustrated the compatibility of target enrichment for the detection and characterization of the SARS-CoV-2 virus (NGS-based Target Capture for SARS-CoV-2 Detection and Characterization) using the standard 16 hour hybridization workflow. To demonstrate the flexibility of the Twist Fast Hybridization system for viral detection and characterization, we assessed several variables using the Twist SARS-CoV-2 Research Panel (PN 102016, 102017 & 102018) to capture the SARS-CoV-2 viral genome.

Here we show the Twist Fast Hybridization system's optimal Fast Wash Buffer 1 temperature, its flexibility in hybridization time, and its compatibility with 8-plex hybridization for detecting and characterizing viral genomes using the Twist SARS-CoV-2 Research Panel.

RESULTS

Optimization of Wash Buffer Temperature

In Appendix A of the Twist Fast Hybridization Target Enrichment Protocol, we highlight the temperature of the Fast Wash Buffer 1 as a tuneable lever for adjusting panel stringency and performance. To determine the optimal Fast Wash Buffer 1 temperature for the SARS-CoV-2 capture (using the Twist SARS-CoV-2 Research Panel), we set a range of temperatures, from 57°C to 70°C, and assessed viral genome recovery (Figure 1). Libraries were made with the Twist SARS-CoV-2 Synthetic RNA Control 1 (PN 102019), at 1,000 and 10,000 copies, and captures were performed using a 2 hour hybridization with the temperature of Fast Wash Buffer 1 ranging from 57°C to 70°C.

Increasing the Fast Wash Buffer 1 temperature led to the reduction of non-specific background capture, increasing the fraction of viral reads at all titer points. Using Fast Wash Buffer 1 at the recommended temperature of 68°C, the SARS-CoV-2 Research Panel gave a ~920,000 fold enrichment of the SARS-CoV-2 genome in the 1,000 titer sample, and a ~165,000 fold enrichment in the 10,000 titer sample (Table 1). When downsampled to 1 million reads per sample, we achieve median coverage at ~1,000x, with ~99.9% of the bases covered at 100x or above.

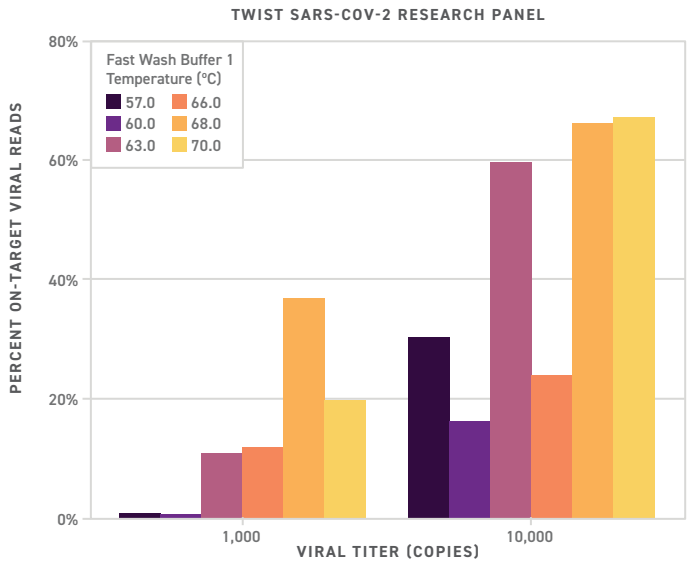

Figure 1: Recovery of viral genomes at different wash temperatures using a hybridization time of 2 hours. The Twist SARS-CoV-2 Research Panel was used to enrich 1,000 and 10,000 copies of SARS-CoV-2 spiked into a human RNA background. Percent of viral reads obtained at different wash buffer temperatures is shown.

VIRAL TITER (COPIES)	% PRE-CAPTURE	% VIRAL READS POST-CAPTURE	FOLD ENRICHMENT	MEDIAN COVERAGE	% BASES AT >= 100X COVERAGE
1,000	0.00004%	36.8%	920662	956x	99.85%
10,000	0.0004%	66.0%	164991	1582x	99.91%

Table 1: Fold-enrichment and percent of the SARS-CoV-2 genome covered at 1x, 10x and 100x for 1,000 and 10,000 viral titers, with 1 million reads after enrichment with the Twist SARS-CoV-2 Research Panel. Wash temperature was 68°C.

Flexibility of Hybridization Time

We next demonstrated the flexibility in hybridization time of the Twist Fast Hybridization system on viral capture. Libraries were made using the Twist SARS-CoV-2 Synthetic RNA Control 1 at 1,000 and 10,000 copies, and target enrichment was completed with the Twist SARS-CoV-2 Research Panel. Captures were performed with a Fast Wash Buffer 1 temperature of 68°C, and hybridization times ranging from 30 minutes to 2 hours.

In as little as 30 minutes, high capture efficiency was achieved (Figure 2). Some fluctuation was seen in on-target viral reads among each hybridization time but, overall, we saw excellent capture efficiency at all hybridization times. This gives flexibility to the workflow, allowing for hybridization times between 30 minutes and 2 hours.

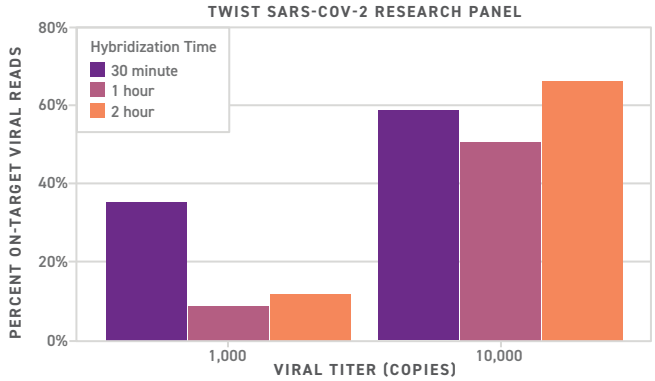

Figure 2: Effect of hybridization time on a single-plex capture of SARS-CoV-2. The Twist SARS-CoV-2 Research Panel was used to enrich viral DNA at 1,000 and 10,000 copies at different hybridization times. Percent of reads matching the viral template is shown.

Use of Multiplexing in Viral Detection

Finally, we determined the compatibility of the Twist Fast Hybridization system using a multiplexed reaction during hybridization. Samples at various viral titers were first indexed during library preparation using Twist UDI barcodes, following the Creating cDNA Libraries using Twist Library Preparation Kit for ssRNA Virus Detection protocol. We then performed an 8-plex capture using hybridization times of 30 minutes and 2 hours, with Fast Wash Buffer 1 at the previously recommended 68°C.

Capture efficiency was similar for both 8-plex and single-plex capture reactions when using the Twist Fast Hybridization system with Fast Wash Buffer 1 at 68°C (Figure 3). Hybridization times of either 30 minutes or 2 hours using the 8-plex system resulted in similar on-target viral reads as to what was seen when using the same conditions in a single-plex.

As shown in Table 2, the entire viral genome was covered at 1x or above using a hybridization time of 30 minutes or 2 hours and a multiplex reaction with as few as 250,000 reads, for both 1,000 and 10,000 copies of the SARS-CoV-2 genome.

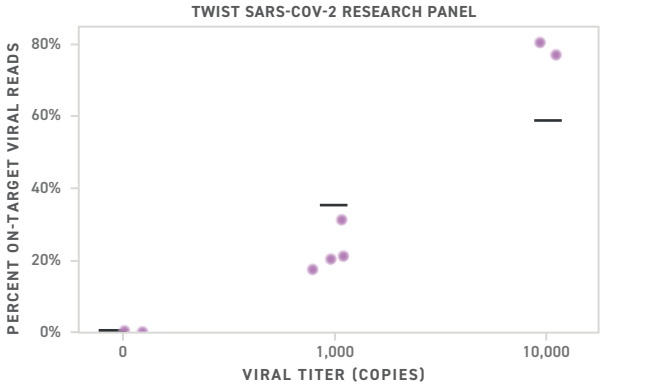

Figure 3: Effect of hybridization time on a multiplexed capture of SARS-CoV-2. The Twist SARS-CoV-2 Research Panel was used to enrich viral DNA at 1,000 and 10,000 copies in an 8-plex reaction. Negative controls were also included in each 8-plex. Data seen here display results for the 30 minute hybridization time, using a 68°C Fast Wash Buffer 1 temperature. The figure shows the fraction of reads that align to the SARS-CoV-2 genome in each condition, compared to alignment in single-plexed captures (black lines).

PANEL NAME	HYBRIDIZATION TIME	VIRAL TITER (COPIES)	READ COUNTS				
			25K	100K	250K	500K	1M
Twist SARS-CoV-2 Research Panel	30 minutes	1,000	99.6%	99.9%	99.9%	99.9%	99.9%
		10,000	99.9%	99.9%	99.9%	99.9%	99.9%
	2 hours	1,000	81.8%	98.8%	99.9%	99.9%	99.9%
		10,000	99.9%	99.9%	99.9%	99.9%	99.9%

Table 2: Percent of genome covered at 1x for 1,000 and 10,000 viral titers, with different read counts, after a 8-plex capture enrichment using the Twist SARS-CoV-2 Research Panel, a wash temperature of 68°C, and a 30 minute or 2 hour hybridization time.

Based on these results, we recommend a hybridization time between 30 minutes and 2 hours when using Twist's Fast Hybridization system to detect and characterize viral genomes.

SUMMARY

Twist's Fast Hybridization Target Enrichment is a flexible system that can be tuned to enhance the success of targeted sequencing efforts. This system can be utilized for panels that detect and characterize viral pathogens of interest. When using this system as a viral detection assay, conditions are most suitable using a hybridization time between 30 minutes and 2 hours, and a Fast Wash Buffer 1 temperature at 68°C. Multiplexing reactions further decrease workflow time, making it possible to monitor viral evolution and rapidly characterize novel viruses.