Twist Synthetic SARS-CoV-2 RNA Controls

The recent Coronavirus pandemic has led to the unprecedented need for diagnostic tests for detecting the presence of the SARS-CoV-2 virus in a variety of sample types. To address this need, laboratories around the world need high quality tools to enable them in catering to this rapidly expanding requirement for testing.

KEY HIGHLIGHTS

- Fully synthetic RNA generated from Twist Gene Fragments
- >99.9% viral genome coverage
- NGS sequence verified
- Positive control for both RT-PCR and NGS-based assays

SPECIFICATIONS

Standard Controls

- Biosafety: Level 1
- Storage Temperature: -70°C to -90°C
- Specification Range: Approximately $1x10^6$ copies/µL
- Physical State: Frozen liquid

Assay Ready Controls

- Biosafety: Level 1
- Storage Temperature: -20°C
- Specification Range: Approximately $2x10^6$ copies/tube
- Physical State: Dried pellet

Positive controls provide quality control measures for a wide range of applications from diagnostic assay development to day-to-day testing including the verification and validation of diagnostic tests of both next-generation sequencing (NGS) and reverse transcription polymerase chain reaction (RT-PCR) assays. Synthetic viral controls are a powerful alternative to “live virus” controls which are viral nucleic acids extracted from either an infected patient or from live virus propagated in cell culture. Synthetic controls created through gene synthesis broaden access across diverse strains while mitigating safety and security concerns.

The Twist Synthetic SARS-CoV-2 RNA controls consist of six non-overlapping 5 kb fragments generated from Twist Gene Fragments then transcribed into ssRNA. These provide coverage of greater than 99.9% of the bases of the viral genome. Standard Controls are supplied in 100 µL at a concentration of approximately one million copies per microliter. Twist also offers certain controls in an Assay Ready Format that are supplied as a desiccated pellet. The Assay Ready Controls are supplied at approximately two million copies per tube.

Twist has created synthetic RNA controls from several sequence variants of the SARS-CoV-2 virus. These variants were selected to cover a wide range of sequence diversity of this evolving virus. Twist Synthetic RNA Control 2, MN908947.3, is one of the initial isolates of SARS-CoV-2 and serves as a reference sequence. As new variants of scientific or human health importance are identified, they will be added to the product offering. For the most up to date listing see the Twist Synthetic RNA Controls Product Listing at twistbioscience.com.

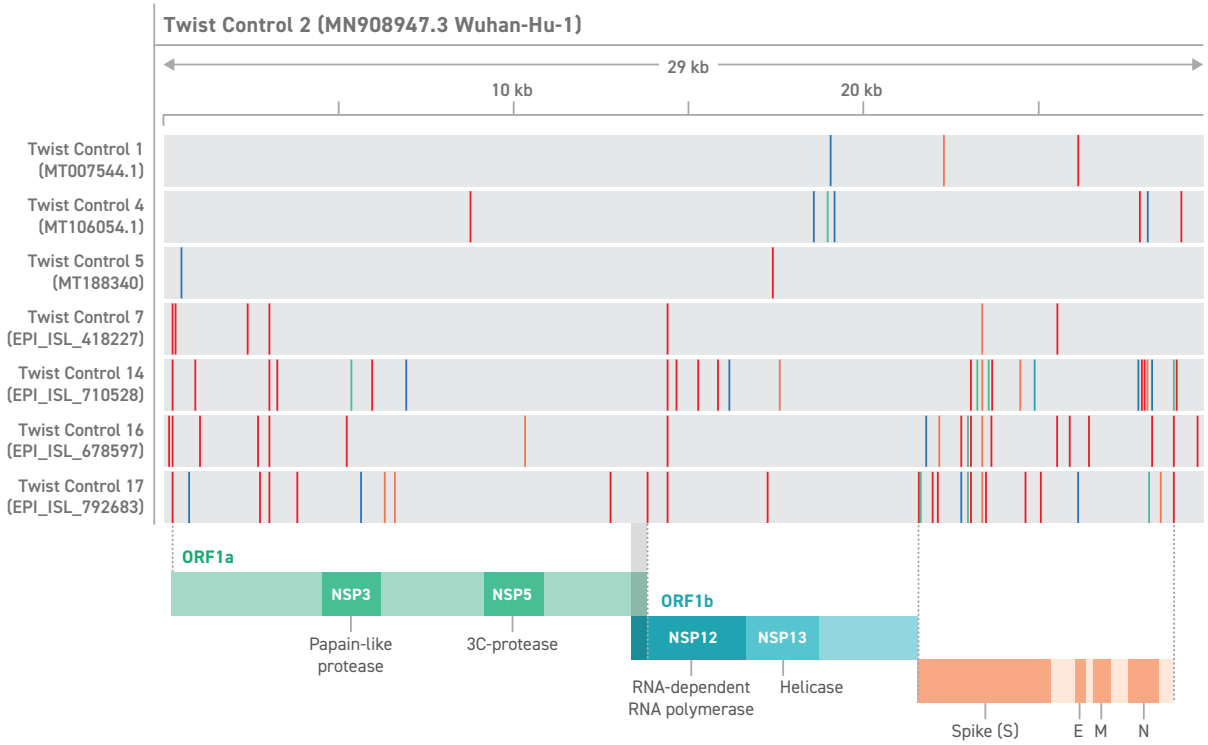

Figure 1: Genome browser view showing the nucleotide variations among the select isolates (all isolates not shown). Seven of the strains were mapped to the reference isolate (MN908947.3). The colored lines indicate SNPs where Red is a "T", Orange is a "G", Green is an "A" and Blue is a "C". The box diagram overlays the approximate location of the SNPs on top of the ORF/Gene structure of the virus. The Twist Controls 14, 16, and 17 represent the B.1.1.7, B.1.351 and P.1 variants respectively.

Over the course of the pandemic, selective pressure has resulted in the emergence of hotspots in the SARS-CoV-2 genome for mutations that have made the virus more contagious. The spike protein region (Figure 1) was the first to emerge, with a key mutation at D614G that appeared to confer greater transmissibility*. Additional mutations, as well as deletions in the spike region were also identified to be widespread as the virus has evolved. Later, a highly contagious lineage (B.1.1.7) was identified from surveillance studies in the United Kingdom, with numerous spike protein mutations, nucleocapsid protein mutations, and other non-structural protein regions NSP3 and NSP12**. Variant sequences differ from each other at several locations along the viral genome as shown in Figure 1.

New variants continue to arise, at present, there are thousands of variants of the SARS-CoV-2 virus available in public repositories. Leveraging our powerful silicon-base writing platform, Twist is able to manufacture and deliver customized synthetic viral genome controls based on viral reference sequences within approximately two weeks. Customers purchasing synthetic controls are subject to Twist's leading biosecurity screening protocols and applicable laws and regulations.

The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity. Lizhou Zhang, Cody B Jackson, Huihui Mou, Amrita Ojha, Erumbi S Rangarajan, Tina Izard, Michael Farzan, Hyeryun Choe. bioRxiv 2020.06.12.148726; doi: https://doi.org/10.1101/2020.06.12.148726

**Rambaut A, Loman N, Pybus O, Barclay W, Barrett J, Carabelli A, et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. Virological; 2020.*

Twist Synthetic SARS-CoV-2 RNA controls are a component of the Twist Infectious Disease portfolio of products.

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