Promega

Product Application

Sanger Sequencing of SARS-CoV-2

Sequence a region of the SARS-CoV-2 genome using ProDye™ Terminator Sequencing System with analysis by capillary electrophoresis on the Spectrum Compact CE System.

Kit: Spectrum Compact CE System (Cat.# CE1304)

Analysis: Capillary electrophoresis

Sample Type: Nucleic acid purified from nasal swabs

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM672 and the Instrument Operating Manual TMD058, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- Spectrum Compact CE System (Cat.# CE1304)
- Spectrum Compact Capillary Array, 4-Capillary, 36cm (Cat.# CE2340)
- Hi-Di™ Formamide (ThermoFisher Scientific, Cat.# 4401457)
- Strip Septa Mat, 8-well (Cat.# CE2308)
- Spectrum Compact Buffer (Cat.# CE2300)
- Spectrum Compact Polymer 7 (Cat.# CE2307)
- MicroAmp Optical 8-Tube Strip, 0.2ml (ThermoFisher Scientific, Cat.# 4316567)
- Absolute Ethanol (ThermoFisher Scientific Cat.# T038181000, or similar)
- 0.5M EDTA (Cat.# V4231)
- ProDye™ Terminator Sequencing System (Cat.# CR4302)

Protocol:

1. Generate an amplicon of the SARS-CoV-2 region of interest by RT-PCR, and clean up the amplification reaction. Measure the concentration of the SARS-CoV-2 amplicon by fluorescent dye quantitation (e.g. QuantiFluor® ONE dsDNA Dye, Cat.# E4871).
2. Follow the ProDye™ Terminator Sequencing System Technical Manual (#TM672) to set up and thermal cycle the sequencing reactions.
3. Clean up sequencing reactions by EDTA/ethanol precipitation as described in the ProDye™ Terminator Sequencing System Technical Manual (#TM672).
4. Resuspend the reactions in 10µl Hi-Di™ Formamide.
5. Analyze samples on the Spectrum Compact CE System with the T_Seq_36_Std protocol. Prior to running samples, the Spectrum Compact CE System and the Capillary Array should be calibrated according to the Spectrum Compact CE System Operating Manual (#TMD058).

Results:

Synthetic SARS-CoV-2 RNA was spiked into total nucleic acid eluates purified from nasal-swab inoculated Universal Viral Transport Medium (BD, Cat.# 220220) with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330). Amplification of a section of the viral spike encoding region of the SARS-CoV-2 genome was carried out with the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) and primers from this reference[1]. The amplicon was cleaned-up with the ReliaPrep™ DNA Clean-up and Concentration System (Cat.# A2892) and diluted to 5ng/µl based on quantification with the QuantiFluor™ ONE dsDNA System (Cat.# E4871) and the Quantus™ Fluorometer (Cat.# E6150). 10ng of the amplicon was sequenced with the ProDye™ Terminator Sequencing System (Cat.# CR4302) in the forward and reverse directions with the same primers used to generate the amplicon. Following clean up by EDTA/ethanol precipitation as described in TM672, the sequencing reactions were resuspended in Hi-Di™ Formamide (Applied Biosystems™, Cat.# 4311320) and analyzed by capillary electrophoresis on the Spectrum Compact CE System (Cat.# CE1304). In Figure 1 below, samples were spiked with Twist synthetic SARS-CoV-2 RNA Control 14 (alpha variant, B.1.1.7; Twist Biosciences, Cat.# 103907) and sequenced as described. The expected point mutations in the SARS-CoV-2 genome, which correspond to characteristic amino acid subsitutions in the spike protein, were observed: A23063T (N501Y), C23271A (A570D), A23403G (D614G), C23604A (P681H), C23709T (T716I). In Figure 2 below, samples were spiked with Twist synthetic SARS-CoV-2 RNA Control 16 (beta variant, P.1; Twist Biosciences, Cat.# 104403) and sequenced as described. The expected point mutations in the SARS-CoV-2 genome, which correspond to characteristic amino acid subsitutions in the spike protein, were observed: G20312A (E484K), A23063T (N501Y), A23403G (D614G), and C23664T (A701V).

References:

1. Jorgensen, T.S. (2021) Sanger sequencing of a part of the SARS-CoV-2 spike protein. https://www.protocols.io/view/sanger-sequencing-of-a-part-of-the-sars-cov-2-spik-bsbdnai6. Accessed 06/2021.

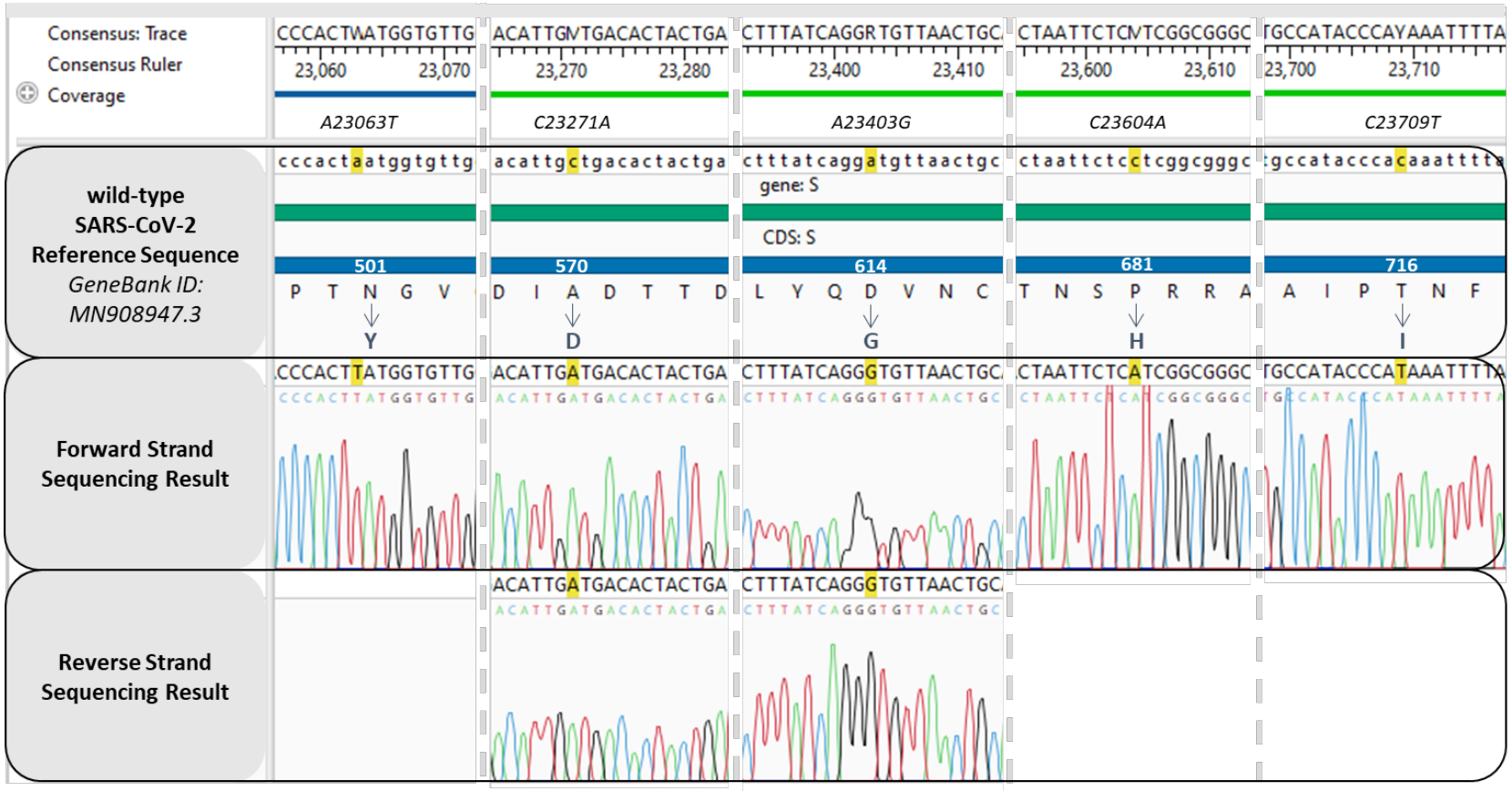

Figure 1. Example forward and reverse strand sequencing traces for amplicon sequencing of the SARS-CoV-2 spike gene (alpha variant) aligned to wild-type SARS-CoV-2 genome. Total nucleic acid was purified with the Maxwell® RSC Viral Total Nucleic Acid Kit (Cat.# AS1330) from Universal Transport Medium for Virus (BD) inoculated with a nasal swab from an uninfected individual. Synthetic SARS-CoV-2 RNA Control 14 (Twist Biosciences, Cat.# 103907) was spiked at 10^5 copies/µl into the resulting nucleic acid eluate. The target region of the spike coding sequence was amplified and sequenced with the ProDye™ Terminator Sequencing System (Cat.# CR4302) as described above. The sequencing reactions were analyzed by capillary electrophoresis on the Spectrum Compact CE System (Cat.# CE1304). The sequencing traces were aligned to the published wild-type SARS-CoV-2 genome sequence (GeneBank ID: MN908947.3) using the DNASTAR SeqMan Ultra® tool. Mutations with respect to the wild-type sequence are indicated by the yellow highlighted bases, and the resulting amino acid changes and their positions within the spike protein are indicated.

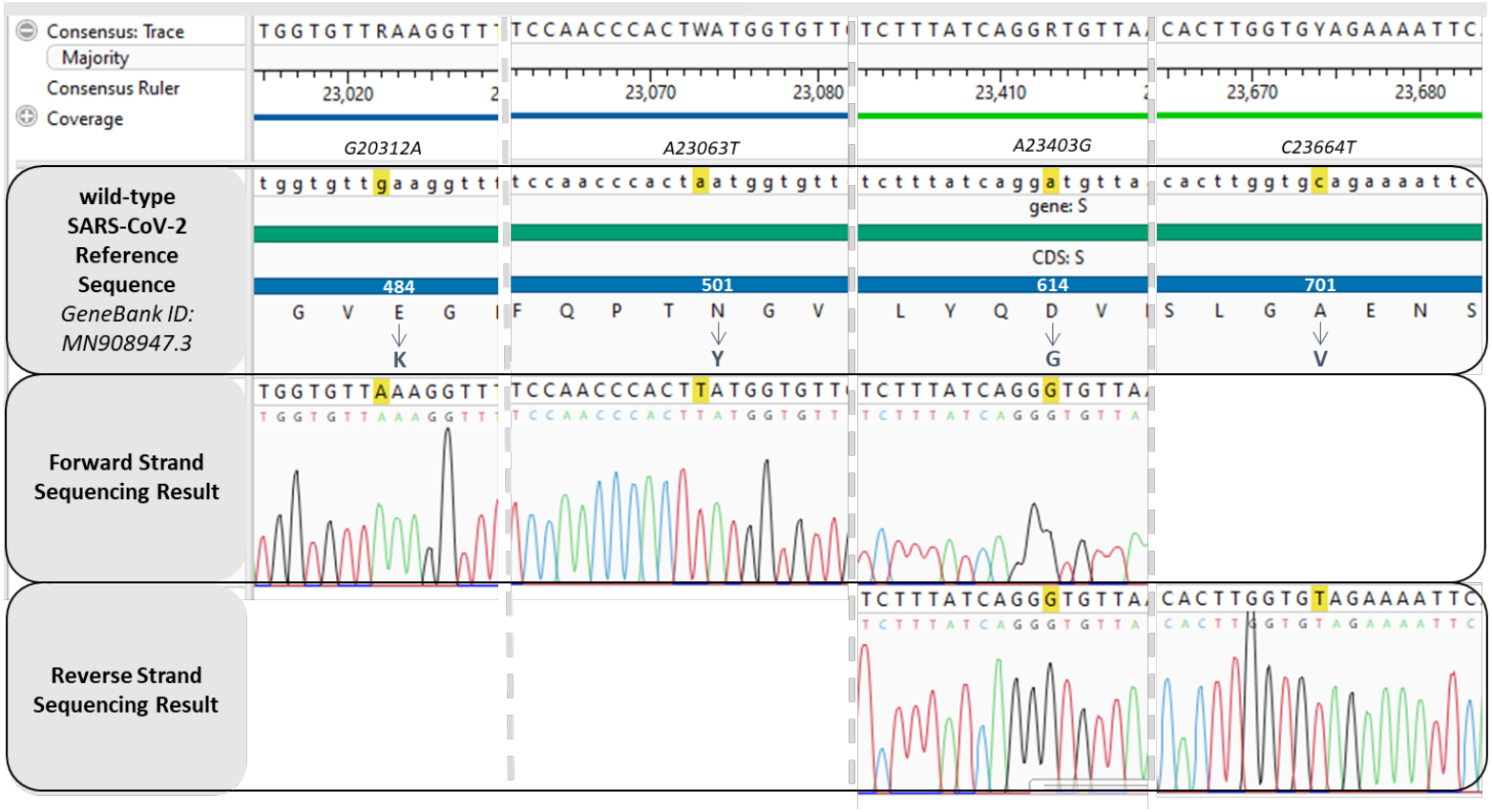

Figure 2. Example forward and reverse strand sequencing traces for amplicon sequencing of the SARS-CoV-2 spike gene (beta variant) aligned to wild-type SARS-CoV-2 genome. Total nucleic acid was purified with the Maxwell® RSC Viral Total Nucleic Acid Kit (Cat.# AS1330) from Universal Transport Medium for Virus (BD) inoculated with a nasal swab from an uninfected individual. Synthetic SARS-CoV-2 RNA Control 16 (Twist Biosciences, Cat.# 104403) was spiked at 10^5 copies/µl into the resulting nucleic acid eluate. The target region of the spike coding sequence was amplified and sequenced with the ProDye™ Terminator Sequencing System (Cat.# CR4302) as described above. The sequencing reactions were analyzed by capillary electrophoresis on the Spectrum Compact CE System (Cat.# CE1304). The sequencing traces were aligned to the published wild-type SARS-CoV-2 genome sequence (GeneBank ID: MN908947.3) using the DNASTAR SeqMan Ultra® tool. Mutations with respect to the wild-type sequence are indicated by the yellow highlighted bases, and the resulting amino acid changes and their positions within the spike protein are indicated.