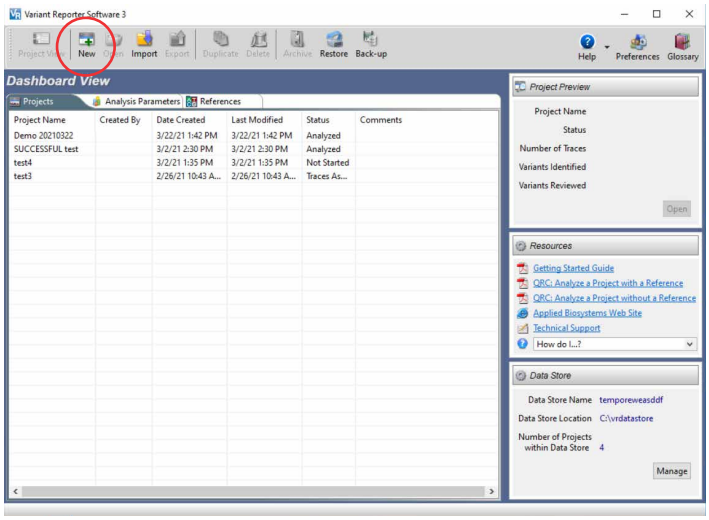
Suggestions for SARS-CoV-2 variant analysis of Sanger sequencing traces using Variant Reporter Software

Detecting and confirming mutations in newly arising SARS-CoV-2 strains is an important strategy for controlling the spread of new viral lineages. Recently we published four protocols that use Sanger sequencing to analyze SARS-CoV-2 isolates (protocols available at **thermofisher.com/sangercoronavirus**). Although the data contained in the sequence files can be compared against known sequences using a BLAST™ search, in some cases a simpler and more definitive workflow is needed. In this protocol, we present options for SARS-CoV-2 variant analysis using Sanger sequencing files and Applied Biosystems™ Variant Reporter™ Software. Although this workflow is not comprehensive, it is presented to help users begin to better understand their results. To take advantage of the complete features of the software, please see the user guide.

Setup of a basic project in Variant Reporter Software generally involves the following steps: importing sequencing traces (.ab1 files), defining a reference sequence, importing known variants of the reference sequence, and importing primer sequences defining the PCR amplicons.

1. Create new project



The Import and Manage Traces page is used to import traces into your project, creating the Amplicon and Specimen Trace Grid. From the application dashboard, click the New icon to create a new project.

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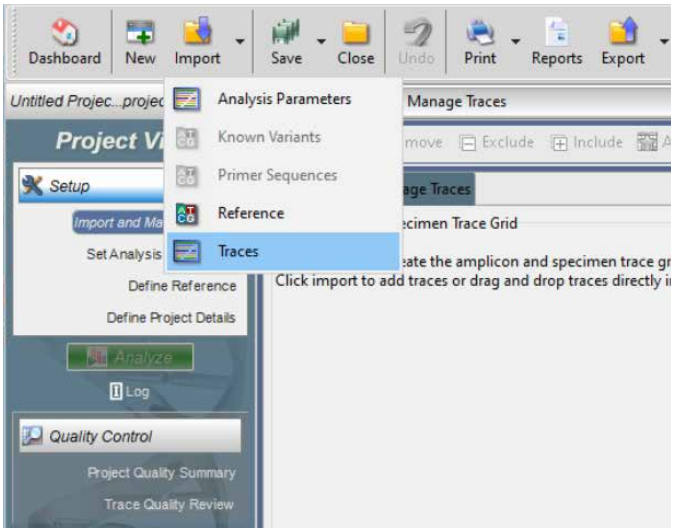
2. Import sequencing traces

A



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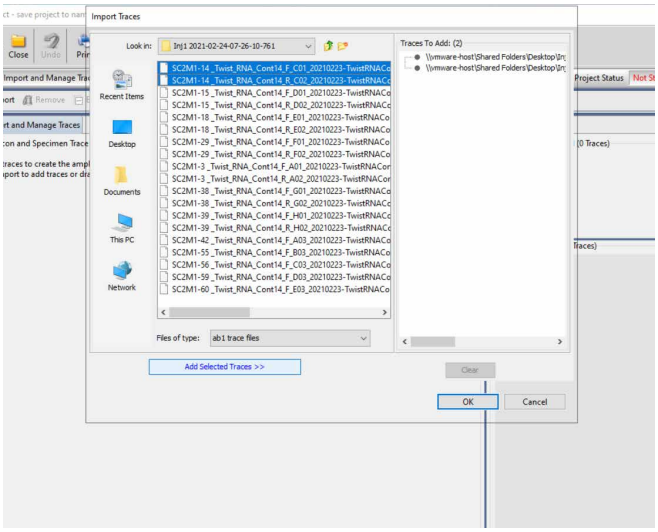

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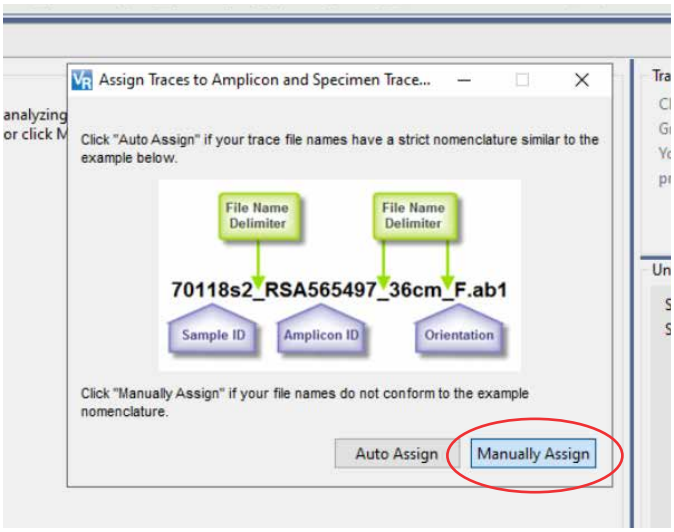

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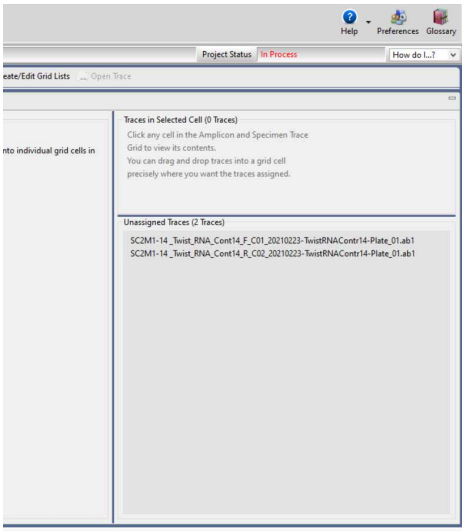
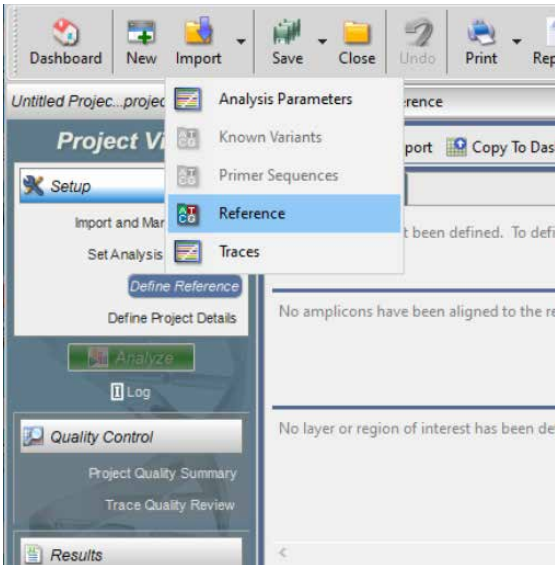


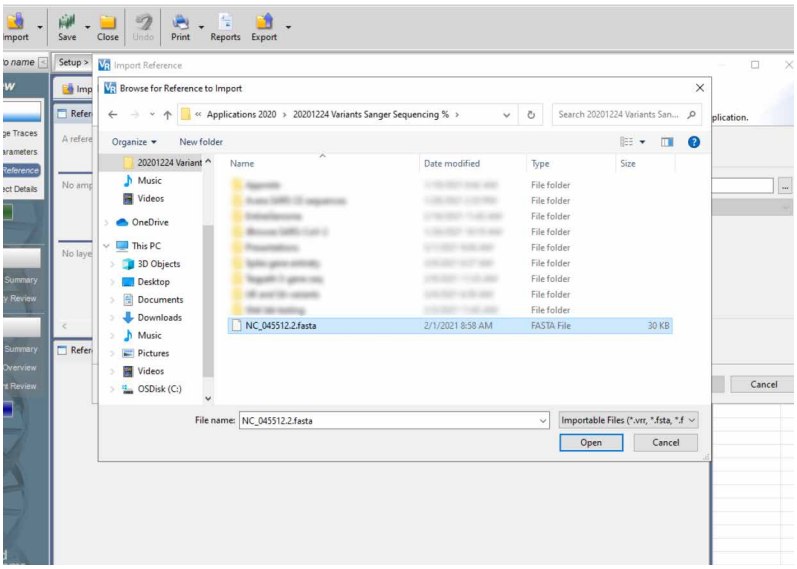
(A) To import the sequencing traces, choose Traces from the Import menu. **(B)** Next, navigate to the folder containing the traces and choose all that are to be imported. Select all files to be analyzed, click Add Selected Traces, then click OK. **(C)** If the file name is in the appropriate format (see example above), the traces can be assigned sample names, amplicons, and sequencing direction automatically. However, for the purposes of this demonstration, the files will be assigned manually by clicking the Manually Assign button. **(D)** Once the button is clicked, traces that have been successfully imported but not yet assigned to amplicons will be listed in the bottom-right quadrant of the subsequent window.

3. Import reference sequence

A



B



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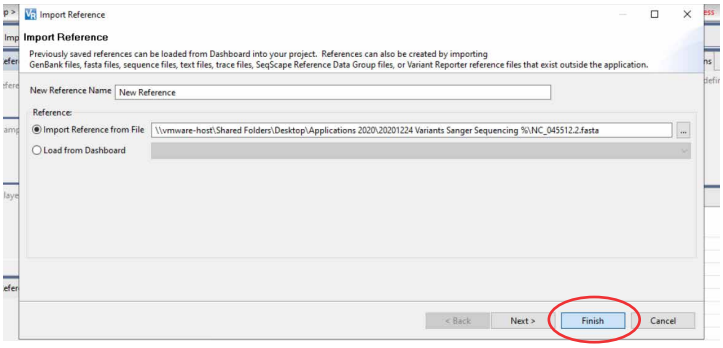

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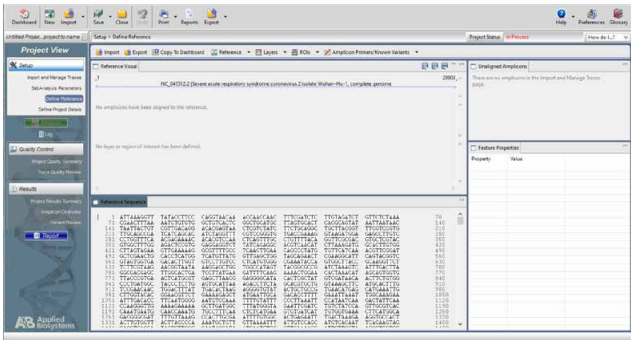
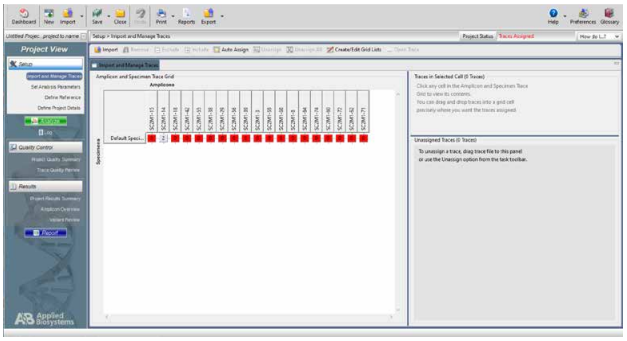


(A) To import the reference sequence, choose Reference from the Import menu. **(B)** Navigate to the reference text file. In this case, the SARS-CoV-2 reference strain NC_045512.2 is used. Variant Reporter Software also supports reference sequences in the .gb file format to include sequence annotations. The FASTA file can be downloaded from **thermofisher.com/sangercoronavirus**. Choose the desired FASTA file and click Open. **(C)** A window appears confirming the location of the file and giving an opportunity to rename it. When completed, click Finish. **(D)** The imported sequence will appear in its own pane at the bottom of the window.

4. Import variants

A



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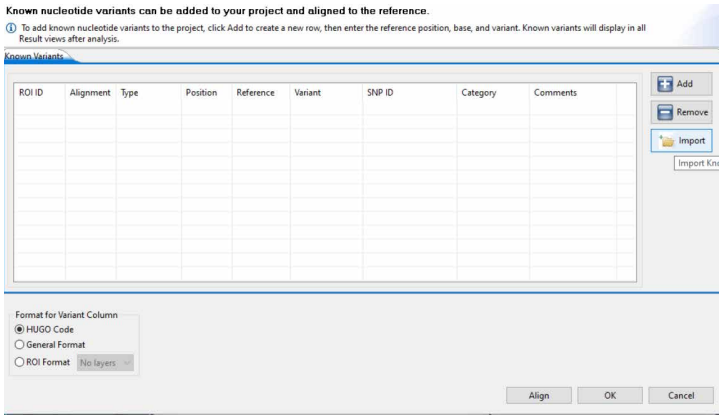

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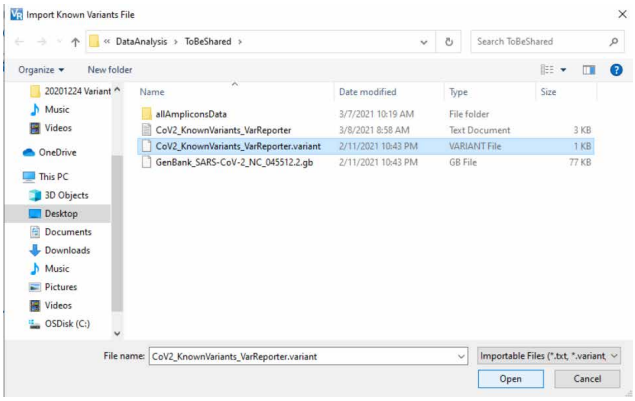

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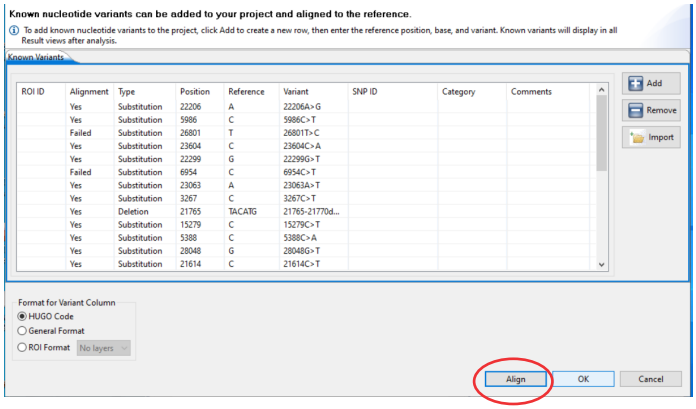
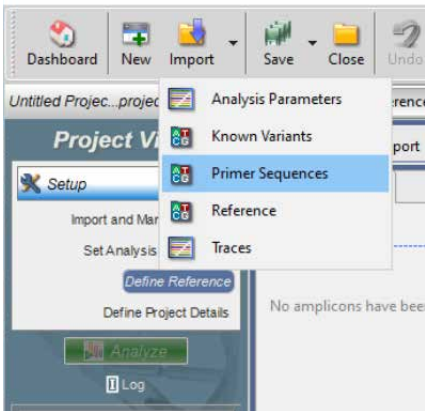


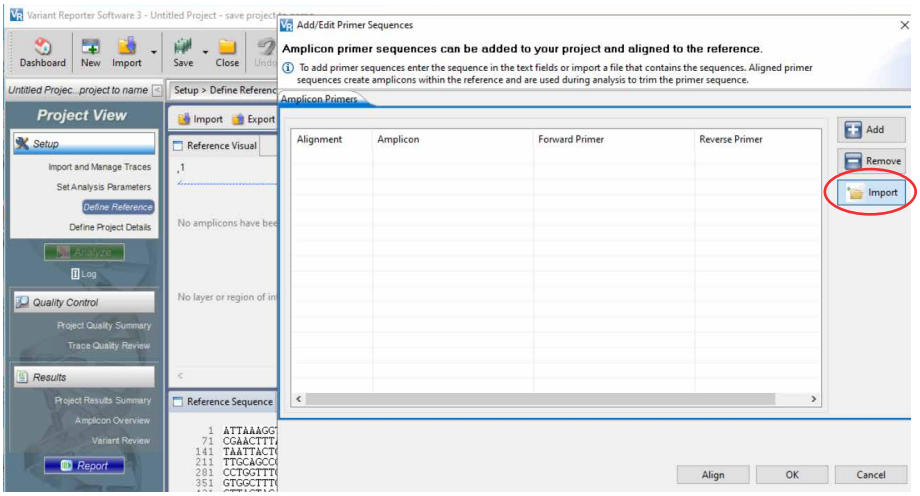
(A) To import the set of known variants, choose Known Variants from the Import menu. **(B)** In the next window, click the Import button and navigate to the location of the variants text file. A text file with a list of mutations found in variants of concern (VOC) can be downloaded from **thermofisher.com/sangercoronavirus**. New variants can be added manually by clicking the Add button in this window. **(C)** Choose the desired variant file (it will have the file extension .variant) and click Open. **(D)** In the final window, click Align to match the variants to the reference sequence, then click OK.

5. Import primer sequences that define amplicons used

A



B



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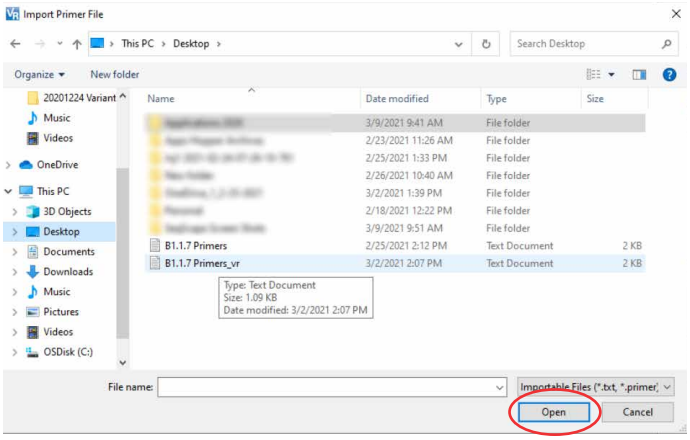

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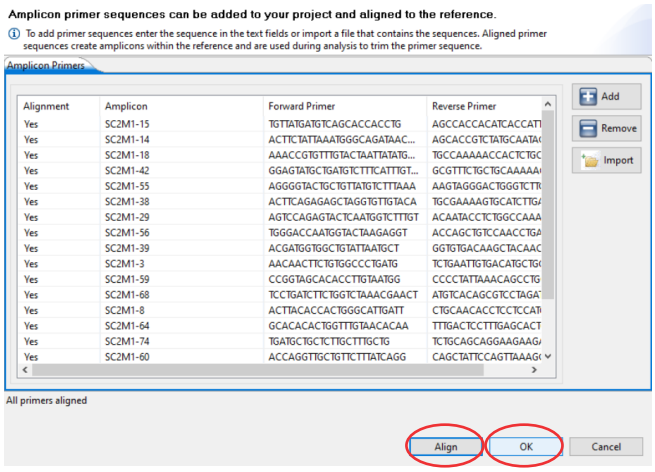
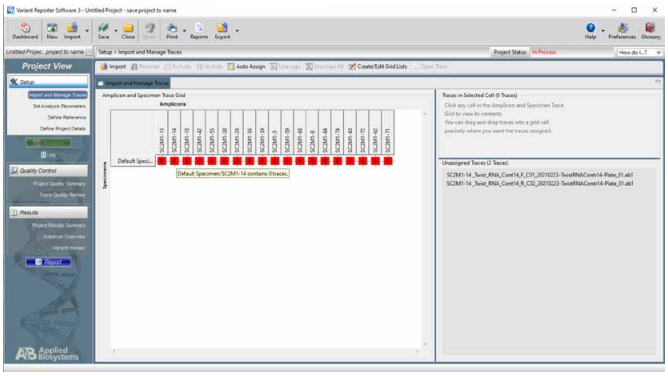


Next, the sequences of the primers defining the amplicons need to be imported. A text file containing primer sequences can be downloaded from **thermofisher.com/sangercoronavirus**. Download the file to a local computer. **(A)** From the Import menu, choose Primer Sequences. **(B)** A window appears; choose Import and navigate to the location of the downloaded text file. **(C)** Choose the file and click Open. **(D)** Once the primers have been imported, align them to the reference sequence by clicking the Align button. If the primers are found in the reference sequence, the alignment column will show Yes. When all primers have been imported successfully, click OK.

6. Assign traces to sequencing primers (manual)

A



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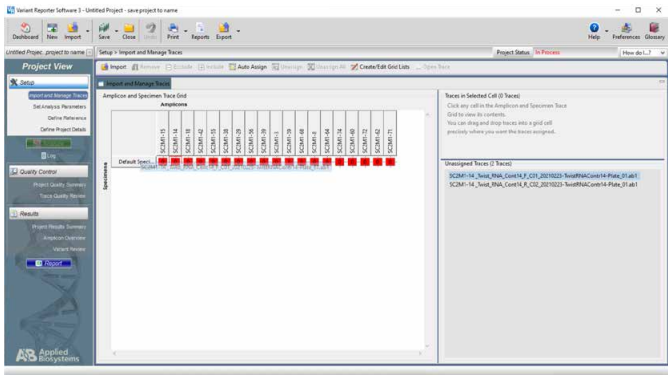

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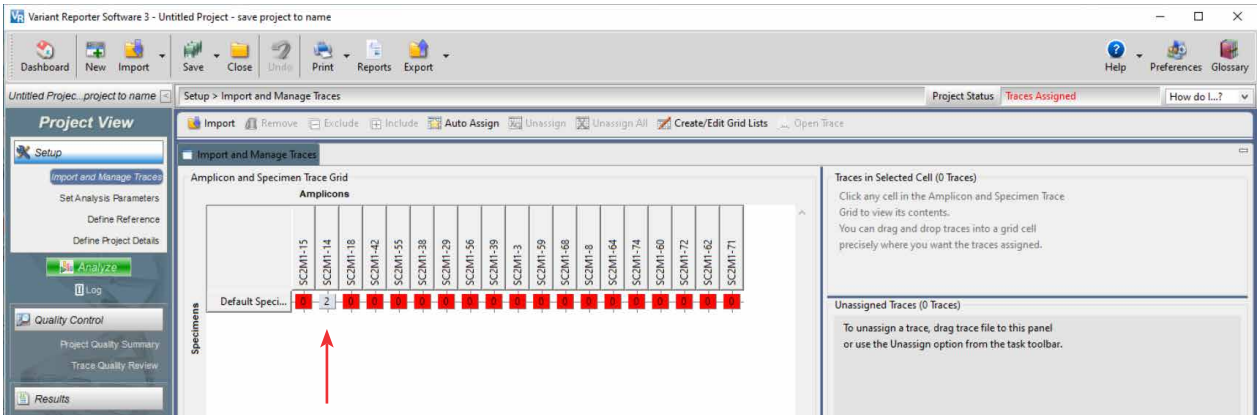
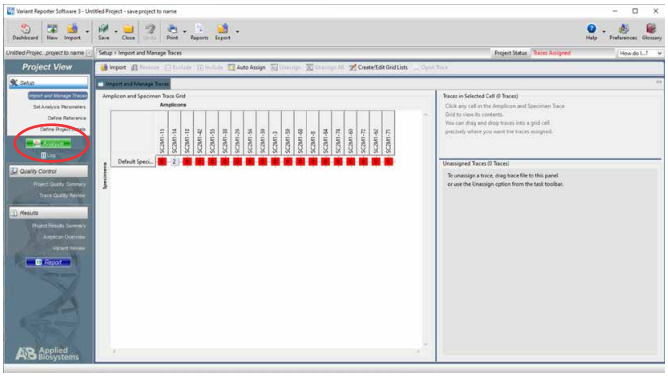


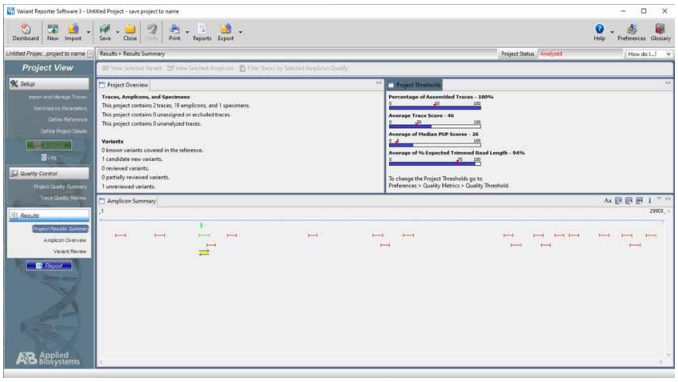
(A) When primers are imported, a grid showing all the primer pairs will appear. Next, sequencing traces will be assigned to the correct primer pairs in the grid. To assign the traces to the primers, drag and drop the file from the list on the right to the correct position in the grid on the left. **(B)** Here, we are moving the file SC2M1-14_Twist_RNA_Cont14_F_C01_20210223-TwistRNAContr14-Plate_01.ab1 to the square under the SC2M1-14 box in the Amplicon and Specimen Trace Grid. **(C)** The color of the box will change from red (empty) to yellow when one trace is added, then gray when the reverse trace is added. Forward and reverse traces from the same amplicon are added to the same square in the grid. Continue until all traces are assigned.

7. Analyze traces

A



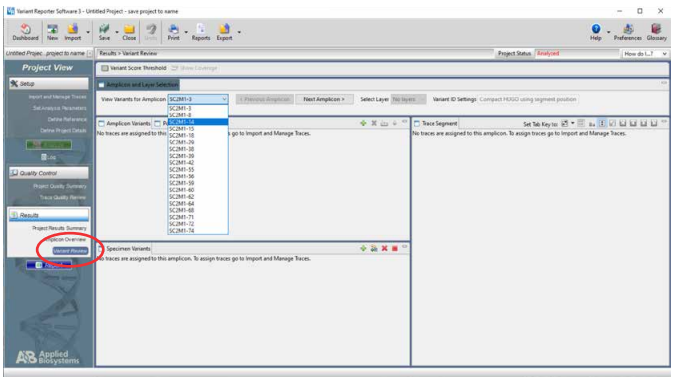
B



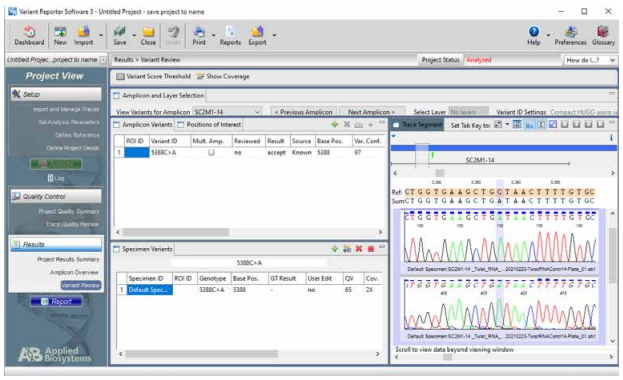
(A) To analyze the traces, click the green Analyze button on the left. **(B)** The position of the amplicon is shown in green, and the traces that were successfully analyzed are shown below the amplicon and highlighted in yellow. In addition, a summary of the analyzed traces is shown on the right side of the window.

8. Choose amplicon to view results

A



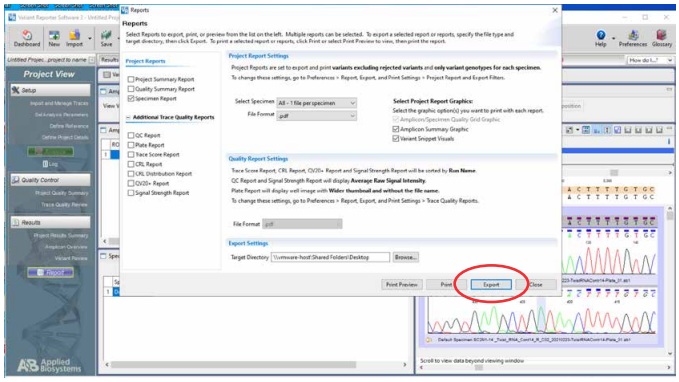
B



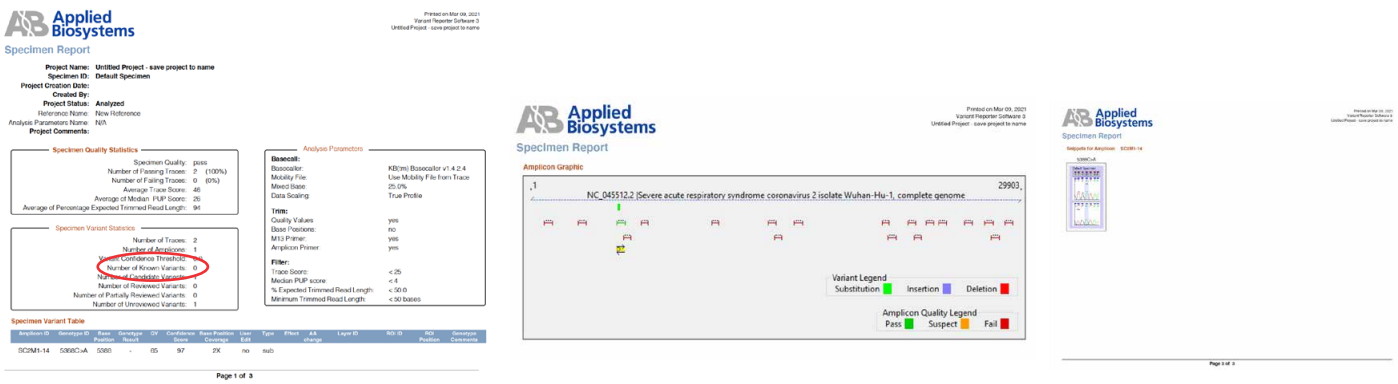
(A) Variants can be visualized by selecting Variant Review from the Project View panel on the left side of the screen (red circle). Then, select the amplicon from the drop-down menu as shown. **(B)** Variants that map to the amplicon will be shown in the next window. Here, the variants within the amplicon and in the sample are listed, as well as the electropherograms showing the sequence change (far right). Navigation between the variants can be done by clicking Next Amplicon at the top of the window.

9. Generate report

A



B



(A) A complete list of the results of the project can be generated by clicking the Reports menu on the main screen. A window with choices for the report appears. The report can be exported by choosing Specimen Report from the choices on the left side of the window, followed by Export. **(B)** Examples of the three pages from the resulting PDF are shown. If any known variants were found in the sample, they will appear on the first page of the report (circled).

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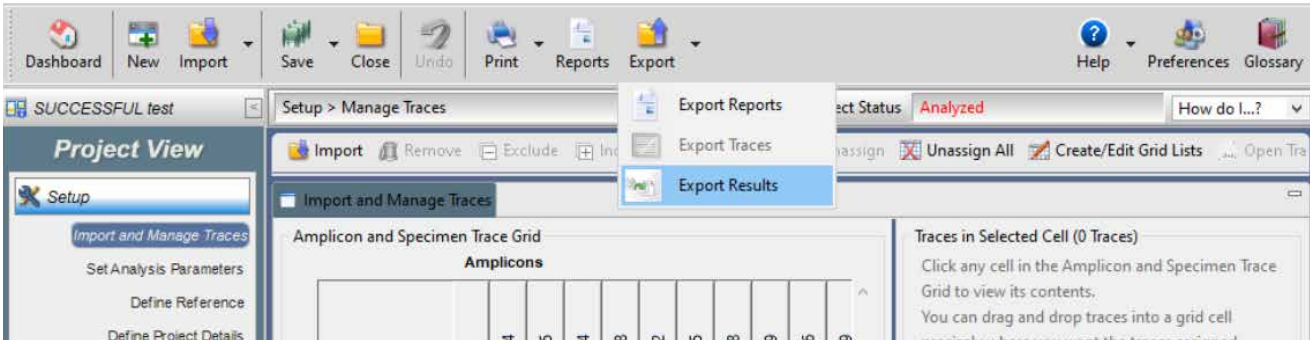
10. Mapping sequences to known strain lineages

A



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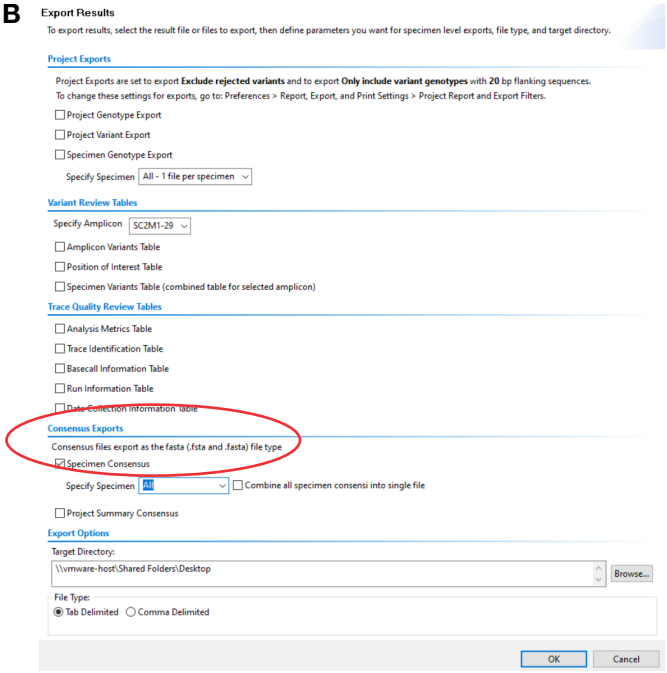

Results can be exported into FASTA files for querying public databases. **(A)** To generate one or more FASTA files, choose Export Results from the Export icon in the toolbar. **(B)** In the window that appears, choose Specimen Consensus near the bottom of the form (red circle). Choose which specimens to export, specify the directory where the exported files will appear, then click OK. A text file containing FASTA files for each of the amplicons in each specimen will be generated. Sequences in this file can be used to query strain lineage databases. One of these, the GISAID database (**epicov.org/epi3/frontend#637476**), provides BLAST search results against all known SARS-CoV-2 strains. Up to 10 FASTA sequences can be queried at once. Registration is required, but use is free. The Coronavirus Typing Tool (**genomedetective.com/app/typingtool/cov**) also accepts FASTA files. Single FASTA files can be queried at no charge, but batch uploading requires premium access.

Find out more at **thermofisher.com/sangercoronavirus**

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