Next-generation Confirmation (NGC) module

Catalog Number A28221

Pub. No. MAN0015891 **Rev.** A.0

Product description

The Applied Biosystems™ Next-generation Confirmation (NGC) module analyzes trace files (*.ab1) generated on Applied Biosystems™ Genetic Analyzers. The NGC module confirms variant identification by comparing trace files to a variants file (*.vcf) generated on a next-generation sequencing (NGS) instrument. The software is available on the Thermo Fisher Cloud.

This document provides a workflow to illustrate how to use the software. For more information, access the *Next-generation Confirmation (NGC) module Help* (Pub. No. MAN0011100) from within the software.

Workflow

"Create a project" on page 2
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Supported browsers and instruments

Component	Supported
Browsers	• Google™ Chrome™ v.25 or later • Microsoft™ Internet Explorer™ v.11 or later • Apple™ Safari™ v.7 or later • Mozilla™ Firefox™ v.16 or later
Applied Biosystems™ instruments	Trace files (*.ab1) from: • 310 Genetic Analyzer • 3130/3130*xl* Genetic Analyzers • 3500 Series Genetic Analyzers • 3730/3730*xl* DNA Analyzers

Recommended naming conventions for *.ab1 files

The Organize function groups *.ab1 files by specimen and amplicon before analysis. For easy grouping, we recommend that you use the following naming conventions for *.ab1 files.

Note: If your *.ab1 files do not follow the naming conventions specified below, the NGC module will still be able to analyze the files.

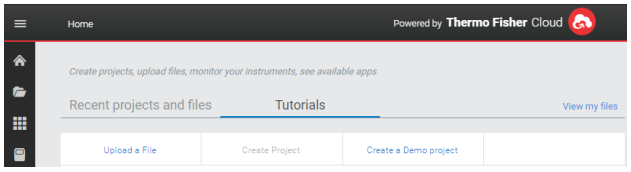
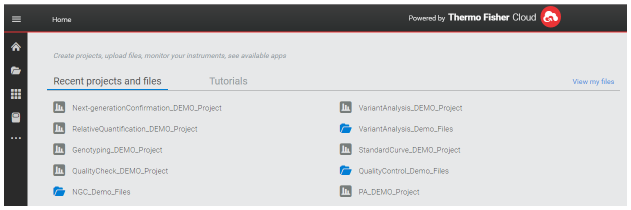
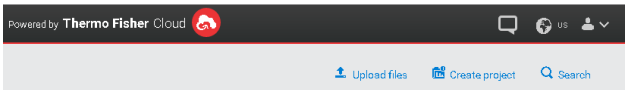
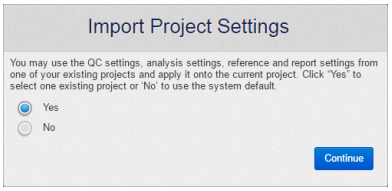
- Include at least:
  - The specimen name
  - The amplicon name (or Assay ID for Primer Designer™ Tool samples)
- (Optional) Add other information as needed, such as the well number and orientation.
- Include delimiters between each piece of information (for example, between the specimen and amplicon names). The Organize function recognizes the following delimiters: - _ $. %.

Examples

File-naming convention	Example
<SpecimenName>_<AmpliconName (or Assay ID)>.ab1	NA19240_328600.ab1
<SpecimenName>_<AmpliconName (or Assay ID)>_<WellNo>_<Orientation>.ab1	NA19240_328600_A01_fwd.ab1

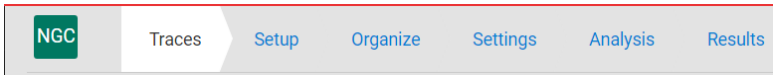
Create a project

Go to **apps.thermofisher.com**, click Sign In, enter your username and password (or click **Create an account**), then:

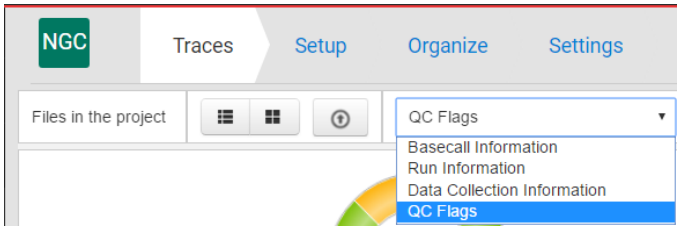
Create a Demo project	*OR*	Create your own project
1. Click **Tutorials** ▸ **Create a Demo project.**  2. At the bottom of the Tutorials screen, click Create a Demo Project, then click **OK**. 3. Click **Recent projects and files**, then click **Next-generationConfirmation_DEMO_Project**.  **Note:** It may take several minutes for the Demo project to appear in the Recent projects and files screen. The module opens.		1. Click **Create project**.  2. Enter a project name, select a save location, then click **OK**. 3. Import trace files (*.ab1). Import from local Import from Thermo Fisher Cloud 4. Click NGC. 5. Select **Yes** to import project settings or **No** to use the system default settings, then click **Continue**. 

View trace quality

1. Click **Traces** in the workflow bar (default view).

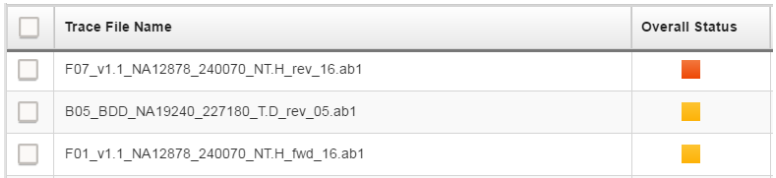


2. Select the information to display: **Basecall Information**, **Run Information**, **Data Collection Information**, or **QC Flags**.



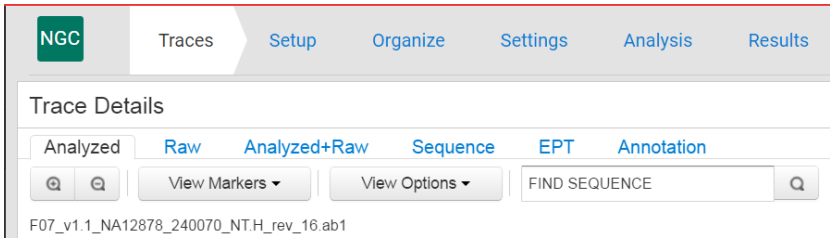
3. Double-click a trace file of interest to open the Trace Details screen.

Note: To compare data across multiple trace files, select two or more trace files in the Traces screen, then click **Actions** ▸ **View Details**.



4. In the Trace Details screen, click a tab to view the data of interest: **Analyzed**, **Raw**, **Analyzed+Raw**, **Sequence**, **EPT**, or **Annotation**.

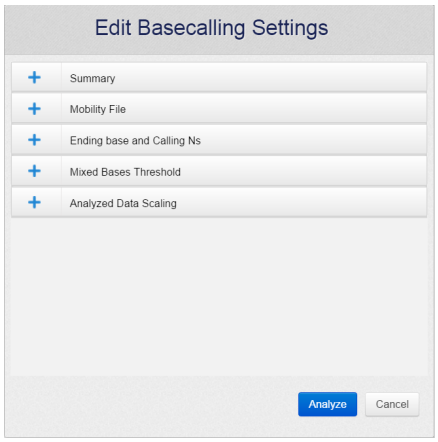
   Note: These tabs are not available if you select multiple trace files in the Traces screen; for multiple trace files, only analyzed data is shown.



Modify data as needed

You can perform any of the following steps as needed to modify the data. You do not need to perform the steps in order.

1. Edit basecalling settings and re-basecall: In the Traces screen, select the checkbox next to each trace file of interest, then click ATGC to open the Edit Basecalling Settings dialog box. Make changes, then click **Analyze** to re-basecall the trace files.



2. Adjust quality flag settings: In the Traces screen, click **Actions** ▸ **Quality Flag Settings** to open the Settings screen. Adjust the settings as needed, then click **Save**.

3. Remove traces: In the Traces screen, select the checkbox next to each trace file of interest, then click **Actions** ▸ **Remove Traces**.

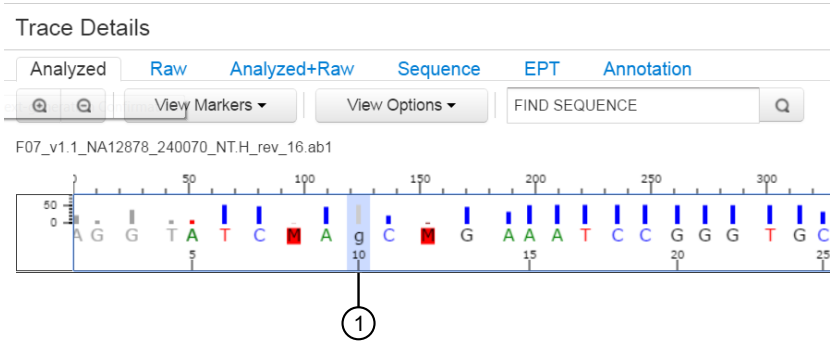
4. Edit bases:

   a. In the Traces screen, double-click the trace file of interest to open the Trace Details screen.

      Note: To edit bases for multiple trace files, select the checkbox next to each trace file of interest, then click **Actions** ▸ **View Details**.

   b. If you selected a single trace file, select the **Analyzed** or **Analyzed+Raw** tab to edit bases.

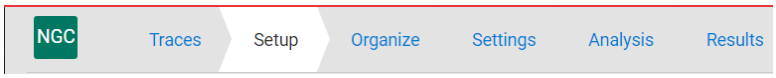
   c. Make changes, then click **Save**. You can insert, delete, or replace bases; changed bases appear in lowercase and the quality values (QVs) are grayed out.



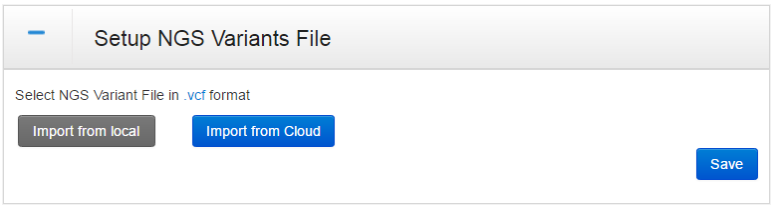
① Edited base

Set up variants and reference files

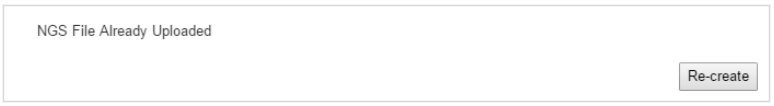
1. Click **Setup** in the workflow bar.



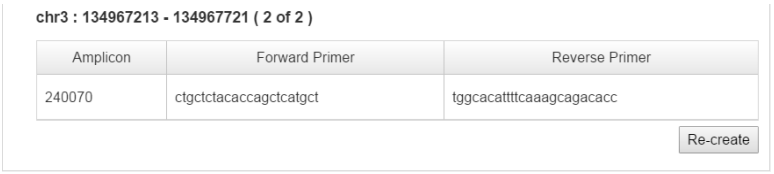
2. *(Optional for NGS confirmation only)* In the **Setup NGS Variants File** pane, import a *.vcf file that contains NGS variants, then click **Save**.



Note: If a variants file already exists, click **Re-create** to import a new file. Alternatively, you can use the existing file and proceed to the next step.



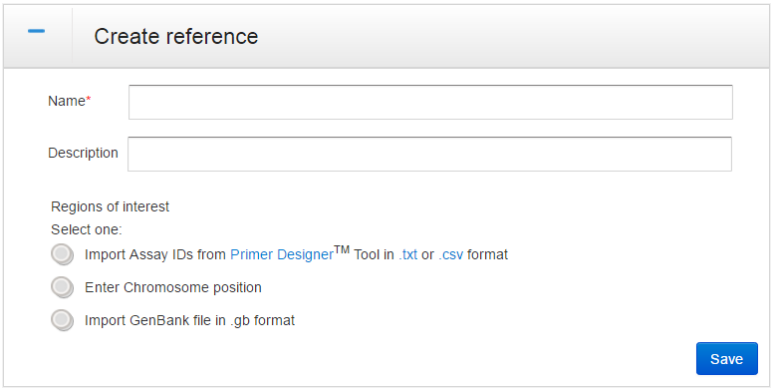
3. *(If a reference already exists)* Click **Re-create** to create a new reference. Alternatively, you can use the existing reference and proceed to "Organize traces" on page 6.



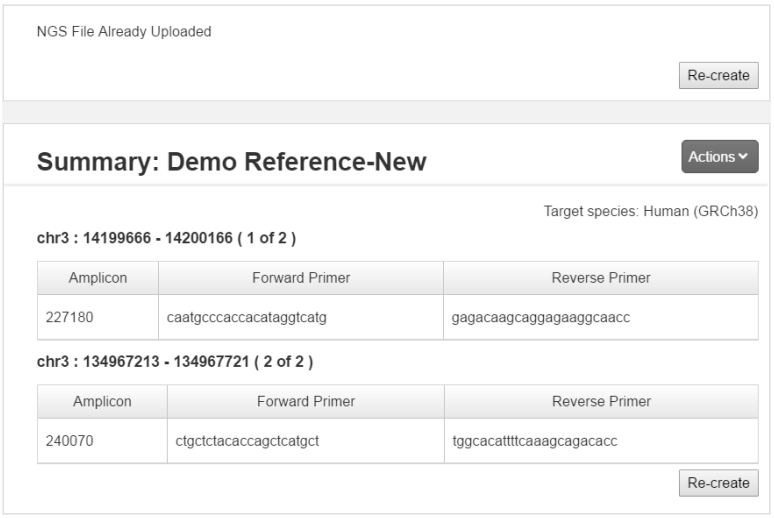
4. Create a reference. In the **Create reference** pane:
   a. Enter a name and description.
   b. For the **Regions of interest**, select one option:
      - Select **Import Assay IDs...**, then click **Choose file**.

        IMPORTANT! To use this option, you must first obtain Assay IDs. Assay IDs are available to customers who are using Thermo Fisher Scientific assay products.

      - Select **Enter Chromosome position**, select a target species, then select a chromosome number and enter start and stop positions. Optionally, click **Choose file** to select a primer file.
      - Select **Import GenBank file...**, then click **Choose file**.
   c. Click **Save**.



5. Review the summary. Click **Actions** ▸ **Export Reference** to export the reference file, or click **Re-create** to discard the reference file and/or NGS variants file and create new ones.

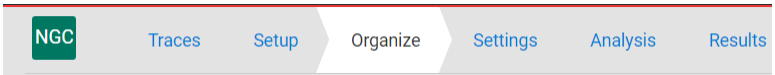


Organize traces

The Organize screen displays folders for the amplicon names specified in the reference file. Use this screen to group together the trace files associated with each amplicon and each specimen.

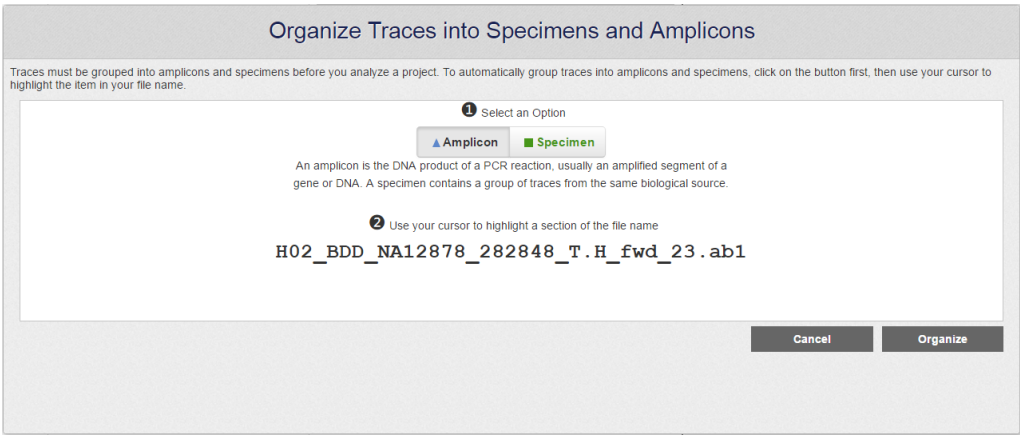
Group traces automatically

1. Click **Organize** in the workflow bar.



2. In the **Un-Grouped Traces** pane, click **Organize**.

3. Group by amplicon and specimen:
   a. Click **Amplicon**, then drag the mouse pointer over the part of the file name that corresponds to the amplicon name.
   b. Click **Specimen**, then drag the mouse pointer over the part of the file name that corresponds to the specimen name.

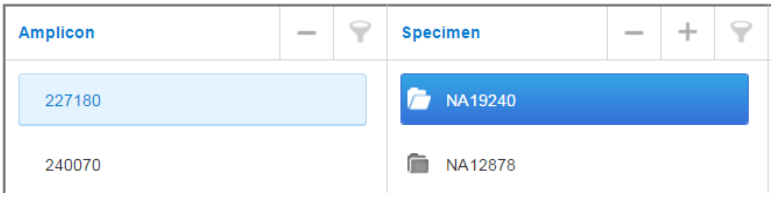


4. Click **Organize**. The software groups all trace files into the appropriate folders.

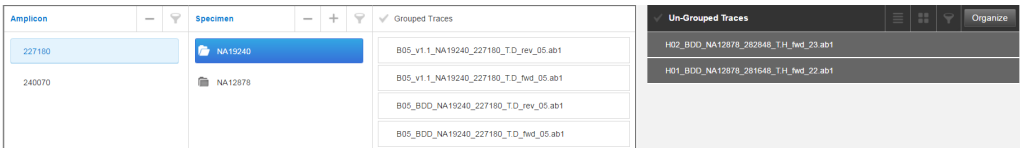
Group traces manually

If any trace files are not automatically grouped, group the trace files manually.

1. In the **Amplicon** and **Specimen** panes, perform the following steps, as needed:
   a. Click to filter the list.
   b. Double-click an amplicon or specimen to rename it.
   c. Click to remove an amplicon.
   d. *(Specimen pane only)* Click to add a specimen.

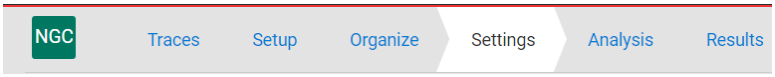


2. Drag trace files from the **Un-Grouped Traces** pane to the appropriate specimen folder in the **Specimen** pane.

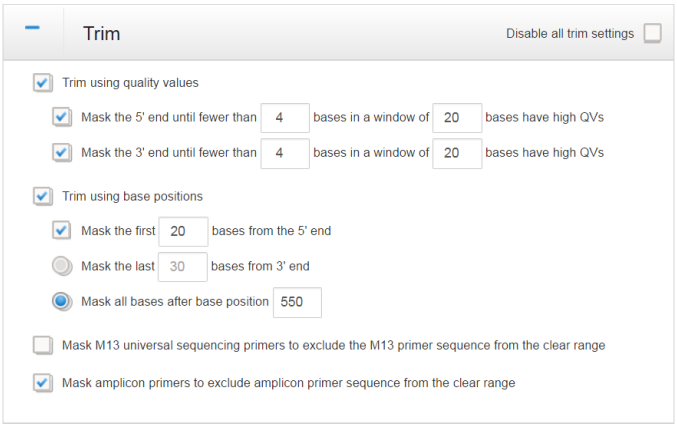


Specify analysis settings

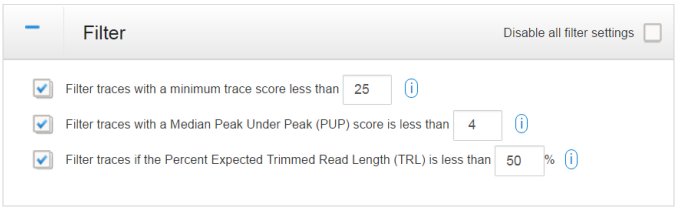
1. Click **Settings** in the workflow bar.



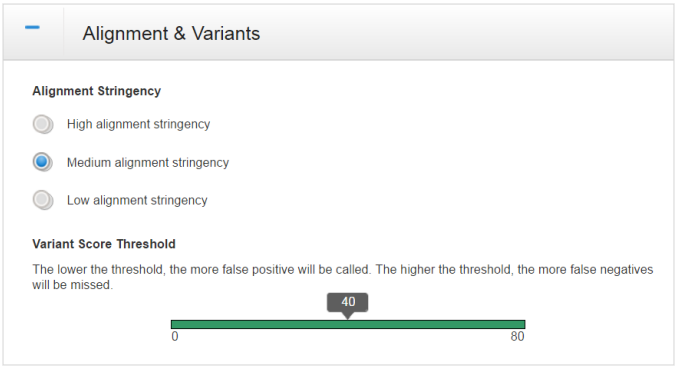
2. Click + to expand the Trim pane, then specify the settings used to trim and mask sequences before analysis.



3. Click + to expand the Filter pane, then specify the settings used to filter out sequences before analysis. Click ⓘ for a description of each setting.



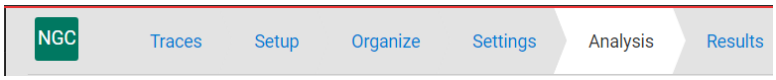
4. Click + to expand the Alignment & Variants pane, then:
   a. Specify the Alignment Stringency to determine the minimum alignment score required for a trace file to be analyzed. Trace files that do not meet the specified stringency are removed from the analysis.
   b. Specify the Variant Score Threshold to control the sensitivity of variant detection.



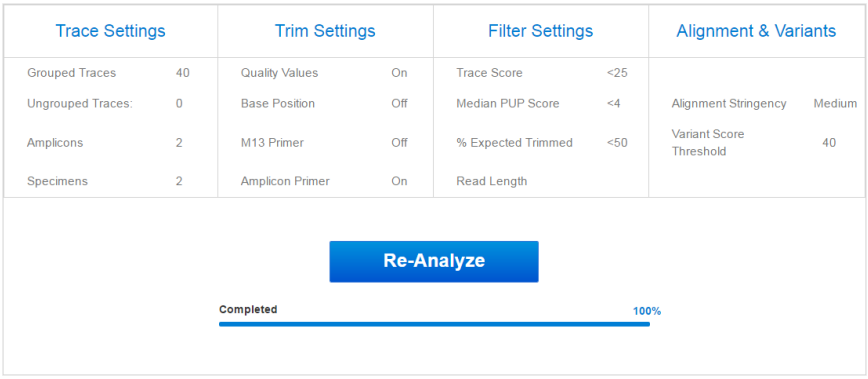
Review the analysis settings and analyze

The Analysis screen displays the analysis settings that you specified in the Settings screen.

1. Click **Analysis** in the workflow bar.



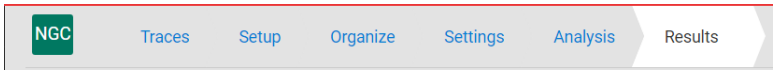
2. Review the analysis settings. If needed, click **Settings** in the workflow bar to make changes.



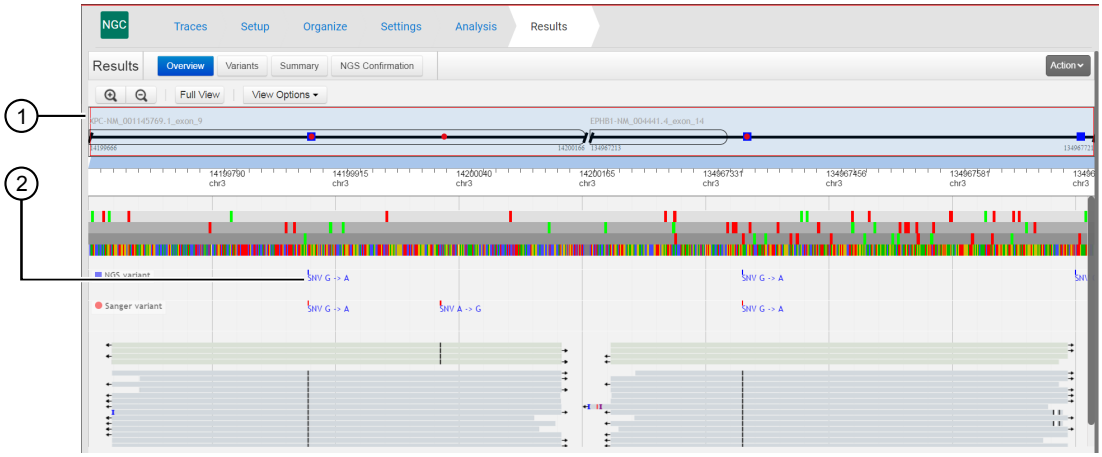
3. Click **Run Analysis** or **Re-Analyze** to analyze the data with the current settings.

Review results and export reports

1. Click **Results** in the workflow bar.

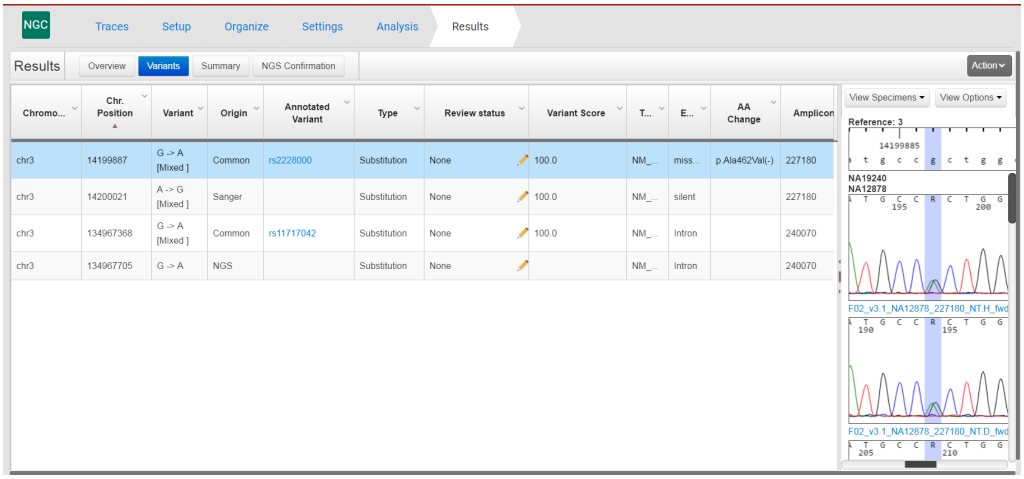


2. Click the **Overview** tab to review alignment coverage and SNP distribution. You can also:
   a. Click Full View to zoom in/out. As you zoom, the selector box (outlined in red) moves to the region of the sequence shown in the zoom area. Click-drag over a region to zoom in on it. Click to the right or left of the box to move to the previous or next region. Use the right or left arrow keys to scroll through regions. As you move the selector box, the blue shading below the box shows the chromosome region selected.
   b. Click **View Options**, then select to view translation and/or specimen information.
   c. Click a variants link to view detailed information about the variant in the **Variants** tab.

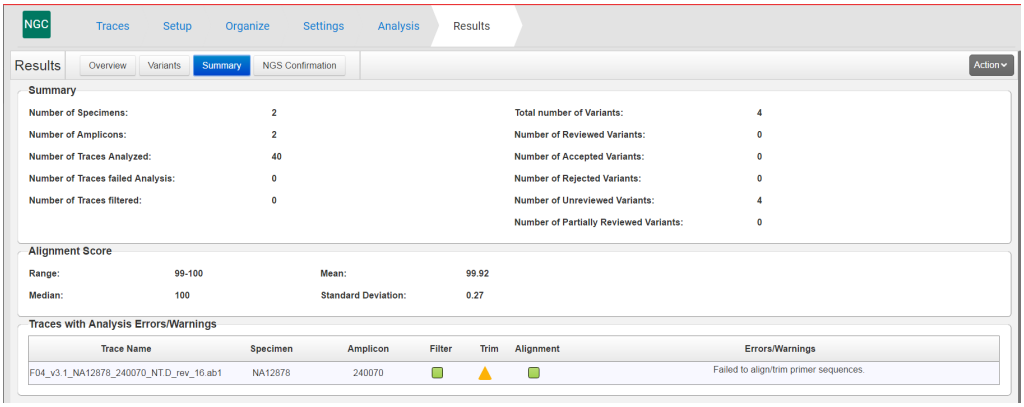


① Selector box
② Variants link

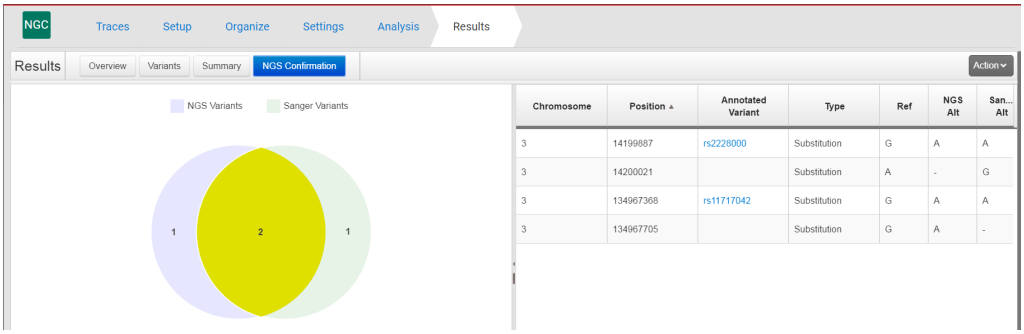
3. Click the **Variants** tab to view all variants found. You can also:
   a. *(For known variants)* Click the links in the **Annotated Variant** column to link to NCBI information on the variant.
   b. Click **View Specimens**, then select the specimens to view.
   c. Click **View Options**, then select electropherogram, basecall, and scaling options.



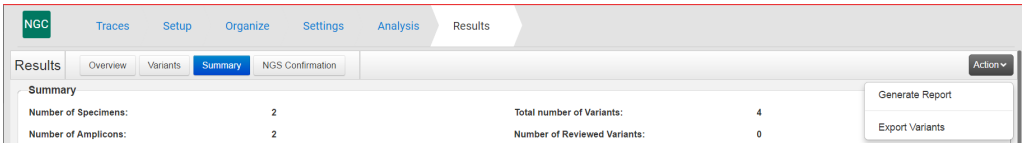
4. Click the **Summary** tab to view a summary of the analysis results.



5. Click the **NGS Confirmation** tab to view the confirmed variants.



6. Export a report from any tab: Click **Actions**, then select:
   - **Generate Report**—Exports a Project Summary Report, Quality Summary Report, or Plate Report (*.pdf file).
   - **Export Variants**—Exports variant information for all NGS variants (*.vcf file) or for Sanger-confirmed NGS variants (*.vcf file).



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Revision history: Pub. No. MAN0015891

Revision	Date	Description
A.0	11 October 2016	New Quick Reference.

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