

Next-generation Confirmation (NGC) module

Catalog Number A28221

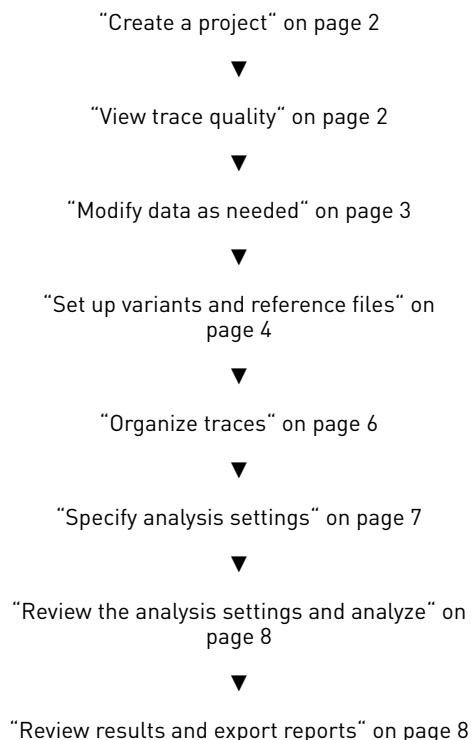
Pub. No. MAN0015891 Rev. A.0

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



Supported browsers and instruments

Component	Supported
Browsers	<ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 310 Genetic Analyzer • 3130/3130x/ Genetic Analyzers • 3500 Series Genetic Analyzers • 3730/3730x/ 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.

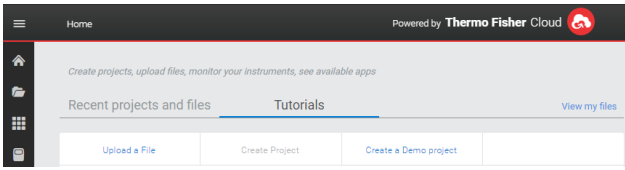
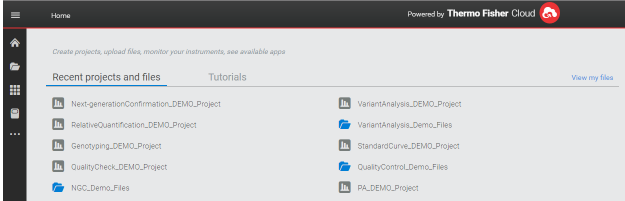
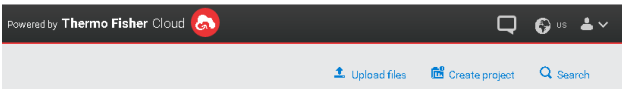

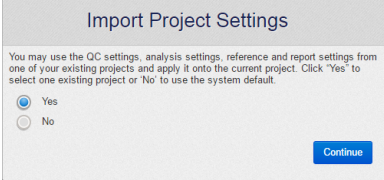
- Include at least:
 - The specimen name
 - The amplicon name (or Assay ID for Primer Designer™ Tool samples)
- (Optional) Add other information as needed, such as the well number and orientation.
- Include delimiters between each piece of information (for example, between the specimen and amplicon names).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

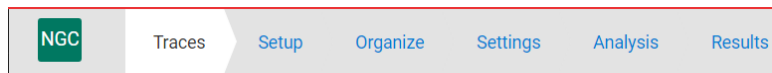
Create a project

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

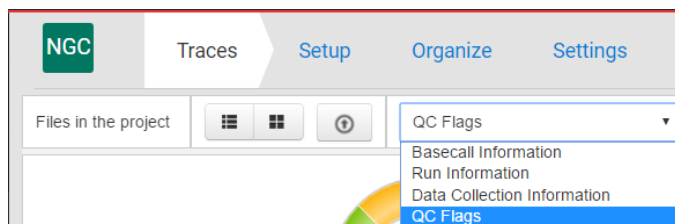
Create a Demo project	OR	Create your own project
<p>1. Click Tutorials ▶ Create a Demo project.</p>  <p>2. At the bottom of the Tutorials screen, click Create a Demo Project, then click OK.</p> <p>3. Click Recent projects and files, then click Next-generationConfirmation_DEMO_Project.</p>  <p>Note: It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

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**.

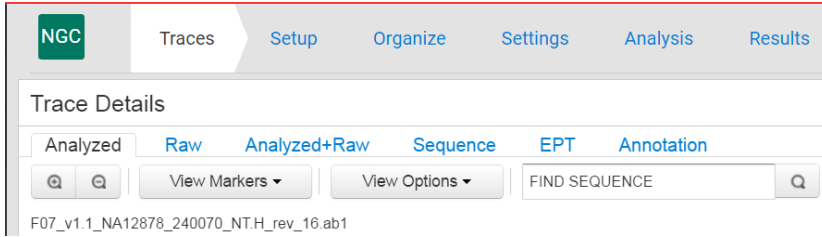


3. Double-click a trace file of interest to open the Trace Details screen.

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


Trace File Name	Overall Status
<input type="checkbox"/> F07_v1.1_NA12878_240070_NT_H_rev_16.ab1	
<input type="checkbox"/> B05_BDD_NA19240_227180_T.D_rev_05.ab1	
<input type="checkbox"/> F01_v1.1_NA12878_240070_NT_H_fwd_16.ab1	

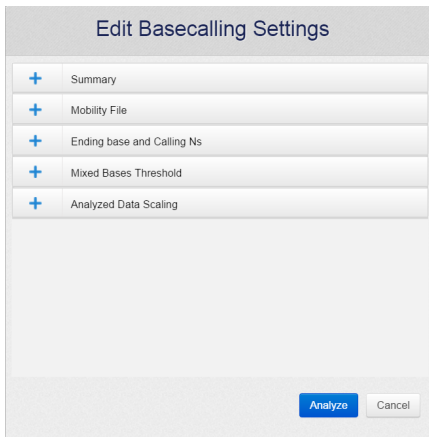
- In the Trace Details screen, click a tab to view the data of interest: **Analyzed**, **Raw**, **Analyzed+Raw**, **Sequence**, **EPT**, or **Annotation**.
Note: These tabs are not available if you select multiple trace files in the Traces screen; for multiple trace files, only analyzed data is shown.



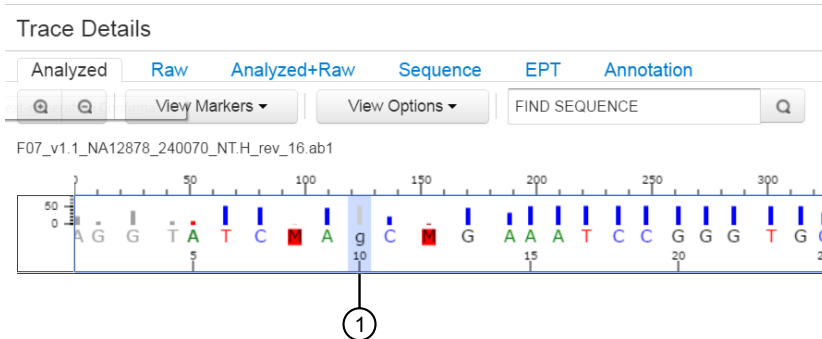
Modify data as needed

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

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



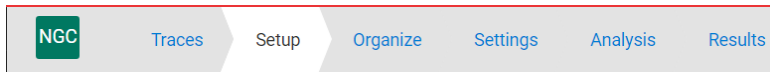
- Adjust quality flag settings: In the Traces screen, click **Actions** ▶ **Quality Flag Settings** to open the Settings screen. Adjust the settings as needed, then click **Save**.
- Remove traces: In the Traces screen, select the checkbox next to each trace file of interest, then click **Actions** ▶ **Remove Traces**.
- Edit bases:
 - 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**.
 - If you selected a single trace file, select the **Analyzed** or **Analyzed+Raw** tab to edit bases.
 - 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.



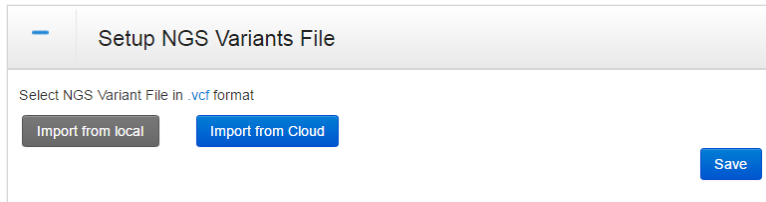
① Edited base

Set up variants and reference files

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



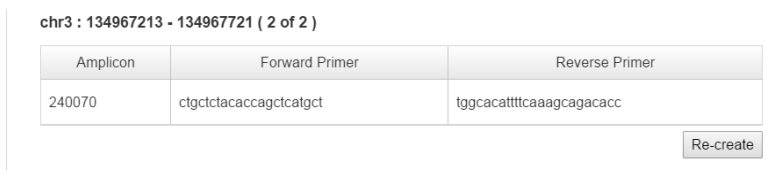
2. (Optional for NGS confirmation only) In the **Setup NGS Variants File** pane, import a *.vcf file that contains NGS variants, then click **Save**.

The screenshot shows a web interface titled 'Setup NGS Variants File'. Below the title, there is a prompt: 'Select NGS Variant File in .vcf format'. There are two buttons: 'Import from local' (grey) and 'Import from Cloud' (blue). A 'Save' button (blue) is located at the bottom right of the pane.

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.

The screenshot shows a message box with the text 'NGS File Already Uploaded'. A 'Re-create' button (grey) is located at the bottom right of the message box.

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.

The screenshot shows a table with primer information for a specific region. The title is 'chr3 : 134967213 - 134967721 (2 of 2)'. The table has three columns: 'Amplicon', 'Forward Primer', and 'Reverse Primer'. The 'Amplicon' column contains the value '240070'. The 'Forward Primer' column contains the sequence 'ctgctctacaccagctcatgct'. The 'Reverse Primer' column contains the sequence 'tggcacattttcaaagcagacacc'. A 'Re-create' button (grey) is located at the bottom right of the pane.

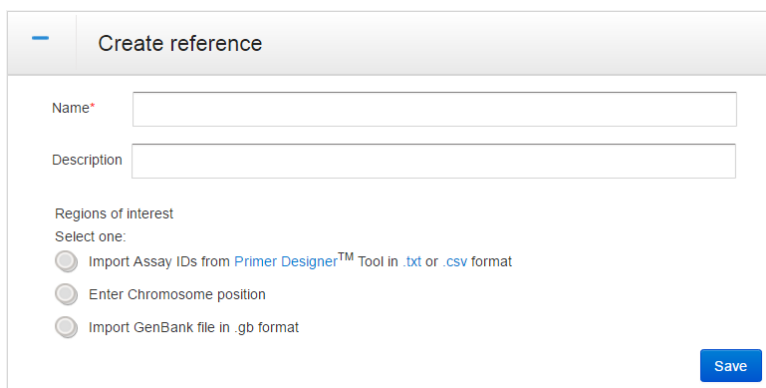
4. Create a reference. In the **Create reference** pane:

- 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**.

The screenshot shows a web interface titled 'Create reference'. It has two text input fields: 'Name*' and 'Description'. Below these fields is a section titled 'Regions of interest' with the instruction 'Select one:'. There are three radio button options: 'Import Assay IDs from Primer Designer™ Tool in .txt or .csv format', 'Enter Chromosome position', and 'Import GenBank file in .gb format'. A 'Save' button (blue) is located at the bottom right of the pane.

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.

NGS File Already Uploaded Re-create

Summary: Demo Reference-New Actions ▼

Target species: Human (GRCh38)

chr3 : 14199666 - 14200166 (1 of 2)

Amplicon	Forward Primer	Reverse Primer
227180	caatgcccccacataggtcatg	gagacaagcaggagaaggcaacc

chr3 : 134967213 - 134967721 (2 of 2)

Amplicon	Forward Primer	Reverse Primer
240070	ctgctctacaccagctcatgct	tggcacatttcaagcagacacc

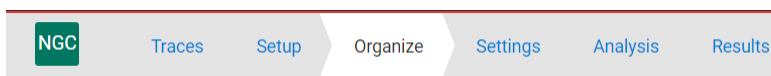
Re-create

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.

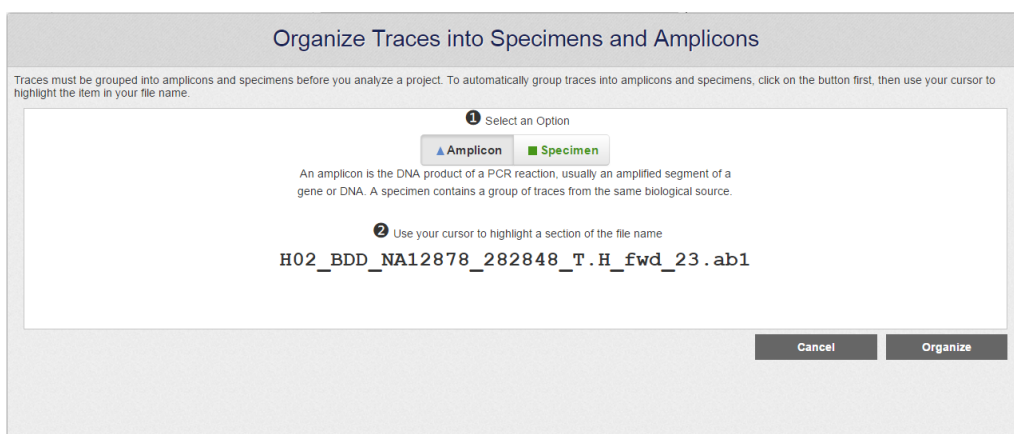


2. In the **Un-Grouped Traces** pane, click **Organize**.



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.

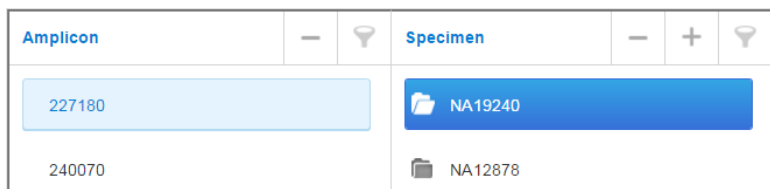


4. Click **Organize**. The software groups all trace files into the appropriate folders.

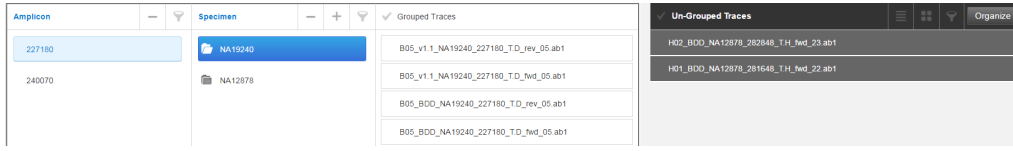
Group traces manually

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.

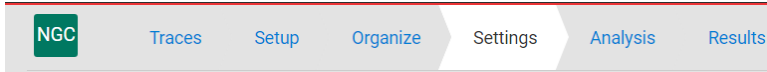


2. Drag trace files from the **Un-Grouped Traces** pane to the appropriate specimen folder in the **Specimen** pane.

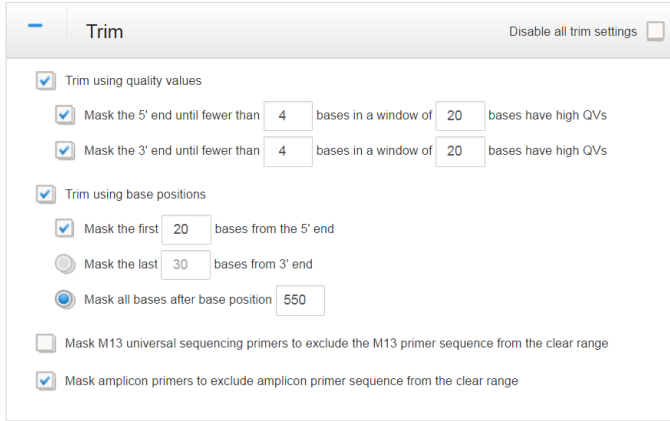


Specify analysis settings

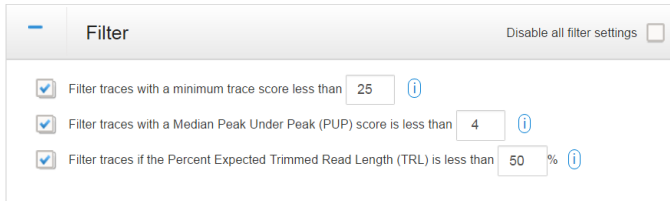
1. Click **Settings** in the workflow bar.



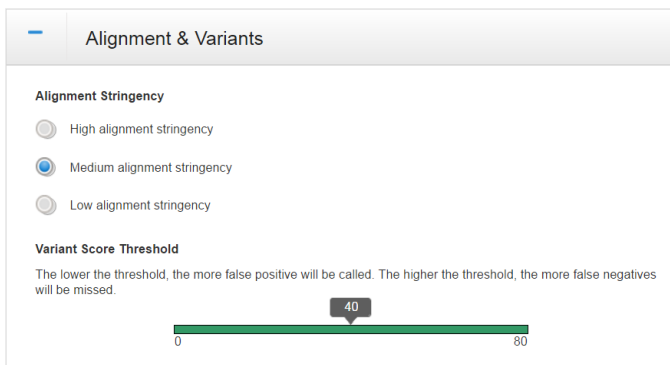
2. Click **+** to expand the Trim pane, then specify the settings used to trim and mask sequences before analysis.



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



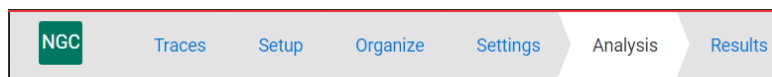
4. Click **+** to expand the Alignment & Variants pane, then:
 - a. Specify the Alignment Stringency to determine the minimum alignment score required for a trace file to be analyzed. Trace files that do not meet the specified stringency are removed from the analysis.
 - b. Specify the Variant Score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

Trace Settings		Trim Settings		Filter Settings		Alignment & Variants	
Grouped Traces	40	Quality Values	On	Trace Score	<25		
Ungrouped Traces:	0	Base Position	Off	Median PUP Score	<4	Alignment Stringency	Medium
Amplicons	2	M13 Primer	Off	% Expected Trimmed	<50	Variant Score Threshold	40
Specimens	2	Amplicon Primer	On	Read Length			

Re-Analyze

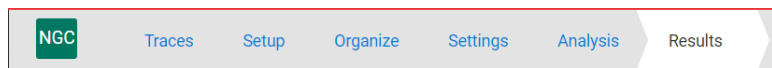
Completed 100%





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

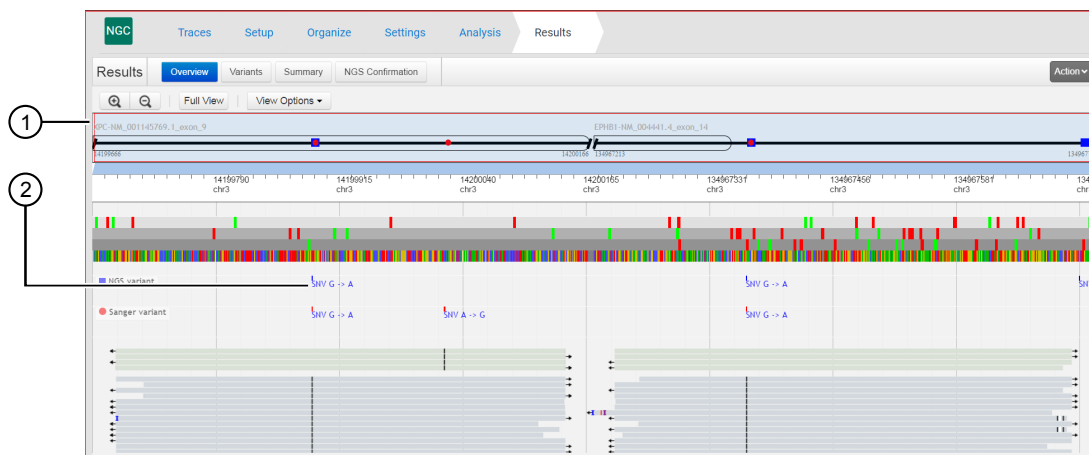
Review results and export reports

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.

The screenshot shows the NGC Variants tab. The table lists the following variants:

Chromo...	Chr. Position	Variant	Origin	Annotated Variant	Type	Review status	Variant Score	T...	E...	AA Change	Amplicon
chr3	14199887	G -> A [Mixed]	Common	rs2228000	Substitution	None	100.0	NM...	miss...	p.A19402(Val-)	227180
chr3	14200021	A -> G [Mixed]	Sanger		Substitution	None	100.0	NM...	silent		227180
chr3	134967368	G -> A [Mixed]	Common	rs11717042	Substitution	None	100.0	NM...	Intron		240070
chr3	134967705	G -> A	NGS		Substitution	None		NM...	Intron		240070

To the right of the table is a sequence viewer showing reference and sample sequences with a highlighted variant region.

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

The screenshot shows the NGC Summary tab with the following statistics:

Summary	
Number of Specimens:	2
Number of Amplicons:	2
Number of Traces Analyzed:	40
Number of Traces failed Analysis:	0
Number of Traces filtered:	0
Total number of Variants:	4
Number of Reviewed Variants:	0
Number of Accepted Variants:	0
Number of Rejected Variants:	0
Number of Unreviewed Variants:	4
Number of Partially Reviewed Variants:	0

Alignment Score

Range:	99-100	Mean:	99.92
Median:	100	Standard Deviation:	0.27

Traces with Analysis Errors/Warnings

Trace Name	Specimen	Amplicon	Filter	Trim	Alignment	Errors/Warnings
F04_v3_1_NA12878_240070_NT.D_rev_16_ab1	NA12878	240070	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Failed to align/trim primer sequences.

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

The screenshot shows the NGC NGS Confirmation tab. On the left is a Venn diagram comparing NGS Variants (purple) and Sanger Variants (green). The intersection contains 2 variants, while each set has 1 unique variant. On the right is a table of confirmed variants:

Chromosome	Position	Annotated Variant	Type	Ref	NGS Alt	San... Alt
3	14199887	rs2228000	Substitution	G	A	A
3	14200021		Substitution	A	-	G
3	134967368	rs11717042	Substitution	G	A	A
3	134967705		Substitution	G	A	-

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).

The screenshot shows the NGC Summary tab with the **Actions** menu open, displaying the following options:

- Generate Report
- Export Variants

Limited product warranty

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Revision history: Pub. No. MAN0015891

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

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