applied biosystems

CarrierScan™ Reporter v1.1

USER GUIDE

Publication Number 703467
Revision 3

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Revision history: Pub No. 703467

Revision	Date	Description
3	June 2019	Version 1.1 release with updated Licensing information.
2	May 2019	Version 1.1 release
1	January 2018	Initial release

Important Software Licensing Information

Your installation and/or use of this CarrierScan Reporter software is subject to the terms and conditions contained in the End User License Agreement (EULA) which is incorporated within the CarrierScan Reporter software, and you will be bound by the EULA terms and conditions if you install and/or use the software.

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1 Introduction

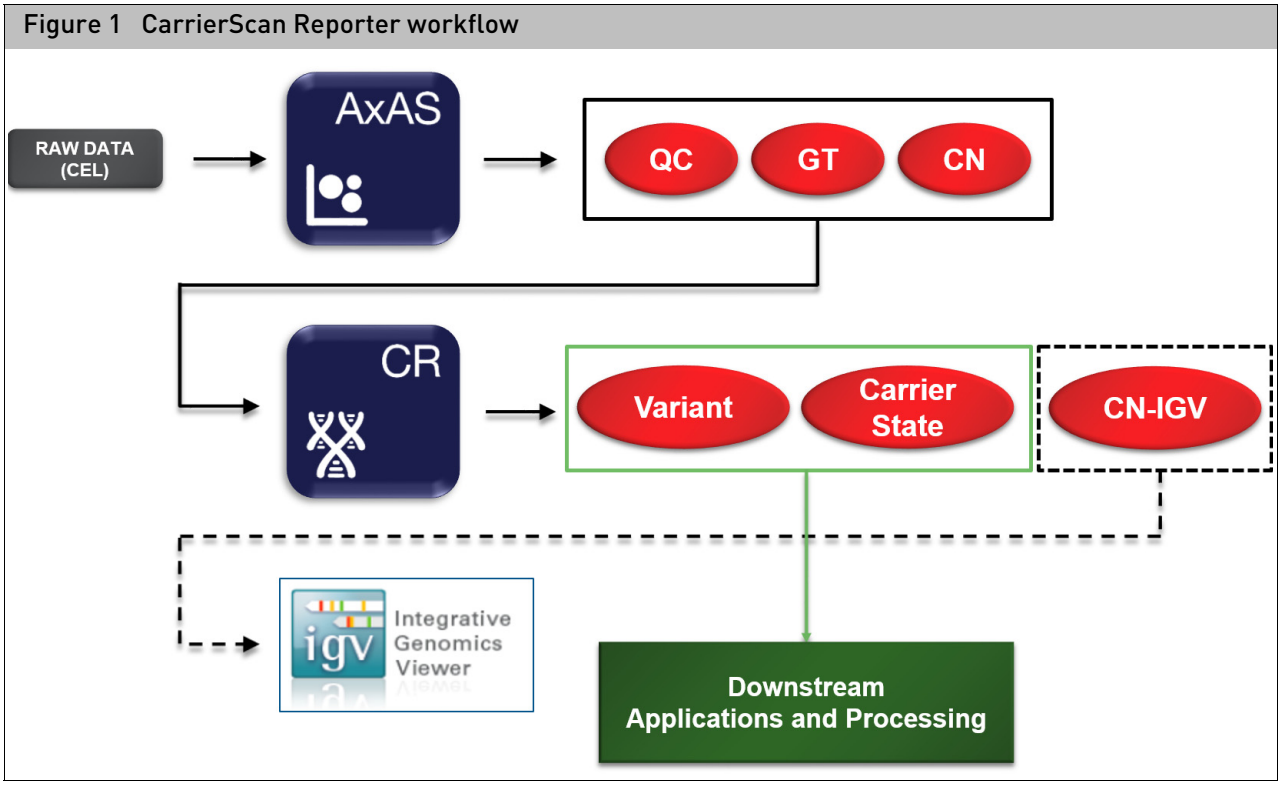
Overview

CarrierScan Reporter is designed to work with Axiom Analysis Suite (AxAS) and its best practices analysis of CarrierScan based arrays. AxAS calculates quality control metrics, as well as assigns genotyping and copy number assessments. It reads in the AxAS results, then determines sample heredity status using phenotypic descriptions found in the public domain (ClinVar, OMIM, etc.).

CarrierScan Reporter also assesses individual and paired samples, then creates customizable reports of the annotated results.

The flowchart (Figure 1) describes the CarrierScan data generation pipeline from analyzing CEL files in Axiom Analysis Suite to CarrierScan Reporter variant and carrier translation. The text outputs can be imported and processed in existing analysis pipelines. In addition, all carrier determinations can be traced back to the original genotype and copy number calls and graphically assessed in AxAS and Integrative Genomics Viewer (IGV), respectively.

Figure 1 CarrierScan Reporter workflow



Features

- Controlled and automated sample carrier state determinations.
- Individual residual chance of carrier occurrence.
- Paired residual chance of phenotype occurrence.
- Compiled information from public databases for associated phenotypes.

New features in v1.1

- CarrierScan Reporter v1.1 uses a new annotation panel containing annotation content for the full array. Output will still be limited to the desired variants based on AxAS results. Custom annotation panels limiting to the desired content are no longer needed. **Note:** CarrierScan Reporter v1.1 requires new library files. See "Downloading library files" on page 8.
- A sample QC metric "Rare Carrier Count" has replaced the QC Het Rate metric. This sample QC identifies samples with >20 rare variants called as non-normal and adjusts the calls to No Call as samples with a high number of rare carrier/ affected calls is indicative of an issue with the sample.
- The requirement to have all annotation columns present in the annotation penal file has been removed. Any annotation column present in the annotation panel will be displayed, but is no longer required.
- Multi-allele variants are now exported on individual lines similar to bi-alleles for easier viewing.
- The link between HBA1 and HBA2 has been removed and will be exported individually.
- The output folder will automatically be named similar to the AxAS Batch name for easier matching of AxAS data and CarrierScan Reporter data for Carrier Reviewer Tool.

Minimum requirements

Operating System	Processor	Memory (RAM)
Windows® 7 (64 bit) Professional with Service Pack 1	2.83 GHz Intel Pentium Quad Core	16 GB [1]
Windows® 10 (64 bit) Professional	2.83 GHz Intel Pentium Quad Core	16 GB [1]

[1] Systems with 8 GB can be used, however it is recommended you limit your processing to <200 samples per analyses.

Installation

1. Go to: thermofisher.com
2. Locate and download the CarrierScan Reporter zipped software package.
3. Unzip the file as you normally would, then double-click on **CarrierScanReporterSetup.exe**.
4. Follow the installer's directions.
5. After the installation is complete, click **Start** → **All Programs** → **Thermo Fisher Scientific** → **CarrierScan Reporter**

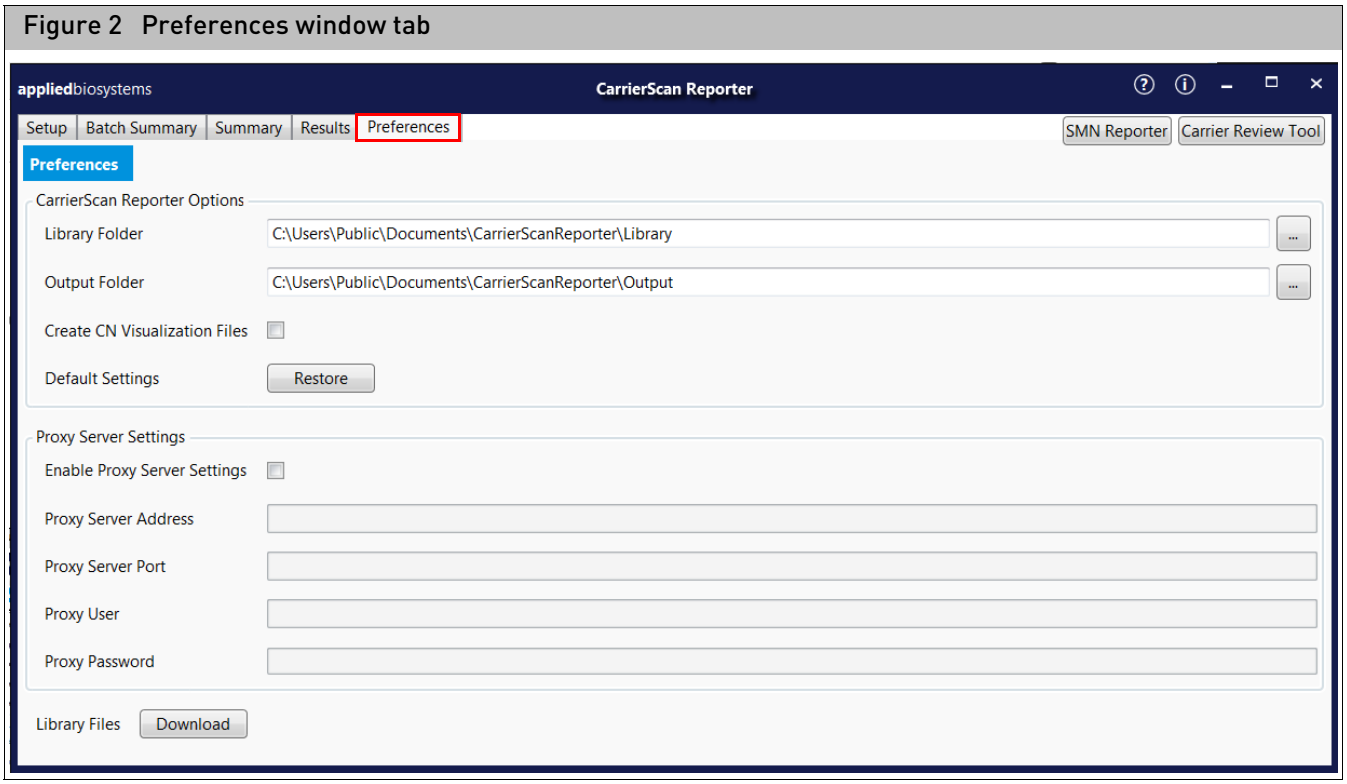
   The application opens.

Start up

The library and output folders are preset to default locations. Before using this software, review or change these folder paths, install required library files, and (if needed) set up access to your site's Proxy Server.

1. Click on the **Preferences** tab. (Figure 2)

Figure 2 Preferences window tab



Locating the library folder

The Library folder's default location is: C:\Users\Public\Documents\CarrierScanReporter\Library. If you want to change this default location, continue to Step 1.

1. Click the Library Folder field's **Browse** button, navigate to the location/folder you want, then click **Select Folder**.

   Your assigned library folder path is now displayed.

   Note: To better organize your data within your library folder, create a sub-folder for each supported array type.

Locating the output folder

This folder stores data your analysis generated. The Output folder's default location is: C:\Users\Public\Documents\CarrierScanReporter\Output. If you want to change this default location, continue to Step 1.

1. Click the Output Folder field's **Browse** button, navigate to the location/folder you want, then click **Select Folder**.

   Your assigned output folder path is now displayed.

(Optional) Creating copy number visualization files

If you want to generate a ***.cn** file that contains log2 ratio data from all files in your analysis, click the **Create CN Visualization Files** check box. After the ***.cn** file is generated, it is saved to your assigned output folder.

This file can be viewed using an Integrative Genomics Viewer (IGV).

To access this viewer, go to: http://software.broadinstitute.org/software/igv/

Setting up custom proxy settings

Follow the steps below if your system has to pass through a Proxy Server before it can access the NetAffx server.

1. Click the **Enable Proxy Settings** check box.

   Note: The proxy user ID and password is NOT the same ID and password used to connect to the NetAffx server.

2. Enter the **Address**, **Port**, **User**, and **Password**. Contact your IT department, if you do not know the proxy settings.

Downloading library files

1. Click the **Download** button.

   The NetAffx User Information window appears.

2. Enter your NetAffx account email and password, then click **OK** or go to netaffx.com and click **Register** to sign up.

   Note: If you are unable to connect to NetAffx, make sure you have an active Internet connection, and/or correct Proxy Server settings.

   The NetAffx Update window appears.

3. Click the corresponding check box(es) of the library file(s) you want to download, then click **OK**.

   An Installing Updates progress bar appears.

4. Click **OK** to close the NetAffx Update window.

   Note: If creating a custom annotation panel file, see *Note* in the full TXT report.

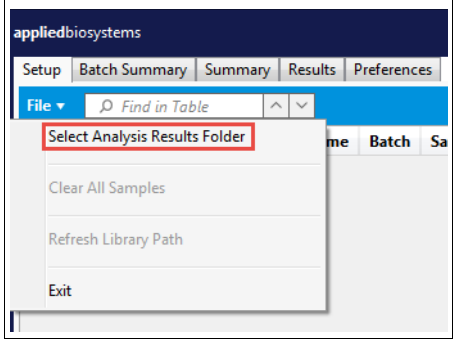
2 Setup

IMPORTANT! Before you can analyze data using CarrierScan Reporter, your CEL files MUST first be processed through the Axiom Analysis Suite 3.1 or higher software application. For instructions on how to do this, refer to the Axiom Analysis Suite User Guide (P/N 703307).

Importing data

1. Click **File** → **Select Analysis Results Folder**. (Figure 3)

Figure 3 File drop-down menu



A window opens.

2. Navigate to the Axiom Analysis Suite output folder.
3. Open the Analysis Results Folder that has the samples you want to analyze.
4. Click **Select Folder**.

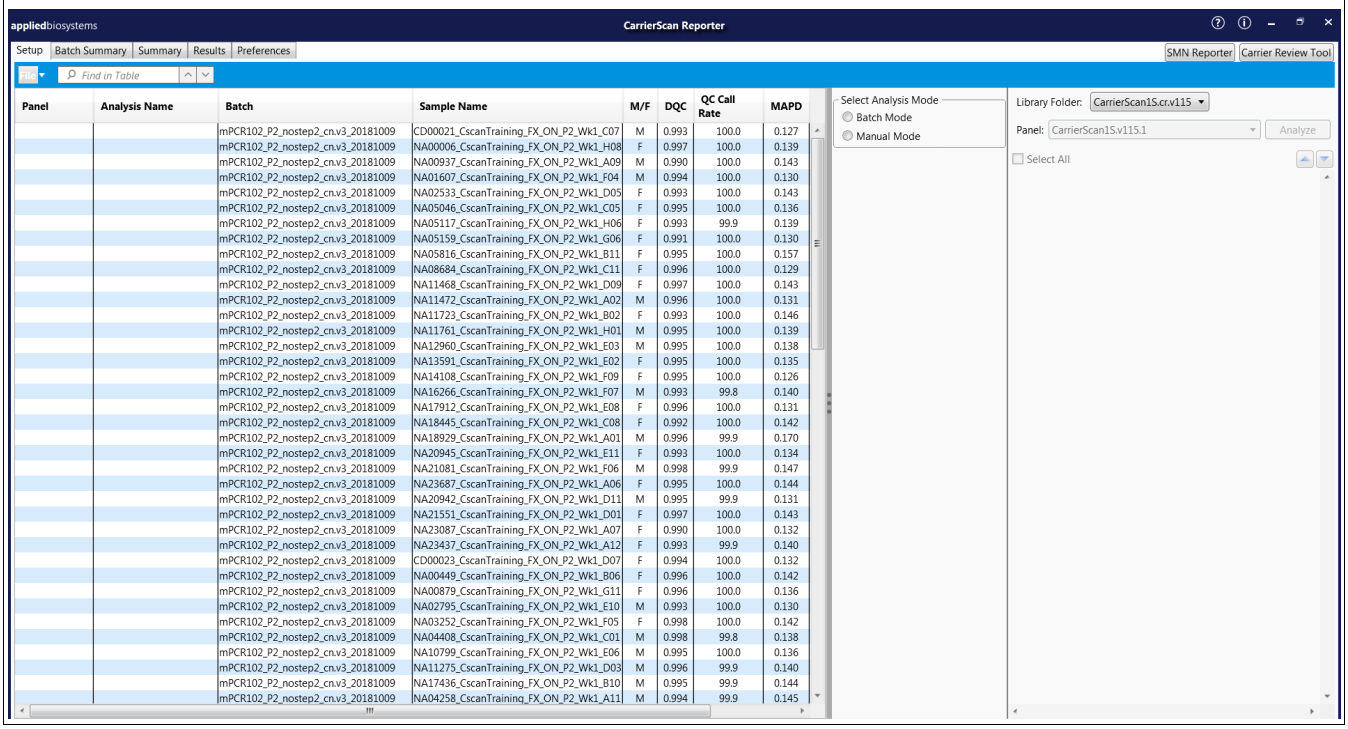
Note: Multiple Analysis Results Folders can be added into the same analysis. To do this, repeat steps 1-4.

IMPORTANT! Analysis Results Folder names must be unique and subsequently loaded results folders must be for the same array type and analysis settings as the first folder.

The sample files from your selected Analysis Results Folder(s) load into the Setup window tab. (Figure 4)

Note: The Setup table (Figure 4) may take longer to populate if you are loading a large file and/or checked the **Create CN Visualization Files** check box (in Preferences).

Figure 4 Loaded data in the Setup window tab



You can sort the data (in ascending or descending order) in each column by clicking on its header. See the table below for column definitions.

Note: Once an analysis has been run and samples are loaded into the table, a QC file is written as **SampleQC.txt**, where all QC values shown on the Setup table are recorded for all samples analyzed.

Column	Description
Panel	Displays the name of the panel used in the analysis.
Analysis Name	Analysis Name provided in the Analysis Mode or the Mapping File.
Batch	Name assigned to the collection of data in the selected analysis results folder.
Sample Name	The assigned sample name (that was designated during its sample registration process in Command Console).
M/F	M = Male, F = Female, U = Unknown
DQC (Dish QC)	DQC is based on intensities of probe sequences for non-polymorphic genome locations (i.e., sites that do not vary in sequence from one individual to the next). When subject to the two-color Axiom assay, probes expected to ligate an A or T base (referred to as AT non-polymorphic probes) produce specific signal in the AT channel and background signal in the GC channel. The converse is true for probes expected to ligate a G or C base (referred to as GC non-polymorphic probes). DQC is a measure of the resolution of the distributions of "contrast" values, where: Distributions of contrast values are computed separately for the AT non-polymorphic probes (which should produce positive contrast values) and GC non-polymorphic probes (which should produce negative contrast values). If sample quality is high, then signal will be high in the expected channel and low in background channel, and the two contrast distributions will be well-resolved. A DQC value of zero indicates no resolution between the distributions of AT and GC probe contrast values, and the value of 1 indicates perfect resolution.
QC Call Rate	Percentage of SNPs assigned a genotype using a subset of probe sets (usually 20,000) that are autosomal.
MAPD	Median of the Absolute values of all Pairwise Differences (MAPD) is a global measure of the variation of all microarray probes across the genome. It represents the median of the distribution of changes in Log2 Ratio between adjacent probes. Since it measures differences between adjacent probes, it is a measure of short-range noise in the microarray data.
MAPDc	MAPD calculation post plate correction.
WavinessSD	A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation.
WavinessSDc	WavinessSD calculation post plate correction.

Selecting an analysis mode

You can setup your analysis in two ways:

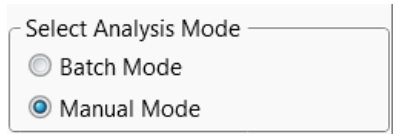
- **Manual Mode** supports individual sample analysis set up, paired sample analysis, and modifications. In this mode, samples can be added one by one or as pairs.
- **Batch Mode** supports quick, pre-determined setup analysis through a structured mapping file.

Either mode enables you to select and run one analysis at a time.

Manual mode

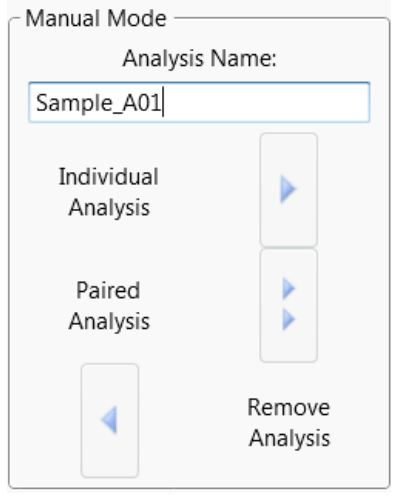
1. Click the **Manual Mode** radio button. (Figure 5)

Figure 5 Select Analysis Mode



The Manual Mode Options window appears. (Figure 6)

Figure 6 Manual Mode options



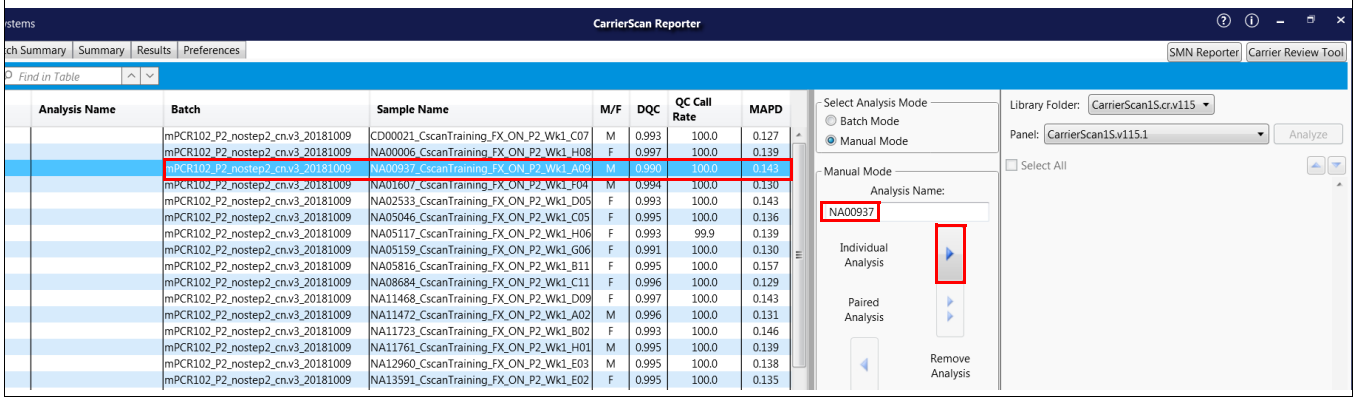
Individual analysis (one sample)

1. Highlight a sample name in the table, as shown in Figure 7.
2. Enter an Analysis Name. If no Analysis Name is provided, the sample name will be used as the Analysis Name.

   Note: "**SA**" is appended to the analysis name to indicate the analysis is a Single Analysis.

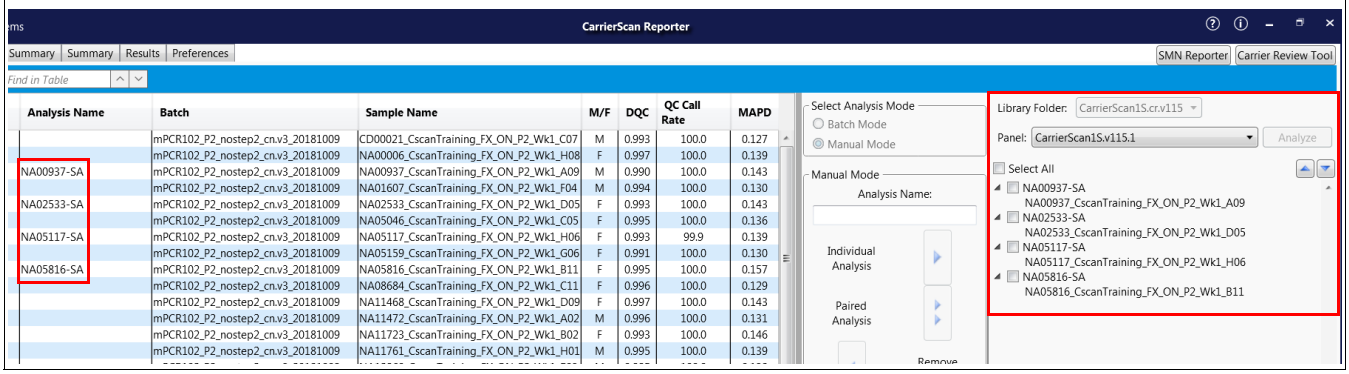
3. Click the newly enabled **Individual Analysis** arrow button (Figure 7) to add the sample to the Analysis Window.

Figure 7 Highlighting a sample name example



The Analysis Name and its associated sample are now added to the far right-hand side of the Analysis Window. The Analysis Name is also added to the Table's **Analysis Name** column, as shown in Figure 8.

Figure 8 Setup window with Analysis Name and Sample added



Adding multiple samples to an individual analysis simultaneously

1. Highlight the desired sample names in the table.
2. Enter an Analysis Name. If no Analysis Name is provided, the sample name will be used as the Analysis Name.
3. Click the newly enabled Individual Analysis arrow to add the sample to the Analysis Window.

   The Analysis Name and its associated sample are added to the far right-hand side of the Analysis Window and are now ready for analysis. The Analysis Name is also added to the Table's **Analysis Name** column, as shown in Figure 8.

Paired analysis (two samples)

1. Click on your first sample, then press the Ctrl key and click on your second sample.

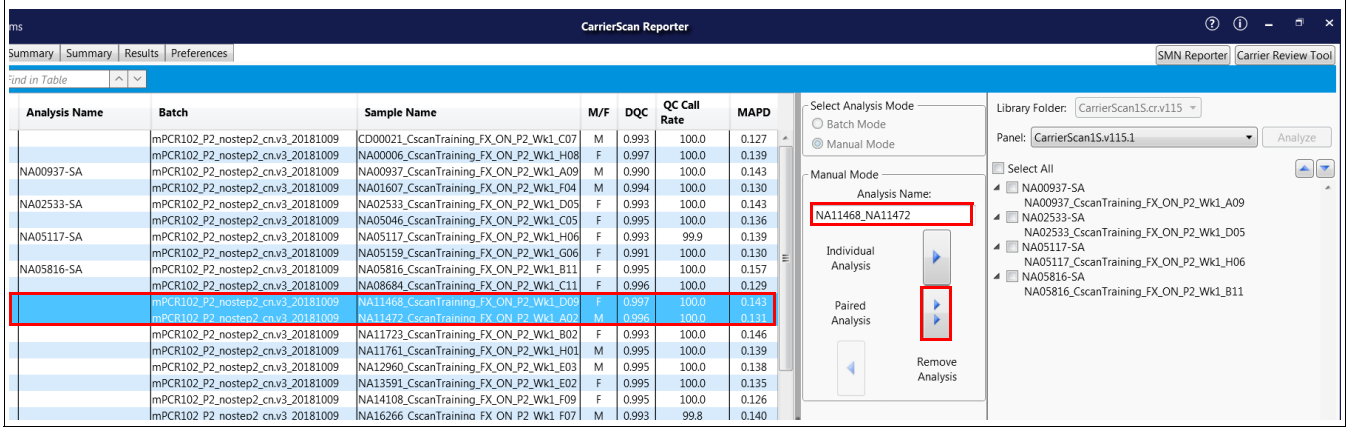
   Both samples are highlighted, as shown in Figure 9.

2. Enter an Analysis Name. If an Analysis Name is not provided, the name of the first sample highlighted for the paired analysis will be used as the Analysis Name.

   Note: PA is appended to the analysis name to indicate the analysis is a Paired Analysis.

3. Click the newly enabled Paired Analysis arrow to add the sample to the Analysis Window, as shown in Figure 9.

Figure 9 Setup window with Paired Analysis Name and Sample added



Removing an analysis name from the setup

1. Check the check box of the Analysis Name you want to remove.
2. Click on the newly enabled **Remove Analysis** button.

Running an analysis

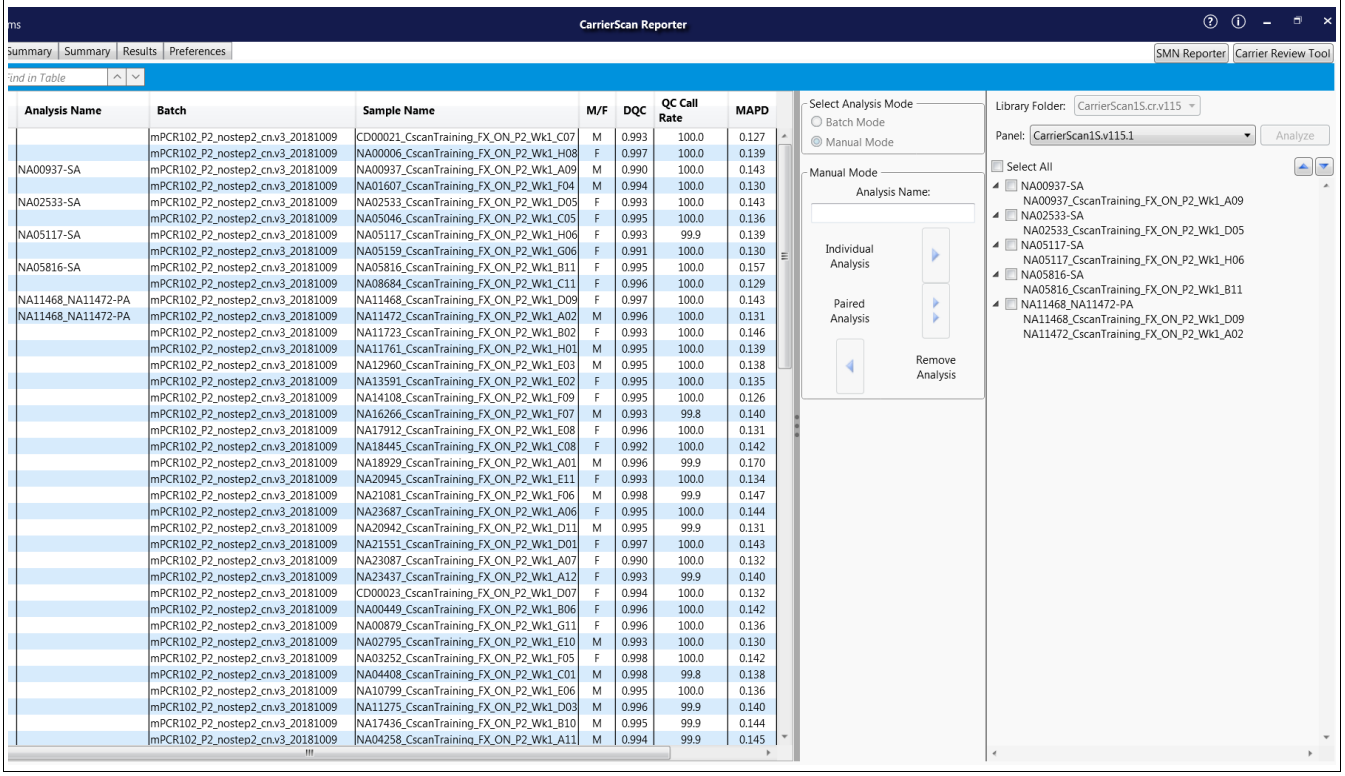
1. Select the appropriate panel from the Panel drop-down.
2. Check the Analysis Name(s) to be analyzed with that panel or click the **Check All** box to include all analysis names in the analysis.
3. Once all analyses have been added, click the **Analyze** button.

IMPORTANT! You can only add one sample per analysis. If you have added a sample that already exists in the analysis you want to run, a window appears detailing why your sample could not be added. Acknowledge the message, then click **OK**.

The analysis process begins and a Please Wait window appears.

After the analysis is complete, the remaining columns in the table are populated, as shown in Figure 10.

Figure 10 Populated Setup window



Batch mode

IMPORTANT! Before using the Batch Mode feature, you must first create a tab-delimited TXT file (Figure 11) to define your individual or paired analysis.

Required batch mode file guidelines

Your Batch Mode Mapping file must contain these six header columns (Figure 11) in the order shown:

[A] Panel - The name of the panel for the analysis.

[B] Analysis Name - The name given to the analysis of that sample(s). **Note:** For a paired analysis, use the same analysis name for both samples in the pair.

[C] Batch - This name must match the batch name provided when loading the samples.

[D] Sample Name - The filename of the samples to be analyzed.

[E] Ethnicity - The assignment of ethnicity only applies to paired analyses. If a valid ethnicity (present on the chosen panel) is assigned to one sample, then the paired sample must also have a valid ethnicity assigned. Ethnicities are used in the pair residual chance of occurrence calculations provided in the full and positive outputs. Ethnicity assignments are not required. **Note:** Column [E] is optional. If you choose not to include it, use your space bar to insert a single blank space in each of its cells to ensure a properly formatted TSV file.

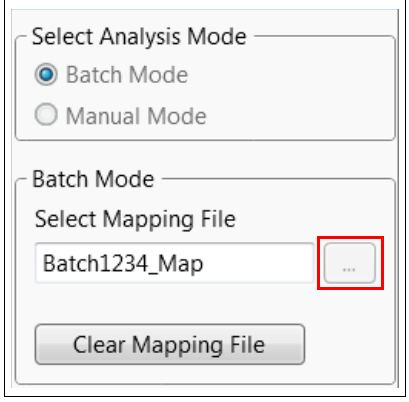
[F] Notes - Use this column to enter any comments you want regarding the samples or analysis. Cells in this column can be left blank, as comments are not required. **Note:** Column [F] is optional. If you choose not to include it, use your space bar to insert a single blank space in each of its cells to ensure a properly formatted TSV file.

Figure 11 Required and optional tab-delimited TXT file example

	A	B	C	D	E	F
1	Panel	Analysis Name	Batch	Sample Name	Ethnicity	Notes
2	CarrierScan.r1	CD00008	TrainingPlate_r1_EX	CD00008_C02		expedite
3	CarrierScan.r1.EX	CD00021_CD00023	TrainingPlate_r1_EX	CD00021_C07	General Population	paired
4	CarrierScan.r1.EX	CD00021_CD00023	TrainingPlate_r1_EX	CD00023_D07	Asian American	paired
5	CarrierScan.r1.CFTR	Run180103_H08	TrainingPlate_r1_EX	NA00006_H08		CFTR only
6	CarrierScan.r1	Sample_NA00059	TrainingPlate_r1_EX	NA00059_A04		
7	CarrierScan.r1.ACOG	NA00244_G09	TrainingPlate_r1_EX	NA00244_G09		ACOG only
8	CarrierScan.r1	NA00449_B06	TrainingPlate_r1_EX	NA00449_B06		rerun
9	CarrierScan.r1.CFTR	Pair-E09_E04	TrainingPlate_r1_EX	NA00649_E09	Caucasian	
10	CarrierScan.r1.CFTR	Pair-E09_E04	TrainingPlate_r1_EX	NA00852_E04	Caucasian	

1. After creating and saving your tab-delimited Batch Mode TXT file, click the **Batch Mode** radio button. (Figure 12)

Figure 12 Select Analysis Mode



If you want to remove samples from the Analysis window, click the **Clear Mapping File** button.

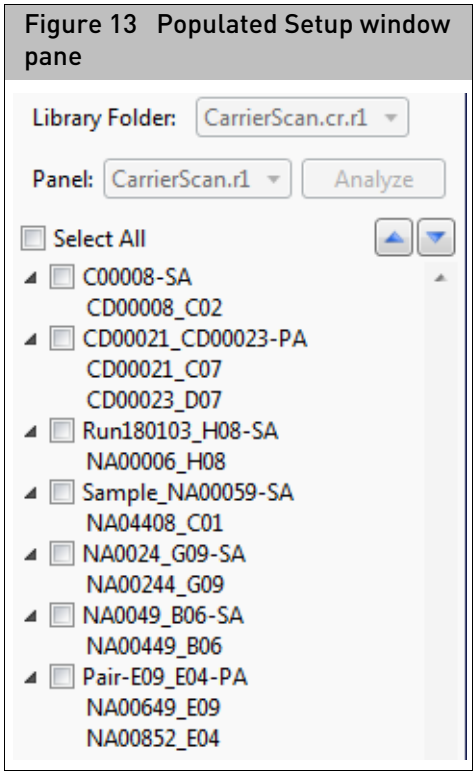
2. Click **Select Mapping File** Browse button.

   An Explorer window appears.

3. Navigate to the Batch Mode file, then click **OK**.

   The Analysis Setup window populates with individual and paired analysis (based on the information in your Batch Mode file), as shown in Figure 13.

Figure 13 Populated Setup window pane



4. Use the check box to check each Analysis Name(s) to be analyzed or click the **Check All** check box to include all the names shown in your analysis.
5. Click the **Analyze** button.

   The analysis process begins and a Please Wait window appears. After the analysis is complete, the remaining columns in the table are populated.

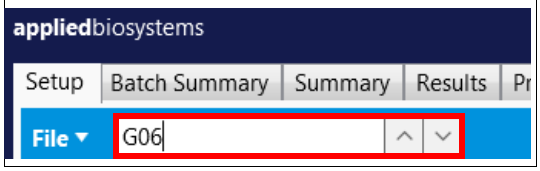
Searching table content

Use this feature to search for batch, gender, and sample names within the table.

Note: Every column, except for the Analysis Completed column is searched.

1. From the Analysis Setup window tab's **Search** box (Figure 14), type the name or portion of the name you want to find.

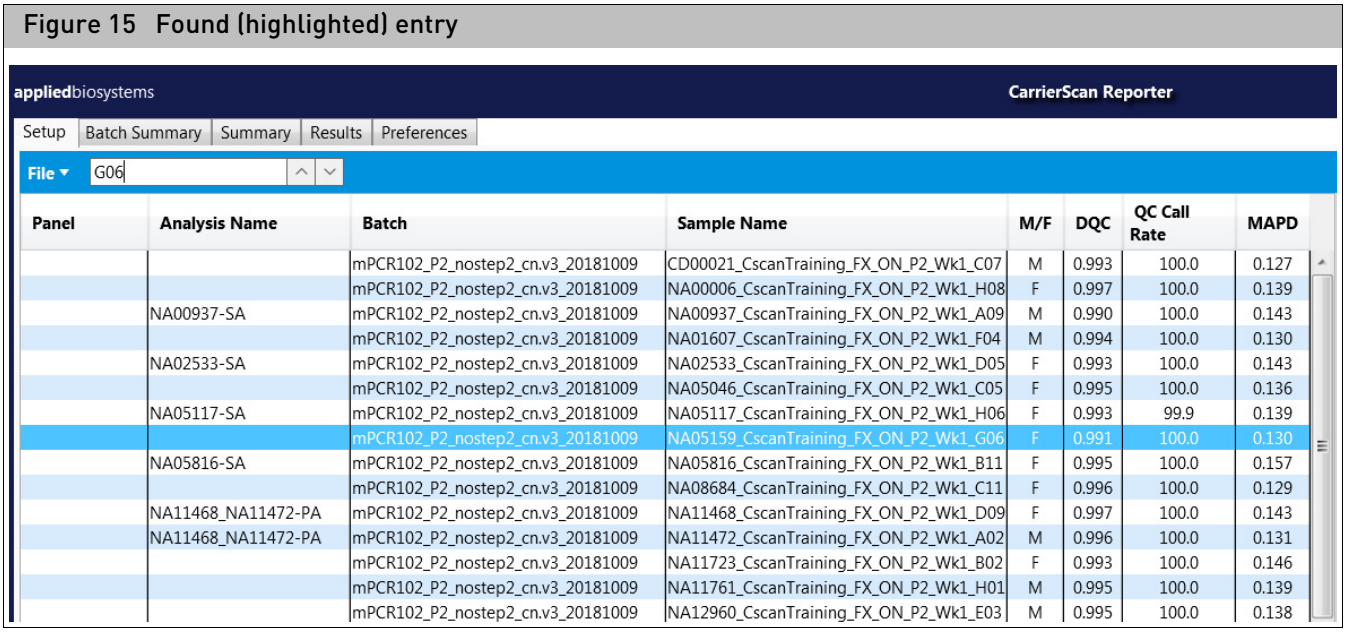
Figure 14 Search box



2. Click the up or down arrow (or press the Enter key).

   If a match is found, it is highlighted in the table, as shown in Figure 15.

Figure 15 Found (highlighted) entry



Panel	Analysis Name	Batch	Sample Name	M/F	DQC	QC Call Rate	MAPD
		mPCR102_P2_nostep2_cn.v3_20181009	CD00021_CscanTraining_FX_ON_P2_Wk1_C07	M	0.993	100.0	0.127
		mPCR102_P2_nostep2_cn.v3_20181009	NA00006_CscanTraining_FX_ON_P2_Wk1_H08	F	0.997	100.0	0.139
	NA00937-SA	mPCR102_P2_nostep2_cn.v3_20181009	NA00937_CscanTraining_FX_ON_P2_Wk1_A09	M	0.990	100.0	0.143
		mPCR102_P2_nostep2_cn.v3_20181009	NA01607_CscanTraining_FX_ON_P2_Wk1_F04	M	0.994	100.0	0.130
	NA02533-SA	mPCR102_P2_nostep2_cn.v3_20181009	NA02533_CscanTraining_FX_ON_P2_Wk1_D05	F	0.993	100.0	0.143
		mPCR102_P2_nostep2_cn.v3_20181009	NA05046_CscanTraining_FX_ON_P2_Wk1_C05	F	0.995	100.0	0.136
	NA05117-SA	mPCR102_P2_nostep2_cn.v3_20181009	NA05117_CscanTraining_FX_ON_P2_Wk1_H06	F	0.993	99.9	0.139
		mPCR102_P2_nostep2_cn.v3_20181009	NA05159_CscanTraining_FX_ON_P2_Wk1_G06	F	0.991	100.0	0.130
	NA05816-SA	mPCR102_P2_nostep2_cn.v3_20181009	NA05816_CscanTraining_FX_ON_P2_Wk1_B11	F	0.995	100.0	0.157
		mPCR102_P2_nostep2_cn.v3_20181009	NA08684_CscanTraining_FX_ON_P2_Wk1_C11	F	0.996	100.0	0.129
	NA11468_NA11472-PA	mPCR102_P2_nostep2_cn.v3_20181009	NA11468_CscanTraining_FX_ON_P2_Wk1_D09	F	0.997	100.0	0.143
	NA11468_NA11472-PA	mPCR102_P2_nostep2_cn.v3_20181009	NA11472_CscanTraining_FX_ON_P2_Wk1_A02	M	0.996	100.0	0.131
		mPCR102_P2_nostep2_cn.v3_20181009	NA11723_CscanTraining_FX_ON_P2_Wk1_B02	F	0.993	100.0	0.146
		mPCR102_P2_nostep2_cn.v3_20181009	NA11761_CscanTraining_FX_ON_P2_Wk1_H01	M	0.995	100.0	0.139
		mPCR102_P2_nostep2_cn.v3_20181009	NA12960_CscanTraining_FX_ON_P2_Wk1_E03	M	0.995	100.0	0.138

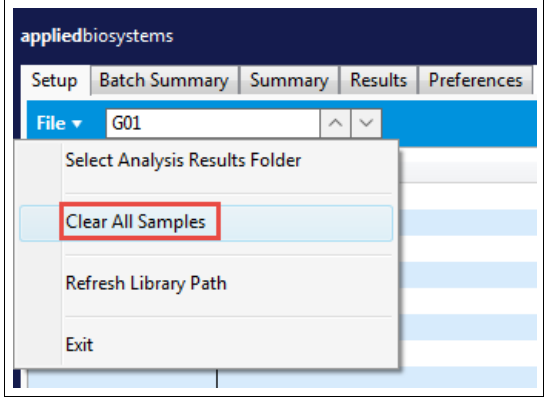
3. Click the up or down arrow (or press the Enter key) to go to the next/previous matching entry.

Removing all samples from the table

1. Click **File** → **Clear All Samples**. (Figure 16)
   All Samples are removed from the table.

IMPORTANT! Any unanalyzed analyses (shown in the right panel) are also removed.

Figure 16 Clear All Samples

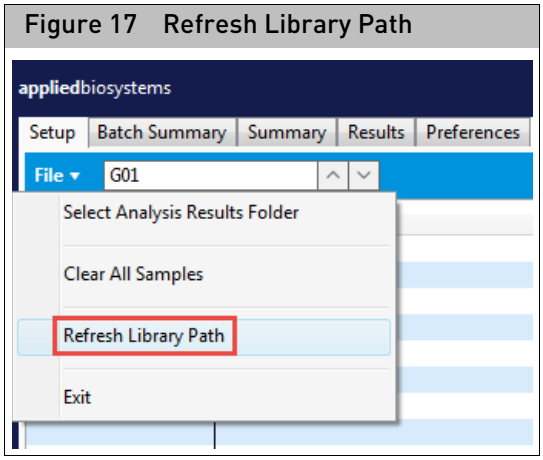


Updating the library folder

To update the contents of your library folder:

1. Click **File** → **Refresh Library Path**. (Figure 17)
   The Library folder is now updated to include your changes (since the application was last opened) and any available library file updates.

Figure 17 Refresh Library Path



3 Summary tables

Batch summary table

After setting up and successfully running analyses for an Axiom Analysis Suite Results Folder as described in Chapter 2, "Setup", click the **Batch Summary** tab to visually inspect variants across all samples analyzed within an Axiom Analysis Suite Results Folder (also referred to as the Batch).

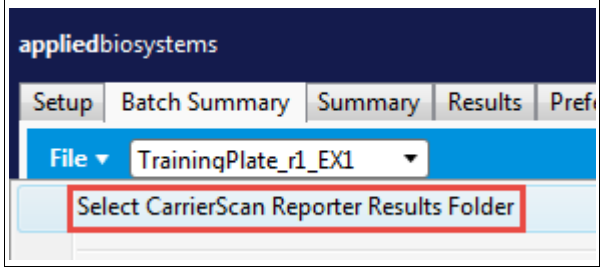
Note: If multiple panels are used with a batch, each panel will generate its own BatchSummaryResults file.

The Batch Summary table contains information for all samples analyzed in a batch. Viewing results for a 96 sample plate is supported in the software, however we advise to view results >96 samples in other software (e.g. Excel) to minimize slowing of software performance.

Selecting previously generated results

1. From the **Batch Summary** window tab, click **File** → **Select CarrierScan Reporter Results Folder**. (Figure 18)

Figure 18 Select a CarrierScan Results Folder



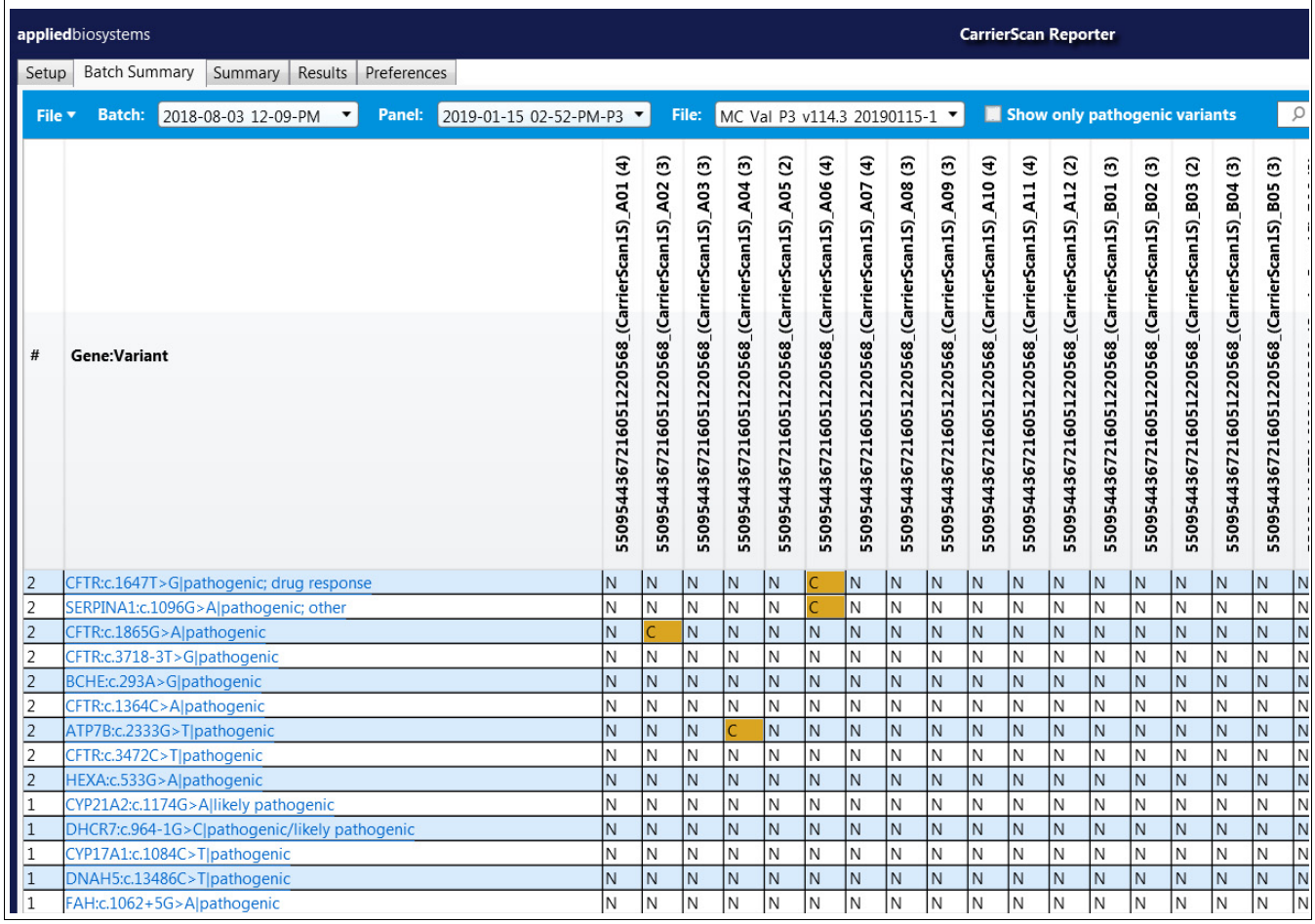
A **Select results folder** window appears.

2. Click to highlight the analysis folder you want to view in detail, then click **Select Folder**.

   Note: Past results can only be viewed if the original files (inside the folder you select) have not been modified.

   A detailed analysis of your selected folder appears, as shown in Figure 19.

Figure 19 Batch Summary table



#	Gene:Variant	5509544367216051220568_(CarrierScan1S)_A01 (4)	5509544367216051220568_(CarrierScan1S)_A02 (3)	5509544367216051220568_(CarrierScan1S)_A03 (3)	5509544367216051220568_(CarrierScan1S)_A04 (3)	5509544367216051220568_(CarrierScan1S)_A05 (2)	5509544367216051220568_(CarrierScan1S)_A06 (4)	5509544367216051220568_(CarrierScan1S)_A07 (4)	5509544367216051220568_(CarrierScan1S)_A08 (3)	5509544367216051220568_(CarrierScan1S)_A09 (3)	5509544367216051220568_(CarrierScan1S)_A10 (4)	5509544367216051220568_(CarrierScan1S)_A11 (4)	5509544367216051220568_(CarrierScan1S)_A12 (2)	5509544367216051220568_(CarrierScan1S)_B01 (3)	5509544367216051220568_(CarrierScan1S)_B02 (3)	5509544367216051220568_(CarrierScan1S)_B03 (2)	5509544367216051220568_(CarrierScan1S)_B04 (3)	5509544367216051220568_(CarrierScan1S)_B05 (3)
2	CFTR:c.1647T>G|pathogenic; drug response	N	N	N	N	N	C	N	N	N	N	N	N	N	N	N	N	N
2	SERPINA1:c.1096G>A|pathogenic; other	N	N	N	N	N	C	N	N	N	N	N	N	N	N	N	N	N
2	CFTR:c.1865G>A|pathogenic	N	C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	CFTR:c.3718-3T>G|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	BCHE:c.293A>G|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	CFTR:c.1364C>A|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	ATP7B:c.2333G>T|pathogenic	N	N	N	C	N	N	N	N	N	N	N	N	N	N	N	N	N
2	CFTR:c.3472C>T|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	HEXA:c.533G>A|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	CYP21A2:c.1174G>A|likely pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	DHCR7:c.964-1G>C|pathogenic/likely pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	CYP17A1:c.1084C>T|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	DNAH5:c.13486C>T|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	FAH:c.1062+5G>A|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Click on a column header to sort the data (in ascending or descending order). See the table below for column definitions.

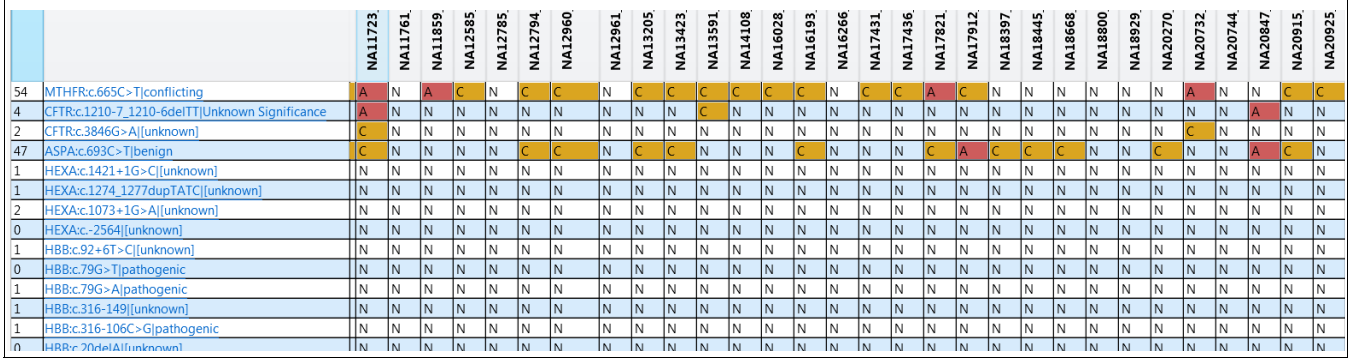
IMPORTANT! As additional samples are run, you must refresh the Batch Summary table to reveal the newly analyzed samples. To do this, click **File** → **Select CarrierScan Results Folder**.

Column	Description
#	Number of samples with Carrier (C), Affected (A), or CNgain statuses.
Gene Variant	Identity of the Variant as the combination of the Gene and Variant Status as found on the panel in their respective columns. **Note:** Clicking on the Gene:Variant auto-launches a ClinVar search result for the Variant (if it exists). For exonic regions, you must perform the ClinVar search manually.
Sample Columns	Sample Names with the number of variants with Carrier (C), Affected (A), Unaffected Normal (N), or CNgain statuses. **Note:** A sample status with an asterisk [*] denotes multiple sample status.

Selecting a sample column will auto-sort the column by its sample status (Figure 20) in the following order:

- **Affected (A)**
- **Carrier (C)**
- **CN gain**
- **NoCall/Unknown (NC)**
- **NRP**
- **Normal (N)**

Figure 20 Sample sorting



		NA11723	NA11761	NA11859	NA12585	NA12785	NA12794	NA12960	NA12961	NA13205	NA13423	NA13591	NA14108	NA16028	NA16193	NA16266	NA17431	NA17436	NA17821	NA17912	NA18397	NA18445	NA18668	NA18800	NA18929	NA20270	NA20732	NA20744	NA20847	NA20915	NA20925
54	MTHFR:c.665C>T|conflicting	A	N	A	C	N	C	C	N	C	C	C	C	C	C	N	C	C	A	C	N	N	N	N	N	N	A	N	N	C	C
4	CFTR:c.1210-7_1210-6delTT|Unknown Significance	A	N	N	N	N	N	N	N	N	N	C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N
2	CFTR:c.3846G>A|[unknown]	C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	C	N	N	N	N
47	ASPA:c.693C>T|benign	C	N	N	N	N	C	C	N	C	C	N	N	N	C	N	N	N	C	A	C	C	C	N	N	C	N	N	A	C	N
1	HEXA:c.1421+1G>C|[unknown]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	HEXA:c.1274_1277dupTATC|[unknown]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	HEXA:c.1073+1G>A|[unknown]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
0	HEXA:c.-2564|[unknown]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	HBB:c.92+6T>C|[unknown]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
0	HBB:c.79G>T|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	HBB:c.79G>A|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	HBB:c.316-149|[unknown]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	HBB:c.316-106C>G|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
0	HBB:c.20del|[unknown]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

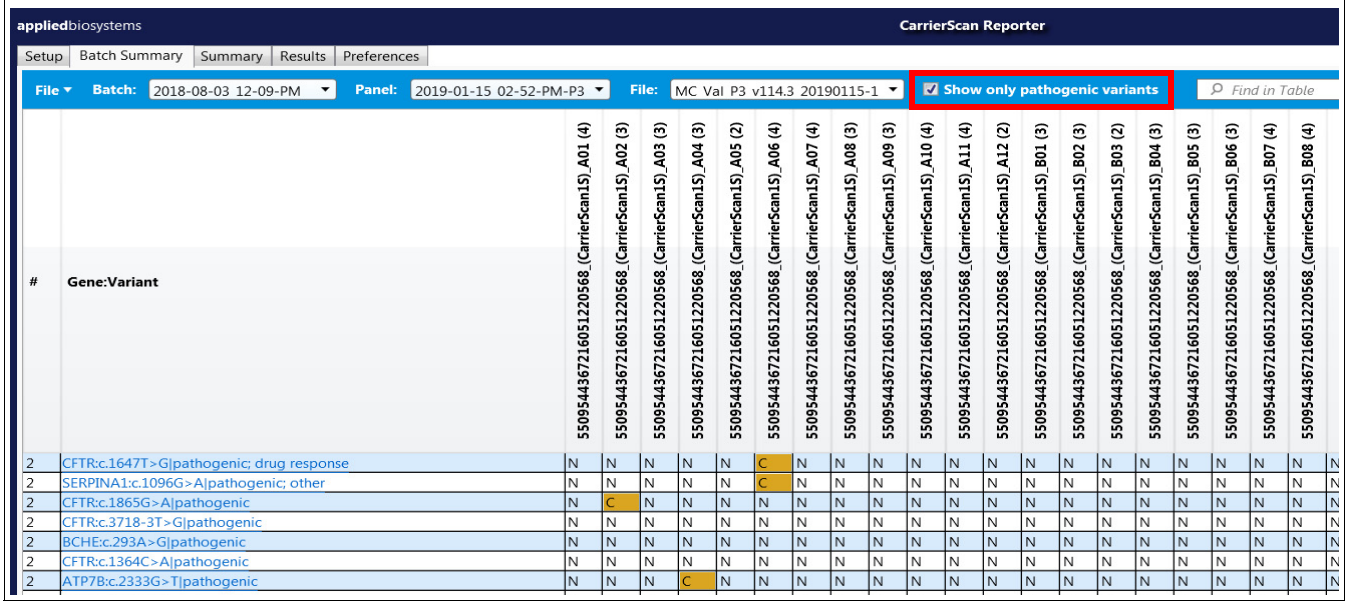
Displaying only pathogenic variants

1. Click the **Show only pathogenic variants** check box.

   Only variants with pathogenic statuses from the panel appear.

   Note: Sample tallies within the column headers are recalculated based on what is displayed, however the # column does NOT recalculate to show which samples have Carrier (C), Affected (A) or CNgain statuses for that variant, as shown in Figure 21.

Figure 21 Show only pathogenic variants



#	Gene:Variant	5509544367216051220568_(CarrierScan1S)_A01 (4)	5509544367216051220568_(CarrierScan1S)_A02 (3)	5509544367216051220568_(CarrierScan1S)_A03 (3)	5509544367216051220568_(CarrierScan1S)_A04 (3)	5509544367216051220568_(CarrierScan1S)_A05 (2)	5509544367216051220568_(CarrierScan1S)_A06 (4)	5509544367216051220568_(CarrierScan1S)_A07 (4)	5509544367216051220568_(CarrierScan1S)_A08 (3)	5509544367216051220568_(CarrierScan1S)_A09 (3)	5509544367216051220568_(CarrierScan1S)_A10 (4)	5509544367216051220568_(CarrierScan1S)_A11 (4)	5509544367216051220568_(CarrierScan1S)_A12 (2)	5509544367216051220568_(CarrierScan1S)_B01 (3)	5509544367216051220568_(CarrierScan1S)_B02 (3)	5509544367216051220568_(CarrierScan1S)_B03 (2)	5509544367216051220568_(CarrierScan1S)_B04 (3)	5509544367216051220568_(CarrierScan1S)_B05 (3)	5509544367216051220568_(CarrierScan1S)_B06 (3)	5509544367216051220568_(CarrierScan1S)_B07 (4)	5509544367216051220568_(CarrierScan1S)_B08 (4)
2	CFTR:c.1647T>G|pathogenic; drug response	N	N	N	N	N	C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	SERPINA1:c.1096G>A|pathogenic; other	N	N	N	N	N	C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	CFTR:c.1865G>A|pathogenic	N	C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	CFTR:c.3718-3T>G|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	BCHE:c.293A>G|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	CFTR:c.1364C>A|pathogenic	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	ATP7B:c.2333G>T|pathogenic	N	N	N	C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

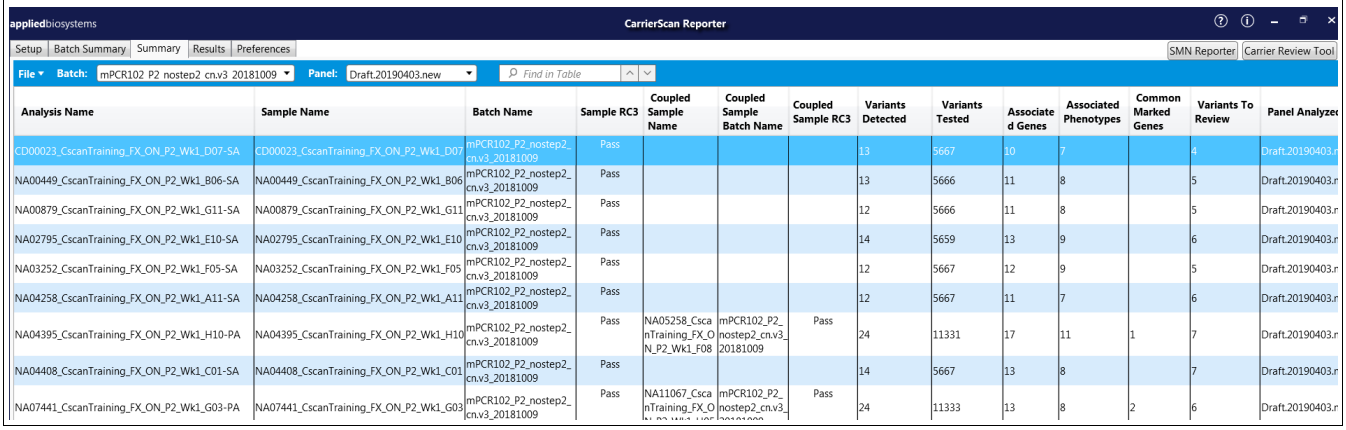
Summary table

After setting up and successfully running an analysis, as described in Chapter 2, "Setup" on page 9, click the **Summary** window tab to view your results. (Figure 22)

The Summary table provides a summary of all analyses run in a session. Each session's analyses are contained within a time-stamped folder. This folder includes the first session's analysis and any subsequent analyses thereafter - until the software is closed.

An **AnalysisSummary.txt** file is auto-generated at the start of each session. This file captures your session's table data, then continues to auto-save any subsequent analyses until you end your session. The file resides in the Output folder you assigned earlier and can be used for reference purposes.

Figure 22 Summary window tab



Click on a column header to sort the data (in ascending or descending order). See the table below for column definitions.

Column	Description
Analysis Name	The name provided for the analysis. SA denotes individual analysis. PA denotes paired analysis.
Sample Name	Displays the filename of the sample.
Batch Name	The name provided for the Batch that contains the sample.
Sample RC3	Sample QC metric based on the number of rare variants determined to be non-normal.
Coupled Sample Name	Only for paired analysis. The other sample filename in the pair.
Coupled Sample Batch Name	Only for paired analysis. The Batch name containing the other filename in the pair.
Variants Detected	Number of non-normal genetic variations detected.
Variants Tested	Total genetic variations analyzed on the selected panel.
Associated Genes	Number of genes associated with the number of genetic variations detected.
Associated Phenotypes	Number of phenotypes associated with the number of genetic variations detected.
Panel Analyzed	Name of the Panel used in the analysis.

Column	Description
Common Marked Genes	Number of common genes with 'pathogenic' genetic aberrations for paired samples. Pathogenic variants are defined by the Variant Status from the panel used for analysis.
Variants to Review	Number of genetic variants on the panel that require follow-up and review. Variants in this category were either Unknown or No Call.
Notes	Notes that were provided on the Mapping File. Additional notes can be added. *To do this:* 1. Click inside a Notes cell. A cursor appears. 2. Enter any additional notes. 3. Click outside the cell to add/save your additional note. **Note:** Your amended notes are not saved unless you click File → Export and save the file using a different name.

Searching table content

1. From the Summary window tab's **Search** box, type the name or portion of the name you want to find.
2. Click the up or down arrow (or press the Enter key).

   If a match is found, it is highlighted in the table.
3. Click the up or down arrow (or press the Enter key) to go to the next/previous matching entry.

   Note: All columns are searched, except for columns containing date and/or timestamps.

Exporting the summary table

1. Click **File** → **Export Summary Results**.

   An Explorer window appears.
2. Navigate to an easily accessible save location, name your export file, then click **Save**.

   Your exported file is saved as a tab-delimited TXT file.

4 Results table

Using the results table

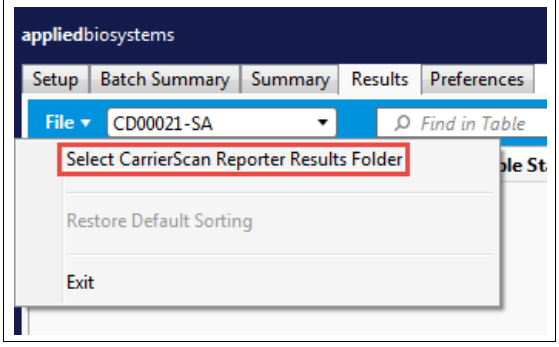
After reviewing your summary, as described in Chapter 3, "Summary tables", you can view more detailed results of your analysis by clicking on the **Results** window tab.

Note: If an analysis has been run in the current session, the default Results view lists your results by Analysis Name (sorted by lowest alphabetical name first).

Selecting a results folder

1. From the **Results** window tab, click **File** → **Select CarrierScan Reporter Results Folder**. (Figure 23)

Figure 23 Select a CarrierScan Results Folder

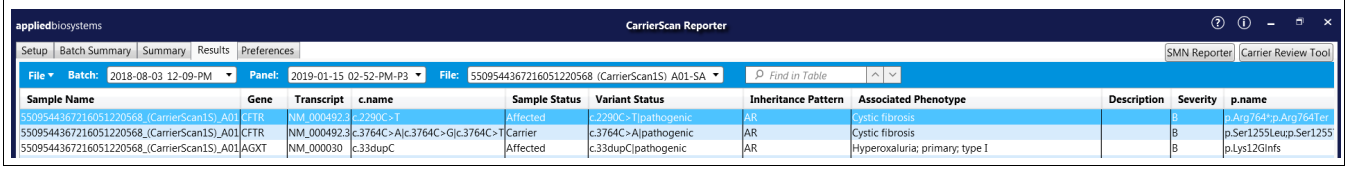


A **Select results folder** window appears.

2. Click to highlight the previously ran analysis folder you want to view in detail, then click **Select Folder**. **Note:** Past results can only be viewed if the original files (inside the folder you select) have not been modified.

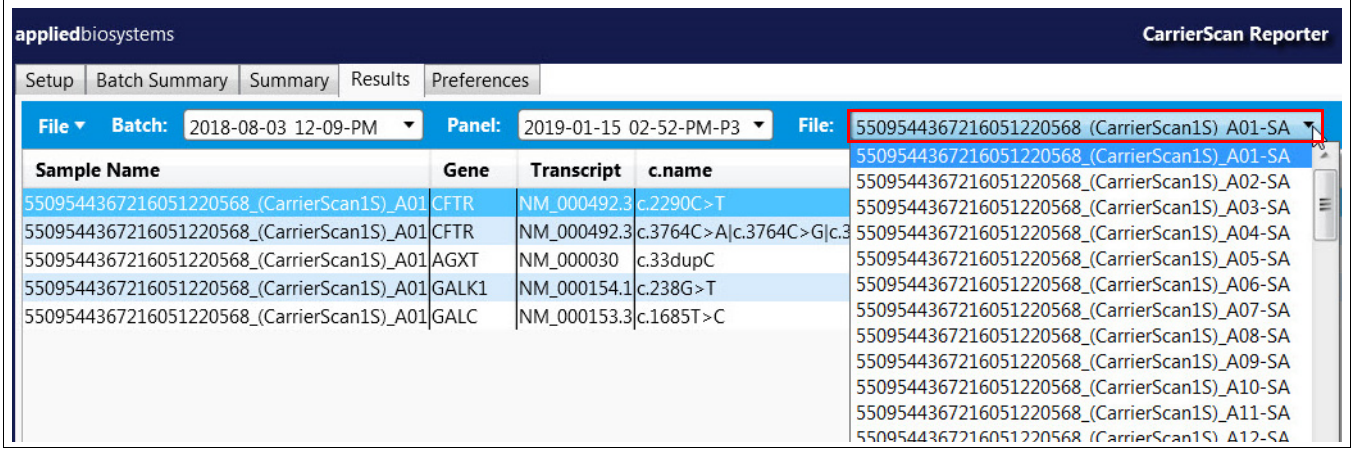
   A detailed analysis of your selected folder appears in the Analysis Results Table, as shown in Figure 24.

Figure 24 Results Table



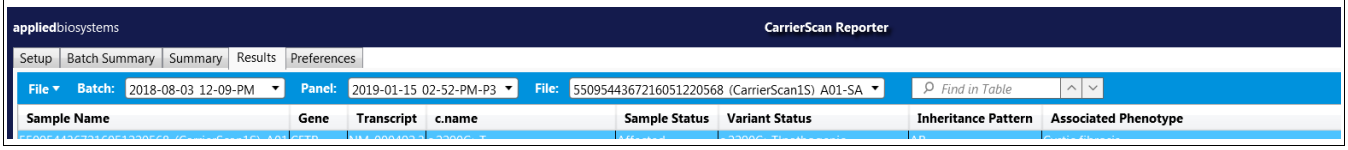
3. Click the **Sample Name** drop-down menu (Figure 25) to select a sample to view.

Figure 25 Selecting a specific Sample Name



The Results table populates with your selected Sample Name's details, as shown in Figure 26.

Figure 26 Analysis details shown in Results Table



See the table below for column definitions.

Column	Description
Sample Name	Displays the filename of the sample.
Gene Name	Name of heritable genetic sequence that encodes proteins.
Transcript c.name	RefSeq transcript ID associated with the c.name.
Sample Status	Hereditary status of the sample for the associated phenotype based on information from the public domain. **Note:** An asterisk [*] indicates that the variant has varying calls and should be reviewed.
Variant Status	Standard classification of variant significance based on ACMG guidelines (pathogenic, likely pathogenic, etc.) **Note:** An asterisk [*] indicates variable or conflicting significance across reported sources. For more information, go to: https://www.ncbi.nlm.nih.gov/pubmed/25741868
Inheritance Pattern	Method of Inheritance (Example: AR (Autosomal Recessive), XLR (X-linked Recessive)
Associated Phenotype	Displays the Phenotype that is associated with the variant.
Description	Detailed information about the Phenotype.

Column	Description
Severity	Severity and impact rating of phenotypes. A = Profound, B = Severe, U = Unclassified, based on systematic classifications. For more information, go to: https://www.ncbi.nlm.nih.gov/pubmed/25494330
c.name	Displays standard variant nomenclature based on coding DNA reference sequences.
p.name	Displays the change in protein translation for the variant.
g.name	Displays the genomic coordinates for the variant.
Alternate Names	Displays the alternative names for the variant.

Searching table content

1. From the **Search** box in the Results window tab, type the name (or portion of the name) you want to locate.
2. Click the up or down arrow (or press the **Enter** key).

   If a match is found, it is highlighted in the table.
3. Click the up or down arrow (or press the **Enter** key) to go to the next/previous matching entry.

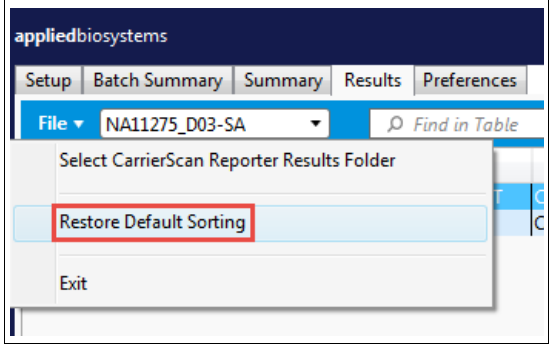
Default row sorting

The Results table (Figure 26) displays Variant Statuses containing 'pathogenic' content first, then 'pseudo'-deficiency related variants.

Click once on a column header to sort its rows (in ascending or descending order) or drag-and-drop a row(s) to its new location within the table.

If you want to return to the default sorting order, click **File** → **Restore Default Sorting**.

Figure 27 Restore Default Sorting



Note: Custom sorting is not saved, as exiting and then reopening the application returns the Results table to its default sorted order.

5 Exporting

Exports

For each analysis run, four exports are auto-generated.

Each of these exports reside in the Output folder you assigned earlier on page 8.

- "Full export"
- "Positive export" on page 30
- "Summary export" on page 30
- "Review export" on page 31

The exports for an Individual analysis provide information pertaining to the single sample in the analysis.

The exports for the Paired analysis provide information pertaining to both samples used in the paired analysis.

Full export

The Full Export details the data for all the markers in the panel whether or not a variant was detected. It also contains all probesets designed to analyze markers in question (especially for genotyping (GT) and Indel markers where multiple probesets are used to check for a particular SNP).

Note: The Full Export column definitions listed below were referenced from the White Paper *PMID: 25730230 DOI: 10.1097/AOG.0000000000000666*. To access this publication, go to: https://www.ncbi.nlm.nih.gov/pubmed/25730230

IMPORTANT! The table below lists the columns included in the CarrierScan Reporter's commercial library file package. By default, all columns are displayed in all outputs and in the tables, but are not essential for the software to run. However, if you are creating a custom annotation panel, the columns noted with an asterisk [*] must be included in your annot.panel file.

Column	Description
Sample Name *	Displays the filename of the sample.
Gene Name *	Name of heritable genetic sequence that encodes proteins.
Transcript c.name	RefSeq transcript ID associated with the c.name.
Sample Status	Hereditary status of the sample for the associated phenotype based on information from the public domain. **Note:** An asterisk [*] indicates that the variant has varying calls and should be reviewed.
Variant Status *	Standard classification of variant significance based on ACMG guidelines (pathogenic, likely pathogenic, etc.) **Note:** An asterisk [*] indicates variable or conflicting significance across reported sources. For more information, go to: https://www.ncbi.nlm.nih.gov/pubmed/25741868

Column	Description
Inheritance Pattern	Method of Inheritance (Example: AR (Autosomal Recessive), XLR (X-linked Recessive)
Associated Phenotype	Displays the Phenotype that is associated with the variant.
Description	Detailed information about the Phenotype.
Severity	Severity and impact rating of phenotypes based on these systematic classifications: A = Profound, B = Severe, U = Unclassified For more information, go to: https://www.ncbi.nlm.nih.gov/pubmed/25494330
c.name *	Displays standard variant nomenclature based on coding DNA reference sequences.
p.name	Displays the change in protein translation for the variant.
g.name	Displays the genomic coordinates for the variant.
Alternate Names	Displays the alternative names for the variant.
Carrier Frequency - Population 1 (Optional)	Proportion of the named Population 1 that has the recessive trait or phenotype. **Note:** This column is included if it is on the annotation panel used for analysis.
Detection Rate Population 1 (Optional)	Theoretical proportion of carriers in the named Population 1 that is identified by this assay. **Note:** This column is included if it is on the annotation panel used for analysis.
Residual Chance of Occurrence Population 1 (Optional)	Remaining chance the sample will be a carrier of the associated phenotype after a negative result considering Population 1's carrier frequency. For more information, see Appendix A, "Chance of occurrence calculation guidelines". **Note:** This column is included if it is on the annotation panel used for analysis.
Marker Type	The type of marker (Indel, SNP, CN).
Affy SNP ID *	Unique Thermo Fisher Scientific generated identifier for the SNP.
Probeset ID *	Unique Thermo Fisher Scientific identifier for the probeset.
Recommended Probeset	A quality control metric determined by SNP Polisher algorithm that chooses the best probesets querying a SNP.
Probe Count	The number of probes used for the call.
Confidence	The number of probesets that agree with determined sample status for that variant.
Analysis Call	Call code for the variant (AA, AB, BB, etc).
Translated Genotype Call	The nucleotide conversion of the genotyping call code.
Ref Code	The Call Code (A,B,C) of the Reference Allele.
Alt Code	The Call Code (A,B,C,D,.....) of the Alternate Allele.
Ref Allele *	The call for the reference allele associated with a normal phenotype.
Alt Allele *	The call for the 1st alternate allele associated with a non-normal phenotype.
Variant Status Alt Allele	Severity status for the variant mapped to Alt Allele.
CN Region	The defined region used for copy number analysis.
CN State	The copy number state of the defined region.
Chromosome *	The chromosomal location of the variant.
Physical Position *	Starting genomic coordinates of the variant bases.

Column	Description
Position End	Ending genomic coordinates of the variant bases.
Plate Barcode	Barcode of the array plate.
Well Position	Position location of the array on the plate.

Positive export

Positive Export details the data for ALL markers in the panel that have a non-normal genetic state (positive result).

Note: Positive Export is a subset of the Full Export, therefore refer to the table of definitions above, as the columns featured in the Full Export and Positive Export are identical.

Summary export

The Summary Export provides summary results for ALL phenotypes in the panel that have at least one non-normal genetic event, including Affected, Carrier, and CNgain sample statuses associated with pathogenic and pseudoallele/pseudodeficiency related variant statuses.

The Summary Export contains the information provided in the Positive Export collated by variant. This export is also read into the software and shown in the Analysis Results Table.

The Positive Export and Summary Export column definitions are listed in the table below.

Column	Description
Sample Name	Displays the filename of the sample.
Gene Name	Name of heritable genetic sequence that encodes proteins.
Transcript c.name	RefSeq transcript ID associated with the c.name.
Variant Status	Standard classification of variant significance based on ACMG guidelines (pathogenic, likely pathogenic, etc.) **Note:** An asterisk [*] indicates variable or conflicting significance across reported sources. For more information, go to: https://www.ncbi.nlm.nih.gov/pubmed/25741868
Sample Status	Hereditary status of the sample for the associated phenotype based on information from the public domain. **Note:** An asterisk [*] indicates that the variant has varying calls and should be reviewed.
Inheritance Pattern	Method of Inheritance (Example: AR (Autosomal Recessive), XLR (X-linked Recessive)
Associated Phenotype	Displays the Phenotype that is associated with the variant.
Description	Detailed information about the Phenotype.
Severity	Severity and impact rating of phenotypes. A = Profound, B = Severe, U = Unclassified, based on systematic classifications. For more information, go to: https://www.ncbi.nlm.nih.gov/pubmed/25494330
c.name	Displays standard variant nomenclature based on coding DNA reference sequences.
p.name	Displays the change in protein translation for the variant.
g.name	Displays the genomic coordinates for the variant.

Column	Description
Alternate Names	Displays the alternative names for the variant.
Plate Barcode	Barcode of the array plate.
Well Position	Position location of the array on the plate.

Review export

The Review Export provides details for all variants that did not have a result on the panel. If a variant has a Sample Status result of Normal, Carrier, Affected, CN gain assessment, then it is considered tested. Otherwise, the variant is recorded in this report to be reviewed.

The Review Export column definitions are listed below.

Column	Description
Sample Name	Displays the filename of the sample.
Gene Name	Name of heritable genetic sequence that encodes proteins.
c.name	Displays standard variant nomenclature based on coding DNA reference sequences.
p.name	Displays the change in protein translation for the variant.
Probeset ID	Unique Thermo Fisher Scientific identifier for the probeset.
CN Region	The defined region used for copy number analysis.
Failure Mode	Reason the Probeset or CN Region failed to give a sample status.
Plate Barcode	Barcode of the array plate.
Well Position	Position location of the array on the plate.

A Chance of occurrence calculation guidelines

Single and paired sample analysis reports contain ethnic panels pre-populated with known frequencies and detection rates.

Note: The Chance of Occurrence Guideline calculations (shown below) were referenced from the European Journal of Human Genetics White Paper *PMID: 18685558 PMCID: PMC2985951 DOI: 10.1038/ejhg.2008.136.* To access this publication, go to: https://www.ncbi.nlm.nih.gov/pubmed/18685558

- Calculations for paired residual chance of occurrence (RCO) are:

  (RCO1) x **(RCO2)** x **1/4** = **paired RCO**

  - **RCO1** = Residual chance of occurrence for sample 1 for the assigned ethnicity and associated phenotype of normal sample status.
  - **RCO2** = Residual chance of occurrence for sample 2 for the assigned ethnicity and associated phenotype of normal sample status.
  - When the sample status is deemed **Carrier** or **Affected**, the values used for RCO are **1** and **2** respectively.

Special handling is performed for Alpha-Thalassemia sample status determination that affects the RCO values used in calculations. Copy number states of HBA1 and HBA2 are considered together (according to guidelines referenced from NCBI GeneReviews). To access this publication, go to: https://www.ncbi.nlm.nih.gov/books/NBK1435

For example, in all reports (exclusive of the BatchSummaryResults) the status for both HBA1 and HBA2 copy number regions will always have the same sample status regardless if the genetic aberration occurs on only 1 region. In the BatchSummaryResults file, the HBA1 and HBA2 copy number regions are assessed independently to allow for review of each variant.

In the scenario of a cis/trans single deletion of HBA1 and HBA2, the cis orientation is assumed as input into the paired RCO calculations.