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UserGuide

OncoScan™ Console 1.3

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Contents

Chapter 1

Installation and First Time Setup

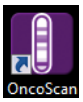
System Requirements

Operating System
Windows® 7 Professional (64-bit) with Service Pack 1 installed

Installing OncoScan Console

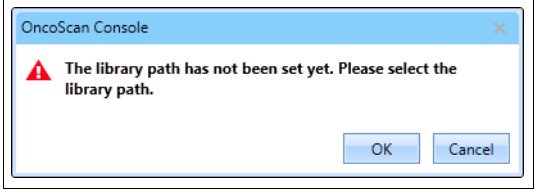
1. Go to **www.affymetrix.com** and navigate to the following location:
   Home > *Products* > *Microarray Solutions* > *Instruments and Software* > *Software* >
2. Locate and download the zipped OncoScan Console software package.
3. Unzip the file, then double-click **OncoScanSetup64.exe** to install it.
4. Follow the directions provided by the installer.

Starting and Setting Up OncoScan Console

1. Locate the OncoScan Console Desktop shortcut, 

then double-click on it.
   The first time you launch OncoScan Console a window appears prompting you to set your Library path. (Figure 1.1)

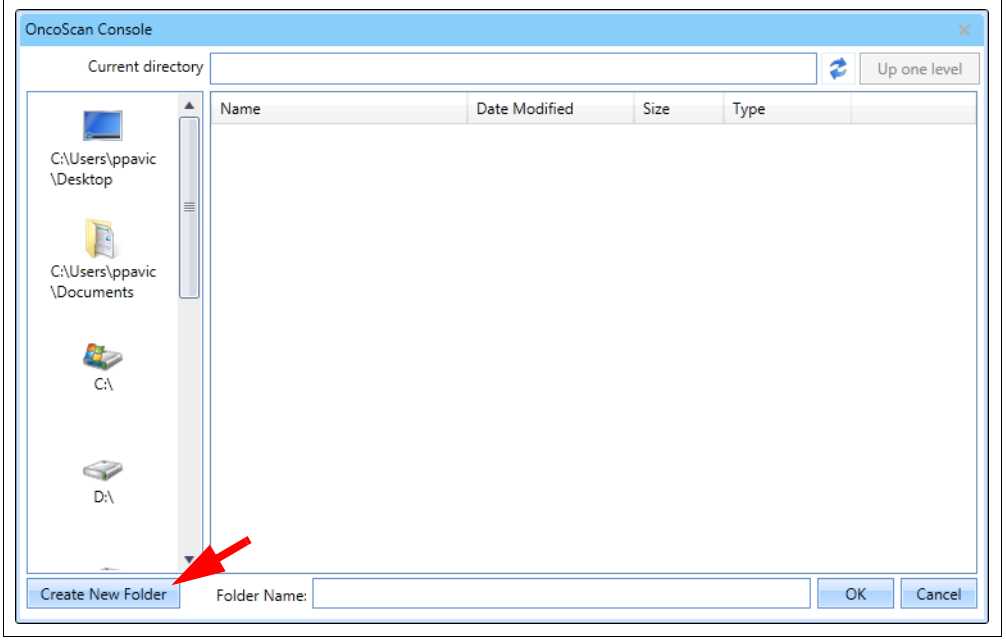
Figure 1.1 Library path message



2. Click **OK**.

The following window appears: (Figure 1.2)

Figure 1.2 Set Library Path Explorer window



Setting a Library Path

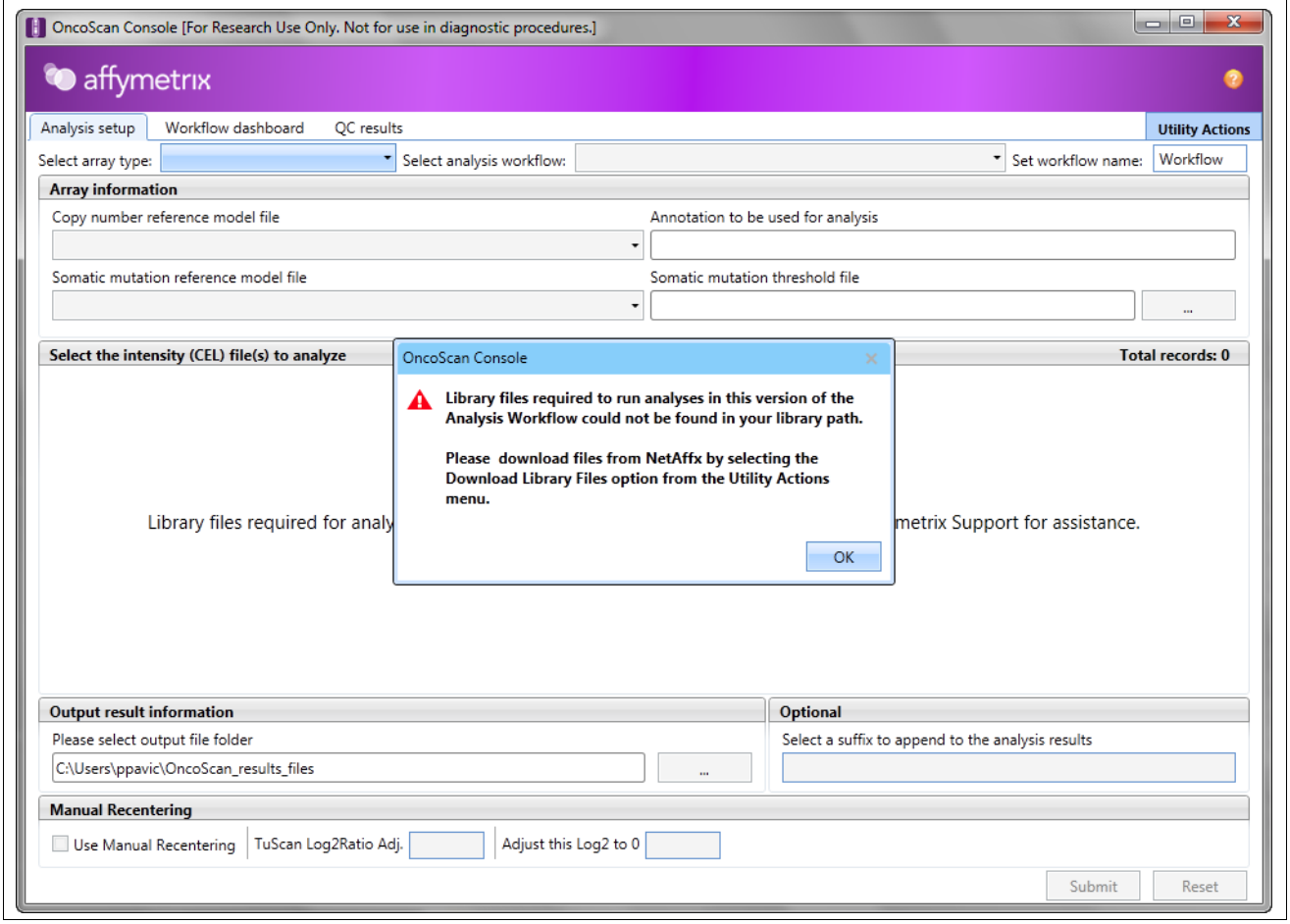
Make sure your assigned Library Path folder is placed in a high-level, easy to access, local directory. (Example: `C:\`)

1. Click the **Library File path** field's browse button.
   An Explorer window appears.
2. Navigate to a high-level, easy to access, local directory.
   (Example: `C:\`)
3. Click **Create New Folder** (lower left) to create a Library Files path folder.
4. In the Create New Folder field, enter a folder name. (Example: `C:\OncoScanLib`)
5. Click **OK**.

NOTE: During the installation process, outdated library files are auto-detected, then automatically moved to an archive folder. Make sure you always download the latest available library files after installing a new version of OncoScan Console.

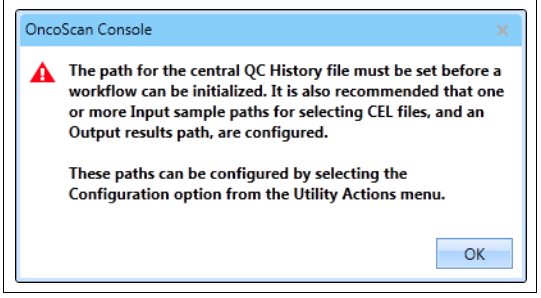
The following window and message appears. (Figure 1.3)

Figure 1.3 Library Files message



6. Acknowledge the message, then click **OK**.
   To download files from NetAffx, go to *Downloading Analysis Files from NetAffx* on page 13.
   The following message appears. (Figure 1.4).

Figure 1.4 Library path message

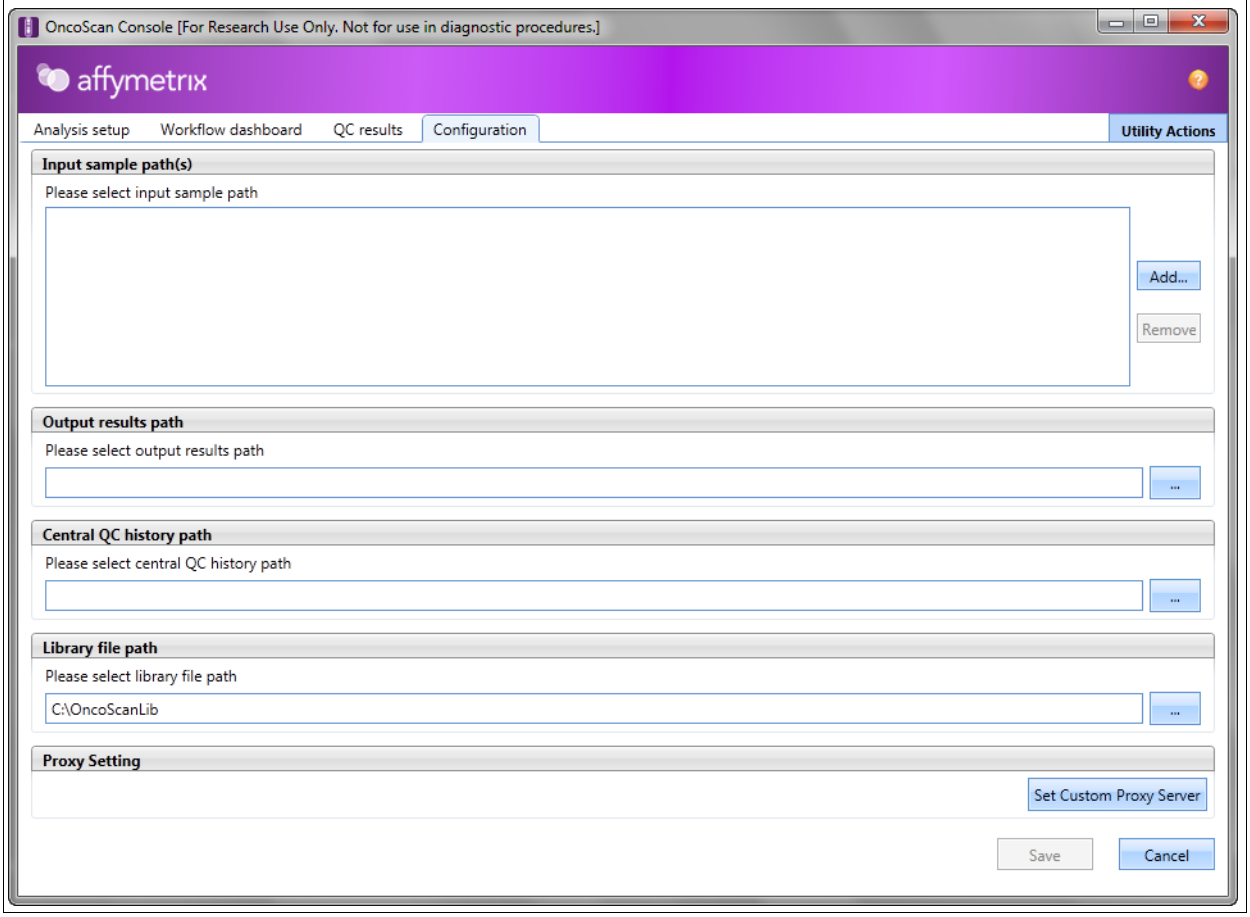


7. Acknowledge the message, then click **OK**.
8. Click the **Utility Actions** button, then click on **Configuration**.

The **Configuration** window tab appears, as shown in Figure 1.5.

NOTE: You only need to perform the following steps once, as the data and selections you enter (throughout this section) are retained for your convenience.

Figure 1.5 Main window



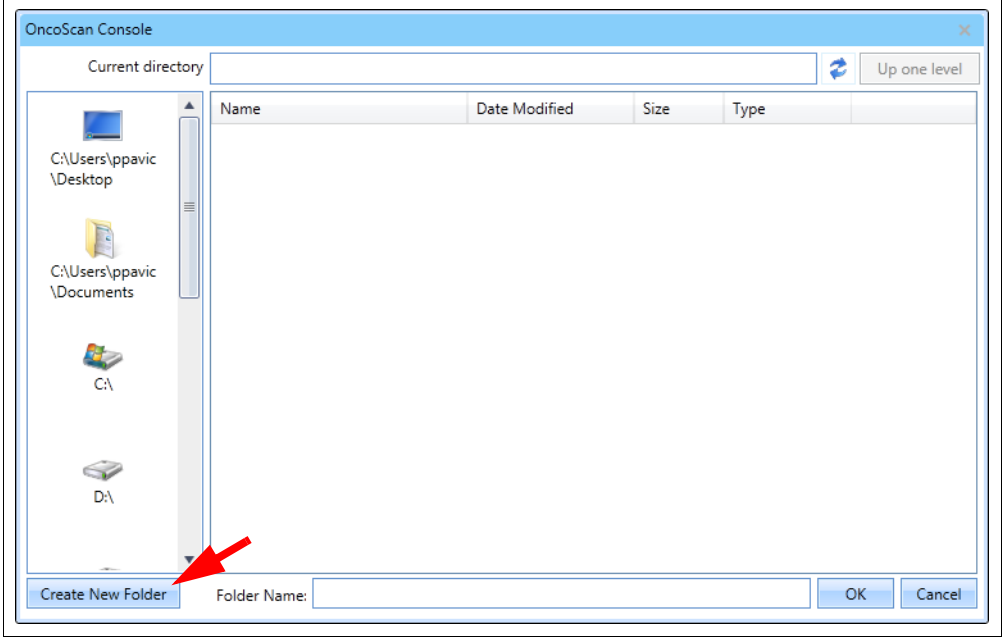
Assigning an Input Sample Path

The Input Sample Path folder is the location you normally store your CEL files.

1. Click **Add**.

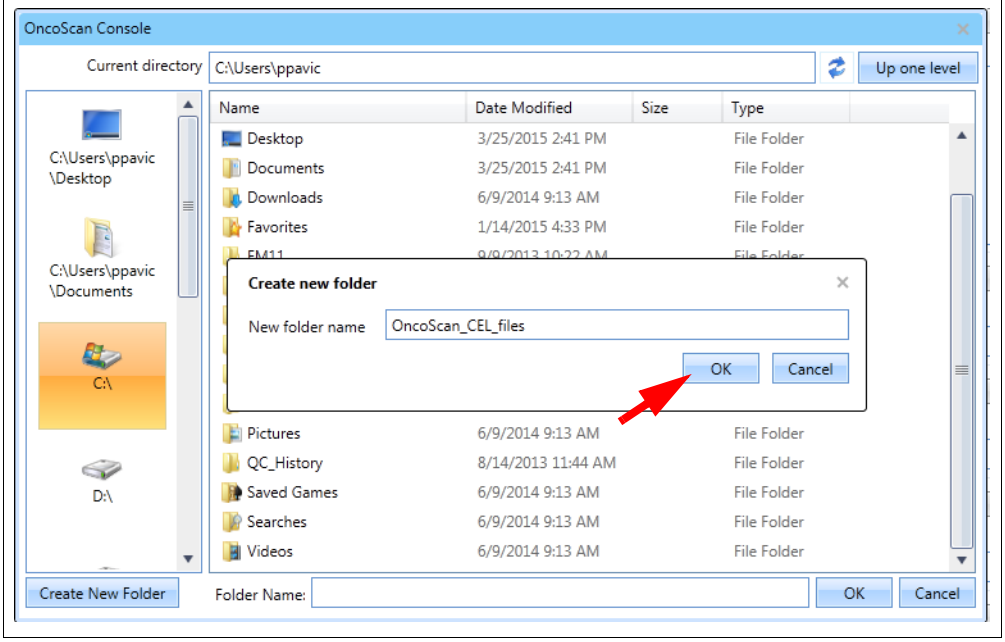
The following window appears: (Figure 1.6)

Figure 1.6 Add Input sample files window



2. Navigate to the recommended **C:\Users** directory, then click the **Create New folder**. (Figure 1.6)
3. In the Create New Folder window field, enter a folder name. (Example: **`C:\Users\YourName\OncoScan_CEL_files`**), then click **OK**. (Figure 1.7)

Figure 1.7 Add Input sample files window



4. Click **OK** to close the window.
   Your new input folder and its path appear, as shown in Figure 1.8.

Assigning an Output Results Path

1. Click the **Output results path** field's browse button.
   An Explorer window appears.
2. Navigate to the recommended **C:\Users** directory, then click **Create New Folder**.
3. In the Create New Folder field, enter a folder name.
   (Example: **`C:\Users\YourName\OncoScan_results_files`**)
4. Click **OK**.
   Your new output folder and its path appear, as shown in Figure 1.8.

Adding Sub-Folders

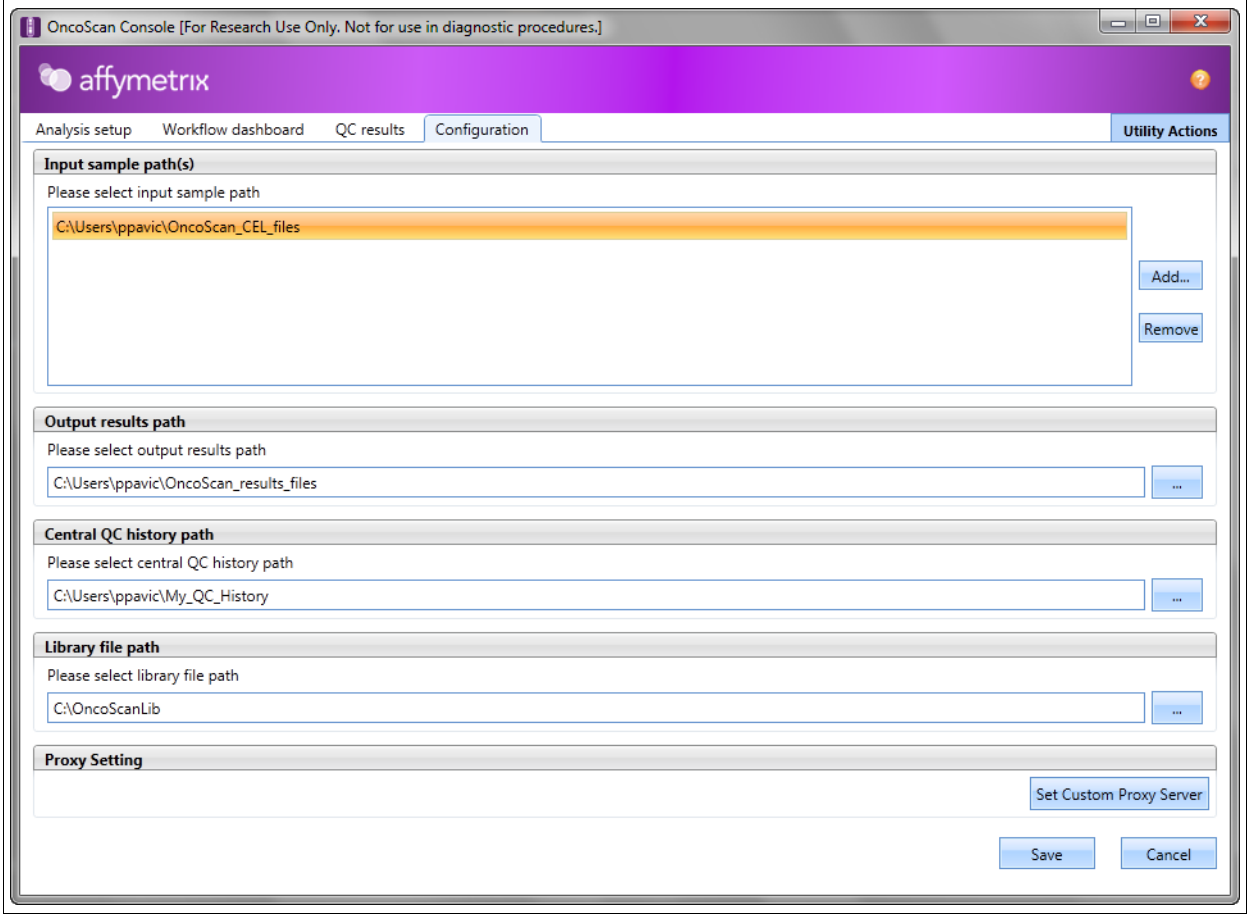
TIP: Add sub-folders to your newly assigned output result path's folder to better organize your output results,

1. The **Output results path** field's browse button to return to your newly assigned output folder.
2. Click **Create New Folder**.
3. Enter a sub-folder name.
4. Click **OK**.
   The newly created sub-folders now appear in the output result information window.
5. Repeat the above steps 1-4 to add more sub-folders.

Assigning a Central QC History Path

1. Click the **Central QC history path** field's browse button.
   An Explorer window appears.
2. Navigate to: **`C:\ProgramData\Affymetrix\OncoScan`**
3. Click **Create New Folder** (lower left) to create a Central QC history path folder.
4. In the Create New Folder field, enter a folder name. (Example: **`My_QC_History`**)
5. Click **OK**, then click **OK** again.
   Your QC History folder now appears in the Central QC History path field, as shown in Figure 1.8.

Figure 1.8 Main window showing assigned paths



Setting Proxy Server Access

This configuration should only be done if the user's system has to pass through a proxy server to access Affymetrix NetAffx server.

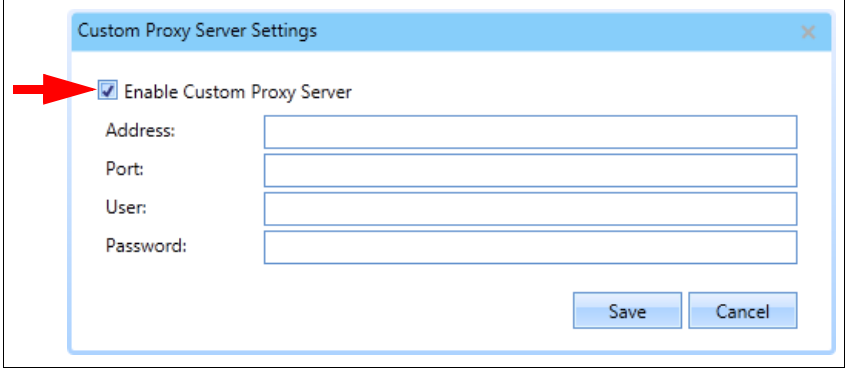
In most cases, when a customer requires the use of a proxy, they can set a system-level proxy using their default Internet browser while keeping the OncoScan Console default setting at **Use System Proxy**.

NOTE: You may need to contact your IT Department for system proxy information.

1. From the Configuration window tab, click Set Custom Proxy Server.

The **Custom Proxy Server Settings** window opens (Figure 1.9).

Figure 1.9 Configuration window



2. Click the **Enable Custom Proxy Server** checkbox, then complete the required fields.

NOTE: This proxy user ID and password is NOT the same ID and password used to connect to the Affymetrix NetAffx server.

3. Click **Save**.
4. Click **Save** to save all your **Configuration** window tab settings and paths.

Downloading Analysis Files from NetAffx

After your Library Path folder is created, you must download the library files that OncoScan Console uses to analyze and annotate the data from NetAffx.

NOTE: You can also download the analysis library file package from directly from *www.affymetrix.com*. After downloading, unzip the contents of the file directly into the Library folder you assigned earlier.

If you go to the website (outside of OncoScan Console) to download the analysis library file package, you must close, then restart OncoScan Console in order for it to recognize the newly downloaded files.

1. Click on **Utility Actions** -> **Download Library Files** or open your Internet browser and go to www.affymetrix.com.
2. Enter your NetAffx user name and password or click **Register Now** to create a NetAffx account.
   The **Choose Files** window opens with a list of array types supported by the software.
3. Click the OncoScan array checkbox.
4. Click **Next**.
   The Download Progress window displays the progress of the downloading and unpacking of the files.

Uninstalling OncoScan Console

1. From the Windows Start Menu, navigate to the Windows Control Panel.
2. Navigate to the **Uninstall or change a program**.
3. Locate the **OncoScan Console** application, then perform the uninstall as you normally would.

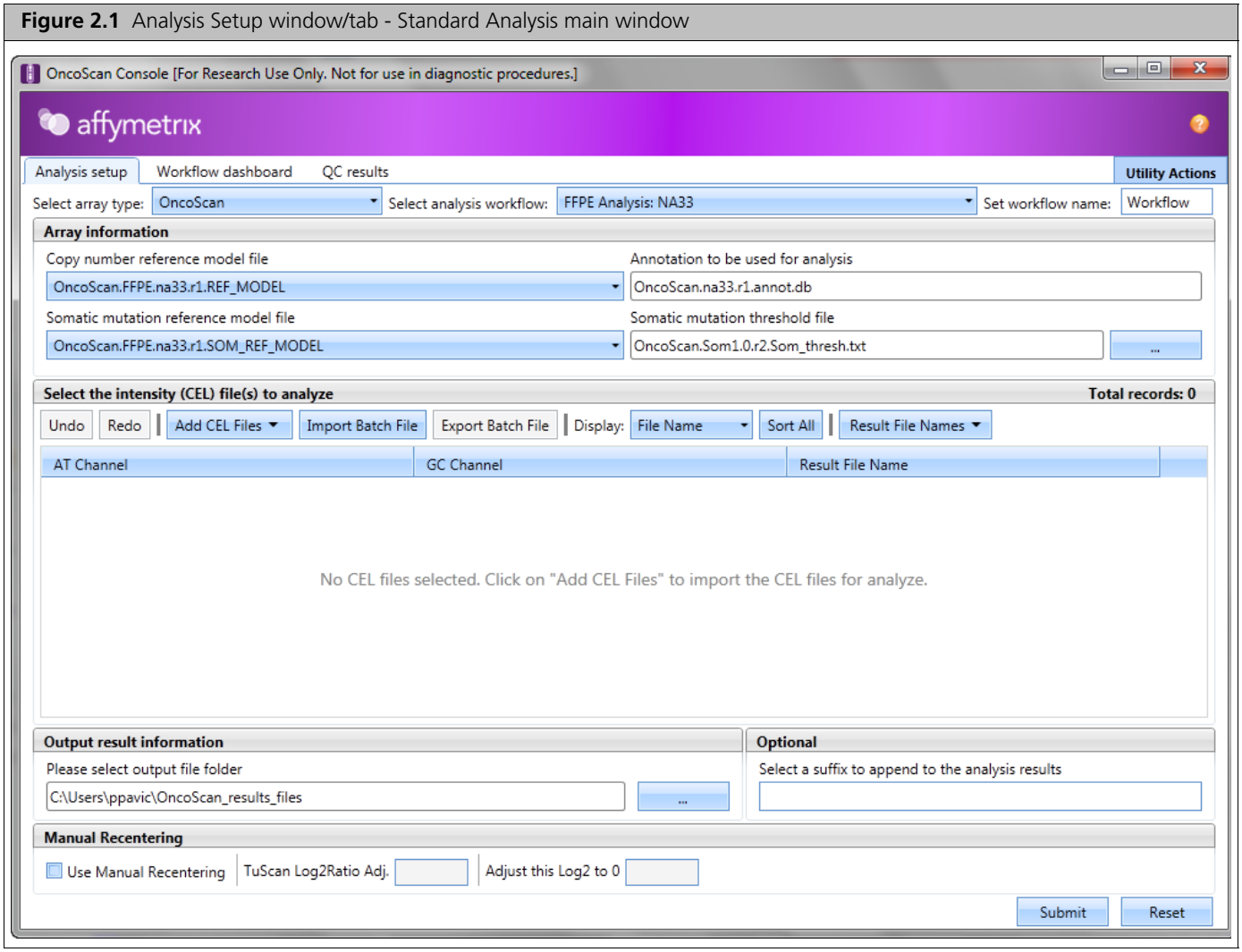
NOTE: Your data and library files are NOT deleted by uninstalling OncoScan Console.

Chapter 2

Standard Analysis Setup

To setup a **Matched Normal Analysis** go to *Matched Normal Analysis Setup* on page 53.

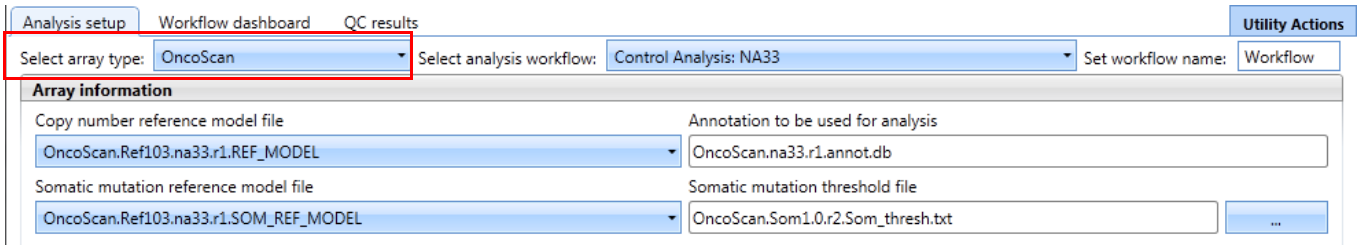
Figure 2.1 Analysis Setup window/tab - Standard Analysis main window



Selecting Array Information

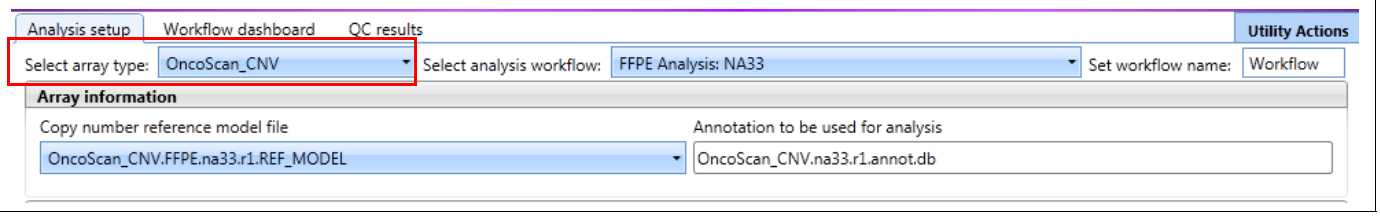
1. From the **Select array type** drop-down list, click to select either **OncoScan** or **OncoScan_CNV**. As long as your library file folder contains the necessary analysis files for the array, your configuration paths are established and your Array Information fields auto-populate, as shown in Figure 2.2.

Figure 2.2 Standard Analysis Configuration - OncoScan array



Somatic mutation file selection is NOT available with the OncoScan_CNV array type, as shown in Figure 2.3.

Figure 2.3 Standard Analysis Configuration - OncoScan_CNV array



NOTE: The *Select array type* drop-down list includes only the array types from the library (analysis) files that have been downloaded from NetAffx or copied from the Library package provided in the OncoScan installation package.

IMPORTANT: After adding new library files to the library file folder, always close and re-launch OncoScan Console to ensure the newly added files are recognized by the software.

2. From the **Select analysis workflow** drop-down list, click to select an analysis workflow.
   - **FFPE Analysis: NA33** - Use this workflow for analyzing FFPE samples.
   - **Non-FFPE Analysis: NA33** - Use this workflow for analyzing Non-FFPE samples.
   - **Control Analysis: NA33** - Use this workflow for analyzing the Ref103 control sample.
   - **FFPE Analysis including Matched Normal: NA33** - Use this workflow when you have DNA from normal and tumor tissue from the same FFPE fixed specimen. To setup a **Matched Normal Analysis** go to page 53.
   - **Reference Generation: NA33** - Select this option when you want to create your own Reference File. See *Appendix A: Custom Reference Files* on page 65.

3. (Optional) Enter a Workflow name. By default, the **Set workflow name** is *Workflow*. Click Workflow (upper right) to enter a different workflow name.

TIP: Customizing a Workflow name can be a useful tool in keeping track of analysis workflows as all the related output files (outside of the OSCHP file) begin with this workflow name.

4. Select a **Copy Number reference model file**. By default, it is set to the most recently used reference model file. If you created your own reference model file, click the drop-down list to select your .REF_MODEL. Check to ensure the reference model file is appropriate for the sample type.

   The Annotation file is automatically selected for you and is based on your selected reference model file. (Example: **OncoScan.na33.v1.annot.db**)

   NOTE: The *Annotation to be used for analysis* field is auto-populated based on your Ref Model file selection. The analysis is not be permitted to run if the appropriate annotation file is not available in your Library folder.

5. Select a **Somatic mutation reference model file.** (OncoScan array only. Not applicable to OncoScan_CNV array.)

   By default, it is set to the most recently used SOM reference model file. If you created your own reference model file, click the drop-down list to select your **.SOM_REF_MODEL**.

6. Check to ensure the somatic mutation reference model file is appropriate for the sample type. If you need to change it, click the **Browse** button, navigate to the appropriate threshold .txt file, then click **OK**.

   IMPORTANT: If the Reference Model File and Somatic mutation Reference Model File were created independently of each other, a warning message appears after you click Submit (to start the Workflow Analysis process). Click OK to acknowledge the message.

Adding CEL Files to Analyze

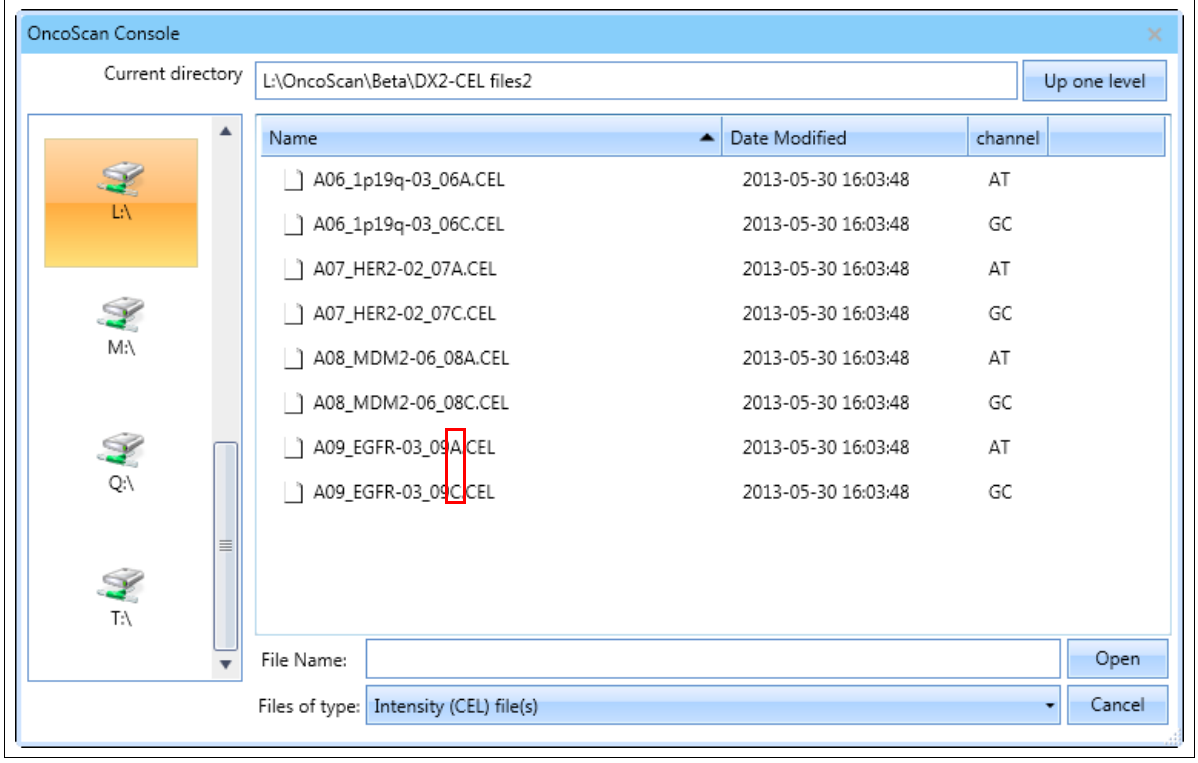
You can manually add CEL files or import them as a tab-delimited text file.

Manually Adding CEL Files to Analyze

To add a batch file containing the list of CEL files, see *Importing CEL Files Using Batch Import* on page 20.

1. From the **Select the intensity (CEL) file(s) to analyze** pane, click the **Add CEL files** drop-down.
2. Click **AT Channel**.
   The CEL file window appears. ((Figure 2.4)).

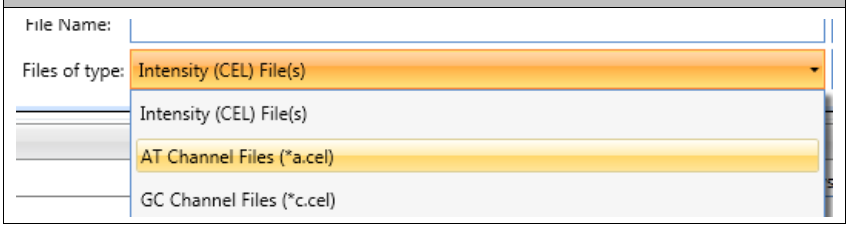
Figure 2.4 CEL file folder -Example



IMPORTANT: Affymetrix recommends using an "A" or "C" as the last character to designate the channel in the CEL file naming convention. Example: "_AS_05A.CEL" is an AT Channel file, while "_AS_05C.CEL" is a GC Channel file. See Figure 2.4.

3. Click any header to sort your files or click the **Files of type** drop-down to filter your CEL files by AT Channel, as shown in Figure 2.5.

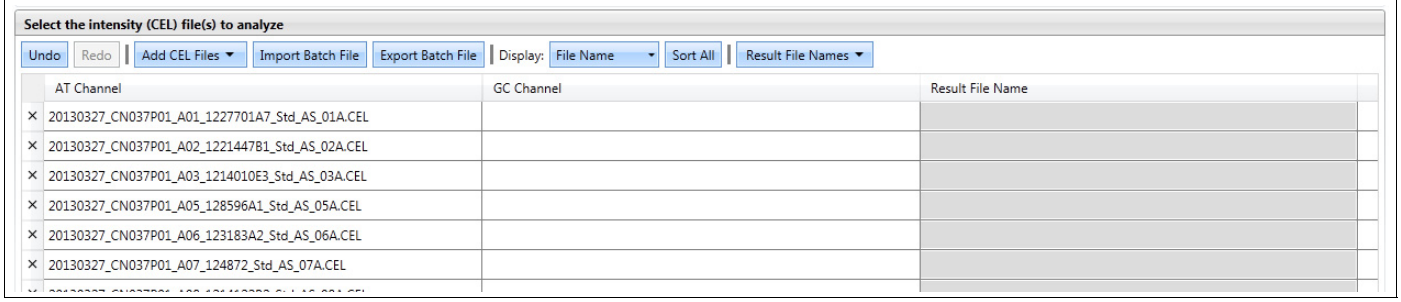
Figure 2.5 Files of type drop-down list



4. Single click, Ctrl click, or Shift click (to select multiple AT Channel files)

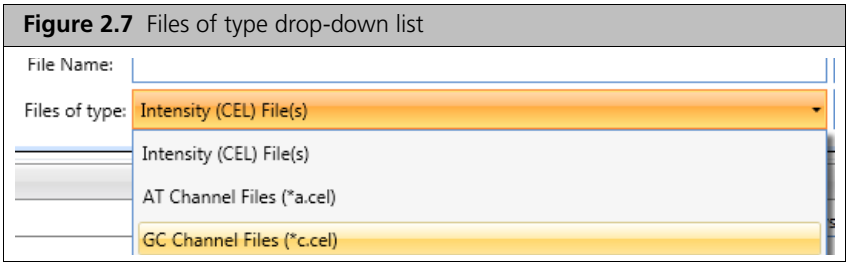
5. Click **Open**.
   The AT Channel fields are now populated. (Figure 2.6)

Figure 2.6 AT Channel file list



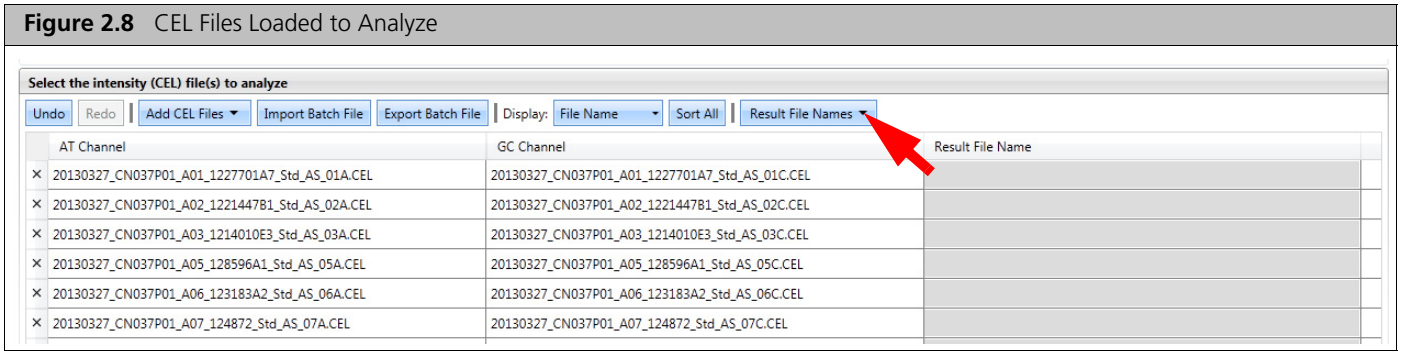
6. Click the **Add CEL files** drop-down.
7. Click **GC Channel**. The CEL file window appears. (Figure 2.4)
8. Click any header to sort your files or click the **Files of type** drop-down to filter your CEL files by GC Channel, as shown in Figure 2.7.

Figure 2.7 Files of type drop-down list



9. Single click, Ctrl click, or Shift click (to select multiple GC Channel files).
10. Click **Open**.
    The GC Channel fields are now populated. (Figure 2.8)

Figure 2.8 CEL Files Loaded to Analyze



CEL File Displaying Options (Optional)

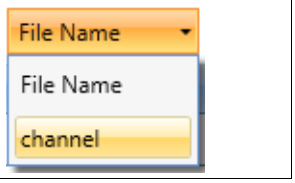
The File Name drop-down list (Figure 2.9) is dynamically populated and based on what attributes are populated in the ARR file.

To use this display option, you must:

1. Provide the appropriate attributes at the time of sample registration in AGCC.

2. The ARR files must reside in the same folder as the CEL files.

Figure 2.9 EXAMPLE: File Name Display Choices



To see "channel" (as an option in the drop down), you must use a template (or the OncoScan template provided in the library files) that contains a "channel" attribute. The resulting ARR file must also reside in the same folder as the CEL files you are analyzing.

You can display one of the attributes from the ARR file in the table. For example, "Channel" can be chosen (Figure 2.9) to confirm the assignment of a CEL file to its appropriate channel.

To select a FIle Name display attribute:

1. Click the **File Name** drop-down button, then click to select the attribute you want displayed along with your CEL file names.

   The two examples (Figure 2.10 and Figure 2.11) show how the table appears with the display set to **Filename**, then to **Channel**.

Figure 2.10 Table with Filename displayed

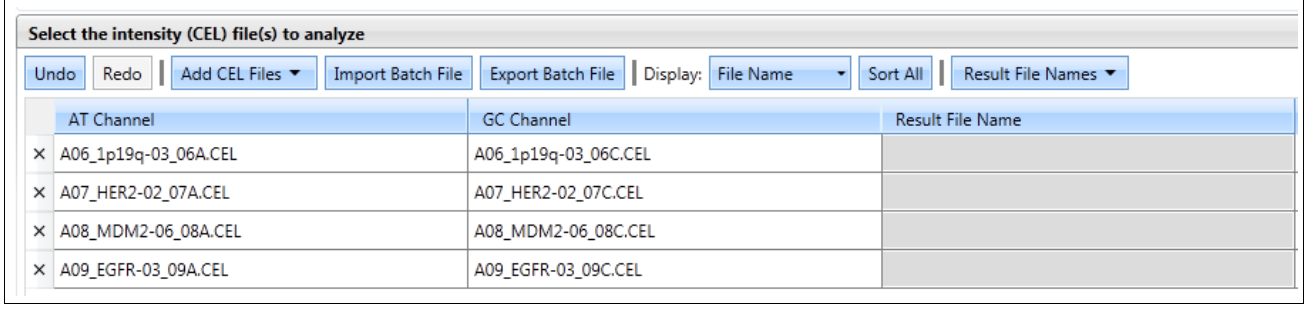
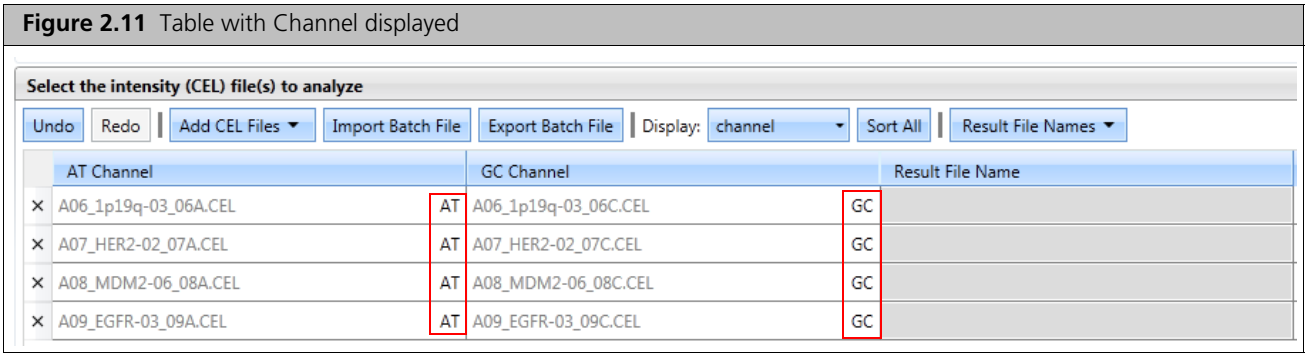


Figure 2.11 Table with Channel displayed



Importing CEL Files Using Batch Import

OncoScan Console allows import of CEL files using a batch file. The batch file must be saved as a text (Tab-delimited) format and include the full directory path for your CEL files (as shown in Figure 2.12).

TIP: The resulting OSCHP files are saved to your output path location, therefore it is not necessary to include a path under RESULT. Simply enter the desired results filename in this column.

The format for this tab-delimited file is 3 columns (A,B, and C) with the headers:

- ATCHANNELCEL
- GCCHANNELCEL
- RESULT

You must provide the full path to the CEL files for **each** Channel column.
(**Example: C:\Desktop\OncoScan\Data\Sample1.cel**)

Figure 2.12 List from Microsoft Excel

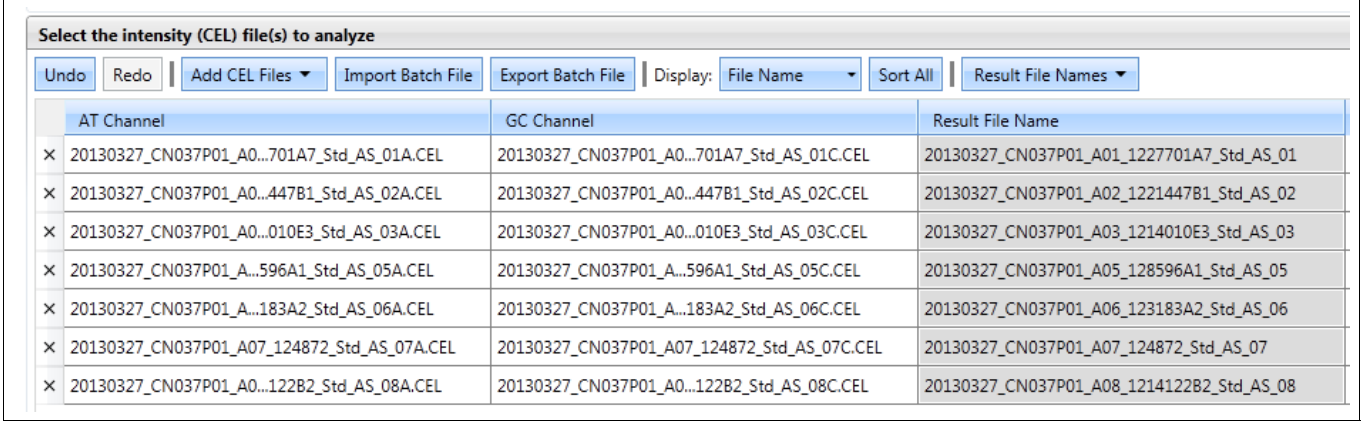
	A	B	C
1	ATCHANNELCEL	GCCHANNELCEL	RESULT
2	C:\Users\ppavic\Desktop\OncoScanData2\A01_1227701A7_Std_AS_01A.CEL	C:\Users\ppavic\Desktop\OncoScanData2\A01_1227701A7_Std_AS_01C.CEL	A01_1227701A7_Std_AS_01
3	C:\Users\ppavic\Desktop\OncoScanData2\A02_1221447B1_Std_AS_02A.CEL	C:\Users\ppavic\Desktop\OncoScanData2\A02_1221447B1_Std_AS_02C.CEL	A02_1221447B1_Std_AS_02
4	C:\Users\ppavic\Desktop\OncoScanData2\A03_1214010E3_Std_AS_03A.CEL	C:\Users\ppavic\Desktop\OncoScanData2\A03_1214010E3_Std_AS_03C.CEL	A03_1214010E3_Std_AS_03
5	C:\Users\ppavic\Desktop\OncoScanData2\A05_128596A1_Std_AS_05A.CEL	C:\Users\ppavic\Desktop\OncoScanData2\A05_128596A1_Std_AS_05C.CEL	A05_128596A1_Std_AS_05
6	C:\Users\ppavic\Desktop\OncoScanData2\A06_123183A2_Std_AS_06A.CEL	C:\Users\ppavic\Desktop\OncoScanData2\A06_123183A2_Std_AS_06C.CEL	A06_123183A2_Std_AS_06
7	C:\Users\ppavic\Desktop\OncoScanData2\A07_124872_Std_AS_07A.CEL	C:\Users\ppavic\Desktop\OncoScanData2\A07_124872_Std_AS_07C.CEL	A07_124872_Std_AS_07
8	C:\Users\ppavic\Desktop\OncoScanData2\A08_1214122B2_Std_AS_08A.CEL	C:\Users\ppavic\Desktop\OncoScanData2\A08_1214122B2_Std_AS_08C.CEL	A08_1214122B2_Std_AS_08

1. Click **Import Batch File**
   A File window appears.
2. Navigate to your text (tab-delimited) file location, then click on the file you want to import.

   IMPORTANT: The Microsoft Excel application must be closed before you import (click Open).

3. Click **Open**.
   The AT, GC, and Result File Name fields are now populated. (Figure 2.13)

Figure 2.13 Tab-delimited text file imported into OncoScan Console



Generating Result File Names

Results File Names can either be entered in manually or OncoScan Console can generate them automatically.

NOTE: If you use the suffix option (*Assigning a Suffix to Append to the Analysis Results* on page 23) and enter your Result File Names manually, your assigned suffix appears in the Results File Name column.

If you auto-generate your Results File Names, your assigned suffix appears in the Results File Name column, but it does get added to your final OSCHP file name(s).

To manually enter a Results File Name:

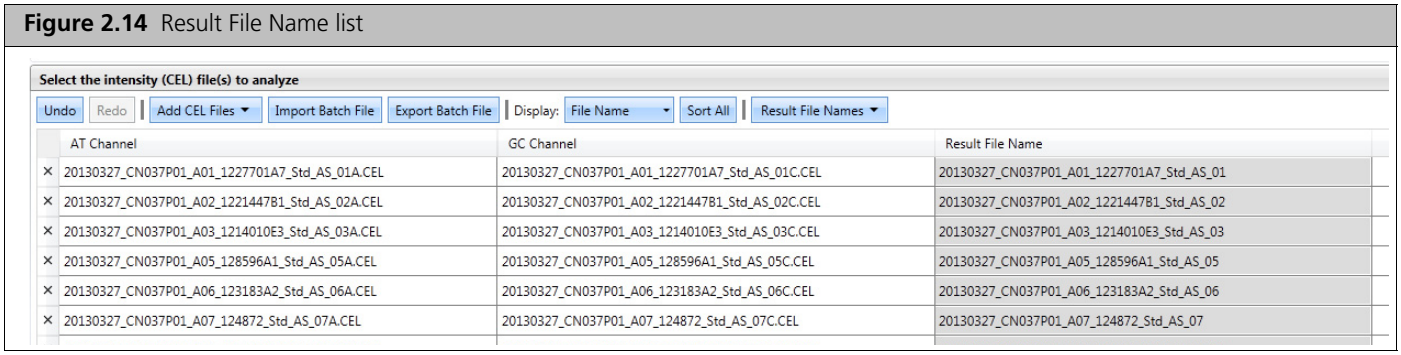
1. Single-click inside the appropriate Results Name File field to produce a cursor, then type in the file name you want.

To auto-generate a suggested Result File Name:

NOTE: During the Result File Name auto-generation process, the file names are compared to identify their common root name for use as a results file name. Generally, the last 5 characters of each CEL file name are ignored, then the remaining root names of the AT and GC file names are compared. If the root names of the AT and GC channel match, then the root name is used in the Results File Name field. The one exception is if your array name "_(OncoScan)" is appended to the file name during registration in Affymetrix GeneChip Command Console (AGCC). In this case, the "_(OncoScan)" is ignored during the comparison, but then added back in the Results File Name field.

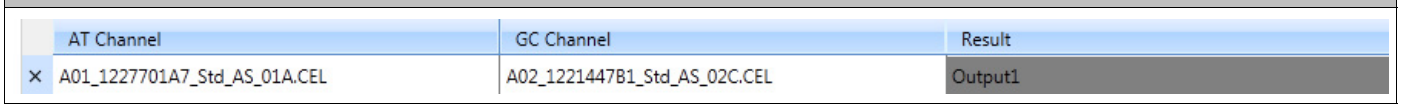
1. After the AT and GC Channel lists are populated, click the **Result File Names** drop-down, then select **Auto Generate Output Name**.
2. The Result File Name column is now populated with suggested filenames for each pairing. (Figure 2.14)

Figure 2.14 Result File Name list



Common root names should be consistent all the way up to the last character of the CEL file name prior to the .cel extension. If there is a paired file mis-match, the Results File Name appears as **Output1**. (Figure 2.15)

Figure 2.15 Result File Name "Output"



If **Output1** or subsequent Outputs (Output 2, Output 3...) appear, investigate the validity of your original pairing. See *Correcting Mismatched CEL File Pairings* on page 22.

To edit an auto-generated Result File Name:

1. Click on the Result File name you want to edit.
2. After the cursor appears, edit the filename as you normally would.
3. Click outside the row to save your edit.

To clear the entire Result File Name column:

1. Click the **Result File Names** drop-down button, then select **Clear Column**.
   The column is now cleared and ready for new Result File Name entries.

Correcting Mismatched CEL File Pairings

If there is a paired file mismatch, the Results File Name appears as **Output1, Output2, Output3, etc**.

A paired file mismatch is most likely caused by an incorrect CEL filename pairing and not a mismatch of your native CEL files.

A simple way to correct mismatches is to sort the AT and GC columns so that files with the same root names are next to each other.

TIP: Common root names should be consistent all the way up to the last character of the cel file name prior to the .cel extension. Affymetrix recommends using an "A" or "C" as the last character to designate the channel in the CEL file naming convention. Example: "_AS_05A.CEL" is an AT Channel file, while "_AS_05C.CEL" is a GC Channel file.

Using the Sorting Features

To sort an individual column:

1. Click on either the AT or GC Channel header.
   The column is now sorted in an ascending order.
2. Click on either the AT or GC Channel header again to reverse the sorting order.

To sort both columns simultaneously:

1. Click **Sort All**.
   The contents of each column are now sorted together in an ascending order.
2. Click **Sort All** again.
   The contents of each column are now sorted together in a descending order.

To swap CEL files between columns:

1. Click and drag a column CEL entry onto another column CEL entry, then release the mouse button.
   The CEL entries have now swapped column positions.

To reorder the CEL files in a column:

1. Click and drag a CEL file to another position within the column, then release the mouse button.
   The CEL file is now at its new position.

To add a cell to a column:

1. Click and drag a column cell to the top or bottom border line of a neighboring cell, then release the mouse button.

Generating a Result File Name after Sorting

1. After your AT and GC Channel lists are properly sorted, click the **Result File Names** drop-down, then select **Auto Generate Output Names**.

   The Result File Name column is now populated with suggested filenames for each pairing.

If OncoScan Console detects an inconsistency between the AT and GC file names to be paired, a Result File Name labeled, "Output n" reappears.

> **IMPORTANT: Confirm that both columns are sorted in the same direction. If they are, examine the files and confirm they are paired correctly. The file names (excluding the last character before the .CEL) MUST match exactly.**

Repeat the sorting steps above, then try to **Auto Generate Output Names** again until a successful Result File Name(s) appears.

Setting your Output Information Location (Optional)

The Output result information path (lower left) is retained from your initial setup.

To select a different folder to store your results:

1. Click the browse button, then navigate to the folder you want. If you want to change the default folder, see *Assigning an Output Results Path* on page 11.

Assigning a Suffix to Append to the Analysis Results

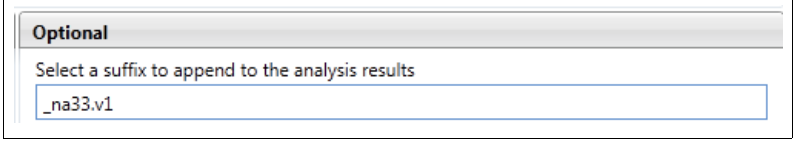
You can append a suffix at the end of all your Results File Names. This is useful when tracking versions of the analysis files used to generate the resulting OSCHP files.

To use an appending suffix:

1. Click inside the **Select a suffix to append to the analysis results** field to enter an appending file suffix. (Figure 2.16)

   Your currently displayed Result Name Files are appended (in real-time) as you type in your suffix.

Figure 2.16 Adding a suffix



> **NOTE: If you are saving the same OSCHP file into the same output file folder that contains your originally run OSCHP file with an identical suffix, a "2" is automatically added to the filename to differentiate the two runs of identical CEL file names.**

Exporting Batch Analysis Files (Optional)

You can export the information shown in the AT, GC, and Results File Names fields to Microsoft Excel as a tab-delimited file for review and/or further batch editing.

> **NOTE: Once an analysis is submitted, a tab delimited file containing the cel file selections is automatically saved in your designated output folder.**

1. Click **Export Batch File**.

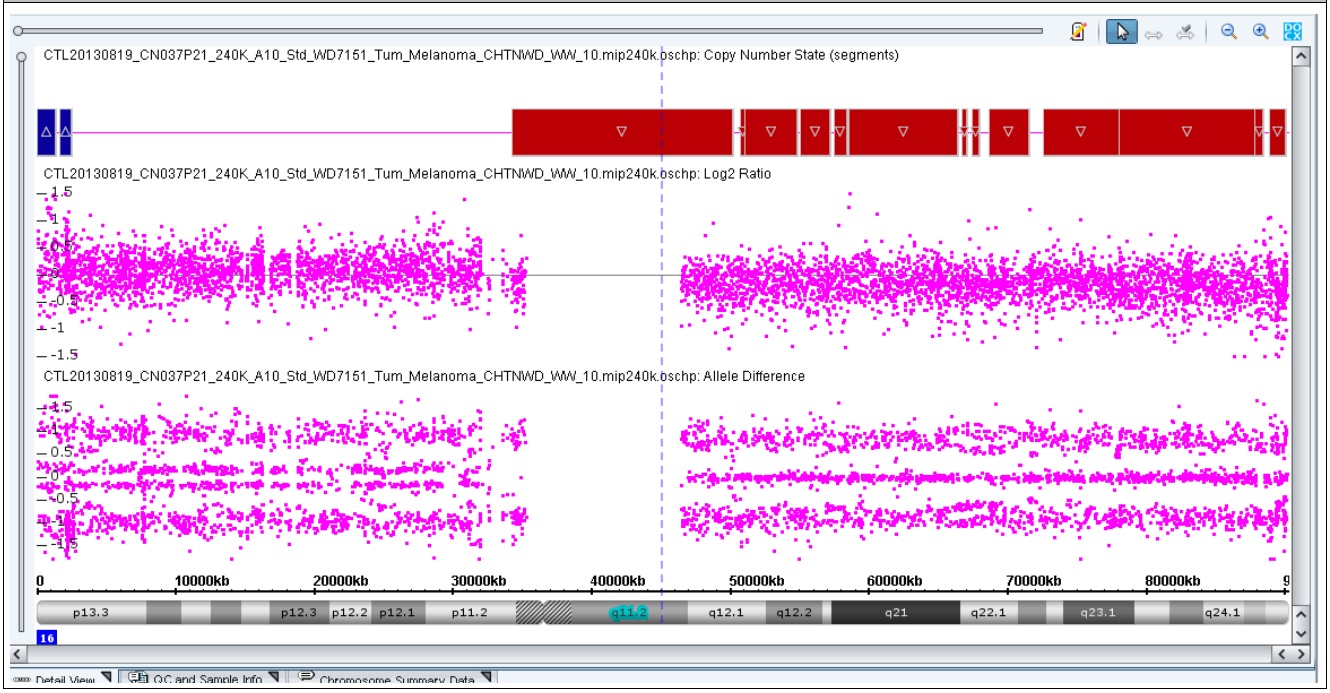
   A File window appears.
2. Navigate to the location where you want to save the file.
3. Make sure the **Files of type** is set to **Tab Delimited File(s)**, then click **Save**.

Recentering OncoScan FFPE Assay and OncoScan CNV Arrays

Due to the complexity and low diploid count in a small fraction of cancer samples, there may be a need to manually assign the diploid region of the sample or "recenter" it.

The graph (Figure 2.17) from ChAS 3.1 shows that Chromosome 16q is called as a loss, the log2 ratio data is shifted downward, but the Allele Difference Graph is displaying three tracks representing AA, AB, BB calls. Having an Allele Difference graph with three tracks means this region must have at least two copies. Since you cannot have three Allele Difference tracks in a region of loss, this sample needs to be recentered. For more information on the Manual Recentering Algorithm, see page 77.

Figure 2.17 Example of 3 Allele Difference tracks in a Region of Loss

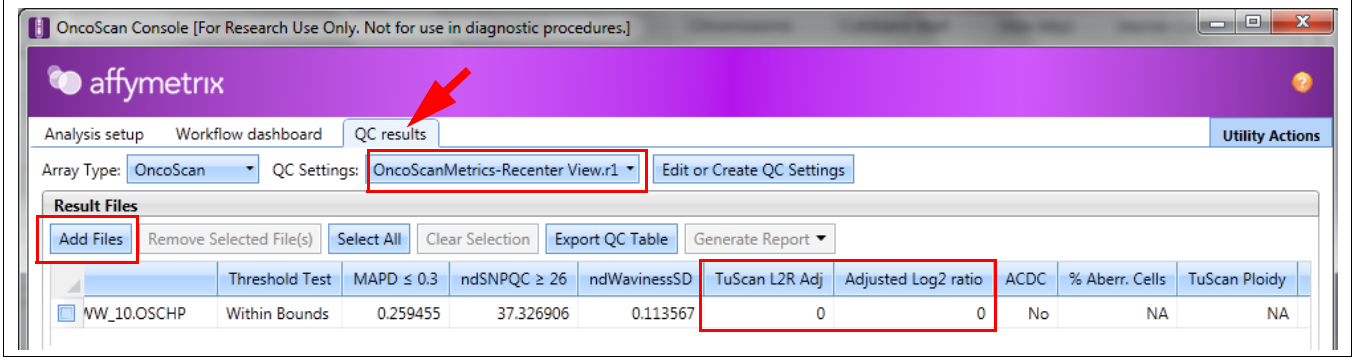


Manually Recentering a File

NOTE: You can only manually recenter one pair of CEL files at a time.

1. Click on the **QC Results** tab. (Figure 2.18)

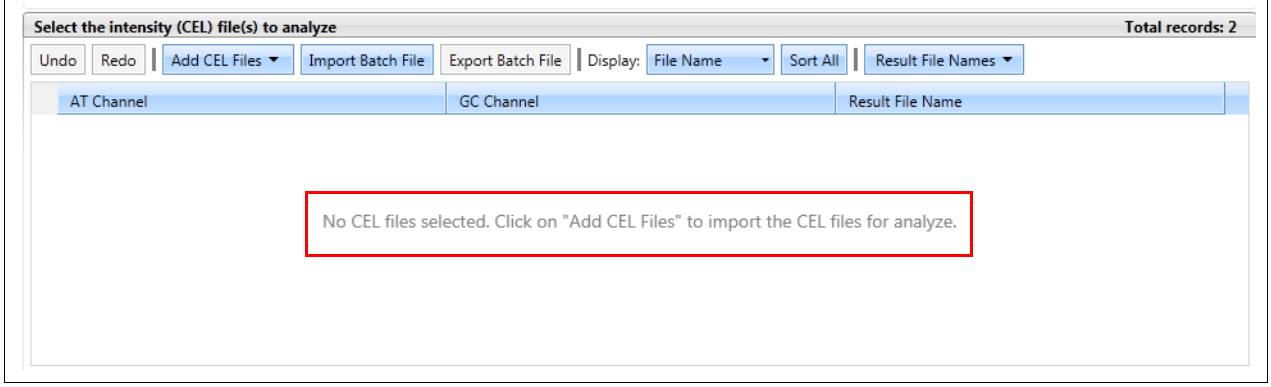
Figure 2.18 QC Results window tab



2. Click on the **Add Files** button, then navigate and select the OSCHP file for the sample you want to manually recenter.

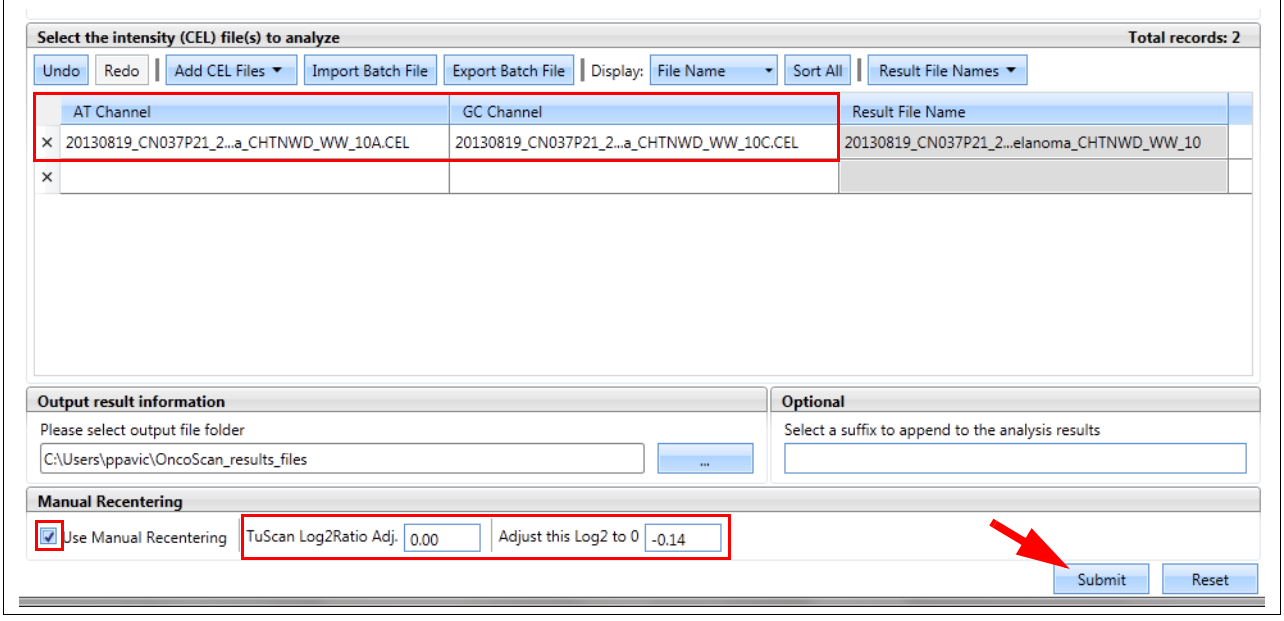
3. From the **QC Settings** drop-down menu, select **Recenter View r1**, then make a note the file's **TuScan L2R Adj** value. You will need to enter this value into the **TuScan L2R Adj** field in the Analysis setup window tab when reprocessing the associated pair of CEL files.
4. Click on the **Analysis setup** tab.
   Make sure there are no CEL files present in the **Select the Intensity (CEL) file(s) to analyze** pane. (Figure 2.19)

Figure 2.19 Empty Select the Intensity (CEL) file(s) to analyze pane



5. Add the AT Channel file and its associated GC Channel file, as described in *Manually Adding CEL Files to Analyze* on page 17.
   The paired CEL files appear, as shown in Figure 2.20.

Figure 2.20 Empty Select the Intensity (CEL) file(s) to analyze pane



6. Click the **Use Manual Recentering** check box.
7. Enter the **TuScan Log2Ratio Adj** value you recorded in Step 3.

8. Enter a **Adjust this Log2 to 0** value. This value is the currently-reported median log2 ratio for the region you would like to call Normal Diploid.

NOTE: Adjusted Log2 ratio in the Recenter View records the amount you manually adjusted the log2 ratios for this analysis. If you did not manually recenter this data, this value will be 0.

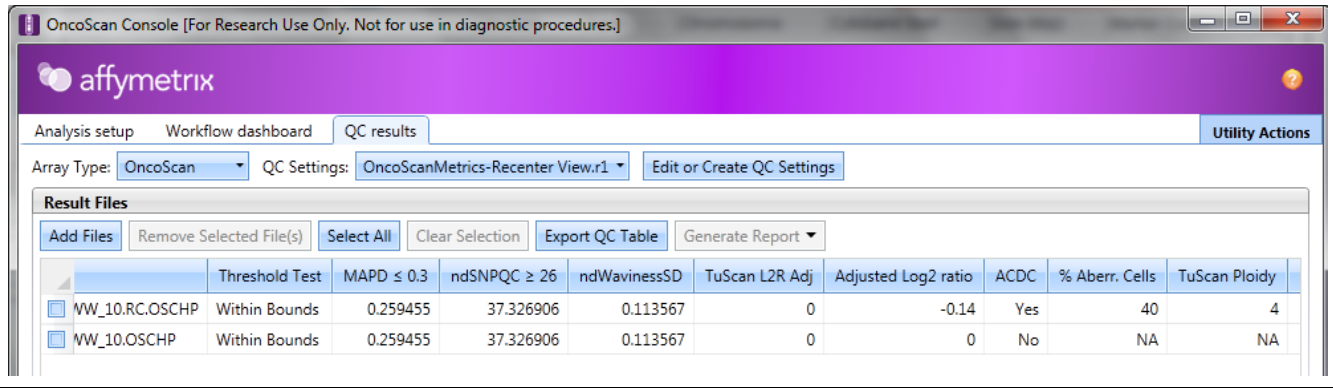
For methods on determining the median log2 ratio for a region, see the ChAS 3.1 User Guide (page 64) available at www.affymetrix.com.

9. Click **Submit**.
10. The **Workflow dashboard** window tab appears and reprocessing begins.

NOTE: An *RC* is automatically appended onto the OSCHP file as it goes through manual recentering process. Example: *RC.OSCHP*

After reprocessing has successfully completed, the QC results of the OSCHP and RC.OSCHP file appear together for comparison, as shown in Figure 2.21.

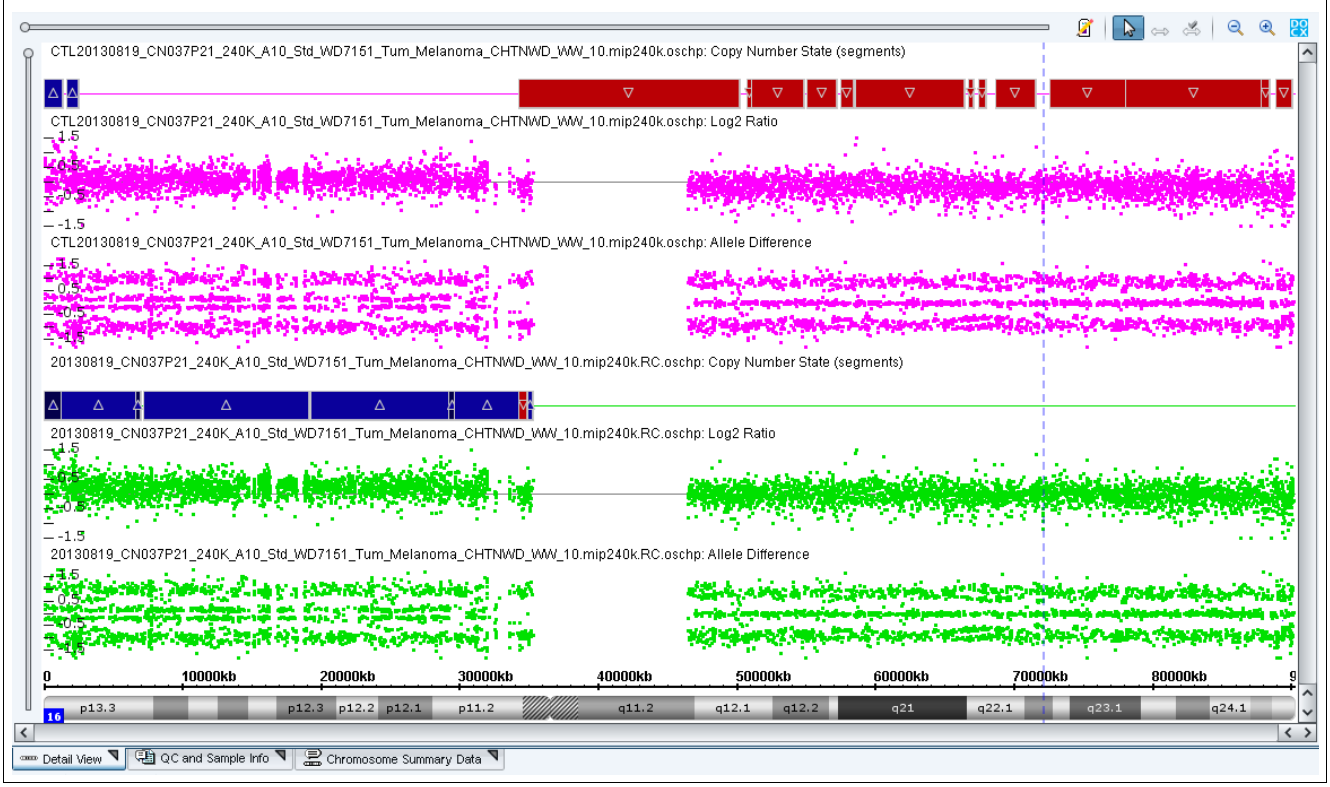
Figure 2.21



The graph (Figure 2.22) from ChAS 3.1, displays the original OSCHP file (pink data) and the manually recentered RC.OSCHP (green data).

By inputting both the TuScan Log2 Ratio value (derived from the algorithm) and the median Log2 Ratio value (for the region you have determined to be diploid, Chromosome 16q for our example), the Recentering Algorithm has recentered the log2 ratio data (for the region determined to be diploid) around 0 and there is no longer a loss segment called in this region.

Figure 2.22 Example original oschp file (pink data) and the manually recentered RC.OSCHP (green data)



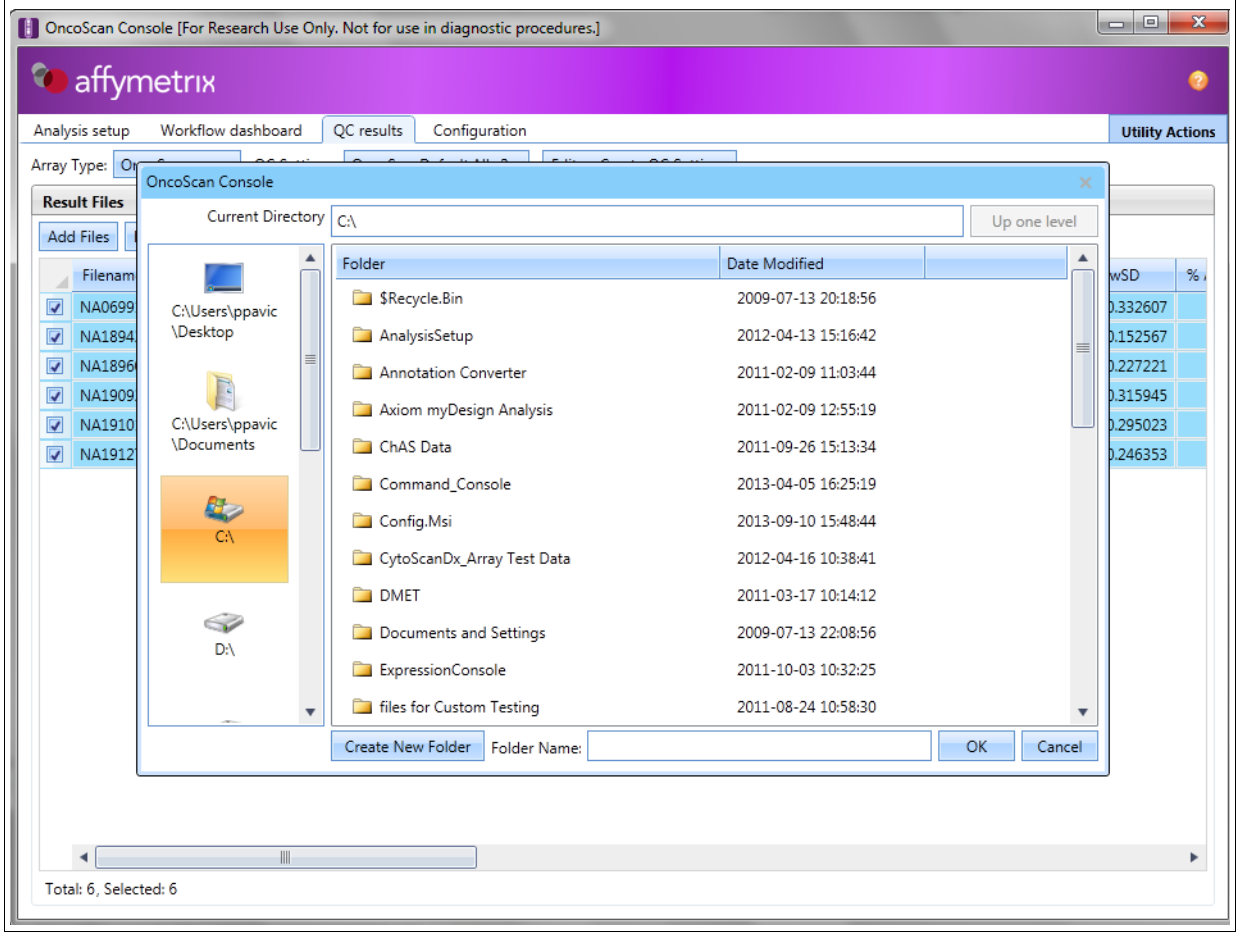
NOTE: For details on how to view .OSCHP and RC.OSCHP files in ChAS, see the ChAS 3.1 User Guide.

Log File Collection

Do the following if you experience any issues or failures with your analysis:

1. Click the **Utilities** button (top right of the OncoScan Console window)
2. Click to select **Log Collection**.
   The following window appears. (Figure 2.23)

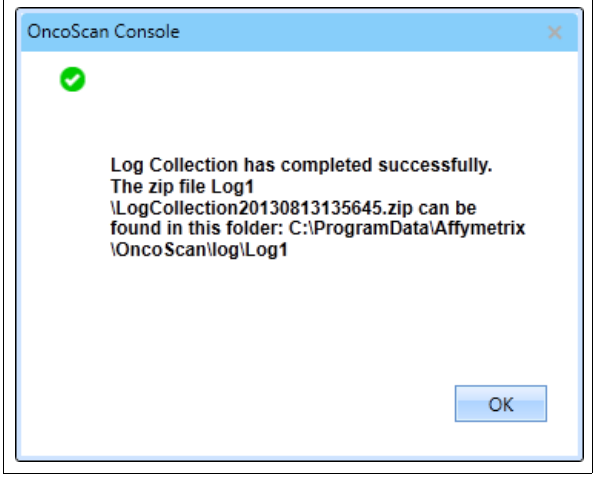
Figure 2.23 Log Collection File window



3. Use OncoScan Console's default location of `C:\` or navigate to a folder location of your choice.
4. Click **Create New Folder**, then enter a folder name for your log.
5. Click **OK**.

The following window appears confirming your log file has been saved as a zip file.

Figure 2.24 Example: Zip file contents of a Log Collection



6. Click **OK** to close the window.

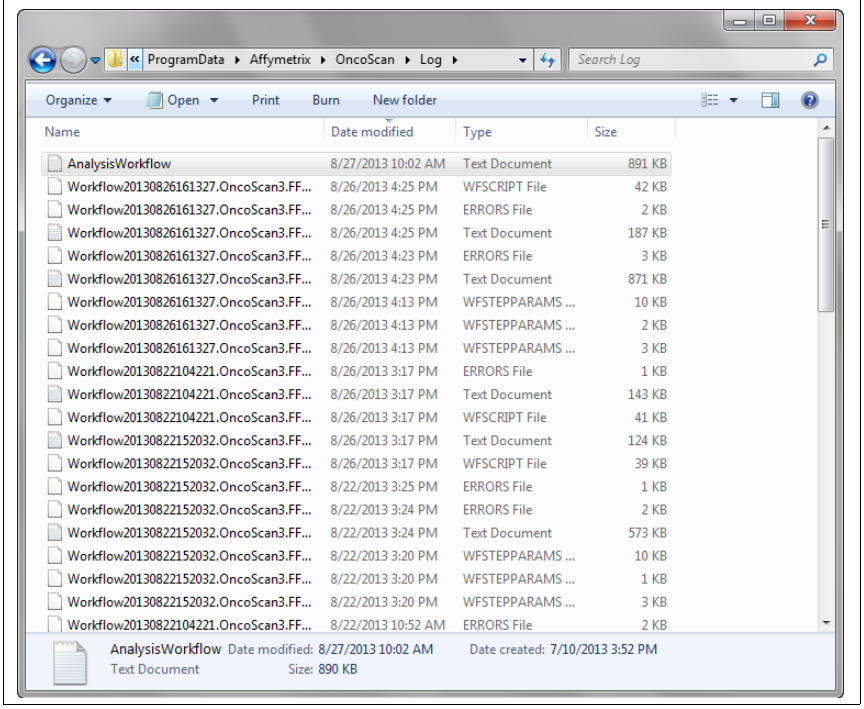
NOTE: The auto-generated log collection zip file contains the full contents of the folder and all QC History log files found in the configured QC History File path. By default, the zip file resides here: *C:\ProgramData\Affymetrix\Oncoscan\log*

Viewing the Log Collection File

1. Use Windows Explorer to navigate to the location.
   (Example: `C:\ProgramData\Affymetrix\OncoScan\log`)
2. Locate the zip folder you created earlier, then double-click on it.
   The folder opens.

3. Extract the zipped folder's contents, as you normally would. (Figure 2.25)

Figure 2.25 Example: Zip file contents of a Log Collection



Log Rollover

When the software determines that the log file for the Analysis Workflow (`C:\ProgramData\Affymetrix\OncoScan\log\AnalysisWorkflow.log`) has reached a defined size (approximately 4MB), the following steps will be completed:

A sub-folder will be created in `C:\ProgramData\Affymetrix\OncoScan\log` called '**Log***' (the '*' denotes the current date and time).

A zip file called **RolledLogFile*.zip** is created in that folder. The '*' is the same date and time used for the folder name. The files in the `C:\ProgramData\Affymetrix\OncoScan\log` folder and all files found in the currently selected QC History Log folder will be included in this zip file.

The Analysis Workflow files that are associated with analysis workflows that are no longer active on the Dashboard will be deleted from: `C:\ProgramData\Affymetrix\OncoScan\log`

A new AnalysisWorkflow.log file will be created here:
`C:\ProgramData\Affymetrix\OncoScan\log`

Chapter 3

Analysis Submission and QC Results

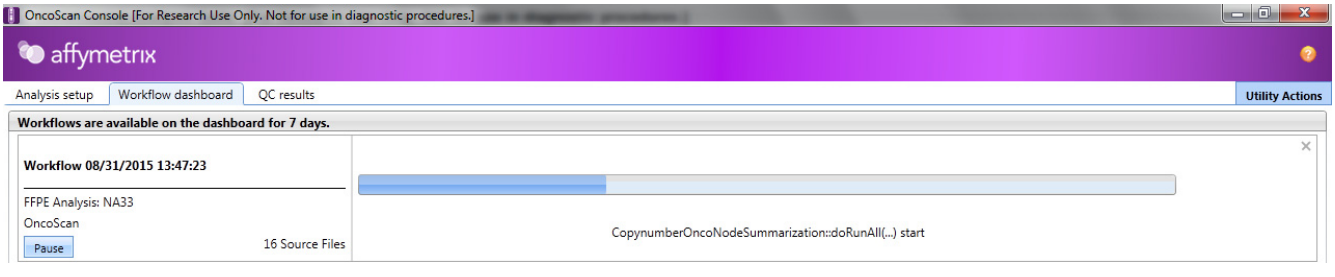
Submitting your Analysis Setup Information

1. After the information in the Analysis Setup window/tab is complete, click **Submit**.
   The **Workflow dashboard** tab appears and processing begins.

Workflow Dashboard

The OncoScan Console Analysis Workflow Dashboard uses a progress bar to track the software's ongoing analysis tasks, then delivers the results of analyses. (Figure 3.1)

Figure 3.1 .CEL file analysis inside the Workflow dashboard



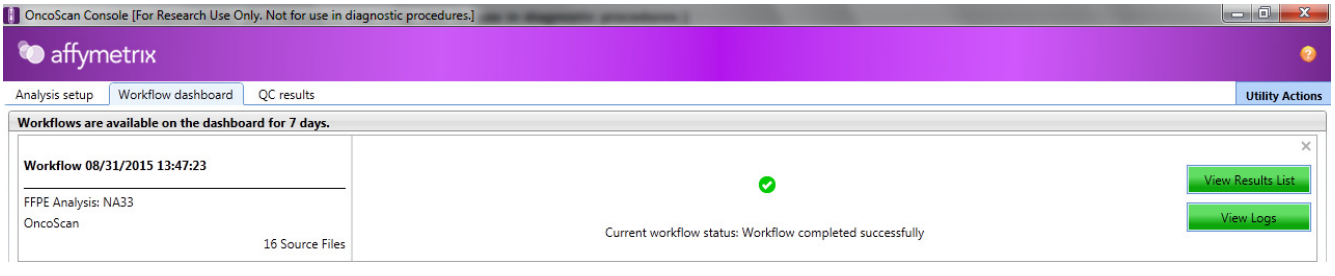
To pause and restart a Workflow analysis in progress:

1. Click **Pause** to stop the Workflow that is in progress.
2. Click **Resume** to restart the Workflow analysis.

To abort the Workflow in progress:

1. Click **Pause** to stop the Workflow that is in progress.
1. Click the **X** (upper right corner) of the Workflow pane.
   A warning message appears.
2. Click **OK** to acknowledge the message.
   After analysis is complete, a Workflow completed successfully message appears. (Figure 3.2)

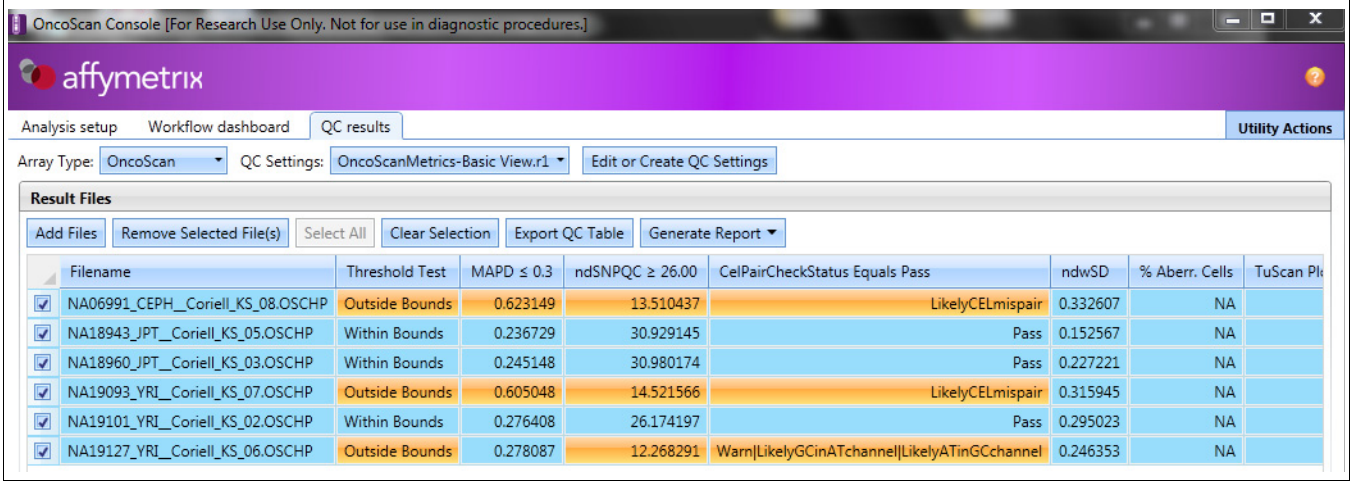
Figure 3.2 Workflow Dashboard example with multiple Single Samples loaded



3. To view the results, click View Results List.

The results appear in the QC Results tab. (Figure 3.3)

Figure 3.3 QC Results window tab



4. Click the checkbox (far left) of each sample you want to include in your report or click **Select All** to auto-select all your samples.

QC Results

Interpreting and Using QC Results

- The column header contains the metric name and its threshold (if a threshold has been defined). See *Customizing QC Metrics and Thresholds* on page 32 to add a threshold.
- A CEL file value that does not pass its threshold test is indicated with an orange background, as shown in Figure 3.3.
- The Threshold Test column displays **Outside Bounds** and is highlighted in orange if any metric in the row fails its threshold test, as shown in Figure 3.3.
- A column labeled **Within Bounds** (as shown in Figure 3.3) indicates that all metrics on the row passed the threshold test (or did not have a threshold applied to them).

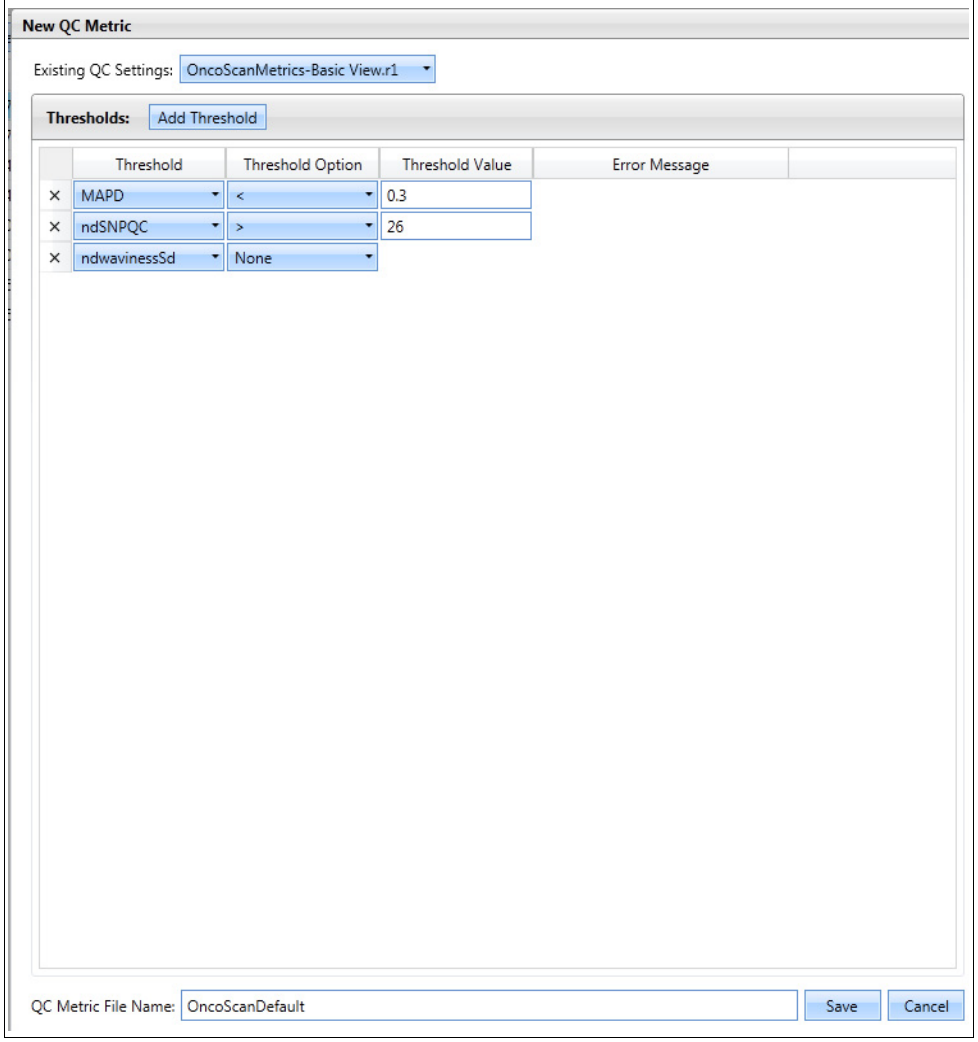
Customizing QC Metrics and Thresholds

To add or remove QC metrics or the threshold associated with the metric:

1. Click **Edit or Create QC Settings.**

The following window appears: (Figure 3.4)

Figure 3.4 Edit or create QC Results window tab



The existing QC Metric **OncoScan.Default** contains the main metrics used in determining whether the array passes or not.

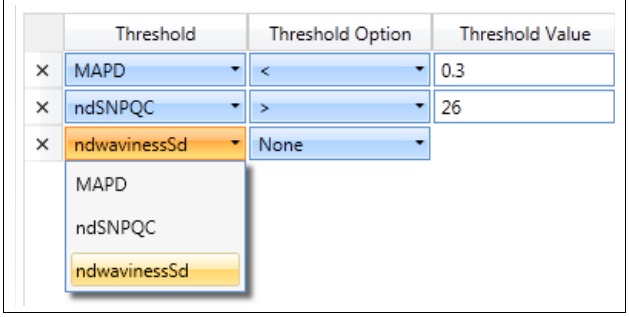
OncoScan.All contains additional algorithm metrics that can be used for advanced troubleshooting.

To view the Thresholds included with OncoScan Console, see *Appendix B: QC Metrics - Definitions* on page 66.

To change a metric:

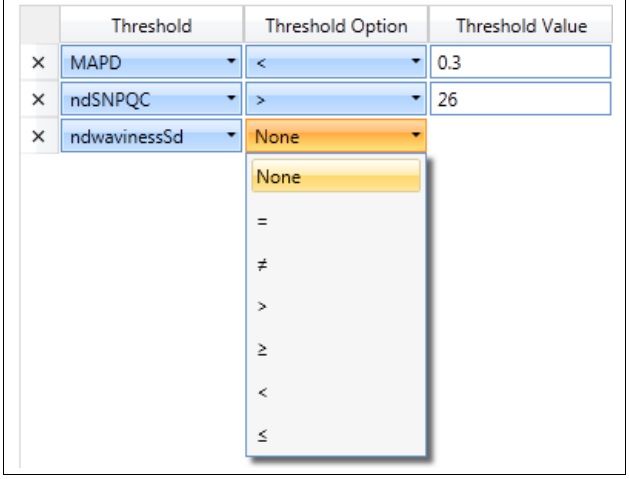
1. Click the Threshold's drop-down to select a different Threshold. (Figure 3.5)

Figure 3.5 Threshold drop-down menu



2. Click the Threshold Option's drop-down, then click to select a different symbol. (Figure 3.6)

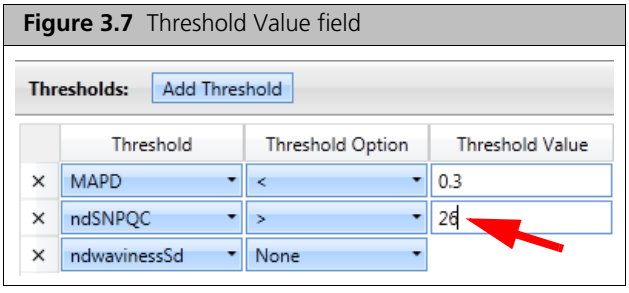
Figure 3.6 Threshold Options drop-down menu



If desired select a threshold value to help determine a prior **Out of bounds** result.

3. Click inside the Threshold Value's field.
4. A cursor appears.
5. Use the backspace key, then enter a new value. (Figure 3.7)

Figure 3.7 Threshold Value field



To add a new a QC Metric(s):

1. Click **Add Threshold**.
   A new Threshold is added to the table.
2. Click the Threshold's drop-down menu to select your new threshold.
   A new Threshold Option is added to the new row.
3. Click the Threshold Option's drop-down, then click to select a symbol.
   A text box for Threshold Value is added to the column.
4. Click inside the Threshold Value's field.
   A cursor appears.
5. Enter a new value
6. You must enter a filename unless you are editing (and plan to overwrite) a previous QC Metric filename
7. Click **Save.**

Exporting the QC Results Table

To export your QC Results table:

1. Click **Export QC Table** to export all the data shown in the table (no checking of the checboxes is required).
   A File window appears.
2. Navigate to the location you want.
3. Enter a File Name or use the default **QCMetrixTable.txt**.
   Make sure the **Files of type** is set to **Tab Delimited File(s)**.
4. Click **Save**.
   The tab-delimited text version of the QC results table is now saved for your records. (Figure 3.8)

Figure 3.8 Exported as a tab-delimited text file

	A	B	C	D	E	F	G	H
1	Filename	Threshold	MAPD	MAPD LessThan 0.3	ndSNPQC	ndSNPQC	ndwavine	NA Version
2	A01_12277(	Outside B	0.223982	Pass	25.88998	Fail	0.065653	33
3	A02_12214(	Within Bo	0.200827	Pass	26.09873	Pass	0.06751	33
4	A03_12140:	Outside B	0.220253	Pass	20.23619	Fail	0.062606	33
5	A05_12859(	Outside B	0.219409	Pass	21.53243	Fail	0.05378	33

Generating and Exporting Reports

To Generate and Export your Results File table(s) as a tab-delimited text file:

1. Click the checkbox next to the Results File(s) you want to generate a report for, or click **Select All**.
2. Click Generate Report to display the report menu options.

- *CelPairCheck Report* on page 36
- *Gene Report* on page 38
- *Probe Level Data Report* on page 41
- *Segment Data Report* on page 44
- *Somatic Mutation Data Report* on page 47
- *Export All Data* on page 51

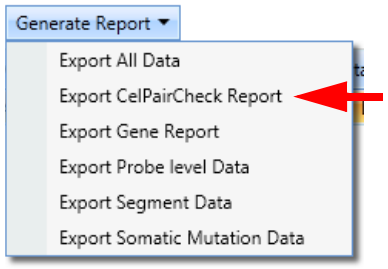
CelPairCheck Report

This report is based off the signature SNPs and indicates whether the cel files selected as the AT and GC files were likely from the same sample and assigned to the correct channel.

Do the following to export a CelPairCheck Report (*aka* SignatureSNP Report):

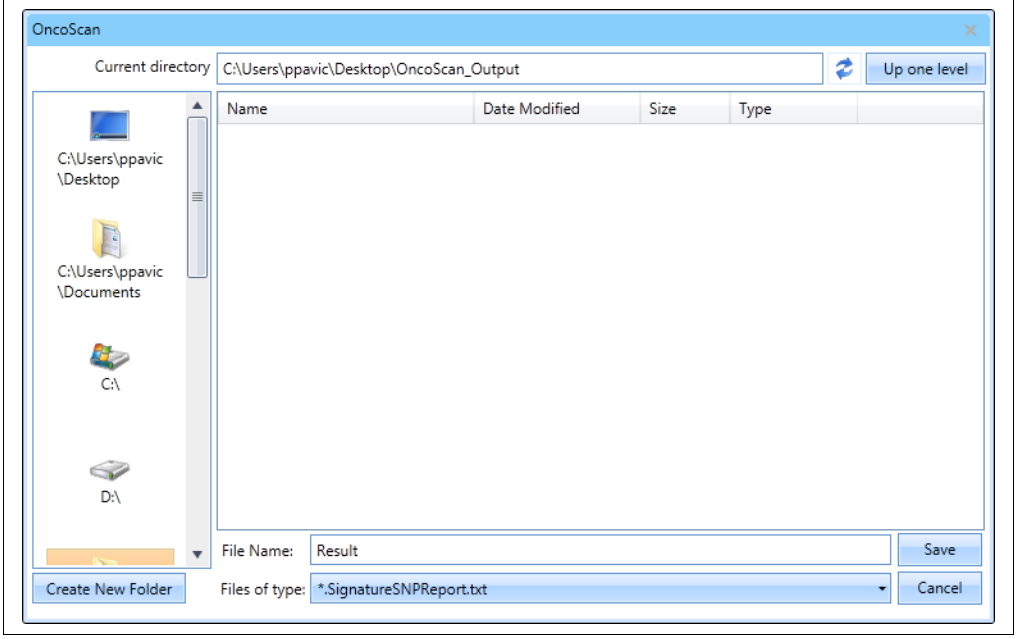
1. Click **Export CelPairCheck Report**. (Figure 3.9)

Figure 3.9 Generate Report drop-down menu



Your previously assigned Output folder file window appears. (Figure 3.10)

Figure 3.10 OncoScan Output folder window

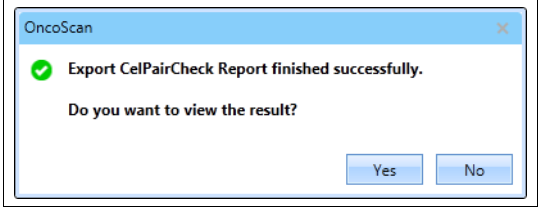


If you have not yet assigned an output folder, see *Assigning an Output Results Path* on page 11.

2. The default root filename is **Result**. Click inside the File Name field to enter a different root filename, then click **Save**.

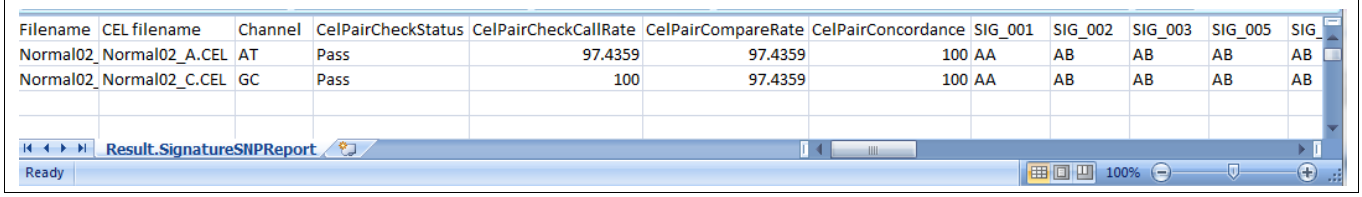
A progress bar appears while your report generates, followed by a report finished successfully message, as shown in Figure 3.11.

Figure 3.11 CelPairCheck Report successful



3. Click **Yes**.
   The OncoScan Output folder window appears.
4. Locate the **SignatureSNP Report** text file, then open it in Microsoft Excel.
   The following window appears. (Figure 3.12)

Figure 3.12 SignatureSNP report



Filename	CEL filename	Channel	CelPairCheckStatus	CelPairCheckCallRate	CelPairCompareRate	CelPairConcordance	SIG_001	SIG_002	SIG_003	SIG_005	SIG_
Normal02_	Normal02_A.CEL	AT	Pass	97.4359	97.4359	100	AA	AB	AB	AB	AB
Normal02_	Normal02_C.CEL	GC	Pass	100	97.4359	100	AA	AB	AB	AB	AB

Filename — Name of the OSCHP file containing the data

CEL Filename — Name of cel file.

Channel — The Channel file from which the signal is measured. "A" is the AT CEL, "C" is the GC CEL.

CelPairCheckStatus — CelPairCheck is a test that inspects each pair of intensity (*.cel) files to determine whether the files have been properly paired and assigned to the correct channel. In addition to accidental mispairing of intensity files while setting up the analysis, a tracking problem during the assay may result in a sample being assigned to the wrong GeneChip array. As a result CelPairCheck ignores file names, and instead inspects the genotypes in the two intensity files to detect file mispairings. To learn more about CelPairCheck Status, see page 73.

CelPairCheckCallRate — CelPairCheckCallRate is the percentage of signature SNPs that make a genotype call for a given CEL file.

CelPairCompareRate — This metric is the percentage of signature SNP control markers whose genotypes are compared between the AT and GC channels.

CelPairConcordance — This metric is the concordance of a set of signature SNP genotypes compared between AT and GC CEL files. If CelPairCheck Compare Rate is high but CelPairCheck Concordance is low, then CelPairCheck Status will report "PossibleCELmispair".

SIG_001..00N — Genotype for signature snp 1..n

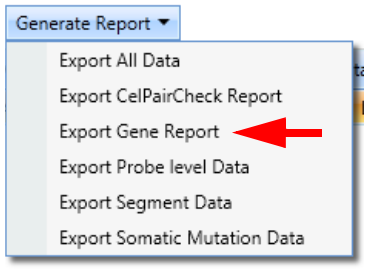
Gene Report

This report summarizes the copy number segments that overlap user defined regions of interest (e.g., Genes) as defined in the selected BED file.

Do the following to export a Gene Report:

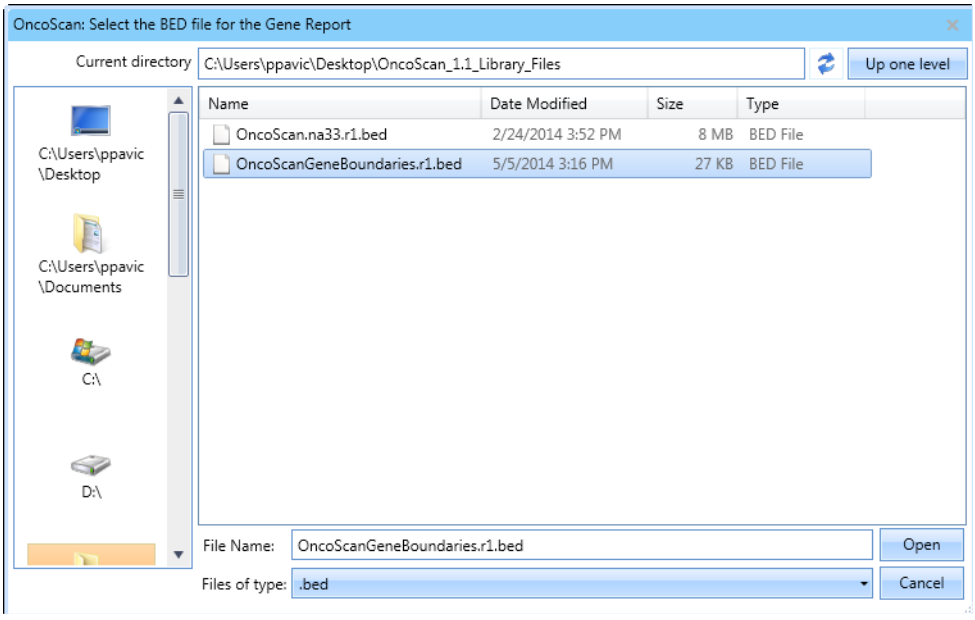
1. Click **Export Gene Report**. (Figure 3.13)

Figure 3.13 Generate Report drop-down menu



The following window appears. (Figure 3.14)

Figure 3.14 Select the BED file for the Gene Report window

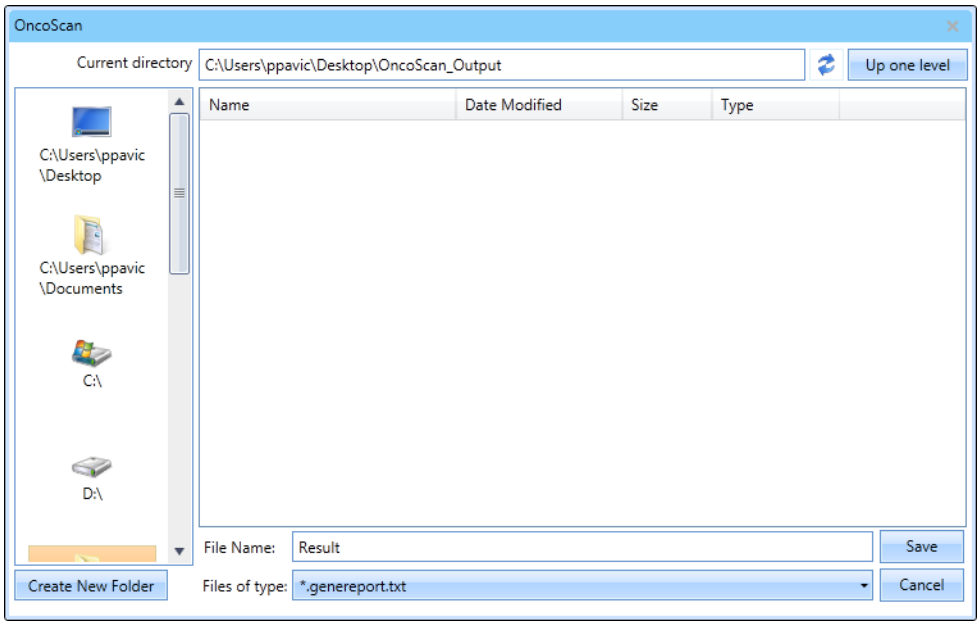


2. Click to select the appropriate BED file, then click **Open**.

NOTE: As shown in Figure 3.14, the default OncoScan-specific BED file for the Gene report is *OncoScanGeneBoundaries.r1.bed*. However, any BED file can be used to generate the Gene Report on any regions of interest contained within the BED file.

Your previously assigned Output folder file window appears. (Figure 3.15)

Figure 3.15 OncoScan Output folder window

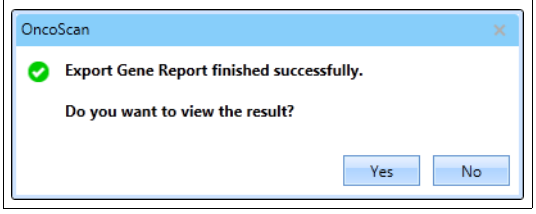


If you have not yet assigned an output folder, see *Assigning an Output Results Path* on page 11.

3. The default root filename is **Result**. Click inside the File Name field to enter a different root filename, then click **Save**.

   A progress bar appears while your report generates, followed by a report finished successfully message, as shown in Figure 3.16.

Figure 3.16 Successful Gene Report



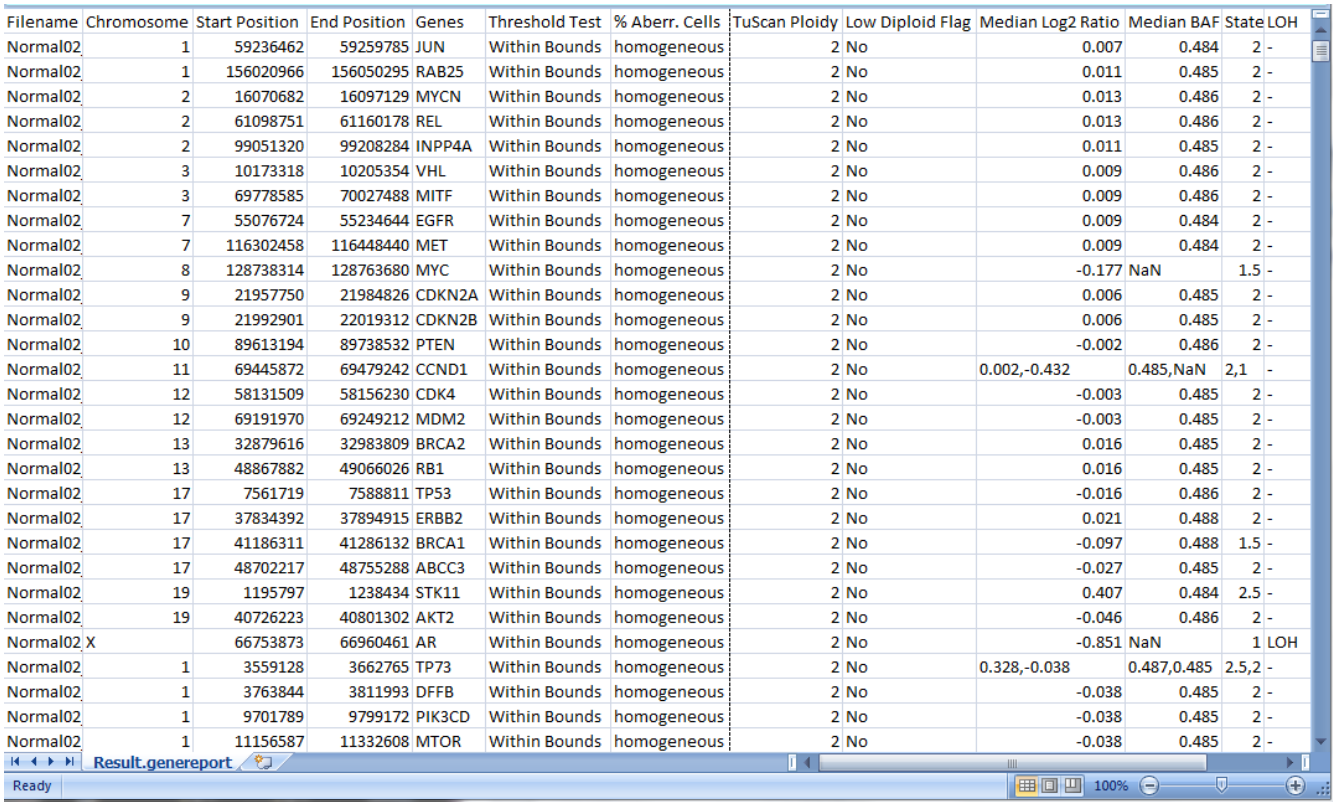
4. Click **Yes**.

   The OncoScan Output folder window appears.

5. Locate the **Gene Report** text file, then open it in Microsoft Excel.

   The following window appears. (Figure 3.17)

Figure 3.17 Gene Report report



Filename	Chromosome	Start Position	End Position	Genes	Threshold Test	% Aberr. Cells	TuScan Ploidy	Low Diploid Flag	Median Log2 Ratio	Median BAF	State	LOH
Normal02	1	59236462	59259785	JUN	Within Bounds	homogeneous	2	No	0.007	0.484	2	-
Normal02	1	156020966	156050295	RAB25	Within Bounds	homogeneous	2	No	0.011	0.485	2	-
Normal02	2	16070682	16097129	MYCN	Within Bounds	homogeneous	2	No	0.013	0.486	2	-
Normal02	2	61098751	61160178	REL	Within Bounds	homogeneous	2	No	0.013	0.486	2	-
Normal02	2	99051320	99208284	INPP4A	Within Bounds	homogeneous	2	No	0.011	0.485	2	-
Normal02	3	10173318	10205354	VHL	Within Bounds	homogeneous	2	No	0.009	0.486	2	-
Normal02	3	69778585	70027488	MITF	Within Bounds	homogeneous	2	No	0.009	0.486	2	-
Normal02	7	55076724	55234644	EGFR	Within Bounds	homogeneous	2	No	0.009	0.484	2	-
Normal02	7	116302458	116448440	MET	Within Bounds	homogeneous	2	No	0.009	0.484	2	-
Normal02	8	128738314	128763680	MYC	Within Bounds	homogeneous	2	No	-0.177	NaN	1.5	-
Normal02	9	21957750	21984826	CDKN2A	Within Bounds	homogeneous	2	No	0.006	0.485	2	-
Normal02	9	21992901	22019312	CDKN2B	Within Bounds	homogeneous	2	No	0.006	0.485	2	-
Normal02	10	89613194	89738532	PTEN	Within Bounds	homogeneous	2	No	-0.002	0.486	2	-
Normal02	11	69445872	69479242	CCND1	Within Bounds	homogeneous	2	No	0.002,-0.432	0.485,NaN	2,1	-
Normal02	12	58131509	58156230	CDK4	Within Bounds	homogeneous	2	No	-0.003	0.485	2	-
Normal02	12	69191970	69249212	MDM2	Within Bounds	homogeneous	2	No	-0.003	0.485	2	-
Normal02	13	32879616	32983809	BRCA2	Within Bounds	homogeneous	2	No	0.016	0.485	2	-
Normal02	13	48867882	49066026	RB1	Within Bounds	homogeneous	2	No	0.016	0.485	2	-
Normal02	17	7561719	7588811	TP53	Within Bounds	homogeneous	2	No	-0.016	0.486	2	-
Normal02	17	37834392	37894915	ERBB2	Within Bounds	homogeneous	2	No	0.021	0.488	2	-
Normal02	17	41186311	41286132	BRCA1	Within Bounds	homogeneous	2	No	-0.097	0.488	1.5	-
Normal02	17	48702217	48755288	ABCC3	Within Bounds	homogeneous	2	No	-0.027	0.485	2	-
Normal02	19	1195797	1238434	STK11	Within Bounds	homogeneous	2	No	0.407	0.484	2.5	-
Normal02	19	40726223	40801302	AKT2	Within Bounds	homogeneous	2	No	-0.046	0.486	2	-
Normal02	X	66753873	66960461	AR	Within Bounds	homogeneous	2	No	-0.851	NaN	1	LOH
Normal02	1	3559128	3662765	TP73	Within Bounds	homogeneous	2	No	0.328,-0.038	0.487,0.485	2.5,2	-
Normal02	1	3763844	3811993	DFFB	Within Bounds	homogeneous	2	No	-0.038	0.485	2	-
Normal02	1	9701789	9799172	PIK3CD	Within Bounds	homogeneous	2	No	-0.038	0.485	2	-
Normal02	1	11156587	11332608	MTOR	Within Bounds	homogeneous	2	No	-0.038	0.485	2	-

Filename Name of the OSCHP file containing the data

Chromosome Chromosome on which the probeset is located.

Start Position Start position of gene or region as defined in the bed file.

End Position End position of gene or region as defined in the bed file.

Genes This column is populated from the name column of the bed file. In most cases, it will contain gene names.

Threshold Test Displays *Outside Bounds* if any of the QC metrics fail to meet a threshold test. For more information on thresholds, see *Customizing QC Metrics and Thresholds* on page 32.

% Aberr.Cells If % AC = 100%, we return "homogeneous" because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.

TuScan Ploidy TuScan Ploidy is the most likely ploidy state of the tumor before additional aberrations occurred. Algorithmically it is the CN state of the markers identified by the algorithm as normal diploid before %AC and ploidy are determined. When a high ploidy is determined the "normal diploid" is deemed to correspond to a higher CN and the log2 ratio gets adjusted appropriately. If ploidy cannot be determined NA (Not Available) is reported.

Low Diploid Flag	An essential part of the algorithm is the identification of "normal diploid" markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that "normal diploid markers" result in a log2 ratio of 0 (e.g. copy number 2). The algorithm might later determine that the "normal diploid" markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of "normal diploid" markers and no "normal diploid calibration occurs. This event triggers "low diploid flag" = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary.
Median Log2 Ratio	Log2 Ratio is the log2 ratio of the normalized intensity of the sample over the normalized intensity of a reference with further correction for sample specific variation. The Median Log2 Ratio is computed for each segment.
Median BAF	B-allele frequency (BAF) is (Signal (B)/{Signal(A) + Signal(B), where signal (A) is the signal from the AT chip and signal (B) is the signal from the G/C chip. Median BAF is reported for each segment and is the median BAF of the markers identified as heterozygous, after mirroring any marker BAFs above 0.5 to the equivalent value below 0.5. If the number of heterozygous markers in the segment is below 10 or the percent of homozygous markers is above 85% no value is reported,
State	This is a comma separated list of the copy number state of the segments that overlap the gene or region.
LOH	Flag to indicate whether the gene or region is in a Loss of Heterozygosity region (0=No, 1=Yes).

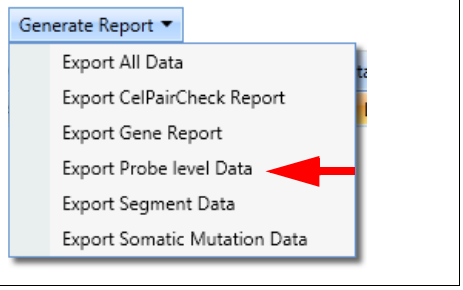
Probe Level Data Report

This report contains base level data for each probeset including the log2ratio and BAF values.

Do the following to export a Probe Level Data Report:

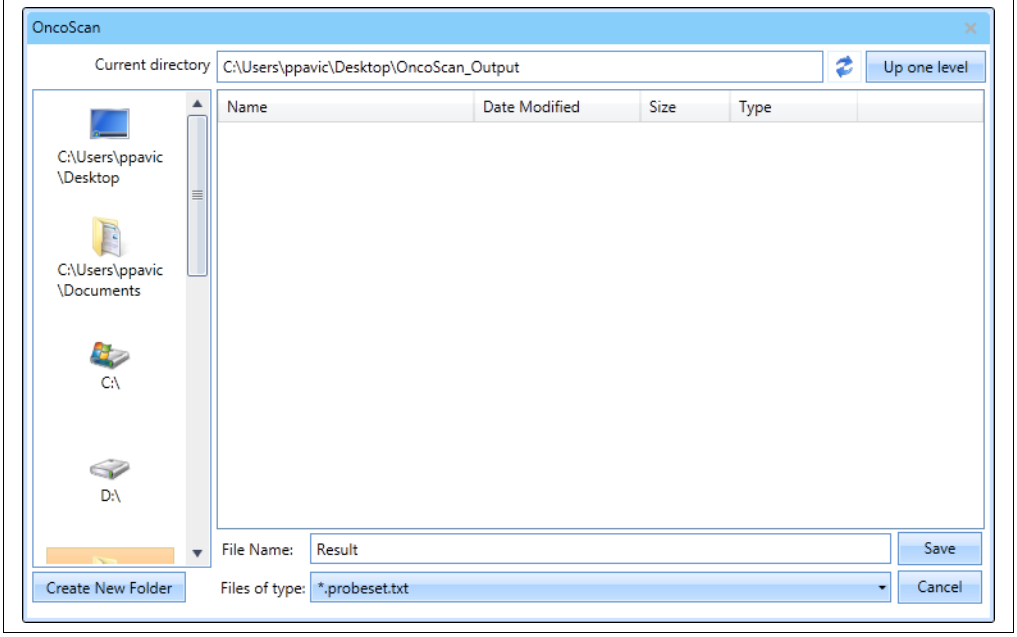
1. Click **Export Probe Level Data.** (Figure 3.18)

Figure 3.18 Generate Report drop-down menu



Your previously assigned Output folder file window appears. (Figure 3.19)

Figure 3.19 OncoScan Output folder window

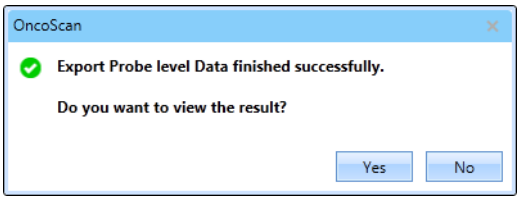


If you have not yet assigned an output folder, see *Assigning an Output Results Path* on page 11.

2. The default root filename is **Result**. Click inside the File Name field to enter a different root filename, then click **Save**.

   A progress bar appears while your report generates, followed by a report finished successfully message, as shown in Figure 3.20.

Figure 3.20 Probe Level Data successful



3. Click **Yes**.

   The OncoScan Output folder window appears.

4. Locate the **Probe Level Report** text file, then open it in Microsoft Excel.

The following window appears. (Figure 3.21)

Figure 3.21 Probe Level report

ProbeSetName	Chromosome	Position	Log2Ratio	WeightedLog2Ratio	AllelicDifference	NormalDiploid	BAF (Norm
S-tag133716	1	754192	-0.331403	-0.2605914	NaN	0	1
S-tag048386	1	757394	-0.115053	-0.1137758	-0.9321401	0	1
S-tag046699	1	790465	0.363814	0.342653	-1.042434	0	1
S-tag282890	1	800830	0.523907	0.4720662	-1.47417	0	1
S-tag133552	1	813034	0.280268	0.05735964	1.429469	0	0
S-tag199817	1	834198	-0.17221	0.1619296	0.173758	0	0
S-tag208223	1	843405	0.181527	0.454184	0.8781084	0	0
S-tag002739	1	852875	1.038033	0.6663697	-1.057539	0	0.03106
S-tag240208	1	866893	0.422378	0.1978381	-1.554223	0	1
S-tag292774	1	882033	0.417244	0.3609293	-2.35094	0	1
S-tag021073	1	887560	0.457381	0.3388038	NaN	0	0
S-tag311686	1	918573	0.095026	0.4948392	-1.441264	0	1
S-tag106064	1	930377	-0.056062	0.2693351	-1.076278	0	1
S-tag107574	1	950677	0.553863	0.547292	-2.707072	0	1
S-tag216847	1	958905	0.484896	0.6355454	2.765048	0	0
S-tag021556	1	978193	1.429911	0.9527115	-2.765048	0	1
S-tag146437	1	987670	0.955917	0.5402005	-1.498609	0	1
S-tag310650	1	1015257	0.655659	0.7424256	-2.585971	0	1
S-tag134516	1	1023788	0.387315	0.5344421	-1.584653	0	0
S-tag264968	1	1037047	-0.941337	0.7465745	NaN	0	1
S-tag308636	1	1049950	0.298342	0.4346693	NaN	0	0
S-tag317658	1	1073251	0.519566	0.6646191	NaN	0	1
S-tag211214	1	1078583	0.6327	0.6893058	-2.406472	0	1
S-tag236616	1	1099437	1.275824	0.9387031	-2.765048	0	1
S-tag242406	1	1110586	0.845567	0.6778119	NaN	0	0.51819
S-tag226434	1	1131581	-0.504062	0.8486567	-0.08207609	0	0.51298
S-tag265103	1	1156131	0.662692	0.6635702	2.609461	0	0
S-tag214679	1	1171377	0.674828	0.8064493	NaN	0	0.44815
S-tag011724	1	1186665	0.26377	0.4801189	0.04518216	0	0.49776
S-tag294018	1	1201640	-0.826599	0.6276051	0.9377512	0	0
S-tag280477	1	1218086	0.238575	0.4574486	-1.736009	0	1
S-tag318697	1	1247494	0.907612	0.5404767	0.8153528	0	0.4347

Result.probeset

Ready

100%

ProbeSet Name Affymetrix identifier for the marker.

Chromosome Chromosome on which the probeset is located.

Position Chromosomal position of the probeset.

Log2 Ratio Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.

WeightedLog2Ratio Contains the Log2 Ratios processed through a Bayes wavelet shrinkage estimator.

AllelicDifference Allele difference is computed based on differencing A signal and B signal, then standardizing based on reference file information.

NormalDiploid Identifies the markers initially designated to be in a normal diploid region Used to select the subset of data for generating the "sample sketch", which is used to quantile normalize the raw intensities prior to further analysis. When the number of Normal Diploid identified falls below a threshold, the "Low Diploid Flag" is set to "yes" and the sample is normalized using all automsomal markers. As a result it is generally not centered correctly, e.g. markers with log2 ratio of 0 may not correspond to CN=2.

BAF BAF is (Signal (B)/{Signal(A) + Signal(B), where signal (A) is the signal from the AT chip and signal (B) is the signal from the G/C chip.

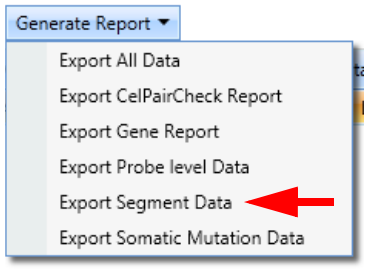
Segment Data Report

This report contains a list of all of the segments of normal and non-normal copy number states and LOH found in the sample.

Do the following to export a Segment Data Report:

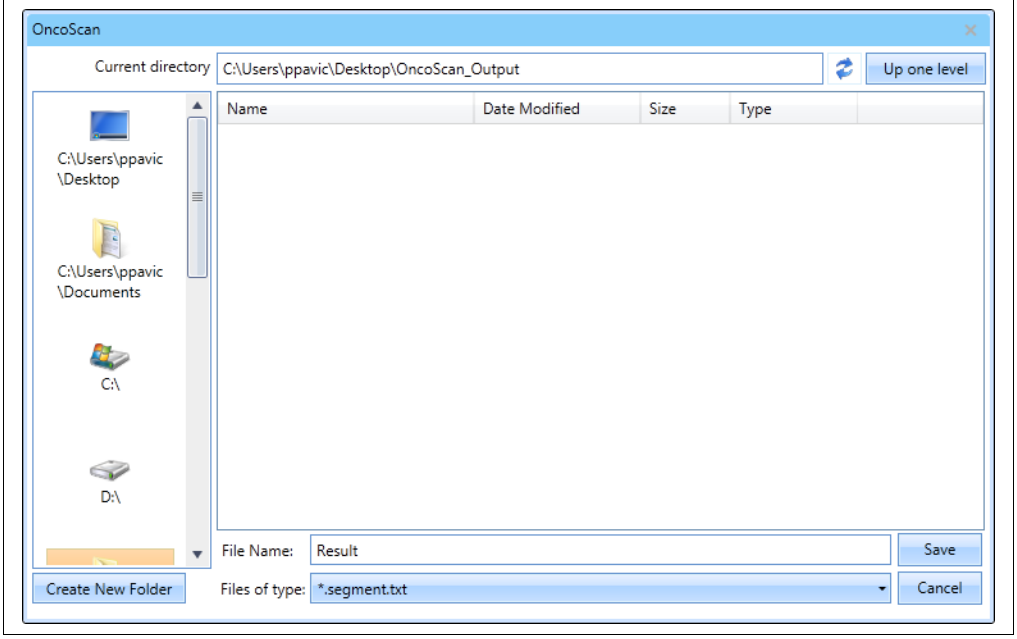
1. Click **Export Segment Data**. (Figure 3.22)

Figure 3.22 Generate Report drop-down menu



Your previously assigned Output folder file window appears. (Figure 3.23)

Figure 3.23 OncoScan Output folder window

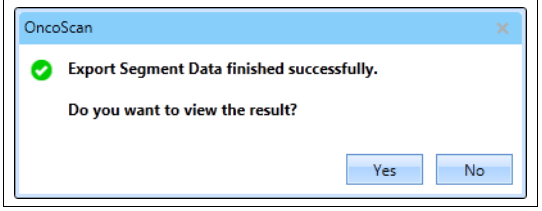


If you have not yet assigned an output folder, see *Assigning an Output Results Path* on page 11.

2. The default root filename is **Result**. Click inside the File Name field to enter a different root filename, then click **Save**.

A progress bar appears while your report generates, followed by a report finished successfully message, as shown in Figure 3.24.

Figure 3.24 Segment Data Report successful



3. Click **Yes**.
   The OncoScan Output folder window appears.
4. Locate the **Segment Data Report** text file, then open it in Microsoft Excel.
   The following window appears. (Figure 3.25)

Figure 3.25 Segment Data report

Chromosome	StartPosition	StopPosition	MarkerCount	Type	State	Median Log2 Ratio	Median BAF	% Aberr. Cells	TuScan Ploidy	FileName
1	754192	3639884	289	TotalCN	2.5	0.328	0.487	homogeneous	2	Normal02_.OSCHP
1	3642248	28863075	2155	TotalCN	2	-0.038	0.485	homogeneous	2	Normal02_.OSCHP
1	28865710	29121185	19	TotalCN	1	-0.558	NaN	homogeneous	2	Normal02_.OSCHP
1	29136686	121350934	7268	TotalCN	2	0.007	0.484	homogeneous	2	Normal02_.OSCHP
1	144009053	145282498	54	TotalCN	2.5	0.215	NaN	homogeneous	2	Normal02_.OSCHP
1	145282799	249212878	8033	TotalCN	2	0.011	0.485	homogeneous	2	Normal02_.OSCHP
2	21494	89125131	6707	TotalCN	2	0.013	0.486	homogeneous	2	Normal02_.OSCHP
2	89138631	91866487	74	TotalCN	4	0.734	NaN	homogeneous	2	Normal02_.OSCHP
2	95429197	243052331	11325	TotalCN	2	0.011	0.485	homogeneous	2	Normal02_.OSCHP
3	63411	197852564	15201	TotalCN	2	0.009	0.486	homogeneous	2	Normal02_.OSCHP
4	69404	689743	41	TotalCN	2	0.073	NaN	homogeneous	2	Normal02_.OSCHP
4	723882	901804	19	TotalCN	3	0.561	NaN	homogeneous	2	Normal02_.OSCHP
4	913437	190915650	13388	TotalCN	2	-0.003	0.486	homogeneous	2	Normal02_.OSCHP
5	38139	180698312	12360	TotalCN	2	0.016	0.486	homogeneous	2	Normal02_.OSCHP
6	204909	31556709	2406	TotalCN	2	0.015	0.486	homogeneous	2	Normal02_.OSCHP
6	31564852	32527746	129	TotalCN	1.5	-0.265	NaN	homogeneous	2	Normal02_.OSCHP
6	32528026	170913051	10188	TotalCN	2	0.001	0.485	homogeneous	2	Normal02_.OSCHP
7	41421	2196551	209	TotalCN	2.5	0.354	0.479	homogeneous	2	Normal02_.OSCHP
7	2227707	159118443	11766	TotalCN	2	0.009	0.484	homogeneous	2	Normal02_.OSCHP
8	172417	39167669	3503	TotalCN	2	-0.013	0.487	homogeneous	2	Normal02_.OSCHP
8	39186857	39439518	18	TotalCN	0	-1.642	NaN	homogeneous	2	Normal02_.OSCHP
8	39458399	128673107	7016	TotalCN	2	0.009	0.485	homogeneous	2	Normal02_.OSCHP
8	128700545	128775144	108	TotalCN	1.5	-0.177	NaN	homogeneous	2	Normal02_.OSCHP
8	128775811	142488553	1033	TotalCN	2	-0.01	0.483	homogeneous	2	Normal02_.OSCHP
8	142504796	146292734	280	TotalCN	2.5	0.253	0.482	homogeneous	2	Normal02_.OSCHP
9	204738	141054761	8538	TotalCN	2	0.006	0.485	homogeneous	2	Normal02_.OSCHP
10	126070	46177093	3107	TotalCN	2	-0.013	0.485	homogeneous	2	Normal02_.OSCHP
10	46965151	47126409	33	TotalCN	3	0.527	NaN	homogeneous	2	Normal02_.OSCHP
10	47127279	133868500	6458	TotalCN	2	-0.002	0.486	homogeneous	2	Normal02_.OSCHP
10	133892178	135434303	97	TotalCN	2.5	0.333	0.491	homogeneous	2	Normal02_.OSCHP
11	192764	2948985	226	TotalCN	2.5	0.404	0.483	homogeneous	2	Normal02_.OSCHP
11	2969242	69474368	4590	TotalCN	2	0.002	0.485	homogeneous	2	Normal02_.OSCHP

Result.segment
Ready
100%

Segment ID	An Affymetrix identifier for the segment.
Chromosome	Chromosome on which the probeset is located.
Start Position	Start position of the segment.
End Position	End position of segment.
Marker Count	Number of markers in the segment.
Type	Indicates if the segment is a copy number segment or an LOH segment.
State	Indicates the copy number state of the segment for copy number segments or if the segment contains LOH for LOH segments (0=No, 1 = Yes).
Median Log2 Ratio	Log2 Ratio is the log2 ratio of the normalized intensity of the sample over the normalized intensity of a reference with further correction for sample specific variation. The Median Log2 Ratio is computed for each segment.
Median BAF	B-allele frequency (BAF) is (Signal (B)/{Signal(A) + Signal(B), where signal (A) is the signal from the AT chip and signal (B) is the signal from the G/C chip. Median BAF is computed for each segment and is the median BAF of the markers identified as heterozygous, after mirroring any marker BAFs above 0.5 to the equivalent value below 0.5. If the number of heterozygous markers in the segment is below 10 or the percent of homozygous markers is above 85% no value is reported.
% Aberr.Cells	If % AC = 100%, we return "homogeneous" because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.
TuScan Ploidy	TuScan Ploidy is the most likely ploidy state of the tumor before additional aberrations occurred. Algorithmically it is the CN state of the markers identified by the algorithm as normal diploid before %AC and ploidy are determined. When a high ploidy is determined the "normal diploid" is deemed to correspond to a higher CN and the log2 ratio gets adjusted appropriately. If ploidy cannot be determined NA (Not Available) is reported.
Filename	Name of the OSCHP file containing the data

Somatic Mutation Data Report

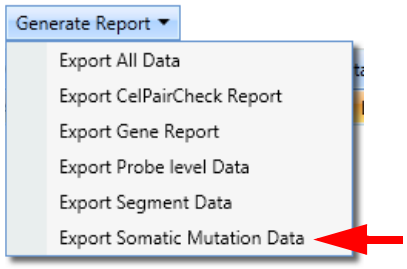
NOTE: This report is not available for OncoScan_CNV.

This report generates two (tab-delimited) text files; the somatic mutation file containing the call for each somatic mutation in the sample, and the somatic mutation annotation file containing annotation information for the somatic mutations assayed.

Do the following to export a Somatic Mutation Data Report:

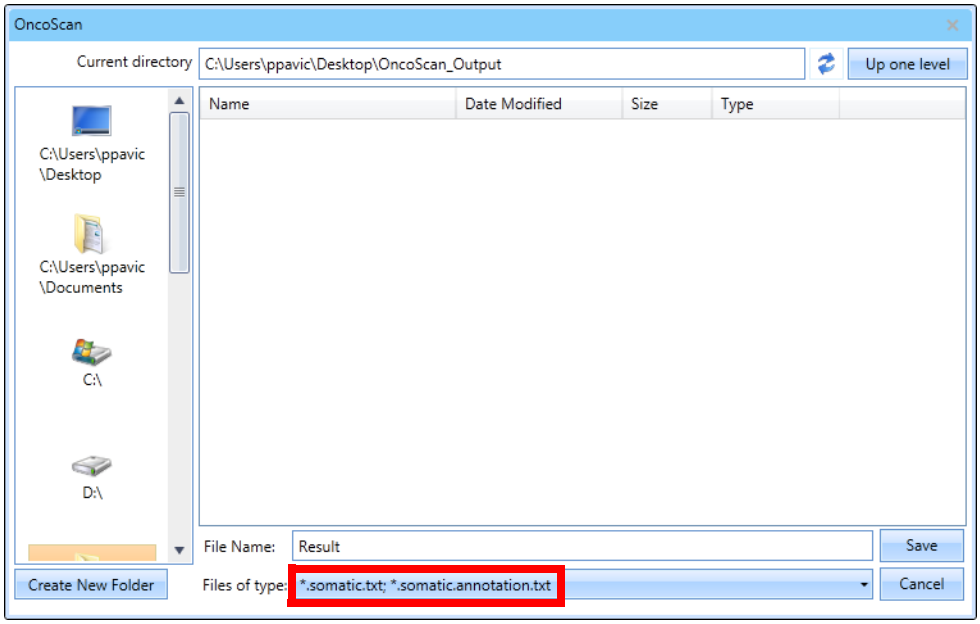
1. Click **Export Somatic Mutation Data**. (Figure 3.26)

Figure 3.26 Generate Report drop-down menu



Your previously assigned Output folder file window appears. (Figure 3.23)

Figure 3.27 OncoScan Output folder window



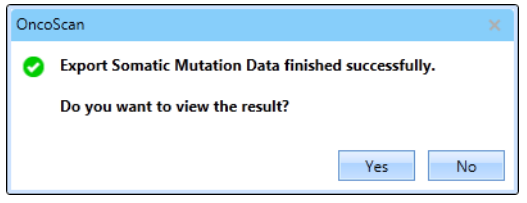
If you have not yet assigned an output folder, see *Assigning an Output Results Path* on page 11.

2. The default root filename is **Result**. Click inside the File Name field to enter a different root filename, then click **Save**.

NOTE: The Export Somatic Mutation Data report produces two separate (tab-delimited) text files, as shown in the *Files of type* field. (Figure 3.27)

A progress bar appears while your report generates, followed by a report finished successfully message, as shown in Figure 3.28.

Figure 3.28 Somatic Mutation Data Report successful



3. Click **Yes**.
   The OncoScan Output folder window appears.
4. Locate the **Somatic Mutation Data Report** (*.somatic), then open it in Microsoft Excel.
   The following window appears. (Figure 3.29)

Figure 3.29 Somatic (*.somatic) Data report

FileName	ProbeSetName	MutCall	MutScore	MutThreshHigh	MutThreshLow
Normal02	93107462A	Undetected	-0.54359	5	2.5
Normal02	93107463C	Undetected	-0.39388	4.4	2.2
Normal02	93107464A	Undetected	-1.21664	3.8	1.9
Normal02	93107466A	Undetected	-0.13264	4.4	2.2
Normal02	93107467C	Undetected	0.539382	4.4	2.2
Normal02	93107469A	Undetected	-0.22148	4.4	2.2
Normal02	93107470A	Undetected	-0.06571	3.2	1.6
Normal02	93107470C	Undetected	-0.39342	3.2	1.6
Normal02	93107471A	Undetected	-0.33453	4.4	2.2
Normal02	93107472C	Undetected	-0.27354	5	2.5
Normal02	93107473C	Undetected	-0.11144	3.8	1.9
Normal02	93107474C	Undetected	-0.76027	4.4	2.2
Normal02	93107475C	Undetected	-1.14571	3.2	1.6
Normal02	93107476C	Undetected	-0.43044	5	2.5
Normal02	93107477C	Undetected	-0.20191	4.4	2.2
Normal02	93107478C	Undetected	-0.36723	3.8	1.9
Normal02	93107479A	Undetected	-0.72974	4.4	2.2
Normal02	93107480C	Undetected	-0.41518	5	2.5
Normal02	93107481C	Undetected	-1.19244	3.2	1.6
Normal02	93107482A	Undetected	0.069109	3.8	1.9
Normal02	93107483A	Undetected	-0.20292	3.8	1.9
Normal02	93107484A	Undetected	-0.79182	3.8	1.9
Normal02	93107487A	Undetected	-0.4768	4.4	2.2
Normal02	93107488A	Undetected	-0.71829	4.4	2.2
Normal02	93107489A	Undetected	-1.01531	3.8	1.9
Normal02	93107490A	Undetected	-0.54622	3.8	1.9
Normal02	93107491C	Undetected	-0.53739	4.4	2.2
Normal02	93107492A	Undetected	-0.71695	3.8	1.9
Normal02	93107493C	Undetected	-0.50434	4.4	2.2
Normal02	93107494A	Undetected	-0.31392	3.8	1.9
Normal02	93107496A	Undetected	-0.62611	3.8	1.9
Normal02	93107497A	Undetected	-0.45933	3.2	1.6

Result.somatic
Ready
100%

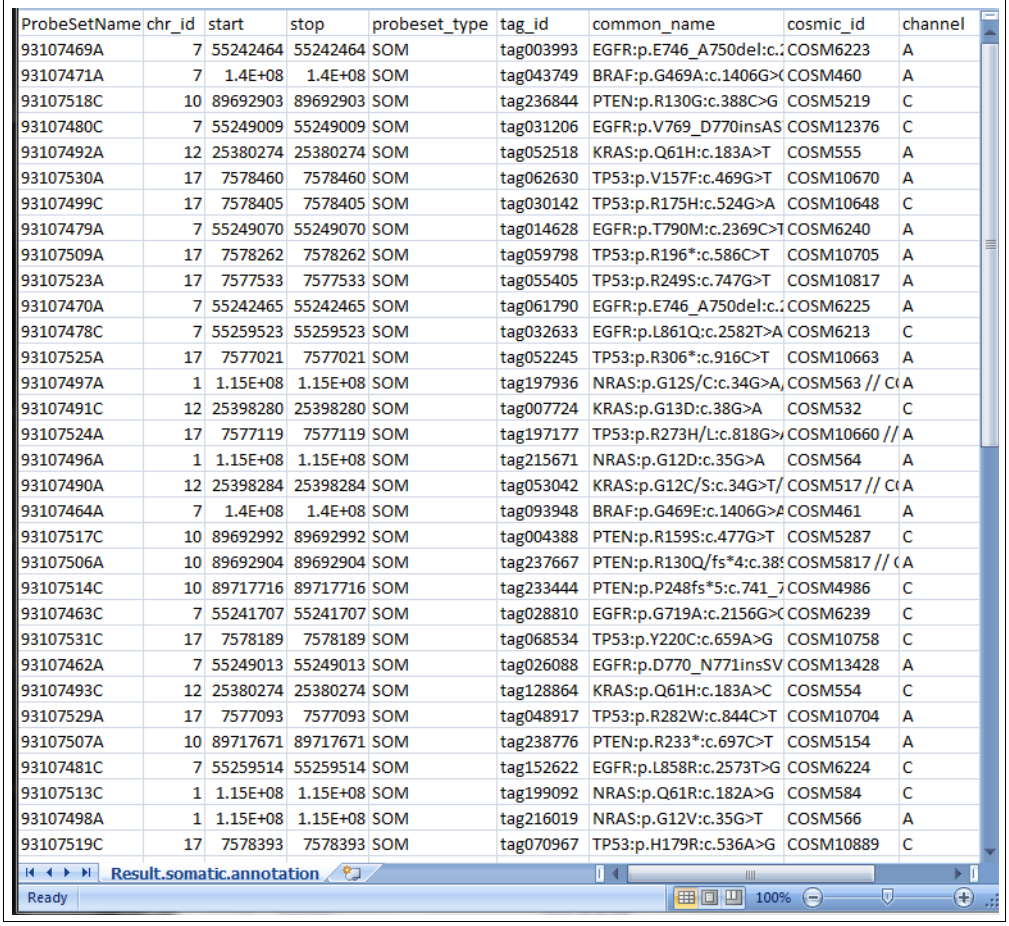
NOTE: For more information on interpreting somatic mutation results, see *Appendix D: Copy Number Effect on Somatic Mutations* on page 78.

Filename	Name of the OSCHP file containing the data
ProbeSetName	Name of the probeset.
MutCall	An indication of the whether the somatic mutation was decteded. A MutCall is displayed as Undetected if the MutScore is below the Low Confidence threshold. A MutCall is reported as HighConfidence if greater than or equal to the High Confidence threshold. If the MutCall is equal to or greater than the Low Confidence threshold and is less than the High Confidence threshold, the MutCall is reported as LowerConfidence. Note: MutCalls from "Outside Bounds" samples are not reliable.
MutScore	Measures somatic mutation probeset response. The stronger the response, the more likely it is that the somatic mutation is present. The MutScore calculation depends on the algorithm version. The newer MutScore calculation also corrects for sample specific effects, and thereby reduces false positive calls, which were sample specific. For algorithm versions 1.0 - 1.2 (ChAS 3.0 and earlier, OncoScan Console 1.2 and earlier): MutScore.old = (measured quantile normalized signal - median signal for this marker in the reference model file) / (95th percentile signal for this marker in the reference model file - median signal for this marker in the reference model file). For algorithm versions 1.3 and newer (ChAS 3.1 and newer, releases of OncoScan Console after 1.2): MutScore.new = (MutScore.old - median MutScore.old for this sample) / standard deviation of MutScore.old for this sample (where standard deviation is calculated for all but the num-out-std strongest MutScore.old for this sample, median is calculated for all but the num-out-med strongest MutScore.old for this sample, and the used median is the maximum of zero and the measured median).
MutThreshHigh	High confidence MutScore threshold. Measurements equal to or greater than this threshold are called "High confidence," describing the likelihood that the mutation is present.
MutThreshLow	Lower confidence MutScore threshold. Measurements with a MutScore below this value are called "Undetected". Measurements equal to or greater than this threshold but less than the High Threshold are called "Lower confidence," describing the likelihood that the mutation is present.

5. From your OncoScan Output folder, locate the second **Somatic Mutation Data Report** (*.somaticannotation) text file, then open it in Microsoft Excel.

The following window appears. (Figure 3.30)

Figure 3.30 Somatic (*.somaticannotation) report



ProbeSetName	chr_id	start	stop	probeset_type	tag_id	common_name	cosmic_id	channel
93107469A	7	55242464	55242464	SOM	tag003993	EGFR:p.E746_A750del:c.2	COSM6223	A
93107471A	7	1.4E+08	1.4E+08	SOM	tag043749	BRAF:p.G469A:c.1406G>(	COSM460	A
93107518C	10	89692903	89692903	SOM	tag236844	PTEN:p.R130G:c.388C>G	COSM5219	C
93107480C	7	55249009	55249009	SOM	tag031206	EGFR:p.V769_D770insAS	COSM12376	C
93107492A	12	25380274	25380274	SOM	tag052518	KRAS:p.Q61H:c.183A>T	COSM555	A
93107530A	17	7578460	7578460	SOM	tag062630	TP53:p.V157F:c.469G>T	COSM10670	A
93107499C	17	7578405	7578405	SOM	tag030142	TP53:p.R175H:c.524G>A	COSM10648	C
93107479A	7	55249070	55249070	SOM	tag014628	EGFR:p.T790M:c.2369C>T	COSM6240	A
93107509A	17	7578262	7578262	SOM	tag059798	TP53:p.R196*:c.586C>T	COSM10705	A
93107523A	17	7577533	7577533	SOM	tag055405	TP53:p.R249S:c.747G>T	COSM10817	A
93107470A	7	55242465	55242465	SOM	tag061790	EGFR:p.E746_A750del:c.2	COSM6225	A
93107478C	7	55259523	55259523	SOM	tag032633	EGFR:p.L861Q:c.2582T>A	COSM6213	C
93107525A	17	7577021	7577021	SOM	tag052245	TP53:p.R306*:c.916C>T	COSM10663	A
93107497A	1	1.15E+08	1.15E+08	SOM	tag197936	NRAS:p.G12S/C:c.34G>A,	COSM563 // C(	A
93107491C	12	25398280	25398280	SOM	tag007724	KRAS:p.G13D:c.38G>A	COSM532	C
93107524A	17	7577119	7577119	SOM	tag197177	TP53:p.R273H/L:c.818G>A	COSM10660 //	A
93107496A	1	1.15E+08	1.15E+08	SOM	tag215671	NRAS:p.G12D:c.35G>A	COSM564	A
93107490A	12	25398284	25398284	SOM	tag053042	KRAS:p.G12C/S:c.34G>T/	COSM517 // C(	A
93107464A	7	1.4E+08	1.4E+08	SOM	tag093948	BRAF:p.G469E:c.1406G>A	COSM461	A
93107517C	10	89692992	89692992	SOM	tag004388	PTEN:p.R159S:c.477G>T	COSM5287	C
93107506A	10	89692904	89692904	SOM	tag237667	PTEN:p.R130Q/fs*4:c.38	COSM5817 // (	A
93107514C	10	89717716	89717716	SOM	tag233444	PTEN:p.P248fs*5:c.741_7	COSM4986	C
93107463C	7	55241707	55241707	SOM	tag028810	EGFR:p.G719A:c.2156G>(	COSM6239	C
93107531C	17	7578189	7578189	SOM	tag068534	TP53:p.Y220C:c.659A>G	COSM10758	C
93107462A	7	55249013	55249013	SOM	tag026088	EGFR:p.D770_N771insSV	COSM13428	A
93107493C	12	25380274	25380274	SOM	tag128864	KRAS:p.Q61H:c.183A>C	COSM554	C
93107529A	17	7577093	7577093	SOM	tag048917	TP53:p.R282W:c.844C>T	COSM10704	A
93107507A	10	89717671	89717671	SOM	tag238776	PTEN:p.R233*:c.697C>T	COSM5154	A
93107481C	7	55259514	55259514	SOM	tag152622	EGFR:p.L858R:c.2573T>G	COSM6224	C
93107513C	1	1.15E+08	1.15E+08	SOM	tag199092	NRAS:p.Q61R:c.182A>G	COSM584	C
93107498A	1	1.15E+08	1.15E+08	SOM	tag216019	NRAS:p.G12V:c.35G>T	COSM566	A
93107519C	17	7578393	7578393	SOM	tag070967	TP53:p.H179R:c.536A>G	COSM10889	C

Result.somatic.annotation

Ready

100%

ProbeSetName Name of the probeset.

chr_id Chromosome on which the somatic mutation is found.

start Start position of the somatic mutation.

stop End position of the somatic mutation.

probeset_type Indicates if the probeset is used for Somatic Mutation analysis (SOM).

tag_id An Affymetrix identifier for the tag associated with the particular probeset.

common_id Abbreviated description of the mutations to which this ProbeSet is known to respond. The name has the form [Gene]:[amino acid change for mutation]:[cDNA change for mutation]. In the event that the ProbeSet cannot differentiate among multiple mutations to which it can respond, the slash (/) delimits the multiple known mutations.

cosmic_id The identifier of the mutation as listed in the COSMIC database, which is a catalogue of somatic mutations in cancer. More information on these mutations can be found at: http://cancer.sanger.ac.uk

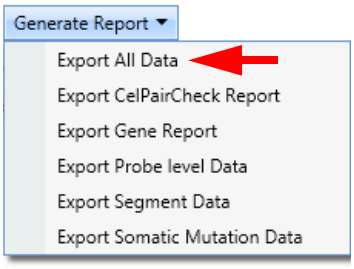
channel The Channel file from which the signal is measured. "A" is the AT CEL, "C" is the GC CEL.

Export All Data

Use this option to generate all the reports (described in detail above) simultaneously.

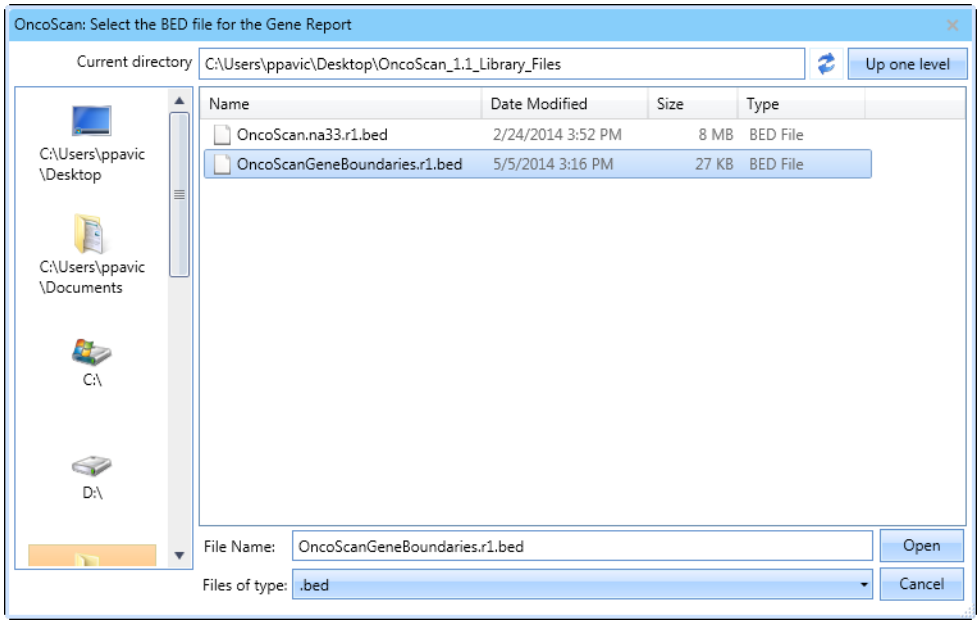
1. Click **Export All Data**. (Figure 3.31)

Figure 3.31 Generate Report drop-down menu



The following window appears. (Figure 3.32)

Figure 3.32 Select the BED file for the Gene Report window



2. Click to select the appropriate BED file, then click **Open**.

NOTE: As shown in Figure 3.32, the default OncoScan-specific BED file for the Gene report is *OncoScanGeneBoundaries.r1.bed*. However, any BED file can be used to generate the Gene Report on any regions of interest contained within the BED file.

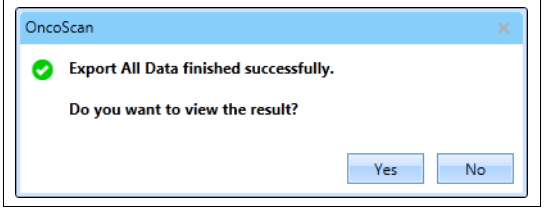
Your previously assigned Output folder file window appears. If you have not yet assigned an output folder, see *Assigning an Output Results Path* on page 11.

NOTE: The default root filename is *Result*. Click inside the File Name field to enter a different root filename.

3. Enter a Root File Name for your text (tab-delimited) Export All Data file, then click **Save**.

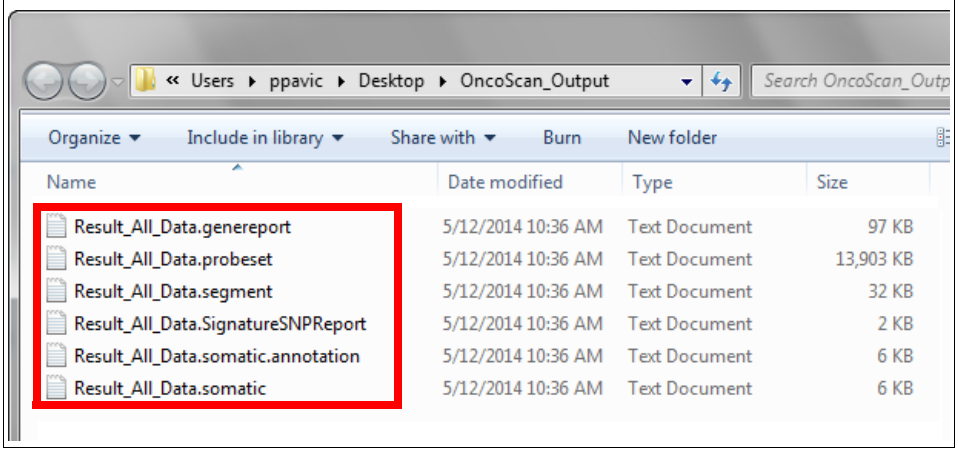
A progress bar appears while your report generates, followed by a report finished successfully message, as shown in Figure 3.33.

Figure 3.33 Export All Data successful



4. Click **Yes**.
   Your OncoScan Output folder window appears and shows all the reports generated from the ***Export All*** option. (Figure 3.34)

Figure 3.34 Output folder window



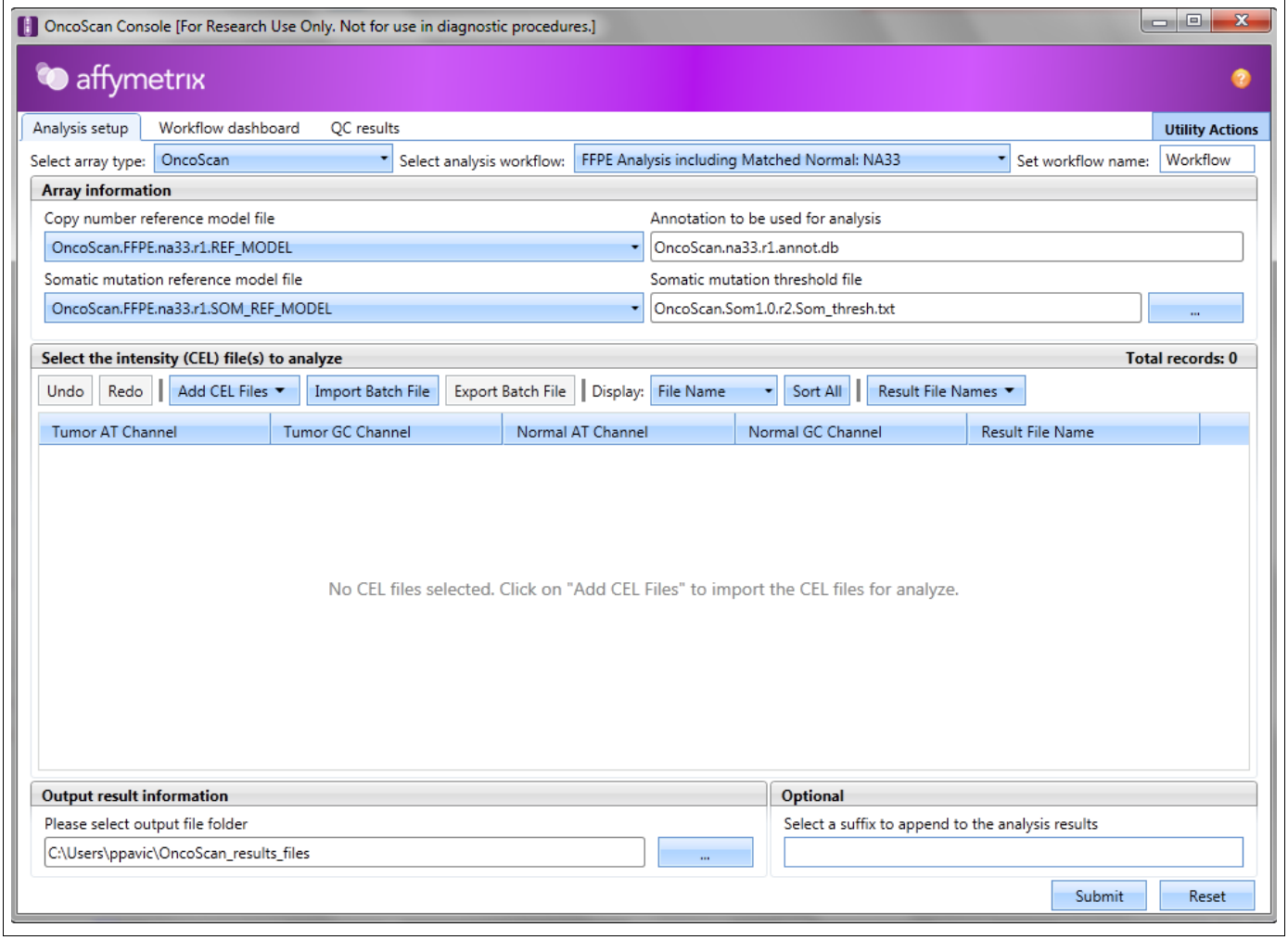
5. Open the text file report you want to view using Microsoft Excel.

Chapter 4

Matched Normal Analysis Setup

To setup a **Standard Analysis** go to *Standard Analysis Setup* on page 14.

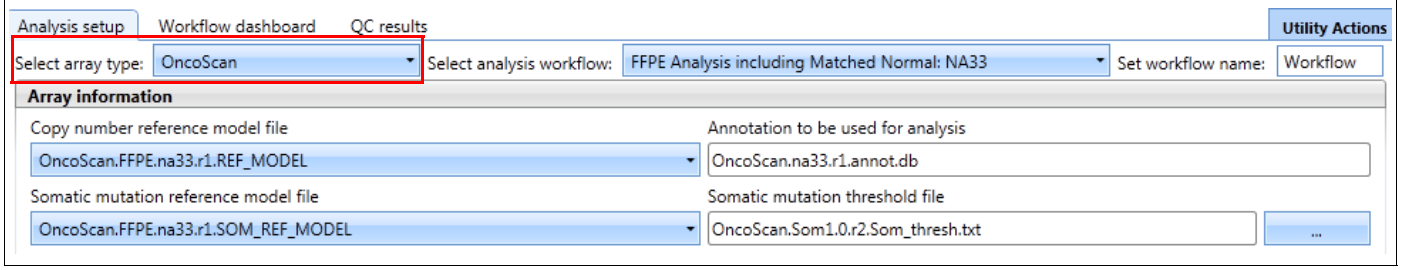
Figure 4.1 Analysis Setup window/tab - Matched Normal Analysis main window



Selecting Array Information

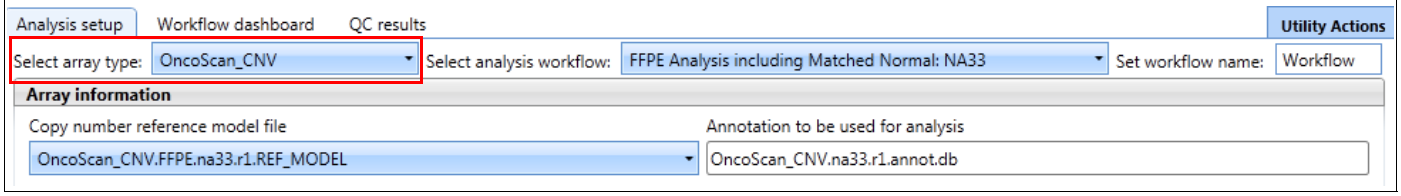
1. From the **Select array type** drop-down list, click to select either **OncoScan** or **OncoScan_CNV**. As long as your library file folder contains the necessary analysis files for the array, your configuration paths are established and your Array Information fields auto-populate, as shown in Figure 4.2.

Figure 4.2 Matched Normal Analysis Configuration - OncoScan array



Somatic mutation file selection is NOT available with the OncoScan_CNV array type, as shown in Figure 4.3.

Figure 4.3 Matched Normal Analysis Configuration - OncoScan_CNV array



NOTE: The *Select array type* drop-down list includes only the array types from the library (analysis) files that have been downloaded from NetAffx or copied from the Library package provided in the OncoScan installation package.

IMPORTANT: After adding new library files to the library file folder, always close and re-launch OncoScan Console to ensure the newly added files are recognized by the software.

2. From the **Select analysis workflow** drop-down list, click to select **FFPE Analysis including Matched Normal NA33**.
3. (Optional) Enter a Workflow name. By default, the **Set workflow name** is *Workflow*. Click Workflow (upper right) to enter a different workflow name.

TIP: Customizing a Workflow name can be a useful tool in keeping track of analysis workflows as all the related output files (outside of the OSCHP file) begin with this workflow name.

The Annotation file is automatically selected for you and is based on your selected reference model file. (Example: **OncoScan.na33.v1.annot.db**)

NOTE: The *Annotation to be used for analysis* field is auto-populated based on your Ref Model file selection. The analysis is not be permitted to run if the appropriate annotation file is not available in your Library folder.

4. Select a **Somatic mutation reference model file**. (OncoScan array only. Not applicable to OncoScan_CNV array.) By default, it is set to the previously used model file. If you created your own reference model file, click the drop-down list to select your **.SOM_REF_MODEL**.

5. Confirm the displayed **Somatic mutation threshold file** to be used is correct. If you need to change it, click the **Browse** button, navigate to the appropriate threshold .txt file, then click **OK**.

> **IMPORTANT: If the Reference Model File and Somatic mutation Reference Model File were created independently of each other, a warning message appears after you click Submit (to start the Workflow Analysis process). Click OK to acknowledge the message.**

Adding CEL Files to Analyze

You can manually add CEL files or import them as a tab-delimited text file.

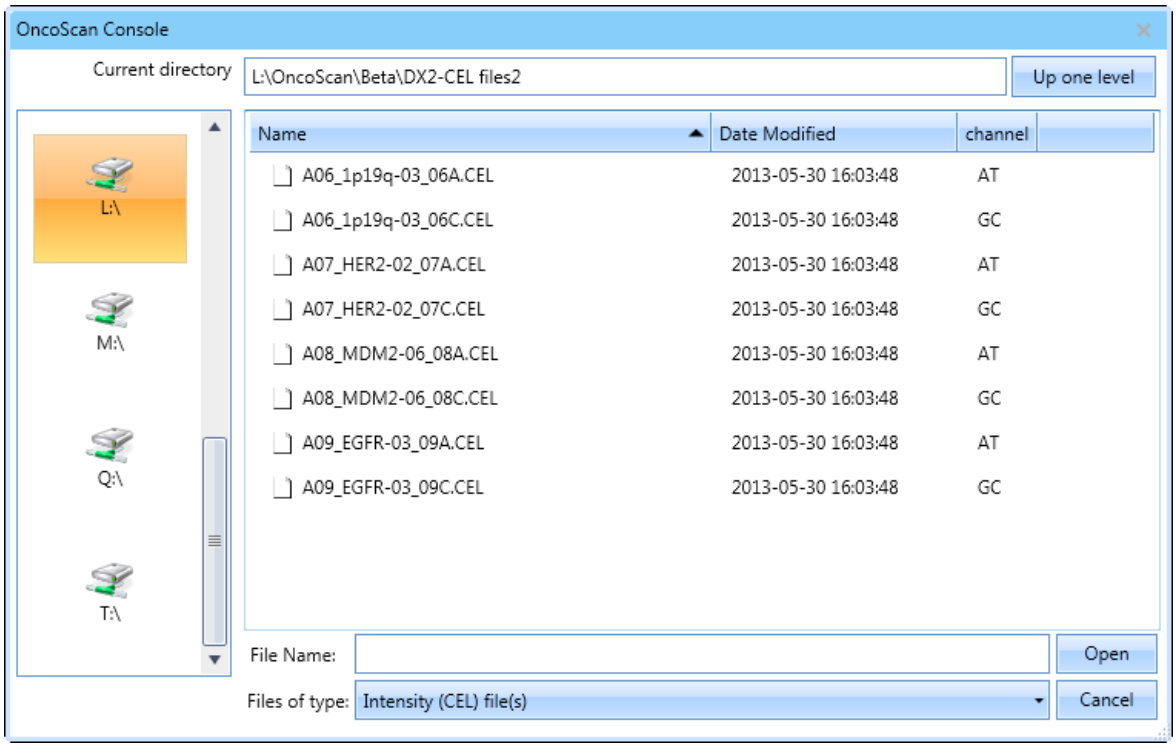
Manually Adding CEL Files to Analyze

To add batch-edited CEL files, see *Importing CEL Files Using Batch Import* on page 58.

To manually add CEL files:

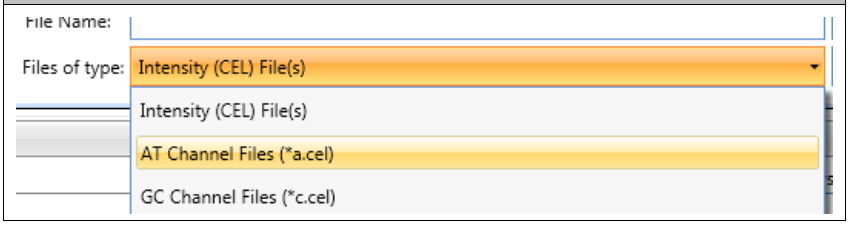
1. At the **Select the intensity (CEL) file(s) to analyze** pane, click the **Add CEL files** drop-down.
2. Click **Tumor AT Channel**.
   The CEL file window appears. (Figure 4.4)

Figure 4.4 CEL file folder -EXAMPLE



3. Click any header to sort your files or click the **Files of type** drop-down to filter your CEL files by AT Channel, as shown in Figure 4.5.

Figure 4.5 Files of type drop-down list

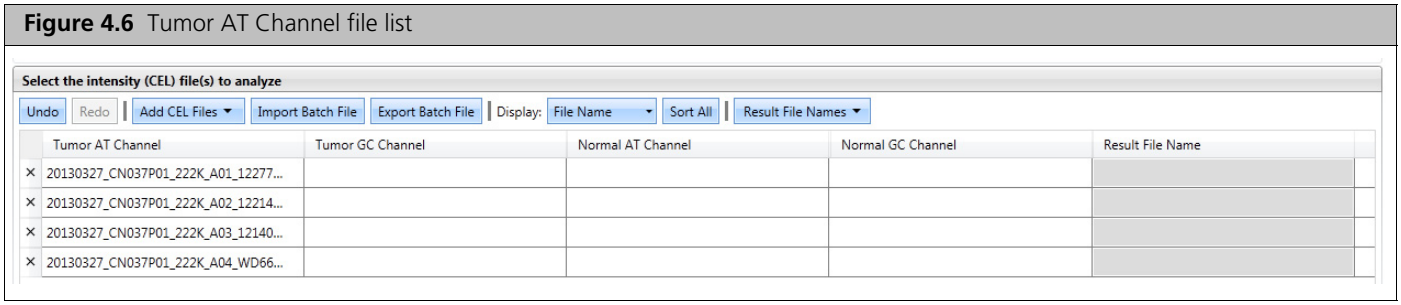


4. Single click, Ctrl click, or Shift click (to select multiple Tumor AT Channel files).

> **IMPORTANT: Affymetrix recommends using an "A" or "C" as the last character to designate the channel in the CEL file naming convention. Example: "_AS_05A.CEL" is an AT Channel file, while "_AS_05C.CEL" is a GC Channel file. See Figure 4.4.**

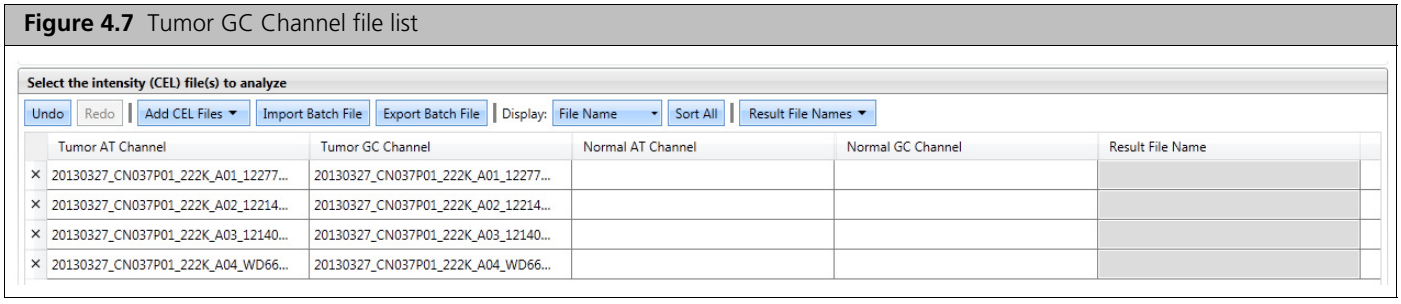
5. Click **Open**.
   The Tumor AT Channel fields are now populated. (Figure 4.6)

Figure 4.6 Tumor AT Channel file list



6. Click the **Add CEL files** drop-down.
7. Click **Tumor GC Channel**. The CEL file window appears. (Figure 4.4 on page 55)
8. Single click, Ctrl click, or Shift click (to select multiple Tumor GC Channel files).
9. Click **Open**.
   The Tumor GC Channel fields are now populated. (Figure 4.7)

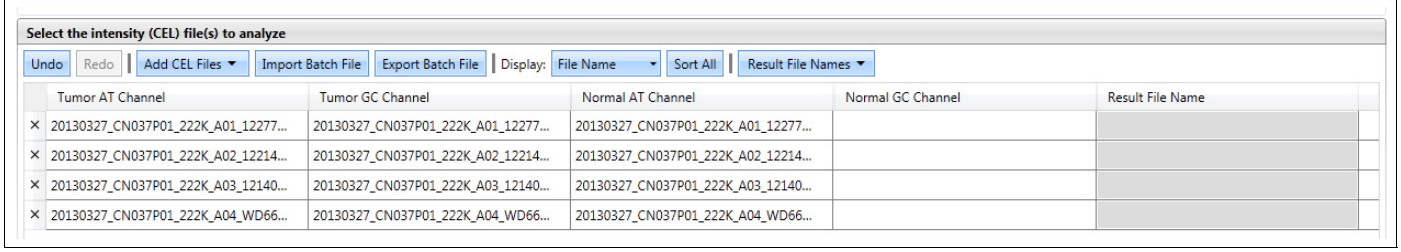
Figure 4.7 Tumor GC Channel file list



10. Click the **Add CEL files** drop-down.
11. Click **Normal AT Channel**. The CEL file window appears. (Figure 4.4)
12. Single click, Ctrl click, or Shift click (to select multiple Normal AT Channel files).
13. Click **Open**.

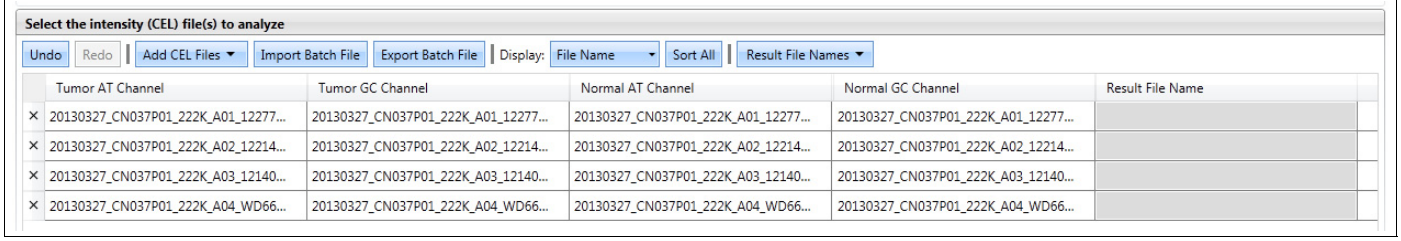
The Normal AT Channel fields are now populated. (Figure 4.8)

Figure 4.8 Normal AT Channel file list



14. Click the **Add CEL files** drop-down.
15. Click **Normal GC Channel**. The CEL file window appears. (Figure 4.4)
16. Single click, Ctrl click, or Shift click (to select multiple Normal GC Channel files).
17. Click **Open**.
    The Normal GC Channel fields are now populated. (Figure 4.9)

Figure 4.9 Normal GC Channel file list



CEL File Displaying Options (Optional)

The File Name drop-down list (Figure 4.10) is dynamically populated and based on what attributes are populated in the ARR file.

To use this display option, you must:

1. Provide the appropriate attributes at the time of sample registration in AGCC.
2. The ARR files must reside in the same folder as the CEL files.

Figure 4.10 EXAMPLE: File Name Display Choices

To see “channel” (as an option in the drop down), you must use a template (or the OncoScan template provided in the library files) that contains a “channel” attribute. The resulting ARR file must also reside in the same folder as the CEL files you are analyzing.

You can display one of the attributes from the ARR file in the table. For example, “Channel” can be chosen (Figure 4.10) to confirm the assignment of a CEL file to its appropriate channel.

To select a FIle Name display attribute:

1. Click the **File Name** drop-down button, then click to select the attribute you want displayed along with your CEL file names.

   The two examples (Figure 4.11 and Figure 4.12) show how the table appears with the display set to **Filename**, then to **Channel**.

Figure 4.11 Table with Filename displayed

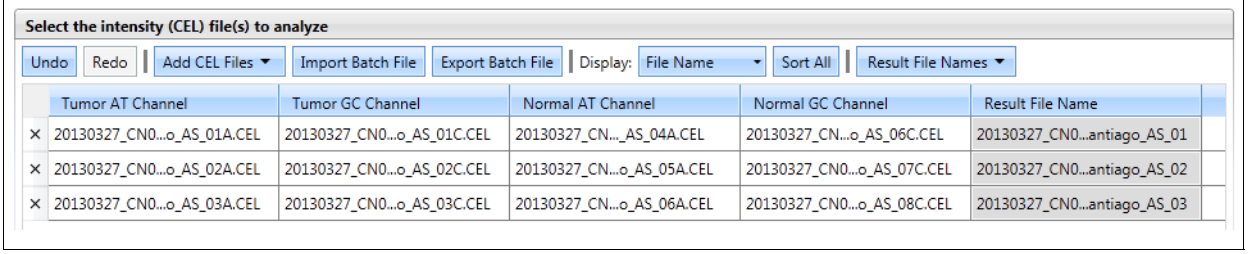
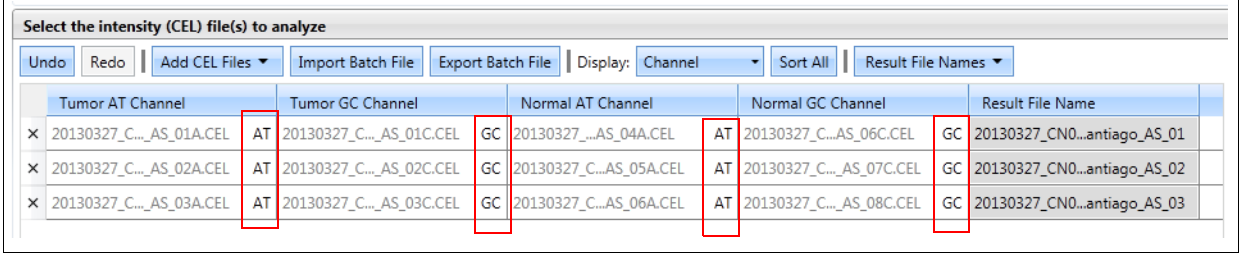


Figure 4.12 Table with Channel displayed



Importing CEL Files Using Batch Import

OncoScan Console allows import of CEL files using a batch file. The batch file must be saved as a text (Tab-delimited) format and include the full directory path for your CEL files (as shown in Figure 4.13).

TIP: The resulting OSCHP files are saved to your output path location, therefore it is not necessary to include a path under RESULT. Simply enter the desired results filename in this column.

The format for this tab-delimited file is 5 columns (A,B, C, D, and E) with the headers:

- ATCHANNELCEL
- GCCHANNELCEL
- ATChannelMatchedNormalCel
- GCChannelMatchedNormalCel
- RESULT

You must provide the full path to the CEL files for each Channel column.
(Example: **C:\Desktop\OncoScan\Data\Sample1.cel**)

Figure 4.13 List from Windows Excel

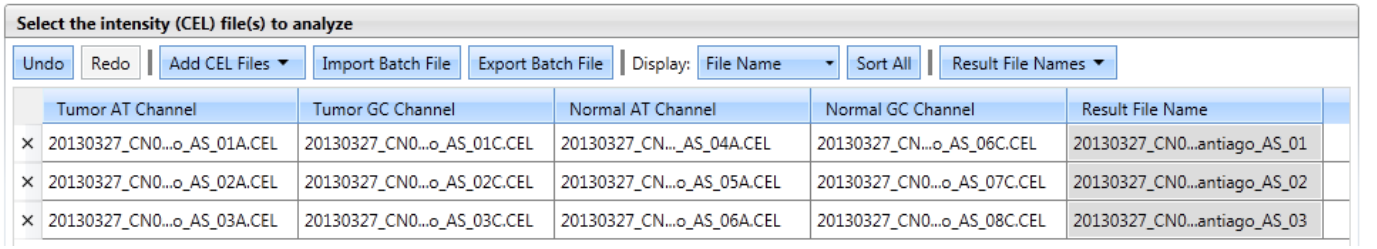
	A	B	C	D	E
1	ATCHANNELCEL	GCCHANNELCEL	ATChannelMatchedNormalCel	GCChannelMatchedNormalCel	RESULT
2	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	20130327_CN037P01_222K_A01_1227
3	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	20130327_CN037P01_222K_A02_1221
4	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	20130327_CN037P01_222K_A03_1214

1. Click **Import Batch File**
   A File window appears.
2. Navigate to your text (tab delimited) file location, then click on the file you want to import.

   IMPORTANT: The Microsoft Excel application must be closed before you import (click Open).

3. Click **Open**.
   The Tumor AT, Tumor GC, Normal AT, Normal GC and Result File Name fields are now populated. (Figure 4.14)

Figure 4.14 Tab-delimited text file imported into OncoScan Console



Generating Result File Names

Results File Names can either be entered in manually or OncoScan Console can generate them automatically.

NOTE: If you use the suffix option (*Selecting a Suffix to Append to the Analysis Results on page 62*) and enter your Result File Names manually, your assigned suffix appears in the Results File Name column.

If you auto-generate your Results File Names, your assigned suffix appears in the Results File Name column, but it does get added to your final OSCHP file name(s).

To manually enter a Results File Name:

1. Single-click inside the appropriate Results Name File field to produce a cursor, then type in the file name you want.

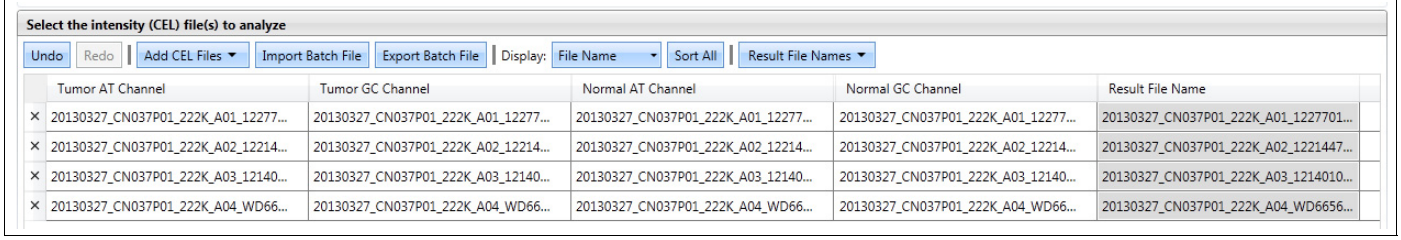
To auto-generate a suggested Result File Name:

NOTE: During the Result File Name auto-generation process, the file names are compared to identify their common root name for use as a results file name. Generally, the last 5 characters of each CEL file name are ignored, then the remaining root names of the AT and GC file names are compared. If the root names of the AT and GC channel match, then the root name is used in the Results File Name field. The one exception is if your array name "_(OncoScan)" is appended to the file name during registration in Affymetrix GeneChip Command Console (AGCC). In this case, the "_(OncoScan)" is ignored during the comparison, but then added back in the Results File Name field.

1. After the 4 Channel lists are populated, click the **Result File Names** drop-down, then select **Auto Generate Output Name**.

2. The Result File Name column is now populated with suggested filenames for each pairing. (Figure 4.15)

Figure 4.15 Matched Normal Results File Name list



Common root names should be consistent all the way up to the last character of the CEL file name prior to the .cel extension. If there is a paired file mis-match, the Results File Name appears as **Output1**. (Figure 4.16)

Figure 4.16 Result File Name "Output"

Tumor AT Channel	Tumor GC Channel	Normal AT Channel	Normal GC Channel	Result File Name
20130327_CN0...o_AS_01A.CEL	20130327_CN...o_AS_06C.CEL	20130327_CN...o_AS_05A.CEL	20130327_CN0...o_AS_01C.CEL	Output1

If **Output1** or subsequent Outputs (Output 2, Output 3...) appear, investigate the validity of your original pairing. See *Correcting Mismatched CEL File Pairings* on page 60.

To edit an auto-generated Result File Name:

1. Click on the Result File name you want to edit.
2. After the cursor appears, edit the filename as you normally would.
3. Click outside the row to save your edit.

To clear the entire Result File Name column:

1. Click the **Result File Names** drop-down button, then select **Clear Column**.
   The column is now cleared and ready for new Result File Name entries.

Correcting Mismatched CEL File Pairings

If there is a paired file mismatch, the Results File Name appears as **Output1, Output2, Output3, etc**.

A paired file mismatch is most likely caused by an incorrect CEL filename pairing and not a mismatch of your native CEL files.

A simple way to correct mismatches is to sort the AT and GC columns so that files with the same root names are next to each other.

TIP: Common root names should be consistent all the way up to the last character of the cel file name prior to the .cel extension. Affymetrix recommends using an "A" or "C" as the last character to designate the channel in the CEL file naming convention. Example: "_AS_05A.CEL" is an AT Channel file, while "_AS_05C.CEL" is a GC Channel file.

Using the Sorting Features

To sort an individual column:

1. Click on a Channel header.
   The column is now sorted in an ascending order.

2. Click on the Channel header again to reverse the sorting order.

To sort all the columns simultaneously:

1. Click **Sort All**.
   The file contents of the 4 columns are now sorted together in an ascending order.
2. Click **Sort All** again.
   The file contents of the 4 columns are now sorted together in a descending order.

To swap CEL files between columns:

1. Click and drag a column CEL entry onto another column CEL entry, then release the mouse button.
   The CEL entries have now swapped column positions.

To reorder the CEL files in a column:

1. Click and drag a CEL file to another position within the column, then release the mouse button.
   The CEL file is now at its new position.

To add a cell to a column:

1. Click and drag a column cell to the top or bottom border line of a neighboring cell, then release the mouse button.

Generating a Result File Name after Sorting

1. After all your columns are properly sorted, click the **Result File Names** drop-down, then select **Auto Generate Output Names**.
   The Result File Name column is now populated with suggested filenames for each pairing.

NOTE: An OSCHP file is created for each pair. The tumor will have "_T" and the normal will have "_N" appended to its root name.

If OncoScan Console detects an inconsistency between the AT and GC file names to be paired, a Result File Name labeled, "Output n" reappears.

IMPORTANT: Confirm that both columns are sorted in the same direction. If they are, examine the files and confirm they are paired correctly. The file names (excluding the last character before the .CEL) MUST match exactly.

Repeat the sorting steps above, then try to **Auto Generate Output Names** again until a successful Result File Name(s) appears.

Setting your Output Information Location (Optional)

The Output result information path (lower left) is retained from your initial setup.

To select a different folder to store your results:

1. Click the browse button, then navigate to the folder you want. If you want to change the default folder, see *Assigning an Output Results Path* on page 11.

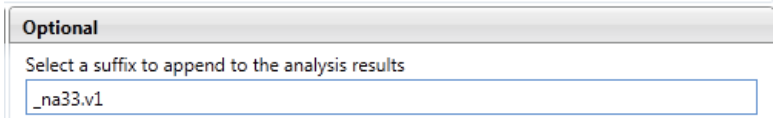
Selecting a Suffix to Append to the Analysis Results

You can append a suffix at the end of all your Results File Names. This is useful when tracking versions of the analysis files used to generate the resulting OSCHP files.

To use an appending suffix:

1. Click inside the **Select a suffix to append to the analysis results** field to enter an appending file suffix. (Figure 4.17)
   Your currently displayed Result Name Files are appended (in real-time) as you type in your suffix.

Figure 4.17 Adding a suffix



NOTE: If you are saving the same OSCHP file into the same output file folder that contains your originally run OSCHP file with an identical suffix, a "2" is automatically added to the filename to differentiate the 2 runs of identical CEL file names.

Exporting Batch Analysis Files (Optional)

You can export the information shown in the Tumor and Normal AT and GC Channels and Results File names fields to Microsoft Excel as a tab-delimited file for review and/or further batch editing.

NOTE: Once an analysis is submitted, a tab delimited file containing the cel file selections is automatically saved in your designated output folder.

1. Click **Export Batch File**.
   A File window appears.
2. Navigate to the location where you want to save the file.
3. Make sure the **Files of type** is set to **Tab Delimited File(s)**, then click **Save**.

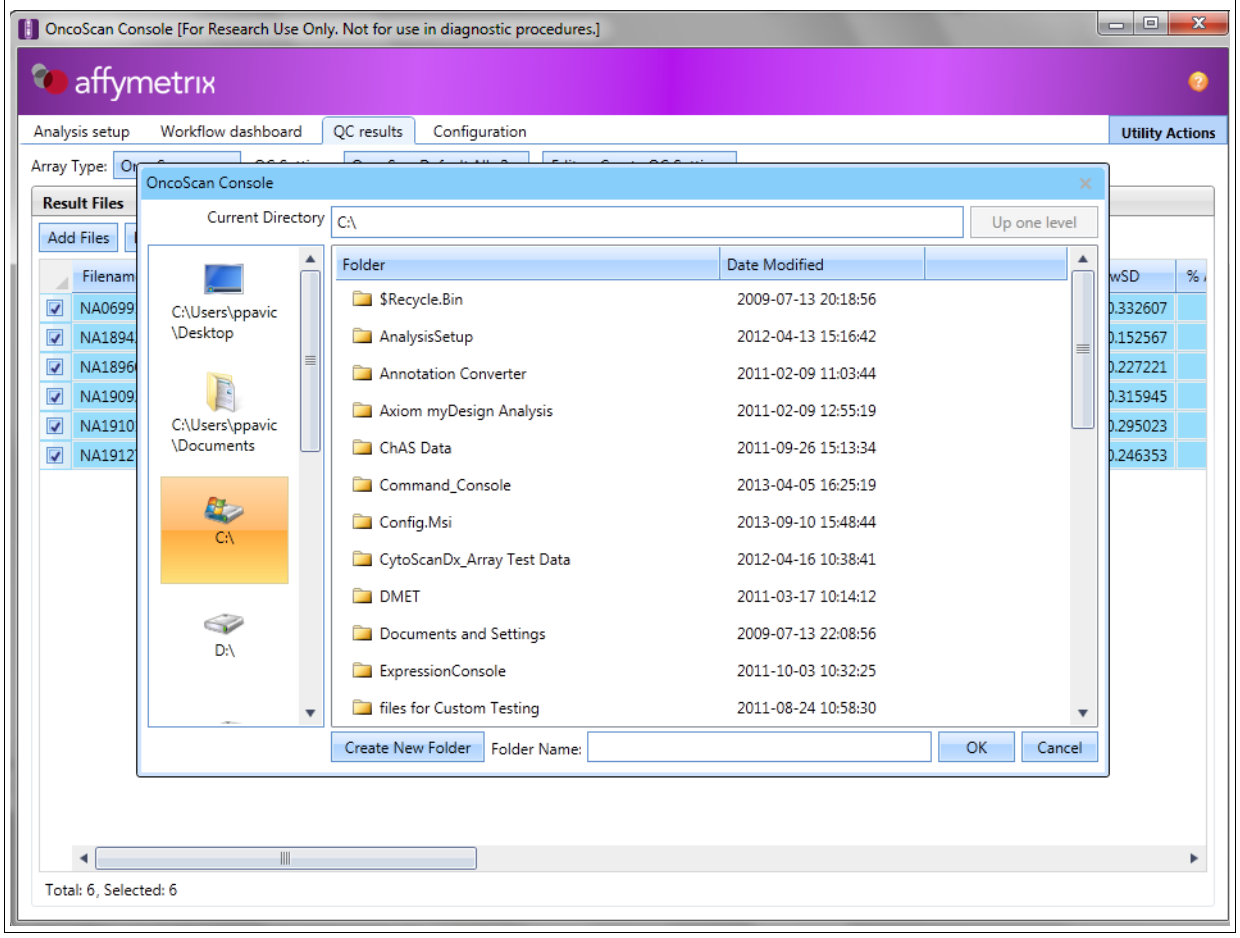
Log File Collection

Do the following if you experience any issues or failures with your analysis:

1. Click the **Utilities** button (top right of the OncoScan Console window)
2. Click to select **Log Collection**.

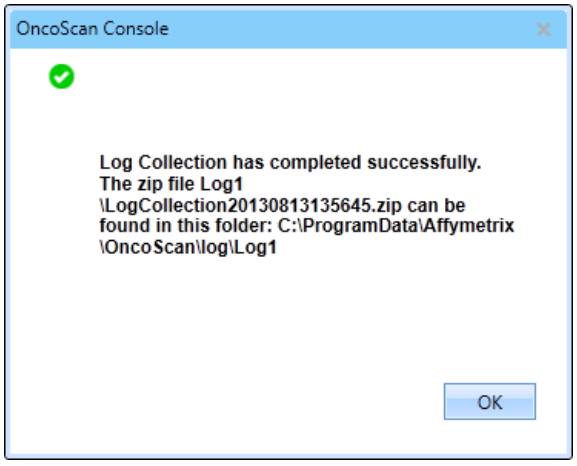
The following window appears. (Figure 4.18)

Figure 4.18 Log Collection File window



3. Use OncoScan Console's default location of `C:\` or navigate to a folder location of your choice.
4. Click **Create New Folder**, then enter a folder name for your log.
5. Click **OK**.
   The following window appears confirming your log file has been saved as a zip file.

Figure 4.19 Example: Zip file contents of a Log Collection



6. Click **OK** to close the window.

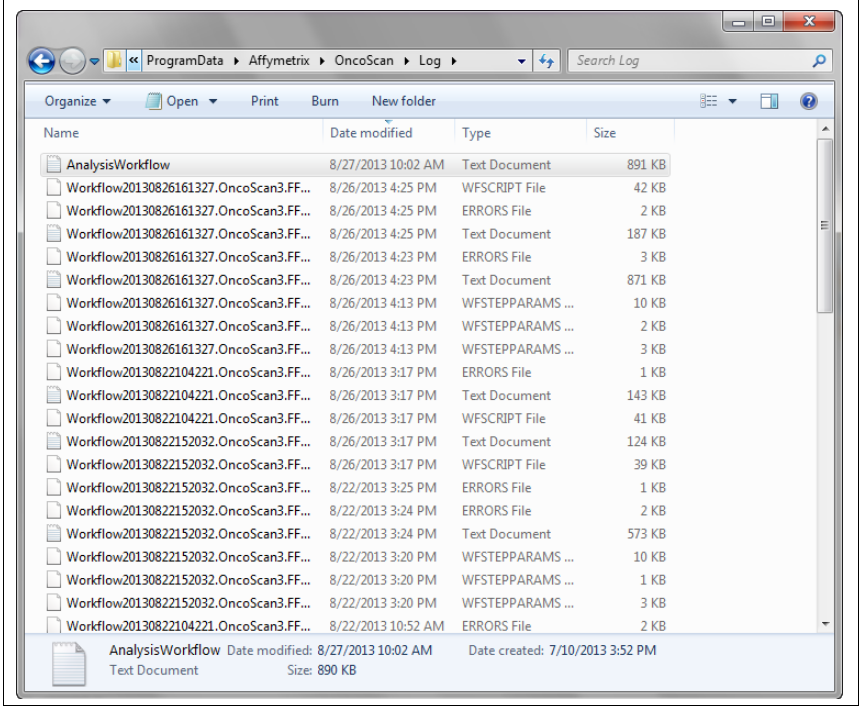
NOTE: The auto-generated log collection zip file contains the full contents of the folder and all QC History log files found in the configured QC History File path. By default, the zip file resides here: *C:\ProgramData\Affymetrix\Oncoscan\log*

Viewing the Log Collection File

To view the log collection file:

1. Use Windows Explorer to navigate to the location.
   (Example: `C:\ProgramData\Affymetrix\OncoScan\log`)
2. Locate the zip folder you created earlier, then double-click on it.
   The folder opens.
3. Extract the zipped folder's contents, as you normally would. (Figure 4.20)

Figure 4.20 Example: Zip file contents of a Log Collection



Log Rollover

When the software determines that the log file for the Analysis Workflow (`C:\ProgramData\Affymetrix\OncoScan\log\AnalysisWorkflow.log`) has reached a defined size (approximately 4MB), the following steps will be completed:

A sub-folder will be created in `C:\ProgramData\Affymetrix\OncoScan\log` called '**Log***' (the '*' denotes the current date and time).

A zip file called **RolledLogFile*.zip** is created in that folder. The '*' is the same date and time used for the folder name. The files in the `C:\ProgramData\Affymetrix\OncoScan\log` folder and all files found in the currently selected QC History Log folder will be included in this zip file.

The Analysis Workflow files that are associated with analysis workflows that are no longer active on the Dashboard will be deleted from: `C:\ProgramData\Affymetrix\OncoScan\log`

A new AnalysisWorkflow.log file will be created here:
`C:\ProgramData\Affymetrix\OncoScan\log`

Appendix A

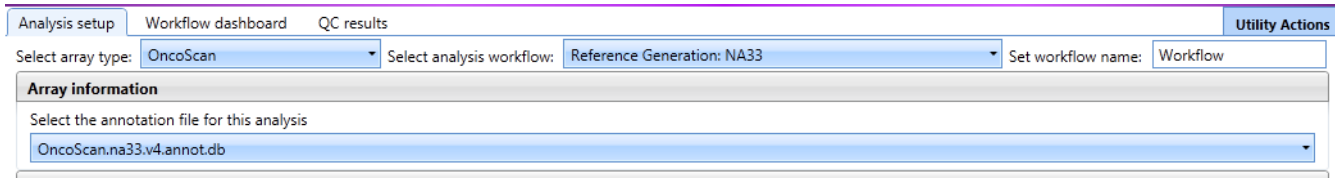
Appendix A: Custom Reference Files

Creating your Own Reference File

IMPORTANT: When creating an OncoScan FFPE Assay reference file, you must use a minimum of six CEL files (including one male and one female). It is recommended that you select at least 44 normal samples of good quality. Ideally, your male and female samples should be of equal numbers (22 and 22).

1. From the Analysis Setup tab **Select array type** drop-down list, click to select an array type (Example: OncoScan) (Figure A.1)

Figure A.1 Reference File creation example



NOTE: The *Select array type* drop-down list includes only the array types for which library (analysis) files have been downloaded from NetAffx or copied from the Library package provided with the installation.

2. From the **Select analysis workflow** drop-down list, click to select **Reference Generation NA33**.
3. By default, the **Set workflow name** is **Workflow**. Click Workflow (upper right) to enter a different workflow name.
4. Select the annotation File for this analysis to be used for analysis. (Example: **OncoScan.na33.v1.annot.db**)

   After you create your reference file, navigate to your library file folder, then open the Signature SNPs Report text file. Confirm that the report shows that your sample pairings and channel IDs are correct.

   If they are not, you must adjust the pairings and channel data in OncoScan Console's Select the intensity (CEL) file(s) to analyze table (see Correcting Mismatched CEL File Pairings on page 18), then re-run the reference file.

Appendix B

Appendix B: QC Metrics - Definitions

Array Data QC Metrics (Overview)

This section provides a high level overview of the key QC metrics used with OncoScan Console.

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. Lower MAPD values are better. For more information, see *Array Data QC Metrics (Detailed Descriptions)* on page 69.

ndSNPQC (SNP Quality Control of Normal Diploid Markers)

ndSNPQC is a measure of how well genotype alleles are resolved in the microarray data. The calculation only uses probesets that appear to be in normal diploid regions. Larger ndSNPQC values are better. For more information, see *ndSNPQC* on page 71.

SNP QC Type (SNP Quality Control Type)

If SNP QC Type is ND, metrics like ndSNPQC, ndWavinessSD, ndSNR_AT, ndSNR_GC, ndRawSNPQC are all based on the performance of the normal diploid marker subset counted by ndCount. If SNP QC Type is non ND, then ndSNPQC and ndRawSNPQC are computed based on a preselected set of 10,000 autosomal markers, while ndWavinessSD are computed on all autosomal markers.

CelPairCheck Status

CelPairCheck is a test that inspects each pair of intensity (*.cel) files to determine whether the files have been properly paired and assigned to the correct channel. In addition to accidental mispairing of intensity files while setting up the analysis, a tracking problem during the assay may result in a sample being assigned to the wrong GeneChip array. As a result CelPairCheck ignores file names, and instead inspects the genotypes in the two intensity files to detect file mispairings. If the CelPairCheck Status is not ***Pass***, see *CelPairCheckStatus* on page 73.

CelPairCheck Compare Rate

This metric is the percentage of signature SNP control markers whose genotypes are compared between the AT and GC channels. The Compare Rate needs to be above a minimum in order for CelPairCheck to determine whether the AT and GC CEL files belong to the same individual.

CelPairCheck Concordance

This metric is the concordance of a set of signature SNP genotypes compared between AT and GC CEL files. If CelPairCheck Compare Rate is high but CelPairCheck Concordance is low, then CelPairCheck Status will report "PossibleCELmispair".

ndWavinessSD (Normal Diploid Waviness Standard Deviation)

ndWavinessSD is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. ndWavinessSD is computed on normal diploid markers.

ndWavinessSD should be used along with Low Diploid Flag, ndCount (the actual number of diploid markers identified) BAFs, and log2 ratio to assess if the log2 ratio is centered correctly. ndWavinessSD can thus help assess if log2 ratios need to be re-centered.

In addition when ndWavinessSD is high, the log2 ratios should be examined for clear breakpoints as opposed to a gradual drift of the log2 ratio. When the latter is observed small aberrations should be examined carefully. When breakpoints are sharp and the ndCount is large a high ndWavinessSD can be ignored.

Y Gender Call

Gender call determined by examining signal on the Y Chromosome.

ndCount

ndCount reports how many normal diploid markers were identified. When ndCount falls below a minimum (typically 2000), the Low Diploid Flag is triggered. ndCount > 10,000 is desired for maximum confidence in ndSNPQC and maximum confidence in the centering of the log2 ratios.

Low Diploid Flag

An essential part of the algorithm is the identification of “normal diploid” markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that “normal diploid markers” result in a log2 ratio of 0 (e.g. copy number 2). The algorithm might later determine that the "normal diploid" markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of “normal diploid” markers and no normal diploid calibration occurs. This event triggers "Low Diploid Flag = Yes." In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary.

ACDC (Aberrant Cell-Derived Copy Number)

Indicates whether the algorithm was able to compute the % Aberr. Cells and measure the Copy Number in the tumor cells only. “ACDC= No” means Copy Number was calculated as an average CN across all cells, “ACDC=Yes” means that Copy Number was calculated only for the tumor cells.

%Aberr. Cells

Algorithmic estimation of the percent of aberrant cells (%AC) in a sample.

When %AC is “NA” it means that the % aberrant cells could not be estimated because the percent is either too low or the sample is heterogeneous and hence is composed of several types of aberrated cells, or the percent varies from one genomic location to another.

When %AC is not NA, (example 60%), it means that across all aberrations, ~60% of the cells were aberrated and contributed to the elevated (or reduced) Copy number.

TuScan Ploidy

TuScan Ploidy is the most likely ploidy state of the tumor before additional aberrations occurred. Algorithmically it is the CN state of the markers identified by the algorithm as normal diploid before %AC and ploidy are determined. When a high ploidy is determined the "normal diploid" is deemed to correspond to a higher CN and the log2 ratio gets adjusted appropriately. If ploidy cannot be determined NA (Not Available) is reported.

Reliability Score

This metric evaluates the fit between the actual and predicted log2 ratios and BAFs, and is only meaningful when ACDC = Yes. The predicted log2 ratios and BAFs are computed from the predicted TuScan Ploidy, %Aberr. Cells, and reported copy number states. Reliability Score ranges from 0 to 1.1, and values above 0.8 represent a good score. If Reliability Score cannot be calculated, "NA" is reported. Samples that are considered Within Bounds but have a low Reliability Score may have less reliable predictions for TuScan Ploidy, %Aberr. Cells, and reported copy number states.

Offset Flag

If the Offset Flag is Yes, the TuScan results required an adjustment to the log2 ratios, as explained under TuScan Ploidy. As a result, allelic peaks, and smooth signal values get rescaled accordingly.

TuScan L2R Adj

TuScan L2R Adj is the constant added by the TuScan algorithm to the log2 ratios, when the Offset Flag = **Yes**. Note: This value does not include any additional user adjustment when performing manual recentering.

Adjusted Log2 Ratio

The median log2 ratio value entered by the user during manual recentering of a chromosome region the user wants to designate as diploid.

Low % Aberrant Cell nGoF

Goodness of fit (GoF) is evaluated when TuScan tries to explain the CN changes by assuming a two state mixture model of normal and aberrant cells. A large nGoF value indicates an estimation of the % aberrant cells cannot be calculated, due to the aberrant cell fraction being below TuScan detection limits.

Hyb Control Intensity_AT

Geometric mean of three array hybridization controls in the AT channel. An unusually low value indicates a problem at the array hybridization and/or washing step.

Hyb Control Intensity_GC

Geometric mean of three array hybridization controls in the GC channel. An unusually low value indicates a problem at the array hybridization and/or washing step.

Q3 Raw Intensity_AT

The 75th percentile of the raw intensity of the AT channel.

Q3 Raw Intensity_GC

The 75th percentile of the raw intensity of the GC channel.

AGR_AT

Antigenomic ratio in the AT channel. Measures non-specific binding to array features in that channel. Smaller values are better.

AGR_GC

Antigenomic ratio in the GC channel. Measures non-specific binding to array features in that channel. Smaller values are better.

ndSNR_AT

This metric is the Signal/Noise Ratio of normal diploid markers in the AT channel. The Signal is the 65th percentile of the measurements used for copy number analysis, minus the median of the antigenomic features. The Noise is the standard deviation of the weakest 15% of measurements used for copy number analysis. A low value indicates poor data quality from the AT CEL file.

ndSNR_GC

This metric is the Signal/Noise Ratio of normal diploid markers in the GC channel. The Signal is the 65th percentile of the measurements used for copy number analysis, minus the median of the antigenomic features. The Noise is the standard deviation of the weakest 15% of measurements used for copy number analysis. A low value indicates poor data quality from the GC CEL file.

ndRawSNPQC

Like ndSNPQC, but calculated on raw intensities.

Call Rate

The percentage of snps for which a genotype could be determined - divided by the total number of snps. The higher the Call Rate, the better. Call rate is based on genotype calls using an algorithm which assumes all autosomal markers are normal diploid. Therefore, this metric is more relevant for normal samples than for aberrated tumor samples.

Matched Normal Compare Rate

If doing FFPE analysis including matched normal (where you supply four CEL files per row during analysis setup), this metric reports the percentage of markers identified as normal diploid in both the resulting Tumor OSCHP file, and its matched Normal OSCHP file. If this metric is unusually low, then the results of the companion metric Matched Normal Concordance may be unreliable.

Matched Normal Concordance

If doing FFPE analysis including matched normal (where you supply four CEL files per row during analysis setup), this metric reports the genotype concordance of the normal diploid markers in common between the Tumor OSCHP file and its matched Normal OSCHP file. If the Matched Normal Compare Rate is reasonably high but Matched Normal Concordance is low, then the normal CEL files you paired with the tumor CEL files may not belong to the same individual. This metric is only reported for the Tumor OSCHP file.

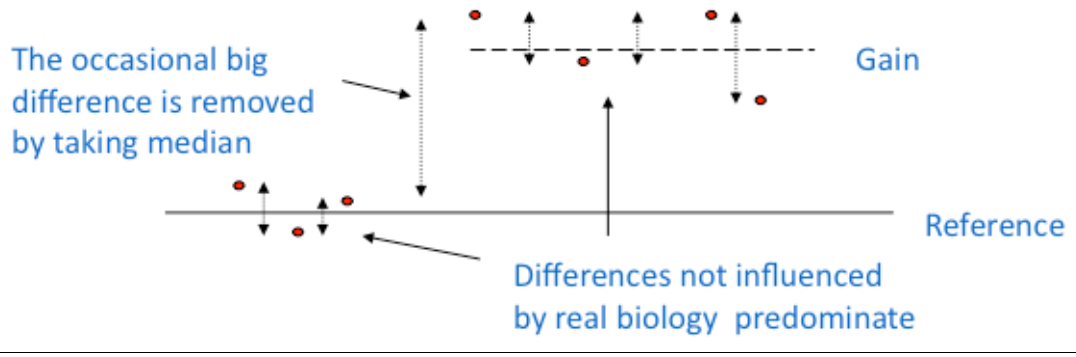
Array Data QC Metrics (Detailed Descriptions)

MAPD

For quality assessment purposes, we define metrics that assess whether the microarray data is of sufficient quality to accurately assess copy number (CN) analysis. One of these metrics is Median of the Absolute values of all Pairwise Differences (MAPD).

MAPD is defined as the Median of the Absolute values of all Pairwise Differences between log2 ratios for a given chip. Each pair is defined as adjacent in terms of genomic distance, with SNP markers and CN markers being treated equally. Hence, any two markers that are adjacent on the genome are a pair. Except at the beginning and the end of a chromosome, every marker belongs to two pairs, as shown in Figure B.1.

Figure B.1 Example: Two pairs to a marker



Formally, if xi: is the log2 ratio for marker i:

MAPD = median($|x_{i-1} - x_i|$, with i ordered by genomic position)

MAPD is a per-microarray estimate of variability, like standard deviation (SD) or interquartile range (IQR). If the log2 ratios are distributed normally with a constant SD, then MAPD/0.96 is equal to SD and MAPD*1.41 is equal to IQR. However, unlike SD or IQR, using MAPD is robust against high biological variability in log2 ratios induced by conditions such as cancer.

Variability in log2 ratios in a microarray arises from two distinct sources:

- Intrinsic variability in the starting material, hybridization cocktail preparation, microarray or scanner
- Apparent variability induced by the fact that the reference may have systematic differences from this microarray

Regardless of the source of the variability, increased variability decreases the quality of CN calls.

Effect of MAPD on Functional Performance

As a measure of performance, we measured copy number gains and loss using over 1000 cancer samples. Arrays with MAPD > 0.3 are out of bounds.

ndWaviness-SD

Waviness refers to an effect seen in all genomic microarrays (see Maroni et al. (2007) Genome Biology 8:R228) where long-range variation is observed, often associated with regional genomic differences like local GC-content changes.

This metric is only computed on markers that have been identified as Normal diploid by the algorithm. When Low Diploid Flag = Yes, ndWaviness-SD is computed for all autosomal markers. For most samples, a ndWaviness-SD value below 0.12 for OncoScan arrays indicates that the long-range variation is within levels that can be accommodated by the algorithms.

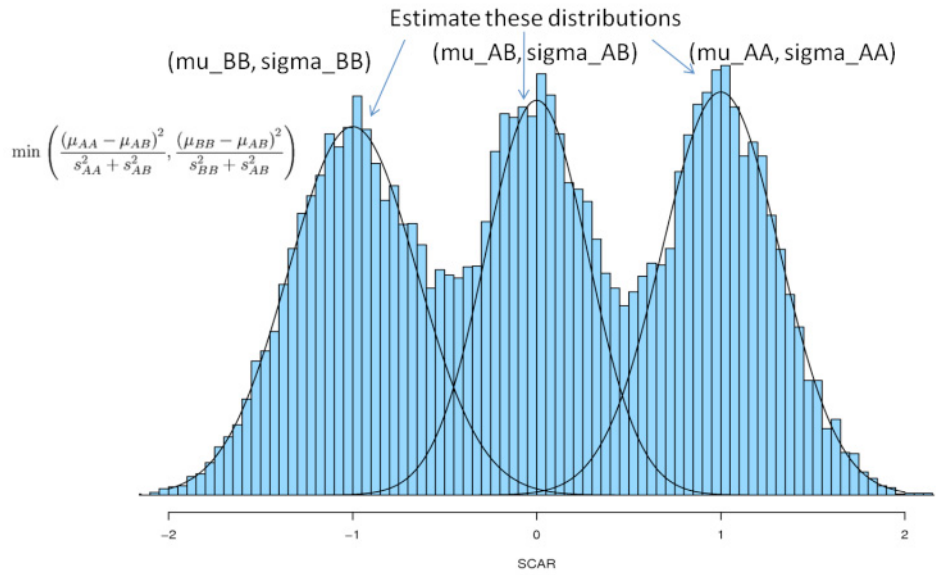
ndWavinessSD should be used along with LowDiploidFlag, ndCount (the actual number of diploid markers identified) BAFs and log2 ratio to assess if the log2 ratio is centered correctly. ndWavinessSD can thus help assess if log2 ratios need to be re-centered.

In addition when ndWavinessSD is high, the log2 ratios should be examined for clear breakpoints as opposed to a gradual drift of the log2 ratio. When the latter is observed small aberrations should be examined carefully. When breakpoints are sharp and the ndCount is large a high ndWavinessSD can be ignored.

ndSNPQC

The ndSNPQC metric estimates the distributions of homozygous AA, heterozygous AB and homozygous BB alleles and calculates the distance between them. The better the separation of these distributions, the better the ability to identify a genotype based on its cluster position, as shown in Figure B.2.

Figure B.2 Example: Genotype based on its cluster position



SNPQC correlates well with genotype performance, as measured by Call Rate and Concordance to published HapMap genotypes.

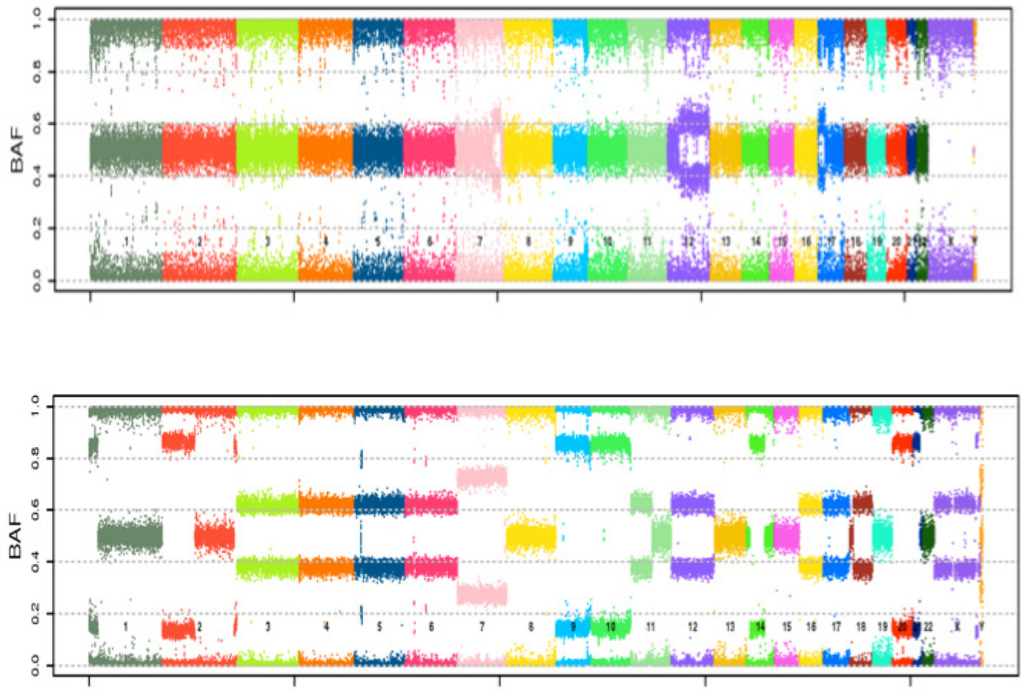
For OncoScan, we use ndSNPQC. This is computed only on the normal diploid markers to assess the quality of the genotypes and the resulting quality of BAFs. When Low Diploid Flag = Yes, ndSNPQC is computed for a preselected set of 10,000 autosomal markers. As a result, values may fall out of bounds although the data quality is good. We therefore recommend always examining the BAF visually when Low Diploid Flag = Yes and ndSNPQC is out of bounds.

Effect of ndSNPQC on Functional Performance

ndSNPQC provides insight into the overall level of data quality from a SNP perspective. The key consideration when evaluating the ndSNPQC value is to ensure the threshold is exceeded. The quality of the SNP allele data is compromised, and is noisier and more difficult to interpret when the ndSNPQC values are below the recommended acceptance threshold as illustrated in the figure below.

When the ndSNPQC value is below 26, the noise within the array is higher than normal which compromises the overall clarity of results. ndSNPQC values above 26 have good data quality and can be relied upon with regards to performance as shown in Figure B.3. Sometimes ndSNPQC values can be as high as 35 (or higher) and provide even better separation of BAFs.

Figure B.3 Examples: (TOP) Sample with Noisier BAFs. (BOTTOM) Sample with clearly delineated BAFs.



CelPairCheckStatus

CelPairCheckStatus message	Description
Pass	No intensity file pairing problem is detected.
PossibleCELmispair	Low genotype concordance exists between data in AT channel and data in GC channel. This is consistent with the data in the two intensity files originating from different individuals. This message may also appear with poor data. Please review the channel-specific sample QC metrics to see if one or both CEL files has a problem.
PossibleGCinATchannel	Data from the GC reaction appears to be assigned to the AT analysis channel.
PossibleATinGCchannel	Data from the AT reaction appears to be assigned to the GC analysis channel.
Warn	Cannot determine whether the two intensity files belong to the same individual. This message may appear with poor data, or with good data where too many of the signature SNPs used for CelPairCheck are in chromosome regions with copy number aberrations.

Most of the probes in the OncoScan assay are designed so that each OncoScan array detects only one of the two alleles for a specific SNP, and so both arrays are needed to measure a full genotype call for that SNP. However a set of control probes are included so that both alleles for a set of "Signature SNPs" can be measured on each array independently.

The pattern of genotype calls for these Signature SNPs is compared between the two intensity files. As the signature SNPs are a set of high minor allele frequency SNPs, under normal assay conditions it is very unlikely that two unrelated tested samples will have the same pattern of Signature SNP genotype calls. Therefore, if the concordance of genotype calls is unusually low between a given pair of intensity files, the CelPairCheck Status message will be "PossibleCELmispair." The concordance check is only done if there are enough Signature SNPs reporting a call in both files. If this is not the case, CelPairCheck Status reports "Warn."

Signature SNP probes are designed to be channel-specific. When an intensity file is assigned to the AT channel, only the Signature SNP probes designed to respond in the assay's AT reaction well are expected to report genotypes. In the case of GC data being assigned to the AT channel for analysis, a low call rate will result in the CelPairCheck Status message "PossibleGCinATchannel." If AT data were assigned to the GC channel, the message would be "PossibleATinGCchannel."

If you need additional help fixing the problems detected by CelPairCheck Status, you can review that Signature SNP metrics and genotypes for each intensity file in the CelPairCheckReport file. This report is created along with result files (*.oschp) for each analysis, or go to OncoScan Console's **QC results** tab, click on the **Generate Report** button, then select **Export [CelPairCheck Report]**. See *Generating and Exporting Reports* on page 35 for more details.

ndWavinessSD

For OncoScan we compute Waviness-SD on markers identified by the algorithm as “normal diploid” markers. It is a QC metric that focuses on measuring long-range effects. As described separately, MAPD is a metric that measures short-range variation, the variation of adjacent probes. The long-range variation measurement is accomplished by calculating the variation in log2 ratios across the whole genome and subtracting out the short-range variation, specifically, for autosomal probes:

Define:

Xi as the log2 ratios of autosomal probes

And Zi as the variance between adjacent probes:

Zi = X2i+1 - X2i

Waviness-SD is the total variance (Xi) minus the local variance (Zi):

Waviness-SD = sqrt(Var(Xi)-Var(Zi)/2)

% Aberrant Cells

If % AC = 100%, we return “homogeneous” because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls.

The cause could be:

1. Low % aberrant cells
2. Low number of diploid regions
3. Data Quality, in particular for all samples for which MAPD is above 0.4 or ndSNPQC falls below 19.
4. Occasionally High/Low Ploidy
5. No Ploidy solution has a good fit to the data.

Low Diploid Flag

An essential part of the algorithm is the identification of “normal diploid” markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that “normal diploid markers” result in a log2 ratio of 0 (e.g. copy number 2). The algorithm might later determine that the "normal diploid" markers identified really correspond to (for example) CN=4. In this case, the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of “normal diploid” markers and no “normal diploid calibration occurs. This event triggers “low diploid flag” = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary.

Appendix C

Appendix C: Algorithms

B-allele Frequencies

B-allele frequencies (BAFs) are a graphical way to show allelic imbalances. BAFs are also used by the algorithm to derive the CN state. Conceptually for each marker we compute (Signal (B)/{Signal(A) + Signal(B), where signal (A) is the signal from the AT chip and signal (B) is the signal from the G/C chip. A homozygous BB SNP will therefore have a value of 1, a homozygous AA SNP a value of zero and a AB SNP a value of 0,5. For SNPS with high Minor allele frequency the BAFs present as three equally thick bands around these values (0, 0.5, 1).

In regions of Loss of heterozygosity the only possible alleles are BB and AA and the middle band (corresponding to AB) is missing.

In regions of Copy Gain the allelic balance is disrupted. With 3 copies it is not possible to have an equal amount of A and B alleles, and the BAF bands are at 0, 1/3, 2/3 and 1.

In cancer samples the additional complication of normal contamination of the tumor cells affects the allelic imbalance further.

When 60% of the cells have CN=3 and 40% of the cells have CN=2 the location of the BAF bands is at (0.6*2+0.4)/(3*0.6+2*0.4) =0.615, while it is at 0.66 (or 2/3) when 100% of the cells have CN=3. In general when the percent aberrated cells is p and the CN for these aberrated cells is 3 then, the middle BAF band shifts up to $(2p+(1-p))/(3p+2(1-p))$ an down to $1-(2p+(1-p))/(3p+2(1-p))$.

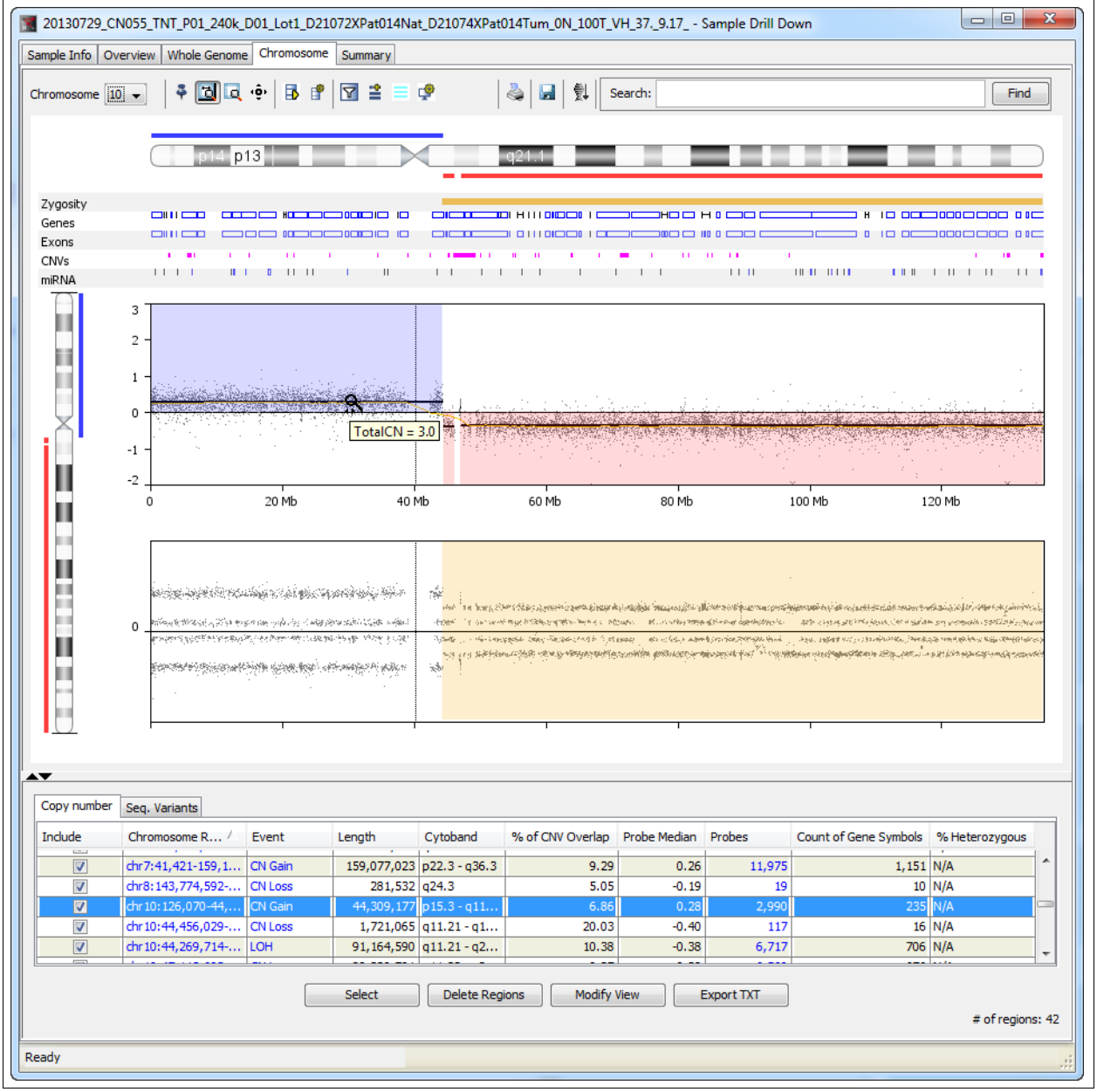
LOH Algorithm

The LOH algorithm uses B-allele frequencies (BAFs) and log2 ratios to find long stretches of homozygosity.

TuScan Algorithm

The TuScan algorithm uses B-allele frequencies (BAFs) and log2 ratios to estimate the ploidy and percentage of aberrant cells in the sample (%AC) which in turn are used to calculate copy number calls (CN). The BAFs and log2 ratios contribute equally to CN determination. TuScan first uses the BAFs and log2 ratio data to identify segments of equal CN. Next TuScan uses the BAFs, log2ratios and segment data to find the combination of %AC and ploidy that best fits the data. When TuScan can successfully determine %AC, the algorithm assigns each aberrant segment an integer copy number representing the copy number in the tumor portion of the sample. This is possible because CN is well approximated by an integer when the tumor is nearly homogeneous. If the tumor is highly heterogeneous (i.e., lacks a dominant clone), or contains a large amount of "normal" cells %AC cannot be determined. In other words, if the percentage of aberrant cells contributing to the various aberrations in the sample varies across all aberrations, %AC and ploidy cannot be determined. When %AC cannot be determined, the segmentation algorithm will still identify segments of equal CN, but the CN in just the aberrant cells cannot be determined. In this case, TuScan bins the copy numbers and returns fractional CN values in 1/3 increments (e.g., 2, 2.33, 2.66, 3 etc.). This fractional copy number is derived from the normal contamination as well as the heterogeneous population of tumor cells; therefore, the fractional CN calls represent the average CN observed for that segment. Users should look at the value of %AC to determine whether the CN value represents the CN in the tumor (%AC= number) or the average CN in the sample (%AC=NA). Tumor heterogeneity also affects the interpretation of the CN number calls when %AC cannot be determined. For example, a TuScan call of 2.33 can result from 40% of the aberrant cells having 3 copies, 10% of aberrant cells having 5 copies, or a more complex heterogeneous mixture of copy numbers. Since nearly every tumor sample will have some amount of normal contamination combined with tumor heterogeneity it is not possible to predict how often TuScan will be able to determine the %AC, it will vary depending on the sample.

Figure C.1 Example: TuScan results as displayed in OncoScan Nexus Express



Manual Recentering Algorithm

TuScan identifies normal diploid markers in a sample of interest, determines the copy number for these markers (2, 4 or 6) and ensures that markers with CN=2 have a log ratio of 0. This is referred to as "centering" the sample.

When no or an insufficient number of normal diploid markers are found, the automatic recentering does not occur. In addition, occasionally the automatic recentering misses the true CN =2 markers and does not correctly center the sample. In these cases, it is advised to center the sample manually to get correct CN calls. Manual recentering is now available through the CHAS software and the recentered sample is re-run through TuScan (described above) to provide integer copy number.

The new RC.OSCHP files can be viewed in ChAS or the BioDiscovery software, Nexus.

To manually recenter samples, an offset (median log2 ratio) is provided that tells the algorithm how much a sample should be pushed up (positive value) or pushed down (negative value) so that this region resides at the log 2 ratio = 0, indicative of normal diploid.

In the example below (Figure C.2) the sample should be centered at chromosome 4q. The median log ratio on 4q is -0.17, therefore the manual recentering adjustment would be given this offset value, resulting in an increment adjustment of 0.17 for all log ratios.

Figure C.2

Appendix D

Appendix D: Copy Number Effect on Somatic Mutations

Somatic mutation probesets in the OncoScan FFPE Assay are designed to selectively respond to the presence of mutation sequences. However, large copy number amplifications spanning the somatic mutation targets can sometimes lead to falsely reporting the presence of mutations in amplified regions.

If the copy number state is greater than ~15, you may observe false positive somatic mutation calls. The only region for which we have observed this problem is the EGFR gene, which is prone to very high copy number in certain cancer types.

In the example below (Figure D.1), the predicted copy number state for the EGFR gene is greater than 30, which affects the somatic mutation score. Another side effect shown in the example below is that three mutations are called in the high Copy Number region, a contradictory event for at least two of these mutations.

Figure D.1