CopyCaller® Software v2.0

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Overview

This CopyCaller® Software v2.0 Quick Reference Card contains abridged procedures for using CopyCaller® Software. For the complete procedures, theory of operation, or for safety and biohazard guidelines, refer to the *CopyCaller® Software v2.0 User Guide* (Part no. 4400042). For information on performing and preparing TaqMan® Copy Number Assay experiments for use with the software, refer to the *TaqMan® Copy Number Assay Protocol* (Part no. 4397425).

Prepare the data using the real-time PCR system software

1 **Analyze the results**

For each TaqMan® Copy Number absolute quantitation experiment that you want to include in the copy number analysis, analyze the real-time PCR data using a manual C_T threshold of 0.2 and automatic baseline.

2 **Export the results**

Assays per plate	Did you specify the placement of the assay(s) using separate targets/ detectors?	Action
One	Not applicable	Export the real-time data to a tab-delimited text (.txt) or comma-separated values (.csv) exported file.
More than one	Yes	Export the real-time PCR data to a single exported file (.csv or .txt) that includes all wells of the plate. CopyCaller® Software uses the target/detector names to distinguish the data from the different assays.
	No	Export the real-time PCR data of each assay to a separate exported file. 1. Select the wells of the plate that contain the data from one of the TaqMan® Copy Number assays. 2. Select **File ▸ Export**, then export the data from the selected wells to an data file. 3. Repeat steps 1 and 2 to export the data from the other assays present on the plate.

Perform the copy number analysis

1 **Import the results files**

a. Start CopyCaller® Software.

b. In the CopyCaller® Software toolbar, click  (Import real-time PCR results file) or select **File ▸ Import**.

c. In the Import dialog box, select one or more real-time PCR files to analyze.

d. Click **Open** to import the data from the selected real-time PCR results.

e. Repeat steps b to d as needed to add additional files to the analysis.

2 **Select and analyze the assay data**

a. In the Assay Selection Table, select one or more assays to analyze. To select an assay, click anywhere in a row of the Assay Selection Table (the software highlights the selected row in blue).

b. In the toolbar, click  (Analysis Settings).

c. In the Analysis Settings dialog box, Calibrator selection panel: specify the calibrator sample settings for the selected assay(s) depending on whether or not a calibrator sample of known copy number is available.

Calibrator present?	Action
Yes	1. Select **With Calibrator Sample**. 2. In the Calibrator Sample Name drop-down list, select or enter a sample to use as the calibrator for the analysis. 3. In the Calibrator Sample Copy Number field, enter the number of copies of the target sequence that are in the calibrator sample. The number of copies must be a whole number greater than zero.
No	1. Select **Without Calibrator Sample**. 2. In the Most Frequent Sample Copy Number field, enter the number of copies of the target sequence expected in the majority of samples. The number of copies must be a whole number greater than zero.

d. (*Optional*) Expand the Advanced settings box to review and/or edit empirical thresholds, or to create copy number bins for confidence estimates:

Exclude wells with VIC C_T greater than	Enter a cycle number to specify the threshold above which CopyCaller® Software excludes samples from the analysis. We recommend a default VIC threshold value of 32.
Zero copy ΔC_T threshold	Enter a ΔC_T value to specify the threshold above which CopyCaller® Software will classify samples as zero copy samples. We recommend a default ΔC_T threshold value of 4.0.
Use copy number bins to estimate confidence	1. Select the number of copy number bins to use for the data analysis (from 2-10). 2. Enter sequential single numbers or range of numbers in each bin box; the final '>=' box must contain a single number value.

e. Click **Apply** to apply the analysis settings and perform copy number analysis using the revised analysis settings.

f. Repeat steps a through e as necessary to analyze any remaining assays.

g. Display the results of the analysis:

1. In the Assay Selection Table, verify that the assays that you want to add to the analysis show "Y" in the (Analysis Status) column. If not, analyze the unanalyzed assay(s) as explained above.
2. In the (Display Analysis Results) column, select the check boxes for up to 10 analyzed assays that you want to display.

Review the results

1 Review the Copy Number Plot

- Samples should have calculated copy number values close to integers and small range bars.
- Review the plot for intermediate copy numbers. The presence of intermediate, calculated copy number values (such as 1.5) can indicate that the calibrator or copy number was specified incorrectly, or a potential problem exists with the associated test sample or calibrator sample
- Review the copy number range bars for each sample. Large bars may indicate that the technical replicates of the associated sample exhibit a broad range of ΔC_T values, possibly indicating that sample data quality is suboptimal

  Note: The copy number range of replicates is frequently larger for samples that have high target copy numbers (>3) as a result of their smaller ΔC_T values.

2 Review the Results Table

- Examine the confidence values and absolute z-score values to assess the reliability of each copy number call.
- Review samples having a predicted copy number of "Undetermined."

A sample is "Undetermined" if CopyCaller® Software cannot analyze the sample because the:

- Reference assay did not amplify sufficiently, possibly indicating low sample quality.
- Replicate data for a sample were conflicting.

- Review samples having a predicted copy number of 0 (zero-copy samples).

  Zero-copy samples produce reference assay amplification (passing VIC) and weak or nonexistent target amplification (generating the NOFAM or DCTET QC flags respectively).

  Note: CopyCaller® Software cannot calculate confidence values for zero-copy-number samples. However, samples that produce no FAM signal are, by definition, high-confidence calls because no target DNA was amplified.

- Review samples having a predicted copy number that is greater than or equal to 1.

  CopyCaller® Software calculates confidence and absolute z-score values for each sample set that has a non-zero predicted copy number value and sufficient data for the estimation.

  Note: The software cannot calculate the confidence and absolute z-score values for sample sets that have fewer than seven samples of a single copy number because the algorithm requires a minimum number of data points.

- Under optimal experimental conditions where samples are of high quality, copy number and reference assays have amplified, and sample replicates have similar C_T and ΔC_T values:
  - Samples that have low copy numbers (1, 2, or 3) commonly have confidence values greater than 95%.
  - As copy numbers increase, confidence progressively decreases due to the decreased separation of ΔC_T subdistribution values of copy numbers.
  - Review samples that have confidence values greater than 95%.

    Samples that have high confidence values can sometimes deviate significantly from the mean copy number for the copy number subdistribution. For sample copy number calls with confidence values greater than 95%, look at the absolute z-scores, then consider accepting or rejecting the copy number call based on the following:

|Z-Score|	Status
< 1.75	Pass
2.65 > *z* ≥ 1.75	Pass with caution
≥ 2.65	Fail

    Note: The thresholds in the table above are based on empirical observations and are provided only as guidelines.

3 (*Optional*) Edit the analysis settings

a. Select one or more assays in the Assay Selection Table, then click (View Analysis Settings) in the toolbar.

b. Revise the analysis settings as needed.

   For example, if the quality of the calibrator data is poor, you can select a different calibrator sample and reanalyze the data.

c. Click **Apply** to perform the copy number analysis using the revised analysis settings.

4 Guidelines for reviewing the Welll Table

Review the Well Table: For each replicate group, review the Flag column for any quality flags generated during the analysis, and determine the source of the warning.

Some quality flags indicate potential issues with wells or samples. For example, wells that generate NOVIC or VICET flags did not amplify the reference assay target properly and may contain low-quantity or poor-quality DNA.

5 (*Optional*) Remove outliers and reanalyze

a. Select the **Well Table** tab.

b. Locate the well(s) that you want to omit from the copy number analysis.

c. For each outlier well, select its corresponding check box in the Omit column.

   Note: After omitting a well, the analysis status of the associated assay in the Assay Selection Table changes from "Y" to "R," indicating that the assay must be reanalyzed.

d. Click ▶ (Analyze) to perform copy number analysis for the assay without using the data from the outlier well(s).

For guidelines on identifying potential outliers, see the *CopyCaller Software v2.0 User Guide* (Part no. 4400042).

6 Review the Analysis Summary

Review the Analysis Summary tab for the summarized results of the copy number analysis. While viewing the summary, you can copy and paste the data into other applications.

7 Review the Statistics Chart

Review the Statistics Chart tab for information about the distribution of copy numbers among the samples in a copy number assay. While viewing the Statistics Chart, you can copy, save, or print it.

8 Review the ΔC_T Plot

Review the ΔC_T Plot tab for information about the distribution of sample ΔC_T values in a copy number assay experiment. While viewing the ΔC_T Plot, you can copy, save, or print it.

Save and export the analysis

1 Save the results

a. Click (Save analysis) or select **File** ▸ **Save As**.

b. If you selected Save As, select the folder to save files in, and optionally edit the ***CopyCaller*® Analysis** file name (keep the .cnv extension).

c. Click **Save**.

2 Export the results to a CopyCaller® file

CopyCaller® Software can export the analyzed results table for all assays selected in the Assay Selection table to Microsoft® Excel® or text files.

To export the analysis results:

a. Click (Export) or select **File** ▸ **Export**.

b. Select the data to export:

Copy Number Results	Saves only the processed data in the Results Table.
Copy Number Results with C_T Data	Saves the processed data in the Results Table plus the C_T Data from the Well Table tab.

c. In the **Export results in** drop-down list, select the file type: a Microsoft Excel file (.xls), comma-separated values file (.csv), or tab-delimited text file (.txt).

d. In the **Export file name** field, edit the file name and browse for a location to save the results.

e. In the **Copy Number Results** section, edit the worksheet name or file name.

f. Click **Export**.

3 Open a saved analysis with CopyCaller® Software

After saving an analysis as a CopyCaller Analysis file, you can resume the saved analysis by opening the (.cnv) file: click (Open) or click **File** ▸ **Open**, select the file in the Open dialog box, then click **Open**.

Note: If you open a CopyCaller® file that contains the analyzed results of multiple assays, CopyCaller® Software displays the individual analyzed assays in the Assay Selection Table.

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.lifetechnologies.com/support

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