



Nucleics

QualTraceTM

User manual

Version 1.2.0

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QualTrace Software

Overview

The QualTrace™ software analyses ABI trace files produced by the ABI3730 and 3730xl DNA sequencers for common sample preparation problems that limit sequence read length. The software generates a logfile reporting the classification of each trace, together with an estimate of the signal to noise ratio at the early, mid, and late trace regions.

The QualTrace software can process either single trace files or a folder of trace files. If a user specifies an input folder then all ABI files found within that folder are processed. Trace files contained within sub-folders can be processed if the `-r` (recursion) option is selected.

The QualTrace logfile contains a record of the analysis process in tab delimited format. The logfile contains the following fields: `QT DATE` (QualTrace run time); `FILE` (trace file name); `QT CLASS` (QualTrace classification of the trace file); `QT CODE` (QualTrace classification number); `MACHINE` (ABI3730 machine name); `RUN` (ABI run name); `RUN DATE` (ABI run time); `TRAY WELL` (Sample plate well); `CAPILLARY` (ABI3730 capillary number); `DYE SIGNAL` (likelihood of dye peaks or dye blobs); `EARLY SIGNAL` (average signal to noise value from the early region of trace); `MID SIGNAL` (average signal to noise value from the middle region of the trace); and `LATE SIGNAL` (average signal to noise value from the late region of trace).

If a QualTrace logfile already exists then the new run messages are appended to the existing logfile. The default logfile name is `qt.log`, but a user may change this name using the `-l` option. The `-nl` option prevents the QualTrace software from writing to the logfile. At the end of each analysis run a summary table is written to the logfile detailing the percentage and total counts of traces that fall into each classifications.

A estimate of the likelihood of dye peaks or dye blobs being present in the trace is reported in the `DYE SIGNAL` field. Values of less than 1 suggest that no dye blob is present, with values between 1 and 5 that a moderate dye blob problem exists, and values above 5 indicating that the trace has very large dye blob present. As a general rule the higher the `DYE SIGNAL` value the worse the dye blob problem is.

Running QualTrace

To use QualTrace, open a Windows command shell and run `QualTrace.exe` using the desired command line parameters and options. To open a command shell select **RUN** from the Windows Start Menu and type `cmd` then press the **OK** button. An example of QualTrace's parameters is shown below:

```
QualTrace.exe -i C:\inputfolder -l C:\my documents\userlog.log -j 2
```

Command line switches and parameters

help -h

Provides the help information for the QualTrace software.

version -v

Provides the version number of the QualTrace software.

input -i [file name|folder name]

Specifies the input file or folder. If a folder is specified then all the ab1 trace files within the folder are processed. Traces contained within subfolders are not processed.

log name -l [logfile name]

Specifies the name of the processing status logfile. This log is always appended to rather than written over. New entries are always appended to the existing logfile.

The default logfile name is `qt.log`, however, both the log name and path can be user defined.

e.g. UNIX: -l /usr/mylogs/userlog.log

 Windows: -l c:\my documents\userlog.log

no log -nl

Disables all writing to the logfile.

Default is OFF.

recursion -r

Search all sub-folders found within the input folder for ab1 trace files. Once the search is completed, QualTrace process all files as a single job and a single analysis summary. The sub-folder search does not follow Windows Shortcuts, nor symbolic links in Unix.

multi-processor -j [integer]

Allows the QualTrace software to run multiple sub-processes. This allows multiple traces from an input folder to be process in parallel. This setting is of utility on multi-processor machines or cluster arrays. On single processor machines it is not recommended that more than one sub-process be used.

Default is ONE.

QualTrace analysis codes

Good 0

Indicates that no problems were identified. Alignable Q20+ bases are usually high to very high.

No signal 1

Indicates that the trace contains very weak or no signal (failed reaction or capillary). Many causes including: no template; loss of sample during clean-up; blocked capillary; or sample contamination with salt. Alignable Q20+ bases are usually very low.

Late Signal Start 2

Indicates that the beginning of the trace peaks is delayed. This test checks whether the start of signal (base 0) has begun later than the normal expected time for base 150. This problem is commonly associated with sample contamination with high levels of salt, protein or template DNA. Alignable Q20+ bases are low to moderate.

Spectral calibration 3

Indicates that the trace file contains significant dye crosstalk is present (>15% in at least one channel). Frequent occurrence of this code indicates that a machine spectral calibration may need to be performed. Alignable Q20+ bases are usually moderate to high depending on the severity of the crosstalk.

Early mixed signal 4

Indicates that the trace contains significant mixed signal before base 350. Commonly caused by primer mis-annealing during sequencing or template amplification. Alignable Q20+ bases are moderate.

Late mixed signal 5

Indicates that the trace contains significant mixed signal after base 350. The usual cause is two of more templates present in the reaction. Alignable Q20+ bases are usually low.

Noisy signal 6

Indicates the trace is weak with poorly resolved and/or mixed signal. Many causes including: incorrect template concentration; excessive sequencing chemistry dilution; machine service

QualTrace errors

The following errors may occur when attempting to process trace files. The various possible causes of these errors and their corresponding code are described together with possible solutions.

Unlicensed machine 20

This error will occur if the machine that produced the trace file has not been licensed for use with QualTrace. Please contact Nucleics to obtain a license for the machine.

Not enough memory 21

This error may occur if too many applications are running. Close nonessential applications and restart QualTrace.

Can't open file 22

This error may occur if the file does not exist or if the file has incorrect access permission settings. Check if file exists and that you read permission to the input folder.

Can't create file 23

This error may occur if you do not have write permission to the selected output folder or if the hard disk is full. If the hard disk is near full please remove excess files and restart QualTrace.

Can't read file 24

This error may occur if an open file was deleted or moved while being used by QualTrace. Do not delete or move trace files after starting QualTrace.

Can't write file 25

This error may occur if you do not have write permission to the selected output folder or if the hard disk is full. Please remove excess files and restart QualTrace and check if you have write permission to the folder selected.

Invalid file 26

This error may occur if the trace file is corrupt. Remove trace from process list.

No raw data in file 29

This error may occur if the trace file is corrupt. Remove trace from process list.

No processed data in file 10

This error may occur if the trace file is corrupt. Remove trace from process list.

No base order in file 11

This error may occur if the trace file is corrupt. Remove trace from process list.

Unsupported machine 32

This error may occur if processing a trace file produced by a sequencing machine type that QualTrace does not support or if the trace file is corrupt. Remove the trace from process list.

Process was aborted 39

This error will occur if a QualTrace sub-process is stopped explicitly. Do not stop the QualTrace sub-processes.

Processing took too long 40

This error occurs if the trace cannot be analysed within one minute. This error indicates a program malfunction. Please contact Nucleics.

Program error 42

This error indicates a program malfunction. Please contact Nucleics.

Technical Support

Nucleics provides expert technical support for the QualTrace™ software.

Please address any questions or comments to: qualtrace@nucleics.com