CounterTrace II Quick Start Guide

Step 1: Installing the CounterTrace software

a. Create a new folder called **CounterTraceII** on your computer.

b. Copy the **CounterTrace.exe** and **ctw.chm** files to the **CounterTraceII** folder.

Step 2: CounterTraceII Spectral Calibration (see the software online-help for more details):

a. Add 3µl of 1000x *CounterTrace Loading Additive* to 997µl of sterile water (3x final conc.)

b. Aliquot 10µl into each well of a 96-well plate, or one quadrant of a 384-well plate. For 48-capillary array ABI3730 use only 48 position wells.

c. Run the plate on an ABI 3730 sequencer using either one of the following modules and run times:

StdSeq36_POP7 module with a run time of **2800** seconds (36cm capillary arrays).

LongSeq50_POP7 module with a run time of **6100** seconds (50cm capillary array).

d. Once the plate run has completed, choose "CounterTrace Spectral Calibration" from the "Process" menu of the CounterTrace software and select the folder containing the calibration (standard only) traces and press "OK". A CounterTrace spectral calibration file called **machines.ctc** will be created after approximately 30 seconds in the **CounterTraceII folder.**

Step 3: Determining the optimal CounterTrace Loading Additive concentration:

a. Perform 30 standard sequencing reactions using a control DNA template (e.g. pGEM-3Z). Clean the reactions using your normal clean-up protocol.

b. For each 10µl of cleaned sequencing reaction add 2µl of *CounterTrace Loading Additive* (CLA) diluted to one of the following concentrations: 6x (1µl CLA + 167µl H_20), 12x (2µl CLA + 166µl H_20), 18x (3µl CLA + 165µl H_20), 24x (4µl CLA + 164µl H_20) and 30x (5µl CLA + 163µl H_20). With each CLA concentration make six reaction replicates (i.e. six of the 6x, 12x, 18x, 24x and 30x dilutions).

c. Run the sequencing reactions using the sequencer run module and times used in Step 2.

d. Once the run has finished, choose "Add Trace Folder" from the "File" menu and select the run folder containing the 30 trace files. Choose the output folder from the "File" menu. Click "Start" in the "Process" menu to process the trace files.

e. After the traces are all processed, look at the status panel and identify the lowest *CounterTrace Loading Additive* concentration at which the fewest "Can't find standard" errors occur. This concentration is the optimal *CounterTrace Loading Additive* concentration for your samples. For example, if 18x, 24x and 30x all gave the lowest number of errors then the 18x concentration is optimal.

Step 4: Running CounterTraceII on your samples

a. Add the optimal *CounterTrace Loading Additive* concentration as determined in Step 3 to your cleaned production sequencing samples and run on the sequencer using the run module and time used in Step 2.

b. Process the trace files with the CounterTrace software as described in the *CounterTraceII help*. Compare the sequencing files before (run folder) and after (output folder) processing to determine the improvement in read-length and sequence quality provide by CounterTraceII.

Further information can be obtained from the CounterTrace software online-help or by contacting Nucleics at info@nucleics.com