

# CounterTrace Phred Guide

## **Introduction**

The CounterTrace software includes a basic base calling module, however, for those users that wish to obtain trace quality scores we recommend the use of the external base caller **phred**. The CounterTrace software processing has been optimised to provide correctly calibrated phred quality scores with traces run on the ABI 3700 and 3730 instruments. One complicating factor with the use of phred is the large number of versions in use as each version gives significantly different base and quality scores, even when run on identical traces. Therefore, if you wish to obtain accurate quality scores we highly recommend that you **ONLY** use **phred version 0.020425.c**. THE USE OF OTHER PHRED VERSIONS WILL RESULT IN UNRELIABLE PHRED QUALITY SCORE ASSIGNMENTS.

To use phred with CounterTrace please use the following steps:

## **Step 1: Process trace files using CounterTrace**

For details on how to process trace files using CounterTrace please see the *CounterTrace Command line manual*, the graphical version *CounterTrace online-help* and the *CounterTrace Quick Start Guide*.

## **Step 2: Modification of phred parameter file**

The CounterTrace software modifies the *.mob* record in each trace if it has been successfully processed. No change is made if the trace is not processed. The new *.mob* record has a "CT\_" prefix added for both the *.abl* and *.scf* output formats (eg. *DT3730POP7{BDv3}.mob* becomes *CT\_DT3730POP7{BDv3}.mob*). This change enables the use of a custom entry in the phred parameter file (*phredpar.dat*).

- a. To add a new record to the *phredpar.dat* file, open the file with a text editor and add a new line for each sequencing chemistry used by your facility with the following format:

CT_[mob type].mob	[chemistry type]	[chemistry used]	MolDyn_MegaBACE
eg. CT_DT3730POP7{BDv3}.mob	terminator	big-dye	MolDyn_MegaBACE

- b. Save the *phredpar.dat* file.

The phred MegaBACE lookup table produces the most accurate agreement between predicted and observed quality scores for CounterTrace processed traces. However, it is **ESSENTIAL** that the latest version of phred is used (0.020425.c), as the quality scores **DO NOT** map accurately with older phred versions.

## **Step 3: Run phred**

For further information on how to use phred, please see the phred help file or visit the phred website <http://www.phrap.org/phrap.docs/phred.html>

Further information can be obtained from the CounterTrace software online-help or by contacting Nucleics ([info@nucleics.com](mailto:info@nucleics.com)).