CounterTrace

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CounterTrace™ is a novel DNA sequencing software and reagent designed to significantly improve the read length and overall quality of DNA sequencing reactions. The CounterTrace DNA sequencing reagent offers the following features –

- *Up to 25% more phred Q20 bases*
- *Compatibility with the ABI 3700 and 3730 DNA sequencing instruments*
- *Easy to use in both large and small DNA sequencing facilities*

The CounterTrace sequencing system

Current state of the art capillary DNA sequencer can produce reads that exceed 1000 bases. However, these long reads typically suffer from excessive peak broadening and poor resolution in the later part of the trace. This latter region is associated with low quality scores and high base call error rates.

CounterTrace uses an internal calibration standard to allow accurate measurement and processing of each sequencing trace (Figure 1). The CounterTrace additive contains DNA fragments of known size labelled with a proprietary fluorophore (CounterTrace standard). This fluorophore has been designed to enable its specific detection in four channel sequence data. Addition of the CounterTrace standard to each sequencing reaction after clean-up provides unique 'landmark' sizing peaks in the raw trace data. The CounterTrace DNA sequencing software uses the location of the standard peaks to enable accurate processing of the trace data. After removal of the standard peaks from the trace the CounterTrace DNA sequencing software output sequencing trace files in either abi or scf format. These trace file are base- and quality-called using any user defined software (e.g. Phred, LifeTrace).

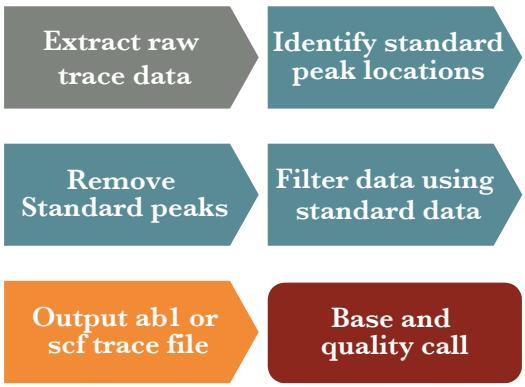

Figure 1. Overview of the CounterTrace process.

Feature and benefits of CounterTrace

Easy implementation

The CounterTrace DNA sequencing system is extremely simple to implement into any DNA sequencing facility. The CounterTrace additive is diluted to the appropriate concentration in the sequencer loading solution used by the facility (e.g. water, 50 µM EDTA, or formamide), and the sequencing sample processed as normal. After collection of the trace data, the CounterTrace software is used to reprocess the traces before base and quality calling with an external sequencing software package (e.g. phred). The CounterTrace software includes an automation feature that allows seamless integration into existing data pipelines.

CounterTrace is compatible with all common sequencing reagents and has been adopted for the

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common DNA sequencer models ABI3700 and ABI 3730 for both the 36 and 50 cm capillary array lengths.

Increased trace read length

The CounterTrace DNA sequencing software allows poorly resolved regions towards the end of the sequencing trace to be accurately processed, thus provides the user with significantly longer and higher quality reads (Figure 2).

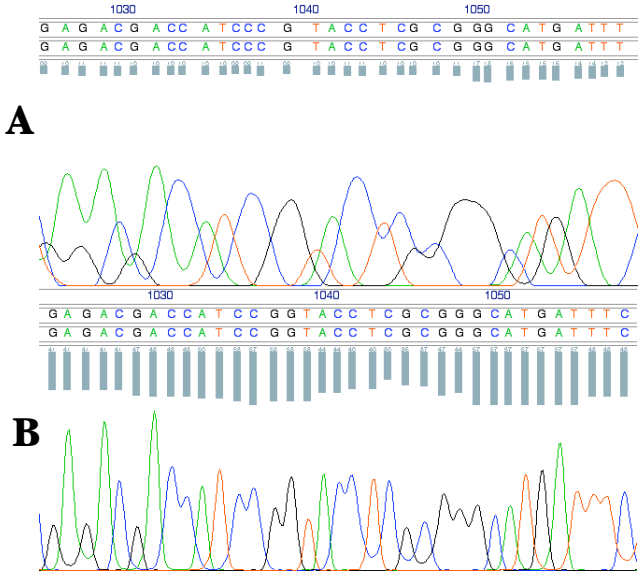

Figure 2. PHRED derived base and quality calls from an ABI3730 50 cm capillary array DNA sequence trace. **A.** Before CounterTrace processing. **B.** After CounterTrace processing.

CounterTrace offers increases in phred Q20+ read lengths of up to 45% for individual traces, with an average increase of 20% in high throughput production environments (Figure 3).

Increased DNA sequencing throughput

CounterTrace processed DNA sequencing traces run on the ABI 3730xl 36cm capillaries provided read lengths previously observed only with 50cm arrays (Figure 3). As these short-array runs take only half the time required for the longer arrays, the throughput can be effectively doubled with CounterTrace without the capital cost involved in purchasing additional instruments.

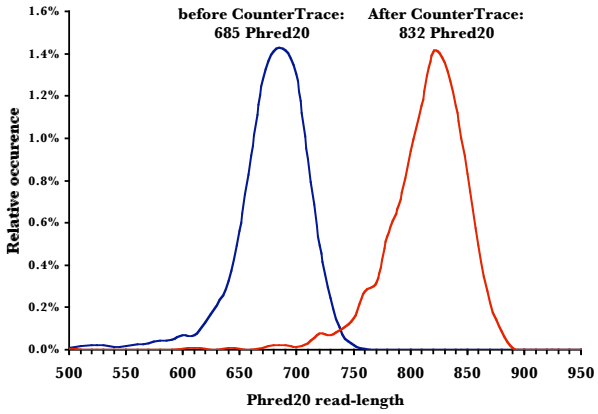

Figure 3. Distribution of phred20 read lengths before and after CounterTrace processing. Results from 3840 traces analysed on a ABI3730xl 36 cm capillary array.

The improved read lengths provided by CounterTrace increases the throughput and lowers the per-base cost. In addition, these longer reads allow for DNA sequencing projects (eg. whole genome, cDNA clones) to be finished at lower coverage levels, offering further cost and time savings.

For further information on the CounterTrace DNA sequencing system please contact:

Nucleics Pty Ltd
12 Mill St
Bendigo VIC 3550
Australia

Ph: 61 3 5444 7361 Fax: 61 3 5444 7476
www.nucleics.com info@nucleics.com