Nucleics

dLUTE SEQ

dLUTE SEQ™ is an unique DNA sequencing reagent developed by Nucleics that allows nanolitre-scale DNA sequencing reactions to be performed using microlitre-scale equipment and protocols. dLUTE SEQ provides the following benefits over existing approaches to reduce sequencing premix chemistry usage:

- *Reactions require as little as 25 nanolitres of BigDye™ DNA sequencing premix reagent (equivalent to a 1:320 dilution)*
- *Reaction substrates are maintained at the optimum concentration resulting in longer reads and more robust DNA sequencing*
- *Avoidance of high precision liquid handling equipment*

dLUTE SEQ DNA sequencing reagent

Ultra-sensitive DNA sequencers, such as the Applied Biosystems 3700 & 3730, and the Amersham Biosciences MegaBACE 1000 & 4000, require only a tiny fraction of the labelled product created by full scale DNA sequencing reactions. Consequently, many DNA sequence facilities have focused on reducing costs by reducing the reaction volumes and/or by using dilutions of the premix reagent. Using this approach, many DNA sequencing facilities have been able limit the amount of DNA sequencing premix chemistry required to as little as 1/320th of that required for a full scale reaction.

While these approaches have yielded very significant cost savings, precision limitations in the equipment used to set up the reactions (especially robotics) has prevented precise matching of the reaction scale to the sensitivity of the DNA sequencing instruments. Furthermore, the use of very high reagent dilutions has a significant negative impact on the read length and success rate of the enzymatic sequencing reaction due to substrate limitations. Because of these problems, sequence reactions are currently performed at scales far in excess of the true sensitivity of modern DNA sequencing instruments.

The dLUTE SEQ DNA sequencing reagent avoids these problems by the use of a novel two-state formulation (Figure 1). In the first state (set-up), the aqueous sequencing reagents are dispersed in a large volume of the inert component of the dLUTE formulation. This effectively dilutes the aqueous reagents and enables pipetting with standard equipment. The dLUTE SEQ DNA sequencing reaction is then induced by heat to enter the second (reaction) state. In this state, the aqueous phase separates from the inert phase forming a small "bubble" (in which the DNA sequencing reaction takes place) overlain by the inert phase.

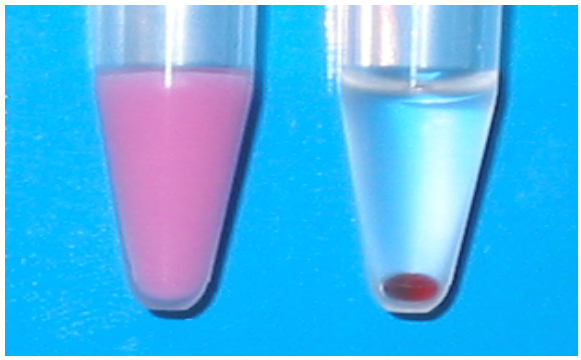

Figure 1. The two phase states of dLUTE SEQ. **Left.** Set-up state. The aqueous phase (red) is dispersed throughout the inert phase. **Right.** Reaction state. The aqueous reaction-phase (red) has separated from the inert phase (clear).

Feature and benefits of dLUTE SEQ

Improved sequencing data

The small volume of a dLUTE SEQ reaction provides true scaled reaction conditions (i.e. the

Nucleics

concentration of the sequencing chemistry is the same in both an undiluted standard reaction and a dLUTE SEQ reaction). The dLUTE SEQ mix maintains the sequencing DNA polymerase with the optimal nucleotide and dye labelled terminator concentrations. This avoids the "ski-slope" effect (i.e. rapid fall off in signal strength) observed with excessive chemistry dilutions (Figure 2A). The dramatic increase in large DNA sequencing product signal can be seen with the dLUTE SEQ reactions, even when using only one quarter (0.1µl) the amount of sequencing chemistry (Figure 2B).

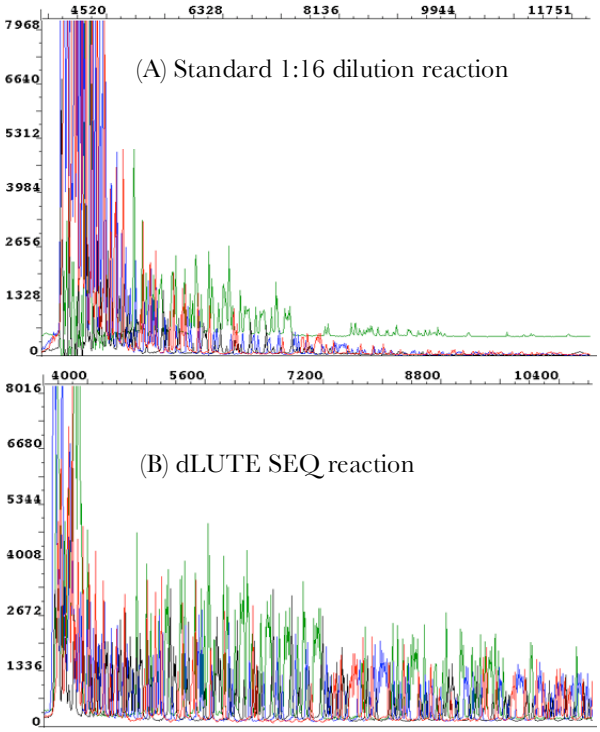

Figure 2. Raw sequencing traces of **(A)** a diluted standard and **(B)** dLUTE SEQ DNA sequencing reaction with 0.1µl of BigDyev3.1 in a total volume of 2.5µl (0.25µl aqueous). Both reactions were purified by alcohol precipitation and analysed on an ABI 3700 DNA sequencer.

The dLUTE SEQ sequencing reagent allows sequencing reactions to be performed in volumes far below the accuracy of the conventional pipetting equipment. For example, an effective 0.25µl sequencing reaction (using 0.1µl of BigDye premix) can be created using an easily handled 2.5µl volume of dLUTE SEQ. In addition, modest DNA sequencing chemistry dilutions can be used enabling as the use of as little as 25 nanolitres of BigDye per reaction.

Ease of implementation

The implementation of dLUTE SEQ into existing production pipelines is straightforward and demands only minor modification to existing protocols. The DNA sequencing chemistry and oligo primer are simply mixed with the dLUTE SEQ reagent to form a stable homogenous, mixture. This mix is aliquoted onto the DNA sequencing template using standard microlitre-scale robotic equipment. In the subsequent thermocycling a temperature induced separation of the two phases occurs and the DNA sequencing reaction proceeds in the small aqueous volume. The inert phase overlays the small reaction volume and prevents sample evaporation. Finally, the DNA sequencing reaction is cleaned using any of a number of compatible methods including SPRI beads, size-exclusion spin columns or plates, or via a simple modified alcohol precipitation protocol.

For further information on the dLUTE SEQ DNA sequencing reagent 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