

UniSeq

UniSeq™ is a universal DNA sequencing technology developed by Nucleics. The UniSeq DNA sequencing system offers the following advantages over current methodologies:

- Provides a fast and cost effective primer walking approach
- Simple and robust
- Compatible with all Sanger DNA sequencing technologies and equipment
- Fully adaptable to high-throughput, large-scale DNA sequencing environments

The UniSeq system

The UniSeq DNA sequencing system utilises a unique methodology to reliably create template specific DNA sequencing primers.

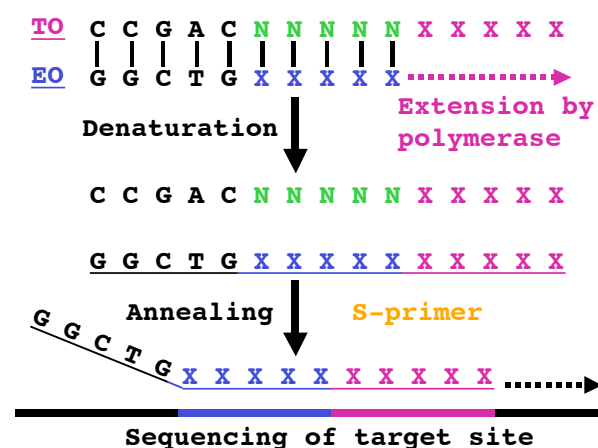


Figure 1. Generation of template specific UniSeq S-primer. The EO hybridises to the TO and gets extended to form the S-primer. N: degenerated oligonucleotide positions. X: specific positions of variable nucleotide value.

The process of generating each UniSeq primer involves the addition of an “E”- and “T”-oligonucleotide, together with a specifically formulated additive mixture, directly to the sequencing

reaction. These oligonucleotides (termed EO and TO) hybridise during the sequencing reaction to produce the template specific primer (S-primer) (Figure 1).

By selecting specific EO and TO oligonucleotides from a small, pre-synthesised library of 768 oligonucleotides, over 131,000 different, template specific primers can be created. This simple combinatorial effect forms the basis for the high specificity and universal applicability of the UniSeq system in DNA sequencing.

The UniSeq system is fully compatible with all common DNA sequencing reagents (e.g. BigDye™ from Applied Biosystems or DYEnamic™ from Amersham Biosciences) and gives excellent results using all modern DNA sequencers (Figure 2).

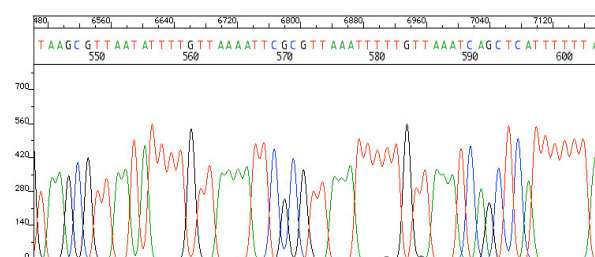


Figure 2. UniSeq reaction sequencing trace. Two-hundred ng of plasmid DNA was sequenced with BigDye™vers.3 and UniSeq primers EO#18 and TO#447. The reaction was purified by ethanol precipitation and analysed on an ABI3700 DNA sequencer.

The UniSelect™ DNA sequencing software is provided for the automated and optimised selection of the optimal EO & TO primer pairs for each DNA template. The UniSelect software also supplies an automated interface to control the robotic pipetting of the UniSeq oligonucleotides,

DNA templates and other required sequencing reagents.

To aid the finishing of Whole Genome Sequencing (WGS) projects, an additional software package (UniFinish™) is available. UniFinish is able to parse ACE format assembly files and select the EO/TO oligonucleotides, together with the appropriate DNA templates, required to close non-physical DNA sequence gaps in the genome assembly.

Features and benefits of UniSeq

Time savings

UniSeq™ provides an extremely competitive alternative to DNA sequencing with custom synthesised oligonucleotides. While many advances have been made in the automation of oligonucleotide synthesis, this process is still complex (eg. requiring high-maintenance machinery) and slow (several hours per synthesis). These limitations have become extremely critical for high throughput DNA sequencing facilities where modern capillary DNA sequencers have reduced the separation time to 1–2 hours. The generation of specific DNA sequencing primers by UniSeq during the DNA sequencing reaction step (i.e. no time-costs) breaks the current bottleneck imposed by custom oligonucleotide synthesis and allows for the most efficient utilisation of this DNA sequencing capacity.

Cost savings

Currently, two major strategies are used in WGS – Random Shotgun DNA Sequencing (RSS) and Primer Walking DNA Sequencing (PWS). Most WGS projects currently employ RSS, especially in

early stages of projects. However, RSS requires a large amount of redundant DNA sequence data (6–15 times the genome size) for assembly. The alternative PWS strategy requires relatively little redundant DNA sequence data, however, it requires a large numbers of custom made oligonucleotide primers. The high costs (US\$3 – 5 per primer) and required synthesis have prevented the general adoption of PWS strategy in WGS Genomics projects.

UniSeq provides the advantages of both WGS strategies. It offers the speed and simplicity of the RSS approach, while providing the data efficiency inherent with the PWS approach. Computer simulations and limited trials have shown that UniSeq DNA sequencing system offers cost and time savings of greater than 80% over current WGS approaches.

Flexibility

Nucleics has extensively tested UniSeq in-house in a number of general DNA sequencing and WGS projects. In addition, Nucleics has formulated novel strategies for the easy and smooth implementation of UniSeq into industrial scale DNA sequencing facilities.

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