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DNA SEQUENCING ON A LI-COR MODEL 4300L DNA ANALYZER USING ATTO-TEC AND DYOMICS DYE-MODIFIED PROBES

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DESCRIPTION

DNA sequencing is a powerful and indispensible technique in basic biological research and widely spread in applied sciences. Several DNA sequencing methods are established but dideoxy-sequencing, a chain-termination based method developed by Sanger, is the most frequently used technique. Dideoxy-sequencing utilizes 2',3'-dideoxynucleotide triphosphates (ddNTPs), molecules that terminate DNA chain elongation. Chain termination occurs because ddNTPs lack a 3'-OH group necessary for the elongation of the synthesized DNA strand by DNA polymerase.

The LICOR Model 4300L DNA Analyzer separates and detects chain-terminated DNA fragments in which the DNA polymerase incorporated either fluorescently labeled nucleotides or primers. This method is easy to use, cheap, and results in reliable DNA sequence information.

AIM

The aim of this Application Note is to evaluate the performance of the fluorescent dyes DY-782, DY-682, and ATTO 700 as alternative labels for oligonucleotides that can be used in DNA sequencing applications. The performance of DY-782, DY-682, and ATTO 700 labels is directly compared to the performance of established IRDye® 800 and IRDye® 700 labels on a 4300L DNA Analyzer.

APPLICATION/METHOD

Numerous DNA sequencing kits are commercially available, many of them optimized for automated DNA sequencing. These kits are based on modified DNA polymerases which accept dNTPs and ddNTPs. The DNA sequencing kit used here is the DNA Cycle Sequencing Kit (Jena Bioscience GmbH, Germany). All reactions were set up as recommended in the kit manual. 200 fmol of a standard DNA plasmid with a 1 kb insert was used as the template for each sequencing reaction and 2 pmol of fluorescently labeled SP6 and/or T7 oligonucleotides were used as sequencing primers.

Separation and detection of labeled DNA fragments were performed on a 4300L DNA Analyzer (LI-COR Biosciences, USA) using gels with a length of 41 cm and a thickness of 0.2 mm. SequaGel® XR (National Diagnostics, USA) was used as separation matrix.

RESULT

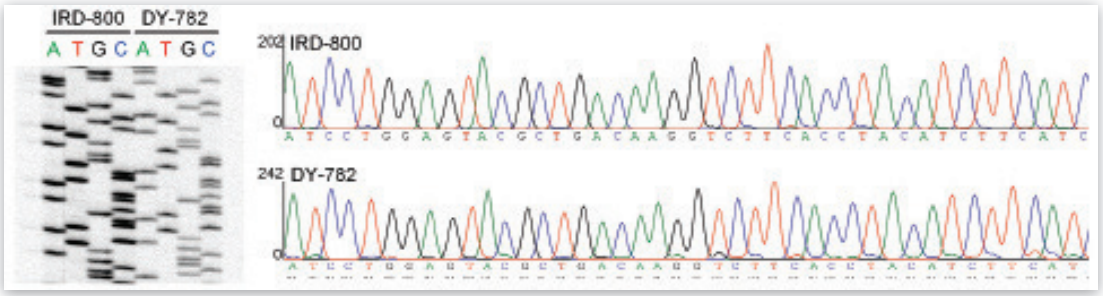
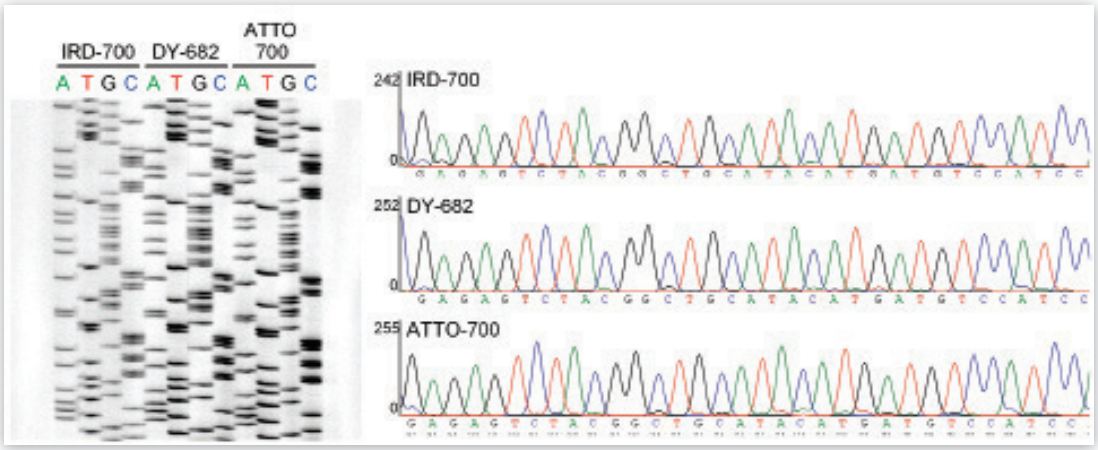


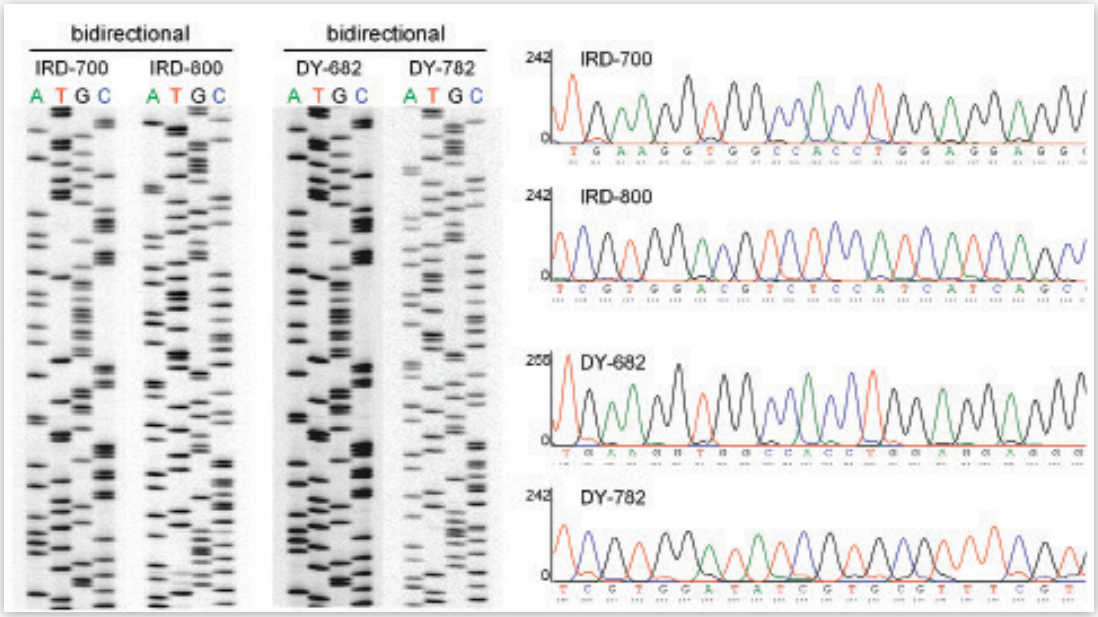
Fig.1 Comparison of IRDye® 800 and DY-782 modified SP6 oligonucleotides.
The two labels show no significant difference in performance, i.e. both dyes show a minimum of background noise as well as uniform and stable signals. The read length was typically 700–900 bp.

Fig.2 Comparison of IRDye® 700, DY-682 and ATTO 700 modified T7 oligonucleotides.



A snapshot of an image containing the raw sequence information is shown on the left. Primer modifications and nucleotide positions are indicated. The raw image was processed using e-Seq™ software (LI-COR) and the extracted DNA sequence information is displayed as a chromatogram on the right. All three labels show very low background noise and lead to comparable robust sequencing results.

Fig.3 Performance of DY-682 and DY-782 modified oligonucleotides in simultaneous bidirectional sequencing reactions.



The 4300L DNA Analyzer is a two channel system that detects infrared fluorescence at about 700 nm and 800 nm. This feature enables the possibility of simultaneous bidirectional sequencing, i.e. sequencing with two primers in one reaction while one primer binds to the sense and one to the antisense strand of the template. This approach doubles the sequence information and leads to longer reads without increasing time and effort.
For comparison T7-IRDye® 700 and SP6-IRDye® 800 or T7-DY-682 and SP6–DY-782 were used in one sequencing reaction and simultaneously analysed. The quality of the sequence information obtained under both conditions was excellent. The low background noise was comparable to that of mono-directional reactions and indicative of good spectral separation of DY-682 and DY-782 dyes. With 700-900 bp the read length per channel was similar to that of mono-directional sequencing reactions.

CONCLUSION

The performance of ATTO-TEC ATTO 700 and Dyomics DY-682 and DY-782 modified oligonucleotides as primers for dideoxy-based DNA sequencing were evaluated and found to be identical to that of IRDye® labels. All three dyes are characterized by high signal intensity, a minimum of background noise and good spectral separation when used in bidirectional sequencing applications. **The new dyes can replace IRDye® labels without further optimisation of the sequencing protocol. In conclusion, ATTO-TEC ATTO 700 and Dyomics DY-682 and DY-782 labeled oligonucleotides are well suited for automated DNA sequencing on LI-COR Model 4300 DNA Analysers.**