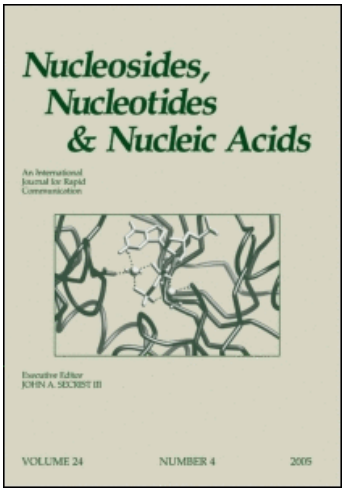

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Negatively Charged, Dye Labeled-Dideoxynucleotides for "Direct-Load" DNA Sequencing

Satyam Nampalli[a]; Patrick J. Finn[a]; Lei Sun[a]; Haiguang Xiao[a]; John R. Nelson[a]; Gregory Grossmann[a]; Parke K. Flick[a]; Carl W. Fuller[a]; Shiv Kumar[ab]
[a] Amersham Biosciences, Piscataway, New Jersey, USA [b] Amersham Biosciences, Piscataway, NJ, USA

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Negatively Charged, Dye Labeled-Dideoxynucleotides for "Direct-Load" DNA Sequencing

Satyam Nampalli, Patrick J. Finn, Lei Sun, Haiguang Xiao, John R. Nelson, Gregory Grossmann, Parke K. Flick, Carl W. Fuller, and Shiv Kumar*

Amersham Biosciences, Piscataway, New Jersey, USA

ABSTRACT

A four-color set of negatively charged, single dye as well as energy transfer dye labeled-ddNTPs were synthesized and evaluated in combination with a novel polymerase in a "direct-load" DNA sequencing, obviating the laborious and time consuming post-reaction work-up.

Key Words: Dye labeled nucleotides; Charged terminators; Direct-load DNA sequencing.

INTRODUCTION

Dye terminator[1] DNA sequencing involves thermal cycling, essentially ending up in a mixture of desired Sanger[2] fragments in addition to unreacted, dye-labeled nucleotides and their break-down products. As the mobility of some of these unreacted dye labeled nucleotides and their break-down products match that of

*Correspondence: Shiv Kumar, Amersham Biosciences, 800 Centennial Avenue, Piscataway, NJ 08855-1327, USA; Fax: 732-980-2877; E-mail: shiv.kumar@amersham.com.

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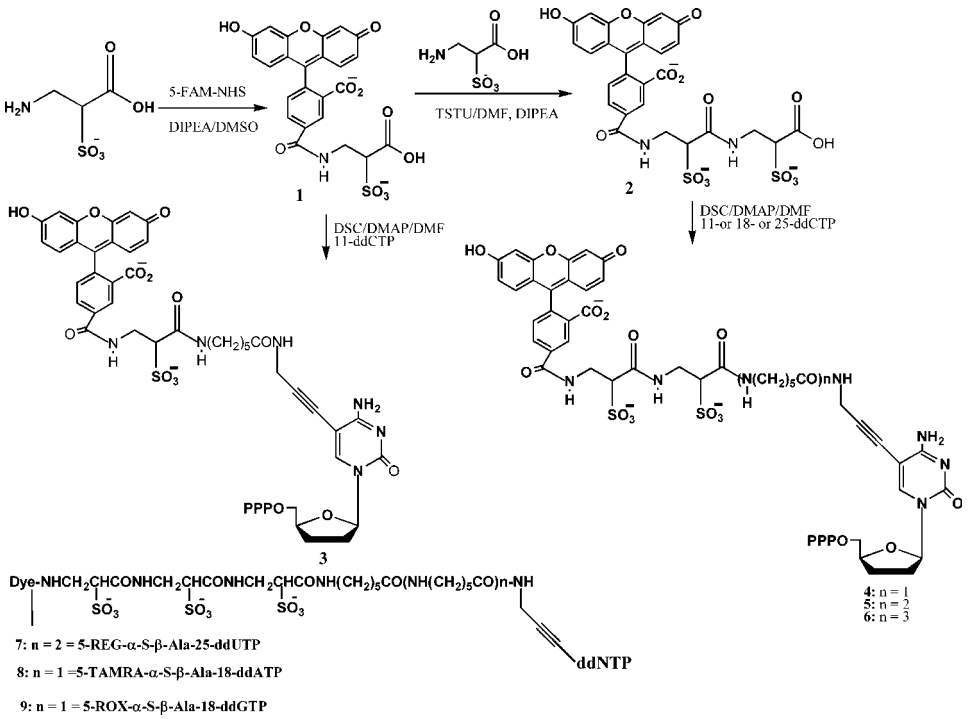
the desired fragments, they obscure the sequence information. A readable sequencing data in the electropherogram can only be obtained after performing a laborious post reaction work-up to get rid of the unreacted, dye-labeled nucleotides and their break-down products. In order to facilitate high throughput DNA sequencing and to eliminate the post reaction work-up, we have synthesized[3] and evaluated a novel series of negatively charged, dye labeled-2′,3′-dideoxynucleoside triphosphates combined with a mutant DNA polymerase, as reagents for ''Direct-Load'' DNA sequencing on a slab gel sequencing platform.

Herein, we present the demonstrated ability of these novel, negatively charged, dye labeled-dideoxynucleotides and their break-down products in migrating well ahead of the sequence information, culminating in a clean readable data without any ''dye-blobs''.

SYNTHESIS AND RESULTS

Commercially available α-sulfo-β-alanine was exploited to serve as a negative charge carrying tether connecting the fluorescent dye and the ddNTP. Initially, compounds **3** and **4** were synthesized (Sch. 1) merely to check how much charge would be necessary enabling unincorporated terminators and the breakdown products migrating ahead of the start of the sequence during electrophoretic migration. Model cycle



Scheme 1. Synthesis of direct-load dye terminators.

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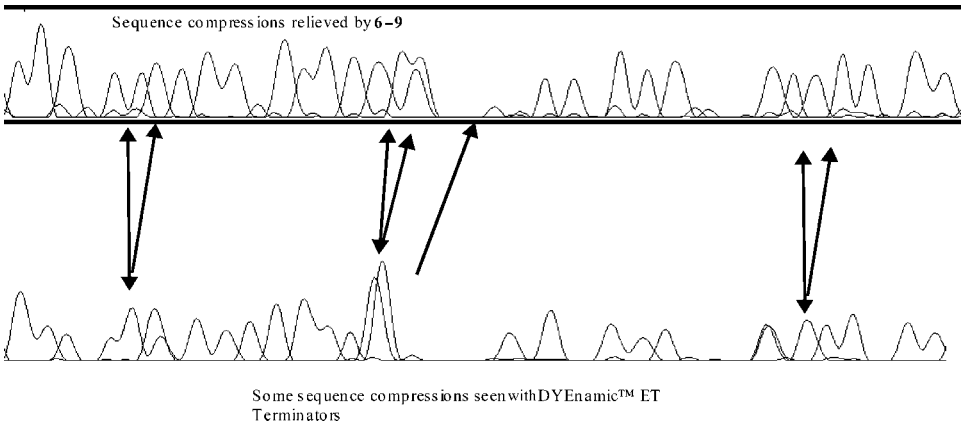

Figure 1. Dye-blobs free and compressions relieved DNA sequence data generated by **6**–**9**.

sequencing reactions were then conducted using **3** and **4** to show that **4** generated dye-blob free sequence data. Incorporation efficiency of compounds **3** and **4** was found to be lower than that of FAM-11-ddCTP by TS-II, DNA polymerase whereas the new, mutant polymerase incorporated several-flods better. In an effort to enhance the rate of incorporation of the terminators with more negative charges, FAM labeled-ddNTPs with much longer tethers were synthesized and evaluated to show enhanced reactivity and "dye-blob" free sequence data. For a direct-load, four-color sequencing reaction (dGTP dye-terminator), on a slab gel sequencing platform, compounds **6**–**9** were employed to generate clearly readable, compressions relieved, "dye-blob" free electropherogram (Fig. 1).

In order to enhance the signal intensity, a four-color energy transfer terminators were synthesized and evaluated in a direct-load experiment to show 2X enhanced signals.

CONCLUSION

Designed, synthesized and demonstrated the direct-load potential of the novel set of negatively charged terminators on a slab gel DNA sequencer, generating "blob-free" and compressions relieved sequence data in the electropherogram.

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