

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

Uniform Band Intensities in Fluorescent Dye Terminator Sequencing

Shiv Kumar^a; C. W. Fuller^a; S. Nampalli^a; M. Khot^a; I. Livshin^a; L. Sun^a; S. Hamilton^a; S. B. Samols^a; J. A. Mamone^a; K. M. Hujer^a; B. F. McArdle^a; J. R. Nelson^a; S. Duthie^a

^a Amersham Pharmacia Biotech, Cleveland, OH, U.S.A.

To cite this Article Kumar, Shiv , Fuller, C. W. , Nampalli, S. , Khot, M. , Livshin, I. , Sun, L. , Hamilton, S. , Samols, S. B. , Mamone, J. A. , Hujer, K. M. , McArdle, B. F. , Nelson, J. R. and Duthie, S.(1999) 'Uniform Band Intensities in Fluorescent Dye Terminator Sequencing', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 4, 1101 – 1103

To link to this Article: DOI: 10.1080/15257779908041659

URL: <http://dx.doi.org/10.1080/15257779908041659>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

UNIFORM BAND INTENSITIES IN FLUORESCENT DYE TERMINATOR SEQUENCING

Shiv Kumar,* C. W. Fuller, S. Nampalli, M. Khot, I. Livshin, L. Sun, S. Hamilton, S. B. Samols, J. A. Mamone, K. M. Hujer, B. F. McArdle, J. R. Nelson & S. Duthie

Amersham Pharmacia Biotech, 26111 Miles Road, Cleveland, OH 44128, U.S.A.

ABSTRACT: The use of Cyanine dye (CyTM 5 and Cy5.5) labeled dideoxy terminators with Thermo SequenaseTM DNA polymerase in DNA sequencing provides uniform band intensity, improved sequence read-length, and accuracy. It also greatly improves the ability to detect single base heterozygotes with dye-terminator sequencing method.

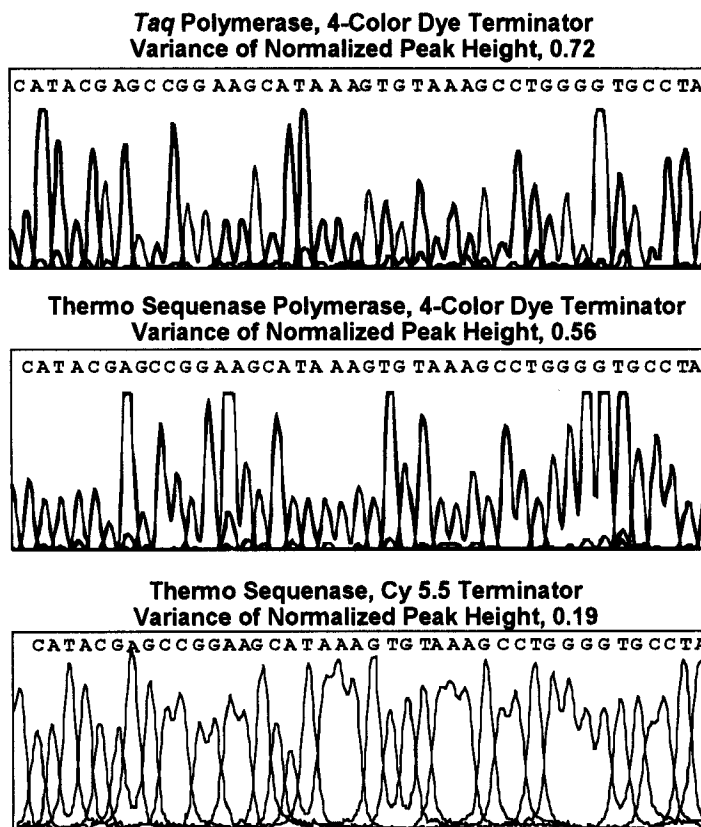
DNA sequencing by the Sanger dideoxy chain termination method requires that one of the components, typically either the primer or dideoxy terminator, be labeled with a radioactive or fluorescent label. With fluorescent DNA sequencing using Taq DNA polymerase, the band intensities are more uniform with dye labeled primers than with the dye labeled terminators originally offered for Taq sequencing with the ABI model 373 sequencing instrument. In this sequencing method, four different dyes are attached to four different dideoxynucleoside triphosphates. Apparently, their rate of incorporation by Taq DNA polymerase is highly dependent on the local sequence environment¹⁻². The uneven sequencing band pattern sometimes makes it difficult to call bases correctly with confidence.

Changing of a single amino acid, phe667 to tyrosine, of Taq DNA polymerase reduces discrimination against ddNTP incorporation by more than 1000-fold. An example of this kind of modified enzyme is Thermo SequenaseTM DNA polymerase (Amersham Pharmacia Biotech). When used for fluorescent DNA sequencing with dye-labeled primers, modified polymerases of this type produce band patterns with uniform peak heights which improve

sequence accuracy and ability to detect mixed-sequence templates³⁻⁴. In contrast, peak heights with the Taq dye-terminators still vary over 20-fold range⁵.

We have been exploring new dye-labeled terminators and new reaction conditions in order to find improved ways of performing dye terminator sequencing with enzymes having this amino acid modification. An example using Cy5.5 labeled ddNTPs is shown in Figure. These produce uniform band intensities and excellent sequence read-length, and accuracy. They also greatly facilitate the ability to detect single-base heterozygotes.

The data presented here were generated using a SEQ 4x4TM fluorescent DNA sequencer (Amersham Pharmacia Biotech). This instrument uses a red laser (676 nm) to excite Cy5.5 labeled DNA. A total of 16 lanes (4 sequencing samples) are available on a 14 X 14 cm, 6% acrylamide gel (50 μ m thick). A typical run takes about 40 minutes and generates read lengths of 350-400 bases. The dyes (Cy5 & Cy5.5) are attached via a normal or extended propargylamino linker at C-5 position of pyrimidines (ddCTP & ddUTP) and C-7 position of deaza purines (ddATP & ddGTP)¹.



REFERENCES

1. Lee, L. G.; Connell, C. R.; Woo, S. L.; Cheng, R. D.; McArdle, B. F.; Fuller, C. W.; Halloran, N. D.; Wilson, R. K. *Nucleic Acids Res.* **1992**, *20*, 2471-2483.
2. Parker, L.T.; Deng, Q.; Zakeri, H.; Carlson, C.; Nickerson, D. A.; Kwok, P.Y. *BioTechniques* **1995**, *19*(1), 116-121.
3. Tabor, S.; Richardson, C.C. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 6339-6343.
4. Fuller, C.W.; Reeve, M. A. *Nature* **1995**, *376*, 796-797.
5. Parker, L.T.; Zakeri, H.; Deng, Q.; Spurgeon, S.; Kwok, P.; Nickerson, D. A. *BioTechniques* **1996**, *21*(4) 694-699.