Rapid Partial Digestion of DNA Fragments by PCR with 5-methyl-dCTP

By Kwong-Kwok Wong, Ph.D.
Molecular Biosciences, Pacific Northwest National Laboratory, Richland, WA

In this report, a method is described for generation of a partial digestion pattern for any fragment that can be amplified by PCR[a]. Using a mixture of 5-methyl-dCTP (5mC) and dCTP in PCR protects some of the restriction sites from digestion with 5-methyl-cytosine-sensitive restriction enzymes. After PCR amplification with 5mC, restriction digestion of the modified PCR products with a 5-methyl-cytosine-sensitive enzyme rapidly produces an array of fragments similar to a partial digestion of unmethylated DNA.

Introduction

The ability to generate partially digested DNA fragments rapidly is highly desirable for applications such as subcloning, restriction mapping and detection of polymorphisms (1,2). We recently reported a novel method of producing a partial digestion pattern of DNA fragments following PCR with 5mC using *Tth* DNA polymerase (3). PCR products generated in the presence of 5mC are partially modified with this nucleotide analog. Many restriction enzymes do not cut 5mC-modified restriction enzyme sites; consequently, when such partially modified PCR products are digested to completion with 5mC-sensitive restriction enzymes, the pattern of restriction digestion will be similar to that of a partial digestion. There is no need to adjust the amount of the enzyme used or the digestion time to obtain a partial digestion pattern. I describe the use of *Pfu* DNA Polymerase[a] (Cat.# M7741) for PCR amplification with and without 5mC in the reaction mixture. After amplification, the PCR products are digested with 5-methyl-cytosine-sensitive enzymes, *Alu* I (Cat.# R6281), *Cfo* I (Cat.# R6241) and *Hpa* II (Cat.# R6311).

Results

Genomic DNA from *Salmonella* strains was prepared from 1.5ml overnight cultures using the Wizard® Genomic DNA Purification Kit (Cat.# A1120). Standard PCR was performed using *Pfu* DNA Polymerase with or without 5mC to amplify a 3.2kb DNA fragment from bacterial genomic DNA. The amount of 5mC added to the PCR mix is one-fourth the amount of the dCTP in the reaction. A previous study (3) has shown that higher amounts of 5mC will increase the abundance of large fragments in the partial digestion pattern obtained.* A 3.2kb PCR product was generated (Figure 1) in PCR amplifications with or without 5mC added. Both PCR products were digested with various amounts of *Cfo* I for two hours. Figure 1 shows that similar partial digestion patterns were obtained for PCR products amplified in the presence of 5mC, regardless of the amount of *Cfo* I used. Conversely, generation of a partial digestion for PCR products amplified in the absence of 5mC requires an optimal amount of *Cfo* I restriction enzyme.

Since the whole length of the PCR product is randomly modified by 5mC, any restriction enzymes that do not cut 5mC-modified sites can be examined simultaneously. Using *Alu* I, *Cfo* I and *Hpa* II restriction enzymes, we digested a 478bp PCR product amplified from various *Salmonella* strains (Figure 2). Partial digestion patterns were obtained for all of these enzymes when 5mC was incorporated into the DNA. Apparently, all five *Salmonella* strains have similar partial restriction digestion patterns for each of the enzymes tested (Figure 2) except *Salmonella* strain #4 using *Alu* I (Figure 2, Panel A).

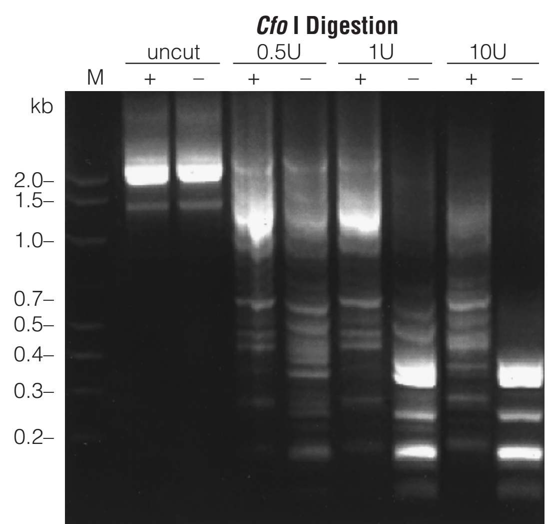


Figure 1. Comparison of *Cfo* I restriction digestion of PCR products amplified with (+) or without (–) 5-methyl-dCTP in the PCR mixture. A 3.2kb fragment was PCR amplified from 100ng *Salmonella* chromosomal DNA, which was extracted with the Wizard® Genomic DNA Purification Kit. In a 100µl PCR amplification, 10µl of *Pfu* DNA Polymerase 10X Reaction Buffer, 1µl of *Pfu* DNA Polymerase (3U/µl), 2µl of dNTP Mix (10mM) and 0.5µM of each *Salmonella* proprietary primer were mixed with 100ng *Salmonella* chromosomal DNA. To one of the 100µl reactions, 0.5µl of 10mM 5-methyl-dCTP (5mC) (Boehringer Mannheim Corporation) was added. The reactions were subjected to 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 5 minutes. The 3.2kb PCR products were amplified in the presence (+) or absence (–) of 5mC. For restriction enzyme digestion, the indicated units of enzyme (in a volume of 1µl) were added directly to 20µl of PCR products and incubated at 37°C for two hours.

*The amount of 5-methyl-dCTP (5mC) used is independent of site density. The ratio of 5mC:dCTP determines the percentage of modified dCTPs that will be protected from digestion. In general, a 1:4 to 1:2 ratio of 5mC:dCTP is used. If the restriction enzyme of interest will cut hemi-methylated (i.e., only one dCTP of the double-stranded site is methylated) but not fully methylated sites, a 1:2 ratio is recommended. If the enzyme will not cut hemi-methylated sites, a 1:4 ratio of 5mC:dCTP is recommended.

E-mail to *kk.wong@pnl.gov*

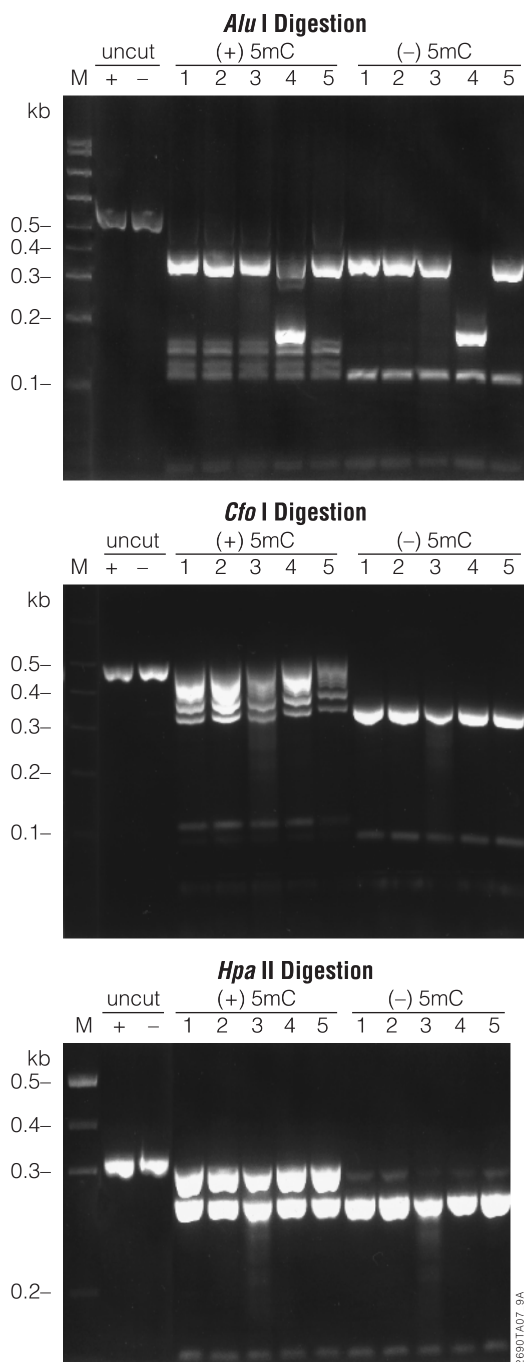


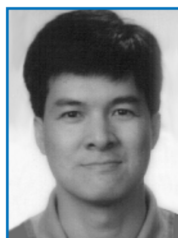
Figure 2. Restriction digestions of 478bp PCR products amplified by *Pfu* DNA Polymerase from five different *Salmonella* strains with (+) and without (–) 5-methyl-dCTP in the reaction mixture as described in Figure 1. **Panel A:** Digestion with *Alu* I. **Panel B:** Digestion with *Cfo* I. **Panel C:** Digestion with *Hpa* II. **Note:** All five strains exhibit similar partial restriction digestion patterns except strain #4 using *Alu* I (Panel A).

Summary

This update shows a reliable and simple method for producing partial digestion patterns by adding 5mC to a standard PCR amplification. As shown here, the ability of restriction enzymes to digest PCR products directly in amplification buffer obviates the need to purify the PCR product (as described previously, see reference 3). This method should be useful with any procedure that requires a partial digestion of DNA, such as subcloning of large DNA fragments (4), detection of restriction site polymorphisms, restriction mapping, sequencing (4) and authentication of PCR products.

References

1. Danna, A.J. (1980) *Meth. Enzymol.* **65**, 449.
2. Nobile, C. and Romeo, G. (1988) *Genomics* **3**, 272.
3. Wong, K.K. *et al.* (1997) *Nucl. Acids Res.* **25**, 4169.
4. Wong, K.K. *et al.* (1998) *Infect. Immun.* **66**, 3365.



Kwong-Kwok Wong

Ordering Information

Product	Size	Cat.#	Price ($)
Pfu DNA Polymerase	100u	M7741	80
	500u	M7745	320
Wizard® Genomic DNA Purification Kit	100 × 300µl	A1120	115
	500 × 300µl	A1125	275
Alu I	500u	R6281	55
Cfo I	3,000u	R6241	77
Hpa II	1,000u	R6311	65
	5,000u	R6315	238

Wizard is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office.

(a)The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.