Inhibition of restriction enzyme cleavage of DNA modified with 7-deaza-dGTP

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DNA templates containing G+C rich regions or stable secondary structures can potentially pose problems for PCR amplification (1) . Incorporation of the structure destabilizing analogue, 7-deaza-dGTP into the PCR reaction allows successful amplification of such template sequences (2). It has been reported that when 7-deaza-dGTP is substituted for dGTP in a DNA-fragment, the cleavage rate by the restriction enzyme EcoRI is reduced (3). We find that EcoRI as well as other commonly used restriction enzymes will not cut DNA containing 7-deaza-dGTP when it is incorporated into PCR product.

Substrates for the restriction enzymes were amplified by PCR using pUC19 and bacteriophage lambda sequences. Each 100 μl reaction contained 200 μM each of dATP, dCTP, dTTP, dGTP or 7-deaza-dGTP, 1 μM of each primer, 2.5 units Taq DNA Polymerase, 1 ng DNA template, 50 mM KCl, 10 mM Tris-HCl, pH 8.3 @ 37°C, 1.5 mM $MgCl_2$, 0.01% gelatin. Five hundred nanograms of the PCR product from reactions containing either 7-deaza-dGTP or dGTP were incubated with commonly-used restriction enzymes. Restriction digests were resolved on 1.2% agarose gels with ethidium bromide staining.

Table 1 summarizes the results of the restriction digests where 7-deaza-dGTP was substituted for dGTP in the PCR reaction. For most enzymes tested, inclusion of 7-deaza-dGTP in the PCR product prevented the restriction enzyme from cutting the DNA. It is not yet clear to us how this inhibition occurs. We assume that the presence of the deaza residue in the recognition sequence affects proper binding and/or cutting of the DNA substrate.

As yet the details of the spatial interaction between the modified G residue and various restriction enzyme active/binding sites is unclear. Inhibition of cutting can occur when the G residues are 5 prime (eg. EcoRI), 3 prime (eg. PstI), immediately adjacent (eg. SalI), or two bases away from the cut site (eg. AccI). Three enzymes (XbaI, HindIII, MaeII) are not inhibited by the presence of modified G residues in their recognition sequence. We have no knowledge of how modified G residues may inhibit cutting from a complementary strand of DNA.

7-deaza-dGTP could be beneficial for applications such as cDNA cloning where multiple internal restriction sites are protected during linker digestion. This would be similar to the strategy of methylating cDNA prior to digestion with methyl-sensitive restriction enzymes. Use of 7-deaza-dGTP to this end may allow the use of a greater number of enzymes.

REFERENCES

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Table 1.

Enzyme	Recognition site	Cut
1. AluI	gcagAG↓CT C	–
2. DdeI	ccctC↓T(N)AG T	–
3.HinfI	ccgtG↓A(N)TC	–
4. MaeII	ggatA↓CGT	+
5. Sau3A	gtgg↓GATC	–
6. AccI	tagaGT↓CGAC	–
7. BamHI	ccggG↓GATCC	–
8. EcoRI	cagtG↓AATTC	–
9. HindIII	atgcA↓AGCTT	+
10. PstI	cgacCTGCA↓Ggcat	–
11. SalI	tagaG↓TCGAC	–
12. SmaI	ggtaCCC↓GGG	–
13. SspI	tttcAAT↓ATT	+
14. XbaI	atccT↓CTAGA	+

Each of the restriction enzymes shown above were incubated separately with 500 ng of unmodified and modified PCR-amplified fragment. A 500 bp fragment of bacteriophage lambda was amplified and digested separately with enzymes 1–5 and a pUC19 1.2 kb fragment was amplified and digested separately with enzymes 6–14. SspI was included as a positive control enzyme because 7-deaza-dGTP is not incorporated into the SspI recognition sequence, allowing for complete cutting of even deaza-modified DNA. Capital letters represent the recognition sequence of the enzyme. Lower case letters represent proximal sequences specific to the lambda and pUC19 templates used in these experiments. Complete cutting of the fragment is indicated by +. Failure to cut is indicated by –. PCR product lacking 7-deaza-dGTP was cut to completion by all enzymes tested (data not shown).

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