Work Smarter Using Isoschizomers and Neoschizomers

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Abstract

New restriction endonucleases are continually being isolated from bacteria and studied for their ability to manipulate DNA. Frequently, newly discovered enzymes are found to cut DNA in patterns identical to those of previously isolated enzymes but have performance advantages that the existing enzymes lack, including cost and/or efficiency benefits. This article explores the benefits of isoschizomers and neoschizomers in the manipulation of DNA by comparing activities of recently isolated enzymes with those of their classic or previously isolated counterparts.

Typically, new iso/neoschizomers are commercialized because they are easily purified, resulting in lower costs, or because they lack the undesirable qualities of the classic enzyme.

Introduction

Over the past three decades more than 3,500 restriction enzymes have been identified, but only about 500 have been commercialized (1). In some cases, the commercialized enzyme may have undesirable qualities (e.g., low yield upon purification, tendency toward star activity, poor stability, etc.), but when it was originally isolated its specificity far outweighed any disadvantages. Another enzyme may be identified later that recognizes the same DNA sequence and either cuts identically to the classic enzyme (isoschizomer) or elsewhere within the same sequence (neoschizomer). The new enzyme may have more desirable traits than the classic enzyme and thus be a more desirable alternative for the same job.

Advantages Provided By Isoschizomers

Ava II and *Sin* I are examples of an isoschizomer pair where the more recently isolated isoschizomer (*Sin* I) offers a clear advantage over the classic enzyme. Both enzymes cleave 5′…G↓G(A/T)CC…3′, but *Ava* II will not cleave if the first C is methylated (Table 1). If the recognition site is followed by TGG, for instance GG(A/T)CCTGG, a *dcm* methylation site (CCTGG) results and the first C will be methylated in *dcm*+ *E. coli* strains, resulting in the sequence GG(A/T)C^{me}CTGG. This sequence cannot be cleaved by *Ava* II but can be cleaved by *Sin* I. (The *dcm* methylation system is found in common *E. coli* strains such as JM109, HB101, DH5α™ and XL1 Blue.) A *dcm*-deficient strain of *E. coli* is required to propagate a plasmid with this site in order to use *Ava* II to cleave the DNA. *Sin* I, on the other hand, is not sensitive to overlapping *dcm* methylation so a *dcm*-deficient strain is not required. *Sin* I is also easier to produce and is, therefore, available in a concentrated form (Cat.# R4144), making it the more economical of the two enzymes.

Another example of an isoschizomer pair is *Mbo* I and *Nde* II. These two enzymes have the same recognition site, 5′...↓GATC…3′, and cleave 5′ of the G. Both *Mbo* I and *Nde* II are sensitive to *dam* methylation. Therefore, neither enzyme will cleave the sequence 5′…G^{me}ATC…3′. (Most *E. coli* strains have an intact *dam* methylation system; however, strain JM110 is *dam*- and *dcm*-deficient.) *Mbo* I is the classic enzyme, while *Nde* II is the newer isoschizomer. *Nde* II is easier to produce and therefore more cost-effective and can be substituted directly into reactions where *Mbo* I is used. Both *Mbo* I and *Nde* II are isoschizomers to *Sau*3A I, which will cleave the same sequence whether the A has been methylated or not.

The enzyme *Dpn* I is an unusual isoschizomer, as it will not cleave unless the A is methylated. The combination of *Mbo* I/*Nde* II, *Dpn* I and *Sau*3A I can be used to examine methylation status of DNA. However, to cleave GATC in a subcloning experiment, *Sau*3A I can be used to avoid any problems with the methylation status of the host *E. coli* strain.

Isoschizomers in Eukaryotic Analysis

For more complex eukaryotic genome analysis, *Mbo* I/*Nde* II and *Sau*3A I perform differently than they do in prokaryotic systems. Some eukaryotes have CpG and CpNpG methylation (Table 1). Methylation of the CG pair is common to mammalian genomes, and both CG and CNG methylation are found in plant genomes. *Sau*3A I is sensitive to overlapping CG and CNG methylation, meaning that if the C in GATC is followed by G or NG, the C can be methylated and will not be cut. *Mbo* I/*Nde* II are not affected by CpG or CpNpG methylation. These enzyme pairs can be used to assess the methylation state of genes of interest from eukaryotic genomes. Another interesting pair of enzymes with differing methylation sensitivities is *Msp* I and *Hpa* II. Both recognize CCGG, but *Msp* I is insensitive to CpG methylation, while *Hpa* II is CpG methylation-sensitive. Both enzymes are sensitive to CpNpG.

Advantages of Neoschizomers

Kpn I$^{(b)}$ and *Acc*65 I are examples of neoschizomers with a clear advantage. Both recognize the sequence, 5′…GGTACC…3′, but *Kpn* I cleaves after the first C, leaving a 3′ overhang, while *Acc*65 I cleaves after the first G, leaving a 5′ overhang (Table 1). *Kpn* I is the classic

Table 1. Comparison of Isoschizomers and Neoschizomers Available from Promega.

	Site	Star Activity[a]	Methylation Sensitivity[b] *dam*	*dcm*	CpG	CpNpG	Activity in MULTI-CORE™ Buffer[c]	Most Cost Effective
Isoschizomers								
Ava II	G↓G(A/T)CC	No	i	s(ol)	s(ol)	s(ol)	25–50%	*Sin* I
Sin I		No	i	i	i	s(ol)	100%	
Mbo I	↓GATC	No	s	i	i	i	<10%	*Nde* II or *Sau* 3A I
Nde II		No	s	i	i	i	25–50%	depending
Sau 3A I		No	i	i	s(ol)	s(ol)	100%	on use
Sph I	GCATG↓C	Yes	i	i	i	i	10–25%	*Bbu* I
Bbu I		No	i	i	i	i	100%	
Hha I	GCG↓C	No	i	i	s	s(ol)	75–100	*Cfo* I
Cfo I		No	i	i	i	i	100%	
*Eco*52 I	C↓GGCCG	No	i	i	s	i	<10%	*BstZ* I
BstZ I[d]		No	i	i	s(ol)	s	10–25%	
Hpa II	C↓CGG	No	i	i	s	s	100%	*Msp* I
Msp I		No	i	i	i	s	25–50%	
Neoschizomers								
Kpn I	GGTAC↓C	Yes	i	i	i	i	75–100%	*Kpn* I
*Acc*65 I	G↓GTACC	No	i	s(ol)	i	i	100%	
Sac I	GAGCT↓C	No	i	i	i	i	100%	no
*Eco*ICR I	GAG↓CTC	No	n/a	n/a	n/a	n/a	100%	preference
Sma I[d]	CCC↓GGG	No	i	i	s	s	100%	*Sma* I
Xma I	C↓CCGGG	No	i	i	i	i	50–75%	
Nae I	GCC↓GGC	No	i	i	s	s	50–75%	no
*Ngo*M IV	G↓CCGGC	Yes	i	i	s	s	100%	preference

[a] Restriction enzymes under nonstandard conditions can demonstrate the ability to cleave DNA at sequences different from their defined recognition sites. For more information see p. 373 of the 2002 Promega Catalog and references 7 and 8.

[b] *dcm*: Prokaryotic, methylates the C5 position of the internal cytosine residue in the sequence 5′...CCTGG...3′

dam: Prokaryotic, methylates the N6 position of the adenine residue in the sequence 5′...GATC...3′

CpG: Eukaryotic, methylates the C5 position of the cytosine residue in the dinucleotide 5′...CG...3′

CpNpG: Eukaryotic, methylates the C5 position of the cytosine residue in the trinucleotide 5′...CNG...3′ (N = any base)

Key: i = insensitive; s(ol) = sensitive when restriction site overlaps methylation sequence; s = sensitive; n/a = no information.

For more information on methylation specificity see p. 381 of the 2002 Promega Catalog and reference 9.

[c] MULTI-CORE™ Buffer is specially formulated for performing double digests with many Promega restriction enzymes. For more information, see pp. 373–75 of the 2002 Promega Catalog. For more information on choosing the best reaction buffer to use for a double digest, see the online *Restriction Enzyme Resource* at www.promega.com/guides/re_guide/default.htm

[d] Restriction digests that occur at temperatures other than 37°C: *BstZ* I at 50°C, *Sma* I at 25°C.

enzyme, one of the first enzymes used commercially. However, care must be exercised when using *Kpn* I, as it is susceptible to star activity, which is relaxation of specificity of the enzyme toward its recognition site.

Adding excess enzyme (>25u/µg) or too much glycerol to a reaction can increase the star activity of an enzyme, resulting in cleavage of other sites within the substrate. The neoschizomer *Acc*65 I does not demonstrate star activity and thus is a smarter alternative to *Kpn* I. *Acc*65 I does, however, have sensitivity to overlapping *dcm* methylation, but this is only a problem if the recognition site is followed by the bases TGG.

A neoschizomer may not necessarily have a performance or cost advantage over the classic enzyme but may instead offer an alternative cleavage and subsequently a different type of end. In the case of *Sac* I and *Eco*ICR I, both lack star activity, have 100% activity in MULTI-CORE™ Buffer and are easy to produce. *Sac* I leaves a 3′ overhang and *Eco*ICR I leaves a blunt end. The 3′ overhang may offer an advantage in subcloning. However, if a *Sac* I/*Eco*ICR I site is the best site to use downstream of an insert prior to in vitro transcription, the *Eco*ICR I enzyme should be chosen because a 3′ overhang could prime aberrant transcription (2).

Nae I and *Ngo*M IV are neoschizomers with some unique characteristics. *Nae* I is a Type IIe restriction enzyme (1) that has two substrate binding sites, the active site and an allosteric site (3). Both sites must be occupied for the enzyme to cleave the substrate in the active site. Effective cleavage by this enzyme is dependent upon the number of cleavage sites present and the amount of enzyme in

the reaction. Reactions with *Nae* I work best with a low enzyme-to-substrate ratio. Too much enzyme will lead to a situation where there are not enough intact substrate molecules to fill both the active site and the allosteric site, thus no further cleavage occurs, resulting in an incomplete reaction. *Ngo*M IV is a Type IIf restriction enzyme (1) and is a tetramer or back-to-back dimer in its native conformation. Each dimer interacts with a substrate site, and both must be engaged for the enzyme to function (4). As is the case for *Nae* I, *Ngo*M IV benefits from low enzyme-to-substrate ratios but may have problems completing a cleavage reaction.

The problem of filling the allosteric site of *Nae* I can be solved by the use of a noncleavable, double-stranded oligonucleotide, ensuring that there is sufficient substrate, or in this case pseudosubstrate, in the allosteric site to allow the active site to function (5,6). This forms the basis of Promega's Turbo™ technology for *Nae* I (Turbo™ *Nae* I[a]) and another Type IIe enzyme, *Nar* I (Turbo™ *Nar* I[a]). Unfortunately, the *Ngo*M IV tetramer requires cleavable substrate at both of its active sites.

Conclusions

New isoschizomers can offer advantages over the existing enzyme. Typically the new enzymes are commercialized because they are easily purified, resulting in lower costs, or because they lack the undesirable qualities of the classic enzyme. Generally, the newer isoschizomer is a better value. Neoschizomers are sometimes developed for the same reasons as isoschizomers—to remove undesirable properties and increase yield. Sometimes, however, the neoschizomer pairs are almost indistinguishable in properties and value. The neoschizomers may simply offer alternative ends upon cleavage, that in and of themselves are advantageous in one reaction or another. Overall, knowledge of the properties of isoschizomers and neoschizomers can help you work smarter.

References

1. Williams, R.J. *et al.* (2002) *Mol. Biotech.,* in press.
2. Schenborn, E.T. and Mierendorf, R.C. (1985) *Nucl. Acids Res.* **13**, 6223–36.
3. Reuter, M. *et al.* (1998) *J. Biol. Chem.* **273**, 8294–8300.
4. Deibert, M. *et al.* (2000) *Nat. Struct. Biol.* **7**, 792–99.
5. Conrad, M. and Topal, M. (1992) *Nucl. Acids Res.* **20**, 5127–30.
6. Senesac, J.H. and Allen, J.R. (1995) *BioTechniques* **19**, 990–3.
7. Barany, F. (1988) *Gene* **65**, 149–65.
8. Brown, T.A., Hames, B.D. and Rickwood, D. (1991) In: *Molecular Biology Lab Fax.* BIOS Scientific Publishers, Ltd., Oxford, U.K.
9. McClelland, M., Nelson, M. and Raschke, E. (1994) *Nucl. Acids Res.* **22**, 3640–59.

Ordering Information

Product	Size	Conc.	Cat.#
*Acc*65 I	1,500u	10u/µl	R6921
Ava II	100u	1–10u/µl	R6131
Bbu I	200u	10u/µl	R6621
*Bst*Z I	500u	10u/µl	R6881
Cfo I	3,000u	10u/µl	R6241
Dpn I	200u	10u/µl	R6231
*Eco*ICR I	1,000u	10u/µl	R6951
Hpa II	1,000u	10u/µl	R6311
Kpn I[b]	2,500u	8–12u/µl	R6341
Mbo I	200u	8–12u/µl	R6711
Msp I	2,000u	10u/µl	R6401
	5,000u	10u/µl	R6315
Nde II	200u	10u/µl	R7291
Sac I	1,000u	10u/µl	R6061
*Sau*3A I	100u	3–10u/µl	R6191
	500u	3–10u/µl	R6195
Sin I	200u	8–12u/µl	R6141
*Ngo*M IV	500u	10u/µl	R7171
Sma I	1,000u	8–12u/µl	R6121
Turbo™ *Nae* I[a]	250u	4u/µl	R7231
Turbo™ *Nar* I[a]	200u	10u/µl	R7261
Xma I	50u	1–5u/µl	R6491

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