Restriction enzyme isoschizomers and key considerations

Abstract

Restriction enzymes are essential and ever-present tools in molecular biology. There are over 4,000 described restriction enzymes, with more than 400 unique specificities. When two or more enzymes recognize the same double-stranded DNA sequence and cleave it in exactly the same way, they are called isoschizomers. These isoschizomers usually originate from different sources, and thus may have optimal activity under different reaction conditions. Here we provide background on restriction enzyme naming conventions and key reaction parameters to consider when evaluating which isoschizomer to select for your research. We demonstrate the equivalency of function when isoschizomers are used under their optimal conditions, and highlight the benefits of the Invitrogen™ Anza™ restriction enzyme collection.

Introduction

Restriction enzymes have long been fundamental tools in molecular biology, enabling the development of recombinant DNA technologies, early methods of DNA sequencing, and DNA forensics and diagnostics. They are ubiquitous in modern biotechnology laboratories, yet they are frequently taken for granted by researchers who use them without much consideration for their selection and application.

The use of restriction enzymes as a research tool began with the discovery of “endonuclease R” by Smith and Wilcox in 1970 [1], and currently over 4,000 different restriction enzymes are described in the Restriction Enzyme DataBASE (REBASE), covering more than 400 unique specificities [2]. Among the described restriction enzymes, a prototype is defined as the first discovered enzyme with a unique recognition site sequence. Two or more restriction enzymes that recognize the same DNA sequence and cleave it with exactly the same specificity are called isoschizomers. Neoschizomers are similar to isoschizomers in that they recognize the same DNA sequence; however, they generate different cleavages at the recognition sequence.

Table 1. Examples of commercially available isoschizomers.

Prototype	Source organism	Recognition sequence	Anza isoschizomer	Source organism
SpeI	*Sphaerotilus species*	5'-A↓CTAGT-3'	Anza 3 BcuI	*Bacillus coagulans VS 29-022*
AgeI	*Agrobacterium gelatinovorum*	5'-A↓CCGGT-3'	Anza 7 BshTI	*Bacillus sphaericus Jo 22-024*
AvrII	*Anabaena variabilis* UW	5'-C↓CTAGG-3'	Anza 15 XmaJI	*Xanthomonas maltophilia Jo 85-025*
AscI	*Arthrobacter species*	5'-GG↓CGCGCC-3'	Anza 21 SgsI	*Streptomyces griseus RFL5*
PmeI	*Pseudomonas mendocina*	5'-GTTT↓AAAC-3'	Anza 24 MssI	*Methylobacterium species Dd 5-732*
SphI	*Streptomyces phaeochromogenes*	5'-GCATG↓C-3'	Anza 25 PaeI	*Pseudomonas aeruginosa*
EcoRV	*Escherichia coli* J62 pLG74	5'-GAT↓ATC-3'	Anza 26 Eco32I	*Escherichia coli RFL32*
ClaI	*Caryophanon latum* L	5'-AT↓CGAT-3'	Anza 30 Bsu15I	*Bacillus subtilis 15*
MfeI	*Mycoplasma fermentas*	5'-C↓AATTG-3'	Anza 31 MunI	*Mycoplasma unidentified*
SapI	*Saccharopolyspora species*	5'-GCTCTTC(1/4) -3'	Anza 33 LguI	*Lysobacter gummosus RFL1*
DdeI	*Desulfovibrio desulfuricans*	5'-C↓TNAG-3'	Anza 41 HpyF3I	*Helicobacter pylori RFL3*
SnaBI	*Sphaerotilus natans*	5'-TAC↓GTA-3'	Anza 43 Eco105I	*Escherichia coli RFL105*
SwaI	*Staphylococcus warneri*	5'-ATTT↓AAAT-3'	Anza 49 SmiI	*Streptococcus milleri S*
HpaI	*Haemophilus parainfluenzae*	5'-GTT↓AAC-3'	Anza 50 KspAI	*Kurthia species N88*

Naming of restriction enzymes is based on the organisms in which they were discovered. The first letter of a restriction enzyme's name represents the first letter of the genus of the source organism. The next two letters usually come from the first two letters of the species name; however, if a three-letter designation has already been used for an enzyme from a different organism, letters that appear later in the species name are selected to create a new unique enzyme name. The three-letter enzyme name is followed by a Roman numeral representing the order of discovery to distinguish restriction enzymes with unique specificities from the same organism. In some cases, abbreviations representing organismal strains may be inserted before the Roman numeral. Hence, a prototype and its isoschizomers carry different names, although they recognize the same DNA sequence and cleave it in the same way (Table 1).

Enzyme selection considerations

There are several parameters to consider in order to obtain optimal restriction enzyme activity and experimental success, including reaction temperature, methylation sensitivity, and recognition site specificity (star activity). These should be evaluated when selecting the appropriate restriction enzyme(s) to use, as well as which isoschizomer is the best fit for the experiment.

Reaction temperature

The correct reaction temperature for optimal enzyme activity is vital, especially when performing simultaneous digestion with multiple enzymes. While the majority of commercially available restriction enzymes are most active at 37°C, those isolated from thermophilic bacteria require higher temperatures (ranging from 50°C to 65°C) for their full activity. On the other hand, some enzymes cleave the substrate better at temperatures lower than 37°C.

Despite sharing the same DNA recognition site, isoschizomers may have different optimal reaction temperatures. Anza 13 EspI has an optimal reaction temperature of 37°C, while its isoschizomer BsmBI works best at 55°C and has only 20% relative activity at 37°C. As another example, SwaI shows 100% activity at 25°C but has only ~50% activity at 37°C. In contrast, its isoschizomer Anza 49 SmiI is fully active at 37°C. When performing digests with two enzymes with different optimal temperatures, it is sometimes necessary to sequentially digest the DNA at two different temperatures in order to obtain complete cleavage. The 128 Anza restriction enzymes are all fully active at 37°C and can be used in simultaneous digestions, making them an excellent choice for simplifying workflows requiring multiple restriction enzymes.

Methylation sensitivity

Over half of all commercially available restriction enzymes have some level of cleavage sensitivity when their target recognition sequence completely or partially overlaps a DNA methylation site. Methylated DNA in the target region can have the following effects on restriction enzyme performance:

- **No effect**—all methylated DNA sequences will be digested
- **Partial inhibition**—some of the methylated DNA sequences will not be digested
- **Complete blocking**—none of the methylated DNA sequences will be digested
- **Activation**—methylation is required for cleavage by the restriction enzyme

Therefore, methylation sensitivity must be carefully considered when evaluating isoschizomers for your experiment. The Anza restriction enzyme collection offers isoschizomers with different methylation sensitivities to aid your workflow. For example, when the DNA sequence 5'-GATC-3' is methylated at adenine (Dam methylation), cleavage by Anza 55 MboI is completely blocked, while its isoschizomer Anza 97 Bsp143I is not affected. On the other hand, the neoschizomer Anza 10 DpnI will cleave this recognition sequence only if it is Dam methylated. Most competent cell lines methylate plasmid DNA during replication, but if you need to propagate unmethylated DNA in order to use Dam or Dcm methylation–sensitive restriction enzymes for cloning, we offer the Dam/Dcm-deficient Invitrogen™ INV110 strain (Cat. No. C717103).

Star activity

Another important property of restriction enzymes is their propensity for star activity, the cleavage of DNA sequences that are similar but not identical to their canonical recognition site, usually differing at a single nucleotide position. Star activity occurs primarily under nonoptimal reaction conditions such as high pH, low ionic strength, higher than 5% glycerol concentration, extremely high enzyme concentrations, and the presence of inorganic solvents in the reaction. Star activity tends to increase with longer digestion times, and is undesirable for cloning applications.

Many traditional restriction enzymes have different optimal reaction conditions and are supplied with optimized reaction buffers. When digestion with multiple enzymes is required, researchers can either perform sequential digestion with intermediate purification steps, leading to potential for decreased sample yield, or execute simultaneous digestion in buffer conditions that are not optimal for at least one of the enzymes. This could lead to incomplete digestion and, subsequently, lower cloning efficiency. Techniques for overcoming this deficiency include adding more of the impaired restriction enzyme or extending the digestion reaction, both of which could lead to increased star activity. Anza restriction enzymes offer the benefit of 100% compatibility in a single proprietary reaction buffer and the flexibility of complete digestion in 15 minutes, or digestion for up to 16 hours with no star activity.

In this paper we examine the DNA cleavage of several isoschizomer pairs, comparing Anza restriction enzymes with other commercially available isoschizomers to demonstrate the advantages of the Anza restriction enzyme collection.

Examination of isoschizomer cleavage efficiencies by gel analysis

By definition, isoschizomers with the same methylation sensitivity share identical DNA recognition sites and cleavage specificity. Therefore, the fragments generated by digesting a specific DNA sample with isoschizomers, when viewed by gel electrophoresis, are expected to be the same. To confirm this premise, Anza restriction enzymes were compared with their isoschizomers of the same methylation sensitivity from a different commercial source. Two types of DNA—lambda phage DNA and linearized bacterial plasmid DNA—were used as substrates. Reactions were set up according to the respective manufacturers' recommendations (Table 2). DNA cleavage patterns were analyzed by agarose gel electrophoresis.

Table 2. Restriction enzyme digestion conditions for Anza enzymes and their isoschizomers.

Anza enzyme	Temp.	Digestion time	Buffer	Isoschizomer	Temp.	Digestion time	Buffer
Anza 7 BshTI	37°C	15 min	1X Anza Buffer	AgeI-HF	37°C	5 min	CutSmart
Anza 25 PaeI				SphI-HF			
Anza 26 Eco32I				EcoRV-HF			
Anza 31 MunI				MfeI-HF			
Anza 33 LguI				SapI		1 hour	
Anza 43 Eco105I				SnaBI			
Anza 50 KspAI				HpaI			
Anza 15 XmaJI				AvrII		15 min	
Anza 3 BcuI				SpeI-HF			

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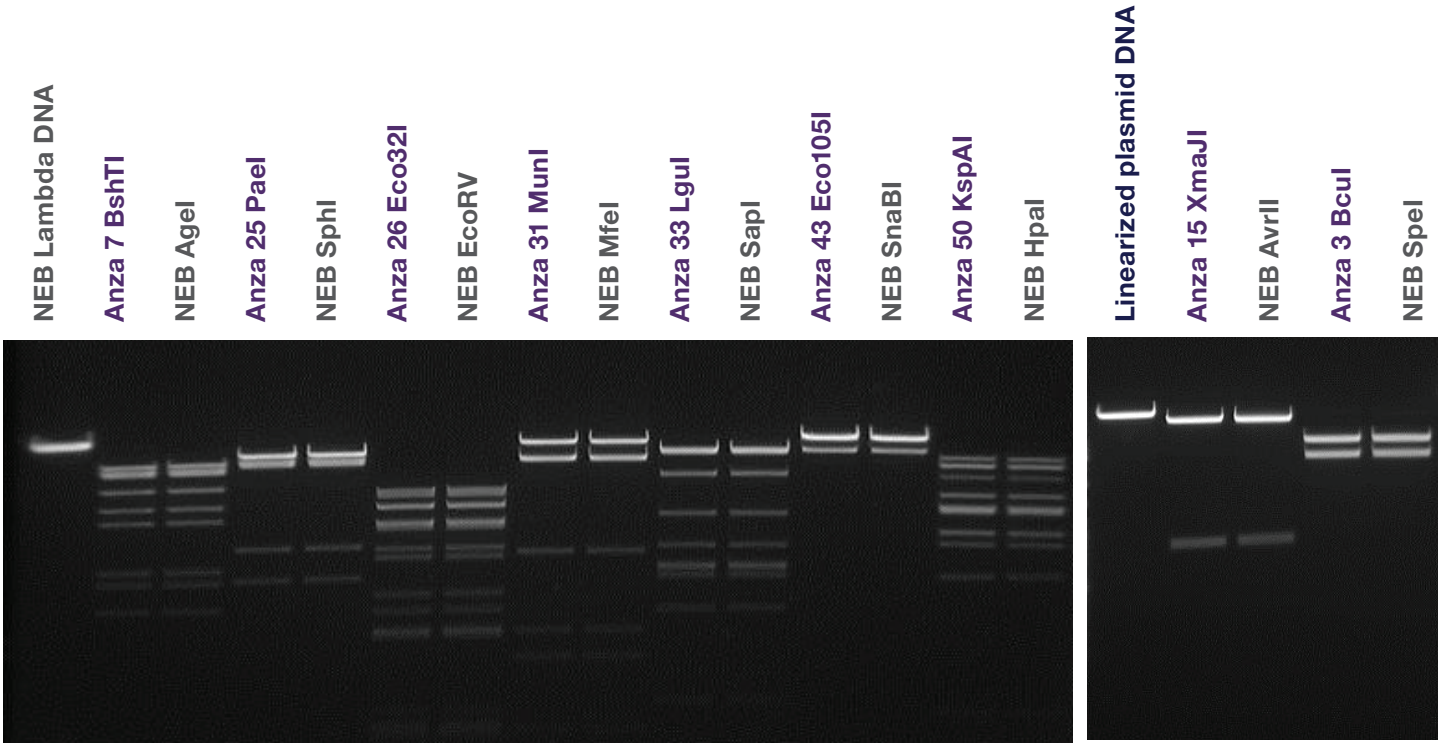

Figure 1. DNA digestions comparing Anza restriction enzymes with commercially available isoschizomers. Reaction conditions were as recommended by the respective manufacturers. Anza enzymes digest the DNA completely in 15 minutes, producing restriction fragment banding patterns identical to those of their isoschizomers. NEB = New England Biolabs.

Complete digestion with identical cleavage patterns was obtained from each pair of isoschizomers, regardless of their source (Figure 1). Anza enzymes provide the convenience and simplicity of a single set of reaction conditions for all enzymes and DNA substrate types, with complete digestion achieved in 15 minutes for all enzymes. Some isoschizomers from other commercial sources (e.g., SapI) require an hour to achieve complete digestion.

Conclusion

Key considerations for restriction enzyme function include optimal reaction temperature, propensity for star activity, and DNA methylation sensitivity. Anza restriction enzymes provide the same function as their commercially available isoschizomers, and have several added benefits: (1) all 128 Anza restriction enzymes are 100% active at 37°C, (2) all are 100% compatible with a single reaction buffer, and (3) they achieve complete digestion in 15 minutes, while providing the flexibility of digesting for up to 16 hours without undesirable star activity. These features cover two of the three main considerations for enzyme selection, temperature and star activity, thus simplifying workflows requiring restriction digests. For more information on the Anza Restriction Enzyme Cloning System, please go to **thermofisher.com/anza**

References

1. Smith HO, Wilcox KW (1970) *J Mol Biol* 51:379–391.
2. Roberts RJ et al. (2015) *Nucleic Acids Res* 43:D298–D299.

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