Cloning with FastDigest restriction enzymes and key performance considerations

Abstract

Restriction enzymes are essential molecular biology tools, and nearly 5,000 restriction enzymes have been described in the literature. Here we discuss restriction enzymes, their naming conventions, and key reaction parameters to consider when selecting restriction enzyme isoschizomers for research. We also demonstrate the functional equivalency of isoschizomers used under optimal conditions and highlight the benefits of using Thermo Scientific™ FastDigest™ restriction enzymes.

Introduction

The use of restriction enzymes for research purposes began in 1970 with the discovery of endonuclease R by Smith and Wilcox [1]. Restriction enzymes enabled the development of recombinant DNA technology, early DNA sequencing methods, DNA forensics and diagnostics, and modern genetic engineering. A wide variety of restriction enzymes are used in biotechnology laboratories today, and nearly 5,000 different enzymes are described in the REBASE restriction enzyme database (**http://rebase.neb.com**).

A prototype restriction enzyme is the first enzyme known to recognize and cleave a specific DNA sequence. Two or more restriction enzymes that recognize the same double-stranded DNA sequence and cleave it in exactly the same way are called isoschizomers. Isoschizomers usually originate from different sources, so they may be optimally active under different reaction conditions. Like an isoschizomer, a neoschizomer of a restriction enzyme recognizes the same target DNA sequence. However, it will cleave differently at the recognition site.

Restriction enzyme nomenclature is based on the organisms in which the enzymes are discovered. The first letter of a restriction enzyme name is the first letter of the genus name of the source organism. The next two letters are usually the first two letters of the species name. The three-letters are followed by a Roman numeral for the order of discovery. Restriction enzymes with unique specificities can thus be distinguished if they are isolated from the same organism. A prototype enzyme and its isoschizomers from different species will have different names even though they recognize the same DNA sequence and cleave it in the same way (**Table 1**).

Considerations for restriction enzyme selection

Several parameters should be considered to achieve optimal restriction enzyme activity. These include the reaction temperature, the methylation sensitivity of the enzyme, its recognition site, and star activity. Each parameter and isoschizomer that would potentially work for a given experiment should be evaluated during restriction enzyme selection.

Table 1. FastDigest restriction enzymes, isoschizomers, source organisms, and recognition sequences.

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

Reaction temperature

Selecting the best reaction temperature is vital for optimal enzyme activity, particularly when performing simultaneous digestion with multiple enzymes. While the majority of commercially available restriction enzymes are most active at 37°C, full activity of enzymes isolated from thermophilic bacteria requires temperatures in the range of 50°C to 65°C. Some FastDigest restriction enzymes cleave substrates more efficiently at temperatures below 37°C, but the isoschizomers of these enzymes may have different optimal reaction temperatures. For example, the optimal reaction temperature for Thermo Scientific™ FastDigest™ Esp3I is 37°C. The optimal reaction temperature for its isoschizomer, BsmBI, is 55°C, and BsmBI displays only 10% activity at 37°C. SwaI (New England Biolabs) is 100% active at 25°C, but it has only ~25% of its maximum activity at 37°C. The optimal activity of Thermo Scientific™ FastDigest™ SmiI, an isoschizomer of SwaI, is observed at 37°C.

When performing digestion with two enzymes that have different optimal temperatures, it is sometimes necessary to sequentially digest the target DNA at two different temperatures to achieve complete cleavage. FastDigest restriction enzymes offer the advantage of all being active in the same reaction buffer and are generally active at the same temperature, so they can be used simultaneously. For example, 172 FastDigest enzymes are fully active in the same buffer, and 162 are fully active at 37°C. FastDigest restriction enzymes can thus simplify the DNA digestion workflow, making them useful alternatives when multiple restriction enzymes are required.

Methylation sensitivity

A DNA methyltransferase is an enzyme that transfers a methyl group from a donor molecule to a cytosine (C) or adenine (A) residue. The most common DNA methyltransferases found in laboratory strains of bacteria include Dam, Dcm, EcoKI, and EcoBI. Methylation of adenine and cytosine by Dam and Dcm are referred to as Dam methylation and Dcm methylation, respectively.

Most restriction enzymes display some level of cleavage activity at methylated recognition sites. If a restriction enzyme has no DNA methylation sensitivity, it will cleave its recognition sites whether they are methylated or not. Some restriction enzymes actually require DNA methylation to activate cleavage of their respective recognition sequences. For other restriction enzymes, however, digestion can be incomplete or blocked entirely if the recognition sequence contains a methylated site. Incomplete digestion by a restriction enzyme indicates it does not always cleave its recognition sequence, which may be due to incomplete methylation or hemimethylation. Partial digestion may also occur when a recognition sequence overlaps a methylated site, or when cleavage of methylated DNA is significantly slower than cleavage of unmethylated DNA. If digestion is completely blocked, no methylated DNA sequence will be cleaved.

Methylation sensitivity is clearly an important consideration when evaluating isoschizomers, and the FastDigest portfolio includes isoschizomers with different methylation sensitivities. For example, methylation of A in the 5′-GATC-3′ recognition sequence of Thermo Scientific™ FastDigest™ MboI completely prevents cleavage. Thermo Scientific™ FastDigest™ Bsp143I, an isoschizomer of MboI, is not affected by Dam methylation, while a neoschizomer, Thermo Scientific™ FastDigest™ DpnI, will cleave only when the A in the recognition sequence is methylated. Most competent cell lines methylate plasmid DNA during replication. Dam- and Dcm-deficient Invitrogen™ One Shot™ INV110 Chemically Competent *E. coli* (Cat. No. C717103) can be used to propagate unmethylated DNA when cloning with restriction enzymes that are sensitive to Dam or Dcm methylation.

Star activity

Another important property of restriction enzymes is their propensity for star activity, which refers to cleavage of a DNA sequence that typically differs from the canonical recognition sequence at a single nucleotide position. Star activity most often occurs when reaction conditions are nonoptimal: examples of these are high pH, low ionic strength, glycerol concentrations above 5%, extremely high enzyme concentrations, and the presence of inorganic solvents. Star activity tends to be more pronounced when digestion times are long, and it is undesirable in cloning applications.

The optimal reaction conditions for many traditional restriction enzymes differ, and these enzymes are frequently sold with optimized reaction buffers. When digestion with multiple enzymes is necessary, researchers can either perform sequential digestion or simultaneous digestion in a buffer that is not optimal for at least one of the enzymes. Sequential digestion requires intermediate purification steps that may reduce sample yield, while use of a nonoptimal buffer can lead to incomplete digestion and ultimately reduce cloning efficiency. Digestion with multiple enzymes may also involve increasing the amount of the less active restriction enzyme or extending the digestion reaction, both of which could lead to higher star activity.

FastDigest restriction enzymes provide the benefit of being fully compatible with a single proprietary reaction buffer. Incubation times for FastDigest enzymes are typically short: complete digestion can often be achieved in 5–15 minutes with no star activity.* In the following section, we compare DNA cleavage by several FastDigest restriction enzymes and commercially available isoschizomers to demonstrate the advantages of the FastDigest enzyme portfolio.

Analysis of isoschizomer cleavage efficiency

By definition, isoschizomers with identical methylation sensitivities have the same DNA recognition sites and cleavage specificities. Digestion of a specific DNA sequence with a restriction enzyme and an isoschizomer is thus expected to generate fragments of the same size. To evaluate this premise, FastDigest restriction enzymes were compared to their prototypes with similar methylation sensitivities, obtained from a different commercial supplier. The enzymes were used to digest bacteriophage lambda (λ) DNA, linearized bacterial plasmid DNA, and circular bacterial plasmid DNA. The reaction conditions were as recommended by the respective suppliers (**Table 2**). Gel electrophoresis was performed using 1% agarose gels with 1X TAE buffer and ethidium bromide stain, to determine whether digestion was complete.

Table 2. Recommended reaction setup for restriction enzymes.

FastDigest restriction enzyme (isoschizomer)	Temperature	Digestion time (min)	Buffer	NEB restriction enzyme (prototype)	Temperature	Digestion time (min)	Buffer
BshTI	37°C	5	Thermo Scientific™ FastDigest™ buffer	AgeI-HF	37°C	5	rCutSmart™ Buffer
SgsI				AscI			
MssI				PmeI			
PaeI				SphI-HF			
Eco32I				EcoRV-HF			
Bsu15I				ClaI			
MunI				MfeI-HF			
BcuI				SpeI-HF			
XmaJI				AvrII			
LguI				SapI		15	
HpyF3I				DdeI			
Eco105I				SnaBI		60	
KspAI				HpaI			
SmiI		20		SwaI	25°C	15	NEBuffer™ r3.1

* Digesting plasmid DNA, genomic DNA, or DNA PCR products with Thermo Scientific™ FastDigest™ AatII, BglII, Eco52I, SmiI, BseMII, Eco57I, FaqI, GsuI, NotI, AlwI44I, BshNI, TauI, ApaI, Eco130I, EcoRI, HindIII, BveI, NdeI, FspBI, MlsI, TatI, XagI, BplI, Bse5I, SspI, Bpu10I, CseI, PstI, SacI, SalI, SfaAI, XmiI, Bse5I, VspI, or SchI generally takes more than 15 minutes.

Results

Complete digestion and identical cleavage patterns were observed with each enzyme and its isoschizomer (Figures 1–4). Most of the FastDigest enzymes afforded the convenience and simplicity of using one set of reaction conditions to digest nearly all of the DNA substrates.

With the exception of SmiI, complete digestion was achieved within 5–15 minutes. It should be noted that complete digestion with the prototype SnaBI takes approximately 1 hour.

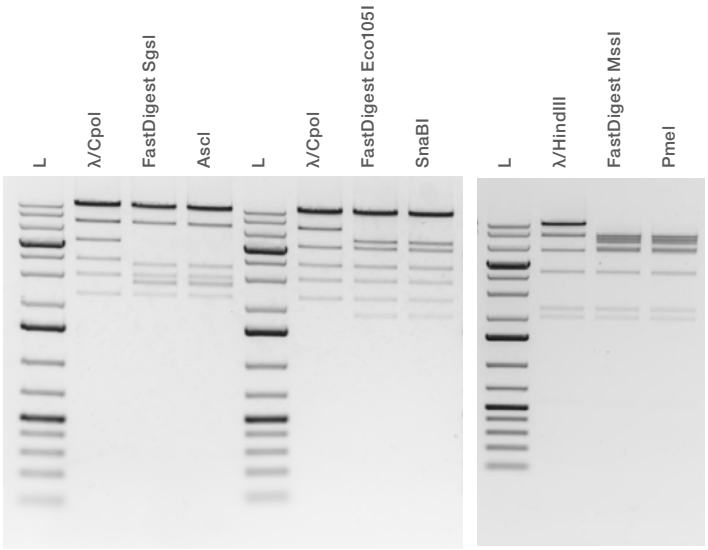

Figure 1. Electrophoresis of lambda phage DNA after digestion with FastDigest restriction enzymes and prototypes from NEB. L: Invitrogen™ GeneRuler™ 1 kb Plus DNA Ladder; AscI, SnaBI, and PmeI: restriction enzymes obtained from NEB. Lambda CpoI DNA (λ/CpoI) was the digestion substrate for AscI, SnaBI, and Thermo Scientific™ FastDigest™ SgsI and Eco105I. Lambda HindIII DNA (λ/HindIII) was the digestion substrate for PmeI and Thermo Scientific™ FastDigest™ MssI.

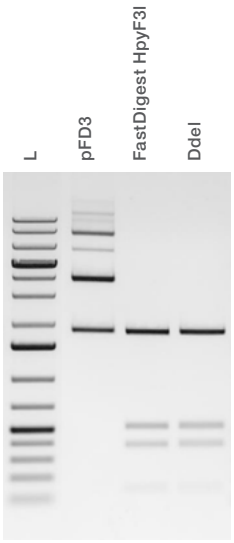

Figure 2. Electrophoresis of supercoiled plasmid DNA (pFD3) after digestion with Thermo Scientific™ FastDigest™ HpyF3I and a prototype, DdeI, from NEB. L: GeneRuler 1 kb Plus DNA Ladder.

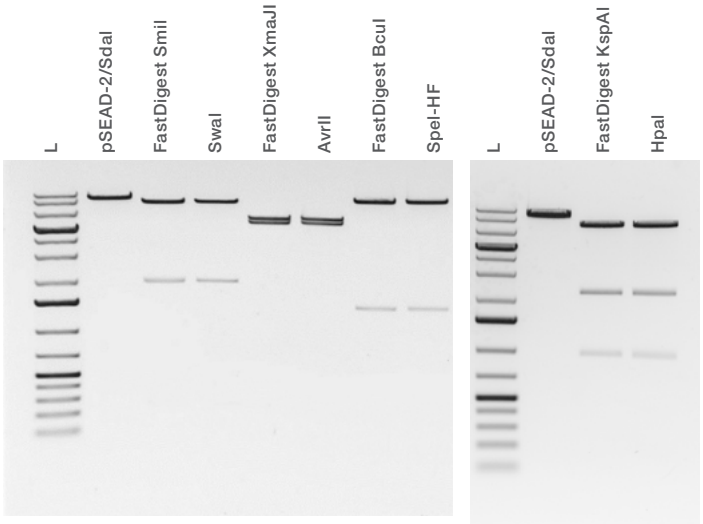

Figure 3. Electrophoresis of linearized plasmid DNA (pSEAD-2/SdaI) after digestion with FastDigest restriction enzymes and prototypes from NEB. L: GeneRuler 1 kb Plus DNA Ladder; Thermo Scientific™ FastDigest™ SmiI, XmaJI, BcuI, and KspAI; SwaI, AvrII, SpeI-HF, and HpaI: restriction enzymes from NEB.

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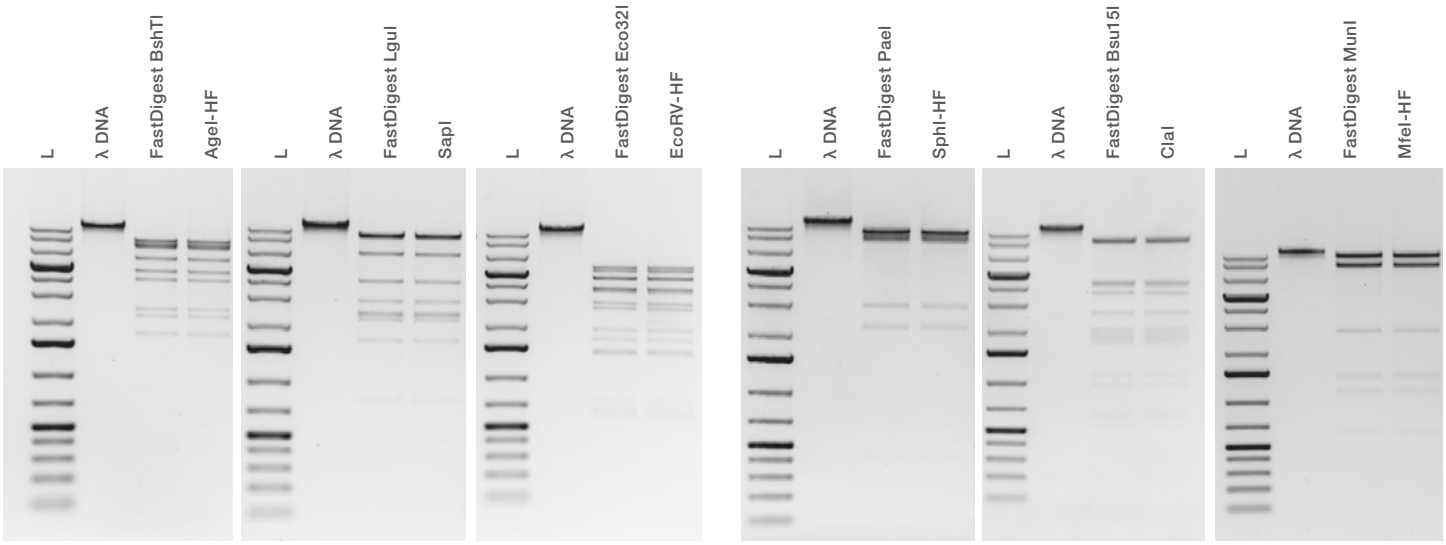

Figure 4. Electrophoresis of lambda phage DNA (λ DNA) after digestion with FastDigest restriction enzymes and prototypes from NEB.
L: GeneRuler 1 kb Plus DNA Ladder; Thermo Scientific™ FastDigest™ BshTI, LguI, Eco32I, PaeI, Bsu15I, and MunI; AgeI-HF, SapI, EcoRV-HF, SphI-HF, ClaI, and MfeI-HF: restriction enzymes from NEB.

Conclusion

Key considerations for optimal restriction enzyme performance include the reaction temperature, the propensity of the enzyme for star activity, and its DNA methylation sensitivity. FastDigest restriction enzymes perform the same functions as their commercially available isoschizomers and have several advantages, including:

- 176 enzymes that are fully compatible with a single reaction buffer and 162 enzymes that are 100% active at 37°C
- 137 enzymes that completely digest plasmid DNA, genomic DNA, and PCR DNA products in 5–15 minutes

The features of FastDigest restriction enzymes facilitate temperature optimization and help minimize star activity to simplify restriction digest workflows.

Reference

1. Smith HO, Wilcox KW (1970) A Restriction enzyme from *Hemophilus influenzae*: I. Purification and general properties. *J Mol Biol* 51(2):379–391.

For more information about FastDigest restriction enzyme cloning systems, please visit **thermofisher.com/fastdigest**

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