

Cleavage Close to the End of DNA Fragments

Annealed 5' FAM labeled oligos were incubated with the indicated enzyme (10 units/1pmol oligo) for 60 minutes at the recommended incubation temperature and NEBuffer. The digest was run on a TBE acrylamide gel and analyzed by fluorescent imaging. The double stranded oligos were designed to have the indicated number of base pairs from the end followed by the recognition sequence and an additional 12 bases. In some cases asymmetric cleavage was observed and interpreted as a negative result. Asymmetric cleavage decreased with increasing base pairs from the end.

Note: As a general rule and for enzymes not listed below, 6 base pairs should be added on either side of the recognition site to cleave efficiently. The extra bases should be chosen so that palindromes and primer dimers are not formed. In most cases there is no requirement for specific bases.

Note: You may download the previous tables, which use a different methodology here: [Cleavage Close to the End of DNA Fragments \(oligonucleotides\)](#) and [Cleavage Close to the End of DNA Fragments \(linearized vector\)](#)

Restriction Enzyme Digest Protocol: Cutting Close to DNA End



When cutting close to the end of a DNA molecule, make sure you know how many bases to add to the ends of your PCR primers.

Chart Legend			
-	0%	+	0-20%
++	20-50%	+++	50-100%
nt	not tested		

* RE-Mix® master mix format available

[A](#) | [B](#) | [C](#) | [D](#) | [E](#) | [F](#) | [H](#) | [K](#) | [M](#) | [N](#) | [P](#) | [R](#) | [S](#) | [X](#)

Enzyme	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
AclI	-	+	+	++	+++
AgeI	+++	+++	+++	+++	+++
AgeI-HF™	++	+++	+++	+++	+++
AluI	-	+++	+++	+++	+++
ApaI	+++	+++	+++	+++	+++
AscI	+++	+++	+++	+++	+++
AvrII	++	++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
BamHI	+	++	+++	+++	+++
BamHI-HF®	+	+	+++	+++	+++
BglII	++	+++	+++	+++	+++
BmtI	+++	+++	+++	+++	+++
BmtI-HF®	+++	+++	+++	+++	+++
BsaI	+++	+++	+++	+++	+++

BsaI-HF®	+++	+++	+++	+++	+++
BsiWI	++	+++	+++	+++	+++
BsmBI	+++	+++	+++	+++	+++
BsrGI	+++	+++	+++	+++	+++
BssHII	+	+++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
ClaiI	-	-	+	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
DdeI	+++	+++	+++	+++	+++
DpnI	-	++	++	nt	nt
DrallI	+++	+++	+++	+++	+++
DrallI-HF®	+++	+++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
EagI	++	+++	+++	+++	+++
EagI-HF®	+	+++	+++	+++	+++
EcoRI	+	+	++	++	+++
EcoRI-HF®	+	+	++	+++	+++
EcoRV	++	++	++	++	+++
EcoRV-HF®	+	++	++	++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
FseI	+	++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
HindIII	-	+	+++	+++	+++
HindIII-HF®	-	+	+++	+++	+++
HpaI	+++	+++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
KpnI	+	+++	+++	+++	+++
KpnI-HF®	+	+++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
MfeI	+	++	+++	+++	+++
MfeI-HF®	+	++	+++	+++	+++
MulI	+	++	+++	+++	+++
MseI	+++	+++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
NcoI	-	++	+++	+++	+++
NcoI-HF®	+	++	+++	+++	+++
NdeI	+	+	+++	+++	+++

NheI	+	++	+++	+++	+++
NheI-HF®	++	++	+++	+++	+++
NlaIII	++	+++	+++	+++	+++
NotI	++	++	++	++	++
NotI-HF®	++	++	++	++	++
NsiI	+	+	+++	+++	+++
NspI	-	-	+	+	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
PacI	+++	+++	+++	+++	+++
PciI	+++	+++	+++	+++	+++
PmeI	+++	+++	+++	+++	+++
PstI	+	+++	+++	+++	+++
PstI-HF®	++	+++	+++	+++	+++
PvuI	+++	+++	++	+++	+++
PvuI-HF®	+++	+++	+++	+++	+++
PvuII	++	++	++	+++	+++
PvuII-HF®	-	++	++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
RsaI	+	+++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
SacI	-	++	+++	+++	+++
SacI-HF®	-	+	+++	+++	+++
SacII	+++	+++	+++	+++	+++
SalI	-	++	+++	+++	+++
SalI-HF®	-	++	+++	+++	+++
SapI	+++	+++	+++	+++	+++
Sau3AI	+++	+++	+++	+++	+++
SbfI	++	+++	+++	+++	+++
SbfI-HF®	++	+++	+++	+++	+++
Scal	+++	+++	+++	+++	+++
Scal-HF®	+	+++	+++	+++	+++
SfiI	+++	+++	+++	+++	+++
SmaI	+++	+++	+++	+++	+++
SpeI	+	++	++	++	++
SpeI-HF®	+	++	++	++	++
SphI-HF®	++	++	+++	+++	+++
SphI	+++	+++	+++	+++	+++
SspI	+	+++	+++	+++	+++
SspI-HF®	+	+++	+++	+++	+++
StuI	+++	+++	+++	+++	+++
StyI	+	++	+++	+++	+++

StyI-HF®	+	+++	+++	+++	+++
Enzyme Back to top	Base Pairs from end				
	1 bp	2 bp	3 bp	4 bp	5 bp
XbaI	++	++	++	++	++
XhoI	++	++	++	+++	+++
XmaI	+++	+++	+++	+++	+++

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