Cleavage Close to the End of DNA Fragments (oligonucleotides)

To test the varying requirements restriction endonucleases have for the number of bases flanking their recognition sequences, a series of short, double-stranded oligonucleotides that contain the restriction endonuclease recognition sites (shown in red) were digested. This information may be helpful when choosing the order of addition of two restriction endonucleases for a double digest (a particular concern when cleaving sites close together in a polylinker), or when selecting enzymes most likely to cleave at the end of a DNA fragment.

The experiment was performed as follows: 0.1 A_{260} unit of oligonucleotide was phosphorylated using T4 polynucleotide kinase and γ-[^{32}P] ATP. 1 µg of 5´ [^{32}P]-labeled oligonucleotide was incubated at 20°C with 20 units of restriction endonuclease in a buffer containing 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM DTT and NaCl or KCl depending on the salt requirement of each particular restriction endonuclease. Aliquots were taken at 2 hours and 20 hours and analyzed by 20% PAGE (7 M urea). Percent cleavage was determined by visual estimate of autoradiographs.

As a control, self-ligated oligonucleotides were cleaved efficiently. Decreased cleavage efficiency for some of the longer palindromic oligonucleotides may be caused by the formation of hairpin loops.

| A | B | C | E | H | K | M | N | P | S | X |

Enzyme	Oligo Sequence	Chain Length	% Cleavage	
			2 hr	20 hr
AccI	GGTCGACC	8	0	0
	CGGTCGACCG	10	0	0
	CCGGTCGACCGG	12	0	0
AflIII	CACATGTG	8	0	0
	CCACATGTGG	10	>90	>90
	CCCACATGTGGG	12	>90	>90
AscI	GGCGCGCC	8	>90	>90
	AGGCGCGCCT	10	>90	>90
	TTGGCGCGCCAA	12	>90	>90
AvaI	CCCCGGGG	8	50	>90
	CCCCCGGGGG	10	>90	>90
	TCCCCCGGGGGA	12	>90	>90
BamHI	CGGATCCG	8	10	25
	CGGGATCCCG	10	>90	>90
	CGCGGATCCGCG	12	>90	>90
BglII	CAGATCTG	8	0	0
	GAAGATCTTC	10	75	>90
	GGAAGATCTTCC	12	25	>90
BssHII	GGCGCGCC	8	0	0
	AGGCGCGCCT	10	0	0
	TTGGCGCGCCAA	12	50	>90
BstEII	GGGT(A/T)ACCC	9	0	10
BstXI	AACTGCAGAACCAATGCATTGG	22	0	0
	AAAACTGCAGCCAATGCATTGGAA	24	25	50
	CTGCAGAACCAATGCATTGGATGCAT	27	25	>90
ClaI	CATCGATG	8	0	0
	GATCGATC	8	0	0
	CCATCGATGG	10	>90	>90
	CCCATCGATGGG	12	50	50

EagI	CGGCCG			
EcoRI	GGAATTCC CGGAATTCCG CCGGAATTCCGG	8 10 12	>90 >90 >90	>90 >90 >90
HaeIII	GGGGCCCC AGCGGCCGCT TTGCGGCCGCAA	8 10 12	>90 >90 >90	>90 >90 >90
HindIII	CAAGCTTG CCAAGCTTGG CCCAAGCTTGGG	8 10 12	0 0 10	0 0 75
KpnI	GGGTACCC GGGGTACCCC CGGGGTACCCCG	8 10 12	0 >90 >90	0 >90 >90
MluI	GACGCGTC CGACGCGTCG	8 10	0 25	0 50
NcoI	CCCATGGG CATGCCATGGCATG	8 14	0 50	0 75
NdeI	CCATATGG CCCATATGGG CGCCATATGGCG GGGTTTCATATGAAACCC GGAATTCCATATGGAATTCC GGGAATTCCATATGGAATTCCC	8 10 12 18 20 22	0 0 0 0 75 75	0 0 0 0 >90 >90
NheI	GGCTAGCC CGGCTAGCCG CTAGCTAGCTAG	8 10 12	0 10 10	0 25 50
NotI	TTGCGGCCGCAA ATTTGCGGCCGCTTTA AAATATGCGGCCGCTATAAA ATAAGAATGCGGCCGCTAAACTAT AAGGAAAAAAGCGGCCGCAAAAGGAAAA	12 16 20 24 28	0 10 10 25 25	0 10 10 90 >90
NsiI	TGCATGCATGCA CCAATGCATTGGTTCTGCAGTT	12 22	10 >90	>90 >90
PacI	TTAATTAA GTTAATTAAC CCTTAATTAAGG	8 10 12	0 0 0	0 25 >90
PmeI	GTTTAAAC GGTTTAAACC GGGTTTAAACCC AGCTTTGTTTAAACGGCGCGCCGG	8 10 12 24	0 0 0 75	0 25 50 >90
PstI	GCTGCAGC TGCACTGCAGTGCA AACTGCAGAACCAATGCATTGG AAAACTGCAGCCAATGCATTGGAA CTGCAGAACCAATGCATTGGATGCAT	8 14 22 24 26	0 10 >90 >90 0	0 10 >90 >90 0
PvuI	CCGATCGG ATCGATCGAT TCGCGATCGCGA	8 10 12	0 10 0	0 25 10
SacI	CGAGCTCG	8	10	10
SacII	GCCGCGGC TCCCCGCGGGGA	8 12	0 50	0 >90

SalI	GTCGACGTCAAAAGGCCATAGCGGCCGC GCGTCGACGTCTTGGCCATAGCGGCCGCGG ACGCGTCGACGTCGGCCATAGCGGCCGCGGAA	28 30 32	0 10 10	0 50 75
ScaI	GAGTACTC AAAAGTACTTTT	8 12	10 75	25 75
SmaI	CCCGGG CCCCGGGG CCCCCGGGGG TCCCCCGGGGGA	6 8 10 12	0 0 10 >90	10 10 50 >90
SpeI	GACTAGTC GGACTAGTCC CGGACTAGTCCG CTAGACTAGTCTAG	8 10 12 14	10 10 0 0	>90 >90 50 50
SphI	GGCATGCC CATGCATGCATG ACATGCATGCATGT	8 12 14	0 0 10	0 25 50
StuI	AAGGCCTT GAAGGCCTTC AAAAGGCCTTTT	8 10 12	>90 >90 >90	>90 >90 >90
XbaI	CTCTAGAG GCTCTAGAGC TGCTCTAGAGCA CTAGTCTAGACTAG	8 10 12 14	0 >90 75 75	0 >90 >90 >90
XhoI	CCTCGAGG CCCTCGAGGG CCGCTCGAGCGG	8 10 12	0 10 10	0 25 75
XmaI	CCCCGGGG CCCCCGGGGG CCCCCCGGGGGG TCCCCCCGGGGGGA	8 10 12 14	0 25 50 >90	0 75 >90 >90

Created in Master PDF Editor - Demo Version