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

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

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