## 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  $\gamma$ -[ $^{32}$ P] ATP.  $1 \ \mu g$  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
AfIIII	CACATGTG	8	0	0
	CCACATGTGG	10	>90	>90
	CCCACATGTGGG	12	>90	>90
AscI	GGCGCGCC	8	>90	>90
	AGGCGCCCT	10	>90	>90
	TTGGCGCCCAA	12	>90	>90
Aval	CCCCGGGG	8	50	>90
	CCCCCGGGGG	10	>90	>90
	TCCCCCGGGGGA	12	>90	>90
BamHI	CGGATCCG	8	10	25
	CGGGATCCCG	10	>90	>90
	CGC <mark>GATCC</mark> GCG	12	>90	>90
BgIII	CAGATCTG	8	0	0
	GAAGATCTTC	10	75	>90
	GGAAGATCTTCC	12	25	>90
BssHII	GGCGCGCC	8	0	0
	AGGCGCCCT	10	0	0
	TTGGCGCGCCAA	12	50	>90
BstEII	GGGT(A/T)ACCC	9	0	10
BstXI	AACTGCAGAACCAATGCATTGG	22	0	0
	AAAACTGCAG <mark>CCAATGCATTGG</mark> AA	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

Hindiii					
CCGANTTCCG	EcoRI	GGAATTCC	8	>90	>90
HaeIII					
HaeIII					
ACCGCCGCT		CCGGAATTCCGG	12	>90	>90
ACCGCCGCT			_		
Hinditi	HaeIII	GGGCCCC	8	>90	>90
HindIII		AGC <mark>GGCC</mark> GCT	10	>90	>90
HindIII		TTGCGGCCGCAA	12	>90	>90
CCA AGCTTGGG		1100000001		, , ,	, , ,
CCA AGCTTGGG	HindIII	CAAGCTTG	8	0	0
CCCA AGCTTGGG	Tilliatti				
KpnI					
MIUI		CCCA <mark>AGCTT</mark> GGG	12	10	75
MIUI			_		
Milui	KpnI	GGGTACCC	8	0	0
Miul   GACGCGTC   8		GGGGTACCCC	10	>90	>90
Miul   GACGCGTC   8		CGGGGTACCCCG	12	>90	>90
CGACGCGTCG		000007700000		, , ,	- 70
Ncol   CCCATGGG	MluI	GACGCGTC	8	0	0
Ncol   CCCATGGG		CGACGCGTCG	10	25	50
Ndel		33/1033133	.0	20	00
Ndel	Ncol	CCCATGGG	8	0	0
Ndel         CCA TATGG         8         0         0           CCCA TATGGG         10         0         0           CCCCA TATGGGG         12         0         0           GGATTICA TATGGAATTCC         18         0         0           GGAATTCCA TATGGAATTCCC         20         75         >90           GGGAATTCCA TATGGAATTCCC         22         75         >90           Nbel         GCTAGCC         8         0         0           CGGCTAGCCG         10         10         25           CTACCTAGCTAG         12         10         50           Notl         TTGCGGCCGCTATAA         12         0         0           AATATAGGGCCGCTATAAA         12         0         10         10           AAGGAAAAAGCGGCCGCAAAAGGAAAA         28         25         >90           Nsil         TGCATGCATGCA         12         10         >90           Pacl         TTAATTAA         8         0         0         0           CCATAGCATTGGTTCTGCAGTT         22         >90         >90         90           Pmel         GTTAAAC         10         0         25           GCTCCAAC         8         0			1.4		
CCCA TATGGG		CATOGCATO	14	50	7.5
CCCA TATGGG	Ndel	CCATATGG	8	0	0
CGCCA TATGGCG   12					
Note   Gegatitica   Gegatiti					
Nhel   GGAATTCCA  TATGGAATTCC   20   75   >90			12	0	
Nhel   GGAATTCCA  TATGGAATTCC   20   75   >90		GGGTTT <mark>CA</mark> TATGAAACCC	18	0	0
Nhel   GGCTAGCC   8				75	>90
Nhel   GdCTAGCC   8					
Noti		GGGAATTCCATATGGAATTCCC	22	75	>90
Noti					
Noti	Nhel	G <mark>G</mark> CTAGCC	8	0	0
Noti		CGGCTAGCCG	10	10	25
Noti			12	10	50
ATTTGC   GGCCGCTTTA		o macina	12	10	30
ATTTGCGGCCGCTTTA	NotI	TTGCGGCCGCAA	12	0	0
AAATATGC GGCCGCTATAAA   20   10   10   10   10   ATAAGAATGC GGCCGCTAAACTAT   24   25   90   90   90   90   90   90   90   9	14011				
ATAAGAATG       GGCCGCTAAACTAT       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         Pmel       GTTTAAAC       8       0       0         GGTTTAAACC       10       0       25         GGGTTTAAACCC       10       0       25         GGGTTTAAACCC       12       0       50         AGCTTTGTTTAAACCGCCGCGCGCG       24       75       >90         PstI       GCTGCAGC       8       0       0         AACTGCAGGAACCAATGCATTGGA       14       10       10         AAAACTGCAGCCAATGCATTGGAA       24       >90       >90         AAAACTGCAGCCAATGCATTGGATGCAT       26       0       0         Pvul       CCGATCGG       8       0       0         ATCGATCGAT       10       10       25         ATCGATCGGA       <					
NSII       TGCATGCATGCA       12       10       >90         PacI       TTAATTAA       8       0       0         GTTAATTAAC       10       0       25         CCTTAATTAAGG       12       0       >90         Pmel       GTTTAAAC       8       0       0         GGTTTAAAC       10       0       25         GGGTTTAAACC       10       0       25         GGGTTTAAACC       12       0       50         AGCTTTGTTTAAACGGCGCGCCGG       24       75       >90         PstI       GCTGCAGC       8       0       0         AACTGCAGAGCCAATGCATTGG       14       10       10       10         AAACTGCAGAACCAATGCATTGGAA       24       >90       >90         AAAACTGCAGAACCAATGCATTGGAA       24       >90       >90         AAAACTGCAGAACCAATGCATTGGATGCAT       26       0       0         Pvul       CCGATCGG       8       0       0         ATCGATCGGA       10       10       10       25         TCGCGATCGCGA       12       0       10         Sacl       CGAGCTCG       8       10       10			20	10	10
NSII       TGCATGCATGCA       12       10       >90         CCAATGCATTGGTTCTGCAGTT       22       >90       >90         PacI       TTAATTAA       8       0       0         GTTAATTAAC       10       0       25         CCTTAATTAAGG       12       0       >90         Pmel       GTTTAAAC       8       0       0         GGTTTAAACC       10       0       25         GGGTTTAAACCC       10       0       25         GGGTTTAAACCC       12       0       50         AGCTTTGTTTAAACCGCGCGCGG       24       75       >90         PstI       GCTGCAGC       8       0       0         AACTGCAGAGCAGTGCA       14       10       10       10         AAAACTGCAGAGCCAATGCATTGGA       22       >90       >90         AAAACTGCAGAACCAATGCATTGGAA       24       >90       >90         CTGCAGAACCAATGCATTGGATGCAT       26       0       0         PvuI       CCGATCGG       8       0       0         ATCGATCGGA       12       0       10         SacI       CGAGCTCG       8       10       10		ATAAGAAT <mark>GO</mark> GGCCGCTAAACTAT	24	25	90
Nsil         TGCATGCATGCA CCAATGCATTGGTTCTGCAGTT         12 22         10 >90         >90           Pacl         TTAATTAA GTTAATTAAC CCCTTAATTAAGG         8 10 10 0 25 0 0         0 25 0 0         0 25 0 0         0 0 25 0 0         0 0 0 25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			28	25	>90
PacI         TTAATTAA         8         0         0           GTTAATTAAC         10         0         25           CCTTAATTAAGG         12         0         >90           PmeI         GTTTAAAC         8         0         0           GGTTTAAACC         10         0         25           GGGTTAAACCC         10         0         25           GGGTTTAAACCC         12         0         50           AGCTTTGTTTAAACGGCGCGCGGG         24         75         >90           PstI         GCTGCAGC         8         0         0           AACTGCAGAACCAGTGCATTGG         22         >90         >90           AAAACTGCAGCCAATGCATTGGAA         24         >90         >90           AAAACTGCAGCCAATGCATTGGAA         24         >90         >90           CTGCAGAACCAATGCATTGGAT         26         0         0           Pvul         CCGATCGG         8         0         0           ATCGATCGCT         10         10         25           TCGCGATCGCGA         12         0         10           Sacl         CGAGCTCG         8         10         10		7.1.1.0.07.1.1.1.1.0.0p.0.0.007.1.1.1.1.0.07.1.1.1.1			, , ,
PacI         TTAATTAA         8         0         0           GTTAATTAAC         10         0         25           CCTTAATTAAGG         12         0         >90           PmeI         GTTTAAAC         8         0         0           GGTTTAAACC         10         0         25           GGGTTAAACCC         10         0         25           GGGTTTAAACCC         12         0         50           AGCTTTGTTTAAACGGCGCGCGGG         24         75         >90           PstI         GCTGCAGC         8         0         0           AACTGCAGAACCAGTGCATTGG         22         >90         >90           AAAACTGCAGCCAATGCATTGGAA         24         >90         >90           AAAACTGCAGCCAATGCATTGGAA         24         >90         >90           CTGCAGAACCAATGCATTGGAT         26         0         0           Pvul         CCGATCGG         8         0         0           ATCGATCGCT         10         10         25           TCGCGATCGCGA         12         0         10           Sacl         CGAGCTCG         8         10         10	Nsil	TGCATGCATGCA	12	10	>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           AACTGCAGGAACCAATGCATTGG         22         >90         >90           AAAACTGCAGCCAATGCATTGGAA         24         >90         >90           CTGCAGAACCAATGCATTGGATGCAT         26         0         0           Pvul         CCGATCGG         8         0         0           ATCGATCGAT         10         10         25           TCGCGATCGCGA         12         0         10           Sacl         CGAGCTCG         8         10         10	14311				
CCTTAATTAAC   10   0   25   25   25   25   25   25   25		CCAATGCATTGGTTCTGCAGTT	22	>90	>90
CCTTAATTAAC   10   0   25   25   25   25   25   25   25	Pacl	TTAATTAA	8	0	0
CCTTAATTAAGG					
PmeI         GTTTAAAC GGTTTAAACC GGGTTTAAACCC         10         0         25           GGGTTTAAACCC GGGTTTAAACCCC         12         0         50           12         0         50         >90           PstI         GCTGCAGC TGCACTGCAGTGCA         8         0         0           AACTGCAGAACCAATGCATTGG         22         >90         >90           AAAACTGCAGCCAATGCATTGGAA         24         >90         >90           CTGCAGAACCAATGCATTGGATGCAT         26         0         0           PvuI         CCGATCGG ATCGATCGCT         8         0         0           SacI         CGAGCTCG         8         10         10					
GGTTTAAACC		CCTTAATTAAGG	12	0	>90
GGTTTAAACC	Dmol	CTTTAAAC	0	0	0
GGGTTTAAACCC	rinei				
AGCTTTGTTTAAACGGCGCGCGG			10	0	25
Pstl   GCTGCAGC   8   0   0     TGCACTGCAGTGCA   14   10   10     AACTGCAGAACCAATGCATTGG   22   >90   >90     AAAACTGCAGCCAATGCATTGGAA   24   >90   >90     CTGCAGAACCAATGCATTGGATGCAT   26   0   0     Pvul		GGGTTTAAACCC	12	0	50
Pstl   GCTGCAGC   8   0   0     TGCACTGCAGTGCA   14   10   10     AACTGCAGAACCAATGCATTGG   22   >90   >90     AAAACTGCAGCCAATGCATTGGAA   24   >90   >90     CTGCAGAACCAATGCATTGGATGCAT   26   0   0     Pvul		AGCTTTGTTTAAACGGCGCGCCGG	24	75	>90
TGCACTGCAGTGCA					
TGCACTGCAGTGCA	PstI	GCTGCAGC	8	0	0
AACTGCAGAACCAATGCATTGG		TGCACTGCAGTGCA			
AAAACTGCAGCCAATGCATTGGAA					
Pvul         CCGATCGG ATCGATCGAT ATCGATCGAT TCGCGATCGCGA         8         0         0           Sacl         CGAGCTCG         8         0         0           8         0         0         10         25           10         10         25         12         0         10           Sacl         CGAGCTCG         8         10         10					
Pvul       CCGATCGG ATCGATCGAT TCGCGATCGCGA       8       0       0         10       10       25         12       0       10         Sacl       CGAGCTCG       8       10       10		AAAA <mark>CTGCAG</mark> CCAATGCATTGGAA	24	>90	>90
ATCGATCGAT       10       10       25         TCGCGATCGCGA       12       0       10         Sacl       CGAGCTCG       8       10       10		CTGCAGAACCAATGCATTGGATGCAT	26	0	0
ATCGATCGAT       10       10       25         TCGCGATCGCGA       12       0       10         Sacl       CGAGCTCG       8       10       10					
TCGCGATCGCGA         12         0         10           Sacl         CGAGCTCG         8         10         10	Pvul	CCGATCGG	8	0	0
TCGCGATCGCGA         12         0         10           Sacl         CGAGCTCG         8         10         10					
Sacl CGAGCTCG 8 10 10					
·		TUGUGATUGUGA	12	U	10
·	SacI	CGAGCTCG	8	10	10
	Cooli	·			_
	Sacii				_
TCCCCGCGGGGA 12 50 >90		TCCCCGCGGGA	12	50	>90

Sall	GCGACGTCAAAAGGCCATAGCGGCCGC	28	0	0
	GCGCGACGTCTTGGCCATAGCGGCCGCGG	30	10	50
	ACGCCCGACGTCGGCCATAGCGGCCGCGGAA	32	10	75
Scal	GAGTACTC	8	10	25
	AAAAGTACTTTT	12	75	75
Smal	CCCGGG CCCCGGGGG CCCCCCGGGGGA	6 8 10 12	0 0 10 >90	10 10 50 >90
Spel	GACTAGTC GGACTAGTCC CGGACTAGTCCG CTAGACTAGTCTAG	8 10 12 14	10 10 0 0	>90 >90 50 50
SphI	GGCATGCC	8	0	0
	CATGCATGCATG	12	0	25
	ACATGCATGCATGT	14	10	50
Stul	AAGGCCTT GAAGGCCTTC AAAAGGCCTTTT	8 10 12	>90 >90 >90	>90 >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 CCCCCGGGGGGG TCCCCCCGGGGGGA	8 10 12 14	0 25 50 >90	0 75 >90 >90