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Cleavage Close to the End of DNA Fragments (linearized vector)

Linearized vectors were incubated with the indicated enzymes (10 units/µg) for 60 minutes at the recommended incubation temperature and NEBuffer for each enzyme. Following ligation and transformation, cleavage efficiencies were determined by dividing the number of transformants from the digestion reaction by the number obtained from religation of the linearized DNA (typically 100-500 colonies) and subtracting from 100%. "Base Pairs from End" refers to the number of double-stranded base pairs between the recognition site and the terminus of the fragment; this number does not include the single-stranded overhang from the initial cut. Since it has not been demonstrated whether these single-stranded nucleotides contribute to cleavage efficiency, 4 bases should be added to the indicated numbers when designing PCR primers. Average efficiencies were rounded to the nearest whole number; experimental variation was typically within 10%. The numbers in parentheses refer to the number of independent trials for each enzyme tested (from Moreira, R. and Noren, C. (1995), *Biotechniques*, 19, 56-59).

Note: As a general rule, enzymes not listed below require 6 bases pairs on either side of their recognition site to cleave efficiently.

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

Enzyme	Base pairs from End	%Cleavage Efficiency	Vector	Initial Cut
AatII	3 2 1	88 (2) 100 (2) 95 (2)	LITMUS 29 LITMUS 28 LITMUS 29	NcoI NcoI PinAI
Acc65I	2 1	99 (2) 75 (3)	LITMUS 29 pNEB193	SpeI SacI
AflII	1	13 (2)	LITMUS 29	StuI
AgeI	1 1	100 (1) 100 (2)	LITMUS 29 LITMUS 29	XbaI AatII
ApaI	2	100 (1)	LITMUS 38	SpeI
AscI	1	97 (2)	pNEB193	BamHI
AvrII	1	100 (2)	LITMUS 29	SacI
BamHI	1	97 (2)	LITMUS 29	HindIII
BglII	3	100 (2)	LITMUS 29	NsiI
BsiWI	2	100 (2)	LITMUS 29	BssHII
BspEI	2 1	100 (1) 8 (2)	LITMUS 39 LITMUS 38	BsrGI BsrGI
BsrGI	2 1	99 (2) 88 (2)	LITMUS 39 LITMUS 38	SphI BspEI
BssHII	2	100 (2)	LITMUS 29	BsiWI
EagI	2	100 (2)	LITMUS 39	NheI
EcoRI	1 1 1	100 (1) 88 (1) 100 (1)	LITMUS 29 LITMUS 29 LITMUS 39	XhoI PstI NheI

EcoRV	1	100 (2)	LITMUS 29	PstI
HindIII	3 2 1	90 (2) 91 (2) 0 (2)	LITMUS 29 LITMUS 28 LITMUS 29	NcoI NcoI BamHI
KasI	2 1	97 (1) 93 (1)	LITMUS 38 LITMUS 38	NgoMIV HindIII
KpnI	2 2 1	100 (2) 100 (2) 99 (2)	LITMUS 29 LITMUS 29 pNEB193	SpeI SacI SacI
MluI	2	99 (2)	LITMUS 39	EagI
MunI	2	100 (1)	LITMUS 39	NgoMIV
NcoI	2	100 (1)	LITMUS 28	HindIII
NgoMIV	2	100 (1)	LITMUS 39	MunI
NheI	1 2	100 (1) 82 (1)	LITMUS 39 LITMUS 39	EcoRI EagI
NotI	7 4 1	100 (2) 100 (1) 98 (2)	Bluescript SK- Bluescript SK- Bluescript SK-	SpeI KspI XbaI
NsiI	3 3 2	100 (2) 77 (4) 95 (2)	LITMUS 29 LITMUS 29 LITMUS 28	BssHII BglII BssHII
PacI	1	76 (3)	pNEB193	BamHI
PmeI	1	94 (2)	pNEB193	PstI
PstI	3 2 1	98 (1) 50 (5) 37 (3)	LITMUS 29 LITMUS 39 LITMUS 29	EcoRV HindIII EcoRI
SacI	1	99 (2)	LITMUS 29	AvrII
SalI	3 2 1	89 (2) 23 (2) 61 (3)	LITMUS 39 LITMUS 39 LITMUS 38	SpeI SphI SphI
SfiI	9 4 1	81 (2) 97 (2) 93 (2)	LITMUS 38 LITMUS 38 LITMUS 38	BamHI MluI EcoRI
SpeI	2 2	100 (2) 100 (2)	LITMUS 29 LITMUS 29	Acc65I KpnI
SphI	2 2 1	99 (1) 97 (1) 92 (2)	LITMUS 39 LITMUS 39 LITMUS 38	SalI BsrGI SalI
XbaI	1 1	99 (2) 94 (1)	LITMUS 29 LITMUS 29	AgeI PinAI
XhoI	1	97 (2)	LITMUS 29	EcoRI
XmaI	2 2	98 (1) 92 (1)	pNEB193 pNEB193	AscI BssHII

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

纽英伦生物技术(北京)有限公司

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限制性内切酶技术资料

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质量控制

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单链DNA的切割
Dam、Dcm和CpG甲基化(新)
CpG甲基化对内切酶的影响
E.coli的dam/dcm甲基化酶
切割后片段的平均长度
创造新的酶切位点
琼脂糖包埋法切割DNA
位点优势效应
大分子DNA图谱

遗传标记
NEB提供的菌株
其他E.coli菌株基因型

遗传密码表
氨基酸结构
DNA碱基对结构
核酸数据
同位素 酸碱 蛋白质数据
常见质粒中的基因产物

寡核苷酸近末端位点的酶切
(Cleavage Close to the End of DNA Fragments (oligonucleotides))

为了解不同内切酶对识别位点以外最少保护碱基数目的要求，NEB采用了一系列含识别序列的短双链寡核苷酸作为酶切底物进行实验。实验结果对于确定双酶切顺序将会有帮助（比如在多接头上切割位点很接近时），或者当切割位点靠近DNA末端时也很有用。在本表中没有列出的酶，则通常需在识别位点两端至少加上6个保护碱基，以确保酶切反应的进行。

实验方法：用γ-[^{32}P]ATP在T4多聚核苷酸激酶的作用下标记0.1A_{260}单位的寡核苷酸。取1 μg已标记了的寡核苷酸与20单位的内切酶，在20°C条件下分别反应2小时和20小时。反应缓冲液含70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM DTT及适量的NaCl或KCl（视酶的具体要求而定）。20%的PAGE（7 M尿素）凝胶电泳分析，经放射自显影确定酶切百分率。

本实验采用自连接的寡核苷酸作为对照。若底物有较长的回文结构，切割效率则可能因为出现发夹结构而降低。

酶	寡核苷酸序列	链长	切割率%	
			2 hr	**20 hr**
Acc I	GGTCGACC CGGTCGACCG CCGGTCGACCGG	8 10 12	0 0 0	0 0 0
Afl III	CACATGTG CCACATGTGG CCCACATGTGGG	8 10 12	0 >90 >90	0 >90 >90
Asc I	GGCGCGCC AGGCGCGCCT TTGGCGCGCCAA	8 10 12	>90 >90 >90	>90 >90 >90
Ava I	CCCCGGGG CCCCCGGGGG TCCCCCGGGGGA	8 10 12	50 >90 >90	>90 >90 >90
BamH I	CGGATCCG CGGGATCCCG CGCGGATCCGCG	8 10 12	10 >90 >90	25 >90 >90

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Bgl II	CAGATCTG GAAGATCTTC GGAAGATCTTCC	8 10 12	0 75 25	0 >90 >90
BssH II	GGCGCGCC AGGCGCGCCT TTGGCGCGCCAA	8 10 12	0 0 50	0 0 >90
BstE II	GGGT(A/T)ACCC	9	0	10
BstX I	AACTGCAGAACCAATGCATTGG AAAACTGCAGCCAATGCATTGGAA CTGCAGAACCAATGCATTGGATGCAT	22 24 27	0 25 25	0 50 >90
Cla I	CATCGATG GATCGATC CCATCGATGG CCCATCGATGGG	8 8 10 12	0 0 >90 50	0 0 >90 50
EcoR I	GGAATTCC CGGAATTCCG CCGGAATTCCGG	8 10 12	>90 >90 >90	>90 >90 >90
Hae III	GGGGCCCC AGCGGCCGCT TTGCGGCCGCAA	8 10 12	>90 >90 >90	>90 >90 >90
Hind III	CAAGCTTG CCAAGCTTGG CCCAAGCTTGGG	8 10 12	0 0 10	0 0 75
Kpn I	GGGTACCC GGGGTACCCC CGGGGTACCCCG	8 10 12	0 >90 >90	0 >90 >90
Mlu I	GACGCGTC CGACGCGTCG	8 10	0 25	0 50
Nco I	CCCATGGG CATGCCATGGCATG	8 14	0 50	0 75
Nde I	CCATATGG CCCATATGGG CGCCATATGGCG GGGTTTCATATGAAACCC GGAATTCCATATGGAATTCC GGGAATTCCATATGGAATTCCC	8 10 12 18 20 22	0 0 0 0 75 75	0 0 0 0 >90 >90
Nhe I	GGCTAGCC CGGCTAGCCG CTAGCTAGCTAG	8 10 12	0 10 10	0 25 50

Not I	TTGCGGCCGCAA ATTTGCGGCCGCTTTA AAATATGCGGCCGCTATAAA ATAAGAATGCGGCCGCTAAACTAT AAGGAAAAAAGCGGCCGCAAAAGGAAAA	12 16 20 24 28	0 10 10 25 25	0 10 10 90 >90
Nsi I	TGCATGCATGCA CCAATGCATTGGTTCTGCAGTT	12 22	10 >90	>90 >90
Pac I	TTAATTAA GTTAATTAAC CCTTAATTAAGG	8 10 12	0 0 0	0 25 >90
Pme I	GTTTAAAC GGTTTAAACC GGGTTTAAACCC AGCTTTGTTTAAACGGCGCGCCGG	8 10 12 24	0 0 0 75	0 25 50 >90
Pst I	GCTGCAGC TGCACTGCAGTGCA AACTGCAGAACCAATGCATTGG AAAACTGCAGCCAATGCATTGGAA CTGCAGAACCAATGCATTGGATGCAT	8 14 22 24 26	0 10 >90 >90 0	0 10 >90 >90 0
Pvu I	CCGATCGG ATCGATCGAT TCGCGATCGCGA	8 10 12	0 10 0	0 25 10
Sac I	CGAGCTCG	8	10	10
Sac II	GCCGCGGC TCCCCGCGGGGA	8 12	0 50	0 >90
Sal I	GTCGACGTCAAAAGGCCATAGCGGCCGC GCGTCGACGTCTTGGCCATAGCGGCCGCGG ACGCGTCGACGTCGGCCATAGCGGCCGCGGAA	28 30 32	0 10 10	0 50 75
Sca I	GAGTACTC AAAAGTACTTTT	8 12	10 75	25 75
Sma I	CCCGGG CCCCGGGG CCCCCGGGGG TCCCCCGGGGGA	6 8 10 12	0 0 10 >90	10 10 50 >90
Spe I	GACTAGTC GGACTAGTCC CGGACTAGTCCG CTAGACTAGTCTAG	8 10 12 14	10 10 0 0	>90 >90 50 50
Sph I	GGCATGCC CATGCATGCATG ACATGCATGCATGT	8 12 14	0 0 10	0 25 50

Stu I	AAGGCCTT GAAGGCCTTC AAAAGGCCTTTT	8 10 12	>90 >90 >90	>90 >90 >90
Xba I	CTCTAGAG GCTCTAGAGC TGCTCTAGAGCA CTAGTCTAGACTAG	8 10 12 14	0 >90 75 75	0 >90 >90 >90
Xho I	CCTCGAGG CCCTCGAGGG CCGCTCGAGCGG	8 10 12	0 10 10	0 25 75
Xma I	CCCCGGGG CCCCCGGGGG CCCCCCGGGGGG TCCCCCCGGGGGGA	8 10 12 14	0 25 50 >90	0 75 >90 >90

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