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 end of 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%.

Note: This data represents the minimum number of bases that will work, but is not recommended for maximum cleavage. 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	88	LITMUS 29	NcoI
	2	100	LITMUS 28	NcoI
	1	95	LITMUS 29	PinAI
Acc65I	2	99	LITMUS 29	SpeI
	1	75	pNEB193	SacI
AflII	1	13	LITMUS 29	StuI
AgeI	1	100	LITMUS 29	XbaI
	1	100	LITMUS 29	AatII
ApaI	2	100	LITMUS 38	SpeI
AscI	1	97	pNEB193	BamHI
AvrII	1	100	LITMUS 29	SacI
BamHI	1	97	LITMUS 29	HindIII
BglII	3	100	LITMUS 29	NsiI
BsiWI	2	100	LITMUS 29	BssHII
BspEI	2	100	LITMUS 39	BsrGI
	1	8	LITMUS 38	BsrGI
BsrGI	2	99	LITMUS 39	SphI
	1	88	LITMUS 38	BspEI
BssHII	2	100	LITMUS 29	BsiWI
EagI	2	100	LITMUS 39	NheI
EcoRI	1	100	LITMUS 29	XhoI
	1	88	LITMUS 29	PstI
	1	100	LITMUS 39	NheI
EcoRV	1	100	LITMUS 29	PstI
HindIII	3	90	LITMUS 29	NcoI
	2	91	LITMUS 28	NcoI
	1	0	LITMUS 29	BamHI
KasI	2	97	LITMUS 38	NgoMIV
	1	93	LITMUS 38	HindIII
KpnI	2	100	LITMUS 29	SpeI
	2	100	LITMUS 29	SacI
	1	99	pNEB193	SacI
MluI	2	99	LITMUS 39	EagI
MunI	2	100	LITMUS 39	NgoMIV
NcoI	2	100	LITMUS 28	HindIII
NgoMIV	2	100	LITMUS 39	MunI

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

* A modified version of LITMUS 38 with an introduced SfiI site was used for this test.