Restriction Enzyme Activity in Promega 10X Buffers, Reaction Temperature and Heat Inactivation.

The 10X Reaction Buffer supplied with each restriction enzyme is optimized to give 100% activity. In many cases good activity is also obtained using one of Promega's 4-CORE® 10X Buffers. Many commonly used cloning enzymes (i.e. restriction sites are found in vector multiple cloning sites) have buffers E and H as their optimal buffer and so we have determined the activity of many of our other restriction enzymes in those buffers as well. This table may be used to select the best buffer for digestion with multiple restriction enzymes. Enzyme activity is expressed as a percent of the activity obtained with the optimal buffer for each enzyme in a one-hour digest. Enzymes with 100% activity (green) perform as well as the optimal buffer. Enzymes with 50–75% or 75–100% (yellow) will give acceptable activity in that buffer. Enzymes with <10%, 10–25%, or 25–50% (pink) activity generally do not have acceptable activity in that buffer. Also, buffers leading to star activity of the enzyme (*; pink) should be avoided. If compatible buffers cannot be identified with acceptable activity for both enzymes, each digest should be performed separately in the optimal buffer for each enzyme.

Promega Enzyme	Cat.#	Buffer Supplied with Enzyme	Activity in							Heat Inactivation	Enzyme Assay Temperature
			A	B	C	D	E	H	MULTI-CORE™		
Aat II	R6541	J	50–75%	10–25%	<10%	<10%	10–25%	<10%	<10%	+	37°C
Acc I	R6411	G	50–75%	25–50%	25–50%	10–25%	<10%	<10%	25–50%	–	37°C
Acc III	R6581	F	<10%	10–25%	25–50%	25–50%	n.d.	n.d.	<10%	–	65°C
*Acc*65 I	R6921	D	10–25%	50–75%	75–100%	100%	75–100%	100–125%**	100%	+	37°C
*Acc*B7 I	R7081	E	10–25%	50–75%	100%*	<10%	100%	n.d.	100%	+	37°C
Age I	R7251	K	25–50%	25–50%	25–50%	50–75%	n.d.	n.d.	100%	+	37°C
Alu I	R6281	B	75–100%	100%	75–100%	10–25%	n.d.	n.d.	10–25%	+	37°C
*Alw*26 I	R6761	C	10–25%	25–50%	100%	10–25%	n.d.	n.d.	75–100%	+	37°C
*Alw*44 I	R6771	C	<10%	25–50%	100%	25–50%	n.d.	n.d.	100%	+	37°C
Apa I	R6361	A	100%	50–75%	50–75%	<10%	10–25%	<10%	75–100%	+	37°C
Ava I	R6091	B	10–25%	100%	50–75%	25–50%	100%	10–25%	<10%	+/–	37°C
Ava II	R6131	C	50–75%	50–75%	100%	25–50%	n.d.	n.d.	25–50%	+	37°C
Bal I	R6691	G	10–25%	<10%	<10%	<10%	n.d.	n.d.	<10%	+	37°C
*Bam*H I	R6021	E	75–100%*	75–100%	75–100%	50–75%	100%	50–75%	75–100%	+	37°C
Ban I	R6891	G	25–50%	25–50%	10–25%	<10%	n.d.	n.d.	100%	–	50°C
Ban II	R6561	E	75–100%	75–100%	75–100%	25–50%	n.d.	n.d.	100%	+	37°C
Bbu I	R6621	A	100%	75–100%	75–100%	<10%	10–25%	10–25%	100%	+	37°C
Bcl I	R6651	C	10–25%	75–100%	100%	50–75%	50–75%	50–75%	10–25%	–	50°C
Bgl I	R6071	D	10–25%	25–50%	75–100%	100%	25–50%	75–100%	100%	+	37°C
Bgl II	R6081	D	25–50%	75–100%	75–100%	100%	n.d.	n.d.	<10%	–	37°C
*Bsa*M I	R6991	D	10–25%	25–50%	50–75%	100%	n.d.	n.d.	25–50%	–	65°C
*Bsp*1286 I	R6741	A	100%	50–75%	25–50%	10–25%	n.d.	n.d.	75–100%	+	37°C
*Bsr*S I	R7241	D	10–25%	25–50%	10–25%	100%	n.d.	n.d.	100%	–	65°C
*Bss*H II	R6831	H	75–100%	50–75%	75–100%	50–75%	n.d.	100%	75–100%	–	50°C
*Bst*98 I	R7141	D	<10%	10–25%	10–25%	100%	n.d.	n.d.	25–50%	–	37°C
*Bst*E II	R6641	D	25–50%	50–75%	50–75%	100%	n.d.	n.d.	100%	–	60°C
*Bst*O I	R6931	C	10–25%	25–50%	100%	25–50%	n.d.	n.d.	<10%	–	60°C
*Bst*X I	R6471	D	<10%	10–25%	25–50%	100%	100%	75–100%	10–25%	+/–	50°C
*Bst*Z I	R6881	D	<10%	<10%	10–25%	100%	10–25%	75–100%	10–25%	–	50°C
*Bsu*36 I	R6821	E	<10%	25–50%	50–75%	25–50%	100%	n.d.	50–75%	–	37°C
Cfo I	R6241	B	75–100%	100%	75–100%	25–50%	n.d.	n.d.	100%	+/–	37°C
Cla I	R6551	C	75–100%	75–100%	100%	75–100%	100%	50–75%	100%	+	37°C
Csp I	R6671	K	<10%	10–25%	25–50%	50–75%	100%	100–125%**	10–25%	+	30°C
*Csp*45 I	R6571	B	25–50%	100%	50–75%	25–50%	100%	25–50%	50–75%	+	37°C
Dde I	R6291	D	25–50%	25–50%	50–75%	100%	n.d.	n.d.	25–50%	+/–	37°C
Dpn I	R6231	B	50–75%	100%	75–100%	50–75%	n.d.	n.d.	100%	+	37°C
Dra I	R6271	B	75–100%	100%	75–100%	50–75%	n.d.	n.d.	25–50%	+	37°C
*Ecl*HK I	R7111	E	<10%	<10%	75–100%	10–25%	100%	n.d.	50–75%	+	37°C
*Eco*47 III	R6731	D	<10%	25–50%	50–75%	100%	n.d.	n.d.	25–50%	+	37°C
*Eco*52 I	R6751	L	<10%	<10%	10–25%	25–50%	25–50%	50–75%	<10%	+	37°C
*Eco*ICR I	R6951	B	10–25%	100%	75–100%	<10%	25–50%	n.d.	100%	+	37°C
*Eco*R I	R6011	H	25–50%	50–75%	50–75%	50–75%	75–100%	100%	100%*	+	37°C
*Eco*R V	R6351	D	10–25%	25–50%	50–75%	100%	25–50%	50–75%	100%	+	37°C
Fok I	R6781	B	75–100%	100%	75–100%	25–50%	n.d.	n.d.	50–75%	+	37°C
Hae II	R6661	B	50–75%	100%	50–75%	10–25%	n.d.	n.d.	100%	–	37°C
Hae III	R6171	C	75–100%	75–100%	100%	50–75%	n.d.	n.d.	100%	–	37°C
Hha I	R6441	C	50–75%	75–100%	100%	50–75%	n.d.	n.d.	75–100%	+	37°C
Hinc II	R6031	B	25–50%	100%	25–50%	50–75%	75–100%	50–75%	100%	+	37°C
Hind III	R6041	E	25–50%	100%	75–100%	10–25%	100%	25–50%	50–75%	+	37°C
*Hin*f I	R6201	B	50–75%	100%	75–100%	75–100%	n.d.	n.d.	50–75%	–	37°C

Restriction Enzyme Activity in Promega 10X Buffers, Reaction Temperature and Heat Inactivation (continued).

Promega Enzyme	Cat.#	Buffer Supplied with Enzyme	Activity in A	B	C	D	E	H	MULTI-CORE™	Heat Inactivation	Enzyme Assay Temperature
Hpa I	R6301	J	25–50%	50–75%	25–50%	10–25%	n.d.	n.d.	100%	–	37°C
Hpa II	R6311	A	100%	50–75%	50–75%	10–25%	n.d.	n.d.	100%	–	37°C
*Hsp*92 I	R7151	F	10–25%	75–100%	50–75%	25–50%	n.d.	n.d.	10–25%	+	37°C
*Hsp*92 II	R7161	K	10–25%	25–50%	25–50%	<10%	n.d.	n.d.	<10%	+	37°C
I-*Ppo* I	R7031	NA	10–25%	25–50%	25–50%	25–50%	n.d.	n.d.	—	+	37°C
Kpn I	R6341	J	100%*	25–50%	25–50%	<10%	25–50%	<10%	75–100%	+/–	37°C
Mbo I	R6711	C	10–25%	75–100%	100%	50–75%	n.d.	n.d.	<10%	+	37°C
Mbo II	R6723	B	10–25%	100%	50–75%	75–100%	n.d.	n.d.	100%	+	37°C
Mlu I	R6381	D	10–25%	25–50%	50–75%	100%	25–50%	100–125%**	10–25%	+/–	37°C
Msp I	R6401	B	75–100%	100%	75–100%	25–50%	n.d.	n.d.	25–50%	+	37°C
*Msp*A1 I	R7021	C	25–50%	100%*	100%	10–25%	n.d.	n.d.	100%	+	37°C
Nae I	R7131	A	100%	50–75%	25–50%	<10%	n.d.	n.d.	50–75%	+	37°C
Nar I	R6861	G	75–100%	50–75%	75–100%	25–50%	n.d.	n.d.	50–75%	+	37°C
Nci I	R7061	B	100%*	100%	25–50%	25–50%	n.d.	n.d.	50–75%	+	37°C
Nco I	R6513	D	50–75%	75–100%	75–100%	100%	100%	100–125%**	75–100%	+	37°C
Nde I	R6801	D	<10%	<10%	25–50%	100%	n.d.	n.d.	25–50%	+	37°C
Nde II	R7291	D	<10%	<10%	10–25%	100%	n.d.	n.d.	25–50%	+	37°C
*Ngo*M IV	R7171	MULTI-CORE™	100%*	100%*	100%*	<10%	n.d.	n.d.	100%	+	37°C
Nhe I	R6501	B	75–100%	100%	75–100%	10–25%	75–100%	10–25%	100%	+	37°C
Not I	R6431	D	<10%	10–25%	25–50%	100%	25–50%	100–125%**	25–50%	+	37°C
Nru I	R7091	K	<10%	<10%	<10%	50–75%	n.d.	n.d.	10–25%	+	37°C
Nsi I	R6531	D	10–25%	50–75%	50–75%	100%	25–50%	>125%**	10–25%	+/–	37°C
Pst I	R6111	H	10–25%	50–75%	50–75%	50–75%	25–50%	100%	25–50%	+	37°C
Pvu I	R6321	D	10–25%	25–50%	50–75%	100%	n.d.	n.d.	<10%	–	37°C
Pvu II	R6331	B	25–50%	100%	50–75%	25–50%	n.d.	n.d.	50–75%	+	37°C
Rsa I	R6371	C	75–100%	75–100%	100%	<10%	n.d.	n.d.	<10%	+	37°C
Sac I	R6061	J	75–100%	25–50%	25–50%	<10%	100%	25–50%	100%	+	37°C
Sac II	R6221	C	100%	50–75%	100%	50–75%	25–50%	>125%**	<10%	+	37°C
Sal I	R6051	D	<10%	10–25%	25–50%	100%	25–50%	25–50%	<10%	+	37°C
*Sau*3A I	R6191	B	25–50%	100%	75–100%	<10%	n.d.	n.d.	100%	+	37°C
Sca I	R6211	K	<10%	100%*	50–75%	75–100%	n.d.	n.d.	10–25%	+	37°C
Sfi I	R6391	B	75–100%	100%	75–100%	25–50%	75–100%	50–75%	75–100%	–	50°C
Sgf I	R7103	C	25–50%	25–50%	100%	<10%	n.d.	n.d.	<10%	+/–	37°C
Sin I	R6141	A	100%	75–100%	50–75%	10–25%	n.d.	n.d.	100%	+	37°C
Sma I	R6121	J	<10%	<10%	<10%	<10%	<10%	<10%	100%	+	25°C
*Sna*B I	R6791	B	50–75%	100%	50–75%	<10%	n.d.	n.d.	100%	–	37°C
Spe I	R6591	B	75–100%	100%	75–100%	75–100%	100%	25–50%	100%	+	37°C
Sph I	R6261	K	75–100%	75–100%	100%*	75–100%	100%	>125%**	10–25%	+	37°C
Ssp I	R6601	E	10–25%	50–75%	50–75%	75–100%	100%	100–125%**	50–75%	+	37°C
Stu I	R6421	B	75–100%	100%	75–100%	50–75%	n.d.	n.d.	50–75%	+	37°C
Sty I	R6481	F	25–50%	75–100%	75–100%	75–100%	10–25%	50–75%	<10%	+	37°C
Taq I	R6151	E	10–25%	25–50%	50–75%	50–75%	100%	n.d.	100%	–	65°C
*Tru*9 I	R7011	F	75–100%	50–75%	75–100%	25–50%	n.d.	n.d.	25–50%	–	65°C
*Tth*111 I	R6841	B	50–75%	100%	75–100%	25–50%	n.d.	n.d.	100%	–	65°C
Vsp I	R6851	D	<10%	25–50%	75–100%	100%	n.d.	n.d.	<10%	+	37°C
Xba I	R6181	D	50–75%	75–100%	75–100%	100%	100%	100–125%**	100%	–	37°C
Xho I	R6161	D	25–50%	75–100%	75–100%	100%	25–50%	100–125%**	10–25%	+	37°C
Xho II	R6811	C	25–50%	25–50%	100%	10–25%	n.d.	n.d.	<10%	+	37°C
Xma I	R6491	B	50–75%	100%	25–50%	<10%	25–50%	<10%	50–75%	+	37°C
Xmn I	R7271	B	75–100%	100%	75–100%	10–25%	n.d.	n.d.	75–100%	+	37°C

* Not recommended due to potential star activity.
** Unit activity is based on recommended buffer. In Buffer H, some enzymes have enhanced activity.
n.d. = Not determined.

Heat Inactivation Key:

+	=greater than 95% inactivation (DNA is undigested)
–	=less than 95% inactivation (DNA digest is complete, i.e., ≥5% of the initial 20 activity units [≥1 unit] remains)
+/–	=partial inactivation (DNA is partially digested)

Heat Inactivation Conditions:
Twenty units of enzyme in 50µl of its optimal buffer were heated at 65°C for 15 minutes.
One microgram of DNA was added and incubated for 1 hour in accordance with the unit definition, then analyzed by agarose gel electrophoresis.

Isoschizomers. **The enzymes in boldface type are available from Promega.**

Enzyme	Isoschizomer(s)	Recognition Sequence
mAat I	***Stu* I**, *Eco*147 I, *Pme*55 I, *Sse*B I	AGG▼CCT
***Aat* II**	—	GACGT▼C
***Acc* I**	*Fbl* I, *Xmi* I	GT▼(A/C)(G/T)AC
***Acc* III**	*Bsp*E II, *Mro* I	T▼CCGGA
***Acc*65 I**	*Asp*718 I	G▼GTACC
	Kpn* I	GGTAC▼C
*Acc*B1 I	***Ban* I**, *Bsh*N I, *Eco*64 I	G▼G(C/T)(G/A)CC
***Acc*B7 I**	*Pfl*M I, *Van*91 I	CCAN$_4$▼NTGG
*Acl*N I	***Spe* I**	A▼CTAGT
*Acl*W I	*Alw* I	GGATCNNNN▼
Acy I	*Bbi* II, *Hin*1 I, ***Hsp*92 I**, *Bsa*H I, *Msp*171 I	G(A/G)▼CG(T/C)C
Acs I	*Apo* I	(G/A)▼AATT(C/T)
Afa I	*Csp*6 I*, ***Rsa* I**	GT▼AC
Afe I	***Eco*47 III**	AGC▼GCT
Afl II	***Bst*98 I**	C▼TTAAGG
***Age* I**	*Pin*A I	A▼CCGGT
Aha III	***Dra* I**	TTT▼AAA
Ahd I	***Ecl*HK I**	GACNNN▼NNGTC
***Alu* I**	—	AG▼CT
Alw I	*Acl*W I	GGATCNNNN▼
***Alw*26 I**[1]	*Bsm*A I	GTCTC(1/5)
***Alw*44 I**	*Apa*L I	G▼TGCAC
Aoc I	***Bsu*36 I**, *Cvn* I	CC▼TNAGG
***Apa* I**	*Bsp*120 I	GGGCC▼C
*Apa*L I	***Alw*44 I**, *Vne* I	G▼TGCAC
Apo I	*Acs* I	(G/A)▼AATT(C/T)
Ase I	***Vsp* I**, *Asn* I	AT▼TAAT
Asn I	***Vsp* I**, *Ase* I	AT▼TAAT
Asp I	***Tth*111 I**	GACN▼NNGTC
*Asp*E I	*Ahd* I, *Eam*1105 I, ***Ecl*HK I**	GACNNN/NNGTC
*Asp*700 I	***Xmn* I**	GAANN▼NNTTC
*Asp*718 I	***Acc*65 I**	G▼GTACC
	Kpn* I	GGTAC▼C
Asu I	*Sau*96 I, *Cfr*13 I	G▼GNCC
Asu II	*Csp*45 I, *Bst*B I	TT▼CGAA
*Asu*HP I	*Hph* I	GGTGAN$_8$▼
***Ava* I**	*Ama*87 I, *Bco* I, *Bso*B I, *Eco*88 I	C▼(C/T)CG(G/A)G
***Ava* II**	***Sin* I**, *Eco*47 I, *Hgi*E I	G▼G(A/T)CC
Axy I	***Bsu*36 I**	CC▼TNAGG
***Bal* I**	*Msc* I, *Mlu*N I	TGG▼CCA
***Bam*H I**	—	G▼GATCC
***Ban* I**	*Acc*BI, *Bsh*N I, *Eco*64 I	G▼G(T/C)(A/G)CC
***Ban* II**	*Eco*24 I	G(A/G)GC(T/C)▼C
Bbe I	—	GGCGC▼C
	Nar* I	GG▼CGCC
*Bbr*P I	*Eco*72 I, *Pml* I	CAC▼GTG
Bbs I[1]	*Bsc*91 I, *Bpi* I	GAAGAC(2/6)
***Bbu* I**	*Pae* I, ***Sph* I**	GCATG▼C
***Bcl* I**	*Bsi*Q I, *Fba* I	T▼GATCA
Bcn I	***Nci* I**	CC▼(C/G)GG
Bfr I	***Bst*98 I**	C▼TTAAG
***Bgl* I**	—	GCCNNNN▼NGGC
***Bgl* II**	—	A▼GATCT
Bmy I	***Bsp*1286 I**	G(G/A/T)GC(C/A/T)▼C
Bpm I	*Gsu* I	CTGGAG(16/14)
*Bsa*H I	***Hsp*92 I**	G(A/G)▼CG(T/C)C
***Bsa*M I**	*Bsm* I	GAATGC(1/–1)
*Bsa*O I	*Bsh*1285 I, *Bsi*E I	CG(A/G)(T/C)▼C
*Bse*A I	***Acc* III**	T▼CCGGA
*Bse*N I	***Bsr*S I**, *Bsr* I	ACTGGN (1/–1)
*Bse*P I	***Bss*H II**, *Pau* I	G▼CGCGC
*Bsh*1285 I	*Bsa*O I	CG(A/G)(T/C)▼CG
*Bsh*N I	***Ban* I**, *Acc*B1 I, *Eco*64 I	G▼G(T/C)(A/G)CC
*Bsh*1365 I	*Bsr*BR I	GATNN▼NNATC
*Bsi*E I	*Bsa*O I	CG(A/G)(T/C)▼CG

Enzyme	Isoschizomer(s)	Recognition Sequence
Bsm I	***Bsa*MI**	GAATGCN▼
*Bsm*A I[1]	***Alw*26 I**	GTCTC(1/5)
*Bso*B I	***Ava* I**, *Ama*87 I, *Bco* I, *Eco*88 I	C(C/T)CG(G/A)G
*Bsp*19 I	***Nco* I**	C▼CATGG
*Bsp*68 I	***Nru* I**	TCG▼CGA
*Bsp*106 I	***Cla* I**, *Bsa*D I	AT▼CGAT
*Bsp*119 I	***Csp*45 I**, *Nsp* V, *Bst* B I	TT▼CGAA
*Bsp*120 I	***Apa* I**	G▼GGCCC
*Bsp*143 I	***Mbo* I**, ***Sau*3A I**, ***Nde* II**	▼GATC
*Bsp*143 II	***Hae* II**	(A/G)GCGC▼(T/C)
***Bsp*1286 I**	*Bmy* I, *Sdu* I	G(G/A/T)GC(C/A/T)▼C
*Bsp*C I	***Pvu* I**	CGAT▼CG
*Bsp*D I	***Cla* I**	AT▼CGAT
*Bsp*E I	***Acc* III**	T▼CCGGA
Bsr I[1]	***Bsr*S I**, *Bse*N I	ACTGGN(1/-1)
***Bsr*S I**[1]	*Bse*N I, *Bsr* I	ACTGGN(1/-1)
***Bss*H II**	*Bse*P I, *Pau* I	G▼CGCGC
***Bst*98 I**	*Afl* II, *Bfr* I	C▼TTAAG
*Bst*B I	***Csp*45 I**, *Nsp* V, *Bsp*119 I	TT▼CGAA
***Bst*E II**	*Bst*P I, *Eco*91 I, *Psp*E I	G▼GTNACC
*Bst*N I	***Bst*O I**, *Mva* I, *Eco*R II	CC▼(A/T)GG
***Bst*O I**	*Bst*N I, *Eco*R II, *Mva* I	CC▼(A/T)GG
***Bst*X I**	–	CCANNNNN▼NTGG
*Bst*Y I	***Xho* II**, *Mfl* I	(A/G)▼GATC(T/C)
***Bst*Z I**	***Eco*52 I**, *Eag* I, *Xma* III, *Ecl*X I	C▼GGCCG
*Bsu*15 I	***Cla* I**	AT▼CGAT
***Bsu*36 I**	*Cvn* I, *Aoc* I, *Eco*81 I	CC▼TNAGG
*Bsu*R I	***Hae* III**, *Pal* I	GG▼CC
***Cfo* I**	***Hha* I**	GCG▼C
	*Hin*6 I	GCG▼C
	*Hin*P1 I*	G▼CGC
*Cfr*9 I	***Xma* I**	C▼CCGGG
	Sma* I	CCC▼GGG
*Cfr*13 I	*Sau*96 I	G▼GNCC
*Cfr*42 I	***Sac* II**	CCGC▼GG
***Cla* I**	*Ban* III, *Bsp*106 I, *Bsp*D I, *Bsu*15 I	AT▼CGAT
Cpo I	***Csp* I**, *Rsr* II	CG▼G(A/T)CCG
***Csp* I**	Cpo I, *Rsr* II	CG▼G(A/T)CCG
*Csp*6 I	***Rsa* I***, *Afa* I*	GT▼AC
***Csp*45 I**	*Bst*B I, *Nsp* V, *Bsp*119 I	TT▼CGAA
Cvn I	***Bsu*36 I**	CC▼TNAGG
***Dde* I**	*Bst*DE I	C▼TNAG
***Dpn* I**[2]	*Dpn* II*	G^{me}A▼TC
Dpn II	***Mbo* I**, ***Sau*3A I**, ***Nde* II**, ***Dpn* I***	▼GATC
***Dra* I**	—	TTT▼AAA
Eag I	***Eco*52 I**, ***Bst*Z I**, *Ecl*X I, *Xma* III	C▼GGCCG
*Eam*1105 I	***Ecl*HK I**, *Ahd* I, *Asp*E I	GACNNN▼NNGTC
*Ecl*136 II	***Eco*ICR I**	GAG▼CTC
	Sac* I	GAGCT▼C
***Ecl*HK I**	*Ahd* I, *Eam*1105 I, *Asp*E I	GACNNN▼NNGTC
*Ecl*X I	***Bst*Z I**, *Eag* I, ***Eco*52 I**, *Xma* III	C▼GGCCG
*Eco*24 I	***Ban* II**, *Fri*O I	G(AG)GC(TC)▼C
*Eco*32 I	***Eco*R V**	GAT▼ATC
*Eco*47 I	***Ava* II**, ***Sin* I**	G▼G(A/T)CC
***Eco*47 III**	*Afe* I	AGC▼GCT
***Eco*52 I**	***Bst*Z I**, *Xma* III, *Eag* I, *Ecl*X I	C▼GGCCG
*Eco*64 I	***Ban* I**, *Bsh*N I, *Eco*64 I	G▼G(TC)(AG)CC
*Eco*81 I	***Bsu*36 I**	CC▼TNAGG
*Eco*88 I	***Ava* I**	C▼(TC)CG(AG)G
*Eco*91 I	***Bst*E II**	G▼GTNACC
*Eco*105 I	***Sna*B I**	TAC▼CTA
*Eco*130 I	***Sty* I**	C▼C(A/T)(T/A)GG
*Eco*147 I	***Stu* I**	AGG▼CCT
***Eco*ICR I**	*Ecl*136 II	GAG▼CTC
	Sac* I	GAGCT▼C
	Sst I*	GAGCT▼C

Isoschizomers (continued). **The enzymes in boldface type are available from Promega.**

Enzyme	Isoschizomer(s)	Recognition Sequence
***Eco*R I**	—	G▼AATTC
*Eco*R II	***Bst*O I**, *Bst*N I, *Mva* I	CC▼(A/T)GG
***Eco*R V**	*Eco*32 I	GAT▼ATC
*Eco*T14 I	***Sty* I**	C▼C(A/T)(A/T)GG
*Eco*T22 I	***Nsi* I**	ATGCA▼T
Ehe I	***Nar* I***	GG▼CGCC
***Fok* I**[2]	—	GGATG(9/13)
***Hae* II**	*Bsp*143 II	(A/G)GCGC▼(T/C)
***Hae* III**	*Bsu*R I, *Pal* I	GG▼CC
Hap II	***Hpa* II**, ***Msp* I**	C▼CGG
*Hgi*E I	*Eco*47 I, ***Sin* I**, ***Ava* II**	G▼G(A/T)CC
***Hha* I**	***Cfo* I**	GCG▼C
	*Hin*P1 I*, *Hin*6 I*	G▼CGC
*Hin*1 I	*Acy* I, ***Hsp*92 I**	G(A/G)▼CG(T/C)C
***Hin*c II**	*Hin*d II	GT(T/C)▼(A/G)AC
*Hin*d II	***Hin*c II**	GT(T/C)▼(A/G)AC
***Hin*d III**	—	A▼AGCTT
***Hin*f I**	—	G▼ANTC
*Hin*P1 I	—	G▼CGC
	Hha* I, ***Cfo* I***	GCG▼C
***Hpa* I**	*Ksp*A I	GTT▼AAC
***Hpa* II**[3]	***Msp* I**, *Hap* II	C▼CGG
***Hsp*92 I**	*Acy* I, *Bsa*H I, *Hin*1 I	G(A/G)▼CG(C/T)
***Hsp*92 II**	*Nla* III	CATG▼
***I-Ppo* I**	—	CTCTCTTAA▼GGTAGC
Kas I	***Nar* I***	GG▼CGCC
***Kpn* I**	—	GGTAC▼C
	Acc*65 I, *Asp*718 I*	G▼GTACC
Ksp I	***Sac* II**	CCGC▼GG
***Mbo* I**	***Sau*3A I**, ***Nde* II**, *Dpn* II	▼GATC
***Mbo* II**[1]	—	GAAGA(8/7)
Mfl I	***Xho* II**	(A/G)▼GATC(T/C)
***Mlu* I**	—	A▼CGCGT
*Mlu*N I	***Bal* I**, *Msc* I	TGG▼CCA
Mro I	***Acc* III**	T▼CCGGA
Msc I	***Bal* I**, *Mlu*N I	TGG▼CCA
Mse I	***Tru*9 I**	T▼TAA
***Msp* I**[3]	***Hpa* II**, *Hap* II	C▼CGG
***Msp*A1 I**	*Nsp*B II	C(A/C)G▼C(G/T)G
Mst II	***Bsu*36 I**	CC▼TNAGG
Mva I	***Bst*O I**, *Eco*R II, *Bst*N I	CC▼(A/T)GG
***Nae* I**	***Ngo*M IV**	G▼CCGGC
***Nar* I**	—	GG▼CGCC
	Ehe I*	GGC▼GCC
	Kas I*	G▼GCGCC
	Bbe I*	GGCGC▼C
***Nci* I**	*Bcn* I	CC▼(C/G)GG
***Nco* I**	*Bsp*19 I	C▼CATGG
***Nde* I**	—	CA▼TATG
***Nde* II**	***Mbo* I**, ***Sau*3A I**, *Dpn* II	▼GATC
***Ngo*M IV**	***Nae* I**	G▼CCGGC
***Nhe* I**	—	G▼CTAGC
Nla III	***Hsp*92 II**	CATG▼
***Not* I**	—	GC▼GGCCGC
***Nru* I**	*Bsp*68 I	TCG▼CGA
***Nsi* I**	*Eco*T22 I, *Mph*1103 I	ATGCA▼T
Nsp V	***Csp*45 I**, *Bst*B I, *Bsp*119 I	TT▼CGAA
*Nsp*B II	***Msp*A1 I**	C(A/C)G▼C(G/T)G
Pae I	***Bbu* I**, ***Sph* I**	GCATG▼C
*Pae*R7 I	***Xho* I**	C▼TCGAG
Pal I	***Hae* III**, *Bsu*R I	GG▼CC
*Pfl*M I	***Acc*B7 I**, *Vau*91 I	CCAN$_4$▼NTGG
*Pin*A I	***Age* I**	A▼CCGGT
***Pst* I**	—	CTGCA▼G
***Pvu* I**	*Bsp*C I	CGAT▼CG
***Pvu* II**	—	CAG▼CTG
***Rsa* I**	*Afa* I	GT▼AC
Rsr II	***Csp* I**, *Cpo* I	CG▼G(A/T)CCG
***Sac* I**	*Sst* I	GAGCT▼C
	*Ecl*136 II*, ***Eco*ICR I***	GAG▼CTC
***Sac* II**	*Sst* II, *Ksp* I, *Cfr*42 I	CCGC▼GG
***Sal* I**	—	G▼TCGAC
***Sau*3A I**	***Mbo* I**, ***Nde* II**, *Dpn* II	▼GATC
*Sau*96 I	*Cfr*13 I	G▼GNCC
***Sca* I**	—	AGT▼ACT
Sdu I	***Bsp*1286 I**	G(G/A/T)GC(C/A/T)▼C
***Sfi* I**	—	GGCCNNNN▼NGGCC
Sfu I	***Csp*45 I**	TT▼CGAA
***Sgf* I**	—	GCGAT▼CGC
***Sin* I**	***Ava* II**, *Eco*47 I	G▼G(A/T)CC
***Sma* I**	—	CCC▼GGG
	Xma* I, *Cfr*9 I*	C▼CCGGG
***Sna*B I**	*Eco*105 I	TAC▼GTA
***Spe* I**	*Acl*N I	A▼CTAGT
***Sph* I**	***Bbu* I**, *Pae* I	GCATG▼C
***Ssp* I**	—	AAT▼ATT
Sst I	***Sac* I**	GAGCT▼C
	Eco*ICR I	GAG▼CTC
Sst II	***Sac* II**	CCGC▼GG
***Stu* I**	*Aat* I, *Eco*147 I	AGG▼CCT
***Sty* I**	*Eco*T14 I	C▼C(A/T)(A/T)GG
***Taq* I**	*Tth*HB8 I	T▼CGA
***Tru*9 I**	*Mse* I	T▼TAA
***Tth*111 I**	*Asp* I	GACN▼NNGTC
*Tth*HB8 I	***Taq* I**	T▼CGA
*Van*91 I	***Acc*B7 I**, *Pfl*M I	CCAN$_4$▼NTGG
Vne I	*Apa*L I, ***Alw*44 I**	G▼TGCAC
***Vsp* I**	*Ase* I, *Asn* I	AT▼TAAT
***Xba* I**	—	T▼CTAGA
***Xho* I**	*Pae*R7 I	C▼TCGAG
***Xho* II**	*Bst*Y I, *Mfl* I	(A/G)▼GATC(T/C)
***Xma* I**	*Cfr*9 I, *Xma*C I,	C▼CCGGG
	Sma* I	CCC▼GGG
Xma III	***Eco*52 I**, ***Bst*Z I**, *Eag* I, *Ecl*X I	C▼GGCCG
*Xma*C I	***Xma* I**	C▼CCGGG
	Sma* I	CCC▼GGG
***Xmn* I**	*Asp*700 I	GAANN▼NNTTC

Key:

N = A, C, G or T
* = neoschizomer

Notes:

1. The locations of cleavage sites falling outside the recognition site are indicated in parentheses. For example, GTCTC(1/5) indicates cleavage at:

   5′...GTCTCN▼...3′
   3′...CAGAGNNNNN▲...5′

2. *Dpn* I is unique among commercially available restriction enzymes in requiring methylation of a nucleotide (adenine) in its recognition sequence in order to cut. Therefore, *Dpn* I cannot be substituted for other enzymes recognizing the GATC sequence (e.g., *Mbo* I and *Sau*3A I).
3. Although *Hpa* II and *Msp* I recognize the same nucleotide sequence, *Hpa* II is sensitive to methylation of either cytosine in its recognition sequence, while *Msp* I is sensitive only to methylation of the external cytosine. These enzymes may not be interchanged for all applications.

Reference

Roberts, R.J. (1991) *Nucl. Acids Res.* **19** (supp), 2077–109.

Compatible Ends.

Promega Restriction Enzymes That Generate 5′ Overhangs

Overhang	Definite Compatible Ends	Possible Compatible Ends
5′-N		*Tth*111 I
5′-S		*Nci* I
5′-W		*Bst*O I
5′-AT	*Acc* I	
5′-CG	*Nar* I, *Msp* I, *Hsp*92 I, *Taq* I, *Cla* I, *Csp*45 I, *Hpa* II	
5′-GN		*Bsr*S I
5′-MK		*Acc* I
5′-TA	*Vsp* I, *Nde* I, *Tru*9 I	
5′-ANT		*Hin*f I
5′-GNC		*Sau*96 I
5′-GWC		*Ava* II, *Csp* I, *Sin* I
5′-TNA		*Dde* I, *Bsu*36 I
5′-AATT	*Eco*R I	
5′-AGCT	*Hin*d III	
5′-CATG	*Nco* I	*Sty* I
5′-CCGG	*Age* I, *Xma* I, *Acc* III, *Ngo*M IV	*Ava* I
5′-CGCG	*Mlu* I, *Bss*H I	
5′-CTAG	*Spe* I, *Nhe* I, *Xba* I	*Sty* I
5′-CWWG		*Sty* I
5′-GATC	*Mbo* I, *Sau*3A I, *Bam*H I, *Bgl* II, *Xho* II, *Bcl* I, *Nde* II	
5′-GCGC	*Ban* I	
5′-GGCC	*Not* I, *Bst*Z I, *Eco*52 I	
5′-GTAC	*Acc*65 I	*Ban* I
5′-GTNAC		*Bst*E II
5′-GYRC		*Ban* I
5′-TCGA	*Sal* I, *Xho* I	*Ava* I
5′-TGCA	*Alw*44 I	
5′-TTAA	*Bst*98 I	
5′-YCGR		*Ava* I

Promega Restriction Enzymes That Generate 3′ Overhangs

Overhang	Definite Compatible Ends	Possible Compatible Ends
N-3′		*Ecl*HK I
AT-3′	*Sgf* I, *Pvu* I	
CG-3′	*Cfo* I, *Hha* I	
CN-3′		*Bsa*M I
GC-3′	*Sac* II	*Bsa*O I
NNN-3′		*Acc*B7 I, *Bgl* I, *Sfi* I
ACGT-3′	*Aat* II	
AGCT-3′	*Sac* I	*Ban* II, *Bsp*1286 I
CATG-3′	*Hsp*92 II, *Sph* I, *Bbu* I	
DGCH-3′		*Bsp*1286 I
GCGC-3′	*Hae* II	
GGCC-3′	*Apa* I	*Ban* II, *Bsp*1286 I
GTAC-3′	*Kpn* I	
NNNN-3′		*Bst*X I
RGCY-3′		*Ban* II, *Bsp*1286 I
TGCA-3′	*Nsi* I, *Pst* I	*Bsp*1286 I
TTAA-3′	I-*Ppo* I	

Key:

D = A or G or T
H = A or C or T
K = G or T
M = A or C
N = A or C or G or T
R = A or G
S = C or G
W = A or T
Y = C or T

Site-Specific Methylation Sensitivity of Promega Restriction Enzymes.

This table lists the sensitivities of several Promega restriction enzymes to site-specific methylation at *dam*, *dcm*, CpG and CpNpG sites (p = phosphoryl group). These four modifications are frequently found in DNA of bacteria, eukaryotes or their viruses. Many strains of *E. coli* contain the site-specific *dam* and *dcm* DNA methylases. Higher eukaryotes contain the site-specific CpG and CpNpG DNA methylases. In mammalian genomes, methylation occurs mainly at the CG dinucleotide. In plant genomes, methylation may occur at both the CG and CNG sequences.

Prokaryotic Methylation

dcm Cytosine methylase mutation—methylates the C5 position of the internal cytosine residue in the sequence 5′...CCTGG...3′.

dam Adenine methylase mutation—methylates the N6 position of the adenine residue in the sequence 5′....GATC...3′.

Eukaryotic Methylation

CpG Methylates the C5 position of the cytosine residue in the dinucleotide recognition sequence 5′...CG...3′.

CpNpGp Methylates the C5 position of the cytosine residue in the trinucleotide recognition sequence 5′...CNG...3′ (N = any base).

For further information regarding site-specific methylation, refer to McClelland, M., Nelson, M. and Raschke, E. (1994) *Nucl. Acids Res.* **22**, 3640–59.

Key:

s = sensitive to this methylation
i = insensitive to this methylation
s(ol) = overlapping (sensitive when restriction site overlaps methylation sequence)
n/a = information not available

Enzyme	Recognition Sequence	*dam*	*dcm*	CpG	CpNpG
Aat II	GACGTC	i	i	s	i
*Acc*B7 I	CCANNNNNTGG	i	s(ol)	i	i
Acc III	TCCGGA	s(ol)	i	i	i
*Acc*65 I	GGTACC	i	s(ol)	i	i
Apa I	GGGCCC	i	s(ol)	s(ol)	i
Ava I	CYCGRG	i	i	s	i
Ava II	GGWCC	i	s(ol)	s(ol)	s(ol)
Bal I	TGGCCA	i	s(ol)	i	s(ol)
*Bam*H I	GGATCC	i	i	i	s(ol)
Ban II	GRGCYC	i	i	i	i
Bbu I	GCATGC	i	i	i	i
Bcl I	TGATCA	s	i	i	i
Bgl I	GCCNNNNNGGC	i	i	s(ol)	s(ol)
Bgl II	AGATCT	i	i	i	s(ol)
*Bsp*1286 I	GDGCHC	i	i	i	i
*Bss*H II	GCGCGC	i	i	s	i
*Bst*E II	GGTNACC	i	i	i	i
*Bst*O I	CCWGG	i	i	i	n/a
*Bst*X I	CCANNNNNNTGG	i	i	i	i
*Bst*Z I	CGGCCG	i	i	s(ol)	s(ol)
Cfo I	GCGC	i	i	s	n/a
Cla I	ATCGAT	s(ol)	i	s	i
Csp I	CGGWCCG	i	i	i	s
*Csp*45 I	TTCGAA	i	i	s	i
Dde I	CTNAG	i	i	i	s(ol)
*Eco*47 III	AGCGCT	i	i	s	i
*Eco*52 I	CGGCCG	i	i	s	i
*Eco*R I	GAATTC	i	i	s(ol)	i
Fok I	GGATC	i	i	i	i
Hae III	GGCC	i	i	i	s(ol)
Hha I	GCGC	i	i	s	s(ol)
Hinc II	GTYRAC	i	i	i	i
Hind III	AAGCTT	i	i	i	i
Hpa II	CCGG	i	i	s	s

Enzyme	Recognition Sequence	*dam*	*dcm*	CpG	CpNpG
Kpn I	GGTACC	i	i	i	i
Mbo II	GAAGA(8/7)	s(ol)	i	i	i
Mlu I	ACGCGT	i	i	s	i
Msp I	CCGG	i	i	i	s
Nae I	GCCGGC	i	i	s	s
Nar I	GGCGCC	i	i	s	i
Nde II	GATC	s	i	i	i
*Ngo*M IV	GCCGGC	i	i	s	s
Nhe I	GCTAGC	i	i	s(ol)	s(ol)
Not I	GCGGCCGC	i	i	s	s
Nru I	TCGCGA	s(ol)	i	s	i
Pst I	CTGCAG	i	i	i	s
Pvu I	CGATCG	i	i	s	s(ol)
Pvu II	CAGCTG	i	i	i	s
Sac I	GAGCTC	i	i	i	i
Sac II	CCGCGG	i	i	s	s
Sal I	GTCGAC	i	i	s	n/a
*Sau*3A I	GATC	i	i	s(ol)	s(ol)
*Sau*96 I	GGNCC	i	s(ol)	s(ol)	s(ol)
Sca I	AGTACT	i	i	i	i
Sfi I	GGCCNNNNNGGCC	i	s(ol)	s(ol)	s(ol)
Sgf I	GCGATCGC	i	i	s	n/a
Sin I	GGWCC	i	i	i	s(ol)
Sma I	CCCGGG	i	i	s	s
*Sna*B I	TACGTA	i	i	s	i
Sph I	GCATGC	i	i	i	i
Stu I	AGGCCT	i	s(ol)	i	s(ol)
Taq I	TCGA	s(ol)	i	i	i
Xba I	TCTAGA	s(ol)	i	i	i
Xho I	CTCGAG	i	i	s	i
Xho II	RGATCY	i	i	i	s(ol)
Xma I	CCCGGG	i	i	i	n/a
Xmn I	GAANNNN	i	i	n/a	n/a

Restriction Enzyme Buffer Composition.

Buffer	pH (at 37°C)	Tris-HCl (mM)	$MgCl_2$ (mM)	NaCl (mM)	KCl (mM)	DTT (mM)
A	7.5	6	6	6	—	1
B	7.5	6	6	50	—	1
C	7.9	10	10	50	—	1
D	7.9	6	6	150	—	1
E	7.5	6	6	100	—	1
F	8.5	10	10	100	—	1
G	8.2	50	5	—	—	—
H	7.5	90	10	50	—	—
J	7.5	10	7	—	50	1
K	7.4	10	10	—	150	—
L	9.0	10	3	100	—	—

MULTI-CORE™ Buffer (1X) = 25mM Tris-acetate (pH 7.5 at 37°C), 100mM potassium acetate, 10mM magnesium acetate, 1mM DTT.

Notes:

1. For each 10°C rise in temperature between 0°C and 25°C, the pH of Tris buffers decreases 0.31 pH units.
2. For each 10°C rise in temperature between 25°C and 37°C, the pH of Tris buffers decreases 0.25 pH units.
3. All of Promega's Restriction enzymes are supplied with 10mg/ml Acetylated BSA. Although BSA is not absolutely required for activity, it has been shown to enhance activity of many restriction enzymes. We recommend adding BSA to all restriction digests at a final concentration of 0.1mg.ml.

Copy Number of Commonly Used Plasmids.

Plasmid	Plasmid Size (approx.)	Origin of Replication*	Copy Number	**Yield per ml of Culture	Reference
pGEM®	2,700bp	mutated pMB1	300–700	1.8–4.1µg	1
pUC	2,700bp	mutated pMB1	500–700	2.9–4.1µg	1
pBR322	4,400bp	pMB1	>25	>0.23µg	2
ColE1	4,500bp	ColE1	>15	>0.15µg	3
pACYC	4,000bp	p15A	~10	~0.09µg	4
pSC101	9,000bp	pSC101	~6	~0.12µg	5
pGL Series	5,000bp	mutated pMB1	300–700	3.3–7.6µg	1
pRL Series	4,000bp	mutated pMB1	300–700	2.7–6.0µg	1
phRL Series	4,000bp	mutated pMB1	300–700	2.7–6.0µg	1
phRG Series	4,000bp	mutated pMB1	300–700	2.7–6.0µg	1
pGEM®-T/ T easy	3,000bp	mutated pMB1	300–700	2.0–4.6µg	1
psiLentGene™ Series	4,000bp	mutated pMB1	300–700	2.7–6.0µg	1
psiCHECK™ 1/2	3,500bp	mutated pMB1	300–700	2.7–5.3µg	1
psiSTRIKE™ Series	4,000bp	mutated pMB1	300-700	2.7–6.0µg	1
pALTER®-1/*Ex*1	5,800bp	pMB1	>25	>0.3µg	3
pALTER®-*Ex*2	5,800bp	p15A	~10	~0.13µg	4
pSP	2,500bp	mutated pMB1	300–700	1.6–3.8µg	1
pCI, pSI	3,600bp	mutated pMB1	300–700	2.4–5.5µg	1

* Plasmids carrying the pMB1, mutated pMBI and ColE1 belong to the same incompatibility group, so they are not compatible with one another, but they are fully compatible with those carrying p15A and pSC101 replicons.

** Theoretical plasmid yields were calculated from the reported copy number and size of each plasmid assuming 2.0×10^9 cells per milliliter of culture grown for 16 hours at 37°C.

References

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4. Birnboim, H.C. and Doly, J. (1979) *Nucl. Acids Res.* **7**, 1513–23.
5. Birnboim, H.C. (1983) *Meth. Enzymol.* **100**, 243–55.

Star Activity.

Restriction enzymes, under nonstandard conditions, can demonstrate the ability to cleave DNA at sequences different from their defined recognition sites. The term "star activity" has been given to this nonsequence-specific cleavage of DNA under nonoptimal reaction conditions. The most common types of altered activity are single-base substitutions, truncation of the outer bases in the recognition sequence and single-strand nicking (1). In general, star activity is not a concern if restriction endonucleases are used in the recommended buffers at the appropriate temperatures. Star activity is evident with a number of restriction enzymes when the following parameters are altered in the reaction environment (2):

- High enzyme concentration (generally >100 units/µg).
- High glycerol content (>5% v/v).
- Substitution of Mn^{2+} for Mg^{2+} (or substitution of other divalent cations).
- Low salt concentration (generally <25mM).
- Extremes of pH, especially pH>8.0.
- Presence of DMSO, ethanol or other organic solvents.

References

1. Barany, F. (1988) *Gene* **65**, 149–65.
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Genotypes of Frequently Used Bacterial Strains.

All genes in the bacterium are presumed to be in the wildtype state, except for those listed, which are mutant alleles carried by that bacterium. Genes listed on the F′ episome, however, represent wildtype alleles unless specified otherwise. Strains are λ– unless specified otherwise. *Strains available from Promega as competent cells are indicated by an asterisk. Strains shown in **bold** are available from Promega as glycerol freezer stocks.

Strain	Genotype
BL21(DE3)	F–, *omp*T, *hsd*S_B (r_B^-, m_B^-), *dcm*, *gal*, λ(DE3)
***BL21(DE3)pLysS**	F–, *omp*T, *hsd*S_B (r_B^-, m_B^-), *dcm*, *gal*, λ(DE3), pLysS (Cm^r)
***BMH 71-18 *mut*S**	*thi*, *sup*E, Δ(*lac-proAB*), [*mutS*::Tn10(tet^r)] [F′, tra D36, *proAB*, *lacI*^{q}ZΔM15]
C600 (1)	*thi*-1, *thr*-1, *leuB*6, *lacY*1, *tonA*21, *supE*44
C600*hfl* (1)	*thi*-1, *thr*-1, *leuB*6, *lacY*1, *tonA*21, *supE*44, *hfl*A150::Tn10(tet^r)
DH1 (2)	*recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17 (r_K^-, m_K+), *supE*44, *relA*1
DH10B	F–, *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15, Δ*lac*X74, *deo*R, *rec*A1, *end*A1, *ara*D139, Δ(*ara*, *leu*)7697, *gal*U, *gal*K, λ-, *rps*L(str^r), *nup*G
DH5α™	ϕ80d*lacZ*ΔM15, *recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17 (r_K^-, m_K+), *supE*44, *relA*1, *deoR*, Δ(*lacZYA-argF*) U169, *pho*A
DM1 (3)	F′, *dam*-13::Tn9(Cm^r) *dcm*, *mcrB*, *hsdr-M*+, *gal*1, *gal*2, ara-, *lac*-, *thr*-, *leu*-, *ton*^{R}, *tsx*^{R}, *Su*^{0}
ES1301 *mut*S	*lacZ*53, *thyA*36, *rha*-5, *metB*1, *deo*C, IN(*rrn*D-*rrn*E), [*mutS*201::Tn5]
*HB101 (4)	*thi*-1, *hsdS*20 (r_B^-, m_B^-), *supE*44, *recA*13, *ara*-14, *leuB*6, *proA*2, *lacY*1, *galK*2, *rpsL*20(str^r), *xyl*-5, *mtl*-1
JM101 (5)	*supE*, *thi*, Δ(*lac-proAB*), F′ (*traD*36, *proAB*, *lacI*^{q}ZΔM15)
***JM109** (5)	*endA*1, *recA*1, *gyrA*96, *thi*-1, *hsdR*17 (r_K^-, m_K+), *relA*1, *supE*44, Δ(*lac-proAB*), [F′, *traD*36, *proAB*, *lacI*^{q}ZΔM15]
JM109(DE3) (5)	*endA*1, *recA*1, *gyrA*96, *thi*-1, *hsdR*17 (r_K^-, m_K+), *relA*1, *supE*44, Δ(*lac-proAB*), [F′, *traD*36, *proAB*, *lacI*^{q}ZΔM15], λ(DE3)
JM110 (5)	*rpsL*(str^r), *thr*, *leu*, *thi*, *hsdR*17 (r_K^-, m_K+), *lacY*, *galK*, *galT*, *ara*, *tonA*, *tsx*, *dam*, *dcm*, *supE*44, Δ(*lac-proAB*), [F′, *traD*36, *proAB*, *lacI*^{q}ZΔM15]
KW251	*supE*44, *galK*2, *galT*22, *metB*1, *hsdR*2, *mcrB*1, *mcrA*, [*argA*81::Tn10(tet^r)], *rec*D1014
LE392 (6)	*hsdR*514, (r_K^-, m_K+), *supE*44, *supF*58, *lacY*1 or Δ(*lacIZY*)6, *galK*2, *galT*22, *metB*1, *trpR*55
NM522 (7)	*supE*, *thi*, Δ(*lac-proAB*), Δ*hsd*5 (r_K^-, m_K^-), [F′, *proAB*, *lacI*^{q}ZΔM15]
NM538 (8)	*supF*, *hsdR* (r_K^-, m_K+), *trpR*, *lacY*
NM539 (8)	*supF*, *hsdR* (r_K^-, m_K+), *lacY*, (P2)
*Select96™	*mcr*A, Δ(*mrr-hsd*RMS-*mcr*BC), ϕ80lacZΔM15, Δ*lac*X74, *rec*A1, *ara*D139 (*ara-leu*)7697, *gal*U, *gal*K, *rsp*L, *end*A1, *nup*G
Stbl2™	F–, *mcr*A, Δ(*mcr*BC-*hsd*RMS-*mrr*), *rec*A1, *end*A1, *gyr*A96, *thi*-1, *sup*E44, *rel*A1, λ–, Δ(*lac-pro*AB)
Stbl4™	*mcr*A, Δ(*mcr*BC-*hsd*RMS-*mrr*), *rec*A1, *end*A1, *gyr*A96, *thi*-1, *sup*E44, *rel*A1, λ–, Δ(*lac-pro*AB), *gal*, F′{*pro*AB+, *lacI*^{q}, ZΔM15, *Tn*10(*tet*^{R})}
SURE®	*e*14–, (*mcr*A–) Δ(*mcr*CB-*hsd*SMR-*mrr*)171, *end*A1, *sup*E44, *thi*-1, *gyr*A96, *rel*A1, *lac*, *rec*B, *rec*J, *sbc*C, *umu*C::Tn5 (*kan*^{r}), *uvr*C, [F′ *pro*AB, *lacI*^{q}ZΔM15::Tn10 (*tet*^{r})]
TOP10	F–, *mcr*A, Δ(*mrr-hsd*RMS-*mcr*BC), ϕ80*lac*ZΔM15, Δ*lac*X74, *deo*R, *rec*A1, *ara*D139, Δ(*ara*, *leu*)7697, *gal*U, *gal*K, *rps*L (str^R), *end*A1, *nup*G
TOP10F′	F′{*lacI*^{q} Tn10 (tet^R)}, *mcr*A, Δ(*mrr-hsd*RMS-*mcr*BC), ϕ80*lac*ZΔM15, Δ*lac*X74, *deo*R, *rec*A1, *ara*D139, Δ(*ara-leu*)7697, *gal*U, *gal*K, *rps*L (str^r), *end*A1, *nup*G
XL1-Blue	*recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17(r_K^-, m_K+), *supE*44, *relA*1, *lac*, [F′, *proAB*, *lacI*^{q}ZΔM15::Tn10(tet^r)]
Y1089 (9)	Δ(*lac*U169), *proA*+, Δ(*lon*), *araD*139, *strA*, *hflA*150, [chr::Tn10(tet^r)], (pMC9)
Y1090 (9)	Δ(*lac*U169), *proA*+, Δ(*lon*), *araD*139, *strA*, *supF*, rpsL(str^r), [*trpC*22::Tn10 (tet^r)], (pMC9), *hsdR* (r_K^-, m_K+)

Miscellaneous

F′ Host contains an F′ episome with the stated features.

λ(DE3) Bacteriophage λ carrying the gene for T7 RNA polymerase is integrated into the host genome.

pMC9 is pBR322 with *lacI*^{q} inserted and confers amp and tet resistance.

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Genetic Markers in *E. coli*.

Symbol	Description	Effect of Mutation
ara-14	Mutation in arabinose metabolism	Blocks arabinose catabolism.
*ara*D	L-ribulose phosphate 4-epimerase mutation; part of an inducible operon araBAD repressed by L-arabinose	Blocks arabinose catabolism.
*arg*A	N-Acetylglutamate synthase mutation; inhibited by the presence of arginine	Arginine required from growth in minimal media.
*cyc*A	Involved in D-alanine, glycine, D-serine and D-cycloserine transport, and an L-alanine carrier	Mutants cannot use D-alanine as a carbon source.
dam	DNA adenine methylase mutation	Blocks methylation of adenine residues in the sequence 5′…G^{m}ATC…3′.
*dap*D	Succinyl-diaminopimelate aminotransferase mutation	Mutant reflects impaired synthesis of succinyl CoA and needs to be supplemented with succinate or lysine + methionine.
dcm	DNA cytosine methylase mutation	Blocks methylation of cytosine in the sequence 5′…C^{m}CAGG…3′ or 5′…C^{m}CTGG…3′.
*deo*C	Deoxyribose-phosphate aldolase mutation	
*deo*R	Regulatory gene mutation allowing constitutive expression of genes for deoxyribose synthesis	Allows efficient propagation of large plasmids.
*dut*1	Mutation of deoxyuridine triphosphatase, which catalyzes dUTP the conversion to dUMP and PPi	Mutants are impaired in conversion of dUTP to dUMP, leading to higher dUTP pools that can lead to misincorporation of uracil instead of thymidine. Stable incorporation of dUTP needs mutation in ung gene.
*end*A1	DNA-specific endonuclease I mutation	Improves quality of plasmid DNA isolations.
*gal*E	Part of the galETK operon that encodes UDP galactose-4-epimerase	Mutant is more resistant to bacteriophage P1 infection.
*gal*K	Galactokinase mutation	Blocks catabolism of galactose.
*gal*T	Galactose-1-phosphate uridylyltransferase mutation	Blocks catabolism of galactose.
*gyr*A96	DNA gyrase mutation	Confers resistance to nalidixic acid.
*hflA*150	Protease mutation that leads to stabilization of cII gene products	Leads to high frequency of lysogeny by λ phages (1).
*hfl*B	Gene encodes a possible protease component	Mutations lead to high frequency of bacteriophage lambda lysogenization.
hsdR (r_K^-, m_K+)	Host DNA restriction and methylation system mutation: Restriction minus, modification positive for the *E. coli* K strain methylation system	Allows cloning without cleavage of transformed DNA by endogenous restriction endonucleases. DNA prepared from this strain can be used to transform r_K+ *E. coli* strains.
*hsdS*20 (r_B^-, m_B^-)	Mutation of specificity determinant for host DNA restriction and methylation system. Restriction minus, modification minus for the *E. coli* B strain methylation system	Allows cloning without cleavage of transformed DNA by endogenous restriction endonucleases. DNA prepared from this strain is unmethylated by the *hsdS*20 methylases.
lacI^{q}	Overproduction of the *lac* repressor protein	Leads to high levels of the *lac* repressor protein, inhibiting transcription from the *lac* promoter.
lacY	Galactoside permease mutation	Blocks lactose utilization.
*lacZ*ΔM15	Partial deletion of β-D-galactosidase gene	Allows complementation of β-galactosidase activity by α-complementation sequence in pGEM®-Z Vectors. Allows blue/white selection for recombinant colonies when plated on X-Gal.
leuB	β-isopropylmalate dehydrogenase mutation	Requires leucine for growth on minimal media.
Δ(*lon*)	Deletion of *lon* protease	Reduces proteolysis of expressed proteins.
*Lys*S	pLysS plasmid is integrated into the host genome	Strains carrying this plasmid will be tet resistant and produce T7 lysozyme, a natural inhibitor of T7 RNA polymerase, thus lowering background transcription of sequences under the control of the T7 RNA polymerase promoter (2).
mcrA	Mutation in methylcytosine restriction system	Blocks restriction of DNA methylated at the sequence 5′…G^{m}CGC…3′.
mcrB	Mutation in methylcytosine restriction system	Blocks restriction of DNA methylated at the sequence 5′…AG^{m}CT…3′.
metB	Cystathionine γ-synthase mutation	Requires methionine for growth on minimal media.
*met*C	Cystathionine beta-lyase mutation; involved in methionine biosynthesis	Methionine required from growth in minimal media.
mtl	Mutation in mannitol metabolism	Blocks catabolism of mannitol.
mutS	Methyl-directed mismatch repair mutation	Prevents repair of the newly synthesized, unmethylated strand.
ompT	Mutation of protease VII, an outer membrane protein	Reduces proteolysis of expressed proteins.
P2	P2 bacteriophage lysogen present in host	λ phages containing the *red* and *gam* genes of λ are growth inhibited by P2 lysogens (3).
*pro*A	γ-glutamyl phoshate reductase mutation	proA/argD mutant will not block proline synthesis, but will be repressed by arginine. Mutants excrete proline on minimal media and are resistant to proline analogs. proA/argD/argR triple mutant grows slowly on minimal media + arginine.
proAB	Mutations in proline metabolism	Requires proline for growth in minimal media.

Genetic Markers in *E. coli* (continued).

Symbol	Description	Effect of Mutation
*recA*1, *recA*13	Mutation in recombination	Minimizes recombination of introduced DNA with host DNA, increasing stability of inserts. Inserts are more stable in *recA*1 than *recA*13 hosts.
recB, *recC* *rec*D	Exonuclease V mutations The Rec BCD trimer (exonuclease V) progressively degrades ssDNA and dsDNA in an ATP-dependent manner to form oligonucleotides; implicated in homologous recombination	Reduces general recombination and affects repair of radiation damage. Allows easier propagation of sequences with inverted repeats.
*rec*F	Recombination and repair mutation	Mutant cannot repair daughter strand gaps (post-replicational repair).
*rel*A	ppGpp synthetase I mutation, a novel nucleotide guanosine 5′-diphosphate-3′-diphosphate produced in response to starvation by relA ribosomal protein sensing uncharged tRNA	Allows RNA synthesis in the absence of protein synthesis.
rha	Utilization of L-rhamnose, a methylpentose	Blocks rhamnose catabolism.
rpsL	Mutation in subunit S12 of 30S ribosome	Confers resistance to streptomycin.
sbcB	Exonuclease I mutation	Allows general recombination in *rec*BC mutant strains.
*str*A	Mutant alters ribosome protein S12	Confers resistance to streptomycin
supB, *supC*, *supG*, *supL*, *supM*, *supN*, *supO*	Suppressor mutations	Suppresses ochre (UAA) and amber (UAG) mutations.
supD, *supE*, *supF*	Suppressor mutations	Suppresses amber (UAG) mutations.
thi-1	Mutation in thiamine metabolism	Thiamine required for growth in minimal media.
thr	Threonine biosynthesis mutation	Mutants are obligate threonine auxotrophs.
*thy*A	Thymidylate synthase; dTTP biosynthesis	Mutants are obligate thymidine auxotrophs.
Tn5	Transposon	Encodes resistance to kanamycin.
Tn10	Transposon	Encodes resistance to tetracycline.
tonA	Mutation in outer membrane protein	Confers resistance to bacteriophage T1.
*traD*36	Transfer factor mutation	Prevents transfer of F′ episome.
*trp*C	Phosphoribosyl anthranilate isomerase mutation; part of tryptophan biosynthesis pathway	
*trp*R	trpR aporepressor; regulates the biosynthesis of tryptophan and its transport	
tsx	T6 and colicin K phage receptor; outer membrane protein involved in specific diffusion of nucleosides; transports the antibotic albicidin	Resistant to bacteriophage T6 and colicin K.
*ung*1	Uracil-DNA N-glycosylase	Allows uracil to exist in plasmid DNA.
xyl-5	Mutation in xylose metabolism	Blocks catabolism of xylose.

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Technical Appendix

Nucleic Acids and Proteins: Calculations.

An online calculator for these values is available in the "tools" section of Promega's Web site at: **www.promega.com/techserv/tools/**

Metric Prefixes

Prefix	Symbol	Factor
kilo	k	10^{3}
centi	c	10^{-2}
milli	m	10^{-3}
micro	µ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}
femto	f	10^{-15}
atto	a	10^{-18}
zepto	z	10^{-21}

Spectrophotometric Conversions

1 A_{260} unit of double-stranded DNA = 50µg/ml
1 A_{260} unit of single-stranded DNA = 33µg/ml
1 A_{260} unit of single-stranded RNA = 40µg/ml

DNA Molar Conversions

1µg of 1,000bp DNA = 1.52pmol (3.03pmol of ends)
1µg of pBR322 DNA = 0.36pmol DNA
1pmol of 1,000bp DNA = 0.66µg
1pmol of pBR322 DNA = 2.8µg

Formulas for DNA Molar Conversions

For dsDNA:

To convert pmol to µg:

$$\text{pmol} \times N \times \frac{660\text{pg}}{\text{pmol}} \times \frac{1\mu\text{g}}{10^{6}\text{pg}} = \mu\text{g}$$

To convert µg to pmol:

$$\mu\text{g} \times \frac{10^{6}\text{pg}}{1\mu\text{g}} \times \frac{\text{pmol}}{660\text{pg}} \times \frac{1}{N} = \text{pmol}$$

where N is the number of nucleotide pairs and 660pg/pmol is the average MW of a nucleotide pair.

For ssDNA:

To convert pmol to µg:

$$\text{pmol} \times N \times \frac{330\text{pg}}{\text{pmol}} \times \frac{1\mu\text{g}}{10^{6}\text{pg}} = \mu\text{g}$$

To convert µg to pmol:

$$\mu\text{g} \times \frac{10^{6}\text{pg}}{1\mu\text{g}} \times \frac{\text{pmol}}{330\text{pg}} \times \frac{1}{N} = \text{pmol}$$

where N is the number of nucleotides and 330pg/pmol is the average MW of a nucleotide.

Dalton (Da) is an alternate name for the atomic mass unit, and kiloDalton (kDa) is 1,000 Daltons. Thus a protein with a mass of 64kDa has a molecular weight of 64,000 grams per mole.

(a) The PCR process is covered by patents issued and applicable in certain countries*. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.
*In the U.S., effective March 29, 2005, U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 will expire. In Europe, effective March 28, 2006, European Pat. Nos. 201,184 and 200,362 will expire.

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