Definition of active unit

A unit is an enzyme activity that completely digests 1 µg of λ DNA in 50 µl of the reaction mixture in 60 minutes. Enzymes that are difficult to test under these conditions are indicated.

Quality control

(1) Mixed Nuclease: It is confirmed that the electrophoresis pattern after reacting 20 units of restriction enzyme with 1 µg of substrate DNA for 5 hours (100-fold overdigestion) matched that of the short-term digestion.

(2) Ligation/Recutting: It is confirmed that the electrophoresis pattern after digesting λDNA with four times units of the restriction enzyme for 2 hours and ligating the fragments with T4 DNA ligase matched that before ligation and recutting.

(3) Nickase: It is confirmed that there is little conversion to form II and III, after reacting 10 - 35 units of restriction enzyme with 1 µg of appropriate supercoiled DNA (e.g., φX174RF I,pBR322)for 5 hours. However, this check is only performed on enzymes that have the appropriate supercoiled DNA.

(4) Phosphatase: It is confirmed that there is little free p-nitrophenol after reacting 20 - 200 units of restriction enzyme with p-nitrophenyl phosphate for 48 hours.

Supplied buffer list

A restriction enzyme reaction buffer is supplied with 1 ml (1 tube) of each restriction enzyme.

Supplied buffer	Label color	Composition	Enzyme name
10 x L Buffer	Yellow	100 mmol/l Tris-HCl (pH 7.9 at 25°C) 100 mmol/l $MgCl_2$ 10 mmol/l DTT	*Alw*44 I (BSA supplied)*, *Apa* I, *Kpn* I, *Mbo* II, *Nar* I, *Nci* I, *Nsp*V, *Sac* I, *Sac* II
10 x M Buffer	Light blue	500 mmol/l NaCl 100 mmol/l Tris-HCl (pH 7.9 at 25°C) 100 mmol/l $MgCl_2$ 10 mmol/l DTT	*Acc* II, *Age* I, *Alu* I, *Ava* I, *Ava* II, *Axy* I, *Dra* I, *Eco*O109 I, *Eco*R II, *Eco*T38 I, *Fok* I, *Hae* II, *Hae* III, *Hin*c II, *Msp* I, *Nhe* I, *Pvu* II, *Rsa* I, *Sau*3A I, *Sau*96 I, *Sfi* I, *Spe* I, *Stu* I, *Xba* I
10 x H Buffer	Red	1,000 mmol/l NaCl 500 mmol/l Tris-HCl (pH 7.9 at 25°C) 100 mmol/l $MgCl_2$ 10 mmol/l DTT	*Ase* I, *Bcl* I, *Bgl* I, *Bgl* II, *Bst*E II, *Bst*X I, *Eco*R I, *Eco*R V, *Hin*f I, *Mlu* I, *Nco* I, *Nde* I, *Not* I (Triton X-100 supplied)*, *Nsi* I, *Pst* I, *Sal* I, *Scr*F I, *Sph* I, *Sty* I, *Swa* I (BSA supplied)*, *Xho* I
10 x A Buffer	Purple	500 mmol/l Potassium acetate 200 mmol/l Tris-acetate(pH 7.9 at 25°C) 100 mmol/l Magnesium acetate 10 mmol/l DTT	*Acc* I, *Afl* II, *Bsp*1286 I, *Fsp* I, *Sma* I, *Taq* I
10 x B Buffer	Gray	1,000 mmol/l NaCl 100 mmol/l Tris-HCl (pH 8.5 at 25°C) 100 mmol/l $MgCl_2$ 10 mmol/l DTT	*Acc* III (BSA supplied)*, *Acy* I, *Bam*H I, *Bsm* I, *Bss*H II, *Hha* I, *Hin*d III, *Ssp* I
10 x Dedicated Buffer	White	10 x concentration of condition for digesting with each restriction enzyme	*Bal* I, *Hpa* I, *Nde* II, *Nru* I, *Sca* I

The supplied reaction buffer is 10 x concentration of reaction condition and 1/10 of the reaction volume (5 µl in the case of 50 µl reaction volume) is used in the enzyme reaction

* *Acc* III, *Alw*44 I and *Swa* I have 1mg/ml BSA separately, and *Not* I has 0.1% TritonX-100 separately. During enzyme reactions, 1/10 of the reaction volume of BSA or Triton X-100 are added

List of Relative Activity by Restriction Enzyme Reaction Buffer

Nippon Gene measures the activity of restriction enzymes using the five restriction enzyme reaction buffers (L, M, H, A, and B) (supplied reaction buffer *[1]: □). The relative activities of restriction enzyme using other reaction buffers are shown below. The activity is 100 % when this enzyme reacts in supplied buffer. ( ) shows reaction buffers that are susceptible to star activity, etc.

For *Bal* I, *Hpa* I, *Nde* II, *Nru* I, and *Sca* I, each dedicated buffer*[1] was used. In addition, the reaction was conducted without adding BSA*[2] for *Acc* III, *Alw*44 I, and *Swa* I, and without Triton X-100 *[3] for *Not* I.

*1 The composition of the supplied reaction buffer and dedicated buffer is 10 x concentration of the enzyme reaction conditions.

*2 The relative activity is 100% when BSA solution is added to a final concentration of 0.1 mg/ml.

*3 The relative activity is 100% when Triton X-100 is added to a final concentration of 0.01%.

Restriction enzyme	L	M	H	A	B	Dedicated
Acc I	50	75	<5	100	<5	—
*Acc*II	(75)	100	10	100	50	—
Acc III	<5	10	50	(5)	75	—
Acy I	<5	10	25	<5	100	—
Afl II	50	50	5	100	25	—
Age I	(75)	100	10	75	25	—
Alu I	100	100	25	150	25	—
*Alw*44 I	75	50	<5	50	25	—
Apa I	100	10	<5	50	<5	—
Ase I	(10)	(50)	100	(25)	100	—
Ava I	10	100	10	25	50	—
Ava II	(75)	100	5	(75)	50	—
Axy I	(100)	100	50	(100)	50	—
Bal I	25	10	<5	25	<5	100
*Bam*H I	(75)	(100)	75	(75)	100	—
Bcl I	(100)	(200)	100	(100)	200	—
Bgl I	(10)	(50)	100	(10)	25	—
Bgl II	(10)	(75)	100	(50)	(150)	—
Bsm I	(25)	(75)	50	(75)	100	—
*Bsp*1286 I	75	50	10	100	10	—
Bss H II	(50)	50	75	(50)	100	—
Bst E II	(25)	(100)	100	(75)	100	—
Bst X I	<5	75	100	25	100	—
Dra I	75	100	10	50	75	—
*Eco*O109 I	(100)	100	10	100	50	—
*Eco*R I	—	—	100	—	(150)	—
*Eco*R II	<5	100	75	75	100	—
*Eco*R V	(10)	75	100	25	150	—
*Eco*T38 I	150	100	5	150	75	—
Fok I	(200)	100	<5	(200)	100	—
Fsp I	25	100	5	100	50	—
Hae II	100	100	25	75	50	—
Hae III	75	100	100	100	100	—
Hha I	(75)	75	25	(100)	100	—
*Hinc*II	50	100	50	100	50	—
*Hin*d III	(<5)	75	10	(25)	100	—
Hinf I	10	75	100	50	150	—

Restriction enzyme	L	M	H	A	B	Dedicated
Hpa I	(25)	(75)	25	(50)	(100)	100
Kpn I	100	50	<5	100	<5	—
Mbo II	100	75	25	75	25	—
Mlu I	10	25	100	10	50	—
Msp I	100	100	25	100	50	—
Nar I	100	5	<5	100	<5	—
Nci I	100	50	<5	100	<5	—
Nco I	(75)	(100)	100	(100)	(150)	—
Nde I	10	25	100	25	100	—
*Nde*II	5	5	25	5	5	100
Nhe I	(150)	100	5	(200)	10	—
Not I	5	25	50	10	50	—
Nru I	<5	5	75	5	25	100
Nsi I	75	100	100	(75)	100	—
*Nsp*V	100	50	<5	150	<5	—
Pst I	(200)	(150)	100	(150)	50	—
Pvu II	(50)	100	5	(50)	5	—
Rsa I	200	100	5	200	50	—
Sac I	100	75	10	100	10	—
Sac II	100	50	5	100	5	—
Sal I	<5	<5	100	<5	5	—
Sau 3A I	(100)	100	25	150	50	—
Sau 96 I	75	100	50	75	150	—
Sca I	(5)	(25)	25	(5)	(100)	100
*Scr*F I	(75)	(100)	100	(100)	(150)	—
Sfi I	25	100	5	75	5	—
Sma I	75	10	<5	100	<5	—
Spe I	(75)	100	10	(75)	75	—
Sph I	(100)	(200)	100	(100)	100	—
Ssp I	(<5)	(75)	10	(50)	100	—
Stu I	100	100	50	100	100	—
Sty I	(10)	(75)	100	25	(75)	—
Swa I	<5	25	75	5	75	—
Taq I	25	50	25	100	100	—
Xba I	50	100	25	150	25	—
Xho I	10	50	100	25	150	—

Effect of salt concentration on restriction enzyme activity

The effect of salt concentration (KCl and NaCl) on restriction enzyme activity was shown below as relative activity. The condition of enzymatic response in this test is 10 mmol/l Tris-HCl (pH7.5), 10 mmol/l $MgCl_2$, 1 mmol/l DTT and various concentrations of KCl or NaCl.
Because EcoRI is prone to star activity under the above condition, the activity of EcoRI was measured under the condition (100 mmol/l Tris-HCl (pH 7.5), 7 mmol/l $MgCl_2$, 7 mmol/l 2-Mercaptoethanol and various concentrations of KCl or NaCl) that does not exhibit star activity .

Relative activity indication :100~70% : 70~40% : 40~0%

Restriction enzyme	Salt	Concentration (mmol/l)									
		0	20	40	60	80	100	120	140	160	180
Acc I	KCl										
	NaCl										
Acc II	KCl										
	NaCl										
Acc III	KCl										
	NaCl										
Acy I	KCl										
	NaCl										
Afl II	KCl										
	NaCl										
Age I	KCl										
	NaCl										
Alu I	KCl										
	NaCl										
Alw44 I	KCl										
	NaCl										
Apa I	KCl										
	NaCl										
Ase I	KCl										
	NaCl										
Ava I	KCl										
	NaCl										
Ava II	KCl										
	NaCl										
Axy I	KCl										
	NaCl										
Bal I	KCl										
	NaCl										
*Bam*H I	KCl										
	NaCl										
Bcl I	KCl										
	NaCl										
Bgl I	KCl										
	NaCl										
Bgl II	KCl										
	NaCl										
Bsm I	KCl										
	NaCl										
*Bsp*1286 I	KCl										
	NaCl										
*Bss*H II	KCl										
	NaCl										
*Bst*E II	KCl										
	NaCl										
*Bst*X I	KCl										
	NaCl										
Dra I	KCl										
	NaCl										
*Eco*O109 I	KCl										
	NaCl										
*Eco*R I	KCl										
	NaCl										
*Eco*R II	KCl										
	NaCl										
*Eco*R V	KCl										
	NaCl										
*Eco*T38 I	KCl										
	NaCl										
Fok I	KCl										
	NaCl										
Fsp I	KCl										
	NaCl										
Hae II	KCl										
	NaCl										
Hae III	KCl										
	NaCl										
Hha I	KCl										
	NaCl										
Hinc II	KCl										
	NaCl										

Restriction enzyme	Salt	Concentration (mmol/l)									
		0	20	40	60	80	100	120	140	160	180
Hind III	KCl										
	NaCl										
Hinf I	KCl										
	NaCl										
Hpa I	KCl										
	NaCl										
Kpn I	KCl										
	NaCl										
Mbo II	KCl										
	NaCl										
Mlu I	KCl										
	NaCl										
Msp I	KCl										
	NaCl										
Nar I	KCl										
	NaCl										
Nci I	KCl										
	NaCl										
Nco I	KCl										
	NaCl										
Nde I	KCl										
	NaCl										
Nde II	KCl										
	NaCl										
Nhe I	KCl										
	NaCl										
Not I	KCl										
	NaCl										
Nru I	KCl										
	NaCl										
Nsi I	KCl										
	NaCl										
Nsp V	KCl										
	NaCl										
Pst I	KCl										
	NaCl										
Pvu II	KCl										
	NaCl										
Rsa I	KCl										
	NaCl										
Sac I	KCl										
	NaCl										
Sac II	KCl										
	NaCl										
Sal I	KCl										
	NaCl										
*Sau*3A I	KCl										
	NaCl										
*Sau*96 I	KCl										
	NaCl										
Sca I	KCl										
	NaCl										
ScrF I	KCl										
	NaCl										
Sfi I	KCl										
	NaCl										
Sma I	KCl										
	NaCl										
Spe I	KCl										
	NaCl										
Sph I	KCl										
	NaCl										
Ssp I	KCl										
	NaCl										
Stu I	KCl										
	NaCl										
Sty I	KCl										
	NaCl										
Swa I	KCl										
	NaCl										
Taq I	KCl										
	NaCl										
Xba I	KCl										
	NaCl										
Xho I	KCl										
	NaCl										

Quality Criteria of Ligation/Re-cutting Efficiency

Nippon Gene's restriction enzymes meet the quality criteria in the following table for ligation/re-cutting tests. As the substrate for ligation, λDNA is used for almost all restriction enzymes. φ105 DNA for *Nar* I and *Sac* I, Ad2 DNA for *Not* I, *Sfi* I, and *Spe* I, M13mp19DNA for Swa I and T7 DNA for Xba I.

Restriction enzyme	Ligation efficiency (%)	Re-cutting efficiency (%)	Restriction enzyme	Ligation efficiency (%)	Re-cutting efficiency (%)	Restriction enzyme	Ligation efficiency (%)	Re-cutting efficiency (%)
Acc I	90	100	*EcoR* I	95	100	*Nsi* I	90	100
Acc II	90	100	*EcoR* II	95	100	*Nsp* V	95	100
Acc III	95	100	*EcoR* V	90	100	*Pst* I	95	100
Acy I	90	100	*EcoT38* I	95	100	*Pvu* II	90	100
Afl II	60	100	*Fok* I	90	100	*Rsa* II	90	100
Age I	95	100	*Fsp* I	80	100	*Sac* I	90	100
Alu I	90	100	*Hae* II	90	100	*Sac* II	95	100
Alw44 I	95	100	*Hae* III	90	100	*Sal* I	80	100
Apa I	95	100	*Hha* I	90	100	*Sau3A* I	90	100
Ase I	80	100	*Hinc* II	80	90	*Sau96* I	90	100
Ava I	90	100	*Hind* III	90	100	*Sca* I	90	100
Ava II	90	100	*Hinf* I	90	100	*ScrF* I	60	95
Axy I *	—	—	*Hpa* I	90	100	*Sfi* I	80	100
Bal I	90	100	*Kpn* I	90	100	*Sma* I	90	100
BamH I	90	100	*Mbo* II	95	100	*Spe* I	80	100
Bcl I	95	100	*Mlu* I	90	100	*Sph* I	95	100
Bgl I	90	100	*Msp* I	90	100	*Ssp* I	90	95
Bgl II	90	100	*Nar* I	95	100	*Stu* I	80	100
Bsm I	90	100	*Nci* I *	—	—	*Sty* I	95	100
*Bsp*1286 I	95	100	*Nco* I	90	100	*Swa* I	75	75
BssH II	95	100	*Nde* I	90	100	*Taq* I	90	100
BstE II	95	100	*Nde* II	90	100	*Xba* I	90	100
BstX I	90	100	*Nhe* I	90	100	*Xho* I	80	100
Dra I	90	100	*Not* I	90	100			
EcoO109 I	80	100	*Nru* I	90	100			

* Fragments cut by *Axy* I and *Nci* I are rarely bound by T4 DNA Ligase

List of Conditions for cutting of chromosomal DNA (Saccharomyces cerevisiae) by Restriction Enzymes

When restriction enzymes are used to cut giant DNA embedded in agarose gels, a large amount of enzyme is often required for complete degradation. Therefore, we measured the minimum amount of enzyme required for complete degradation of the chromosomal DNA of yeast (*Saccharomyces cerevisiae**1), a eukaryotic organism, by reacting each restriction enzyme at different enzyme levels for 5 and 20 hours, and by performing pulsed-field electrophoresis.

Restriction enzyme	Recognition sequence*2	Reaction buffer	Reaction temperature (℃)	Total enzyme requirement (unit)	
				5-hour reaction	20-hour reaction
Bgl I	GCCNNNNNGGC	H	37	5	5
BssH II	GCGCGC	B	50	5	5
Dra I	TTTAAA	M	37	25	25
Fsp I	TGCGCA	A	37	5	5
Mlu I	ACGCGT	H	37	5	5
Nhe I	GCTAGC	M	37	50	10
Not I	GCGGCCGC	H + Triton *3	37	20	20
Nru I	TCGCGA	Dedicated	37	50	20
Nsp V	TTCGAA	L	37	5	5
Sal I	GTCGAC	H	37	5	5
Sma I	CCCGGG	A	30	> 50	> 50
Spe I	ACTAGT	M	37	100	100
Ssp I	AATATT	B	37	5	5
Xba I	TCTAGA	M	37	50	50
Xho I	CTCGAG	H	37	5	5

*1 The ranges of molecular weight of *Saccharomyces cerevisiae* are from 245 to 2,500 kbp

*2 N denotes any of the bases A, C, G, T

*3 0.01% Triton X-100 is added to the reaction solution for Not I

List of heat inactivation conditions for restriction enzymes

Heat treatment is often used to stop restriction enzyme reactions. The following table shows the residual activity after incubation of restriction enzymes at 65°C for 30 minutes or at 70°C for 30 minutes. 30 units of restriction enzyme digest 2 µg of appropriate substrate DNA in 40 µl of reaction solution for 1 hour, and then were incubated at 65°C for 30 minutes or at 70°C for 30 minutes. 20 µl of the DNA solution was added 1 µg of DNA and was digested for additional 150 minutes. The residual activity in such solution was investigated by agarose gel electrophoresis.

-: Residual activity < 5% +: residual activity ≥ 5%

Restriction enzyme	65°C 30 minutes	70°C 30 minutes	Restriction enzyme	65°C 30 minutes	70°C 30 minutes	Restriction enzyme	65°C 30 minutes	70°C 30 minutes
Acc I	+	+	*EcoR* I	+	—	*Nsi* I	—	
Acc II	+	—	*EcoR* II	—		*Nsp* V	+	+
Acc III	+	+	*EcoR* V	+	+	*Pst* I	+	+
Acy I	+	—	*EcoT38* I	—		*Pvu* II	+	+
Afl II	—		*Fok* I	—		*Rsa* I	+	+
Age I	—		*Fsp* I	—		*Sac* I	—	
Alu I	—		*Hae* II	—		*Sac* II	—	
Alw44 I	—		*Hae* III	+	—	*Sal* I	—	
Apa I	—		*Hha* I	+	—	*Sau3A* I	—	
Ase I	—		*Hinc* II	—		*Sau96* I	+	—
Ava I	—		*Hind* III	+	+	*Sca* I	+	—
Ava II	—		*Hinf* I	+	—	*ScrF* I	—	
Axy I	—		*Hpa* I	—		*Sfi* I	+	+
Bal I	—		*Kpn* I	—		*Sma* I	—	
BamH I	—		*Mbo* II	—		*Spe* I	+	—
Bcl I	+	+	*Mlu* I	+	+	*Sph* I	—	
Bgl I	—		*Msp* I	+	—	*Ssp* I	—	
Bgl II	+	+	*Nar* I	—		*Stu* I	+	+
Bsm I	+	+	*Nci* I	—		*Sty* I	—	
*Bsp*1286 I	—		*Nco* I	—		*Swa* I	—	—
BssH II	—		*Nde* I	—		*Taq* I	+	+
BstE II	+	+	*Nde* II	—		*Xba* I	—	
BstX I	—		*Nhe* I	—		*Xho* I	+	+
Dra I	+	—	*Not* I	—				
EcoO109 I	—		*Nru* I	+	—			

Heat resistance varies depending on the enzyme. Considering the DNA denaturation temperature, Heat treatment alone may not be enough to completely inactivate. Phenol treatment is recommended to ensure complete inactivation. Enzymes with a residual activity of 5% or more after heat treatment at 70°C for 30 minutes are not inactivated by heat treatment. The phenol treatment is required to inactivate such enzymes.

Listing of Cutting Conditions for Plasmids by Restriction Enzymes

Cutting a supercoiled plasmid with a restriction enzyme requires more enzymes than cutting generally a stranded DNA, such as λ DNA. Concerning the most commonly used restriction enzymes for cloning, the following table shows the numbers of units required for complete digestion of 1 µg of pBR322 DNA and pUC19 DNA under Nippon Gene restriction enzyme reaction conditions.

pBR322

Restriction enzyme	Number of cutting sites	Total enzyme requirement (unit)	Restriction enzyme	Number of cutting sites	Total enzyme requirement (unit)	Restriction enzyme	Number of cutting sites	Total enzyme requirement (unit)
Ase I	1	1	*Nru* I	1	> 10	*Fsp* I	2	3
Ava I	1	1	*Pst* I	1	1	*Hinc* II	2	7
Bal I	1	> 10	*Pvu* II	1	1	*Alw44* I	3	10
BamH I	1	1	*Sal* I	1	6	*Bgl* I	3	2
Bsm I	1	1	*Sca* I	1	1	*Dra* I	3	1
EcoR I	1	2	*Sph* I	1	1	*Rsa* I	3	2
EcoR V	1	2	*Ssp* I	1	1	*Nar* I	4	1
Hind III	1	1	*Sty* I	1	5	*EcoO109* I	4	3
Nde I	1	1	*Acc* I	2	1			
Nhe I	1	2	*EcoT38* I	2	2			

pUC19

Restriction enzyme	Number of cutting sites	Total enzyme requirement (unit)	Restriction enzyme	Number of cutting sites	Total enzyme requirement (unit)	Restriction enzyme	Number of cutting sites	Total enzyme requirement (unit)
Acc I	1	1	*Nar* I	1	> 10	*Bgl* I	2	2
Ava I	1	2	*Nde* I	1	1	*Pvu* II	2	1
BamH I	1	2	*Sac* I	1	3	*Acy* I	3	1
EcoO109 I	1	5	*Sal* I	1	9	*Alw44* I	3	> 10
EcoR I	1	3	*Sca* I	1	2	*Ase* I	3	1
EcoT38 I	1	1	*Sma* I	1	2	*Dra* I	3	3
Hinc II	1	5	*Sph* I	1	2	*Hae* II	3	2
Hind III	1	2	*Ssp* I	1	1	*Rsa* I	3	1
Kpn I	1	> 10	*Xba* I	1	1	*Fsp* I	4	10
Pst I	1	1	*Ava* II	2	1	*Taq* I	4	1

Classification of restriction enzymes by recognition sequence

I Recognizing palindromes of 4, 5, and 6 base

	AATT	ACGT	AGCT	ATAT	CATG	CCGG	CGCG	CTAG	GATC	GCGC	GGCC	GTAC	TATA	TCGA	TGCA	TTAA
▼OOOO									*Sau* 3AI *Nde* II							
O▼OOO		*Mae* II				*Msp* I *Hpa* II		*Mae* I		*Sci* NI				*Taq* I		
OO▼OO			*Alu* I				*Acc* II		*Dpn* I		*Hae* III	*Rsa* I				
OOO▼O										*Hha* I						
OOOO▼					*Nla* III											
▼OONOO												(*Mae* III)				
O▼ONOO								(*Dde* I)	(*Hinf* I)		(*Cfr* 13 I) (*Sau* 96 I)					
OO▼NOO						(*Scr* F I)				(*Fnu* 4H I)						
OON▼OO																
OONO▼O																
OONOO▼																
▼OO^{A}/$_{T}$OO						(*Eco* R II)										
O▼O^{A}/$_{T}$OO											(*Ava* II) (*Eco* 47 I)					
OO▼^{A}/$_{T}$OO						(*Bst* N I) (*Mva* I)										
OO^{A}/$_{T}$▼OO																
OO^{A}/$_{T}$O▼O																
OO^{A}/$_{T}$OO▼																
▼OO^{G}/$_{C}$OO																
O▼O^{G}/$_{C}$OO																
OO▼^{G}/$_{C}$OO						(*Nci* I)										
OO^{G}/$_{C}$▼OO						(*Ban* I)										
OO^{G}/$_{C}$O▼O																
OO^{G}/$_{C}$OO▼																
A▼OOOOT			*Hind* III		(*Afl* III)	*Age* I	(*Afl* III) *Mlu* I	*Spe* I	(*Bgl* II) (*Xno* II)							
AO▼OOOT														*Ban* III *Cla* I		*Ase* I
AOO▼OOT				*Ssp* I						*Eco* 47 III	*Aat* I *Hae* I *Stu* I	*Sca* I				
AOOO▼OT																
AOOOO▼T					(*Nsp* I)					(*Hae* II)					*Ava* III *Eco* T22 I *Nsi* I	
C▼OOOOG					*Nco* I	(*Ava* I) *Xma* I		*Avr* II			(*Cfr* I) *Eco* 52 I *Xma* III			(*Ava* I) *Xho* I		*Afl* II
CO▼OOOG				*Nde* I												
COO▼OOG		*Pma* C I	(*Nsp* B II) *Pvu* II			*Sma* I	(*Nsp* B II)							*Sci* I		
COOO▼OG							*Sac* II *Sst* II		*Pvu* I *Xor* II							
COOOO▼G															*Pst* I	
G▼OOOOC	*Eco* R I					(*Cfr* 10 I)	*Bss* H II	*Nhe* I	*Bam* H I *Bst* I (*Xho* II)	(*Ban* I)		*Asp* 718 I (*Ban* I)		*Sal* I	*Alw* 44 I	
GO▼OOOC		(*Acy* I)								(*Acy* I) *Nar* I			(*Acc* I)	(*Acc* I)		
GOO▼OOC				*Eco* R V		*Nae* I								(*Hinc* II)		(*Hinc* II) *Hpa* I
GOOO▼OC																
GOOOO▼C		*Aat* II	(*Eco* T38 I) (*Hgi* A I) *Sac* I		(*Nsp* I) *Sph* I					*Bbe* I (*Hae* II)	*Apa* I (*Eco* T38 I)	*Kpn* I			(*Hgi* A I)	
T▼OOOOA						(*Acc* III) *Mro* I		*Xba* I	*Bcl* I		(*Cfr* I)					
TO▼OOOA														*Nsp* V		
TOO▼OOA		*Sna* B I					*Nru* I			*Fsp* I	*Bal* I (*Hae* I)					*Dra* I
TOOO▼OA																
TOOOO▼A																

( )The enzymes in parentheses recognize multiple base sequences. Enzymes sold by Nippon Gene

II Recognizing palindromes of 7,8 bases

CC▼TNAGG	*Axy* I	*Eco*81 I
CG▼G(A/T)CCG	*Rsr* II	
GC▼TNAGC	*Esp* I	
GG▼TNACC	*Bst*E II	
PuG▼G(A/T)CCPy	*Ppu*M I	
PuG▼GNCCPy	EcoO109 I	
GC▼GGCCGC	*Not* I	

III Recognizing an interrupted palindrome

CACNNN▼GTG	*Dra* III
C▼CNNGG	*Sec* I
CCANNNNN▼NTGG	*Bst*X I
GAANN▼NNTTC	*Xmn* I
GACN▼NNGTC	*Tth*111 I
GCCNNNN▼NGGC	*Bgl* I
GGN▼NCC	*Nla* IV
GGCCNNNN▼NGGCC	*Sfi* I

IV Recognizing sequences that are not palindromes

ACCTGC(N)4▼ TGGACG(N)8▲	*Bsp*M I	CTCCAG(N)14▼ GAGGTC(N)10▲	*Gsu* I	GACGC(N)5▼ CTGCG(N)10▲	*Hga* I	GGATC(N)4▼ CCTAG(N)5▲	*Bin* I
CAAPuCA(N)4▼ GTTPyGT(N)9▲	*Tth*111 II	GAAGA(N)8▼ CTTCT(N)7▲	*Mbo* II	GCAGC(N)8▼ CGTCG(N)12▲	*Bbv* I	GGATG(N)9▼ CCTAC(N)13▲	*Fok* I
CCTC(N)7▼ GGAG(N)7▲	*Mnl* I	GAATGCN▼ CTTAC▲GN	*Bsm* II	GCATC(N)5▼ CGTAG(N)9▲	*Sfa*N I	GGTGA(N)8▼ CCACT(N)7▲	*Hph* I

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V Recognizing multiple sequences

AC▼ACGT	AfI III	AG▼GACCT	*Eco*O109 I	CA▼GCGG	*Bsp*B II	GAGCA▼C	*Bsp*1286 I
AC ATGT		AG GACCC	(*Dra* II)	CA GCTG		GAGCC C	
AC GCGT		AG GCCCT		CC GCGG		GAGCT C	
AC GTGT		AG GCCCC		CC GCTG		GGGCA C	
		AG GGCCT				GGGCC C	
ACATG▼C	*Nsp*C I	AG GGCCC		C▼CAAGG	*Sty* I	GGGCT C	
ACATG T		AG GTCCT		C CATGG		GTGCA C	
GCATG C		AG GTCCC		C CTAGG		GTGCC C	
GCATG T		GG GACCT		C CTTGG		GTGCT C	
		GG GACCC					
A▼GATCC	*Xho* II	GG GCCCT		C▼CCGAG	*Ava* I	GAGCC▼C	*Eco*T38 I
A GATCT		GG GCCCC		C CCGGG		GAGCT C	(*Ban* II)
G GATCC		GG GGCCT		C TCGAG		GGGCC C	
G GATCT		GG GGCCC		C TCGGG		GGGCT C	
		GG GTCCT					
AGCGC▼C	*Hae* II	GG GTCCC		C▼GGCCA	*Cfr* I	G▼GCACC	*Ban* I
AGCGC T				C GGCCG		G GCGCC	
GGCGC C		AGGAC▼CT	*Pss* I	T GGCCA		G GTACC	
GGCGC T		AGGAC CC		T GGCCG		G GTGCC	
		AGGCC CT					
AGG▼CCA	*Hae* I	AGGCC CC		C▼GGCCG	*Gdi* II	GT▼AGAC	*Acc* I
AGG CCT		AGGGC CT		T GGCCG		GT ATAC	
TGG CCA		AGGGC CC				GT CGAC	
TGG CCT		AGGTC CT		GA▼CGCC	*Acy* I	GT CTAC	
		AGGTC CC		GA CGTC			
AG▼GACCT	*Ppu*M I	AGGAC CT		GG CGCC		GTC▼AAC	*Hinc* II
AG GACCC		AGGAC CC		GG CGTC		GTC GAC	
AG GTCCT		AGGCC CT				GTT AAC	
AG GTCCC		AGGCC CC		GAGCA▼C	*Hgi*A I	GTT GAC	
GG GACCT		AGGGC CT		GAGCT C			
GG GACCC		AGGGC CC		GTGCA C			
GG GTCCT		AGGTC CT		GTGCT C			
GG GTCCC		AGGTC CC					

Enzymes sold by Nippon Gene

List of DNA methylation and restriction enzyme reactivity

Escherichia coli has two types of methylases that recognize and methylate specific sites in the DNA. One is dam methylase, which recognizes "GATC" and methylates N6 of adenine. The other is dcm methylase, which recognizes the "CC(A/T)GG" and methylates the inner cytosine C5. Therefore, DNA-such as plasmids prepared from Escherichia coli is methylated by the two methylases. Restriction enzymes whose recognition sites match or overlap with the recognition sites of these methylases cannot cut the DNA methylated by these methylases. However, because these methylations are incomplete, some of the DNA is cut and a partial digestion pattern is obtained.

Restriction enzyme	Recognition sequence	Methylases that exert influence and their recognition sequences	Cleavage-inhibited base sequence
Acc I	GT↓(A/C)(T/G)AC	M. *Taq* I M TCGA	* GTCGAC
Acc III	T↓CCGGA	*dam* M GATC	* TCCGGATC
Alu I	AG↓CT	M. *Alu* I M AGCT	* AGCT
		M. *Pst* I M CTGCAG	* CTGCAGCT
Apa I	GGGCC↓C	M. *Hae* III M GGCC	* GGGCCC
Apy I	CC↓(A/T)GG	*dcm* M CC(A/T)GG	m CC(A/T)GG
Ava I	C↓(T/C)CG(A/G)G	M. *Taq* I M TCGA	* CTCGAG
		M. *Hpa* II M CCGG	* CCCGGG
Ava II	G↓G(A/T)CC	*dcm* M CC(A/T)GG	* GG(A/T)CC(A/T)GG
		M. *Hpa* II M CCGG	* GG(A/T)CCGG
Bal I	TGG↓CCA	M. *Hae* III M GGCC	* TGGCCA
*Bam*H I	G↓GATCC	M. *Bam*H I M GGATCC	* GGATCC
		M. *Msp* I M CCGG	* GGATCCGG
Ban II	G(A/G)GC(C/T)↓C	M. *Alu* I M AGCT	* GAGCTC
		M. *Hae* III M GGCC	* GGGCCC
Bcl I	T↓GATCA	*dam* M GATC	* TGATCA
Bcn I	CC(G/C)↓GG	M. *Bcn* I M CC(G/C)GG	* CC(G/C)GG
		M. *Msp* I M CCGG	* CCCGGG
		M. *Hpa* II M CCGG	* CCGGG
Bgl I	GCCN4↓NGGC	M. *Hae* III M GGCC	* GGCCN5GGCC
*Bsp*1286 I	G(A/G)GC(A/C)↓C	M. *Alu* I M AGCT	* GAGCTC
		M. *Hae* III M GGCC	* GGGCCC
*Cfr*13 I	G↓GNCC	*dcm* M CC(A/T)GG	* GGNCC(A/T)GG
		M. *Hpa* II M CCGG	* GGNCCGG
		M. *Msp* I M CCGG	* GGNCCCGG
Cla I	AT↓CGAT	M. *Cla* I M ATCGAT	* ATCGAT
		M. *Taq* I M TCGA	* ATCGAT
		dam M GATC	* ATCGATC
Dde I	C↓TNAG	M. *Alu* I M AGCT	* AGCTNAG
Dpn I	GA↓TC	*dam* M GATC	m GATC
Dpn II	GA↓TC	M. *Cla* I M ATCGAT	* ATCGATC
		M. *Taq* I M TCGA	* TCGATC
		dam M GATC	* GATC
Eae I	(T/C)↓GGCC(A/G)	*dcm* M CC(A/T)GG	* (T/C)GGCCAGG
		M. *Hap* II M CCGG	* (T/C)GGCCGG
*Eco*O109 I	PuG↓GNCCPy	*dcm* M CC(A/T)GG	* PuGGNCCTGG
*Eco*R I	G↓AATTC	M. *Eco*R I M GAATTC	* GAATTC
		M. *Msp* I M CCGG	* GAATTCCGG
*Eco*R II	↓CC(A/T)GG	*dcm* M CC(A/T)GG	* CC(A/T)GG
*Eco*R V	GAT↓ATC	M. *Taq* I M TCGA	* TCGATATC
Hae II	(A/G)GCGC↓(T/C)	M. *Hha* I M GCGC	* (A/G)GCGC(T/C)
Hae III	GG↓CC	M. *Hae* III M GGCC	* GGCC
		M. *Msp* I M CCGG	* GGCCGG
Hha I	GCG↓C	M. *Hha* I M GCGC	* GCGC
		M. *Msp* I M CCGG	* GCGCCGG
Hinc II	GT(T/C)↓(A/G)AC	M. *Hind* III M GC(T/C)(A/G)AC	* GC(T/C)(A/G)AC
		M. *Taq* I M TCGA	* GTCGAC
Hind III	A↓AGCTT	M. *Hind* III M AAGCTT	* AAGCTT
*Hin*f I	G↓ANTC	M. *Taq* I M TCGA	* GANTCGA
Hpa I	GTT↓AAC	M. *Hpa* I M GTTAAC	* GTTAAC
Nde II	↓GATC	*dam* M GATC	* GATC
		M. *Cla* I M ATCGAT	* ATCGATC
		M. *Taq* I M TCGA	* TCGATC
Mbo II	GAAGAN8↓	*dam* M GATC	* GAAGATC
Mfl I	(A/G)↓GATC(T/C)	*dcm* M GATC	* (A/G)GATC(T/C)
Msp I	C↓CGG	M. *Msp* I M CCGG	* CCGG
		M. *Hae* III M GGCC	* GGCCGG
		M. *Bam*H I M GGATCC	* GGATCCGG
Nci I	CC↓(G/C)GG	M. *Bcn* I M CC(G/C)GG	* CC(G/C)GG
Nco I	C↓CATGG	M. *Hae* III M GGCC	* GGCCATGG
Nru I	TCG↓CGA	*dcm* M GATC	* TCGCGATC
Pst I	CTGCA↓G	M. *Alu* I M AGCT	* AGCTGCAG
Sal I	G↓TCGAC	M. *Taq* I M TCGA	* GTCGAC
*Sau*3A I	↓GATC	M. *Msp* I M CCGG	* GATCCGG
		M. *Bam*H I M GGATCC	* GGATCC
*Sau*96 I	G↓GNCC	*dcm* M CC(A/T)GG	* GGNCC(A/T)GG
		M. *Hpa* II M CCGG	* GGNCCGG
		M. *Hae* III M GGCC	* GGGCC
		M. *Msp* I M CCGG	* GGNCCCGG
*Scr*F I	CC↓NGG	M. *Hpa* II M CCGG	* CCGGG
		M. *Msp* I M CCGG	* CCCGG
Sma I	CCC↓GGG	M. *Hpa* II M CCGG	* CCCGGG
Stu I	AGG↓CCT	*dcm* M CC(A/T)GG	* AGGCCTGG
Taq I (*Tth*HB8 I)	T↓CGA	M. *Taq* I M TCGA	* TCGA
		dam M GATC	* TCGATC
		M. *Cla* I M ATCGAT	* ATCGAT
Xba I	T↓CTAGA	*dam* M GATC	* TCTAGATC
Xho I	C↓TCGAG	M. *Taq* I M TCGA	* CTCGAG
Xho II	(A/G)↓GATC(C/T)	M. *Msp* I M CCGG	* (A/G)GATCCGG
Xma I	C↓CCGGG	M. *Msp* I M CCGG	* CCCGGG
Xma III	C↓GGCCG	M. *Hae* III M GGCC	* CGGCCG
		M. *Msp* I M CCGG	* CGGCCGC
Xmn I	GAAN2↓N2TTC	M. *Taq* I M TCGA	* TCGAAN4TTC

m: If it is not methylated, it will not be cleaved

*: Inhibited by methylation

↓: Cutting site

M: Base to be methylated

The sequence affected by methylation represents only the base sequence of one DNA strand.

For example, in the case of Alu I-M.Pst I, in addition to the sequence 5'...CTGCAGCT...3', the sequence 5'...AGCTGCAG...3' is also included.

List of star activity of restriction enzymes

Certain restriction enzymes are known to cut sequences that are similar to, but not identical to, their fixed recognition sequences. Fluctuations in the specificity of this recognition sequence are called star activity. In general, star activity is caused by changes in reaction conditions, such as the use of large amounts of enzymes relative to the substrate, the presence of different metal ions, lower salt concentrations, pH higher than the standard and the addition of organic solvents such as Glycerol or DMSO. The relatively well-known star activity is shown on the table.

Restriction enzyme	Normal recognition sequence	Recognition sequence for star activity	cause	References
Ava I	C ↓ PyCGPuG		Excessive enzyme Increase in glycerol concentration	1
*Bam*H I	G ↓ GATCC	GGNTCC GGANCC GPuATCC	Excessive enzyme Increase in glycerol concentration Substitution of Mg^{2+} and Mn^{2+} Decrease in salt concentration	1,2,3,4
Bst I	G ↓ GATCC	NGATCN	Excessive enzyme Increase in glycerol concentration	5
*Bsu*R I	GG ↓ CC	NGCN	Excessive enzyme Increase in glycerol concentration High pH	6
Dde I	C ↓ TNAG		High pH Decrease in salt concentration	7
*Eco*R I	G ↓ AATTC	NAATTN	Excessive enzyme Increase in glycerol concentration Substitution of Mg^{2+} and Mn^{2+} High pH Decrease in salt concentration	4,8,9,10,11
*Eco*R V	GAT ↓ ATC	PuATATC GNTATC GANATC GATNTC GATANC GATNPy	Addition of DMSO	12
Hae III	GG ↓ CC		Excessive enzyme Increase in glycerol concentration	1
Hha I	GCG ↓ C		Excessive enzyme Increase in glycerol concentration Addition of DMSO	4
*Hin*d III	A ↓ AGCTT	PuAGCTT $A(^{A}G_{T})GCTT$ $AA(^{G}{}_{T})CTT$ AAGCNT AAGCTPy	Substitution of Mg^{2+} and Mn^{2+} Addition of DMSO	10,13
Hpa I	GTT ↓ AAC		Excessive enzyme Increase in glycerol concentration	1
Kpn I	GGTAC ↓ C			_
*Pae*R 7	C ↓ TCGAG		Increase in glycerol concentration Decrease in salt concentration	14
Pst I	CTGCA ↓ G		Excessive enzyme Increase in glycerol concentration Addition of DMSO	1,4
Pvu II	CAG ↓ CTG	CCGCTG CATCTG CAGATG CAGGTG CAGCGG	Excessive enzyme Increase in glycerol concentration Addition of DMSO	15
Sal I	G ↓ TCGAC		Excessive enzyme Increase in glycerol concentration Addition of DMSO	4
*Sau*3A I	↓ GATC	GAGC CATC	Excessive enzyme Increase in glycerol concentration	16
Sca I	AGT ↓ ACT			_
Sst I	GAGCT ↓ C		Excessive enzyme Increase in glycerol concentration Addition of DMSO	4
Sst II	CCGC ↓ GG		Excessive enzyme Increase in glycerol concentration	1
*Tth*111 I	GACN ↓ NNGTC	NACNNNGTC GACNNNNTC GACNNNGNC	Substitution of Mg^{2+} and Mn^{2+} High pH Increase in salt concentration	17
Xba I	T ↓ CTAGA		Excessive enzyme Increase in glycerol concentration Addition of DMSO	1,4

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List of restriction enzymes available for multicloning sites of M13 phage, plasmid pUC

Restriction enzymes for cloning sites	Sequence of the cleavage site*1)	Restriction enzymes that produce ligatable cleavage sites	
		Restriction enzyme	Recognanition sequence
*Eco*R I	↓AATT	*Eco*R I	G↓AATTC
Sac I	AGCT↓	*Sac* I	GAGCT↓C
		Sst I	GAGCT↓C
Kpn I	GTAC↓	*Kpn* I	GGTAC↓C
Xma I	↓CCGG	*Xma* I	C↓CCGGG
		*Cfr*9 I	C↓CCGGG
		Xcy I	C↓CCGGG
		*Cfr*10 I	Pu↓CCGGPy
*Bam*H I	↓GATC	*Bam*H I	G↓GATCC
		Ali I	G↓GATCC
		Bst I	G↓GATCC
		Bcl I	T↓GATCA
		Bgl II	A↓GATCT
		Xho II	Pu↓GATCPy
		*Bce*243 I	↓GATC
		Cpf I	↓GATC
		*Fnu*C I	↓GATC
		*Fnu*E I	↓GATC
		Nde II	↓GATC
		*Sau*3A I	↓GATC
Xba I	↓CTAG	*Xba* I	T↓CTAGA
		Avr II	C↓CTAGG
		Nhe I	G↓CTAGC
		Spe I	A↓CTAGT
Sal I	↓TCGA	*Sal* I	G↓TCGAC
		*Hgi*C III	G↓TCGAC
		*Hgi*D II	G↓TCGAC
		Nop I	G↓TCGAC
		Blu I	C↓TCGAG
		*Pae*R 7	C↓TCGAG
		Pan I	C↓TCGAG
		Xho I	C↓TCGAG
		Xpa I	C↓TCGAG
Acc I	↓CG	*Acc* I	GT↓$(^{A}_{C})(^{G}_{T})$AC
		Aha II	GPu↓CGPyC
		Aos II	GPu↓CGPyC
		*Ast*W I	GPu↓CGPyC
		Asu III	GPu↓CGPyC
		*Hgi*D I	GPu↓CGPyC
		*Hgi*G I	GPu↓CGPyC
		*Hgi*H II	GPu↓CGPyC
		Nar I	GG↓CGCC
		Nda I	GG↓CGCC
		Nun II	GG↓CGCC
		Asu II	TT↓CGAA
		Fsp II	TT↓CGAA
		Cla I	AT↓CGAT
		Hpa II	C↓CGG
		Mno I	C↓CGG
		Msp I	C↓CGG
		*Hin*P I	G↓CGC
		*Sci*N I	G↓CGC
		Taq I	T↓CGA
		*Tth*HB8 I	T↓CGA
		Mae II	A↓CGT

Restriction enzymes for cloning sites	Sequence of the cleavage site*1)	Restriction enzymes that produce ligatable cleavage sites	
		Restriction enzyme	Recognanition sequence
Pst I	TGCA↓	*Pst* I	CTGCA↓G
		*Sal*P I	CTGCA↓G
		Sfi I	CTGCA↓G
		Nsi I	ATGCA↓T
Sph I	CATG↓	*Sph* I	GCATG↓C
		*Nsp*7524 I	PuCATG↓Py
		*Esp*H I	PuCATG↓Py
		Nla III	CATG↓
Hind III	↓AGCT	*Hind* III	A↓AGCTT
		*Eco*V III	A↓AGCTT
		Hsu I	A↓AGCTT
Sma I	blunt(CCC↓GGG)	*Sma* I	CCC↓GGG
Hinc II	blunt(GTC↓GAC)	*Hinc* II	GTPy↓PuAC
		*Hin*JC I	GTPy↓PuAC
		Hind II	GTPy↓PuAC
		Hpa I	GTT↓AAC
		Aos I	TGC↓GCA
		Fdi II	TGC↓GCA
		Mst I	TGC↓GCA
		Bal I	TGG↓CCA
		Aha III	TTT↓AAA
		Dra I	TTT↓AAA
		Nru I	TCG↓CGA
		SnaB I	TAC↓GTA
		Gdi I	AGG↓CCT
		Hae I	$(^{A}_{T})$GG↓CC$(^{T}_{A})$
		Sca I	AGT↓ACT
		Stu I	AGG↓CCT
		*Eco*R V	GAT↓ATC
		Nae I	GCC↓GGC
		Nla IV	GGN↓NCC
		Pvu II	CAG↓CTG
		*Nsp*B II	C$(^{A}_{C})$G↓C$(^{T}_{G})$G
		*Bsp*R I	GG↓CC
		Clt I	GG↓CC
		*Fun*D I	GG↓CC
		Hae III	GG↓CC
		Sfa I	GG↓CC
		Alu I	AG↓CT
		Acc II	CG↓CG
		Tha I	CG↓CG
		Rsa I	GT↓AC
		*Asp*700	GAANN↓NNTTC
		Xmn I	GAANN↓NNTTC

*1)Sequence of the cleavage site

ex. *Bam*H I
↓GATC

5' G|GATC C 3'
3' C CTAG|G 5'

*Bam*H I →

5'|GATC C 3'
|G

The base sequence of the BamH I restriction site (↓GATC) shows the bolded protruding portion.

□ Enzymes sold by Nippon Gene.