

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 λ DNA 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	Alw44 I (BSA supplied)*, Apa I, Kpn I, Mbo II, Nar I, Nci I, NspV, 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, EcoO109 I, EcoR II, EcoT38 I, Fok I, Hae II, Hae III, Hinc II, Msp I, Nhe I, Pvu II, Rsa I, Sau3A I, Sau96 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, BstE II, BstX I, EcoR I, EcoR V, Hinf I, Mlu I, Nco I, Nde I, Not I (Triton X-100 supplied)*, Nsi I, Pst I, Sal I, ScrF I, Sph I, Sty I, Swa I (BSA supplied)*, Xba 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, Bsp1286 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, BamH I, Bsm I, BssH II, Hha I, Hind 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, Alw44 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, *Alw44* 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%.

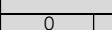
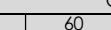
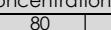
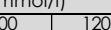
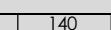
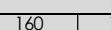
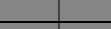
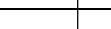
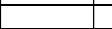
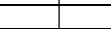
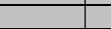
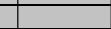
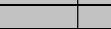
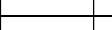
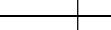
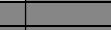
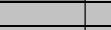
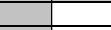
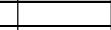
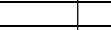
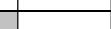
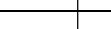
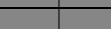
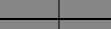
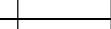
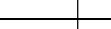
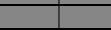
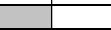
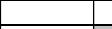
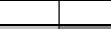
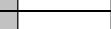
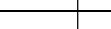
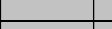
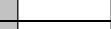
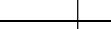
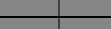
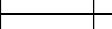
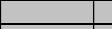
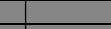
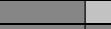
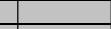
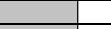
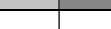
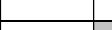
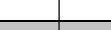
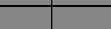
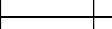
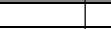
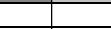
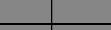
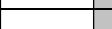
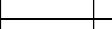
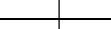
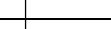
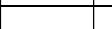
Restriction enzyme	L	M	H	A	B	Dedicated	Restriction enzyme	L	M	H	A	B	Dedicated
<i>Acc</i> I	50	75	<5	100	<5	—	<i>Hpa</i> I	(25)	(75)	25	(50)	(100)	100
<i>Acc</i> II	(75)	100	10	100	50	—	<i>Kpn</i> I	100	50	<5	100	<5	—
<i>Acc</i> III	<5	10	50	(5)	75	—	<i>Mbo</i> II	100	75	25	75	25	—
<i>Acy</i> I	<5	10	25	<5	100	—	<i>Mlu</i> I	10	25	100	10	50	—
<i>Afl</i> II	50	50	5	100	25	—	<i>Msp</i> I	100	100	25	100	50	—
<i>Age</i> I	(75)	100	10	75	25	—	<i>Nar</i> I	100	5	<5	100	<5	—
<i>Alu</i> I	100	100	25	150	25	—	<i>Nci</i> I	100	50	<5	100	<5	—
<i>Alw44</i> I	75	50	<5	50	25	—	<i>Nco</i> I	(75)	(100)	100	(100)	(150)	—
<i>Apa</i> I	100	10	<5	50	<5	—	<i>Nde</i> I	10	25	100	25	100	—
<i>Ase</i> I	(10)	(50)	100	(25)	100	—	<i>Nde</i> II	5	5	25	5	5	100
<i>Ava</i> I	10	100	10	25	50	—	<i>Nhe</i> I	(150)	100	5	(200)	10	—
<i>Ava</i> II	(75)	100	5	(75)	50	—	<i>Not</i> I	5	25	50	10	50	—
<i>Axy</i> I	(100)	100	50	(100)	50	—	<i>Nru</i> I	<5	5	75	5	25	100
<i>Bal</i> I	25	10	<5	25	<5	100	<i>Nsi</i> I	75	100	100	(75)	100	—
<i>Bam</i> H I	(75)	(100)	75	(75)	100	—	<i>Nsp</i> V	100	50	<5	150	<5	—
<i>Bcl</i> I	(100)	(200)	100	(100)	200	—	<i>Pst</i> I	(200)	(150)	100	(150)	50	—
<i>Bgl</i> I	(10)	(50)	100	(10)	25	—	<i>Pvu</i> II	(50)	100	5	(50)	5	—
<i>Bgl</i> II	(10)	(75)	100	(50)	(150)	—	<i>Rsa</i> I	200	100	5	200	50	—
<i>Bsm</i> I	(25)	(75)	50	(75)	100	—	<i>Sac</i> I	100	75	10	100	10	—
<i>Bsp</i> 1286 I	75	50	10	100	10	—	<i>Sac</i> II	100	50	5	100	5	—
<i>Bss</i> H II	(50)	50	75	(50)	100	—	<i>Sal</i> I	<5	<5	100	<5	5	—
<i>Bst</i> E II	(25)	(100)	100	(75)	100	—	<i>Sau</i> 3A I	(100)	100	25	150	50	—
<i>Bst</i> X I	<5	75	100	25	100	—	<i>Sau</i> 96 I	75	100	50	75	150	—
<i>Dra</i> I	75	100	10	50	75	—	<i>Sca</i> I	(5)	(25)	25	(5)	(100)	100
<i>Eco</i> O109 I	(100)	100	10	100	50	—	<i>Scr</i> F I	(75)	(100)	100	(100)	(150)	—
<i>Eco</i> R I	—	—	100	—	(150)	—	<i>Sfi</i> I	25	100	5	75	5	—
<i>Eco</i> R II	<5	100	75	75	100	—	<i>Sma</i> I	75	10	<5	100	<5	—
<i>Eco</i> R V	(10)	75	100	25	150	—	<i>Spe</i> I	(75)	100	10	(75)	75	—
<i>Eco</i> T38 I	150	100	5	150	75	—	<i>Sph</i> I	(100)	(200)	100	(100)	100	—
<i>Fok</i> I	(200)	100	<5	(200)	100	—	<i>Ssp</i> I	(<5)	(75)	10	(50)	100	—
<i>Fsp</i> I	25	100	5	100	50	—	<i>Stu</i> I	100	100	50	100	100	—
<i>Hae</i> II	100	100	25	75	50	—	<i>Sty</i> I	(10)	(75)	100	25	(75)	—
<i>Hae</i> III	75	100	100	100	100	—	<i>Swa</i> I	<5	25	75	5	75	—
<i>Hha</i> I	(75)	75	25	(100)	100	—	<i>Taq</i> I	25	50	25	100	100	—
<i>Hinc</i> II	50	100	50	100	50	—	<i>Xba</i> I	50	100	25	150	25	—
<i>Hind</i> III	(<5)	75	10	(25)	100	—	<i>Xho</i> I	10	50	100	25	150	—
<i>Hinf</i> I	10	75	100	50	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 MgCl₂, 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 MgCl₂, 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										
BamH I	KCl										
	NaCl										
Bcl I	KCl					<img alt="dark gray					

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, λ DNA is used for almost all restriction enzymes. ϕ 105 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 (%)
<i>Acc</i> I	90	100	<i>EcoR</i> I	95	100	<i>Nsi</i> I	90	100
<i>Acc</i> II	90	100	<i>EcoR</i> II	95	100	<i>Nsp</i> V	95	100
<i>Acc</i> III	95	100	<i>EcoR</i> V	90	100	<i>Pst</i> I	95	100
<i>Acy</i> I	90	100	<i>EcoT38</i> I	95	100	<i>Pvu</i> II	90	100
<i>Afl</i> II	60	100	<i>Fok</i> I	90	100	<i>Rsa</i> II	90	100
<i>Age</i> I	95	100	<i>Fsp</i> I	80	100	<i>Sac</i> I	90	100
<i>Alu</i> I	90	100	<i>Hae</i> II	90	100	<i>Sac</i> II	95	100
<i>Alw44</i> I	95	100	<i>Hae</i> III	90	100	<i>Sal</i> I	80	100
<i>Apa</i> I	95	100	<i>Hha</i> I	90	100	<i>Sau3A</i> I	90	100
<i>Ase</i> I	80	100	<i>Hinc</i> II	80	90	<i>Sau96</i> I	90	100
<i>Ava</i> I	90	100	<i>Hind</i> III	90	100	<i>Sca</i> I	90	100
<i>Ava</i> II	90	100	<i>Hinf</i> I	90	100	<i>ScrF</i> I	60	95
<i>Axy</i> I *	—	—	<i>Hpa</i> I	90	100	<i>Sfi</i> I	80	100
<i>Bal</i> I	90	100	<i>Kpn</i> I	90	100	<i>Sma</i> I	90	100
<i>BamH</i> I	90	100	<i>Mbo</i> II	95	100	<i>Spe</i> I	80	100
<i>Bcl</i> I	95	100	<i>Mlu</i> I	90	100	<i>Sph</i> I	95	100
<i>Bgl</i> I	90	100	<i>Msp</i> I	90	100	<i>Ssp</i> I	90	95
<i>Bgl</i> II	90	100	<i>Nar</i> I	95	100	<i>Stu</i> I	80	100
<i>Bsm</i> I	90	100	<i>Nci</i> I *	—	—	<i>Sty</i> I	95	100
<i>Bsp</i> 1286 I	95	100	<i>Nco</i> I	90	100	<i>Swa</i> I	75	75
<i>BssH</i> II	95	100	<i>Nde</i> I	90	100	<i>Taq</i> I	90	100
<i>BstE</i> II	95	100	<i>Nde</i> II	90	100	<i>Xba</i> I	90	100
<i>BstX</i> I	90	100	<i>Nhe</i> I	90	100	<i>Xho</i> I	80	100
<i>Dra</i> I	90	100	<i>Not</i> I	90	100			
<i>EcoO109</i> I	80	100	<i>Nru</i> 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 (°C)	Total enzyme requirement (unit)	
				5-hour reaction	20-hour reaction
<i>Bgl</i> I	GCCNNNNNGGC	H	37	5	5
<i>BssH</i> II	GCGCGC	B	50	5	5
<i>Dra</i> I	TTTAAA	M	37	25	25
<i>Fsp</i> I	TGCGCA	A	37	5	5
<i>Mlu</i> I	ACGCGT	H	37	5	5
<i>Nhe</i> I	GCTAGC	M	37	50	10
<i>Not</i> I	GCGGCCGC	H + Triton *3	37	20	20
<i>Nru</i> I	TCGCGA	Dedicated	37	50	20
<i>Nsp</i> V	TTCGAA	L	37	5	5
<i>Sal</i> I	GTCGAC	H	37	5	5
<i>Sma</i> I	CCCGGG	A	30	> 50	> 50
<i>Spe</i> I	ACTAGT	M	37	100	100
<i>Ssp</i> I	AATATT	B	37	5	5
<i>Xba</i> I	TCTAGA	M	37	50	50
<i>Xho</i> 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.

Restriction enzyme	65°C 30 minutes		Restriction enzyme	65°C 30 minutes		Restriction enzyme	65°C 30 minutes	
	65°C 30 minutes	70°C 30 minutes		70°C 30 minutes	65°C 30 minutes		70°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	—	—
Bsp1286 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	—	—	—

Restriction enzyme

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									Sau3A I Nde II							
O▼OOO		Mae II				Msp I Hpa II		Mae I		Sci N I			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						(SceF I)				(Fnu 4H I)						
OON▼OO																
OONO▼O																
OONOO▼																
▼OO^/hOO						(Eco R II)										
O▼O^/hOO											(Avr II) (Eco 47 I)					
OO▼^/hOO						(Bst N I) (Mva I)										
OO^/t^OO																
OO^/hO▼O																
OO^/hOO^																
▼OO^/cOO																
O▼O^/cOO																
OO▼^/cOO						(Nci I)										
OO^/c▼OO							(Ban I)									
OO^/cO▼O																
OO^/cOO▼																
A▼OOOOT		Hind III		(Afl III)	Age I	(Afl III) Mlu I	Spe I	(Bgl II) (Xba II)						Ban III		
AO▼OOT														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			(Avr 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 Xba II									
COOOO▼G													Pst I			
G▼OOOOOC	Eco R I				(Cfr 10 I)	BssH II	Nhe I	Bam H I Bst I (Xba II)	(Ban I)		Asp 718 I (Ban I)		Sai I	Alw 44 I		
GO▼OOOC		(Acy I)					Nae I				(Acy I) Nar I		(Acc I)	(Acc I)		
GOO▼OOC			Eco R V											(Hinc II)		(Hpa I)
GOOO▼OC																
GOOOO▼C																
T▼OOOOA																
TO▼OOOA														Nsp V		
TOO▼OOA																
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	<i>Axy</i> I	<i>Eco81</i> I
CG▼G(^T)CCG	<i>Rsr</i> II	
GC▼TNAGC	<i>Esp</i> I	
GG▼TNACC	<i>BstE</i> II	
PuG▼G(^T)CCPy	<i>PpuM</i> I	
PuG▼GNCCPy	<i>EcoO109</i> I	
GC▼GGCCGC	<i>Not</i> I	

III Recognizing an interrupted palindrome

CACNNN▼GTG	<i>Dra</i> III	
C▼CNNGG	<i>Sec</i> I	
CCANNNNN▼NTGG	<i>BstX</i> I	
GAANNN▼NNTTC	<i>Xmn</i> I	
GACN▼NNGTC	<i>Tth111</i> I	
GCCNNNN▼NGGC	<i>Bg</i> I	
GGN▼NCC	<i>Nla</i> IV	
GGCCNNNN▼NGGCC	<i>Sfi</i> I	

IV Recognizing sequences that are not palindromes

ACCTGC(N) ₄ ▼ TGGACG(N) ₈ ▲	<i>Bsp</i> M I	CTCCAG(N) ₁₄ ▼ GAGGTC(N) ₁₀ ▲	<i>Gsu</i> I	GACGC(N) ₅ ▼ CTGCG(N) ₁₀ ▲	<i>Hga</i> I	GGATC(N) ₄ ▼ CCTAG(N) ₅ ▲	<i>Bin</i> I
CAAPuCA(N) ₄ ▼ GTTPyGT(N) ₉ ▲	<i>Tth111</i> II	GAAGA(N) ₈ ▼ CTTCT(N) ₇ ▲	<i>Mbo</i> II	GCAGC(N) ₈ ▼ CGTCG(N) ₁₂ ▲	<i>Bbv</i> I	GGATG(N) ₉ ▼ CCTAC(N) ₁₃ ▲	<i>Fok</i> I
CCTC(N) ₇ ▼ GGAG(N) ₇ ▲	<i>Mnl</i> I	GAATGCN▼ CTTAC▲GN	<i>Bsm</i> II	GCATC(N) ₅ ▼ CGTAG(N) ₉ ▲	<i>Sfa</i> N I	GGTGA(N) ₈ ▼ CCACT(N) ₇ ▲	<i>Hph</i> I

□ Enzymes sold by Nippon Gene

V Recognizing multiple sequences

AC▼ACGT	Afl III	AG▼GACCT	<i>Eco</i> O109 I (<i>Dra</i> II)	CA▼GCGG	<i>Bsp</i> B II	GAGCA▼C	<i>Bsp</i> 1286 I
AC ATGT		AG GACCC		CA GCTG		GAGCC C	
AC GCGT		AG GCCCT		CC GCGG		GAGCT C	
AC GTGT		AG GCCCC		CC GCTG		GGGCA C	
		AG GGCT				GGGCC C	
ACATG▼C	<i>Nsp</i> C I	AG GGGCC		C▼CAAGG	<i>Sty</i> 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	<i>Xba</i> II	GG GCCCT		C▼CCGAG	<i>Ava</i> I	GAGCC▼C	
A GATCT		GG GCCCC		C CCCGG		GAGCT C	
G GATCC		GG GG CCT		C TCGAG		GGGCC C	
G GATCT		GG GGCCC		C TCGGG		GGGCT C	
		GG GT CCT					
AGCGC▼C	<i>Hae</i> II	GG GTCCC		C▼GGCCA	<i>Cfr</i> I	G▼GCACC	<i>Ban</i> I
AGCGC T				C GGCG		G GCGCC	
GGCGC C		AGGAC▼CT	<i>Pss</i> I	T GGCCA		G GTACC	
GGCGC T		AGGAC CC		T GGCG		G GTGCC	
		AGGCC CT					
AGG▼CCA	<i>Hae</i> I	AGGCC CC		C▼GGCCG	<i>Gdi</i> II	GT▼AGAC	<i>Acc</i> I
AGG CCT		AGGGC CT		T GGCG		GT ATAC	
TGG CCA		AGGGC CC				GT CGAC	
TGG CCT		AGGTC CT		GA▼CGCC	<i>Acy</i> I	GT CTAC	
		AGGTC CC		GA CGTC			
AG▼GACCT	<i>Ppu</i> M I	AGGAC CT		GG CGCC		GTC▼AAC	<i>Hinc</i> II
AG GACCC		AGGAC CC		GG CGTC		GTC GAC	
AG GT CCT		AGGCC CT				GTT AAC	
AG GTCCC		AGGCC CC		GAGCA▼C	<i>Hgi</i> A I	GTT GAC	
GG GACCT		AGGGC CT		GAGCT C			
GG GACCC		AGGGC CC		GTGCA C			
GG GT CCT		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	Restriction enzyme	Recognition sequence	Methylases that exert influence and their recognition sequences	Cleavage-inhibited base sequence
<i>Acc</i> I	GT↓(↑ _c)↑ _d AC	M. <i>Taq</i> I TC ^M GA	GT [*] CGAC	<i>Hind</i> III	A↓AGCTT	M. <i>Hind</i> III AG ^M CTT	AAGCTT
<i>Acc</i> III	T↓CCGGA	dam G ^M ATC	TCCGGATC	<i>Hinf</i> I	G↓ANTC	M. <i>Taq</i> I TC ^M GA	GANTCGA
<i>Alu</i> I	AG↓CT	M. <i>Alu</i> I AG ^M CT M. <i>Pst</i> I CTG ^M CAG	AGCT * CTG ^M CAGCT	<i>Hpa</i> I	GTT↓AAC	M. <i>Hpa</i> I GT ^M TAAC	GTTAAC
<i>Apa</i> I	GGGCC↓C	M. <i>Hae</i> III GG ^M CC	GGGCC	<i>Nde</i> II	↓GATC	M. <i>Cla</i> I AT ^M GAT	GATC
<i>Apy</i> I	CC↓(↑ _T)GG	dcm CC ^M (↑ _T)GG	CC ^M (↑ _T)GG			M. <i>Taq</i> I TC ^M GA	ATCGATC
<i>Ava</i> I	C↓(↑ _c)CG(↑ _d)G	M. <i>Taq</i> I TC ^M GA M. <i>Hpa</i> II CC ^M GG	CTCGAG * CCCGGG	<i>Mbo</i> II	GAAGAN ₈ ↓	dam G ^M ATC	GAAGATC
<i>Ava</i> II	G↓G(↑ _T)CC	dcm CC ^M (↑ _T)GG M. <i>Hpa</i> II CC ^M GG	GG(↑ _T)CC(↑ _T)GG * GG(↑ _T)CCGG	<i>Mfl</i> I	(↑ _d)↓GATC(↑ _c)	dcm G ^M ATC	(↑ _d)GATC(↑ _c)
<i>Bal</i> I	TGG↓CCA	M. <i>Hae</i> III GG ^M CC	TGGCCA	<i>Msp</i> I	C↓CGG	M. <i>Msp</i> I CC ^M GG	CCGG
<i>Bam</i> H I	G↓GATCC	M. <i>Bam</i> H I GGATCC M. <i>Msp</i> I CC ^M GG	GGATCC * GGATCCGG			M. <i>Hae</i> III GG ^M CC	* GGCCGG
<i>Ban</i> II	G(↑ _d)GC(↑ _c)↓C	M. <i>Alu</i> I AG ^M CT M. <i>Hae</i> III GG ^M CC	GAGCTC * GGGCCC	<i>Nru</i> I	TCG↓CGA	dcm G ^M ATC	TCGCGATC
<i>Bcl</i> I	T↓GATCA	dam G ^M ATC	TGATCA	<i>Sal</i> I	G↓TCGAC	M. <i>Taq</i> I TC ^M GA	GTCGAC
<i>Bcn</i> I	CC(↑ _c)↓GG	M. <i>Bcn</i> I CC ^M (↑ _c)GG M. <i>Msp</i> I CC ^M GG M. <i>Hpa</i> II CC ^M GG	CC ^M (↑ _c)GG * CCCGGG * CCGGG	<i>Sau</i> 3A I	↓GATC	M. <i>Msp</i> I CC ^M GG	GATCCGG
<i>Bgl</i> I	GCCN ₄ ↓NGGC	M. <i>Hae</i> III GG ^M CC	GGCCN ₅ GGCC			M. <i>Bam</i> H I GGATCC	* GGATCC
<i>Bsp</i> 1286 I	G(↑ _d)GC(↑ _c)↓C	M. <i>Alu</i> I AG ^M CT M. <i>Hae</i> III GG ^M CC	GAGCTC * GGGCCC	<i>Sau</i> 96 I	G↓GNCC	dcm CC ^M (↑ _T)GG	GGNCC(↑ _T)GG
<i>Cfr</i> 13 I	G↓GNCC	dcm CC ^M (↑ _T)GG M. <i>Hpa</i> II CC ^M GG M. <i>Msp</i> I CC ^M GG	GGNCC(↑ _T)GG * GGNCCGG * GGNCCCGG			M. <i>Hpa</i> II CC ^M GG	* GGNCCGG
<i>Cla</i> I	AT↓CGAT	M. <i>Cla</i> I AT ^M GAT M. <i>Taq</i> I TC ^M GA dam G ^M ATC	ATCGAT * ATCGAT * ATCGATC	<i>Stu</i> I	AGG↓CCT	dcm CC ^M (↑ _T)GG	AGGCTTG
<i>Dde</i> I	C↓TNAG	M. <i>Alu</i> I AG ^M CT	AGCTNAG			M. <i>Taq</i> I TC ^M GA	TCGA
<i>Dpn</i> I	GA↓TC	dam G ^M ATC	GATC	<i>Xba</i> I	T↓CTAGA	dam G ^M ATC	TCTAGATC
<i>Dpn</i> II	GA↓TC	M. <i>Cla</i> I AT ^M GATC M. <i>Taq</i> I TC ^M GA dam G ^M ATC	ATCGATC * TCGATC * GATC	<i>Xho</i> I	C↓TCGAG	M. <i>Taq</i> I TC ^M GA	CTCGAG
<i>Eae</i> I	(↑ _c)↓GGCC(↑ _d)	dcm CC ^M (↑ _T)GG M. <i>Hpa</i> II CC ^M GG	(↑ _c)GGCCAGG * (↑ _c)GGCCGG	<i>Xho</i> II	(↑ _d)↓GATC(↑ _c)	M. <i>Msp</i> I CC ^M GG	(↑ _d)GATCCGG
<i>Eco</i> O109 I	PuG↓GNCPy	dcm CC ^M (↑ _T)GG	PuGGNCTGG	<i>Xma</i> I	C↓CGGG	M. <i>Msp</i> I CC ^M GG	CCCGGG
<i>Eco</i> R I	G↓AATTG	M. <i>Eco</i> R I GAATT ^M C M. <i>Msp</i> I CCGG	GAATTC * GAATTCCGG			m: If it is not methylated, it will not be cleaved	
<i>Eco</i> R II	↓CC(↑ _T)GG	dcm CC ^M (↑ _T)GG	CC ^M (↑ _T)GG			*: Inhibited by methylation	
<i>Eco</i> R V	GAT↓ATC	M. <i>Taq</i> I TC ^M GA	TCGATATC			↓: Cutting site	
<i>Hae</i> II	(↑ _d)GC ₂ ↓(↑ _c)	M. <i>Hha</i> I GC ^M CG	(↑ _d)GC ^M CG(↑ _c)			M: Base to be methylated	
<i>Hae</i> III	GG↓CC	M. <i>Hae</i> III GG ^M CC M. <i>Msp</i> I CC ^M GG	GGCC * GGCCGG			The sequence affected by methylation represents only the base sequence of one DNA strand.	
<i>Hha</i> I	GCG↓C	M. <i>Hha</i> I GC ^M CG M. <i>Msp</i> I CC ^M GG	GC ^M CG * GCGCCGG			For example, in the case of <i>Alu</i> I-M. <i>Pst</i> I, in addition to the sequence 5'...CTGCAGCT...3', the sequence 5'...AGCTGCAG...3' is also included.	
<i>Hinc</i> II	GT(↑ _c)↓(↑ _d)AC	M. <i>Hind</i> III GC(↑ _c)↑ ^M AC M. <i>Taq</i> I TC ^M GA	GC(↑ _c)↑ ^M AC * GTCGAC				

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

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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 enzymes for cloning sites	Sequence of the cleavage site*1)	Restriction enzymes that produce ligatable cleavage sites	
		Restriction enzyme	Recognition sequence			Restriction enzyme	Recognition sequence
EcoR I	↓ AATT	EcoR I	G ↓ AATT	Pst I	TGCA ↓	Pst I	CTGCA ↓ G
Sac I	AGCT ↓	Sac I	GAGCT ↓ C	Sst I	GAGCT ↓ C	Sa/P I	CTGCA ↓ G
Kpn I	GTAC ↓	Kpn I	GGTAC ↓ C			Sf I	CTGCA ↓ G
Xma I	↓ CCGG	Xma I	C ↓ CCGGG	Sph I	CATG ↓	Sph I	GCATG ↓ C
		Cfr9 I	C ↓ CCGGG			Nsp7524 I	PuCATG ↓ Py
		Xcy I	C ↓ CCGGG			EspH I	PuCATG ↓ Py
		Cfr10 I	Pu ↓ CCGGPy			Nla III	CATG ↓
Bam H I	↓ GATC	Bam H I	G ↓ GATCC	Hind III	↓ AGCT	Hind III	A ↓ AGCTT
		Al I	G ↓ GATCC			EcoV III	A ↓ AGCTT
		Bst I	G ↓ GATCC			Hsu I	A ↓ AGCTT
		Bcl I	T ↓ GATCA	Sma I	blunt(CCC ↓ GGG)	Sma I	CCC ↓ GGG
		Bgl II	A ↓ GATCT	Hinc II	blunt(GTC ↓ GAC)	Hinc II	GTPy ↓ PuAC
		Xho II	Pu ↓ GATCPy			HinJ C I	GTPy ↓ PuAC
		Bce243 I	↓ GATC			Hind II	GTPy ↓ PuAC
		Cpf I	↓ GATC			Hpa I	GTT ↓ AAC
		FnuC I	↓ GATC			Aos I	TGC ↓ GCA
		FnuE I	↓ GATC			Fdi II	TGC ↓ GCA
		Nde II	↓ GATC			Mst I	TGC ↓ GCA
		Sau3A I	↓ GATC			Bal I	TGG ↓ CCA
Xba I	↓ CTAG	Xba I	T ↓ CTAGA			Aha III	TTT ↓ AAA
		Avr II	C ↓ CTAGG			Dra I	TTT ↓ AAA
		Nhe I	G ↓ CTAGC			Nru I	TCG ↓ CGA
		Spe I	A ↓ CTAGT			SnaB I	TAC ↓ GTA
Sal I	↓ TCGA	Sal I	G ↓ TCGAC			Gdi I	AGG ↓ CCT
		HgiC III	G ↓ TCGAC			Hae I	(^A T)GG ↓ CC(^T A)
		HgiD II	G ↓ TCGAC			Sca I	AGT ↓ ACT
		Nop I	G ↓ TCGAC			Stu I	AGG ↓ CCT
		Blu I	C ↓ TCGAG			EcoR V	GAT ↓ ATC
		PaeR 7	C ↓ TCGAG			Nae I	GCC ↓ GGC
		Pan I	C ↓ TCGAG			Nla IV	GGN ↓ NCC
		Xho I	C ↓ TCGAG			Pvu II	CAG ↓ CTG
		Xpa I	C ↓ TCGAG			NspB II	C(^A C)G ↓ C(^T C)G
Acc I	↓ CG	Acc I	GT ↓ (A ^G) ^{(G})AC			BspR I	GG ↓ CC
		Aha II	GPu ↓ CGPyC			Clt I	GG ↓ CC
		Aos II	GPu ↓ CGPyC			FunD I	GG ↓ CC
		AstW I	GPu ↓ CGPyC			Hae III	GG ↓ CC
		Asu III	GPu ↓ CGPyC			Sfa I	GG ↓ CC
		HgiD I	GPu ↓ CGPyC			Alu I	AG ↓ CT
		HgiG I	GPu ↓ CGPyC			Acc II	CG ↓ CG
		HgiH II	GPu ↓ CGPyC			Tha I	CG ↓ CG
		Nar I	GG ↓ CGCC			Rsa I	GT ↓ AC
		Nda I	GG ↓ CGCC			Asp700	GAANN ↓ NNNTTC
		Nun II	GG ↓ CGCC			Xmn I	GAANN ↓ NNNTTC
		Asu II	TT ↓ CGAA				
		Fsp II	TT ↓ CGAA				
		Cla I	AT ↓ CGAT				
		Hpa II	C ↓ CGG				
		Mbo I	C ↓ CGG				
		Msp I	C ↓ CGG				
		HinP I	G ↓ CGC				
		Scn N I	G ↓ CGC				
		Tag I	T ↓ CGA				
		TthHB8 I	T ↓ CGA				
		Mae II	A ↓ CGT				

*1) Sequence of the cleavage site

ex. **BamH I** 5' G**GATC C** 3'
↓ GATC 3' C CTAGG 5'

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



Enzymes sold by Nippon Gene.