

# RESTRICTION ENZYME PROPERTIES

## Composition of Takara Universal Restriction Enzyme Buffers

<b>10X L</b>	100 mM	Tris-HCl (pH 7.5)
	100 mM	MgCl <sub>2</sub>
	10 mM	Dithiothreitol
<b>10X M</b>	100 mM	Tris-HCl (pH 7.5)
	100 mM	MgCl <sub>2</sub>
	10 mM	Dithiothreitol
	500 mM	NaCl
<b>10X H</b>	500 mM	Tris-HCl (pH 7.5)
	100 mM	MgCl <sub>2</sub>
	10 mM	Dithiothreitol
	1,000 mM	NaCl
<b>10X K</b>	200 mM	Tris-HCl (pH 8.5)
	100 mM	MgCl <sub>2</sub>
	10 mM	Dithiothreitol
	1,000 mM	KCl
<b>10X T*</b>	330 mM	Tris-acetate (pH 7.9)
	100 mM	Magnesium acetate
	5 mM	Dithiothreitol
	660 mM	Potassium acetate
<b>10X Loading Buffer</b>	1%	SDS
	0.05%	Bromophenol blue
	50%	Glycerol

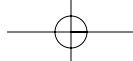
\*BSA should be added to a final concentration of 0.01% to obtain 100% activity.

## Restriction Enzyme-Universal Buffer Compatibility

L	M	H	K	T+BSA
<i>Afa</i> I	<i>Acc</i> I	<i>Ban</i> II	<i>Afa</i> I	<i>Aat</i> II
<i>Alu</i> I	<i>Acc</i> II	<i>Bgl</i> II	<i>Aor</i> 13H I*	<i>Acc</i> I
<i>Aor</i> 51H I	<i>Afa</i> I	<i>Bmg</i> T120 I	<i>Bam</i> H I	<i>Acc</i> II
<i>Apa</i> I	<i>Afl</i> II*	<i>Bsp</i> T107 I	<i>Ban</i> II	<i>Afa</i> I
<i>Apa</i> L I	<i>Alu</i> I	<i>Bss</i> H II	<i>Bcn</i> I	<i>Afl</i> II
<i>Ava</i> II	<i>Aor</i> 51H I	<i>Bst</i> P I	<i>Bln</i> I	<i>Alu</i> I
<i>Bsp</i> T104 I	<i>Ava</i> I	<i>Bst</i> X I	<i>Bme</i> T110 I	<i>Aor</i> 13H I
<i>Bsp</i> 1286 I	<i>Ava</i> II	<i>Bst</i> 1107 I	<i>Bsp</i> T107 I	<i>Aor</i> 51H I
<i>Bss</i> H II	<i>Bsp</i> T104 I	<i>Cla</i> I	<i>Bst</i> 1107 I	<i>Apa</i> L I
<i>Cfr</i> 13 I	<i>Bsp</i> 1407 I	<i>Cpo</i> I	<i>Cfr</i> 13 I	<i>Ava</i> I
<i>Dra</i> I	<i>Bss</i> H II	<i>Dra</i> I	<i>Cla</i> I	<i>Ava</i> II
<i>Eae</i> I	<i>Bst</i> 1107 I	<i>Eco</i> 065 I*	<i>Cpo</i> I	<i>Avi</i> II
<i>Eco</i> 0109 I	<i>Cfr</i> 13 I	<i>Eco</i> R I	<i>Dra</i> I	<i>Bcn</i> I
<i>Hae</i> II	<i>Cla</i> I	<i>Eco</i> R V	<i>Eco</i> T14 I	<i>Bpu</i> 1102 I
<i>Hae</i> III	<i>Dra</i> I	<i>Eco</i> T14 I	<i>Fba</i> I	<i>Bsp</i> T104 I
<i>Hap</i> II	<i>Eae</i> I	<i>Eco</i> T22 I	<i>Hae</i> II	<i>Bsp</i> 1286 I
<i>Hha</i> I	<i>Eco</i> 0109 I	<i>Hae</i> III	<i>Hae</i> III	<i>Bsp</i> 1407 I
<i>Hinf</i> I	<i>Eco</i> 81 I	<i>Hha</i> I	<i>Hha</i> I	<i>Bss</i> H II
<i>Kpn</i> I	<i>Fok</i> I*	<i>Hinf</i> I	<i>Hind</i> III	<i>Cfr</i> 13 I
<i>Mbo</i> II	<i>Fse</i> I	<i>Mbo</i> I	<i>Hinf</i> I	<i>Cla</i> I
<i>Mfi</i> I	<i>Hae</i> II	<i>Mlu</i> I	<i>Hpa</i> I	<i>Dra</i> I
<i>Mlu</i> I	<i>Hae</i> III	<i>Mva</i> I	<i>Mbo</i> I	<i>Eae</i> I
<i>Msp</i> I	<i>Hap</i> II	<i>Nde</i> I	<i>Msp</i> I	<i>Eco</i> 0109 I
<i>Nae</i> I	<i>Hha</i> I	<i>Not</i> I**	<i>Mva</i> I	<i>Eco</i> 81 I
<i>Pma</i> C I	<i>Hinc</i> II	<i>Pst</i> I	<i>Nco</i> I*	<i>Fse</i> I
<i>Sac</i> I	<i>Hind</i> III	<i>Sal</i> I	<i>Nde</i> I	<i>Hae</i> II
<i>Stu</i> I	<i>Hinf</i> I	<i>Sau</i> 3A I	<i>Psh</i> A I	<i>Hae</i> III
	<i>Hin</i> 1 I*	<i>Sca</i> I	<i>Pst</i> I	<i>Hap</i> II
	<i>Kpn</i> I	<i>Spe</i> I	<i>Pvu</i> I*	<i>Hha</i> I
	<i>Mbo</i> II	<i>Sph</i> I	<i>Spe</i> I	<i>Hinc</i> II
	<i>Mfi</i> I	<i>Stu</i> I	<i>Sph</i> I	<i>Hinf</i> I
	<i>Mfu</i> I	<i>Swa</i> I	<i>Stu</i> I	<i>Hin</i> 1 I
	<i>Msp</i> I	<i>Taq</i> I	<i>Taq</i> I	<i>Mbo</i> II
	<i>Mun</i> I*	<i>Van</i> 91 I	<i>Tth</i> 111 I	<i>Mfi</i> I
	<i>Nhe</i> I	<i>Vpa</i> K11B I	<i>Van</i> 911 I	<i>Mlu</i> I
	<i>Pma</i> C I	<i>Xho</i> I	<i>Xho</i> I	<i>Msp</i> I
	<i>Psp</i> 1406 I		<i>Xsp</i> I	<i>Mun</i> I
	<i>Pvu</i> II			<i>Nae</i> I
	<i>Sac</i> I			<i>Nde</i> I
	<i>Sau</i> 3A I			<i>Pma</i> C I
	<i>Sfi</i> I			<i>Pst</i> 1406 I
	<i>Spe</i> I			<i>Psh</i> A I
	<i>Sse</i> 8387 I*			<i>Sac</i> I
	<i>Stu</i> I			<i>Sac</i> II
	<i>Taq</i> I			<i>Sfi</i> I
	<i>Tth</i> 111 I			<i>Sma</i> I
	<i>Xba</i> I*			<i>Stu</i> I
	<i>Xho</i> I			<i>Taq</i> I
	<i>Xsp</i> I			<i>Xba</i> I
				<i>Xho</i> I
				<i>Xsp</i> I

\* +0.01% BSA \*\* +0.01% BSA+0.01% Triton X-100





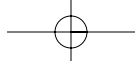
# RESTRICTION ENZYME PROPERTIES



## Recommended Buffers for Double Digests

Takara provides optimized buffers with each restriction enzyme and has determined the relative activity of each enzyme in these buffers (see page 7-6). In some situations, it may be necessary to perform a digestion with more than one restriction enzyme simultaneously or sequentially. This table provides information on the optimal buffer for a “double digest” application. The recommended buffer is shown for each combination of enzymes commonly used in cloning procedures. Some enzymes will function optimally at buffer concentrations other than 1X; these concentrations can be achieved by dilution or by adding more buffer as indicated.

Enzyme	Acc I	BamH I	Bgl II	Cla I	EcoR I	EcoR V	Hinc II	Hind III	Kpn I	Nco I	Nde I	Not I	Pst I	Pvu I	Sac I	Sal I	Sma I	Spe I	Sph I	Xba I	Xho I
Supplied Buffer	10 x M	10 x K	10 x H	10 x M	10 x H	10 x H	10 x M	10 x M	10 x L	10 x K +BSA	10 x H	10 x H +BSA +Triton	10 x H	10 x K +BSA	10 x L	10 x H	10 x T +BSA	10 x M	10 x H	10 x M +BSA	10 x H
Acc I	—	0.5 x K	1 x T	1 x M	1 x M	0.5 x K	1 x M	1 x M	1 x M	1 x M +BSA	1 x T	0.5 x K +BSA	1 x M	0.5 x K	1 x M	1.5 x T	1 x T +BSA	1 x M	0.5 x K	1 x M	1 x M
BamH I	0.5 x K	—	1 x K	1 x K	1 x K	1 x K	0.5 x K	1 x K	0.5 x K	1 x K +BSA	1 x K	0.5 x K +BSA	1 x K	1 x K	0.5 x K	1.5 x T	0.5 x T +BSA	1 x K	1 x K	0.5 x K	1 x K
Bgl II	1 x T	1 x K	—	1 x H	1 x H	1 x H	2 x K	1 x K	1 x T	1 x K +BSA	1 x H	1 x H +BSA	1 x H	1 x K	0.5 x K	1 x H	1 x T +BSA	1 x H	1 x H	2 x T	1 x H
Cla I	1 x M	1 x K	1 x H	—	1 x H	1 x H	1 x M	1 x M	1 x M	1 x K +BSA	1 x H	1 x H +BSA	1 x H	1 x K	1 x M	1 x H	1 x T +BSA	1 x M	1 x H	1 x M	1 x H
EcoR I	1 x M	1 x K	1 x H	1 x H	—	1 x H	1 x M	1 x M	1 x M	1 x K +BSA	1 x H	1 x H +BSA	1 x H	1 x K	1 x M	1 x H	1 x T +BSA	1 x H	1 x H	1 x M	1 x H
EcoR V	0.5 x K	1 x K	1 x H	1 x H	1 x H	—	2 x T	1 x K	0.5 x K	1 x K +BSA	1 x H	1 x H +BSA	1 x H	1 x K	0.5 x K	1 x H	0.5 x K +BSA	1 x H	1 x H	2 x T	1 x H
Hinc II	1 x M	0.5 x K	2 x K	1 x M	1 x M	2 x T	—	1 x M	1 x M	1 x M +BSA	1 x T	0.5 x K +BSA	1 x M	0.5 x K	1 x M	1.5 x K	1 x T +BSA	1 x M	2 x T	1 x M	1 x M
Hind III	1 x M	1 x K	1 x K	1 x M	1 x M	1 x K	1 x M	—	1 x M	1 x K +BSA	1 x K	0.5 x K +BSA	1 x M	1 x K	1 x M	1.5 x K	1 x T +BSA	1 x M	1 x K	1 x M	1 x M
Kpn I	1 x M	0.5 x K	1 x T	1 x M	1 x M	0.5 x K	1 x M	1 x M	—	0.5 x K +BSA	1 x T	0.5 x K +BSA	1 x M	0.5 x K	1 x L	1.5 x T +BSA	1 x T +BSA	1 x M	0.5 x K	1 x M	1 x M
Nco I	1 x M +BSA	1 x K +BSA	1 x K +BSA	1 x K +BSA	1 x K +BSA	1 x K +BSA	1 x M +BSA	1 x K +BSA	0.5 x K +BSA	—	1 x K +BSA	0.5 x K +BSA	1 x K +BSA	1 x K +BSA	0.5 x K +BSA	1.5 x T +BSA	1 x T +BSA	1 x K +BSA	1 x K +BSA	1 x M +BSA	1 x K +BSA
Nde I	1 x T	1 x K	1 x H	1 x H	1 x H	1 x H	1 x T	1 x K	1 x T	1 x K +BSA	—	1 x H +BSA	1 x H	1 x K	1 x T	1 x H	1 x T +BSA	1 x H	1 x H	1 x T	1 x H
Not I	0.5 x K +BSA	0.5 x K +BSA	1 x H +BSA	1 x H +BSA	1 x H +BSA	1 x H +BSA	0.5 x K +BSA	0.5 x K +BSA	0.5 x K +BSA	0.5 x K +BSA	1 x H +BSA	—	1 x H +BSA	2 x K +BSA	0.5 x K +BSA	1 x H +BSA	0.5 x T +BSA	1 x H +BSA	1 x H +BSA	0.5 x K +BSA	1 x H +BSA
Pst I	1 x M	1 x K	1 x H	1 x H	1 x H	1 x H	1 x M	1 x M	1 x M	1 x K +BSA	1 x H	1 x H +BSA	—	1 x K	1 x M	1 x H	0.5 x T +BSA	1 x H	1 x H	1 x M	1 x H
Pvu I	0.5 x K	1 x K	1 x K	1 x K	1 x K	1 x K	0.5 x K	1 x K	0.5 x K	1 x K +BSA	1 x K	2 x K +BSA	1 x K	—	0.5 x K	1.5 x K +BSA	1 x K +BSA	1 x K	1 x K	0.5 x K	1 x K
Sac I	1 x M	0.5 x K	0.5 x K	1 x M	1 x M	0.5 x K	1 x M	1 x M	1 x L	0.5 x K +BSA	1 x T	0.5 x K +BSA	1 x M	0.5 x K	—	1.5 x T +BSA	1 x T +BSA	1 x M	0.5 x K	1 x M	1 x M
Sal I	1.5 x T	1.5 x T	1 x H	1 x H	1 x H	1 x H	1.5 x K	1.5 x K	1.5 x T +BSA	1.5 x T +BSA	1 x H	1 x H +BSA	1 x H	1.5 x K +BSA	1.5 x T +BSA	—	1.5 x T +BSA	1 x H	1 x H	1.5 x T	1 x H
Sma I	1 x T +BSA	0.5 x T +BSA	1 x T +BSA	1 x T +BSA	1 x T +BSA	0.5 x K +BSA	1 x T +BSA	1 x T +BSA	1 x T +BSA	1 x T +BSA	1 x T +BSA	0.5 x T +BSA	0.5 x T +BSA	1 x K +BSA	1 x T +BSA	1.5 x T +BSA	—	1 x T +BSA	0.5 x T +BSA	1 x T +BSA	1 x T +BSA
Spe I	1 x M	1 x K	1 x H	1 x M	1 x H	1 x H	1 x M	1 x M	1 x M	1 x K +BSA	1 x H	1 x H +BSA	1 x H	1 x K	1 x M	1 x H	1 x T +BSA	—	1 x H	1 x M	1 x H
Sph I	0.5 x K	1 x K	1 x H	1 x H	1 x H	1 x H	2 x T	1 x K	0.5 x K	1 x K +BSA	1 x H	1 x H +BSA	1 x H	1 x K	0.5 x K	1 x H	0.5 x T +BSA	1 x H	—	2 x T	1 x H
Xba I	1 x M	0.5 x K	2 x T	1 x M	1 x M	2 x T	1 x M	1 x M	1 x M	1 x M +BSA	1 x T	0.5 x K +BSA	1 x M	0.5 x K	1 x M	1.5 x T	1 x T +BSA	1 x M	2 x T	—	1 x M
Xho I	1 x M	1 x K	1 x H	1 x H	1 x H	1 x H	1 x M	1 x M	1 x M	1 x K +BSA	1 x H	1 x H +BSA	1 x H	1 x K	1 x M	1 x H	1 x T +BSA	1 x H	1 x H	1 x M	—



# RESTRICTION ENZYME PROPERTIES



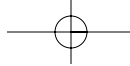
## Residual Enzyme Activity Following Inactivation Treatment

Heat treatment is generally applied to inactivate restriction enzymes after completion of a reaction. Heat tolerance varies depending on the kind of enzyme; however, in some cases heat treatment is insufficient to inactivate restriction enzymes completely. Therefore, four kinds of inactivation treatment were used for each enzyme, and the residual enzyme activity was measured to determine the best conditions for complete inactivation.

Restriction Enzyme	60°C 15 min.	70°C 15 min.	Ethanol Precipitation	Phenol Extraction
<i>Aat</i> II	+	-	-	NT
<i>Acc</i> I	+	-	-	NT
<i>Acc</i> II	+	+	-	NT
<i>Acc</i> III	NT	-	-	NT
<i>Afa</i> I	+	-	+	-
<i>Afl</i> II	-	NT	-	NT
<i>Alu</i> I	-	NT	+	-
<i>Aor</i> 13H I	NT	+	+	-
<i>Aor</i> 51H I	-	NT	-	NT
<i>Apa</i> I	-	NT	-	NT
<i>Apa</i> L I	+	-	-	NT
<i>Ava</i> I	+	-	-	NT
<i>Ava</i> II	+	-	+	-
<i>Avi</i> II	+	-	+	-
<i>Bal</i> I	-	NT	+	-
<i>Bam</i> H I	-	NT	-	NT
<i>Ban</i> II	-	NT	-	NT
<i>Bbe</i> I	-	NT	+	-
<i>Bcn</i> I	+	+	-	NT
<i>Bgl</i> I	-	NT	+	-
<i>Bgl</i> II	+	-	+	-
<i>Bln</i> I	+	+	-	NT
<i>Bpu</i> 1102 I	+	+	+	-
<i>Bsp</i> 1286 I	-	NT	-	NT
<i>Bsp</i> 1407 I	+	-	+	-
<i>Bsp</i> T107 I	-	NT	-	NT
<i>Bss</i> H II	+	-	-	NT
<i>Bst</i> P I	NT	+	-	NT
<i>Bst</i> X I	-	NT	-	NT
<i>Bst</i> 1107 I	+	+	+	-
<i>Cfr</i> 10 I	+	+	+	-
<i>Cfr</i> 13 I	-	NT	-	NT
<i>Clal</i>	-	NT	-	NT
<i>Cpo</i> I	-	NT	-	NT
<i>Dra</i> I	-	NT	-	NT
<i>Eae</i> I	-	NT	-	NT
<i>Eam</i> 1105 I	-	NT	+	-
<i>Eco</i> 0109 I	-	NT	-	NT
<i>Eco</i> 065 I	+	-	+	-
<i>Eco</i> R I	-	NT	-	NT
<i>Eco</i> R V	+	-	+	-
<i>Eco</i> T14 I	-	NT	-	NT
<i>Eco</i> T22 I	-	NT	-	NT
<i>Eco</i> 52 I	-	NT	+	-
<i>Eco</i> 81 I	+	-	-	NT
<i>Fba</i> I	+	+	-	NT
<i>Fok</i> I	-	NT	+	-
<i>Fse</i> I	-	NT	-	NT
<i>Hae</i> II	-	NT	-	NT
<i>Hae</i> III	+	-	-	NT
<i>Hap</i> II	+	-	+	-

Restriction Enzyme	60°C 15 min.	70°C 15 min.	Ethanol Precipitation	Phenol Extraction
<i>Hha</i> I	+	-	-	NT
<i>Hinc</i> II	+	-	-	NT
<i>Hind</i> III	+	-	-	NT
<i>Hinf</i> I	+	-	-	NT
<i>Him</i> I	-	NT	+	-
<i>Hpa</i> I	-	NT	-	NT
<i>Kpn</i> I	-	NT	-	NT
<i>Mbo</i> I	+	-	-	NT
<i>Mbo</i> II	+	-	-	NT
<i>Mfl</i> I	+	+	+	-
<i>Mlu</i> I	+	+	-	NT
<i>Msp</i> I	-	NT	+	-
<i>Mun</i> I	+	-	+	-
<i>Mva</i> I	-	NT	+	-
<i>Nae</i> I	-	NT	-	NT
<i>Nco</i> I	+	-	+	-
<i>Nde</i> I	+	-	-	NT
<i>Nhe</i> I	+	-	-	NT
<i>Not</i> I	+	+	+	-
<i>Nru</i> I	-	NT	+	-
<i>Pma</i> C I	-	NT	-	NT
<i>Psh</i> A I	-	NT	-	NT
<i>Psh</i> B I	+	-	-	NT
<i>Psp</i> 1406 I	-	NT	+	-
<i>Pst</i> I	-	NT	-	NT
<i>Pvu</i> I	-	NT	-	NT
<i>Pvu</i> II	+	+	+	-
<i>Sac</i> I	-	NT	-	NT
<i>Sac</i> II	+	-	+	-
<i>Sal</i> I	-	NT	-	NT
<i>Sau</i> 3A I	+	-	-	NT
<i>Sca</i> I	-	NT	-	NT
<i>Sfi</i> I	+	-	+	-
<i>Sma</i> I	-	NT	+	-
<i>Sna</i> B I	-	NT	-	NT-
<i>Spe</i> I	-	NT	-	NT
<i>Sph</i> I	-	NT	-	NT
<i>Sse</i> 8387 I	-	NT	-	NT
<i>Ssp</i> I	-	NT	-	NT
<i>Stu</i> I	-	NT	+	-
<i>Swa</i> I	-	NT	+	-
<i>Tth</i> H8 I	NT	+	+	-
<i>Tth</i> 111 I ( <i>Taq</i> I)	NT	+	-	NT
<i>Var</i> 91 I	-	NT	+	-
<i>Vpa</i> K11B I	-	NT	-	NT
<i>Xba</i> I	-	NT	+	-
<i>Xho</i> I	+	+	-	NT
<i>Xsp</i> I	+	+	+	-

+ : Residual activity remaining  
 - : No residual activity  
 NT: Not tested



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## Star Activity of Restriction Enzymes

Some restriction enzymes may cleave different base sequence than their recognition sequence, under sub-optimal reaction conditions. These conditions generally include high salt concentrations, impure templates, or a large excess of enzyme compared to substrate DNA. This reduced specificity is called star activity. The table below lists enzymes for which star activity may be observed together with the experimental conditions required to induce star activity. The alternative base sequences cleaved are noted where these data are available.

Restriction Enzyme	Normal recognition Sequence	Reaction conditions*	Recognition Sequence**	Reference
<i>Aat</i> II	GACGT C	D		22
<i>Aor</i> 13H I	T CCGGA	A, C, D		22
<i>Ava</i> I	C YCGRG	A, D		1
<i>Ava</i> II	G GWCC	A, D		22
<i>Avi</i> II	TGC GCA	C, D		22
<i>Bam</i> H I	G GATCC	A, B, D, E	GRATCC GGNTCC GGANCC GGATYC	1,2,3,4
<i>Ban</i> II	GRGCY C	A, D, E		22
<i>Bgl</i> I	GCCNNNN NGGC	E		22
<i>Bgl</i> II	A GATCT	D		22
<i>Bsp</i> T104 I	TT CGAA	A, D		22
<i>Bst</i> P I	G GTNACC	A, E		22
<i>Bst</i> 1107 I	GTA TAC	E		22
<i>Eam</i> 1105 I	GACNNN NNGTC	A, C, E		22
<i>Eco</i> 065 I	G GTNACC	D, E		22
<i>Eco</i> R I	G AATTC	A, B, D, E	NAATTN	4,8,9,10,11
<i>Eco</i> R V	GTA ATC	D	RATATC GNTATC GANATC GATNTC GATANC GATATY	12,22
<i>Eco</i> T22 I	ATGCA T	E, G		22
<i>Fba</i> I	T GATCA	A, C, D, E		22
<i>Hae</i> III	GG CC	A		1
<i>Hha</i> I	GCG C	A, D		4
<i>Hinc</i> II	GTY RAC	D		22
<i>Hind</i> III	A AGCTT	B, D	RAGCTT ANGCTT AAKCTT AAGMTT AAGCNT AAGCTY	10,13,22
<i>Hpa</i> I	GTT AAC	A, D		1,22
<i>Kpn</i> I	GGTAC C	D		22
<i>Mun</i> I	C AATTG	A, E		22
<i>Nco</i> I	C CATGG	A, D		22
<i>Nhe</i> I	G CTAGC	A, C, D, E		22
<i>Psp</i> 1406 I	AA CGTT	D		22
<i>Pst</i> I	CTGCA G	A,D		1,4,22

### \* Reaction Conditions

A: high levels of glycerol; B: in the presence of Mn<sup>2+</sup>; C: alkaline pH; D: in the presence of DMSO; E: low ionic strength; F: high ionic strength; G: in the presence of 2-mercaptoethanol

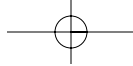
### \*\* Key

M = A or C; K = G or T; N = A, C, G, or T;  
R = A or G; Y = C or T; W = A or T; S = G or C

Restriction Enzyme	Normal recognition Sequence	Reaction conditions*	Recognition Sequence**	Reference
<i>Pvu</i> II	CAG CTG	A, D	NAGCTG CNGCTG CANCTG CAGNTG CAGCNG CAGCTN	15,16,22
<i>Sac</i> I	GAGCT C	A D		22
<i>Sal</i> I	G TCGAC	A, D		4,22
<i>Sau</i> 3A I	GATC	A, D	SATC GMTC GAKC GATS	17,22
<i>Sca</i> I	AGT ACT	B, C, E		21
<i>Sfi</i> I	GGCCNNNN NGGCC	B, D		22
<i>Spe</i> I	A CTAGT	D, E		22
<i>Sse</i> 8387 I	CCTGCA GG	D, E		22
<i>Ssp</i> I	AAT ATT	A, C, D, E		22
<i>Swa</i> I	ATTT AAAT	D, E		22
<i>Tth</i> HB8 I	T CGA	C, D, E		22
<i>Tth</i> 11 I	GACN NNGTC	B, C, F	NACNNGTC GNCNNGTC GANNNGTC GACNNNGTC GACNNNGNC GACNNNGTN	19
<i>Var</i> 9 I	CCANNNN NTGG	A		22
<i>Vpa</i> K11B I	G GWCC	A, C, D, E		22
<i>Xba</i> I	T CTAGA	A, D		1,4

### References

- Nath, K. and Azzolina, B. A. (1981) *Gene Amplif. Anal.* **1**:113-30.
- George, J., *et al.* (1980) *J. Biol. Chem.* **255**:6521-4.
- George, J. and Chirikjian, J.G. (1982) *Proc. Natl. Acad. Sci. USA* **79**:2432-6.
- Malyguine, E. *et al.* (1980) *Gene* **8**:163-77.
- Clarke, C.M. and Hartley, B.S. (1979) *Biochem. J.* **177**:49-62.
- Heininger, K. *et al.* (1977) *Gene* **1**:291-303.
- Makula, R.A. and Meagher, R.B. (1980) *Nucl. Acids Res.* **8**:3125-31.
- Polisky, B. *et al.* (1975) *Proc. Natl. Acad. Sci. USA* **72**:3310-4.
- Tikhonenko, T.I. *et al.* (1978) *Gene* **4**:195-212.
- Hsu, M. and Berg, P. (1978) *Biochemistry* **17**:131-8.
- Woodbury, C.P. *et al.* (1980) *J. Biol. Chem.* **255**:11534-46.
- Halford, S.E. *et al.* (1986) *Gene* **41**:173-81.
- Nasri, M. and Thomas, D. (1986) *Nucl. Acids Res.* **14**:811-22.
- Gingeras, T.R. and Brooks, J.E. (1983) *Proc. Natl. Acad. Sci. USA* **80**:402-6.
- Nasri, M. *et al.* (1985) *FEBS Lett.* **185**:101-4.
- Nasri, M. and Thomas, D. (1987) *Nucl. Acids Res.* **15**:7677-87.
- Pech, M. *et al.* (1979) *Cell* **18**:883-93.
- Barany, F. (1988) *Gene* **65**:149-65.
- Shinomiya, T. *et al.* (1982) *J. Biochem.* **92**:1823-32.
- Kessler, C., unpublished observations.
- Grosskopt, R. and Kessler, C., unpublished observations.
- Takara Shuzo Co., Ltd., unpublished observations.



# RESTRICTION ENZYME PROPERTIES

## Restriction Enzyme Sensitivity to CpG Methylation

Many prokaryotes and eukaryotes contain enzymes known as DNA methyltransferases, which modify DNA by methylating cytosine in the CpG sequence. While these enzymes are known to play a role in protecting host DNA from cleavage by corresponding restriction endonucleases in prokaryotes, their role in eukaryotes is not clear. However, several restriction enzymes will be unable to cleave eukaryotic DNA at sites that have been methylated by CpG methylation. These Takara enzymes are listed below. Methylation may occur at CpG sequences either within the recognition site (indicated by boldface color type), or overlapping it (indicated by boldface type).

Enzyme	Site	Enzyme	Site	Enzyme	Site
<i>Aat</i> II	GAC <b>GC</b> T	<i>Cfr</i> 13 I	GGN <b>CC</b>	<i>Pma</i> CI	CAC <b>GTG</b>
<i>Acc</i> I	GTM <b>KAC</b>	<i>Cla</i> I	ATCGAT	<i>Psh</i> AI	GACN <sub>2</sub> <b>GTC</b> <sup>2</sup>
<i>Acc</i> II	C <b>GGG</b>	<i>Cpo</i> I	C <b>GGWCCG</b>	<i>Psp</i> 1406 I	AAC <b>GTT</b>
<i>Afa</i> I	GT <b>AC</b>	<i>Eae</i> I	YGG <b>CCR</b> <sup>1</sup>	<i>Pvu</i> I	C <b>GATCG</b>
<i>Aor</i> 13H I	T <b>CCGGA</b>	<i>Eco</i> 52 I	C <b>GGCCG</b>	<i>Sac</i> II	CCG <b>CGG</b>
<i>Aor</i> 51H I	AG <b>CGCT</b>	<i>Eco</i> RI	GAAT <b>CGG</b>	<i>Sal</i> I	GT <b>CGAC</b>
<i>Apa</i> I	GGG <b>CCC</b>	<i>Fse</i> I	GGC <b>CGGCC</b>	<i>Sau</i> 3A I	G <b>ATC</b>
<i>Apa</i> L I	GTG <b>CAC</b>	<i>Hae</i> II	RGC <b>GCY</b>	<i>Sfi</i> I	GGCCN <sub>2</sub> <b>GGCC</b> <sup>3</sup>
<i>Ava</i> I	CY <b>CGRG</b>	<i>Hap</i> II	C <b>GGG</b>	<i>Sma</i> I	CC <b>CGGG</b>
<i>Ava</i> II	GG <b>WCC</b>	<i>Hha</i> I	G <b>CGC</b>	<i>Sna</i> BI	TAC <b>GTA</b>
<i>Avi</i> II	TG <b>CGCA</b>	<i>Hin</i> 1 I	GR <b>CGYC</b>	<i>Vpa</i> K11B I	GG <b>WCCG</b>
<i>Bbe</i> I	GGC <b>GCC</b>	<i>Mlu</i> I	AGC <b>GGT</b>		
<i>Bgl</i> I	GCCN <sub>2</sub> <b>GGC</b>	<i>Nae</i> I	GCC <b>GGC</b>		
<i>Bss</i> H II	G <b>CGGC</b>	<i>Nhe</i> I	GCT <b>AGC</b>		
<i>Bst</i> 1107 I	GTAT <b>ACG</b>	<i>Not</i> I	G <b>CGGCCG</b>		
<i>Cfr</i> 10 I	R <b>CCGGY</b>	<i>Nru</i> I	TCG <b>CGA</b>		

<sup>1</sup>only when R = G

<sup>2</sup>methylation at multiple sites required to block cleavage

<sup>3</sup>internal site methylated only when the adjacent N = G

## Restriction Enzyme Sensitivity to *dam* and *dcm* Methylation

DNA prepared from most common strains of *E. coli* may prove resistant to cleavage by certain restriction enzymes. This can often be caused by site-specific methylation. Two types of methylases are commonly encountered: the *dam* methylase, which methylates the N<sup>6</sup> position of the adenine residues in the sequence GATC, and the *dcm* methylase, which methylates the internal cytosine residues in the sequences CCWGG at the C<sup>5</sup> position. The sensitivity of Takara's enzymes to these types of methylation is listed below.

### Enzymes blocked by overlapping *dam* methylation

Enzyme	Site
<i>Acc</i> III	TCCGG <b>ATC</b>
<i>Cla</i> I	ATCGAT(C)
<i>Fba</i> I	TG <b>ATCA</b>
<i>Mbo</i> I	G <b>ATC</b>
<i>Mbo</i> II	GA <b>AGA</b> (TC)
<i>Mfi</i> I	RG <b>ATCY</b>
<i>Nru</i> I	TCG <b>CGA</b> (TC)
<i>Tth</i> HB8 I	TC <b>GA</b> (TC)
<i>Xba</i> I	TCT <b>AGA</b> (TC)

### Enzymes NOT blocked by overlapping *dam* methylation

Enzyme	Site
<i>Bam</i> HI	GG <b>ATCC</b>
<i>Bgl</i> II	AG <b>ATCT</b>
<i>Pvu</i> I	CG <b>ATCG</b>
<i>Sau</i> 3A I	G <b>ATC</b>

M = A or C; K = G or T; N = A, C, G, or T;

R = A or G; Y = C or T; W = A or T; S = G or C

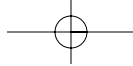
### Enzymes blocked by overlapping *dcm* methylation

Enzyme	Site
<i>Apa</i> I	GGG <b>CCC</b> (WGG)
<i>Ava</i> II	GG <b>WCC</b> (WGG)
<i>Bal</i> I	TGG <b>CCA</b> (GG)
<i>Cfr</i> 13 I	GGN <b>CC</b> (WGG)
<i>Eae</i> I	YGG <b>CCA</b> (GG)
<i>Eco</i> 0109 I	RGGN <b>CCT</b> (GG)
<i>Sfi</i> I	GGCCN <sub>2</sub> <b>GGCC</b> (WGG) or GGCCWGGN <sub>2</sub> <b>GGCC</b>
<i>Stu</i> I	AGG <b>CCT</b> (GG)
<i>Var</i> 91 I	CCAGGN <sub>2</sub> <b>TGG</b>
<i>Vpa</i> K11B I	GG <b>WCCWGG</b>

### Enzymes NOT blocked by overlapping *dcm* methylation

Enzyme	Site
<i>Bam</i> HI	GG <b>ATCC</b>
<i>Bbe</i> I	GGC <b>GCC</b>
<i>Bst</i> PI	GGT <b>NACC</b>
<i>Bst</i> XI	CCAN4 <b>CCTGG</b>
<i>Eco</i> 065 I	GGT <b>NACC</b>
<i>Hae</i> III	GG <b>CC</b>
<i>Hin</i> 1 I	GR <b>CGYC</b>
<i>Kpn</i> I	GG <b>TACC</b>
<i>Mva</i> I	CC <b>WGG</b>





# RESTRICTION ENZYME PROPERTIES



## Restriction Enzyme Activity in Takara Buffers

The table below displays the activity of Takara's restriction enzymes in each of the buffers supplied by Takara. The activity in each of the standard Takara buffers (L, M, H, K, or T) is expressed relative to the specific buffer supplied with the enzyme, indicated by boldface type. Fourteen enzymes (*Acc* III, *Bal* I, *Bbe* I, *Bcn* I, *Bgl* I, *Bpu* 1102 I, *Cfr* 10 I, *Eam* 1105 I, *Eco* 52 I, *Nru* I, *Psh* B I, *Sna* B I, *Ssp* I and *Vpa* K 11B I) are supplied with customized "basal" buffers, whose composition varies with the enzyme. In general, when performing double digests, select a buffer in which both enzymes have close to optimal activity. However, some enzymes may display star activity in certain buffers (indicated by parentheses), and these buffers should be avoided.

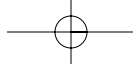
Restriction Enzyme	Relative Activities (%)					
	L	M	H	K	T+BSA	Basal
<i>Aat</i> II	<20	<20	<20	<20	<b>100</b>	120
<i>Acc</i> I	20	<b>100</b>	<20	(<20)	160	80
<i>Acc</i> II	(260)	<b>100</b>	<20	20	200	160
<i>Acc</i> III	(<20)	(<20)	20	(80)	(<20)	<b>100</b>
<i>Afa</i> I	60	60	40	60	<b>100</b>	100
<i>Afl</i> II	20	<b>80*</b>	<20	<20	140	120
<i>Alu</i> I	<b>100</b>	100	<20	40	200	120
<i>Aor</i> 51H I	80	<b>100</b>	<20	20	120	120
<i>Apa</i> I	<b>100</b>	<20	<20	<20	<20	120
<i>Apa</i> L I	<b>100</b>	20	<20	<20	120	120
<i>Ava</i> I	(<20)	<b>100</b>	20	40	100	120
<i>Ava</i> II	80	<b>100</b>	<20	20	100	100
<i>Avi</i> II	<20	40	<b>100</b>	(140)	100	200
<i>Bal</i> I	20	20	<20	<20	40	<b>100</b>
<i>Bam</i> HI	(<20)	<20	40	<b>100</b>	(<20)	80
<i>Ban</i> II	(120)	(120)	<b>100</b>	80	(100)	100
<i>Bbe</i> I	<20	<20	<20	<20	<20	<b>100</b>
<i>Bcn</i> I	<20	20	40	(60)	(60)	<b>100</b>
<i>Bgl</i> I	<20	<20	20	40	<20	<b>100</b>
<i>Bgl</i> II	<20	20	<b>100</b>	(100)	(60)	100
<i>Bln</i> I	<20	20	40	<b>100</b>	20	120
<i>Bpu</i> 1102 I	<20	<20	<20	40	(60)	<b>100</b>
<i>Bsp</i> 1286 I	<b>100</b>	20	<20	<20	60	100
<i>Bsp</i> 1407 I	20	60	20	20	<b>100</b>	100
<i>Bsp</i> T107 I	<20	20	80	100	20	<b>100</b>
<i>Bss</i> HI	100	<b>100</b>	60	20	140	100
<i>Bst</i> PI	(<20)	(60)	<b>100</b>	(100)	(100)	100
<i>Bst</i> XI	<20	40	<b>100</b>	<20	<20	120
<i>Bst</i> XI 107 I	(<20)	60	100	<b>100</b>	40	100
<i>Cfr</i> 10 I	(<20)	(<20)	(<20)	40	(20)	<b>100</b>
<i>Cfr</i> 13 I	60	80	<20	<b>100</b>	80	100
<i>Cla</i> I	40	<b>100</b>	120	100	60	100
<i>Cpo</i> I	<20	<20	80	<b>100</b>	<20	100
<i>Dra</i> I	100	<b>100</b>	60	100	80	80
<i>Eae</i> I	60	<b>100</b>	<20	<20	120	160
<i>Eam</i> 1105 I	(<20)	(40)	20	40	40	<b>100</b>
<i>Eco</i> 065 I	(20)	(60)	<b>60*</b>	40	40	100
<i>Eco</i> 0109 I	<b>100</b>	60	<20	<20	100	160
<i>Eco</i> RI	(20)	(100)	<b>100</b>	(120)	(80)	120
<i>Eco</i> RV	(<20)	(40)	<b>100</b>	(120)	(40)	100
<i>Eco</i> T14 I	(<20)	(40)	<b>100</b>	120	(60)	100
<i>Eco</i> T22 I	<20	20	<b>100</b>	(140)	(20)	120
<i>Eco</i> 52 I	<20	<20	<20	<20	<20	<b>100</b>
<i>Eco</i> 81 I	<20	<b>100</b>	<20	<20	100	160
<i>Fba</i> I	(<20)	(<20)	(80)	<b>100</b>	(20)	100
<i>Fok</i> I	(20)	<b>60*</b>	<20	<20	(200)	100
<i>Fse</i> I	(120)	<b>100</b>	<20	<20	80	100
<i>Hae</i> II	80	<b>100</b>	<20	80	140	100
<i>Hae</i> III	60	<b>100</b>	100	60	100	100
<i>Hap</i> II	<b>100</b>	60	<20	<20	100	80
<i>Hha</i> I	80	<b>100</b>	100	120	120	100

Restriction Enzyme	Relative Activities (%)					
	L	M	H	K	T+BSA	Basal
<i>Hin</i> I	40	80	<20	20	60	<b>160</b>
<i>Hinc</i> II	20	<b>100</b>	20	40	100	80
<i>Hind</i> I	(60)	<b>100</b>	<20	200	(100)	80
<i>Hind</i> III	(60)	<b>100</b>	<20	200	(100)	80
<i>Hinf</i> I	80	100	<b>100</b>	160	60	100
<i>Hpa</i> I	<20	(40)	20	<b>100</b>	(80)	100
<i>Kpn</i> I	<b>100</b>	60	<20	<20	(100)	80
<i>Mbo</i> I	20	40	60	<b>100</b>	40	100
<i>Mbo</i> II	<b>100</b>	60	<20	<20	60	100
<i>Mfl</i> I	<b>100</b>	80	<20	<20	80	100
<i>Mlu</i> I	60	60	<b>100</b>	(100)	60	100
<i>Msp</i> I	80	80	<20	100	100	100
<i>Mun</i> I	(200)	<b>100*</b>	<20	<20	160	100
<i>Mva</i> I	(<20)	(40)	80	<b>100</b>	(20)	120
<i>Nae</i> I	<b>100</b>	<20	<20	<20	100	120
<i>Nco</i> I	(40)	(60)	20	<b>60*</b>	(60)	160
<i>Nde</i> I	<20	40	<b>100</b>	100	80	100
<i>Nhe</i> I	(120)	<b>100</b>	<20	<20	(160)	100
<i>Not</i> I	(<20)	(<20)	<b>20**</b>	<20	(<20)	100
<i>Nru</i> I	0	<20	20	20	<20	<b>100</b>
<i>Pma</i> CI	<b>100</b>	80	<20	<20	100	100
<i>Psh</i> A I	20	40	<20	<b>100</b>	60	160
<i>Psh</i> B I	(20)	(40)	20	40	40	<b>100</b>
<i>Psp</i> 1406 I	20	60	<20	<20	<b>100</b>	100
<i>Pst</i> I	(<20)	(60)	<b>100</b>	80	(20)	80
<i>Pvu</i> I	(<20)	(20)	(40)	<b>80*</b>	(40)	120
<i>Pvu</i> II	(80)	<b>100</b>	40	<20	(40)	<b>120</b>
<i>Sac</i> I	<b>100</b>	60	<20	<20	80	80
<i>Sac</i> II	40	20	<20	<20	<b>100</b>	100
<i>Sal</i> I	<20	<20	<b>100</b>	(20)	<20	120
<i>Sau</i> 8A I	(60)	80	<b>100</b>	<20	(80)	100
<i>Sca</i> I	(<20)	(<20)	<b>100</b>	(60)	(<20)	<b>100</b>
<i>Sfi</i> I	(40)	<b>100</b>	<20	<20	100	<b>100</b>
<i>Sma</i> I	<20	<20	<20	<20	<b>100</b>	<b>100</b>
<i>Sna</i> B I	(20)	(40)	<20	<20	(40)	<b>100</b>
<i>Spe</i> I	(80)	<b>100</b>	80	100	(80)	<b>100</b>
<i>Sph</i> I	(20)	(40)	<b>100</b>	120	(20)	100
<i>Sse</i> 6387 I	(120)	<b>60*</b>	<20	<20	(60)	100
<i>Ssp</i> I	(<20)	(60)	40	(100)	(80)	<b>100</b>
<i>Stu</i> I	60	<b>100</b>	60	80	140	100
<i>Swa</i> I	<20	<20	100	20	<20	<b>100</b>
<i>Tth</i> HB8 I ( <i>Taq</i> I)	40	80	60	60	80	100
<i>Tth</i> 111 I	(20)	80	40	<b>100</b>	(80)	120
<i>Van</i> 91 I	<20	(20)	60	<b>100</b>	(60)	100
<i>Vpa</i> K 11B I	<20	<20	60	(40)	<20	<b>100</b>
<i>Xba</i> I	<20	<b>80*</b>	20	<20	120	120
<i>Xho</i> I	<20	60	<b>100</b>	160	60	100
<i>Xsp</i> I	<20	60	<20	100	160	100

\* BSA should be added to a final concentration of 0.01% to obtain 100% activity.

\*\* BSA and TRITON® X-100 should be added to a final concentration of 0.01% each to obtain 100% activity.





# RESTRICTION ENZYME PROPERTIES

## Restriction Enzyme Isoschizomers and Neoschizomers

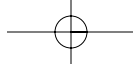
Isoschizomers are restriction enzymes having the same recognition sequence specificity. Neoschizomers (denoted by an asterisk) are restriction enzymes that recognize the same site but cleave at different locations within the recognition sequence. Not all enzymes have a commercially available isoschizomer (indicated by NA). Takara and enzymes are denoted by boldface type.

<b>Aat</b> II	Zra I
<b>Acc</b> I	Fbl I Xmi I
<b>Acc</b> II	Bsh1236 I BstFN I BstU I Mvn I Tha I
<b>Acc</b> III	BseA I Bsm I Bsp13 I BspE I Mro I Kpn2 I
Acc65I	Asp718 I <b>Kpn I*</b>
AccB7I	PfiM I AccB7 I <b>Var91 I</b>
AccI	<b>Psp1406 I</b>
<b>Afa</b> I	<b>Rsa</b> I Csp6 I*
<b>Afl</b> II	Bfr I BspT I Bst98 I MspC I Vha464 I
Ahd I	AspE I <b>Eam1105 I</b> EclHK I NruG I
<b>Alu</b> I	NA
Alw44 I	<b>Apal</b> I Vne I
<b>Aor51H</b> I	Eco47 III Fun I Afe I
<b>Apa</b> I	Bsp120 I* PspOM I*
<b>Apal</b> I	Alw44 I Vne I
Ase I	Asn I <b>PshB</b> I Vsp I
<b>Ava</b> I	Ama87 I Bco I BsoB I Eco88 I Nsp III
<b>Ava</b> II	Bme18 I Eco47 I HgiE I
<b>Avi</b> II	Acc16 I Fsp I Nsb I
Avr II	<b>Bln</b> I BspA2 I Xmal I

<b>Bal</b> I	Mis I MluN I Msc I Msp20 I
<b>BamH</b> I	NA
<b>Ban</b> I	AccB1 I BshN I <b>BspT107 I</b> Eco64 I
<b>Ban</b> II	Eco24 I EcoT38 I Fro I
<b>Bbe</b> I	Eng I* Ehe I* Kas I Mly113 I* Nar I* Sfo I*
<b>Bbu</b> I	Pae I SpaH I <b>Sph</b> I
<b>Bcn</b> I	AsuC2 I Nci I
<b>Bgl</b> I	NA
<b>Bgl</b> II	NA
<b>Bln</b> I	Avr II BspA2 I XmaJ I
<b>Bpu1102</b> I	Blp I Bsp1720 I Cei II
<b>Bsp1286</b> I	Bmy I Sdu I
<b>Bsp1407</b> I	Aau I BsrG I SspB I
<b>BspT107</b> I	AccB II Ban I BshN I Eco64 I
<b>BssH</b> II	BseP I PaeR71 Pau I Sfr2741 Sla I Tii I <b>Xho</b> I
<b>Bst1107</b> I	BssNA I BstZ17 I
<b>BstP</b> I	BstE II Eco91 I <b>Eco065 I</b> PspE I
<b>BstX</b> I	NA
<b>Cfr10</b> I	Bse118 I BsrF I BssA I

<b>Cfr13</b> I	AspS9 I BstZ I PspP I Sau96 I
<b>Cla</b> I	Ban III Bsa29 I Bsc I BseC I BstX I Bsp106 I BspD I BspX I Bsu15 I BsuTU I Zho I
<b>Cpo</b> I	Csp I Rsr II Rsr2 I
<b>Dra</b> I	NA
<b>Eae</b> I	Cfr I
<b>Eam1105</b> I	Ahd I AspE I EclHK I NruG I
<b>Eco52</b> I	BseX3 I BstZ I Eag I EclX I Xma III
<b>Eco81</b> I	Aoc I Axy I Bse21 I Bsu36 I Cvri I
<b>Eco065</b> I	BstE II <b>BstP</b> I Eco91 I PspE I
<b>Eco0109</b> I	Dra II
<b>EcoR</b> I	NA
<b>EcoR V</b>	Eco32 I
<b>EcoT14</b> I	Bsst1 I Eco130 I Erh I Sty I
<b>EcoT22</b> I	BfrB I Mph1103 I Nsi I Ppu10 I* Zsp2 I
<b>Fba</b> I	Bcl I BstQ I Ksp22 I
<b>Fok</b> I	BseG I* BstF5 I*
<b>Fse</b> I	NA
<b>Hae</b> II	Bsp143 II BstH2 I





# RESTRICTION ENZYME PROPERTIES



<b>Hae III</b>	<i>Bsh</i> I <i>BsuR</i> I <i>Pal</i> I
<b>Hap II</b>	<i>BstS</i> I <i>BstF</i> I <i>Hpa</i> II <b><i>Msp</i> I</b>
<b>Hha I</b>	<i>AspLE</i> I <i>BstHH</i> I <i>Cfo</i> I <i>Hin6</i> I <i>HinP1</i> I <i>HspA</i> I
<b>HinI I</b>	<i>Acy</i> I <i>BsaH</i> I <i>BstAC</i> I <i>Hsp92</i> I <i>Msp17</i> I
<b>Hinc II</b>	<i>Hind</i> II
<b>Hind III</b>	NA
<b>Hinf I</b>	NA
<b>Hpa I</b>	<i>BstHP</i> I <i>KspA</i> I
<b>Kpn I</b>	<i>Acc65</i> I* <i>Asp718</i> I*
<b>Mbo I</b>	<i>BscF</i> I <i>Bsp143</i> I <i>BstEN</i> II <i>BstKT</i> I <i>Dpn</i> II <i>Kzo9</i> I <i>Nde</i> II <b><i>Sau3A</i> I</b>
<b>Mbo II</b>	NA
<b>Mfi I</b>	<i>BstX2</i> I <i>BstY</i> I <i>Psu</i> I <i>Xho</i> II
<b>Mlu I</b>	NA
<b>Msp I</b>	<i>BstS</i> I <b><i>Hap</i> II</b> <i>Hpa</i> II
<b>Mun I</b>	<i>Mfe</i> I
<b>Mva I</b>	<i>BseB</i> I <i>BstL</i> I <i>BstN</i> I <i>BstO</i> I <i>Bst2U</i> I <i>EcoR</i> II* <i>PspG</i> I*
<b>Nae I</b>	<i>MroN</i> I* <i>NgoA</i> IV* <i>NgoM</i> IV* <i>Pdi</i> I
<b>Nco I</b>	<i>Bsp19</i> I
<b>Nde I</b>	<i>FauND</i> I
<b>Nhe I</b>	<i>AsuNH</i> I
<b>Not I</b>	<i>CcN</i> I

<b>Nru I</b>	<i>Bsp68</i> I
<b>PmaC I</b>	<i>Acv</i> I <i>BbrP</i> I* <i>Eco72</i> I <i>Pml</i> I
<b>PshA I</b>	<i>Box</i> I <i>BstPA</i> I
<b>PshB I</b>	<i>Ase</i> I <i>Asn</i> I <i>Vsp</i> I
<b>Psp1406 I</b>	<i>AcI</i> I
<b>Pst I</b>	<i>BstMA</i> I
<b>Pvu I</b>	<i>BspC</i> I <i>Ple19</i> I
<b>Pvu II</b>	NA
<b>Rsa I</b>	<b><i>Afa</i> I</b> <i>Csp6</i> I*
<b>Sac I</b>	<i>Ecl136</i> I* <i>EcoCR</i> I* <i>Psp124B</i> I <i>Sst</i> I
<b>Sac II</b>	<i>Cfr42</i> I <i>Ksp</i> I <i>SgrB</i> I <i>Sfr303</i> I <i>Sst</i> II
<b>Sal I</b>	NA
<b>Sau3A I</b>	<i>BscF</i> I <i>Bsp143</i> I <i>BstEJN</i> II <i>BstKT</i> I <i>Dpn</i> II <i>Kzo9</i> I <b><i>Mbo</i> I</b> <i>Nde</i> II
<b>Sca I</b>	<i>Acc113</i> I
<b>Sfi I</b>	NA
<b>Sma I</b>	<i>Cfr9</i> I* <i>PspA</i> I* <i>Xma</i> I* <i>XmaC</i> I*
<b>SnaB I</b>	<i>BstSN</i> I <i>Eco105</i> I
<b>Spe I</b>	<i>AcN</i> I <i>Bcu</i> I
<b>Sph I</b>	<i>Bbu</i> I <i>Pae</i> I <i>SpaH</i> I
<b>Sse8387 I</b>	<i>Sbf</i> I <i>Sda</i> I
<b>Ssp I</b>	NA
<b>Stu I</b>	<i>Aat</i> I <i>Eco147</i> I <i>Pce</i> I <i>SseB</i> I
<b>Swa I</b>	<i>Smi</i> I
<b>TthHB8 I</b>	<i>Taq</i> I

<b>Tth111 I</b>	<i>Asp</i> I <i>PfiF</i> I <i>Psy</i> I <i>TeI</i> I
<b>Van91 I</b>	<i>AccB7</i> I <i>PfiM</i> I
<b>VpaK11B I</b>	<i>Afi</i> I <i>Ava</i> II <i>Bme18</i> I <i>Eco47</i> I <i>HgE</i> I <i>Sin</i> I
<b>Xba I</b>	NA
<b>Xho I</b>	<i>BssH</i> I <i>PaeR7</i> I <i>Sfr274</i> I <i>Sla</i> I <i>Tii</i> I