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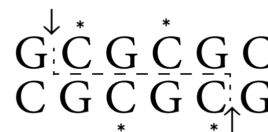
Roche

Restriction Endonuclease BssH II

From *Bacillus stearothermophilus* H3

Cat. No. 11 168 851 001
200 units (10 U/µl)



Version 20
Content version: July 2017
Store at −15 to −25°C

Stability/Storage The undiluted enzyme solution is stable when stored at −15 to −25°C until the control date printed on the label. Do not store below −25°C to avoid freezing.

Sequence specificity *Bss*H II recognizes the sequence G/CGCGC and generates fragments with 5´-overhanging ends (1). The enzyme is classified as a rare-cutter enzyme. Since the most rarely occuring trinucleotides in bacterial genomic DNA are CCG and CGG, the enzyme cleaves bacterial DNA to produce large fragments, on average between 20-50 kb in size depending on the G+C content. Yeast DNA. which is rich in A and T sequences, is cleaved by BssH II to produce fragments approx. 30 kb in size. Mammalian genomic DNA is cleaved by BssH II to produce fragments in the range of 100 kb due to the low occurence in the DNA of the dinucleotide CG.

Compatible ends The enzyme generates compatible ends to Mlu I.

Enzyme with compatible ends	Recognition sequence	New sequence if *BssH*II is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		*BssH*II-Enzyme	Enzyme–*BssH*II	
BssH II	G/CGCGC	G/CGCGC	G/CGCGC	BssH II, Cfo I, Mlu I, Mvn I
Mlu I	A/CGCGT	A/CGCGT	A/CGCGC	Cfo I, Mvn I

Isoschizomers *Bss*H II is not known to have isoschizomers.

Methylation sensitivity *Bss*H II is inhibited by 5'-methylcytosine as indicated (*).

Storage buffer 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg bovine serum albumin, 50% Glycerol (v/v), pH approx. 7.8 (at 4° C).

Incubation buffer, 10x 330 mM Tris-acetate, 660 mM K-acetate, 100 mM Mg-acetate, 5 mM Dithioerythritol, pH 7.9 (at 37° C), (= SuRE/Cut Buffer **A)**

Activity in SuRE/Cut Buffer System Bold face printed buffer indicates the recommended buffer for optimal activity:

A	B	L	M	H
100%	100%	75-100%	100%	75-100%

Incubation temp. **50°C**

Unit definition One unit is the enzyme activity that completely cleaves the 1 µg λDNA in 1 h at **50°C** in the SuRE/Cut buffer A in a total volume of 25 µl.

Typical experiment

Component	Final concentration
DNA	1 µg
10 × SuRE/Cut Buffer **A**	2.5 µl
Repurified water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at **50°C** for 1 h.

Heat inactivation The enzyme can not be heat inactivated by heating to 65°C for 15 min.

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
6	52	0	1	0	0	0	0

PFGE tested *Bss*H II has been tested in Pulsed-Field Gel Electrophoresis (test system bacterial chromosomes).For cleavage of genomic DNA (*E. coli* C600) embedded in agarose for PFGE analysis 10 units enzyme/µg DNA and 4 h incubation time at **50°C** are recommended.

Activity in PCR buffer Relative activity in PCR mix (Taq DNA Polymerase buffer) is **100%**. The PCR mix contained λ target DNA, primers, 10 mM Tris-HCl (pH 8.3, 20°C), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

Troubleshooting A critical component is the DNA substrate. Many compounds used in the isolation of DNA such as phenol, chloroform, ethanol, SDS, high levels of NaCl, metal ions (*e.g.*, Hg^{2+}, Mn^{2+}) inhibit or alter recognition specificity of many restriction enzymes. Such compounds should be removed by ethanol precipitation followed by drying, before the DNA is added to the restriction digest reaction. Appropriate mixing of the enzyme is recommended.

Quality control Lot-specific certificates of analysis are available at www.lifescience.roche.com/certificates.

Absence of unspecific endonuclease activities 1 µg λDNA is incubated for 16 h in 50 µl SuRE/Cut buffer A with excess of *Bss*H II. The number of enzyme units which do not change the enzyme-specific pattern is stated in the certificate of analysis.

Absence of exonuclease activity Approx. 5 µg [^{3}H] labeled calf thymus DNA are incubated with 3 µl *Bss*H II for 4 h at 37°C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH approx. 7.5. The release of radioactivity is calculated as a percentage value of liberated to input radioactivity per unit of enzyme (stated in the certificate of analysis).

Ligation and recutting assay *Bss*H II fragments obtained by complete digestion of 1 µg λDNA are ligated with 1 U T4-DNA ligase in a volume of 10 µl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM ATP, pH 7.5 (at 20°C).

The percentage of ligation and subsequent recutting with *Bss*H II which yields the typical pattern of λ × *Bss*H II fragments are determined and stated in the certificate of analysis.

References

1 Kessler, C. & Manta, V. (1990) *Gene* **92,** 1–248.
2 Rebase The Restriction Enzyme Database: http://rebase.neb.com

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Ordering Information

Product	Application	Packsize	Cat. No.
Rapid DNA Ligation Kit	Ligation of sticky- or blunt-ended DNA fragments in just 5 min at 15 - 25 °C.	Kit (40 DNA ligations)	11 635 379 001
T4 DNA Ligase	Ligation of sticky- and blunt- ended DNA fragments.	100 U 500 units (1 U/µl)	10 481 220 001 10 716 359 001
SuRE/Cut Buffer Set for Restriction Enzymes	Incubation buffers A, B, L, M and H for restriction enzymes	1 ml each (10× conc. solutions)	11 082 035 001
SuRE/Cut Buffer A	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 959 001
SuRE/Cut Buffer B	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 967 001
SuRE/Cut Buffer H	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 991 001
SuRE/Cut Buffer L	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 975 001
SuRE/Cut Buffer M	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 983 001
Water, PCR Grade	Specially purified, double-distilled, deionized, and autoclaved	100 ml (4 vials of 25 ml) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001

Changes to previous version
Editorial changes

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Commonly used bacterial strains

Strain	Genotype
BL21	*E. coli B F⁻ dcm ompT hsdS(r_B- m_B-) gal* (Studier, F.W. *et al* (1986) *J. Mol. Biol.*, **189,** 113.)
C600[e]	*supE44 hsdR2 thi-1 thr-1 leuB6 lacY1 tonA21*; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
DH5α	*supE44* Δ(*lac*U169 (φ80d*lac*ZΔM15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
HB101	*supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*; (Hanahan, D., (1983) *J. Mol. Biol.* **166**, 557.)
JM108	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB); (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lac*I^q *lac*ZΔM15]; (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
JM110	*rpsL (Str^r) thr leu thi-l lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) F'[traD36proAB$^+$, lacI^q lacZΔM15];* (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
K802	*supE hsdR gal metB;* (Raleigh, E. et al., (1986) Proc.Natl. Acad.Sci USA, 83, 9070.; Wood, W.B. (1966) J. Mol. Biol., **16**, 118.)
SURE[r]	*recB recJ sbc C201 uvrC umuC::Tn5(kan^r) lac* , Δ(hsdRMS) endA1 gyrA96 thi relA1 supE44 F'[proAB$^+$ lacI^q lacZΔM15 Tn10 (tet^r); (Greener, A. (1990) Stratagies, **3**, 5.)
TG1	*supE hsd Δ5 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lac*I^q *lac*ZΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue[r]	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F'[*proAB*$^+$, *lac*I^q *lac*ZΔM15 Tn10 *(tet^r)*]; (Bullock *et al.,* (1987) BioTechniques, 5, 376.)

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