



FauI

HaeIII |  
SacII | |  
BstUI | | |  
MspAlI | | |  
ApaI | | | |  
BanII | | | |  
BsaJI | | | |  
BseSI | | | |

FauI

NciI	Bsp1286I	HhaI
ScrFI	BtgI	BstUI
SmaI	Cac8I	HinPII
BanII	BsmFI	Cac8I
BsaJI	HaeIII	BbeI
Bsp1286I	Sau96I	HaeII
HpaII	EcoO109I	HhaI
NciI	BstNI	SfoI
ScrFI	ScrFI	BsaHI
AvaI	BssKI	HinPII
BsaJI	PspGI	NarI
BssKI	AvaII	BanI
XmaI	Sau96I	PspOMI
BssKI	BseRI	Sau96I
		BslI
		BsmFI

241 GAGGAGCCCCGGGGAGAGGGACCAGGAGGGCCCCGCGCCCTCGCAGGGGCGCCCGCGCCCCACCCCTGCCCCCGCCAGC 320

CTCCTCGGGCCCCCTCTCCCTGCTCCTCCCGGGCGCCGGAGCGTCCCCGCGGGCGCGGGGGTGGGGACGGGGCGGTGC

a E E P G G E G P G G A R G L A G A P A P P P L P P P A -

AvaII	AvaII	MboII	HinPII
Sau96I	Sau96I	XcmI	MspAlI
HpaII	HpaII	HpyCH4V	Cac8I
AgeI	NciI	NlaIII	BbvI BbvI TseI
BsaWI	ScrFI		
BsrFI	ScrFI		
AvaII	BslI		
RsrII	BsaJI		
Sau96I	BssKI		

321 GGACCGGTCCCCACCCCGGTCCTTCCACC**ATG**CACCTTGGTGGGCTTCTTCTCTGTGGCGTGTCTCTGCTCGCCGCTG 400

CCTGGCCAGGGGGTGGGGCCAGGAAGGTGG**TAC**TGAACGACCCGAAGAAGAGACA**CCGCACAAGAGACGAGC**GGCGAC

3237 51031

a D R S P T P G P S T **M** H L L G F F S V A C S L L A A A -

AvaII	EcoO109I	PpuMI	Sau96I	NciI	MwoI	ScrFI	Cac8I	SmaI	BbeI	TaqI	HpaII	MlyI	NciI	HhaI	PleI	ScrFI	SfoI	HinfI	AvaI	BsaHI	HpaII	BsaJI	HinPII	BsaWI	BssKI	NarI	BspEI	XmaI	BanI	MlyI	BanII	BssKI	KasI	MwoI	PleI	HhaI	BstUI	MwoI	HinfI	TseI	NruI	FauI	TaqI	Hpy188I	BstUI	HgaI	FauI
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**11280**  
**TTCGAGTCCGGACTCG**

**10263**  
**GAGTCCGGACTCGACC**

401 CGTGCTCCCGGGTCTCTCGGAGGGCGCCCGCCGCGCCGCTTC**GAGTCCGGACTCGACC**TCTCGGACGCGGAGGCC 480

GCGACGAGGGCCAGGAGCGCTCCGCGGGCGCGGGCGGCGGAAGCTCAGGCCTGAGCTGGAGAGCCTGCGCCTCGG

a L L P G P R E A P A A A A A **X** F E S G L D L S D A E P -

BsaJI  
 Cac8I BtgI DpnI BpmI  
 BslI | HaeIII | BglII | BseRI |  
 BstUI | HgaI | | Cac8I BstYI | Bst4CI | |  
 Hpy99I | MwoI | | HpyCH4V | MboI | MaeIII | | | BsrI TspRI BspHI  
 | | | | | | | | | | | | | | | |

5855!/5857! 3231  
 481 GACGCGGGCGAGGC **CACGGCTTATGCAAGCAAAG**ATCTGGA **GGAGCAGTTACGGTCTGTGTC**CAGTGTAGATGAACTCAT  
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 560  
 CTGCGCCCGCTCCGGTGCCGAATACGTTTCGTTTCTAGACCTCCTCGTCAATGCCAGA **CACAGGTCACATCTACTTGAGTA**  
 3236

a D A G E A T A Y A S K D L E E Q L R S V S S V D E L M -

RsaI  
 Csp6I | RsaI  
 Bst4CI | | Csp6I | Bpu10I  
 TatI | | BstXI BsrGI | | DdeI  
 NlaIII | | SspI | | TatI | | AluI | | Cac8I HaeIII  
 | | | | | | | | | | | | | | |

5914 5916/3664  
 561 GACTGTACTCTACCCAGAATATTGAAAAATGTACAAGTGTACAGCTAAGGAAAGGAGGC **TGGCAACATAACAGAGAACAGG**  
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 640  
 CTGACATGAGATGGGTCTTATAACCTTTTACATGTTCCAGTCGATTCCTTCTCCGACCGTTGTATTGTCTCTTGTC

a T V L Y P E Y W K M Y K C Q L R K G G W Q H N R E Q A -

PstI  
 ApoI HpyCH4V | DpnI  
 MboII TseI | BglII |  
 BsmAI Tsp509I SfcI | | BbvI BstYI |  
 SmlI EarI | BbvI | TseI | | PsiI | MboI |  
 | | | | | | | | | | | | |

3457,12488 12597  
 4047 12253  
 641 **CCAACCTCAACTCAAGGACAGAAGAGACTATAAAAATTTGCTG** **CAGCACATTATAATACAG**AGATCTTGAAAAGTATTGAT  
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 720  
**GGTTGGAGTTGAGTTCCCTGT**CTTCTCTGATATTTTAAACGACGTCGTGTAATATTATGTCTCTAGAACTTTTTCATAACTA  
 3415

a N L N S R X T E E T I K F A A X A H Y N T E I L K S I D -

BsaJI  
 BtgI  
 NlaIII |  
 NspI |  
 SphI |  
 Cac8I | |  
 NsiI | | MlyI  
 HpyCH4V | | | PleI |  
 MlyI Ppu10I | | | BstUI | |  
 PleI HinfI | | | BplI HinfI NruI | |  
 | | | | | | | | | |

3250 15323 6049  
 721 AATGAGTGGAGAAAGACTCAATGCATGCC **CGGGAGGTGTGTATAGATGTGGGGAAGGAGTTTGGAGTCGCGACAAACAC**  
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 800  
 TTACT **CACCTCTTTCTGAGTTACGT**ACGGTGCCCTCCACACATATCTACCCCCTTCCTCAAACCTCAGCGCTGTTTGTC  
 51025

a N E W R K T Q C M P R E V C I D V G K E F G V A T N T -

NlaIII  
 BtsI |  
 TspRI |  
 HpyCH4V | |  
 PstI | | |  
 BbvI HpyCH4V | | | |  
 HpyCH4V SfcI | | | |  
 DraI SfcI HpyCH4V  
 MseI | NlaIII AccI | BbvI TseI | TseI | | | | |  
 | | | | | | | | | |

801 CTCTTTAAACCTCCATGTGTGTCCGTCTACAGATGTGGGGTTGCTGCAATAGTGAGGGGCTGCAGTGCATG **AACACCA**  
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 880  
 GAAGAAATTTGGAGGTA **CACACAGGCAGATGTCTACACC**CCAACGACGTTAT **CACTCCCCGACGTCA**CGTACT **TTGTTGGT**

3458 11287!,15206! 5840  
 a F F K P P C V S V Y R C G G C C N S E G L Q C M N T S -  
 C156→S mutation

MwoI Tsp509I HaeIII  
 BbvCI | BseMII | EcoO109I |  
 Bpu10I | TaiI | TspRI | Sau96I | MaeIII  
 BssSI AluI DdeI | MaeII | Bst4CI | SmlI | BsrI |

5964!  
 3395 15393  
 881 GCACGAGCTACCTCAGCAAGACGTTATTTGAAATTACAGTGCCTCTCTCTCAAGGCCCAAAACCAGTAACAATCAGTTTT 960  
 CGTGCTCGATGGAGTCTGTTCTGCAATAAACTTTAATGTACACGGAGAGACAGTTCCGGGGTTTGGTCAATTGTTAGTCAAAA  
 3251 6050 GGTTTGGTCATTGTTAGTCAAAA  
 5966! 11288! ,15207!  
 a T S Y L S K T L F E I T V P L S Q G P K P V T I S F -

NlaIII  
 NspI  
 NsiI |  
 HpyCH4V | | BstF5I | TaiI  
 SfaNI Ppu10I | | BsrI | FokI | MaeII | Cac8I

13761!  
 ATTATTAGACGTTCCCTGCC  
 3397 10879!  
 961 GCCAATCACACTTCCTGCCGATGCATGTCTAAACTGGATGTTTACAGACAAGTTCATTCATTATTAGACGTTCCCTGCC 1040  
 CGGTTAGTGTGAAGGACGGCTACCGTACAGATTTGACCTACAAATGTCTGTTCAAGTAAAGGTAATAATCTGCAAGGGACGG  
 CGGTTAGTGTGAAGGACGGCTACGTAC 3252 51026  
 4048CAGATTTGACCTA  
 1521510272  
 GGCTACCGTACAGATTTGACCTA  
 1248912261!  
 CTACGTACAGATTTGACCTA  
 12598  
 a A N H T S C R C M S K L D V Y R Q V H S I I R R X S L P -

BstNI  
 ScrFI  
 BssKI |  
 PspGI |  
 NlaIII  
 TseI | AflIII | PstI | |  
 TspRI | Tsp509I | NspI | HpyCH4V | | |  
 Bst4CI | BbvI | BspMI | PciI | SfaNI SfcI | | | |

3623 51027  
 1041 AGCAACACTACCACAGTGTGAGGCAGCGAACAAAGACCTGCCCCACCAATTACATGTGGAATAATCACATCTGCAGATGCC 1120  
 TCGTTGTGATGGTGTACAGTCCGTCGCTTGTCTGGACGGGGTGGTTAATGTACACCTTATTAGTGTAGACGTCTACGG  
 11289!  
 a A T L P Q C Q A A N K T C P T N Y M W N N H I C R C L -

Hpy188I  
 BslI BsaJI |  
 Bpu10I | MboII | | MlyI BpmI  
 DdeI | SfaNI | | PleI FokI HinfI XcmI AvaII  
 EcoNI | | BseMII | | BstF5I | HinfI TfiI NlaIII | Sau96I

51030  
 1121 TGGCTCAGGAAGATTTTATGTTTTCTCGGATGCTGGAGATGACTCAACAGATGGATTCCATGACATCTGTGGACCAAAAC 1200  
 ACGAGTCCCTTCTAAAATAAAAAGGAGCCTACGACCTCTACTGAGTTGTCTACCTAAGGTACTGTAGACA CCTGGTTTG  
 3398 3168  
 a A Q E D F M F S S D A G D D S T D G F H D I C G P N -

AvaII  
 Sau96I  
 BsrBI AluI |  
 BstF5I PstI | MspAII |  
 BsaI | HpyCH4V | | PvuII |  
 BsmAI | FauI | | | Cac8I | |  
 EarI | | MboII SfcI | | | Cac8I | | |  
 AluI | | FokI | TspRI | | | HaeIII | | | BfaI

1201 AAGGAGCTGGATGAAGAGACCTGTCAAGTGTCTGCAGAGCGGGGCTTCGGCCTGCCAGCTGTGGACCCACAAAGAACT 1280  
 TTCCTCGACCTACTTCTCTGGACAGTCACACAGCTCTCGCCCCGAAGCCGGTGCACACCTGGGGTGTCTTCTTGA  
 3624  
 a K E L D E E T C Q C V C R A G L R P A S C G P H K E L -

BsrI HaeIII  
MslI Sau96I | ApoI  
NlaIII| TspRI MboII EarI BslI | | Tsp509I  
| | | | | | |

AGACAGAACTCATGCCAGTGTGTCTGTAAAAACAAAC**5864**CTTCCCCAGCCAATGTGGGGCCAACCGAGAATTTGATGAAA  
1281 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1360  
TCTGTCTTTGAGTACGGTCACACAGACATTTTGTGTTGAGAAGGGGTCTGGTTACACCCCGTTGGCTCTTAAACTACTTT  
**5863 3167**  
a D R N S C Q C V C K N K L F P S Q C G A N R E F D E N -

BsrI BstNI RsaI  
MslI ScrFI Csp6I|  
NlaIII| BssKI | BsrGI||  
NspI| TspRI BspMI PspGI | TatI||  
| | | | | | |

ACACATGCCAGTGTGTATGTAAAAGAACCTGCCCCAGAAATCAACCCCTAAATCCTGGAAAATGTGCCTGTGAATGTACA  
1361 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1440  
TGTGTACGGTCACACATACATTTTCTTGGACGGGGTCTTTAGTTGGGGATTTAGGACCTTTTACACGGACACTTACATGT  
a T C Q C V C K R T C P R N Q P L N P G K C A C E C T -

MaeIII  
AluI |  
MspA1I | RsaI  
PvuII | Csp6I|  
HpyCH4V | NlaIII|  
NlaIII | | HaeIII | |  
NspI | | EaeI | |  
TseI | | BbvI | | | |

MslI MseI  
| |

GAAAGTCCACAGAAATGCTTGTAAAAGGAAAGAAGTCCACCACCAACATGCAGCTGTTACAGACGGC**3169/3853**CATGTACGAA  
1441 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1520  
CTTT**51032**CAGGTGCTTTACGAACAATTTTCTTTCTTCAAGGTGGTGGTGTGTACGTCGACAATGTCTGCCGGTACATGCTT  
a E S P Q K C L L K G K K F H H Q T C S C Y R R P C T N -

BstNI  
ScrFI  
BssKI |  
BslI PspGI | BsmFI MboII  
| | | | |

CCGCCAGAAAGCTTGTGAGCCAGGATTTTCATATAGTGAAGAAGTGTGTCTGTTGTGCCCTTCATATTGGAAAAGACCAC  
1521 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1600  
GGCGGTCTTCCGAACACTCGGTCTTAAAGTATATCACTTCTTACACAGCAACACAGGGAAGTATAAC**3827**CTTTTCTGGTG  
a R Q K A C E P G F S Y S E E V C R C V P S Y W K R P Q -

Bst4CI  
RsaI |  
DdeI Csp6I| | ClaI  
AluI| TatI|| | BsrI TaqI Bst4CI Bst4CI Bst4CI  
| | | | | | |

AAATGAGC**TAA**GATTGTACTGTTTTCCAGTTCATCGATTTTCTATTATGGAAAAGTGTGTTGCCACAGTAGAACTGTCTG  
1601 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1680  
**5856**TTTACTCGATTCTAACATGACAAAAGGTCAAGTAGCTAAAAGATAATACCTTTTGACACAA**5865**CGGTGTCATCTTGACAGAC  
a M S \* D C T V F Q F I D F L L W K T V L P Q \* N C L -

BsaI AvaII  
BsmAI Sau96I NlaIII PshAI NlaIII  
| | | | |

TGAACAGAGAGACCC**51029**TTGTGGGTCCATGCTAACAAAGACAAAAGTCTGTCTTTCTGAACCATGTGGATAACTTTA**10615**CAGA  
1681 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1760  
ACTTGTCTCTCTGGGAACACCCAGGTACGATTGTTTCTGTTTTTCAGACAGAAAGACTTGGTACACCTATTGAAATGTCT  
a \* T E R P L W V H A N K D K S L S F L N H V D N F T E -

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                HpyCH4V
                BanII |
                BsiHKAI |
                Bsp1286I |
                SacI |
                AluI | | BpmI
                Ecl136II | | HaeIII|
                BsrI| | | StuI| | BsrI
                || | | | || |
AATGGACTGGAGCTCATCTGCAAAGGCCTCTTGTAAGACTGGTTTTCTGCCAATGACCAAACAGCCAAGATTTTCCTC
1761 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1840
TTACCTGACCTCGAGTAGACGTTTTTCCGGAGAACATTTCTGACCAAAAGACGGTTACTGGTTTGTTCGGTTCTAAAAGGAG
                5965
a      M D W S S S A K G L L * R L V F C Q * P N S Q D F P L -
                DraI
                MseI| | Tsp509I | SspI BsmI
                || | | | | | HpyCH4V |
                TTGTGATTTCTTTAAAAGAATGACTATATAATTTATTTCCACTAAAAATATTGTTTCTGCATTCATTTTTATAGCAACAA
1841 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1920
AACACTAAAGAAATTTTCTTACTGATATATAAATAAAGGTGATTTTTATAACAAAGACGTAAGTAAAAATATCGTTGTT
a      V I S L K E * L Y N L F P L K I L F L H S F L * Q Q -
                DpnI
                TspRI|
                BclI||
                MfeI MboI|| HpyCH4V DraI
Tsp509I Bst4CI ||| SspI NlaIII MseI| Tsp509I
                | | | | | | | |
3416
CAATTGGTAAAACACTCACTGTGATCAATATTTTATATCATGCAAATATGTTTAAAATAAAAATGAAAATGTATTATAAA
1921 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2000
GTTAACCATTTTGAGTGACACTAGTTATAAAAATATAGTACGTTTTTACAAATTTTATTTTACTTTTAACATAATTTT
a      Q L V K L T V I N I F I S C K I C L K * N E N C I -
                NotI
                |
AAAAAAAAAAAAAAAAAGCGGCCGC
2001 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2023
TTTTTTTTTTTTTTTTTCGCCGCG

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Enzymes that do cut and were not excluded:

AccI	AflIII	AgeI	AluI	ApaI	ApoI	AvaI	AvaII	BanI	BanII	BbeI
BbvI	BbvCI	BclI	BfaI	BglII	BplI	BpmI	Bpu10I	BsaI	BsaHI	BsaJI
BsaWI	BseMII	BseRI	BseSI	BsiHKAI	BslI	BsmI	BsmAI	BsmFI	Bsp1286I	BspEI
BspHI	BspMI	BsrI	BsrBI	BsrFI	BsrGI	BssKI	BssSI	Bst4CI	BstF5I	BstNI
BstUI	BstXI	BstYI	BtgI	BtsI	Cac8I	Clal	Csp6I	DdeI	DpnI	DraI
EaeI	EarI	Ecl136II	EcoNI	EcoO109I	FauI	FokI	HaeII	HaeIII	HgaI	HhaI
HinPI	HinFI	HpaII	HphI	Hpy99I	Hpy188I	HpyCH4V	KasI	MaeII	MaeIII	MboI
MboII	MfeI	MlyI	MseI	MslI	MspAI	MwoI	NarI	NciI	NlaIII	NruI
NsiI	NspI	PciI	PleI	Ppu10I	PpuMI	PshAI	PsiI	PspGI	PspOMI	PstI
PvuII	RsaI	RsrII	SacI	SacII	Sau96I	ScrFI	SfaNI	SfcI	SfoI	SmaI
SmlI	SphI	SspI	StuI	TaiI	TaqI	TatI	TfiI	TseI	Tsp509I	TspRI
XcmI	XmaI									

Enzymes that do not cut:

AatII	Acc65I	AclI	AfeI	AflII	AhdI	AlwI	AlwNI	ApaLI	AscI	AseI
AvrII	BaeI	BamHI	BbsI	BcgI	BciVI	BglI	BlpI	BmrI	BsaAI	BsaBI
BsgI	BsiEI	BsiWI	BsmBI	BsrDI	BssHII	BstAPI	BstBI	BstEI	BstZ17I	Bsu36I
BtrI	DraIII	DrdI	EagI	EciI	Eco57I	EcoRI	EcoRV	FseI	FspI	HincII
HindIII	HpaI	KpnI	MluI	MscI	NaeI	NcoI	NdeI	NgoMIV	NheI	NotI
PacI	PflMI	PmeI	PmlI	PvuI	SalI	SanDI	SapI	SbfI	ScaI	SexAI
SfiI	SgfI	SgrAI	SnaBI	SpeI	SrfI	StyI	SwaI	Tsp45I	Tth111I	XbaI
XhoI	XmnI									

Enzymes excluded; MinCuts: 1 MaxCuts: 16

AccI	CviJI	Fnu4HI	MnlI	NlaIV
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