Sequence of pTYB12 from Web -June1, 2005

Plasmid pTYB12

Update 3/26/04

Features:

140-1000	beta-lactamase (bla; amp-r) CDS (start 140)
140- 208	beta-lactamase signal peptide CDS (start 140)
1042-1555	M13 origin of replication (- +)
1666-2254	pMB1 origin of replication (clockwise) (RNAII -35 to RNA/DNA switch point)
2814-2623	rop CDS (start 2814, complementary strand)
4453-3371	lacI CDS (start 4453, complementary strand)
4840-4857	T7 promoter (transcript start 4840 clockwise)
4840-4859	T7 universal primer sequence (NEB #S1248S)
4859-4883	lac operator
4913-4919	RBS for expression CDS
4928-6562	expression CDS (start 4928)
4928-4972	malE fragment (translational start)
4973-6502	Sce VMA intein with embedded CBD
4973-5791	Sce VMA intein 5' region
5798-5950	chitin binding domain (CBD)
5963-6502	Sce VMA intein 3' region
6503-6559	multiple cloning site (NdeI-SmaI)

→ The target gene was inserted using Nde I and Eco RI sites

6362-6385	Intein forward primer sequence (NEB #S1263S)
6582-6704	T7 Tphi transcription terminator With 252 enzymes: *

June 1, 2005 15:40 ..

```
                  BccI
               BccI  |
          Hpy188III|  |
         BstF5I   ||  |  BstF5I
         CviJI|   ||  |  CviJI|   FokI
        HaeIII|   ||  | HaeIII| TspGWI
             ||   ||  |      ||      |
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      1 ---------+---------+---------+---------+---------+---------+ 60
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a       R  R  P  S  *  R  M  A  F  L  R  F  Y  K  L  F  L  F  I  F   -
b        E  G  H  P  D  G  W  P  F  C  V  S  T  N  S  F  C  L  F  F  -
c         K  A  I  L  T  D  G  L  F  A  F  L  Q  T  L  F  V  Y  F  S -

                                 NlaIII
                                 BciVI|
                              CviAII ||
                           Hpy188III ||
                              BspHI| ||
                               FatI| ||
                            BsmAI || ||
                            BsrBI || ||
                           AciI | || ||
                              | | || ||
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     61 ---------+---------+---------+---------+---------+---------+ 120
        GATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTAT

a       L  N  T  F  K  Y  V  S  A  H  E  T  I  T  L  I  N  A  S  I   -
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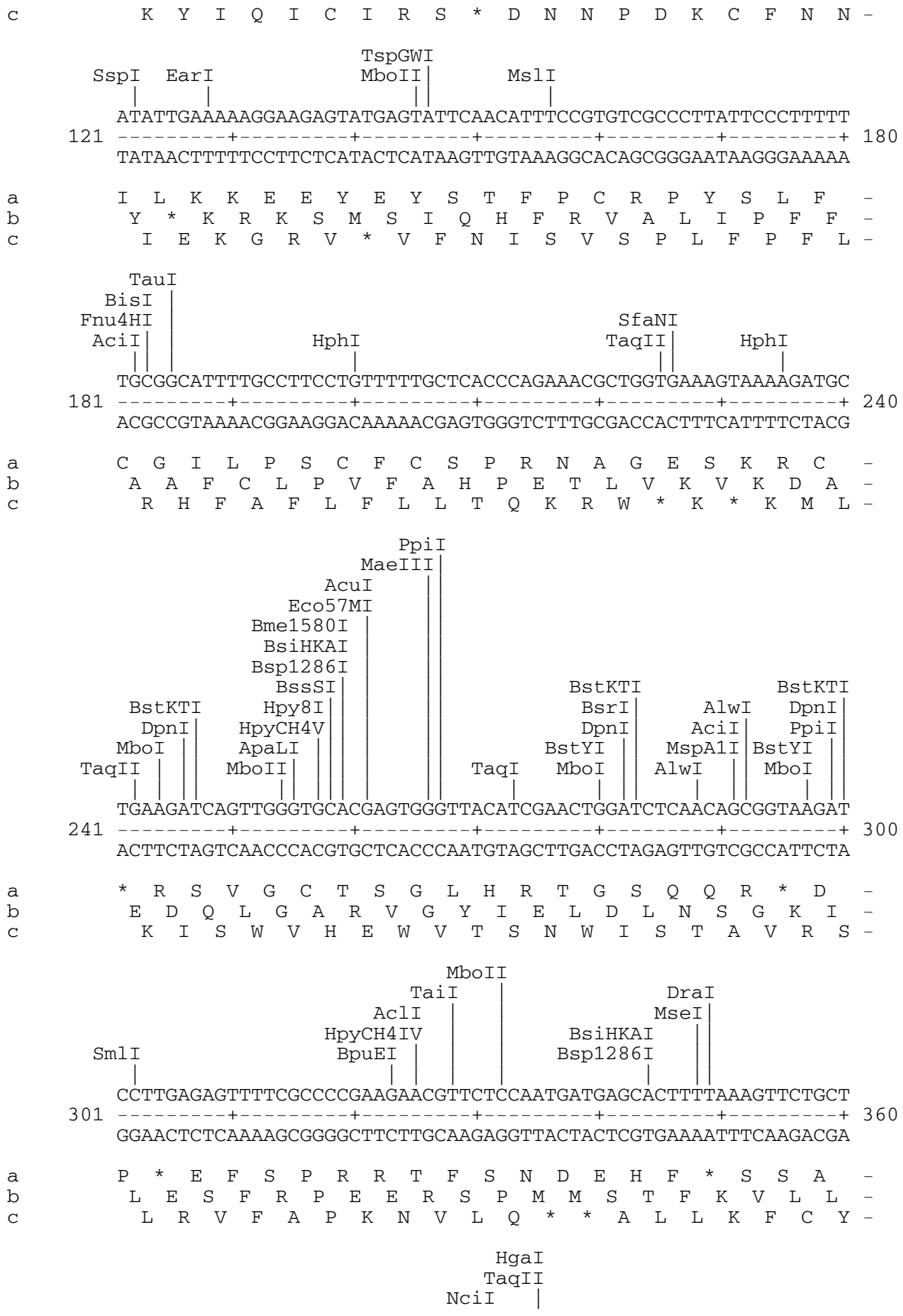
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SspI EarI MboII MslI
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b Y * K R K S M S I Q H F R V A L I P F F -
c I E K G R V * V F N I S V S P L F P F L -
TauI
BisI
Fnu4HI
AciI HphI SfaNI TaqII HphI
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181 240
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b A A F C L P V F A H P E T L V K V K D A -
c R H F A F L F L L T Q K R W * K * K M L -
PpiI
MaeIII
AcuI
Eco57MI
Bme1580I
BsiHKAI
Bsp1286I
BssSI BstKTI BstKTI
BstKTI Hpy8I BsrI AlwI DpnI
DpnI HpyCH4V DpnI AciI PpiI
MboI ApaLI BstYI MspA1I BstYI
TaqII MboII TaqI MboI AlwI MboI
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241 300
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b E D Q L G A R V G Y I E L D L N S G K I -
c K I S W V H E W V T S N W I S T A V R S -
MboII
TaiI DraI
AclI MseI
HpyCH4IV BsiHKAI
SmlI BpuEI Bsp1286I
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301 360
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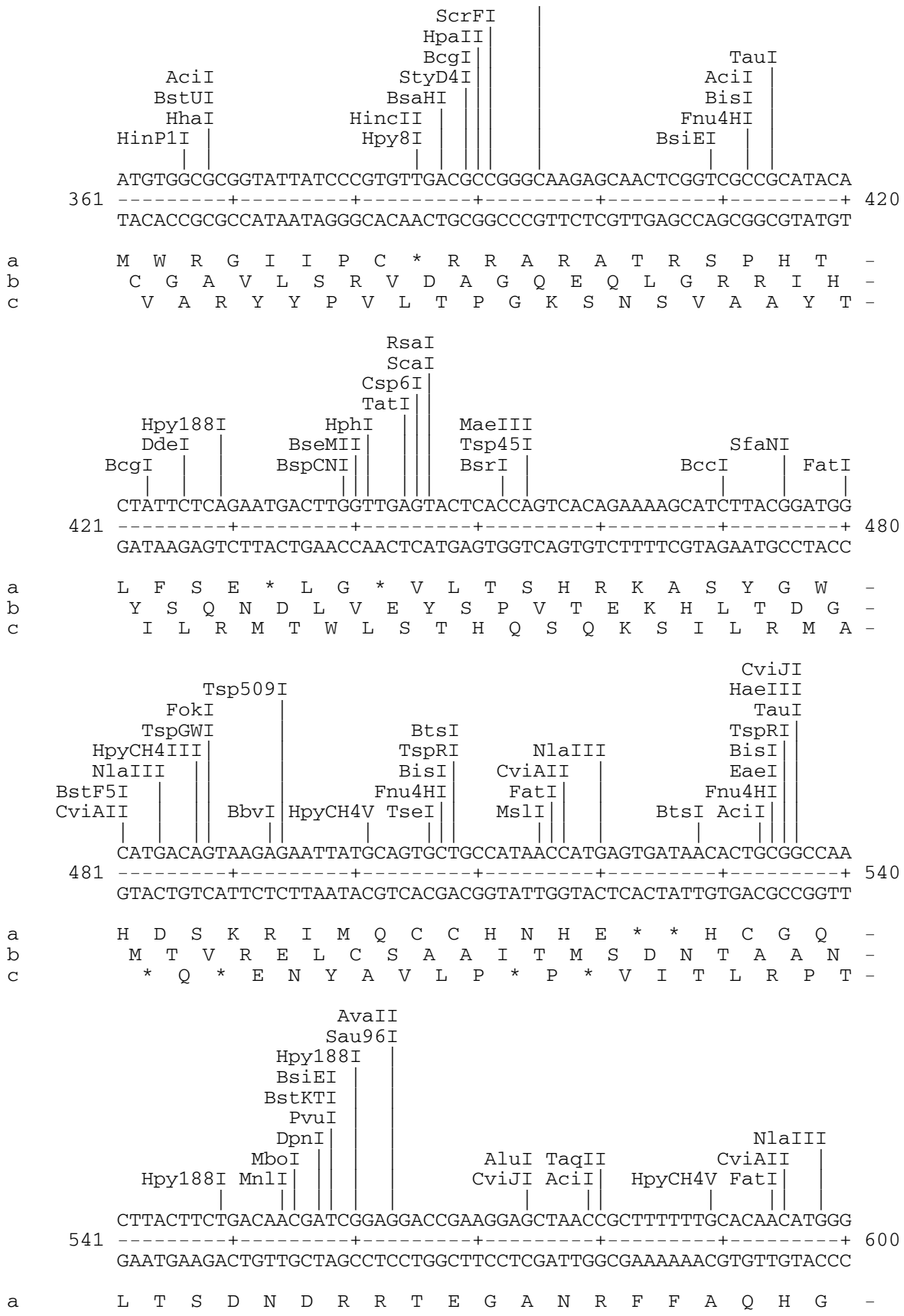
TaqII
NciI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b C G A V L S R V D A G Q E Q L G R R I H -
c V A R Y Y P V L T P G K S N S V A A Y T -
RsaI
ScaI
Csp6I
TatI
Hpy188I
HphI
MaeIII
DdeI
BseMII
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BccI
FatI
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CviJI
HaeIII
Tsp509I
TauI
FokI
TspRI
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BtsI
TspRI
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EaeI
BstF5I
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BtsI
AciI
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540
a H D S K R I M Q C C H N H E * * H C G Q -
b M T V R E L C S A A I T M S D N T A A N -
c * Q * E N Y A V L P * P * V I T L R P T -
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Sau96I
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DpnI
NlaIII
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AluI
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CviAII
Hpy188I
MnlI
CviJI
AciI
HpyCH4V
FatI
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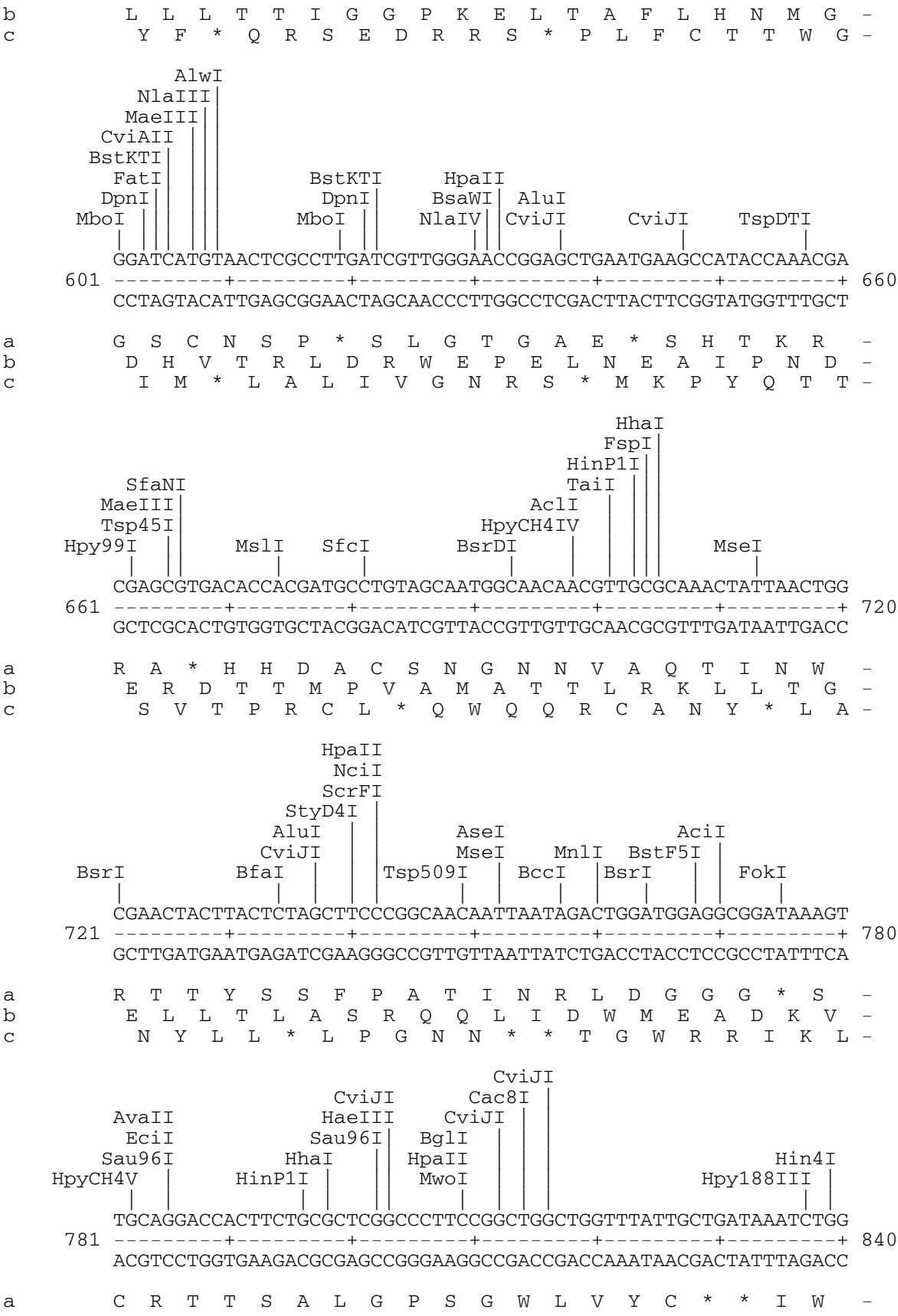
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CviAII
BstKTI
FatI
DpnI
MboI
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HpaII
BsaWI
AluI
NlaIV
CviJI
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FspI
HinP1I
TaiI
SfaNI
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Hpy99I
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SfcI
BsrDI
MseI
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NciI
ScrFI
StyD4I
AluI
CviJI
AseI
MseI
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BsrI
BfaI
Tsp509I
BccI
BsrI
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c N Y L L * L P G N N * * T G W R R I K L -
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CviJI
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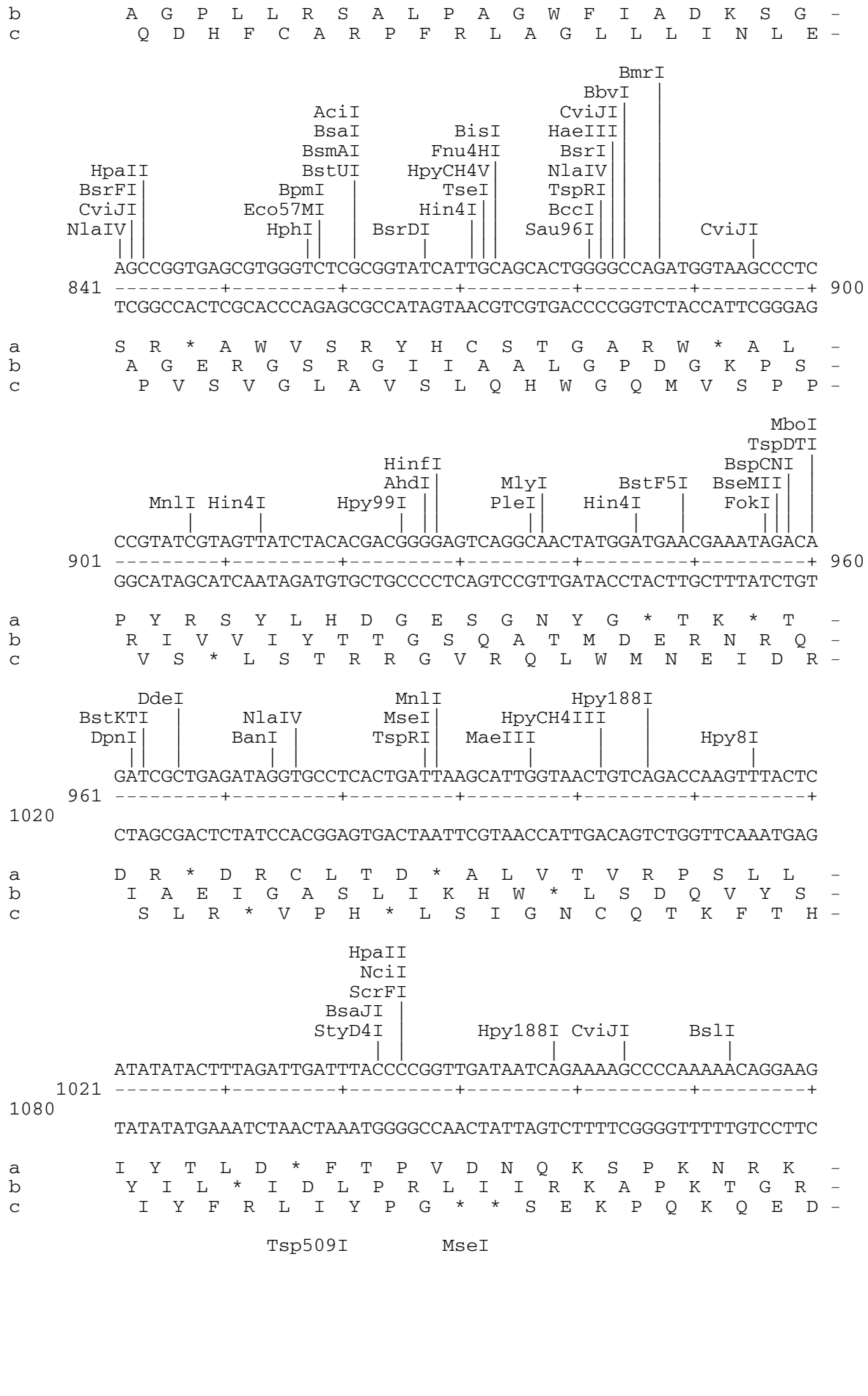
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BisI
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HpyCH4IIII
DpnI
BanI
TspRI
MaeIII
Hpy8I
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1020
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c S L R * V P H * L S I G N C Q T K F T H -
HpaII
NciI
ScrFI
BsaJI
StyD4I
Hpy188I CviJI
BslI
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1021 ---------+---------+---------+---------+---------+---------+
1080
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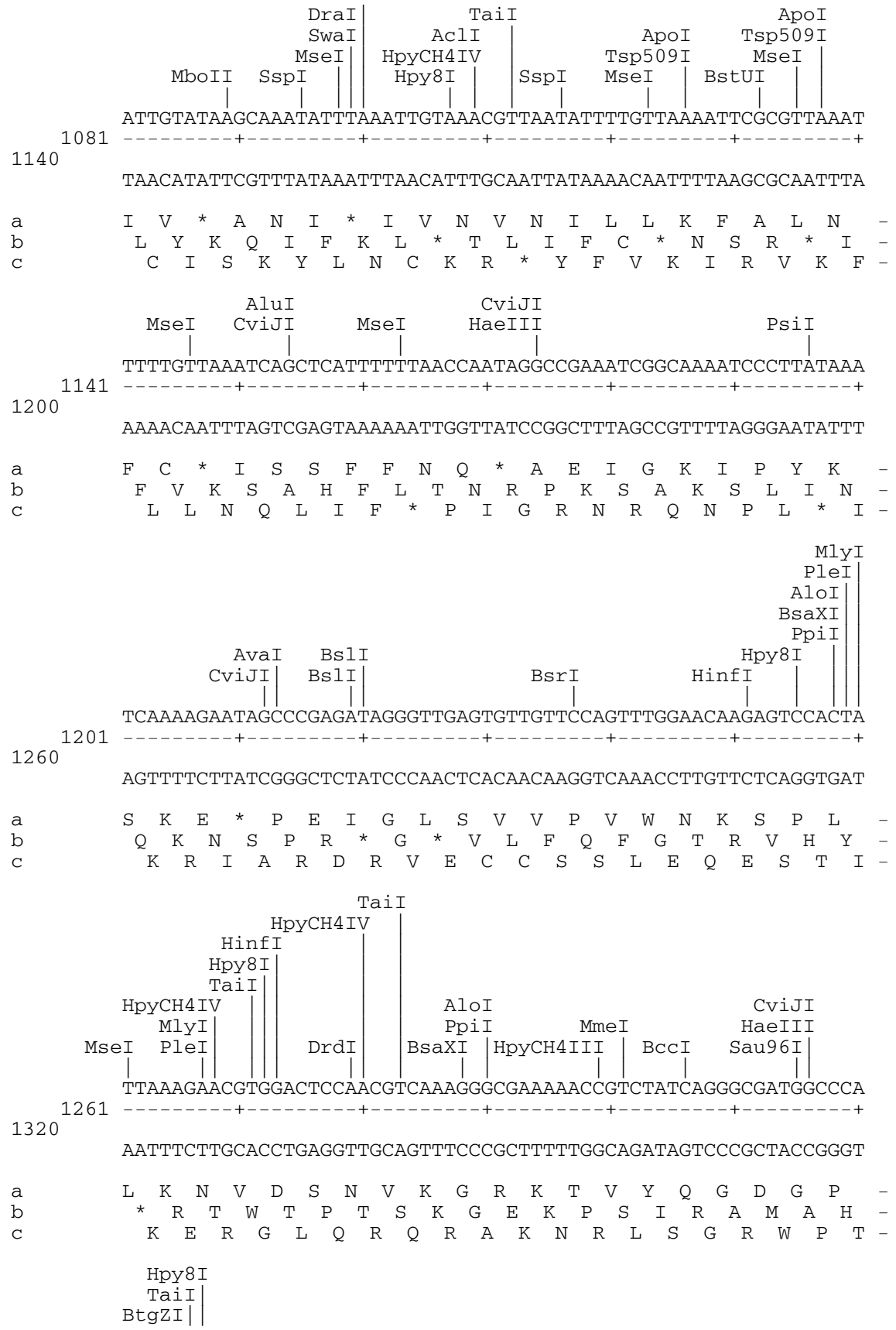
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TaiI
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1081
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c C I S K Y L N C K R * Y F V K I R V K F -
AluI
CviJI
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PleI
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c K R I A R D R V E C C S S L E Q E S T I -
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HpyCH4IV
HinfI
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TaiI
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MseI
PleI
DrdI
AloI
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b * R T W T P T S K G E K P S I R A M A H -
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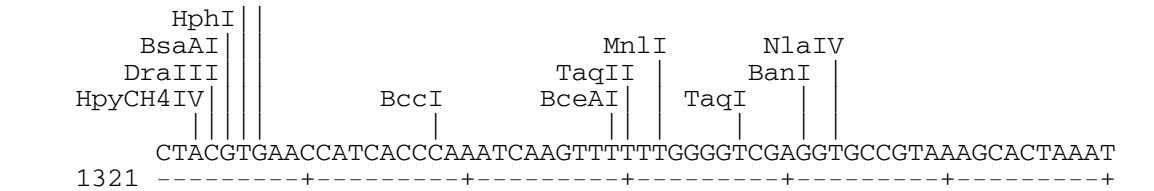

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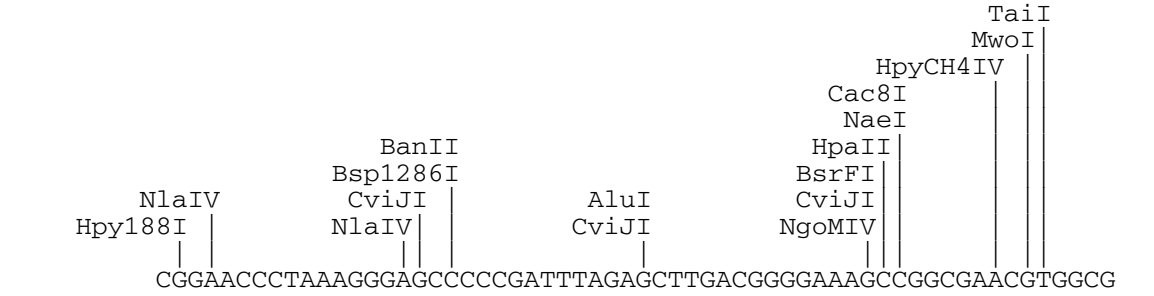
c T * T I T Q I K F F G V E V P * S T K S -



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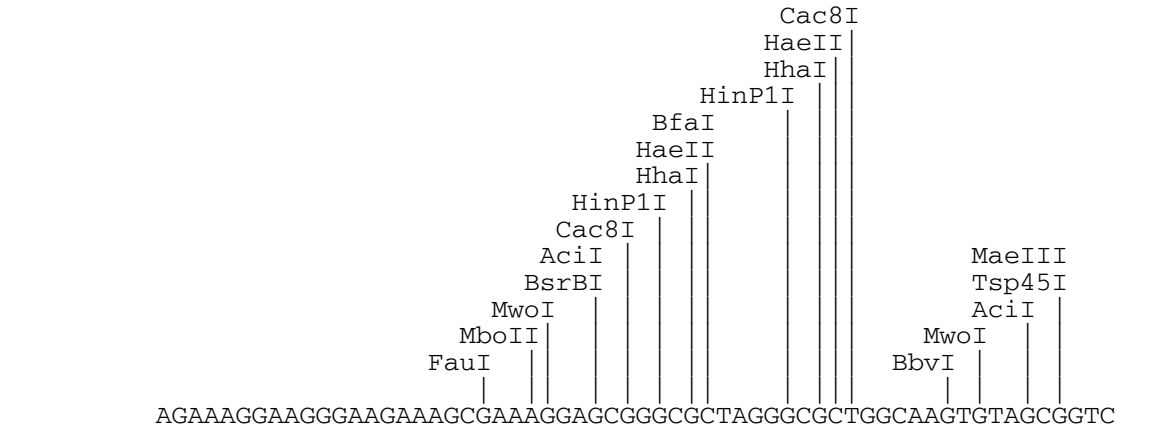

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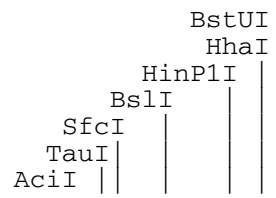

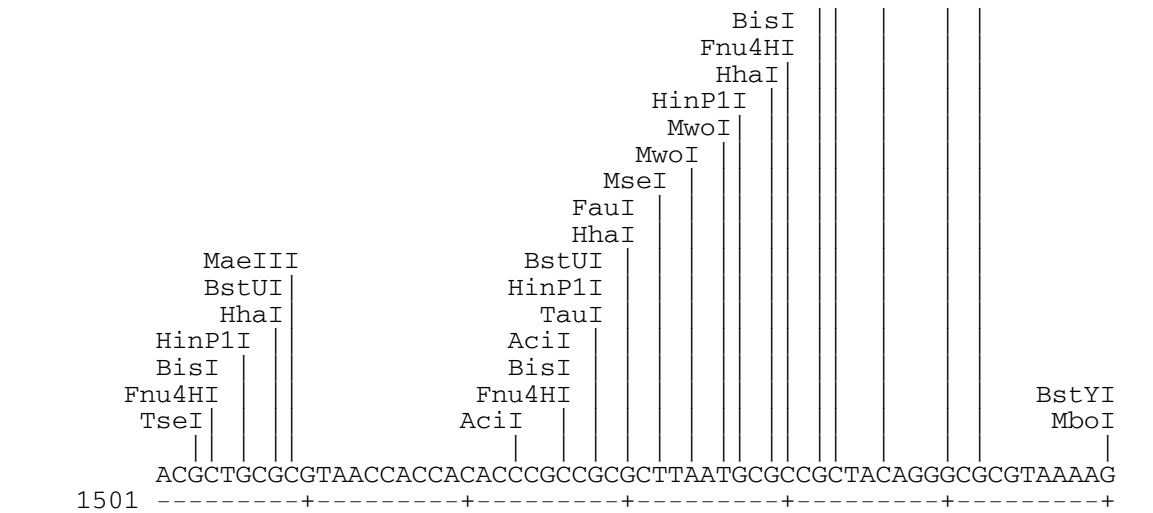
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AciI
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MboI
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1560
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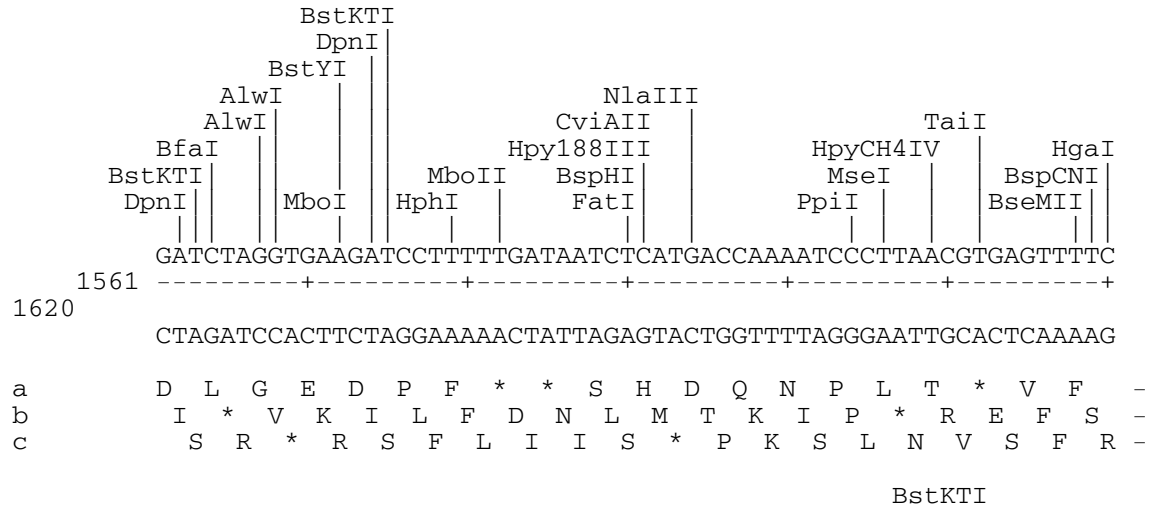
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BstKTI
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BspHI
MseI
BspCNI
DpnI
MboI
HphI
FatI
PpiI
BseMII
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1561 ---------+---------+---------+---------+---------+---------+
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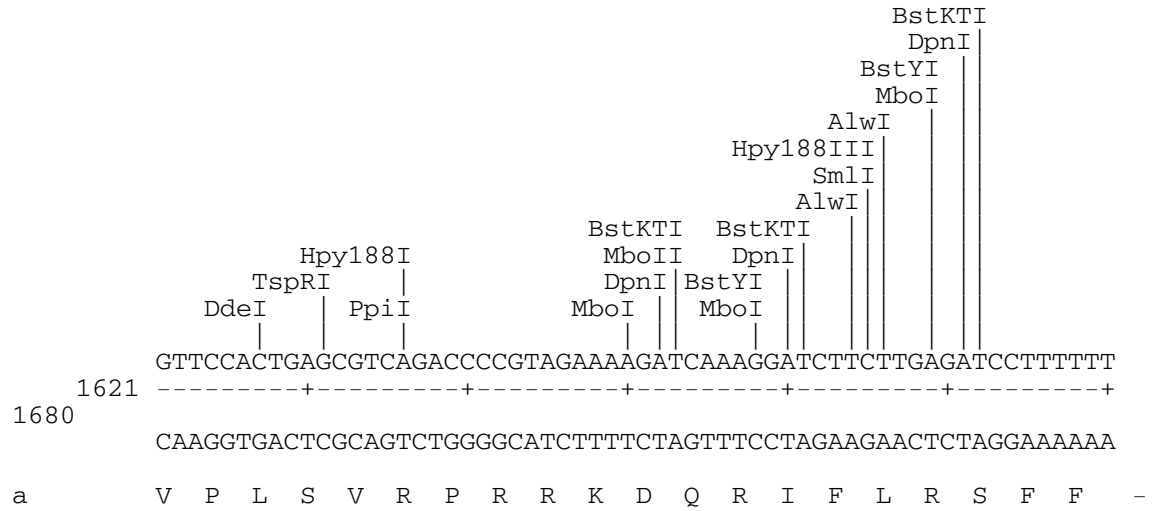
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DdeI
PpiI
MboI
MboI
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1621 ---------+---------+---------+---------+---------+---------+
1680
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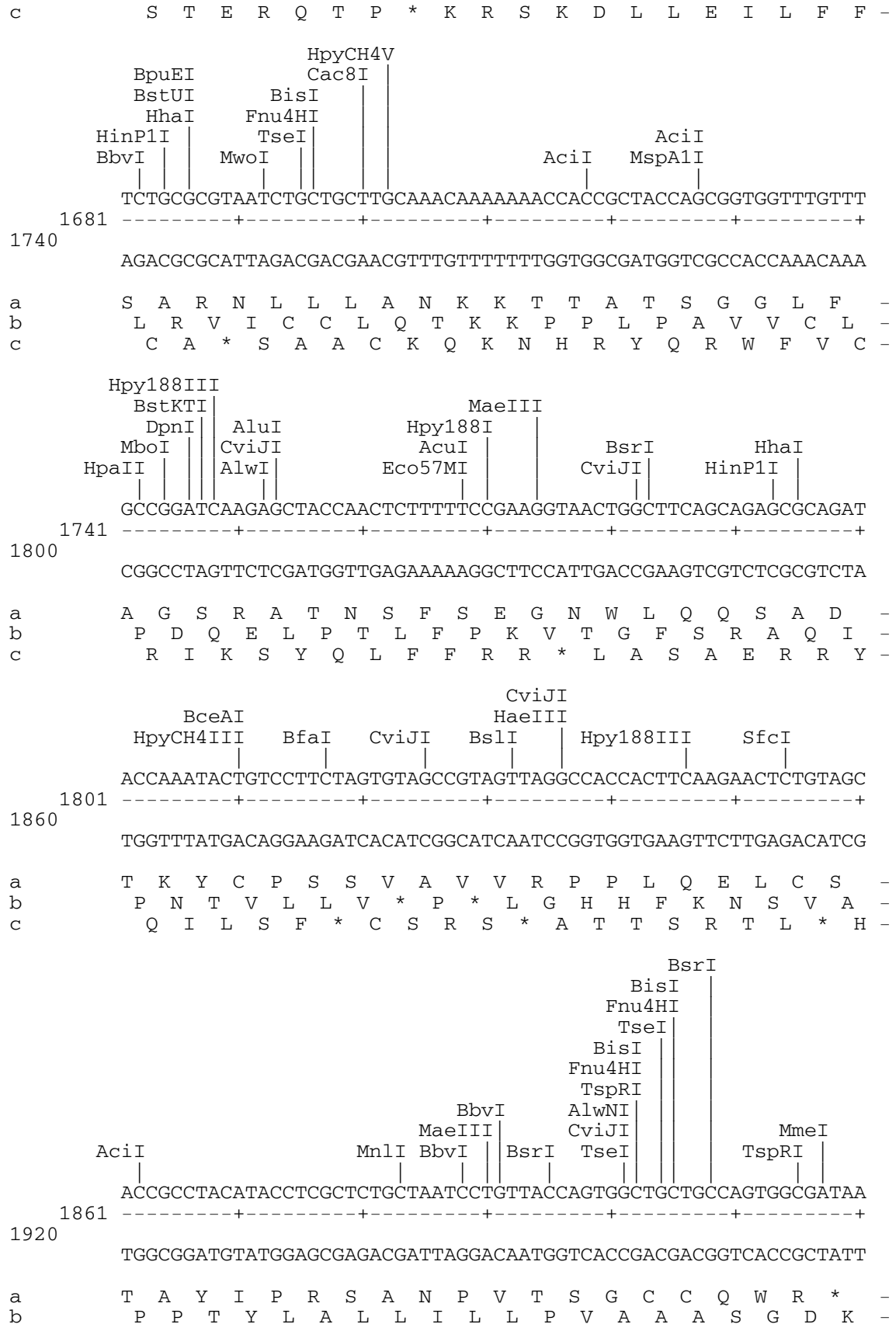
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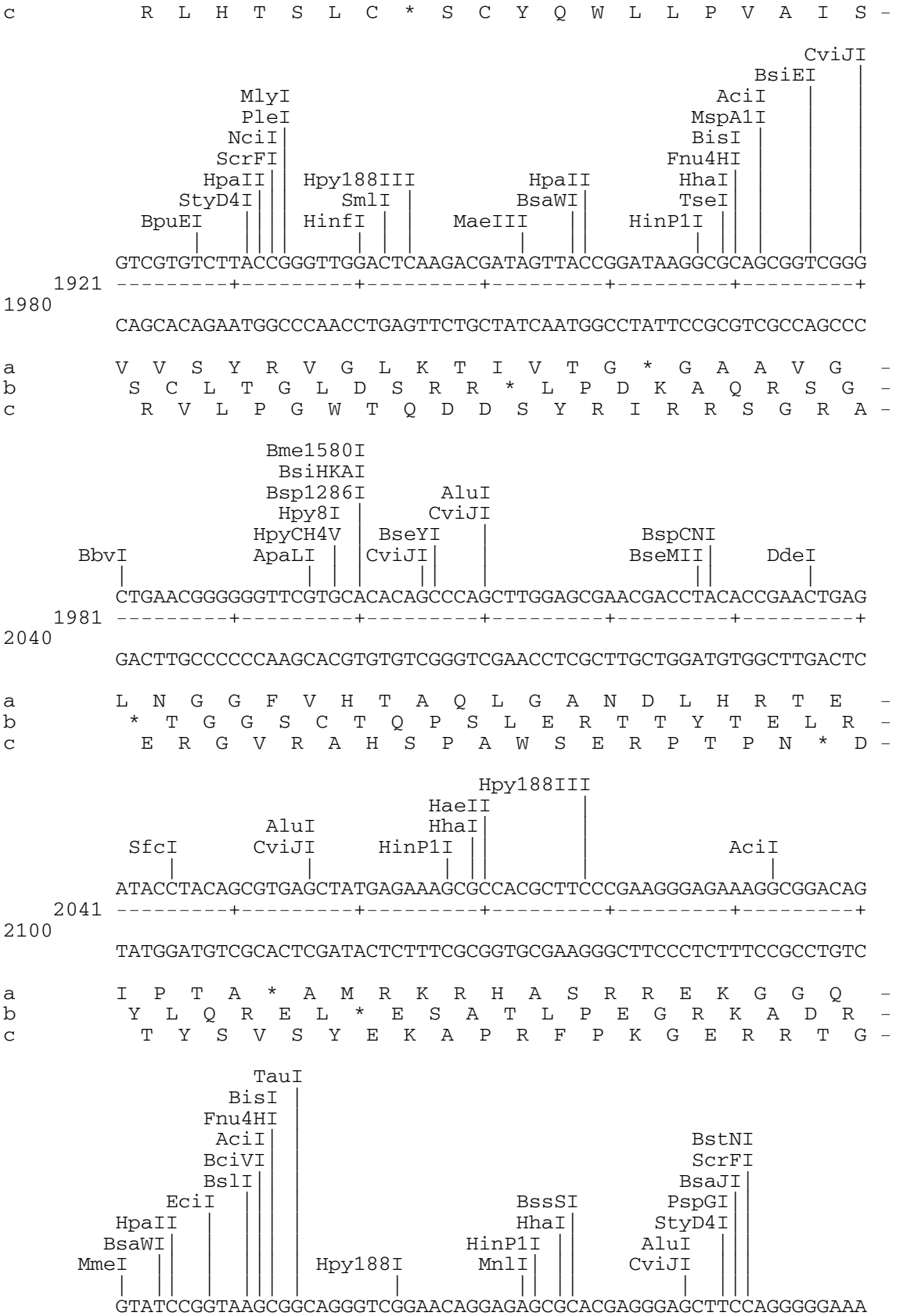
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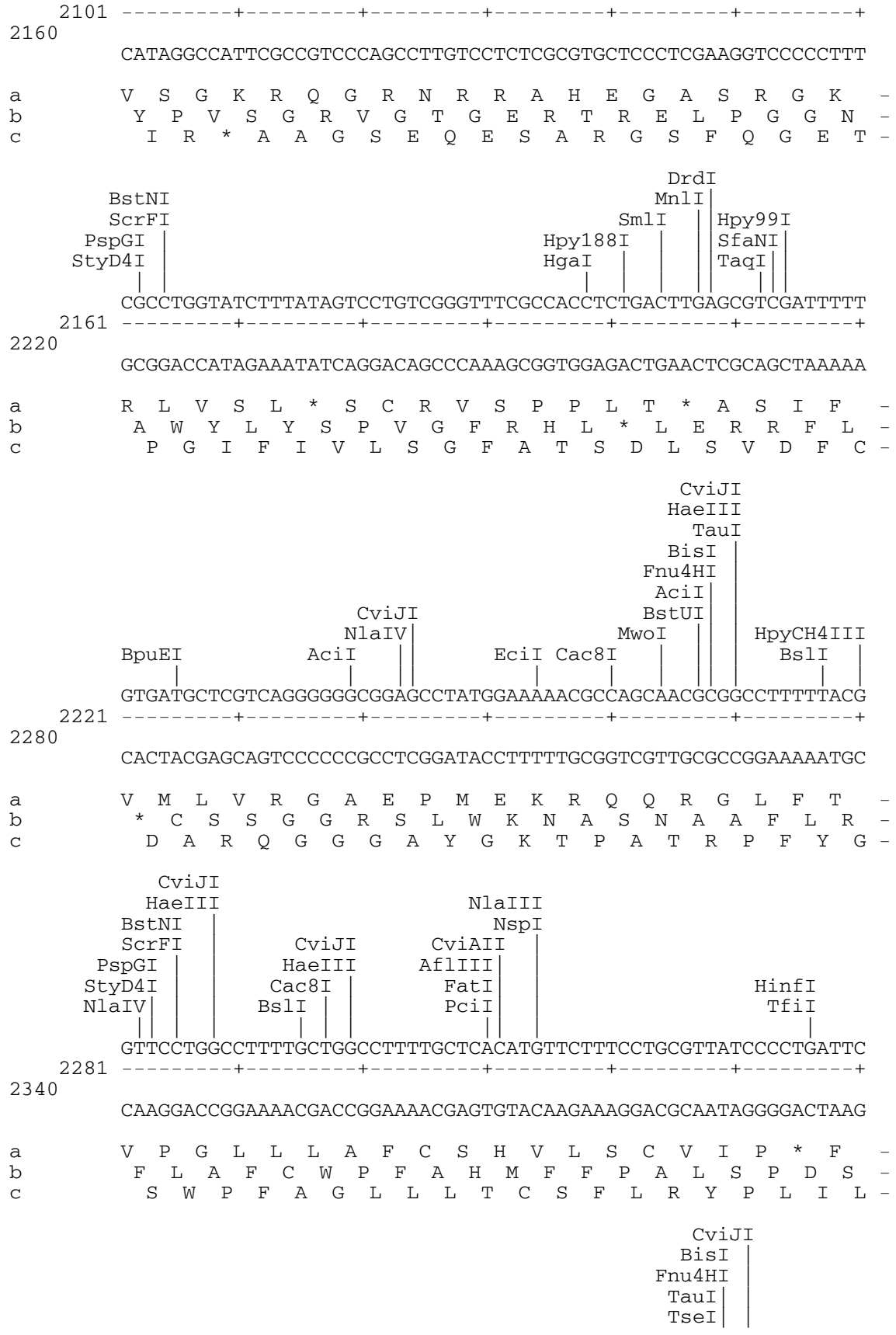
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TauI
BisI
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CviJI BstUI
NlaIV MwoI HpyCH4III
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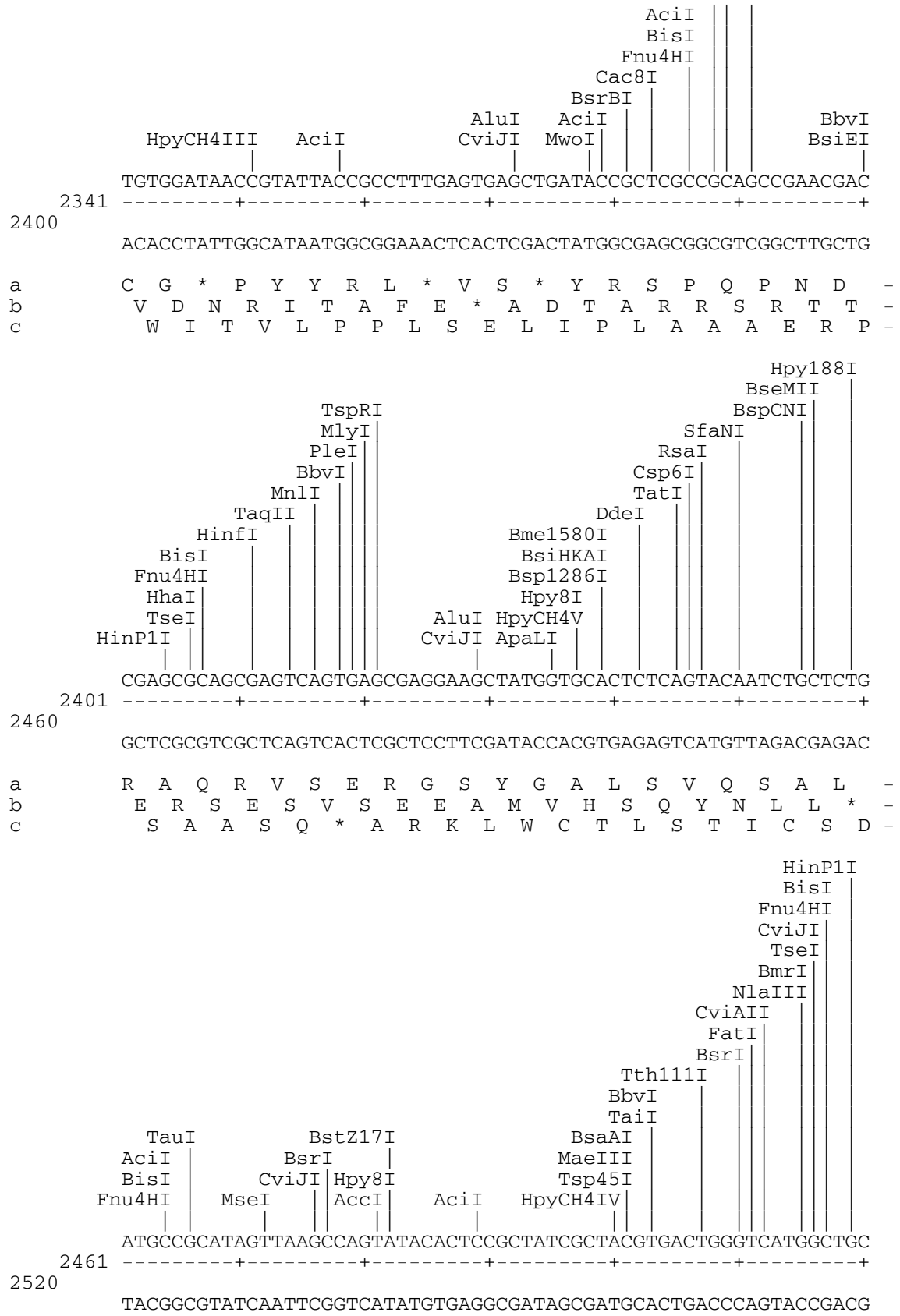
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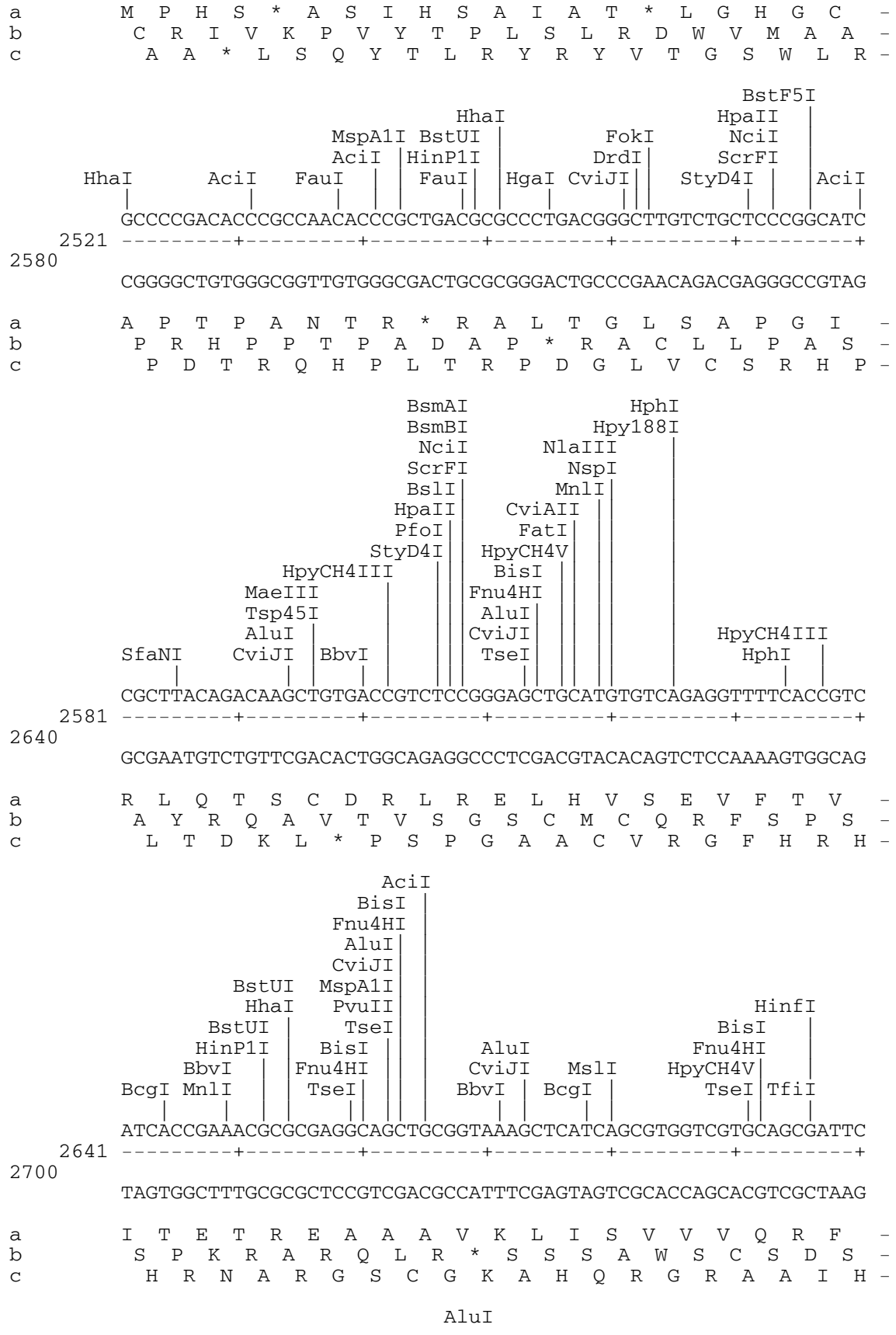
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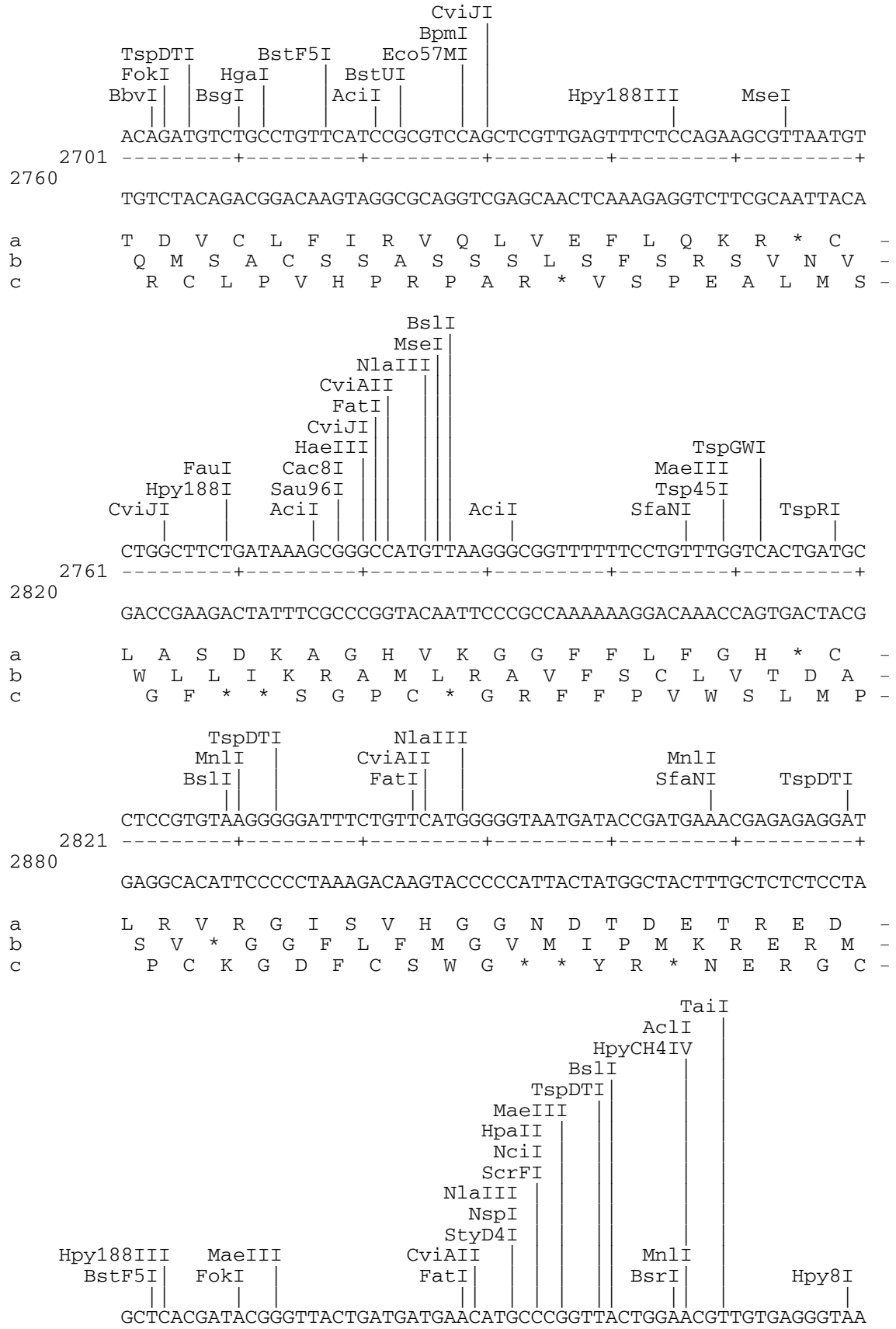
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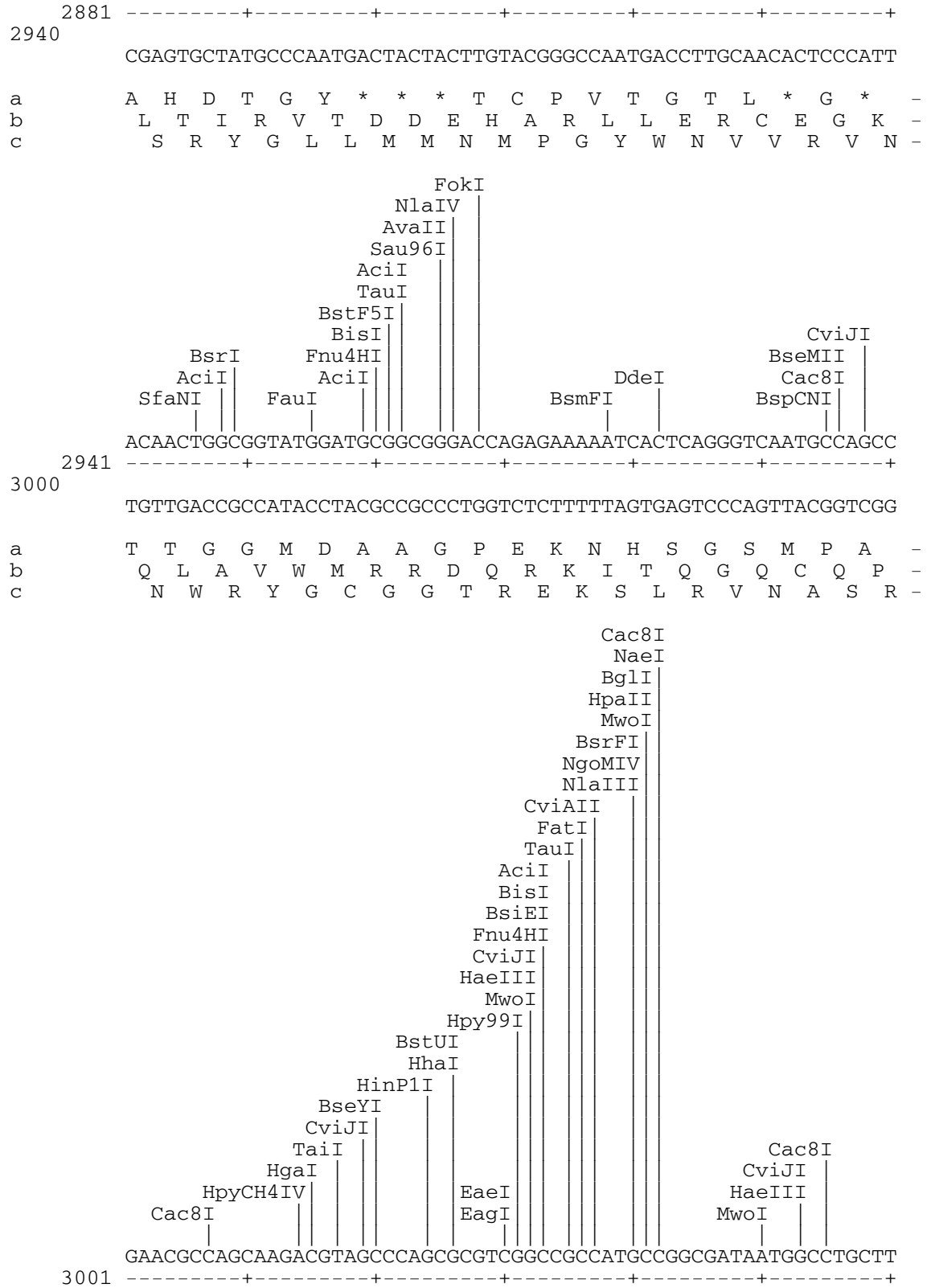
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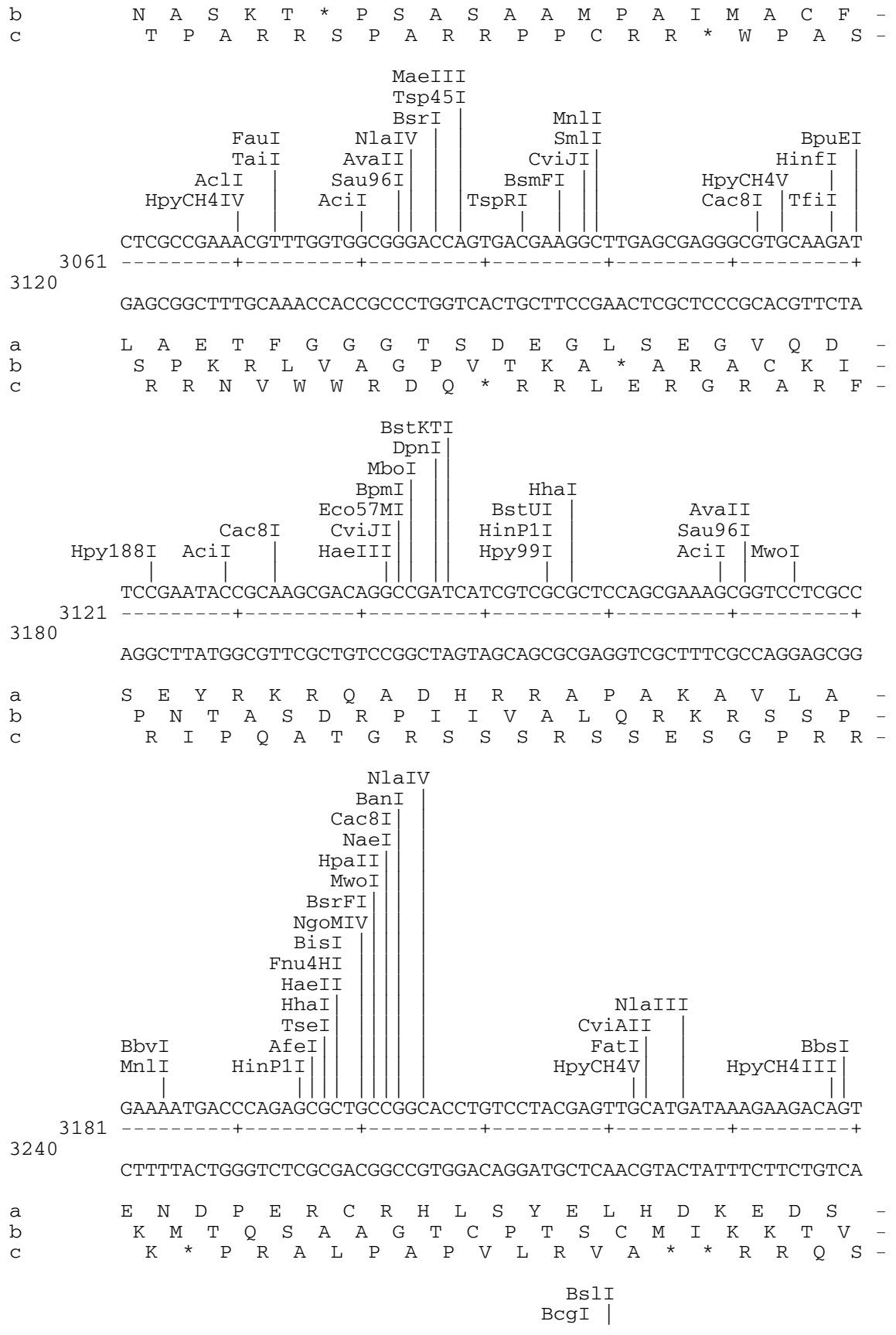
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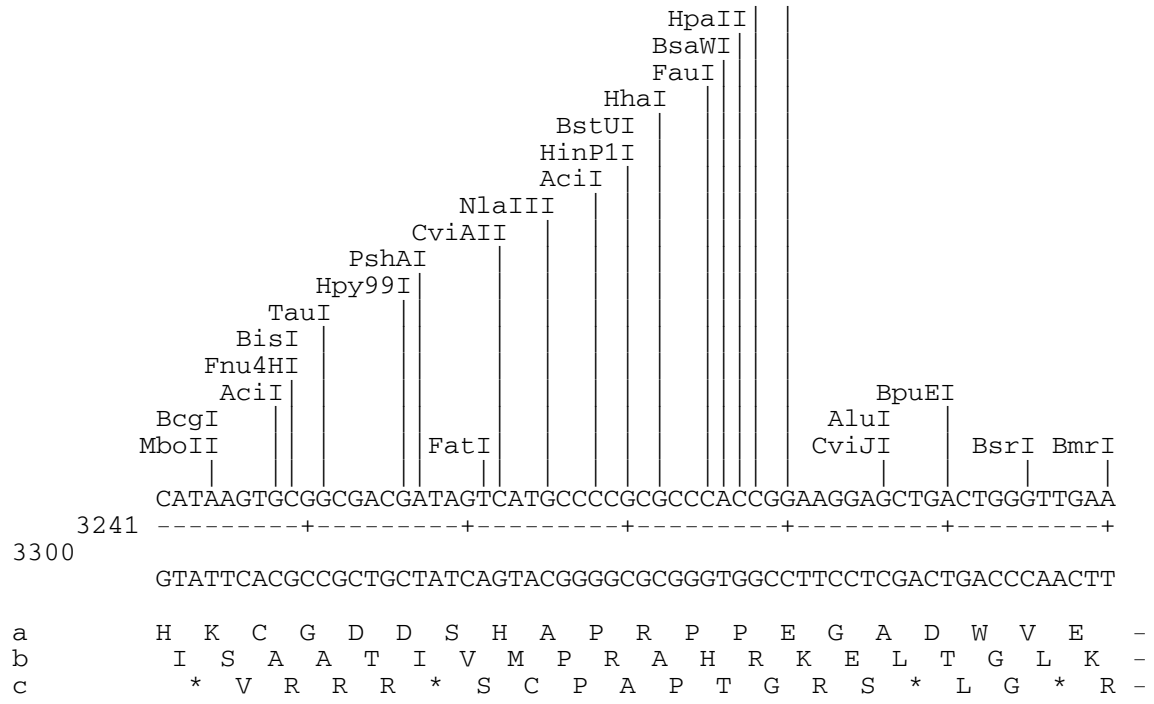
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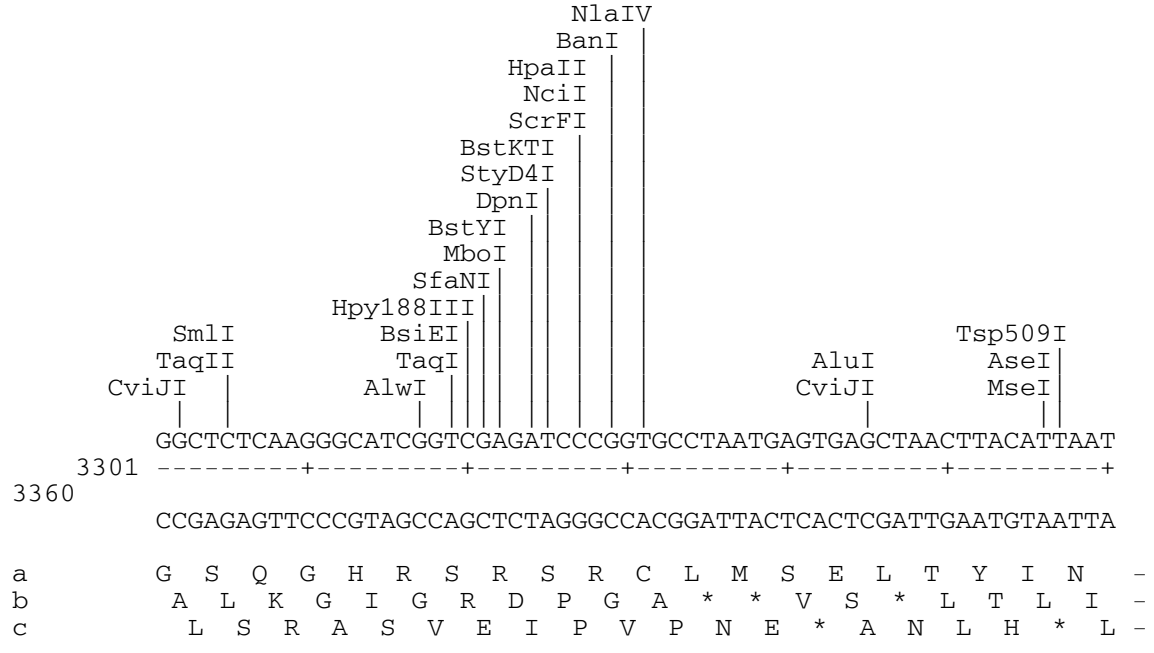
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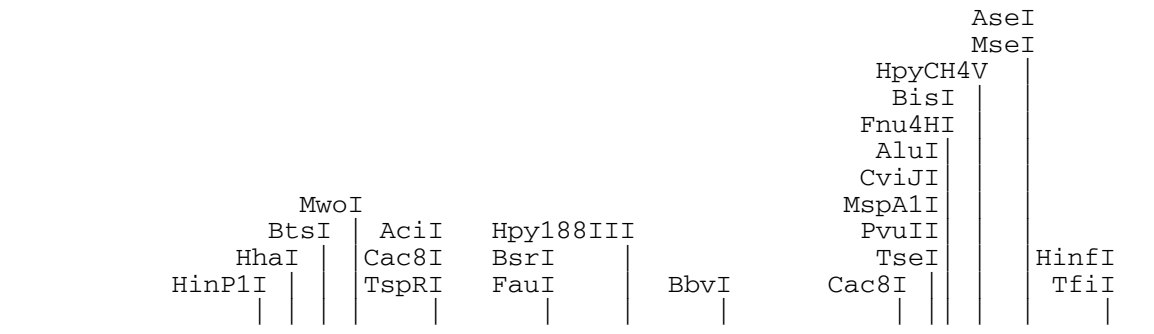
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BtsI
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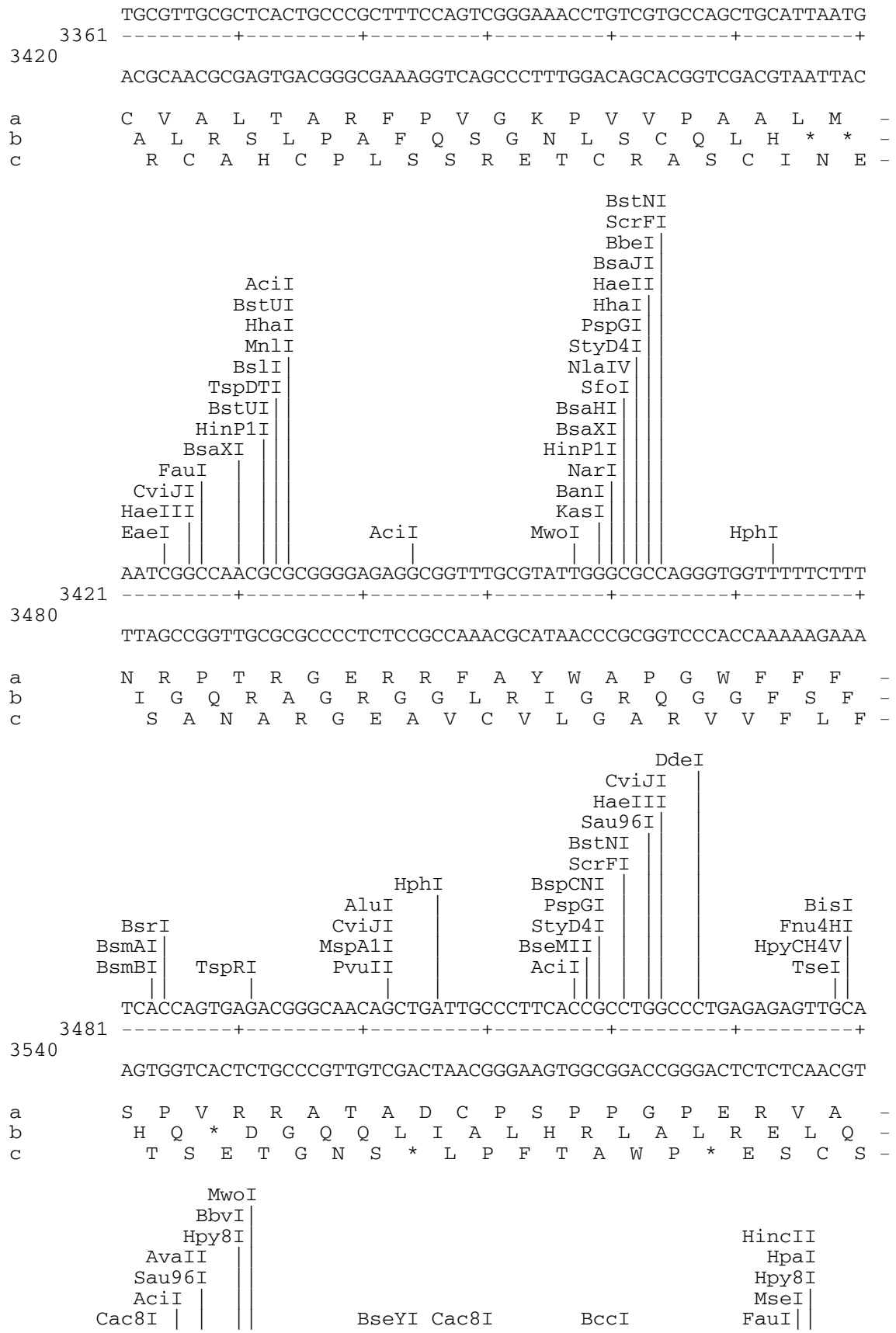
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c R C A H C P L S S R E T C R A S C I N E -
BstNI
ScrFI
BbeI
BsaJI
AciI
HaeII
BstUI
HhaI
HhaI
PspGI
MnlI
StyD4I
BslI
NlaIV
TspDTI
SfoI
BstUI
BsaHI
HinP1I
BsaXI
BsaXI
HinP1I
FauI
NarI
CviJI
BanI
HaeIII
KasI
EaeI
AciI
MwoI
HphI
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3480
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b I G Q R A G R G G L R I G R Q G G F S F -
c S A N A R G E A V C V L G A R V V F L F -
DdeI
CviJI
HaeIII
Sau96I
BstNI
ScrFI
HphI
BspCNI
AluI
PspGI
BisI
BsrI
CviJI
StyD4I
Fnu4HI
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BseMII
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AciI
TseI
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c T S E T G N S * L P F T A W P * E S C S -
MwoI
BbvI
Hpy8I
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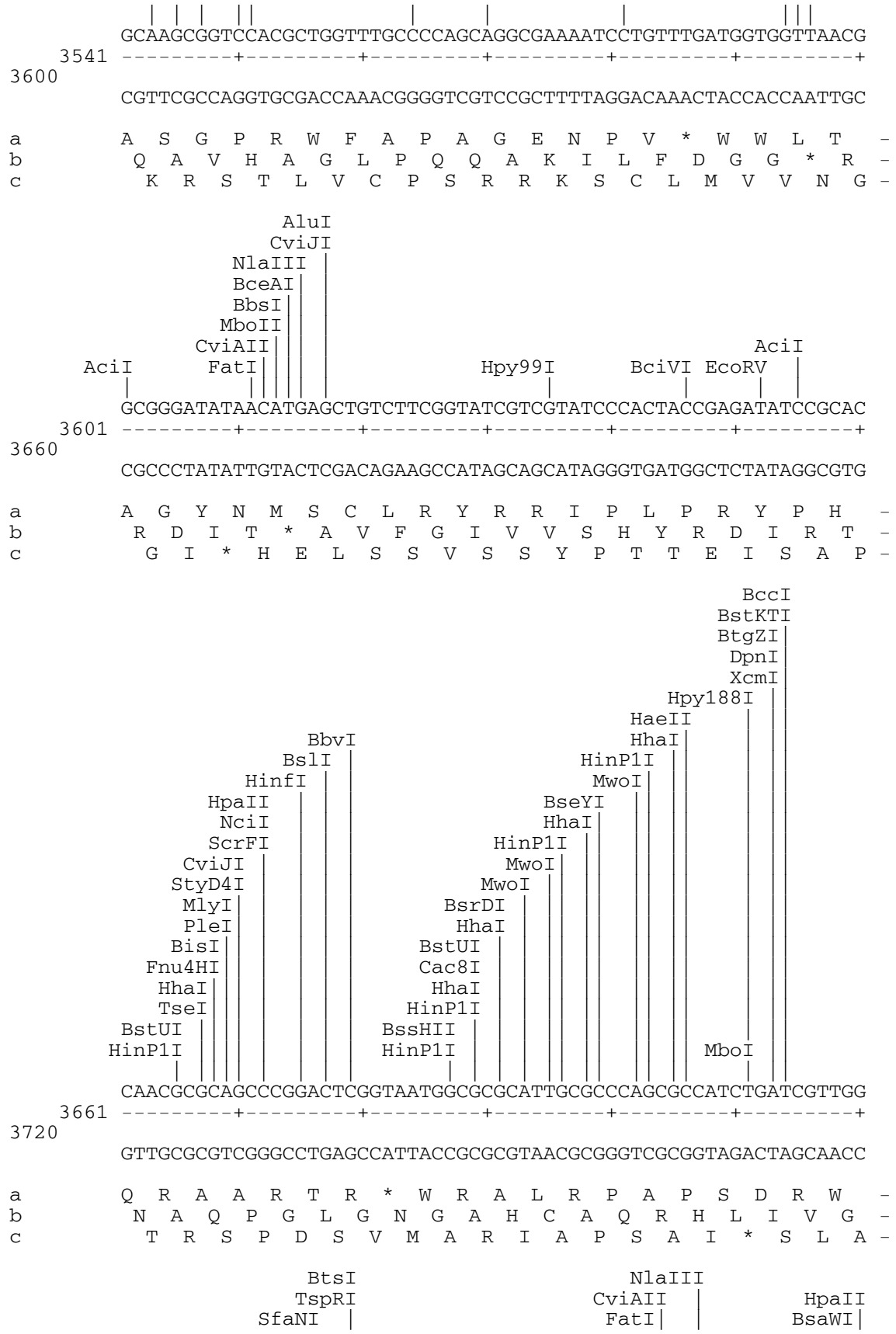
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c K R S T L V C P S R R K S C L M V V N G -
AluI
CviJI
NlaIII
BceAI
BbsI
MboII
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FatI
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c G I * H E L S S V S S Y P T T E I S A P -
BccI
BstKTI
BtgZI
DpnI
XcmI
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HhaI
BbvI
BslI
HinP1I
HinfI
MwoI
HpaII
BseYI
NciI
HhaI
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MwoI
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Cac8I
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c T R S P D S V M A R I A P S A I * S L A -
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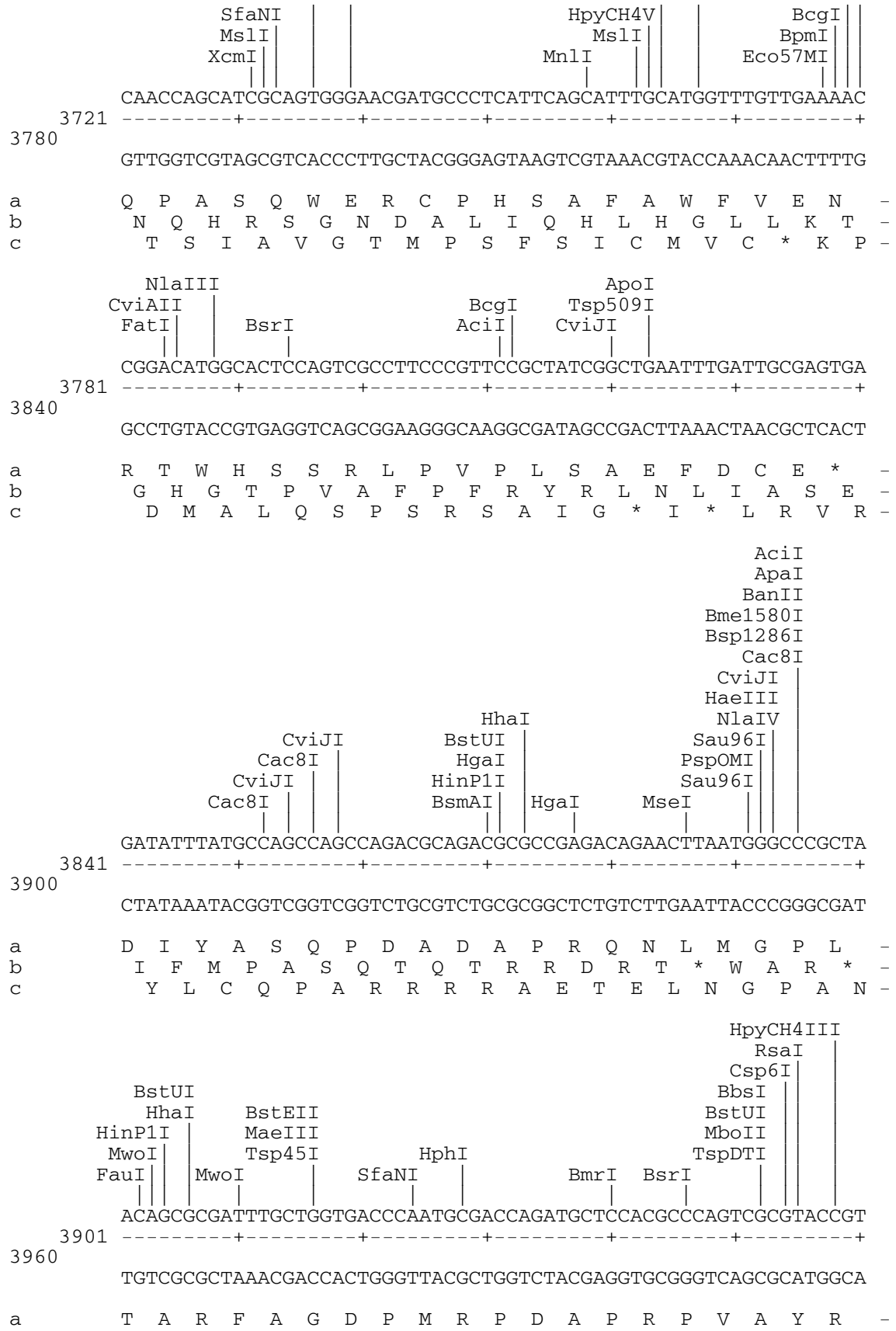
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c T S I A V G T M P S F S I C M V C * K P -
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FatI
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c Y L C Q P A R R R R A E T E L N G P A N -
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Csp6I
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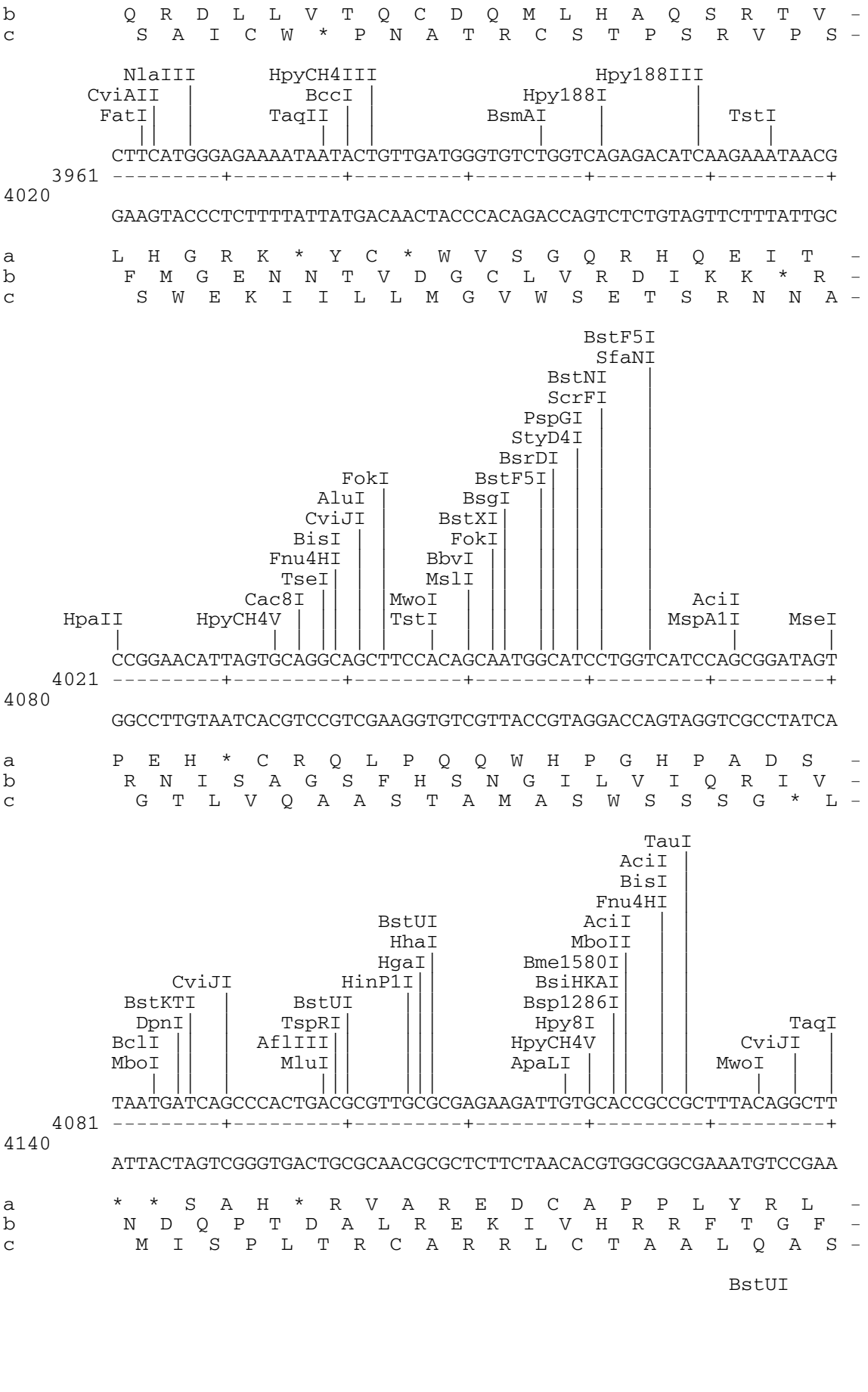
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Fnu4HI
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c G T L V Q A A S T A M A S W S S S G * L -
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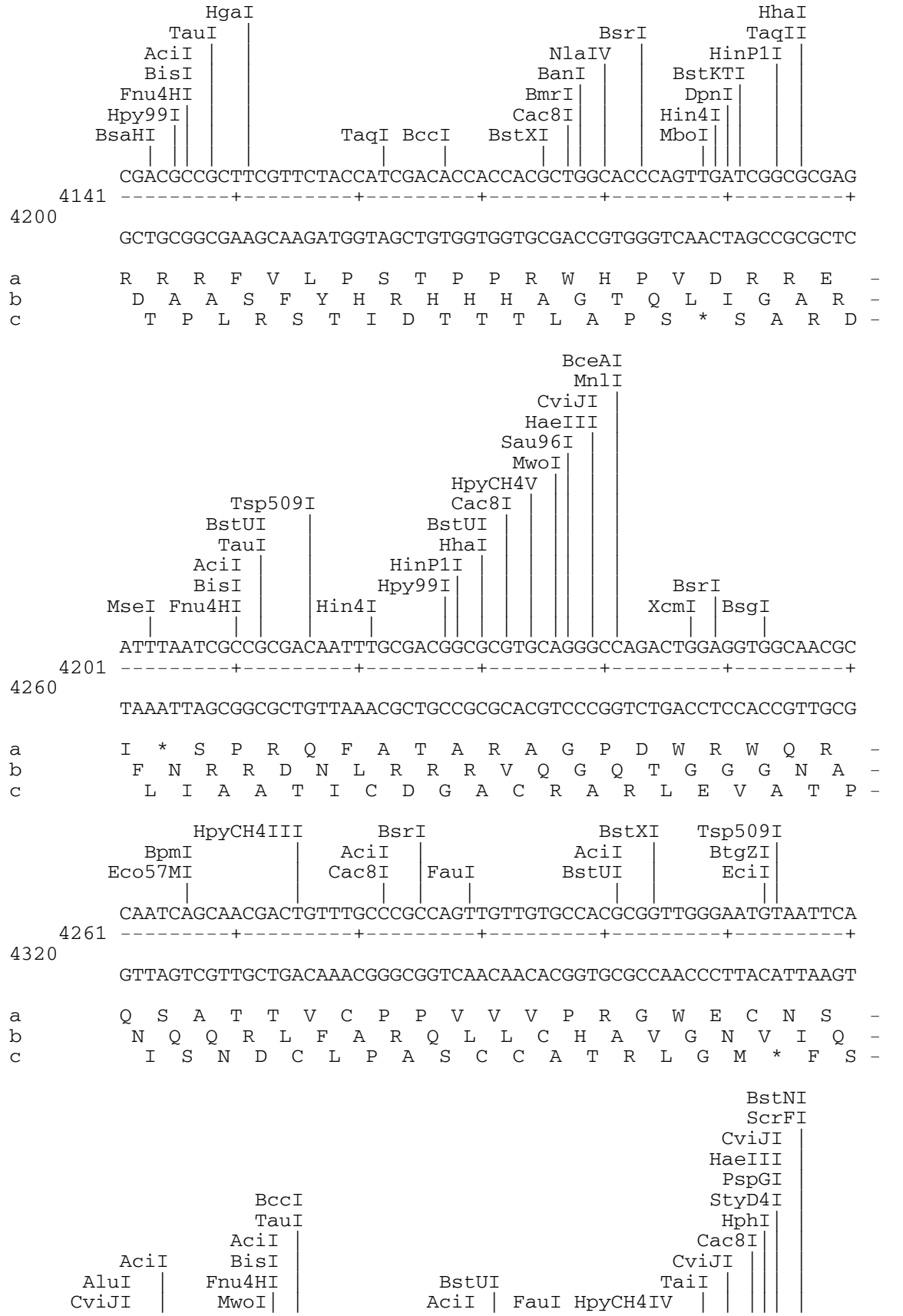
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BmrI
Cac8I
HhaI
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c T P L R S T I D T T T L A P S * S A R D -
BceAI
MnlI
CviJI
HaeIII
Sau96I
MwoI
HpyCH4V
Cac8I
Tsp509I
BstUI
TauI
AciI
BisI
MseI Fnu4HI
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b F N R R D N L R R R V Q G Q T G G G N A -
c L I A A T I C D G A C R A R L E V A T P -
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BsrI
BstXI
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c I S N D C L P A S C C A T R L G M * F S -
BstNI
ScrFI
CviJI
HaeIII
PspGI
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BccI
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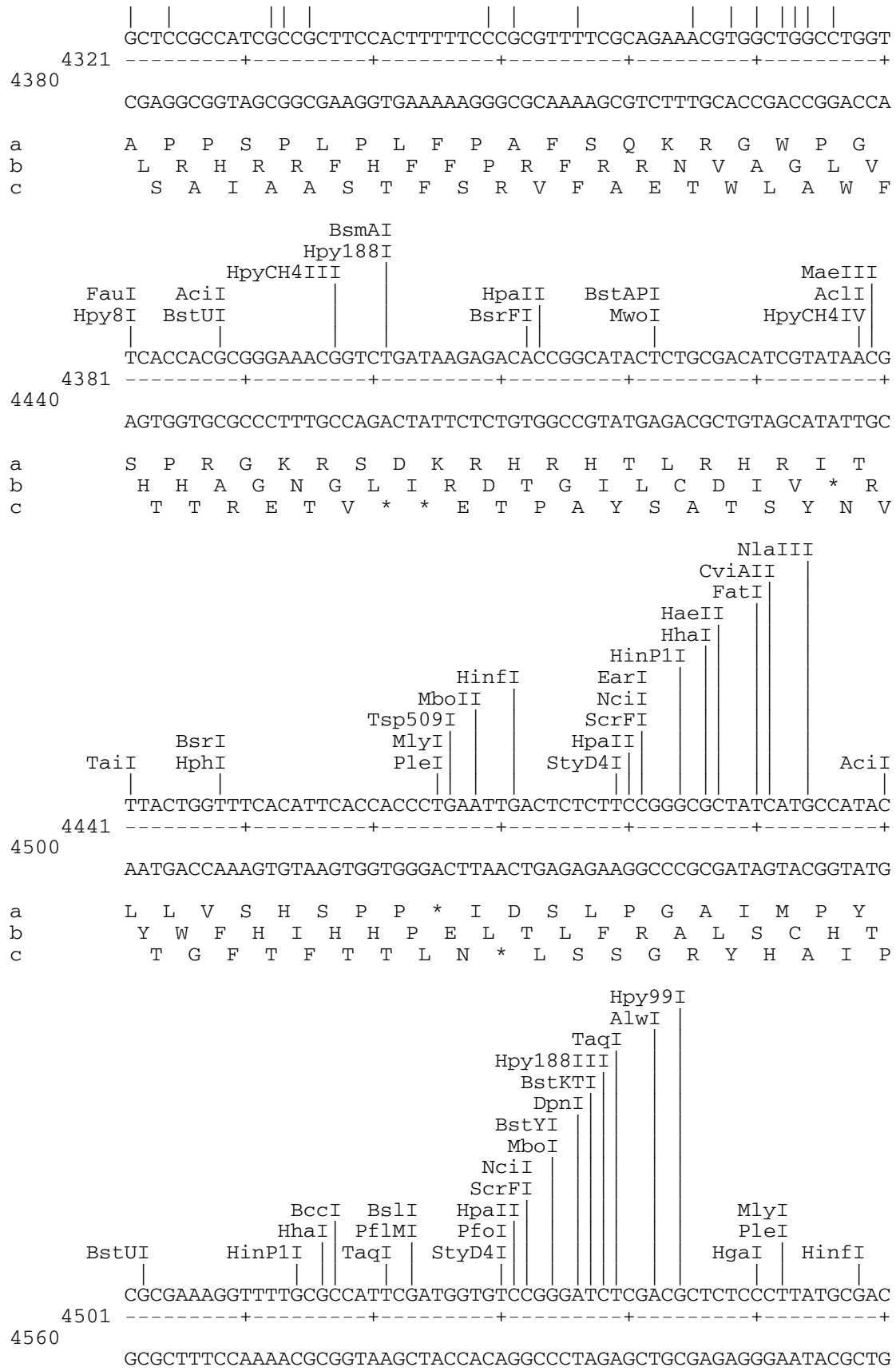
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c T T R E T V * * E T P A Y S A T S Y N V -
NlaIII
CviAII
FatI
HaeII
HhaI
HinP1I
HinfI
EarI
MboII
NciI
Tsp509I
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BsrI
MlyI
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TaiI
HphI
PleI
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AlwI
TaqI
Hpy188III
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MboI
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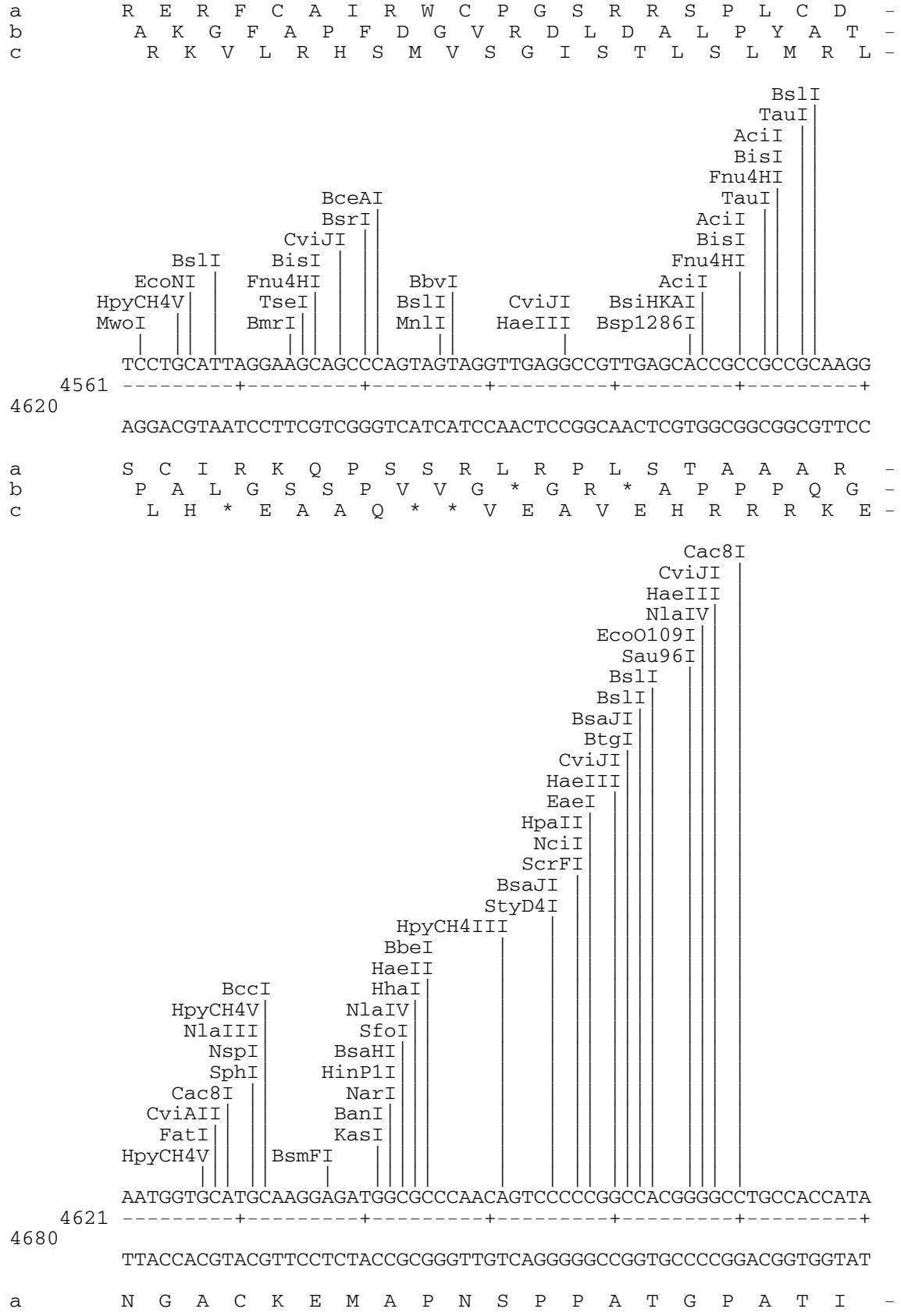
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c L H * E A A Q * * V E A V E H R R R K E -
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BccI
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HpyCH4V
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NlaIII
SfoI
NspI
BsaHI
SphI
HinP1I
Cac8I
NarI
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FatI
KasI
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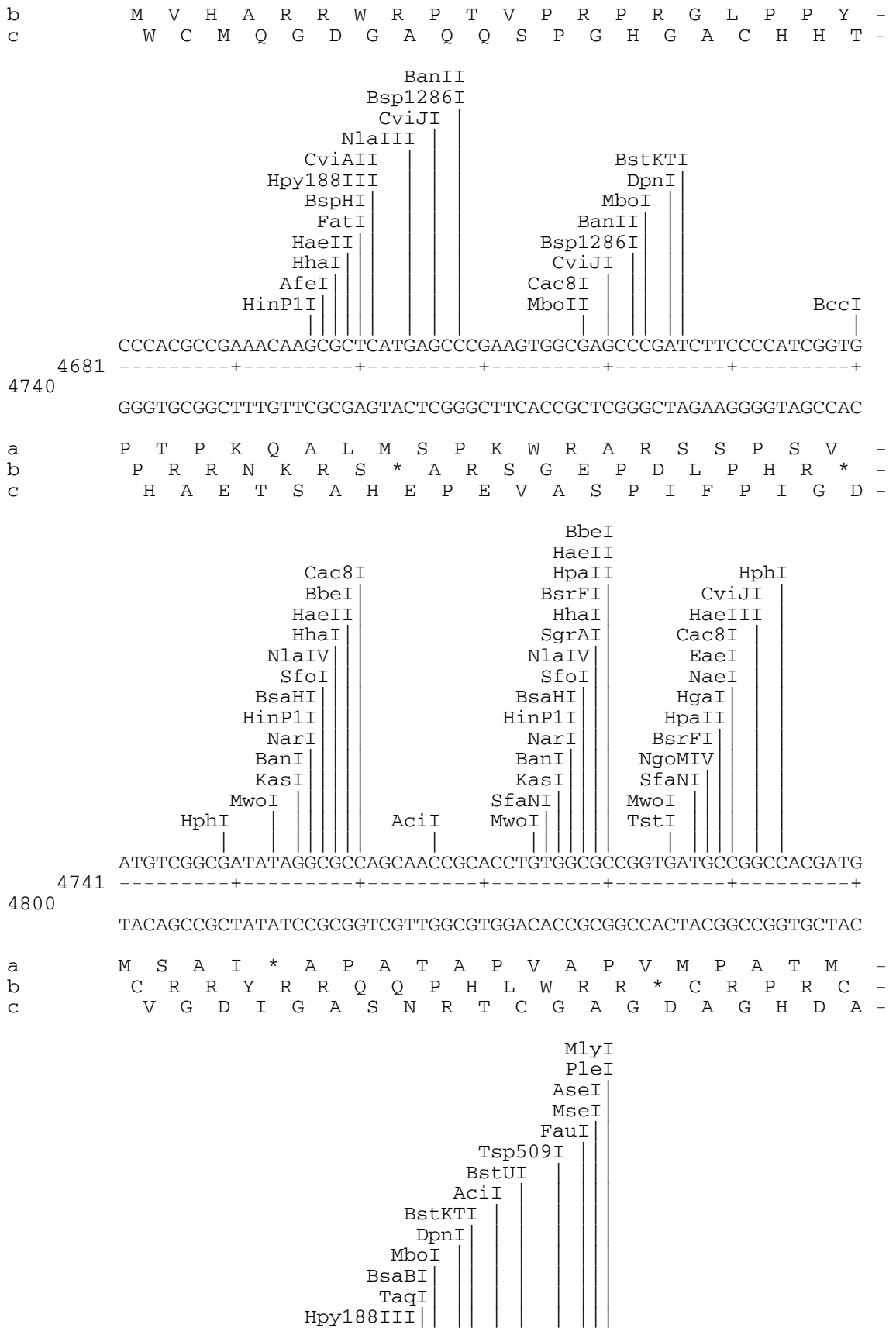
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HaeII
Cac8I
HpaII
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HaeIII
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SfoI
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HpaII
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AciI
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c V G D I G A S N R T C G A G D A G H D A -
MlyI
PleI
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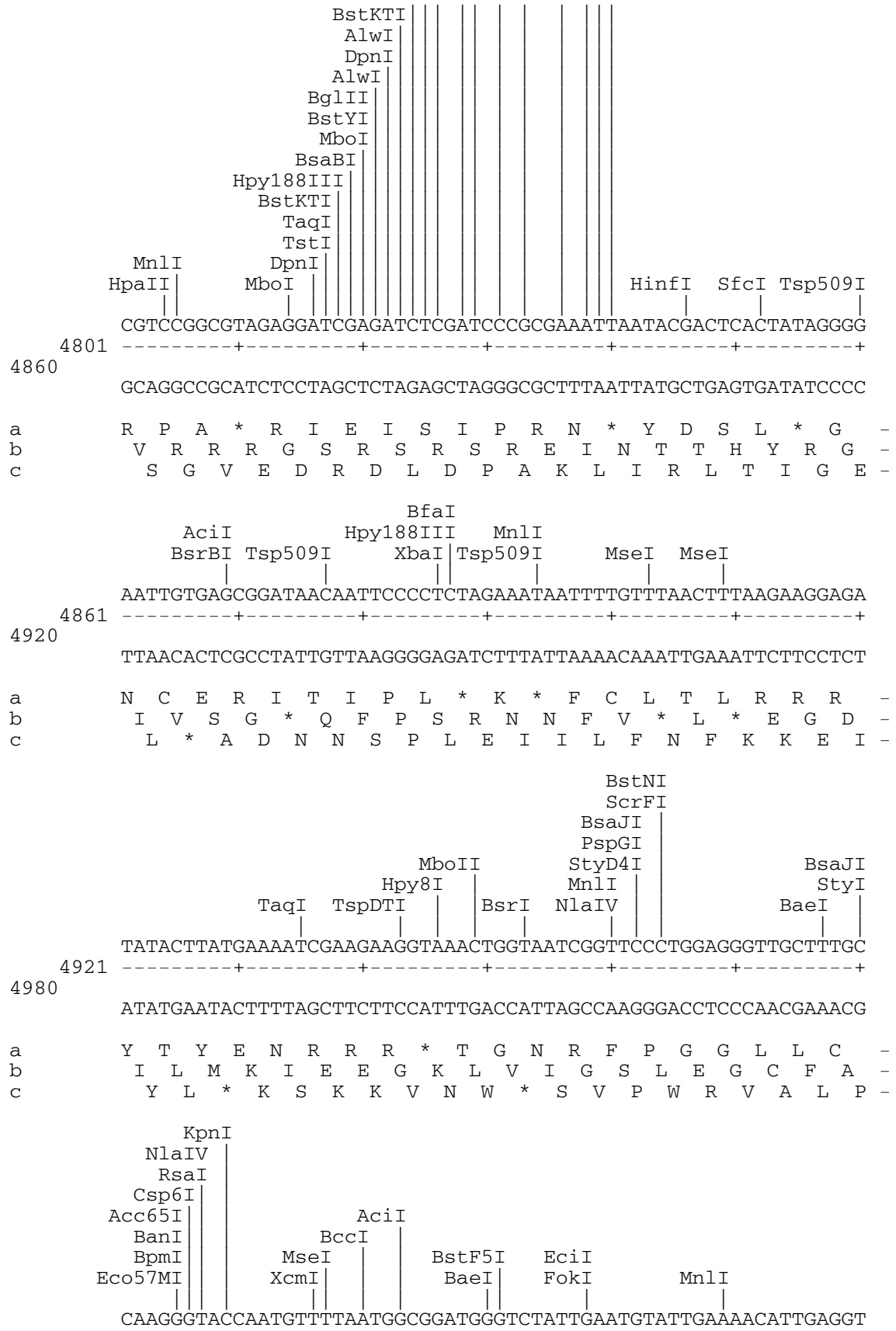
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AciI
Hpy188III
MnlI
BsrBI
Tsp509I
XbaI
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MseI
MseI
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c L * A D N N S P L E I I L F N F K K E I -
BstNI
ScrFI
BsaJI
PspGI
MboII
StyD4I
BsaJI
Hpy8I
MnlI
StyI
TaqI
TspDTI
BsrI
NlaIV
BaeI
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b I L M K I E E G K L V I G S L E G C F A -
c Y L * K S K K V N W * S V P W R V A L P -
KpnI
NlaIV
RsaI
Csp6I
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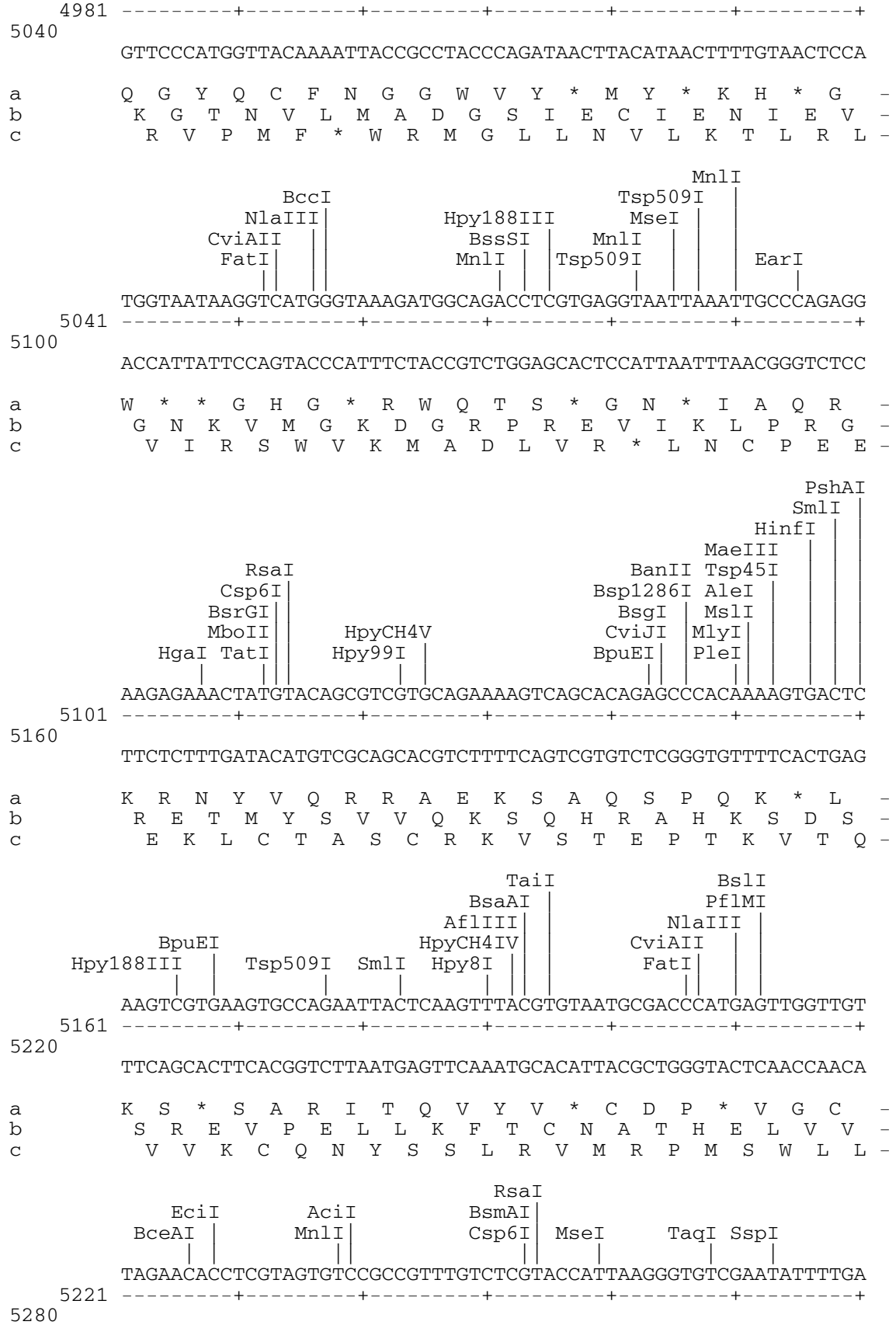
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c V I R S W V K M A D L V R * L N C P E E -
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BsaAI
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c V V K C Q N Y S S L R V M R P M S W L L -
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EciI
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5280

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b         R  T  P  R  S  V  R  R  L  S  R  T  I  K  G  V  E  Y  F  E  -
c          E  H  L  V  V  S  A  V  C  L  V  P  L  R  V  S  N  I  L  K -

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                         HaeIII            HpyCH4III   |      AluI
                BccI   Sau96I |      CviJI        |   |     CviJI
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c          L  L  L  L  R  W  A  K  R  K  P  P  T  V  E  L  L  S  L  S -

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                                             CviJI |
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                                            NlaIV| |
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                                          Sau96I|| |
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5400
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b         K  E  V  S  K  S  Y  P  I  S  E  G  P  E  R  A  N  E  L  V  -
c          R  K  F  Q  R  A  T  Q  Y  L  R  G  L  R  E  P  T  N  *  * -

                                                                 DpnI
                                                              BglII |
                                   AluI                       BstYI |
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b         E  S  Y  R  K  A  S  N  K  A  Y  F  E  W  T  I  E  A  R  D  -
c          N  P  I  E  R  L  Q  I  K  L  I  L  S  G  L  L  R  P  E  I -

                           NlaIII      BsaXI
                        CviAII  |      Hin4I
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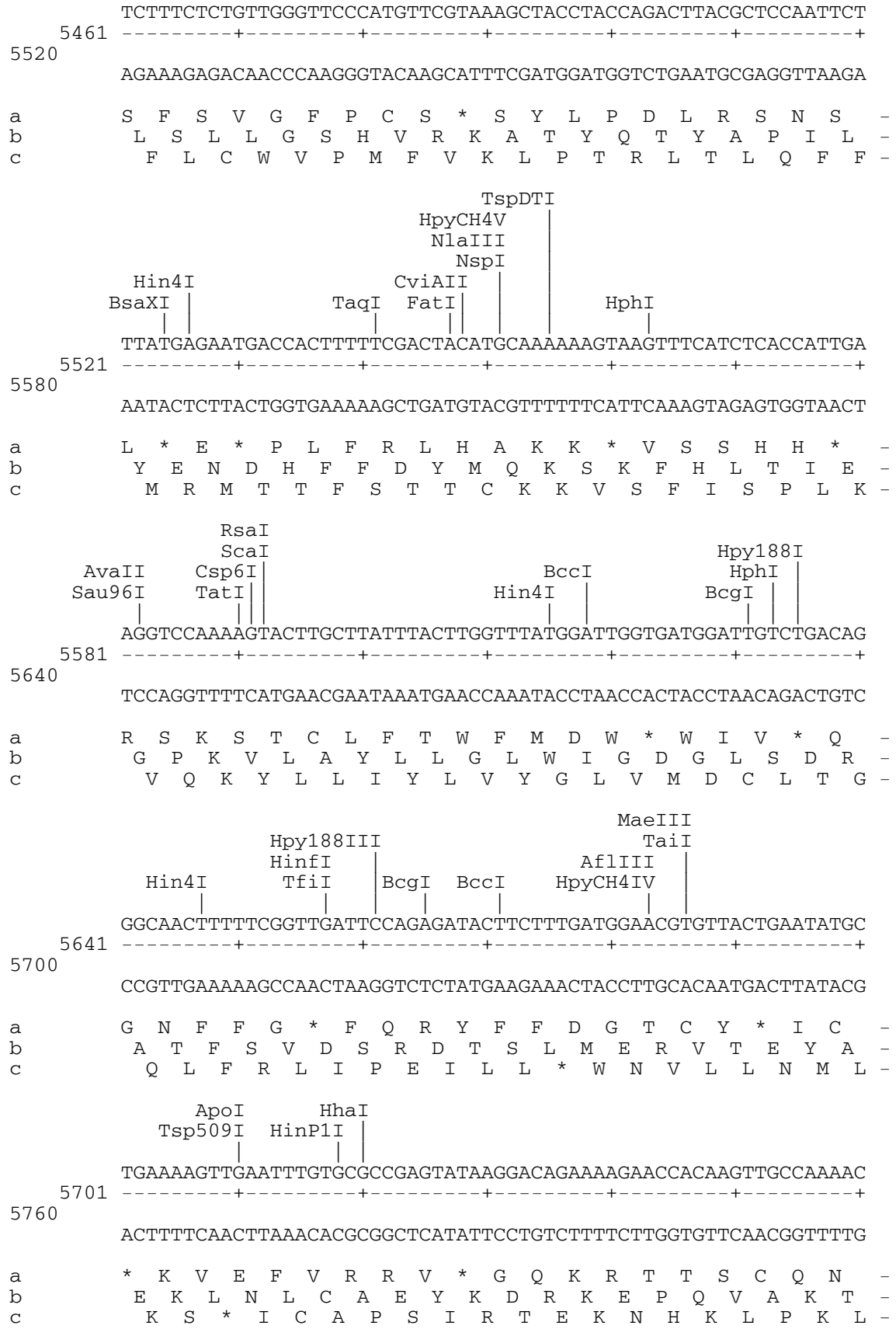
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c M R M T T F S T T C K K V S F I S P L K -
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c V Q K Y L L I Y L V Y G L V M D C L T G -
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BcgI
BccI
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c Q L F R L I P E I L L * W N V L L N M L -
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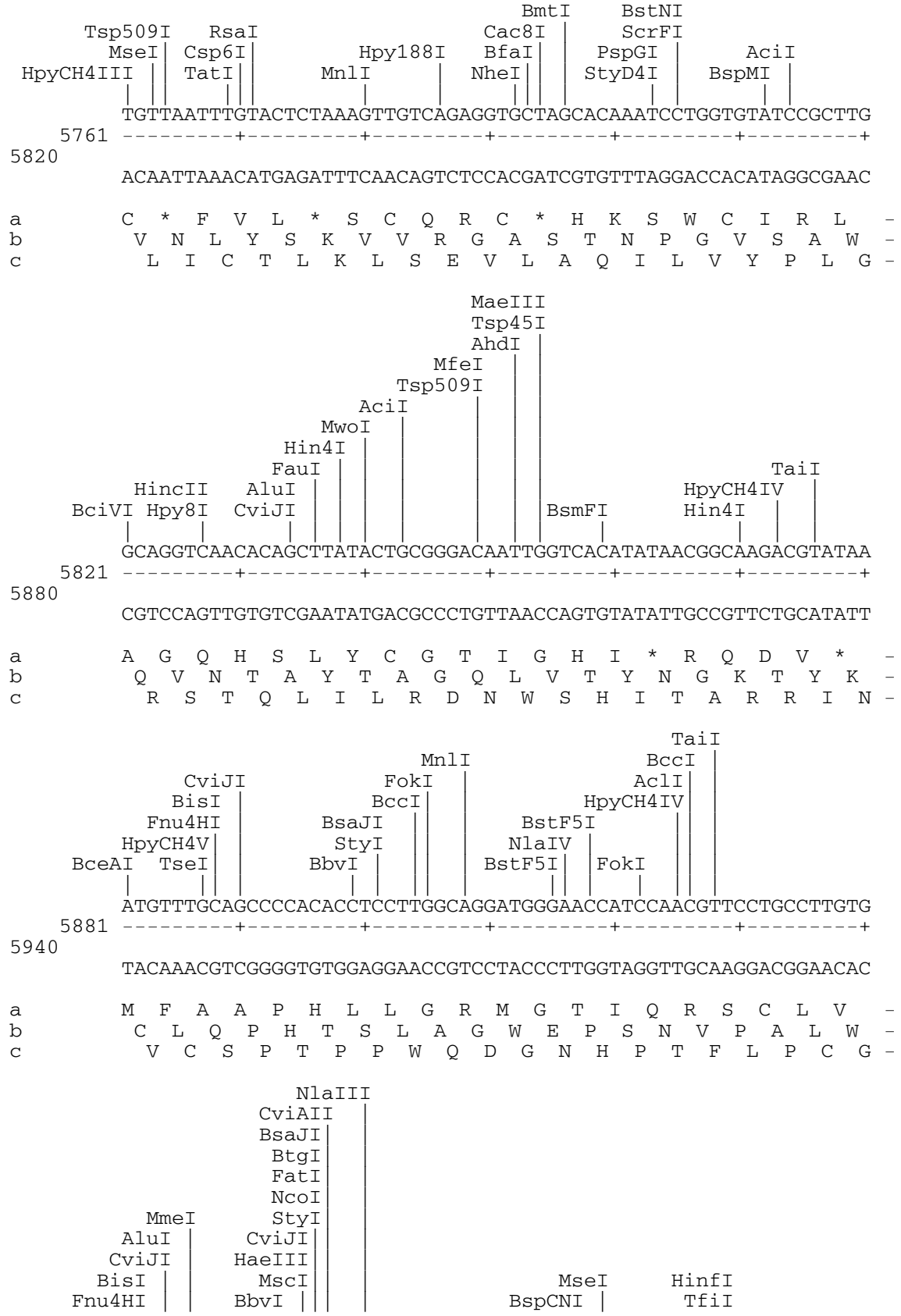
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MwoI
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TaiI
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BciVI Hpy8I CviJI BsmFI Hin4I
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TaiI
MnlI BccI
CviJI FokI AclI
BisI BccI HpyCH4IV
Fnu4HI BsaJI BstF5I
HpyCH4V StyI NlaIV
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CviAII
BsaJI
BtgI
FatI
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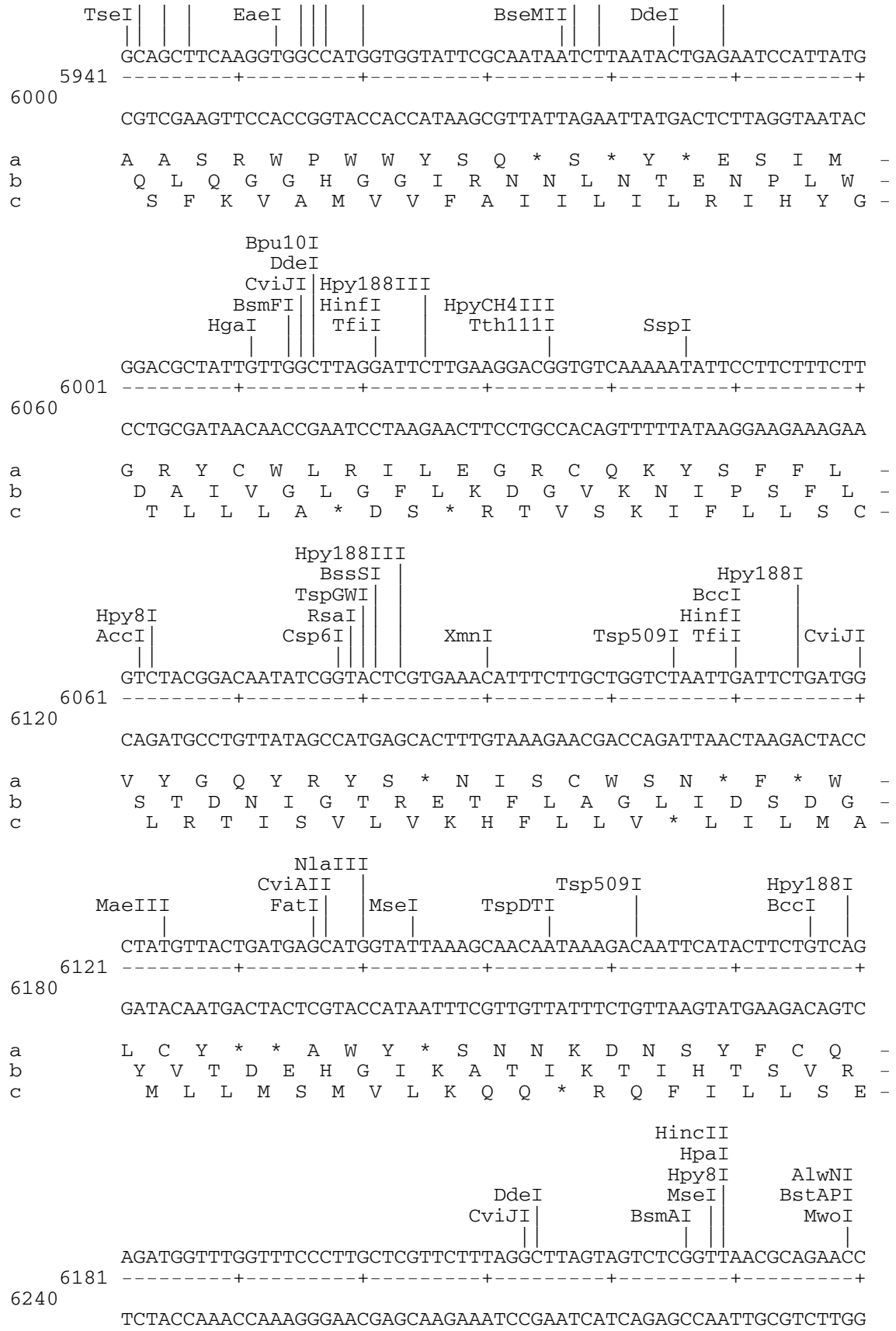
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c T L L L A * D S * R T V S K I F L L S C -
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TspGWI
RsaI
Csp6I
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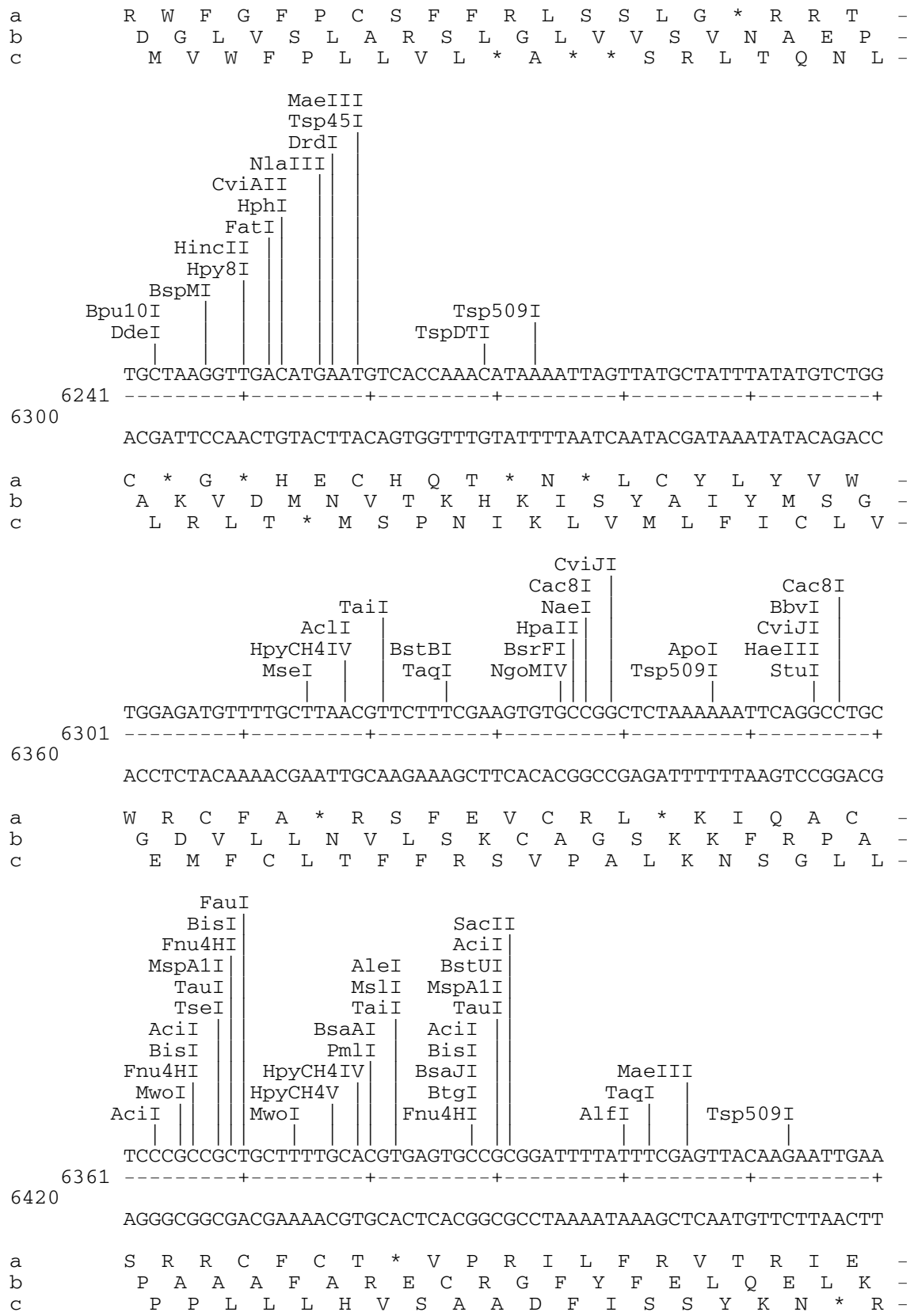
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DrdI
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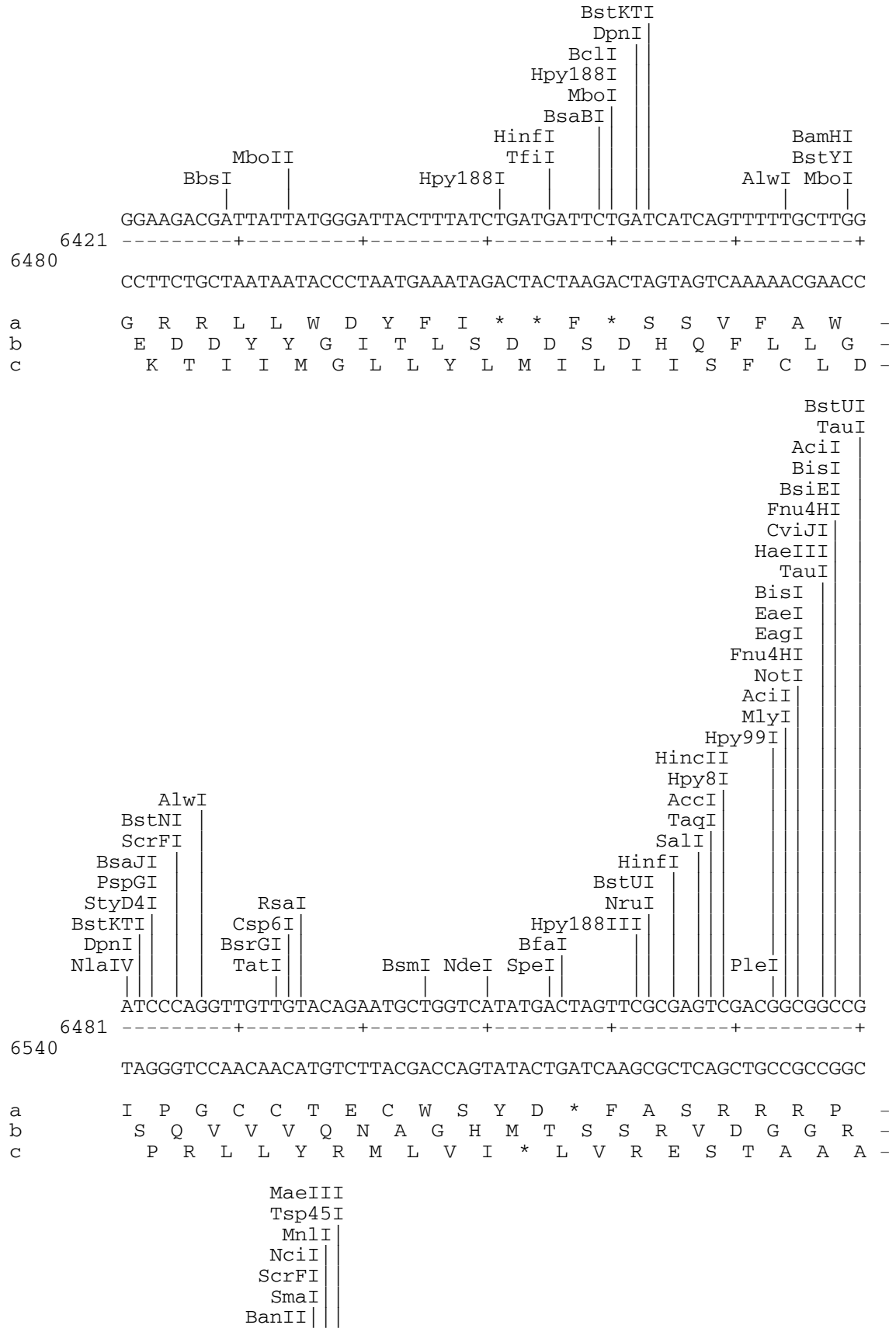
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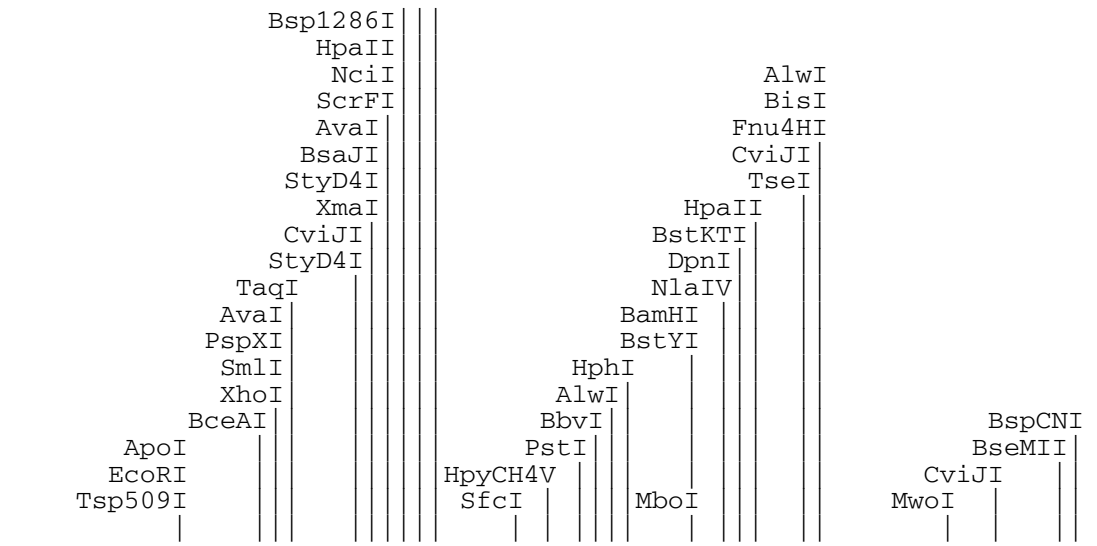
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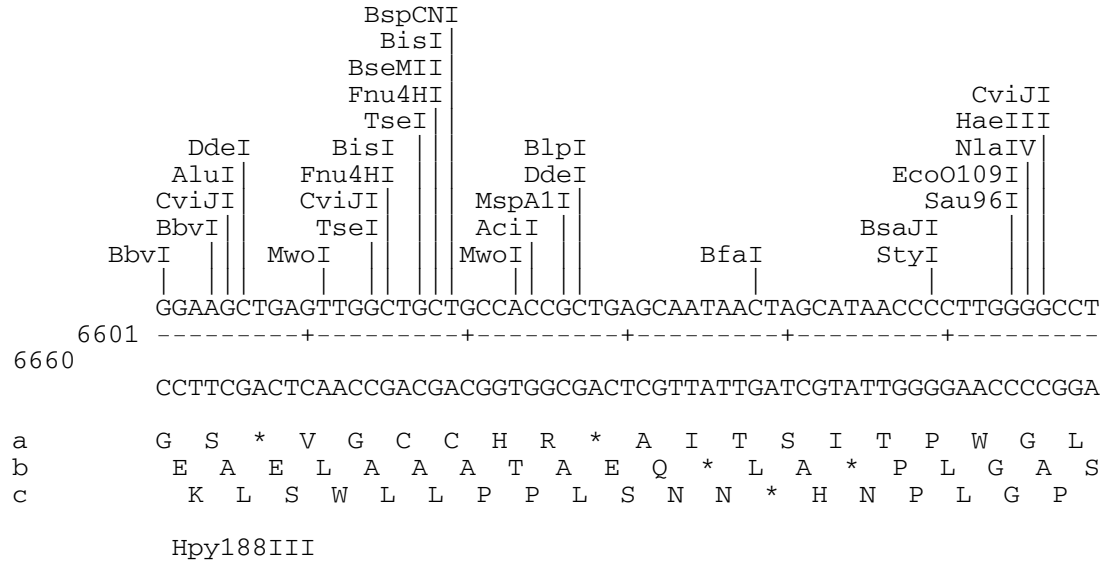
c N S S S P G D C R K G I R L L T K P E R -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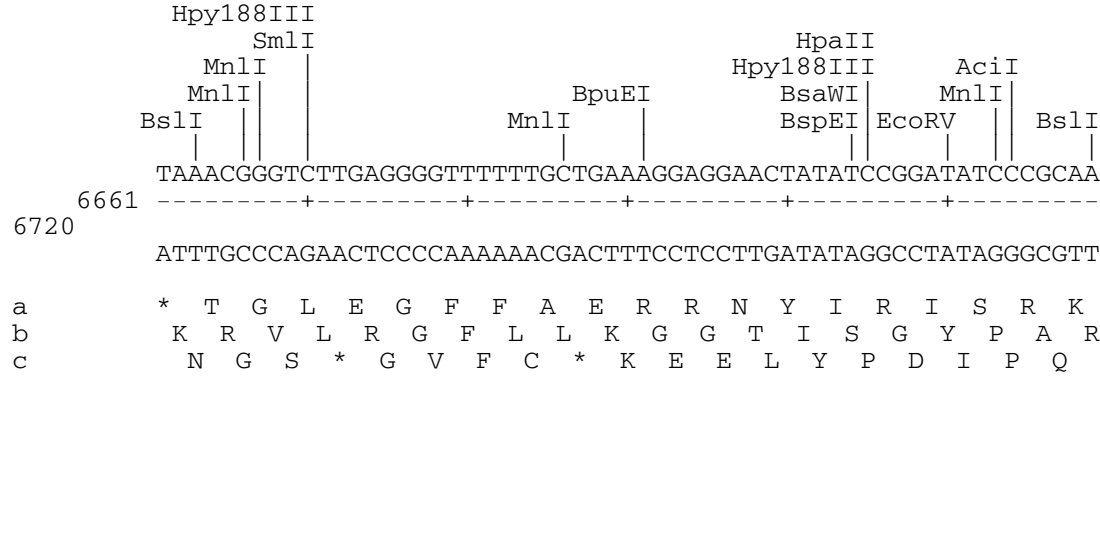
BisI
BseMII
Fnu4HI
TseI
CviJI
HaeIII
NlaIV
EcoO109I
Sau96I
BsaJI
StyI
DdeI
AluI
CviJI
BbvI
BbvI
BisI
Fnu4HI
CviJI
TseI
MwoI
BlpI
DdeI
MspA1I
AciI
MwoI
BfaI
GGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTC
6601
6660
CCTTCGACTCAACCGACGACGGTGGCGACTCGTTATTGATCGTATTGGGGAACCCCGGAG
a G S * V G C C H R * A I T S I T P W G L -
b E A E L A A A T A E Q * L A * P L G A S -
c K L S W L L P P L S N N * H N P L G P L -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MnlI
MnlI
BslI
BpuEI
MnlI
HpaII
Hpy188III
BsaWI
BspEI
AciI
MnlI
EcoRV
BslI
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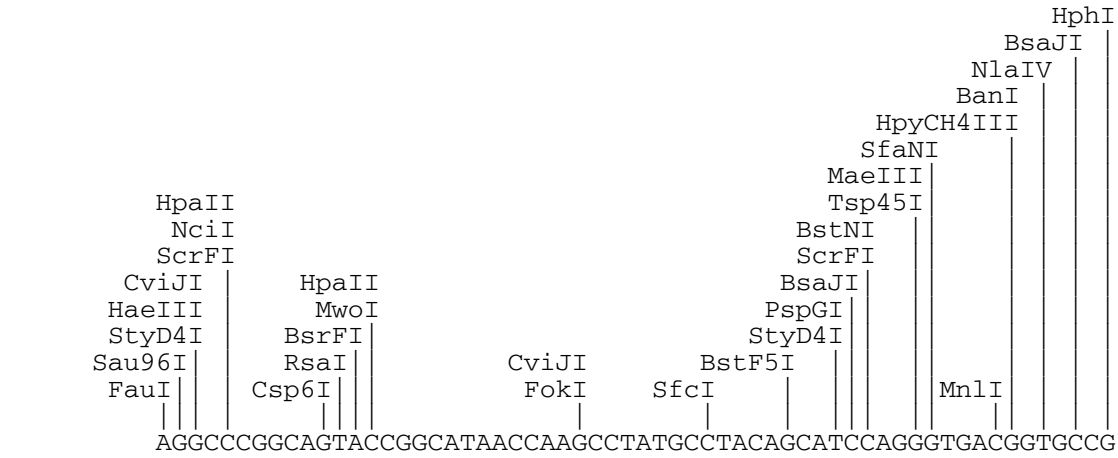
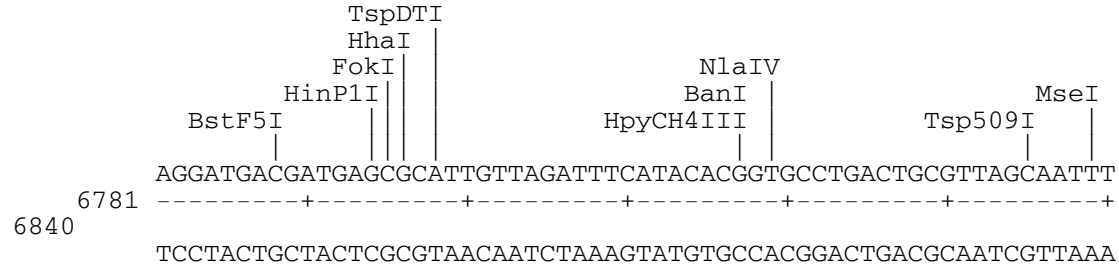
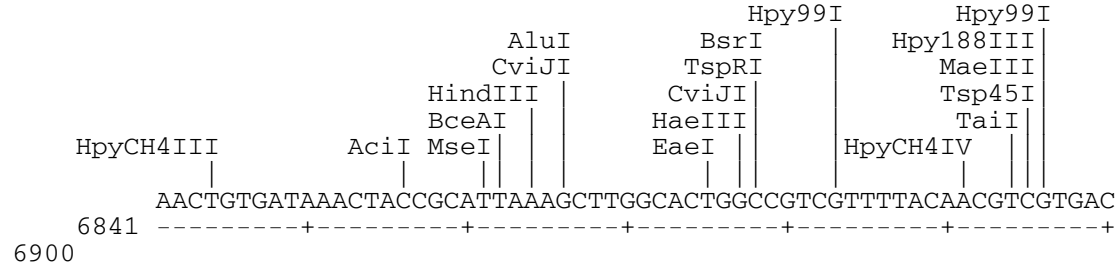
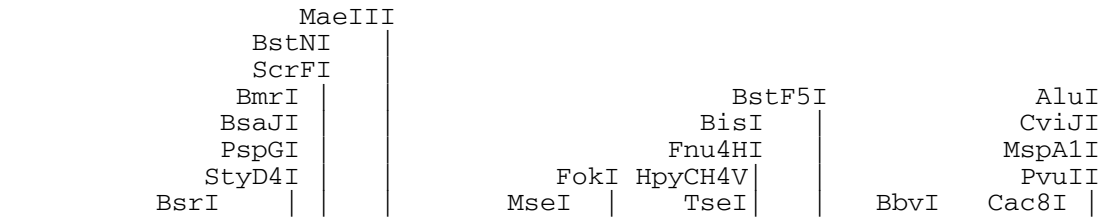
6661
6720
ATTTGCCCAGAACTCCCCAAAAAACGACTTTCCTCCTTGATATAGGCCTATAGGGCGTTC
a * T G L E G F F A E R R N Y I R I S R K -
b K R V L R G F L L K G G T I S G Y P A R -
c N G S * G V F C * K E E L Y P D I P Q E -









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                                                                                HphI
                                                                             BsaJI |
                                                                          NlaIV | |
                                                                         BanI | | |
                                                                   HpyCH4III | | |
                                                                  SfaNI    | | | |
                                                               MaeIII|    | | | |
        HpaII                                                  Tsp45I|    | | | |
         NciI                                                BstNI  ||    | | | |
        ScrFI                                                ScrFI  ||    | | | |
      CviJI |    HpaII                                     BsaJI|  ||    | | | |
     HaeIII |     MwoI                                   PspGI||  ||    | | | |
     StyD4I |   BsrFI|                                  StyD4I||  ||    | | | |
    Sau96I| |   RsaI||        CviJI       BstF5I  |||  ||    | | | |
     FauI|| | Csp6I|||         FokI    SfcI    |  |||  ||MnlI| | | |
        ||| |     ||||            |       |    |  |||  ||   || | | |
        AGGCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGACGGTGCCG
    6721 ---------+---------+---------+---------+---------+---------+
6780
        TCCGGGCCGTCATGGCCGTATTGGTTCGGATACGGATGTCGTAGGTCCCACTGCCACGGC

a       R  P  G  S  T  G  I  T  K  P  M  P  T  A  S  R  V  T  V  P   -
b        G  P  A  V  P  A  *  P  S  L  C  L  Q  H  P  G  *  R  C  R  -
c         A  R  Q  Y  R  H  N  Q  A  Y  A  Y  S  I  Q  G  D  G  A  E -

                    TspDTI
                    HhaI |
                   FokI| |                NlaIV
                HinP1I|| |               BanI |                MseI
          BstF5I     ||| |          HpyCH4III |         Tsp509I   |
               |     ||| |                  | |                |   |
        AGGATGACGATGAGCGCATTGTTAGATTTCATACACGGTGCCTGACTGCGTTAGCAATTT
    6781 ---------+---------+---------+---------+---------+---------+
6840
        TCCTACTGCTACTCGCGTAACAATCTAAAGTATGTGCCACGGACTGACGCAATCGTTAAA

a       R  M  T  M  S  A  L  L  D  F  I  H  G  A  *  L  R  *  Q  F   -
b        G  *  R  *  A  H  C  *  I  S  Y  T  V  P  D  C  V  S  N  L  -
c         D  D  D  E  R  I  V  R  F  H  T  R  C  L  T  A  L  A  I  * -

                                                  Hpy99I       Hpy99I
                                AluI        BsrI    |   Hpy188III|
                               CviJI       TspRI    |      MaeIII|
                          HindIII |      CviJI|    |      Tsp45I|
                          BceAI | |     HaeIII|    |       TaiI||
    HpyCH4III        AciI MseI| | |     EaeI ||    |HpyCH4IV  |||
            |            |    || | |        | ||    |       |  |||
        AACTGTGATAAACTACCGCATTAAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGAC
    6841 ---------+---------+---------+---------+---------+---------+
6900
        TTGACACTATTTGATGGCGTAATTTCGAACCGTGACCGGCAGCAAAATGTTGCAGCACTG

a       N  C  D  K  L  P  H  *  S  L  A  L  A  V  V  L  Q  R  R  D   -
b        T  V  I  N  Y  R  I  K  A  W  H  W  P  S  F  Y  N  V  V  T  -
c         L  *  *  T  T  A  L  K  L  G  T  G  R  R  F  T  T  S  *  L -

                 MaeIII
              BstNI   |
              ScrFI   |
             BmrI |   |                     BstF5I             AluI
            BsaJI |   |                   BisI   |            CviJI
            PspGI |   |                 Fnu4HI   |           MspA1I
           StyD4I |   |           FokI HpyCH4V|   |            PvuII
        BsrI    | |   |        MseI  |    TseI|   |   BbvI   Cac8I |
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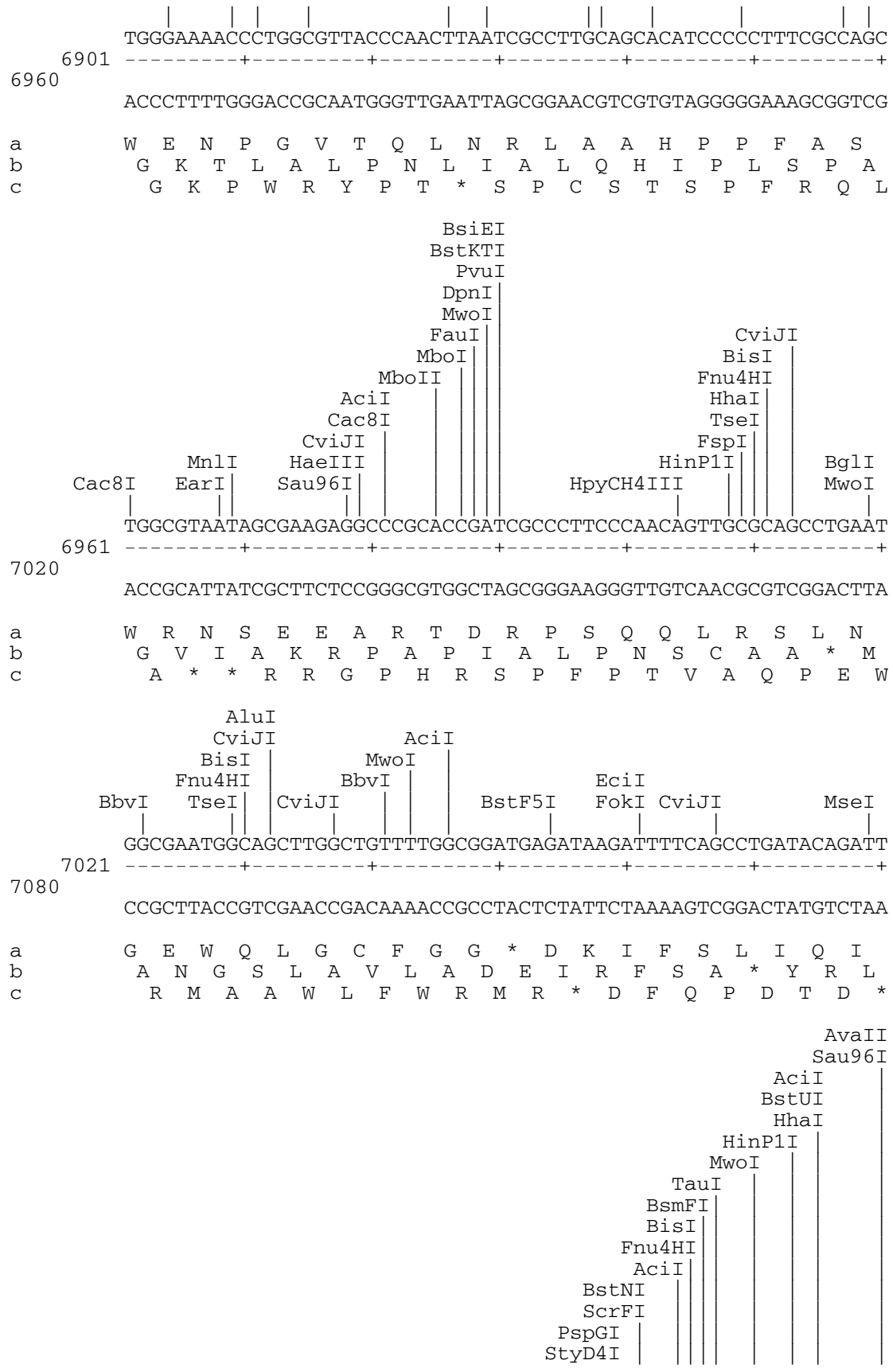
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6901 ---------+---------+---------+---------+---------+---------+
6960
ACCCTTTTGGGACCGCAATGGGTTGAATTAGCGGAACGTCGTGTAGGGGGAAAGCGGTCG
a W E N P G V T Q L N R L A A H P P F A S -
b G K T L A L P N L I A L Q H I P L S P A -
c G K P W R Y P T * S P C S T S P F R Q L -
BsiEI
BstKTI
PvuI
DpnI
MwoI
FauI
MboI
MboII
AciI
Cac8I
CviJI
MnlI
HaeIII
Cac8I
EarI
Sau96I
HpyCH4III
CviJI
BisI
Fnu4HI
HhaI
TseI
FspI
HinP1I
BglI
MwoI
TGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAAT
6961 ---------+---------+---------+---------+---------+---------+
7020
ACCGCATTATCGCTTCTCCGGGCGTGGCTAGCGGGAAGGGTTGTCAACGCGTCGGACTTA
a W R N S E E A R T D R P S Q Q L R S L N -
b G V I A K R P A P I A L P N S C A A * M -
c A * * R R G P H R S P F P T V A Q P E W -
AluI
CviJI
BisI
Fnu4HI
AciI
MwoI
BbvI
BbvI
TseI
CviJI
EciI
BstF5I
FokI
CviJI
MseI
GGCGAATGGCAGCTTGGCTGTTTTGGCGGATGAGATAAGATTTTCAGCCTGATACAGATT
7021 ---------+---------+---------+---------+---------+---------+
7080
CCGCTTACCGTCGAACCGACAAAACCGCCTACTCTATTCTAAAAGTCGGACTATGTCTAA
a G E W Q L G C F G G * D K I F S L I Q I -
b A N G S L A V L A D E I R F S A * Y R L -
c R M A A W L F W R M R * D F Q P D T D * -
AvaII
Sau96I
AciI
BstUI
HhaI
HinP1I
MwoI
TauI
BsmFI
BisI
Fnu4HI
AciI
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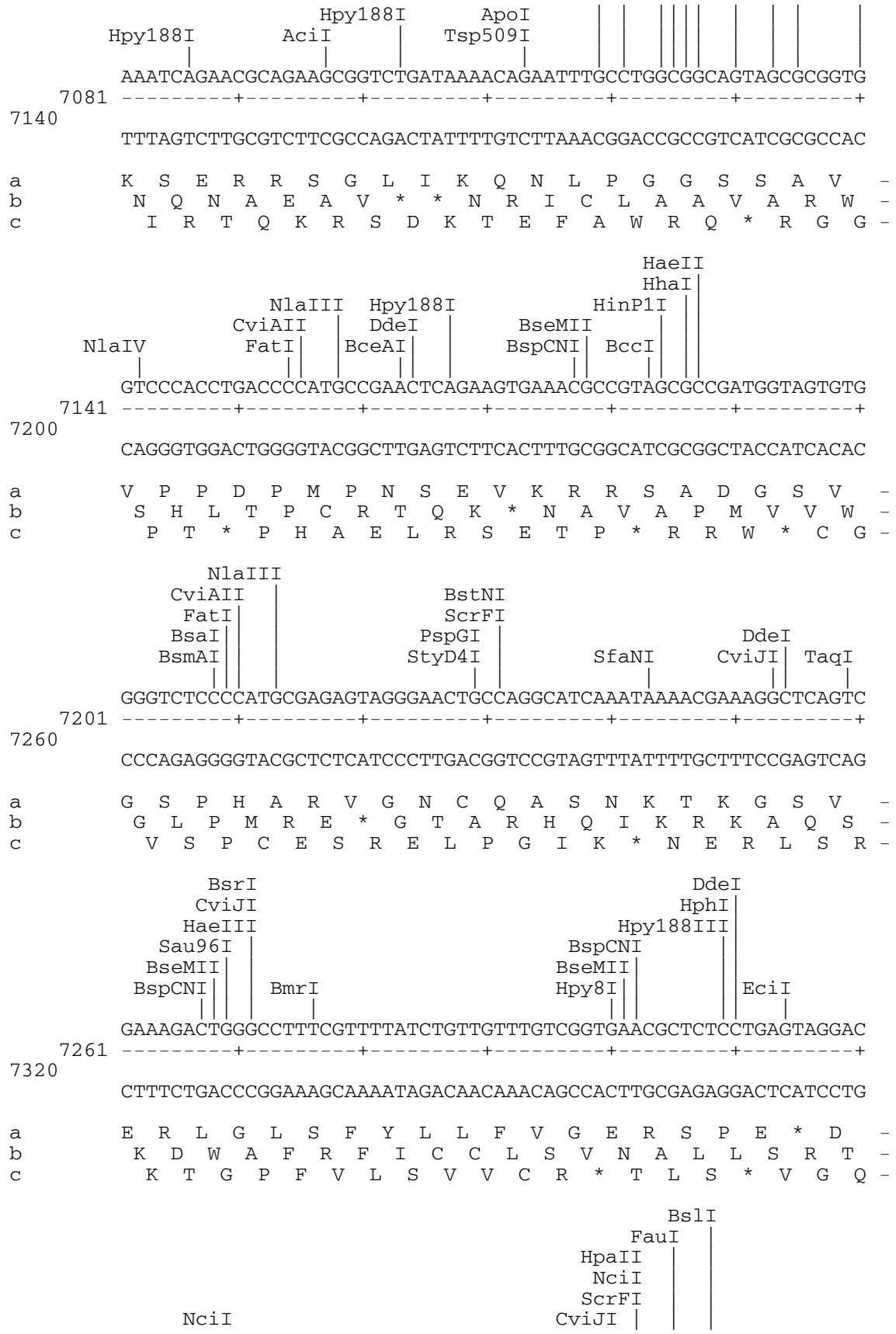
PspGI
StyD4I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ApoI
Hpy188I
AciI
Tsp509I
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7081
7140
TTTAGTCTTGCGTCTTCGCCAGACTATTTTGTCTTAAACGGACCGCCGTCATCGCGCCAC
a K S E R R S G L I K Q N L P G G S S A V -
b N Q N A E A V * * N R I C L A A V A R W -
c I R T Q K R S D K T E F A W R Q * R G G -
HaeII
HhaI
NlaIII
Hpy188I
HinP1I
CviAII
DdeI
BseMII
NlaIV
FatI
BceAI
BspCNI
BccI
GTCCCACCTGACCCCATGCCGAACTCAGAAGTGAAACGCCGTAGCGCCGATGGTAGTGTG
7141
7200
CAGGGTGGACTGGGGTACGGCTTGAGTCTTCACTTTGCGGCATCGCGGCTACCATCACAC
a V P P D P M P N S E V K R R S A D G S V -
b S H L T P C R T Q K * N A V A P M V V W -
c P T * P H A E L R S E T P * R R W * C G -
NlaIII
CviAII
BstNI
FatI
ScrFI
BsaI
PspGI
DdeI
BsmAI
StyD4I
SfaNI
CviJI
TaqI
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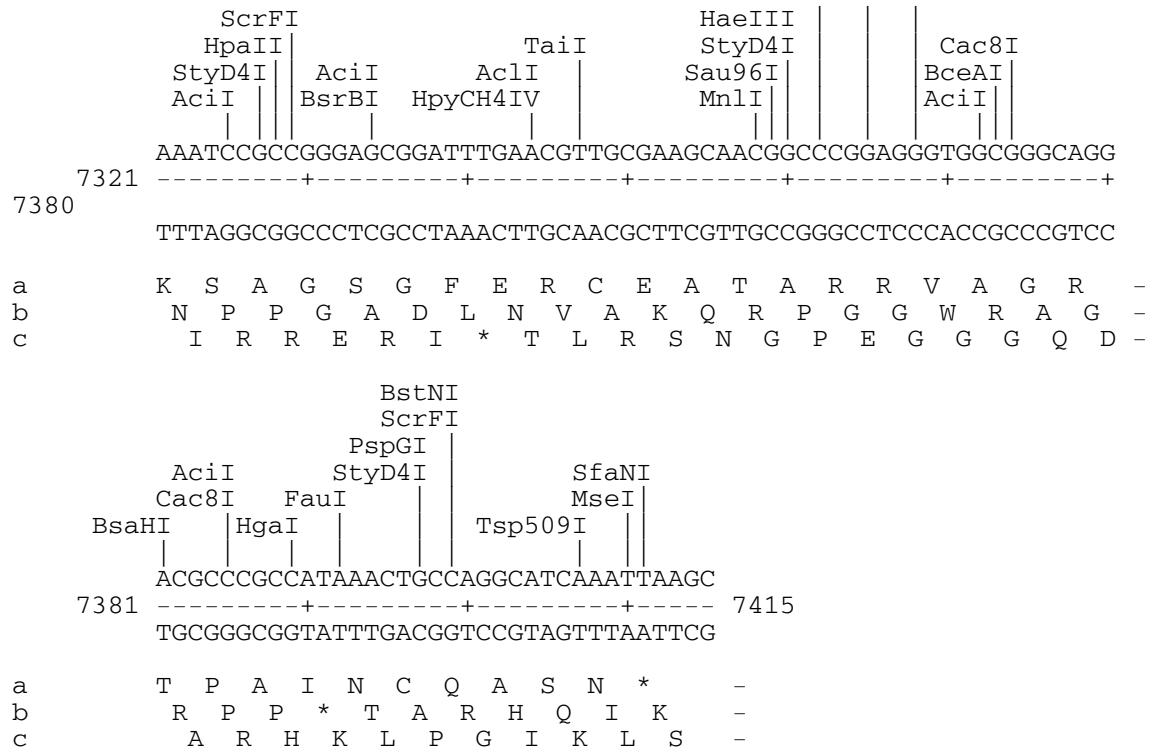
7201
7260
CCCAGAGGGGTACGCTCTCATCCCTTGACGGTCCGTAGTTTATTTTGCTTTCCGAGTCAG
a G S P H A R V G N C Q A S N K T K G S V -
b G L P M R E * G T A R H Q I K R K A Q S -
c V S P C E S R E L P G I K * N E R L S R -
BsrI
DdeI
CviJI
HphI
HaeIII
Hpy188III
Sau96I
BspCNI
BseMII
BseMII
BspCNI
Hpy8I
BmrI
EciI
GAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGAC
7261
7320
CTTTCTGACCCGGAAAGCAAAATAGACAACAAACAGCCACTTGCGAGAGGACTCATCCTG
a E R L G L S F Y L L F V G E R S P E * D -
b K D W A F R F I C C L S V N A L L S R T -
c K T G P F V L S V V C R * T L S * V G Q -
BslI
FauI
HpaII
NciI
ScrFI
NciI
CviJI



Enzymes that do cut:

AccI	Acc65I	AciI	AclI	AcuI	AfeI	AflIII	AhdI
AleI	AlfI	AloI	AluI	AlwI	AlwNI	ApaI	ApaLI
ApoI	AseI	AvaI	AvaII	BaeI	BamHI	BanI	BanII
BbeI	BbsI	BbvI	BccI	BceAI	BcgI	BciVI	BclI
BfaI	BglI	BglII	BisI	BlpI	Bme1580I	BmrI	BmtI
BpmI	Bpu10I	BpuEI	BsaI	BsaAI	BsaBI	BsaHI	BsaJI
BsaWI	BsaXI	BseMII	BseYI	BsgI	BsiEI	BsiHKAI	BslI
BsmI	BsmAI	BsmBI	BsmFI	Bsp1286I	BspCNI	BspEI	BspHI
BspMI	BsrI	BsrBI	BsrDI	BsrFI	BsrGI	BssHII	BssSI
BstAPI	BstBI	BstEII	BstF5I	BstKTI	BstNI	BstUI	BstXI
BstYI	BstZ17I	BtgI	BtgZI	BtsI	Cac8I	Csp6I	CviAII
CviJI	DdeI	DpnI	DraI	DraIII	DrdI	EaeI	EagI
EarI	EciI	Eco57MI	EcoNI	EcoO109I	EcoRI	EcoRV	FatI
FauI	Fnu4HI	FokI	FspI	HaeII	HaeIII	HgaI	HhaI
Hin4I	HinP1I	HincII	HindIII	HinfI	HpaI	HpaII	HphI
Hpy8I	Hpy99I	Hpy188I	Hpy188III	HpyCH4III	HpyCH4IV	HpyCH4V	KasI
KpnI	MaeIII	MboI	MboII	MfeI	MluI	MlyI	MmeI
MnlI	MscI	MseI	MslI	MspA1I	MwoI	NaeI	NarI
NciI	NcoI	NdeI	NgoMIV	NheI	NlaIII	NlaIV	NotI
NruI	NspI	PciI	PflMI	PfoI	PleI	PmlI	PpiI
PshAI	PsiI	PspGI	PspOMI	PspXI	PstI	PvuI	PvuII
RsaI	SacII	SalI	Sau96I	ScaI	ScrFI	SfaNI	SfcI
SfoI	SgrAI	SmaI	SmlI	SpeI	SphI	SspI	StuI
StyI	StyD4I	SwaI	TaiI	TaqI	TaqII	TatI	TauI
TfiI	TseI	Tsp45I	Tsp509I	TspDTI	TspGWI	TspRI	TstI
Tth111I	XbaI	XcmI	XhoI	XmaI	XmnI		

Enzymes that do not cut:

AarI	AatII	AflII	AgeI	AscI	AsiSI	AvrII	BbvCI
BfrBI	BmgBI	BplI	BseRI	BsiWI	Bsu36I	ClaI	CspCI
EcoICRI	FalI	FseI	FspAI	NsiI	PacI	PasI	PmeI

PpuMI PsrI RsrII SacI SanDI SapI SbfI SexAI
SfiI SnaBI SrfI ZraI