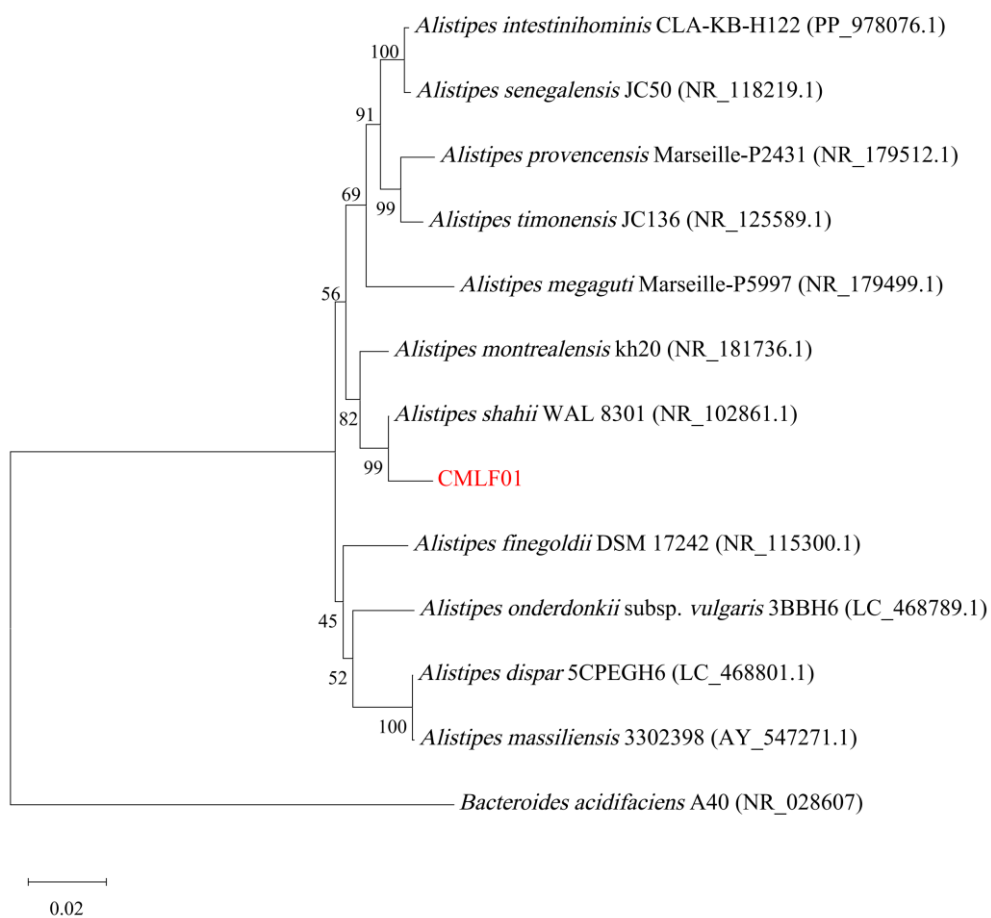
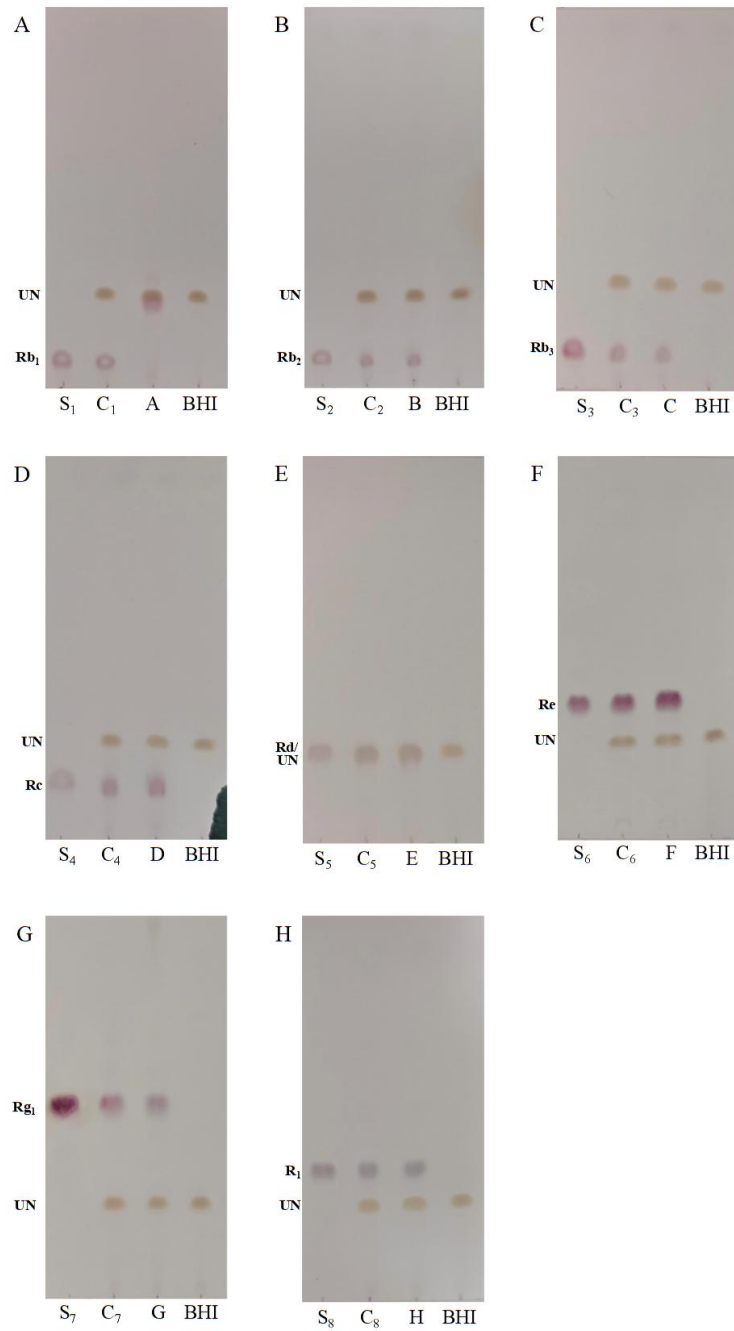




附图 1 CMLF01 与另枝菌属菌株基于 16S rDNA 序列的系统发育树

Supplementary Fig. 1 Phylogenetic tree of 16S rDNA sequences from CMLF01 and *Alistipes* strains.

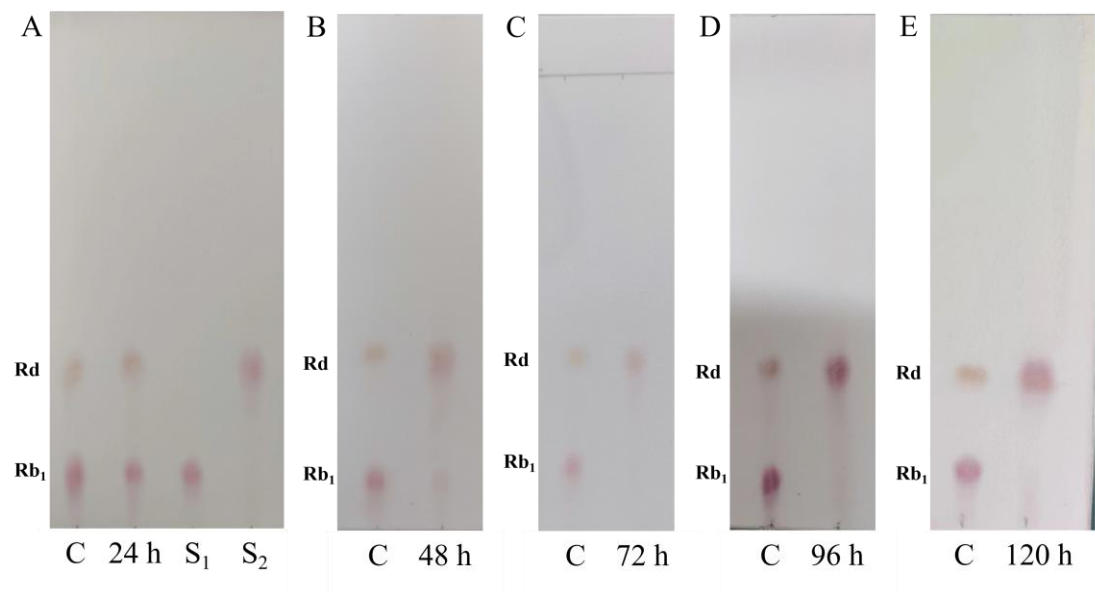


A–H: 分别为 *Alistipes shahii* CMLF01 培养 48 h 后人参皂苷 Rb₁、Rb₂、Rb₃、Rc、Rd、Re、Rg₁ 及三七皂苷 R₁ 的转化结果。S₁–S₈: 各种皂苷标准品; C₁–C₈: 添加相应皂苷的 BHI 培养基 (不含 CMLF01); BHI: 接种 CMLF01 的 BHI 培养基 (图 1 中的对照); UN: BHI 培养基中的未知物质。

A-H: Transformation of ginsenosides Rb₁, Rb₂, Rb₃, Rc, Rd, Re, Rg₁, and notoginsenoside R₁ by *Alistipes shahii* CMLF01 over a 48 h incubation, respectively. S₁–S₈: various saponin standards; C₁–C₈: BHI medium supplemented with various saponins (without CMLF01); BHI: BHI medium inoculated with CMLF01 (the control in Fig. 1); UN: unknown substance in BHI medium.

附图 2 CMLF01 转化皂苷的薄层色谱 (TLC) 分析

Supplementary Fig. 2 TLC analysis of saponins transformed by CMLF01.

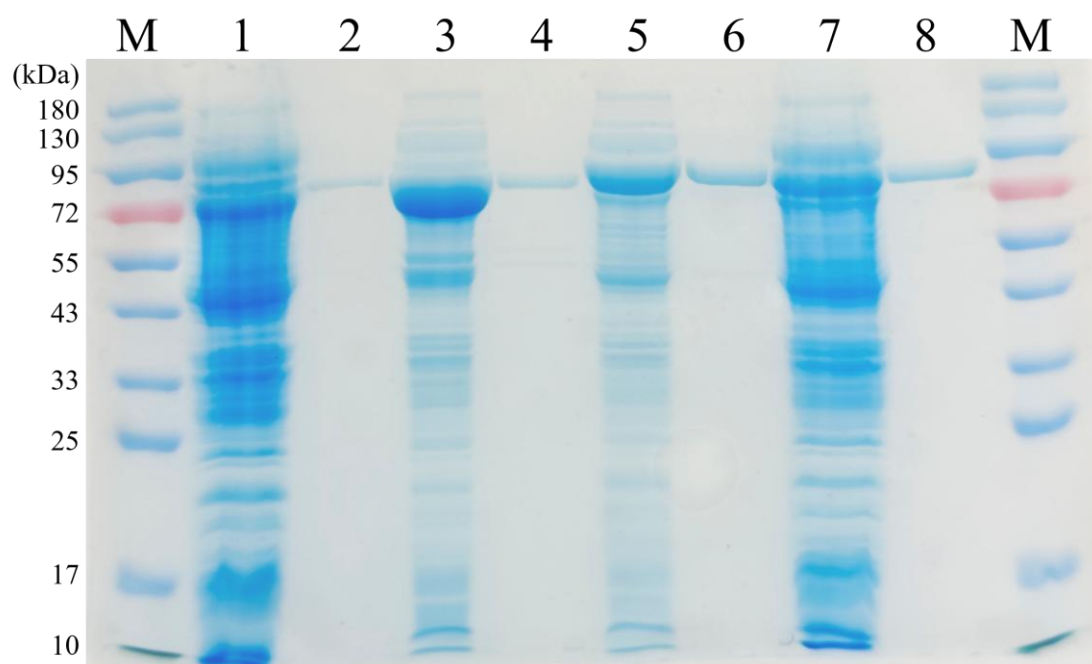


A-E: 分别为 *A. shahii* CMLF01 培养 24、48、72、96、120 h 后人参皂苷 Rb₁ 的转化结果。S₁-S₂: 人参皂苷 Rb₁ 和 Rd 标准品; C: 添加相应皂苷的 BHI 培养基 (不含 CMLF01)。结果显示 Rb₁ 在 48 h 内完全转化为 Rd, 且 120 h 内 Rd 条带无明显变化, 表明 Rd 未被进一步代谢。

A-H: Ginsenoside Rb₁ transformation by *A. shahii* CMLF01 at 24, 48, 72, 96, and 120 h. S₁-S₂: Ginsenoside Rb₁ and Rd standards; C: BHI medium supplemented with various saponins (without CMLF01).

附图 3 菌株 CMLF01 转化人参皂苷 Rb₁ 的 TLC 分析 (0-120 h)

Supplementary Fig. 3 TLC analysis of ginsenosides Rb₁ transformed by CMLF01 (0-120 h)

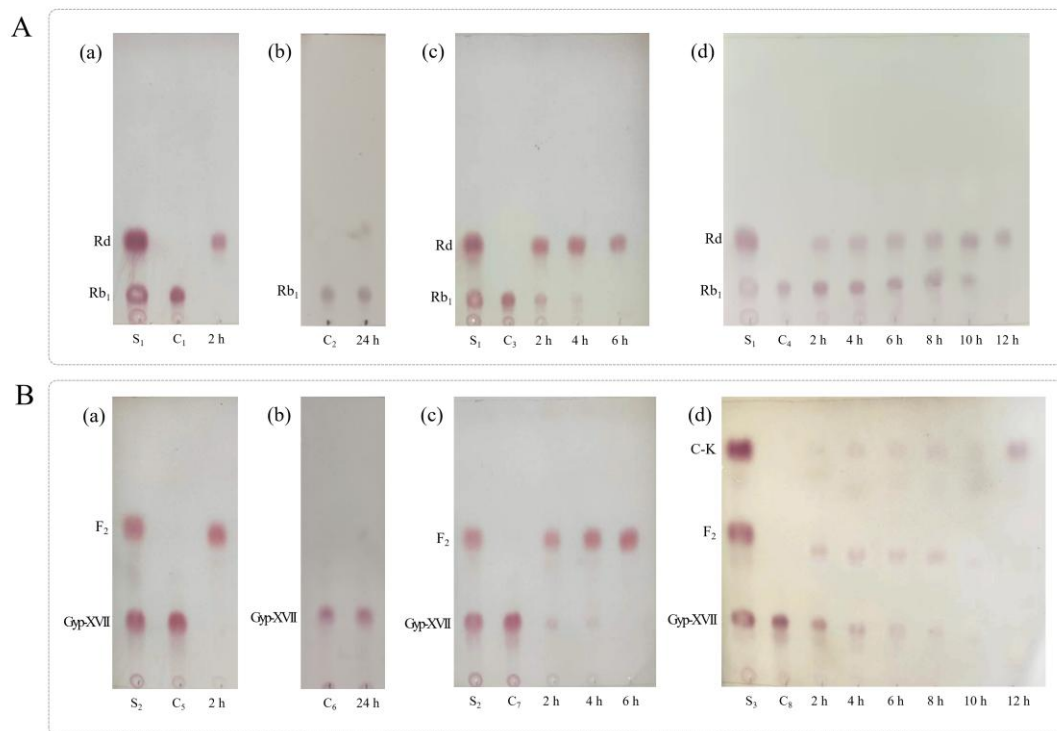


泳道 M: 蛋白质分子量标记; 泳道 1、3、5、7 分别为 AsBgl767、AsBgl1806、AsBgl2409 和 AsBgl2410 细胞超声破碎后的粗提液; 泳道 2、4、6、8 分别为对应上述蛋白的纯化产物。

Lane M: protein molecular mass marker; lanes 1, 3, 5, and 7 are crude extracts of AsBgl767, AsBgl1806, AsBgl2409, and AsBgl2410 after ultrasonic cell disruption, respectively; lanes 2, 4, 6, and 8 are the purified products of the corresponding proteins, respectively.

附图 4 重组酶的 SDS-PAGE 分析

Supplementary Fig. 4 SDS-PAGE analysis of recombinant enzymes

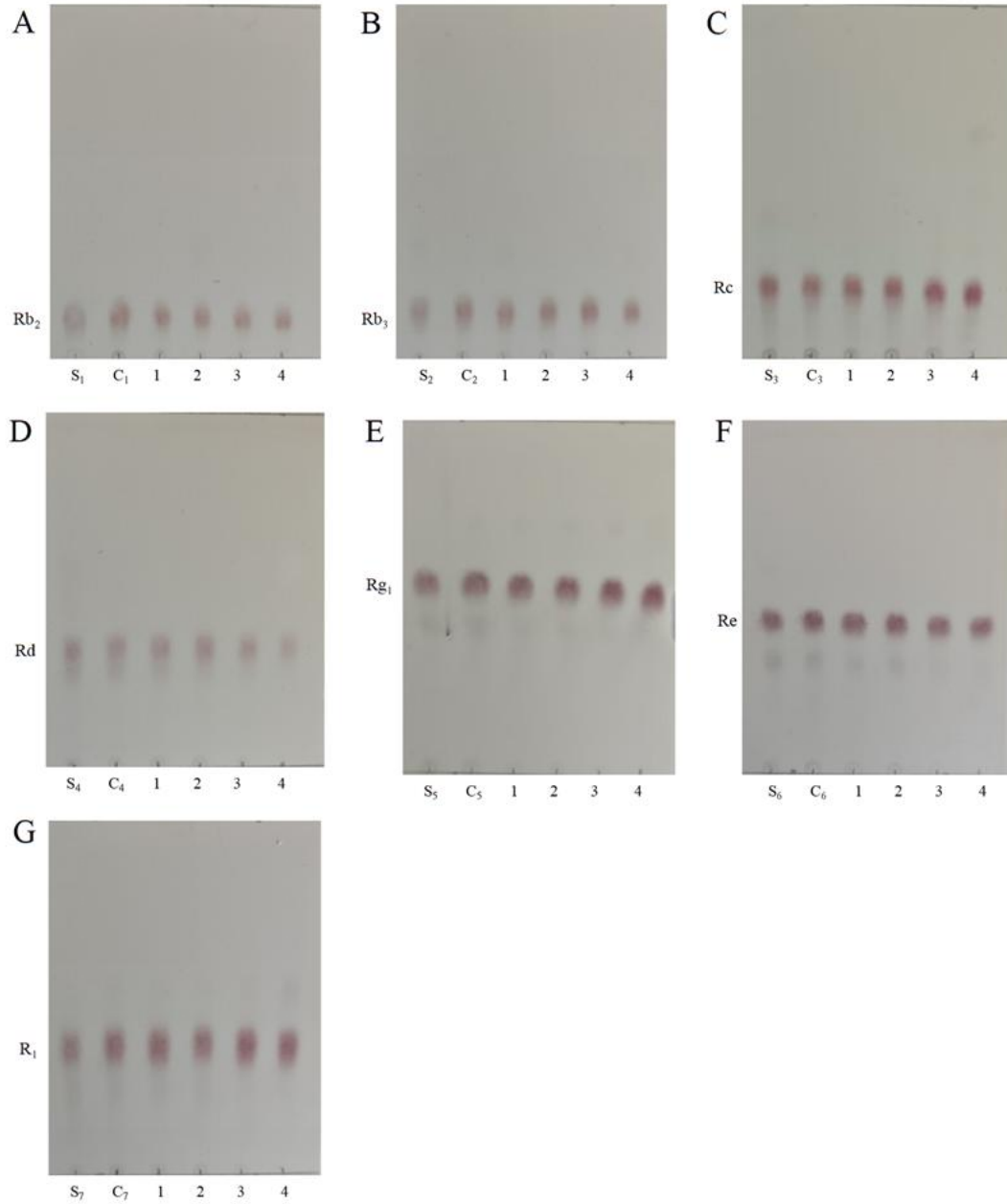


A: 重组酶转化人参皂苷 Rb₁; B: 重组酶转化 Gyp-XVII。AsBgl767 (a)、AsBgl2409 (c) 和 AsBgl2410 (d) 能分别将 Rb₁ 和 Gyp-XVII 完全转化为 Rd 和 F₂/C-K, 而 AsBgl1806 (b) 即使在孵育 24 h 后仍表现出极弱的转化活性。S₁-S₃: 皂苷标准品; C₁-C₈: 对照。

A: Transformation of ginsenoside Rb₁ by recombinant enzymes; B: transformation of Gyp-XVII by recombinant enzymes. AsBgl767 (a), AsBgl2409 (c), and AsBgl2410 (d) were able to completely convert Rb₁ and Gyp-XVII into Rd and F₂/C-K, respectively, whereas AsBgl1806 (b) exhibited very weak conversion activity even after 24 hours of incubation. S₁-S₃: saponin standards; C₁-C₈: controls.

附图 5 重组酶转化皂苷的 TLC 分析

Supplementary Fig. 5 TLC analysis of saponins converted by recombinant enzymes.



A–G: 分别为 AsBgl767 (1)、AsBgl1806 (2)、AsBgl2409 (3) 和 AsBgl2410 (4) 孵育 24 h 后人参皂苷 Rb₂、Rb₃、Rc、Rd、Rg₁、Re 及三七皂苷 R₁ 的转化结果。四种重组酶对上述皂苷均未检测到水解活性。S₁–S₇: 皂苷标准品; C₁–C₇: 对照。

A-G: Transformation of ginsenosides Rb₂, Rb₃, Rc, Rd, Rg₁, Re, or notoginsenoside R₁ by AsBgl767 (1), AsBgl1806 (2), AsBgl2409 (3), and AsBgl2410 (4) over a 24 h incubation, respectively. The four recombinant enzymes exhibited no detectable hydrolytic activity toward the aforementioned saponins. S₁–S₇: saponin standards; C₁–C₇: controls.

附图 6 重组酶未转化皂苷的 TLC 分析

Supplementary Fig. 6 TLC analysis of saponins not converted by recombinant enzymes.

附录：4 个酶的基因序列与氨基酸序列

> AsBgl767 野生型基因序列

ATGAAAAATAAAATTCTGTTGAAGAGTCTCGCCGGGCTGTGCGCCCTGGCGGGCGGTGCGCTGCGGAG
GCGGGCCGGGGCCGCAGGGCAGCGTCGCCGTCTACCTCGACGAGTCGCAGCCGATCGAAAAGCGGG
TCGAAGACGCCCTTTCGCGCATGACCCCTGAAGAGAAGGTCGCCATCCTGCATGCGCAGTCGAAATTC
TCGTGCGGCGGGCGTTCCGCGCCTGGGCATTCCCGAGGTGTGGTGCACCGACGGCCCTCACGGCATCC
GCCCCGAGGTCTGTGGGACGAATGGGACCAGGCCGGCTGGACGAACGACTCCTGCACGGCTTTCCC
GGCGCTGACGTGCCTTGCCGCGACGTGGAATCCCGAAATGTCGGCGCTGTATGGCAAATCCATCGGCG
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CAACGGCCGCAATTTGAGTACATGGGCGAGGACCCCTACCTCTCTTCGCGGATGGTCGTTCCCTACAT
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CACCGTCACGGCATCGACGTCGAGGTGACGACCGTGCCTTGAACGAGATCTACCTCCCGGCGTTTAA
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CTCCGACTGGGGCGGCACGCACGACACGAAGCAGGCCGCCGAGAACGGTCTCGACATGGAGTTGCG
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ACCTCGATATGCTCCGAAGGGCGAGGCTTCGACGGCGACGCTCGACGACAAGGCGCGCCGGGTGCT
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GCATGCGCTGGGCGAATACCCGGGCGCGGACAAGGTGAAGTATAACGAGAGCATCTTCGTGCGTTACC
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CGCCGTCGGCAACGTGAAGGCCGACCGTACGACGCTCGCCCCGAACGGCAGCATCCGTATTTGCGCC
GACGTGACCAACACGGGCGACCGTGCGGGCGCCGAGGTGCTGCAGCTTTACATCGGCGACGAGCAGT
CGTCGCTGCCGCGTCCCGTGAAGGAGCTGAAAGGTTTTCAGAAAGTTTCGCTGAATCCCGGGCAGAC
GCGGACCGTGACTTTCGAGATCACCTCCGGGATGTTGCAGTACTACGACGACGCAAAGGGTGCGTGG
GTGGCCGAGCCGGGCGCGTTACGGCCTATGTGGGCGCCGCGTCGGACGATATTCGCGGCACGGTGG
AATTCGAGTTGAAATGA

> AsBgl767 优化后基因序列

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AAAACGCGTGGAAGATGCGCTGAGCCGCATGACCCTGGAAGAGAAAGTGGCGATTCTGCATGCGCAG
AGCAAATTTAGCAGCGCGGGCGTGCCGCGCTTAGGCATTCCGGAAGTGTGGTGTACTGATGGTCCGCA
TGGCATTGCCCCGGAAGTGCTGTGGGATGAATGGGATCAGGCGGGCTGGACCAACGATAGCTGCACC
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GCGAACACTGCTGCCATAACCGCTATCTGCTGAACGATATTCTGAAACGCGATTGGGCGTTTGATGGCG
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TGTGACCAACACCGGCGATCGCGCAGGTGCAGAAAGTGGTGCAACTGTATATTGGCGATGAACAGAGC
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CGCACCGTGACCTTTGAAATTACCAGCGGCATGCTGCAGTATTATGATGATGCGAAAGGCGCGTGGGT
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> AsBgl767 氨基酸序列

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KDILLGPGVNIYRTPLNGRNFEYMGEDPYLSSRMVVPYIEEVQKNGVAACVKHFALNNQEAHRHGIDVEV
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> AsBgl1806 野生型基因序列

ATGAAAACCTCCGTAAAACTTTTGCTCGCCTTCGCAACGGCAGCCGCGGCGATCGCACCGGCCGCCCG

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CCACGCTGCCCCTCTCGGTGAAATAA

> AsBgl1806 优化后基因序列

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GCGCGCTGCATGAACTGTATCTGCCGCCGTTTAAAGCGGGCATTGATGCGGGCGTGGGCTATGTGATG
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TTCGCACCGCGGTGGAAAGCAACCCGAACACCATTGTGCTGGTGAACAGCGGCAGCGGCATTTCGCAT
GGGCGGCTGGAACGATAAAGCAGCAGCAGTGATTATGGCTGGTATCCGGGCCAGAGCGGCTTTACCG
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GGTGAATTTGAACTGGATCGCCAGGCGTTAGCGTTTGGGATACCCAGAGCCGCGGCTGGAAAGTG
AACCCGGGCGAATACACCCTGAGCTTAGGCACCAGCAGCGCGGATATTGCGGCGACCTTACCGGTGA
GCGTGAAACACCACCACCACCACCTAA

> AsBgl1806 氨基酸序列

MKTSVKLLLLAFATAAAAIAPAAAQQVVAWDEAYRQADARIAQLTLDEKISFMRGYSSFFFYGVPEKGMPY
IYLSDATQGVHMRDNLSDPTMVRQLERSTAFPCISLAATFNPSLAYRYAEAVGEECRAGGIEVLLGPGMNI
YRNSQNGRNF EYMGEDPLLASAMVAEYVKGMQSTGTVACLKHFICNETEFYRRRSNSIVDERALHELYLP
PFKAGIDAGVGYVMTSYNRLNGEWAGQNADLIRRLRGELGFRGCVMSDWSSVNDTEKVVKSGQNVEM
PGRKEFFGEVRALLKEGRITEKDIEKMIRPNIATSIAGFLYDREKYVPALLEKFP AHKQTGYDVAAEGIVLL
RNNGILPLAEGRKILLTGRFTEQIPRTGGSTSSSAEVLGYDNVTLTALREVYGD A VRVVEKPSREELAAAD
VVLLSTGTIDVESFERPFALPKEEEAFIRTAVESNPNTIVLVNSGSGIRMGGWNDKAAAVIYGWYPGQSGFT
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TKGIAPLYPFGFLSYTTFELSKPRAPKAF PADKPLKVS VTLTNTGDREGAEVVQLYVTENAPT VLRPKKE
LKGFRKVT LAPGKS AVVEFELDRQALAFWDTQSRGWK VNPGEYTL SLGTSSADIAATLPVSVK*

> AsBgl2409 野生型基因序列

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TTTGAATAGGACAGCCCCGCGAAGTTTGGCTTCGGGTATCAATAAGATCCAGCAGCGCTCGCTCGA
CGAACTGGGAATCCCCTGTATCTACGGCGTGGATCAGATTACGGTGCCTCTATACCGTCGGTGCAAC
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GATCCTCGCTGGCCTCGGATGTGGGAGAGCTACGGAGAGGATGTCTGCATCAATACGCGTATGGCCGT
AGCCTCAGTATCCGGCATGCAGGGCGACGATCCCAACCACATTGCGCCGAACCGGGTGGCCGCCTGTC

TAAAGCATTTTCATGGCCTATGGCGTACCGGTTTCGGGACAGGACAGAACCCCGTCGTCGGTAACCCGC
AATGCATTGCGCGAGAAATATTTTCATTCCTTTATGGAATCGATTTCGGGAGGGAGCTTTGTCGCTGATG
GTAAATTCGGCAAGCAACGACGGCATGCCGTTTCACGCTAACCACGAACTGCTTACCGGCTGGCTCAA
AGAGGAATTAGACTGGGACGGACTGATCGTAACGGACTGGAACGACATCTATAACCTTTGTGATCGGG
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GTGATCTGGATTCCGGGCGTGGAGTACGGAACCTCCGAAGAGAACTGGCAGACGGAGTATGTCACGG
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TTTGACCGTAACTGGGAAACCCATGATTTTGATTCTGAACGAGGGCCGACCGCGACTCGTCCGCGAAA
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AATTTGCTGGCCGGGAATGCAAACCTTCAGCGGACGGTTACCGTTTACCTATCCCCGCTGGCCCGATGC
CCTGGCTACATACGACTTCAAACCTGTCAACAGATGGGACCCATGGAGGGCGAATACAATTACGACG
CCGTAATGGACGTGCAATGGCCCTTCGGTTATGGTTTGAGCTATACACGATTACCTATTCGAACTTCA
AGACCAAATGCAGCGAATTCGAGACGGAGATTGTCTCACTTTTTTCGGTCGACGTGACCAATACGGGA
AATCGAAGCGGGAAAGAAGTCGTGATGCTGTGGTCGAGTGATTGGTGGCGAGTCTACCCCCGACG
TGATCCGACTGCGCAATTTGAGAAGATCGAACTCGCTCCCGGTGAAACCCGCACCGTGACTCTTCA
ATCCCCGCATCAGATCTCGCTTTTGTGGCTACGACGGTCGTTGGCGGCTTGAAGCAGGTGAATCCG
GATCCGAATGGGGTCGGAAGCGACCGTAATCCGCTGTAGCGAGACTCGAATCTACGAAACTCCTAATC
TTCCCCAAGTTTGA

> AsBgl2409 优化后基因序列

ATGTGCAGCCATCTGAACCCGCAGACCTTTCGCGAGCGATCGCGAAATTGAAGCGCGCATTGAAAAGAT
TCTGGGCGGCCTGACCCTGGAAGAAAAGGTGGGCCAGATGTGCCAGCTGACTGTGGGCATGGTGATT
GATAACAGCGATCCGCAGAACCCGTTTATTAGCGATGCGCTGCTGGACACCGTGATTGGCCATTACAA
AGTGGGCAGCATTCTGAACATTCCGTTTGGCATTGGCCAGCCGCGCGAAGTGTGGCTGCGCGTGATTA
ACAAAATTCAGCAGCGCAGCCTGGATGAACTGGGCATTCCGTGCATTTATGGCGTGGATCAGATTTCATG
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GATCTGATGCGCCGACGACGCGAAATTACCGCGTATGAAACCCGCGCGTGCGGCATTCCGTGGAACCT
TGCGCCGGTTATGGATCTGGGTGCGGATCCGCGTTGGCCACGTATGTGGGAAAGCTATGGCGAAGATG
TGTGCATTAACACCCGCATGGCGGTGGCGAGCGTGAGCGGCATGCAAGGTGATGATCCAAACCATATT
GCGCCGAACCGCGTGGCGGCGTGCCTGAAACATTTTATGGCGTATGGCGTGCCGGTGAGCGGTCAAGA
TCGACCCCCATCTAGCGTTACCGCAACGCGTTACGCGAAAAATATTTTATTCCGTTTATGGAAAGCATT
CGCGAAGGCGCGCTGAGCCTGATGGTGAACAGCGCGAGCAACGATGGCATGCCGTTTCATGCGAACC
ATGAACTGCTGACCGGCTGGCTGAAAGAAGAACTGGATTGGGATGGCCTGATTGTGACCGATTGGAA
CGATATTTATAACCTGTGCGATCGCGATCATTTGCGGCGACCCGCAAAGAAGCGGTGGCGCTGGCAAT
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GCAGGAACGCGTGGTGCCGATGAGCCGCAATTGATGATGCGGTTTCGTGCGGTTCTGCGCCTGAAAATGC
GCCTGGGCTGTTTAAAGAACCCTTTATTAAAGCGGAAGAATTCGATCGCTTTGCGTGCGATGAATTTA

GCGCGGTGGCGCTGCAGGCGGCAGAAGAGAGCATGATTCTGCTGAAAAACGAAAGCGATCTGCTGCC
GCTGAAACGCAGCACCCGCATTCTGCTGACCGGTCCAAACGCACATAGCATGCGCTGCCTGAACGGC
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AACCCGCACCGTGACCTTAAGCATTCCAGCATCTGATCTGGCGTTTGTGGGCTATGATGGCCGCTGGCG
CCTGGAAGCGGGTGAATTTGCGATTGCGATGGGCAGTGAAGCGACCGTGATTCGCTGCAGCGAAACC
CGCATTTATGAAACCCCGAACCTGCCGCGAGGTGCACCACCACCACCACCTAA

> AsBgl2409 氨基酸序列

MKNLFYLLICALGWSSCSHLNPQTFRSDREIEARIEKILGGLTLEEKVGQMCQLTVGMVIDNSDPQNPFI
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GASLNC DLMRRSSEITAYETRACGIPWNFAPVMDLGRDPRWPRMWESYGEDVCINTRMAVASVSGMQGD
DPNHIAPNRVAACLKHFMAYGVPVSGQDRTPSSVTRNALREKYFIPFMESIREGALSLMVNSASNDGMPF
HANHELLTGWLKEELDWDGLIVTDWNDIYNLCDRDHIAATRKEAVALAINAGIDMAMVPSDWKFC DYL
RELVQERVVPMSRIDDAVRRVRLKMR LGLFKEPFKAEEFDRFACDEFSAVALQAAEESMILLKNESDLLP
LKRSTRILLTGPNASHMRCLNGGWSYSWQGDRCDEFAGEYHTIYEALNRKFDHVIWIPGVEYGTSEENW
QTEYVTGIDRAVAAAHA DVIIACVGENSYCETPGNINDLNLSSNQQLVRALTVTGKPMILILNEGRPRL
VREIEPLVQAIVDILLPGNYGGDALANLLAGNANFSGRLPFTYPRWPDALATYDFKPCQQMGPMEGEYNY
DAVMDVQWPFGYGLSYTRFTYSNFKTKCSEFRDGDCLTFSVDVTNTGNRSGKEVVMLWSSDLVASLTPD
VIRLRNFEKIELAPGETRTVTL SIPASDLAFVGYDGRWRLEAGEFRIRMGSEATVIRCSETRIYETPNLPQV*

> AsBgl2410 野生型基因序列

ATGCATCAATCGCACCTTGATTTCATCAAACAGCGGATGTGCGGACTGCTGAGTCTGCTGCTCCCTCTG
GAGAACCTTGCTACTGATGTGCCGAGCTGGGCAAGGATTCGGTTCAACGGATCGTAAAGTCCATGAC
CCTCGAGGAGAAGGCTTCGCTACTGGTCGGCTTATCAATGGAGGATTACGGCGGGGAGGGATCCGTAG
CGGGTCACACGCTCCGTTATATCCCGGCTCGGCGGGAACGACCGCCCCGATGCCCCGATATGGGATTG
CGCCGACGGTTATGGCCGACGCCCCGCGCGGATTGCGGATTGATCCCGTACGAAAAGCGGATTGCTG
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CAAATGCGGAGCTGCTATGGTTGCGGTATTTCAGTCGCAGGGCGTGGGTGCATCGATCAAACACTTTG
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GAAATACGGCTTAAATTGCCCTTAAAGATACCGGCTACGCGCCGTGCGGTACACAACGTGCTTAATCCC
CAACAGCCGATCTGTGTTCCGGACTTTC AACGTGAAAACGTTGGATCATTCCGTCCGGTCCGTGGTTT
CTGA

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ATGACCGATGTGCCGACGTGGGCAAAGATAGCGTGCAGCGCATTGTGAAAAGCATGACCCTGGAAG
AAAAAGCGAGCCTGCTGGTGGGCCTGAGCATGGAAGATTATGGCGGCGAAGGCAGCGTGGCAGGTCA
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CGTGATGGCGGATGGTCCGGCGGGTTACGCATTGATCCGGTTCGCAAAGGCGATAGCCTGACCTATTA
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GAACAACCAGGAAGGCCTGCGCCTGCAGAATGATGCGCATGTGAGCCAGCGCGCATTACGCGAAATT
TATCTGCGCGGCTTTGAAATTGCGGTGCGCGAAGGCAAACCGTGACCGTGATGAGCAGCTATAACCG
CCTGAACCGCAGCTATACCCAGGAAAACCGCGAACTGCTGACCACCGTGCTGCGCGATGAATGGGGC
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CGATCTGATGATGCCGGGCGAACCGAGCCAGAGCGAAGAAATTATTCGCGCGGTGCGCGAAGGCCGC
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CAGAAGGCATTGTGCTGTTAAGCAACCGCCAGAAAACCTGCCGCTGCCGCCGGAAGCCGTATTGC
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GCATTTATACCCCGGAAAACAGCCTGACCCCGGAATTTATTGAAGCGCGCGCGGGCGACCAACGATATT
GCGGTGATTAGCTTTGGCCGCCTGAGCGGCGAAGGCAACGATCGCCCAACCCTGGATTTTATCTGAG
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TGATCCTGAACATTGGCGGCGTGATTGAAACCGATAGCTGGCATGCGCTGCCGGATGCGATTCTGGTG
GGCTGGCAAGGTGGTCAAGAAGGTGGCTGCGCGACCGTGGATGTGTTAAGCGGCAAAGTGAGCCCGT
CTGGTCGCCTGCCGATGACCTTTCCGAAAGATTATACCGATCATCCGAGCAGCCAGAACTATCCGCTGA
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ACCGATTATGAGGAAGATATCTGGGTGGGCTATCGCTATTTTAACACCTGGGCGAGCGATCGCATTGTG
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CTGTTTGTGGCGGCGCCGGGCAGCACTCTGCCAAAACCGGTGCGTGAATAAAAGCGTTTGCGAAAA
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TTTGATCCGGCGAGCGCGAGCTTTGTGACTGATGCGGGCACCTATACCATGAGCTGGCGAGCAGCGC
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ACCCGCAACAGCCAATTTGCGTGCCGGATTTTCAGCGCGAAAACGTGGGCAGCTTTTCGCCCGGTGGG
TGGCTTTCACCACCACCACCACCACTAA

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MHQSHLDFIKQRMCGLLSLLLPLENLATDVPQLGKDSVQRIVKSMTLEEKASLLVGLSMEDYGGEGSVAG
HTLRYIPGSAGTTAPMPRYGIPPTVMADGPAGLRIDPVRKGDSLTYCYTAFVGTMLASTWNTKLIEQVGS
AIGNEVLEYGCDVILGPGMNIHRNPLCGRNFEYYSDDLSSGKCGAAMVRGIQSQGVGASIKHFAANNQE
GLRLQNDAHVSQRALREIYLRGFEIARVREGKPWTVMSSYNRLNGSYTQENRELLTTVLRDEWGYKGIVV
TDWIGRRNTVAQVHAGNDLMMPGEPSSQSEIIRAVREGRLDEKEVDICVERMLKYIMRTPFRFRYTPSNRP
DFTKNATIRQAAAEGIVLLSNRQKTLPPESRIALFGVHGYDYIGCGTGAGYVYAPYMTLNRSFTEAG
FRLDSIVDNLYAQYRSFGEILYAEQNRKLVGNRIYTPENSLTPEFIEARAATNDIAVISFGRLSGEGNDRPTL
DFYLSDDERTLLERVCTIFHAAGRKVVVILNIGGVIEDTSWALPDAILVGWQGGQEGGCATVDVLSGKV
SPSGRLPMTFPKDYTDHPSSQNYPLNYSYRGDWADNTPERKFRNLGYTDYEEDIWVGYYRYFNTWASDR
IVFPFGFLSYTTFEWSNAALKLSRDECLVTLQVTNSGTYPAKEVIELFVAAPGSTLPKPVRELKAFKTRM
LEPGESTMIRLCFRVNDLASFPASASFVTDAGTYTIELASSANEIRLKLPLKIPATRRRVHNVLPQQPICV
PDFQRENVGSFRPVGGF*