

Produkce rekombinantních proteinů

Úkol 1

Náš známý student dostal za úkol vyzkoušet produkci několika hypotetických/nových proteinů v expresním systému *E. coli*. Je stále stejně zbrklý a začal s experimenty, aniž by si o proteinech cokoliv zjistil. Takže když narazil na problémy, je úplně ztracený. Zkuste mu poradit, co může způsobovat potíže při produkci proteinů.

A) Hypotetický protein z Gram-negativní bakterie

MGSASSLHAQAQITGRPEWIWLALGTALMGLGTLYFLVKGMWIAIGTGLMALGTLYFIVKGMVWLVCN
HQQFQLFMSINPVPTSVMWPHHHMALQGTMGMTIFNNCHAPNWSGVCAFHILIHHSGFWTCTHLLFWM
SCSMVPQGGWIWNGMGHAFFFTNVSLNFWLFTLCVACVWNNACPIIMSVQCFGTMSVLQPWLWHVSPH
LTSQAAMPPIPQQCWNQPPIASSANCTITCMQNAWQIAFLGGSVTGFNHIVTMIATGCWLALGTALMG
LGTLYFLVKGMWIAIGTGLMALGTLYFIVKGM

Problém: buňky málo a pomalu rostou!

B) Nově identifikovaný protein z byliny používané v tradičním čínském léčitelství.

MNIKMRYRCEIKHRAAGIPRYREADFTRRIWRAIMTKTCPKLPVCSPIDKQLPRWNPEQPYDNLIIN
PLMNSVKFPHRQHHRKLRPMFLITRCDNRAMIYNEPDAQIRRFKVIDFITFYQRPCCNWQYPCLAQSY
VIQPRIIRRTIVPRHTVGGEQRIILIEEMCLNFMRPHELWLLRARLCFRHTRHTIVEVCWYRRHHQSGP
QRLEVWGKINQLPIEYCLCLWVIQVRNGVRLNLELFPDIAQRLNEYHWTRYDRTPVWRFISLLRKRQI
CFRWPMSDWRKHDVRYGSPARRSLDGGKPPIRSQPCIPRLGNPYRNRTRRRCDPFAPHSIRGSITALR
PEWEYKCSFEVLLRSLRPIPIRVMKAMSPPGYDIHMQFDIAWMPARRFRGASMMRRYWGTDRRSGDY
PKTMAWFLRVHHTMMERGSRNTPKRDKPCFN

Problém: protein není produkován! Ani v nerozpustné formě (inkluze)!

C) Hypotetický protein z Gram-negativní bakterie

MVTVNSRVDSARASHITLNVMTVLDVGAILDDIKAQEKRLGETPDTGTSIGHKYIYMSSDDPRGWSVG
NDPGNITINAHVGETLSFACASTSENSEYAAFLYRLTGDPHLDPSHVEVIKLQNAAQPTPSNGYPFT
TAPVAFSSCDAKVAQQGQAKNFYVWAGLFTLDESKEVEVLAAYVWKEPTLVNGSGGLSGLSWERTNET
YQTLGLGLNDVDGTEQMFKKPSYSDRTALMDVSQIEASLASFAEIVDFHAIMLRLKPCALRAKLDYL
ARRVSKIEIEVPHVNVNIGGPFKNHNGGKEVPIIAQ

Problém: protein je produkován, ale všechen pouze ve formě inkluzí!

D) Nově izolovaný protein z patogenní houby, optimalizované kodony, ověřené exony/introny, studentova kolegyně dosáhla pěkných výtěžků!

MTQPHCICKGHDNAFYLVCRSWQKVDQCNVQRNMWWFNCSSLMMTYAMYANGRFPAHAHEHEAKRPQYE
QEHNGMDVCGETMEARWCITWPCCESIRKFINSAWLDIFQYTKDKLLYIDKKVSLLVFHRQNFNYEG
CLTYVQSFHGYTMEPTDRDPVRLFPFNYCSKGMPIHTGESWPHAMWDAIIPSRVWDMQHCCKVL

Problém: všechny buňky (12 lahví! Student připravoval medium půl dne!) v průběhu kultivace umřely...

Úkol 2

Student dostal za úkol odhadnout, zda se v proteinech, se kterými pracuje, tvoří disulfidické můstky. Pomozte mu!

Protein 1

FDSKLIKEDKVAQVASEFRCVEIDVLLPVKFERNMTVGLLKGSPHHQAQHVIDLAEAKKI
NCVGMYSVKGAEPLEWTPINIVIAVGRFNTRIAFYHYGKSTGELDRNLKLFALCDQEPG
NTLDDAGLMRYRMSQALLPFEIISLSASSTVGKTSGAIAATDYTQPFLDGPRVSRNWRG
VESTLKRNHQGILIDEPQTM

Protein 2

AWKGEVLANNEAGQVTSIIYNPGDVITIVAAGWASYGPTQKWGPQGDREHPDQGLICHDAFC
GALVMKIGNSGTIPVNTGLFRWVAPNNVQGAILIYNDVPGTYGNNSGSFSVNIIGKDQS

Úkol 3

Student, poučen předchozími nezdary, se rozhodl vybrat si proteiny, se kterými bude pracovat. Využijte nástroj **PROSO** a pomozte mu...

Protein 1

AWKGEVLANNEAGQVTSIIYNPGDVITIVAAGWASYGPTQKWGPQGDREHPDQGLICHDAFCGALVMK
IGNSGTIPVNTGLFRWVAPNNVQGAILIYNDVPGTYGNNSGSFSVNIIGKDQS

Protein 2

LVIVDAVTLLSAYPEASRDPAAPTVIDGRHLYVVS PGDAAQLGHNDSRLFTGLSPGDQLHLRETALAL
RAEVS VLFIRFALKDAGIVAPIELEVRDAATAVPDADDLLHPSCRPLKDHYWRS DVLAAGATTCTADF
AVCDRDGTVSGYFRWETSIEIAGSQPDTKQPGFKPSSDRNGNFS LPPNTAFKAI FYANAADRQDLKLF
IDDAPEPAATFVGNSEDGVR LFTLNSKGGKIRIEASANGRQSATDARLAPLSAGDTVWLGWLGAEDGA
DADYNDGIVILQWPIT

Protein 3

PLLSASIVSAPVVTSETYVDI PGLYLDVAKAGIRDGKLVQVILNVPTPYATGNNFPGIYFAIATNQG
VADGCFYSSKVPESTGRMPFTLVATI DVGSGVTFVKGQWKS SVRGSAMHIDS YASLSAIWGTAA
PSSQG SGNQGAETGGT GAGNIGGGGERDGT FNLPPIKFGVTAL THAANDQTI DIYIDDDPKPAAT
FKGAGAQ DQNLGTKVLDSGNRVRVIVMANGRPSRLGSRQVDIFKKS YFGIIGSEDGADDDYNDGIV
FLNWPLG

Protein 4

MGSASSLHAQAQITGRPEWIWLALGTALMGLGTYFLVKGMWIAIGTGLMALGTYFIVKGMVWLV
CNHQQFQLFMSINPVPTSVMWPHHMLQGTMGMTIFNNCHAPNWSGVCAFHILIHSGFWTCTHLLF
WMSCSMVPQGGWIWNGMGHAFFFTNVS LNFLWFTLCVACVWNNACPIIMSVQCFGTMSVLQ
PWLWHVSPH LTSQAAMPPI PQQCWNPQPIASSANCTITCMQNAWQIAFLGGSVTGFNHIVT
MIATGCWLALGTALMGLGTYFLVKGMWIAIGTGLMALGTYFIVKGM

Úkol 4

Student se rozhodl výsledky ověřit pomocí jiného predikčního nástroje. Predikujte rozpustnost proteinů z úkolu 3 s využitím dalšího programu. Výsledky porovnejte.

Úkol 5

Analyzujte následující geny a určete četnost využitých kodonů v *E. coli* a *Saccharomyces cerevisiae*.

Gen 1

ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTC
TATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGGATGACGCAGCGGGTCAGGCGATTGCTAACCGTTTACCT
CTAACATTAAGGCCTGACTCAGGCGGCCCGTAACGCCAACGACGGTATCTCCGTTGCGCAGACCACCGAAGGCGCGCTGTCC
GAAATCAACAACAACCTTACAGCGTGTGCGTGAACGTACGGTACAGGCCACTACCGGTACTAACTCTGAGTCTGATCTGTCTTC
TATCCAGGACGAAATTAATCCCGTCTGGATGAAATTGACCGCGTATCTGGTCAGACCCAGTTCAACGGCGTGAACGTCTGG
CAAAAAATGGCTCCATGAAAATCCAGGTTGGCGCAAATGATAACCAGACTATCACTATCGATCTGAAGCAGATTGATGCTAAA
ACTCTTGGCCTTGATGTTTTAGCGTTAAAAATAACGATACAGTTACCACTAGTGCTCCAGTAACCTGTTTTGGTGCTACCAC
CACAAACAATATTAACCTTACTGGAATTACCCTTTCTACGGAAGCAGCCACTGATACTGGCGGAACTAACCCAGCTTCAATTG
AGGGTGTTTTACTGATAATGGTAATGATTACTATGCGAAAATCACC GGTTGATAACGATGGGAAGTATTACGCAGTAACA
GTTGCTAATGATGGTACAGTGACAATGGCGACTGGAGCAACGGCAAATGCAACTGTAACCTGATGCAAACTACTACTAAAGCTAC
AACTATCACTTCAGCGGTACACCTGTTGAGATTGATAATACTGCAGGTTCCGCAACTGCCAACCTTGGTGCTGTTAGCTTAG
TAAACTGCAGGATCCAAGGGTAATGATACCGATACATATGCGCTTAAAGATACAAATGGCAATCTTTACGCTGCGGATGTG
AATGAACTACTGGTGCTGTTTTCTGTTAAACTATTACCTATACTGACTCTTCCGGTGCCGCCAGTTCTCCAACCGCGGTCAA
ACTGGCGGAGATGATGGCAAACAGAAGTGGTCGATATTGATGGTAAAACATACGATCTGCCGATTTAAATGGCGGTAATC

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TGCAACAGGTTTTGACTGCTGGTGGTGGGCTCTGACTGCTGTTGCAATGGTAAAACCACGGATCCGCTGAAAGCGCTGGAC
GATGCTATCGCATCTGTAGACAAATTCGGTTCTTCCCTCGGTGCGGTGCAAAACCGTCTGGATTCCGCGGTACCAACCTGAA
CAACACCCTACCAACCTGTCTGAAGCGCAGTCCCGTATTTCAGGACGCCGACTATGCGACCGAAGTGTCCAATATGTCGAAAG
CGCAGATCATCCAGCAGGCCGTAACCTCCGTGTTGGCAAAAGCTAACAGGTACCGCAGCAGGTTCTGTCTCTGCTGCAGGGT
TAA

Gen 2

ATGGATACCTCTCACGAAATTCATGATAAAATACCCGATACATTAAGAGAGCAGCAGCAGCATTGCGCCAAAAAGAGTCGGA
AGGGTGCATAACAACATTGAAAGATCTTAATGTACCTGAGACGAAGAACTTTCCCTCCGTCTTACATGGCAGAAAGGCGAGTA
CATATTTAAGAATTTTAGAGATGATGAATGTTTAGCCGATAACAACAATGGTGTAGATAGCAATAATGGCGGGTCCGTGACA
TGCGCGGACAAAATAACAAGGTCAGAAGCAACTCCCAATCGGTACCAGAAGGACTACAAGTTTCTGAAAAAAGAATAACCC
AGATACTTTGTCTCTTTCATTGTCAAGTTTCATTTTATCTAACCCAGGAGCGCGCAATTAAGCCCAATAAACACCGTGGCGC
ATAGAAATAATATTACTGAGACAGGTCAGGCTCTGGTGGAGATATCGCAAAACAACAATCACACCAACCACAGGTGTTACAC
CACCAGACTTCTTTAAAGCCAATTCAGAATGTTGATGAGGGATGATTTTCGCTAAGTCCACTTATCAGGAGAGCTTACATGG
AATATCGGAGGACTTAACGTTAAAGCCTGTTTCTTCTGCAACATACTATCCTCACAAAAGTAAAGCAGATTCTGGTTATGAAG
AAAAGGATAAAATGAAAAATGATATTGATACTATTCAGCCTGCCACCATAAATTTGTGCCCTCTGGTATAGCGACACTTCTTAGT
TCATATAACCGACATACTTTCAAAGTTAAGACATATTCAACTTTATCACAATCCCTAAGGCAAGAAAACGTCAACAATCGTAG
TAACGAAAAAAAACCTCAACAGTTTGTGCCACATAGCGAATCAATTAAGAAAAACCAATACGTTTGAACAAGATAAAGAAG
GCGAGCAAGCGGACGAAGAAGAGGATGAAGGTGATAATGAACATAGGGAATATCCCTTAGCAGTTGAACTAAAACCAATTTACG
AATAGAGTTGGAGGTCACACTGCAATATTTCAGATTTTCGAAGAGAGCGGTTTGTAAAGGCTCTAGTGAATAGAGAAAAACAGGTG
GTATGAGAATATAGAGCTGTGCCATAAAGAATTATTGCAATTCATGCCACGTTATATTGGTGTCTGAATGTTAGGCAACATT
TTCAGTCAAAAGATGACTTTTTAAGCGATTTGGATCAAGAAAAACACGGTAAAAATGATACCAGTAATGAAAACAAAGACATA
GAAGTCAATCATAACAATAACGACGATATTGCGCTTAATACAGAACCACGGGGACCCCTTAACACACATACATTCCCTCCC
CTTGGAACATTCGTCGAGACAAGTACTTGAAAAAGAGCATCCTGAAATTTGAGTCTGTCCATCCACACGTGAAAAGATCACTGT
CAAGTTCCAACCACTTCTTGTGCCCCGAGGTTGATTAATGATAACAGGCATATTTAA

Úkol 6

Analyzujte geny z různých organismů a určete, které z nich je možné použít pro expresi v *E. coli* bez optimalizace kodonů. Za jakých podmínek?

Gen 1 (*Saccharomyces cerevisiae*)

ATGTCATTTCGACGACTTACACAAAGCCACTGAGAGAGCGGTCATCCAGGCCGTTGGACCAGATCTGCGACGATTTTCGAGGTTAC
CCCCGAGAAGCTGGACGAATTAACGTCTACTTTCATCGAACAAATGGAAAAAGGTCTAGCTCCACCAAAGGAAGGCCACACAT
TGGCTTCGGACAAAGTCTTCCATGATTCCGGCGTTCGTCACCGGGTCAACCAACGGGACGGAGCGCGGTGTTTTACTAGCC
GCCGACCTGGGTGGTACCAATTTCCGTATATGTTCTGTAACTTGCATGGAGATCATACTTTCTCCATGGAGCAAATGAAGTC
CAAGATTCCCGATGATTTGCTAGACGATGAGAACGTCACATCTGACGACCTGTTTGGGTTTCTAGCAGCTCGTACACTGGCCT
TTATGAAGAAGTATACCCGGACGAGTTGGCCAAGGTTAAAGACGCCAAGCCCATGAACTGGGGTTCACTTTCTCATAACCTT
GTAGACCAGACCTCTCTAAACTCCGGGACATTGATCCGTTGGACCAAGGGTTTCCGCATCGCGGACACCGTTCGAAAGGATGT
CGTGCATTTGTACCAGGAGCAATTAAGCGCTCAGGGTATGCCTATGATCAAGGTTGTTGCATTAACCAACGACACCGTTCGGAA
CGTACCTATCGCATTGCTACACGTCGGATAACACGGACTCAATGACGTCGGGAGAAATCTCGGAGCCGTCATCGGATGATATT
TTCGTTACCGGTACCAATGGGTGCTATATGGAGGAGATCAACAAGATCACGAAGTTGCCACAGGAGTTGGGTGACAAGTTGAT
AAAGGAGGGTAAGACACACATGATCATCAATGTGCAATGGGGTCTTTCGATAATGAGCTCAAGCACTTGCCCTACTACTAAGT
ATGACGTCGTAATTGACCAACCTGTCAACGAACCCGGGATTTCACTTGTTTGAAAAACGTGTCTCAGGGATGTTCTTGGGT
GAGGTGTTGCGTAACATTTAGTGGACTTGCCTCGCAAGGCTTGTCTTTGCAACAGTACAGGTCCAAGGAACAACACTCCCTCG
CCACTTGACTACACCTTTCCAGTTGTCATCCGAAGTGTGTCGATATTGAAATTTGACGACTCGACAGGTTACGTTGAAACAG
AGTTGTCATTATTACAGAGTCTCAGACTGCCACCCTCAACAGAGCGTGTTCAAATTCAAAAATTTGGTGCAGCGGATTTCT
AGGAGATCTGCGTATTTAGCCGCGCTGCCGCTTGGCCGATATTGATCAAGACAAATGCTTTGAACAAGAGATATCATGTTGA
AGTCGAGATCGGTTGATGTTCCGTTGTGGAATACTACCCGTTTCAGATCTATGCTGAGACACGCTTAGCCTTGTTCAC
CCTTGGGTGCCGAGGGTGGAGGAAGGTGCACTTGAAGATTGCCAAGGATGGTTCCGGAGTGGGTGCCGCTTGTGTGCGCTT
GTAGCATGA

Gen 2 (*Burkholderia* sp.)

ATGCTACTGGTGTGCAAACTAAAGCTGTGCCGGGCGCGGCCAGCACGCCGATGGACCAGGCTGCTGGCCGACATCGGCGG
TACCAATGCGCGTTTCGCGCTCGAGACGGGCCCGCGAAATCGGCTCGGTGCAGGTCTATCCCTGCGCCGAGTATCCGAGCG
TCGCCGACGTCATCAAGAAGTACCTGAAGGACACGAAGATCGGCCGCTCAATCACCGCGGATCGCGATTGCGAACCCGGTTC
GACGCGACAGGTCAGCATGACCAATCACGACTGGACCTTTTCGATCGAAGCGACGCGCCGACGCTCGGCTTCGACACGCT
GCTCGTCAACGACTTCAACCGCGCTCGCGATGGCGCTGCCCGGCTCACCGACACGCGAGCGCTGCAAGTTCGGCGCGGCC
AGCGCCGGCCGAACAGCGTGTATCGGCTGCTCGGCCCGGACCCCGCATGGGCGTCTCGGGCTGATCCCGCGACGACCCG
TGGATCGCGCTCGGACGAGGCGCCACGCACTTCCGCGCCCGCAGCAGCGGAGAGATCTGCTGCACTACGCGCG
CAAGAAGTGGTTCGACGTTTCGTTCCGACGGGTGGCCGCGGCCCGGCTCGAGGTGATCTATCGCGGCTCGCGGGCCGCG
ACAAGAAACGCGTTCGGGCCAGCGTCAACCCGGCCGATGTGGTCAAGCGCGCACGAAGGCGAGCCGCTCGCGGCCGAATCG
GTCGACGTTCTGCGGATTTCTCGGCACCTTCCGCCGCAACATCGCGGTGACGCTCGGCGGCTCGGCGGCATCTATATCGG
CGGCGGTGTCGTCGCGCTCTCGGCGAGTTGTTCCGCGCTTTCGCTTCCGCCAGCGCTTCGAGGCGAAGGGCCGCTTCGAGG
CGTATCTGCAGAATGTGCCGACCTATGTGATCACCGCCGAATACCCGGCTTCTCGGCGTATCGGCGATTCTCGCGGAGCAG
CTGTCAACCGCGCGGCCGCGAGCTCGTCCGCGGTGTTTCGAGCGGATTCGCCAGATGCGCGACGCGCTGACGCCGGCCGAGCG

CCGCGTCGCGGATCTCGCGCTGAACCATCCGCGTTCGATCATCAACGATCCGATCGTCGACATCGCGCGCAAGGCCGACGTCAGTCAAGCCGACCGTGCATCCGCTTCTGCCGCTCGCTCGGCTGCCAGGGGTTGTTCGGATTTCAAGCTGAAGCTCGCGACCGGTTGACGGCAGCATTCCGGTCAAGCCAGCCAGGTGCATCTCGGGGACACCGCGACCGACTTCGGCGCGAAGGTGCTCGACAACACCGTTCGGCGATCCTGCAGTTGCGCGAGCATCTGAACCTTCGAGCACGTGGAACGCGCGATCGACCTGCTGAACGGAGCAGGC GCATCGAGTTCTACGGGCTCGGCAATTCGAACATCGTCGCGCAGGACGCGCACTACAAGTTCTTCCGTTTCGGTATTCCGACC ATCGCGTACGGTGACCTGTACATGCAGGCAGCCTCGGCCGCGCTGCTCGGCAAGGGCGACGTGATCGTCGCGGTGTCGAAGTC GGGGCGCGCGCCGAGCTGCTGCGCGTGTCTGACGTCGCGATGCAGGCCGGCGCGCAGGTGATCGCGATCACCTCGAGCAACA CGCCGCTCGCGAAGCGCGGACGGTTCGCGCTGGAAACCGATCACATCGAGATTTCGCGAGTTCGCGAGTTCGATGATCTCGCGC ATCTGACCTCGTGTGATCGACATTCTCGCGGTTCGGTGTGGCGATTTCGTCGCGCGGTGCCGAGCGATGAAGTGGCCGAGAC CGTCGAGAAAGCGCGCAAGGGCGCCGACGACGCGACTGCCGTACTCGACTGGCTGAGCCACGGCGCGGCTTCGTCGCGCG CCGACTGA

Gen 3 (neznámý organismus)

ATGAATATTAATAAGAGATATAGATGTGAAATTAACATAGAGCTGCTGGAATCCAAGATATAGAGAAGCTGATTTCACTAG AAGAAATTTGGAGAGCTATTATGACTAAAACCTTGCCAAAACCTTCCAATTTGTTTGTCTCCAATTTGATAAAACAACCTCCAAGAT GGAATCCAGAACAACCATATGATAATCTTATTATTAATCCACTTATGAATTTCTGTTAAATTTCCACATAGACAACATCATAGA AAACCTCCAAGAATGTTTCTTATTACTAGATGTGATAATAGAGCTATGATTTATAATGAACCAGATGCTCAAATTTAGAAGATT CAAAGTTATTGATTTCACTTCTTATCAAAGACCATGTTGTAATTTGGCAATATCCATGTCTTGTCTCAATCTTATGTTATTC AACCAAGAATTAGAAGAATTTGTTCCAAGACACTGTTGGAGGAGAACAAGAATTATTCTTATTGAAGAATGTGTCTT AATTTTCATGAGACCACATGAACCTTTGGCTTCTTAGAGCTAGACTTTGTTTTCAGACATACTAGACATACTATTGTTGAAGTTTG TTGGTATAGAAGACATCATCAATCTGGACCACAAAGACTTGAAGTTTGGGGATGGAATAATCAACTTCCAATTTGAATATT GTCTTTGTCTTTGGGTTATTCAAGTTAGAAATGGAGTTAGACTTAATCTTGAACCTTTCCAATTTGCTCAAAGACTTAATGAA TATCATTGGACTAGATATGATAGAATCCAGTTTGGAGATTCATTTCTTCTTAGAAAAAGACAAATTTGTTTTCAGATGGCC AATGTCTGATTGGAGAAAACATGATGTTAGATATGGATCTCCAGCTAGAAGATCTCTTGTATGGAGAAAACCAACATTTAGAT CTCAACCATGTATTCCAAGACTTGGAAATCCATATAGAAAATAGAACTAGAAGAAGATGTGATCCATTCGCTCCACATTTCTATT AGAGGATCTATTACTGCTCTTAGACCAGAATGGGAATATAAATGTTCTTTCGAAGTTCTTCTTAGATCTCTTAGACCAATTC AATTAGAGTTATGAAAGCTATGTCTCCACCAGGATATGATATTATGCAATTCGATATTGCTTGGATGCCACCAGCTAGAA GATTCAGAGGAGCTTCTATGATGAGAAGATATTGGGAACTGATAGAAGATCTGGAGATTATCCAAAACCTATGGCTTGGTTC CTTAGAGTTCTACTCATATGATGGAAAGAGGATCTAGAAATACTCCAAAAGAGATAAACCATGTTTCAAT

Úkol 7

Optimalizujte následující geny/proteiny pro produkci v hostitelských organismech.

1

MAQVINTNSLSLITQNNINKNQALSSS IERLSSGLRINS AKDDAAGQAIANRFTSNIKGLTQAARNANDGISVAQTTEGALS EINNNLQRVRELTQVQATTGTNSEDLSS IQDEIKSRLDEIDRVSGQTQFNGVNVLAKNGSMKIQV GANDNQITITIDLKQIDAK TLGLDGF SVKNNNDVTTSAPVTAFGATTTNNIKLTGITLSTEAAATDTGGTNPASIEGVYTDNGNDY YAKITGGDNDGKYAVT VANDGTVTMATGATANATVTDANTTKATTITSGGT PVQIDNTAGSATANLGA VSLVKLQDSKGNDDT YALKDTNGNLYAADV NETTGAVSVKTIITYTDSSGAASSPTAVKLGDDGKTEVVDIDGKTYDSADLNGGNLQ TGLTAGGEALTAVANGKTTDPLKALD DAIASVDKFRSSLGAVQNRLDSAVTNLNNTTNLSEAQSRIQDADYATEVSNMSKAQIIQQAGNSVLAKANQVPQQVLSLLQG

Pro produkci v *E. coli*.

2

MDTSHEIHKIPD TLREQQQHLRQKESEGCITTLKDLNVPETKKLSSVLHGRKASTYLRI FRDDECLADNNGVDSNNGGSVT CADK ITRSEATPKSVPEGLQVSEKKNPDTLSSLSLSSF ILSNHEEPAIKPNKHVAHRNNITETGQSGEDI AKQQSHQPQVLH HQTSLKPIQNVDEGCISPKSTYQESLHGISED LTLKPVSSATYYPHKS KADSGYEKDKMENDIDTIQPATINCASGIATLPS SYNRHTEFKVKTYS TLSQSLRQENNVNRSNEKPKQFVPHSESIKEKPNTFEQDKEGEQADEEEDEGDNEHREYPLAVELKPFTRVGGHTAIFRFSKRAVCKALVNREN RWYENIELCHKELLQFMPRYI GVLNVRQHFQSKDDFLSDLDQENNGKNDTSNENKDI EVNHNND DIALNTEPTG TPLTHIHSFPLEHSSRQVLEKEHPEIESVHPHVKRSLS SSNQPSLLPEVVLNDRHI

Pro produkci v *E. coli*. Nesmí obsahovat štěpící místa pro *HindIII* a *NdeI*!

3

ATGGCAACACAAGGAGTGTTCACCTTCCCGCCAACACCCGGTTCGCGCTCACC GCCTTCGCCAACCTCGTCCGGAACCCAGAC GGTGAACGTGCTGGTCAACAACGAGACGGCCGCGACCTTCAGCGGGCAAAGCACC AATAACGCCGTCATCGGCACCCAGGTGC TCAACTCCGGCAGCAGTGGCAAGGTACAGGTCCAGGT CAGCGTCAACGGCCGCCCTCGGATCTGGTCTCGGCACAGGTAATC CTGACCAACGAGCTGAACCTCGCCCTGGTTCGCTCTGAAGACGGCACCGACAACGACTACAACGACGCCGCTCGTGGTGTATCAA CTGGCCGCTCGGCTAG

Pro produkci v HEK 293.

