Klonování genu pro glukosa oxidasu z *A. niger*

>AF234246.2:486-2303 Aspergillus niger glucose oxidase (GO2) gene, complete cds

ATGCAGACTCTCCTTGTGAGCTCGCTTGTGGTCTCCCTCGCTGCGGCCCTGCCACACTACATCAGGAGCA
ATGGCATTGAAGCCAGCCTCCTGACTGATCCCAAGGATGTCTCCGGCCGCACGGTCGACTACATCATCGC
TGGTGGAGGTCTGACTGGACTCACCACCGCTGCTCGTCTGACGGAGAACCCCAACATCAGTGTGCTCGTC
ATCGAAAGTGGCTCCTACGAGTCGGACAGAGGTCCTATCATTGAGGACCTGAACGCCTACGGCGACATCT
TTGGCAGCAGTGTAGACCACGCCTACGAGACCGTGGAGCTCGCTACCAACAATCAAACCGCGCTGATCCG
CTCCGGAAATGGTCTCGGTGGCTCTACTCTAGTGAATGGTGGCACCTGGACTCGCCCCCACAAGGCACAG
GTTGACTCTTGGGAGACTGTCTTTGGAAATGAGGGCTGGAACTGGGACAATGTGGCCGCCTACTCCCTCC
AGGCTGAGCGTGCTCGCGCACCAAATGCCAAACAGATCGCTGCTGGCCACTACTTCAACGCATCCTGCCA
TGGTGTTAATGGTACTGTCCATGCCGGACCCCGCGACACCGGCGATGACTATTCTCCCATCGTCAAGGCT
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TGTCCATGTTCCCCAACACCTTGCACGAAGACCAAGTGCGCTCCGATGCCGCTCGCGAATGGCTACTTCC
CAACTACCAACGTCCCAACCTGCAAGTCCTGACCGGACAGTATGTTGGTAAGGTGCTCCTTAGCCAGAAC
GGCACCACCCCTCGTGCCGTTGGCGTGGAATTCGGCACCCACAAGGGCAACACCCACAACGTTTACGCTA
AGCACGAGGTCCTCCTGGCCGCGGGCTCCGCTGTCTCTCCCACAATCCTCGAATATTCCGGTATCGGAAT
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CAGACCACCGCTACCGTCCGCTCCCGCATCACCTCTGCTGGTGCAGGACAGGGACAGGCCGCTTGGTTCG
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GTGGGCCGAAGAGGCCGTCGCCCGTGGCGGATTCCACAACACCACCGCCTTGCTCATCCAGTACGAGAAC
TACCGCGACTGGATTGTCAATCACAACGTCGCGTACTCGGAACTCTTCCTCGACACTGCCGGAGTGGCCA
GCTTCGATGTGTGGGACCTTCTGCCCTTCGACCGAGGATACGTCCACATCCTCGACAAGGACCCCTACCT
CCACCACTTTGCCTACGACCCTCAGTACTTCCTCAACGAGCTCGACCTGCTCGGTCAGGCTGCCGCTACT
CAGCTGGCCCGCAACATCTCCAACTCCGGTGCCATGCAGACCTACTTCGCTGGGGAGATACTCCCCGGTG
ATAACCTCGCGTATGATGCCGATTTGAGCGCCTGGACTGAGTACATCCCGTACCACTTCCGTCCTAACTA
CCATGACGTAGGTACTTGCTCCATGATGCCGAAGGAGATGGGCAGTGTTGTTGATAATGCTGCCCGTGTG
TATGGTGTGCGGGGACTGCGTGTCATTGATGGTTCTATTCCTCCTACGCAAATGTCGTCCCATGTCATGA
CGGTGTTCTATGCCATGGCTTTGAAAATTTCGGATGCTATCTTGGAAGATTATGCTTCCATGCAGTGA

N-HIS,T7-GO2-HIS

*BamH*1(GGATCC)
*Xho*I(CTCGAG)
5` TCGCGGATCCCTGCCACACTACATCAGGAGCA
5` GGTGCTCGAGCTGCATGGAAGCATAATCTTCCA

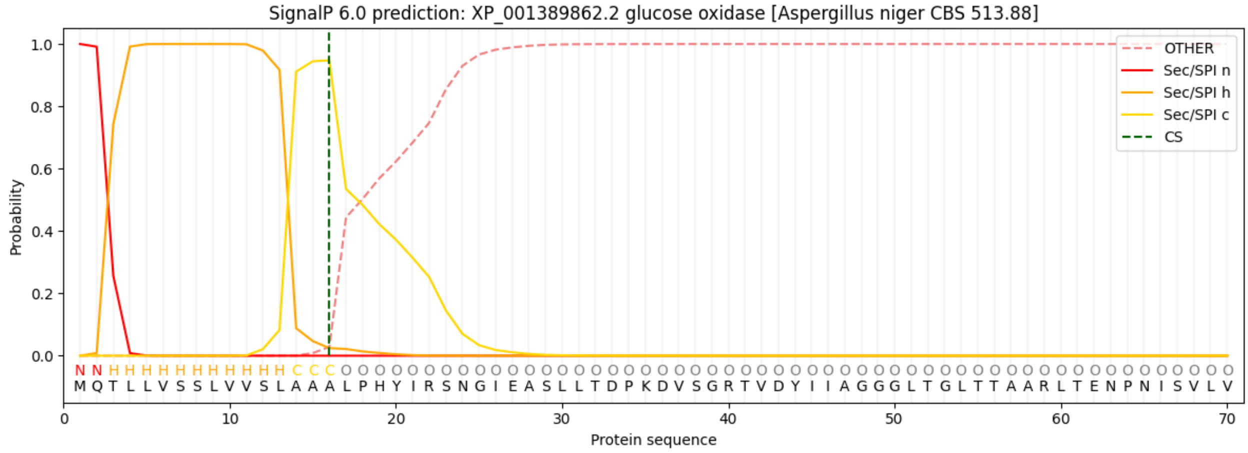
N-HIS,T7-GO2

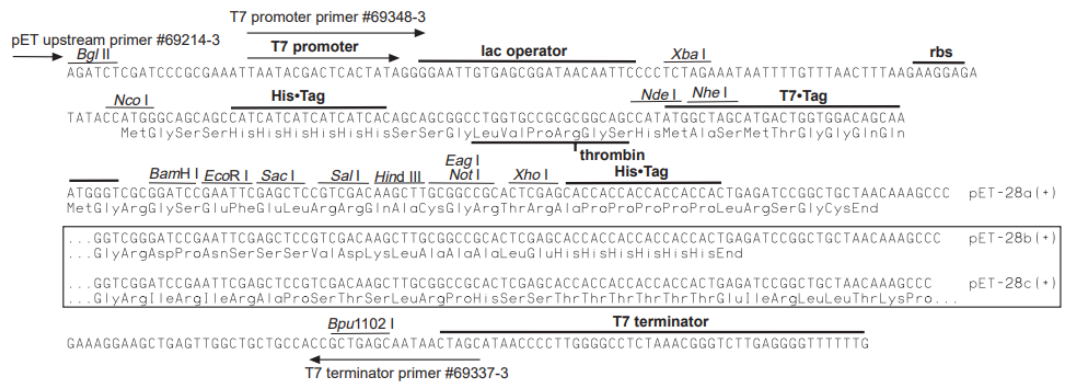
*BamH*1(GGATCC)
*Xho*I(CTCGAG)
5` TCGCGGATCCCTGCCACACTACATCAGGAGCA
5` GGTGCTCGAGTCACTGCATGGAAGCATAATCTTC

N-HIS-GO2

NdeI(CATATG)
*Xho*I(CTCGAG)
5` TATGCATATGCTGCCACACTACATCAGGAGCA
5` GGTGCTCGAGTCACTGCATGGAAGCATAATCTTC

>AAF59929.2 glucose oxidase [Aspergillus niger]
MQTLLVSSLVVSLAAALPHYIRSNGIEASLLTDPKDVSGRTVDYIIAGGGLTGLTTAARLTENPNISVLV
IESGSYESDRGPIIEDLNAYGDIFGSSVDHAYETVELATNNQTALIRSGNGLGGSTLVNGGTWTRPHKAQ
VDSWETVFGNEGWNWDNVAAYSLQAERARAPNAKQIAAGHYFNASCHGVNGTVHAGPRDTGDDYSPIVKA
LMSAVEDRGVPTKKDFGCGDPHGVSMFPNTLHEDQVRSDAAREWLLPNYQRPNLQVLTGQYVGKVLLSQN
GTTPRAVGVEFGTHKGNTHNVYAKHEVLLAAGSAVSPTILEYSGIGMKSILEPLGIDTVVDLPVGLNLQD
QTTATVRSRITSAGAGQGQAAWFATFNETFGDYSEKAHELLNTKLEQWAEEAVARGGFHNTTALLIQYEN
YRDWIVNHNVAYSELFLDTAGVASFDVWDLLPFDRGYVHILDKDPYLHHFAYDPQYFLNELDLLGQAAAT
QLARNISNSGAMQTYFAGEILPGDNLAYDADLSAWTEYIPYHFRPNYHDVGTCSMMPKEMGSVVDNAARV
YGVRGLRVIDGSIPPTQMSSHVMTVFYAMALKISDAILEDYASMQ





pET-28a-c(+) cloning/expression region

Amplifikace genu GO2 z gDNA *Aspergillus niger*

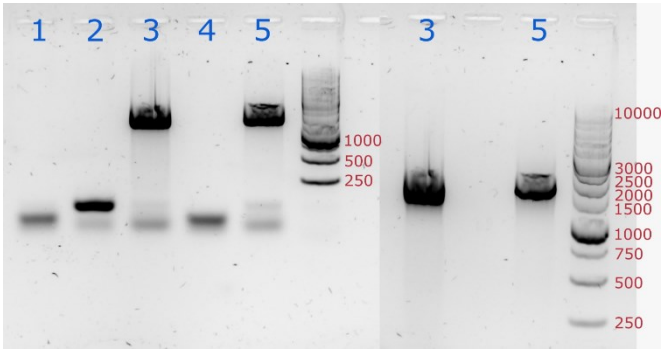
Materiál:
Elizyme HS ROBUST MIX RED (Elisabeth Pharmacon)
Primery (viz. přiložený Datasheet):
N-HIS,T7-GO2-HIS (T7-GO2-HIS_FW/GO2-HIS_REV)
N-HIS,T7-GO2 (T7-GO2-HIS_FW/GO2_REV)
N-HIS-GO2 (HIS-GO2_FW/GO2_REV)

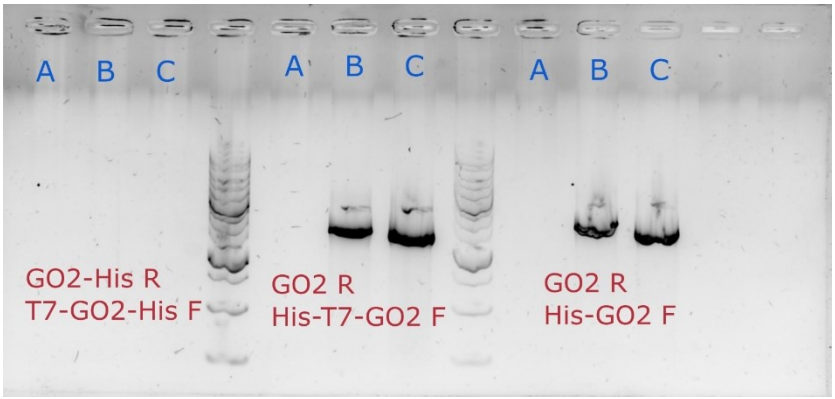
Složení PCR reakční směsi (50 ul)

HS ROBUST MIX RED	25.0 ul
FW primer (10 uM)	2.0 ul
REV primer (10 uM)	2.0 ul
PCR voda	16.0 ul
gDNA	5.0 ul

Teplotní protokol:

95°C	2:30	1 x
95°C	10s	
60°C	20s	40 x
72°C	50s	
72°C	5 min	1 x





Přečištění PCR produktu

Materiál: Monarch® PCR & DNA Cleanup Kit (5 µg) (https://international.neb.com/-/media/nebus/files/protocols/t1030_quick_protocol_card_monarch_pcrdna_cleanup.pdf?rev=a562d8f1f21741b0ac6d59ea9020cef3&hash=E4845F80E8B2BE925A09DAD6562A41EC

Restrikční analýza

Materiál:
Restriktázy
*Nde*I (https://international.neb.com/products/r0111-ndei#Product%20Information)
*Xho*I (https://international.neb.com/products/r0146-xhoi#Product%20Information)
*BamH*I-HF (https://international.neb.com/products/r3136-bamhi-hf#Product%20Information)
Vektor pET28a

Složení směsi RA (50 ul)

CutSmart buffer (10x)	5.0 ul
PCR produkt/vektor	15 ul
PCR voda	28 ul
NcoI-HF/ BamHI-HF	1.0 ul (10U)
XhoI	1.0 ul (10U)

Teplotní protokol: Inkubace při 37°C po dobu 3 hodin

Izolace naštěpeného PCR produktu a plasmidu z agarózového gelu

Materiál: Monarch® DNA Gel Extraction Kit (https://international.neb.com/-/media/nebus/files/protocols/t1020_quick_protocol_card_monarch_dna_gel_extraction.pdf?rev=09308c01500f43c6a8589b01845765d9&hash=0FD4350A9DE2AEAC84913DB2A00140AF)

Ligace PCR produktu do vektoru pET28a

Materiál: Instant Sticky-end Ligase Master Mix
(https://international.neb.com/protocols/2012/08/27/protocol-transfer-master-mix-to-ice-prior-to-reaction-set-up-mix-tube-by-finger-flicking-before-u)

Protokol:
1. Transfer master mix to ice prior to reaction set up. Mix tube by finger flicking before use.
2. Combine 100 ng of vector pET28a with a 3-fold molar excess of insert and adjust volume to 5 µl with dH2O.
3. Add 5 µl of Instant Sticky-end Ligase Master Mix, mix thoroughly by pipetting up and down 7-10 times, and place on ice. The sample is now ready to be used for transformation.

Transformace vektoru po ligaci do NEB 5-alpha E. coli

Materiál:

NEB 5-alpha Competent E. coli (High Efficiency) (https://international.neb.com/products/c2987-neb-5-alpha-competent-e-coli-high-efficiency#Product%20Information)

Protokol:

1. Thaw a tube of NEB 5-alpha Competent E. coli cells on ice for 10 minutes.
2. Add 2 µl of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Spread 50-100 µl of each dilution onto a selection plate and incubate overnight at 37°C.