Generation of Str-KDEL-IRES-MannosidaseII-mScarlet-i

Str-KDEL_ManII-SBP-mCherry (Plasmid #65253) from Franck Perez was used as a template.

The insert EcoRI_mScarlet-i_FseI was synthesised as gBlocks® Gene Fragment by Integrated DNA technologies (724bp):

taagcagaattccATGGTGAGCAAGGGCGAGGCAGTGATCAAGGAGTTCATGCGGTTCAAGGTGCACATGGA
GGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCC
AGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCTCCTGGGACATCCTGTCCCCTCAG
TTCATGTACGGCTCCAGGGCCTTCATCAAGCACCCCGCCGACATCCCCGACTACTATAAGCAGTCCTT
CCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGCCGTGACCGTGACCCAGG
ACACCTCCCTGGAGGACGGCACCCTGATCTACAAGGTGAAGCTCCGCGGCACCAACTTCCCTCCTGAC
GGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAAGCGTCCACCGAGCGGTTGTACCCCGAGGACGG
CGTGCTGAAGGGCGACATTAAGATGGCCCTGCGCCTGAAGGACGGCGGCCGCTACCTGGCGGACTTCA
AGACCACCTACAAGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACAACGTCGACCGCAAGTTGGAC
ATCACCTCCCACAACGAGGACTACACCGTGGTGGAACAGTACGAACGCTCCGAGGGCCGCCACTCCAC
CGGCGGCATGGACGAGCTGTACAAaggccggcctTAAgca

Cloning procedure:

- Double digest of template and insert with EcoRI-HF and FseI (to remove the SBP and mCherry-tags from the template)
- digested template was dephosphorylated (antarctic phosphatase NEB), and gel purified
- both fragments were ligated using T4 ligase (Promega) into the final product of 6146bp.

Sequencing revealed the complete sequence as the following:

[…]tggttttcctttgaaaaacacgatgataagcttgccacaacccgggaggcgcgccATGAAGTTAA
GTCGCCAGTTCACCGTGTTTGGCAGCGCGATCTTCTGCGTCGTAATCTTCTCACTCTACCTGATGCTG
GACAGGGGTCACTTGGACTACCCTCGGGGCCCGCGCCAGGAGGGCTCCTTTCCGCAGGGCCAGCTTTC
AATATTGCAAGAAAAGATTGACCATTTGGAGCGTTTGCTCGCTGAGAACAACGAGATTATCTCAAATA
TCAGAGACTCAGTCATCAACCTGAGCGAGTCTGTGGAGGACGGCCCGCGGGGGTCACCAGGCAACGCC
AGCCAAGGCTCCATCCACCTCCACTCGCCACAGTTGGCCCTGCAGGCTGACCCCAGAGACTGTTTGGA
TCCCACCGGTCGCCACCGCaattccATGGTGAGCAAGGGCGAGGCAGTGATCAAGGAGTTCATGCGGT
TCAAGGTGCACATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGC
CCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCTCCTGGGA
CATCCTGTCCCCTCAGTTCATGTACGGCTCCAGGGCCTTCATCAAGCACCCCGCCGACATCCCCGACT
ACTATAAGCAGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGCC
GTGACCGTGACCCAGGACACCTCCCTGGAGGACGGCACCCTGATCTACAAGGTGAAGCTCCGCGGCAC
CAACTTCCCTCCTGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAAGCGTCCACCGAGCGGT
TGTACCCCGAGGACGGCGTGCTGAAGGGCGACATTAAGATGGCCCTGCGCCTGAAGGACGGCGGCCGC
TACCTGGCGGACTTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACAACGT
CGACCGCAAGTTGGACATCACCTCCCACAACGAGGACTACACCGTGGTGGAACAGTACGAACGCTCCG
AGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAaggtgcttaaGGccggcctaaggcctc
gagggccaattaattaactctagataactgatcataa[…]

Seq. Primers (5’ -> 3’):

IRES_fwd	TGGCTCTCCTCAAGCGTATT	mScarlet-i_m2_fwd	CCCCGACTACTATAAGC
ManII_m1_fwd	ACCCCAGAGACTGTTTGG		