

Sequences:

SpCas9 WT (2x SV40 NLS):

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR
LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDEV
AYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSVDKLFQILVQ
TYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNFKS
NFDLAEDAQLQSKDQYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS
ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE
KMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILT
FRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEK
VLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYF
KKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE
RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQ
LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKILQTVKVDELVKVMGRHKPEN
IVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYLQNGR
DMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKN
YWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKY
DENDKLIREVKVITLKSCLVSDFRKDFQFYKVVREINNYHHAHDAYLNAVVG TALIKKYPKLESE
FVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE
IVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYG
GFDSPTVAYSVLVAKVEKKGSKKLSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLII
KLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLGSPEDNEQKQL
FVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGDAYPYDVPDYASLGSGSP
KKKRKVEDPKKRKVD

N-Cas9_N-Intein_v1:

MGPKKRKRKVAADYKDDDDKGIHGVPAAADKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVL
GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFF
HRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMI
KFRGHFLIEGDLNPDNSVDKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLI
AQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDAQLQSKDQYDDDLDNLLAQIGDQYAD
LFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD

QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE
EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS
GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECLSYETEILTVEYGLLPKIVEKRIECTVYSV
DNNGNIYTQPVAQWHDRGEQEVFEYCLEDGSLIRATKDHKFM TVDGQMLPIDEIFEREDL
MRVDNLPN

C-Intein_C-Cas9_v1:

MIKIATRKYLGKQNVYDIGVERDHNFALKNGFIASCFDSVEISGVEDRFNASLGTYHDLLKIIK
DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSR
KLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANL
AGSPAIKKILQTVKVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEEGIK
ELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDS
IDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELD
KAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFY
SNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEK GKSKKLKSVKELL
GITIMERSSEKNPIDFLEAKGYKEVKKDLIILPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFYLYLASHYEKLGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL
SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLY
ETRIDLSQLGGDAYPYDVPDYASLGSGSPKKKRKVEDPKKKR KVD

N-Cas9_N-Intein_v2:

MGPKKKRKVAADYKDDDDKGIHG VPAADKKYSIGLDIGTNSVGVAVITDEYKVPSKKFKVL
GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDD SFF
HRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLR LIYLALAHMI
KFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLI
AQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYAD
LFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD
QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE
EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS
GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDKD
FLDNEENEDILEDIVLTLTLFEDREMIEERLKCLSYETEILTVEYGLLPKIVEKRIECTVYSVD

NNGNIYTQPVAQWHDRGEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERE₂DLM
RVDNLPN

C-Intein_C-Cas9_v2:

MIKIATRKYLGKQNVYDIGVERDHNFALKNGFIAS₂YAHLFDDKVMKQLKRRRYTGWGRLSR
KLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANL
AGSPAIKKGILQTVKVVDLVKVMGRHHPENIVIE₂MARENQTTQKGQKNSRERMKRIEEGIK
ELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDS
IDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELD
KAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS₂LVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVG₂TALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFY
SNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL₂SMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPK₂KYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELL
GITIMERS₂SFEKNPIDFLEAKGYKEVKKDLI₂KLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNF₂LYLASHYEKLGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL
SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ₂SITGLY
ETRIDLSQLGGDAYPYDVPDYASLGGSPKKK₂RVEDPKKKR₂KVD

crTLR#1 gRNA (20 bp protospacer + gRNA scaffold):

GTTGCAGCTCGAACTTCACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGT
CCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT

Rosa26#1 (20 bp protospacer + gRNA scaffold):

GCTCCAGTCTTTCTAGAAGATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGT
CCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT

Rosa26#3 (20 bp protospacer + gRNA scaffold):

GACTCCAGTCTTTCTAGAAGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGT
CCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT

Rab38#2 (19 bp protospacer + gRNA scaffold):

GAACTTCTCCTCGCACTACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC
CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT

***Fus* gRNA (G + 19 bp protospacer+ gRNA scaffold):**

GTCGGCCCCGGTAGCCGCCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGT
CCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT

Traffic light reporter. Reporter (promoter and terminator sequence underlined):

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCC
ATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCA
ACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG
ACTTTCCATTGACGTCAATGGGTGGATATTTACGGTAAACTGCCCACTTGGCAGTACATC
AAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCC
TGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTA
TTAGTCATCGCTATTACCATGGTTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCAT
CTCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTTAATTATTTTGTGCAGCG
ATGGGGGCGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGG
GCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTC
CGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCG
CGCGGCGGGGCGGAGTCGCTGCGTTGCCTTCGCCCGTGCCCGCTCCGCGCCGCC
TCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTACTCCACAGGTGAGCGGGCGG
GACGGCCCTTCTCTCCGGGCTGTAATTAGCGCTTGGTTAATGACGGCTCGTTTCTTT
TCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGA
GCGGCTCGGGGGTGCCTGCGTGTGTGTGCGTGGGAGCGCCGCGTGC GGCCCCG
CGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGGGGCTTTGTGCGCTCCGC
GTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGCGGTGCGGGGGGCTGC
GAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGT
GGGCGCGGCGGTTCGGGCTGTAACCCCCCTGCACCCCCCTCCCCAGTTGCTGAGC
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGCGCGGGGCTCGCCGTG
CCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCTCGGG
CCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCGGAGCGCCGGCGGCTGTGCA
GGCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGGCGCAGGGA
CTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCCGCGCACCCCCTC
TAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGG
GCCTTCGTGCGTCGCCGCGCCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCC
GCAGGGGGACGGCTGCCTTCGGGGGGACGGGGCAGGGCGGGGTTTCGGCTTCTGGC
GTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTTTCTAC
AGATCCTTAATTAAGCCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGT

GGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCC
GGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATCTGCACC
ACGGGCAAGCTGCCCGTGCCCTGGCCACCCTCGTGACCACCCTGGGCTACGGCCTG
CAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCA
TGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAA
GACCCGCGCCGAGGTGAAGTTCGAGCTGCAACTCCAGTCTTTCTAGAAGATGGGCGGG
AGTCTTCTGGGCAGGCTTATATCAAGCGCTATGTGCACCAAACTTCTCCTCGCACTAC
CGGGCCACCATTGGTGATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTC
AAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAG
AACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACC
AGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT
CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAGACGCGTTG
GCCACGAACTTCTCTCTGTTAAAGCAAGCAGGAGATGTTGAAGAAAACCCCGGGCCTAT
GGTGTCTAAGGGCGAAGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAG
GGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACG
AGGGCACCCAGACCATGAGAATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTT
CGACATCCTGGCTACCAGCTTCATGTACGGCAGCAGAACCTTCATCAACCACACCCAGG
GCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACC
ACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGC
TGCCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGAT
GCAGAAGAAAACACTCGGCTGGGAGGCCAACACCGAGATGCTGTACCCCGCTGACGG
CGGCCTGGAAGGCAGAAGCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGAT
CTGCAACTTCAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCG
GCGTCTACTATGTGGACCACAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACCTA
CGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACCTGGG
GCACAAACTTAATTGAGCGATCGCTACGAGATCTGAGCTCCCTGGCGGAATTGCGTAAA
TGATTGCAGATCCACTAGTTCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTT
GCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCTTCTTGACCCTGGAAGGTGCCACT
CCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAT
TCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAAT
AGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCA

Traffic light reporter. Donor vector:

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTG
GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCC
ACCTACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCCTGCCCT
GGCCACCCTCGTGACCACCCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCG
ACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGA
GCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC
GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGAC
GGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCAC
CGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAG
GACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGC
CCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACC
CCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCAC
TCTCGGCATGGACGAGCTGTACAAGTAGACGCGTTGGCCACGAACTTCTCTGTAAA
GCAAGCAGGAGATGTTGAAGAAAACCCCGGGCCTATGGTGTCTAAGGGCGAAGAGCTG
ATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTT
CAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAAT
CAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTC
ATGTACGGCAGCAGAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCA
GTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTG
CTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGA
TCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTG
GGAGGCCAACACCGAGATGCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAAGCGA
CATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTTCAAGACCACATAC
AGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACCACA
GACTGGAAAGAATCAAGGAGGCCGACAAAGAGACCTACGTGAGCAGCAGGAGGTGG
CTGTGGCCAGATACTGCGACCTCCCTAGCAAACCTGGGGCACAACTTAATTGA