pCCM935:(unc-22 GUIDE with U6 promoter and 3’ UTR)

Magenta=unc-22 guide sequence

Target sequence: GAACCCGTTGCCGAATACAC**AGG** (PAM in bold)

Yellow=scaffold sequence

This 840 bp long fragment is subcloned by TOPO cloning into pCR-Blunt II-TOPO vector (Kan resistance: from Invitrogen)

**Transform and plate on Kanamycin plate (not Amp!)**

**Recommend preparing DNA by MIDI prep kit, or by Zyppy miniprep kit from Zymo Research (QIAGEN Mini-prep kit does not work well for CRISPR in our hands.)**

CTCCAAGAACTCGTACAAAAATGCTCTGAAGTAGGTCTCGAGATCAATACTGGGAAGACGAAAGTCTTGCGAAACCGATTCGCTGACCCCAGTAAAGTCTACTTCGGTAGCCCTTCCCCCACCACCCAGCTCGACGACGTCGACGAGTACATCTACCTCGGTCGTCAAATCAACGCCCAAAACAACTTGATGCCGGAAATCCACCGAAGACGTCGAGCAGCCTGGGCTGCATTCAATGGAATCAAGAATGCCACCGACTCCATCACCGACAAGAAGATTCGTGCGAATCTGTTCGACTCAATTGTCCTTCCAGCGCTCACCTACGTTTCAGAAGCCTGGACATTCACCAAAGCTCTATCCGAACGAGTACGAATCACACATGCCTCCCCCACAAATTATTGATCTACATCCCCATTTGGTATAGTGTCTTACAGGACTCTCAATATGTCAGCTGCATGATAAATGGTCCCTATATAATTGAATTGCAAATCTAAATGTTTGAACCCGTTGCCGAATACACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCAATAATATTCTAGATATCCCTTTTTGAAACTAAAATGAAATACTCTAAATTTTTGAGTTCAATGTTTCAACTAATCTTGGTTAAAGGTAAATTCCCGGAGGGACGATTTTAAAGTTGCCAGAGTTGATTAAATTGGTCCAAAAAATTTTATTAGGAAACTCGAAAAAAAATTGATTTTTTTTGACAATATTCCAAAGAAAATTACAAAATCTCGACGCCAAACATAGTCGGCTGTG

Suggested Co-CRISPR Injection Mix for testing a new guide:

unc-22 guide 50 ng/l

new guide 50 ng/l

eft-3-Cas9 50 ng/l

pRF4 50 ng/l

Contact for any questions

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