pCCM936:(avr-14 GUIDE with U6 promoter and 3’ UTR)

Magenta=avr-14 guide sequence

Target sequence: GATTGGAGAGTTAGACCACG**TGG** (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.)**

CTCCAAGAACTCGTACAAAAATGCTCTGAAGTAGGTCTCGAGATCAATACTGGGAAGACGAAAGTCTTGCGAAACCGATTCGCTGACCCCAGTAAAGTCTACTTCGGTAGCCCTTCCCCCACCACCCAGCTCGACGACGTCGACGAGTACATCTACCTCGGTCGTCAAATCAACGCCCAAAACAACTTGATGCCGGAAATCCACCGAAGACGTCGAGCAGCCTGGGCTGCATTCAATGGAATCAAGAATGCCACCGACTCCATCACCGACAAGAAGATTCGTGCGAATCTGTTCGACTCAATTGTCCTTCCAGCGCTCACCTACGTTTCAGAAGCCTGGACATTCACCAAAGCTCTATCCGAACGAGTACGAATCACACATGCCTCCCCCACAAATTATTGATCTACATCCCCATTTGGTATAGTGTCTTACAGGACTCTCAATATGTCAGCTGCATGATAAATGGTCCCTATATAATTGAATTGCAAATCTAAATGTTTGATTGGAGAGTTAGACCACGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCAATAATATTCTAGATATCCCTTTTTGAAACTAAAATGAAATACTCTAAATTTTTGAGTTCAATGTTTCAACTAATCTTGGTTAAAGGTAAATTCCCGGAGGGACGATTTTAAAGTTGCCAGAGTTGATTAAATTGGTCCAAAAAATTTTATTAGGAAACTCGAAAAAAAATTGATTTTTTTTGACAATATTCCAAAGAAAATTACAAAATCTCGACGCCAAACATAGTCGGCTGTG