**Appendix B: primers, sgRNA’s and other DNA templates used**

**Appendix B1: pMT 12.5 plasmid**

Every sgRNA that was designed (See Appendix B2, B3), was inserted into the pMT 12.5 plasmid (Fig. B1.1). The GFP was removed from the plasmid using the *BtsI* restriction enzyme that creates sticky ends for the annealed oligo’s to bind to (See “note” Appendix B2, B3, B5). The plasmid also contains a ampicillin resistance cassette for selection in *E. coli* and an hygromycin resistance cassette for selection in *A. niger*.

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**Figure B1.1: Schematic overview of the pMT12.5 plasmid.**

**Appendix B2: sgRNA’s and primers used for *amyR* deletion and verification**

*Legend:*

* *Brown:* Primer binding site used for PCR amplification.
* *Red:* protospacer adjacent motif (PAM)
* *Blue:* sgRNA binding site
* *Green:* target gene

Note: the colours function solely as a locus indicator, the designed primers/oligos do not fully resemble the marked region. The sgRNA was designed to be ordered in two separate oligo’s (sense and the complimenting anti-sense sequence), while also containing 2 extra nucleotides at the end for successful integration into the pMT 12.5 plasmids (GT for forward, GA for reverse oligo). The downstream primer was ordered in anti-sense to allow PCR amplification.

CTGGTTTGTAAAGCAGGTGGCACTCAATCTTGCTTCTAAACCTAAAATGATGAGCACTGAAGGCGGGGACGAATGAAGATGGGTTTCGATTAATGAAGACACTTACCAGGGCCCCTGACAGCGAGGCATTTAATGGCTTTGATAATCATCTCATTTGACTAAGTAGGTCACAAGTCTCGGCTTCCCGAGTGGGATGTTCACCAGTGTTACGTGAAAGCTCTTTCGGACCGTCATTAGCATGAATCCCATCGTCAGCGAGCCTCAGACTCTGTCAGCTTTTGACATAAGAAAACACATTGACAATGCTTTGTGCAGGGGTGCTGATTCTCCGAAAGTACTCCGTAGCGCATGGATGATGCCCTTGGTCCCAATGATGGATCCTTCAGGCCGGCCCAACAACTCATGGCATGCCCACACCACGTACACGCAAAGAGTCTCTAAATGATTCGAGTCGTCTACCGGATATACCGAGGCAATATCTGTGATGATGGCTCCGTGACATATCATGGTTCAGCCTACGCTTCTCTCGCACTTGAAGGACATGGCTTCGGCCTCCTGTCTGGAGTATACCTGACCCGCGTCATGGCTCGGGCGGAGGGTCCACCTGCTAAACCAGGAAGCCATCCTGGGACTAAAAGACTAGTAGCTCCAGTCGAGGTGATACCCCCTTTGGCTCGAGCAACGGGTTGAAATGAACAGGCCAGTCGTAGCAGGTTAATGGAGGTGGCTCATACCGCGGGGTCCGGGTAGCCTGGAGGTGCCGACTTTCTCACGGTGTCGCTTCCCCGCCGCCCTTCCCCAGATTTCTCTCCTTTGGTCTCCCCTCCTTTAGCAATCGCATGATTTTCGGTGACTGCTCACCTTGTGGCCATCTTGACCACCGAAACTTGTCTCACGAGTATCGTTATTTCTCAAAATACAATGAAGTCTCCAGAGTCTCGCTCCACTGCCGCTGCTACTGCTAAGTTCTGACGGGAGTCACACTTGTCTCCGCATACAAATGGACTCTCATCCTTCCCCTACCAAACAAAAGGCCTCCAAGCAAGCCTGCGACAACTGTCGTCGGCGTAAGATCAAGTGCTCGAGAGAGCTTCCCTGCGATAAGTGTCGACGCCTTCTTCTTTCTTGTTCCTACAGTGATGTGCTCCGGCGCAAAGGCCCCAAGTTCCGCACCTTATATCCACTTGCGCCCATTCATCCCCTGGTATCACGACAACAGAATACATACCAACAGAATTCGTCACAAAATCCGTTAAACAAGCAATGGACTGCAGATGGAGTGGGCTATCCGTTAAGCTCACTAATGTCGCCTTCTTTCACAGTGGCAGACCCTCAATACTTACCCCATGACGCTCCCGAGCCGTTCTCTCAGTTTCCTCCGCCAGAGCTAGTCTCCTCACCCGACTCAACCAATTCATTGTCAGACTCTAGTATGGCACTAGTGCGCCCTTATGCACGACGCCTGTCTGCTCCGGTGCTACTTGCTCATGTGAATGTTTATCTAAAATATCTGTTCCCCATCATGCCTGTGGTGCGGAAGGAGGAGCTTCAACAAGATTGCCACCAGCCTGAACGGTTATCGCCCCAACGATATGCCTTTCTTGTCGCCCTATGCGCAGCCACACACATCCAGCTGAAACTAGATGGCACAGCATCTGTCCCAGAACCTTCACACCTTCAAGCCGGGATTGACGGGCATTCCTGGATGTCCGGCGAAGAGTTGCTGGCTGAAGCAGTACGCGCAAGGAAGGATTGCGACCCAGTAGACGGCATGAACATAGAAAGCCTTCTTACGTCTTTCTTCCTGTTTGCTTCGTATGGTAACCTGGACAAACAGGACCATGCCTGGTTCTACCTTTGTCAGGCAACATCCATGGTCTTCACGCTGGCACTCCACCGAGAGTCAAGTTATGTGGATCTGAGTACCGAAGAAGCAGAGGAACGACGCAGGGTGTTTTGGCTACTGTTTGTCACCGAAAGGTAGGTGCATCCTATGAAAGCATTGCCCATTTTTCGACTAATGTGAATCAGGGGCTACGCACTTCAACAATCGAAACCGGTAATGCTGCGTAATTCAATCCGCAAGCCTCAAGTACTTTGCTCCGAAGATCCTATCTTGGCCTACGGTTTCATCAATCTTATTAGCATCTTTGAGAAACTGACCGTCAACCTTTATGACTGGGTCTCTGCGGGAGGCATGGACGGCTCGTCCGAGATGCCCCCTACATCTGCTATTCAGTCTAGTCTCTGCAACGCAATTTCGGTCGACGGAGTCTCGGAGATTCAAAAGGTCGACATACTCATCACCCAGCAATGGCTACAGACAGTAATGTGGAAACTTTCTATGACTCGTGCTACTCAGCCTGGATCTCGCGATGAGGCGGTTCTTCCCTTTCATCTCCCCGTTCTTGTCGGAAAAGCCGTTATGAATGTTATTGGTGCTGCATCCCAGGGAGCTGTTGATGCTCACGGCATTGGCATGGTCAGTATCTTAGTTCCGGCGCAGCACATGCCAATACCATTTACTAACAACATAACAGGAACAAAAATTATTCGACCTGGGTTCCTCAGTTGCAGACGTGGCACGATCACTCAACTCCAAAGCTGCGCACCGCCTTACTGAAGCGGCCGTCGATCCTCGCGAACTCCTTTGGGGCATTCTTACCACCTTATCACGCATCCGCGGCTCTCAGTCCTACCTCTTTCCTTCATTGTTGGAGCGATGCAAAGGCGCCCTGGACTTTACCTCCCCCACGTCGATGGGCAACTTCCTCCCTCCATTATCCACTGCTTCCACATGGGAAGAGGAAACGGGGCTCGCCGTGGTTTCTGTCCCGGAGAATCCTGACCCTCATGAACAAGAAGCAGCATTAATGGAGCCGCTATCGCAGCTCTTACCAACTCCACAGGTGCAATTCCCGGAAAATAATCTTTTGAACTAGGAAGGCAACTACGACGATGACGATGCTAGCATTAATCCGCGTTTGTTACGACTTTGCTTATGTTATGATACCATTTATCCGGAGACTATGTGGCAATGTATACGGCATGAAGGTGAACTGTATGTAATAAAGCATGTATATAGGTGATGAGACTATCTTATATGATTACCGATCGTGGTCGAATATTAGCTGAGCCACCATGCATTGCATTGGACTATTCAGCCATCACACTTCTGTTATTCTGAAAAGGTAGCATTAATTTTTTGTGGAATCTTTCTTTATTTCAGTCTAACTGCGCTATACAAAGCAACACAACCGGGTCAATCCGAGAATAAATGTACAATTGGTATGAAAGTGTAAGAATGTAAGAAACCCAAGACTATGTACCTGTATCGCGCACGAATCTGATATCATCCCAAGCTAAACAATCAAGTCAAGTTTCATGATCTATGCGCCGTAAGAAAGAAGGAAAATTATAAACGTGGCTAGCGTAGAAGAGGGAGTGAAAGGAATATCCCTCTTCATATATGAGTGAGCAAGTCGGGGGGGTGGACATGAGATAGAACCTGTAGCTTGAAGAAGCAGTACAGGTGATTTTGATGGTGGTGGAAGGCGAAAATATGAGGGCGGAGGGGAATGGGAGGGATTGCGCAAATGGTAGACGCGCAATGCAGACAGGCGGCCAATCTTAGGCTGTCCGCCAGATCAACTCTAGAATTTGATCACCTTTCTCTGGGTCACGGTGGACGTACCATTGACGGACGCGGTTTCCGTCAATGATGGCGTCCTCATGCAGGTAGCTGATCTTCCCCTCGTCCTTTAAGCGGAGAGGACCCAGCTCATGCAGGTATGACTCGAAAACTGCACCGTTGTGGCTGTCCCACGTGAGCTCCATGCTTCTGTCGCCTGGCTTTTTCTCGCTGCTATACTTCACCACCGAGAAGCCGTTGGTGAGTATCCAGCCGTCTCCAAAGTGCCATTTCCGCAGGAGACATGAATC

**Appendix B3: sgRNA’s and primers used for *glaA* deletion and verification**

*Legend:*

* *Brown:* Primers used for PCR amplification.
* *Red:* protospacer adjacent motif (PAM)
* *Blue:* sgRNA binding site
* *Green:* target gene

Note: the colours function solely as a locus indicator, the designed primers/oligos do not fully resemble the marked region. The sgRNA was designed to be ordered in two separate oligo’s (sense and the complimenting anti-sense sequence), while also containing 2 extra nucleotides at the end for successful integration into the pMT 12.5 plasmids (GT for forward, GA for reverse oligo). The downstream primer was ordered in anti-sense to allow PCR amplification.

CCTCTCGTATGCAGAGGAAATCTCCCCTGATCTTCCGAACTGGTCGTACCTGGCGACCTATGACTATGGCACCCCAGTTCTGGGGACCTTCCACGGAAGTGACCTGCTGCAGGTGTTCTATGGGATCAAGCCAAACTATGCAGCTAGTTCTAGCCACACGTACTATCTGAGCTTTGTGTATACGCTGGATCCGAACTCCAACCGGGGGGAGTACATTGAGTGGCCGCAGTGGAAGGAATCGCGGCAGTTGATGAATTTCGGAGCGAACGACGCCAGTCTCCTTACGGATGATTTCCGCAACGGGACATATGAGTTCATCCTGCAGAATACCGCGGCGTTCCACATCTGATGCCATTGGCGGAGGGGTCCGGACGGTCAGGAACTTAGCCTTATGAGATGAATGATGGACGTGTCTGGCCTCGGAAAAGGATATATGGGGATCATAATAGTACTAGCCATATTAATGAAGGGCATATACCACGCGTTGGACCTGCGTTATAGCTTCCCGTTAGTTATAGTACCATCGTTATACCAGCCAATCAAGTCACCACGCACGACCGGGGACGGCGAATCCCCGGGAATTGAAAGAAATTGCATCCCAGGCCAGTGAGGCCAGCGATTGGCCACCTCTCCAAGGCACAGGGCCATTCTGCAGCGCTGGTGGATTCATCGCAATTTCCCCCGGCCCGGCCCGACACCGCTATAGGCTGGTTCTCCCACACCATCGGAGATTCGTCGCCTAATGTCTCGTCCGTTCACAAGCTGAAGAGCTTGAAGTGGCGAGATGTCTCTGCAGGAATTCAAGCTAGATGCTAAGCGATATTGCATGGCAATATGTGTTGATGCATGTGCTTCTTCCTTCAGCTTCCCCTCGTGCAGATGAGGTTTGGCTATAAATTGAAGTGGTTGGTCGGGGTTCCGTGAGGGGCTGAAGTGCTTCCTCCCTTTTAGACGCAACTGAGAGCCTGAGCTTCATCCCCAGCATCATTACACCTCAGCAATGTCGTTCCGATCTCTACTCGCCCTGAGCGGCCTCGTCTGCACAGGGTTGGCAAATGTGATTTCCAAGCGCGCGACCTTGGATTCATGGTTGAGCAACGAAGCGACCGTGGCTCGTACTGCCATCCTGAATAACATCGGGGCGGACGGTGCTTGGGTGTCGGGCGCGGACTCTGGCATTGTCGTTGCTAGTCCCAGCACGGATAACCCGGACTGTATGTTTCGAGCTCAGATTTAGTATGAGTGTGTCATTGATTGATTGATGCTGACTGGCGTGTCGTTTGTTGTAGACTTCTACACCTGGACTCGCGACTCTGGTCTCGTCCTCAAGACCCTCGTCGATCTCTTCCGAAATGGAGATACCAGTCTCCTCTCCACCATTGAGAACTACATCTCCGCCCAGGCAATTGTCCAGGGTATCAGTAACCCCTCTGGTGATCTGTCCAGCGGCGCTGGTCTCGGTGAACCCAAGTTCAATGTCGATGAGACTGCCTACACTGGTTCTTGGGGACGGCCGCAGCGAGATGGTCCGGCTCTGAGAGCAACTGCTATGATCGGCTTCGGGCAGTGGCTGCTTGTATGTTCTCCACCCCCTTGCGTCTGATCTGTGACATATGTAGCTGACTGGTCAGGACAATGGCTACACCAGCACCGCAACGGACATTGTTTGGCCCCTCGTTAGGAACGACCTGTCGTATGTGGCTCAATACTGGAACCAGACAGGATATGGTGTGTTTGTTTTATTTTAAATTTCCAAAGATGCGCCAGCAGAGCTAACCCGCGATCGCAGATCTCTGGGAAGAAGTCAATGGCTCGTCTTTCTTTACGATTGCTGTGCAACACCGCGCCCTTGTCGAAGGTAGTGCCTTCGCGACGGCCGTCGGCTCGTCCTGCTCCTGGTGTGATTCTCAGGCACCCGAAATTCTCTGCTACCTGCAGTCCTTCTGGACCGGCAGCTTCATTCTGGCCAACTTCGATAGCAGCCGTTCCGGCAAGGACGCAAACACCCTCCTGGGAAGCATCCACACCTTTGATCCTGAGGCCGCATGCGACGACTCCACCTTCCAGCCCTGCTCCCCGCGCGCGCTCGCCAACCACAAGGAGGTTGTAGACTCTTTCCGCTCAATCTATACCCTCAACGATGGTCTCAGTGACAGCGAGGCTGTTGCGGTGGGTCGGTACCCTGAGGACACGTACTACAACGGCAACCCGTGGTTCCTGTGCACCTTGGCTGCCGCAGAGCAGTTGTACGATGCTCTATACCAGTGGGACAAGCAGGGGTCGTTGGAGGTCACAGATGTGTCGCTGGACTTCTTCAAGGCACTGTACAGCGATGCTGCTACTGGCACCTACTCTTCGTCCAGTTCGACTTATAGTAGCATTGTAGATGCCGTGAAGACTTTCGCCGATGGCTTCGTCTCTATTGTGGTAAGTCTACGCTAGACAAGCGCTCATGTTGACAGAGGGTGCGTACTAACAGAAGTAGGAAACTCACGCCGCAAGCAACGGCTCCATGTCCGAGCAATACGACAAGTCTGATGGCGAGCAGCTTTCCGCTCGCGACCTGACCTGGTCTTATGCTGCTCTGCTGACCGCCAACAACCGTCGTAACTCCGTCGTGCCTGCTTCTTGGGGCGAGACCTCTGCCAGCAGCGTGCCCGGCACCTGTGCGGCCACATCTGCCATTGGTACCTACAGCAGTGTGACTGTCACCTCGTGGCCGAGTATCGTGGCTACTGGCGGCACCACTACGACGGCTACCCCCACTGGATCCGGCAGCGTGACCTCGACCAGCAAGACCACCGCGACTGCTAGCAAGACCAGCACCAGTACGTCATCAACCTCCTGTACCACTCCCACCGCCGTGGCTGTGACTTTCGATCTGACAGCTACCACCACCTACGGCGAGAACATCTACCTGGTCGGATCGATCTCTCAGCTGGGTGACTGGGAAACCAGCGACGGCATAGCTCTGAGTGCTGACAAGTACACTTCCAGCGACCCGCTCTGGTATGTCACTGTGACTCTGCCGGCTGGTGAGTCGTTTGAGTACAAGTTTATCCGCATTGAGAGCGATGACTCCGTGGAGTGGGAGAGTGATCCCAACCGAGAATACACCGTTCCTCAGGCGTGCGGAACGTCGACCGCGACGGTGACTGACACCTGGCGGTAGACAATCAATCCATTTCGCTATAGTTAAAGGATGGGGATGAGGGCAATTGGTTATATGATCATGTATGTAGTGGGTGTGCATAATAGTAGTGAAATGGAAGCCAAGTCATGTGATTGTAATCGACCGACGGAATTGAGGATATCCGGAAATACAGACACCGTGAAAGCCATGGTCTTTCCTTCGTGTAGAAGACCAGACAGACAGTCCCTGATTTACCCTTGCACAAAGCACTAGAAAATTAGCATTCCATCCTTCTCTGCTTGCTCTGCTGATATCACTGTCATTCAATGCATAGCCATGAGCTCATCTTAGATCCAAGCACGTAATTCCATAGCCGAGGTCCACAGTGGAGCAGCAACATTCCCCATCATTGCTTTCCCCAGGGGCCTCCCAACGACTAAATCAAGAGTATATCTCTACCGTCCAATAGATCGTCTTCGCTTCAAAATCTTTGACAATTCCAAGAGGGTCCCCATCCATCAAACCCAGTTCAATAATAGCCGAGATGCATGGTGGAGTCAATTAGGCAGTATTGCTGGAATGTCGGGGCCAGTTGGCCCGGTGGTCATTGGCCGCCTGTGATGCCATCTGCCACTAAATCCGATCATTGATCCACCGCCCACGAGGCGCGTCTTTGCTTTTTGCGCGGCGTCCAGGTTCAACTCTCTCTGCAGCTCCAGTCCAACGCTGACTGACTAGTTTACCTACTGGTCTGATCGGCTCCATCAGAGCTATGGCGTTATCCCGTGCCGTTGCTGCGCAATCGCTATCTTGATCGCAACCTTGAACTCACTCTTGTTTTAATAGTGATCTTGGTGACGGAGTGTCGGTGAGTGACAACCAACATCGTGCAAGGGAGATTGATACGGAATTGTCGCTCCCATCATGATGTTCTTGCCGGCTTTGTTGGCCCTATTCGTGGGATGCGATGCCCTCGCTGTGCAGCAGCAGGTACTGCTGGATGAGGAGCCATCGGTCTCTGCACGCAAACCCAACTTCC

**Appendix B4: Repair templates**

Using 3 bp into the sgRNA binding site from the PAM, 45 bp upstream of the upstream cutting site and 45 bp downstream of the downstream cutting side was used to create 90 bp long repair sequence so the DNA could be repaired using homologous recombination.

*glaA repair sequence:*

TTCCTTCAGCTTCCCCTCGTGCAGATGAGGTTTGGCTATAAATTGTCTTTCCTTCGTGTAGAAGACCAGACAGACAGTCCCTGATTTACC

*amyR repair sequence:*

CATGATTTTCGGTGACTGCTCACCTTGTGGCCATCTTGACCACCGTCGTGGTCGAATATTAGCTGAGCCACCATGCATTGCATTGGACTA

**Appendix B5: *kusA* deletion information.**

*Legend:*

* *Brown:* Primers used for PCR amplification.
* *Red:* protospacer adjacent motif (PAM)
* *Blue:* sgRNA binding site
* *Green:* target gene

Note: the colours function solely as a locus indicator, the designed primers/oligos do not fully resemble the marked region. The sgRNA was designed to be ordered in two separate oligo’s (sense and the complimenting anti-sense sequence), while also containing 2 extra nucleotides at the end for successful integration into the pMT 12.5 plasmids (GT for forward, GA for reverse oligo). The downstream primer was ordered in anti-sense to allow PCR amplification.

GTGAGGCGCAGTCTCCGGGTTGATCTTGTCCAGCAGCTTGCACATTTCCTCGCATTGGTATTCCGACCATTTTCTTATGGGTGAGCCTCCACCGATGTCCGCATACTGTTTTTGAATCTTGGGTGTGCGTCGTTTCGAAATAAGAGGCCCGAGGTAATGCTGGAACTTGCCAAGAGGAATCAAATCGCCGTCGGCCTTGAATAGAAGTAGAATGTTAGAAACGGAGCAACCAAAATGACAGCTTGCCATAGTCGGAGACGTACAAAGAGCCGGCTGAGGAAATCTTCTACTTCGTCTGTCGTCGAGGGCCCTCCCATGTTCAGGAAGACCATGGCTGTAGGGCCCTTAGAGCCTGTTGCATCCTGGGTAACCGGAGGCACTGTTGTCGCCAGCCCACATCTTTGTTCTTGCTTGTATCCGAACAGGGTGCGAGAAGCCGGTCGCAGCAATTGCCGGGGAAGGGTAAACGGGCGGCGGAGAGCCATGACAGGTAATTGTACTGAATTCGGTTGACCTAGTCAATGGGGGTATAAGAAAAGACCGTTCGTATCGCGCAAGCAGATGAACTATTCAAGCCCGCATTCAATACTTAAAAGATAGACGAGTGGCAAGAACAGGTAGTGGGTGTATGCAACAGCGCAAGGCCTTCTGGAAGCTGAAAAGTCCAGAACGGCTTGATGACGGAGCACCGAGACCACGACCAACTCCGACTCCCGACAGCCAATGACCGGCCAGCTAGCGTCATCAATTACCGGGCGGACATCACATGATGTTCGTGTCTCCCCGCGTCTTTCTGCCCACCGGTTTGATCGCGTCCCTCGCGACCGGATCCAGTGACGATATAGATCTCCCCTCGGCTGCAGGCAGCAGAGGCCAAACAGGCAGACACAACAGCCCCACTTGTTCCTGGTTACGATTCAAGTTGTCTTAACCTTTATACTTCCCTCTTTCAATTTCGATAATATCTTGATTGCTTTAAACGATTCCACAACATTCTACTATGGCGGACGGTAACCCACATCGGGAAGATGAGGCGGCCGAGGAAGAAGAGGAGATTGATGAGACTGTACGCAAATTTACCCATGAACTTGGACTGGAACTCTGGAACTGACAATAAGATCAGAGCTACAAACCAGTCAAAGATGCGGTCCTCTTCGCAATCGATGTCAGCGATTCCATGTTGACGCCGCGCCCCTCGGCAGATCCTAAGAAACACACCCAAGAATCACCCACCACGGCAGCGCTCAAATGCGCCTATCACTTCATGCAACAACGAATCATATCAAATCCACAAGACATGATGGGTGTTTTGCTGTTCGGGACCCAGGCGTCCAAGTTCTTTGAAGAAGATGAAGACAGTCGGGGAGACCTGTCCTACCCCAACTGCTACCTCTTCACTGATCTGGATGTTCCTTCGGCTCATGAGGTCAAAGAACTTCGAGCACTGGTAGATGATGAAGGAGACTCAAGGGAGGTTCTATCTCCAGCGAAAGAGCAGGTCTCTATGGCAAACGTCCTATTTTGCGCCAACCAGATATTCACATCCAGAGCGCCAAATTTCCTCTCCCGGCGTTTGTTCATCATAACCGACAATGACAACCCCCATGGTGATGATAAAACCCTGCGGTCAGCGGCGACTGTACGTGCTAAGGATCTTTACGATCTTGGTGTCACAATTGAGCTGTTTCCGATCTCACGCCCTGAGCATGAGTTCAAGAACAGCAAGTTCTATGACGTAAGCTATCATACTCTATAGCAAAGTGGCAGGGGTCGATACTCACTACAGATACAAAGGATATTATCTACAAGTCATTGCCCAGCGATCCAGAGGCGCCTGCATATCTACAATCTGATTCAAAAGCGGCGACTGCGACCGGGGACGGGATTTCACTCCTCAACACGCTTCTGTCCAGTATTAATTCGAGAACGGTTCCGCGTCGCACTCATTTTTCGAACATGCCTTTAGAACTTGGCCCAGACTTCAGAATTTCGGTATCGGGCTATATACTCTTACGAAGGCAAGCGCCCGCTAGAAACTCCTTCATCTGGCTGAACGGCGAGAAGCCTGTGGTCGCGAAAGGAGTGACTTCCCACTCCGCAGATGATACTGGCCGGACTGTCGAGAAATGGGAGATCAGAAAGGCATATAAGTTCGGTGGCGACCAAGTAACCTTTTCGCCTGATGAGCAGAAGGCGCTTAGGGATTTCGGTGAGCCAGTAATCCGGGTTATTGGGTTCAAGCCTATCACTGCGCTTCCATTCTGGGCAAACGTCAAGCACCCATATTTTATCTATCCATCCGAGGAAGACTATGTAGGCTCCTCGCGAGTATTTTCCGCATTGCATCAGACTCTTTTGCGTTCCAAGAAGATGGCACTCGTCTGGTTCATTGCGCGCAAGGGTGCTGGCCCCGTTCTCGCCGCTATGATCGCAGGCGAAGAAAAGCTTGATGAGAATGGCGTACAAAAATACCCTCCTGGCATGTGGATTCTTCCCCTCCCCTTCGCAGACGATATCCGGCAGAACCCCGAAACAACGTTGAATGTCGCCCCGGAGTCATTGATTGATCAGATGCGCGTGGTCGTCCAGCAACTGCAGCTGCCGAAGGGAGTGTACGAGCCTCTCAAATACCCCAATCCATGTAAGTCACTGCTGTCTTGCATTGCTCGTATACGATGAACGAGAAGTTGACAGCCCGTGATCAGCCCTTCAATGGCATTACCGCATCCTACAAGCTCTCGCATTAGACGAAGATCTCCCTGAAAAACCAGAAGACAAAACCATTCCGAAATACCGCCAAATCGACAAGGTAAAACCACTACACCCAAGAAACAACCCTCCACGCATTCAACCTACTGACAATTGCACCGCAGCGCGCCGGTGACTACGTATTATCCTGGGCCGACGAACTCGAAAAGCAATACGCCAAAACCTCAGCAGCGGCCCCTCGCCCAACCAGCACCCTCGTGAAACGAGGATCAAAAGACCGAGCAAGCGAAACCGAGGACTCCAAGCCATCGAAAAAGATCAAGGTTGAGGAAGACTCTGGAAGCCTAGAGGAGGAAGTCCGCAGGCATCACAAGAAGGGAACGCTATCCAAGGTAAGCCACCACAGGCTTTCTACACGTCCTCGTGATGGCAAATATGACATCGTATTAACCGGCGGTTTTCTAGCTTACGGTCGCTATCCTCAAGGACTTCTTGACTTCCAATGGACGCTCAAATGCCGGTAAGAAGGCGGATCTTATTGAGCGGGTAGAGGAGTTCTTGGAGCAGTGACATGGCGGGATTGTTGGATTCGCTAGTGCGCTTCTGTTGGTGGATGTCGTTATGTGGTGTCTTATCTCGGGTTAGGCGTTCGTGACCTGAGGACATGAGCTTGTAATTAATGATGGGTTGGATGTCGCGGTATTCGTTCTTCAGCGAAACGTAATGGACACGTATTTTAGGCGATGTACAGTTATAAAAATCGAATTCGCTGGGCTAGCCGGACATGTCAAAACGAAGAGTATTAGGAGAGACATCAGGTCCAAGTGCTATTTTTCAAACCAGTCGCTTAAGACCACCGAGGCCTTTATCTCCAGAAAATATACCGGTTCAGCAGGTGCGCGTATCCCGAATTCAAATTAATATTGGAACGATCGTAAATAACCGCCCAGATTCGCCGTAAAACGATAGTAGTCAGGCTTTGCCGCCGACAGAAGGGGACGAGTATGTCAACTGAGTCAACTTGAACCGAGCAGCCCCTCTAAACAACGCCACGCTGTTTGTAATATCCCTTTAGAAACGTGTTGTCGCTGGCAATTATCCACAAAAAATGAGTCTAAACGGGCGAAAAAAGTCACCAAAATGGGAGAATATGTGGAAAGAAGAAAGAAAGAGAGACCAAAGCAAGAGAGCGCCGAAAGGAAGCTATCGTAATATATACAAGTAGAAGCCGTGGGTATTTTTATGAAAGCAGAAACGTTAACGGTATGCGTACAATGATCAACATTGTCCATAAACTTGACAGTAGCAGACTTCTTCGTCGGGACAGCTGAGAGTAGCGAAGTGTTAGTATTTAGGACGCATTCAGCAGGTAGACGGGGGAGGTGTGCAAAGGCAACATACTATATTGATTCTTTGCCGAATATGACATGCCAGAGAAATTCCATGACACGGCCACTACTGGCGTCATCCTTGTCGGTATCGATTATCCACTGGCGGATCTTGATGTAGTCCTCTCGTGGTCGTTGGTGGACCTGCTCCCGGGACACGGCGAATTGCGCACAGCACGCCG

*kusA repair sequence:*

TGATCGCGTCCCTCGCGACCGGATCCAGTGACGATATAGATCTCCCGAAGGCAAGCGCCCGCTAGAAACTCCTTCATCTGGCTGAACGGC

**PCR check:**

Same protocol as appendix A3 can be used.

*Adjustments:*

Extension time: 35 seconds

Annealing temperature: 52 °C

*PCR result on 0.6% agarose gel:*

Band length of a confirmed deletion: roughly 1150 bp

Band length of a failed deletion attempt: roughly 2300 bp