

**Table S1: DNA and protein sequences of FokI nuclease domain variants**

<b>FokI_WT nuclease domain</b>
<b>DNA Sequence:</b> CAACTAGTCAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTATG AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATC TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCAGA TGAAATGCAACGATATGTGAAGAAAATCAAACACGAAACAAACATATCAACCCTAATGAATGGTGGAAAGTCT ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC ACGATTAATCATATCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGAT TAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA
<b>Protein Sequence:</b> QLVKSELEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG VIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITN CNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF

### FokI nuclease domain mutant DA (Cathoman Lab)<sup>1</sup>

#### DNA Sequence:

CAACTAGTCAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT  
ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCCGATC  
TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCAGA  
TGAAATGCAAGATTATGTGCAAGAAAATCAAACACGAAACAAACATGCCAACCTAATGAATGGTGGAAAGTCT  
ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC  
ACGATTAATCATATCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGAT  
TAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA

#### Protein Sequence:

QLVKSELEEKSELRHLKLYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VIVDTKAYSGGYNLPIGA**A**ADEM**QD**YVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITN  
CNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF

### FokI nuclease domain mutant RV (Cathoman Lab)<sup>1</sup>

#### DNA Sequence:

CAACTAGTCAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT  
ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCCGATC  
TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCACG  
TGAAATGCAACGATATGTGCAAGAAAATCAAACACGAAACAAACATATCAACCCTAATGAATGGTGGAAAGTCT  
ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC  
ACGATTAATCATGTCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGAT  
TAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA

#### Protein Sequence:

QLVKSELEEKSELRHLKLYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VIVDTKAYSGGYNLPIG**QA**RE**M**QRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNH**V**T  
CNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF

## FokI nuclease domain mutant EL (Sangamo)<sup>2</sup>

### DNA Sequence:

CAACTAGTCAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT  
ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATC  
TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCAGA  
TGAAATGGAACGATATGTGCAAGAAAATCAAACACGAAACAAACATCTCAACCCTAATGAATGGTGGAAAGTCT  
ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC  
ACGATTAATCATATCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGAT  
TAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA

### Protein Sequence:

QLVKSELEEKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VIVDTKAYSGGYNLPIGQADEMERYVEENQTRNKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITN  
CNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF

## FokI nuclease domain mutant KK(Sangamo)<sup>2</sup>

### DNA Sequence:

CAACTAGTCAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT  
ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATC  
TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCAGA  
TGAAATGCAACGATATGTCAAAGAAAATCAAACACGAAACAAACATATCAACCCTAATGAATGGTGGAAAGTCT  
ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC  
ACGATTAATCATAAACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGAT  
TAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA

### Protein Sequence:

QLVKSELEEKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHKT  
CNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF

### FokI nuclease domain mutant REL (PBPL/JHU)<sup>3</sup>

#### DNA Sequence:

CAACTAGTCAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT  
ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATC  
TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCACG  
TGAAATGGAACGATATGTGCAAGAAAATCAAACACGAAACAAACATCTCAACCCTAATGAATGGTGGAAAGTCT  
ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC  
ACGATTAATCATATCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGAT  
TAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA

#### Protein Sequence:

QLVKSELEEKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VIVDTKAYSGGYNLPIGQAREMERYVEENQTRNKHLPNNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITN  
CNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

### FokI nuclease domain mutant DKK (PBPL/JHU)<sup>3</sup>

#### DNA Sequence:

CAACTAGTCAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT  
ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATC  
TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCAGA  
TGAAATGCAAGATTATGTCAAAGAAAATCAAACACGAAACAAACATATCAACCCTAATGAATGGTGGAAAGTCT  
ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC  
ACGATTAATCATAAACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGAT  
TAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA

#### Protein Sequence:

QLVKSELEEKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VIVDTKAYSGGYNLPIGQADEMQDYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHKT  
CNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

### FokI nuclease domain mutant RVEL (PBPL/JHU)<sup>3</sup>

#### DNA Sequence:

CAACTAGTCAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT  
ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATC  
TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCACG  
TGAAATGGAACGATATGTCAAGAAAATCAAACACGAAACAAACATCTCAACCCTAATGAATGGTGGAAAGTCT  
ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC  
ACGATTAATCATgTCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGATT  
AAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA

#### Protein Sequence:

QLVKSELEEKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VIVDTKAYSGGYNLPIGQAREMERYVEENQTRNKH~~L~~NPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNH~~V~~  
NCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

### FokI nuclease domain mutant DAKK (PBPL/JHU)<sup>3</sup>

#### DNA Sequence:

CAACTAGTCAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATAT  
ATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATC  
TCCTATTGATTACGGTGTGATCGTGGATACTAAAGCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCAGA  
TGAAATGCAAGATTATGTCAAAGAAAATCAAACACGAAACAAACATGCCAACCTAATGAATGGTGGAAAGTCT  
ATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTAC  
ACGATTAATCATAAACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGAT  
TAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGATAAACTTTTAA

#### Protein Sequence:

QLVKSELEEKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VIVDTKAYSGGYNLPIGA~~A~~DEM~~Q~~DYV~~K~~ENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNH~~K~~  
NCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

**FokI nuclease domain variant with StsI segment  
containing REL mutations (PBPL/JHU)<sup>3</sup>**

**DNA Sequence:**

GGACTAGTCAAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATA  
TATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCCGGATC  
TCCTATTgattgtgcaattattcttgattcaaaagcttactcagaaggctttcactcactgcctcccacacacagagctatggaaagatattgagg  
caatttacagagcgaaaagaagaactcaagccaacgtgggtgggatattgctccagaacatttagacaatacatatttcgcttacgtttctgggagttt  
tcgggtaattataaggaacagttacaaaaatttaggcaagatACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTA  
ATTGGTGGAGAAATGATTAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGA  
TAAACTTTTAA

**Protein Sequence:**

QLVKSELEEKSELRHKLKYPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VILDSKAYSEGFP**L**TASHT**R**AMERYL**R**QF**T**ERKEE**L**KPTWWDIAPEHLDNTYFAYVSGSFSG**NYKEQLQKFRQDT**NCN  
GAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF

**FokI nuclease domain variant with StsI segment  
containing DKK mutations (PBPL/JHU)<sup>3</sup>**

**DNA Sequence:**

GGACTAGTCAAAAAGTGAAGTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTGAAATATGTGCCTCATGAATA  
TATTGAATTAATTGAAATTGCCAGAAATCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATG  
AAAGTTTATGGATATAGAGGTAAACATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCCGGATC  
TCCTATTgattgtgcaattattcttgattcaaaagcttactcagaaggctttcactcactgcctcccacacagatgctatgggagattattgaaac  
aatttacagagcgaaaagaagaataaagccaacgtgggtgggatattgctccagaacatttagacaatacatatttcgcttacgtttctgggagttt  
tcgggtaattataaggaacagttacaaaaatttaggcaaaaaACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTA  
ATTGGTGGAGAAATGATTAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAATTTAATAACGGCGAGA  
TAAACTTTTAA

**Protein Sequence:**

QLVKSELEEKSELRHKLKYPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYG  
VILDSKAYSEGFP**L**TASHT**D**AM**G**DY**L**KQF**T**ERKEE**I**KPTWWDIAPEHLDNTYFAYVSGSFSG**NYKEQLQKFRQ**K****NCN  
GAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF

Helices  $\alpha 4$  and  $\alpha 5$  are highlighted in yellow color; StsI segment incorporated into FokI nuclease domain is shown in blue.

<sup>1</sup>Szcepek et al 2007; <sup>2</sup>Miller et al 2007; <sup>3</sup>This article