B351 T0 ENG1 contigs

T0 amplicons

```
ACGCTTCCAGGCCGG----------------CCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCA--T-CTACCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCAT------CCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCATCT-CTACCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGAC
ACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGAC
```

wildtype reference `ACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGAC`

t3 PAM

B373 T0 ENG1 contigs

T0 amplicons

```
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCC---------------CACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCT--TACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCT-CTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCAT---CTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCC---------CCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC
```

wildtype reference `GACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCC`

t3 PAM

B374 T0 ENG1 contigs

T0 amplicons

```
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCAT---CTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCT-CTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCG---------------------------ACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC
```

wildtype reference `GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACC`

t3 PAM

B389 T0 ENG1 contigs

T0 amplicons

```
AGGTCGGTGATGGGGTAG---ATGGGCGGCGCCGGCCTGGAAG
AGGTCGGTGATGGGGTAG---ATGGGCGGCGCCGGCCTGGAAG
AGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAG
AGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAG
AGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAG
AGGTCGGTGATGGGGTAG---ATGGGCGGCGCCGGCCTGGAAG
```

wildtype reference `AGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAG`

t3 PAM

B393 T0 ENG1 contigs

```
                     GAGGTCGGTGATGGGGTAG---ATGGGCGGCGCCGGCCT
                     GAGGTCGGTGATGGGGTAG---ATGGGCGGCGCCGGCCT
T0 amplicons         GAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCT
                     GAGGTCGGTGATGGGGTAG---ATGGGCGGCGCCGGCCT
                     GAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCT
wildtype reference   GAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCT
                                         t3 PAM
```

B395 T0 ENG1 contigs

```
                     CGGTGATGGGGTA---AGATGGGCGGCGC
                     CGGTGATGGG----------GGGCGGCGC
                     CGGTGATGGGGTA---AGATGGGCGGCGC
T0 amplicons         CGGTGATGGGGTAGGAAGATGGGCGGCGC
                     CGGTGATGGGG-------ATGGGCGGCGC
                     CGGTGATGGGGTAG--AGATGGGCGGCGC
                     CGGTGATAAGGTAGGGAGATGGGCGGCGC
wildtype reference   CGGTGATGGGGTAGG-AGATGGGCGGCGC
                                       t3 PAM
```

T1 ENG1 contigs B395.1 (cas9 negative)

```
                     CGGTGATGGGGTAG-AGATGGGCGGCGC
                     CGGTGATGGGGTAG-AGATGGGCGGCGC
                     CGGTGATGGGGTAG-AGATGGGCGGCGC
T1 amplicons         CGGTGATGGGGTAG-AGATGGGCGGCGC
                     CGGTGATGGGGTAG-AGATGGGCGGCGC
                     CGGTGATGGGGTAG-AGATGGGCGGCGC
wildtype reference   CGGTGATGGGGTAGGAGATGGGCGGCGC
                                     t3 PAM
```

B396 T0 ENG1 contigs

```
                    CGGTGATGGGGTAGGAGATGGGCGGCGC(
                    CGGTGATGGGGTAG---ATGGGCGGCGC(
                    CGGTGATGGGGTAG---ATGGGCGGCGC(
T0 amplicons        CGGTGATGGGGTAGGAGATGGGCGGCGC(
                    CGGTGATGGGGTAG---ATGGGCGGCGC(
                    CGGTGATGGGGTAGGAGATGGGCGGCGC(
wildtype reference  CGGTGATGGGGTAGGAGATGGGCGGCGC(
                                       t3 PAM
```

T1 ENG1 contigs B396.1 (cas9 positive)

```
                    CGGTGATGGGGTAG---ATGGGCGGCGC
                    CGGTGATGGGGTAG---ATGGGCGGCGC
                    CGGTGATGGGGTAG---ATGGGCGGCGC
T1 amplicons        CGGTGATGGGGTAG---ATGGGCGGCGC
                    CGGTGATGGGGTAG---ATGGGCGGCGC
                    CGGTGATGGGGTAG---ATGGGCGGCGC
wildtype reference  CGGTGATGGGGTAGGAGATGGGCGGCGC
                                       t3 PAM
```

T1 ENG1 contigs B396.2 (cas9 negative)

```
                    CGGTGATGGGGTAGGAGATGGGCGGCGC(
                    CGGTGATGGGGTAG---ATGGGCGGCGC(
                    CGGTGATGGGGTAG---ATGGGCGGCGC(
T1 amplicons        CGGTGATGGGGTAGGAGATGGGCGGCGC(
                    CGGTGATGGGGTAG---ATGGGCGGCGC(
                    CGGTGATGGGGTAG---ATGGGCGGCGC(
wildtype reference  CGGTGATGGGGTAGGAGATGGGCGGCGC(
                                       t3 PAM
```

B410 T0 ENG1 contigs

T0 amplicons

wildtype reference

```
GCCCATCT--T--CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA--GCCGCT
GCCCATCTC-TACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA--GCCGCT
GCCCATCTC-TACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA--GCCGCT
GCCCATCT--T--CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCATCGCCGCT
GCCCATCTC-TACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA--GCCGCT
GCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCAC-GCCGCT
```

t3 PAM   tA PAM

B411 T0 ENG1 contigs

T0 amplicons

wildtype reference

```
CGGCGGGA--------------------------------------------------------------------AGCGTCGAACGGCGG
CGGCGGGA--------------------------------------------------------------------AGCGTCGAACGGCGG
CGGCGGGA--------------------------------------------------------------------AGCGTCGAACGGCGG
CGGCGGGA--------------------------------------------------------------------AGCGTCGAACGGCGG
CGGCGGGA--------------------------------------------------------------------AGCGTCGAACGGCGG
CGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGC-------------------------AGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
CGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGC-------------------------AGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
CGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGC-------------------------AGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
CGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
```

tA PAM   t3 PAM

T1 ENG1 contigs B411.1 (cas9 negative)

T1 amplicons

wildtype reference

```
GCCGTTCGACGCT-----------------------------------CCCATCT------------------------------TCCCGCCGCGG
GCCGTTCGACGCT-----------------------------------CCCATCT------------------------------TCCCGCCGCGG
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCT----------------------------GCCGCTCCCCTCTCCCGGGGCGCCGCTCCCGCCGCGG
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCT----------------------------GCCGCTCCCCTCTCCCGGGGCGCCGCTCCCGCCGCGG
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCT----------------------------GCCGCTCCCCTCTCCCGGGGCGCCGCTCCCGCCGCGG
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCT----------------------------GCCGCTCCCCTCTCCCGGGGCGCCGCTCCCGCCGCGG
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCT----------------------------GCCGCTCCCCTCTCCCGGGGCGCCGCTCCCGCCGCGG
GCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCACGCCGCTCCCCTCTCCCGGGGCGCCGCTCCCGCCGCGG
```

t3 PAM   tA PAM

T1 ENG1 contigs B411.2 (cas9 negative)

T1 amplicons

```
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
AGGCCGGCGCCGCCCATCT-----------------------------------------------GCCGCTCCCCTCTCCCGGGGCGC
```

wildtype reference
```
AGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCACGCCGCTCCCCTCTCCCGGGGCGC
```
t3 PAM   tA PAM

B413 T0 ENG1 contigs

T0 amplicons

```
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
AGGGGAGCGGCG------------------------------------------------------AGATGGGCGG
```

wildtype reference
```
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGG
```
tA PAM   t3 PAM

T1 ENG1 contigs B413.1

T1 amplicons

```
GGGAGAGGGGAGC----TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTA--AGATGGGCGGCGCCGG
GGGAGAGGGGAGC----TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTA--AGATGGGCGGCGCCGG
GGGAGAGGGGAGC----TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTA--AGATGGGCGGCGCCGG
GGGAGAGGGGAGC----TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTA--AGATGGGCGGCGCCGG
GGGAGAGGGGAGC----TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTA--AGATGGGCGGCGCCGG
GGGAGAGGGGAGC----TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTA--AGATGGGCGGCGCCGG
```

wildtype reference
```
GGGAGAGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGG
```
tA PAM   t3 PAM

T1 ENG1 contigs B413.2 (cas9 negative)

```
            AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
            AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
            AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
            AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
T1 amplicons AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
            AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
            AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
            AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
            AGAGGGGAGCGGCG--------------------------------------------------------------------AGATGGGCGGCGCCGGC
wildtype reference AGAGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGC
                          tA PAM                                                                    t3 PAM
```

B420 T0 ENG1 contigs

```
T0 amplicon      GGCGCCGCCCATCT-------------------------------------------------------------------------CGCCGCTCCCCTCTC
wildtype reference GGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCACGCCGCTCCCCTCTC
                       t3 PAM                                                                        tA PAM
```

```
            GCCGCCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA-GCCGCTCC
            GCCGCCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA-GCCGCTCC
T0 amplicons GCCGCCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA-GCCGCTCC
            GCCGCCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA-GCCGCTCC
wildtype reference GCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCACGCCGCTCC
                   t3 PAM                                                                       tA PAM
```

```
T0 amplicon      GAGCGGC-TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAG-----GGGCGGCG
wildtype reference GAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCG
                        tA PAM                                                                           t3 PAM
```

T1 ENG1 contigs B420.1

```
            GAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGC
            GAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGC
            GAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGC
T1 amplicons GAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGC
            GAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGC
            GAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGC
wildtype reference GAGCGGCG-TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGC
                       tA PAM                                                                        t3 PAM
```

T1 ENG1 contigs B420.2 (cas9 negative)

```
GGAGCGGC--TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGC
GGAGCGGC--TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGC
GGAGCGGC--TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGC
GGAGCGGC--TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGC
GGAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGCGC
GGAGCGGC--TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGC
GGAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGCGC
GGAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGCGC
GGAGCGGCGGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGA----AGATGGGCGGCGC
GGAGCGGCG-TGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGC
```
           tA PAM   **t3 PAM**

B421 T0 ENG1 contigs

```
              CCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCATCGCCGCTCC
              CCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCATCGCCGCTCC
              CCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA-CGCCGCTCC
T0 amplicons  CCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCATCGCCGCTCC
              CCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCATCGCCGCTCC
              CCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCATCGCCGCTCC
              CCCATCTT----CCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA-CGCCGCTCC
wildtype reference CCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA-CGCCGCTCC
```
   t3 PAM   **tA PAM**

B422 T0 ENG1 contigs

```
                   GGGAGAGGGGAGCGGC--TGGAGGCGCGGTTGAA
                   GGGAGAGGGGAGCGGC--TGGAGGCGCGGTTGAA
                   GGGAGAGGGGAGCGGC--TGGAGGCGCGGTTGAA
T0 amplicons       GGGAGAGGGGAGCGGCG-TGGAGGCGCGGTTGAA
                   GGGAGAGGGGAGCGGCG-TGGAGGCGCGGTTGAA
                   GGGAGAGGGGAGCGGCGTTGGAGGCGCGGTTGAA
                   GGGAGAGGGGAGCGGCG-TGGAGGCGCGGTTGAA
wildtype reference GGGAGAGGGGAGCGGCG-TGGAGGCGCGGTTGAA
                                      tA PAM
```

B423 T0 ENG1 contigs

```
                   GGGAGCGGC-TGGAGGCGCGGTTGAAGGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTA-GAGATGGGCGGCGCCC
                   GGGAGCGGC-------------------------------------------------------------------------------------------GAGATGGGCGGCGCCC
T0 amplicons       GGGAGCGGC-TGGAGGCGCGGTTGAAGGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTA-GAGATGGGCGGCGCCC
                   GGGAGCGGC-------------------------------------------------------------------------------------------GAGATGGGCGGCGCCC
wildtype reference GGGAGCGGCGTGGAGGCGCGGTTGAAGGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCC
                             tA PAM                                                                                   t3 PAM
```

B426 T0 ENG1 contigs

```
                   CGGAGAGGT-------------------GGCGGCGCCGG
                   CGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGG
                   CGGAGAGGT-------------------GGCGGCGCCGG
T0 amplicons       CGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGG
                   CGGAGAGGT-------------------GGCGGCGCCGG
                   CGGAGAGGT-------------------GGCGGCGCCGG
                   CGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGG
wildtype reference CGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGG
                                               t2 PAM
```

B427 T0 ENG1 contigs

```
              GGGGTAGGAG-----GCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
              GGGGTAGGAGAT-GGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
              GGGGTAGGAGATTGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
              GGGGTAGGAGAT-GGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
T0 amplicons  GGGGTAGGAGAT-GGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
              GGGGTAGGAGA-------------------------ACGGCGG
              GGGGTAGGAGAT-GGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
              GGGGTAGGAGAT-GGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
              GGGGTAGGAGAT-GGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
wildtype reference  GGGGTAGGAGAT-GGGCGGCGCCGGCCTGGAAGCGTCGAACGGCGG
                                t2 PAM
```

B428 T0 ENG1 contigs

```
              GGTGATGGGGTAG-AGATGGGCGGCGC(
              GGTGATGGGGTAG-AGATGGGCGGCGC(
              GGTGATGGGGTAGGAGATGGGCGGCGC(
              GGTGATGGGGTAGGAGATGGGCGGCGC(
T0 amplicons  GGTGATGGGGTAGGAGATGGGCGGCGC(
              GGTGATGGGGTAGGAGATGGGCGGCGC(
              GGTGATGGGGTAGGAGATGGGCGGCGC(
              GGTGATGGGGT----GATGGGCGGCGC(
wildtype reference  GGTGATGGGGTAGGAGATGGGCGGCGC(
                            t3 PAM
```

B429 T0 ENG1 contigs

```
              AGGTCGGTGATGGGGTA---AGATGGGCGGCGCCGGCCT
              AGGTCGGTGATGGGGTAGG-AGATGGGCGGCGCCGGCCT
              AGGTCGGTGATGGGGTAGGTAGATGGGCGGCGCCGGCCT
T0 amplicons  AGGTCGGTGATGGGGTA---AGATGGGCGGCGCCGGCCT
              AGGTCGGTGATGGGGTAGG-AGATGGGCGGCGCCGGCCT
              AGGTCGGTGATGGGGTAGG-AGATGGGCGGCGCCGGCCT
              AGGTCGGTGATGGG-------------CGGCGCCGGCCT
wildtype reference  AGGTCGGTGATGGGGTAGG-AGATGGGCGGCGCCGGCCT
                                t3 PAM
```

B433 T0 ENG1 contigs

```
T0 amplicon        GCGCCCCGGGAGA-----------------------------------------------------------------TGGGCGGCGCCGGCC
wildtype reference GCGCCCCGGGAGAGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCC
                                       tA PAM                                                                                    t3 PAM
```

```
                   TCGGTGATGGG------------CGGCGCCGGC(
T0 amplicons       TCGGTGATGGG------------CGGCGCCGGC(
                   TCGGTGATGGG------------CGGCGCCGGC(
wildtype reference TCGGTGATGGGGTAGGAGATGGGCGGCGCCGGC(
                                 t3 PAM
```

B435 T0 ENG1 contigs

```
                   TGGGGTAGGAGA--------------------GCGTCGAACGGCG
                   TGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCG
T0 amplicons       TGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCG
                   TGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCG
wildtype reference TGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGCG
                                      t2 PAM
```

B436 T0 ENG1 contigs

```
                   CATGACCCTGC-------------------------------------------------------CCTACCCCATCA
                   CATGACCCTGC-------------------------------------------------------CCTACCCCATCA
T0 amplicons       CATGACCCTGC-------------------------------------------------------CCTACCCCATCA
                   CATGACCCTGC-------------------------------------------------------CCTACCCCATCA
                   CATGACCCTGCCCGCCGCGGACGACGCCGCCGCCTCCGAAGGCGAGCGGCGCGACTGGGAGCCGCCGTTCGACGCTTCCAGGCCGGCGCCGCCCTCT-CCTACCCCATCA
                   CATGACCCTGCCCGCCGCGGACGACGCCGCCGCCTCCGAAGGCGAGCGGCGCGACTGGGAGCCGCCGTTCGACGCTTCCAGGCCGGCGCCGCCCTCT-CCTACCCCATCA
wildtype reference CATGACCCTGCCCGCCGCGGACGACGCCGCCGCCTCCGAAGGCGAGCGGCGCGACTGGGAGCCGCCGTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCA
                                                                                                     t2 PAM
```

B438 T0 ENG1 contigs

T0 amplicons

wildtype reference

```
GTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA----CGCTCCCCTCTC
GTTCGACGCTTCC-GGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCAACGCCGCTCCCCTCTC
GTTCGACGCTT-CAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA--GCCGCTCCCCTCTC
GTTCGACGCTTCT-GGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCA--GCCGCTCCCCTCTC
GTTCGACGCTTCCAGGCCGGCGCCGCCCATCTCCTACCCCATCACCGACCTCTCCGCGCTCGCCTCCCGCTCCTACCTCTCCGAGGCCTCCAACTTCCACCTCCCCTTCAACCGCGCCTCCAC-GCCGCTCCCCTCTC
```

t1 PAM   tA PAM

B439 T0 ENG1 contigs

T0 amplicons

wildtype reference

```
AGCGGCGCCCCGG-------------------------------------------------------AAGCGTCGAACGGC
AGCGGCGCCCCGGGA----------------------------------------------------TGGGCGGCGCCGGCCTGGAAGCGTCGAACGGC
AGCGGCGCCCCGGGAGA-------------------------------------------------TGGGCGGCGCCGGCCTGGAAGCGTCGAACGG
AGCGGCGCCCCGGGAGA-------------------------------------------------TGGGCGGCGCCGGCCTGGAAGCGTCGAACGG
AGCGGCGCCCCGGGAGA-------------------------------------------------TGGGCGGCGCCGGCCTGGAAGCGTCGAACGG
AGCGGCGCCCCGGGAGA-------------------------------------------------TGGGCGGCGCCGGCCTGGAAGCGTCGAACGG
AGCGGCGCCCCGGGAGA-------------------------------------------------TGGGCGGCGCCGGCCTGGAAGCGTCGAACGG
AGCGGCGCCCCGGGAGA-------------------------------------------------TGGGCGGCGCCGGCCTGGAAGCGTCGAACGG
AGCGGCGCCCCGGGAGAGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACGGC
```

tB PAM   t3 PAM

B440 T0 ENG1 contigs

T0 amplicons

wildtype reference

```
AGGGGAGCGG-----AGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCC-GGAAGCGTCGAACG
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACG
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCC---AAGCGTCGAACG
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACG
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACG
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACG
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACG
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACG
AGGGGAGCGGCGTGGAGGCGCGGTTGAAGGGGAGGTGGAAGTTGGAGGCCTCGGAGAGGTAGGAGCGGGAGGCGAGCGCGGAGAGGTCGGTGATGGGGTAGGAGATGGGCGGCGCCGGCCTGGAAGCGTCGAACG
```

tA PAM   t1 PAM

B442 T0 ENG1 contigs

T0 amplicons

wildtype reference

```
GCGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGCC
GCGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGCC
GCGGCGGGAGC---------GGAGAGGGGAGCGGCC
GCGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGCC
GCGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGCC
GCGGCGGGAGCGGCGCCCCGGGAGAGGGGAGCGGCC
```

t2 PAM