

Supplementary Information

Promoter RNA sequencing (PRSeq) for the massive and quantitative promoter analysis *in vitro*

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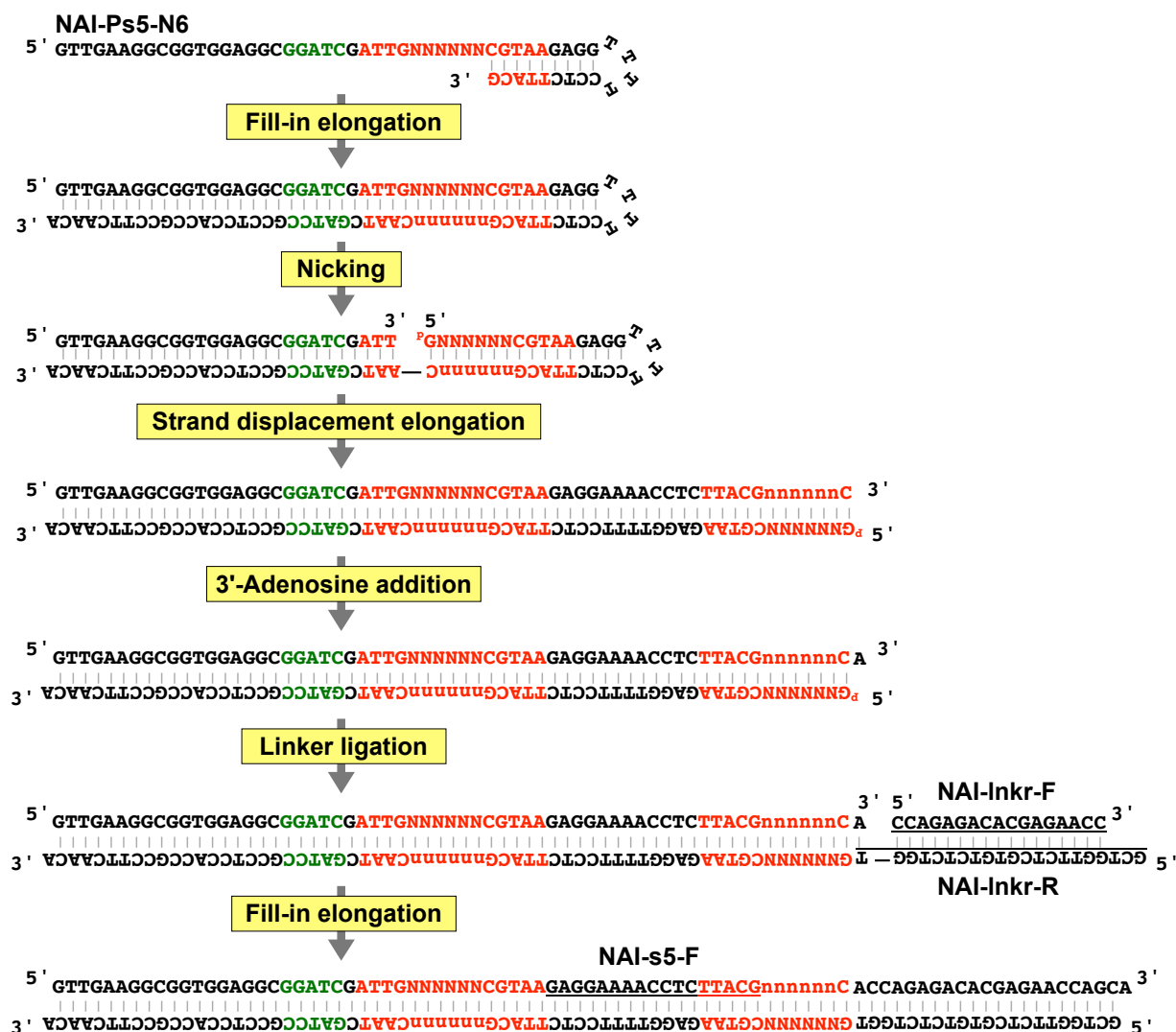
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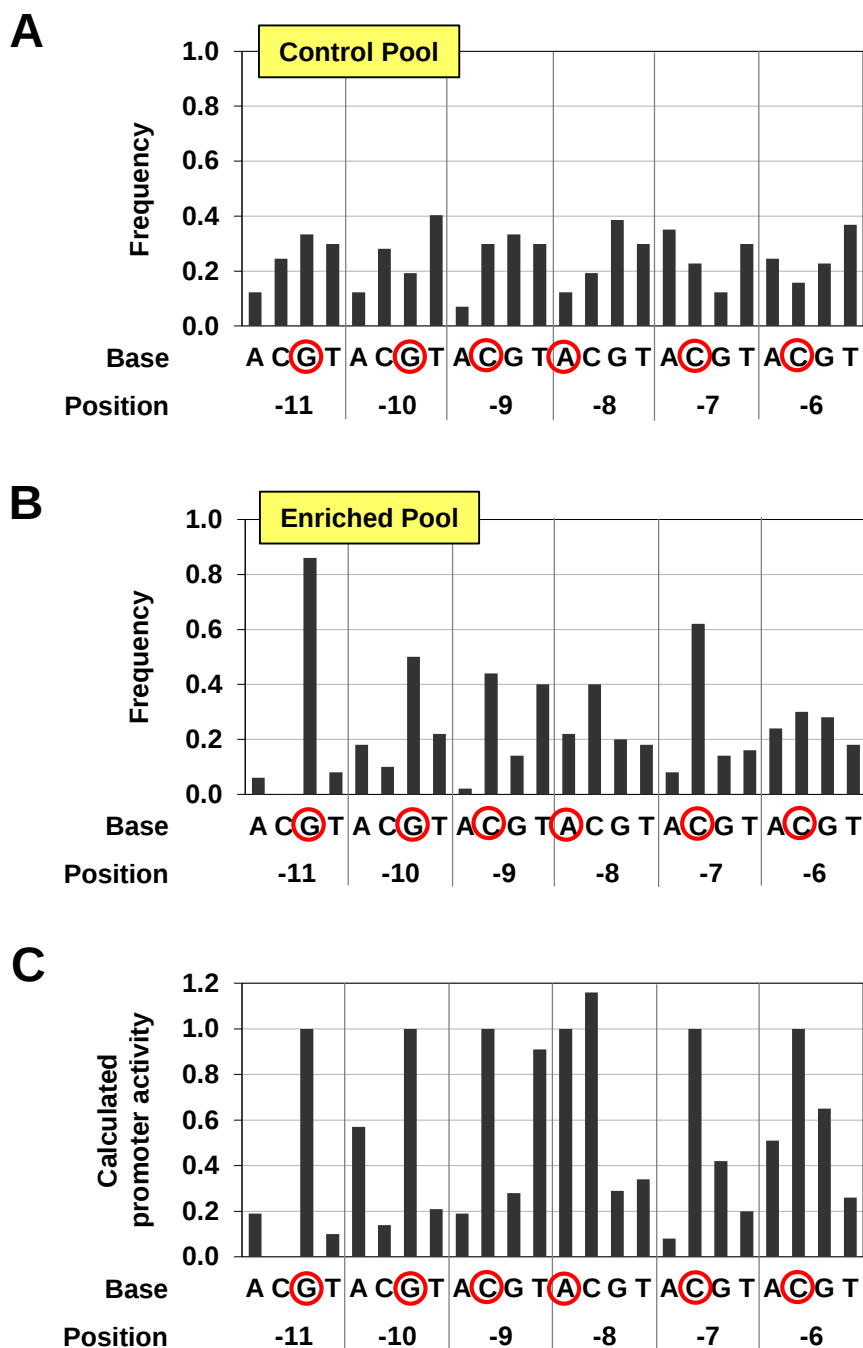
Supplemental Table S4. Sequences of clones from template DNA pool for Syn5 promoter analysis.

Supplemental Table S5. Sequences of clones from the control Syn5 promoter pool.

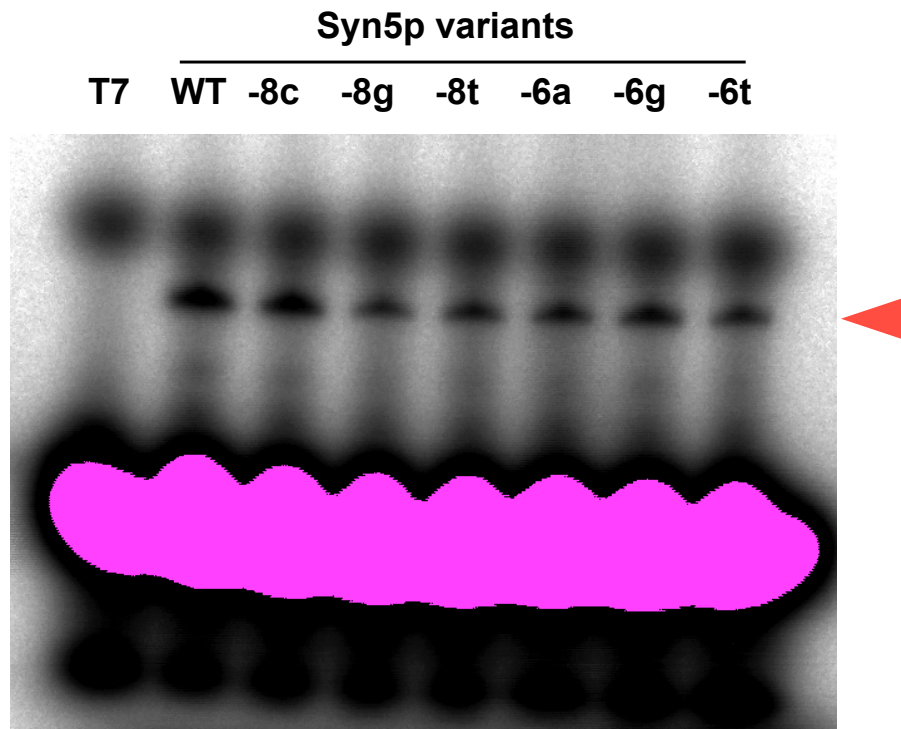
Supplemental Table S6. Sequences of clones from the enriched Syn5 promoter pool.



For the Figure explanation, see Supplemental Figure S1.



Supplemental Figure S3. Syn5 promoter analysis by PRSeq. (A) Sequence frequency of randomized -11 to -6 region of Syn5 promoter in the control pool. After the control reaction, variants in the pool were cloned, and 57 clones were randomly picked out for sequencing. The wild-type promoter sequence is emphasized by red circles. The clone sequences are listed in Supplemental Table S2. **(B)** Sequence frequency of randomized -11 to -6 region of Syn5 promoter in the enriched pool. After the PRSeq employing Syn5 RNAP, variants in the pool were cloned, and 50 clones were randomly picked out for sequencing. The clone sequences are listed in Supplemental Table S3. **(C)** Promoter activities of Syn5 promoter variants calculated according to the sequence frequencies of above figures.



Supplemental Figure S4. Full-length gel image for the validation of promoter activities of Syn5 promoter variants.

The full-length image of the gel displayed in Figure 3A is shown. Template DNAs with six single mutations of Syn5 promoter were subjected for IVTX. As a negative control and positive standard, the reactions employing the DNAs with T7 promoter (T7) and wild-type Syn5 promoter (WT), respectively, were also carried out. Mean values of three independent duplicate experiments were shown under the gel image. Mutations A(-8)C, A(-8)G, A(-8)T, C(-6)A, C(-6)G, and C(-6)T are indicated as -8c, -8g, -8t, -6a, -6g, and -6t, respectively. The position of the corresponding transcripts is indicated by red arrowhead.

Supplemental Table S1. Sequences of template DNAs for the evaluation of mutations on Syn5 promoter

Name	Sequence (5'-to-3') ^a	Promoter
T7p-D01	<u>TAATACGACT</u> <u>CACTATA</u> GGG ATCGACAGGG CTCCCGATTG GCTCGGCCGA GATCGCCTGT ACTGTGAAA GCCGAGTCGT G	T7 promoter (negative control)
Syn5p-D01	GGT <u>ATTGGC</u> <u>ACCCGTA</u> AGG GATCGACAGG GCTCCCGATT GGCTCGGCCGA AGATCGCCTG TACTGTGAA AGCCGAGTCG TG	Wild-type Syn5 promoter
Syn5p(-8c)-D01	GGTATTGGGC <u>CCCCGTA</u> AGG GATCGACAGG GCTCCCGATT GGCTCGGCCGA AGATCGCCTG TACTGTGAA AGCCGAGTCG TG	A(-8)C mutant of Syn5 promoter
Syn5p(-8g)-D01	GGT <u>ATTGGC</u> <u>qCCCCGTA</u> AGG GATCGACAGG GCTCCCGATT GGCTCGGCCGA AGATCGCCTG TACTGTGAA AGCCGAGTCG TG	A(-8)G mutant of Syn5 promoter
Syn5p(-8t)-D01	GGTATTGGGC <u>tCCCCGTA</u> AGG GATCGACAGG GCTCCCGATT GGCTCGGCCGA AGATCGCCTG TACTGTGAA AGCCGAGTCG TG	A(-8)T mutant of Syn5 promoter
Syn5p(-6a)-D01	GGT <u>ATTGGC</u> <u>ACaCGTA</u> AGG GATCGACAGG GCTCCCGATT GGCTCGGCCGA AGATCGCCTG TACTGTGAA AGCCGAGTCG TG	C(-6)A mutant of Syn5 promoter
Syn5p(-6g)-D01	GGTATTGGGC <u>ACqCGTA</u> AGG GATCGACAGG GCTCCCGATT GGCTCGGCCGA AGATCGCCTG TACTGTGAA AGCCGAGTCG TG	C(-6)G mutant of Syn5 promoter
Syn5p(-6t)-D01	GGT <u>ATTGGC</u> <u>ACtCGTA</u> AGG GATCGACAGG GCTCCCGATT GGCTCGGCCGA AGATCGCCTG TACTGTGAA AGCCGAGTCG TG	C(-6)T mutant of Syn5 promoter

a) Promoter sequences are emphasized by underlines, and mutations are indicated by lowercase letters.

Supplemental Table S2. Sequences of clones from template DNA pool for T7 promoter analysis^a

GGTGAGGCCGTTGAGGCGGATCTAATA	TTTCGA	ACTATAGGGAACCCTATAGT	TCGAAA	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GGCATC	ACTATAGGGAACCCTATAGT	GATGCC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GTGAGT	ACTATAGGGAACCCTATAGT	ACTCAC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GACGTT	ACTATAGGGAACCCTATAGT	AACGTC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	CTTGCT	ACTATAGGGAACCCTATAGT	ACCAAG	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GTTTGG	ACTATAGGGAACCCTATAGT	CCAAC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GACAGC	ACTATAGGGAACCCTATAGT	GCTGTC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GTCCGG	ACTATAGGGAACCCTATAGT	CCGGAC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	AGCCTG	ACTATAGGGAACCCTATAGT	CAGCCT	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	AGTTGA	ACTATAGGGAACCCTATAGT	TCAACT	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	TTGAAT	ACTATAGGGAACCCTATAGT	ATTCAA	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	AAATGA	ACTATAGGGAACCCTATAGT	TCATTT	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	TTCGTT	ACTATAGGGAACCCTATAGT	AACGAA	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	CGTGTC	ACTATAGGGAACCCTATAGT	GACACG	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	CGGTGG	ACTATAGGGAACCCTATAGT	CCACC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	CTTCGG	ACTATAGGGAACCCTATAGT	CCGAAG	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	TTTCTT	ACTATAGGGAACCCTATAGT	AAGAAA	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	AGAGTG	ACTATAGGGAACCCTATAGT	CACCTCT	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GCTCCT	ACTATAGGGAACCCTATAGT	AGGAGC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	ATTGAG	ACTATAGGGAACCCTATAGT	CTCAAT	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	TTTTTC	ACTATAGGGAACCCTATAGT	GAAAAA	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GCATTT	ACTATAGGGAACCCTATAGT	AAATGC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	GACGTG	ACTATAGGGAACCCTATAGT	CACGTC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	CCGTGG	ACTATAGGGAACCCTATAGT	CCACGC	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	CTGGCG	ACTATAGGGAACCCTATAGT	CGCCAG	TACCAAGATCCCGCTCCAGCTCA
GGTGAGGCCGTTGAGGCGGATCTAATA	CTTTGA	ACTATAGGGAACCCTATAGT	TCAAAAG	TACCAAGATCCCGCTCCAGCTCA

Wild-type T7 promoter: TAATA CGACTC ACTATA

a) The sequences are represented as 5'-to-3' orientation. The six nucleotides between spaces indicate the randomized region. The transcribed region is emphasized by *Italics*. For reference, the wild-type T7 promoter sequence is shown at the bottom of the table.

Supplemental Table S3. Sequences of clones from the enriched T7 promoter pool^a

TGAGCTGGAGCGGGATCGTGGTA	GAGCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CGACGC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	TGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	AGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CGCCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GATCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	AGTCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	AGTATC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GGCCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CATCAG	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CGTCAG	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CGCCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GGCCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	AGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CGCTCG	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GGTCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GCCCGA	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	TGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GGGCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	TGCCTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	AAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	GAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CGACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TGAGCTGGAGCGGGATCGTGGTA	CAACTC	ACTATAGGGTTTTCCCTGTGAAGTGAA
TAATA CGACTC		ACTATA: Wild-type T7 promoter

a) The sequences are represented as 5'-to-3' orientation. The six nucleotides between spaces indicate the randomized region (positions -12 to -7). For reference, the wild-type T7 promoter sequence is shown at the bottom of the table.

Supplemental Table S4. Sequences of clones from template DNA pool for Syn5 promoter analysis^a

GTTGAAGGCGGTGAGGCGCGATCGATTG	TGTGTC	CGTAAAGAGGAAAACTCTTTACG	GACACA	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	AGGGT	CGTAAAGAGGAAAACTCTTTACG	ACCCCT	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GCCCTG	CGTAAAGAGGAAAACTCTTTACG	CAAGGC	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GACACC	CGTAAAGAGGAAAACTCTTTACG	GGTGTc	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GCTGGC	CGTAAAGAGGAAAACTCTTTACG	GCCAGC	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GTGTAG	CGTAAAGAGGAAAACTCTTTACG	CTACAC	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GGTCAG	CGTAAAGAGGAAAACTCTTTACG	CTGACC	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	TGTTAT	CGTAAAGAGGAAAACTCTTTACG	ATAACA	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GGCTCC	CGTAAAGAGGAAAACTCTTTACG	GGA GCC	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	TGTTGA	CGTAAAGAGGAAAACTCTTTACG	TCAACA	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GGCCCT	CGTAAAGAGGAAAACTCTTTACG	AGGCC	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	TCACAC	CGTAAAGAGGAAAACTCTTTACG	GTGTGA	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	CTTCTC	CGTAAAGAGGAAAACTCTTTACG	GAGAAG	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	TCGGGA	CGTAAAGAGGAAAACTCTTTACG	TCCCGA	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	CATTTC	CGTAAAGAGGAAAACTCTTTACG	GAAATG	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	AACTAC	CGTAAAGAGGAAAACTCTTTACG	GTA GTT	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	TGCCAT	CGTAAAGAGGAAAACTCTTTACG	ATGGCA	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GCTCCA	CGTAAAGAGGAAAACTCTTTACG	TGGAGC	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	CTTGTA	CGTAAAGAGGAAAACTCTTTACG	TACAAG	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	CCTCGC	CGTAAAGAGGAAAACTCTTTACG	GCGAGG	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	CCGTAT	CGTAAAGAGGAAAACTCTTTACG	ATA CGG	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	TGTCAC	CGTAAAGAGGAAAACTCTTTACG	GTGACA	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	GTCCCA	CGTAAAGAGGAAAACTCTTTACG	TGGGAC	CACCAAGACACGAGAACCAGCA
GTTGAAGGCGGTGAGGCGCGATCGATTG	TTCTAA	CGTAAAGAGGAAAACTCTTTACG	TTAGAA	CACCAAGACACGAGAACCAGCA

Wild-type Syn5 promoter:ATTG GGCACC CGTAA

a) The sequences are represented as 5'-to-3' orientation. The six nucleotides between spaces indicate the randomized region. The transcribed region is emphasized by *Italics*. For reference, the wild-type Syn5 promoter sequence is shown at the bottom of the table.

Supplemental Table S5. Sequences of clones from the control Syn5 promoter pool^a

GCTGGTTCTCGTGTCTCTGGTG	CCGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CCTGTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	ACCTTT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGTCTA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GCGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	ATGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTTAAA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CCCGGG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TTGTCT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TTCTTA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TCCGTC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCCAA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTTCAA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CTCTGG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CATTTA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CCGCAC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TCCTCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTCGGG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CTTTCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGAACG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GAGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CTTAAC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	ATGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CCCCCT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CTTCTC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CTCTTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TCACTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGTACT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CTCTGC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGTTGG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTGCTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TATTTA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGCCTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GAGGTT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GCTTTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CCGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	AAT'TGA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TATACT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTATCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TTCGCT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCATA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	ACGGCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TCCCGC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GCATTT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	ACTCCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	AGTTAC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GAGGTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTCGAC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	CTGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGT'TCT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TTCACC	CGTAAGAGGAAAACCTCGGC
ATTG	GGCACC	CGTAA: Wild-type Syn5 promoter

a) The sequences are represented as 5'-to-3' orientation. The six nucleotides between spaces indicate the randomized region (positions -11 to -6). For reference, the wild-type Syn5 promoter sequence is shown at the bottom of the table.

Supplemental Table S6. Sequences of clones from the enriched Syn5 promoter pool^a

GCTGGTTCTCGTGTCTCTGGTG	GACGCC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTCCT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTCACC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	ATTGTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTCCG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	ATGTAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTCTCT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTTCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCTCG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCCTC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GCAGCC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GATGGG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GCTGCC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTCACT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GCCCGT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTCACC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTACC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTCTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCACG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCTCC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTCGA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GCCCCG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGGGAT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTCACC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTCATC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GACACT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTACA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCGCG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCTCC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTGCCG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GACCCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GATTTA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	AGGAGC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCGCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TTTCGA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GATCCA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGGCCG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCGCC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTCTT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TAGGAG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTTCAA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCCTA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GCTCCC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GACTCC	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GATACG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGTCTG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCTCG	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GTGCGT	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	GGCCGA	CGTAAGAGGAAAACCTCGGC
GCTGGTTCTCGTGTCTCTGGTG	TGTCCA	CGTAAGAGGAAAACCTCGGC
<u>ATTG GGCACC CGTAA: Wild-type Syn5 promoter</u>		

a) The sequences are represented as 5'-to-3' orientation. The six nucleotides between spaces indicate the randomized region (positions -11 to -6). For reference, the wild-type Syn5 promoter sequence is shown at the bottom of the table.