# Development of a transformation method for *Metschnikowia borealis* and other CUG-serine yeasts.

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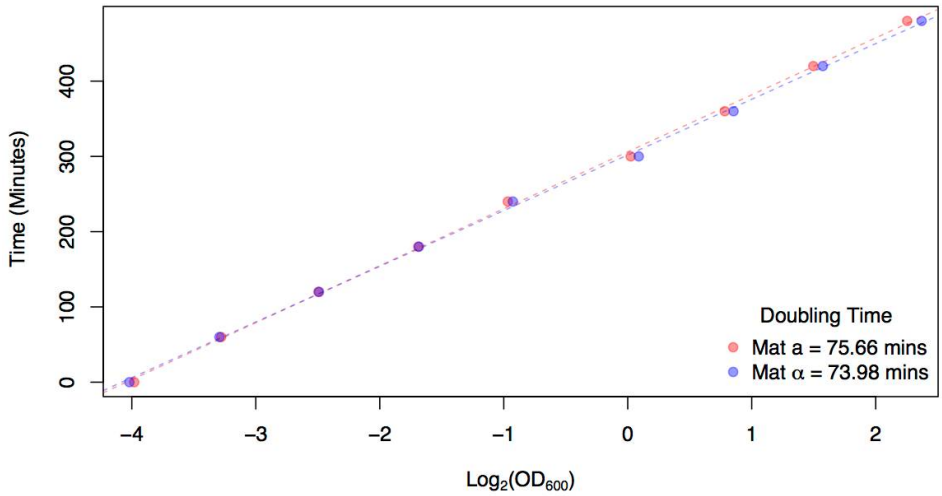
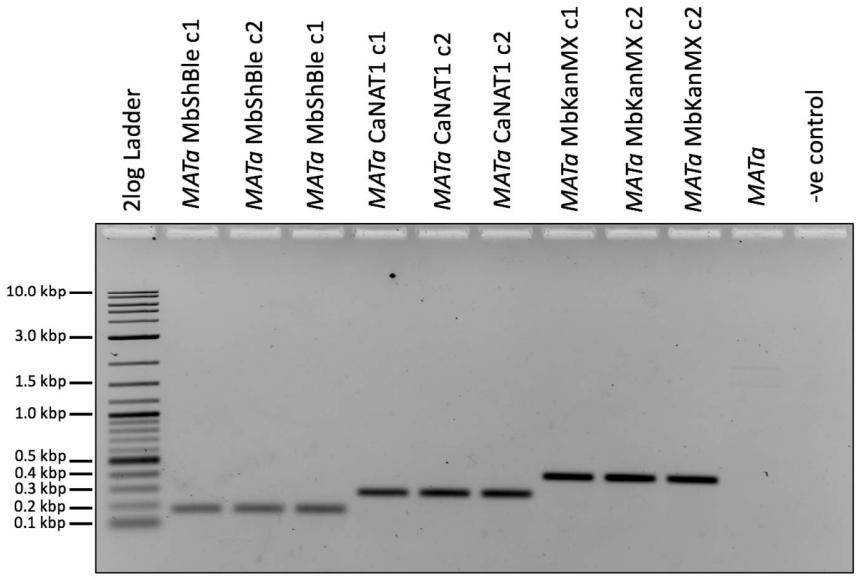
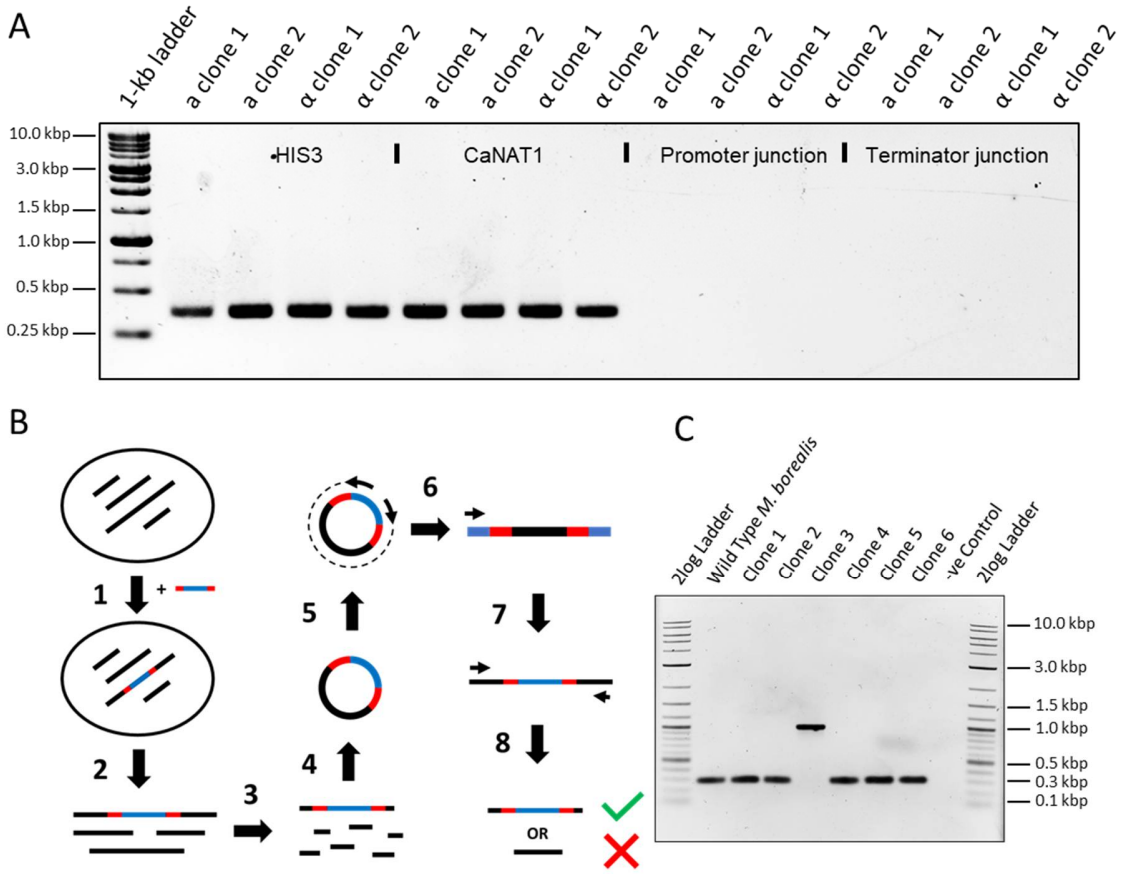
**Supplementary Figures and Tables:**




**Supplementary Figure S1. Growth rate of *M. borealis MATa* and *MATα*.** Three cultures of each mating type of *MATa* and *MATα* were grown to mid-log phase ($OD_{600}$ = 1.0), and diluted in 50 mL of YPAD to an optical density of 0.065. Each culture was then grown at 30ºC with shaking at 225 rpm, and $OD_{600}$ was recorded at 1-hr time points for 8 hours. The average $OD_{600}$ of the three cultures for each mating type was recorded at each time point, and the doubling times were calculated as the slopes of the lines of best fit of $Log_2(OD_{600})$ versus each time point in minutes.




**Supplementary Figure S2. Genotyping transformants.** Three colonies of *M. borealis MATa* that were transformed with MbShBle, CaNAT1, and MbKanMX (Figure 2) were genotyped by Multiplex PCR (Quiagen) using primers that amplify each selectable marker. Expected sizes were 185 base-pairs (MbShBle), 283 base-pairs (CaNAT1), and 404 base-pairs (MbKanMX). Two negative controls include PCR performed with DNA isolated with untransformed MATa strain as well as without any DNA (-ve control). The PCR was run for 28 cycles with all three sets of primers present in each reaction.




**Supplementary Figure S3. Identification of insertion sites.** *M. borealis* mating types a and *α* were transformed with a PCR-linearized CaNAT1 in yeast alternative nuclear code, flanked by 60 base-pair sequences of the *M. borealis HIS3* promoter and terminator by electroporation. (A) Two colonies of a and *α* mating types were screened by PCR to look for a targeted knockout of *HIS3*. Lanes 2-5 used primers that bind within the *M. borealis HIS3* gene (expected size 416 base pairs), lanes 6-9 used primers that bind within the CaNAT1 marker (expected size 388 base pairs), lanes 10-14 used primers to amplify across the *HIS3* promoter-CaNAT1 insertion junction (expected size 682 base-pairs), and lanes 14-17 used primers to amplify across the CaNAT1-HIS3 terminator junction (expected size 779 base-pairs). (B) Schematic of the protocol used to identify the insertion site: 1) Lithium acetate/electroporation to transform *M. borealis* with the marker DNA for insertion; 2) Alkaline lysis to isolate *M. borealis* DNA; 3) Restriction digest with CfoI; 4) Ligate sticky ends with T4 ligase; 5) PCR amplify the adjacent genomic DNA; 6) Sequence PCR product; 7) Design primers ~150 base-pairs upstream and downstream of the insertion site; 8) PCR amplify expected insertion site. (C) Confirmation of one insertion site. Primers were designed to amplify across the insertion site identified in clone 3, and the site was PCR-amplified in wildtype *M. borealis*, as well as transformants 1-6. Expected size of the site is ~300 base-pairs (wildtype) and ~1000 base-pairs (with the CaNAT1 vector insertion).

**Supplementary Table S1.** Identification of antibiotic sensitivity. Cultures of *M. borealis MATa* and *MATα* were grown to $OD_{600}$ of 1.5, concentrated to $OD_{600}$ = 3.0, and three dilutions were plated onto YPAD with various concentrations of zeocin or nourseothricin or combination of these two, or geneticin (G418). Plates were incubated for 2-4 days, and colonies were counted. Zeo = zeocin; NTC = nourseothricin; G418 = geneticin; NG = no growth; C = confluent growth.

| | ***MATa*** | | | | | | ***MATα*** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Growth Time | 2 days | | | 4 days | | | 2 days | | | 4 days | | |
| Dilution Factor | $10^0$ | $10^1$ | $10^2$ | $10^0$ | $10^1$ | $10^2$ | $10^0$ | $10^1$ | $10^2$ | $10^0$ | $10^1$ | $10^2$ |
| Zeo 50 mg $L^{-1}$ | 1 | NG | NG | 7 | 2 | NG | 2 | NG | NG | 8 | 3 | 1 |
| Zeo 75 mg $L^{-1}$ | NG | NG | NG | 1 | NG | NG | NG | NG | NG | 1 | 1 | NG |
| Zeo 100 mg $L^{-1}$ | NG | NG | NG | 1 | NG | NG | NG | NG | NG | NG | NG | NG |
| Zeo 125 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| NTC 50 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| NTC 75 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| NTC 100 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| NTC 125 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| Zeo 25 mg $L^{-1}$ NTC 25 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| Zeo 25 mg $L^{-1}$ NTC 50 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| Zeo 50 mg $L^{-1}$ NTC 25 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| Zeo 50 mg $L^{-1}$ NTC 50 mg $L^{-1}$ | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| G418 200 mg $L^{-1}$ | C | C | NG | C | C | C | C | C | NG | C | C | C |
| G418 300 mg $L^{-1}$ | NG | NG | NG | 8 | 1 | NG | NG | NG | NG | 12 | 1 | NG |
| G418 400 mg $L^{-1}$ | NG | NG | NG | 2 | NG | NG | NG | NG | NG | 1 | NG | NG |

**Supplementary Table S2. Transformation efficiencies for *M. borealis*.** Cultures of *MATa* and *MATα* were transformed with CaNAT1 by electroporation and lithium acetate methods alongside negative controls in triplicate, plated on YPAD with 75 mg $L^{-1}$ nourseothricin, and incubated for 48 hrs at 30ºC for colonies to appear. No colonies grew on any of the control plates. Efficiency is given as colony forming units per μg of DNA for $10^8$ cells. Av = average efficiency for each strain; Electro = electroporation; LiOAc = lithium acetate.

| Strain | Method | # Cells | Vector DNA (μg) | Plated | Colonies | Efficiency | Av. |
|---|---|---|---|---|---|---|---|
| *MATa* | Electro. | $10^8$ | 1 | 2% | 45 | 2250 | |
| | Electro. | $10^8$ | 1 | 2% | 13 | 650 | 1600 |
| | Electro. | $10^8$ | 1 | 2% | 38 | 1900 | |
| *MATα* | Electro. | $10^8$ | 1 | 2% | 9 | 450 | |
| | Electro. | $10^8$ | 1 | 2% | 19 | 950 | 817 |
| | Electro. | $10^8$ | 1 | 2% | 21 | 1050 | |
| *MATa* | LiOAc | $10^8$ | 1 | 100% | 13 | 13 | |
| | LiOAc | $10^8$ | 1 | 100% | 4 | 4 | 9 |
| | LiOAc | $10^8$ | 1 | 100% | 9 | 9 | |
| *MATα* | LiOAc | $10^8$ | 1 | 100% | 5 | 5 | |
| | LiOAc | $10^8$ | 1 | 100% | 1 | 1 | 4 |
| | LiOAc | $10^8$ | 1 | 100% | 5 | 5 | |

**Supplementary Table S3. Transformation results for additional yeast strains.** An additional 19 yeast strains were transformed by electroporation using the PCR-linearized CaNAT1 gene (in standard code or yeast alternative nuclear code) flanked by 60-base-pair *ADH1* promoter and terminator sequences from *M. borealis*. Transformants were plated on YPAD with 75-200 mg $L^{-1}$ nourseothricin and incubated at 30ºC for 2 days until colonies appeared. *M. bicuspidata* and *M. orientalis* were incubated at 27ºC for 4 days until colonies appeared. NTC = nourseothricin; Alt. = CaNAT1 in yeast alternative nuclear code; Std. = CaNAT1 in standard code; Ctrl. = negative control.

| | | | Number of Colonies | | |
|---|---|---|---|---|---|
| **Strain** | **NTC (mg $L^{-1}$)** | **Plated** | **Alt.** | **Std.** | **Ctrl.** |
| *Candida* aff *bentonensis* | 75 | 40% | 27 | 30 | 0 |
| *Candida bromeliacearum* | 100 | 10% | 54 | 0 | 0 |
| *Candida intermedia* | 100 | 100% | 15 | 0 | 0 |
| *Candida saopaulonensis* | 200 | 10% | 42 | 0 | 0 |
| *Candida tolerans* | 100 | 10% | 25 | 0 | 0 |
| *Candida ubatubensis* | 100 | 20% | 41 | 0 | 0 |
| *Clavispora lusitaniae* | 75 | 40% | 16 | 0 | 0 |
| *Metschnikowia agaves* | 100 | 10% | 25 | 0 | 0 |
| *Metschnikowia bicuspidata* | 75 | 40% | 4 | 0 | 0 |
| *Metschnikowia caudate* | 100 | 20% | 9 | 0 | 0 |
| *Metschnikowia drosophilae* | 100 | 40% | 4 | 0 | 0 |
| *Metschnikowia gelsemii* | 100 | 10% | 93 | 0 | 0 |
| *Metschnikowia gruessii* | 100 | 10% | 37 | 0 | 0 |
| *Metschnikowia lunata* | 100 | 10% | 64 | 0 | 0 |
| *Metschnikowia orientalis* | 100 | 100% | 13 | 0 | 0 |
| *Metschnikowia pulcherrima* | 100 | 10% | 112 | 0 | 0 |
| *Metschnikowia rancensis* | 100 | 10% | 27 | 0 | 0 |
| *Metschnikowia reukaufii* | 200 | 10% | 121 | 0 | 0 |
| *Saccharomyces cerevisiae* | 100 | 10% | 32 | 29 | 0 |

**Primers used in this study:**

| | |
|---|---|
| Primers to amplify CaNAT1 (with *ADH1* promoter and terminator elements) | |
| BK420F | TCTTTCTTCACTATTCAAACATACATTGAATACAACCAAGCATCAATTAAGAAAA ATGTCTACTACTTTGGATGATACTG |
| BK420R | AGAAATGCAATGAACGATGAACTATTTATTGTGTATTGGGAGGGGGTCAAAGAG TTTATGGACATGGCATAGACATATAC |
| Primers to amplify CaNAT1 (with *HIS3* promoter and terminator elements) | |
| BK398F | ATTATGACTCTTCCCCAATCTTTTCTCACTCATTACCAATACTAACAGATCAACCC CAAAATGTCTACTACTTTGGATGA |
| BK398R | CTTCGTATCTATGATTCCTACACCGCATATAGTGGTCACTTAAATAATTCTATATG TCGCTTATGGACATGGCATAGACA |
| Primers to genotype attempted *HIS3* knock-outs – amplifying *HIS3* | |
| BK402F | CATGCTCTAGCCAAGCACTCGGGCT |
| BK402R | CTGATTGCCTCCTTTATGGCGATAG |
| Primers to genotype attempted *HIS3* knock-outs – amplifying CaNAT1 | |
| BK364F | TGTTCCAGGTGATGCTGAAG |
| BK364R | CAACCACAAATGACCAGCAC |
| Primers to genotype attempted *HIS3* knock-outs – promoter junction | |
| BK402F | CATGCTCTAGCCAAGCACTCGGGCT |
| BK364R | CAACCACAAATGACCAGCAC |
| Primers to genotype attempted *HIS3* knock-outs – terminator junction | |
| BK364F | TGTTCCAGGTGATGCTGAAG |
| BK402R | CTGATTGCCTCCTTTATGGCGATAG |
| Primers to genotype *M. borealis MATα* clones – amplifying insertion sequences | |
| BK476F | GTGCTGGTCATTTGTGGTTG |
| BK478R | TCAATGGTGGATCAACTGGA |
| Primers to genotype *M. borealis MATα* clones – confirming insertion site in gDNA | |
| BK523F | AGAGCTGGGCCAATAAGGAG |
| BK523RA | TTGTCACATCAAGTTTCCTTGG |
| Multiplex genotyping primers for presence of MbShBle gene | |
| BK578_F | TTGCTGGTGCTGTTGAGTTC |
| BK578_R | CTCAGCGTACAACTCGTCCA |
| Multiplex genotyping primers for presence of CaNAT1 gene | |
| BK579_F | TCCAGTTGATCCACCATTGA |
| BK579_R | CAACCACAAATGACCAGCAC |
| Multiplex genotyping primers for presence of MbKanMX gene | |
| BK580_F | GACGTTACCGACGAGATGGT |
| BK580_R | TCACCGTGGGTAACAACAGA |

**Gene sequences:**

**Wild-type NAT:**

ATGACCACTCTTGACGACACGGCTTACCGGTACCGCACCAGTGTCCCGGGGGACGCCGAGGCCA
TCGAGGCACTGGATGGGTCCTTCACCACCGACACCGTCTTCCGCGTCACCGCCACCGGGGACGG
CTTCACCCTGCGGGAGGTGCCGGTGGACCCGCCCCTGACCAAGGTGTTCCCCGACGACGAATCG
GACGACGAATCGGACGCCGGGGAGGACGGCGACCCGGACTCCCGGACGTTCGTCGCGTACGGG
GACGACGGCGACCTGGCGGGCTTCGTGGTCGTCTCGTACTCCGGCTGGAACCGCCGGCTGACCGT
CGAGGACATCGAGGTCGCCCCGGAGCACCGGGGGCACGGGGTCGGGCGCGCGTTGATGGGGCT
CGCGACGGAGTTCGCCCGCGAGCGGGGCGCCGGGCACCTCTGGCTGGAGGTCACCAACGTCAAC
GCACCGGCGATCCACGCGTACCGGCGGATGGGGTTCACCCTCTGCGGCCTGGACACCGCCCTGT
ACGACGGCACCGCCTCGGACGGCGAGCAGGCGCTCTACATGAGCATGCCCTGCCCCTGA

**CaNAT1:**

ATGTCTACTACTTTGGATGATACTGCTTATAGATACAGAACTTCTGTTCCAGGTGATGCTGAAGCT
ATTGAAGCTTTGGATGGTTCTTTCACTACCGATACTGTTTTTAGAGTTACTGCTACTGGTGATGGTT
TCACTTTGAGAGAAGTTCCAGTTGATCCACCATTGACTAAGGTTTTCCCAGATGATGAATCCGAT
GATGAATCCGATGCTGGTGAAGATGGTGATCCAGATTCTAGAACTTTCGTTGCTTATGGTGATGA
TGGTGATTTGGCTGGTTTCGTTGTTGTTTCTTATTCTGGTTGGAACAGAAGATTGACTGTTGAAGAT
ATTGAAGTTGCTCCAGAACATAGAGGTCATGGTGTTGGTAGAGCTTTGATGGGTTTGGCTACTGA
ATTCGCCAGAGAAAGAGGTGCTGGTCATTTGTGGTTGGAAGTTACCAATGTTAATGCTCCAGCTA
TTCATGCTTATAGAAGAATGGGTTTCACTTTGTGTGGTTTGGATACTGCTTTATACGATGGTACTGC
TTCCGATGGTGAACAAGCTTTGTATATGTCTATGCCATGTCCATAA

**CaNAT1 with CUG codons:**

ATGTCTACTACTTTGGATGATACTGCTTATAGATACAGAACTTCTGTTCCAGGTGATGCTGAAGCT
ATTGAAGCTCTGGATGGTTCTTTCACTACCGATACTGTTTTTAGAGTTACTGCTACTGGTGATGGTT
TCACTCTGAGAGAAGTTCCAGTTGATCCACCACTGACTAAGGTTTTCCCAGATGATGAATCCGAT
GATGAATCCGATGCTGGTGAAGATGGTGATCCAGATTCTAGAACTTTCGTTGCTTATGGTGATGA
TGGTGATCTGGCTGGTTTCGTTGTTGTTTCTTATTCTGGTTGGAACAGAAGACTGACTGTTGAAGA
TATTGAAGTTGCTCCAGAACATAGAGGTCATGGTGTTGGTAGAGCTTTGATGGGTTTGGCTACTG
AATTCGCCAGAGAAAGAGGTGCTGGTCATTTGTGGCTGGAAGTTACCAATGTTAATGCTCCAGCT
ATTCATGCTTATAGAAGAATGGGTTTCACTTTGTGTGGTCTGGATACTGCTCTGTACGATGGTACT
GCTTCCGATGGTGAACAAGCTTTGTATATGTCTATGCCATGTCCATAA

**Wild-type Sh *ble*:**

ATGGCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTCGCCGGAGCGGTCGAGTT
CTGGACCGACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTTCGCCGGTGTGGTCCGGG
ACGACGTGACCCTGTTCATCAGCGCGGTCCAGGACCAGGTGGTGCCGGACAACACCCTGGCCTG
GGTGTGGGTGCGCGGCCTGGACGAGCTGTACGCCGAGTGGTCGGAGGTCGTGTCCACGAACTTC
CGGGACGCCTCCGGGCCGGCCATGACCGAGATCGGCGAGCAGCCGTGGGGGCGGGAGTTCGCC
CTGCGCGACCCGGCCGGCAACTGCGTGCACTTCGTGGCCGAGGAGCAGGACTGA

**MbShBle:**

ATGGCTAAGTTGACCTCTGCTGTTCCAGTTTTGACCGCTAGAGACGTTGCTGGTGCTGTTGAGTTC
TGGACCGACAGATTGGGTTTCTCTAGAGACTTCGTTGAGGACGACTTCGCTGGTGTTGTTAGAGA
CGACGTTACCTTGTTCATTTCTGCTGTTCAAGACCAAGTTGTTCCAGACAACACCTTGGCTTGGGT
TTGGGTTAGAGGTTTGGACGAGTTGTACGCTGAGTGGTCTGAGGTTGTTTCTACCAACTTCAGAGA
CGCTTCTGGTCCAGCTATGACCGAGATTGGTGAGCAACCATGGGGTAGAGAGTTCGCTTTGAGAG
ACCCAGCTGGTAACTGTGTTCACTTCGTTGCTGAGGAGCAAGACTGA

**MbShBle with CUG codons:**

ATGGCTAAGTTGACCTCTGCTGTTCCAGTTTTGACCGCTAGAGACGTTGCTGGTGCTGTTGAGTTC
TGGACCGACAGATTGGGTTTCTCTAGAGACTTCGTTGAGGACGACTTCGCTGGTGTTGTTAGAGA
CGACGTTACCCTGTTCATTTCTGCTGTTCAAGACCAAGTTGTTCCAGACAACACCCTGGCTTGGGT
TTGGGTTAGAGGTCTGGACGAGCTGTACGCTGAGTGGTCTGAGGTTGTTTCTACCAACTTCAGAG
ACGCTTCTGGTCCAGCTATGACCGAGATTGGTGAGCAACCATGGGGTAGAGAGTTCGCTCTGAG
AGACCCAGCTGGTAACTGTGTTCACTTCGTTGCTGAGGAGCAAGACTGA

**Wild-type KanMX:**

ATGGGTAAGGAAAAGACTCACGTTTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATA
TGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGA
AGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGAT
GAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCG
TACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAG
AAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATT
CGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCAC
GAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAA
CAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGA
TTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGT
CGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTC
ATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTC
ATTTGATGCTCGATGAGTTTTTCTAA

**MbKanMX:**

ATGGGTAAGGAGAAGACCCACGTTTCTAGACCAAGATTGAACTCTAACATGGACGCTGACTTGT
ACGGTTACAAGTGGGCTAGAGACAACGTTGGTCAATCTGGTGCTACCATCTACAGATTGTACGGT
AAGCCAGACGCTCCAGAGTTGTTCTTGAAGCACGGTAAGGGTTCTGTTGCTAACGACGTTACCGA
CGAGATGGTTAGATTGAACTGGTTGACCGAGTTCATGCCATTGCCAACCATCAAGCACTTCATCA
GAACCCCAGACGACGCTTGGTTGTTGACCACCGCTATCCCAGGTAAGACCGCTTTCCAAGTTTTG
GAGGAGTACCCAGACTCTGGTGAGAACATCGTTGACGCTTTGGCTGTTTTCTTGAGAAGATTGCA
CTCTATCCCAGTTTGTAACTGTCCATTCAACTCTGACAGAGTTTTCAGATTGGCTCAAGCTCAATC
TAGAATGAACAACGGTTTGGTTGACGCTTCTGACTTCGACGACGAGAGAAACGGTTGGCCAGTTG
AGCAAGTTTGGAAGGAGATGCACAAGTTGTTGCCATTCTCTCCAGACTCTGTTGTTACCCACGGT
GACTTCTCTTTGGACAACTTGATCTTCGACGAGGGTAAGTTGATCGGTTGTATCGACGTTGGTAGA
GTTGGTATCGCTGACAGATACCAAGACTTGGCTATCTTGTGGAACTGTTTGGGTGAGTTCTCTCCA

TCTTTGCAAAAGAGATTGTTCCAAAAGTACGGTATCGACAACCCAGACATGAACAAGTTGCAAT
TCCACTTGATGTTGGACGAGTTCTTCTAA

**MbKanMX with CUG codons:**

ATGGGTAAGGAGAAGACCCACGTTTCTAGACCAAGATTGAACTCTAACATGGACGCTGACTTGT
ACGGTTACAAGTGGGCTAGAGACAACGTTGGTCAATCTGGTGCTACCATCTACAGATTGTACGGT
AAGCCAGACGCTCCAGAGTTGTTCCTGAAGCACGGTAAGGGTTCTGTTGCTAACGACGTTACCGA
CGAGATGGTTAGATTGAACTGGCTGACCGAGTTCATGCCATTGCCAACCATCAAGCACTTCATCA
GAACCCCAGACGACGCTTGGTTGTTGACCACCGCTATCCCAGGTAAGACCGCTTTCCAAGTTTTG
GAGGAGTACCCAGACTCTGGTGAGAACATCGTTGACGCTCTGGCTGTTTTCCTGAGAAGATTGCA
CTCTATCCCAGTTTGTAACTGTCCATTCAACTCTGACAGAGTTTTCAGATTGGCTCAAGCTCAATC
TAGAATGAACAACGGTTTGGTTGACGCTTCTGACTTCGACGACGAGAGAAACGGTTGGCCAGTTG
AGCAAGTTTGGAAGGAGATGCACAAGTTGTTGCCATTCTCTCCAGACTCTGTTGTTACCCACGGT
GACTTCTCTTTGGACAACTTGATCTTCGACGAGGGTAAGTTGATCGGTTGTATCGACGTTGGTAGA
GTTGGTATCGCTGACAGATACCAAGACTTGGCTATCTTGTGGAACTGTTTGGGTGAGTTCTCTCCA
TCTTTGCAAAAGAGATTGTTCCAAAAGTACGGTATCGACAACCCAGACATGAACAAGTTGCAAT
TCCACTTGATGTTGGACGAGTTCTTCTAA

***M. borealis ADH1*** **promoter:**

TTGTCATTGTCAGCAAAGTACATTGATCTGTATTCTTCCAAACCCAAGACATTCTTGACGAAAGCT
TGGATTCTTATAACACTAGCACTTCATGCTTCCACTGCATGCTTCTTCACCAGAATCAATGAAAGT
GGAGAGATCCAAGTAATTGAGCACCTCAAAAATGATGAGACGGTGAAATCCATAGGATTCCTTA
CTCCGTGCCATTCAACACCATGGCATAGTATGTTGCATCGTCCTGAAATCGTTGACTCATCATGGT
TTTTGACATGTGAACCACCATTGCATTTGACCTTTGGTAATATCAACAATGTCAAAGAGTACCGA
GACGAACTGGATCGCTTCTTCGACAATCCCGTGGAGTTTGTACGAAACTTGCCGTATGAGTGGCC
CTCACATCTTGTTGTGTTCGAACCAATGGAGTACTTAGTGACACAGGAATTGCCTCAGTACCATG
AATGCCACAGGTATTTCAATAGCTACTTTCATTGGGACTCACGTCGTCAAGGAGATTTGATTGTGT
TCTGCAAAACAATCTGAGGTGTTGCTTCGTGAATAAATTTCACGTGGAGAAGCGACTTATCTAAA
CGCATAAATGTGTCGCGCATTGAAACACGCATCAAATGCGCTCGTCGGCTAATGTCGGAAAGGC
CGCTCTCGCTCTTCTAAATCTTGTAATCTATCGGGAAATAACTGATATCAAATCATGCCACCCGAC
AATTGCAGCAGATCTGAGACCTGCATAATTATGAGTCAAGAAATATCATAAAATGCGTGCATTGT
ACTTAACTTTAAAGTCTACTCTTTCACTAAAAACCTTAGCATCCTCCTCTGCATGAGTATGCGCTT
AAGTGTCGCAACAAAGCCAGAAATCACACCACGCACATAGAAGCAGCAAACATTCGTGACTAT
AAATATGATGCTTCGCCGGACTCCAGCAATTCTTCTTTCTTCACTATTCAAACATACATTGAATAC
AACCAAGCATCAATTAAGAAAA

***M. borealis ADH1*** **Terminator:**

ACTCTTTGACCCCCTCCCAATACACAATAAATAGTTCATCGTTCATTGCATTTCTTCTCCAACTCTG
CTATGCGTTCTCTCATCACGTTCTGCTGGATCTGGGTGATCAATTGCTCATCATTCAACACGTCAA
AAAGCTTATTTTTCTCTGCCAACGCATCGCTGATGTTCGCTAGTTTCGAATTCAACCTTTTCATTTT
CATATCCTTCTCTGCCAAGGCCTTTGTCAACTCGTTCACCCGGAGGTTGGCATTCTCCAACTTCAT
GGTTTGTTGATTGAGCCGGGTAATAAGCGATTCCGTTTCTTCATGCAAATGCGAATTTTCTTTAAT
AAGCCGTTCGTTATCAGCATGGCCTGCTGATGTTTTTACATATGCTAATTGCTCAAAAGCCTTCAC
GAAGTCTGCGTCTCGCTTCTCTATCTGGTCACGTTTGTTCAACTGGTCTAGTATTGCATTCCACGGC
TCATTCATTTTGGTTAAAAATGTGAGTCTCCAAGCCCCAATATGGTAATTTGGATGTGTGTTTCAA

GTTTCCTATGTGTCAAATCAGGCTCAGAATGACACATTGCAAGTATCTGGGGTCTTAAATTGCGA
GTGCACAGTGCCACGGTTGGCGCGCTTGATAAGCTTACGACAGAGCATATAACCTCAAACCGAA
ATACACCATCCTAGAATATACTGTCCTATAATATTGACCATTTGAAGAGTCAGTTTTGCTTCTTCA
CTGGTCTAAGAATTGCATTGCACTGCTGCACTAATTGTCTTTAACTCACTTAATTGCCTGTGTATTG
ACATCTGCAAACATCTTCCCCTGTTGATTAGCAAAGAAGCAGGTGAACCGATCGTCTCCTTAGAA
TGGCTGAGCCTTCTTCTTCATGGTGGTCTCTGTTATATCGCTCCGAATCTACCAAACAAGCTGATA
TTTCTTCAAAGGCCACTTCCCCTGGGACACTACCTCCTGCCAACTCTAAAAAGGAAACGAATACT
CAAAAGGCCTC

***M. borealis HIS3* promoter:**

TGTCTAGCTCTAACACCTTGATGGCCACAACCTTTTCAGTTTTCTTGTGATACCCTTTGTAGACGAC
ACCGAACTTGCCCTGGCCGATGACCTTTGTTCTTTGGTAAGAAGAAGTACTTAGCATGGTGAAAG
TGGAGGAGTGTTCTCAAGGCCAAGGACCTAGGGGAATTTGAGATCGAGTACTTTCTCTTGCATGG
AAAGAGAGTTCAGAAGAACGCCTTTGGAACTTCTGCCAATGTTTATAAGCGCTTGAAAACTAAGT
ATGGCGTACAGCGATATCCTGCAGAGTTGATCCGATGGATTATGGTGAAGGCCAGCACGTTGGG
CGTCCAAAACAGAGATCGGGTCGTGAAGGAAAGTGTACCAGTATGAAAATTGAAGGCGCGGTA
GCCAGGATCTGCAGCTGAATACGGTGGGTCGCGCCTGAGAAACAAGATGCACCGAATGCGGAG
GAAGCTTCAAGTAGAGAGCAGAAAGCAAAGATGCTAATTTACGACGAAGGGGTTTTCGAAGGGT
TCGGAGCGTTCGATGCGTTTGCCCAGCCAAATTTGCCAACTAATTCGAAAGCCACCATTGTTAAT
CACCAAAAGGTCAGCTGCCTGAATGGTCAGGTGTACATGTGCAGTCAGTAGACAATAGTGGTGG
ACTTTTGGATTTTCAGGCAAAGAAGTGCGAAGTAAGCTATCAAATGACCAGTGTGCCAACACAC
CATGCAAGAAATTCGGCCACAAGCAAAAAGAGTTGAATTTTCGCCGGCGATGCAAATTAAAAAA
AAAAAAAAAAAGACCCAAAAAACTTAGCCTTCTGTTCCAAAATCAGAAATTGAAAGGTCAGTCT
TTTGTTTGCTCGAATCGGGTACTTCCGAGTTTGCTGCTGCACTGGTCGGGCCATTTTGGTGTGGATG
GAACTTTGGTGAACGGAATTTGCCAGAGATATGCGCATTATGACTCTTCCCCAATCTTTTCTCACT
CATTACCAATACTAACAGATCAACCCCAAA

***M. borealis HIS3* terminator:**

GCGACATATAGAATTATTTAAGTGACCACTATATGCGGTGTAGGAATCATAGATACGAAGCGAA
AAGTCAGAGGTGCGCATTACAACTTTCGGGGCACCGCTTTCGATGCGCATCTCTTCACTACTACC
ATGAGAAGGTTCCACACTAGTGGTATCCGCCAAGTCATCAAGCCAGTGTTCAAACTGCATGATCT
GAAGAAAGGACTCAAGAAATTTGAAGATTCCTTCAATGCAGGTAGTAACCGGAAGCTGGAGCA
GAAAATATGGGACAAGTTGAATATCTCCAAGCACGAGTTTTTCATACGGAAATATGGCAACATTT
CGCCCGAAAAACGAAAACAGTTGGATGACAAAGTCACCCGACAAAGGTCGCTCCGCGAGCAGA
GAAGGAAGAACGAAATGGGCGACGACTACGAATCTTACAGGAAGCCCCGGGCGGCGTTGAACC
CTCTTGCTGAGTACCTCTTTGGCACTCACGCAGTCATGTCCGCATTGACTGCAGGTAAGCGAGAG
GCCTTCAGCACCCTCTACATCCAAAAGTCTAAGGACAGTGTGCGTCAGGTTCTCCTGCTTGCGAA
GAAATATGGAGTCCGGGTCGTGGAGAAGGGATCTAAAGGTGAGATGAACACCTTAAGCTCCAAC
GGTGTCCACAATGGCGTTGTCTTGGAAACCAAGCCCTTGCGCATTCCAGAGGTGTACGAACTTGA
CAAAACTCATGACGGAACCGAAGGACTGTACAATGTTAAAGTGTACGATGAGGAGACGGATTCG
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CTAGGTGCAGATTTCCTTGTGATTCCCAATGCAGAGTCTGCTCGTCTAGGCCCCGTTGCTGCCAAA
GCATCAGCCGGCGCGCTTGACCTCATGCCCATAT