

Figure S1: Construction of G13. (A) Genomic DNA was used as a template to amplify 500bp targeting arms flanking the URA3 ORF of *K. marxianus*. P1 and P2 were used to amplify the left arm, while P3 and P4 were used to amplify the right arm. (B) In the second round of PCR, the left and right arm was joined by 30bp overlap using P1 and P4. (C) The final PCR of a total volume of 600 $\mu$ l (1 $\times$  Phusion master mix, 1 $\mu$ l of prior PCR products, and 0.2 $\mu$ M of each primer) was purified yielding 18 $\mu$ g after gel purification (Qiagen Qiaquick). Either 1 or 5 $\mu$ g of the targeting construct was electroporated into *K. marxianus*. The cells were grown to mid log phase, washed several times with ice cold water and further treated with LiAC and DTT at 30C for 45 min (final concentrations of 80mM LiAC, 20mM DTT, 10mM Tris-HCl (pH 8.0), 1mM EDTA). Cells were further washed with ice cold water and resuspended in 1M sorbitol in water as an electroporation buffer. One hundred  $\mu$ ls of cells were mixed with DNA in a 0.4mm gap electroporation cuvette and pulsed at 2.5kV, 25 $\mu$ F, and 200 ohms in a BioRad Gene Pulser Xcell electroporation apparatus. Cells were recovered in YPDS (YPD plus 1M sorbitol) overnight at 30°C and spread on 5-FOA plates the next day (SDA-Ura Powder, 500mls, Sunrise Science products catalog number 1704-300, plus 25mg uracil, Sunrise Science products catalog number 1906-10, and 0.5 g of 5-FOA, Gold Biotechnology catalog number F-230-10). P5 and P6 were primers used for screening positive transformants based on size differences.

TGAGAGTGCACCACGCTTTCAATTCCAATTCATCATTTTTTTTTTATTCTTTTTTTGATTTCCGTTTCTT  
TGAAATTTTTTGGATTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAAGGAGCACAGACTTAGA  
TTGGTATATATACGCATATGTAGTGTTGAAGAAACATGAAATTGCCAGTATTCTTAACCCAACTGCA  
CAGAACA AAAACCTGCAGGAAACGAAGATAAATCATGTCGAAAGCTACATATAAGGAACGTGCTG  
CTACTCATCCTAGTCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAACTTGTG  
TGCTTCATTGGATGTTCTGACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAT  
TTGTTTACTAAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCT  
AAAGGCATTATCCGCCAAGTACAATTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAA  
TACAGTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAATG  
CACACGGTGTGGTGGGCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCAGAAGAAGTAACAAA  
GGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCTACTGGAGAATA  
TACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTATCGGCTTTATTGCTCAAAG  
AGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGACACCCGGTGTGGGTTTAGAT  
GACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGATCTGA  
CATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGAACGT  
TACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAACTAAaaaactgtattataa  
gtaaatgcatgtataactcaaaattagagcttcaatttaattatcagttattaccctg

Figure S2: Sequence of *ScURA3* used in transformation

Bold: *ScURA3* ORF. Underscore: Start and Stop codons. Primers used for *ScURA3* amplification are in blue.

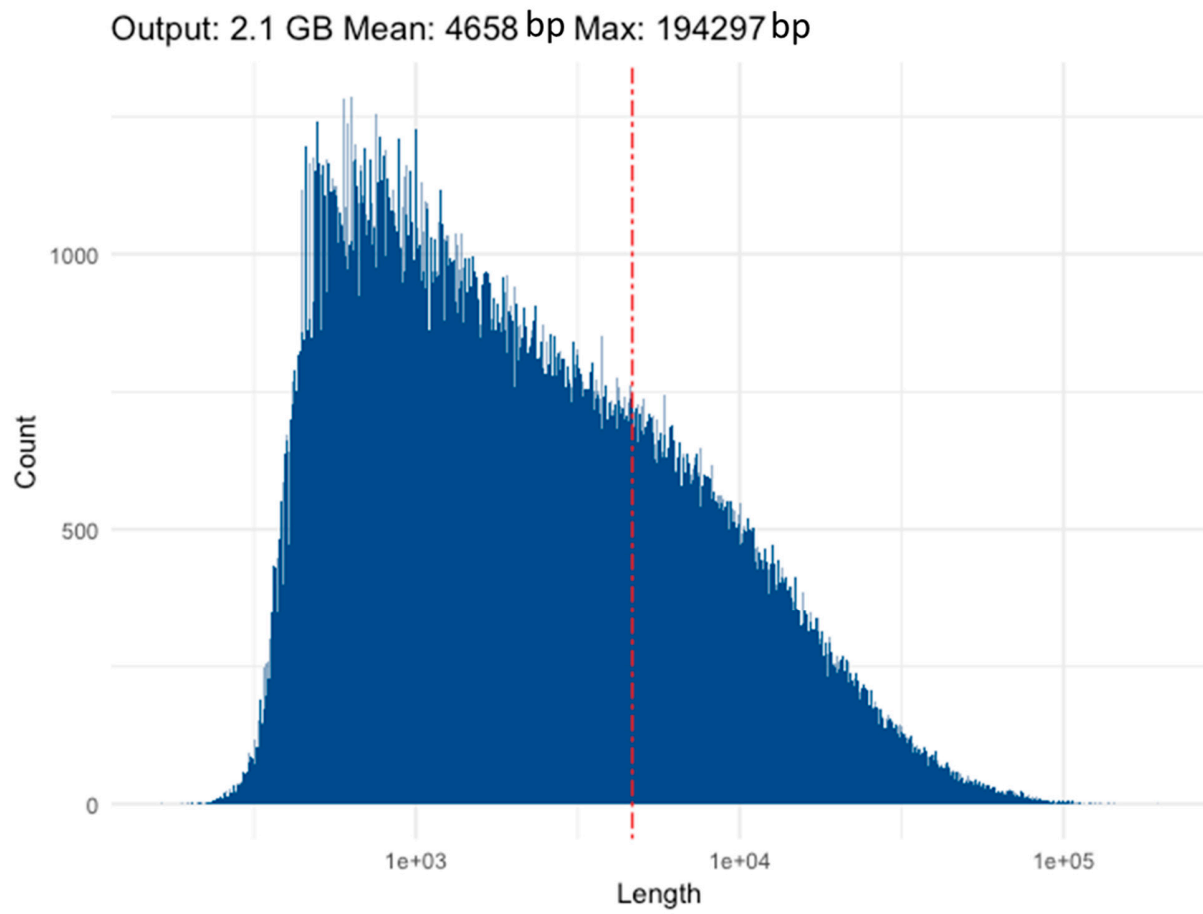
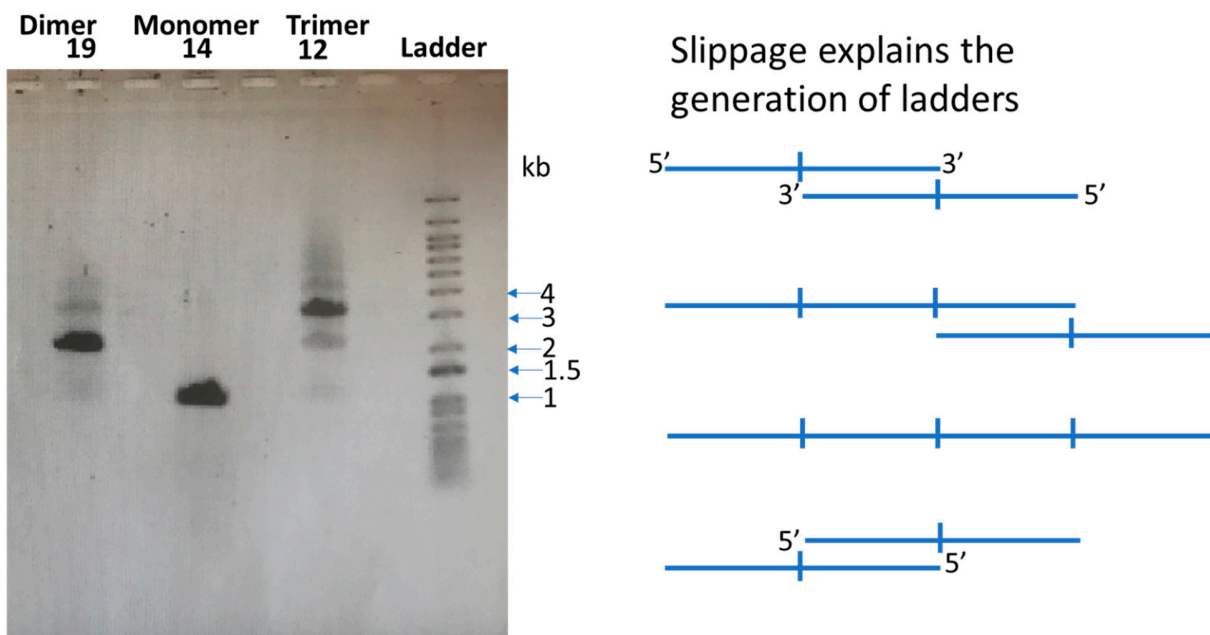


Figure S3. Distribution of Oxford Nanopore sequencing read lengths for the 24-clone pool DNA of *K. marxianus* *ScURA3* cassette DNA transformants.



**Figure S4.** Gel electrophoresis of PCR amplified *K. marxianus* *ura3* monomer (clone 14), dimer (clone 19) and trimer (clone 12) transformants using flanking primers. The dimer and trimer lanes show ladders of bands produced by slippage during the annealing step of each cycle.

Clone 6 PS9 intergenic-intergenic

PS15 for deleted region clone6

AAAACCAAAAAAAAAACCATAAAAACCAAAACA TGAGAGTGCACCACGCTTTTCAA

chr4 714164mi

CAATTTAATTATATCAGTTATTACCCTG AACGACTTGATGATGTACATAG

chr4 762150mi

Nanopore monomer out2 read 005,117,134(both ends), 320(both ends), 538(both ends), 597(both ends), 688(both ends)

> read134

AAAACATCCGCACCTGCAAATTACGCCTTCCAGTGACTGTCTAATCGCTCAACAACTCCTCTCC  
CACTGTCTCAGATACTCGACTTTGTTGCGATGAGCATAGGTTTCGGGAATTTCAATGGATGTATA  
TCTGTGTAATTAACGCGATATTTAAATCACGTGTAAGGAAAAACCAAAAAATATAAAAAGCAA  
ACA chr4 714164

**TGAGAGTGCACCACGCTTTTCAA** TTCATCATTTTTTTTTATTCTTTTTTTGATTTTCAGTTTTTCATGAAA  
TTTTTGGATTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAGCACAGACTTAGATTGGTATAT  
ATACGCATATGTAGTGTGAAGAACTAAAATTGCCAGTATTCTTAATAACTGCACAGAACAA  
AACCTGCAGGAAACGAAGATAAAATCATGTCAGAAAGCTACATATAAGGAACGTGCTGCTACT  
CATCCTAGTCTGTGCTGCCAAAGCTATTTAATATCATGCCACGAAAAGCAAACAACTTGGCC  
ATTGGATGTTTCGTACCACAAGGAATTACAGATTGGTTGGAAACGTGGGTCCCAAAATTTGTTTAC  
TAAAACACATGTGGATATCTTGACTGATTTTCCATGGAGGGCACAGTTAAACCGCTAAAGGGCAT  
TTATCCGCCAAGAAATTACAGTGCCTCTTCCAAGACAGAAAGTTGCTGACATTGGTAATACAGT  
CAAATTGCAGTACTCTGCGGTGTATACAGAATAGCAGAATGGGCAGACGTGGAATACACGTTG  
TGGTGAGCCAGGTATTAGCGGTTTGAAGCAGGCGCAGAAGAAGTAACAAAGGAACCTAGAGG  
CCTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCTCTGCTGGAGAATATACTAAGGGTACTGTT  
GACGTGCGAAAGAGCGACAGATTTTGTATCGGCCTTTATTGCTCAAAGAGACATGGGTGGAAA  
GATGAAGGTTACGATTGGTTGATTATGACTTGGTGGCGTTTAGGTGAACAGGGGGAGCGCATTGG  
GTCCAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAA  
GAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCT  
GGGAAGCATATTTGAAGAAGATGCGGCCAGCAAAATAAAAGGCGGTATAGTAAGAAATGCATG  
TATACTAACTCACAAATTAGAGCTTCAATTTAA**TTATATCAGTTATTGCCTG**

chr4 762150

AACGACTTGATGATGTACATAGACTTCTGTAGGAGATTTACTGGATACACAGGATGGGGTGGTAA  
AAGGGCTGGGAGATAATTTATTGGCTTTTACAGAGCCCGGTAGATCAAGATTCCGTAGTGTGTGA  
GGGTAAATAAGTAAACAACCGGACGGGTTGGCGTATAGTAGAGAGTAAGTAGTTGTGATAGCAC  
T

**Yellow** highlight indicates the flanked the *ScURA3* fragment

**Figure S5. Clone 6 Oxford Nanopore sequencing read**

MiSeq (mi), Nanopore (nox)

**Clone 7**            **PS7**                            **intergenic-intergenic**  
 AATCAGAATCTTGAAGAGGAAAAGCATACCA TGAGAGTGCACCACGCTTTTCAA  
 chr3 953765 mi  
 CAATTTAATTATATCAGTTATTACCCTG ATGCTGGCATGAGGGGGGGAAGTC    chr3  
 824416 mi  
 Nanopore monomer out2 read 594, 131(both ends),407(both ends),442(both ends),506(both ends),594(both  
 ends),715  
 > read131  
 TCATCGGAAGAAGATCGGAACGGCTGCAGGTTGTATCGATTTTGTATTGGCTTGCATTGTTATGCG  
 TGTTTTGAGTTTTCGCTGGAATGGTGCATTACCCTTCGTGGGCTTCTATTATTATTGTTTCCTTAT  
 AATAGCGATATTTGTTGCATGTTTCTTGTACATCAGATCAAGTCTTGAAGAGGAAAAGCATACCA  
                                  **chr3 953765**  
**TGAGGTGCACCACGCTTTTCAA** TTCATCATTTTTTTTTTTTTTATTCTTTTTTGATTTTCGGTTTCCTTGAA  
 ATTTTTGATTCGGTAATCTCCGAACAGAAGAACGAGAAGAACACAGACTTAGATTGGTATATATA  
 CGCATATGTAATTTGAAGAAACATTAGAAATTGCTTGATAT **TCTTAACCCA**ACTGCACACAAGAC  
 AAAGAGCAGAACGAGATAAATCATGTCAGAAAGCTACATATAAGGAACGTGCTGTACTCATCCC  
 TAGTCCTATTTGCTGCCAAACTATTTAATATCATGCACGAAAAGCAAACAACATTTTCATTGGAT  
 GTTCGTACCACCAAGGAATTACTGGAGTTCGGTTGACATTGGGTCCCAAAGTTTGTTTTACCTAAA  
 ACACATGTGGATATCTTATTCAGTTTTTCCATGGAGGCACAGTTAAGCCGCTAAAAGGCATTACC  
 TCCGCCAAGTACAATTTTTTACTCTTTCGAAGACAGAAGGATTTGCTTTGACATTGGTATTACAGTC  
 CAGAATTGCGGTACTCTGCAGATTGGTATACAGAATAGCAGAATGGGCAGGGCATTACAAGAAT  
 ACAGTGGTGAGCCCAGAGTATTATTAAGCGGTTTGAAGCAGGCGGCAGAAGAAGTAACAAAAG  
 GAACAGAGGCCTTTTGATGTTATAGAATTAATTGTCATACAAGGAGCTCCCTATCTACTGGAGAA  
 TATACTAGGGTACTGTTGACATTGCGAAAGAGCGACAAAGGAATTTTGTATCAGCTTTATTGCTC  
 CAAAGAGACATAGATTGGAAAGATAGAAGGTTACGATTGGTTGATTATGACACCCGGTGTGAGT  
 TTAGATGACAAAGGGGAGCCGCATTAGGTCAACAGTATAGAACCGTGGATGATGTGGTCTCTGG  
 GATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGG  
 GTGAACGATTACAGAAAAGCAAGAGCTGGGAAGCATATTTGAAAGATGCGTAGCAAACTAAA  
 AACTGTATTATAGAAATGAAATGCATGTATACTAACTCACAATTGAAACTTCCAAGATTTAAT  
 TA **TATCAGTTATTACCTG**  
**chr3 824416**  
 ATGCTGGCATGAAGTCAGTGCTATATATATGTTTGTACTACGCTATGATGGGTACGTGGAGGTGT  
 GTGTGAAAGGCGCGCTGGCCAGACCCATCGCCCTGATTTCCAAGTACCCAGCCCCTAGAGCCC  
 CATCCATCATTGTTGGATCAGCACCAACCCCATCCACCATGATCTTGTATACGTATACGTTTTGA  
 AGC

**Yellow** highlight indicates the flanked the *ScURA3* fragment

**Figure S6. Clone 7 Oxford Nanopore sequencing read**  
 MiSeq (mi), Nanopore (nox)

Clone 8 PS5

SG4EUKG584656 -intergenic

TCCAAAGTCAAACCTTCCAAGTCGACG TGAGAGTGCACCACGCTTTTCAA chr1 377332 mi

CAATTTAATTATATCAGTTATTACCCTG GTAATGCAAATTTATACAGGTCAAAA chr1 375438 mi

Nanopore monomer read283 (read 283 is a ura3 monomer followed by non ura3 and then followed by 900 bp ura3 missing 200 bp of head end)

PS5a TCCAAAGTCAAACCTTCCAAGTC(GACG) chr1 377332 mi

PS5b TTTTGACCTGTATAAATTTGCATTAC chr1 375438 mi

1894 bp inversion size

With inversion, the PCR product is ura3 size of about 1.2kb.

CAATTTAATTATATCAGTTATTACCCTG GGCAGCAGTAGTGGATATGCAGC chr1 375422 mi,nox

Read 283(both ends)

Nanopore monomer read468(tail end),649 (??one end 377318),708b, 175

> read283

chr1 377460 PS 283top

ATAGACTCAATCCTGACGGTACCGTTGACTGTGTTTTGGGAAGGGTTGGATCATGGATCTGACG TATTC ACTCCTGGAGTGAAGTAGGAACCGTTGTGCAAGAGTCTGGAGTAAGTTGGGGTGACAATT GGTCTCTCAAGTGGCGAGACTTCTTGTCCAAAGTCAAGCCTTCAAGTCTGACG PS5a>

TGAGTGCACCACGCTTTCAA TTCATCATTTTTTTTTATTCTTTTTTGATTTCCGTTTTTCCTTGAAATTTT TGATTCGGTAATCTCCGAACAGAAGAGACGAAGGGAAGGCACGGAGCTTAAATTGGGTATATAT ACGCATATGTAGTGTGAAGAAGCGTGAAATTGCCAGTATATTCTTAATAGCACGCAGGGCAA AAACCTTGCAGGAAACGAAGATAAATCATGTCAAGCTACATATAAGGAACGTGCTGCTACTCAT CTAGTCCTGTTGCTGCCAAGCGTTTAATAATGCACGAAAAAGCAAAACAAACTTGTGTACTTCAT TGATGTTCCGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGTTTA CTAAAACACGTGGATATCTTGACTGATTTTTTCCATGGAGGGCTGGATTCGGCACTTCAAAGGCA TTATCGCCGTACAATTTTACTCTTCGAAGACAAGAAAATTTGCTGACATTAGTATACAGTCAAATT GCAGTACTCTGCGGGTGTATACAGAATAACCAAGAATGGGCAGACATTACGAATGCACACGGTG TGGTGGGTAGGTATTGATGTTAGCGGTTTGAAGCAGGC GGCAGAAAGAAGTAACAAAAGACAC AGAGGCCTTTTGATGTTGGGAATTATGTATTACAAAGAGGCTCCCTATCTGGAGAATATACTAAG GAGTGCTGTTGACATTGCGAAGAGCGACAAAAGATTTTGTATCGGCTTTATTGCTCAAAGAACA TGGTGAAGATAGAAAGGTTACGATTGGTTGATTATGACACCCGGTGTGAGTTTTAGATGACAAAG GGAACGCATTGGGTCAACGAGTATAGAACCGTGGATGATGTGGTCTCTGGGATCTGACGTGGC AGTATTTGAAGAGGACTATTTGCAAAGGAGGTGCTAGGGTAGAGAGATTGAACGTTACAGAAGA AAAGCAAGCTGGGAAGCATATTTGAAGATGCGGTATGTAAAGAGCTAAAAAACTGTATTATAAG TAAATGCATGTATGCTAAACTCACAAATTAGAGCTGTTAATTATATAGTTATTACCCTG

Chr1 375438

PS5b<

GTAATGCAAATTTATACAGGTCAAAA GGCATCCTTAACAGGTCGCGCGCTCGCTCTAACTCAAT TTTTTTTTTTATTCCGCACCGCACACAAGATTTACTCTCTGTGGCCATTAATGCCTAAACTGGAAA CTCTCCCTTTCTAAGGCATAAGGCATTCAAAAAGAAACGATATTGCAACCCGTACACCTGGTTTTA TTTCTTTTTCTTGTTTTTCTGCTTTCCCTATTTACAACCGGGTAACTCGTCTTCTCTTGCACTTAGA GAACACAAGCCAAAACGCTGAAGCGCAAGAACACAAGAAACCCAGCATCAGCCAGTTATTG TCAAGCTAATTGAAAGTAGCGATCGTCCTTGTGACTACCGCCATAATATTCGTGATGATTCAAAA CCTCAAAGATGAGCCAGTTTGAACCTTGGAACTTTAATTCAGGGTTTTCCAGCACAGTTTAATT TACAGTTTTAGTGATATATTTTGGACCGCATGGAAATCTTTTTTTTCAATCACTTGTTTTTAAATT TCTGTGAGTGTATTTAGTTATGGATGGGTACCACCAAAACAACAATTCACAGAGGTGGGCAT

TTGGGCTCTAAGTGCTAGTCGTCAGTGTAGGGATATGAGTCATTTTTATTTTTCTTTTCGCTGTCTT  
GCTTGTGTTGAGTGTAGGTAAGAGTCAGAAACCAGTGATACAGACTAATGCTGTAGCATTGCCTGT  
TGATAAAATTAATTAAGATGCCCATCGAAAATGAGTAAAAGTTGGAGAAAACTTTAGACGGTAT  
ATAAATGGTTTCGGAGAGTGGAAACAAATATGTGGAGGAAGTAAAGTTCTCTTGCGTATGGTTGTAA  
TAAGCTAATGTATATATTTGTTAGTGTGTATAGAAGCTAAGCAATGTAGATTGATAGTGGAAAGGA  
TAGTTGCAACAGGGAAGAGCGATTTCACGCAAGAGCATAGATCTAAGTAGTTTACAAAAATGTC  
TAAAAGGTAAGGTTTGTGGCATACTCCGGTGGTTGGAATACCTCTGTGATTTTGGCGTTGTTGGA  
ACAGGAATTGCAAAGTTGTTACCATTTGCAGCCAATGTTGGTCCAGAAGACTGAGGCCGCGAAA  
ACTTTGCTTGTGGTGCCACCAAAGTTGTTCTAGTGGACTGAGAGAAGAAATTTTCAGGACGTTT  
TGTTCCAGCTTCTGTGCAAGTTAACGTTTGTATACGAATGTGTACCTGTGGAACATCTTTGTGA  
ACCAGTGATTGCCAAGGCCCAAATTGACGTGGCCAAGCAAGAGTTGTTTGCCGTTGCGCACGGG  
TGTACCGGTAGGGTAACGATAAAATCAACGTTTGGAACTAGGGTTCTACGCCTGAAGCCAGAC  
GTCCAGTCATTGCTCCATGGAGAGACCCATCTTTCTTCAACTTAGATTTTGTGTTAGAAAGGAT  
CTTTTTGGCGCTGCTGAAAGACATTCCAGTGGTCAAACCTGCTTCAAGCCATGGTCTACCGATGA  
GAACTTGGCCACATAACCAGGAAGCCGGTATTCTAGAAGATCGACCAAACCACCAAAGGAC  
ATGTGGAAGTTGATCACCGACCCACAAGATGCCCCAGACCAACCACAAGACTTGGACATTGGGC  
GAAAAGGGTTTGGCAGTCAGAGTTGGCGTACAAGGATGCCAGGTCGGTAAAGGGAAATCACA  
GCCACAACCCATTGGACATTTCTTGACCGCTGCCAAGTTGGCCAGAGCTAACGGTGTGGCAGAA  
TCGAGCATTGTCGAAAACCGTTACATT

AACCTAAAGTCCAGAGGCTGTTACGAACAGAAATGTTGTCGTCTCTGGCAACTGCACAGAACA  
AAACCTGCGGGAAACCGAAGATAAATCATGTGCAAGCTGCATATAAGGAACGTGCTGCTGCGCA  
AATGCGATCCTGTTGCTGCCAAGCTGTTAATATCATGCACGAAAAGCGGCAAAACTTGTGTGCT  
TCGTGGATGTTGCTCCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAATTTGT  
TTACTAAAGCACATGTGGATATCTTGACTGATTTTTTTCTGTGGAGAAGTGGATTAAGCCGCTA  
AAAGGCATTATCCGCCAAGTACAATTTTACTCTTGAAGACAGAAAATTTGCTGACATTGGTAA  
TACAGTCAAATTGCAGTACTCTGCGGGTATACAGAATAGCAGATGGGCAGACATTACGAATGCA  
CACACGGTGTGAGGTGGGCCGAGGGCAGTATTGCTTGGTTTGAAGCAGCCGCGCAGAAGAAGTA  
ACAAAGGAACCTGAGGCAGCACAGTGTAGCAGAATTGTCATGCAAGGGCTCCCTATCTACTGG  
AGAATATACTAAAGAATTTACTGTTGACATTGCGAAACGACAAAGATTTTGTATCATTTATTGCT  
CAAAGAGACATGGGTGGAAAGAGATGAAGGTGCATTGGTTGCGGCCACCCGGTGTGGGTTTAGA  
TGACAAAGGGAGACGCATTTGGGTCAAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGAT  
CGACATTATTATTGTTGGAAGAGGACTGTTGCAAAGGGATGCTAAGGTAGAGAGATTGAACGTT  
ACAGAAAAGCAGGCTGGGAAGCATGTTGAGAGAATGCGTAACAAACTAAAACTATGTTATAA  
GTAAATGTATGTACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCTG

Chr1 375422 PS6b<

GGGCAGCAGTAGTGATATACAGCTGTCCCGGGCGTTAGAGTAAAAAATTAGAGTGGAAAGAATA  
GAAAAAGAAAGAATATTGTGTAACGTTTGCAGTATCTATTTATCTTATGTAGATGGGTAAAGCTT  
TAGTTTATTTGTCGGATGACAGCTAGCTCTCGCTAGTCGTATGTGATTTTCTCGAATATCTTGCTT  
CAAT PS 283 bottom

Yellow highlight indicates the flanked the *ScURA3* fragment

Green highlight indicates partial *ura3\_TestF*

### Figure S7. Clone 8 Oxford Nanopore sequencing read

MiSeq (mi), Nanopore (nox)



**Clone 5 PScl5 intergenic chr7--intergenic chr6 Dimer**

Miseq

TAACTTCGGAATATTGAATTAATCGTCTCC TGAGAGTGCACCACGCTTTTC chr7

24526 mi

CAATTTAATTATATCAGTTATTACCCTG TATATTATATTAATAAAAAATAAT chr6

375003 mi

**Found in chr6 Nanopore assembly, but not in MiSeq assembly**

Nanopore sequence read 17015, 008a,

302,311,329,355,383,390,439,480,532,550,563,581,622,626,629,668,678,685,712,721,724

>read355 reverse complement

TGCTGGACTAACTATAGGGTAGTTCGTAGCTGTGATCTTGATTGTCAGTTTTGCTCAAATAAATAT  
TATATATATATATAGAAAGAAGTGGGTAGACCACATTGTTTACAATACGTCTTAAATTGTGTTCCG  
ATGAGCATGTAGACAAAATTAGAGTAGAAGTGTGGGTTCTAACTTCGAATATTGGAATTAATCG  
TTCC chr7

GAGAGTGCGCACCACGCTTTTCAATTCATCATATTTTTTTTATTCTTTTTTGATTTTCGGTTTCTTGAAA  
TTTTTTTGATTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAAGGAGCACAGACTTAGATTG  
GTATATATACGCATATGTAGTGTGAAGAAACATGAAATTGCCCAGTATTCTTAACCCAACCTGCA  
CAGAACAAACCCGCAGGAAAACCTGAAGATGCTAAATCATGTGCGAAAGCTACATAAGGAACGTG  
CTGCTACCATCCCAGTCTGTAAAGCTGCTGCCATTTATCATCATGCACGAAAAGCAAACAACCTT  
GTGTGCTTCATTGGATGTTTCGTACCACCGCTGAACTACTGAGTTAGTTGAAGGCCATTAGGTCCCA  
AAATTTGTTTACTAAAACACACATGTGGATATCTGACTGATTTCTTTCCATGAGGGCACAGTTAAG  
CCGCTAAAGGCATTATCCGCCAGCACAAATTTTACTCGAAGACAAGAAATTTGCTGACATTGGTA  
ATACAGTCAAATTGGTAGTACTCTGCGTGGTAGTATACAGAATAGTGCAGAATAAGACATTACG  
AATGCACACGGTGTGGTGGGCCAGGTATTGTTATGGTTTGAAGCAGGCGCGGTAGAAGTAAAT  
GAAACCTAGAGGCCTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCTCATTCTACTGGAGAAGG  
AATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATCGGCTTTATTGCTC  
AAAGAGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTGTGACACCCGGTGTGGGTTT  
AGATGACAAGGAGACGCATTGGGTCAACAACAGTATAGAACCGTGGGATGATGTGGTCTCTACA  
GGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGTGCTAAGGTAGA  
GGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAACCTA  
AAAACCTGTATTATAAGTAAATGTACGTATACTAAACTCACAAATTAGAGCTCTAATTTAATT**TAT**  
**CAGTTATTACCCTG**

TGAGAGTGCACCACGCTTTCAATTCATCATTTTTTTTATTCTTTTTTGATTTTCGGTTTTCGAAATTTTTTTG  
ATTCGGTAATTCCGAACAGAAGGAAGAACGAAGGAAGGAGCATGACTTAGATTGGTATATATAC  
ATATGTAGTGTGAAGAAACATGAAATTGCCCAGTATTCTTAACCCAACCTGCACAGAACAAAA  
CCTGCAGGAAACGAAGATAAATCATGTGCGAGTTACATATAAGGAACGTGCTGCTACTCATCCTA  
GTCCTGTTGCCGCTAAGCTATTTAATATCATGCACGAAAAGCAACAAATCCCAGGTAAGCCTATT  
GGATGTTTCGTACCACCAAGGCACTACTGGAGTTAAGTTGAAGCATTAGGTCCCAAAATTTGTTTA  
CTAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGGCACAGTTGCCGCTAAAGGCA  
TTATCCGCCAAGTACAATTTTACTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAAT  
TGGCAGTACTTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAACACACGGTGT  
GGTGGGCCAGGCATTGTTAGCGGTTTGAAGCAGGCGGCAGAAGAAGTAAATCGTAGCGAACCC  
AGAGGCCTTTGATGTTAGTGCAGAATTGTCATGCAAGCGGCCCTCGTATCTGGAGAATACTAAG  
GGTACTGTTGACATTGCATTGTTAATAAAGATTTTGTTATCGGCTTCATTGCTCAAAGACATTAC

GGGTGAAAAGAGATGAAGGTTACGATTGGTTGATTATGACACCCCGTGTGCTTTAGAATAGCCGC  
GAGACGCATTGGGTCAAATTGCATAGAACCGTGGATGATGCGGTCTCTGCCATGATCTTGACATT  
ATTGCTGGAAGAGGACCATTTGCAAAGGAAGGATGTGCTAAGGTAGAGGGTGAACGTTACAGA  
AAAAGCAGGCTGGAAGCTATTATTTGAGAAGATGCGGCCAGCAAACCAAAAAAACTGTATTATAA  
GTAAATGCATGTATACTAAACTCACAAATTAATAGAGCTTCAATTTAATTATATATCAGTTATTAC  
CCTA

**chr6**

TATTATATTAATAAAAATAATACTGATTAACAATAAAGTTCATAAATAATTAATAAATGATA  
TTAATTACTTGTATTAATTACAAGCATATTTATACTCATCATCTTGGGCATTGAGAGGGAATCAAT  
CAACTTACAGCATGGGCAGATGTTCTATATGAAAATCAGACAAACAAAGCTAAGAGCAACGTA  
CGC

**Yellow** highlight indicates the flanked the *ScURA3* fragment

**Figure S8. Clone 5 Oxford Nanopore sequencing read**

MiSeq (mi), Nanopore (nox)

Clone 15 PS10 intergenic-intergenic  
 AAAAAAATTATTATGGAACAATTTGTGTG tgagagtgaccaATTA**CGCTTTTCAATTC** chr1  
 1098288 mi,nox  
 CAATTTAATTATATCAGTTATTACCCtg **CATACGCGAAACTCAGGTGCTGCA** chr5  
 1165171 mi,nox  
 CAATTTAATTATATCAGTTATTACCCtg  
 CATACGCGAAACTCAGGTGCTGCAATCTATATTTTTTTTTTTTTCCCTCTTACCATTCTGAAAAGAC  
 TTAAATAA  
 CAATTTAATTATATCAGTTATTACCCtg  
 CATACGCGAAACTCAGGTGCTGCAATCTATATTTTTTTTTTTTTCCCT  
 chr5 1172530 single site Present in mi assembly  
 Nanopore monomer read 566 rc Both junctions are on same read.  
 Nanopore 086,454b,541b,566,629

>read566 reverse complement out2  
 TAGAACTAAATAAAATGTCTATAATGAGATTACACAAGGTATTAACACAACCTTACCATTAAATATATG  
 AGTTAGATCTCCCAATCATCGCGCACTTTTCTTTTAATATCTTTTCATTTTTCCATACTAACTCTACTGCT  
 AAATGTAGTGAAGCAACAAAAAATTATTATGGAACAATT **chr1**

**TGTGTGATTACGCTTTTCAATTC**ATCATTTTTTTTATTCTTTTTTTTGATTTTCGGTTTCCTTGAAATTTTTGATTC  
 GGTAATCTCCGAACAGAAGGAAGAACGAAAGCTGAAGCTGAGCACAGACTTAGATTGGTATATATACG  
 CATATGTAGTGTGAAGAAACATGAAATTGCCAGGCATTCTAACCCAAACAGAACAAAAACCTGCAGG  
 AAACGCAAAGAGTTAAATCATGTGCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTT  
 GCTGCCAAGCTGGGTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGATGTCGTA  
 CACCAAGGAATTACTGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGCTTTGCCAAACACATGTGGATAT  
 CTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCTGCTAAAGGCATTATCCGTCATACAATTTTTTACTC  
 TTCGAAGACAGAAAAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTACCTGCGGGTGTATACAGA  
 ATAGCAGAATGGGCAGACATTACGAATGCACACGGTGTGGTGGGCCAGGTATTGTTAGCGGTTTGAAG  
 CAGGCGGCAGAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGCCGCGCAAGGG  
 CTCCCATCTACTGAGAATATACTAAGGGTACTGTTGATTGCGAAGAGCGACAAAGATTTGTTATCGGCC  
 ATTGCTCAAAGCATATATAAAGTGGGAAGAGATGAAGGTTACGATTGGTTGATTATGCACACCCGGTGTG  
 GCTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGAT  
 TCGACATTATTATTGTTGGAAGAGGACGGTTTGTAAAGGAAGGGATGCTAAGGTAGAGGGTGAACGTTA  
 CAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAACTAAAACTGTATTATAAGT  
 AAATGCATGCATACTAACTCACAAATTAGAGCTTCAATTTA**ATTATATCAGTTATTACCC**  
**chr 5**  
 CATACGCGAAACTCACAGGTGCTGCAATCTATATTTTTTTTTTTTTCCCTCTTACCATTCTTCTGAAAAGACTT  
 AACAATCATATAGGATAAGTACTGCAGCGGCTGTTCTAAACAAATATGCAAGCCCACATAAGGCACGT  
 AGCACAATATTAATAGACCATATCTCTACTTAGTGAAAGTCATGAGCGGCTAAGAACG

**Yellow** highlight indicates the flanked the *ScURA3* fragment

**Figure S9. Clone 15 Oxford Nanopore sequencing read**

MiSeq (mi), Nanopore (nox)

**Clone 23 PS8 SG4EUKG586913-intergenic**

TATACACGAGATATTAGTCGAGTGGGA GTCCCATGCACCACGCTTTTCAA chr4 101355 mi

TTATATCAGTTATTACCCTG GTATCTTCGCTTTGTCTCTTAGCTTCC chr6 7292 mi

Nanopore monomer out2 read718, 421.067, 147, 164 (both ends),191,194(both ends),253,417(both ends),421(both ends),718(both ends), In chr6 partial on nox  
> read164

TAGCCTTGTTGGCAAGATGGTATTATATACACACATGGCTAATAGTGTGCATCCGGTGGAGATTA  
GGTACAGTGGAAAGTCGGATAACTCCGAAAACCGGAGACCCATGGAACGTCATCTCGCCATCGG  
TGTCGCTGAACAGCTGCAATGAGTA **TATACACGAGATATTAGT**CGAGTGGGA **chr4**

**ATTCTATGCACCACGCTTTTCAA**TTTCATCATTTTTTTTTTATTCTTTTTTTTGATTTCCGGTTTCCTTGAAAT  
TTTTTGATTCGGTAATCTCCGAACAGAAGGAAGAACTTTGAGAGAATACAGACTTAGATTGAGTA  
TATACGCATATGTAGTGGTGAAAACATGAAATTGCCAGTATTAATAGCGCTACTGGGAACAAA  
ACCTGCAGGAAACGAAGATAAATCATGTCAGCTACATATAAGGAACGTGCTGCTGTCATCCTAG  
TCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAACTTGTGTGCTTCATTGG  
ATGTTTCGTACCACCAAGGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAATTTGTTTACTA  
AAACACATGTGGATGTCATGACTGATTTTTTCCATGGAGGGCACAGTTAAGCCGCTGAGGCATTA  
TCCGCCAAGTAAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAA  
TTGCAGTACTCTTGCGGGTGTACCTAGAATAGCAGAATGGGCAGACGTACGAATGCACACGGTG  
TGGTGGGCCAGGTATTGTTAGCGTTTTGAAGCAGGCGGCAGAAGTAACAAAGGGAACCTAGAG  
CCTTTTGATGTTAGCAGAATTGTCATGCAAGGGGCTCTATACTGGAGAATATACTAAGGGTACTG  
TTGACATTGCGAAGAGCGACAAAGATTTTTGTTATCGGCTTTATTGCTCAAAGAGACATGGGTGG  
AAGAGATGAAGGTTACGATTGGTTGATTATGACACCCGGTGTGGTTTTAGATGACAAGGGCGC  
GTTAGGTGCGGCTTAGTAGAACCGTGGATGATGTGGTCTCACCACCAGGATCTGACATTATTAT  
TATTTAGGAAGGACTGTTGCGAAAGGGATGCTAAAGGTAGAGGTGAACGCGTTACAGAAAAGC  
AGGCTGGGAGCATATTTGAGAAGATGCGGCCAGCAAACTAAACTGTATTATAAGTAAATGCA  
TGTATACTAAACTACAAATTAGAGCTTCAATTTAATTA **TATCAGTTATTACCCTG**

GTATCTTCGCTTTGTCTCTTAGCTTCCATGTAAAGTGGTTACAAGTAACTAGAAACCAAACTCAA  
ACAAATAAAACCACAACTTCAGAAACAGAACTTTCAAGTTGAAAAAGTTCTCCTCTCTTTTCTC  
CTTCTATACTTTTAATTGGAGAAAGATATTTGGTATTTCAATTTGATTGACCTCTGGTTTTGCTTGA  
TA **chr6**

**Yellow** highlight indicates the flanked the *ScURA3* fragment  
Red indicates sequence from the read is different from *ScURA3* fragment

**Figure S10 Clone 23 Oxford Nanopore sequencing read**  
MiSeq (mi), Nanopore (nox)

Primer name	Sequence 5'→3'
P1	GTATAGACGCATGAAGTCCTTCATTTGCTTTTGTTCACA
P2	gagttctccgagaacaagcagagggtcgagTGTACTCGGA
P3	ctcgaacctctgcttgtctcggagaactcCCTCCTTTGATTAGTTTATGGGGCTAGCTG
P4	TAGGTGCCTGTCACGGCTCTTTTTTTACTGTACCTGTGAC
P5	TTGGAAGTTAGCTTAGCAGAGGTAAGTCG
P6	GGAAAGACCATTCTGCTTACTTTTAGAGC
P7	atatcctaaattgcatgagggtgagATGTCGAAAGCTACATATAAGGAACGTGCTGCTAC
P8	tgccatgtaaaacatacggatagTTAGTTTTGCTGGCCGCATCTTCTCAAATATGCTTC
P9	CAGCAAACTAAActatccgatgttttacatggcaattcctaatttgcaatcatgcac
P10	aggcgaggaagaggaaggtggaaatgtgaaaagttg
P11	ACCTTCGAACCAACCAtatcctaaattgcatgagggtgag
P12	gatgfactgcattcaatgacggtttggctcttctcgtatttaacgggttcga
P13	CAATGGTTGGGCATTGCAATTCCTACGTTCTAATTAGTTTCATAGGATATGTTGCCAC
P14	tatacgttctcaaaactcagagatggatgaaggcgaggaagaggaag
P15	GCATGAGGGTGAGcatggcaattcctaatttgcaatcatgcacaaatgatcgctag
P16	gcaaaattaggaattgccatgCTCACCTCATGCAATTTAGGATATGGTTGGTTCGAAG
P17	CACAACTTCATAAGCGTCCCAAGGTTTCGACC
PS1a	GCTAAGCTGTTGCAATGACCCAC
PS1b	CCCCGGTTCCTTATATTTCCCTATTACTACA
PS2a	TCTCAATACCAAATTCGTCGTCTAGAG
PS2b	TTGTTTGGCTCCTTTGGTGAAGG
PS3a	CCTATTGAGAAAAAACCAGAACTATTT
PS3b	GCTTAGAGTGTGTTATTTAGTCAAGAATT
PS4a	TGATGATGATGATAAGATAGTGAAGAAGG
PS4b	TCATTTTACTTTCCAAACAAGACTAGATTCT
PS5a	TCCAAAGTCAAACCTTCCAAGTC
PS5b	CCTTTTGACCTGTATAAATTTGCATTAC
PS6a	GGAATTTGCCAAGTCCAGAGG
PS6b	GCTGCATATCCACTACTGCTGC
PS7a	AATCAGAATCTTGAAGAGGAAAAG
PS7b	GACTTCCCCCCTCAT
PS8a	TATACACGAGATATTAGTCGAGTGG
PS8b	GGAAGCTAAGAGACAAAGCG
PS9a	AAAACCAAAAAAACCATAAAAAACC
PS9b	CTATGTACATCATCAAGTCGTT
PS10a	AAAAAAAATTATTATGGAACAATTTGTGTGAT
PS10b	TGCAGCACCTGAGTT
PS11a	TGTGGCTAAGGTAACGG
PS11b	ACCAAGTGACCTGGC
PS12a	TGAAAAAATGATCCTGAAAAGA
PS12b	GGTGTTCATGTGAC

PS13a	GATATACCAGTACAAAGGTAGTGA
PS13b	CGCTTCGTCCGTTTC
PS14a	TACAGTGGAAAGTCGGATAACT
PS14b	ACTGAGCTGACTGCATG
PS15a	ACCACTGTTACGTCGG
PS15b	TTTGTATAAGAAGACCTTGCG
PS16a	TTTTTCGATTTAATTAATATATATTATATAACAAC
PS16b	CAAACATATCGTCTTGGTATCTTTA
PS17a	CATGCCGGCCGAGTAACCGAGAGAA
PS17b	CGCATAAGAATCGATCCGA
PS18a	TGTACTIONCTACGAAGGGC
PS18b	GAAAAAAAAAATAAACTTTTTTTTCTCAAAAGCT
PS19a	ACTTCTAAATCGGATAGATTCTGA
PS19b	ACTCTGGCATTTAATTTTGAGTC
PS20a	GGCGTCGGGGTAACTA
PS20b	CGAAAAAGAAACGCCCTCAG
PS21a	CCCTGCCGTATATACCTG
PS21b	GATCTGGGATTTAAGAGGCG
PS22a	CAATAAAACACTAAAATAAAAATAAAATAAATTGAGAAAAAATCGA
PS22b	TTTTCCATTATTTTTTATATTATATTAGATATTCCATGC
PS23a	CGACATTTTTTTTCGATTTAATTAATATATATTATATAACAAC
PS23b	CAAACATATCGTCTTGGTATCTTTAG
PS24a	TGTGGCTAAGGTAACGG
PS24b	ACCAAGTGACCTGGC
PS25a	GCCGTAACCTCTTTTTTATTG
PS25b	CGGAAAAAAAAACGAAAAAAAAAAAAAAAAAATA
PS26a	TTCAAAGTGGAAAAAACACCA
PS26b	ACGATTTACAGGATTATCTAACG
PS27a	CATTAGCACCAAACCCACG
PS27b	TTGAGACCTGGTGTTTTATTAGGA
PS28a	TCCAAAGTCAAACCTTCC
PS28b	TTTGACCTGTATAAATTTGCATTAC
PS29a	GGAATTTGCCAAGTCCAGA
PS29b	GCTGCATATCCACTACTGC
PS30a	ACCGTGCATTAACGGT
PS30b	GGAAGAATCCGAGACACT
PS31a	TCTCAATTTATTTTATTTTATTTTAGTGTTTTATTG
PS31b	GGAAATATCTAATATAATATAAAAAAATAATGGAAAAA
PS32a	GCATCATTAGTAGTTATTGCATGG
PS32b	ACTCATTCCACTAGAATCTTCTCA
PS36a	TTTTCAAAGAACCGTGCATTAAC
PS36b	TGGGCAAGTTTTCCCG
PS37a	TGTACTIONCTACGAAGGGC
PS37b	GGAATTATAAACACTTACATACGCTGA
PS283top	TTGGATCATGGATCTGACGTATT
PS283bottom	CTAGCTGTCATCGGACAAATAAACTAAAAG

PS4-6F	TACAGTGGAAAGTCGGATAACT
PS4-6R	AGAATGGTGTGATTTAGGTTTCG
PSchr4delF	CCTGCAAATTACGCCTTC
PSchr4delR	GCCATACCAAGTGCG
PSade1F	CCAAGGTATACGTTGGTATA
PSade1R	CTATTACTCGTTGGTTCCTAG
PSadenewF	AGGAACTATCGTTAAAACCAAGGTATACGTT
PSadenewR	GACTTGGATTTGTATAAGTATTCCACTTCCCA
PSchr8a	ACTTCTAAATCGGATAGATTCTGA
PSchr8b	ACTCTGGCATTTAATTTTGAGTC
PScl5a	TTCGGAATATTGAATTAATCGTCTCC
PScl5b	AATTAATATTATTTTTATTTAATATAATATAATTATTTATGAACTTTATTGTTAATC
T33	CAGAAACGGGAAGAATCTG
T34	TTCAACACCGTTCGACA
T35	CTAGGCTATCACACACACTA
ura3-F	TGAGAGTGCACCACGCTTTTCAATTC
ura3-R	CAGGGTAATAACTGATATAATTAATTAATTG
ura3+720	GGGAAGGGATGCTAAGGTAGAGGGT
ura3+720c	ACCCTCTACCTTAGCATCCCTTCCC
URA3-R1-1-DN	CGGCCAGCAAAACTAAAAAACTG
ura3+61c	GCTTGGCAGCAACAGGACTAGGATG
ura3_TestF	GTTAGCGGTTTGAAGCAGGC
ura3_TestR	GCCTGCTTCAAACCGCTAAC

**Table S1. Primers used in PCR reactions. F: forward. R: reverse. DN: downstream. chr: chromosome. Del: deletion. Lower case letters such as a, c, and c are given as a way to keep tracking the number of primers for each testing locus.**