

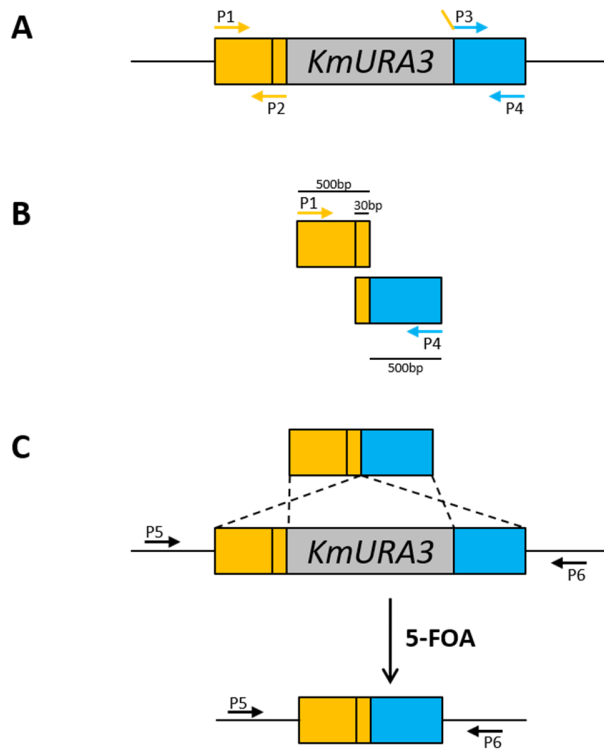


Figure S1: Construction of G13. (A) Genomic DNA was used as a template to amplify 500bp targeting arms flanking the *URA3* ORF of *K. marxianus*. P1 and P2 were used to amplify the left arm, while P3 and P4 were used to amplify the right arm. (B) In the second round of PCR, the left and right arm was joined by 30bp overlap using P1 and P4. (C) The final PCR of a total volume of 600 μ l (1x Phusion master mix, 1 μ l of prior PCR products, and 0.2 μ M of each primer) was purified yielding 18 μ gs after gel purification (Qiagen Qiaquick). Either 1 or 5 μ gs 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 μ ls of cells were mixed with DNA in a 0.4mm gap electroporation cuvette and pulsed at 2.5kV, 25 μ 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.

TGAGAGTGCACCACGCTTTCAATTCAATTCATCATTTTTTTTTTATTCTTTTTTTGATTTCCGTTTCTT
 TGAAATTTTTTTGATTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAAGGAGCACAGACTTAGA
 TTGGTATATATACGCATATGTAGTGTTGAAGAAACATGAAATTGCCAGTATTCTTAACCCAACTGCA
 CAGAACAAAAACCTGCAGGAAACGAAGATAAATC**ATG**TCGAAAGCTACATATAAGGAACGTGCTG
CTACTCATCCTAGTCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAACTTGTG
TGCTTCATTGGATGTTCTGACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAT
TTGTTTACTAAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCCGCT
AAAGGCATTATCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAA
TACAGTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAATG
CACACGGTGTGGTGGGCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCAGAAGAAGTAACAAA
GGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCCTATCTACTGGAGAATA
TACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTATCGGCTTTATTGCTCAAAG
AGACATGGGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGACACCCGGTGTGGGTTTAGAT
GACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGATCTGA
CATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGAACGT
TACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAACTTAA****aaaactgtattataa
 gtaaatgcatgtataactaaactcacaattagagcttcaatttaattatatcagttattaccctg

Figure S2: Sequence of *ScURA3* used in transformation

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

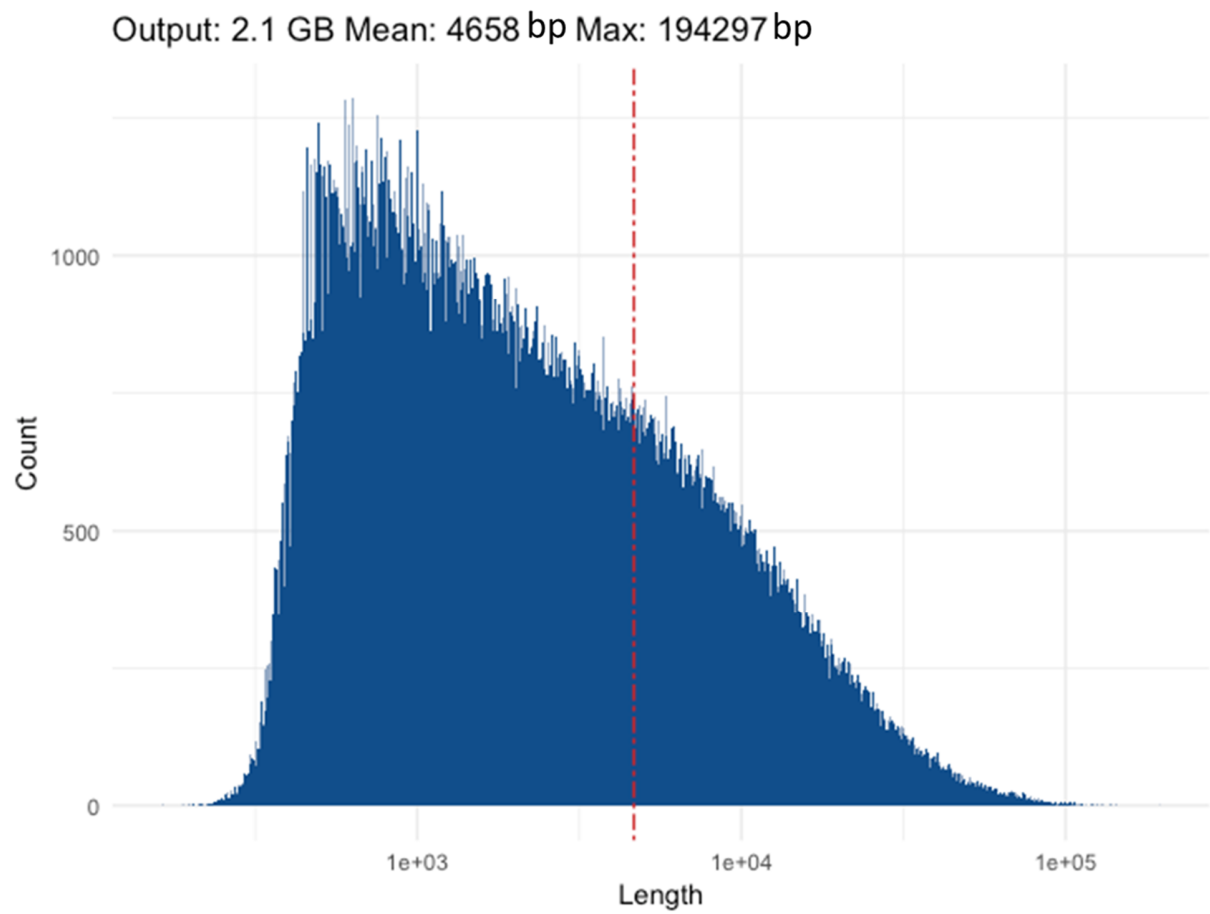


Figure S3. Distribution of Oxford Nanopore sequencing read lengths for the 24-clone pool DNA of *K. marxianus* *ScURA3* cassette DNA transformants.

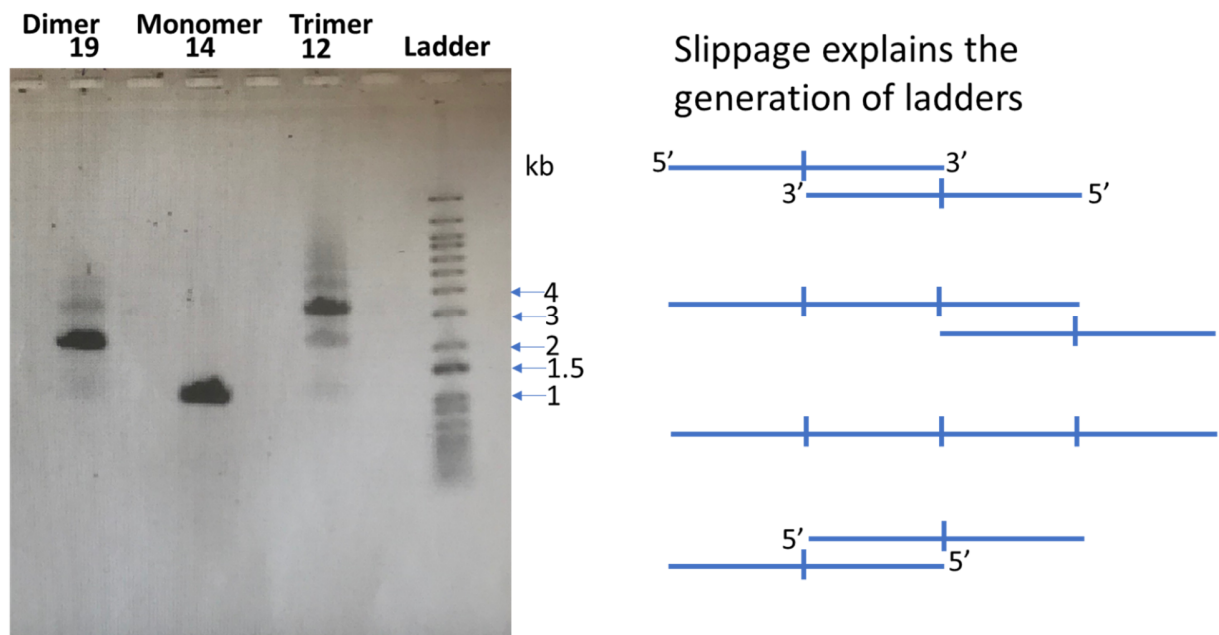


Figure S4. Gel electrophoresis of PCR amplified *K. marxianus* ura3 monomer (clone 14), dimer (clone 19) and trimer (clone 12) transformants using flanking primers. The dimer and trimer lanes show ladders of bands produced by slippage during the annealing step of each cycle.

Clone 6 PS9 * intergenic-intergenic

PS15 for deleted region clone6

AAAACCAAAAAAAAAACCATAAAAACCAAAACA TGAGAGTGCACCACGCTTTTCAA

chr4 714164mi

CAATTTAATTATATCAGTTATTACCCTG AACGACTTGATGATGTACATAG

chr4 762150mi

Nanopore monomer out2 read005,117,134(both ends),320(both ends),538(both ends),597(both ends),688(both ends)

> read134

AAAACATCCGCACCTGCAAATTACGCCTTCCAGTGACTGTCTAATCGCTCAACAACTCCTCTCC
CACTGTCTCACGATACTCGACTTTGTTGCGATGAGCATAGGTTTCGGGAATTTCAATGGATGTATA
TCTGTGTACTTAACGCGATATTTAAATCACGTGTAAGGAAAAAACCAAAAAATATAAAAAGCAA
AACA chr4 714164

TGAGAGTGCACCACGCTTTTCAA TTCATCATTTTTTTTATTCTTTTTTTGATTTTCAGTTTTTCATGAAA
TTTTTGGATTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAGCACAGACTTAGATTGGTATAT
ATACGCATATGTAGTGTTGAAGAACTAAAATTGCCAGTATTCTTAATAACTGCACAGAACAA
AAACCTGCAGGAAACGAAGATAAAATCATGTGAGAAAGCTACATATAAGGAACGTGCTGCTACT
CATCCTAGTCCTGTTGCTGCCAAAGCTATTTAATATCATGCCACGAAAAGCAAACAACTTGGCC
ATTGGATGTTTCGTACCACAAGGAATTACAGATTGGTTGGAAACGTGGGTCCCAAAATTTGTTTAC
TAAAACACATGTGGATATCTTGACTGATTTTCCATGGAGGGCACAGTTAAACCGCTAAAGGGCAT
TTATCCGCCAAGAAATTACAGTGCCTCTTGAAGACAGAAAGTTGCTGACATTGGTAATACAGT
CAAATTGCAGTACTCTGCGGTGTATACAGAATAGCAGAATGGGCAGACGTGGAATACACGTTG
TGGTGAGCCAGGTATTAGCGGTTTGAAGCAGGCGGCAGAAGAAGTAACAAAGGAACCTAGAGG
CCTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCCTCTGCTGGAGAATATACTAAGGGTACTGTT
GACGTGCGAAAGAGCGACAGATTTTGTTATCGGCCTTTATTGCTCAAAGAGACATGGGTGGAAA
GATGAAGGTTACGATTGGTTGATTATGACTGGTGGCGTTTAGGTGAACAGGGGGAGCGCATTGG
GTCCAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGAAA
GAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCT
GGGAAGCATATTTGAAGAAGATGCGGCCAGCAAAATAAAAGGCGGTATAGTAAGAAATGCATG
TATACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTGCCTG
chr4 762150
AACGACTTGATGATGTACATAGACTTCTGTAGGAGATTTACTGGATACACAGGATGGGGTGGTAA
AAGGGCTGGGAGATAATTTATTGGCTTTTACAGAGCCCGGTAGATCAAGATTCCGTAGTGTGTGA
GGGTAAATAAGTAAACAACCGGACGGGTGGCGTATAGTAGAGAGTAAGTAGTTGTGATAGCAC
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

TGTTTTGAGTTTTGCGCTGGAATGGTGCATTACCCTTCGTGGGCTTCTATTATTATTTGTTTCCTTAT

AATAGCGATATTTGTTGCATGTTTCTTGTACATCAGATCAAGTCTTGAAGAGGAAAAGCATACCA

chr3 953765

TGAGGTGCACCACGCTTTTCAA TTCATCATTTTTTTTTTTTTTTTATTCTTTTTTGATTTTCGGTTTCCTTGAA

ATTTTTGATTTCGGTAATCTCCGAACAGAAGAACGAGAAGAACACAGACTTAGATTGGTATATATA

CGCATATGTAATTTGAAGAAACATTAGAAATTGCTTGATAT TCTTAACCCAAC TGCACACAAGAC

AAAGAGCAGAACGAGATAAATCATGTCAGAAAGCTACATATAAGGAACGTGCTGTACTCATCCC

TAGTCCTATTTGCTGCCAAACTATTTAATATCATGCACGAAAAGCAAACAAACATTTTCATTGGAT

GTTTCGTACCACCAAGGAATTACTGGAGTTCGGTTGACATTGGGTCCCAAAGTTTGTTTTACCTAAA

ACACATGTGGATATCTTATTCAGTTTTTCCATGGAGGCACAGTTAAGCCGCTAAAAGGCATTACC

TCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGAAGGATTTGCTTTGACATTGGTATTACAGTC

CAGAATTGCGGTACTCTGCAGATTGGTATACAGAATAGCAGAATGGGCAGGGCATTACAAGAAT

ACAGTGGTGAGCCCAGAGTATTATTAAGCGGTTTGAAGCAGGCGGCAGAAGAAGTAACAAAAG

GAACAGAGGCCTTTTGATGTTATAGAATTAATTGTCATACAAGGAGCTCCCTATCTACTGGAGAA

TATACTAGGGTACTGTTGACATTGCGAAAGAGCGACAAAGGAATTTTGTTATCAGCTTTATTGCTC

CAAAGAGACATAGATTGGAAAGATAGAAGGTTACGATTGGTTGATTATGACACCCGGTGTGAGT

TTAGATGACAAAGGGGAGCCGCATTAGGTCAACAGTATAGAACCGTGGATGATGTGGTCTCTGG

GATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGG

GTGAACGATTACAGAAAAGCAAGAGCTGGGAAGCATATTTGAAAGATGCGTAGCAAACTAAA

AACTGTATTATAGAAATGAAATGCATGTATACTAACTCACAAATTGAAACTTCCAAGATTTAAT

TA TATCAGTTATTACCTG

chr3 824416

ATGCTGGCATGAAGTCAGTGCTATATATATGTTTGTACTACGCTATGATGGGTACGTGGAGGTGT

GTGTGAAAGGCGCGCTGGCCAGACCCATCGCCCTGATTCCAAGTAGCCCAGCCCCTAGAGCCC

CATCCATCATTGTTGGATCAGCACCAACACCCATCCACCATGATCTTGTATACGTATACGTTTGA

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 GTAATGCAAATTTATACAGGTCAAA 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

ATAGACTCAATCCTGACGGTACCGTTGACTGTGTTTTGGGAAGGGT**TTGGATCATGGATCTGACG**
TATTCACTCCTGGAGTGAAGTAGGAACCGTTGTGCAAGAGTCTGGAGTAAGTTGGGGTGACAATT
GGTCTCTCAAGTGGCGAGACTTCCTTGTCCAAAGTCAAGCCTTCAAGTCTGACG PS5a>

TGAGTGCACCACGCTTTCAATTTCATCATTTTTTTTATTCTTTTTTGATTTCCGTTTTCTTGAAATTTT
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ACGCATATGTAGTGTTGAAGAAGCGTGAAATTGCCAGTATATTCTTAATAGCACGCAGGGCAA
AAACCTTGCAGGAAACGAAGATAAATCATGTCAAGCTACATATAAGGAACGTGCTGCTACTCAT
CTAGTCCTGTTGCTGCCAAGCGTTTAATAATGCACGAAAAAGCAAAACAACTTGTGTACTTCAT
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AGTATTTGAAGAGGACTATTTGCAAAGGAGGTGCTAGGGTAGAGAGATTGAACGTTACAGAAGA
AAAGCAAGCTGGGAAGCATATTTGAAGATGCGGTATGTAAAGAGCTAAAAAACTGTATTATAAG
TAAATGCATGTATGCTAAACTCACAAATTAGAGCTGTTTAATT**ATATAGTTATTACCCTG**

Chr1 375438

PS5b<

GTAATGCAAATTTATACAGGTCAAAAGGCGATCCTTAACAGGTCGCGCGCTCGCTCTAACTCAAT
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TTCTTTTTTCTTGTTTTTCCTGCTTTCCCTATTTACAACCGGGTAACCTCGTCTTCTCTTGCACTTAGA
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TACAGTTTTAGTGATATATTTTGGACCGCGATGGAAATCTTTTTTTTCAATTCATTGTTTTTAATT
TCTGTGAGTGTATTTTAGTTATGGATGGGTCAACCACCAAAACAACAATTCACAGAGGTGGGCAT

TTGGGCTCTAAGTGCTAGTCGTCAGTGTAGGGATATGAGTCATTTTTATTTTCCTTTCGCTGTCTT
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ACTTTGCTTGTGGTGCCACCAAAGTTTGTCTAGTGGACTGAGAGAAGAAATTTTCAGGACGTTT
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ACCAGTGATTGCCAAGGCCCAAATTGACGTGGCCAAGCAAGAGTTGTTTGGCGTTGCGCACGGG
TGTACCGGTAGGGTAACGATAAAATCAACGTTTGGAACTAGGGTTCTACGCCTGAAGCCAGAC
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GAACTTGGCCCAACATACCACGAAGCCGGTATTCTAGAAGATCGACCAAACCACCAAAGGAC
ATGTGGAAGTTGATCACCGACCCACAAGATGCCCCAGACCAACCACAAGACTTGGACATTGGGC
GAAAAGGGTTTGCCAGTCAGAGGTTGGCGTACAAGGATGCCAGGTCGGTAAAGGGAAATCACA
GCCACAACCCATTGGACATTCTTGACCGCTGCCAAGTTGGCCAGAGCTAACGGTGTGGCAGAA
TCGAGCATTGTCGAAAACCGTTACATT

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TCGTGGATGTTGCTCCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGT
TTACTAAAGCACATGTGGATATCTTGACTGATTTTTTTCTGTGGAGAAGTGGATTAAAGCCGCTA
AAAGGCATTATCCGCCAAGTACAATTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAA
TACAGTCAAATTGCAGTACTCTGCGGGTATACAGAATAGCAGATGGGCAGACATTACGAATGCA
CACACGGTGTGAGGTGGGCCGAGGGCAGTATTGCTTGTTTGAAGCAGGCAGGAGAGAAGTA
ACAAAGGAACCTGAGGCAGCACAGTGTAGCAGAATTGTCATGCAAGGGCTCCCTATCTACTGG
AGAATATACTAAAGAATTTACTGTTGACATTGCGAAACGACAAAGATTTTGTATCATTTATTGCT
CAAAGAGACATGGGTGGAAAGAGATGAAGGTGCATTGGTTGCGGCCACCCGGTGTGGGTTTAGA
TGACAAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGAT
CGACATTATTATTGTTGGAAGAGGACTGTTGCAAAGGGATGCTAAGGTAGAGAGATTGAACGTT
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Chr1 375422 PS6b<
GGGCAGCAGTAGTGGA TATACAGCTGTCCCGGGCGTTAGAGTAAAAAATTAGAGTGGAAGAATA
GAAAAAGAAAGAATATTGTGTAACGTTTGCAGTATCTATTTATCTTATGTAGATGGGTAAAGCTT
TTAGTTTATTGTCCGATGACAGCTAGCTCTCGCTAGTCGTATGTGATTTTCTCGAATATCTTGCTT
CAAT PS 283 bottom

Yellow highlight indicates the flanked the *ScURA3* fragment

Figure S7. Clone 8 Oxford Nanopore sequencing read

MiSeq (mi), Nanopore (nox)

Clone 5 PSc15 intergenic chr7--intergenic chr6 Dimer

Miseq

TAAC TTCGGAATATTGAATTAATCGTCTCC TGAGAGTGCACCACGCTTTTC chr7

24526 mi

CAATTTAATTATATCAGTTATTACCCtg TATATTATATTAAATAAAAAATAAT 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

TGCTGGACTAACTATAGGGTAGTTCGTAGCTGTGATCCTTGATTGTCAGTTTTGCTCAAATAAATAT
TATATATATATATAGAAAGAAGTGGGTAGACCACATTGTTTACAATACGTCTTAAATTGTGTTCCG
ATGAGCATGTAGACAAAATTAGAGTAGAAGTGTGGTTCTAACTTCGAATATTGGAATTAATCG
TTCC chr7

GAGAGTGCACACCACGCTTTTCAAATTCATCATATTTTTTTATTCTTTTTTGATTTCGGTTTCTTGAAA
TTTTTTTGATTTCGGTAATCTCCGAACAGAAGGAAGAACGAAGGAAGGAGCACAGACTTAGATTG
GTATATATACGCATATGTAGTGTGAAGAAACATGAAATTGCCAGTATTCTTAACCCAACTGCA
CAGAACAAACCCGCAGGAAAACCTGAAGATGCTAAATCATGTGCGAAAGCTACATAAGGAACGTG
CTGCTACCATCCCAGTCTGTAAAGCTGCTGCCATTTATCATCATGCACGAAAAGCAAACAACCTT
GTGTGCTTCATTGGATGTTTCGTACCACCGCTGAACTACTGAGTTAGTTGAAGGCCATTAGGTCCTA
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CCGCTAAAGGCATTATCCGCCAGCACAAATTTTTACTCGAAGACAAGAAATTTGCTGACATTGGTA
ATACAGTCAAATTGGTAGTACTCTGCGTGGTAGTATACAGAATAGTGCAGAATAAGACATTACG
AATGCACACGGTGTGGTGGGCCAGGTATTGTTATGGTTTGAAGCAGGCGCGGTAGAAGTAAAT
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AATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTTATCGGCTTTATTGCTC
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AGATGACAAGGAGACGCATTGGGTCAAACAACAGTATAGAACCGTGGGATGATGTGGTCTCTACA
GGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGTGCTAAGGTAGA
GGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAACTA
AAAACCTGTATTATAAGTAAATGTACGTATACTAACTCACAAATTAGAGCTCTAATTTAATTAT
CAGTTATTACCTG

TGAGAGTGCACCACGCTTTCAAATTCATCATTTTTTTATTCTTTTTTGATTTCGGTTTCGAAATTTTTTG
ATTTCGGTAATTCCGAACAGAAGGAAGAACGAAGGAAGGAGCATGACTTAGATTGGTATATATAC
ATATGTAGTGTGAAGAAACATGAAATTGCCAGTATTCTTAACCCAACTGCACAGAACAACAAA
CCTGCAGGAAACGAAGATAAATCATGTGCGAGTTACATATAAGGAACGTGCTGCTACTCATCCTA
GTCCTGTTGCCGCTAAGCTATTTAATATCATGCACGAAAAGCAACAAATCCCAGGTAAGCCTATT
GGATGTTTCGTACCACCAAGGCACTACTGGAGTTAAGTTGAAGCATTAGGTCCCAAAATTTGTTA
CTAAAACACATGTGGATATCTTGACTGATTTTCCATGGAGGGGCACAGTTGCCGCTAAAGGCA
TTATCCGCCAAGTACAATTTTACTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAAT
TGGCAGTACTTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAACACACGGTGT
GGTGGGCCCAGGCATTGTTAGCGGTTTGAAGCAGGCGGCAGAAGAAGTAAATCGTAGCGAACCC
AGAGGCCTTTGATGTTAGTGCAGAATTGTCATGCAAGCGGCCCTCGTATCTGGAGAATACTAAG
GGTACTGTTGACATTGCATTGTTAATAAAGATTTTGTATCGGCTTCATTGCTCAAAGACATTAC
GGGTGAAAAGAGATGAAGGTTACGATTGGTTGATTATGACACCCCGTGTGCTTTAGAATAGCCGC
GAGACGCATTGGGTCAAATTGCATAGAACCGTGGATGATGCGGTCTCTGCCATGATCTTGACATT

ATTGCTGGAAGAGGACCATTGCAAAGGAAGGATGTGCTAAGGTAGAGGGTGAACGTTACAGA
AAAAGCAGGCTGGAAGCTATTATTTGAGAAGATGCGGCCAGCAAACCAAAAACTGTATTATAA
GTAAATGCATGTATACTAACTCACAAATTAATAGAGCTTCAATTTAATTATATA**TCAGTTATTAC**
CCTA

chr6

TATTATATTAAATAAAATAATACACTGATTAACAATAAAGTTCATAAATAATTAAAATAATGATA
TTAATTACTTGTATTAATTACAAGCATATTTATACTCATCATCTTGGGCATTGAGAGGGAATCAAT
CAACTTCACAGCATGGGCAGATGTTCTATATGAAAATCAGACAAACAAAGCTAAGAGCAACGTA
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

AAAAAAAAATTATTATGGAACAATTTGTGTG tgagagtgcaccaATTA 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

TAGAACTAAATAAAATGTCTATAATGAGATTACACAAGGTATTAAACACAACCTTACCATTAAATATATG
AGTTAGATCTCCCAAATCATCGCGCACTTTTCTTTTAATATCTTTTCATTTTCCATACTAACTCTACTGCT
AAATGTAGTGAAGCAACAAAAAAAATTTATTATGGAACAATT chr1

TGTGTGATTACGCTTTTCAATTCATCATTTTTTTTATTCTTTTTTTGATTTTCGGTTTCCTTGAAATTTTTGATTC
GGTAATCTCCGAACAGAAGGAAGAACGAAAGCTGAAGCTGAGCACAGACTTAGATTGGTATATATACG
CATATGTAGTGTTGAAGAAACATGAAATTGCCAGGCATTCTAACCCAAACAGAACAAAAACCTGCAGG
AAACGCAAAGAGTTAAATCATGTGCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTT
GCTGCCAAGCTGGGTTTAATATCATGCACGAAAAGCAAACAACTTGTGTGCTTCATTGGATGTCGTA
CACCAAGGAATTACTGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGCTTTGCCAAACACATGTGGATAT
CTTGACTGATTTTTCCATGGAGGGCACAGTTAAGCTGCTAAAGGCATTATCCGTCATACAATTTTTTACTC
TTCGAAGACAGAAAAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTACCTGCGGGTGTATACAGA
ATAGCAGAATGGGCAGACATTACGAATGCACACGGTGTGGTGGGCCAGGTATTGTTAGCGGTTTGAAG
CAGGCGGCAGAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGCCGCGCAAGGG
CTCCCCATCTACTGAGAATATACTAAGGGTACTGTTGATTGCGAAGAGCGACAAAGATTTGTTATCGGCC
ATTGCTCAAAGCATATATAAAGTGGGAAGAGATGAAGGTTACGATTGGTTGATTATGCACACCCGGTGTG
GCTTAGATGACAAGGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTCTCTACAGGAT
TCGACATTATTATTGTTGGAAGAGGACGGTTTGTAAAGGAAGGGATGCTAAGGTAGAGGGTGAACGTTA
CAGAAAAGCAGGCTGGGAAGCATATTTGAGAAGATGCGGCCAGCAAACTAAAACTGTATTATAAGT
AAATGCATGCATACTAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCC

chr 5

CATACGCGAAACTCACAGGTGCTGCAATCTATATTTTTTTTTTCCCTCTTACCATTCTTCTGAAAAGACTT
AACAATCATATAGGATAAGTACTGCAGCGGCTGTTCTTAAACAAATATGCAAGCCACATAAGGCACGT
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 **GTCCCAT**TGCACCACGCTTTTCAA 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
GGTACAGTGGAAGTCGGATAACTCCGAAAACCGGAGACCCATGGAACGTCATCTCGCCATCGG
TGTCGCTGAACAGCTGCAATGAGTA **TATACACGAGATATTAGT**CGAGTGGGA **chr4**

ATTCTATGCACCACGCTTTTCAATTTCATCATTTTTTTTATTCTTTTTTTGATTTCGGTTTCCTTGAAAT
TTTTTGATTTCGGTAATCTCCGAACAGAAGGAAGAACTTTGAGAGAATACAGACTTAGATTGAGTA
TATACGCATATGTAGTGGTGAACATGAAATTGCCAGTATTAATAGCGCTACTGGGAACAAA
ACCTGCAGGAAACGAAGATAAATCATGTCAGCTACATATAAGGAACGTGCTGCTGTCATCCTAG
TCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAACTTGTGTGCTTCATTGG
ATGTTTCGTACCACCAAGGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAATTTGTTTACTA
AAACACATGTGGATGTCATGACTGATTTTTTCCATGGAGGGCACAGTTAAGCCGCTGAGGCATTA
TCCGCCAAGTAAATTTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAA
TTGCAGTACTCTTGCGGGTGACCTAGAATAGCAGAATGGGCAGACGTACGAATGCACACGGTG
TGGTGGGCCCAGGTATTGTTAGCGGTTTGAAGCAGGCGGCAGAAGTAACAAAGGGAACCTAGAG
CCTTTTGATGTTAGCAGAATTGTCATGCAAGGGGCTCTATACTGGAGAATATACTAAGGGTACTG
TTGACATTGCGAAGAGCGACAAAGATTTTGTATCGGCTTTATTGCTCAAAAGAGACATGGGTGG
AAGAGATGAAGGTTACGATTGGTTGATTATGACACCCGGTGTGGGTTTTAGATGACAAGGGCGC
GTTAGGTGCGGCTTAGTAGAACCGTGGATGATGTGGTCTCACCACCCAGGATCTGACATTATTAT
TATTTAGGAAGGACTGTTGCGAAAGGGATGCTAAAGGTAGAGGTGAACGCGTTACAGAAAAGC
AGGCTGGGAGCATATTTGAGAAGATGCGGCCAGCAAACTAAACTGTATTATAAGTAAATGCA
TGTATACTAAACTCACAAATTAGAGCTTCAATTTAATTA **TATCAGTTATTACCCTG**

GTATCTTCGCTTTGTCTCTTAGCTTCCATGTAAAGTGGTTACAAGTAACTAGAAACCAAACTCAA
ACAAATAAAACCACAACTTCAGAAACAGAACTTTCAAGTTGAAAAAGTTCTCCTCTCTTTTCTC
CTTCTATACTTTTAATTGGAGAAAGATATTTGGTATTTCAATTTGATTGACCTCTGGTTTTGCTTGA
TA **chr6**

Yellow highlight indicates the flanked the *ScURA3* fragment

Figure S10 Clone 23 Oxford Nanopore sequencing read

MiSeq (mi), Nanopore (nox)

Primer name	Sequence 5'→3'
P1	GTATAGACGCATGAAGTCCTTCATTTGCTTTTGTCCACA
P2	gagttctccgagaacaagcagaggttcgagTGTACTCGGA
P3	ctcgaacctctgcttgttctcggagaactcCCTCCTTTGATTAGTTTATGGGGCTAGCTG
P4	TAGGTGCCTGTCACGGCTCTTTTTTACTGTACCTGTGAC
P5	TTGGAAGTTAGCTTAGCAGAGGTAAAGTCG
P6	GGAAAGACCATTCTGCTTTACTTTTAGAGC
P7	atatcctaaattgcatgaggggtgagATGTCGAAAGCTACATATAAGGAACGTGCTGCTAC
P8	tgccatgtaaaaacatacggatagTTAGTTTTGCTGGCCGCATCTTCTCAAATATGCTTC
P9	CAGCAAAACTAAActatccgtatgtttttacatggcaattcctaattttgcaatcatgcac
P10	aggcgaggaagaggaaggtggaaatgtgaaaagtttg
P11	ACCTTCGAACCAACCAtatcctaaattgcatgaggggtgag
P12	gatgtactgcattcaatgacggttttggctcttccttctgatttaacgggtttcga
P13	CAATGGTTGGGCATTGCAATTCCTACGTTCTAATTAGTTTCATAGGATATGTTGCCCAC
P14	tatacgttctcaaaactcagagatgggtgatgaaggcgaggaagaggaag
P15	GCATGAGGGTGAGcatggcaattcctaattttgcaatcatgcacaaatgatcgctag
P16	gcaaaattaggaattgccatgCTCACCCCTCATGCAATTTAGGATATGGTTGGTTCGAAG
P17	CACAAACTTCATAAGCGTTCCCAAGGTTGACC
PS1a	GCTAAGCTGTTGCAATGACCCAC
PS1b	CCCCGGTTCTTATATTTTCCCTATTACTACA
PS2a	TCTTCAATACCAAATTCGTCGTCTAGAG
PS2b	TTGTTTGGCTCCTTTGGTGAAGG
PS3a	CCTATTGAGAAAAAAACCGAAACTATTT
PS3b	GCTTAGAGTGTGTTATTTAGTCAAGAATTT
PS4a	TGATGATGATGATAAGATAGTGAAGAAGG
PS4b	TCATTTTACTTTCCAAACAAGACTAGATTCT
PS5a	TCCAAAGTCAAACCTTCCAAGTC
PS5b	CCTTTTGGACCTGTATAAATTTGCATTAC
PS6a	GGAATTTGCCAAGTCCAGAGG
PS6b	GCTGCATATCCACTACTGCTGC
PS7a	AATCAGAATCTTGAAGAGGAAAAAG
PS7b	GACTTCCCCCCTCAT
PS8a	TATACACGAGATATTAGTCGAGTGG
PS8b	GGAAGCTAAGAGACAAAGCG
PS9a	AAAACCAAAAAAAACCATAAAAAACC
PS9b	CTATGTACATCATCAAGTCGTT
PS10a	AAAAAAAATTATTATGGAACAATTTGTGTGAT
PS10b	TGCAGCACCTGAGTT
PS11a	TGTGGCTAAGGTAACGG
PS11b	ACCAAGTGACCTGGC
PS12a	TGAAAAAATGATCCTGAAAAGA
PS12b	GGTGTGTCATGTGAC
PS13a	GATATACCAGTACAAAGGTAGTGA
PS13b	CGCTTCGTCCGTTTC
PS14a	TACAGTGGAAAGTCGGATAACT

PS14b	ACTGAGCTGACTGCATG
PS15a	ACCACTGTTACGTCGG
PS15b	TTTGTATAAGAAGACCTTGCG
PS16a	TTTTTCGATTTAATTAATATATATTATATAACAAC
PS16b	CAAACATATCGTCTTGGTATCTTTA
PS17a	CATGCCGGCCGAGTAACCGAGAGAA
PS17b	CGCATAAGAATCGATCCGA
PS18a	TGTACTTCTACGAAGGGC
PS18b	GAAAAAAAAAATAAACTTTTTTTTCTCAAAAGCT
PS19a	ACTTCTAAATCGGATAGATTCTGA
PS19b	ACTCTGGCATTTAATTTTGAGTC
PS20a	GGCGTCGGGGTAACTA
PS20b	CGAAAAAGAAACGCCCTCAG
PS21a	CCCTGCCGTATATACCTG
PS21b	GATCTGGGATTTAAGAGGCG
PS22a	CAATAAAACACTAAAATAAAATAAAATAAATTGAGAAAAAATCGA
PS22b	TTTTTCCATTATTTTTTTATATTATATTAGATATTTCCATGC
PS23a	CGACATTTTTTTTCGATTTAATTAATATATATTATATAACAAC
PS23b	CAAACATATCGTCTTGGTATCTTTAG
PS24a	TGTGGCTAAGGTAACGG
PS24b	ACCAAGTGACCTGGC
PS25a	GCCGTAACCTCTTTTTTATTG
PS25b	CGGAAAAAAAAACGAAAAAAAAAAAAAAAAATA
PS26a	TTCAAAGTGGAACCAACACCA
PS26b	ACGATTTTACAGGATTATCTAACG
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	TGGGCAAGTTTCCCG
PS37a	TGTACTTCTACGAAGGGC
PS37b	GGAATTATAAACACTTACATACGCTGA
PS283top	TTGGATCATGGATCTGACGTATTC
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	AATTAATATTATTTTATTTAATATAATATAATTATTTATGAACTTTATTGTTAAT C
T33	CAGAAACGGGAAGAATCTG
T34	TTCAACACCGTCGACA
T35	CTAGGCTATCACACACACTA
ura3-F	TGAGAGTGCACCACGCTTTTCAATTC
ura3-R	CAGGGTAATAACTGATATAATTAATG
ura3+720	GGAAGGGATGCTAAGGTAGAGGGT
ura3+61c	GCTTGGCAGCAACAGGACTAGGATG
ura3_TestF	GTTAGCGGTTTGAAGCAGGC
ura3_TestR	GCCTGCTTCAAACCGCTAAC

Table S1. Primers used in PCR reactions.