

Table S1. Primers used to construct cut14 mutants

Oligo sequence (5' to 3')	Oligo name
CCCCAGCAAAGTTGAAGCAACTTGCTAAAGATCGC	cut14-L478P-F
TGCTTCAACTTTTGCTGGGGTACATCTTCACCTGT	cut14-L478P-R
AGCAAAGTTGAAGCAACCTGCTAAAGATCGCGGC	cut14-L485P-F
AGGTTGCTTCAACTTTTGCTGGAGTACATCTTCACC	cut14-L485P-R
ATCGCGGCAATCCGTTAAATGAACTTGATGCTTTA	cut14-L492P-F
ATTTAACGGATTGCCGCGATCTTTAGCAAGTTG	cut14-L492P-R
AACTTGATGCTCCAAAAGCAAACCTTGCTTATATGG	cut14-L499P-F
GCTTTTTGGAGCATCAAGTTCATTTAACAAATTGCC	cut14-L499P-R
AAAGAATTGGTGGCTCAATTGCTAACCTTGAATGA	cut14-G524E-F
AATTGAGCCACCAATTCTTTCACCTTTGAGCGATC	cut14-G524E-R
AAAGGACCGGTGGCTCAATTGCTAACCTTGAATGA	cut14-L525P-F
AATTGAGCCACCGGTCCTTTCACCTTTGAGCGATC	cut14-L525P-R
AAGGATTGGTGGCTCAACCGCTAACCTTGAATGAAG	cut14-L529P-F
CGGTTGAGCCACCAATCCTTTCACCTTTGAGCGATC	cut14-L529P-R
GCTGAAGGCCGCCCTTTATAATCTCATTGTTGAAACC	cut14-G548E-F
TTATAAAGGCGGCCTTCAGCTGTAATTTCTAATGC	cut14-G548E-R
GCTGGTGAACGCCCTTTATAATCTCATTGTTGAAACC	cut14-G549E-F
TTATAAAGGCGTTCACCAGCTGTAATTTCTAATGC	cut14-G549E-R
GTGGCCGCCCTTATAATCTCATTGTTGAAACCGAG	cut14-L551P-F
GAGATTATAAGGGCGGCCACCAGCTGTAATTTCTAA	cut14-L551P-R
AAAATTGAAGCACAATTGCTTCAAAAAGGAAACCTG	cut14-G562E-F
AGCAATTGTGCTTCAATTTTCTCGGTTTCAACAAT	cut14-G562E-R
CAACCGCTTCAAAAAGGAAACCTGAAGAGGAGAGT	cut14-L565P-F
TTTCCTTTTTGAAGCGGTTGTGCACCAATTTTCTC	cut14-L565P-R
CAATTGCCTCAAAAAGGAAACCTGAAGAGGAGAGT	cut14-L566P-F
TTTCCTTTTTGAGGCAATTGTGCACCAATTTTCTC	cut14-L566P-R
AAAAAGAGAACCTGAAGAGGAGAGTTACTATTATTCCC	cut14-G569E-F
CCTCTTCAGGTTCTCTTTTTGAAGCAATTGTGCACC	cut14-G569E-R
AAAAAGGAAACCCGAAGAGGAGAGTTACTATTATTCCC	cut14-L571P-F
CCTCTTCGGGTTTCTTTTTGAAGCAATTGTGCACC	cut14-L571P-R
ATTATTCCCCCAACAAAATTACCAGCTTCGTAGC	cut14-L580P-F
ATTTTGTGGGGGGAATAATAGTAACTCTCCTCTT	cut14-L580P-R
TCCGGAATTAATCGGTTATGACGATGAGCTTTTGCC	cut14-L608P-F
CATAACCGATTAATTCCGGAGCAAGTTGGGCTTTAT	cut14-L608P-R
TTTGAACCAATCGGTTATGACGATGAGCTTTTGCC	cut14-L610P-F
CATAACCGATTGGTTCCAAAGCAAGTTGGGCTTTAT	cut14-L610P-R
ATGACGATGAGCCTTTCCTGCAATGCAGTATGTT	cut14-L617P-F
AGGCAAAGGCTCATCGTCATAACCGATTAATTCCAA	cut14-L617P-R
TTTGAATCCACATTAGTCTGTGATACCCCTGAATCG	cut14-G626E-F
CAGACTAATGTGGATTCAAAAACATACTGCATTGC	cut14-G626E-R
TTTTTGGCTCCACACCAGTCTGTGATACCCCTGAA	cut14-L629P-F
GACTGGTGTGGAGCCAAAACATACTGCATTGCAGG	cut14-L629P-R

AATTA AAAAGTGT CACGCCTGATGGTGATGTTTATG	cut14-L653P-F
AGGCGTGACACTTTTTAATTTAACTGAAGGATGAAA	cut14-L653P-R
GAAGATGTTTATGACCCATCTGGTACCCTTACTGGT	cut14-G655E-F
GATGGGTCATAAACATCTTCATCAAGCGTGACACT	cut14-G655E-R
GAAGGGTCTGTGAATAAATCTGCCGGTCCTTTACTA	cut14-G666E-F
GATTTATTCACAGACCCTTCAGTAAGGGTACCAGA	cut14-G666E-R
GGTGAGTCTGTGAATAAATCTGCCGGTCCTTTACTA	cut14-G667E-F
GATTTATTCACAGACTCACCAGTAAGGGTACCAGA	cut14-G667E-R
GAACCTTTACTACAGATACAAAAGCTGAACAGT	cut14-G674E-F
TGTATCTGTAGTAAAGGTTCCGGCAGATTTATTCAC	cut14-G674E-R
CCTCCACTACAGATACAAAAGCTGAACAGTCTGCAG	cut14-L676P-F
CTTTTGTATCTGTAGTGGAGGACCGGCAGATTTATT	cut14-L676P-R

Table S2. Primers used to construct cut3 mutants

Oligo sequence (5' to 3')	Oligo name
AAAGCTCCTTTAAGTTCTTCTAGATCTCGTGGAAT	cut3-S627P-F
GAAGAACTTAAAGGAGCTTTCATCTCCTCCAACCT	cut3-S627P-R
AAAGCTTCTCCAAGTTCTTCTAGATCTCGTGGAAT	cut3-L628P-F
GAAGAACTTGGAGAAGCTTTCATCTCCTCCAACCT	cut3-L628P-R
AGACCTCGTGGAATGTGTTAGAAAGTCTTCAAAGA	cut3-S633P-F
AACACATTTCCACGAGGTCTAGAAGAACTTAAAGAAGC	cut3-S633P-R
AGATCTCGTGAGAATGTGTTAGAAAGTCTTCAAAGA	cut3-G635E-F
AACACATTTCTCACGAGATCTAGAAGAACTTAAAGAAGC	cut3-G635E-R
AATGTGCCAGAAAGTCTTCAAAGACTTCATGAATCT	cut3-L638P-F
TGAAGACTTTCTGGCACATTTCCACGAGATCTAGA	cut3-L638P-R
AATGTGTTAGAAAGTCTCAAAGACTTCATGAATCT	cut3-L641P-F
TGAGGACTTTCTAACACATTTCCACGAGATCTAGA	cut3-L641P-R
ATGAACCTGATAATTTAAATGGTTTCTTTGGACGT	cut3-S647P-F
ATTTAAATTATCAGGTTTCATGAAGTCTTTGAAGAC	cut3-S647P-R
ATAATTTAAATGAATTCTTTGGACGTCTTGGTGAT	cut3-G652E-F
AAAGAATTCATTTAAATTATCAGATTCATGAAGTC	cut3-G652E-R
TGAACGTCTTGGTGATTTGGCTACAATTGACGAGGC	cut3-G655E-F
CCAAATCACCAAGACGTTCAAAGAAACCATTTAAATT	cut3-G655E-R
TGGACGTCCTGGTGATTTGGCTACAATTGACGAGGC	cut3-L657P-F
CCAAATCACCAAGACGTTCAAAGAAACCATTTAAATT	cut3-L657P-R
TGGACGTCCTGAGGATTTGGCTACAATTGACGAGGC	cut3-G658E-F
CCAAATCCTCAAGACGTTCAAAGAAACCATTTAAATT	cut3-G658E-R
TGGACGTCCTGGTGATCCGGCTACAATTGACGAGGC	cut3-L660P-F
CCGGATCACCAAGACGTTCAAAGAAACCATTTAAATT	cut3-L660P-R
CTTATGATGTGGCCATTCCAACCTGCCTGCTGCT	cut3-S672P-F
TGGAATGGCCACATCATAAGCCTCGTCAATTGTAGC	cut3-S672P-R
CTGCCTGTCTGCTCCAAATCATATTGTAGTTGAC	cut3-L678P-F
ATTTGGAGCAGGACAGGCAGTTGAAATGGCCACAT	cut3-L678P-R
AATATTGAACTGAACAGAAATGTGTGGCTTTTCTG	cut3-G689E-F
TTCTGTTCAAGTTTCAATATTGTCAACTACAATATG	cut3-G689E-R
GCTCCAATAACCTAGAGCGTGCTAGTTTTATTATTC	cut3-G702E-F
ACGCTCTAGGTTATTGGAGCGCAGAAAAGCCACAC	cut3-G702E-R
CTAGTTTTATTATTCCCAAAGAACTGGCGCAGAAA	cut3-L709P-F
TTTGGGAATAATAAAACTAGCACGGCCTAGGTTAT	cut3-L709P-R
CCCGGCCCTTCGACTTACTTCGGTTTAATGATCAG	cut3-L729P-F
AAGTAAGTCAAGGGCCGGGGAACATTCTCAGGTG	cut3-L729P-R
CCCGGCTCTTCGACCCACTTCGGTTTAATGATCAG	cut3-L732P-F
AAGTGGGTCAAGAGCCGGGGAACATTCTCAGGTG	cut3-L732P-R
TTCCGCAGAATACCTTAGTTGCTAAAAATTTGGAAC	cut3-L748P-F
AACTAAGGTATTCTGCGGAACGTTATAAAACGCAGG	cut3-L748P-R
TTTTGCAGAATACCCAGTTGCTAAAAATTTGGAAC	cut3-L752P-F
AACTGGGGTATTCTGCAAAACGTTATAAAACGCAGG	cut3-L752P-R

AAAAATCCGGAACAGGCTAATAGAATTGCGTATGGC	cut3-L757P-F
TTAGCCTGTTCCGGATTTTTAGCAACTAAGGTATTC	cut3-L757P-R
CACCAAGTGGTCAGTTAATTGATAAATCGGGCACA	cut3-L775P-F
AATTAAGTACCCTTGGTGTAACTCTCCAACG	cut3-L775P-R
GAACAGTTAATTGATAAATCGGGCACAATGACT	cut3-G777E-F
TTTATCAATTAAGTCTTCACTTAGTGTAACAAC	cut3-G777E-R
TTAATTGATAAACCGGGCACAATGACTGGAGGTGG	cut3-S783P-F
GTGCCCGGTTTATCAATTAAGTACCCTTAGTGT	cut3-S783P-R
GATAAATCGGAAACAATGACTGGAGGTGGTACTCGA	cut3-G784E-F
GTCATTGTTTCCGATTTATCAATTAAGTACCCT	cut3-G784E-R
TGAAGGTGGTACTCGAGTAAAGAAAGGCGGAATGT	cut3-G788E-F
TTACTCGAGTACCACCTTCAGTCATTGTGCCCGAT	cut3-G788E-R
GGAGAAGGTACTCGAGTAAAGAAAGGCGGAATGT	cut3-G789E-F
TTACTCGAGTACCTTCTCCAGTCATTGTGCCCGAT	cut3-G789E-R
TGGAGGTGAACTCGAGTAAAGAAAGGCGGAATGT	cut3-G790E-F
TTACTCGAGTTTACCTCCAGTCATTGTGCCCGAT	cut3-G790E-R
AAGAAAGAAGGAATGTCCTCAGCTATAACGAGTGAT	cut3-G796E-F
GAGGACATTCCTTCTTTCTTTACTCGAGTACCACC	cut3-G796E-R
AAGAAAGGCGAAATGTCCTCAGCTATAACGAGTGAT	cut3-G797E-F
GAGGACATTCGCCTTTCTTTACTCGAGTACCACC	cut3-G797E-R

A Reaction setup for the 1st round of PCR:

Component	Volume
10x Buffer for DOD-Plus-Neo	5 μ l
2mM dNTPs	5 μ l
25mM MgSO ₄	3 μ l
10 μ M Primer #1	1 μ l
10 μ M Primer #2	1 μ l
Template DNA (genomic DNA)	1 μ l
Water	33 μ l
KOD-Plus-Neo (1.0 U/ μ l)	1 μ l
Total reaction volume	50 μ l

B Reaction setup for the 2nd round of PCR:

Component	Volume
10x Buffer for DOD-Plus-Neo	5 μ l
2mM dNTPs	5 μ l
25mM MgSO ₄	3 μ l
10 μ M Primer #1	1 μ l
10 μ M Primer #2	1 μ l
Template #1*	1 μ l
Template #2*	1 μ l
Water	32 μ l
KOD-Plus-Neo (1.0 U/ μ l)	1 μ l
Total reaction volume	50 μ l

*:Template #1 and Template #2 are the purified PCR product of 1st round PCR

C Cycling conditions used for PCRs:

<3-step cycle>	conditions
Pre-denaturation:	94°C, 2 min
Denaturation:	98°C, 10 sec
Annealing:	55°C, 30 sec
Extention:	68°C, 4 min

} 35 cycles

Figure S1. PCR conditions used to introduce mutations into target genes. (A) Reaction setup for the 1st round of PCR as illustrated in **Figure 2A**. Two fragments of the target gene, containing the designed mutation, were amplified from genomic DNA. (B) Reaction setup for the 2nd round of PCR as illustrated in **Figure 2A**. Purified products of the 1st round of PCR were used as template. Entire gene with the designed mutation introduced was the product of the 2nd round of PCR.

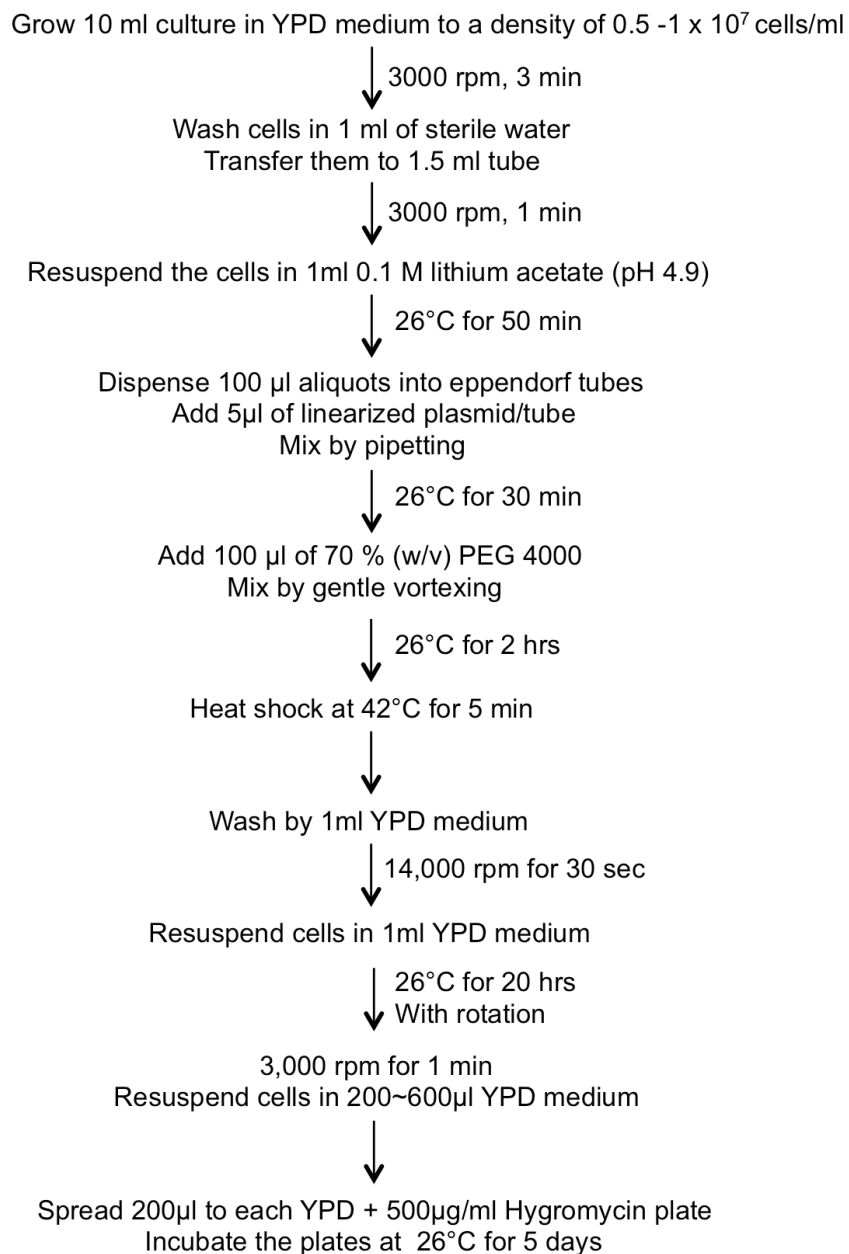


Figure S2. Transformation protocol. Lithium acetate method was used to transform linearized plasmids described in **Figure 2B** into fission yeast genome.