

## pCORE chromosomal modification system

See *Storici et al. Methods Enzymol. 409:329-345, 2006.*

Use pCORE plasmid for amplification of KIURA3-KanMX4 integration cassette.  
Not including 45-50 base overhangs:

**Primer 1:** GAGCTCGTTTTTCGACACTGG (231bp downstream of pCORE KANMX)  
**Primer 2:** TCCTTACCATTAAGTTGATC (322bp downstream of pCORE URA3)

The amplicon (not including overhangs) is 3195bp (sequence below).

Use the thermal cycler program JJS > RD16.

The conditions are:

Calculated

94degC 5:00

Repeat 32x:

94degC 0:45

50degC 0:45

72degC 1:00

72degC 10:00

4degC forever

end

The primers we have used have an annealing temp of 60 degC.

Use primers with 45-50 bases of homologous sequence on either side to allow for targeting of CORE cassette by recombination. Set up 3 50-100 microliter PCR reactions, check product on agarose gel, combine, ethanol precipitate, resuspend in 20 microliters TE and use 10 microliters for transformation. After transformation, select for growth on SC-URA plates and confirm integration by PCR.

To introduce the desired sequence changes, transform the CORE integrant with 25-100 micrograms of 80-100 bp double-stranded, completely overlapping sequences. Plate overnight on YPD, then replica plate to FOA. Confirm loss of core cassette by PCR, then verify mutation by sequencing.

**pCORE plasmid 5' – 3'**; note that KANMX (1157-348) and URA3 (2148-2951) are on different strands

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1  aacctggccttatcgaaattaatacgcactcactatagggagaccggcagat
51  ccgcgccgcgataggccactagtggatctgatatcatcgatgaattcgag
101 ctcgttttcgacactggatggcggcggttagtatcgaatcgacagcagtat
151 agcgaccagcattcacatacgcattgacgcgatgatattactttctgcgcac
201 ttaacttcgcacatctgggcagatgatgtcgcagggcgaaaaaaaaatataaatc
251 acgctaacatttgattaaaaatagaacaactacaatataaaaaaactatac
301 aaatgacaagtctctgaaaacaagaatctttttattgtcagtagtactgatta
351 gaaaaactcatcgagcatcaaatgaaactgcaatttattcatatcaggat
401 tatcaataccatatttttgaaaaagccgtttctgtaatgaaggagaaaaac
451 tcaccgaggcagttccataggatggcaagatcctgggtatcgggtctgcgat
501 tccgactcgtccaacatcaatacaaacctattaatttcccctcgtcaaaaa
551 taaggttatcaagtgagaaatcacatgagtgacgactgaatccggtgag
601 aatggcaaaagcttatgcatttctttccagacttggtcaacaggccagcc
651 attacgctcgtcatcaaatcactcgcacatcaaccaaacggttattcattc
701 gtgattgcgcctgagcgcagacgaaatacgcgatcgcgtgtaaaaggacaa
751 ttacaaacaggaatcgaatgcaaccggcgcaggaacactgccagcgcac
801 aacaatattttcacctgaatcaggatattcttctaataacctggaatgctg
851 ttttgccgggggatcgcagtggtgagtaacctatgcacatcaggagtagcgg

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901 ataaaatgcttgatggctcggaagaggcataaattccgtcagccagtttag  
 951 tctgaccatctcatctgtaacatcattggcaacgctacctttgccatggt  
 1001 tcagaaacaactctggcgcatcgggcttcccatacaatcgatagattgtc  
 1051 gcacctgattgcccacattatcgcgagccatttatacccatataaatc  
 1101 agcatccatggttgaatttaatcgcggcctcgaaacgtgagtcctttcct  
 1151 tacccatggtttgttatgttcggatgtgatgtgagaactgtatcctagca  
 1201 agattttaaaaggaagtatatgaaagaagaacctcagtgggcaaatcctaa  
 1251 ccttttatatttctctacaggggcgcgggcggtggggacaattcaacgcgtc  
 1301 tgtgagggggagcgtttccctgctcgcaggctcgcagcgaggagccgtaat  
 1351 ttttgcttcgcgcccgtgcccacatcaaatgtatggatgcaaatgattat  
 1401 acatggggatgtatgggctaaatgtacggggcgacagtcacatcatgcccc  
 1451 tgagctcgcacgctcaagactgtcaaggagggtattctgggcctccatgt  
 1501 cgctggccgggtgacccggcggggacgaggcaagctaaacagatctggcg  
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 1601 aaagcgggcagtgagcgaacgcaattaatgtgagttacctcactcatta  
 1651 ggcaccaccaggtttacactttatgcttcgggctcctatgttgtgtggaa  
 1701 ttgtgagcggataacaatttcacacaggaaacagctatgacctgattac  
 1751 gccaagctcgggaattaaccctcactaaaggggaacaaaagctgggtaccgC  
 1801 GGAGACAATCATATGGGAGAAGCAATTGGAAGATAGAAAAAAGGTACTCG  
 1851 GTACATAAATATATGTGATTCTGGGTAGAAGATCGGTCTGCATTGGATGG  
 1901 TGGTAACGCATTTTTTTTACACACATTACTTGCCTCGAGCATCAAATGGTG  
 1951 GTTATTTCGTGGATCTATATCACGTGATTTGCTTAAGAATTGTCGTTCATG  
 2001 GTGACACTTTTAGCTTTGACATGATTAAGCTCATCTCAATTGATGTTATC  
 2051 TAAAGTCATTTCAACTATCTAAGATGTGGTTGTGATTGGGCCATTTTGTG  
 2101 AAAGCCAGTACGCCAGCGTCAATACACTCCCGTCAATTAGTTGCACCATG  
 2151 TCCACAAAATCATATACCAGTAGAGCTGAGACTCATGCAAGTCCGGTTGC  
 2201 ATCGAACTTTTACGTTTAATGGATGAAAAGAAGACCAATTTGTGTGCTT  
 2251 CTCTTGACGTTTCGTTTCGACTGATGAGCTATTGAACTTGTGAAACGTTG  
 2301 GGTCCATACATTTGCCTTTTGAACACACAGTTGATATCTTGGATGATTT  
 2351 CAGTTATGAGGGTACTGTCGTTCCATTGAAAGCATTGGCAGAGAAATACA  
 2401 AGTTCCTTGATATTTGAGGACAGAAAATTCGCCGATATCGGTAACACAGTC  
 2451 AAATTACAATATACATCGGGCGTTTACCGTATCGCAGAATGGTCTGATAT  
 2501 CACCAACGCCCACGGGGTTACTGGTGCTGGTATTGTTGCTGGCTTGAAAC  
 2551 AAGGTGCGCAAGAGGTCACCAAAGAACCAAGGGGATTATTGATGCTTGCT  
 2601 GAATTGTCTTCCAAGGGTTCTCTAGCACACGGTGAATATACTAAGGGTAC  
 2651 CGTTGATATTGCAAAGAGTGATAAAGATTTGTTATTGGGTTTATTGCTC  
 2701 AGAACGATATGGGAGGAAGAGAAGAAGGGTTTGATTGGCTAATCATGACC  
 2751 CCAGGTGTAGGTTTAGACGACAAAGGCGATGCATTGGGTGAGCAGTACAG  
 2801 AACCGTCGACGAAGTTGTAAGTGGTGGATCAGATATCATCATTGTTGGCA  
 2851 GAGGACTTTTCGCCAAGGGTAGAGATCCTAAGGTTGAAGGTGAAAGATAC  
 2901 AGAAATGCTGGATGGGAAGCGTACCAAAGAGAATCAGCGCTCCCCATTA  
 2951 ATTATACAGGAACTTAATAGAACAAATCACATATTTAATCTAATAGCCA  
 3001 CCTGCATTGGCACGGTGCAACACTCACTTCAACTTCATCTTACAAAAGAT  
 3051 CACGTGATCTGTTGTATTGAACTGAAAATTTTTGTTTGCTTCTCTCTCT  
 3101 CTCTTTTATTATGTGAGAGTTTAAAAACCAGAACTACATCATCGAAAAA  
 3151 GAGTTTAAACCATTACAACCATTGCGATAAGCCCTCTCAAACCTTCTTCCA  
 3201 gactgtttgtcatccaattggtaagattaattatcatataccccggataa  
 3251 gaaaagaagcaagcacacaatg **gatcaacttaatggtaagga** acaacaag  
 3301 agccaccgcggtgGAGCTCGAATTCATCGATGATATCAGATCCACTAGTG  
 3351 GCCTATGCGGCCGCGGATCTGCCGGTCTCCCTATAGTGAGTCGTATTAAT  
 3401 TTCGATAAGCCAGGTTAACCTGCATTAATGAATCGGCCAACGCGCGGGGA  
 3451 GAGGCGGTTTGCATTTGGGCGCTCTTCCGCTTCCCTCGCTCACTGACTCG  
 3501 CTGCGCTCGGTTCGTTCCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGC  
 3551 GGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGT  
 3601 GAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTG  
 3651 GCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACG  
 3701 CTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGT

3751 TTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTT  
3801 ACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCA  
3851 ATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGC  
3901 TGGGCTGTGTGCACGAACCCCCGTTTCAGCCCCGACCGCTGCGCCTTATCC  
3951 GGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACT  
4001 GGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTG  
4051 CTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACA  
4101 GTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGT  
4151 TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTT  
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4251 CCTTTGATTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACG  
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4351 TTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAA  
4401 ACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGC  
4451 GATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGA  
4501 TAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATA  
4551 CCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCC  
4601 AGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCA  
4651 TCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTT  
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4751 CTCGTCGTTTTGGTATGGCTTCATTTCAGCTCCGGTTCCTAACGATCAAGGC  
4801 GAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGT  
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