### Table 53: Construction of chromosomal gene disruptions

<table>
<thead>
<tr>
<th>Allele</th>
<th>PCR Primer Name</th>
<th>PCR Primer Sequence</th>
<th>PCR Template *a</th>
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<tr>
<td><strong>pol2Δ::kanMX</strong></td>
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<td>Pol2-kanMXkoF</td>
<td>5′-ATGATGTTTGGGAAAGAAAAAAAAACGAGGAGGATCTCCACTGCAAGATATTCTGGCTGCTG-3′</td>
<td>pUG6 (Guldener et al. 1996)</td>
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<td>Pol2-kanMXkoR</td>
<td>5′-TCATATGGCAAACTCAGAACTAATATACATATCAAAACCCGTAATAC TGCTTACACTGAGTTAGGGTGTCG-3′</td>
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<td><strong>pol2Δ::natMX</strong></td>
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<td>Pol2::nat1-for2</td>
<td>5′-AGAGATGTTTGGGAAAGAAAAACGAGGAGGATCTCCACTGCAAGATATTCTGGCTGCTG-3′</td>
<td>pFvL99</td>
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<td>Pol2::nat1-rev2</td>
<td>5′-TTTTTTTTTTTTTTTTTTTCTTGAATTATATATGATATC GGCTAATCCGCGTGTGTATATAC TGCTTACACTGAGTTAGGGTGTCG-3′</td>
<td>(Stulemeijer et al. 2011)</td>
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<tr>
<td><strong>pol3Δ::natMX</strong></td>
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<td>Pol3MXF</td>
<td>5′-ATGATGTTTGGGAAAGAAAAACGAGGAGGATCTCCACTGCAAGATATTCTGGCTGCTG-3′</td>
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<td>Pol3MXR</td>
<td>5′-GCAAAAAAGCTTGAACCTTGTTTTATATATGATATC GGCTAATCCGCGTGTGTATATAC TGCTTACACTGAGTTAGGGTGTCG-3′</td>
<td>(Stulemeijer et al. 2011)</td>
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<tr>
<td><strong>msh2Δ::HIS3 or msh2Δ::TRP1</strong></td>
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<td>Msh2U</td>
<td>5′-AAAAAATCTTCTTCTGCGACTAATCTAATACATATCAGCACAGCAAACTTCTACTAATAGAGTGA TGTATGAGCTGCAC-3′</td>
<td>pRS413 or pRS414 (Brachmann et al. 1998)</td>
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<td>Msh2D</td>
<td>5′-TTTAAACCAACAGGCTTTTTATATATATGATATC GGCTAATCCGCGTGTGTATATAC TGCTTACACTGAGTTAGGGTGTCG-3′</td>
<td>(Brachmann et al. 1998)</td>
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<td><strong>msh2Δ::MET15</strong></td>
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<td>Msh2::Met15F</td>
<td>5′-AAAAAATCTTCTTCTGCGACTAATCTAATACATATCAGCACAGCAAACTTCTACTAATAGAGTGA TGTATGAGCTGCAC-3′</td>
<td>pRS411</td>
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<td>Msh2::Met15R</td>
<td>5′-TTTAAACCAACAGGCTTTTTATATATATGATATC GGCTAATCCGCGTGTGTATATAC TGCTTACACTGAGTTAGGGTGTCG-3′</td>
<td>(Brachmann et al. 1998)</td>
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<td><strong>msh6Δ::HIS3 or msh6Δ::TRP1</strong></td>
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<td>Msh6U</td>
<td>5′-TTTAAATGGGAACAGCACTAGATAATTTGGAAGAAAAACGAAATCCACGAGGAACTTGTTCTACTGAGCTGCTG-3′</td>
<td>pRS413 or pRS414 (Brachmann et al. 1998)</td>
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<td>Msh6D</td>
<td>5′-ACTTAAAAAAATAAGTAATAAATCTTACATACATCGTTAAAATGAAATACAC GAAATTTATATAGGTTAGGGTGTCG-3′</td>
<td>(Brachmann et al. 1998)</td>
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<td><strong>mlh1Δ::HIS3 or mlh1Δ::TRP1</strong></td>
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<td>Mlh1U</td>
<td>5′-ATGATGTTTGGGAAAGAAAAACGAGGAGGATCTCCACTGCAAGATATTCTGGCTGCTG-3′</td>
<td>pRS413 or pRS414 (Brachmann et al. 1998)</td>
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<td>Mlh1D</td>
<td>5′-CTCACAGAAAACAACTTTGGTATACAGCCAACACGTATTAAAAATACAC AACACCCCTCAAAAAAAAATATCTGCGGT ATTTCACACCG-3′</td>
<td>(Brachmann et al. 1998)</td>
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<tr>
<td><strong>pms1Δ::HIS3 or pms1Δ::TRP1</strong></td>
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<td>Pms1U</td>
<td>5′-GAAGCGGGAAGAAAAAGCAGGCTCTCTTACTTTATAATTCATAGTGCGATAAAT TTTTATACCACTGAGGAACTTGTTCTACTGAGCTGCTG-3′</td>
<td>pRS413 or pRS414 (Brachmann et al. 1998)</td>
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<td>Pms1D</td>
<td>5′-TGATATGTTTGGTATATATATATGGAATCATAAATCTAATATCATGAGGAGATCTGACGACGACGAC-3′</td>
<td>(Brachmann et al. 1998)</td>
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<tr>
<td>Mutant</td>
<td>Primers and Conditions</td>
<td>Template</td>
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<tr>
<td>mlh3::MET15 or mlh3Δ::TRP1</td>
<td>Mlh3KO upstream 5′-ACATAAACACGGAGGCTTTCCAAGGAAGAATGAAGCCTGAACTCGTCAACTC AAAAGAAAGATTTGACTGAGAGTGCAC-3′</td>
<td>pRS411 or pRS414 (Brachmann et al. 1998)</td>
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<tr>
<td>Mlh3KO downstream 5′-TGCATATCCGGCAATTTAAATGCAGGCGACAAACCTTGGATTCCAGGATTAA GGTTCCTCTGTGCGGTATTTCCACCG-3′</td>
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<td>msh3::MET15 or msh3Δ::LEU2</td>
<td>Msh3KO upstream 5′-GTACTTTTGAGGCGAAAAAGCAGGCGAATAGTTTTTTGAAATCTATT AAACAAAGATTTGACTGAGAGTGCAC-3′</td>
<td>pRS411 or pRS415 (Brachmann et al. 1998)</td>
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<td>Msh3KO downstream 5′-TCAGTGGATATCCAATGATAGTAATTTTCGCGAGTTTATCCGTTGCTTATAT TATCTGTGCGGTATTTCCACCG-3′</td>
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<td>rev3Δ::TRP1</td>
<td>Rev3U 5′-TTACCAATCATATATAGATTAATGCTTCTTCCCTTTGAACAGATTGACTTGT GCGGTATTTCCACCG-3′</td>
<td>pRS414 (Brachmann et al. 1998)</td>
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<td>Rev3D 5′-TTACCAATCATATATAGATTAATGCTTCTTCCCTTTGAACAGATTGACTTGT GCGGTATTTCCACCG-3′</td>
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<td>rad30Δ::TRP1</td>
<td>Rad30U 5′-TAGGCACGGCGTCTCTTTTGAACGGCCTTTGTGATAAAAAGAAGACAAAGCGG ATTGTACTGAGAGTGCAC-3′</td>
<td>pRS414 (Brachmann et al. 1998)</td>
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<td>Rad30D 5′-TCATTTTTCTTTGTAATAAGATATGTTTTTGGAAGATGAATCTTCTG TGCAGTATTTCCACCG-3′</td>
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* pRS411 was used as template for gene replacement with MET15; pRS413 with HIS3; pRS414 with TRP1; pRS415 with LEU2.

Mutations were introduced into yeast using PCR products generated with the indicated primers and template DNAs. The PCR conditions for all primers used here were: 98°C, 1 min.; 30x (98°C, 10 sec.; 55°C, 30 sec.; 72°C, 90 sec.); 72°C, 60 sec.