

**S3 Table. Plasmids used in this study.****A. *MLH3* mutagenesis plasmids**


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	allele
pEAI254	<i>MLH3</i>
pEAI325	<i>mlh3-1</i>
pEAI326	<i>mlh3-2</i>
pEAI327	<i>mlh3-3</i>
pEAI328	<i>mlh3-4</i>
pEAI329	<i>mlh3-5</i>
pEAI330	<i>mlh3-6</i>
pEAI331	<i>mlh3-7</i>
pEAI332	<i>mlh3-8</i>
pEAI333	<i>mlh3-9</i>
pEAI334	<i>mlh3-10</i>
pEAI335	<i>mlh3-11</i>
pEAI336	<i>mlh3-12</i>
pEAI337	<i>mlh3-13</i>
pEAI338	<i>mlh3-14</i>
pEAI339	<i>mlh3-15</i>
pEAI340	<i>mlh3-16</i>
pEAI341	<i>mlh3-17</i>
pEAI342	<i>mlh3-18</i>
pEAI343	<i>mlh3-19</i>
pEAI344	<i>mlh3-20</i>
pEAI345	<i>mlh3-21</i>
pEAI346	<i>mlh3-22</i>
pEAI347	<i>mlh3-23</i>
pEAI348	<i>mlh3-24</i>
pEAI349	<i>mlh3-25</i>
pEAI350	<i>mlh3-26</i>
pEAI351	<i>mlh3-27</i>
pEAI352	<i>mlh3-28</i>
pEAI353	<i>mlh3-29</i>
pEAI354	<i>mlh3-30</i>
pEAI355	<i>mlh3-31</i>
pEAI356	<i>mlh3-32</i>
pEAI357	<i>mlh3-33</i>
pEAI358	<i>mlh3-34</i>
pEAI359	<i>mlh3-35</i>
pEAI360	<i>mlh3-36</i>
pEAI361	<i>mlh3-37</i>
pEAI362	<i>mlh3-38</i>
pEAI363	<i>mlh3-39</i>
pEAI364	<i>mlh3-40</i>
pEAI365	<i>mlh3-41</i>
pEAI366	<i>mlh3-42</i>
pEAI367	<i>mlh3-43</i>
pEAI368	<i>mlh3-44</i>
pEAI369	<i>mlh3-45</i>
pEAI370	<i>mlh3-46</i>

pEAI371	<i>mlh3-47</i>
pEAI372	<i>mlh3-48</i>
pEAI373	<i>mlh3-49</i>
pEAI374	<i>mlh3-50</i>
pEAI375	<i>mlh3-51</i>
pEAI376	<i>mlh3-52</i>
pEAI377	<i>mlh3-53</i>
pEAI378	<i>mlh3-54</i>
pEAI379	<i>mlh3-55</i>
pEAI380	<i>mlh3-56</i>
pEAI394	<i>mlh3-57</i>
pEAI395	<i>mlh3-58</i>
pEAI396	<i>mlh3-59</i>
pEAI397	<i>mlh3-60</i>
pEAI252	<i>mlh3-D523N</i>

#### B. Two-hybrid plasmids

	relevant genotype	
pBTM116	<i>pADH1-lexA</i> , dummy vector	2 $\mu$ , TRP1
pEAM105	<i>pADH1-lexA-MLH1<sub>SK1</sub></i>	2 $\mu$ , TRP1
pGAD424	<i>pADH1-GAL4AD</i>	2 $\mu$ , LEU2
pEAM234	<i>pADH1-GAL4-AD-MLH3<sub>SK1</sub></i>	2 $\mu$ , LEU2
pEAM235	<i>pADH1-GAL4-AD-mlh3-39</i>	2 $\mu$ , LEU2
pEAM236	<i>pADH1-GAL4-AD-mlh3-40</i>	2 $\mu$ , LEU2
pEAM237	<i>pADH1-GAL4-AD-mlh3-41</i>	2 $\mu$ , LEU2
pEAM238	<i>pADH1-GAL4-AD-mlh3-42</i>	2 $\mu$ , LEU2
pEAM241	<i>pADH1-GAL4-AD-mlh3-45</i>	2 $\mu$ , LEU2
pEAM242	<i>pADH1-GAL4-AD-mlh3-48</i>	2 $\mu$ , LEU2
pEAM244	<i>pADH1-GAL4-AD-mlh3-54</i>	2 $\mu$ , LEU2
pEAM245	<i>pADH1-GAL4-AD-mlh3-60</i>	2 $\mu$ , LEU2

#### C. 2 $\mu$ URA3 plasmids

	Relevant genotype
pRS426 (pEAO34)	Dummy vector
pEAM266	<i>SGS1</i>
pEAM270	<i>sgs1-K706A (sgs1-hd)</i>

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A. All *MLH3* mutagenesis plasmids are derived from pEAI254, a 7.8 kb *MLH3<sub>SK1</sub>::KANMX* integrating vector. pEAI254 was mutagenized by QuickChange to create the alleles listed. The DNA sequence of the entire ORF, and 70 bp upstream and 150 bp downstream, were confirmed by DNA sequencing using primers EAO318, EAO319, EAO1778 and EAO321. B. For the two-hybrid analysis, pEAM105 contains the entire *MLH1* gene derived from the SK1 strain inserted immediately after the *lexA* binding domain in pBTM116. All *GAL4* activating domain-*mlh3* plasmids are derived from pEAM234, which contains DNA sequence encoding SK1 *MLH3* amino acids 481 to 715 inserted immediately after the *GAL4* activating domain in pGAD424. C. *SGS1* was cloned into pRS426 to make pEAM266 as described in the Methods. Site directed mutagenesis of pEAM266 was performed to make pEAM270 carrying the *sgs1* helicase defective allele as described in the Methods.