

Supplemental Table 2. Oligos used in this study. Coordinates are taken from the *Saccharomyces* Genome Database, strain S288c, and list chromosome followed by nucleotide range.

Oligo name	Sequence (5'→3')	Coordinates	Use
ENA6-F	GTTGGAAAGCTCACGGTGAT	IV: 529277-529260 ¹	PCR-RFLP of <i>ENA</i> locus; digested with <i>RsaI</i>
ENA6-R	ACCCACAAAGTCCCCAGTTAA	IV: 528664-528684 ¹	PCR-RFLP of <i>ENA</i> locus; digested with <i>RsaI</i>
EHD3-2F	ACCACCAGCACAGAACGAAC	IV: 524414-524433	PCR-RFLP to screen <i>EHD3</i> CRISPR transformants; digested with <i>AluI</i> ; paired with KRS1-R
KRS1-R	GGCGGCTTTGACATTATCTT	IV: 525469-525450	PCR-RFLP to screen <i>EHD3</i> CRISPR transformants; digested with <i>AluI</i>
GPD1-F	TGTCTGCTGCTGCTGATAGA	IV: 411826-411845	PCR-RFLP to screen <i>EHD3</i> CRISPR transformants; digested with <i>DdeI</i>
GPD1-R	CTCGCCTCTGAAATCCTTTG	IV: 412451-412432	PCR-RFLP to screen <i>EHD3</i> CRISPR transformants; digested with <i>DdeI</i>
ADR1-F	GTCCATTTGAAAAATCGTTTG	IV: 897146-897166	PCR-RFLP to screen <i>EHD3</i> CRISPR transformants; digested with <i>AluI</i>
ADR1-R	CGATTCGATCCAAAAACACA	IV: 897870-897851	PCR-RFLP to screen <i>EHD3</i> CRISPR transformants; digested with <i>AluI</i>
GIN4-F	GGGAGCACTGGTAAAGTCCA	IV: 1465705-1465686	PCR-RFLP to screen <i>EHD3</i> CRISPR transformants; digested with <i>AluI</i>
GIN4-R	TCCATCTTCCTCGTCAAAGG	IV: 1465079-1465098	PCR-RFLP to screen <i>EHD3</i> CRISPR transformants; digested with <i>AluI</i>
ENA-LPCR-F	TCTCGTGGGCAAAGGTTTAG	IV: 540048-540029	Primer for long PCR to amplify <i>ENA</i> locus region
ENA-LPCR-R	CAAGGTGATGACGAAGCTCA	IV: 527009-527028	Primer for long PCR to amplify <i>ENA</i> locus region
EHD3-24-cr-oligo1	GATCATTTAAAGTAATAACTCTAGGTTTTAGAGCTAG	IV: 524555-524574	crRNA targeting the SSP24 allele at <i>EHD3</i> ; oligo 1 of 2, includes 5' and 3' non-specific sgRNA regions.
EHD3-24-cr-oligo2	CTAGCTCTAAAACCTAGAGTTATTACTTTAAAT	IV: 524555-524574	crRNA targeting the SSP24 allele at <i>EHD3</i> ; oligo 2 of 2 (complement)

EHD3v2-253-cr-oligo1	GATCGCGTCGGTGACATTTAGCTGGTTTTAGAGCTAG	IV: 524607-524626	crRNA targeting the SSP253 allele at <i>EHD3</i> ; oligo 1 of 2, includes 5' and 3' non-specific sgRNA regions.
EHD3v2-253-cr-oligo2	CTAGCTCTAAAACCAGCTAAATGTCACCGACGC	IV: 524607-524626	crRNA targeting the SSP253 allele at <i>EHD3</i> ; oligo 2 of 2 (complement)
MAL31-253-cr-oligo1	GATCACCAGAGTCTCCATGGTGCGTTTTAGAGCTAG	II: 803600-803581	crRNA targeting the SSP253 allele at <i>MAL31</i> ; oligo 1 of 2, includes 5' and 3' non-specific sgRNA regions.
MAL31-253-cr-oligo2	CTAGCTCTAAAACGCCACCATGGAGACTCTGGT	II: 803600-803581	crRNA targeting the SSP253 allele at <i>MAL31</i> ; oligo 2 of 2 (complement)
MAL31-24-cr-oligo1	GATCTATAGCCTAGATCTGAGTTAGTTTTAGAGCTAG	II: 803662-803681	crRNA targeting the SSP24 allele at <i>MAL31</i> ; oligo 1 of 2, includes 5' and 3' non-specific sgRNA regions.
MAL31-24-cr-oligo2	CTAGCTCTAAAACCTAACTCAGATCTAGGCTATA	II: 803662-803681	crRNA targeting the SSP24 allele at <i>MAL31</i> ; oligo 2 of 2 (complement)
MAL33-U-F	TTGAGCACAATTTTCGATTGC	II: 800605-800624	PCR-RFLP of <i>MAL33</i> locus; digested with <i>MboI</i>
MAL33-U-R	TGAGCATTATTGGCGTTTGA	II: 801595-801576	PCR-RFLP of <i>MAL33</i> locus; digested with <i>MboI</i>
MAL-5-24-253-F	TGTTCTTTCAAGTGATCTCCTC	II: 803531-803552	PCR-RFLP of <i>MAL31</i> locus; digested with <i>RsaI</i>
MAL-6-24-253-R	CCTGGCAAATCGGTCTATGT	II: 804033-804014	PCR-RFLP of <i>MAL31</i> locus; digested with <i>RsaI</i>

¹There are three locations in the S288c genome that match this oligo.