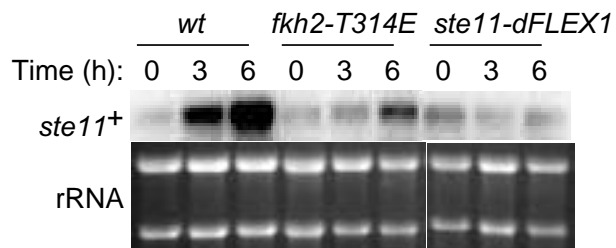
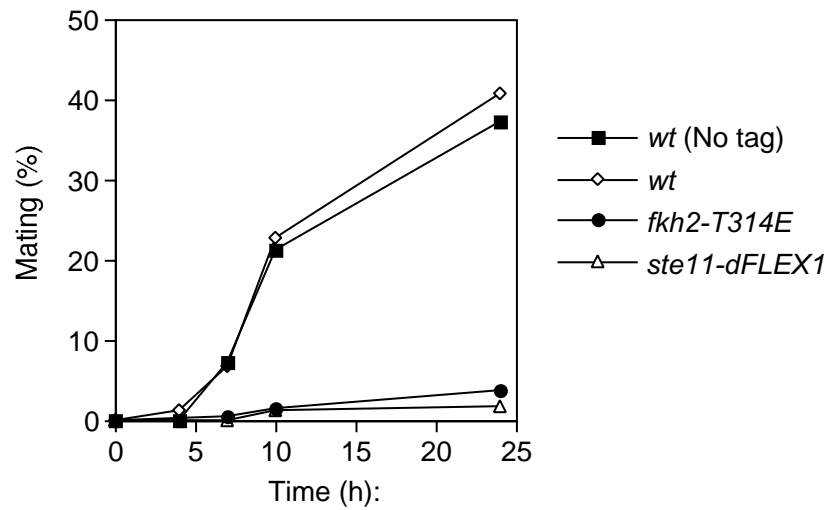
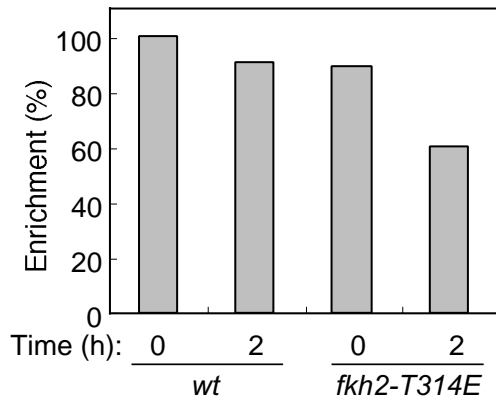


Supplementary Figure 1. DNA content of forkhead mutant cells.

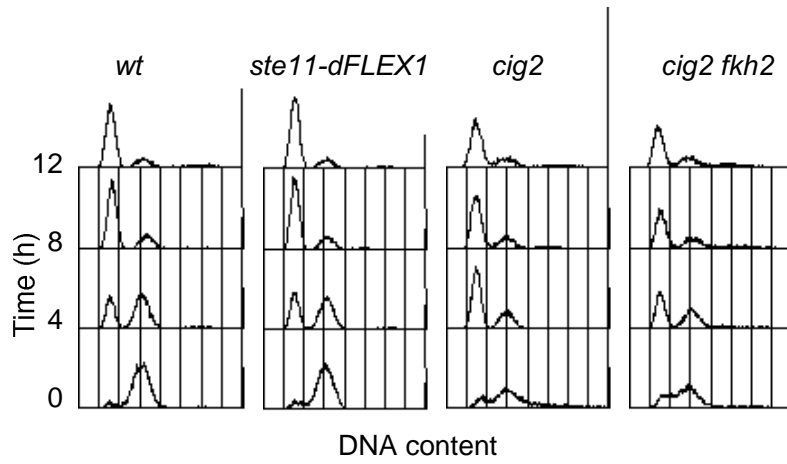
wt (HM6), *fkh2* (HM4843), *fhl1* (HM4837), *mei4* (HM50), *fkh2 fhl1* (HM4887), *fkh2 mei4* (HM5515), or *fkh2 fhl1 mei4* (HM5544) cells were grown in EMM2 medium to a density of 1×10^7 cells/ml, washed, and resuspended at a density of 2×10^7 cells/ml in EMM2 medium lacking nitrogen. They were then cultured at 30°C, and samples were collected at the indicated times for determination of DNA content by staining with propidium iodide and flow cytometric analysis.



Supplementary Figure 2. Mating and *ste11*⁺ expression in GFP-tagged Fkh2p cells
wt (No tag, HM6), *wt* (HM5719), *fkh2-T314E* (HM5912) or *ste11-dFLEX1* (HM6124) cells were grown and treated as in Figure 1. Mating efficiency was determined as in Figure 1. Total RNA was extracted and the abundance of *ste11*⁺ mRNA was examined by Northern blot analysis. Ethidium bromide staining of rRNA is shown as a loading control.

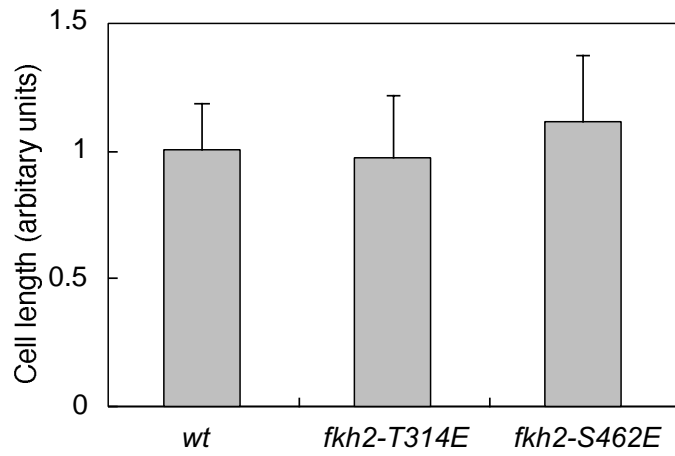


Supplementary Figure 3. ChIP analysis around the *cdc15*⁺ FLEX region. Cells expressing GFP-tagged Fkh2p (*wt*; HM5719 and *fkh2-T314E*; HM591 2) were treated as in Figure 2C. After nitrogen deprivation for 2 h at 30°C, cells were collected and analyzed by ChIP with antibodies to GFP and with the primer sets around the *cdc15*⁺ FLEX region.



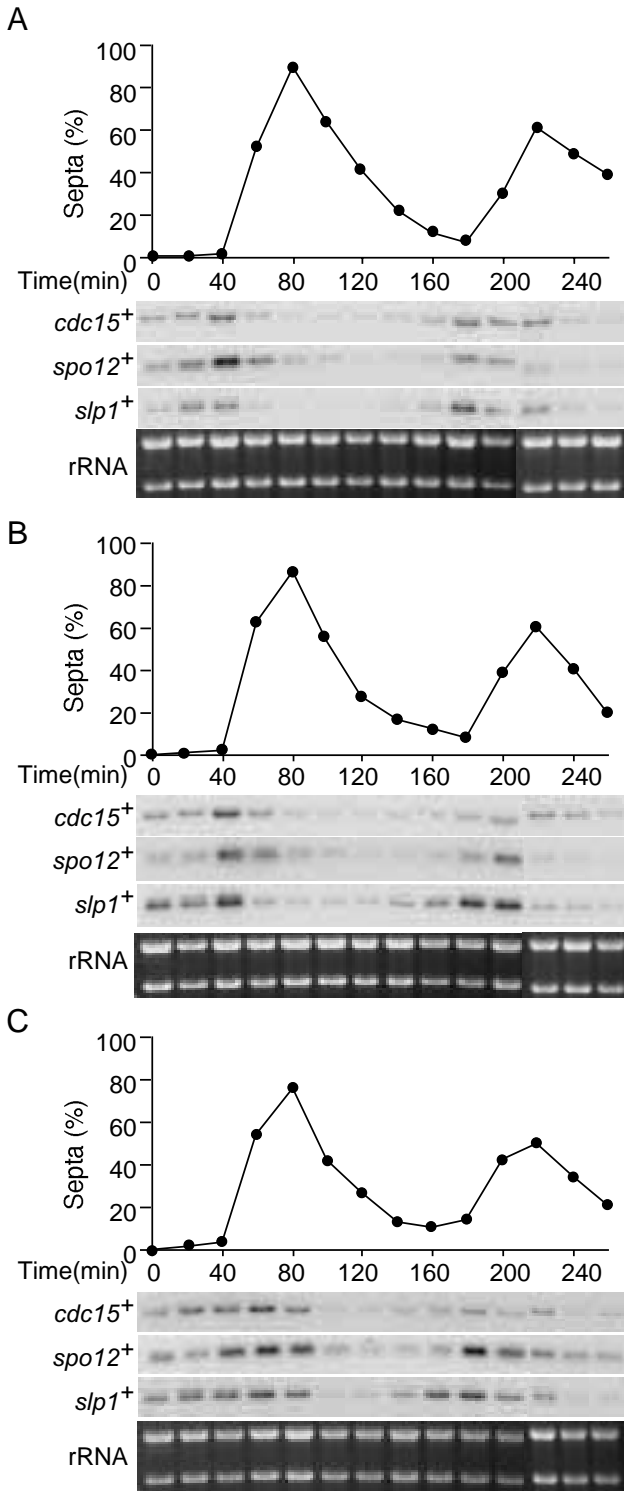
Supplementary Figure 4. DNA content of *ste11-dFLEX1*, *cig2*, or *cig2 fkh2* mutant cells.

wt (HM6), *ste11-dFLEX1* (HM5832), *cig2* (HM5555), or *cig2 fkh2* (HM5701) cells were induced to mate and analyzed for DNA content as in Supplementary Figure 1.



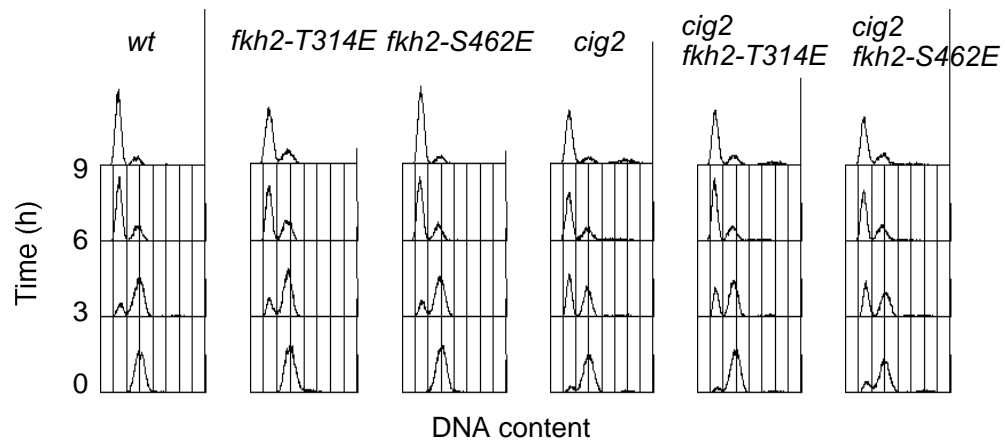
Supplementary Figure 5. Cell length

Cells (*wt*, HM5145; *fkh2-T314E*, HM5910; *fkh2-S462E*, HM5911) were grown to log phase. Cell length was measured and expressed as a proportion of *wt* cells. The *bars* indicate the average data. *Error bars* indicate the S.D.



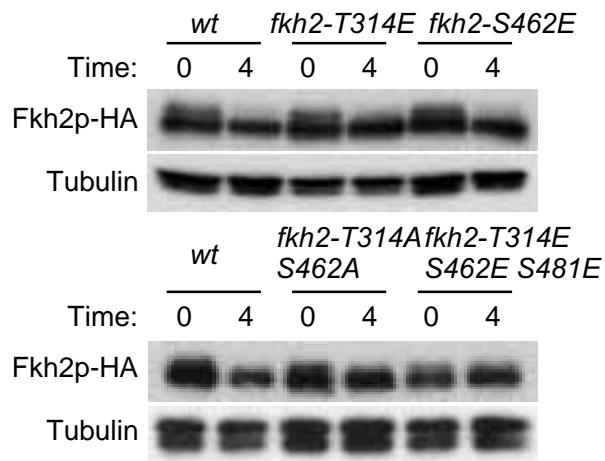
Supplementary Figure 6. Periodic expressions of *cdc15*⁺, *spo12*⁺ and *slp1*⁺ mRNAs.

cdc25^{ts} (HM6095) (A), *cdc25^{ts} fkh2-T314E* (HM6099) (B), and *cdc25^{ts} fkh2-S462E* (HM6101) (C) cells were synchronized by transient temperature arrest and samples taken every 20 min upon release to the permissive temperature. Total RNA was extracted from cells treated as in Figure 1, and the abundance of *cdc15*⁺, *spo12*⁺ and *slp1*⁺ mRNA was examined by Northern blot analysis. Ethidium bromide staining of rRNA is shown as a loading control. Septation indices were counted microscopically and are plotted to indicate the synchrony of the culture.



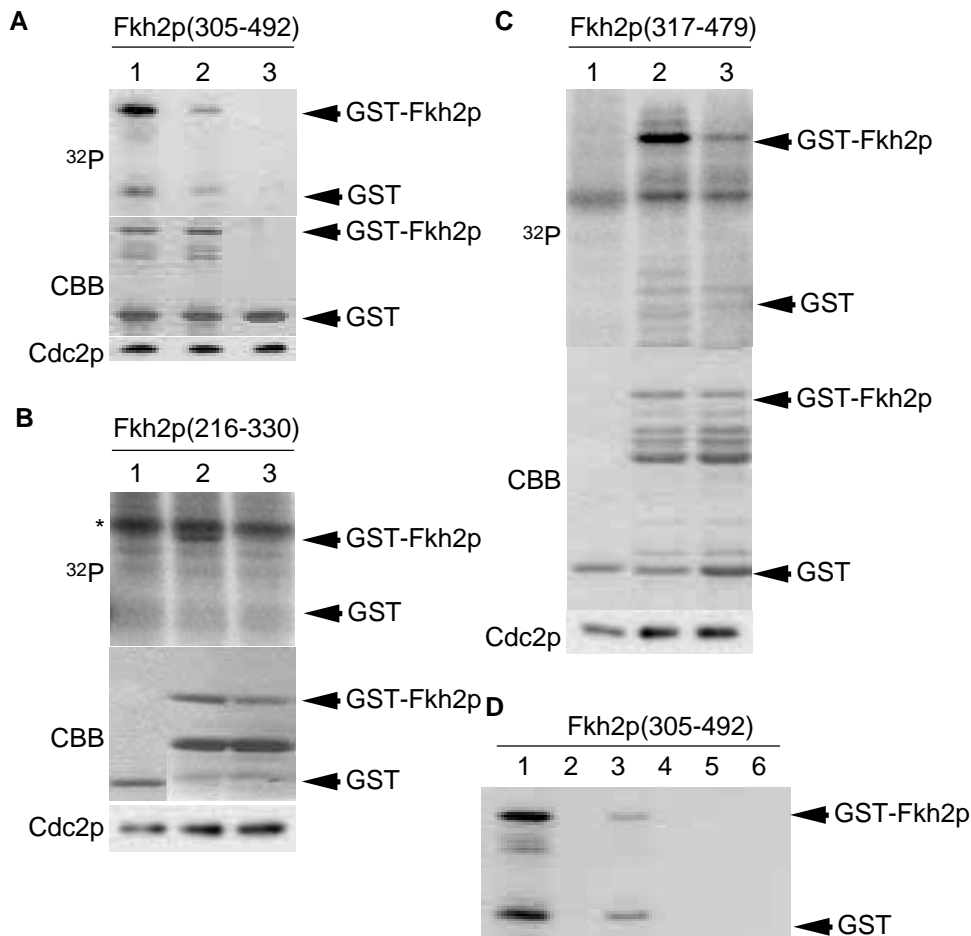
Supplementary Figure 7. DNA content of various *fkh2* and *cig2* mutant cells.

wt (HM5145), *fkh2-T314E* (HM5910), *fkh2-S462E* (HM5911), *cig2* (HM5530), *cig2 fkh2-T314E* (HM5924), or *cig2 fkh2-S462E* (HM5925) cells were induced to mate and analyzed for DNA content as in Supplementary Figure 1.



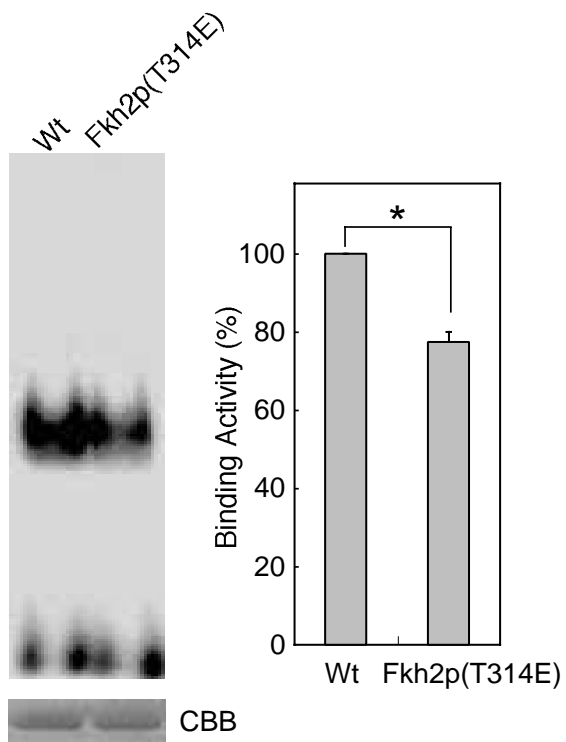
Supplementary Figure 8. Expression of wild-type and mutant forms of Fkh2p.

Cells of the indicated genotypes (*wt*, HM5145; *fkh2-T314E*, HM5910; *fkh2-S462E*, HM5911; *fkh2-T314A S462A*, 5722; *fkh2-T314E S462E S481E*, 5827) expressing hemagglutinin epitope (HA)-tagged forms of Fkh2p were induced to mate for 0 or 4 h as in Supplementary Figure 1. Cell lysates were prepared by the 'boliling method' and were then subjected to immunoblot analysis with antibodies to HA and to α -tubulin (loading control).



Supplementary Figure 9. Phosphorylation of Fkh2p on T314 and S462 by Cdc2p in vitro.

(A–C) Kinase assays were performed with Cdc2p precipitates prepared from protein extracts of exponentially growing *wt* (HM6) cells with Suc1p-coated beads. Substrates (lanes 1 to 3, respectively) included GST-Fkh2p(305–492), GST-Fkh2p(305–492) containing T314A, S462A, and S481A mutations, or GST alone (A); GST, GST-Fkh2p(216–330), or GST-Fkh2p(216–330) containing the T314A mutation (B); and GST, GST-Fkh2p(317–479), or GST-Fkh2p(317–479) containing the S462A mutation (C). Reaction mixtures were separated by SDS-polyacrylamide gel electrophoresis, and proteins were detected by staining with Coomassie brilliant blue (CBB) and autoradiography (^{32}P). Arrows indicate GST and the GST-Fkh2p fusion proteins. The asterisk indicates a non-specific band. The Cdc2p input into each reaction mixture was also examined separately by Western blotting. (D) Kinase assays were performed with recombinant human Cdc2-CyclinB1 complex (lanes 1, 3, 5) or human kinase-mutant Cdc2-CyclinB1 complex (lanes 2, 4, 6). Substrates included GST-Fkh2p(305–492)(lanes 1, 2), GST-Fkh2p(305–492) containing T314A, S462A, and S481A mutations (lanes 3, 4), or GST alone (lanes 5, 6).



Supplementary Figure 10. EMSA analysis for Fkh2p(T314E)

Recombinant GST fusion proteins containing wild-type (Wt) or T314E mutant forms of Fkh2p(216–330) were subjected to EMSA analysis with a ^{32}P -labeled FLEX1 probe as in Figure 2B. A representative autoradiogram and the region of the Coomassie brilliant blue–stained gel containing the fusion proteins are shown in the left panel. Quantitation by densitometric analysis of the binding activity of the mutant protein expressed relative to that of the wild-type protein is shown in the right panel; data are means \pm SE of values from three independent experiments. * $P < 0.0002$ (Student's t test).

Supplementary Table 1. Yeast strains used in the study.

Strain	Genotype
HM6	<i>h⁹⁰ leu1-32</i>
HM50	<i>h⁹⁰ ura4-D18 leu1-32 mei4::ura4⁺</i>
HM4837	<i>h⁹⁰ leu1-32 fhl1::kan^r</i>
HM4843	<i>h⁹⁰ leu1-32 fkh2::kan^r</i>
HM4887	<i>h⁹⁰ leu1-32 fhl1::kan^r fkh2::kan^r</i>
HM5145	<i>h⁹⁰ leu1-32 fkh2-HA-kan^r</i>
HM5146	<i>h⁻ leu1-32 fkh2-HA-kan^r</i>
HM5444	<i>h⁹⁰ leu1-32 fkh2-HA-kan^r cdc2-L7</i>
HM5515	<i>h⁹⁰ leu1-32 ura4-D18 fkh2::kan^r mei4::ura4⁺</i>
HM5530	<i>h⁹⁰ leu1-32 ura4-D18 cig2::ura4⁺ fkh2-HA-kan^r</i>
HM5544	<i>h⁹⁰ leu1-32 ura4-D18 fkh2::kan^r fhl1::kan^r mei4::ura4⁺</i>
HM5554	<i>h⁻ leu1-32 fkh2-HA-kan^r ura4-D18 cdc13::ura4⁺ nmt41-cdc13⁺</i>
HM5555	<i>h⁹⁰ leu1-32 ura4-D18 cig2::ura4⁺</i>
HM5657	<i>h⁹⁰ leu1-32 ura4-D18 fkh2::ura4⁺</i>
HM5701	<i>h⁹⁰ leu1-32 ura4-D18 cig2::ura4⁺ fkh2::kan^r</i>
HM5702	<i>h⁹⁰ leu1-32 ura4-D18 cig2::ura4⁺ fkh2::kan^r fhl1::kan^r</i>
HM5703	<i>h⁹⁰ leu1-32 ura4-D18 cig2::ura4⁺ fkh2::kan^r mei4::ura4⁺</i>
HM5704	<i>h⁹⁰ leu1-32 ura4-D18 cig2::ura4⁺ fkh2::kan^r mei4::ura4⁺ fhl1::kan^r</i>
HM5719	<i>h⁹⁰ leu1-32 nmt41-GFP-fkh2-kan^r</i>
HM5722	<i>h⁹⁰ leu1-32 fkh2-T314A S462A S481A -HA-kan^r</i>
HM5827	<i>h⁹⁰ leu1-32 fkh2-T314E S462E S481E-HA-kan^r</i>
HM5832	<i>h⁹⁰ leu1-32 ste11-dFLEX1</i>
HM5910	<i>h⁹⁰ leu1-32 fkh2-T314E-HA-kan^r</i>
HM5911	<i>h⁹⁰ leu1-32 fkh2-S462E-HA-kan^r</i>
HM5912	<i>h⁹⁰ leu1-32 nmt41-GFP-fkh2T314E-kan^r</i>
HM5913	<i>h⁹⁰ leu1-32 nmt41-GFP-fkh2S462E-kan^r</i>
HM5924	<i>h⁹⁰ leu1-32 ura4-D18 cig2::ura4⁺ fkh2-T314E-HA-kan^r</i>
HM5925	<i>h⁹⁰ leu1-32 ura4-D18 cig2::ura4⁺ fkh2-S462E-HA-kan^r</i>

HM6095 *h⁻ cdc25-22 fkh2-HA-kan^r leu1-32*

HM6099 *h⁻ cdc25-22 fkh2-T314E-HA-kan^r leu1-32*

HM6101 *h⁻ cdc25-22 fkh2-S462E-HA-kan^r leu1-32*

HM6107 *h⁺ cdc10-V50 fkh2-HA-kan^r leu1-32*

HM6118 *h⁻ cdc2-L7<<cdc2-HA(ura4⁺) ura4-D18 leu1-32*

Supplementary Table 2. Oligonucleotides used as primers or probes.

Oligonucleotide	Sequence (5' to 3')
1002	CGGGATCCGAGCTGAATATGGTGATAATAA
1003	CCGCTCGAGCTTTTTTGCTAATAATGGCA
1038	CACGTTGAACGGCGACAGG
1088	TACTTTGACTTATTTTCACTGACTATTTCTAAGTTTAATTTGTTGATTAATTTTTATTACATCTCTT CTTTTCTTTATAGACTTAAACTTTTTTTTCTTC
1089	GTTAATTAGTAGAGAGAAAG
1141	CTTTATAGTAAACAGACTTA
1142	TAAGTCTGTTTACTATAAAG
1143	TTGAATTTCTTTGTTTGATT
1144	AATCAAACAAGAAATTCAA
1167	GACGAAAGCACTATAAAG
1168	GTTTACGCGGAGCTTTTCTAGTCTTTGC
1169	GACTAGAAAAGCTCCGCGTAAACGCTCG
1170	GAGAGTATATCAAGTGACTATTGACCGGATTTCTGTAAGGAGCAATT GTGTCATATGAACCTACT
1171	CAATAGTCACTTGATATACTCTCAAATACAGCAATCTGCTCCGAAAA AGATTGATG
1172	ACTGTTTGTGAAGTTG
1270	GTTTACGCGGCTCTTTTCTAGTCTTTGC
1271	GACTAGAAAAGAGCCGCGTAAACGCTCG
1272	GAGAGTATATCAAGTGACTATTGACCGGATTTCTGTAAGGCTCAA TTGTGTCATATGAACCTACT
1273	GGTCAATAGTCACTTGATATACTCTCAAATACAGCAATCTGAGCC GAAAAAGATTGATG
1325	TGTCTTGATTGCAGCTTGTCG

1326	AGAATGGGAGGAGGCAAGCTA
1327	CTCGGTTTGTGCTGTTTCGC
1328	TTCGCAGTCAGAAAGAACGAAG
1329	GATG TTCAGCACCGTCCCTTT
1330	CTCGGGAAGGCAATCAAGAA
1373	GCCTACACATGCTGCATTATC
1411	CGGGATCCCCAAACGCTCGCCCTCCTCT
1412	CCGCTCGAGTTGCTGTATTTGAGAGTATAT
