



The Complete Pathway for Thiosulfate Utilization in Saccharomyces cerevisiae

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ABSTRACT Saccharomyces cerevisiae is known to grow with thiosulfate as a sulfur source, and it produces more ethanol when using thiosulfate than using sulfate. Here, we report how it assimilates thiosulfate. S. cerevisiae absorbed thiosulfate into the cell through two sulfate permeases, Sul1 and Sul2. Two rhodaneses, Rdl1 and Rdl2, converted thiosulfate to a persulfide and sulfite. The persulfide was reduced by cellular thiols to H₂S, and sulfite was reduced by sulfite reductase to H₂S. Cysteine synthase incorporated H₂S into O-acetyl-L-homoserine to produce L-homocysteine, which is the precursor for cysteine and methionine in S. cerevisiae. Several other rhodaneses replaced Rdl1 and Rdl2 for thiosulfate utilization in the yeast. Thus, any organisms with the sulfate assimilation system potentially could use thiosulfate as a sulfur source, since rhodaneses are common in most organisms.

IMPORTANCE The complete pathway of thiosulfate assimilation in baker's yeast is determined. The finding reveals the extensive overlap between sulfate and thiosulfate assimilation. Rhodanese is the only additional enzyme for thiosulfate utilization. The common presence of rhodanese in most organisms, including *Bacteria*, *Archaea*, and *Eukarya*, suggests that most organisms with the sulfate assimilation system also use thiosulfate. Since it takes less energy to reduce thiosulfate than sulfate for assimilation, thiosulfate has the potential to become a choice of sulfur in optimized media for industrial fermentation.

KEYWORDS sulfur starvation, thiosulfate permease, rhodanese, cysteine synthesis, budding yeast

Sulfur is an essential element in all organisms, being present in organic compounds like μ-cysteine. Animals obtain organic sulfur from food (1), while plants and microorganisms can assimilate sulfate (2–4). The pathway for sulfate assimilation has been well characterized. In *Saccharomyces cerevisiae* (yeast), sulfate is transported into the cell by two H⁺-dependent symporters, Sul1 and Sul2 (5, 6), and reduced to H₂S, which is incorporated into *O*-acetyl-μ-homoserine to produce μ-homocysteine, which is the precursor for cysteine and methionine (7). Yeast has recently been reported to produce more biomass and ethanol when growing with thiosulfate than with sulfate, because less energy is needed to reduce it to H₂S (8).

Thiosulfate assimilation has been comprehensively characterized in *Escherichia coli*. It is transported into the cell through thiosulfate permease, a sulfate/thiosulfate ABC-type transporter (9). Inside the cell, it is directly combined with *O*-acetyl-L-serine to generate *S*-sulfocysteine by cysteine synthase B (CysM) (10), and the product is then reduced to cysteine and sulfite by glutaredoxin (11). *E. coli* also contains cysteine synthase A (CysK) (12, 13). CysK and CysM are homologous and both synthesize cysteine from sulfide and *O*-acetyl-L-serine, but only CysM can use thiosulfate as a substrate (10). It is unknown whether the yeast cysteine synthase (Met15) could use thiosulfate as a substrate to synthesize *S*-sulfohomocysteine, which could be converted

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to L-homocysteine by glutaredoxin. Alternatively, yeast may reduce thiosulfate to sulfide and sulfite by thiosulfate reductase (14). The latter was recently shown to be a rhodanese, also known as thiosulfate sulfurtransferase, that converts thiosulfate and glutathione (GSH) to oxidized glutathione (GSSH) and sulfite (15). GSSH spontaneously reacts with GSH to produce H₂S and glutathione disulfide (GSSG) (15). Met15 potentially can use the produced sulfide for the synthesis of L-homocysteine.

Since it has been both theoretically and practically proven that thiosulfate is a better sulfur source than sulfate for yeast during the production of biomaterials (8), it is important to have a detailed understanding of how thiosulfate is used by yeast. In the present work, both genetic and biochemical data support that S. cerevisiae BY4742 $(MAT\alpha\ his3\Delta 1\ leu2\Delta 0\ lys2\Delta 0\ ura3\Delta 0)$ uses sulfate permeases, Sul1 and Sul2 (5, 16), to transport thiosulfate into the cell. Inside the cell, the rhodaneses RdI1 and RdI2 convert thiosulfate to sulfide and sulfite; the latter is also reduced to sulfide by sulfite reductase. Met15 uses sulfide for L-homocysteine synthesis. The pathway of thiosulfate assimilation extensively overlaps with that of sulfate assimilation. The only additional enzyme is a rhodanese that is widely present in Bacteria, Archaea, and Eukarya. Thus, organisms with the sulfate assimilation ability may also use thiosulfate as a sulfur source for growth.

RESULTS

S. cerevisiae uses thiosulfate as a sole source of sulfur. S. cerevisiae grew at similar rates to an optical density at 600 nm (OD $_{600}$) of 2.8 \pm 0.1 in the modified SD medium with either 100 μ M Na₂S₂O₃ or Na₂SO₄ as a sole sulfur source, but it grew to an OD_{600} of 1.4 \pm 0.1 in the sulfur-free medium, indicating that *S. cerevisiae* can utilize thiosulfate as a sole sulfur source for growth (see Fig. S1 in the supplemental material). S. cerevisiae was further transferred twice in the medium without added sulfur, and the final OD $_{600}$ values were 0.85 \pm 0.03 and 0.61 \pm 0.02 for the 2nd and 3rd transfers, respectively, suggesting that S. cerevisiae carries over some intracellular sulfur, and the medium may contain trace amounts of sulfur. When 100 μ M thiosulfate and 100 μ M sulfate were both included in the medium, yeast grew to an ${\rm OD}_{\rm 600}$ of 2.8 in 21 h, and it consumed 25 \pm 2 μ M thiosulfate and did not consume sulfate (Fig. S2), suggesting that yeast prefers to use thiosulfate instead of sulfate.

Phenotypic study of SUL1- and SUL2-disrupted mutants. Thiosulfate and sulfate are structurally similar (Fig. 1A). Whether sulfate permeases (Sul1 and Sul2) could transport thiosulfate in S. cerevisiae was tested. The genes were disrupted. When the WT, $\Delta sul1$, $\Delta sul2$, and $\Delta sul1$ $\Delta sul2$ strains grew in the modified SD medium with 100 μ M thiosulfate, the mutants bearing disruptions in SUL1 or SUL2 grew as well as the wild type, and only the double mutant ($\Delta sul1 \Delta sul2$) did not grow (Fig. 1B), indicating that both Sul1 and Sul2 transported thiosulfate, and one of them was sufficient for thiosulfate uptake. The lack of growth by the $\Delta sul1$ $\Delta sul2$ strains also suggests that the mutant contained minimal intracellular sulfur, and the modified SD medium without added sulfur contained some Na₂S₂O₃ or Na₂SO₄, which was likely from the impurities of the used chemicals and was able to support minimal growth of the wild-type strain (Fig. S1). However, the $\Delta sul1 \Delta sul2$ strain was able to grow in the modified SD medium with 500 μ M thiosulfate (Fig. 1C). The $\Delta sul1 \Delta sul2 \Delta soa1$ triple mutant did not grow with 1 mM thiosulfate but grew with 5 and 10 mM thiosulfate (Fig. S3). The data implied that Sul1 and Sul2 were high-affinity transporters, Soa1 was a low-affinity transporter, and at very high concentrations thiosulfate still was able to enter the organism either via diffusion or mediated by another transporter.

The uptake of thiosulfate via Sul1p and Sul2p in yeast. WT, \(\Delta sul1, \(\Delta sul2, \) and Δsul1 Δsul2 strains were subjected to sulfur starvation for 2 days, and the cells were harvested and suspended at an OD₆₀₀ of 2 in 50 mM potassium phosphate buffer (pH 6) with 2% glucose. Thiosulfate was added to the cell suspensions at 200 μ M. The wild type was the most efficient in absorbing thiosulfate, removing about 100 μ M thiosulfate from the medium in 90 min, while the $\Delta sul1 \Delta sul2$ strain did not take up thiosulfate (Fig. 2). The cellular concentration of the absorbed thiosulfate in the wild type was

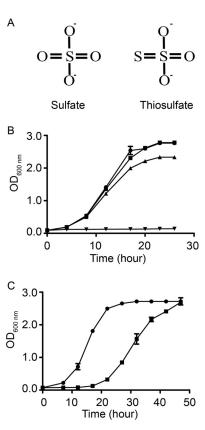


FIG 1 Growth of S. cerevisiae and its mutants on thiosulfate. (A) Chemical structure of sulfate and thiosulfate. (B) Growth with 100 µM thiosulfate. Wild-type (●), ∆sul1 (■), ∆sul2 (▲), and ∆sul1 ∆sul2 (▼) strains are shown. (C) Growth on 500 μ M thiosulfate. Wild-type (\bullet) and $\Delta sul1 \Delta sul2$ (\blacksquare) strains are shown. The optical density was measured via a spectrophotometer. All data are averages with standard deviations (error bars) from at least three cultures.

calculated to be \sim 890 μ M by using a reported haploid cell volume of 50 fl (17, 18). The $\Delta sul1$ single-gene deletion mutant absorbed more thiosulfate than the $\Delta sul2$ mutant did, indicating Sul2 was the major thiosulfate permease in yeast (Fig. 2).

Thiosulfate-mediated downregulation of SUL1 and SUL2 transcription and thiosulfate uptake. Sulfur-starved yeast cells strongly expressed SUL1 and SUL2. A sharp drop in both SUL1 and SUL2 mRNA levels occurred shortly after the addition of 1 mM thiosulfate to the cells, indicating a tight negative regulation of the two genes at the transcriptional level coupled with mRNA breakdown (Fig. 3A). Moreover, upon the addition of thiosulfate to sulfur-starved cells, the thiosulfate uptake rate also rapidly decreased, with a 50% reduction in 10 min and 90% reduction in 30 min, and then remained constant (Fig. 3B).

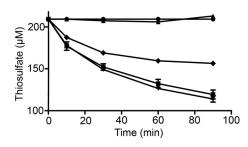
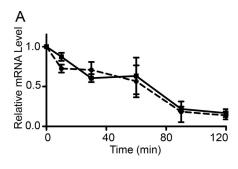


FIG 2 Uptake of thiosulfate by S. cerevisiae and its mutants. The sulfur-starved cells were suspended in 50 mM potassium phosphate buffer, pH 6. The amounts of thiosulfate remaining in the medium were determined. Wild-type (\P) , $\Delta sul1$ (\blacksquare) , $\Delta sul2$ (\spadesuit) , and $\Delta sul1$ $\Delta sul2$ (\spadesuit) strains, as well as a control (\blacksquare) , are



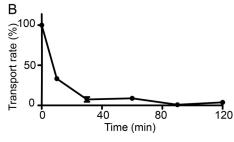


FIG 3 Effects of thiosulfate on Sul1 and Sul2 transcription and thiosulfate uptake rates. (A) Relative RNA levels of *SUL1* (●) and *SUL2* (■) after 1 mM thiosulfate addition to sulfur-starved cells. (B) The uptake rate of thiosulfate by the wild type decreased after the addition of 1 mM thiosulfate to sulfur-starved cells. The cells were harvested at the indicated time point, and the uptake rate was then measured with a 1-h uptake assay.

CYS3 and MET15 were essential genes for thiosulfate utilization. The MET15 deletion strain did not grow in the modified SD medium with 100 μ M thiosulfate, indicating that MET15 was an essential gene for thiosulfate utilization (Fig. S4A). CYS3 encodes cystathionine- γ -lyase, which is involved in converting homocysteine to cysteine (19), and its deletion strain did not grow in the modified SD medium with 100 μ M thiosulfate either (Fig. S4B). The complementation restored the growth of the mutants (Fig. S4). The results show that cysteine synthesis from thiosulfate involves homocysteine and cystathionine as intermediates.

Met15p could not directly use thiosulfate as a substrate. We tested whether Met15 could use thiosulfate directly. The recombinant protein with a His tag was produced in *E. coli* and purified (Fig. S5A). Met15 used sulfide and *O*-acetyl-homoserine to produce homocysteine (Fig. S5B); however, the enzyme did not catalyze the reaction between thiosulfate and *O*-acetyl-homoserine. Due to the lack of *O*-acetyl-serine in yeast (20), we did not try *O*-acetyl-serine as a substrate. Met15 shared 29% and 57% sequence identity with *E. coli* CysM and CysK, respectively (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Further bioinformatics analysis revealed that the sequenced fungal genomes from GenBank contained homologous cysteine synthases that grouped together; they were closer to CysK than CysM in terms of sequence similarity (Fig. S6).

The role of rhodaneses in thiosulfate utilization. *S. cerevisiae* has five rhodaneses, *RDL1*, *RDL2*, *TUM1*, *YCH1*, and *UBA4*. Single-deletion mutants grew well in the SD medium containing sulfate except the $\Delta uba4$ strain (data not shown), perhaps due to its involvement in the thiolation of the wobble base of tRNAs (21, 22). The reduced growth rate prevented testing the mutation for its function in thiosulfate utilization. Only the single deletion of *RDL1* ($\Delta rdl1$ mutant) showed growth retardation in the modified SD medium containing 100 μ M thiosulfate (Fig. 4). The other three single-deletion strains showed the same growth as the wild type in the modified SD medium with thiosulfate (Fig. S7), while the double deletion mutant of *RDL1* and *RDL2* grew more poorly than the $\Delta rdl1$ mutant and the $\Delta rdl1$ $\Delta rdl2$ $\Delta tum1$ $\Delta ych1$ (4k) strain grew more poorly than the $\Delta rdl1$ $\Delta rdl2$ strain (Fig. 4). On the other hand, the $\Delta rdl1$ $\Delta rdl2$ $\Delta tum1$ strain grew the same as the $\Delta rdl1$ $\Delta rdl2$ strain (Fig. S7). Thus, *RDL1* is mainly responsible for thiosulfate utilization in yeast; *RDL2* is dispensable but can partially replace the role of *RDL1*.

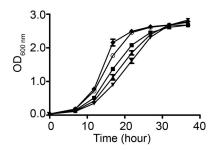


FIG 4 Growth curves of S. cerevisiae and its rhodanese mutants. Growth of the wild-type and its rhodanese mutant strains in the modified SD medium with 100 µM thiosulfate. Wild-type (○), ∆rdl1 (■), $\Delta rdl1:RDL1$ (\spadesuit), $\Delta rdl1$ $\Delta rdl2$ (\spadesuit), and $\Delta rdl1$ $\Delta rdl2$ $\Delta tum1$ $\Delta ych1$ (4k) (\blacktriangledown) strains were used. All data are averages with standard deviations (error bars) from at least three cultures.

The participation of sulfite reductase (Met5) in thiosulfate utilization. A singledeletion mutant of MET5 showed growth retardation in the modified SD medium containing 100 μ M thiosulfate compared with the wild-type strain (Fig. 5A). The double deletion mutant of RDL1 and MET5 ($\Delta rd11 \Delta met5$) resulted in further growth retardation (Fig. 5A). The grown cells were then harvested and resuspended in modified SD

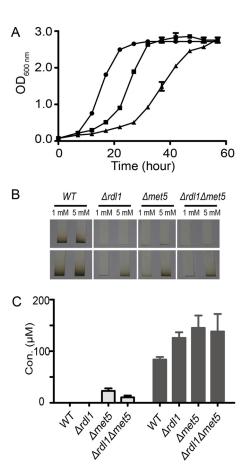


FIG 5 Yeast used both sulfur atoms of thiosulfate. (A) Growth of the parental and different mutant strains on 100 μ M thiosulfate. Wild-type (\blacksquare), $\Delta met5$ (\blacksquare), and $\Delta rdl1$ $\Delta met5$ (\blacktriangle) strains were used. All data are averages with standard deviations (error bars) from at least three cultures. (B) The parental and different mutant strains were subjected to sulfur starvation for 2 days. A concentration of 1 mM or 5 mM thiosulfate was added to 3-ml cell cultures (OD_{600} of 1) and H_2S accumulation was detected with lead-acetate paper strips, with incubation for 2 h (top) or 24 h (bottom). (C) The production of sulfite by the MET5 mutant. The parental and mutant cells were diluted to an OD_{600} of 10 in 50 mM potassium phosphate buffer (pH 6), and 150 μ M thiosulfate was added. After incubating for 60 min, the levels of sulfite (light gray bar) and thiosulfate (dark gray bar) remaining in the medium were determined.

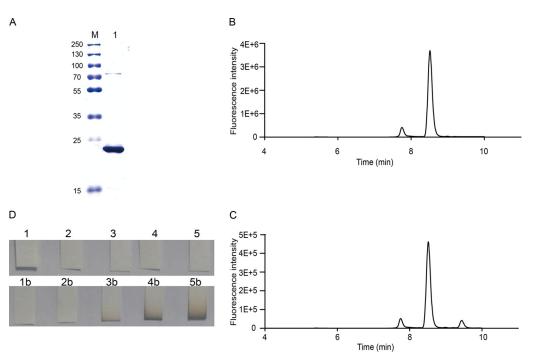


FIG 6 Rdl2p catalyzes the reaction of thiosulfate and GSH to produce sulfite. (A) SDS-PAGE analysis of purified Rdl2p. The SDS-12% polyacrylamide gel was stained with Coomassie blue staining solution. Lane M, molecular markers; lane 1, 2 μ l of purified Rdl2p. Measurements are in kDa. (B) HPLC analysis of sulfite production. The reaction mixture (0.5 ml) contained 50 mM potassium phosphate buffer (pH 8), 1 mM thiosulfate, 2 mM GSH, and 3 μ g/ml Rdl1p, and it was incubated at 30°C for 15 min. One hundred microliters of sample was derivatized with monobromobimane and analyzed via HPLC, and the retention time of sulfite was 8.51 min. (C) HPLC experiment with 30 μ M sulfite as the control. (D) Three milliliters of the enzyme reaction mixture was incubated at 30°C for 60 min, and H₂S accumulation was detected with lead-acetate paper strips. (Top) Control with different concentrations of GSH in the absence of Rdl2p. (Bottom) Reaction mixture contained Rd12p at 3 μ g/ml. Gels: 1 and 1b, 2 mM GSH; 2 and 2b, 1 mM thiosulfate; 3 and 3b, 1 mM thiosulfate plus 2 mM GSH; 4 and 4b, 1 mM thiosulfate plus 4 mM GSH; 5 and 5b, 1 mM thiosulfate plus 6 mM GSH.

medium without added sulfur and incubated for 2 days. When the sulfur-starved cells were tested to release H_2S in the modified SD medium with 1 or 5 mM thiosulfate, the wild type released about 100 μ M H_2S , the $\Delta met5$ mutant released about 10 μ M H_2S , and the $\Delta rdl1$ and $\Delta rdl1$ $\Delta met5$ strains did not release any H_2S after a 2-h incubation (Fig. 5B). After a 24-h incubation, the $\Delta rdl1$ and $\Delta rdl1$ $\Delta met5$ strains also released some H_2S in the medium with 5 mM thiosulfate (Fig. 5B). Further, the *MET5* deletion strain accumulated sulfite in the medium when thiosulfate was added to the cell suspension, while the wild type did not (Fig. 5C). These results suggest that both sulfur atoms of thiosulfate are used by the wild type.

Recombinant Rdl2p exhibited thiosulfate sulfurtransferase activity. The *RDL2* gene was cloned into pET30a, and the recombinant protein with an N-terminal His tag was produced in *E. coli* and purified (Fig. 6A). Rdl2 had apparent thiosulfate sulfurtransferase activity, producing 231 \pm 30 μ M sulfite (Fig. 6B and C) and releasing about 50 μ M H₂S (Fig. 6D) in 50 mM potassium phosphate buffer (pH 8) with 1 mM thiosulfate and 4 mM GSH at 30°C after 1 h of incubation. Rdl2 likely catalyzed the reaction of thiosulfate with GSH to produce GSSH (equation 1), which is further reduced by GSH to produce H₂S (equation 2). We tested the possibility of GSSH formation with a bacterial persulfide dioxygenase, *Cupriavidus pinatubonensis* JMP134 Pdo2 (WP_011299714), that oxidizes GSSH to sulfite (23). When CpPdo2 was added to the reaction mixture, H₂S was not produced and 966 \pm 70 μ M sulfite was formed, confirming the production of GSSH as an intermediate during the conversion of thiosulfate to H₂S in the reaction mixture containing Rdl2.

$$SO_3 S^- + GS^- \rightleftharpoons SO_3^- + GSS^- \tag{1}$$

$$2H^{+} + GSS^{-} + GS^{-} \rightleftharpoons GSSG + H_{2}S \tag{2}$$

TABLE 1 Doubling times of different strains^a

Strain	Doubling time (h)
WT	1.4 ± 0.1
4k	2.4 ± 0.2
4k::PSPE	1.2 ± 0.1
4k::GLPE	1.1 ± 0.1
4k::RDHA	1.1 ± 0.1
4k::SSEA	1.3 ± 0.2
4k::DUF442	1.1 ± 0.1

^aThe doubling time was derived from the length of the exponential growth phase. The data are averages with standard deviations from three cultures. The P values (by t test) for the 4k strain versus others were all <0.05.

GSH could be replaced by other small thiols, such as cysteine, dithiothreitol, and coenzyme A, in the enzymatic conversion of thiosulfate to H₂S and sulfite (Fig. S8A). Thus, most small organic thiols, either natural or nonnatural, can serve as sulfane sulfur acceptors. Dithiothreitol was the best acceptor, as the reaction with it accumulated the most H₂S. Cys106 in Rdl2 was predicted to be the catalytic residue via sequence comparison, and the purified Rdl2 C106A or Rdl2 C106S was inactive (Fig. S8B). Further, iodoacetamide, a thiol blocking agent, inactivated Rdl2 (data not shown). In conclusion, both Rdl1 and Rdl2 have thiosulfate sulfurtransferase activities, and they share similar predicted three-dimensional structures (Fig. S9).

Rdl1, Tum1, Uba4, and Ych1 were also purified. Rdl1 had strong rhodanese activity, as reported previously (15). Tum1 and Uba4 showed low activity for releasing $\rm H_2S$ from thiosulfate and GSH, but Ych1 displayed no activity (Fig. S10A). Although the $\Delta rdl1$ $\Delta rdl2$ strain and the 4k mutant grew poorly at similar rates in the SD medium modified with thiosulfate, the expression of TUM1 or UBA4 in the 4k mutant allowed the mutant to growth in the SD medium modified with thiosulfate (Fig. S10B and C). Perhaps the physiological levels of Tum1 and Uba4 were not sufficient to support thiosulfate utilization in its natural state. The expression of Ych1 in the 4k mutant did not help with growth on thiosulfate (Fig. S10D).

Rhodaneses from other organisms also participate in thiosulfate utilization. Since rhodaneses are common in most organisms (23), their potential role in helping other organisms to use thiosulfate was tested. Several bacterial rhodaneses were selected. They were PspE, GlpE, and SseA from *E. coli* MG1655, RdhA from *Pseudomonas aeruginosa*, and Duf442 from *Cupriavidus pinatubonensis* JMP134 (24–27). When they were cloned and expressed in the 4k strain, all restored the mutant's growth on thiosulfate (Table 1). They were also cloned with a His tag, produced in *E. coli*, and purified. All of them catalyzed the conversion of thiosulfate to H_2S in the presence of GSH (Fig. S11). PspE, GlpE, RdhA, and Duf442 produced about 30 μ M H_2S , and SseA generated about 10 μ M H_2S under the same conditions (see the legend to Fig. S11). Thus, rhodanese involvement in thiosulfate utilization is likely a common phenomenon.

DISCUSSION

We deciphered the pathway for thiosulfate utilization in *S. cerevisiae* (Fig. 7A). It extensively overlaps that of sulfate utilization. The two pathways share the same substrate transporters, Sul1 and Sul2, sulfite reductase, cysteine synthase, and cystathionine- γ -lyase. *S. cerevisiae* uses cysteine synthase to produce homocysteine, which is then converted to cysteine via cystathionine (20). Sul1 and Sul2 are high-affinity H⁺-dependent symporters for sulfate uptake (5), and we showed that they also transport thiosulfate. Soa1, another H⁺-dependent symporter for organic sulfonate uptake, also transports both sulfate and thiosulfate (28). Here, we showed that Sul1 and Sul2 are high-affinity thiosulfate transporters and Soa1 is a low-affinity thiosulfate transporter (Fig. 1; see also Fig. S3 in the supplemental material). These findings are in agreement with an ABC-type transporter that transports both thiosulfate and sulfate in *E. coli* (9). Therefore, sulfate and thiosulfate are often transported into the cell by the same transporters.

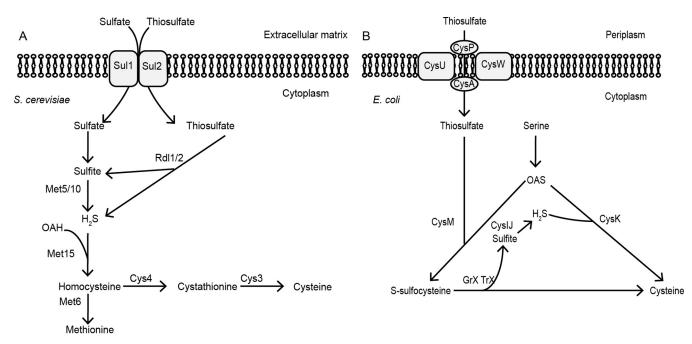


FIG 7 Thiosulfate assimilation pathways. (A) A proposed thiosulfate assimilation pathway in *S. cerevisiae*. (B) The thiosulfate assimilation pathway in *E. coli* (11).

The difference between sulfate and thiosulfate assimilation in yeast occurs in the initial steps. For sulfate, it is first reduced to sulfite by three enzymes. For thiosulfate, a rhodanese is required. It may be a common phenomenon that a rhodanese takes part in thiosulfate utilization; even E. coli 3-mercaptopyruvate sulfurtransferase (SseA) and yeast Uba4p with a rhodanese domain and low rhodanese activity (27, 29) replaced Rdl1 or Rdl2 in yeast for thiosulfate utilization (Fig. S10E and F). It appears that any fungi, bacteria, or plants with the sulfate utilization pathway also are able to assimilate thiosulfate; however, the physiological level of rhodanese in some organisms may not be sufficient to support thiosulfate utilization; for instance, the native level of Tum1 or Uba4 was insufficient to support the $\Delta rdl1$ $\Delta rdl2$ mutant to properly use thiosulfate (Fig. S7). The physiological level of rhodanese activities is also not sufficient to support thiosulfate utilization in an E. coli cysM mutant that resumed growth with the overexpression of the E. coli rhodanese GIPE (30). Thus, any organisms with the sulfate assimilation system may use thiosulfate as a sulfur source with the help of a rhodanese. For example, tobacco cells have been shown to use thiosulfate as a sulfur source (31), and rhodanese may play a role in the utilization.

Rhodaneses are widely present in all domains of life (32). They share evolutionary relationships with a conserved catalytic Cys residue and extensive heterogeneity at different levels, including the sequence, active-site loop length, and domain arrangement (33). They play a number of important biological roles, such as cyanide detoxification, tRNA thiolation, and the synthesis of iron-sulfur centers (24, 25, 32). They may also be involved in managing stress and maintaining redox homeostasis (34-37). Further, they participate in sulfur oxidation, catalyzing the conversion of GSSH and sulfite to thiosulfate, which is a step during sulfide oxidation by heterotrophic bacteria and by human mitochondria (26, 38, 39). The reversible reaction has been reported with yeast Rdl1 and the rhodanese domain of a bacterial persulfide dioxygenase (15, 40), which show thiosulfate sulfurtransferase activity, implying Rdl1 participates in thiosulfate metabolism in vivo. Rdl1 transfers the sulfane sulfur from thiosulfate to other thiols, such as GSH, coenzyme A, cysteine, and dithiothreitol (Fig. S8A), to form persulfides (15); however, GSH is likely the physiological sulfane sulfur acceptor due to high concentrations, up to 10 mM, in yeast cells (41). In yeast, Rdl1 and Rdl2 were the primary rhodaneses for thiosulfate utilization, and they can be replaced by rhodaneses

from various sources. Thus, another common function of rhodaneses is to catalyze the reaction of thiosulfate with GSH to produce GSSH.

SUL1 and SUL2 are overexpressed when yeast is under sulfur starvation (42), and sulfur starvation causes an \sim 10,000-fold increase in the sulfate influx rate mediated by Sul1p and Sul2p (43). The addition of sulfate causes a rapid decrease in sulfate transport without mRNA degradation (43). The sulfur-starved cells also rapidly accumulated thiosulfate, and the addition of thiosulfate quickly decreased the rate of thiosulfate uptake. Further, thiosulfate induced rapid degradation of SUL1 and SUL2 mRNA. Sul1 and Sul2 are transceptors that not only transport but also sense extracellular sulfate and signal via the protein kinase A pathway to regulate sulfur-utilizing genes (44). Further research is required to understand their sensing and response to thiosulfate.

In E. coli the CysM pathway for thiosulfate assimilation is preferred, probably due to the lack of free sulfide during cysteine biosynthesis (Fig. 7B). However, Met15 did not use thiosulfate. Sequence analysis showed that CysM homologues are found mainly in bacteria and are not found in sequenced fungal genomes, in which Met15 homologues are more common and are closely related to CysK. This suggests that fungi use the yeast pathway for thiosulfate utilization. For organisms that do not have a CysM-type cysteine synthase, the yeast system can be used for thiosulfate assimilation. As presented here, yeast can efficiently use thiosulfate (Fig. S2), which is in agreement with a previous report that S. cerevisiae uses thiosulfate more effectively than sulfate during the production of useful materials (8). Thiosulfate contains a sulfane sulfur that requires one NADPH equivalent for sulfide production, while the conversion of sulfate to sulfide consumes 2 ATPs and 4 NADPHs (45). Therefore, microorganisms may preferentially use thiosulfate as a sulfur source for growth in nature. Thiosulfate is common in the environment, as heterotrophic bacteria can actively produce sulfide and then oxidize it to thiosulfate during growth on organic compounds (26, 46). Further, thiosulfate has the potential to become the choice of sulfur source in optimized media for industrial fermentation because less energy is required for its reduction than sulfate reduction.

MATERIALS AND METHODS

Materials and media. Thiosulfate, sulfate, O-acetyl-serine (OAS), O-acetyl-homoserine (OAH), and pyridoxal-5-phosphate (PLP) were purchased from Sigma Chemical (St. Louis, MO). The enzymes used in the DNA manipulations were from Thermo Fisher (Waltham, MA). Enzymes for PCR were from Toyobo (Osaka, Japan).

A modified SD medium (per liter: 20 g glucose, 10 g NH₄Cl, 1.42 g KH₂PO₄, 0.25 g MgCl₂, 142 mg NaCl, 142 mg CaCl₂, 567 mg niacin, 567 mg pyridoxine, 567 mg thiamine-HCl, 2.8 mg folic acid, 2.8 mg biotin, 283 mg p-aminobenzoic acid, 567 mg calcium pantothenate, 283 mg riboflavin, 2.8 mg inositol, 708 mg boric acid, 567 mg MnCl₂, 567 mg ZnCl₂, 57 mg CuCl₂, 283 mg FeCl₃, 283 mg Na₂MoO₄, and 142 mg Kl, pH 6) had no added sulfur but might contain trace amounts of Na_2SO_4 or Na_2SO_3 as impurities from the added chemicals, which did not support normal growth of the yeast.

Strains and plasmids. General cloning procedures, sequencing, and PCR were carried out according to standard procedures (47). The yeast strains used in this study were derived from S. cerevisiae BY4742 $(MAT\alpha \ his3\Delta 1 \ leu2\Delta 0 \ lys2\Delta 0 \ ura3\Delta 0)$, which was kindly provided by W. J. Guan (Zhejiang University, China). Escherichia coli strain DH5 α served as the host strain for all plasmid constructions and was grown in lysogeny both (LB) medium with 50 μ g/ml ampicillin or kanamycin if necessary. *S. cerevisiae* strains were grown at 30°C in yeast extract-peptone-dextrose (YPD) medium (1% yeast extract, 2% peptone, and 2% glucose) or in modified SD medium left unsupplemented or supplemented with a sulfur compound and with compounds necessary to meet auxotrophic requirements. Gene disruptions were carried out by one-step PCR-mediated gene disruption in BY4742 (48).

The TEF1 promoter, a strong constitutive promoter, was amplified from yeast genomic DNA by PCR, and the PCR product was cloned into YEplac195 (digested with HindIII and BamHI) to obtain YEplac195-TEF1p. The CYC1 terminator was amplified from the pYES2 plasmid, and the PCR product was cloned into YEplac195-TEF1p (digested with SacI and EcoRI) to obtain YEplac195-TEF1p-CYC1t. The DNA fragment (MET15) encoding bifunctional cysteine synthase was amplified from yeast genomic DNA by PCR, and the PCR product was cloned into YEplac195-TEF1p-CYC1t (digested by BamHI and KpnI) to obtain YEplac195-TEF1p-MET15-CYC1. The construct was sequenced and confirmed to be correct. The rhodanese gene from other organisms was incorporated into the YEplac195-TEF1p-CYC1t plasmid. The plasmids were transformed into yeast strains by using the LiAc/SS carrier DNA/polyethylene glycol method as reported previously (49).

The strains and plasmids used in this study are listed in Table 2. All primers are given in Table 3.

Measurement of S. cerevisiae growth curve with thiosulfate or sulfate as the sulfur source. Fresh cells of S. cerevisiae strains were inoculated in 5 ml of YPD and grown overnight at 30°C. Cells were collected by centrifugation (11,000 rpm, 5 min), washed twice with sterile water, and suspended in the

TABLE 2 Strains and plasmids used this study

TABLE 2 Strains and plasmids	used this study	
Strain or plasmid	Relevant characteristic(s)	Source
S. cerevisiae		
BY4742	MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0	Laboratory stock
E. coli		Laboratory stock
DH5α	supE44 ΔlacU169(φ80dlacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1	Laboratory Stock
BL21(DE3)	F^- ompT hsdS _B (r_B^- m _B $^-$) gal(λ 1857 ind1 sam7 nin5 lacUV5 T7 gene 1) dcm	Laboratory stock
BY4742 Δ <i>sul1</i>	$\Delta sul1$::BLE	This study
BY4742 $\Delta sul2$	$\Delta sul2$::LEU2	This study
BY4742 $\Delta sul1 \Delta sul2$	Δsul1::BLE Δsul2::LEU2	This study
BY4742 <i>Amet5</i>	Δ met5::loxP Δ rdl1::BLE	This study
BY4742 Δ met5 Δ rdl1	Δmet5::BLE	This study
BY4742 Δ <i>cys3</i>	Δcys3::BLE	This study
Δcys3::CYS3	Δcys3::YEplac195-CYS3	This study
Δmet15	Δmet15::BLE	This study
Δ met15::MET15	$\Delta met15$::YEplac195- <i>MET15</i>	This study
Δrdl 1	Δrdl 1::BLE	This study
$\Delta rdl2$	$\Delta rdl2$::BLE	This study
Δ tum1	Δtum1::HIS5	This study
Δ ych1	Δmet15::LEU2	This study
Δ uba4	Δuba4::kanMX	This study
$\Delta rdl1 \ \Delta rdl2$	$\Delta rdl1::loxP$ $\Delta rdl2::BLE$	This study
$\Delta rdl1 \Delta rdl2 \Delta tum1$	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2	This study
4k	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3	This study
4k::RDL1	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3 YEplac195-RDL1	This study
4k:: <i>RDL2</i>	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3 YEplac195-RDL2	This study
4k::TUM1	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3 YEplac195-TUM1	This study
4k:: <i>YCH1</i>	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3 YEplac195-YCH1	This study
4k::PSPE	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3 YEplac195-PSPE	This study
4k::GLPE	ArdI1::loxP ArdI2::BLE Atum1::LEU2 Aych1::HIS3 YEplac195-GLPE	This study
4k::RDHA 4k::DUF442	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3 YEplac195-RDHA	This study
4k::SSEA	Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3 YEplac195-DUF442 Δrdl1::loxP Δrdl2::BLE Δtum1::LEU2 Δych1::HIS3 YEplac195-SSEA	This study This study
Plasmid		
YEplac195	Multicopy plasmid for expression in yeast	Laboratory stock
pSH47	Cre expression plasmid	Laboratory stock
pUG66	BLE template plasmid ^a	Laboratory stock
pUG6	kanMX template plasmid ^b	
pFA6a-GFP(S65T)-His3MX6	HIS5 template plasmid ^c	Laboratory stock
pYE242ws-fapr-fapo17	LEU2 template plasmid ^d	Laboratory stock
YEplac195-MET15	MET15 in YEplac195, control by TEF1 promoter	This study
YEplac195-CYS3	CYS3 in YEplac195, control by own promoter	This study
YEplac195- <i>RDL1</i>	RDL1 in YEplac195, control by own promoter	This study
YEplac195-RDL2	RDL2 in YEplac195, control by own promoter	This study
YEplac195-TUM1	TUM1 in YEplac195, control by own promoter	This study
YEplac195-YCH1	YCH1 in YEplac195, control by own promoter	This study
YEplac195-PSPE	PSPE in YEplac195, control by TEF1 promoter	This study
YEplac195-GLPE	GLPE in YEplac195, control by TEF1 promoter	This study
YEplac195-RDHA	RDHA in YEplac195, control by TEF1 promoter	This study
YEplac195- <i>DUF442</i>	DUF442 in YEplac195, control by TEF1 promoter	This study
YEplac195-SSEA	SSEA in YEplac195, control by TEF1 promoter	This study
pET30a	Expression plasmid in <i>E. coli</i>	Lab stock
pET30a- <i>MET15</i>	MET15 in pET30a, control by IPTG-induced lac promoter	This study
pET30a- <i>RDL2</i>	RDL2 in pET30a, control by IPTG-induced <i>lac</i> promoter CpPDO2 in pET30a,control by IPTG-induced <i>lac</i> promoter	This study
pET30a- <i>PDO2</i> pET30a- <i>RDL2</i> 106A	RDL2 in pET30a, 106 site was mutated to Ala, control by IPTG induced lac promoter	Laboratory stock This study
pET30a- <i>RDL2</i> 106A pET30a- <i>RDL2</i> 106S	RDL2 in pET30a, 106 site was mutated to Aia, control by IPTG induced lac promoter RDL2 in pET30a, 106 site was mutated to Ser, control by IPTG-induced <i>lac</i> promoter	This study
pET30a-RDL2 1003 pET30a-PSPE	PSPE in pET30a, control by IPTG-induced <i>lac</i> promoter	This study This study
pET30a- <i>GLPE</i>	GLPE in pET30a, control by IPTG-induced lac promoter	This study This study
pET30a-GLPE pET30a-RDHA	RDHA in pET30a, control by IPTG-induced <i>lac</i> promoter	This study This study
pET30a-NDF1A pET30a-DUF442	CpDUF442 in pET30a, control by IPTG-induced <i>lac</i> promoter	This study
pET30a-SSEA	SSEA in pET30a, control by IPTG-induced <i>lac</i> promoter	This study

^aBLE, Zeocin resistance gene.

bkanMX, G418 resistance gene.

^cHIS5 encodes a key enzyme for histone synthetase.

dLEU2, encoding a key enzyme for leucine synthetase.

Sequence	TABLE 3 Oligonucleotide primers used for pl	asmid construction
SULI 10 R CTARACCTICCATTTAGAAAAATCGGATATCAAAAAACGGTA CAATTCGACTCTGTTAAAC SULI 20 R ATGTCCAGGGAGAGGTTACCAAACTTGGACTACAAACTGAAATCCAAACTTGAAAAATCCAAACTTGACTCTCTAACTTCAACTTTAGACCTGAACTTAGAACTTACCAACTTGACAACTTCAAACTTGACAACTTAGAACTAACT	Name	Sequence
SULZ DO F SOLZ DO R CINGADITICCATITACAMACTICGACTITACAMAGGINALANACGICATICAMAGGINAL ACTORICATIONAGGINAL CONTROLOGY METS DO F ATGACTICITACCECTITGACCECTITGACCECTICACACGICATICACGICTICGTACCETICACACGIC METS DO R THAGACATICACACACACACACACTICACACACGICTICAGACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICTICACACGICACACACACACACACACACACACACACACACA	SUL1 ko F	ATGTCACGTAAGAGCTCGACTGAATATGTGCATAATCAGGAGGATGCTGA TGTTTAGCTTGCCTCGTCC
SULZ DO R METS NO F ATGACTECTICALACTICACGACTACTAGGACANACGTACATTACCATCACTCGACAGCT METS NO R THANTAGGACATCTTCAGACCTCTCAACCTCTCAACCTCCAATTACCCTCGACGCTC WEST NO R THANTAGGACATCTTCAGACCACACTTCTAGGACGAACACTATTACCCTCGAGGCCATTACCGAGGCC WEST NO R THANTAGGACACTCTCTTCAGGACCACATTATACCCTCGGTGGCCAATTACCGAACCGCCTCT CYSS NO F TAGGACATTCACCACACACCACTCTCATGCGAACCACATTACCCCAATTACCCAATACCGAAACCGCCTCT CYSS NO R THATCGTACTTAAAAAGGTCCGGCTGAACACACAATTACCCTCGAGGCCCAATTACCCAAACCGCCTCT WEST NO R THATCGTACTTAAAAAAGGTCCGGCTGAACACACACTACCCCAATTACCCAAAACCGCCTCT WEST NO R CACCACCACACACACACACCACACCCCTCAACCACCACCA	SUL1 ko R	CTAAACGTCCCATTTAGAAAAATCGGGTATATCGATATGAAAAAACGGTA GAATTCGAGCTCGTTTAAAC
METS NO F ATSACTGGTTCTGACCTCTGACGCTCCACATTTTGGCTCGTACCGTCGACGCTC METS NO F TAMAGCGATTTCTGACCTACACACCTCTTGACGCACTCACTTGGATGCCAAAACCCACTC CYSS NO F TAGCACTTTGCACCTATACATATACACACACCCCTCTGGATGCCACAAACCCCACTC CYSS NO R TATCGACTTTGACACTATACATATACACACCCCCGCCCAAAAACCAAAACCAACC	SUL2 ko F	ATGTCCAGGGAAGGTTATCCAAACTTTGAAGAGGTAGAAATTCCTGACTT AACTGTGGGAATACTCAGGT
METS NO R THAMTAGGCATCTICAGACCATUTICATIGGAAGTATTICACCTICGATGCCATACCCCATACCCCTT (75) NO F TAGACATTICGACCTITACATATACAAAAAAAAAAAAAAA	SUL2 ko R	CTAGATATCCCATTTAGCAAAATCTGGGATATCGATATGGAAGAAAGGTAATATCGACTACGTCGTTAAGG
CFSI NO F TAGACATITICACCTITATACATATACACACAGACAAAAACCAAAAACCAGCTC WEFIS NO F TATCGTACTTAAAAAGCTCCGTGCAAGCCACTGCGGCCACCAGCCACACCACCACCCAC	MET5 ko F	ATGACTGCTTCTGACCTCTTGACGCTCCCACAATTGTTGGCGCAATATTCGCTTCGTACGCTGCAGGTC
CR33 NR METIS NO F ATTACCATACTTAAAAAGGTCCGGTCGAAGGCAGGAGCATGACCACATACCCCACTCATACTCCTCATTCCATTCCATCACGCCGCCACAAAACCGCCTCT METIS NO R TCATGGTTTTTGGCCAGCGGAAAACAGTTTCAAAAATCCCATTCCATTCCATTCCATCCT METIS OBR F CATAGGTTTTTGGCCAGCGAAAACCGCTCT METIS OBR F CATAGGTTTTTGGCCAGCGAAAACCGCTCT METIS OBR F CATAGGTTTTTGGCCAGCGAAAACCGCTCT METIS OBR F CATAGGTTTTTTGGCCAGCGAAAACCGCCTCT METIS OBR F CATATCACTGAGGACCACGAGCACAACCGCCTCATGGTTTTTGGCCAGCGAA METIS his R CGACCACAATAGGCCATGGGTTCACTCCATTCCATCCTATCCATCTCATCCATC	MET5 ko R	TTAATAGGCATCTTCAGACACATCTTCATGGAAGTATTTACCCTCGGTGGCCAATACGCAAACCGCCTCT
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MET15 OR P TCATGGTTITTGGCCAGCGAAAACAGTTICAAAAGATTGCTGCAAGTCTG CCAATACGCAAACCGCCTCT MET15 OBP R MET15 OBP R CTAATTACAATTGCAATTCCACTCCTCATTCCATTTCGATTCTAATTG MET15 bis F CGACCACGACAAGAGCTCGGTCATGGTTTTTGGCCAGCGAA MET15 bis F CGACCACGACAAGAGCTCGGTCATCAATTGTTTTTGGCCAGCGAA MET15 bis R CCACCACGACAAGAGCATCGCTCAATTGGTTTTTTGGCCAGCGAA SUL1 in R ACGGCACTATTAGGGAATCTG SUL1 out R AATAGGTGGGCACGATTGACTCATTCATTCTCAATCTGACT SUL1 out R AATAGGTCCAAGGGAATTGAC SUL2 out R GGACCATCTAACTTTGATC SUL2 out R GGACCATCTACTTTTTTC SUL2 out R GGACCATCTAACTTATT SUL2 in R GGACCATCTACCTATTTTT SUL2 in R CCAGTTGATTGAAGAGGAAGTTATC SUL2 in R CCAGTTGATTGATTGCAGGGAAGGTTATC SUL2 in R CCAGTTGATTGATTGCAGGGAAGGTTAC SUL2 in R CCAGTTGATTGATTGCAGGGAAGGTTATC SUL2 in R CCAGTTGATTGATTGCAGGGAAGGTTATC SUL2 in R CCAGTTGATTGATTGAGAGAGGTTAC SUL2 in R CCAGTTGATTGATTGAGAGGGTTCATTCCCTTATCTTTAGTTGCCATTCGCTCGC		TTATCGTACTTAAAAAGGTCCGGTCGAAGGCAGAGACGTGGCACTGGCGACCAATACGCAAACCGCCTCT
MET15 ORF F AICTAGATTTACATACAGACTCGCTATTCGATACTG MET15 bis F CACACCACCACACAGCCAGCTGCTCATTCGATTTCGATACTG MET15 bis F CACTGGTGGTGGTGGTGCTCCAGTCATTCATTCCATACACACAC		
MET15 bits F CGACCAGCAGAAGCCTGGGTCATGCATTCATTCCATCAGATC MET15 bits R CAGCAGCAGCAGCAGCCAGGCCAGCCATCATCATCTCTTTCAGCT MET15 bits R CAGCAGCAGCAGCAGCCAGCAGCAGCCAGCAGCAGCAGCA		
MEP15 bis F CACGACGACAMAGGCATIGGCTGATCCATTCTATTCAATACTGTTCAACT MEP15 bis F CACTGGTGGTGGTGTGCTCCCAGTCCAGTCATGGTTTTTGGCCAGCGAA SULT in F ACCGCACATIAGGGGATCTG SULT out F ACCGCACATIAGGGGATCTG SULT out F TGCACATGGTGTTGTGTGTG SULT out F AAATAGGCTCAAGCTCAAGTTAAC SULT out F GACATGGTCCAGGTAAC SULT out F GATATTGTCAGGGAAGGTTATAC SULZ out F GATATTGTCAGGGAAGGTTATC SULZ out F GGACATCCTACACCTATATTT SULZ in F CAGTTGATCCAGGGAAAGATTATC SULZ in F CAGTTGATCAGGGAAAGATTATC SULZ in F CAGTTGATCAGGGAAAGATATTTT SULZ in F CAGTTGATCAGGGAAAGATACGAATTGAGAAAAGGCCCAATTATCGCTTCGTACGCTGCAGGTC SOAT in C CAGTTGAATAAACCATATATCACAGACGCTCATTATCTTTAGTTGCCAATACGCAAACCGCCTCT SOAI in F ATTGTGAAAAAAGCACAATTATCCAGGACACCTCATTATCTTTAGTTGCCAATACGCAAACCGCCTCT SOAI in F ATTGTGAAAAAGGACCCAATTATC SOAI out F ATTGTAGAAAAAGCCCAATTATCCAGGACGTCATTCCCTTACTTTAGTTGCCAATACGCAAACCGCCTCT SOAI out F ATTGTAGAAAAAGCACACATTATCCCGAAACCGCCTCTT GATATACACTATAATCAAGACGC MEPS in F ATTGACTGCTTCGACCTCTT MEPS out F AAAGAAACCTATAATCAAGACGC MEPS in F ATTGTAGAAAAACCTATAATCAAGACGC MEPS in F ATTGTAGAAAACCTATAATCAAGACGC MEPS in F ATTGTAGAAAACCTATAATCAAGACGC MEPS in F ACAAAACCTATAATCAAAACCTC MEPS out F AAAGTAAACCTATATCAATCACGCAACCAC MEPS out F AAAGTAAACATTATACAAGACCG MEPS in F CACATCCCGTACAAACTAC MEPS out F AAAGTAAACATTATACAAGACCG MEPS in F CACATCCCGTACAAACTAC MEPS out F AAAACCTATATCACCTCGT MEPS out F AAAACCTATATCACCTCGT MEPS out F AAAACCTATATCACCAAGTCCC MEPS out F AAAACCTATATCACCAAGTCCC MEPS out F AAAACCTATATCACCAAGTCCC MEPS out F AAAACCTATACCACAGTCCGC MEPS out F AAAACCTATACCACAGTCCCCCAAACAACAA MEPS in F ACAAACCTATACCACAGTCCGACAACAACAACAACAACAACAACAACAACAACAACAA		
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SULT IN F ATCACTICAGEACAGATATA SULT OUT F TOCACATGATETTISTACT SULT OUT F TOCACATGATETTISTACT SULT OUT F AAATTAGGGCACAGATAAC SULT OUT F GATATGCAGGCACAGATTAC SULT OUT F GATATGCAGGCACAGATTAC SULT OUT F GATATGCAGGGAAAGATTATC SULT OUT F GATATGCAGGGAAAGATTATT SULT IN F GATATGCCAGGGAAAGATTATT SULT IN F GATATGCCAGAAAAGAAAACCATATATCAGAACCAGCTCATTATGCATATGGCTACAGCTCCAGGTC SOAI IN F GATATGTAGAAAAGGCCCAATTAT SOAI IN F CAAGTATAGAAATTGCCAGGG SOAI OUT F GATAGACACAGACACACCACTTAT SOAI OUT F GATAGAACAGAACACACACCACTTAT GATAGAAAAGGCCCAATTAT GATAGAAAAGGCCCAATTAT GATAGAAAAGGCCCAATTAT GATAGAAAAGGCCCAATTAT GATAGAAAAGGCCCAATTAT GATAGAAAAGGCCCAATTAT GATAGAAAAGGCCCAATTAT GATAGAAAAGGCCCAATTAT GATAGAAAAGGCCCAATTAT GATAGAAAACAGATTATACAAAGACG METS IN F GATAGACAGATATACAAAACAGACCA METS IN F CACACTACCACATTATCACAACACCACTCC METS OUT F GATAGACAGATACCAAATTACACAACACACACCACACACA		
SULT OUT F TOCACATGGATCATTGTACT SULT OUT R SULT OUT R SAPATAGGCTCAAGCTCACTATTATTC SULZ IN F GATATGTCAGGAAAAAAC SULZ IN R CACAGTTAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA		
SULT OUT F MATTAGGCTCAAGCTCAAGTTAAC SULZ OUT F GATATGTCAGGGAAGGTTATC SULZ OUT R GGCACCATCCTACTATTT SULZ IN F GGACCATGAAGCTCACGTTATC SULZ IN F GGACCATGTCACTATTTT SULZ IN F GGACCATGTCACTATTTT SULZ IN F GGACCATGTCAGTGATGGGAAAAGCTTATC SULZ IN F GCACCATGTAGTGATGGGAAAAGCTTATC SULZ IN F GCACCATGTAGTGATGAAAACGAACGATTATTGTAGAAAAGGCCCAATTATGGCTGCAGGTC SOAT IN F CAGTGTACAAAAACAACCATATATCAGAACGAGTCATTATGTTAGAAAAGGCCCAATTATGGCAATACGCTAAGCGCCTCT SOAT IN F ATGTGAGAAAAACCAATTATCAGAACAGGTCATTATCATTTAGTTGCCAATACGCAAACCGCCTCT SOAT IN F ATGTGAGAAAAGGCCCAATTAT SOAT IN F CAGTGAACACATTATCAGAACAGGTCAATTATCAGAACAGGCTCAATTATGCAATACGCAAACCGCCTCT ATGTGAAAAAGGCCCAATTAT SOAT IN F ATGTGAGAAAAGGCCCAATTAT SOAT IN F ATGTGAGAAAAGGCCCAATTAT AGATGAACAGATTATACAAGACGC METS IN F ATGTGTGAAAAAGGCCCAATTAT AGATGAACAGATTATACAAGACGC METS IN F ATGTGTGAAAAACGAATATCACAAGTCCC METS OUT F AAGTAAAACCTATAAACAGATACACAATTACAACAGATTACACAAGTCGC CVS3 IN F CCACTCCCGCTACAAACTAC CVS3 IN F CACACTCCACACACAGTCGC CVS3 IN F CACACACTCTACACAAGTCCC CVS3 IN F CACACACTCTACACAAGTTACACACAGTCCC CVS3 IN F CACACACTCTACACACAGTTACACACATTACACACAGTCCC CVS3 IN F CACACACTCTACACACAGTTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACATTACACACACATTACACACACATTACACACACATAC		
SUL2 OUT R GATASTICCAGGGAAGGTATATC SUL2 OUT R GGCACCATCCTACCTATATT SUL2 IN R GGCACCATCCTACCTATATTT SUL2 IN R GCACCATCCTACCTATATTT SUL2 IN R GCACCATCCTACCTATATTT SUL2 IN R CCAGTTGATGCAGGAAGGAAGACACCCCCT SUL3 IN R CCAGTTGATCAGGGAAGACTATC CCAGTTGATCACCAATTATC SUL3 IN R CCAGTTGATCACAAMACAAAACAAAACCCAATTTCCCTTATCTTTAGAAAAGGCCCAATTATCGCTCCAAGGTC CTAGTCAAAAACCTATAATCCAAATAATCCAATTATCCCTTTATCTTTAGATTGCCAATACGCCAATCACCCCTC SOA1 IN R CAAGTTATAGAAAACCTATAATCCAATTAT SOA1 OUT F ATTGTAGAAAAGCCCCAATTAT SOA1 OUT F AATGTAGAAAAACGCCCAATTAT SOA1 OUT R GAATTAAACCAATTATCCAGG WETS IN R TCTCTTCGTTAGCCAATCAT WETS OUT R AATGTAGAAAACGCCCCAATTAT CCAGTTCACCCCCTT WETS OUT R AAAGTAAACCATTAATCCAAGACGC WETS IN R CTCTTCTCTTTAGCCACTCCTT CCT33 IN R CAAGATTATTCTTCACCTCCGTT CCT33 IN R CAGAGATTTCTAACCAAGTCCT CCT33 OUT R CATCTAAACCAATTAACCAAGTAC CCT33 OUT R CATCTAAACCAATTAACCAAGTAC CCT33 OUT R CATCTAAACCAATTCAACCAATCAC CCT33 OUT R CATCTAAACCAATTCAACAATCAC CCT33 OUT R CATCTAAACCAATTCAACAATCAC CCT33 OUT R CATCTAAACCAATTCAAATTACCAATCAC CCT33 OUT R CATCTAAACCAATTCAAACCAATTAC METTS OUT F GAACACCCTCCAATTCAA METTS OUT F GAACACCCTCCAATTCACAATTCAC METTS OUT R METTS OUT R CACCCCCAATTCAAATTTAACCCAATTCAC METTS OUT R CACCCCCAATTCAAATTTAACCCAACTCAATTCAC METTS OUT R CACCCCCAATTCAAATTTAACCCACCCC METTS OUT R CACCCCCCACACCACCATTCAACCCC CCCCCCCCCCC		
SUL2 DULF GGACATCTCCTACTATTATT SUL2 IN F GGACCATCTCACCTATTATT SUL2 IN F GGACCATCCACCTACCACTATTATT SUL2 IN F GGACCATCCACCTACCACTATTATT SUL2 IN F GGACCATCCACCACTACGGGAAAGC SOAI NO F CAGTIGAGACAAGAAAC SOAI NO F CAGTIGAGACAAAAC SOAI IN F CAGTIGAGACAAAAC SOAI IN F CAGTIATAGACACCGCTTCATTCCCTTATCTTTAGTTGCCAATACCGCAGCCCTC SOAI IN F CAGTIATAGAAACCTATATCCAGACCGCTTCATTCCCCTTATCTTTAGTTGCCAATACCGCAGACCGCCTCT SOAI IN R CAGTIATAGAAATATTGTCCGGG SOAI OUT F ATTGTAGAAAACCCCAATTAT SOAI OUT F CAGTIATAGAAATATTGTCCGGG METS OUT R GATTAAACCCTATAATCAAGACCG METS OUT R CAGTICTCAGCACTCCT METS IN R CAGTICTCAGCACTCCT METS IN R CAGTICTCAGCACTCCC CYS3 IN R CAGTICTAACCAGATGACAC CYS3 OUT F CAGTICACAAGTACAC METS IN R CAGTICACAACTACACCAATTAC METS IN R CAGTICACAACTACACCAATTAC METS IN R CAGTICACAACTACACCCCGAACCAC METS OUT R CAGTICACAACTACACCCCCACACCACATTAC METS IN R CACCAGACCTGAACCAATTAC METS OUT R CAGTICACAACTACACCCCCCACACCACACCACACCACAC		
SUL2 in F GATASTICCAGGGAAAGATATC SUL2 in R CCAGTTGATCAGGGAAAGATATC SUL2 in R CCAGTTGATCAGGGAAAAGATACGATATTGAGAAAAGGACCCCAATTATCGCTTCGTACGCTGCAGGTC SOAT in R CTAGTGAATAAACCTATAATCAGACGCTTCATTCCCTTATCTTTAGTTGCCAATACGCAGACCGCTC SOAT in R CAGTATAGACAAAGAAAAGAACAGCCCTTCATTCCCTTATCTTTAGTTGCCAATACGCAAACCGCCTC SOAT in R CAAGTATAGAACTATTCATCCAGG SOAT out R ATGTTAGAAAAGGCCCAATTAT SOAT out R GAATAAACCTATAATCAGACGC METS in F TCTCTTGATTAGACAAAGGCCCATTAT METS out F AAAGTACACTATAATCAGACGC METS in R CCAGTTCACCCCCTCT METS out F AAAACTACACTAGGAACG CCAGAATTAATCTCACCACTCTT CCY33 in F CCAGAGTTCATCTCACCCTCTT CCY33 in F CCAGAGTTCATCTCACCCAGTCCTC CCY34 in R CCAGAGTTCATCTACCAAGTCGT CCY35 in R CCAGAGTTCATCTAACCAAGTCGT CCY35 in C CCAGAGTTGATTTTGAA CCY35 out F CGTGCCAGATTGAATTTGAA CCY35 out F CGTGCCAGATTGAATTTGAA CCY35 out F CGTGCCAGATTGAATTTGAA CCY35 out F CGTGCCAGATTGAATTTGAA CCY35 out F CGTGCCAGATTGAATTTGAC CCY35 in C CCAGAGTTCATCTAACCAAGTCGTC CCY36 out F CGTGCCAGATTGAATTTGAA CCY36 out F CGTGCCAGATTGAATTTGAA CCY37 out F CGTGCCCCACCACAATTTAC METTS in F CACCAGTGAAACACCCCCCACCACCATTA CCY37 out F CGGAATCCCCCCACCACCACTA CCY37 out F CCAGACTTGATCCCCCACCACCACTA CCY37 R CCAGATTCCCCCACCACCACTAT CCY37 R CCAGATTCCCCCACCACCACTAT CCY37 R CCAGATTCCCTATTATATATAACTTGAGTTAGATCC CCY37 R CCAGATTCCCTCACACCCCTATACC CCGGAATTCCCACACCACCATTA CCY37 R CCAGATTCCCTCACACCCCTATACC CCGGAATTCCCCCACCACCACCATTA CCY37 R CCAGATTCCCTCACACCCCTATACC CCGGAATTCCCCCACCACCACCACCACCACCACCACCACCACCA		
SUL2 in F SUL2 in R CAGTIGATICATICAGGGAAGGTTATC SUL2 in R CAGTIGATICATICAGGAAAAAC SOA1 to F ATGTCCGTACAAAAAGAAAACA SOA1 to F SOA1 to F SOA1 to R CTAGTGAATAAACCTATAATCAAGACGCTTCATTCGTTGACGCTGCAGGTC SOA1 in F SOA1 in F SOA1 in F SOA1 in R CAGTATAAACCTATAATCAAGACGCTTCATTCCTTAGTTGCCAATACGCAAACCGCCTCT SOA1 in R CAGTATAGAATATTGTCCGGG SOA1 in R CAGTATAGAATAGCCCTCTT METS in F CAGTAGAAAAGGCCCAATTAT SOA1 out F ATGATCAGCTTCTGACCTCTT METS in R CTCTTCGTTTAGCAATCTCC METS out F CAGTACAAGATAGCGAAACG METS out F CAGTACAAGATAGCACCAAGTAC CYS3 in R CAGAGATTCTAACCAAGTAGG METS out F CAGTACAAGATTGAAC CYS3 out F CGTGCCAAGATTGAATCAC CYS3 out F CGTGCCAAGATTGAATTGAA METS in R CACTAGACACAATTTAC METS in R CACTAGACACAATTTAC METS out F CACTAGACACTTGAATTACA METS out F CAACAGCTTGATCCAACACAATTTAC METS out F CAACAGCTTGATCAAAAA METS out F CAACAGCTTGATCCACCACACACTA METS out F CAACAGCTTGATCAAAAAA METS out F CAACAGCTTGATCAAAAA METS out F CAACAGCTTGATCCACCACACACTA METS out F CAACAGCTTGATATAACTTTAACCTCGATCACACACTA TEF1 F CCAACGCTTGATATAACTTTAACCTCGATCACACACTA TEF1 F CCAGGATTCCACTAATAAACTTTAACCTCCACACACCATA METS out F CAACAGCTTGATATTACTGCCCCCACACACCATA METS out F CAACAGCTTGATTAAACTTTAAACTTTAACTTCACCCCCACACACCATA TEF1 F CCAGGATTCGATTAAAACTTTAAACTTTAACCTTCGAGC SUL1 RT F CACTAGCTTCATTAAACTTTAACCTTCGAGC SUL1 RT F CACTAGCTTCATTAATACTTCCCCCCACACACCATA CCATTCATAGCTTCCAGTTGAATTAACCTTCCAGCC CCCACACACACACACTACCCATACACACACTACCCACTACACACACTACCCACTACACCAC		
SULZ IN R CCAGTIGATCATGGGAANAAC SOAT NO F ATGTCCGTACAAAAAGAACAAATACCATATTIGAGAAAAGGCCCAATTATCGCTTCGTACGCTCCAGGTC SOAT NO R CTAGTGAATAAAACTATAATCAAGACGCTTCATTCCCTTATCTTTAGTTGCCAATACCGCACCCTCT SOAT IN R CAAGTATAGAATATTCCCGG SOAT OUT F ATTGTAGAAAAAGGCCCAATTAT SOAT OUT R GAATAAACCTATAATCCAGGC SOAT OUT R GAATAAACCATATATTCCGGG SOAT OUT R GAATAAACCTATAATTCAGGCTCCATTAT SOAT OUT R GAATAAACCATTAGATATACAGACGC METS IN R CTCTTCTGTAGCAATCCC METS OUT R METS OUT R GATTTATTCTCACCCTCGTT CYS3 IN R CCACTCCGCTTACCAAAGTAC CYS3 IN R CCACCCGCTACAAAATAC CYS3 IN R CCACCAGTTCAAACTAGCCTCCT WETS OUT R GATTCTATCCATTCTCACCCAGTCCT CYS3 OUT F CCACCCCGTACCAAACTAC CYS3 OUT F CCACCAGTTCAAATTTTGAC CYS3 OUT F CCACCAGTTCAAATTTTGAC WETTS IN F GATCCAGAACTTTCAACCTAGTTTAGCA WETTS IN F GATCCAGAACTTTCAACCTAGTTTACC WETTS OUT R METTS OUT R GATCCAGACGTTTCAATTTTACC WETTS OUT R GATCCAGACATTTCAATTTTACC WETTS OUT R GATCCAGACTTTAACCAAGTCGTC CYS3 OUT F CCACCTCGATACCAAATTACA WETTS OUT R GAACACGCTCGATACCAATTTAC WETTS OUT R GAACACGCTCGATACCAATTTAC WETTS OUT R CTGGAGCACAATTTAAATTACCCTCCGATACC WETTS OUT R CTGGAGCTCCTTTGTAATTAAAACTTACCT CYC1 R CCGGATTCCAATTCCC CYC1 R CCGGATTCCTATTCAATTTAACCTCCCCACACCATTA CCCCTCACCACCACCACCATT SULJ RT R CCACTCTCACTCCCCACACCACCATT SULJ RT R CCACTCTCACTCCCTCACACCACCACTA CCCGGAATTCCCCCACACCACTAT CCCGGAATTCCCCCACACCACTAT CCCGTCAACCCCCACACCACTAT CCCGTCAACCCCCACACCACTAT CCCGTCAACCCCCACACCACTAT CCCGTCAACCCCCACACCACTAT CCCGTCAACACCCCCACACCACTACCCCACACCACTACCCCCCC		
SOAI NO F ATGTCCSTACAAAAAAGAAGAATACCATATTGTAGAAAAGGCCCAATTATCGCTTGAGGCTGCAGGTC SOAI IN F SOAI IN F SOAI IN F SOAI OU F ATTGTAGAAAAGGCCCAATTAT CAAGTTAGAATTTTTCCCGG SOAI IN F CAAGTTAGAATATTTCTCCGGG SOAI OU F ATTGTAGAAAAGGCCCAATTAT SOAI OU F ATTGTAGAAAAAGGCCCAATTAT SOAI OU F ATTGTAGAAAAGGCCCAATTAT SOAI OU F ATTGTAGAAAAAGGCCCAATTAT SOAI OU R GAATAAACCTATAATCAAGACGC METS IN F ATGACTGACTTCTGACCTCTT METS IN F ATGACTGACTTCTGACCTCTT METS OU R CYS3 IN R CYS3 IN R CAAGTATACACTAGGAACG METS OU R CYS3 OU F CAGGAATTCAAATTTCACAAGTAG CYS3 OU R CATCTAACACTAGGAACAG METS OU R CAGGAATTCAAATTTGAA CYS3 OU R CATCTAACACAATTGAAATTTGAA METS OU R CATCTAACACAGTAGGAACAG METS OU R CATCTAACACAGTAGGAACAG METS OU R CATCTAACACAGTAGGAACAG METS OU R CATCTAACACAGTAGGAAAAAA METS OU R METS OU R CACAACTAGACAAATTAAATTTAGATTAC METS OU R METS OU R CACAACTAGACAAAAAAAAAAAAAAAAAAAAAAAAAAA		
SOA1 in F SOA1 in F SOA1 in F SOA1 in F SOA1 in R SOA1 out F SOA1 out F SOA1 out F SOA1 out R SOA1 out		
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SOA1 out F ATTGTACAATTATCCCGGG SOA1 out R GAATAAACCTATAATTCCAGACCC METS in F ATGACTACTTCCACCTCTT SOA1 out R GAATAAACCTATAATCAAGACGC METS in R TCCTTCGTACCCTCTT METS out P AAAGTAACACTACCCGTTCCT METS out R GATTTATCTCACCCCTT CYS3 in F CCACTCCGCTACAACATAC CYS3 in F CCACTCCGCTACAACATAC CYS3 out F CAGACAATTCTACCAAGACCG CYS3 out F CAGACAATTCAACCAAGATCAC CYS3 out F CAGCACATTCAACCAAGATCAC CYS3 out F CATCTAACCAATTCAACCAAGATCAC CYS3 out F CACTCACCACATTCAC METS out F CATCTAACCAATTCAACCAAGATCAC CYS3 out F CATCTAACCAAATTCAACCAATTCAACCAAGATCACCACCATTTAC METS in F GATCCACACCATTTAC METS out F GAACACCACTCATTAC METS out F GAACACCACCACCATTAC METS out F CACACACCATTAAC METS out F CACACACCATCATCA METS out F CACACCACCACCATA METS OUT F CACACCCTCATTCAACCAAGATCACC CYC1 F CTTGAGCTCCATGAACCAACCACCCATA TEF1 F CACACCCTCATTGTAATTAAAACTTAGATTACC CYC1 F CTTGAGCTCCTCATTGTAATTAAGTTTACC CYC1 F CTTGAGCTCCTCATTGTAATTAAACCTTCGACC SULI RT F CACTGGTTGGCTATACCTG SULI RT F CACTGGTTGGCTAACCACCACCACACACACACACACCACCACCACCACCA		
SOA1 out F SOA1 out R GATAGACAAGGCCCATTAT SOA1 out R GATAGACCTATAATCAAGACG MET5 in F ATGACTGCTTCTGACCTCTT MET5 in R TCTCTTCGTTAGCAATCTCC MET5 out F AAAGTAACAGTAGGGAACGG MET5 out F GATTHATTCTTCACCTCGTT CV53 in F CCACTCCGGTACAAAGTAC CV53 in R CAGAGATTCTACCCAAAGTAC CV53 in R CAGAGATTCTACCCAGATTGAACGTAGACG MET5 out R CATCTAACACGATTGAATTTGAA CV53 out F CGTGCCAGATTGAATTTGAA CCAGAGATTCTAACCAAGTCGTC CV53 out R CATCTAACACGATTGAATTTGAA MET15 in F GATCCAGAGTTGAATTTTGA MET15 in R ACAATCTAGCCTCGATACC MET15 in R ACAATCTAGCCTCGATACC MET15 out F GAACACGCTCGATGAAAAAA MET15 out F GAACACGCTCGATGAAAAAA MET15 out F CCAGACTTGATCCCACATTTAC MET15 out F CCAGACTTGATCCCCACACCCATA MET15 out F CCAGACTCTTGTAATTTAAAACTTAGATTTGATTTGC CCAGATTCGCAAATTTAAC MET15 out R CCAGATTCGCAAATTTAAACTTAGATTAGATTGCT CVC1 F CCGGATCCTTGTTAATTAAAACTTAGATTAGATTGC CVC1 F CCGGATTCGCAAATTTAAACCTTCGAGC SUL1 RT F CACTGGGTAACTGC SUL2 RT F CACTGGGTAACACAA CTT RT F ATGAGACCCGAATTTGACC ACTT RT F ATGACCAGTTGATTAGAAACCACACCATTAAAAACCTTTTAAAACCTTCCTAGACCACACCATTACGCAAAACCACTTTTTATTTCCAGGTTTAACACACTTTAAAACCTTCGTAACAAAAACCTTTCTTATTTTCAGGGTTTAGACTCAGAAAAACCACTTTTAACACTTTTAAAACCTTCCTT		
SOA1 OUT R GARTAAACCTATAATCAAGAGC MET5 IN R TCTCTTCGTTAGCCATCTT MET5 IN R TCTCTTCGTTAGCAATCTCC MET5 OUT F AAAGTAACAGTTAGGGAACCGG MET5 OUT R GATTITATTCTTCACCTCGTT CYS3 IN F CCACTCCGCTACAAAGTAC CYS3 IN F CCAGTCCAGAATCTAC CYS3 OUT F CCAGTCCAGAATCTAC CYS3 OUT F CCAGTCCAGAATCTACCAAAAGTAC CYS3 OUT F CCAGTCCAGATTGAATCTTAGA MET15 IN F GATCCAGAGTTGAATCTAC MET15 IN F GATCCAGAGTTGAATCAGC MET15 OUT R MET15 OUT R ACAAATCTAGCCTCGATACC MET15 OUT F GACACCCCCGCTAGAAAAAA MET15 OUT F GACACCCCCCGATACACAGTGATC MET15 OUT F GACACCCCCCAGACACCACTA MET15 OUT F CCAGACTCTGATCAGAAAAA MET15 OUT F CCAGCTCGATCAGAAAAA MET15 OUT F CCAGCTCGATCAGAAAAA MET15 OUT F CCAGCTCGATCAGAAAAA MET15 OUT F CCAGCTCGATCAGAACAA MET15 OUT F CCAGCTCGATCAGACCACACCCATA TEF1 F CCAGCTCGATCAGACCACACCCATA TEF1 R CGGGATCCTTGTAATTAAAACTTAGATTAGATTGCT CYC1 F CTTGAGCTGAAATAAAACTTAGATTAGATTAGATTGCT CYC1 F CTTGAGCTCCAATTAAAACTTAGATTAGATTAGATTGCT CYC1 R CCGGAATTCGCAAAATTAAAACCTTCGAGC SULI RT F CACTGGGTTGGGTATACTGC SULI RT F CACTGGGTTGGGTATACTGC SULI RT F CACGGTAGAAGAGACCATGTA CTI RT F ATGGACCCGAAATTTAAACCCTTCGAGC ACTI RT F ATGGTCGGTATGGGTATACTGC ACTI RT F ATGGTCGGTATGGGTAAAAA ACTI RT R CCAGTGAAGAGCCGAAATTTATTTCC MIT F ATGGTCGGTATGGGTCAAAA ACTI RT R TCCATATCGTCCCAGTTGGT RDL1 IN F CACTGTAGTAGTGGGAAAAC ACTI RT R TCCATATCGTCCCAGTTGGT RDL1 IN F CAGTGTAGAATCTGGAAACC ACTI RT F ATGGTCGGTATGGGTCAAAA ACTI RT R TCCATATCGTCCCAGTTGGT RDL1 IN F CAGTGTAGAATCTGGAAACCCGCAATTTAAAGCCTTCGTACCCCAGAACCCCCCTC CCTGGTAGAATCACTCAGAAACCCCACTTTGGAATCACAGAGAACACCGCCATTACCGCAAACCCGCCCTC CCTGTAGAATCATCTGGAAT RDL1 IN F CAGTGTAGAATCATCTGGAAT RDL1 IN F CAGCGGTGAGAACTAACAGGGTACTAACCAAGGGTAATACAAGGCCAATACGCAAACCGCCCTCT CCTGTAGAATCATCTCAACAAATGCGCATTAACAGAAACACCGCCTCT CCTGTAGAATCATTACGAAACACGCCTTTTATACCTCAACAAACCAGCCTTCTAACCAAAGCCCAATACCCCAATACCCCAAACCGCCTCT CCTGTAGAATCTCTCAACAAATGCGAACACAACAC		
MET5 in F MET5 in R TCTCTTCGTAGCACTCT MET5 OUT F AAAGTAACAGTAGGGAACGG MET5 OUT F AAAGTAACAGTAGGGAACGG MET5 OUT R GATTITATICTICACCTCGTT CYS3 in F CCACTCCGGTACAAAGTAC CYS3 IN R CAGAGATTCTAACCAAGTCGTC CYS3 OUT R CATCACACAGATTGATTGAGC MET5 IN R CAGACAGTTGTAACCAAGTCGTC CYS3 OUT R CATCACACAGATTGATTGAGC MET15 IN R ACAATCTACCAGTTGATTGATGAGC MET15 IN R ACAATCTAGCCTCGATACC MET15 OUT R GACCCGTCCAAGAAAAAA MET15 OUT R GACCAGCTTGATCCAATTTAC MET15 OUT R CTGGCAAGAGAACAGAACAAAAAA MET15 OUT R CCAGGCTTTTTTTAC TEF1 F CCAGCTCTTTTGAATTTAC TEF1 R CGGGATCCTTTTTTTTAACCACTCACCCCACACCATA TEF1 R CGGGATCCTTTTTTTTAACACTTTACCCCCCCACACCACTA TEF1 R CGGGATCCTTTTTTTTAATACACTTACGCTCCCCCCACACCACACCACACCACACCACACCACACCAC		
METS OUT F AAAGTAACAGTAGGAACGG METS OUT R GATTTTATTCTTCACCTCGTT CYS3 IN F CCACTCCGCTTACAAAGTAC CYS3 OUT F CAGAGATTCTAACCAAGTCGTC CYS3 OUT F CAGAGATTCTAACCAAGTCGTC CYS3 OUT F CATCTAACACGATTGATTGAGC MET15 IN F GATCCAGAGCTGTTACCAATTTAC MET15 IN F GACACGAGCTGATACC MET15 OUT F GAACACGCTCGATACC MET15 OUT F GAACACGCTCGATACC MET15 OUT R CTTGTGAGAGAAAAAAA MET15 OUT R CTTGTGAGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA		
MET5 out F AAAGTAACAGTAGGGAAACGG MET5 out R GATITITATICTITCACCTGTT CYS3 in F CCACTCCGCTACAAAGTAC CYS3 out F CGTGCCAGATTGAATTGAAA CYS3 out R CATCTAACAGATTGATTGAG MET15 in F GATCCAGAGTGTACCAATTTAC MET15 in R ACAAATCTAGCCTCGATACC MET15 out F GAACACGCTCGATCCAATTTAC MET15 out F GAACACGCTCGATGAAAAAA MET15 out R CTTGTGAGAGAAAGTAGGTTTATAC TEF1 F CCAGACCTTGATTGAATTATATTATTATTATTATTATTATTATTATT		
METS OUT R CYS3 IN F CCACTCCGCTACAAAGTAC CYS3 IN F CCACTCCGCTACCAAGTAC CYS3 OUT F CGTGCCAGATTGAATTTTGAA CYS3 OUT R CAGAGATTCTAACCAAGTCGTC CYS3 OUT R CATCAACAGGATTGATTTTGAA CXS3 OUT R CATCAACAGGATTGAATTTTGAAC METTS IN F GATCCAGAGCTGTACCCAATTTAC METTS OUT F GAACAGCTCGATACC METTS OUT F GAACAGCTGCATGAAAAAA METIS OUT R CTTGTGAAGAGAAAAAA METIS OUT R CCAGAGTCCTTTGTAAATTAAAACTTAAGATTAGATTGCT CYC1 F CTGAGACTCCCACACACACAT TEF1 R CGGGATCCTTTGTAATTAGAACTTAGATTAGATTGCT CYC1 F CTGAGACTCTCATGTAATTAGATTAGATTAGATTGCT CYC1 R CGGAATTCCGCAAAATTAAAGCTTCCAGC SUL1 RT F SUL2 RT R ATGAGAGCCGGAATTGACC SUL2 RT R CACGTGAAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAA		
CYS3 in F CYS3 in R CYS3 out F CAGAGATCTAACCAAGTCGTC CYS3 out F CAGTCCACATTGAATTTGAA CYS3 out F CATCTAACACGATTTAATCAACTCAAGTCC CYS3 out F CATCTAACACGATTTAATCACCAATTTAC MET15 in F GATCCAGACTCGATCACC MET15 out F GAACACGCTCGATCACC MET15 out R CTTGTGACAGAAACTAACC MET15 out R CTTGTGACAGAAACTAGACTTAAC MET15 out R CAGACTCGATCCACCACACCATA TEF1 R CAGACTCTATAACTAACCCCCCACACCACAC CYC1 R CCGGATTCGTTAATTAAACTTTAGGTCT CYC1 F CTTGAGACGAAACTAACCCCCCCACACCACACCATA CYC1 R CCGGATTCGTTAATTATATTATGTCACGCT CYC1 R CACGGGTAACGCAACTCAAACCCCCCCCCCCCCCCCCCC		
CYS3 OUT F CYS3 OUT F CGTGCCAGATTGAACCAAGTCGTC CYS3 OUT R CATCTAACCAGATTGATTTGAAC MET15 IN F GATCCAGAGCTGTACCAATTTAC MET15 IN R ACAAATCTAGCCTCGATGAACAA MET15 OUT F GAACACGCTCGATGAAAAAA MET15 OUT R CTGTGAGAGAAAGTAGGTTTATC MET18 OUT R CGGGATCCTTGATCACCACACCATA MET19 OUT R CGGGATCCTTGATCACCACACCATA MET19 OUT R CGGGATCCTTGATTAAAACCTTAGATTGACT CYCI F CCAAGCTTGATTGATTAAAACCTTAGATTAGATTGCT CYCI F CCGGAATTCGATATTAGATTAAAACCTTCGAGC SULI RT R CACTGGGTTGGGTATACTGC SULI RT R ATGAGAGCCGGAATTTGACC SULI RT R CCAGTGAAAGAGAGCAGTGTA SULI RT R CCAGTGAAAGAGAGACAGTCGA AAACCATGTTA SULI RT R CCAGTGAAAGAGACAGTCTA CCAGTTGAAGAAAAAAAAC CTI RT F ATGGCGGTATGGGTCAAAA ACTI RT R ATGGTCGGTATGGGTCAAAA ACTI RT R CCATTCGTTCATGTTATTTTCAGGGTTTGTGACCAAGACAGAC		
CYS3 out F CYS out F CYS out R CATCHACACGATTGATTTGAC CYS3 out R CATCHACACGATTGATTGAC CYS5 out R CATCHACACGATTGACCATTTAC MET15 in F ACAAATCTAGCCTCGATACC MET15 out F GACCAGAGCTGTACCAATTTAC MET15 out R CTTGTGAGAGAAAAAA MET15 out R CTTGTGAGAGAAAGTAGGTTTATAC TEF1 F CCAACCTTGATCCCCCCACACACCATA CYC1 F CTTGAGCTCTCATGATATTAAACTTAGATTGCT CYC1 F CTTGAGCTCTCATGTAATTAGTTATGTCACGCT CYC1 R CCGGATCCTTTGATTCAAAACTTAGACTTCAGC SUL1 RT R ATGAGAGCCGGAATTTGACC SUL2 RT F CACTGGGTTGGGTATACTGC SUL2 RT R ATGAGAGCCGGAATTTGACAAAAAAC ACT1 RT R ATGGTCGGTATAGGGTCAAAA ACT1 RT R ATGGTCGGTATGGGTCAAAAA ACT1 RT R ATGGTCGGTATGGGTCAAAA ACT1 RT R TCCATATCGCTCAGTTGGT RDL1 ko F AATCTTCCCTCTAGTTATTTTTCAGGGTTACTAGAAAACCGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 ko F AATCTTCCCCCAGTTGGATACTGGAAAAACC RDL1 in F CCGGATGATGATACTAGAAAAACCCGCCTCT RDL1 in R ACCCCCCATGAGAAGCCGGAATTGGACAAAACC RDL1 out F GAGAGTGGAGACTTAATCAA RDL1 out R AACGGTGGACATAACACACGCCTTTTATTGCCCCATGAGAACCGCCCTCT RDL1 ko R RDL1 self F TATCACCCCCATGAGAAACC RDL1 out R ACCGTGTGACATTAACAA RDL1 out R ACCGTGTGACATTAATCAA RDL1 in R ACCGTGTGACATTAATCAC RDL1 in R ACCGTGGACATTAATCAC RDL1 in R ACCGTGTGACATTAATCAC RDL1 in R ACCGTGTGACATTAATCACACAAATCCCCCCATGAAGCCCACTCT RDL1 in R ACCGTGTGACATTAATCACCCCCATTTTATTGCCCCCATGAAGCCCATTATCACCCCCATGAG RDL1 in R ACCGTGTGACATTAATCACACAAATCGCCATTATAAAGCTTAACCCCCCATGAG RDL1 in R ACCGTGTGACATTAATCACACAAATGGCCTTTATAAAGTCACACAAACCCACCTTC RDL2 in R ACAGTGTGACATTAATCACCCCCATTTATTAACTCCACCATGAACCACCCCCTTC RDL2 in R BDL2 in R BDL2 in R BAACCAGTGTTACACCACAAAAAACC ACAGACTGACACACACAAAAACCCACCCTTC ACAGATCACTTACACAAAAAAAACC ACAGACACACACACAAAAAACC ACAGACACACAC		
CYS3 OUT R MET15 IN F GATCCAGAGCTGTATCACCANTTCA MET15 IN R ACAAATCTAACCCAGAGCTGTATACC MET15 OUT F GACACGCTCGATGAAAAAA MET15 OUT R CTIGTGAGAGAAAGTAGGTTTATAC MET15 OUT R CTIGTGAGAGAAAGTAGGTTTATAC MET15 OUT R CTIGTGAGACAAAAAAA MET15 OUT R CTIGTGAGACAAAAAAA MET15 OUT R CTIGTGAGACAAAAAAAA MET15 OUT R CTIGTGAGACAAAAAAAA MET15 OUT R CTIGTGAGACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA		
MET15 in F GATCCAGAGCTGTACCAATTTAC MET15 out F ACAAATCTAGCCTCGATACC MET15 out F GAACACGCTCGATACAAAAA MET15 out R CTTGTGAGAGAAAAAAA MET15 out R CTTGTGAGAGAAAGTAGGTTTATAC TEF1 F CCAAGCTTGATCCCCCACACACCATA TEF1 R CGGGATCCTTTGTAATTAAACTTAGATTAGTTGCT CYC1 F CTTGAGCTCTCATGTAATTAAACTTAGATTAGTC CYC1 R CCGGAATTCGCAAATTAAAGCCTTCGAGC SUL1 RT R CATGGGTTGGGTATACTGC SUL2 RT F CACGGTGAAAGGAGCATGTA SUL2 RT F CACGGTGAAAGGAGCATGTA SUL2 RT R ATGAGAGCCGGAATTTGACC SUL2 RT R CACGGTGATAGGGGAAAAA ACT1 RT R ATGGTGGTATGGGTCAAAA ACT1 RT R ATGCTGATGAGTGGGTCAAAA ACT1 RT R ATCATATCGTCCCAGTTGGT RDL1 ko R GTTACTAGCTTACGAAAATACACAGGGTACATACCTAGAGATATACAAGGCCAATACCGCAGCCTCT RDL1 in F CCGTGATGAATGCTTGGAAT RDL1 in R AACGGTGTGACATAAATGCTTGGAATACCTAGAGAACC RDL1 out R AACGGTGTGACATAAATGCC RDL1 in R GTCACCTGCAGGCATGCATGATGAGCAGAGACAAG RDL1 in S F CAGGACGACAGAGCCATGACT		
MET15 in R ACAAATCTAGCCTCGATACC MET15 out F GAACACCTCGATTGAANAAA MET15 out R CTTGTGAGAGAAAGTAGGTTTATAC TEF1 F CCAAGCTTGATCCCCCACACACCATA TEF1 R CGGACTCCTTTGTAATTAAAACTTAGATTAGATTAGCT CYC1 F CTTGAGCTCTCATGTAATTAGATATGTATGCT CYC1 R CCGGAATTCGCAAATTAAAGCCTTCGAGC SUL1 RT F CACTGGGTTGGGTATACTGC SUL2 RT F CACGGGTGAAAGGAGCATGTA SUL2 RT R CACGGTGAAAGGAGCATGTA SUL2 RT R CACTGGTATGAGTCAAAA ACT1 RT R TCCATTGATTGATTGAGCAAAA ACT1 RT R TCCATATCGTCCCAGTTGGT RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 ko R GTTACTAGCTTACGAAAAATACACAGGGTACATACCTAAGAGAAACGAAACCGCCTCT RDL1 in R GTTACTAGCTTACGAAAAATACACAGGGTACATACCTAAGAGTATACAAAGCCCAAAACCGCCTCT RDL1 in R TATCACCCCCATGAGAAACC RDL1 out F GAGAGTGGAGCCTTAATCAA RDL1 out R AACGGTGACAATACGCCATTTTATTTTGGCGCATAGACAAG RDL1 in R TATCACCACTGATTTATTACGCCATTTTTATTTGGCGCATAGACTAGC RDL1 self R GTCGACCTGCAGGCATGCATGAGGGTTTCGACTTAGAGTTT RDL1 bis		
MET15 out F GAACACGCTCGATGAAAAAA MET15 out R CTTGTGAGAGAAAATGAGTTTATAC TEF1 F CCAAGCTCTGATCCCCCACCACCACATA TEF1 R CGGGATCCTTGTAATTAGATTAGATTAGATTGCT CYC1 F CTTGAGCTCTCATGTAATTAGTTATGTCACGCT CYC1 R CCGGAATTCAAAGCCTTCGAGC SUL1 RT F CACTGGGTTGGGTATACTGC SUL2 RT F CACGGGGAAAGGACATGA SUL2 RT F CACGGTGAAAGGAGCATGA SUL2 RT R CCAGTTGATGGTGCAAAA ACT1 RT R ATGGTCGGTATGGGTCAAAA ACT1 RT R ATGCTTCCCAGTTGGT RDL1 ko F AATTCTTTCCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 ko R GTTACTAGCTTACGAAAATACACCAGGGTACATACCTAGAGTATACAAAGGCCAATACGCAAACCGCCTCT RDL1 in R TATCACCCCCATGAGAAATACACCAGGGTACATACCTAGAGTATACAAAGGCCAATACGCAAACCGCCTCT RDL1 out F GAGAGTGGAGGCTTAATCAA RDL1 out R AACGGTGGAGAGCATTAATCAA RDL1 self R TATGACCATGATTACGCCATTTATTATGGCGCATTGGAGTT RDL1 self R GAGAGTGGAGCTTAATCAGCCATTTATTAGGCATTGGAGTTGGATGATGC RDL1 his F CGACGACAAGGCCATGGCTGATTGGAAGCCATGAATTCACCCCCATGAG RDL2 in F CAGGTTGTATGTATACAGCGAGACAAGAGCAAAAGAAAAAACCCCCCCC		
MET15 out R CTTGTGAGAGAAAGTAGGTTTATAC TEF1 F CCAAGCTTGATCCCCCACACACCATA TEF1 R CGGGATCCTTGTAATTAAAACTTAGATTAGTTTGCT CYC1 F CTTGAGCTCTCATGTAATTAGTTATGTCACGCT CYC1 R CGGGATTCGCAAATTAAAGCCTTCGAGC SUL1 RT F CACTGGGTTGGGTATACTGC SUL2 RT F CACGGGAATTTGACC SUL2 RT F CACGGTGAAAGGAGCATGTA SUL2 RT R CCAGTTGATGATGATGAGGAAAAAC ACT1 RT F ATGGTCGCTAGTGGT RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGATTACCAAGGGTACATACCTAGAGTATACAAGGCCAATACGCAAACCGCCTCT RDL1 in F CCGTGATGAAGCTTACGAAAATACCAAGGGTACATACCTAGAGTATACAAAGGCCAATACGCAAACCGCCTCT RDL1 in R TATCACCCCCATGAGAAAC RDL1 out F GASAGTGGAGCCTTAATCAA RDL1 out R AACGGTGGACGCTTAATCAA RDL1 self F TATGACCATGATTAACGCCATTTTATTGGCGCATAGACAAG RDL1 self F GTAGCAGACAAGGCCATGCATGGGTGTCGACTAGGCTGATGAATGC RDL1 his R GCGACCAGACAAGGCCATGCATTGGAAGCCGTGATGAATGC RDL1 his R CAGTGGTGGTGGTGGTGGTGCTCGATTTAAAGCCAAGAACAAAACCGCCTTCCTACAGGTC RDL2 ko R AAAGGTTT		
TEF1 F TEF1 R CGGATCCTTIGATCCCCCACACACCATA TEF1 R CGGATCCTTIGTAATTAGATTAGATTGCT CYC1 F CTTGAGCTCTCATGTAATTAGATTAGATTGCT CYC1 R CCGGAATTCGCAAATTAAAACCTTCGAGC SUL1 RT F CACTGGGTTGGGTATACTGC SUL1 RT R ATGAGAGCCGGAATTTGACC SUL2 RT F CACGGTGAAAGGAAGCATGTA SUL2 RT R CCAGTTGATGATGGGTAAACGAACACACGACATGTA SUL2 RT R ATGGTCGGTATGGGTCAAAA ACT1 RT F ATGGTCGGTATTGGGTCAAAA ACT1 RT R ATGGTCGGTATTGGGTCAAAA ACT1 RT R ATGGTCGGTATTGGGTCAAAA ACT1 RT R ATGGTCGGTATTGGTCCAGTTTGT RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGGTTTGGACTAAGAAACCGCCTCT RDL1 in F CCGGTGAAGAATACACAGGGTACATACCAAGAGCAATATCAAAGCCCAATACCGCAAACCGCCTCT RDL1 in R ATACCACCCCCATGAGAAAC RDL1 out F GAGAGTGGAGGCTTAATCAA RDL1 out F GAGAGTGGAGGCTTAATCAA RDL1 self F TATGACCATGATTACGAATATCGC RDL1 ks R GTCAACTGATTACGCAATACGCATAGGTT RDL1 his F CGACCATGAATACGCCATTTTATTTGGCGCATAGACAAG RDL1 self R GTCAACTGCATGATTACGCAATAGTCGACTAGGTT RDL1 his F CGACCACGACAAGCCCATTGGATGGTTCGACTAGGTT RDL1 his R CAGTGGTGGTGGTGGTGGTGGTGGTTCGACTAGGTT RDL1 ks R CGACCACGACAAGCCCATTGGATGGGTGTTCGACTAGGTT RDL1 ks R CGACCACGACAAGGCCATGGGTGGTTCGACTAGGTT RDL1 ks R CGACCACGACAAGGCCATGGGTGGTTCGACTAGGTT RDL1 ks R CGACCACGACAAGGCCATGGGTGGTTCGACTAGGTT RDL1 ks R CGACCACGACAAGGCCATGGGTGGTTCGACTAAGTTAACACCCCCCATGAG RDL2 ko R AAAGGTTGCTATATACAGGATATATCCAATTATACTTGTTTCTTTTTGGC CCAATACGCCAAACCGCCTCT RDL2 ko R AAAGGTTGCTATATACAGGATATATACCAATTGGTTCTTTTTTGGC CCCAATACGCCAAACCGCCCTCT RDL2 ko R AAAGGTTGCTATATACAGGATATATACCAGTTATACTTTTTTTT		
TEF1 R CGGGATCCTTTGTAATTAAAACTTAGATTGCT CYC1 F CTTGAGCTCTCATGTAATTAGTTAGTTAGCT CYC1 R CCGGAATTCGCAAAATTAGATTAGTCACGCT CYC1 R SUL1 RT F CACTGGGTTGGGTATACTGC SUL2 RT F CACGGTGAAAGGACATGTA SUL2 RT R CCAGTGATGATGAGGAAAAC ACT1 RT F ATGCGTATTGGGTCAAAAA ACT1 RT R ATGCGGTATAGGGTCAAAAA ACT1 RT R ATGCTGCTATTGGTCCAGGTTGGT RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 ko R GTTACTAGCTTACGAAAATACACAGGGTACATACCAAGAGACAACCGCCTCT RDL1 in R RDL1 out F GAGACTGGAAGAACC RDL1 out R AACGTTGTACACAAAATACACAGGGTAGACAAG RDL1 self F TATGACCCCCATGAGGAAACC RDL1 is self F TATGACCATGATTTACGCCATTTTATTTTCGCCCATAGACAAG RDL1 is self R RDL1 his F CGACGACGACAACACGCATGCATGGGTGTTCGAACGCATAGCAAAG RDL1 is R RDL1 is R GTCGACTGAGGAGACACAAGGCCATAGCCAAGG RDL1 is R GTCGACTGCAGGCATGACTGGAGGTTTTCGAACGCATTATACACGCGTTAGACAAG RDL1 is R GTCGACCTGCAGGCATGCATGGAGGAACC RDL1 his R GTCGACCTGCAGGCATGCATGGAGGAACCAAGGCCATAGCCAAGG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCAAGAAAAAAAAAAA		
CYC1 F CYC1 R CCGGAATTCGCAAATTAAGTTATGTCACGCT CYC1 R CCGGAATTCGCAAATTAAAGCCTTCGAGC SUL1 RT F CACTGGGTTGGGTATACTGC SUL2 RT F CACGGTGAAAGGAGCCGGAATTTGACC SUL2 RT F CACGGTGAAAGGAGCCGGAATTGACC SUL2 RT R CCAGTTGATGATGGGTCAAAA ACT1 RT F ATGGTCGGTATGGGTCAAAA ACT1 RT R ATGGTCGGTATGGGTCAAAA ACT1 RT R ATGGTCGGTATGGGTCAAAA ACT1 RT R ATGGTCCAATATCGTCCAGTTGGT RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGGTTTGTAACAAAACCAATATTAAAGCTTCGTACGCTGCAGGTC RDL1 ko R GTTACTAGCTTACGAAAATACACAGGGTACATACCTAGAGTATACAAGGCCAATACGCAAACCGCCTCT RDL1 in R CCGTGATGAATGCTTGGAAT RDL1 in R AACGGTGTGACATAACAA RDL1 out F GAGAGTGGAGGCTTAATCAA RDL1 out R AACGGTGTGACATAAATGGC RDL1 self F TATGACCATGATTACGCCATTTTATTGGCGCATAGACAAG RDL1 self F GTCACCTCCAGGACATGCCTATTCGAACCATGGTT RDL1 his F CGACGACCACAACAGCCATGCTGTTGGAATGC RDL1 his F CGACGACCACACAAGCCATGCTGTATTCGAACCACGCTTGATGCACAGG RDL1 his R CACTGGTGGTGGTGTGTGTGTCTCGATTGGAAAGCCATTATACCCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGACAAGAAAAAAAAAA		
CYC1 R SUL1 RT F CACTGGGTTGGCAAATTAAAGCCTTCGAGC SUL1 RT R ATGAGAGCCGGAATTTGACC SUL2 RT F CACGGTGAAAGGAGCATGTA SUL2 RT R CCAGTTGATGATGAGC SUL2 RT R CCAGTTGATGATGGGTCAAAA ACT1 RT F ATGGTCGGTATGGGTCAAAA ACT1 RT R TCCATATCGTCCAGTTGT RDL1 ko F ATTCTTTCTCGTTTATTTTCAGGGTTTGGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 ko R GTTACTAGCTTACGAAAATCACAGGGTACCTAGAGAAACCAACC		
SUL1 RT F CACTGGGTTGGGTATACTGC SUL2 RT F ATGAGAGCCGGAATTTGACC SUL2 RT F CACGGTGAAAGGAGCATGTA SUL2 RT R CCAGTTGATGGGAAAAAC ACT1 RT F ATGGTCGGTATGGGTCAAAA ACT1 RT R TCCATATCGTCCCAGTTGGT RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 in F GTTACTAGCTTAACGAAAATACACAGGGTACATACCTAGGAGTATACAAAGGCCAATACGCAAACCGCCTCT RDL1 in R TATCACCCCCATGAGAAACC RDL1 out F GAGAGTGGAGGCTTAATCAA RDL1 out R AACGGTGTGACATAAATGGC RDL1 self F TATGACCATGATTACCACATGATTTATTGGCGCATAGACAAG RDL1 self R GTCGACCTGCAGGCATGCATGGGGTGTTCGACTAGGTT RDL1 his F CGACGACGACAAGGCCATGGATGGATGGATGAATGC RDL1 his R CAGTGGTGGTGGTGGTGGTGGTGGTGGTGATTATACACCCATTTATACACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAACCAAGAGACAGAAGAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 in F CAAGATCGCCTACATTGGT RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 in R GATACCAGTGTTTTTCTACCC RDL2 out F TGGAAGCGAGACAGAAGAAAA		
SUL1 RT RATGAGAGCCGGAATTTGACCSUL2 RT FCACGGTGAAAGGAGCATGTASUL2 RT RCCAGTTGATGATGGGAAAAACACT1 RT FATGGTCGGTATGGGTCAAAAACT1 RT RTCCATATCGTCCCAGTTGGTRDL1 ko FAATTCTTTCTCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTCRDL1 ko RGTTACTAGCTTACGAAAATACACAGGGTACATACCTAGAGTATACAAGGCCAATACGCAAACCGCCTCTRDL1 in FCCGTGATGAATGCTTGGAATRDL1 out FGAGAGTGGAGGCTTAATCAARDL1 out RAACGGTGTGACATAAATGGCRDL1 self FTATGACCCATGAGAAACCRDL1 self FTATGACCATGATTTATTGGCGCATAGACAAGRDL1 self RGTCGACCTGCAGGCATGCATGGGTTTCGACTAGGTTRDL1 his FCGACGACCAAGGCCATGGCTGATTGGAAGGCCGTGATGAATGCRDL1 his RCAGTGGTGGTGGTGGTGGTGGTGGTGACAGATTTATACGCCCATTTATACAGCAAGGTTRDL2 ko FGCGATAACTCTCAACAAATGGAAGCAGAAGAAAAAAAGACCAACGCTTCGTACGCTGCAGGTCRDL2 ko RAAAGGTTGCTATATACACAGATTATCGATTATACTTGTTTCTTTTTTGGC CCAATACGCAAACCGCTCTRDL2 in FCAAGATCGCCTACATTGGTRDL2 in RGATACCAGTGTTTTCGTACCCRDL2 in RGATACCAGTGTTTTCGTACCCRDL2 out FTGGAAGCGAGACAGAAAAAAAAAAAAAAAAAAAAAAAAA		
SUL2 RT FCACGGTGAAAGGAGCATGTASUL2 RT RCCAGTTGATGATGGGAAAAACACT1 RT FATGGTCGGTATGGGTCAAAAACT1 RT RTCCATATCGTCCCAGTTGGTRDL1 ko FAATTCTTTCTCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTCRDL1 ko RGTTACTAGCTTACGAAAATACACAGGGTACATACCTAGAGTATACAAGGCCAATACGCAAACCGCCTCTRDL1 in FCCGTGATGAATGCTTGGAATRDL1 in RTATCACCCCCATGAGAAACCRDL1 out FGAGAGTGGAGGCTTAATCAARDL1 self FTATGACCATGATTACGCCATTTTATTGGCGCATAGACAAGRDL1 self RGTCGACCTGCAGGCATGCATGGGTGTTCGACTAGGTTRDL1 his FCGACGACGACAAGGCCATGCATGGAGTGTTCGACTAGGTTRDL1 his RCAGTGGTGGTGGTGGTGGTGGTGGTGATGAAGCAGGRDL1 his RCAGTGGTGGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATCACCCCCATGAGRDL2 ko FGCGATAACTCTCAACAAATGGAACGAGACAGAAGAAAAAAGACCCAACGCTTCGTACGCTGCAGGTCRDL2 ko RAAAGGTTGCTATATACAGGATATATCGATTATACTTGTTTCTTTTTTGGC CCAATACGCAAACCGCCTCTRDL2 in FCAAGATCGCCTACATTGGTRDL2 in RGATACCAGTGTTTTCGTACCCRDL2 in RGATACCAGTGTTTTCGTACCCRDL2 in RGATACCAGTGTTTTCGTACCCRDL2 in RGATACCAGTGTTTTCGTACCCRDL2 in RGATACCAGTGTTTTCGTACCCRDL2 in RGATACCAGTGTTTTCGTACCC		
SUL2 RT RCCAGTTGATGATGATGGGAAAAACACT1 RT FATGGTCGGTATGGTCAAAAACT1 RT RTCCATATCGTCCCAGTTGGTRDL1 ko FAATTCTTTCTGTTTATTTTCAGGGTTAGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTCRDL1 in FGTTACTAGCTTACGAAAATACACAGGGTACATACCTAGAGTATACAAGGCCAATACGCAAACCGCCTCTRDL1 in RCCGTGATGAATGCTTGGAATRDL1 out FGAGAGTGGAGGCTTAATCAARDL1 out RAACGGTGTGACATAAATGGCRDL1 self FTATGACCATGATTACGCCATTTTATTGGCGCATAGACAAGRDL1 self RGTCGACCTGCAGGCATGCATGGGTGTTCGACTAGGTTRDL1 his FCGACGACGACAAGGCCATGGCTGATTGGAAGGCCGTGATGAATGCRDL1 his RCAGTGGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATACACCCCCATGAGRDL2 ko FGCGATAACTCTCAACAATGGAAGCGAGAAGAAAAAGACCAACGCTTCGTACGGTCRDL2 ko RAAAGGTTGCTATATAACAGGATATATCGATTATACTTGTTTCTTTTTTGGC CCAATACGCAAACCGCCTCTRDL2 in FCAAGATCGCCTACATTGGTRDL2 out FTGGAAGCGAGACAGAAAAAAAAAAAAAAAAAAAAAAAAA		
ACT1 RT F ATGGTCGGTATGGGTCAAAA ACT1 RT R TCCATATCGTCCCAGTTGGT RDL1 ko F RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 in F CCGTGATGAATGCTTGGAAAT RDL1 in R RDL1 out F GAGAGTGGAGGCTTAATCAA RDL1 out R RDL1 self F TATGACCATGATGATTACGCATTGGTTTATTTATGGCGCATAGGTT RDL1 his F CGACGACGACAACGCATTTTATTTGGCGCATAGATACAAG RDL1 his F CGACGACGACAAGGCCATGGTTGGACATAAATGC RDL1 his R CAGTGTGGTGTGTGTGTTGATTGAAGTTTGAAGTTTATACACCCCCATGAG RDL1 his R CAGTGTGTGTGTGTGTGTGTGTTGAATGAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCCAAGAAAAAAAAAA		
ACT1 RT R RDL1 ko F AATTCTTTCTCGTTTATTTTCAGGGTTTGTACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 ko R GTTACTAGCTTACGAAAATACACAGGGTACATACCTAGAGTATACAAGGCCAATACCCAAACCGCCTCT RDL1 in F CCGTGATGAATGCTTGGAAT RDL1 in R TATCACCCCCATGAGAAACC RDL1 out F GAGAGTGGAGGCTTAATCAA RDL1 out R AACGGTGTGACATAAATGGC RDL1 self F TATGACCATGATTACGCCATTTTATTTATTGGCGCATAGACAAG RDL1 self R GTCGACCTGCAGGCATGCATGGAGTGTTCGACTAGGTT RDL1 his F CGACGACGACAAAGGCCATGCATGGATTGGAAGGCCGTGATGAATGC CAGTGGTGGTGGTGGTGGTGGTGTCGACTAGGTT RDL1 his R GCACGACGACAAAGGCCATGGATTATAAGTCAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGACCAACGCTTCGTTACTTTGTTTCTTTTTGGC CCAATACGCAAACCGCCTCT RDL2 in F CAAGATCGCCTACATTGGT RDL2 out F GGAAGCGAGAACAGAAAAAAAAAAAAAAAAAAAAAAA		
RDL1 ko F RDL1 ko R GTTACTAGCTTACTTTCTCGTTTATTTTCAGGGTTTGTGACTAAGAAACGATATTAAAGCTTCGTACGCTGCAGGTC RDL1 in F CCGTGATGAATGCTTGGAAT RDL1 in R RDL1 out F RDL1 out R RDL1 self F RDL1 self R RDL1 his F CGACGACGACATGCTGAGGTTCAATGCATGGTT RDL1 his F CGACGACGACACAAGGCCATGATGATGCATGGTT RDL1 his R RDL2 ko F RDL2 ko F RDL2 in F RDL2 in F RDL2 out F RDL3 out F RDL3 out F RDL4 out F RDL5 out F RDL6 out F RDL7 out R RDC6 occarragattacgccattgattgacacacacacacacacacacac		
RDL1 ko R GTTACTAGCTTACGAAAATACACAGGGTACATACCTAGAGTATACAAGGCCAATACGCAAACCGCCTCT RDL1 in F CCGTGATGAATGCTTGGAAT TATCACCCCCATGAGAAACC RDL1 out F RDL1 out R AACGGTGTGACATAAATGGC RDL1 self F TATGACCATGAGTTACGCAATACAGGTTTATTAGGCGCATAGACAAG RDL1 self R GTCGACCTGCAGGCATGCATGGGTGTTCGACTAGGTT RDL1 his F CGACGACGACAAGGCCATGATTGGAAGGCCGTGATTGAAATGC RDL1 his R CAGTGGTGGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCGAGACAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 in F RDL2 in F GATACCAGTGTTTTTCGTACCC RDL2 out F GATACCAGTGTTTTCGTACCC TGGAAGCGAGACAAGAAAAAA GTTACCACAAATGGAAAAAAAAAA		
RDL1 in F CCGTGATGAATGCTTGGAAT RDL1 in R TATCACCCCCATGAGAAACC RDL1 out F RDL1 out R RDL1 self F RDL1 self R GTCGACCTGAGGACATGACTGGGTTTCGACTGGTTTCGACTGGTTTCGACTGGTTTCGACGTGGTTTCGACTGGTTTTATTCGCCCTGAGGTTTTTATTCGCCGTGATGAATGC RDL1 his F CGACGACGACCAAGGCCATGGTTGGAAGGCCTGATTGGAAGGCCGTGATGAATGC RDL1 his R CAGTGGTGGTGGTGGTGGTGGTGGTGCTCGATTAAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCCAGAAGAAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 in F RDL2 in F GATACCAGTGTTTTCGTACCC RDL2 out F GATACCAGTGTTTTCGTACCC RDL2 out F GATACCAGTGTTTTCGTACCC TGGAAGCGAGACAGAAAAAAAAAA		
RDL1 in R RDL1 out F GAGAGTGGAGGCTTAATCAA RDL1 out R RDL1 self F TATGACCATGATTACGCCATTTTATTGGCGCATAGACAAG RDL1 self R GTCGACCTGCAGGCATGCATGCTTTCGATTGGTTTCGATTGGATGGTTT RDL1 his F CGACGACGACAAGGCCATGGTGATTATAAGTCAAGTTTATTCACCCCATGAGG RDL2 ko F RDL2 ko R AAAGGTTGTCTCTAACAAATGGAAGCCAGAAGAAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 in F RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 out F GATACCAGTGTTTTCGTACCC GATACCAGAGAAAAAAAAAA		
RDL1 out F RDL1 out R RDL1 self F RDL1 self R RDL1 self R RDL1 self R RDL1 his F CGACGACGACACAAGGCCATGGTGACATGACGCATTATACGCCATGATGACAAG RDL1 his R RDL2 ko F RDL2 ko R RDL2 in F RDL2 out F RDL2 out F RDL2 out F RDL3 self R GAGAGTGGAGGCTGATGCATGGGTGTTCGACTAGGTT CGACGACGACGACGACGACGAGGCCATGGCTGATTGGAAGGCCGTGATGAATGC CAGTGGTGGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATCACCCCCATGAG RDL2 ko R AAAGGTTGTCTATATACAGGATATATACTTGTTTCTTTTTGGC CCAATACGCAAACCGCTCCT RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 out F GATACCAGTGTTTTCGTACCC RDL2 out F		
RDL1 out R AACGGTGTGACATAAATGGC RDL1 self F TATGACCATGATTACGCCATTTTATTGGCGCATAGACAAG RDL1 self R GTCGACCTGCAGGCATGCATGGGGTGTTCGACTAGGTT RDL1 his F CGACGACGACAAGGCCATGGCTGATTGGAAGGCCGTGATGAATGC RDL1 his R CAGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCGAGACAGAAGAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 ko R AAAGGTTGTCTATATACAGGATATATCGATTATACTTGTTTCTTTTTGGC CCAATACGCAAACCGCCTCT RDL2 in F CAAGATCGCCTACATTGGT RDL2 out F TGGAAGCGAGACAGAAGAAA		
RDL1 self F TATGACCATGATTACGCCATTTTATTGGCGCATAGACAAG RDL1 self R GTCGACCTGCAGGCATGCATGGGGTGTTCGACTAGGTT RDL1 his F CGACGACGACAAGGCCATGGCTGATTGGAAGGCCGTGATGAATGC RDL1 his R CAGTGGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCAAGAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 ko R AAAGGTTGTCTATATACAGGATATATCGATTATACTTGTTTCTTTTTGGC CCAATACGCAAACCGCCTCT RDL2 in F CAAGATCGCCTACATTGGT RDL2 out F TGGAAGCGAGACAGAAAA		
RDL1 self R GTCGACCTGCAGGCATGCATGGGGTGTTCGACTAGGTT RDL1 his F CGACGACGACAAGGCCATGGCTGATTGGAAGGCCGTGATGAATGC RDL1 his R CAGTGGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCAAGAAGAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 ko R AAAGGTTGTCTATATACAGGATATATCGATTATACTTGTTTCTTTTTGGC CCAATACGCAAACCGCCTCT RDL2 in F CAAGATCGCCTACATTGGT RDL2 out F TGGAAGCGAGACAGAAGAAA		
RDL1 his F CGACGACGACAAGGCCATGGCTGATTGGAAGGCCGTGATGAATGC RDL1 his R CAGTGGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCGAGACAGAAGAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 ko R AAAGGTTGTCTATATACAGGATATATCGATTATACTTGTTTCTTTTTGGC CCAATACGCAAACCGCCTCT RDL2 in F CAAGATCGCCTACATTGGT RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 out F TGGAAGCGAGACAGAAGAAA		
RDL1 his R CAGTGGTGGTGGTGGTGGTGCTCGATTATAAGTCAAGTTTATCACCCCCATGAG RDL2 ko F GCGATAACTCTCAACAAATGGAAGCGAGACAGAAGAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 ko R AAAGGTTGTCTATATACAGGATATATCGATTATACTTGTTTCTTTTTGGC CCAATACGCAAACCGCCTCT RDL2 in F CAAGATCGCCTACATTGGT RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 out F TGGAAGCGAGACAGAAGAAA		
RDL2 ko F GCGATAACTCTCAACAAATGGAAGCGAGACAGAAGAAAAAGACCAACGCTTCGTACGCTGCAGGTC RDL2 ko R AAAGGTTGTCTATATACAGGATTATACTTGTTTCTTTTTGGC CCAATACGCAAACCGCCTCT RDL2 in F CAAGATCGCCTACATTGGT RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 out F TGGAAGCGAGACAGAAAA		
RDL2 ko R AAAGGTTGTCTATATACAGGATATATCGATTATACTTGTTTCTTTTTGGC CCAATACGCAAACCGCCTCT RDL2 in F CAAGATCGCCTACATTGGT RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 out F TGGAAGCGAGACAGAAAA		
RDL2 in F CAAGATCGCCTACATTGGT RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 out F TGGAAGCGAGACAGAAA		
RDL2 in R GATACCAGTGTTTTCGTACCC RDL2 out F TGGAAGCGAGAAAA		
RDL2 out F TGGAAGCGAGACAGAAGAAA		

(Continued on next page)

TABLE 3 (Continued)

Name	Sequence
RDL2 self F	TATGACCATGATTACGCCAGAACCATCTGAGTACTCGATT
RDL2 self R	GTCGACCTGCAGGCATGCAGAAAAAGTCTGAGAAACGTAAAAGT
RDL2 his F	CGACGACACGCCATGGCTGATTTCAAGCATACAGTACAG
RDL2 his R	CAGTGGTGGTGGTGGTGCTCGATTATTTTTTGGGCTTAACGTCAG
TUM1 ko F	ATGCCATTATTTGATCTTATTTCTCCAAAAGCGTTTGTTAAGTTAGTGGC TGTTTAGCTTGCCTCGTCC
TUM1 ko R	TAATCTCTGTTTTCAGCAATCCACTCGGGCCCGGATTTCAAGACCCACTGAATTCGAGCTCGTTTAAAC
TUM1 in F	CCAGCTTTTCATACTTCTACC
TUM1 in R	TCTGTACCTTCGAATCTACCG
TUM1 out F	CCAGCTTTCATACTTTCTACC
TUM1 out R	GGTTTAATCGTGTTGGAATTTCG
TUM1 self F	TATGACCATGATACGCCACTTGATCCCATTGTTGC
TUM1 self R	GTCGACCAGGAGGAGGAGCATCGATCATTCATTCATCATCATATTCAGGAAAAG
TUM1 his F	CGACGACGACAAGGCCATGGATCAATTAATTTGATCTTATTTCTCCAAAAG
TUM1 his R	CAGTGGTGGTGGTGGTGCTCGATTAATCTCTGTTTTCAGCAATCCA
YCH1 ko F	GGACTCGTACTCAATAACAAACGTAAAATACCTGGATCCGACTGAATTAACTGTGGGAATACTCAGGT
YCH1 ko R	TCAACGCCACAGATCGGGTAGGTAACCCGCCGTAACGCTCTCGTCGTCACATATCGACTACGTCGTTAAGG
YCH1 in F	CACTACTACGCTGAGGGAG
YCH1 in R	AAACCTGTCAACGCCACA
YCH1 out F	GTGTCTGCTGTGTACTA
YCH1 out R	AAACCTGTCAACGCCACA
YCH1 self F	TATGACCATGATTACGCCAGTGTCTGCTGTGCTTTTGTACTA
YCH1 self R	GTCGACCTGCAGGCATGCACATGCGTACATATGTGGCT
YCH1 his F	CGACGACGACAAGGCCATGGCTGATGACTCGTACTCAATAACAAACGTAAAAATAC
YCH1 his R	CAGTGGTGGTGGTGGTGCTCGA AAACCTGTCAACGCCACA
UBA4 ko F	ATGAATGACTACCATCTCGAGGATACCACGTCTGAACTTGAAGCATTAAG GCTTCGTACGCTGCAGGTC
UBA4 ko R	CTAATATTTAGGAATGGTTTGATCAATATCGTCTATGTATTTGAAGTATC CCAATACGCAAACCGCCTCT
UBA4 in F	CTGCAAAAGGAAGTAAATAGAAG
UBA4 in R	GGGTTCAGTTTCGTGATATA
UBA4 out F	CTGCAAAAGGAAGTAAATAGAAG
UBA4 out R	TATTTAGGAATGGTTTGATCAAT
UBA4 self F	TATGACCATGATTACGCCATTTTACAGCTTTAGACACAGG
UBA4 self R	GTCGACCTGCAGGCATGCAAAATATTTCCATCATGCGAC
UBA4 his F	CGACGACGACAAGGCCATGGCTGATAATGACTACCATCTCGAGG
UBA4 his R	CAGTGGTGGTGGTGGTGCTCGACTAATATTTAGGAATGGTTTGATCAATA
RDL2 106A F	GAATTGATTTTCTTgcTGCGAAAGGAGTAAGAGC
RDL2 106A R	GCTCTTACTCCTTTCGCagcAAGAAAATCAATTC
RDL2 106S F	GAATTGATTTTCTTtcTGCGAAAGGAGTAAGAGC
RDL2 106S R	GCTCTTACTCCTTTCGCagaAAGAAAAATCAATTC
PspE F	ATCTAAGTTTTAATTACAAAATGTTTAAAAAAAGGCTTACTTGCTCT
PspE R	CTAATTACATGAGAGCTCGG TTAACCTTTGACCTTCGGCATTG
GlpE F	ATCTAAGTTTTAATTACAAAATGGATCAGTTCGAATGTATTAACGTTGCCGAC
GlpE R	CTAATTACATGAGAGCTCGG TTACGCGCCGTACGCCACC
RdhA F	ATCTAAGTTTTAATTACAAA ATGAGCGACACCTTCCAGCG
RdhA R	CTAATTACATGAGAGCTCGG CTACTCCGCTTCGCCGCTG
SseA F	ATCTAAGTTTTAACAAA ATGTCCGTTTTCTCCGACC
SseA R	CTAATTACATGAGAGCTCGG TCAAACCTCTACAGGGGTATCG

modified SD culture. Equal amounts of cells (OD $_{600}$ of 0.05) were cultured in 400 μ l of the modified SD medium containing sulfate or thiosulfate at 30°C with shaking for 24 h or longer, and growth was monitored at OD₆₀₀ or by a Microplate Reader (BioTek, Synergy H1). Yeast inoculated into the modified SD medium without sulfur was used as the control. The relationship of plate data and OD_{600} is shown in Fig. S12.

Thiosulfate uptake assay. Fresh S. cerevisiae cells were inoculated in 30 ml of the YPD medium and grown overnight at 30°C. Cells were collected by centrifugation (11,000 rpm, 5 min), washed twice with sterile water, and suspended in the modified SD medium without sulfur to the same volume. The cells were incubated at 30°C with shaking for 1 day, and the cells were harvested, washed, and resuspended in the same medium and incubated for another day. The sulfur-starved cells were then harvested and resuspended in sterile 50 mM potassium phosphate (pH 6, 2% glucose) at an OD₆₀₀ of 10. Thiosulfate was added to a final concentration of 200 μM to initiate the reaction. One milliliter of cell suspension was centrifuged (13,000 rpm, 5 min) at various time intervals, and thiosulfate in the supernatant was measured by using the cyanide method (26, 50).

Measurement of thiosulfate uptake by S. cerevisiae strains. Thiosulfate was added to a final concentration of 1 mM in the sulfur-starved cell suspensions. One milliliter of the cell suspension was centrifuged (12,000 \times g, 2 min) at various time intervals with one wash in H_2O . The pellets were suspended in 50 mM potassium phosphate (pH 6, 2% glucose), and thiosulfate was added to 200 μ M to initiate the reaction. After 1 h of incubation, the thiosulfate concentration in the supernatant was measured and the rate was calculated (51).

 H_2S measurement. H_2S production by yeast cells was measured with 3 ml of the sulfur-starved yeast cells in 50 mM potassium phosphate (pH 6) at an OD_{600} of 1 in a 15-ml glass tube. The tube was sealed with a rubber stopper, and a lead-acetate paper strip was placed in the gas phase. The cells were incubated at 30°C. When H_2S is produced and evaporated into the gas phase, sulfide reacts with lead to produce a dark stain on the paper strip (52). H_2S production by enzymes (in 50 mM potassium phosphate, pH 8) was detected in the same way.

To obtain a standard curve, different concentrations of NaHS were added to 3 ml of a specific buffer and incubated at 30°C for 1 h; the paper strips then were taken out to measure the darkness via densitometer (Fig. S13).

Real-time PCR analysis. The sulfur-starved cells were harvested at specific time intervals before and after the addition of 1 mM thiosulfate. Total RNA was isolated by using the total RNA extract kit (R6834-01; Omega), and the *SUL1* and *SUL2* mRNAs were analyzed by real-time PCR normalized against the *ACT1* mRNA according to a reported method (53).

Recombinant protein production and purification. *E. coli* BL21(DE3) cells with the expression plasmid pET30a-*MET15* were incubated in the LB medium with 50 μ g/ml kanamycin and cultured to an OD₆₀₀ of 0.6 at 25°C; 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was then added to induce the production of the recombinant proteins. The cultures were further incubated with shaking for 8 h. Cells were harvested by centrifugation and disrupted by a pressure cell homogenizer (SPCH–18; Stansted Fluid Power Ltd., UK) in ice-cold buffer I (20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, 0.2 mM PLP, pH 8). The lysate was centrifuged at 12,500 × g for 10 min to remove cell debris. The target protein was purified via nickel-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen, Shanghai, China) according to the supplier's recommendations. The final buffer was exchanged to buffer II (20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, 0.2 mM PLP, pH 8), and then 50% glycerol was added to give a final concentration of 10% before storage at -80° C (10). The concentration of purified Met15p was estimated via its molar extinction coefficient ($\epsilon_{280} = 51,800 \text{ M}^{-1} \text{ cm}^{-1}$), calculated with the ProParam tool (http://web.expasy.org/protparam/) (54, 55). Other recombinant proteins were produced and purified in the same way.

Analysis of Met15 activities. Met15p was incubated at 25°C for 10 min prior to the experiment. For reaction experiments, O-acetyl-homoserine (HCl form) was first dissolved in 50 mM potassium phosphate buffer (pH 8), and the pH of the mixture was then adjusted to 7.5 with 5 M NaOH. All experiments were carried out in 1.5-ml microcentrifuge tubes. In a typical experiment, a 0.1-ml portion of enzyme solution was added to 0.9 ml of a reaction mixture containing 0.5 to 5 mM OAH, 0.0125 to 0.2 mM $Na_2S_2O_3$ or NaHS, and 0.1 mM PLP in 50 mM potassium phosphate buffer (pH 7.4). The final Met15 concentration was 2 nM. At appropriate time points, 0.3 ml of the reaction mixture was withdrawn and added to 0.03 ml of 1.0 M HCl solution to stop the reaction, followed by measuring the concentrations of thiosulfate and cysteine by using a reported high-performance liquid chromatography (HPLC) method (26, 56, 57).

Thiosulfate sulfurtransferase assay. Thiosulfate sulfurtransferase activity was estimated by measuring the production of sulfite (58). Briefly, the reactions were initiated by adding the enzyme to 1 ml of the assay mixture containing 50 mM potassium phosphate buffer (pH 8), 20 mM thiosulfate, and 20 mM GSH at 30°C. The reactions were quenched after 2 min by heating at 100°C and centrifuged. The produced sulfite in the supernatant was assayed by using a monobromobimane-derived method (26).

Potential CysK, CysM, and CysO from sequenced fungal genomes. A fungal genomic protein sequence set from NCBI, updated through 10 January 2018, was downloaded as a preliminary database for sorting cysteine synthases consisting of three types: CysK, CysM, and CysO. This database contains all proteins from 17 completely annotated genomes and 176 chromosomes. The reported sequences belonged to cysteine synthases (CysK, CysM, and CysO), cystathionine beta-synthase (CBS; EC 4.2.1.22), threonine dehydratase (THDH; EC:4.2.1.16), and aminocyclopropane-1-carboxylate deaminase (ACCD; EC:3.5.99.7). They were used to establish a phylogenetic tree by using a neighbor-joining analysis with the MEGA version 7.0 program, running a pairwise deletion, p-distance distribution, and bootstrap analysis of 1,000 repeats. The reported CysK from *Helicobacter pylori* (UniProt identifiers P56067 and Q9ZMW6) was found to be in the same clade as CBS. Thus, these two sequences were removed. The rest of the CysKs were found to be in the same clade. The reported CysO and CysM were grouped in the same clade. The reported CBS, THDH, and ACCD were used as outgroups.

The grouped CysK, CysM, and CysO proteins were used as queries for BLAST searches of the total GenBank fungal genomes with conventional criteria (E value of $\leq 1e-10$, coverage of $\geq 60\%$, identity of $\geq 35\%$) via standalone BLASTP algorithm, resulting in 460 candidates. Redundancy was removed from these candidates by using CD-HIT with >99% identity within each group. The remaining 196 candidates were further tested via phylogenetic tree analysis with known CysK, CysM, CysO, proteins and the outgroups mentioned above. One hundred twenty-eight proteins were found to be in the same clade with CysK. No candidates were found to be in the same clade with CysM and CysO. The rest of the proteins all were distributed in the CBS clade.

The annotated 128 proteins were grouped into 67 subgroups by using CD-hit with 80% identity, and 67 representative proteins were combined with the known CysK, CysM, and CysO proteins and the outgroups to generate a phylogenetic tree.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM 01241-18

SUPPLEMENTAL FILE 1, PDF file, 10.1 MB.

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