Using yeast synthetic lethality to inform drug combination for malaria

Suvitha Subramaniam\textsuperscript{a,b}, Christoph D. Schmid\textsuperscript{a,b,c}, Xue Li Guan\textsuperscript{d}, Pascal Mäser\textsuperscript{a,b#}

Swiss Tropical and Public Health Institute, 4051 Basel, Switzerland\textsuperscript{a}, University of Basel, 4002 Basel, Switzerland\textsuperscript{b}; SIB Swiss Institute of Bioinformatics, 4000 Basel, Switzerland\textsuperscript{c}, Nanyang Technological University, 639798 Singapore\textsuperscript{d}

Running head: Learning from yeast for combinatorial chemotherapy

#address correspondence to Pascal Mäser, pascal.maeser@swisstph.ch
Abstract

Combinatorial chemotherapy is imperative for the treatment of malaria. However, finding a suitable partner drug for a new candidate is challenging. Here we develop an algorithm that identifies all the gene pairs of *Plasmodium falciparum* which possess orthologues in yeast that have a synthetic lethal interaction, but are absent in humans. This suggests new options for drug combinations, in particular for inhibitors of targets like *P. falciparum* calcineurin, cation ATPase 4, or phosphatidylinositol 4-kinase.
There is a persistent need for new antimalarials due to the evolution of drug-resistant parasites. Under the auspices of the Medicines for Malaria Venture, new drug candidates are being developed that are active also against artemisinin-resistant isolates of *Plasmodium falciparum*. The frontrunners are artefenomel, KAF156, cipargamin, DSM265, MMV390048, ferroquine and tafenoquine (1, 2). However, the choice of the right partner drug will be critical for the success of these new molecules, as the WHO enforces antimalarials to be applied in combination therapy (3). In addition to protecting each other from drug resistance, two molecules to be combined need to be compatible for co-formulation, should have matching pharmacokinetics, and must not have unfavorable polypharmacology (4-6). Ideally, the two molecules will potentiate each other, thereby decreasing the duration of treatment and the required doses. Thus, combinatorial chemotherapy can not only reduce the risk of drug resistance but also enhance drug safety and drug efficacy, enabling the ambitious goal of ‘Single Exposure Radical Cure’ (7, 12).

Here we propose to support the matchmaking of antimalarial candidates by learning from yeast reverse genetics. *Saccharomyces cerevisiae* is probably the best studied of all eukaryotes. Only about 20% of its protein-coding genes are essential for growth on rich medium (8). High-throughput crossing experiments have shown that many of the *S. cerevisiae* viable gene deletion mutants possess synthetic phenotypes, i.e. growth defects that become apparent only in the absence of another non-essential gene. The concept of genetic synthetic lethality can be adopted to combination chemotherapy (9-12). The principal idea is to infer from synthetic lethal gene pairs in...
S. cerevisiae to orthologous pairs of genes in P. falciparum, assuming that the combined inhibition of the respective gene products will produce a synergistic effect. However, this seemingly straightforward approach is complicated by the fact that S. cerevisiae is more closely related to H. sapiens than to P. falciparum (13). Thus, a drug combination inferred from yeast synthetic genetic lethality might enhance the toxicity to humans rather than the antimalarial efficacy. To avoid such a scenario, we developed an algorithm to exclude gene pairs that are conserved in H. sapiens.

Yeast synthetic lethal gene pairs were obtained from BioGRID v3.4 (14), pairs and groups of orthologous genes from OrthoMCL-DB v5, based on the similarity of the derived protein sequences (15, 16). Mining the OrthoMCL database with the 16,217 synthetic lethal gene pairs of S. cerevisiae identified in BioGRID, we found that only 1,505 (9.3%) pairs had direct orthologues in P. falciparum for both gene products (Figure 1). From this set, we tested all the proteins for the presence of an orthologue in the human proteome, again referring to the downloaded OrthoMCL database. This included direct, pairwise orthology between the P. falciparum or S. cerevisiae protein and a H. sapiens protein, or indirect orthology where either the malaria protein or its yeast orthologue belonged to an OrthoMCL group that also contained a human protein (Figure 1). All the P. falciparum gene pairs of which both gene products tested positive for direct or indirect human orthology were eliminated. This yielded 37 pairs composed of 55 unique P. falciparum proteins which fulfilled the condition that (i) their direct orthologues in S. cerevisiae are synthetic lethal and (ii) at least one of the two proteins neither has a direct nor an indirect orthologue in the human proteome.
We therefore suggest these pairs as targets for combinatorial chemotherapy. The comparative genomics pipeline (Figure 1) is built with self-developed Python scripts that are available for download at the GitHub repository (https://github.com/suvisubra/SynthLeth).

The final set of 37 pairs was enriched in druggable proteins (Table 1). Of the 55 proteins in the set, 30 had either been validated as drug targets or had a positive 'druggability index' as predicted by TDRtargets (17). Some of the suggested combinations affected the same pathway, e.g. the pyridoxal kinase-like protein and pyridoxine biosynthesis protein involved in vitamin B6 metabolism, or NAD(P)H-dependent glutamate synthase and NADP-specific glutamate dehydrogenase. The latter is selectively inhibited by isophthalic acid (18), while glutamate synthase had been suggested as a target based on comparative genomics (19). Calcineurin subunit B paired with the cation/H+ antiporter PfCHA, which is sensitive to known inhibitors like KB-R7943 (20). Hubs of inferred interactions were apurinic/apyrimidinic endonuclease PfAPN1 and the U5 small nuclear ribonucleoprotein PfSNU114 of the spliceosome, both involved in the processing of nucleic acids. Two proteins in the target set were of particular pharmacological interest: the Ca2+-ATPase PfATP4 and phosphatidylinositol 4-kinase (PfPI4K). Either protein is targeted by new antimalarial candidates (21-27). PfATP4 is the target of cipargamin and paired with PfCHA (Table 1), suggesting to test for potential synergy between cipargamin and KB-R7943. PfPI4K, the target of imidazolopiperazines and MMV390048, paired with ubiquitin-conjugating enzyme E2 (Table 1). An inhibitor of Atg8-Atg3 interaction was identified.
from the MMV Malaria Box (28), and ubiquitin-protein ligase E3 was proposed as an antimalarial target (29). The inferred link between PI4K and ubiquitination suggests to test for potential synergy between PfPI4K inhibitors and *P. falciparum* proteasome inhibitors (30-32).

The present approach critically depends on the existence of *S. cerevisiae* genes that (i) possess synthetic lethal phenotypes and (ii) are orthologous to known *P. falciparum* drug target genes. This seems contradictory: by definition, drug targets are essential and genes with synthetic phenotypes are non-essential. However, we show here that several validated drug targets of *P. falciparum* possess orthologues in *S. cerevisiae* that are non-essential (Table 1). Phosphoethanolamine methyltransferase and phosphatidylinositol 4-kinase (Table 1), for instance, have been demonstrated to be essential enzymes in *P. falciparum* (24, 33). Most of the genes that are conserved between *S. cerevisiae* and *P. falciparum* also have an orthologue in *H. sapiens* (OrthoMCL-DB only contains 80 yeast genes with an orthologue in *P. falciparum* but not in *H. sapiens*). We speculate that of the conserved genes which are essential in yeast, many might also be essential in *H. sapiens* and their products not suitable as drug targets. On the other hand, conserved genes that are devoid of synthetic phenotypes in yeast might also be disposable in *P. falciparum*, and thus not suitable either. The set of conserved genes that have synthetic lethal phenotypes in yeast might be the pharmacologically most interesting.

The proposed algorithm strongly narrows down the target space for antimalarial drug combinations by including potentially synergistic interactions
regarding efficacy against *P. falciparum* as well as toxicity against *H. sapiens*. The fact that it relies on genome-scale experimental data from *S. cerevisiae* rather than *P. falciparum* makes the algorithm straightforward and unbiased, but also difficult to validate experimentally. Presently the experimental testing of the identified target pairs in Table 1 is precluded by the lack of inhibitors for most of the proposed targets. However, we think that the presence of targets like PfPI4K or PfATP4 in Table 1 validates the algorithm and hope that it will inform future combinations of antimalarial molecules that potentiate each other.
Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. We are grateful to Andrew M. Waterhouse for help with Python programming and to Matthias Rottmann for critical reading of the manuscript.
References

1. MMV drug development portfolio. https://www.mmv.org/research-development/mmv-supported-projects.

2. White NJ. 2016. Can new treatment developments combat resistance in malaria? Expert Opin Pharmacother 17:1303-7.

3. WHO. 2010. Guidelines for the treatment of malaria. WHO library.

4. Wells TN, Hooft van Huijsduijnen R, Van Voorhis WC. 2015. Malaria medicines: a glass half full? Nat Rev Drug Discov 14:424-42.

5. Kremsner PG, Krishna S. 2004. Antimalarial combinations. Lancet 364:285-94.

6. Drugs mCGo. 2011. A research agenda for malaria eradication: drugs. PLoS Med 8:e1000402.

7. Diagana TT. 2015. Supporting malaria elimination with 21st century antimalarial agent drug discovery. Drug Discov Today 20:1265-70.

8. Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, Arkin AP, Astromoff A, El-Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Guldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kotter P, LaBonte D, Lamb DC, Lan N, Liang H, Liu L, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, et al. 2002. Functional profiling of the Saccharomyces cerevisiae genome. Nature 418:387-91.

9. Fügi MA, Kaiser M, Tanner M, Schneider R, Mäser P, Guan XL. 2015. Matchmaking for posaconazole through systems thinking. Trends Parasitol 31:46-51.

10. Kaelin WG, Jr. 2005. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer 5:689-98.

11. Roemer T, Boone C. 2013. Systems-level antimicrobial drug and drug synergy discovery. Nat Chem Biol 9:222-31.
12. Conde-Pueyo N, Munteanu A, Sole RV, Rodriguez-Caso C. 2009. Human synthetic lethal inference as potential anti-cancer target gene detection. BMC Syst Biol 3:116.

13. Adl SM, Simpson AG, Farmer MA, Andersen RA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup O, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW, Taylor MF. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J Eukaryot Microbiol 52:399-451.

14. Stark C, Breitkreutz BJ, Reguly T, Boucher L, Breitkreutz A, Tyers M. 2006. BioGRID: a general repository for interaction datasets. Nucleic Acids Res 34:D535-9.

15. Chen F, Mackey AJ, Stoeckert CJ, Jr., Roos DS. 2006. OrthoMCL-DB: querying a comprehensive multi-species collection of ortholog groups. Nucleic Acids Res 34:D363-8.

16. Fischer S, Brunk BP, Chen F, Gao X, Harb OS, Iodice JB, Shanmugam D, Roos DS, Stoeckert CJ, Jr. 2011. Using OrthoMCL to assign proteins to OrthoMCL-DB groups or to cluster proteomes into new ortholog groups. Curr Protoc Bioinformatics Chapter 6:Unit 6 12 1-19.

17. Aguero F, Al-Lazikani B, Aslett M, Berriman M, Buckner FS, Campbell RK, Carmona S, Carruthers IM, Chan AW, Chen F, Crowther GJ, Doyle MA, Hertz-Fowler C, Hopkins AL, McAllister G, Nwaka S, Overington JP, Pain A, Paolini GV, Pieper U, Ralph SA, Riechers A, Roos DS, Sali A, Shanmugam D, Suzuki T, Van Voorhis WC, Verlinde CL. 2008. Genomic-scale prioritization of drug targets: the TDR Targets database. Nat Rev Drug Discov 7:900-7.

18. Aparicio IM, Marin-Menendez A, Bell A, Engel PC. 2010. Susceptibility of Plasmodium falciparum to glutamate dehydrogenase inhibitors—a possible new antimalarial target. Mol Biochem Parasitol 172:152-5.
19. Ludin P, Woodcroft B, Ralph SA, Maser P. 2012. In silico prediction of antimalarial drug target candidates. Int J Parasitol Drugs Drug Resist 2:191-9.

20. Salcedo-Sora JE, Ward SA, Biagini GA. 2012. A yeast expression system for functional and pharmacological studies of the malaria parasite Ca(2)(+)/H(+) antiporter. Malar J 11:254.

21. Rottmann M, McNamara C, Yeung BK, Lee MC, Zou B, Russell B, Seitz P, Plouffe DM, Dharia NV, Tan J, Cohen SB, Spencer KR, Gonzalez-Paez GE, Lakshminarayana SB, Goh A, Suwanarusk R, Jegla T, Schmitt EK, Beck HP, Brun R, Nosten F, Renia L, Dartois V, Keller TH, Fidock DA, Winzeler EA, Diagana TT. 2010. Spiroindolones, a potent compound class for the treatment of malaria. Science 329:1175-80.

22. Goldgof GM, Durrant JD, Ottile S, Vigil E, Allen KE, Gunawan F, Kostylev M, Henderson KA, Yang J, Schenken J, LaMonte GM, Manary MJ, Murao A, Nachon M, Stanhope R, Prescott M, McNamara CW, Slayman CW, Amaro RE, Suzuki Y, Winzeler EA. 2016. Comparative chemical genomics reveal that the spiroindolone antimalarial KAE609 (Cipargamin) is a P-type ATPase inhibitor. Sci Rep 6:27806.

23. Jimenez-Diaz MB, Ebert D, Salinas Y, Pradhan A, Lehane AM, Myrand-Lapierre ME, O’Loughlin KG, Shackleford DM, Justino de Almeida M, Carrillo AK, Clark JA, Dennis AS, Diep J, Deng X, Duffy S, Endsley AN, Fedewa G, Guiguemde WA, Gomez MG, Holbrook G, Horst J, Kim CC, Liu J, Lee MC, Matheny A, Martinez MS, Miller G, Rodriguez-Alejandro A, Sanz L, Sigal M, Spellman NJ, Stein PD, Wang Z, Zhu F, Waterson D, Knapp S, Shelat A, Avery VM, Fidock DA, Gamo FJ, Charman SA, Mirmalis JC, Ma H, Ferrer S, Kirk K, Angulo-Barturen I, Kyle DE, DeRisi JL, Floyd DM, Guy RK. 2014. (+)-SJ733, a clinical candidate for malaria that acts through ATP4 to induce rapid host-mediated clearance of Plasmodium. Proc Natl Acad Sci U S A 111:E5455-62.

24. McNamara CW, Lee MC, Lim CS, Lim SH, Roland J, Simon O, Yeung BK, Chatterjee AK, McCormack SL, Manary MJ, Zeeman AM, Dechering KJ, Kumar
TS, Henrich PP, Gagaring K, Ibanez M, Kato N, Kuhen KL, Fischli C, Nagle A, Rottmann M, Plouffe DM, Bursulaya B, Meister S, Rameh L, Trappe J, Haasen D, Timmerman M, Sauermann RW, Suwaranurk R, Russell B, Renia L, Nosten F, Tully DC, Kocken CH, Glynne RJ, Bodenreider C, Fidock DA, Diagana TT, Winzeler EA. 2013. Targeting Plasmodium PI(4)K to eliminate malaria. Nature 504:248-253.

Spillman NJ, Allen RJ, McNamara CW, Yeung BK, Winzeler EA, Diagana TT, Kirk K. 2013. Na(+) regulation in the malaria parasite Plasmodium falciparum involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. Cell Host Microbe 13:227-37.

Dembele L, Ang X, Chavchich M, Bonamy GMC, Selva JJ, Lim MY, Bodenreider C, Yeung BKS, Nosten F, Russell BM, Edstein MD, Strainer J, Fidock DA, Diagana TT, Bifani P. 2017. The Plasmodium PI(4)K inhibitor KDU691 selectively inhibits dihydroartemisinin-pretreated Plasmodium falciparum ring-stage parasites. Sci Rep 7:2325.

Paquet T, Le Manach C, Cabrera DG, Younis Y, Henrich PP, Abraham TS, Lee MCS, Basak R, Ghidelli-Disse S, Lafuente-Monasterio MJ, Bantscheff M, Ruecker A, Blagborough AM, Zakutansky SE, Zeeman AM, White KL, Shackelford DM, Mannila J, Morizzi J, Scheurer C, Angulo-Barturen I, Martinez MS, Ferrer S, Sanz LM, Gamo FJ, Reader J, Botha M, Dechering KJ, Sauermann RW, Tungtaeng A, Vanachayangkul P, Lim CS, Burrows J, Witty MJ, Marsh KC, Bodenreider C, Rochford R, Solapure SM, Jimenez-Diaz MB, Wittlin S, Charman SA, Donini C, Campo B, Birkholtz LM, Hanson KK, Drewes G, Kocken CHM, Delves MJ, Leroy D, Fidock DA, et al. 2017. Antimalarial efficacy of MMV390048, an inhibitor of Plasmodium phosphatidylinositol 4-kinase. Sci Transl Med 9.

Hain AU, Bartee D, Sanders NG, Miller AS, Sullivan DJ, Levitskaya J, Meyers CF, Bosch J. 2014. Identification of an Atg8-Atg3 protein-protein interaction inhibitor from the medicines for Malaria Venture Malaria Box active in blood and liver stage Plasmodium falciparum parasites. J Med Chem 57:4521-31.
29. Jain J, Jain SK, Walker LA, Tekwani BL. 2017. Inhibitors of ubiquitin E3 ligase as potential new antimalarial drug leads. BMC Pharmacol Toxicol 18:40.

30. LaMonte GM, Almaliti J, Bibo-Verdugo B, Keller L, Zou BY, Yang J, Antonova-Koch Y, Orjuela-Sanchez P, Boyle CA, Vigil E, Wang L, Goldgof GM, Gerwick L, O’Donoghue AJ, Winzeler EA, Gerwick WH, Ottolie S. 2017. Development of a potent inhibitor of the Plasmodium proteasome with reduced mammalian toxicity. J Med Chem doi:10.1021/acs.jmedchem.7b00671.

31. Li H, O’Donoghue AJ, van der Linden WA, Xie SC, Yoo E, Foe IT, Tilley L, Craik CS, da Fonseca PC, Bogyo M. 2016. Structure- and function-based design of Plasmodium-selective proteasome inhibitors. Nature 530:233-6.

32. Tschan S, Brouwer AJ, Werkhoven PR, Jonker AM, Wagner L, Knittel S, Aminake MN, Pradel G, Joanny F, Liskamp RM, Mordmuller B. 2013. Broad-spectrum antimalarial activity of peptido sulfonyl fluorides, a new class of proteasome inhibitors. Antimicrob Agents Chemother 57:3576-84.

33. Bobenchik AM, Witola WH, Augagneur Y, Nic Lochlainn L, Garg A, Pachikara N, Choi JY, Zhao YO, Usmani-Brown S, Lee A, Adjalley SH, Samanta S, Fidock DA, Voelker DR, Fikrig E, Ben Mamoun C. 2013. Plasmodium falciparum phosphoethanolamine methyltransferase is essential for malaria transmission. Proc Natl Acad Sci U S A 110:18262-7.
Figure legend

Figure 1. Graphic representation of the algorithm with numbers of *P. falciparum* gene pairs that passed the filters; the final 37 are shown in Table 1 (yellow, *S. cerevisiae*; blue, *P. falciparum*; red, *H. sapiens*).
Table 1. Pairs of *P. falciparum* proteins suggested as targets for combinatorial chemotherapy based on synthetic lethal genetic interaction in *S. cerevisiae*.

| Gene1 ID     | Gene1 product                                      | Gene2 ID     | Gene2 product                                      |
|--------------|----------------------------------------------------|--------------|---------------------------------------------------|
| PF14_0492    | Calcineurin subunit B                              | PFF0170w     | Cation/H+ antiporter PFCHA                         |
| PF0590c      | Non-SERCA-type Ca2+ transporting P-ATPase 4        | PFF0170w     | Cation/H+ antiporter PFCHA                         |
| PF0485w      | Phosphatidylinositol 4-kinase                      | PFF0305c     | Ubiquitin-conjugating enzyme E2                    |
| PF08_0311    | Dicarboxylate/tricarboxylate carrier               | mal_mito_2   | Cytochrome c oxidase subunit 1                     |
| PFF1105c     | Chorismate synthase                                | PF14_0511    | Glucose-6-phosphate dehydrogenase                  |
| PFL2465c     | Thymidylate kinase                                 | PF13_0176    | Apurinic/apyrimidin endonuclease                    |
| MAL13P1.346  | DNA repair endonuclease                            | PF13_0176    | Apurinic/apyrimidin endonuclease                    |
| PF0160w      | ERCC1 nucleotide excision repair protein           | PF13_0176    | Apurinic/apyrimidin endonuclease                    |
| PFF0715c     | Endonuclease III homologue                         | PF13_0176    | Apurinic/apyrimidin endonuclease                    |
| PFD0865c     | Cdc2-related protein kinase 1                      | PFF0165c     | Conserved plasmodium protein, unknown function     |
| PFL1635w     | Sentrin-specific protease 1                        | PF10_0092    | Metallopeptidase                                   |
| PF13_0251    | DNA topoisomerase 3                                | PF10_0092    | Metallopeptidase                                   |
| PFF0750w     | Pyridoxal kinase-like protein                      | PFF1025c     | Pyridoxine biosynthesis protein                    |
| PFL1407w     | Histone acetyltransferase                          | PF10_0041    | US small nuclear ribonucleoprotein                 |
| PFL2440w     | DNA repair protein                                 | MAL7P1.94    | Prefoldin subunit 3                               |
| PF11_0087    | DNA repair protein                                 | PF10_0041    | US small nuclear ribonucleoprotein                 |
| PF00445c     | ATP-dependent RNA helicase                         | PF10_0041    | US small nuclear ribonucleoprotein                 |
| PF0925c      | ATP-dependent RNA helicase                         | PF10_0041    | US small nuclear ribonucleoprotein                 |
| PF10_0294    | Pre-mRNA-splicing factor ATP-dependent RNA helicase| PF10_0041    | US small nuclear ribonucleoprotein                 |
| PFC0365w     | Pre-mRNA-processing factor 17                      | PF10_0041    | US small nuclear ribonucleoprotein                 |
| PFD0685c     | Structural maintenance of chromosomes protein 3    | PF10_0041    | US small nuclear ribonucleoprotein                 |
| MAL13P1.214  | Phosphoethanolamine N-methyltransferase            | PFA0455c     | Fatty acid elongation protein GNS1/SUR4 family     |
| MAL13P1.214  | Phosphoethanolamine N-methyltransferase            | PFF1180w     | Anaphase-promoting complex subunit 3               |
| MAL8P1.17    | Protein disulfide isomerase                         | PF10_0092    | Metallopeptidase                                   |
| MAL8P1.17    | Protein disulfide isomerase                         | PF080920w    | DNAJ protein                                       |
| PF07_0029    | Heat shock protein 86                              | MAL13P1.139  | Mitochondrial fission 1 protein                    |
| PF07_0029    | Heat shock protein 86                              | PF0300w      | Vacular protein sorting-associated protein 46      |
| PF14_0068    | rRNA 2'-O-methyltransferase fibrillarin            | PF1180w      | Anaphase promoting complex subunit                 |
| PF14_0261    | Proliferation-associated protein 2g4               | PF14_0612    | Zinc finger protein                                |
| PF14_0286    | NADP-specific glutamate dehydrogenase              | PF14_0334    | NAD(P)H-dependent glutamate synthase               |
| PF14_0401    | tRNA import protein                                | PF13_0257    | Glutamate-tRNA ligase                              |
| PFC0510w     | E3 ubiquitin-protein ligase                        | PF0300w      | Vacular protein sorting-associated protein 46      |
| PFE0750c     | Pre-mRNA-splicing factor                           | PF14_0688    | Pre-mRNA-splicing factor ISY1                      |
| PFF1385c     | Conserved Plasmodium protein                       | PB0920w      | DNAJ protein                                       |
| PFL1140w     | Vacular iron transporter                           | PFL0725w     | Thioredoxin peroxidase 2 (Trx-Px2)                 |
S. cerevisiae synthetic lethal gene pair

Gene A  Gene B

Both genes have an orthologue in P. falciparum

Gene A'  Gene B'

Not more than one has a direct orthologue in H. sapiens

Gene A''

Not more than one has an indirect orthologue in H. sapiens

A  or  A'

16217

1505

64

37