國立臺灣大學生命科學院基因體與系統生物學學位學程

碩士論文

Genome and Systems Biology Degree Program

College of Life Science

National Taiwan University

Master Thesis

酵母菌種間之雜種優勢：溫度耐受範圍的擴展

Extended range of temperature tolerance as a form of hybrid vigor in *Saccharomyces* yeast

陳聖安

Samuel Sheng-An Chen

指導教授：呂俊毅 博士

Advisor: Jun-Yi Leu, Ph.D.

中華民國 105 年 7 月

July 2016

doi:10.6342/NTU201602586

國立臺灣大學碩士學位論文
口試委員會審定書

酵母菌種間之雜種優勢：溫度耐受範圍的擴展

Extended range of temperature tolerance as a form of hybrid vigor in *Saccharomyces* yeast

本論文係陳聖安君（R03B48005）在國立臺灣大學基因體與系統生物學學位學程完成之碩士學位論文，於民國 105 年 07 月 21 日承下列考試委員審查通過及口試及格，特此證明

口試委員：

______________________（簽名）

（指導教授）

__________ __________

__________ __________

__________ __________

基因體與系統生物學學位學程主任 ______________（簽名）

doi:10.6342/NTU201602586

致謝

When I was an undergraduate, I felt that doing fundamental research is the most attractive job in the world. Similar to astronauts exploring the unknown regions of the universe, researchers also explore the unknowns of fundamental sciences, a career deemed highly noble and dreamy by naïve students like me. Without much difficulties, I managed to get into the Genomics and Systems Biology degree program of the best university in Taiwan. Being overconfident, I was eager to begin my work, and determined to have enough results to tell an amazing story by the end of my master degree.

What actually followed were long periods of continuous struggles and generation of doubts against myself. The difficulty of doing research is beyond my imagination, and I could not have made it to the end without the help and support from the kind people around me. Some provided knowledge when I was lost doing experiments, while others encouraged when I was mentally exhausted. Discouragements were also well appreciated, for they urged me to think deeper and work harder beyond my limit. Whether or not I will continue to pursue an academic career in the future, the lessons I have learned in these two years were priceless, and I am forever grateful to those who have mentored me. A big thank you to my parents, who have allowed their son to pursue what he desires freely. A big thank you to my committee members, who have provided good suggestions toward this project. Finally, a big thank you to the members of N411 lab, who are the brilliant people that have influenced me the most in these two years.

Samuel Chen

2016.8.12

doi:10.6342/NTU201602586

酵母菌種間之雜種優勢：溫度耐受範圍的擴展

Samuel Sheng-An Chen[†*], and Jun-Yi Leu[†*]

[†]Institute of Molecular Biology, Academia Sinica
[*]Genome and Systems Biology Degree Program, National Taiwan University

摘要

雜種優勢(Hybrid vigor)是指不同物種間的雜交種(Hybrid)之生物特性優越於其父母。 雜種優勢的現象在植物和動物間相當普遍，然而，目前尚未有研究能徹底地解釋雜種優勢的分子機制。在此研究中，我們利用 *Saccharomyces cerevisiae* 及 *Saccharomyces bayanus* 此二種酵母菌來探討其雜種優勢。儘管這兩個物種已經由演化過程適應了不同的溫度範圍，但相較於親代，雜交種卻有更寬廣的溫度耐受範圍。溫度耐受性的擴展可被視為雜種優勢的一種。我們推測在不同的溫度下，雜交種擁有與親代不同的轉錄組(transcriptome)，而部分基因在雜交種裡的獨特表現可能是造成溫度耐受範圍擴展的原因。藉由 DNA 晶片(microarray)，我們比較雜交種與親代在不同溫度下的轉錄組。在高溫下，參與呼吸作用及粒線體轉譯機制的基因有著獨特的雜交種表現量；低溫下，與轉譯機制相關的基因有獨特的表現量。進一步的分析讓我們發現，在雜交種中有獨特表現量的基因，其大部分在親代的表現量已經有所差異，而且兩個親代之表現量差異性多為 trans regulatory divergence 所造成的。然而，雜交種獨特的基因表現與溫度耐受性的擴展間之連結需要進一步的實驗證明。

doi:10.6342/NTU201602586

Extended range of temperature tolerance as a form of hybrid vigor in *Saccharomyces* Yeast

Samuel Sheng-An Chen[†*], and Jun-Yi Leu[†*]

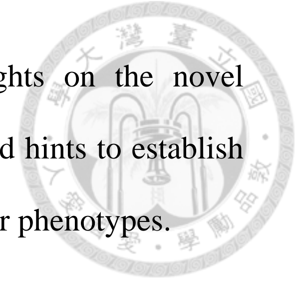
[†]Institute of Molecular Biology, Academia Sinica
[*]Genome and Systems Biology Degree Program, National Taiwan University

ABSTRACT

Hybrid vigor refers to the outperformance of intra- or interspecific hybrids compared to both parents in biological qualities, and is widespread in plants and animals. However, the molecular bases of hybrid vigor remain widely unexplored. Here, we studied the interspecific hybrid between mesophilic yeast *Saccharomyces cerevisiae* and cryophilic yeast *Saccharomyces bayanus*. While the two species have diverged in their fitness at distinct temperatures, the hybrid performed equally well as the better parent at two ends of the temperature spectrum. Furthermore, hybrid vigor was displayed as an extended range of temperature tolerance when compared to the parents. We speculated the novel gene expression patterns of hybrid at different temperatures were responsible for the extended temperature tolerance. Transcriptome comparison between hybrid and the two parents demonstrated that the hybrid displayed different sets of novel expression patterns at distinct temperatures: hybrid-specific expressions were enriched in genes relating to respiration and mitochondrial translation at high temperature, and in genes relating to translational machinery at low temperature. Majority of the hybrid-specific expressions belonged to genes with conditional trans-regulatory divergence between the parental species, suggesting the divergence in temperature sensing of the parents gave rise to novel expression of hybrid. Further evidence is required to demonstrate the importance of hybrid-specific expression in the

doi:10.6342/NTU201602586

hybrid vigor phenomena described. Our results provided insights on the novel regulatory changes upon hybridization of two different species, and hints to establish the connection between these regulatory changes to the hybrid vigor phenotypes.



doi:10.6342/NTU201602586

目　錄

口試委員會審定書-- i

誌謝-- ii

中文摘要--- iii

英文摘要--- iv~v

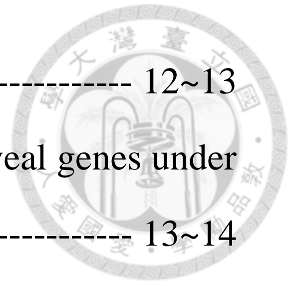
目錄--- vi~vii

Chapter 1. Introduction--- 1~4

Chapter 2. Results-- 4~15

- Interspecific hybrid exhibits extended range of temperature tolerance-- 4
- Hybrid has a selective advantage upon fluctuating temperatures-- -- 4~5
- Hybrid displayed overlapped and distinct sets of mis-regulation at different temperatures-- 5~6
- Mis-regulation at 30°C is associated with respiration and mitochondrial translation--- 6~7
- Mis-regulation at 18°C is associated with translation and ribosome biogenesis--- 7
- Hybrid vigor in respiratory condition at high temperatures-8~9
- Minimal inhibitory concentration of cycloheximide under various temperatures--- 16~17
- Classification of cis and trans regulatory divergence at different temperatures--- 10~12
- Azf1p is a possible transcription factor causing hybrid mis-

doi:10.6342/NTU201602586



regulation at 30°C and 18°C------------------------------------ 12~13

- Compensating and enhancing cis-trans effects reveal genes under diversifying and stabilizing selection------------------------- 13~14
- Hybrid vigor at salt stress condition--------------------------- 14~15

Chapter 3. Discussion--- 16~18

Chapter 4. Materials and Methods------------------------------------ 18~23

- Parental strains-- 18
- Hybrid strain construction-- 19
- Growth rate determination and spot assay at various temperatures-- 19
- Competition assay between hybrid and two parents-------- 19~20
- Transcriptome profiling of hybrid and parents by two-color microarray-- 20~21
- Classification of hybrid mis-regulation--------------------------- 21
- Classification of cis and trans effects--------------------------- 21~22
- GO enrichment analysis-- 22~23
- Motif identification and annotation-------------------------------- 23
- Generation of petite cells via EtBR treatment--------------------- 23
- Real-Time PCR-- 23

Chapter 5. References-- 24~27

Figures-- 28~40

Supplementary figures-- 41~48

doi:10.6342/NTU201602586

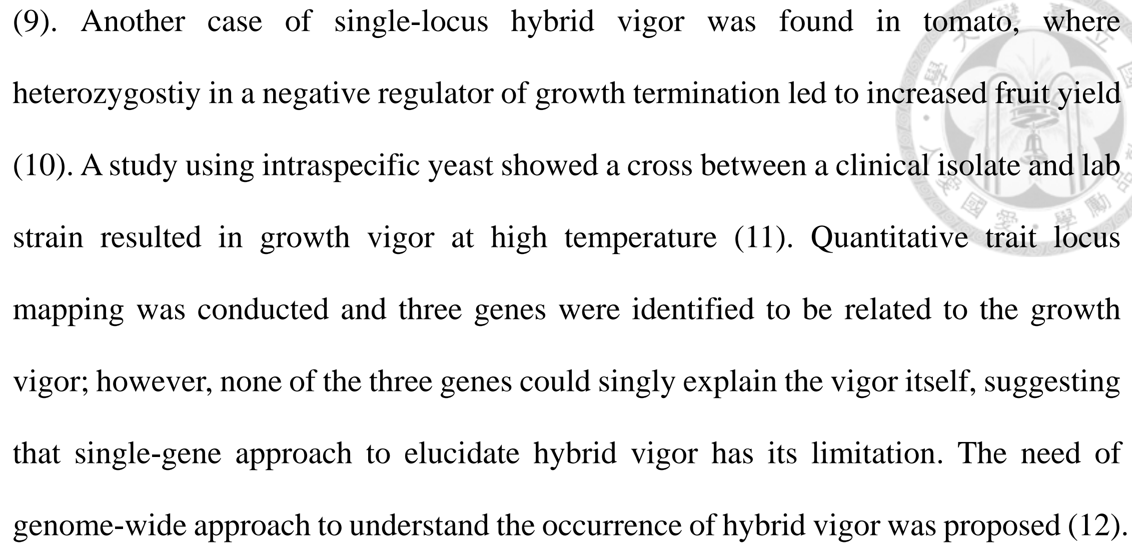
Introduction

In biological terms, species is defined as a group of organisms capable of producing fertile progeny. Hybrid is the result of cross-fertilization between two different species or distant strains, and has been widely reported in animals and plants (1). The interspecific hybrids are often sterile, as a form of reproductive isolation to prevent the rise of new species due to hybridization. Hybrid vigor refers to the superior levels of biomass, growth rate, stress tolerance, or other biological qualities when compared to the parents (2). Hybrid vigor has been studied extensively in plants and revolutionized plant breeding. The use of hybrid maize in the 1930s has steadily increased the yield by six-fold and more (3), for maize hybrid displays increased seed size and grain yield when compared to the parents (4). To this day, we still lack the basic knowledge on the occurrence of hybrid vigor.

Two genetic models have been developed and debated for over a century to explain the molecular bases of hybrid vigor. The first is the dominance model (5), which suggests that the two parental species each contains homozygous, deleterious alleles that inhibit overall performance. Hybrid vigor occurs through masking of deleterious alleles in the hybrid environment. The second is the overdominance model (6), stating that the novel allelic interactions in the hybrid lead to superior performance over the two parents, which are in homozygous states. So far, neither of the two models can on its own fully explain the phenomena of hybrid vigor. For example, studies using similar sets of rice hybrids have shown that both dominance and overdominace models were associated with the growth vigor (7 & 8).

Both single-gene and genome-wide approaches have been used to elucidate the molecular mechanisms of hybrid vigor. An early study using maize demonstrated that heterodimer of the enzyme alcohol dehydrogenase led to growth vigor: heterodimer was shown to be more stable and enzymatically active than both sets of homodimers

doi:10.6342/NTU201602586

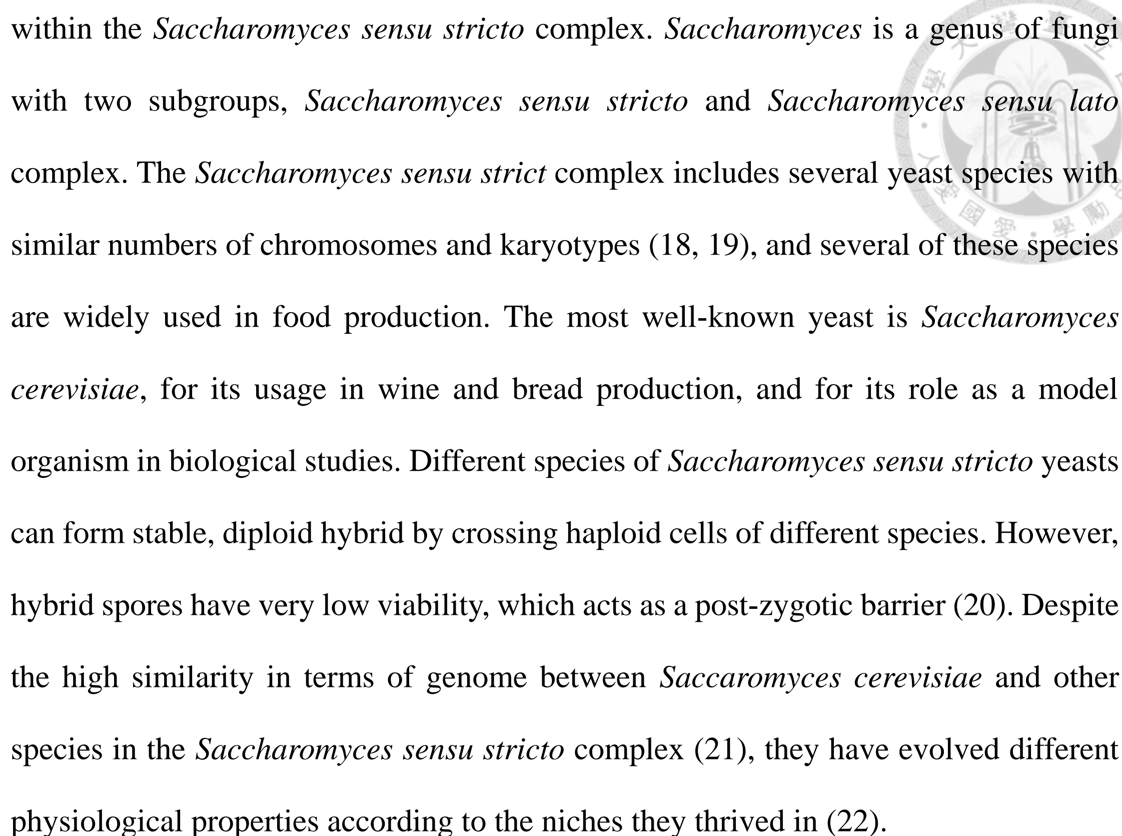


(9). Another case of single-locus hybrid vigor was found in tomato, where heterozygostiy in a negative regulator of growth termination led to increased fruit yield (10). A study using intraspecific yeast showed a cross between a clinical isolate and lab strain resulted in growth vigor at high temperature (11). Quantitative trait locus mapping was conducted and three genes were identified to be related to the growth vigor; however, none of the three genes could singly explain the vigor itself, suggesting that single-gene approach to elucidate hybrid vigor has its limitation. The need of genome-wide approach to understand the occurrence of hybrid vigor was proposed (12).

Genome-wide studies using intra- and interspecific hybrids, including transcriptomic and proteomic approaches, have provided insights into the occurrence of hybrid vigor. First of all, hybrid's novel gene expressions corresponded to alternations of biological networks and pathways. For example, it has been shown in interspecific hybrid of *Arabidopsis*, genes enriched in energy, metabolism, and stress response have altered their expressions relative to the parents (13). In another study using *Arabidopsis thaliana* intraspecific hybrid, genes involved in photosynthesis have altered expressions relative to the parental strains, and this was correlated with the increased photosynthetic capacity of the hybrid. (14) Secondly, genome-wide studies have explored the regulation of hybrid-specific expression. It was suggested that altered gene expressions in the hybrid might arise from trans-regulatory divergence between the parental species (15, 16). Trans-regulatory divergence between the parents led to novel cis-trans interactions in the hybrid environment and resulted in altered gene expression and phenotype of hybrid (16), a reminiscent of overdominace model.

Because of the resourceful genome-wide data provided, yeasts in the *Saccharomyces sensu stricto* clade are suitable materials for research of functional genomics (17), including studies on hybrid vigor where complicated regulation of gene expression are involved (12). Interspecific hybrid can form between yeast species

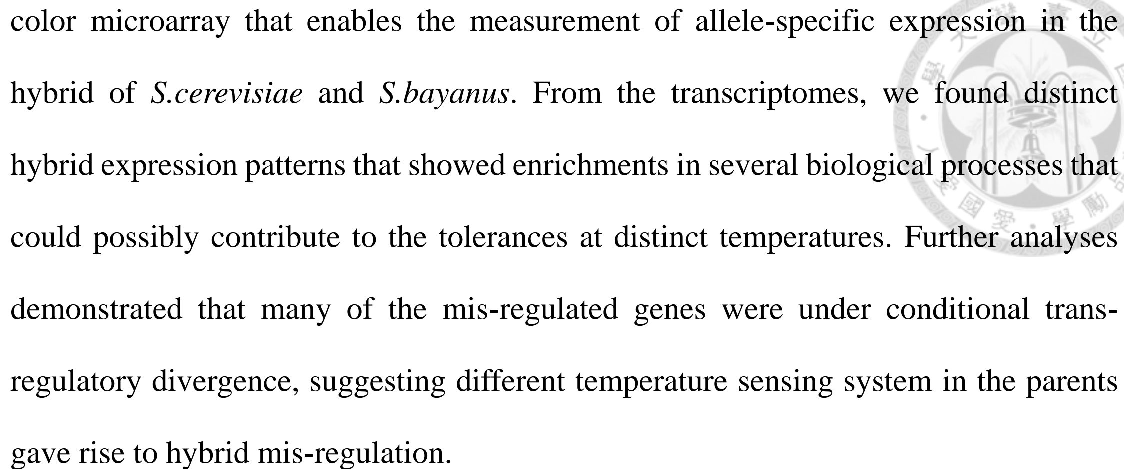
doi:10.6342/NTU201602586



within the *Saccharomyces sensu stricto* complex. *Saccharomyces* is a genus of fungi with two subgroups, *Saccharomyces sensu stricto* and *Saccharomyces sensu lato* complex. The *Saccharomyces sensu strict* complex includes several yeast species with similar numbers of chromosomes and karyotypes (18, 19), and several of these species are widely used in food production. The most well-known yeast is *Saccharomyces cerevisiae*, for its usage in wine and bread production, and for its role as a model organism in biological studies. Different species of *Saccharomyces sensu stricto* yeasts can form stable, diploid hybrid by crossing haploid cells of different species. However, hybrid spores have very low viability, which acts as a post-zygotic barrier (20). Despite the high similarity in terms of genome between *Saccaromyces cerevisiae* and other species in the *Saccharomyces sensu stricto* complex (21), they have evolved different physiological properties according to the niches they thrived in (22).

Interspecific hybrids between different *Saccharomyces sensu stricto* species have been reported in both natural and domestic environments (23, 24). In this study, we specifically looked into the interspecific hybrid between mesophilic yeast *Saccharomyces cerevisiae* and cryophilic yeast *Saccharomyces bayanus*. It is well known that *S.cerevisiae* exhibits higher growth rate than *S.bayanus* at intermediate temperatures, while the reverse is true at low temperature (22). We demonstrated that the interspecific hybrid between *S.cerevisiae* and *S.bayanus* displayed equivalent growth as the better parent throughout the temperature spectrum, and showed an extended range of temperature tolerance than both the parents. Viewing this observation as a form of hybrid vigor, we therefore set out to find the molecular mechanism underlying this phenomenon. We speculated that hybrid-specific expression, or expression that deviates from parental values, could contribute to hybrid's tolerance at distinct temperatures. To search for novel expression patterns in the hybrid, transcriptome comparisons between hybrid and two parents were carried out using two-

doi:10.6342/NTU201602586



color microarray that enables the measurement of allele-specific expression in the hybrid of *S.cerevisiae* and *S.bayanus*. From the transcriptomes, we found distinct hybrid expression patterns that showed enrichments in several biological processes that could possibly contribute to the tolerances at distinct temperatures. Further analyses demonstrated that many of the mis-regulated genes were under conditional trans-regulatory divergence, suggesting different temperature sensing system in the parents gave rise to hybrid mis-regulation.

Results

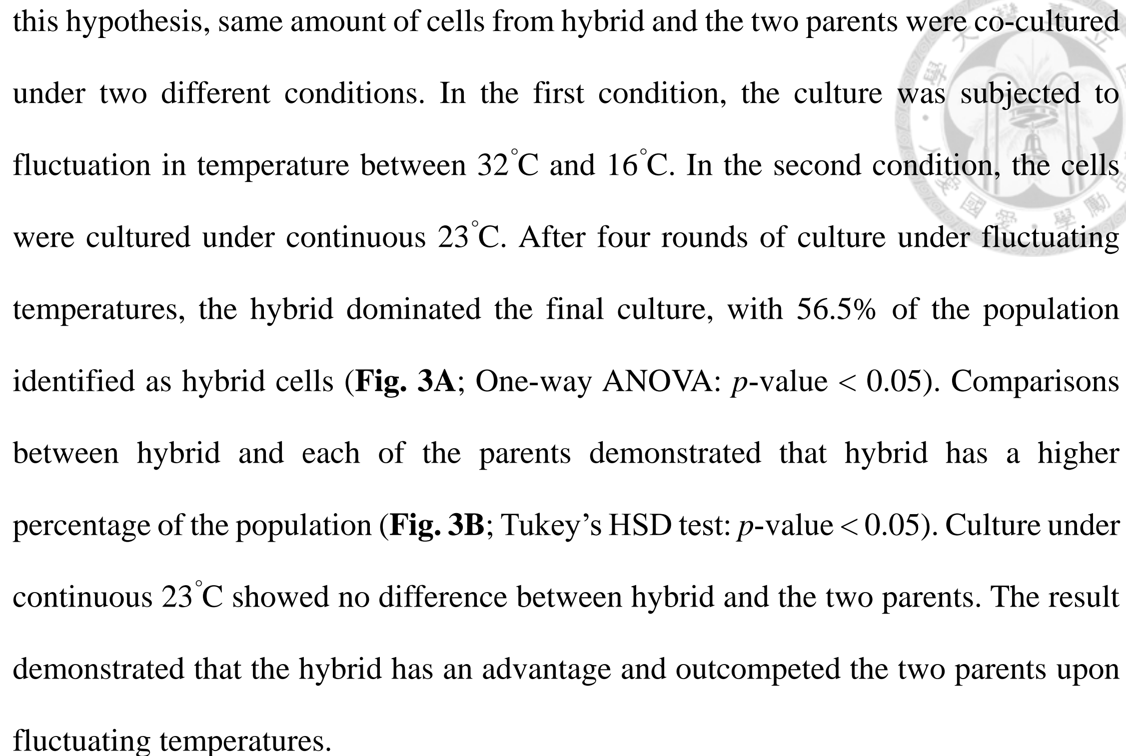
Interspecific hybrid exhibits extended range of temperature tolerance

Hybrid strain was generated by crossing *S.cerevisiae* lab strain BY4741 with *S.bayanus* wild isolate JYL1026 (**Fig. S1**). Hybrid and parents were tested for growth at different temperatures with spot assay; furthermore, the absolute growth rates of hybrid and parents at different temperatures were also measured. Both spot assay and measurements of growth rate demonstrated that the hybrid has equal growth rate as the better-performing parent at both ends of the temperature range (**Fig. 1**). Furthermore, we showed that the hybrid has an extended range of temperature tolerance than both parental species. (**Fig. 2**) In addition, we also tested the interspecific hybrid formed by crossing a wild *S.cerevisiae* strain UWOPS05-217.3 with *S.bayanus*; the resulting hybrid displayed similar growth as the hybrid formed using the lab strain *S.cerevisiae* BY47471 at different tempertures (**Fig S2**).

Hybrid has a selective advantage upon fluctuating temperatures

Because the hybrid has equal fitness as the better-performing parent at two ends of the temperature range, we hypothesized that hybrid should have a selective advantage upon fluctuating temperatures when competing with the two parents. To test

doi:10.6342/NTU201602586

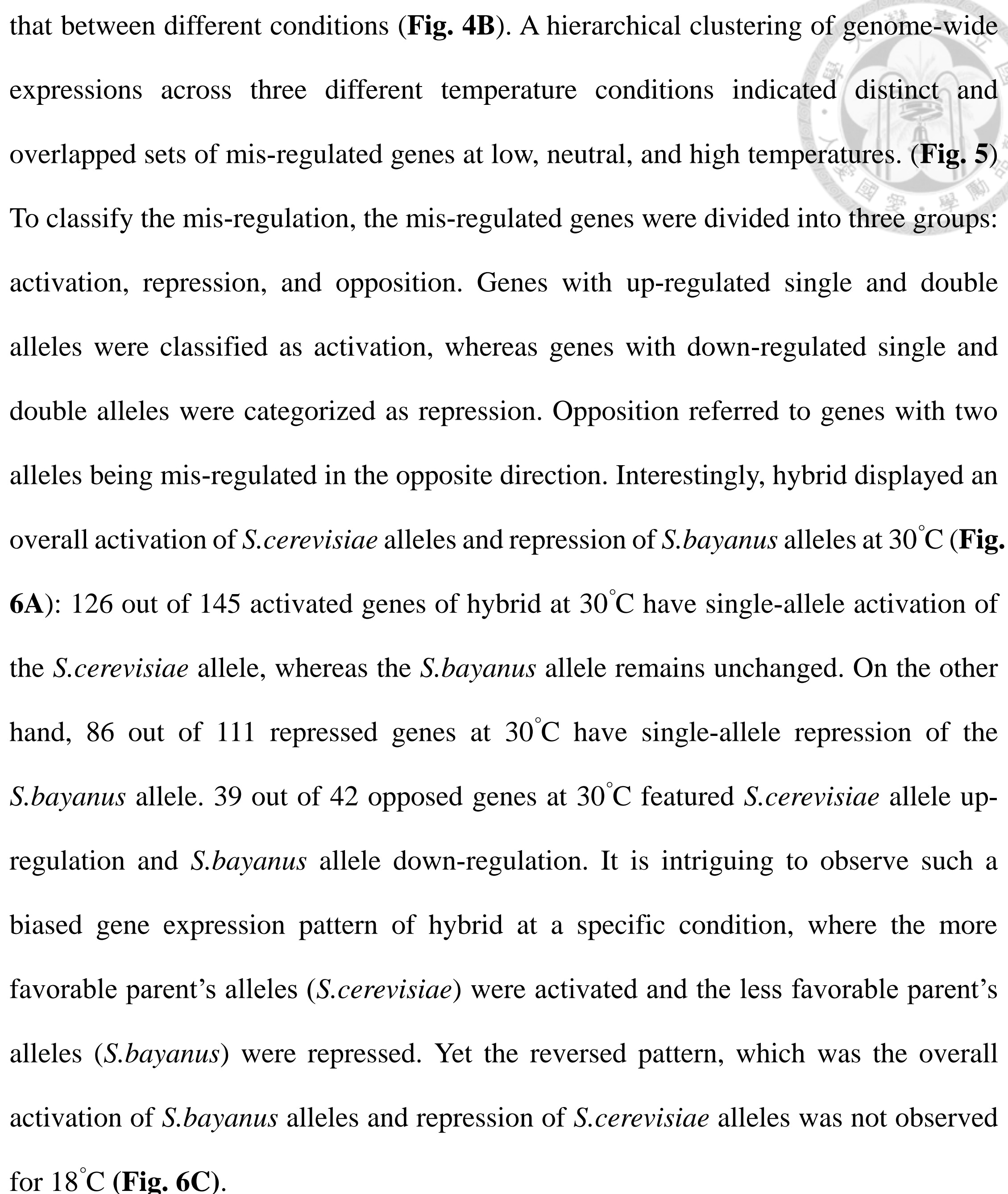


this hypothesis, same amount of cells from hybrid and the two parents were co-cultured under two different conditions. In the first condition, the culture was subjected to fluctuation in temperature between 32°C and 16°C. In the second condition, the cells were cultured under continuous 23°C. After four rounds of culture under fluctuating temperatures, the hybrid dominated the final culture, with 56.5% of the population identified as hybrid cells (**Fig. 3A**; One-way ANOVA: p-value < 0.05). Comparisons between hybrid and each of the parents demonstrated that hybrid has a higher percentage of the population (**Fig. 3B**; Tukey's HSD test: p-value < 0.05). Culture under continuous 23°C showed no difference between hybrid and the two parents. The result demonstrated that the hybrid has an advantage and outcompeted the two parents upon fluctuating temperatures.

Hybrid displayed overlapped and distinct sets of mis-regulation at different temperatures

To investigate the genome-wide expression of hybrids at different temperatures, both hybrid and parental species were cultured in three distinct temperatures: low (18°C), neutral (23°C), and high (30°C) temperature. At early log-phase, total RNA samples were isolated from four independent, isogenic hybrid clones and two parental clones (one each for *S.cerevisiae* and *S.bayanus*). A commercial Agilent gene-expression array was used to simultaneously monitor expression of *S.cerevisiae* and *S.bayanus* alleles for approximately 5000 pairs of orthologs between the two species. A two-color microarray design was used to compare the hybrid trancriptome relative to that of both parents at three different temperatures (**Fig. 4A**). A gene is defined as "mis-regulated" if the fold change between hybrid and parental intensities is equal to or larger than two and the adjusted p-value is less than 0.05 (Details described in material and methods). Correlations between replicates within the same condition were higher than

doi:10.6342/NTU201602586



that between different conditions (**Fig. 4B**). A hierarchical clustering of genome-wide expressions across three different temperature conditions indicated distinct and overlapped sets of mis-regulated genes at low, neutral, and high temperatures. (**Fig. 5**) To classify the mis-regulation, the mis-regulated genes were divided into three groups: activation, repression, and opposition. Genes with up-regulated single and double alleles were classified as activation, whereas genes with down-regulated single and double alleles were categorized as repression. Opposition referred to genes with two alleles being mis-regulated in the opposite direction. Interestingly, hybrid displayed an overall activation of *S.cerevisiae* alleles and repression of *S.bayanus* alleles at 30°C (**Fig. 6A**): 126 out of 145 activated genes of hybrid at 30°C have single-allele activation of the *S.cerevisiae* allele, whereas the *S.bayanus* allele remains unchanged. On the other hand, 86 out of 111 repressed genes at 30°C have single-allele repression of the *S.bayanus* allele. 39 out of 42 opposed genes at 30°C featured *S.cerevisiae* allele up-regulation and *S.bayanus* allele down-regulation. It is intriguing to observe such a biased gene expression pattern of hybrid at a specific condition, where the more favorable parent's alleles (*S.cerevisiae*) were activated and the less favorable parent's alleles (*S.bayanus*) were repressed. Yet the reversed pattern, which was the overall activation of *S.bayanus* alleles and repression of *S.cerevisiae* alleles was not observed for 18°C **(Fig. 6C)**.

<u>Mis-regulation at 30°C is associated with respiration and mitochondrial translation</u>

Conditional-specific mis-regulated genes were input to GOrilla online gene ontology tool (25) for search of biological process enrichment. Interestingly, most of the enrichments at 30°C were related to mitochondria (**Table 1A**): for conditional-specific activation at 30°C, the enrichments were related to the respiratory electron

doi:10.6342/NTU201602586

transport chain; for repression and opposition, the enrichments were found in the mitochondrial translation machinery. Such biased expression patterns of genes related to mitochondria made us question which species' mitochondria does the interspecific hybrid contain, and whether if the hybrid has altered performance of mitochondrial function when compared to the parents.

When we lowered the fold change criteria for selection of mis-regulation from 2 to 1.5 fold at 30°C, a new enrichment for repression termed "negative regulation of mitotic cell cycle" was found; most genes within the enrichment showed single-allele down-regulation of the *Sb*-allele. It is possible that *S.bayanus* induces genes that negatively regulate the mitotic cell cycle at 30°C, which is a stressful condition for *S.bayanus*. However, in the hybrid environment, these negative regulators were lowered in expression to promote overall cell proliferation. Whether or not such repression contributed to the hybrid fitness at 30°C and higher temperatures requires further evidence.

Mis-regulation at 18°C is associated with translation and ribosome biogenesis

At 18℃, the gene ontology enrichments for conditional-specific activation were found in the translation machinery (**Table 1B**); a large part of the enriched genes belong to components of ribosome; for repression, we observed enrichments for amino acid catabolism and lysosomal microautophagy. Previous studies have shown *S.cerevisiae* responded to low temperature (10°~18°C) by up-regulating genes related to the translational machinery and the synthesis of ribosomes (26). A large part of hybrid activation at 18°C was associated with translation, rRNA processing, and ribosome biogenesis. Further experiment is needed to elucidate the importance of such activation.

doi:10.6342/NTU201602586

Hybrid vigor in respiratory condition at high temperatures

Based on the gene ontology enrichment observed, we investigated the component of hybrid mitochondrial DNA and compared the performance of growth on non-fermentable condition with hybrid and parents. Hybrid containing species-specific mitochondrial DNA was acquired by crossing one parent with another "petite" parent. Petite is a term used to describe cells without mitochondrial DNA. Petite parents were acquired by treating haploid parents with ethium bromide overnight, which induced the loss of mitochondrial DNA (27). At low (18℃) and neutral temperature (23℃), there was no difference in growth between hybrids containing either *S.cerevisiae* (*Sc*-M) or *S.bayanus* (*Sb*-M) mitochondrial DNA (**Fig. 7B**). However, at a high temperature (34℃), *Sb*-M hybrid began to show growth defect. The *Sc*-M hybrid behaved similarly to the hybrid used in all previous experiments, including transcriptome profiling. Thus, it is likely that the hybrid preferentially retained the *Sc* but not *Sb* mitochondrial DNA during the process of hybridization despite the fact that we did not purposely select for one certain mitochondria.

We used glycerol as the non-fermentable carbon source for testing respiratory growth. Hybrid displayed similar respiratory growth as *S.bayanus* in the temperature range 18℃ to 23℃ (**Fig. 7A**). Nonetheless, when the temperature condition was 30℃ and higher, hybrid displayed better growth than both parents. From the transcriptome comparison, we observed genes in the respiratory electron transport chain complex were activated with single-allele up-regulation of the *Sc*-allele. However, the samples used for transcriptome comparison were cultured in YPD medium, where the carbon source is the fermentable glucose. With the presence of glucose, budding yeasts preferentially use fermentation to produce energy. It is possible that the same genes were mis-regulated in the same direction in YPGlycerol as in YPD, and such mis-regulation gave rise to the hybrid vigor observed in non-fermentable condition.

doi:10.6342/NTU201602586

When hybrids with distinct mitochondria were tested for respiratory growth, it was obvious that *Sc*-M hybrid performs better than *Sb*-M hybrid (**Fig. 7B**). However, when the temperature was lowered to 10℃, the *Sb*-M hybrid showed superior growth compared to *Sc*-M hybrid in both fermentable and non-fermentable condition.

Minimal inhibitory concentration of cycloheximide under various temperatures

Activated genes for hybrid at 18°C were enriched in biological processes related to translation and ribosome biogenesis. However, it is not clear whether if such mis-regulations contribute to hybrid growth at the low temperatures. In order to gain insights on this phenomenon, we measured the resistance of hybrid and parents toward an inhibitor of translation, cycloheximde. Cycloheximide (CHX) is an inhibitor of protein synthesis in eukaryotic organisms, and it acts by interfering with the translocation step during translation. Resistance towards cyclohexmide was determined by measuring the minimal inhibition concentration of hybrid and parents. Minimal inhibition concentration, or MIC, is the lowest concentration of a drug or chemical that prevents the visible growth of a microbe. We measured the MIC of cycloheximide with hybrid and parents at two distinct temperatures, 16°C and 32°C (**Fig. S3**). The result was puzzling. At high temperature (32°C), *S.bayanus* showed the least resistance with a MIC of 0.025 ug/ml (CHX), and hybrid with intermediate MIC of 0.1 ug/ml; *S.cerevisiae* was the most resistant with a MIC of 0.4 ug/ml. It is not surprising to find that the hybrid showed an intermediate resistance toward a translational inhibitor, for it suggested hybrid has only half the amount of well-performing translational machinery when compared to the better-performing parent, which is *S.cerevisiae*. Nonetheless, the MICs observed at low temperature (16°C) did not support this idea: at 16°C, hybrid and the two parents all displayed the same MIC of 0.2 ug/ml. When comparing the growth of hybrid and parents at lower concentrations of cycloheximide (0.025 and 0.05 ug/ml),

doi:10.6342/NTU201602586

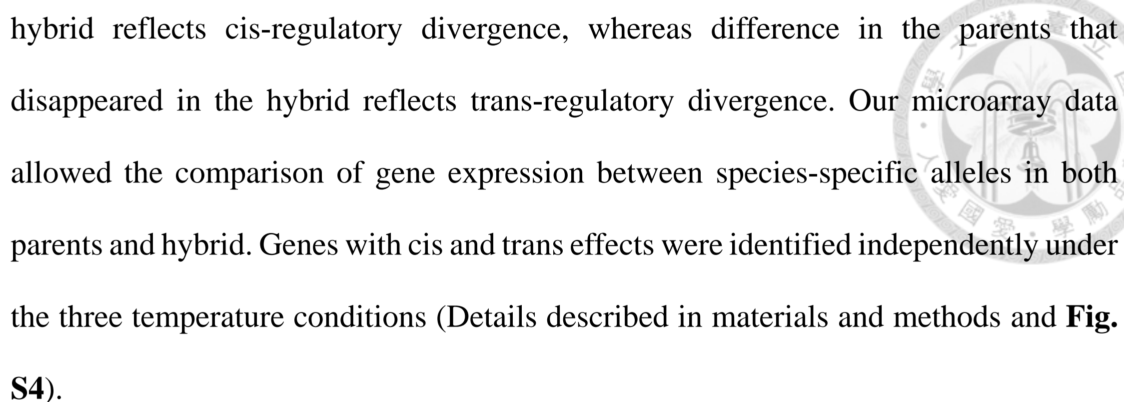
we observed that hybrid displayed similar growth as *S.bayanus*, and *S.cerevisie* had the poorest growth. An intriguing point here was the minor effect of increasing cycloheximide concentration on *S.cerevisiae* at low temperature. Based on this experiment, we cannot draw any conclusive statements regarding the hybrid mis-regulations at 18°C.

Classification of cis and trans regulatory divergence at different temperatures

Genome-wide changes in gene expression in intra- or interspecific hybrid can be attributed to the cis and trans regulatory divergence between strains or species. Gene expression divergence can be characterized by the location of causative mutations. Cis-regulatory divergence can result from mutations in the regulatory sequence, such as promoter elements, or in the coding sequence. Trans-regulatory divergence can result from mutations elsewhere in the genome, such as the upstream transcriptional regulators (13, 14). A study using *Saccharomyces* yeast hybrid suggested hybrid-specific expressions occured through novel cis-trans interactions in the hybird's nuclear environment (13). In another study using F1 allotetraploids of *Arabidopsis thaliana* and *Arabidopsis arenosa*, it was found that *A.arenosa* trans factors tend to up-regulate *A.thaliana* alleles, leading to overall activation of *A.thaliana* alleles and repression *A.arenosa* phenotype in the allotetraploid (14). Therefore, it is of great interest to investigate the relative contribution of cis and trans-regulatory divergence on the mis-regulation observed in the interspecific hybrid under different temperatures.

When comparing the parental expressions, the difference between the two parental alleles may be due to cis, trans, or combinations of the two effects. Within the interspecific hybrid, alleles from the two parents are under the same nuclear environment, implying that trans effects should be masked in the hybrid. Therefore, difference of expression between alleles observed in the parents that maintained in the

doi:10.6342/NTU201602586

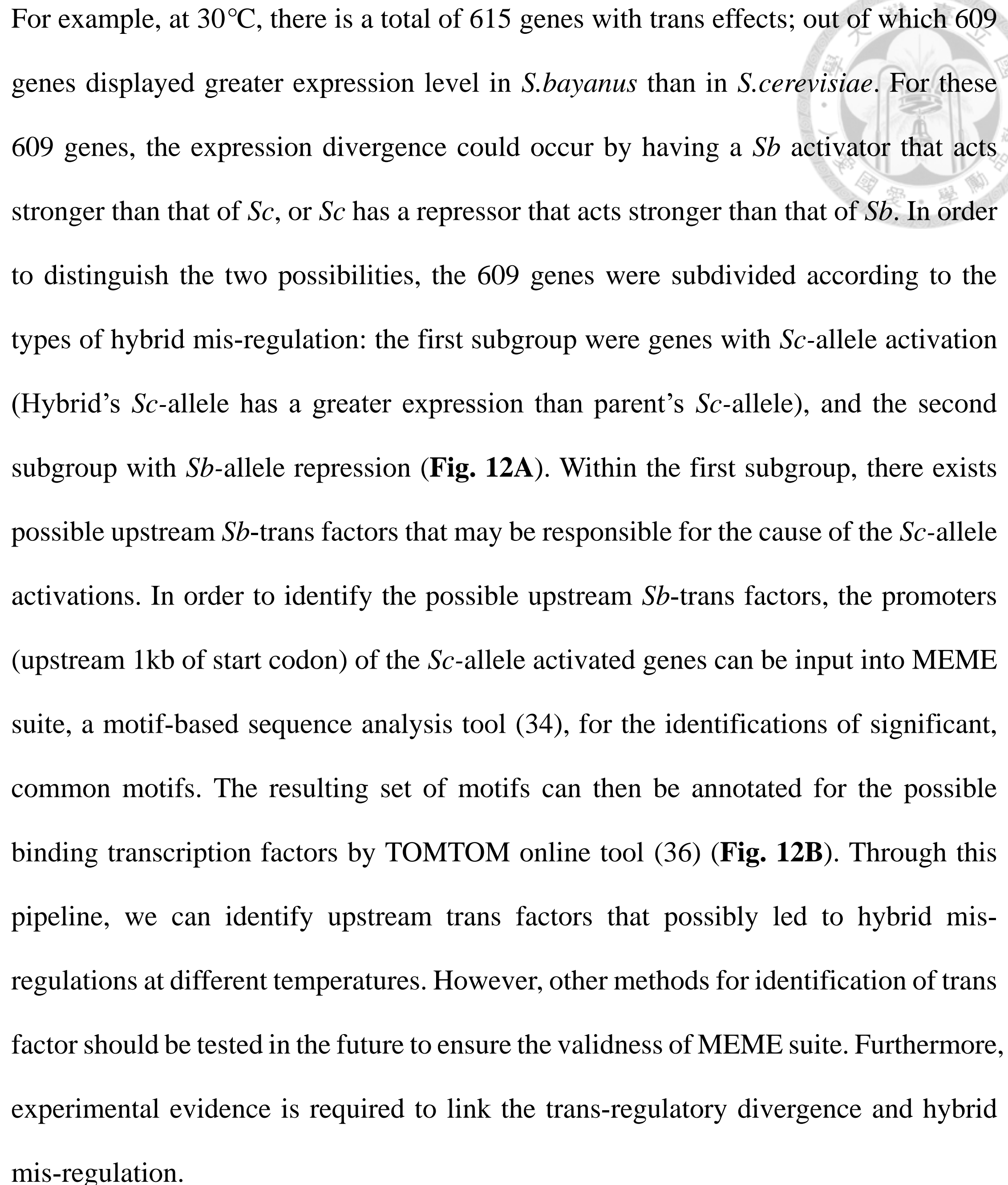


hybrid reflects cis-regulatory divergence, whereas difference in the parents that disappeared in the hybrid reflects trans-regulatory divergence. Our microarray data allowed the comparison of gene expression between species-specific alleles in both parents and hybrid. Genes with cis and trans effects were identified independently under the three temperature conditions (Details described in materials and methods and **Fig. S4**).

In three different temperatures, the relative contribution of cis and trans effects to gene expression divergence varied (**Fig. 8A**). Consistent with previous studies, cis effects dominated in all conditions (13, 14, 24). Furthermore, trans effects were more condition-dependent than cis effects (**Fig. 9**). Next, we asked whether if the mis-regulated genes were enriched for cis or trans effects. At both 30℃ and 18℃, more than 50% of the total mis-regulated genes under two fold change criteria showed trans or cis-trans effects (**Fig. 10**). However, at 23℃, only 21% of the total mis-regulated genes showed trans or cis-trans effects. A comparison between the proportion of cis and trans among mis-regulated genes and genome-wide background (Pearson's chi-squared test: p-value < 0.05) indicated that frequency of having a gene with trans effect in mis-regulated genes was significantly higher than that in genome-wide background; whereas having a gene with cis effect in mis-regulated genes was significantly lower. Here we observed that a majority of the hybrid mis-regulation was associated with trans or cis-trans combined effects at conditions that were stressful for one of the parents. However, this association was weakened at a neutral temperature (23˚C) for both parents. Such finding suggested conditional trans effects at 30˚C and 18˚C gave rise to hybrid mis-regulation, and implied divergence of temperature sensing between the two parents was the cause of hybrid-specific expression.

In order to identify the upstream trans factors causing hybrid mis-regulation, the genes with trans effect were first classified according to parental expression divergence.

doi:10.6342/NTU201602586

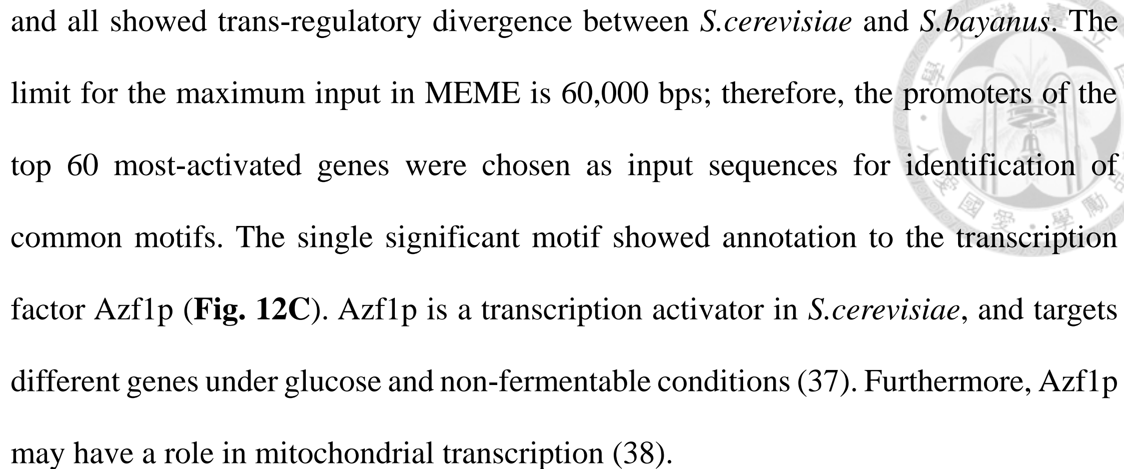


For example, at 30℃, there is a total of 615 genes with trans effects; out of which 609 genes displayed greater expression level in *S.bayanus* than in *S.cerevisiae*. For these 609 genes, the expression divergence could occur by having a *Sb* activator that acts stronger than that of *Sc*, or *Sc* has a repressor that acts stronger than that of *Sb*. In order to distinguish the two possibilities, the 609 genes were subdivided according to the types of hybrid mis-regulation: the first subgroup were genes with *Sc*-allele activation (Hybrid's *Sc*-allele has a greater expression than parent's *Sc*-allele), and the second subgroup with *Sb*-allele repression (**Fig. 12A**). Within the first subgroup, there exists possible upstream *Sb*-trans factors that may be responsible for the cause of the *Sc*-allele activations. In order to identify the possible upstream *Sb*-trans factors, the promoters (upstream 1kb of start codon) of the *Sc*-allele activated genes can be input into MEME suite, a motif-based sequence analysis tool (34), for the identifications of significant, common motifs. The resulting set of motifs can then be annotated for the possible binding transcription factors by TOMTOM online tool (36) (**Fig. 12B**). Through this pipeline, we can identify upstream trans factors that possibly led to hybrid mis-regulations at different temperatures. However, other methods for identification of trans factor should be tested in the future to ensure the validness of MEME suite. Furthermore, experimental evidence is required to link the trans-regulatory divergence and hybrid mis-regulation.

Azf1p is a possible transcription factor causing hybrid mis-regulation at 30°C and 18°C

With the pipeline described previously, possible transcription factors that gave rise to hybrid mis-regulation were identified at different temperatures. At 30°C, only one significant motif were returned from MEME suite with 60 genes as input (MEME suite; E-value < 0.05). The 60 genes were all activated in the hybrid with *Sc*-allele activation,

doi:10.6342/NTU201602586



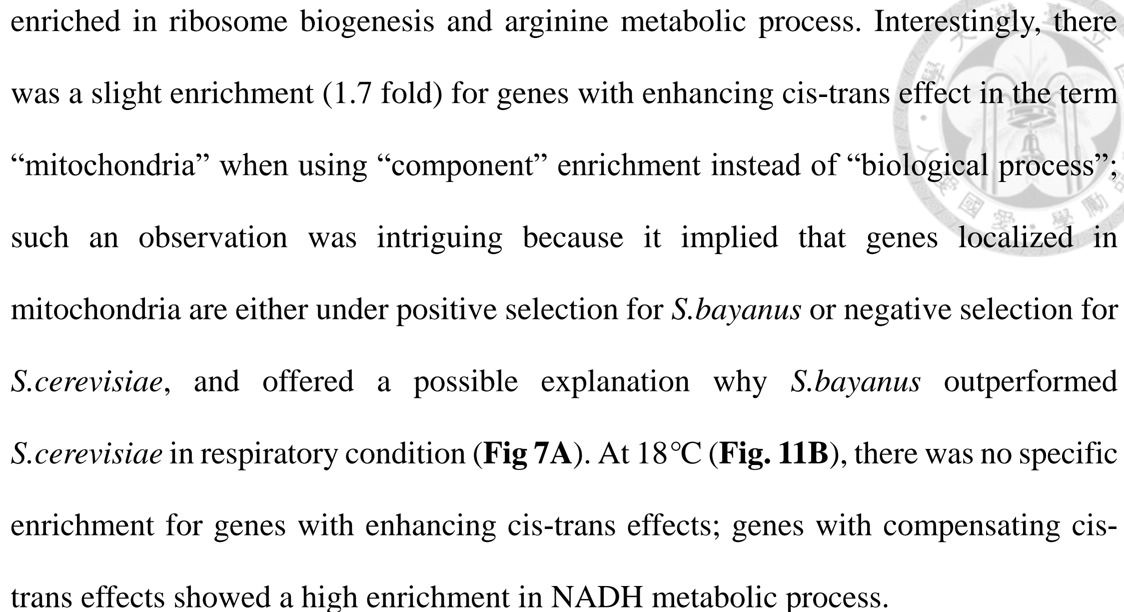
and all showed trans-regulatory divergence between *S.cerevisiae* and *S.bayanus*. The limit for the maximum input in MEME is 60,000 bps; therefore, the promoters of the top 60 most-activated genes were chosen as input sequences for identification of common motifs. The single significant motif showed annotation to the transcription factor Azf1p (**Fig. 12C**). Azf1p is a transcription activator in *S.cerevisiae*, and targets different genes under glucose and non-fermentable conditions (37). Furthermore, Azf1p may have a role in mitochondrial transcription (38).

Surprisingly, we also found Azf1p to be the possible transcription factor causing hybrid mis-regulation at 18°C when using the same approach. It was possible that the two input gene-lists from 30°C and 18 °C have overlapped. Indeed, genes related to mitochondrial translation existed in both lists. Therefore, it is possible that Azf1p acts as a major transcription factor leading to novel gene expression in hybrid at both temperatures.

Compensating and enhancing cis-trans effects reveal genes under diversifying and stabilizing selection

When a gene is under stabilizing selection, cis and trans effects can act in the opposite direction, leading to compensating cis-trans effect that reduces the expression divergence between species or strains. In contrary, a gene is under diversifying selection when cis and trans effects act in the same direction to increase expression divergence, which is termed enhancing cis-trans effect. By examining genes with compensating or enhancing cis-trans effects, we can gain insights on what kind of selection pressures gave rise to expression buffering or divergence between *S.cerevisiae* and *S.bayanus* under various temperature conditions. Gene ontology enrichment was conducted on the genes with compensating and enhancing cis-trans effects at 30℃ and 18℃. At 30℃ (**Fig. 11A**), terms relating to rRNA and mRNA processing were highly enriched in genes with compensating cis-trans effect; genes with enhancing cis-trans effect were

doi:10.6342/NTU201602586



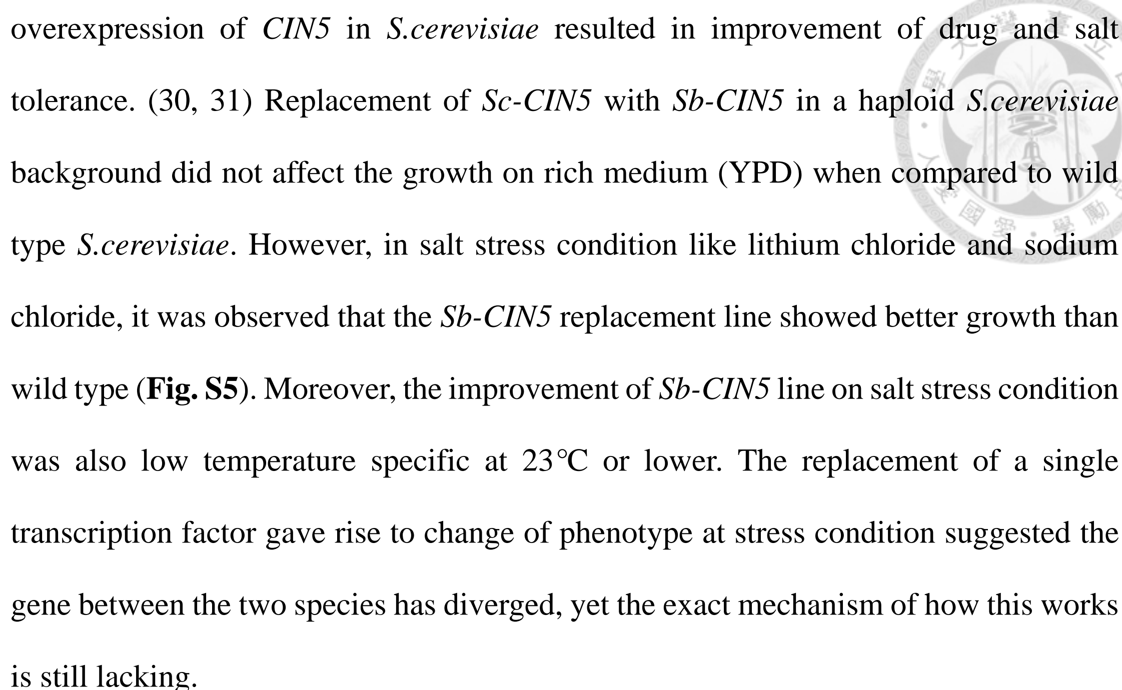
enriched in ribosome biogenesis and arginine metabolic process. Interestingly, there was a slight enrichment (1.7 fold) for genes with enhancing cis-trans effect in the term "mitochondria" when using "component" enrichment instead of "biological process"; such an observation was intriguing because it implied that genes localized in mitochondria are either under positive selection for *S.bayanus* or negative selection for *S.cerevisiae*, and offered a possible explanation why *S.bayanus* outperformed *S.cerevisiae* in respiratory condition (**Fig 7A**). At 18℃ (**Fig. 11B**), there was no specific enrichment for genes with enhancing cis-trans effects; genes with compensating cis-trans effects showed a high enrichment in NADH metabolic process.

<u>Hybrid vigor at salt stress condition</u>

Divergence of transcriptional regulation plays an important role in generating distinct transcriptional networks and possibly novel phenotypes between different species (29). Transcriptional regulation may diverged through the mutations occurring on the transcription factors, or on the promoter and other regulatory elements. Either ways, the mutations can lead to stronger activation or repression of the gene being controlled. Thus, when replacing a species' transcription factor with the ortholog from another species, one can expect a novel expression pattern that may result in hybrid incompatibility or hybrid vigor. A previous project in our lab focused on the hybrid incompatibility caused by divergence of transcriptional regulation between *S.cerevisiae* and *S.bayanus*: possible incompatible transcription factors were selected by those having a high evolving rate (high Ka/Ks) ratio and targeting many other transcription factors. Whether if there exists incompatibility between the *S.cerevisiae* and *S.bayanus* transcription factor was tested by replacing the *Sc* copy of the transcription factor with that of *Sb*.

CIN5 is a transcription factor with structural motif of leucine zipper, and

doi:10.6342/NTU201602586

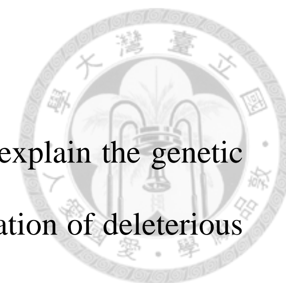


overexpression of *CIN5* in *S.cerevisiae* resulted in improvement of drug and salt tolerance. (30, 31) Replacement of *Sc-CIN5* with *Sb-CIN5* in a haploid *S.cerevisiae* background did not affect the growth on rich medium (YPD) when compared to wild type *S.cerevisiae*. However, in salt stress condition like lithium chloride and sodium chloride, it was observed that the *Sb-CIN5* replacement line showed better growth than wild type (**Fig. S5**). Moreover, the improvement of *Sb-CIN5* line on salt stress condition was also low temperature specific at 23℃ or lower. The replacement of a single transcription factor gave rise to change of phenotype at stress condition suggested the gene between the two species has diverged, yet the exact mechanism of how this works is still lacking.

We speculated that the interspecific hybrid (*Sc x Sb*) may also displayed a different phenotype at salt stress condition when compared to the parents. Spot assay was performed to observe the growth of hybrid and the parents under different salt stress conditions at various temperatures. A very obvious hybrid vigor event was found (**Fig. S6**): in sodium chloride condition, the hybrid displayed superior growth than both parents at 23˚C and 16˚C; as for lithium chloride condition, the hybrid also displayed better growth at 16℃. Whether if the heterozygous state of *CIN5* was responsible for the hybrid vigor observed was examined by deleting *CIN5* in the hybrid background: both *Sc-CIN5* and *Sb-CIN5* were independently deleted from the hybrid genome. There deletions of both copies reduced the hybrid growth in both NaCl and LiCL conditions at low temperatures (**Fig. S7**): the reduction in NaCl condition was not obvious with spot assays, quantification of growth rate is necessary to measure the difference; the reduction in LiCl condition was obvious at 16˚C, nonetheless, the reduction disappeared when a new batch of LiCl plates was prepared. Therefore, we cannot yet draw the conclusions on the contribution of *CIN5* towards hybrid vigor at salt condition.

doi:10.6342/NTU201602586

Discussion



Almost a century ago, two genetic models were proposed to explain the genetic basis of hybrid vigor. Domiance model proposed that complementation of deleterious recessive alleles in the hybrid gave rise to the vigor (4). Overdomiance model stated the presence of heterozygosity resulted in the vigor (5). Since then, evidences from studies of hybrids supported the notion that neither of the two models could fully explain hybrid vigor on its own. Yet, few have provided the detailed molecular mechanism on the occurrence of hybrid vigor. Our study focused on a case of hybrid vigor observed in the interspecific hybrid between budding yeasts *Saccharomyces cerevisiae* and *Saccharomyces bayanus*: the interspecific hybrid displayed an extended range of temperature tolerance than both parental species. Transcriptome profiling of hybrid and parents at distinct temperatures were performed. Recent transcriptomic studies on hybrid provided possible links between novel phenotype of hybrid and hybrid-specific expression patterns. We also made the same speculation: could hybrid-specific expressions at distinct temperatures be responsible for the extended range of tolerance? The hybrid mis-regulation we identified did provide possible links between hybrid expression and hybrid phenotype: at 30°C, *Sc*-alleles related to respiration and mitochondria were up-regulated in hybrid when compared to *S.cerevisiae* parent, and we observed that hybrid displayed better growth than both parents in non-fermentable condition. In addition, *Sb*-alleles related to negative regulation of mitosis were down-regulated in hybrid when compared to *S.bayanus* parent at 30°C, suggesting that hybrid mitosis proceeded normally by suppressing these *Sb*-alleles. At 18°C, we observed that *Sc*-alleles related to the translation machinery were up-regulated. It is well known that *S.cerervisiae* responses to cold stress by up-regulating genes related to the translation machinery. Therefore, such up-regulations could be meaningful for the hybrid to adapt

doi:10.6342/NTU201602586

to cold stress. Although we have characterized the hybrid mis-regulation at different temperatures, further experimental evidence is required to demonstrate the contribution of mis-regulation to hybrid fitness under different temperatures.

In order to elucidate the importance of hybrid mis-regulation, we must first understand how the mis-regulation occurred. We suspected that divergence of trans-regulatory signaling is a major cause of mis-regulation. Therefore, genome-wide analyses were performed to classify the genes with cis- and trans-regulatory divergence using the microarray data from three different temperatures. Two interesting observations can be seen from the analyses on cis- and trans-regulatory divergence. The first interesting observation was that a large portion of the mis-regulated genes displayed trans-regulatory divergence; this was the most obvious at 30°C, where 34% of total mis-regulated genes were under trans effects. The second observation was that trans effects were more condition-dependent than cis effects. By combining the two observations together, we could generate a hypothesized scenario on how the hybrid mis-regulation occurred: the two parents have already diverged in their response to specific temperatures, such as 30°C. At 30°C, *S.bayanus* generates conditional trans factors that are not present in *S.cerevisiae*, for this temperature is a heat stress for *S.bayanus* but not *S.cerevisiae*. When in a hybridized nuclear environment, all the *Sb* conditional trans factors can act upon the cis-elements of *Sc*-alleles and lead to mis-regulation of *Sc*-allele.

Despite the seemingly large contribution of trans-regulatory divergence toward hybrid mis-regulation, many mis-regulated genes showed no trans or cis-trans effect according to our analyses. One possibility is that the trans effect does exist but was not classified by our pipeline due to technical errors. Another possibility is that there are other factors that led to hybrid mis-regulation other than trans-regulatory divergence. One intriguing hypothesis is that the newly organized chromatin structures in the hybrid

doi:10.6342/NTU201602586

environment caused specific regions of the genome to express differentially from parents.

What are some other possibilites for hybrid's extended range of temperature tolerance? An early study using maize tetraploid hybrid showed that there is a dosage component in hybrid vigor: hybrid tetraploids with constitution of AAAB or ABBB were less vigorous than AABB. A similar scenario could be true in *Sc* x *Sb* interspecific hybrid's extended range of temperature tolerance. Perhaps one ploidy of *S.cerevisiae* in the hybrid genome is sufficient to allow the hybrid cell to grow at 30°C and higher temperatures, and the reverse is true for low temperatures. In order to test this idea, we should construct tetraploid hybrids with different ploidy of *S.cerevisiae* and *S.bayanus* geonmes, and test the different tetraploid hybrids at various temperatures. If the dosage hypothesis is correct, we should observe the different performances of hybrids with different genomic compositions.

Materials and Methods

Parental strains

The parental *Saccharomyces cerevisiae* strain is a cross between lab strains BY4741 (*MAT*a *HIS3 leu2Δ0 LYS2 met15Δ0 URA3*; a cassette carrying mCherry fluorescent protein and *URA3*-selection marker has been inserted) and BY4742 (*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0*). The parental *Saccharomyces bayanus* strain is a cross between wild isolates JYL1026 (*MAT*α *his3Δ leu2Δ URA3*) and JYL1027 (*MAT*a *his3Δ leu2Δ URA3*) collected by Dr. Duccio Cavalieri (University of Florence, Italy). The F1 interspecific hybrid strain is a cross between BY4741 and JYL1026. The details about the parents and hybrid strain are labeled in the supplementary data (**Table S1**).

doi:10.6342/NTU201602586

Hybrid strain construction

S.cerevisiae BY4741 strain (*MAT*a) and *S.bayanus* JYL1026 strain (*MAT*α) were used to generate the F1 interspecific hybrid. BY4741 strain contains a *HIS3* selectable marker. JYL1026 strain contains a *URA3* selectable marker The BY4741 and JYL1026 strains were grown in liquid YPD medium to log-phase, mixed in equal amounts and spotted on YPD agar plate at 28°C for 3 hours for mating. The cells were then spread on CSM agar plate without addition of histidine (CSM-His) and uracil (CSM-Ura) for the selection of interspecific hybrids. Individual clones were picked and examined with halo assay to confirm the mating type. In addition, species-chromosome specific primers were used to confirm the presence of species-specific chromosomes.

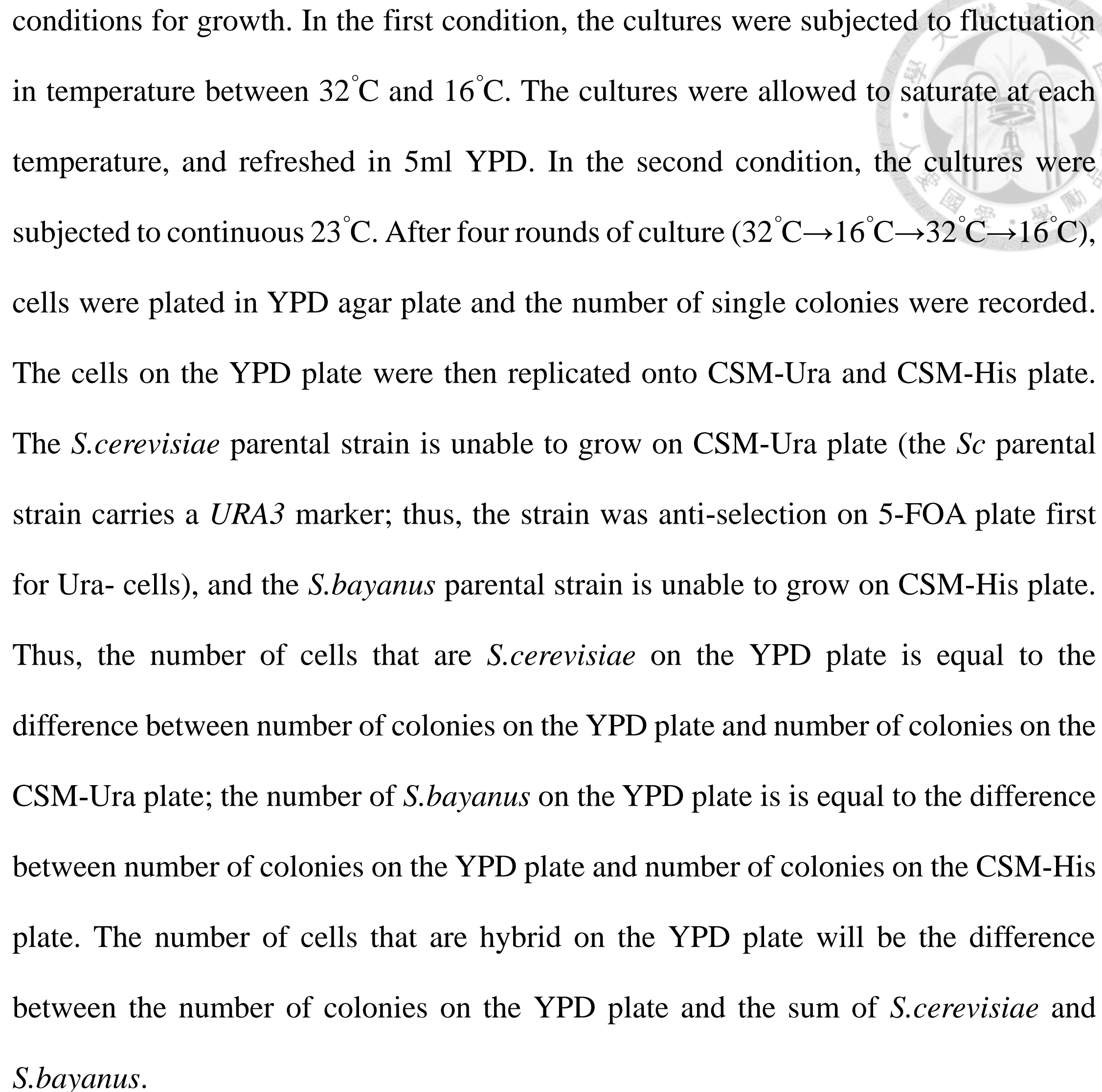
Growth rate determination and spot assay at various temperatures

Overnight culture of hybrid and parent were refreshed in YPD medium at various temperatures. Absorbance at OD600 were measured at one hour intervals for eight continuous hours by spectrophotometer. (At low temperatures, the absorbance was recorded every two hours for 16 continuous hours.) Data points within log-phase (OD600 range: 0.2~1) were applied for least square fitting method for the calculation of absolute doubling time and growth rate. Spot assay were conducted first by diluting the overnight culture into 5 different 10-fold serial dilutions (concentrations ranging from $1x10^6$/ml to $1x10^2$/ml), and spotted on agar plates.

Competition assay between hybrid and two parents

Hybrid and parents were cultured in YPD medium overnight at 23˚C. Equal amount of cells from hybrid and parents from the overnight culture were refreshed in the same culture (5ml YPD) to reach a beginning OD_{600} of 0.1. The cultures (four independent hybrid clones) were then placed under two different temperature

doi:10.6342/NTU201602586



conditions for growth. In the first condition, the cultures were subjected to fluctuation in temperature between 32°C and 16°C. The cultures were allowed to saturate at each temperature, and refreshed in 5ml YPD. In the second condition, the cultures were subjected to continuous 23°C. After four rounds of culture (32°C→16°C→32°C→16°C), cells were plated in YPD agar plate and the number of single colonies were recorded. The cells on the YPD plate were then replicated onto CSM-Ura and CSM-His plate. The *S.cerevisiae* parental strain is unable to grow on CSM-Ura plate (the *Sc* parental strain carries a *URA3* marker; thus, the strain was anti-selection on 5-FOA plate first for Ura- cells), and the *S.bayanus* parental strain is unable to grow on CSM-His plate. Thus, the number of cells that are *S.cerevisiae* on the YPD plate is equal to the difference between number of colonies on the YPD plate and number of colonies on the CSM-Ura plate; the number of *S.bayanus* on the YPD plate is is equal to the difference between number of colonies on the YPD plate and number of colonies on the CSM-His plate. The number of cells that are hybrid on the YPD plate will be the difference between the number of colonies on the YPD plate and the sum of *S.cerevisiae* and *S.bayanus*.

<u>Transcriptome profiling of hybrid and parents by two-color microarray</u>

Overnight culture of hybrid and parents were refreshed in YPD medium for 12 hours at various temperatures (18°C, 23°C, and 30°C) to reach early log-phase (OD_{600} range: 0.2~0.5) for total RNA isolation. Total RNA was isolated using the RNeasy Mini Kit (Qiagen). The total RNA product was treated with TURBO™ DNase (Life technologies) to remove genomic DNA contamination. The genomic DNA free RNA was then subjected to Nanodrop for checking the $OD_{260/280}$ and $OD_{260/230}$ ratios. Furthermore, the RNA quality was checked with Agilent Bioanalyzer. Invitrogen SuperScript™ kit was then used to reverse transcribed the total RNA to cDNA. Next,

doi:10.6342/NTU201602586

Invitrogen Indirect cDNA Labeling was conducted to label the hybrid cDNA with Alexa 647 (Cy5) and parental cDNA with Alexa 555 (Cy3). Quality of dye-labeled cDNA was checked by loading the labeled cDNA products alongside lambda DNA marker labeled with the same dyes, and running on 1% agarose gel; gel after electrophoresis was scanned by Typhoon FLA 9000.

A commercial Agilent gene expression array (Agilent e-array ScSb) was used to compare the transcriptome of hybrid to that of both parents at different temperatures. The array contains probes that distinctly recognizes *S.cerevisiae* and *S.bayanus* orthologs. Four biological clones of *S.cerevisiae* x *S.bayanus* interspecific hybrids were used in the microarray study. For each temperature condition, equal amount of hybrid sample (10 μg labeled cDNA) and parental sample (*Sc*: 5 μg + *Sb*: 5 μg) were pooled together for hybridization. The hybridization and washing of the microarray was done following Agilent Gene Expression Hybridization and Wash Kits. Scanning of the arrays was done by Agilent DNA Microarray Scanner at the recommended setting for two-color gene expression microarray. The raw signal intensities of the arrays were extracted via Agilent Feature Extraction software.

Classification of hybrid mis-regulation

The raw signal intensities were subjected to quantile and lowess normalization to remove systematic bias between and within arrays. An allele is considered to be “mis-regulated” if it is expressed more than two fold differently between the hybrid and parent with an adjusted p-value less than 0.05 (Benjamini-Hochberg FDR correction).

Classification of cis and trans effects

Ratios between alleles of the same ortholog in parental and hybrid comparison were generated using normalized fluorescent signals from the microarray. Log_2-transformed ratios were used to classify the genes based on cis or trans effects. Cis with

doi:10.6342/NTU201602586

trans effects were derived from the comparison between alleles in the two parents (Parental ratio → A: $\log_2$ (Sc-P/Sb-P)). Cis effects were derived from the comparison between alleles within the hybrid (Hybrid ratio → B: $\log_2$ (Sc-H/Sb-H)), whereas trans effects were derived by subtracting cis effects from that of cis with trans (A-B). Student's t-tests were performed to examine the difference between parental expression ratio (A), hybrid expression ratio (B), and the difference between the two (A – B). The p-values were corrected using Benjamini-Hochberg FDR correction.

A gene is considered to have only cis effect if parental ratio (A) is equal to hybrid ratio (B), and absolute value of hybrid ratio (B) is greater than 1. A gene is considered to have only trans effects if parental ratio does not equal to hybrid ratio, and the absolute value of hybrid ratio is smaller than 1.

If the gene's parental ratio (A) is not equal to hybrid ratio (B), but the absolute value of hybrid ratio (B) is greater than 1, then the gene can be considered as having a combination of cis and trans effects. Genes with cis and trans effects can be further classified into compensating or enhancing cis and trans effects. A gene is under compensating cis-trans effect if the hybrid ratio (B) is greater than the parental ratio (A), such that [{$B > 0$ and $(A-B) < 0$}] or [{$B < 0$ and $(A-B) > 0$}]; enhancing cis-trans effect is described if the hybrid ratio (A) is smaller than the parental ratio (B), such that [{$B > 0$ and $(A-B) > 0$}] or [{$B < 0$ and $(A-B) < 0$}].

GO enrichment analysis

GO enrichment analysis was conducted using the online service of GOrilla (Gene Ontology enRIchment anaLysis and visuaLizAtion tool) (21). All gene lists used for GO enrichment analysis were compared to a whole genome-list (background) containing 4973 genes (the number of total orthologs that can be identified between *S.cerevisiae* and *S.bayanus* using the microarray). A FDR p-value < 0.05 was used for

doi:10.6342/NTU201602586

significant enrichment of gene ontology term.

Motif identification and annotation

Motif identification was implemented using the MEME suite. (21) The 1 kb upstream sequences of target genes were input into MEME for significant motifs identification using zero or one occurrence per sequence model up to 10 motifs; in the model, it is assumed that each sequence may contain at most one occurrence of each motif. The resulting motifs were then submitted to the TOMTOM tool, using YEASTRACT database, for the annotation of motifs. (23)

Generation of petite cells via EtBR treatment

Overnight culture of non-petite cells were refreshed in YPD with EtBR (10 μg/ml) medium for 12 hours and spread on YPD agar plate. Single colonies were selected and tested for respirational growth on YEP + glycerol plate; clones that showed no growth on YEP + glycerol plate were selected as petite cells for further analysis.

Real-Time PCR

Total RNA of samples were extracted by RNeasy Mini Kit (Qiagen). The total RNA product was treated with TURBO™ DNase (Life Technologies) to remove genomic DNA contamination, and quality control was as described previously for the microarray samples. Reverse transcription of total RNA to cDNA was done by High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Relative quantitation of mRNA expressions between samples were carried out by ABI Fast-7500 machine (Applied Biosystems) using SYBR-Green labeling. Analyses of the raw data were done using 7500 Software v2.0.6 (Applied Biosystems).

doi:10.6342/NTU201602586

Reference

1. Chen, Z. Jeffrey. "Molecular mechanisms of polyploidy and hybrid vigor."*Trends in plant science* 15.2 (2010): 57-71.

2. Shull, George Harrison. "What is" heterosis"?." *Genetics* 33.5 (1948): 439.

3. Crow, James F. "90 years ago: the beginning of hybrid maize." *Genetics* 148.3 (1998): 923-928.

4. Riedelsheimer, Christian, et al. "Genomic and metabolic prediction of complex heterotic traits in hybrid maize." *Nature genetics* 44.2 (2012): 217-220.

5. Shull, George H. "The composition of a field of maize." *Journal of Heredity* 1 (1908): 296-301.

6. East, Edward M. "Heterosis." *Genetics* 21.4 (1936): 375.

7. Xiao, Jinhua, et al. "Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers." *Genetics* 140.2 (1995): 745-754.

8. Li, Zhi-Kang, et al. "Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield."*Genetics* 158.4 (2001): 1737-1753.

9. Schwartz, Drew. "Single gene heterosis for alcohol dehydrogenase in maize: the nature of the subunit interaction." *Theoretical and Applied Genetics* 43.3-4 (1973): 117-120.

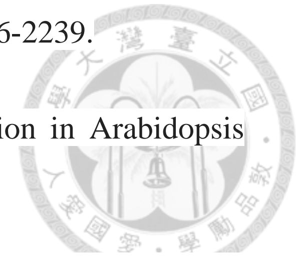
10. Krieger, Uri, Zachary B. Lippman, and Dani Zamir. "The flowering gene SINGLE FLOWER TRUSS drives heterosis for yield in tomato." *Nature genetics* 42.5 (2010): 459-463.

11. Steinmetz, Lars M., et al. "Dissecting the architecture of a quantitative trait locus in yeast." *Nature* 416.6878 (2002): 326-33

12. Birchler, James A., Donald L. Auger, and Nicole C. Riddle. "In search of the

doi:10.6342/NTU201602586

molecular basis of heterosis." *The Plant Cell* 15.10 (2003): 2236-2239.



13. Wang, Jianlin, et al. "Genomewide nonadditive gene regulation in Arabidopsis allotetraploids." *Genetics* 172.1 (2006): 507-517.

14. Fujimoto, Ryo, et al. "Heterosis of Arabidopsis hybrids between C24 and Col is associated with increased photosynthesis capacity." *Proceedings of the National Academy of Sciences* 109.18 (2012): 7109-7114.

15. Tirosh, I., Reikhav, S., Levy, A. A., & Barkai, N. (2009). A yeast hybrid provides insight into the evolution of gene expression regulation. *Science*,*324*(5927), 659-662.

16. Shi, X., Ng, D. W., Zhang, C., Comai, L., Ye, W., & Chen, Z. J. (2012). Cis-and trans-regulatory divergence between progenitor species determines gene-expression novelty in Arabidopsis allopolyploids. *Nature communications*, *3*, 950.

17. Borneman, Anthony R., and Isak S. Pretorius. "Genomic insights into the Saccharomyces sensu stricto complex." *Genetics* 199.2 (2015): 281-291.

18. Barnett, J. A. "The taxonomy of the genus Saccharomyces meyen ex Reess: a short review for non-taxonomists." *Yeast* 8.1 (1992): 1-23.

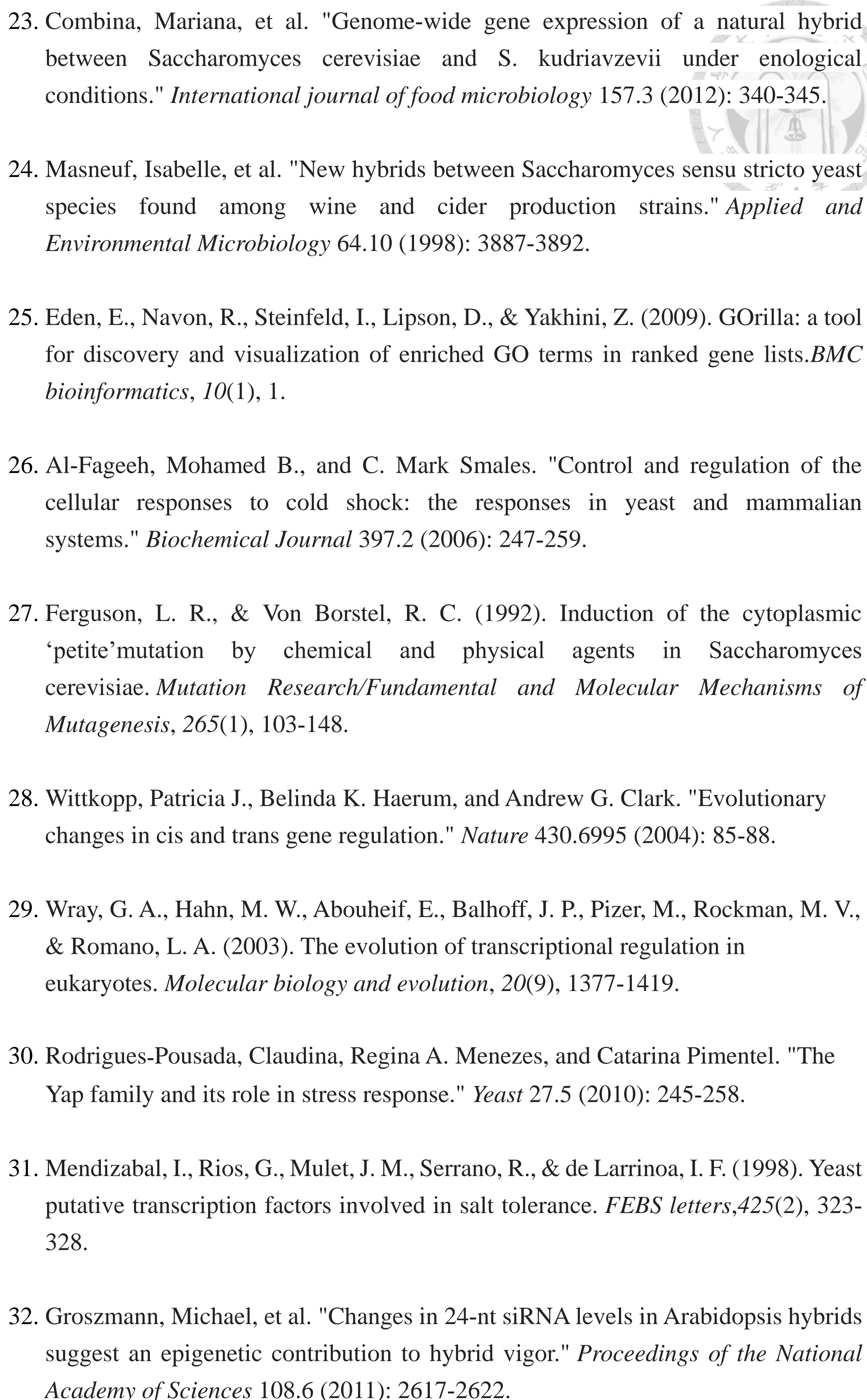
19. Petersen, Randi Føns, Torsten Nilsson-Tillgren, and Jure Piškur. "Karyotypes of Saccharomyces sensu lato species." *International Journal of Systematic and Evolutionary Microbiology* 49.4 (1999): 1925-1931.

20. Lee, Hsin-Yi, et al. "Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species." *Cell* 135.6 (2008): 1065-1073.

21. Naumov, Gennadi I., et al. "Genetic homology between Saccharomyces cerevisiae and its sibling species S. paradoxus and S. bayanus: electrophoretic karyotypes." *Yeast* 8.8 (1992): 599-612.

22. Kishimoto, Munekazu. "Fermentation characteristics of hybrids between the cryophilic wine yeast Saccharomyces bayanus and the mesophilic wine yeast Saccharomyces cerevisiae." *Journal of fermentation and bioengineering* 77.4 (1994): 432-435.

doi:10.6342/NTU201602586



23. Combina, Mariana, et al. "Genome-wide gene expression of a natural hybrid between Saccharomyces cerevisiae and S. kudriavzevii under enological conditions." *International journal of food microbiology* 157.3 (2012): 340-345.

24. Masneuf, Isabelle, et al. "New hybrids between Saccharomyces sensu stricto yeast species found among wine and cider production strains." *Applied and Environmental Microbiology* 64.10 (1998): 3887-3892.

25. Eden, E., Navon, R., Steinfeld, I., Lipson, D., & Yakhini, Z. (2009). GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists.*BMC bioinformatics*, *10*(1), 1.

26. Al-Fageeh, Mohamed B., and C. Mark Smales. "Control and regulation of the cellular responses to cold shock: the responses in yeast and mammalian systems." *Biochemical Journal* 397.2 (2006): 247-259.

27. Ferguson, L. R., & Von Borstel, R. C. (1992). Induction of the cytoplasmic ‘petite’mutation by chemical and physical agents in Saccharomyces cerevisiae. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *265*(1), 103-148.

28. Wittkopp, Patricia J., Belinda K. Haerum, and Andrew G. Clark. "Evolutionary changes in cis and trans gene regulation." *Nature* 430.6995 (2004): 85-88.

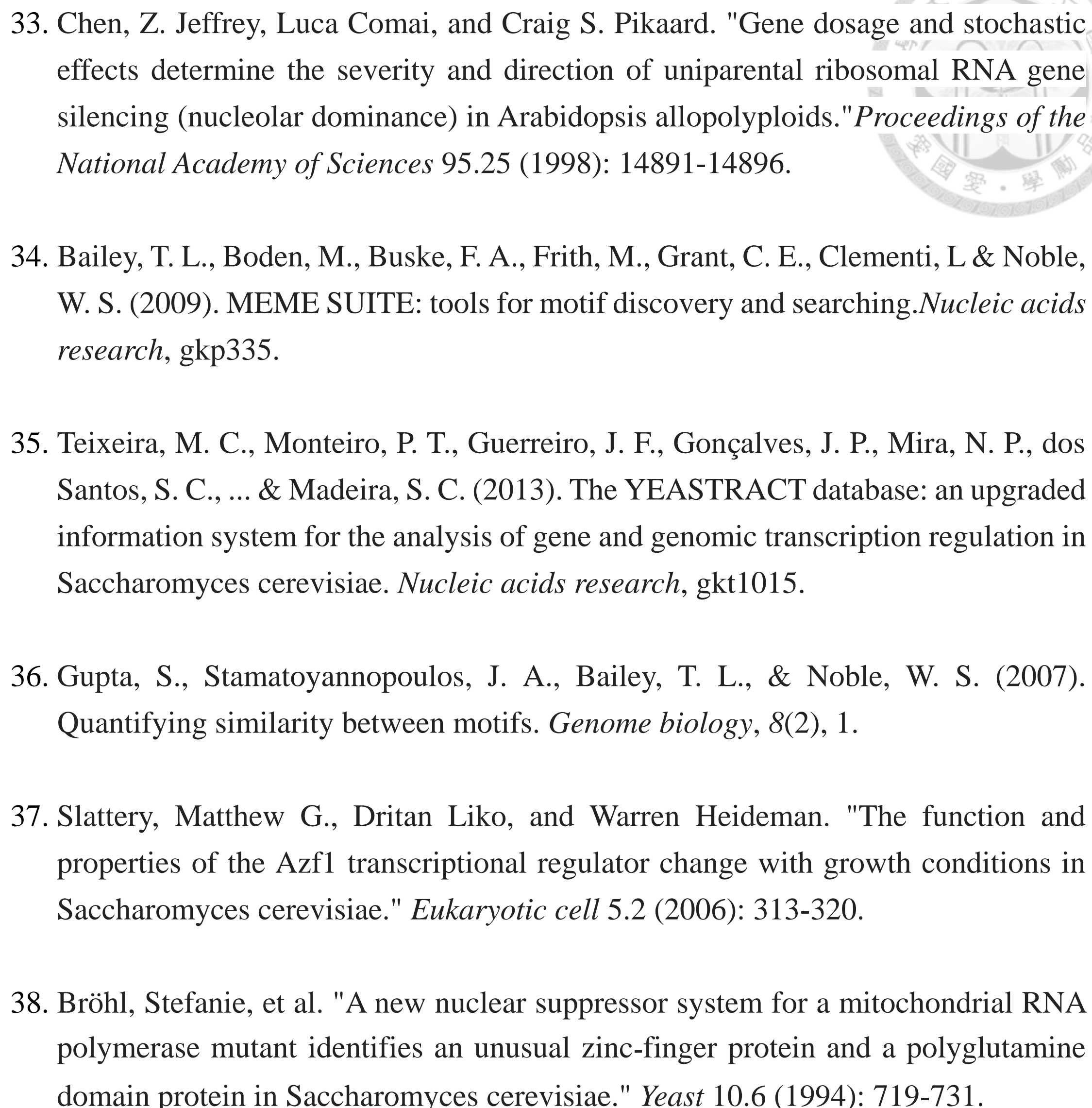
29. Wray, G. A., Hahn, M. W., Abouheif, E., Balhoff, J. P., Pizer, M., Rockman, M. V., & Romano, L. A. (2003). The evolution of transcriptional regulation in eukaryotes. *Molecular biology and evolution*, *20*(9), 1377-1419.

30. Rodrigues-Pousada, Claudina, Regina A. Menezes, and Catarina Pimentel. "The Yap family and its role in stress response." *Yeast* 27.5 (2010): 245-258.

31. Mendizabal, I., Rios, G., Mulet, J. M., Serrano, R., & de Larrinoa, I. F. (1998). Yeast putative transcription factors involved in salt tolerance. *FEBS letters*,*425*(2), 323-328.

32. Groszmann, Michael, et al. "Changes in 24-nt siRNA levels in Arabidopsis hybrids suggest an epigenetic contribution to hybrid vigor." *Proceedings of the National Academy of Sciences* 108.6 (2011): 2617-2622.

doi:10.6342/NTU201602586



33. Chen, Z. Jeffrey, Luca Comai, and Craig S. Pikaard. "Gene dosage and stochastic effects determine the severity and direction of uniparental ribosomal RNA gene silencing (nucleolar dominance) in Arabidopsis allopolyploids."*Proceedings of the National Academy of Sciences* 95.25 (1998): 14891-14896.

34. Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L & Noble, W. S. (2009). MEME SUITE: tools for motif discovery and searching.*Nucleic acids research*, gkp335.

35. Teixeira, M. C., Monteiro, P. T., Guerreiro, J. F., Gonçalves, J. P., Mira, N. P., dos Santos, S. C., ... & Madeira, S. C. (2013). The YEASTRACT database: an upgraded information system for the analysis of gene and genomic transcription regulation in Saccharomyces cerevisiae. *Nucleic acids research*, gkt1015.

36. Gupta, S., Stamatoyannopoulos, J. A., Bailey, T. L., & Noble, W. S. (2007). Quantifying similarity between motifs. *Genome biology*, *8*(2), 1.

37. Slattery, Matthew G., Dritan Liko, and Warren Heideman. "The function and properties of the Azf1 transcriptional regulator change with growth conditions in Saccharomyces cerevisiae." *Eukaryotic cell* 5.2 (2006): 313-320.

38. Bröhl, Stefanie, et al. "A new nuclear suppressor system for a mitochondrial RNA polymerase mutant identifies an unusual zinc-finger protein and a polyglutamine domain protein in Saccharomyces cerevisiae." *Yeast* 10.6 (1994): 719-731.

doi:10.6342/NTU201602586

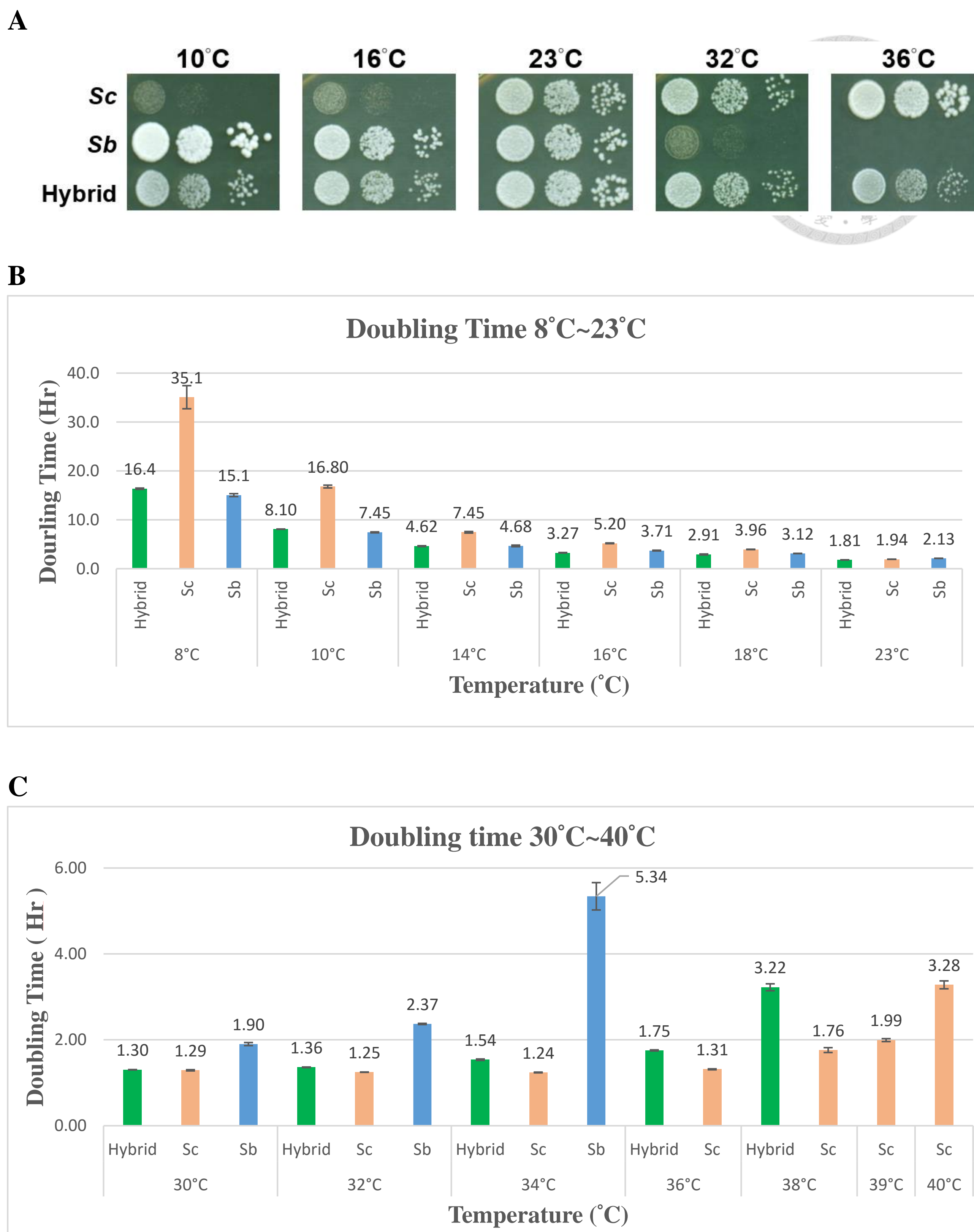


Figure 1. (A) Spot assay of *S.cerevisiae* (*Sc*), *S.bayanus* (*Sb*) and hybrid on YPD (rich medium) agar plates at various temperatures. **(B)** Measurement of doubling time (hour) of parents and hybrid in a temperature range of 8℃~23℃. Three independent clones were used for hybrid and each parent. **(C)** Measurement of doubling time (hour) of parents and hybrid in a temperature range of 30℃~40℃.

doi:10.6342/NTU201602586

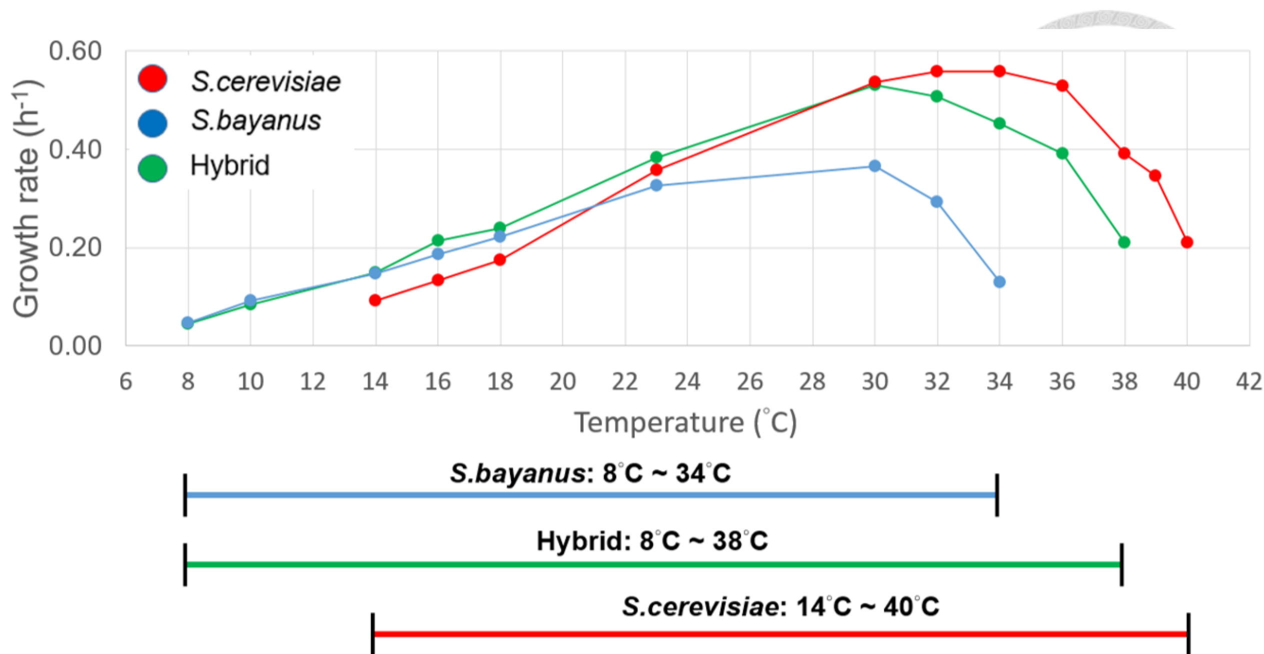
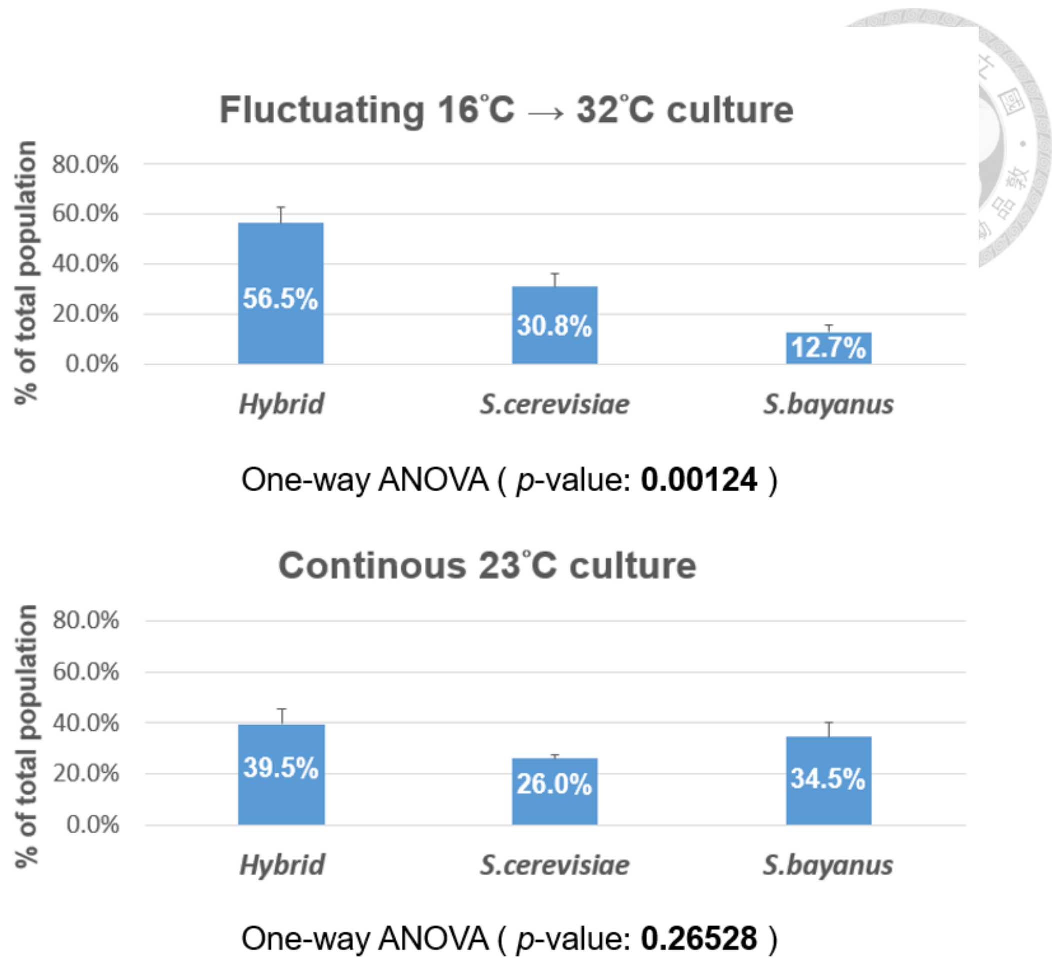


Figure 2. Comparison of growth rates between *S.cerevisiae*, *S.bayanus*, and hybrid across different temperatures. Range of temperature growth is terminated under two criteria: (i) No increase in OD600 after 8 hours of culturing. (ii) Doubling time is greater than 16 hours. Following the criteria, the hybrid displayed an extended range of temperature tolerance when compared to the parents.

doi:10.6342/NTU201602586

A



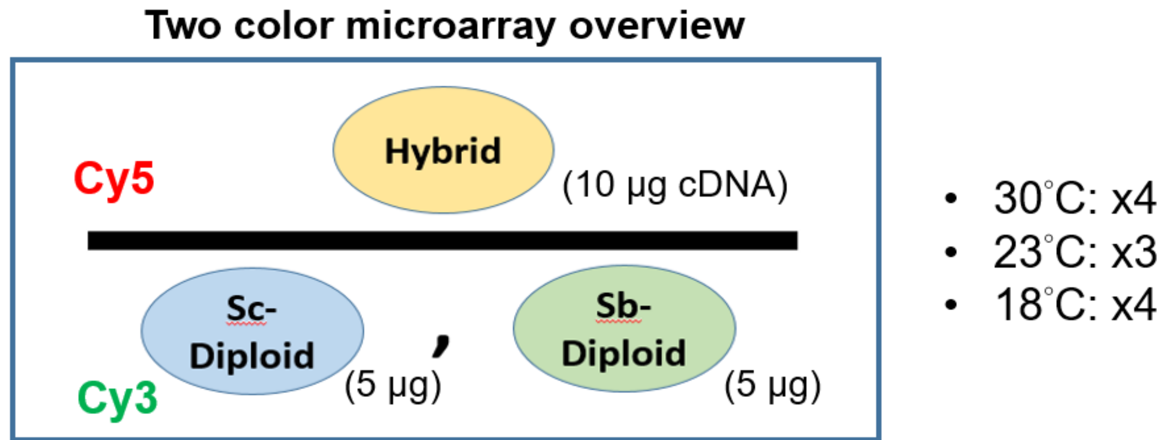
B

Flucutating 16°C→32°C culture		
Pairs	**Tukey HSD p-value**	**Tukey HSD Significance**
Hybrid vs *Sc*	0.024991	* $p<0.05$
Hybrid vs *Sb*	0.001033	** $p<0.01$
Sc vs *Sb*	0.118841	Insignificant

Figure 3. (A) Competition assay between hybrid and the two parents under fluctuating (16°C→32°C) and continuous, neutral (23°C) temperature. Four independent clones were used for hybrid. Significant difference between means of groups were compared using one-way ANOVA (p-value < 0.05). Error bars are +1 standard error. **(B)** *Post-hoc* Tukey HSD test were used to compare means between hybrid and the two parents (p-value < 0.05).

doi:10.6342/NTU201602586

A



B

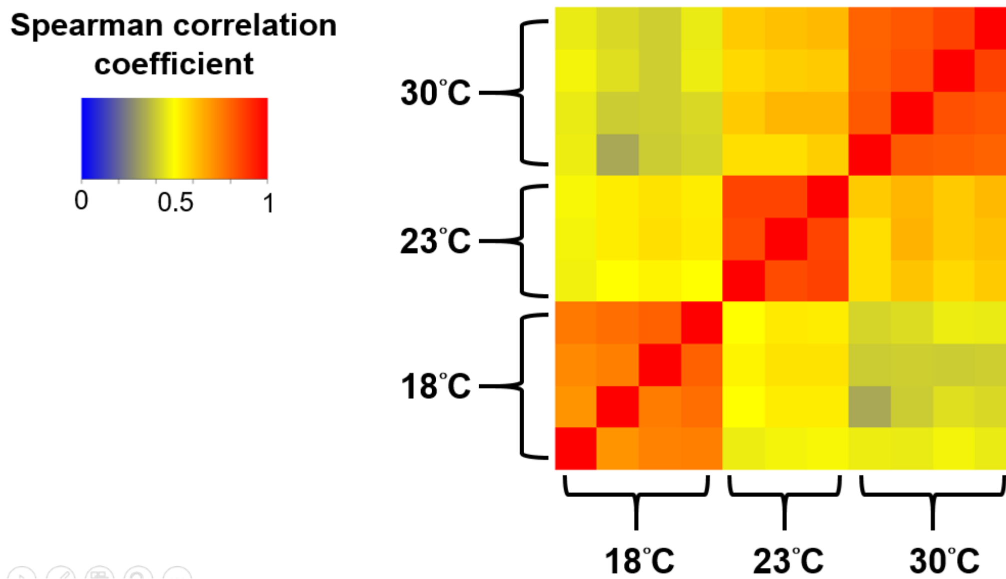


Figure 4. (A) An overview of the experimental design for two color, gene expression microarray. Total RNA samples were isolated independently from hybrid and parents at 30°C, 23°C, and 18°C, and then labeled with different fluorescent dyes when converted to cDNA. Equal amounts of hybrid and parental cDNA were hybridized to the microarray. **(B)** Genomde-wide Spearman correlation of $\log_2$(Hybrid/Parent) across replicates from the same and different conditions.

doi:10.6342/NTU201602586

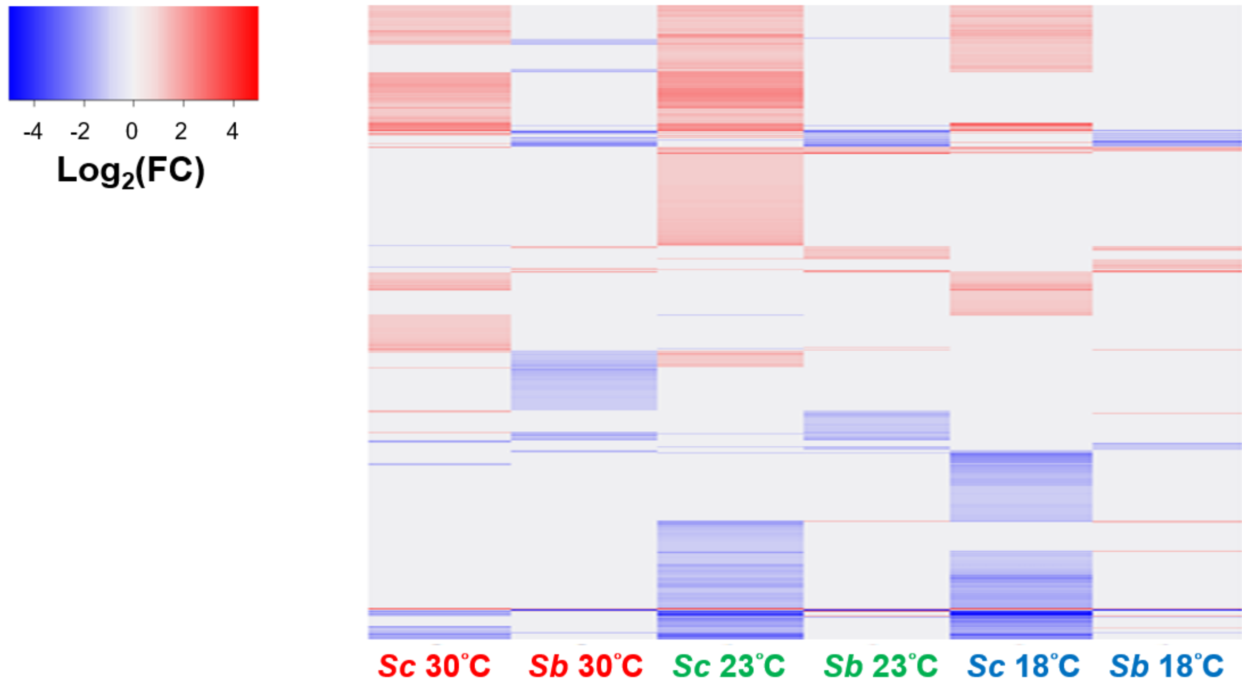
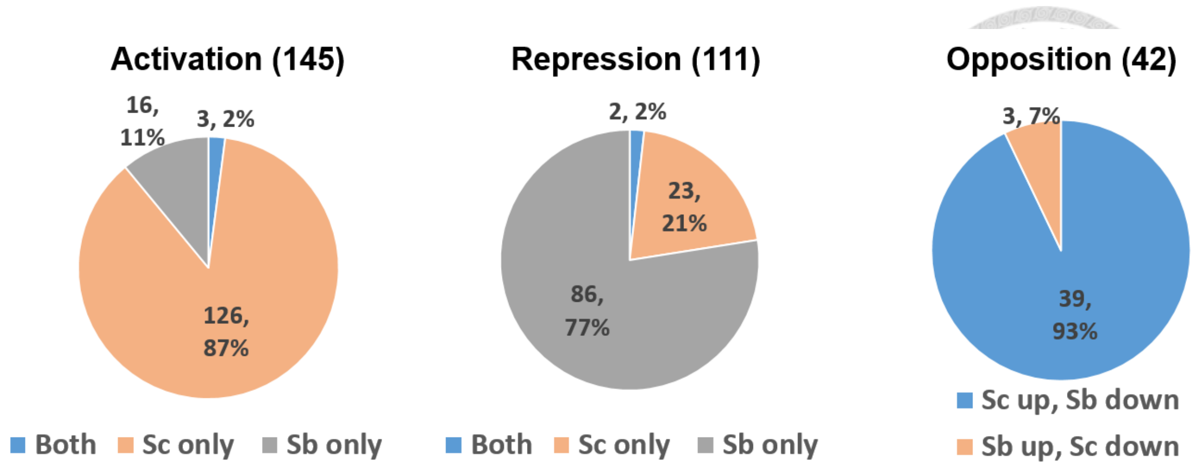


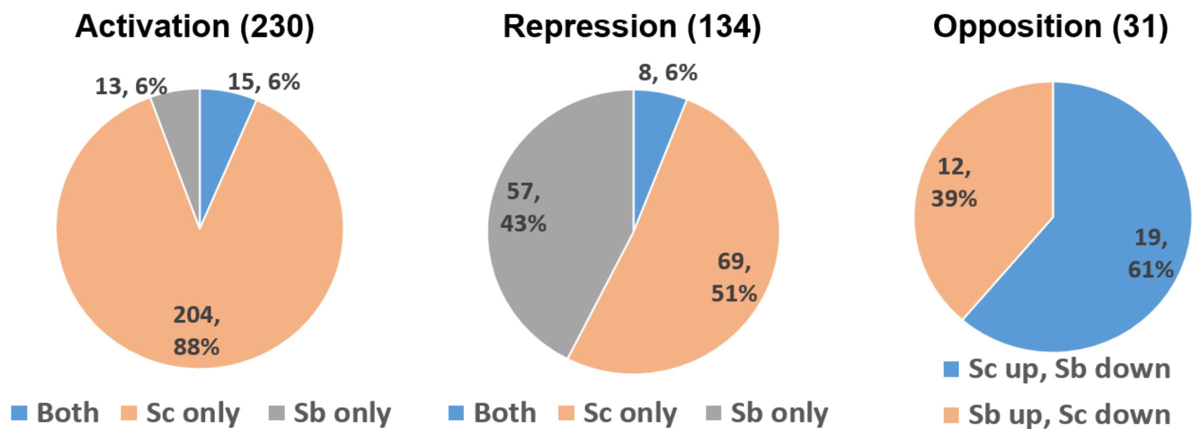
Figure 5. An overview of hybrid mis-regulation across different temperatures. Expression of species-specific allele in hybrid was independently compared with that of the parent's. An allele is considered mis-regulated if the fold change between hybrid and parental expression is equal to or larger than 2, and adjusted *p*-value following false discovery rate correction is less than 0.05.

doi:10.6342/NTU201602586

A



B



C

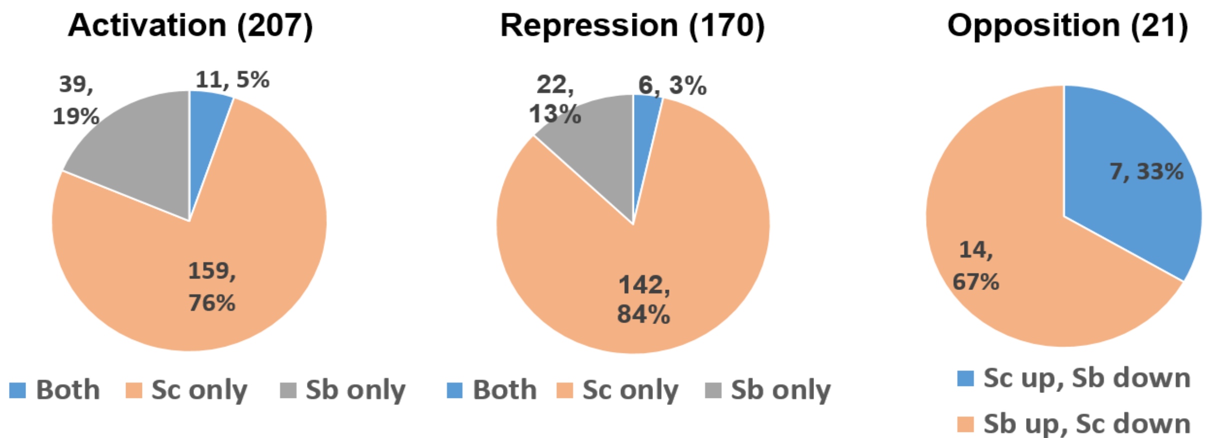


Figure 6. Hybrid mis-regulation at 30°C (**A**), 23°C (**B**), and 18°C (**C**). Number inside parenthesis is the total number of genes under this group. Activation refers to genes with single- or double-allele up-regulations. Repression refers to genes with single- or double-allele down-regulations. Opposition refers to genes with both alleles mis-regulated, but in opposite direction.

doi:10.6342/NTU201602586

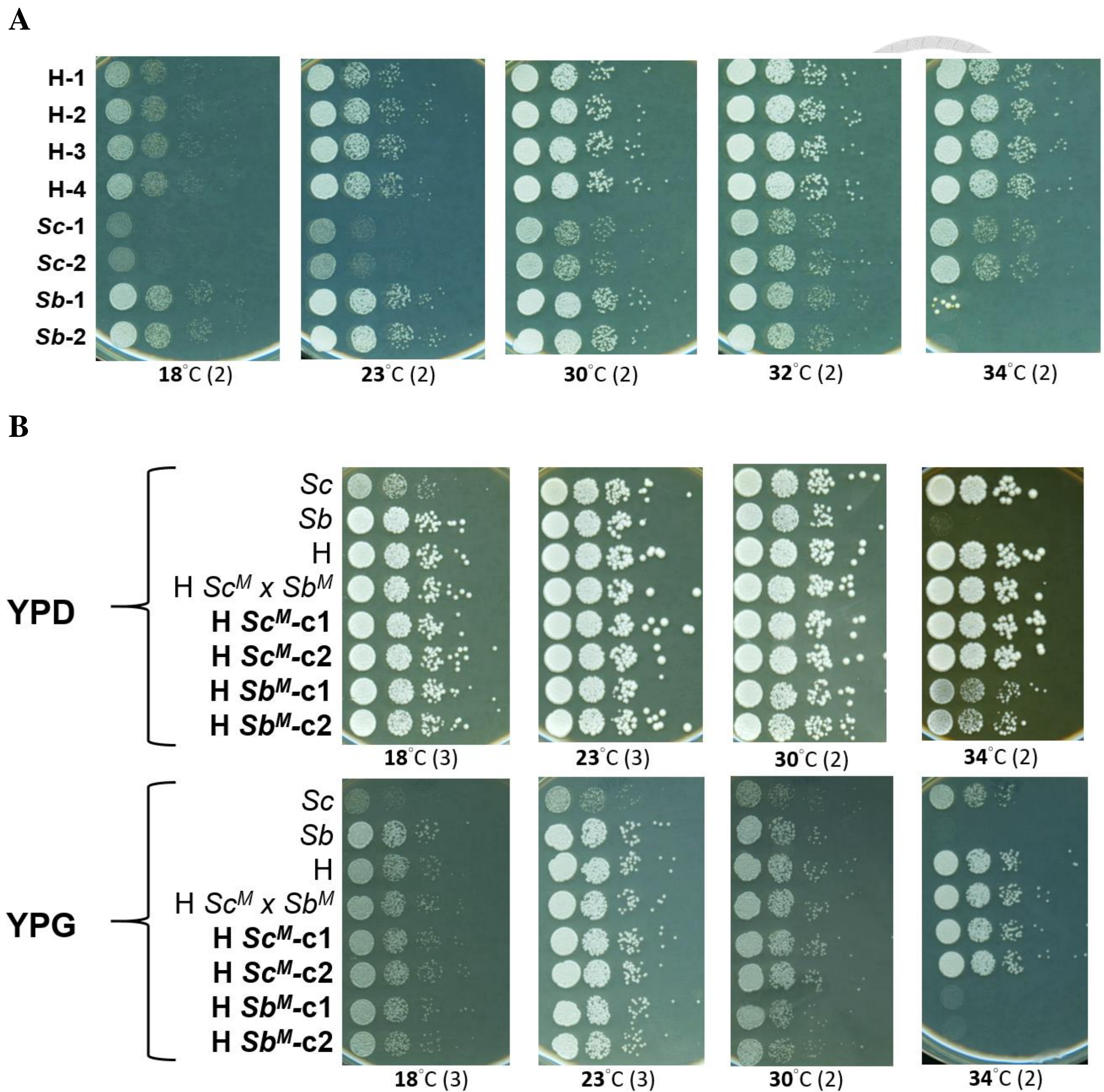
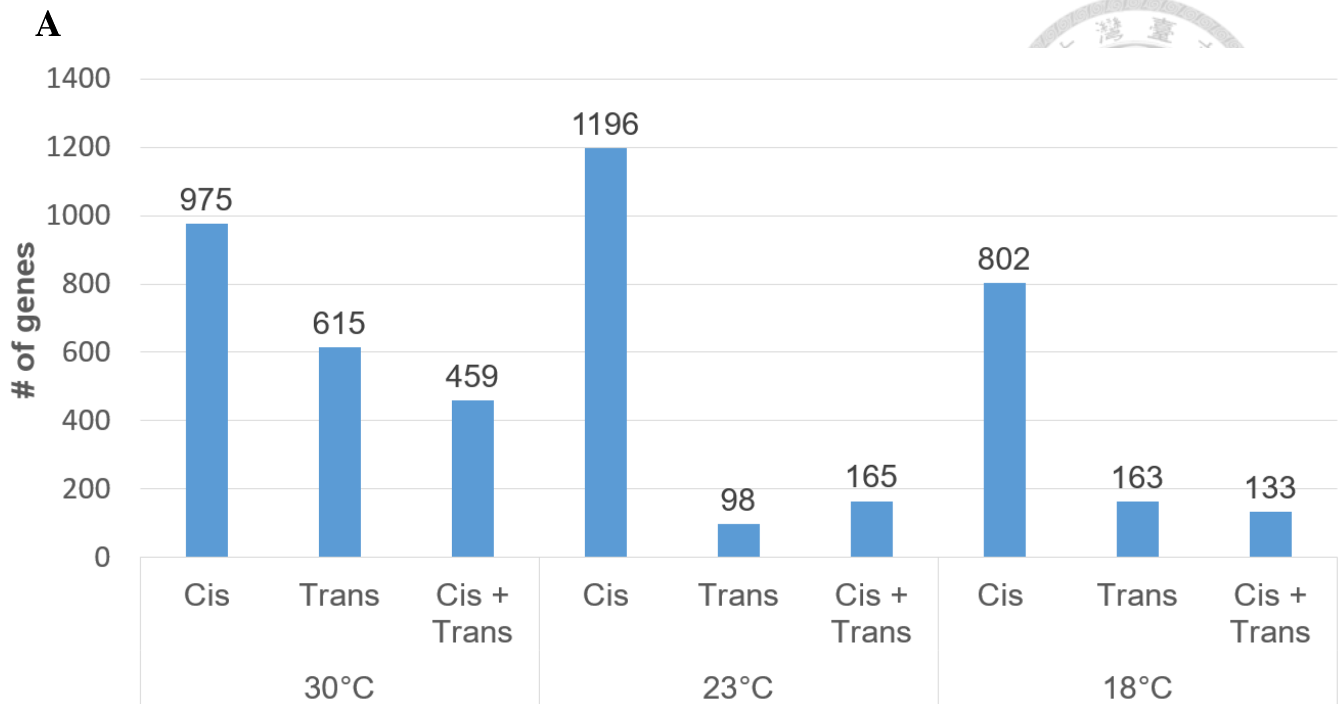


Figure 7. (A) Spot assay of *S.cerevisiae* (*Sc*), *S.bayanus* (*Sb*) and hybrid on YPGlycerol (non-fermentable carbon source) agar plates at various temperatures. **(B)** Spot assay of parents, hybrid, and hybrids with species-specific mitochondrial DNA (H Sc^{M}: hybrid with *Sc*'s mitochondrial DNA; H Sb^{M}: hybrid with *Sb*'s mitochondrial DNA) on YPD and YPGlycerol agar plates at various temperatures.

doi:10.6342/NTU201602586

A



B

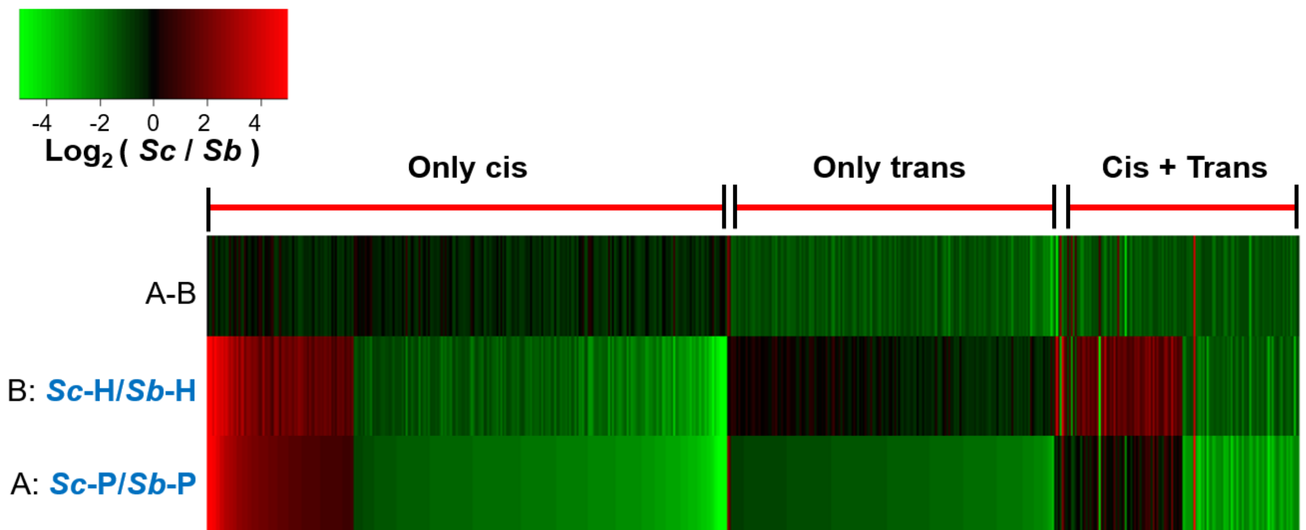


Figure 8. (A) Number of genes with significant cis, trans, and combined cis-trans effect at the three different temperatures. (**B**) A heatmap of genes with significant cis, trans, and cis-trans effect at 30°C. Genes with cis effect showed expression divergence in both parents and hybrid (fold change > 2); genes with trans effect showed divergence in parents but not in hybrid background (*Sc*-P: *Sc*-allele expression in parent; *Sb*-P: *Sb*-allele expression in parent; *Sc*-H: *Sc*-allele expression in hybrid; *Sb*-H: *Sb*-allele expression in hybrid).

doi:10.6342/NTU201602586

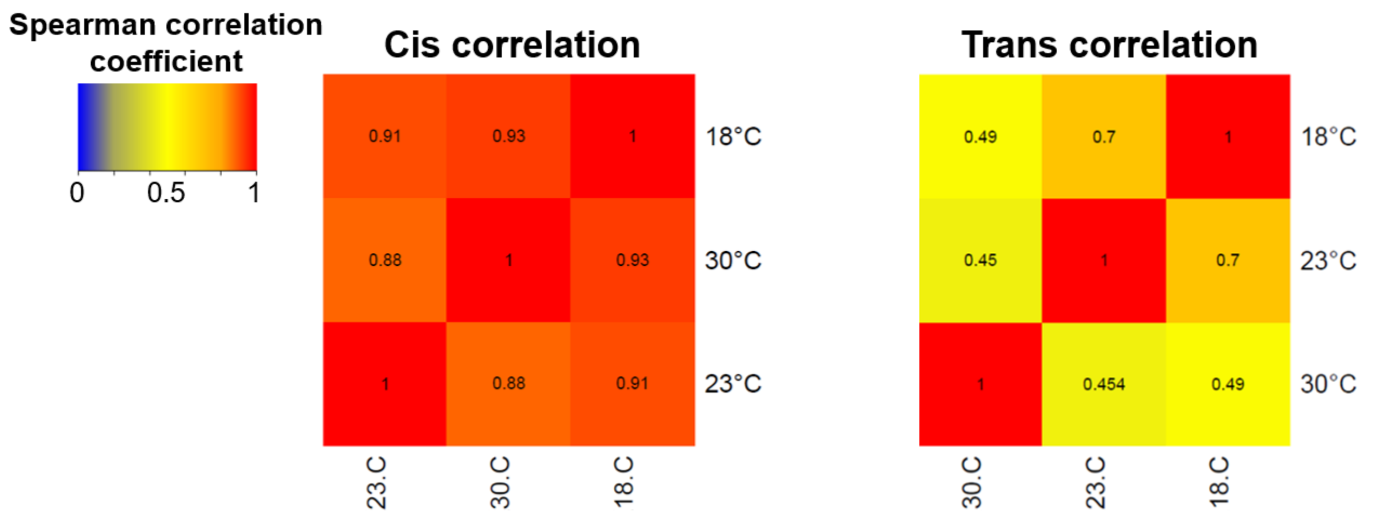
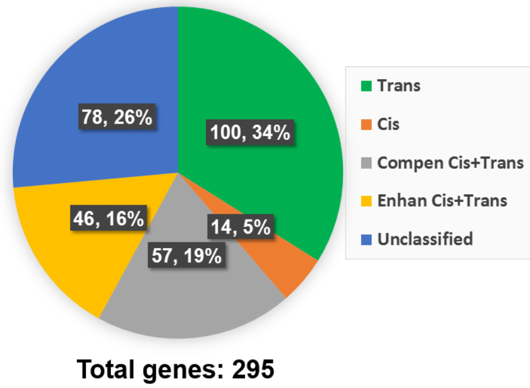


Figure 9. Spearman correlation of cis and trans effects across conditions. A total of 1614 genes identified with cis effects across the conditions were used as input, and the magnitude of cis effect (B: $\log_2$(*Sc*-H/*Sb*-H)) was used for correlation. For trans correlation, a total of 703 genes identified with trans effects across the conditions were used as input, and the magnitude of trans effect (A-B; A: $\log_2$(*Sc*-P/*Sb*-P)) was used for correlation.

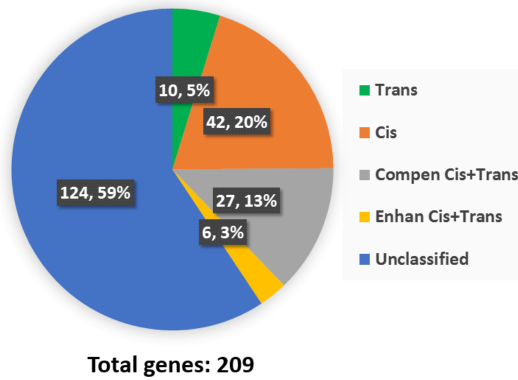
doi:10.6342/NTU201602586

A



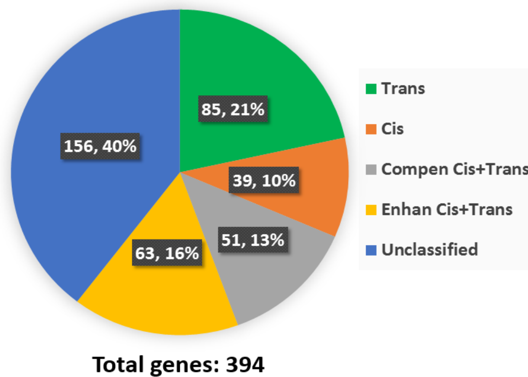
	Mis-regulation (295)	Genome-wide (4973)
Cis	5%	20%
Trans	34%	12%
Comp. Cis+Trans	19%	5%
Enha. Cis+Trans	16%	4%

B



	Mis-regulation (394)	Genome-wide (4973)
Cis	20%	24%
Trans	5%	2%
Comp. Cis+Trans	13%	2%
Enha. Cis+Trans	3%	1%

C

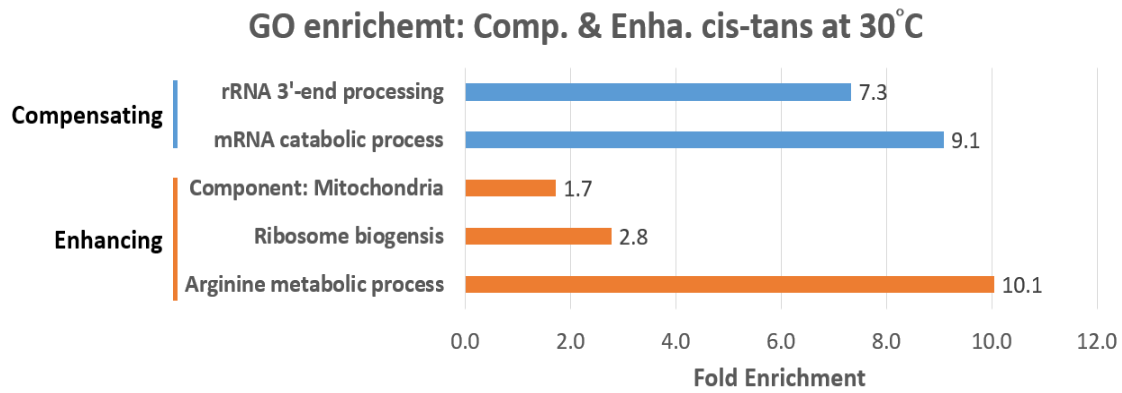


	Mis-regulation (394)	Genome-wide (4973)
Cis	10%	17%
Trans	21%	3%
Comp. Cis+Trans	13%	2%
Enha. Cis+Trans	16%	3%

Figure 10. Cis and trans effects of hybrid mis-regulation at 30°C (**A**), 23°C (**B**), and 18°C (**C**). Percentages of genes with cis, trans, and cis-trans effect from hybrid mis-regulation and genome-wide background were compared. (Pearson's chi-square test: p-value < 0.05)

doi:10.6342/NTU201602586

A



B

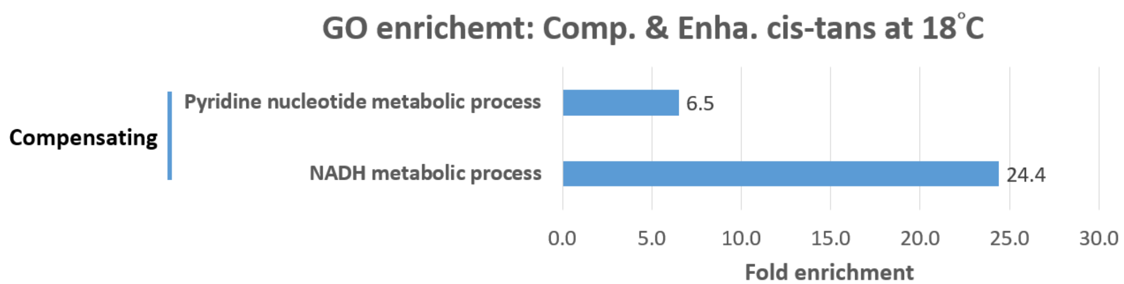
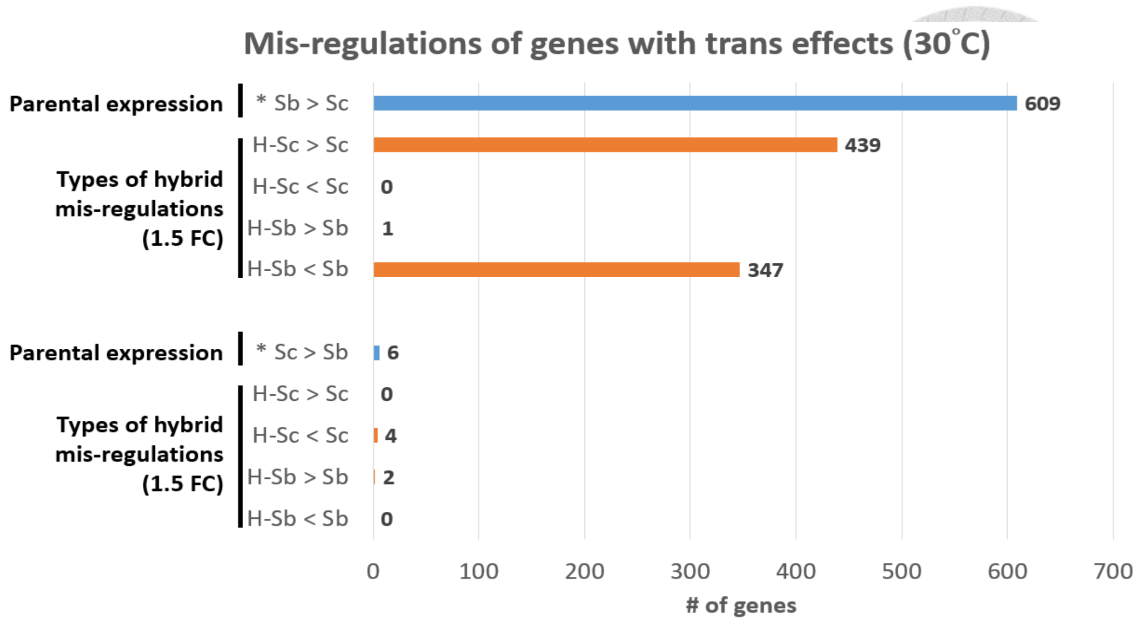


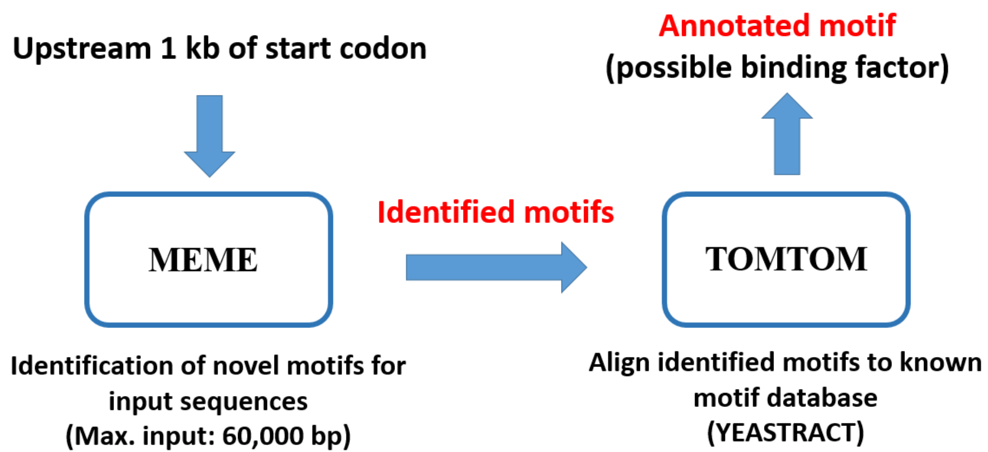
Figure 11. Gene ontology enrichment of genes with compensating and enhancing cis-trans effects at 30°C (**A**) and 18°C (**B**). Gene ontology enrichment was calculated by GOrillla. Gene lists of interest were compared to genome-wide background of 4973 genes. A FDR q-value < 0.05 was used for significant enrichment of GO term.

doi:10.6342/NTU201602586

A



B



C

Figure. 12 (**A**) Genes with trans effects at 30°C subdivided according to the type of hybrid mis-regulation. (**B**) Pipeline for identification of upstream trans factor that led to hybrid mis-regulation using MEME and TOMTOM suite. (**C**) Sequence logo of transcription factor Azf1p.

doi:10.6342/NTU201602586

Table 1. Enriched gene ontology terms for hybrid mis-regulated genes at 30°C (**A**) and 18°C (**B**). Gene ontology enrichment was calculated by GOrillla. Gene lists of interest were compared to genome-wide background of 4973 genes. A FDR q-value < 0.05 was used for significant enrichment of GO term.

Table 1A. Gene ontology enrichment for hybrid mis-regulation at 30°C

Activation	**Fold enrichment**	**# of genes**	**FDR q-value**
Respiratory electron transport chain	14.91	9	5.57E-06
ATP metabolic process	6.18	8	1.82E-02
Repression			
Cellular amino acid metabolic process	3.32	17	4.91E-02
Mitochondrial translation	4.7	10	5.28E-02
Opposition			
Mitochondrial translation	14.91	12	4.09E-08
Component: Mitochondrial part	4.37	21	6.01E-07

Table 1B. Gene ontology enrichment for hybrid mis-regulation at 18°C

Activation	**Fold enrichment**	**# of genes**	**FDR q-value**
Ribosome biogenesis	4.01	30	6.88E-08
ncRNA processing	2.45	33	4.47E-04
Metal ion transport	4	12	5.72E-03
Translation	2.59	26	1.43E-03
Mitochondrial translation	3.8	15	1.55E-03
Repression			
Cellular amino acid catabolic process	8.33	13	4.68E-06
Lysosomal microautophagy	5.64	9	8.12E-03

doi:10.6342/NTU201602586

Tabl1 S1. Strains used in this study

Table S1		
Strain name	**Description**	**Genotype**
C4 M3-1 Z3	*Sc x Sb* hybrid Cross between BY4741 and JYL1026	*MATa/α HIS3/his3Δ leu2Δ0/leu2Δ0 LYS2/LYS2 MET15/MET15 URA3/URA3*
C2 M3-1 Z1	*Sc* diploid parent Cross between BY4741 and BY4742	*MATa/α HIS3/his3Δ1 leu2Δ0/leu2Δ0 LYS2/ lys2Δ0 met15Δ0/MET15 URA3/ura3Δ0*
N8 Z2	*Sb* diploid parent Cross between JYL1026 and JYL1027	*MATa/α his3Δ/his3Δ leu2Δ/leu2Δ URA3/URA3*
N15	*Sc x Sb* hybrid Cross between JYL1743 and JYL1026	*MATa/α HIS3/his3Δ lys2Δ/LYS2 met15Δ/MET15 ura3::KanMX/URA3*
N11	*Sc x Sb* hybrid with *Sc*'s mito.DNA Cross between BY4741 and JYL1026 (ρ-)	*MATa/α HIS3/his3Δ leu2Δ0/leu2Δ0 URA3/URA3*
N12	*Sc x Sb* hybrid with *Sb*'s mito.DNA Cross between BY4741 (ρ-) and JYL1026	*MATa/α HIS3/his3Δ leu2Δ0/leu2Δ0 URA3/URA3*

doi:10.6342/NTU201602586

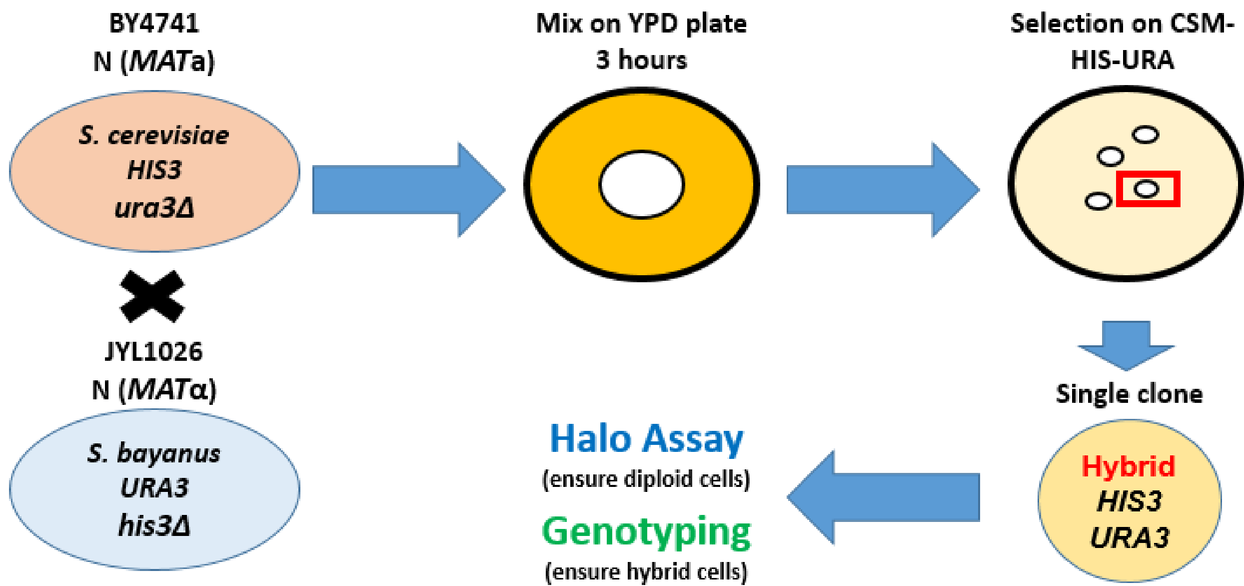


Figure S1. Generation of interspecific hybrid between *S.cerevisiae* and *S.bayanus*. Haploid, parental cells from the two species contain complementary selection markers for each other. The parental cells of different mating types were mixed on YPD agar plate and then spread on double-selection plate (CSM-HIS-URA) for selecting singe clones of hybrid. The selected hybrid clones were then tested for their ploidy with halo assay and for their hybridized genomes with species-specific primers.

doi:10.6342/NTU201602586

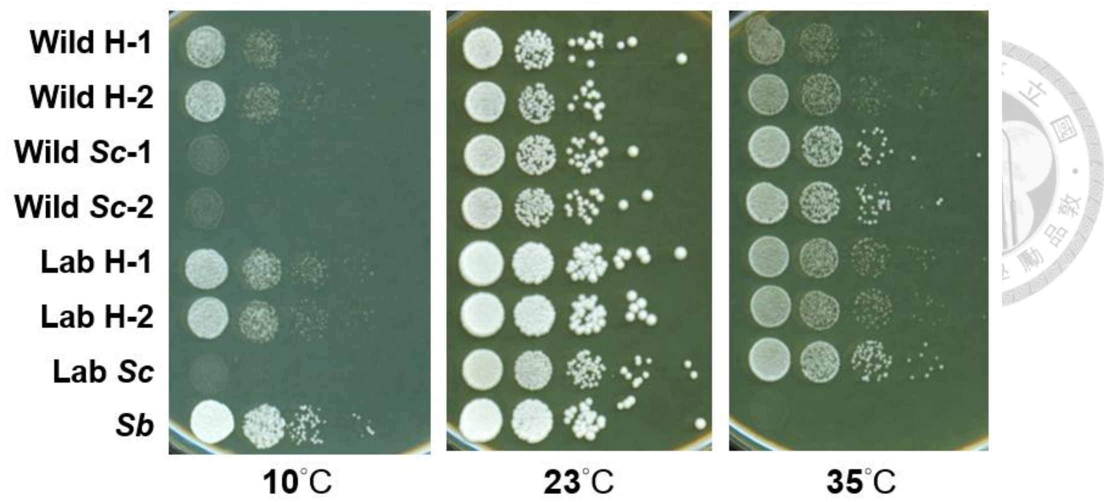
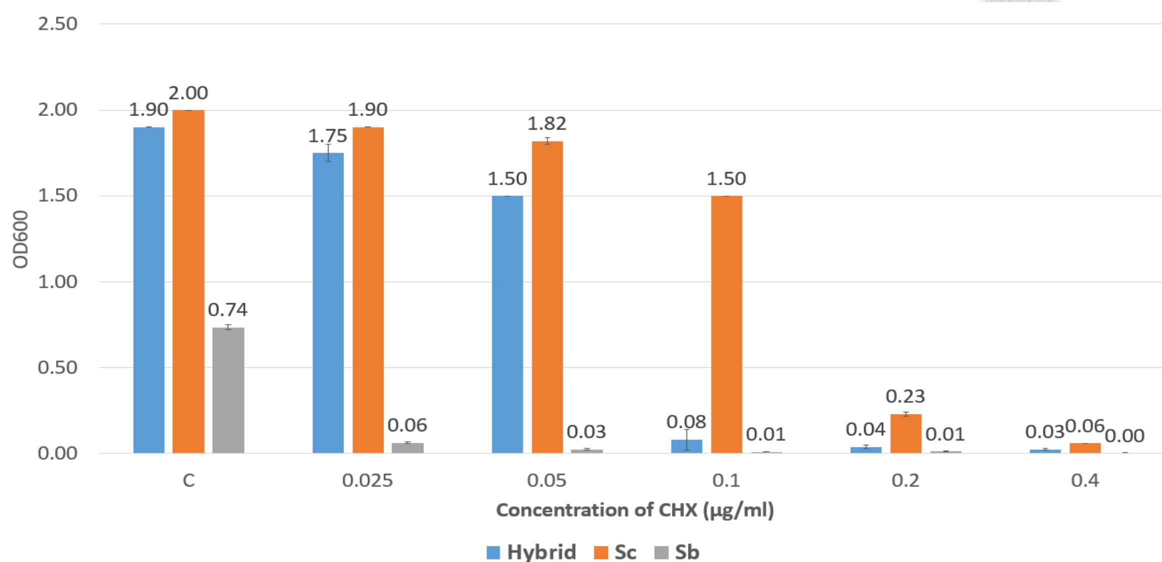


Figure S2. Spot assay of two hybrid strains (Wild H: *S.cerevisiae* UWOPS05-217.3 x *S.bayanus* JYL1026; lab H: *S.cerevisiae* BY4741 x *S.bayanus* JYL1026) and parents (Wild *S.cerevisiae* strain: UWOPS05-217.3; Lab *S.cerevisiae* strain: BY4743; *S.bayanus*: JYL1026 x JYL1027) at various temperatures.

doi:10.6342/NTU201602586

A



B

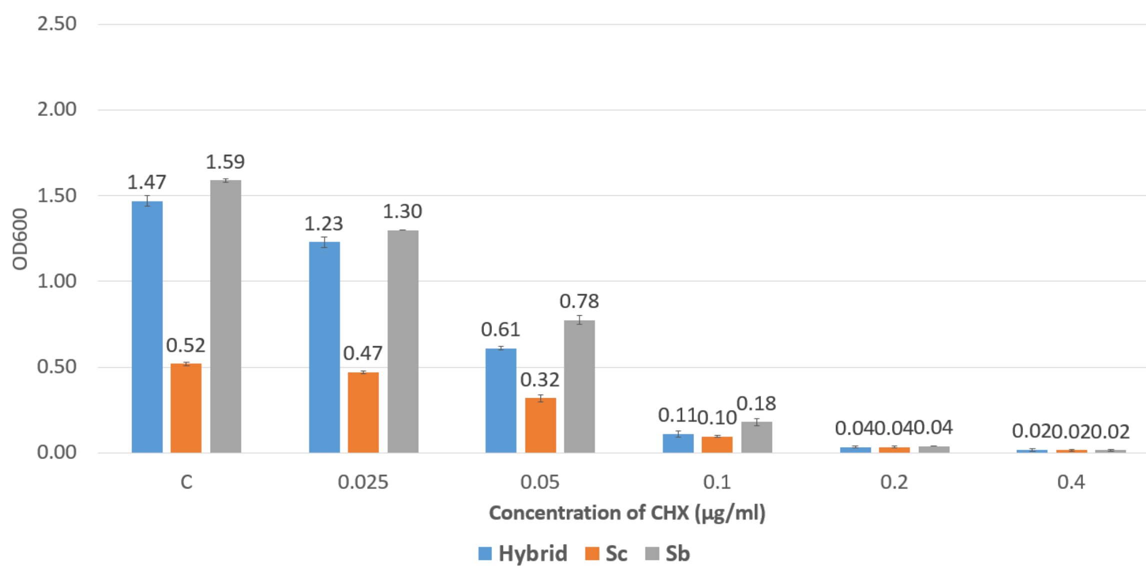


Figure S3. Measurement of minimal inhibitory concentration (MIC) for cycloheximide at various temperatures. Pre-cultured cells were refreshed in YPD media with different concentrations of cycloheximide (C: control without cycloheximide). OD600 was measured after 24 hours of culture at 32°C (**A**) and 16°C (**B**). A concentration is considered to be inhibitory if the OD600 is less than 0.1 after 24 hours of culture.

doi:10.6342/NTU201602586

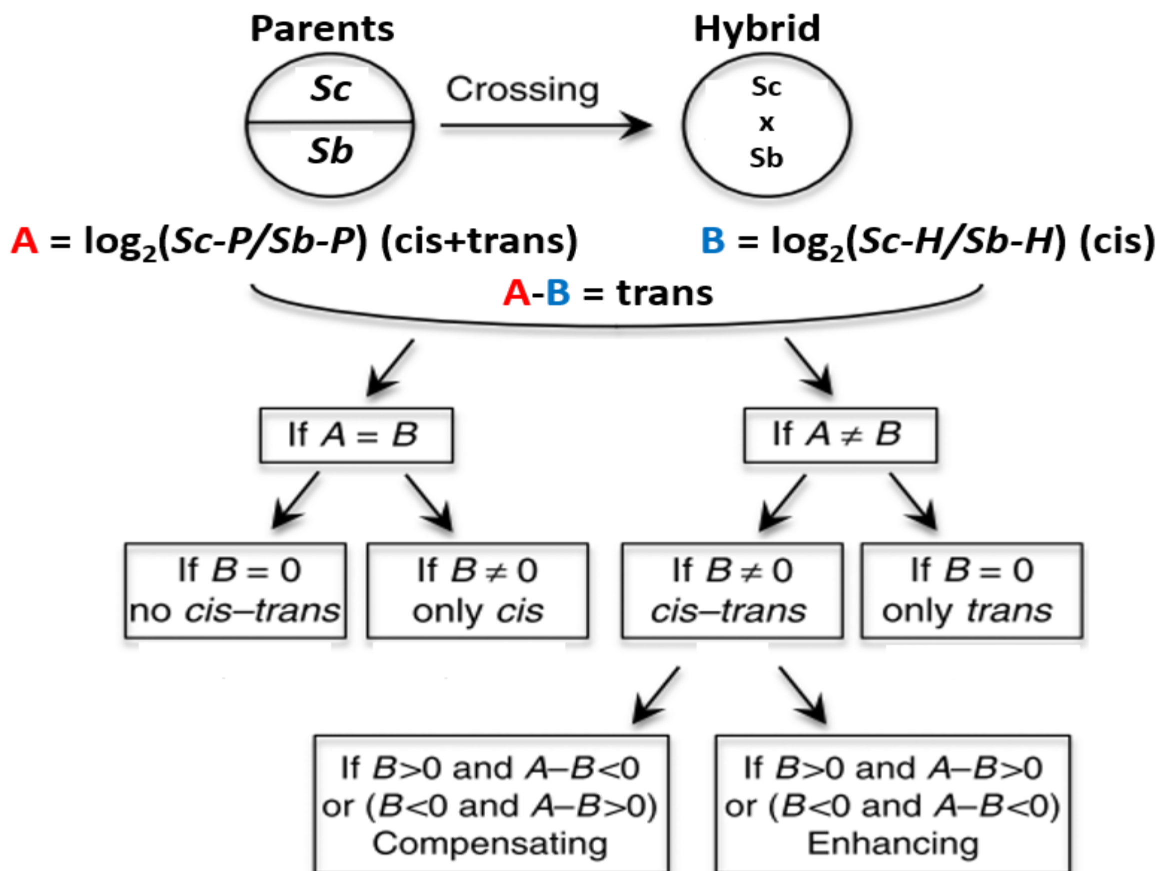


Figure S4. Pipeline for determination of genes under cis, trans, and cis-trans combined effects and quantification of the effects (14). Ratios between alleles of the same ortholog in parents (A) and hybrid (B) were generated using normalized fluorescent signals from the microarray. A gene is considered to have only cis effect if parental ratio (A) is equal to hybrid ratio (B), and absolute value of hybrid ratio (B) is greater than 1. A gene is considered to have only trans effects if parental ratio does not equal to hybrid ratio, and the absolute value of hybrid ratio is smaller than 1.

If the gene's parental ratio (A) is not equal to hybrid ratio (B), but the absolute value of hybrid ratio (B) is greater than 1, then the gene can be considered as having a combination of cis and trans effects. A gene is under compensating cis-trans effect if the hybrid ratio (B) is greater than the parental ratio (A), such that [{B > 0 and (A-B) < 0}] or [{B < 0 and (A-B) > 0}]; enhancing cis-trans effect is described if the hybrid ratio (A) is smaller than the parental ratio (B), such that [{B > 0 and (A-B) > 0}] or [{B < 0 and (A-B) < 0}].

doi:10.6342/NTU201602586

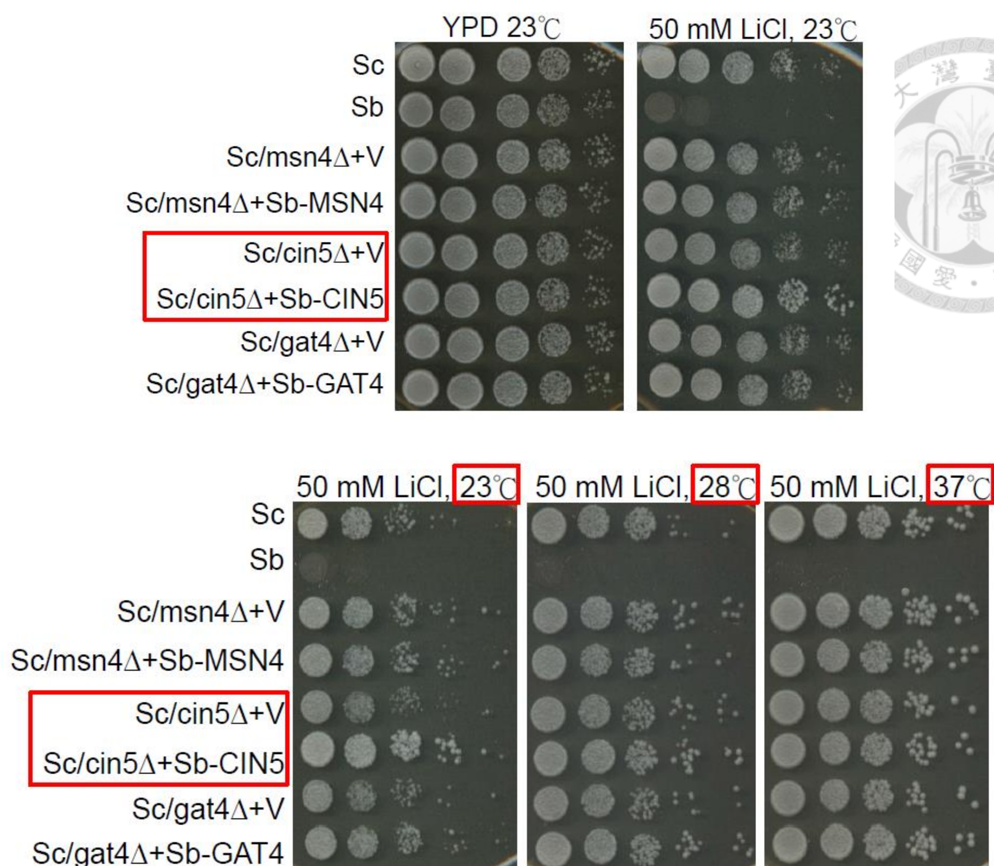


Figure S5. Spot assay of *S.cerevisiae*, *S.bayanus*, *Sc-CIN5* deletion line (*Sc/cin5*Δ+V) and *Sb-CIN5* replacement line (*Sc/cin5*Δ+*Sb-CIN5*) in YPD and 50 mM LiCl agar plates at various temperatures. *Sb-CIN5* in *Sb-CIN5* replacement line were expressed through a single-copy vector, while *Sc-CIN5* deletion line carried an empty vector as control.

doi:10.6342/NTU201602586

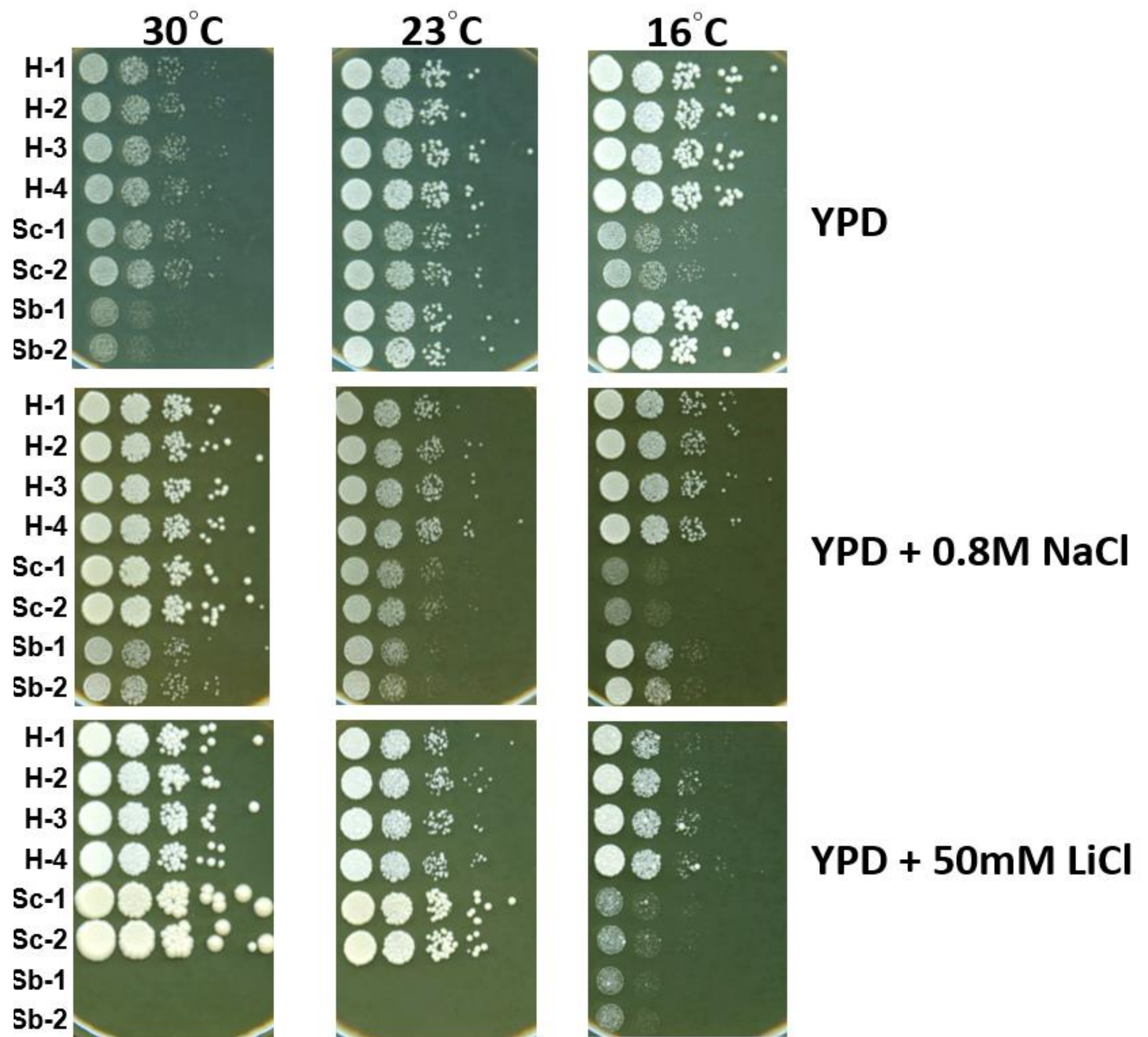
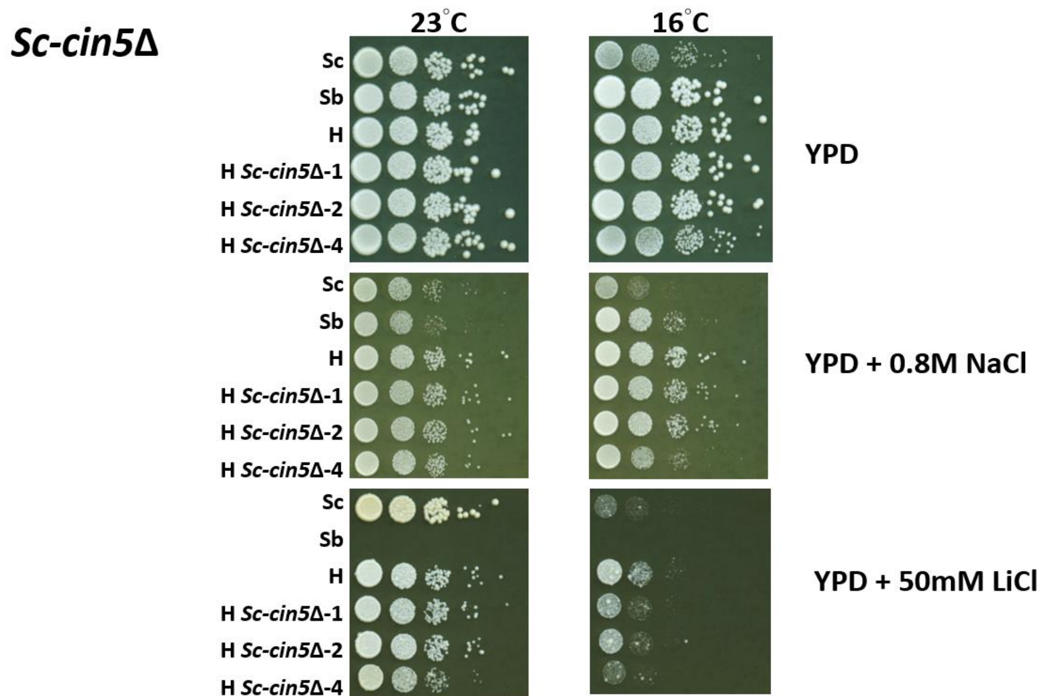


Figure S6. Spot assay of *S.cerevisiae* (*Sc*), *S.bayanus* (*Sb*) and hybrid (H) in YPD, NaCl, and LiCl agar plates at various temperatures. Four independent clones of hybrid and two clones for each parents were used in the experiment.

doi:10.6342/NTU201602586

A



B

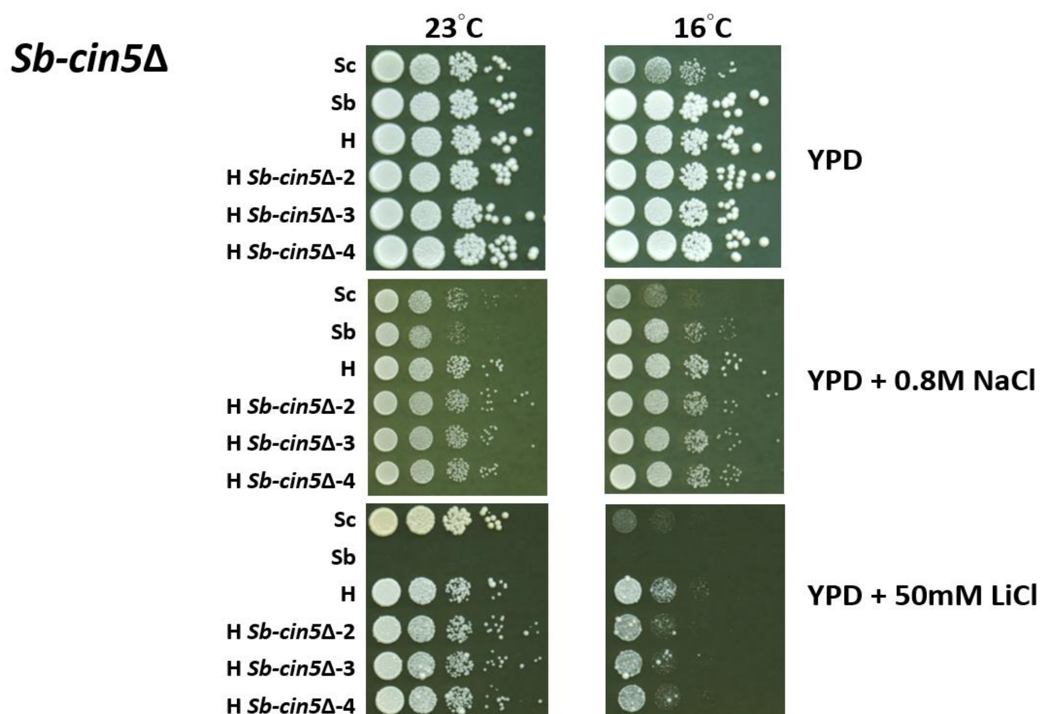


Figure S7. (**A**) Spot assay of *S.cerevisiae* (*Sc*), *S.bayanus* (*Sb*), hybrid (H), and hybrid with *Sc-CIN5*(H *Sc-cin5*Δ) or (**B**) *Sb-CIN5* (H *Sb-cin5*Δ) deletion in YPD, NaCl, and LiCl agar plates at 16℃ and 23℃. Three independent clones of hybrid with *Sc-cin5*Δ and *Sb-cin5*Δ deletion were used.

doi:10.6342/NTU201602586