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Construction of the first compendium of chemical-genetic profiles in the fission yeast *Schizosaccharomyces pombe* and comparative compendium approach

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ABSTRACT

Genome-wide chemical genetic profiles in *Saccharomyces cerevisiae* since the budding yeast deletion library construction have been successfully used to reveal unknown mode-of-actions of drugs. Here, we introduce comparative approach to infer drug target proteins more accurately using two compendiums of chemical-genetic profiles from the budding yeast *S. cerevisiae* and the fission yeast *Schizosaccharomyces pombe*. For the first time, we established DNA-chip based growth defect measurement of genome-wide deletion strains of *S. pombe*, and then applied 47 drugs to the pooled heterozygous deletion strains to generate chemical-genetic profiles in *S. pombe*. In our approach, putative drug targets were inferred from strains hypersensitive to given drugs by analyzing *S. pombe* and *S. cerevisiae* compendiums. Notably, many evidences in the literature revealed that the inferred target genes of fungicide and bactericide identified by such comparative approach are in fact the direct targets. Furthermore, by filtering out the genes with no essentiality, the multi-drug sensitivity genes, and the genes with less eukaryotic conservation, we created a set of drug target gene candidates that are expected to be directly affected by a given drug in human cells. Our study demonstrated that it is highly beneficial to construct the multiple compendiums of chemical genetic profiles using many different species. The fission yeast chemical-genetic compendium is available at http://pombe.kaist.ac.kr/compendium.

1. Introduction

In terminology of chemical-genetic profile, 'chemical' means FDA approved drugs, bioactive compounds, or natural extracts and 'genetic' means the library of genome-wide deletion strains in a species. 'Profile' indicates quantitative growth defect of each strain in the library. Usually, the profiles of deletion strains can be made by high-throughput spot assay [1–3] and DNA-chip [4–8], and are used to find the mode-of-action of drugs on cells [9]. Especially DNA-chip based chemical-genetic profiles require huge resources. First, the deletion strains should be constructed on a genome scale. Second, each deletion strain needs to have unique DNA barcodes which are finally used as molecular probe for the DNA-chip based measurement. Third, the DNA microarray chip assay should be customized to measure unique DNA barcodes inserted in each strain. All tasks needed to satisfy these requirements are time-consuming, labor-intensive, cost-expensive resources. In *Saccharomyces cerevisiae*, such resources have been established from 2002 [6], and thousands of chemical-genetic profiles have been generated for a couple of years and are nowadays publicly available [7,10].

The main concept of our comparative compendium approach in this study is to compare more than two compendiums of chemical-genetic profiles generated from different species to find a drug's target proteins and target pathway. As a prerequisite, the construction of a genome-wide set of diploid deletion mutants in *Schizosaccharomyces pombe* had completed by our group in 2010 [11], which is the second genome-wide deletion library in the

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world. These deletion strains have unique DNA barcodes that can be measured by customized an Affymetrix chip like those of *S. cerevisiae*, and ~4836 strains are currently available [12]. For the first time, we present a compendium of chemical-genetic profiles composed of more than 4000 heterozygous deletion strains and 47 chemical compounds in *S. pombe* which have already been tested in *S. cerevisiae* [5,7,8].

In a fission yeast compendium experiment, the hypersensitive genes to a given drug were named as the putative Drug Target genes for a given drug (DT). Then, this fission yeast compendium was compared with that of budding yeast (*S. cerevisiae*). In this comparison, if both genes were DT for an orthologous gene pair, we called these genes evolutionarily conserved DT (ecDT); if only one of them was DT, we called it evolutionarily diverged DT (edDT). Furthermore, we presented further analysis of ecDTs from two yeasts to show that the comparative compendium study is beneficial to infer the putative drug target in humans.

2. Materials and methods

2.1. Selection of chemicals

We prepared the stock solutions of drugs as follows: First, we listed up the available compounds of 110 drugs. Second, we searched the solubility of the selected drugs via the database of DrugBank [13]. Third, we determined the amounts of each drug, and types and volumes of solvents such as water, ethanol and DMSO for high concentration of the stock solutions. Third, we selected 75 drugs used more than once in previous genome-wide chemical-genetic studies in *S. cerevisiae* [4,5,8]. Fourth, we finally selected 47 drugs after growth inhibition test addressed as described in the following section.

2.2. Determination of a drug dose

To determine the optimal concentrations of drugs to be tested, we adopted the pool fitness as the measure of the pool growth inhibition by the drug treatment [8]. Here, the pool means the pooled deletion strains. The pool fitness (PF) can be defined as:

$$\mathrm{PF} = 1 + \left(\frac{\log\left(\frac{\mathrm{OD}_{600}^{treated}}{\mathrm{OD}_{600}^{untreated}}\right)}{\log(2) \times g} \right)$$

where OD_{600} is optical density measured at 600 nm and g is the generations of the untreated pool at the measurement of OD_{600}. To measure growth inhibitions according to the serial diluted doses of each drug, the pooled heterozygous deletion strains in deep 48 well were cultured for ~48 h (~10 generations) at 25 °C, 1200 rpm using Deep Well Maximizer (Model: BNMBR-022UP, Bionex Company). We performed the two sets of serial dilution experiments to determine the optimal concentration. In the first 5-fold serial dilution experiment, the pool was treated with the drug doses in the broad range of 5.0×10^{-5} M to 1.0×10^{-9} M. In the second 2-fold serial dilution experiment, we treated the pool with the different dose ranges by each drug. From the first experiment, we were able to determine more effective inhibition dose range of each drug by observing the pellet size of each well after the centrifugation of deep 48-well plates (Supplementary Fig. S1). Finally, we measured OD using (device) from this second experiment, and calculated PF by each dose of each drug and determined the drug dose showing $0.85 < \mathrm{PF} < 0.95$.

2.3. Pooled growth of heterozygous deletion strains in S. pombe

We adopted the pooled growth strategy reported in [14]. It was performed with a series of two 50 ml cultures in flask at 25 °C (Supplementary Fig. S2). We usually sampled the drug-treated pool around 20 generations. Exceptionally actinomycin, dyclonin, and dipyridamole had severe growth inhibitions in our pool growth condition, which raised the sampling error (Supplementary Fig. S3). In sampling at 10 and 20 generations, drugs tested make the doubling time of the no-drug-treated pool decrease 5–30% compared with the drug-treated pool as expected in PF (Supplementary Table S1 and Fig. S4). The pool fitness by doubling time ($\mathrm{PF}_{\mathrm{DT}}$) is defined as

$$\mathrm{PF}_{\mathrm{DT}} = \frac{\textit{doubling time of the controlled pool}}{\textit{doubling time of the drug treated pool}}$$

2.4. Generation of the first S. pombe compendium

2.4.1. Measurement of TAGs inserted into each strain

We isolated genomic DNA from the heterozygous deletion pools sampled at 20 generations. The short DNA barcode sequences of 20 bp, called "TAG" were inserted into the corresponding genomic DNAs in each strain and were amplified using the universal primers flanking all of TAGs. Those amounts of the amplified TAGs were regarded as the amounts of each strain exposed in the same condition, and measured by the customized Affymetrix chip with the complementary sequences of TAGs. These experiments of DNA isolation, PCR amplification, and chip hybridization followed the protocols already established in [14].

2.4.2. Preprocessing of TAG intensities regarded as the amounts of each strain

Raw intensities of Affymetrix's cel file were preprocessed as follows: first, raw intensities of each probe set were summarized as mean-values of them without background correction, normalization, PM (perfect match) correction in the probe-level. Second, those intensities were corrected using the below correction function to prevent underestimation of the relative fitness by the saturated intensities

$$I_{corrected} = I_{raw} \times \exp(-I_{raw}\alpha)$$

where $I_{corrected}$ and I_{raw} are corrected and raw intensity, and the parameter α of correction function was estimated from the same serial dilution chip experiments as Pierce et al. did [14]. The estimated α of UPTAG and DNATAG were 0.0003412759 and 0.0002767581, respectively. Third, the constant-based normalization was applied to those corrected raw intensities of each array. For such normalization, the grand mean value of intensities of five chip experiments with pooled deletion strains at the glycerol stock was used. Lastly, those normalized intensities of each array were log2-transformed for post-analysis.

2.4.3. Calculation of fitness scores of each strain

We used the error model to calculate fitness scores of each strains treated with a drug by the method developed by others [7]. In this calculation, we excluded TAGs with 30% of poor intensities in 54 chip experiments (*Supplementary* Table S1). The poor signal was defined as a signal four times less than the mean of intensities of negative TAGs which did not exist in TAGs inserted in strains. A compendium of competitive growth experiment for different drugs was referred to as reference set. The reference set can be viewed as a matrix as a matrix P with rows of $1 \ldots i \ldots N$ strains and columns of $1 \ldots j \ldots M$ drugs. We calculated the mean and variance of the reference set of each strain as follows

$$Avg_{i,ref} = \frac{1}{M}\sum_{j}^{M} P_{ij}, \quad Var_{i,ref} = \frac{1}{M-1}\sum_{j}^{M}(P_{ij} - Avg_{i,ref})^2$$

where i is the index of strains, j is the index of drug, and reference set ('ref') denotes the set of all data points across drug j to strain i. When we calculated the statistics of the reference set, we trimmed the top 5% and the bottom 10% of outlying experiments yielding TAG intensities furthest from the average because it reduced drug-specific effects in the reference set. We finally obtained Z scores and p-values of 4400 heterozygous deletion strains under 47 drugs, called '*S. pombe* compendium' in this study (see Supplementary methods and Table S1).

3. Results and discussion

3.1. Drug-induced haploinsufficiency profile as a toolbox to find direct drug target proteins

As the first *S. pombe* compendium, we generated a drug-induced haploinsufficiency (DIHI) profile (Fig. 1A). Haploinsufficiency (HI) is the phenomenon that the reduced level of gene product causes abnormal phenotype (i.e. growth defect) in an organism in normal condition. Similarly, drug-Induced (DI)-HI is the phenomenon that a reduced level of a gene product causes growth defect in an organism under a given drug. Therefore, DIHI phenomenon-related gene products can be screened by a DNA chip-based measuring of quantitative growth defect of genome-wide heterozygous deletion strains under a bioactive compound. As a reference, the *S. cerevisiae* compendium including 220 unique chemicals and 5200 heterozygous deletion mutants is currently available [7].

The following properties of bioactive compounds make them suitable for DIHI screening. First, a bioactive compound binds directly to a protein. Second, the homozygous deletion strain of the corresponding gene show lethality when not treated with chemicals. In this case, the growth defect of heterozygous deletion strain of the corresponding gene under the given compound can be explained as growth inhibition by direct binding of protein to the compound. Otherwise, it is difficult to interpret the compound-protein binding as the cause of the growth defect. Typically such growth defects result from strains with abnormal drug influx/efflux (multi-drug sensitive) or strains with unknown secondary mutations (Fig. 1B).

Therefore, the analysis of bioactive compounds through DIHI profile screening has been limited to proteins which display phenotypes with lethality. In *S. cerevisiae* and *S. pombe*, there are 1200 and 1260 proteins of essential genes that show lethality in a homo deletion strain phenotype, respectively. In this regard, DIHI screening with the heterozygous deletion library of *S. pombe* will enable us to extend the chemical space to find direct protein targets of bioactive compounds. Consequently, the increased size of library by using two yeasts will give us a better opportunity to find direct protein targets of bioactive compounds (Table 1).

3.2. Evolutionarily conserved Drug Targets (ecDTs) as more confident direct drug target candidates

If two yeast's heterozygous deletion strains of the gene orthologous between *S. pombe* and *S. cerevisiae* [15] are hypersensitive to a given drug (see Supplementary method for calculation and threshold setup of p-value), such orthologous gene was defined as evolutionarily conserved DTs (ecDTs) in this study. We assume that ecDTs are more likely to be directly affected under the compound because their defects, due to their similar function are consistently observed across two yeasts. Schematic procedure is shown in Fig. 2A. Furthermore, by filtering out ecDTs with no essentiality (i.e. inviable phenotype in homozygous deletion of the gene), multi-drug sensitivity and less eukaryotic conservation, we finally infer drug target gene candidates that are more likely to be directly affected by a given drug as well as testable in human cells (Fig. 2B).

We tested 47 drugs in the *S. pombe* compendium, and used 36 drugs to infer ecDTs by comparing it with *S. cerevisiae* one. After

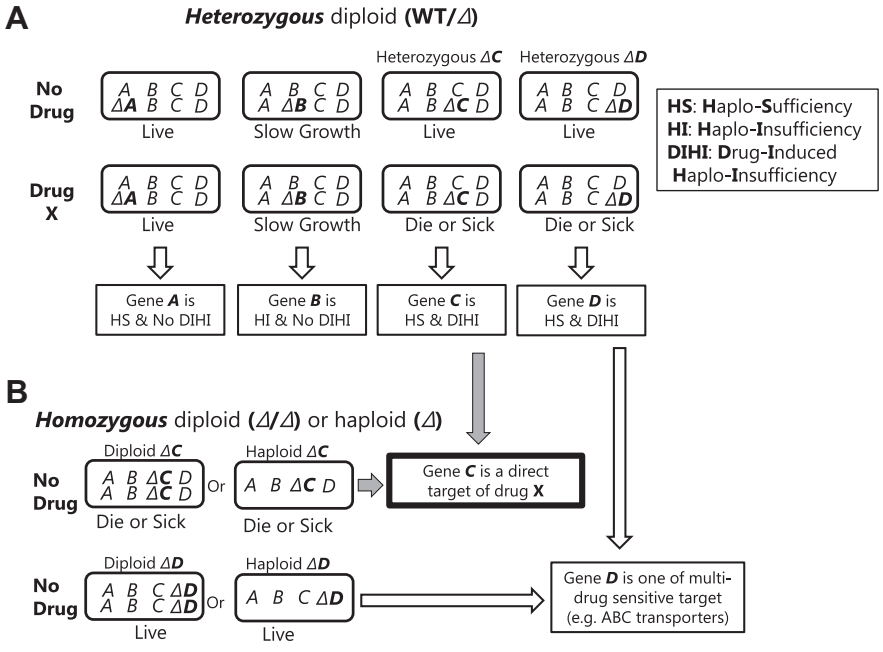

Fig. 1. Drug-induced haploinsufficiency profile. (A) Haploinsufficiency (HI) is the abnormal phenomenon in the half of the gene dosage. In *S. pombe*, most of genes are generally haploinsufficient (gene A), but ~3% of the genes (gene B) are haploinsufficient in terms of the cell growth. When this HI is induced by a drug, it is called drug-induced haploinsufficiency (DIHI). This DIHI profile is used for finding drug-target proteins (Gene C or D). (B) One of interpretations on the drug-sensitive strains in DIHI profiles. Suppose that some heterozygous deletion strains have significant growth defect in the treatment of a drug X (i.e. gene C and D in figure A). When the corresponding homozygous deletion strain also has lethality or shows significant growth defects (gene C), it is likely that such drug X sensitive heterozygous deletion strain occurs by the direct drug effect. Otherwise (in case of gene D), it is likely that the indirect drug effect (e.g. multi-drug sensitive genes) make the heterozygous deletion strain to be sensitive.

Table 1
The comparison of one-to-one orthologous essential genes between two yeasts.

S. pombe	*S. cerevisiae*	Number
Essential	Essential	669
Viable	Essential	266
Essential	Viable	150

orthologous mapping between DTs in the two yeasts, the ecDTs remained in 13 drugs. Then, we filtered out the ecDTs with weak evidences on direct drug effect. Finally, we obtained the set of ecDTs for 9 drugs (Fig. 3).

Supplementary Table 2 shows the list of the ecDTs which is consistent with known primary direct target proteins under three fungicides and bactericides: ERG1 of terbinafine, ALG7 of tunicamycin and ERG11 of clotrimazole. Whereas the ecDTs in other six drugs are not quite consistent to primary tentative target pathways known in the literature (Supplementary Table 3). They seem to be more likely to be involved in a secondary drug effect in human cells when we consider eukaryotic conservation of such ecDTs. For example, doxorubicin is known to interact with DNA by intercalation and inhibit the progression of the topoisomerase II [16], but also have many side effects in human. For example, doxorubicin interacts with iron and then generates reactive oxygen species. These can damage the myocytes (heart cells), causing myofibrillar loss and cytoplasmic vacuolization [17]. Actually, the link between this side effect and the deleterious components (vma1 and vma7) in V-Type ATPase is so far not clear, but might be testable in human cells. In *S. pombe*, all components in V-Type ATPase are essential genes and seem to be more sensitive to doxorubicin than those in *S. cerevisiae* (Supplementary Fig. S5). Similarly, ecDTs in other drugs are needed to be investigated in terms of their secondary direct target proteins related to the side effects in human cells (Supplementary Table 3).

3.3. Biological meaning and limitation of evolutionarily conserved and diverged drug target genes

Basically orthologous genes across species are typically defined on the basis of their high sequence homology and similar protein structure, and supposed to have the same biological function. Therefore, if a compound binds directly to a protein in *S. cerevisiae*, the compound also would bind directly to the orthologous protein in *S. pombe* because of their structural similarity. Furthermore, if a heterozygous deletion strain of the gene of such protein in *S. cerevisiae* has a significant growth defect under the compound, the heterozygous deletion strain of the orthologous gene of such protein in *S. pombe* is also thought to have growth defect under the same compound because of their similar functional role in each yeast. On this rationale, we proposed that the evolutionarily conserved Drug Targets (ecDTs) would be more confident direct drug target candidates.

However, percentages of ecDTs among DTs are less than 10% (7.41% and 2.78% in *S. pombe* and *S. cerevisiae*, respectively). Whereas, more than 90% (92.59% and 97.22%) of heterozygous deletion strains of orthologous genes are only hypersensitive in one of two yeasts under the same drug (hereafter, called evolutionarily diverged DT (edDT; Supplementary Tables S4 and S5). From this observation, we speculated a couple of reasons in biological and technical perspectives.

In a biological sense, the contribution of orthologous genes to a significant cell growth phenotype would be different across species because of different survival conditions the two yeasts experienced in long evolutionary history even though their molecular functional roles in a local biological pathway are similar. For example, the responses to erythromycin in two yeasts are significantly different. The mode-of-action of erythromycin is known to bind to 50 S subunit of the bacterial 70 S rRNA complex in prokaryotes [18], and to inhibit mitochondrial protein synthesis in *S. pombe* and *S. cerevisiae* [19,20]. The GO terms of 'mitochondrion' ($p <$

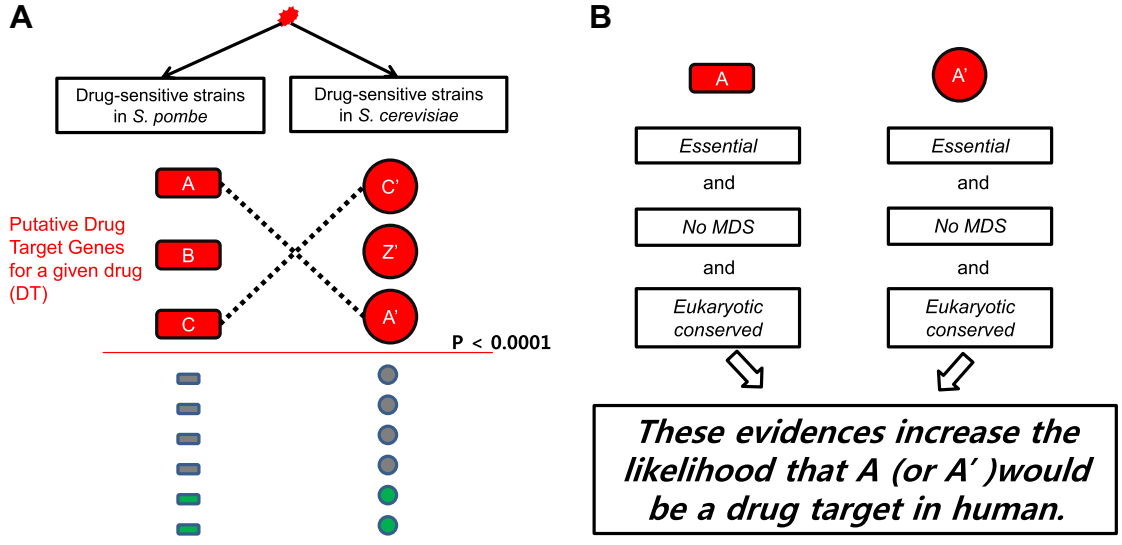

Fig. 2. If orthologous gene pairs from two yeasts are hypersensitive to a given drug, the corresponding ortholog in human is also highly likely to be a target to the drug. (A) The red squares (*S. pombe*, Sp) or circles (*S. cerevisiae*, Sc) stand for hypersensitive genes set for a given drug (p-value <0.0001) in DIHI profiles. We called this set the putative Drug Target genes for a given drug (DT).The dotted line denotes the orthologous relationship between two yeasts. For an orthologous gene pair, if both are DT, we call them evolutionarily conserved DT (ec-DT); if only one of them is DT, we call it evolutionarily diverged DT (ed-DT). Here A (or A′) and C (or C′) are ec-DT, B and Z′ are ed-DT. (B) In this example, both A (Sp gene) and A'(Sc gene) are ec-DT. Furthermore if both A and A′ fulfill all of the following requirements; both having the same essentiality, both showing no multi drug sensitivity and both having eukaryotic conservation, they can be highly expected to be possible targets of a given drug in human.

Num	*S. pombe*	*S. cerevisiae* (2004)	*S. cerevisiae* (2008)
1	5-Fluorouracil	5-Fluorouracil	5-fluorouracil
2	Actinomycin D	Actinomycin D	
3	Brefeldin A		Brefeldin A
4	Clotrimazole	Clotrimazole	Clotrimazole
5	Camptothecin	Camptothecin	Camptothecin
• • •			
45	Terbinafine	Terbinafine	Terbinafine
46	Tunicamycin	Tunicamycin	Tunicamycin
47	Valproic acid	Valproic Acid	

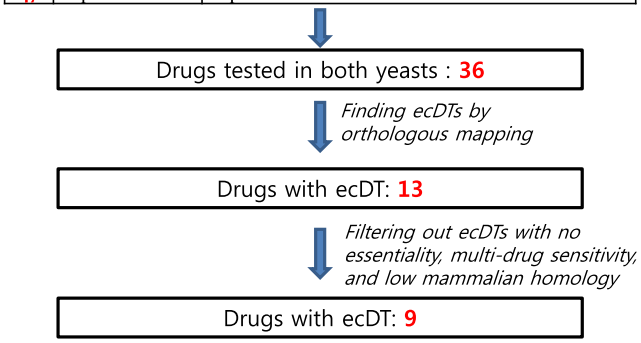

Fig. 3. The schematic procedure of selecting the evolutionarily conserved Drug Target genes (ecDT) using *S. pombe* compendium (in this work) and *S. cerevisiae* compendiums [7,8]. Among 47 drugs, 11 drugs were not tested in *S. cerevisiae* compendium. The 36 drugs, therefore, were used in comparative genetic analysis. For a given drug, first, gene sets hypersensitive to the drug (p-value <0.0001) were obtained from both compendiums. Second, orthologous mapping was carried out with the resulting gene sets for the given drug. Third, if there was no corresponding ortholog in this mapping, then the given drug was ruled out in further analysis. After this process, the remaining 13 drugs designate that these drugs are having common drug targets simultaneously in both yeasts for a given drug. Among 13 drugs, 4 drugs were ruled out because their target genes for a given drug had inappropriate features such as non-essential genes, multi-drug sensitivity, and low mammalian homology. For each of final 9 drugs, the drug target genes, from this analysis of two yeast compendiums, are expected to be directly affected by the drug in human cells (for more details, see Supplementary Tables S2 and S3).

10^{-7}; based on hyper geometric distribution) and 'mitochondrial small ribosomal subunit' ($p < 10^{-6}$) are enriched in the top 100 of erythromycin-sensitive genes (Supplementary Fig. S6) in *S. pombe* compendium while there are no enriched GO terms in corresponding top 100 in *S. cerevisiae* compendium. This could be due to mitochondrial dependency for normal cell growth in *S. pombe* [21]. In another biological sense, the inhibition effect of a drug could be masked by other proteins which have redundant function in one of two yeasts (i.e. paralogous proteins in a species).

In an experimental point of view, sub-culturing the pooled heterozygous deletion strains and PCR-based chip experiment to measure growth defect of each strain in a massively parallel way make the accumulation of random noise enhanced especially for deletion strains with subtle growth defect. More replicated experiments in two yeasts are needed to reduce the false positive DTs, which help to find more meaningful ecDTs or edDTs.

From a statistical point of view, comparative analysis using different types of fitness scoring schemes to rank drug effects of each deletion strain has some drawbacks. In *S. cerevisiae*, there are two main z-scoring schemes to calculate the statistical distance of the strain fitness at the test condition from the control condition. One of them (denoted as Hillenmeyer2008) uses 10–40 experiments with no drug as the control [7], and the other (denoted as Lum2004) uses a very diverse drug-treated chip experiments as the control [8]. We used the scoring scheme of Lum2004. Therefore, when the *S. pombe* compendium is compared with Hillenmeyer2008 compendium, there would be some discrepancy because of a different scoring scheme. In the Lum2004 compendium, its scoring scheme is the same as that of *S. pombe* compendium, but it included only heterozygous deletion strains on the half of the genome scale. Consequently, the comparison of *S. pombe* and Lum2004 compendiums also has some discrepancy by half of missing strains. These limitations would be one of the reasons to decrease percentages of the ecDTs to find more drug targets applicable to human cells. For more accurate and meaningful comparison, the unified scoring method [22] combined with more replicated experiments is needed to be developed and applied.

3.4. Web implementation

The compendium of fission yeast chemical-genetic profile and related information is freely accessible at http://pombe.kaist.ac.kr/compendium. This web page allows users to browse the compendium and look into a profile of a single drug. The page of each profile provides Z-score and p-value of drug sensitivity of *S. pombe* strains, and the interface allows sorting the profile by each kind of value. We also provide cross-reference links and orthologous information of each gene.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.05.138.

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