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Research Article

Tiled ChrI RHS collection: a pilot high-throughput screening tool for identification of allelic variants

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Abstract

Reciprocal hemizygosity analysis is a genetic technique that allows phenotypic determination of the allelic effects of a gene in a genetically uniform background. Expanding this single gene technique to generate a genome-wide collection is termed as reciprocal hemizygosity scanning (RHS). The RHS collection should circumvent the need for linkage mapping and provide the power to identify all possible allelic variants for a given phenotype. However, the published RHS collections based on the existing genome-wide haploid deletion library reported a high rate of false positives. In this study, we report *de novo* construction of a RHS collection that is not based on the yeast deletion library. This collection has been constructed for the shortest yeast chromosome, ChrI. Using this ChrI RHS collection, we identified 13 allelic variants for the previously mapped loci and novel allelic variants for the growth differences in different environments. A few of these novel variants, which were fine mapped to a gene level, identified novel genetic variation for the previously studied environmental conditions. The availability of a genome-wide RHS collection would thus help us uncover a comprehensive list of allelic variants and better our understanding of the molecular pathways modulating a quantitative trait. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: **quantitative traits; reciprocal hemizygosity scanning; high-throughput; genetic tool**

Introduction

Budding yeast, *Saccharomyces cerevisiae,* has emerged as a prime model organism for studying genetic and molecular basis of quantitative trait variation [Liti and Louis, 2012; Fay, 2013]. Linkage mapping methods have identified quantitative trait loci (QTL) regulating variation in a trait. Despite a decade of yeast quantitative trait analyses, a comprehensive identification of genes contributing to trait variation has been difficult. This has been mainly because the identified QTL typically span 40–60 kb [Bloom *et al.,* 2013; Yang *et al.,* 2013], having several candidate genes for further fine mapping.

One of the most precise genetic tools used for fine mapping is reciprocal hemizygosity analysis (RHA), which identifies allelic variants contributing to variation in a phenotype [Steinmetz *et al.,* 2002]. Expansion of a single gene RHA to a genome-wide scale to generate a reciprocal hemizygosity scanning (RHS) collection has been proposed [Steinmetz and Davis, 2004] as a method for the comprehensive identification of allelic variants contributing to trait variation. This RHS collection would not only circumvent the need for linkage mapping, but also provide high-resolution identification of variants and minor-effect QTL. Kim *et al.* [2012] implemented a quantitative non-complementation assay (half complement RHS), which was a collection of hybrid strains constructed by crossing multiple haploid strains to the commercially available haploid genome-wide deletion library. Phenotypic analysis for allelic differences identified recessive alleles of the non-essential genes contributing to trait

variation [Kim *et al.,* 2012]. While this screen allowed for the high-throughput identification of variants, it identified a limited set of alleles from only one parental background, due to the design of the collection. Wilkening *et al.* [2014], constructed the first single-gene resolution RHS collection for hybrids between a soil isolate (SK1) with lab strain (S288c). The reciprocal hemizygous strains were constructed by deleting the genes with drug cassettes, with DNA barcodes, amplified from the yeast deletion library [Wilkening *et al.,* 2014]. This genome-wide RHS collection had the ability to detect both recessive and dominant allelic variants of the essential and non-essential genes, overcoming the previous shortcomings [Kim *et al.,* 2012]. However, in this SK1/S288c RHS collection [Wilkening *et al.,* 2014] multiple incidences of chromosomal aberrations, aneuploidies and secondary site mutations were observed, which was attributed to the high rates of false positive detection.

In the present study, we report the construction of a RHS collection that seeks to overcome the above limitations. The previous RHS collections [Kim *et al.,* 2012; Wilkening *et al.,* 2014] employed the used of the haploid strains and the yeast deletion library for the construction of their collection. Furthermore, the yeast deletion collection has been shown to have several secondary site mutations that have phenotypic effects [Teng *et al.,* 2013; Giaever and Nislow, 2014]. In order to overcome these shortcomings, three strategies were employed. Firstly, the yeast deletion library was not used for construction of the collection. Secondly, genetically and phenotypically divergent diploid strains were used for the construction of a ChrI RHS collection. The diploid strains, whose genomes are more stable [Nishant *et al.*, 2010], have been known to be more robust to the phenotypic effects of secondary site mutations that can be induced during transformations [Pierce *et al.,* 2007]. Lastly, the entire ChrI RHS collection was generated in triplicates, ensuring a more confident identification of variants. Since this RHS collection was not based on the yeast deletion library, *de novo* deletions were done for 35 tiled 5 kb genomic fragments that spanned 80% of the entire length of the ChrI. In contrast, to the single gene deletion library based collections, this tiled design covered both coding and non-coding regions of the chromosome, thus having a potential to study ncRNA-dependent phenotypic variation.

Material and methods

The strains used in this study were diploid derivatives of laboratory strain, S288c (denoted as 'S') [Mortimer and Johnston, 1986] and a clinical isolate YJM145 (denoted as 'Y') [McCusker *et al.,* 1994]. The strains for the RHS collection and subsequent RHA analysis were hybrid (denoted as 'Y/S') which were generated by crossing the heterozygous deletions in S288c or YJM145 strains with the wild type parent of the opposite genetic background. The heterozygous deletions in the diploids were constructed for 5 kb long genomic fragments. Three independent deletions were constructed for the each fragment or the gene, *i.e.*, each deletion was constructed in triplicate, such that the each replicate was generated in a single transformation experiment. Tiled deletions were made from 6,793 to 222,270 bp of ChrI, excluding the two telomeres (5' telomere: 1 to 6,792 bp and 3' telomere: 222,271 to 230,218 bp) and the centromere (146,821 to 152,256 bp). To delete 5 kb fragments, deletion cassette [Goldstein and McCusker, 1999] containing a hygromycin resistance marker (*hph*MX4) was used to delete the S allele and a nourseothricin resistance marker (*nat*MX4) was used for the Y allele deletion. These deletion cassettes were transformed into the yeast using the high-throughput lithium acetate method in 96-deep well plates [Giaever *et al.,* 2002]. The heterozygous deletions in the parental strains were confirmed by a colony PCR. A list of the ChrI RHS fragments, all the ChrI RHS strains and the primers, fine mapping strains and the primers used in this study are mentioned in the Supplementary File 1 (available at the companion website http://www.tifr.res.in/~dbs/faculty/hsinha/RHS/).

DNA barcodes [Winzeler *et al.,* 1999] flanked each 5 kb fragment deletion in the collection to allow fitness estimation in a high-throughput analysis [Pierce *et al.,* 2007]. The forward and reverse primers, which included the unique DNA barcodes, for each of these deletions were designed as described previously [Winzeler *et al.,* 1999; Giaever *et al.,* 2002]. Single gene deletion primers for the RHA confirmations, did not include the barcodes and were designed to delete the entire ORF (see Supplementary File 1 available at companion website).

Isogenic hemizygous strains for the RHS collection and the RHA were constructed as described earlier [Steinmetz *et al.,* 2002]. In brief, the three independently generated heterozygous deletions

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were sporulated and their spores were crossed with those of the diploid wild type parent of the opposite genetic background. The sporulated cultures of both the genotypes were spotted on YPD plates: YJM145 *lys5/lys5* x S288c *FRAGN/ΔfragN::hphMX4 lys2/lys2* and YJM145 *FRAGN/ΔfragN::natMX4 lys5/lys5* x S288c *lys2/lys2* (where N is the fragment number) and the hybrids were selected for LYS prototrophy on SDC-LYS plates followed by screening for the respective drug cassette on YPD + Drug plates. The genotypes of the hybrid strains obtained after this process were: *FRAGN-145/ΔfragN::hphMX4* (Y allele present) and *ΔfragN::natMX4/FRAGN-288* (S allele present).

The base media used for all growth and phenotyping assays was the standard YP media (1% yeast extract, 2% bacto peptone) supplemented with 2% carbon source. Minimal media was made by mixing 0.67% nitrogen base (without amino acid), 0.087% complete amino acid mix (in case of SDC-LYS, lysine drop out amino acid mix was used) and 2% glucose in water. All the sterile stock solutions of various chemicals were made in milliQ water except menadione and 4-NQO, which were made in DMSO. List of all the environmental conditions tested is given in Table 1.

Growth was assayed in a 96-well format [Perlstein *et al.,* 2007]. Growth was estimated by measuring OD_{600} at 24, 48 and 72 h using TECAN Finite M200 multi-plate reader. Experiments were performed with the three biological replicates of each strain and an average growth was measured across the experiments done on three separate days (N =3, n =3). Student's *t*-test was performed on the samples to test for significance between a given pair of strains at p-value <0.05. Only the RHS strains showing consistent differences across the three experiments for a given time point were further considered and have been listed in Table 2.

Results

The reciprocal hemizygous strains were constructed for 5 kb long tiled genomic fragments. This tiled design had two advantages. Firstly, it reduced the total number of strains to be constructed and secondly, it covered both coding and non-coding regions of the genome. Compared to the single-gene RHS collections [Kim *et al.,* 2012; Wilkening *et al.,* 2014], our RHS design reduced the number of strains to be constructed for the ChrI from 102 to 45. On a genome-wide scale, such a design would reduce the number of strains from ~6,000 to 2,400 pairs. The telomeric ends and the centromere were not deleted to maintain the structural stability of the chromosome.

On an average, the 230 kb long ChrI has 19.7 SNPs per kb between S and Y strains, which is the highest compared to the other chromosomes in the genome [Wei *et al.,* 2007]. Due to this high sequence heterogeneity, confirmation primers were redesigned specific to the Y genome for four fragments. A highly polymorphic region spanning the fragments F37 (182,263 to 187,263 bp) and F38 (187,264 to 192,264 bp) was found to be difficult to delete. Thus, to delete this entire region (182,263 to 192,264 bp), a single 15 kb fragment spanning F36-F38 (177,262 to 192,264 bp) was deleted. This reduced the strains for the ChrI RHS collection from 45 to 43. Of these 43 RHS strains, despite multiple attempts, a few fragments could not be deleted in either both (F1, F30, F42, F45) or one of the parents (F3, F4, F5, F6), maybe due to their close proximity to the telomeres or the centromere (coordinates of the fragments are described in the Supplementary File 1 available at companion website). Thus, finally, we have been successful in constructing a RHS collection in which there were 35 hemizygous pairs which covered slightly more than 80% of the collection, spanning 185 kb of the ChrI.

A few previous studies have reported QTL on the ChrI for growth differences in various environments [Perlstein *et al.,* 2007; Ehrenreich *et al.,* 2010; Bloom *et al.,* 2013; Gagneur *et al.,* 2013]. *FLO1,* identified for variation in cell aggregation [Li *et al.,* 2013], is the only gene on the ChrI which has been fine mapped for a complex trait. The pairs of strains of the RHS collection are genetically identical except at the locus/fragment of interest. This genetic uniformity allows for robust identification of the minor-effect variants. These variants can be missed in the linkage mapping due to the genetic heterogeneity of the segregants that leads to a high background noise. To assess the ability of this RHS collection to identify novel and/or minor variants, we tested the growth of the ChrI RHS collection strains in 15 different growth environments (Table 1). Of the tested environments, a QTL on the ChrI has been reported only for growth in

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Table 1. Environments tested on the ChrI RHS collection

Environment	QTL position on ChrI	Reference
Minimal medium (SDC)	NA	
YP dextrose (2%)	27 915–83 285 bp	Bloom *et al.,* 2013
YP ethanol (2%)	NA	
YP fructose (2%)	NA	
YP galactose (2%)	31 636–83 285 bp	Bloom *et al.,* 2013
YP glycerol (2%)	NA	
YP lactose (2%)	NA	
YP maltose (2%)	NA	
YPD +0.05% SDS	NA	
YPD +0.25 μg/ml 4-NQO	NA	
YPD +350 mM NaCl	NA	
YPD +1 M NaCl	NA	
YPD +1 M sorbitol	NA	
YPD +100 μM menadione	NA	
YPD +6 mM H_2O_2	Yes (position unknown)	Ehrenreich *et al.,* 2010

Various media tested for the ChrI RHS collection. Positions of the various QTLs detected in the previous yeast QTL studies are indicated.
YPD, YP dextrose (2%) medium; NA, a QTL on the ChrI has been reported.

galactose and glucose [Bloom *et al.,* 2013] and hydrogen peroxide [Ehrenreich *et al.,* 2010], using RM and BY (derivative of S288c, which is isogenic to one of the parental strain used in the current RHS collection) strains. Consistent with these reports, we found variants on the ChrI contributing to growth differences in galactose at 72 h (F8 and F13) and hydrogen peroxide at 72 h (F8, Table 2). In addition to the reported QTL in the previous study [Bloom *et al.,* 2013], we found more variants at 24, 48 and 72 h in galactose. Furthermore, for glucose, we identified a different genomic locus at 72 h (Table 2). These additional loci detected in this RHS collection could result from the differences in phenotypic growth assays or due to the different genetic backgrounds of the strains used [Ehrenreich *et al.,* 2010; Bloom *et al.,* 2013]. To find if the additional loci identified above could have been a result of strain differences, we compared the identified variants from

Table 2. List of fragment variants identified in the various environments at different time points

	Fragment variants showing growth difference		
Condition	**24 h**	**48 h**	**72 h**
Minimal medium (SDC)	F8 (S)		
YP dextrose (2%)			F34 (Y)
YP ethanol (2%)	F18 (S), F23 (S), F31 (S)	F7 (Y), F18 (S), F23 (S), F31 (S)	F23 (S)
YP fructose (2%)		F20 (S)	F20 (S)
YP galactose (2%)	F22 (S), F23 (S), F25 (S)	F20 (S), F22 (S), F23 (S)	F8 (Y), F13 (S), F20 (S), F22 (S), F23 (S), F32 (Y)
YP lactose (2%)			F18 (Y), F23 (Y)
YP maltose (2%)	F21 (S), F22 (S), F23 (S) F24 (S)	F21 (S), F22 (S), F23 (S), F24 (S)	F22 (S), F23 (S), F25 (S)
YPD +0.05% SDS		F12 (S)	
YPD +0.25 μg/ml 4-NQO		F24 (S)	
YPD +1 M NaCl			F7 (S), F22 (Y)
YPD +1 M sorbitol	F8 (S)		
YPD +6 mM H_2O_2			F8 (Y)

The fragment variants showing significant growth differences, with the better-performing parental allele indicated in the brackets.

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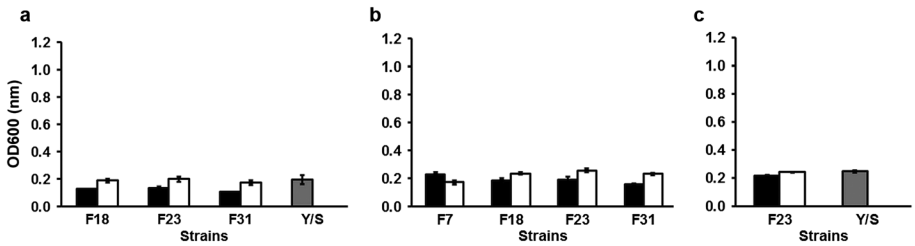

Figure 1. Fragments identified for growth differences in YP Ethanol (2%) (a) 24 h; (b) 48 h; (c) 72 h. Histogram bars represent the mean and error bars are SEM (N =3, n =3). Black bars show the growth of the diploid hemizygous strains with the Y allele (Y/Δ), white bars of the S allele (Δ/S) and gray bar of the wild type diploid hybrid (Y/S). The mean differences for all the shown fragments are significant at $p < 0.05$

the RHS collection to those found in a recent study by Gagneur *et al.* [2013]. In ChrI, only a single QTL for growth differences in rapamycin (which was not tested) was identified by Gagneur *et al.* [2013]. However, using the ChrI RHS collection, a total of 13 novel variants were identified in 12 environments (see below). This suggested that the ChrI RHS collection had a higher power for detecting variants than mapping.

Screening the entire ChrI RHS library of 35 hemizygous strains for growth at 24, 48 and 72 h (see Supplementary File 2, Supplementary Table 1 and Supplementary Table 2 available at companion website), identified a total of 13 fragments showing the growth differences across 12 environments (Figure 1, Figure 2 and Table 2). This result, unlike what has been reported previously [Gagneur *et al.,* 2013], suggested that the ChrI harboured multiple loci, possibly minor effect, that were causative for the phenotypic variations and could have been missed in the linkage mapping. The F8 and F23 RHS strains showed growth differences in the maximum number (4) of conditions whereas the RHS pairs for F12, F13, F32 and F34 showed growth differences only in one environment (Table 2). Results from the previous studies [Kim *et al.,* 2012; Wilkening *et al.,* 2014] indicated a high rate of false positive detection. In order to validate our findings by fine mapping, we constructed single gene RHA strains for the three positive fragments (F7, F22 and F23) to fine map the causative gene(s), among the 2–3 genes within each fragment. To prioritize the candidate gene for deletion within a fragment, we first checked if the gene overlapped across two identified fragments, *e.g.* F22 and F23. Followed by, occurrence of non-synonymous SNPs in the genes and finally checking for any functional or phenotypic relationship to the tested environment. We constructed RHA strains for identification of the causative genes within fragments F7, F22 and F23.

Fragment F7 contains 4 genes (*GDH3*, *BDH2* (overlapping SUT, *SY0604*), *BDH1* and *ECM1*; Figure 3a and see Supplementary Table 1 available at companion website). Fragment F7 RHS strains showed growth differences in ethanol (Figure 3b) and 1 M NaCl (Figure 3c), but the allelic effects were in the opposite direction. *ECM1* gene was not considered for fine mapping as the F8 did not show these phenotypic differences. The RHA strains were constructed for *GDH3, BDH2* and *BDH1* for identification of the underlying causative gene. RHA of

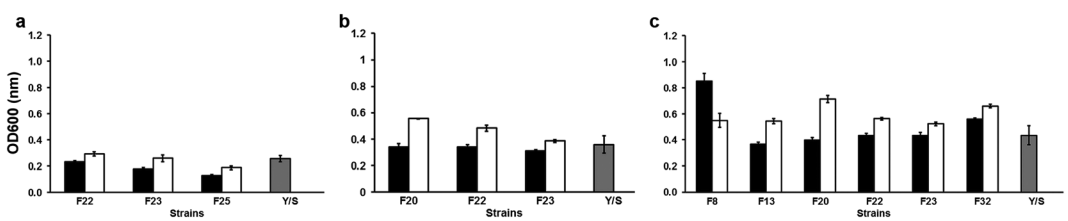

Figure 2. Fragments identified for growth differences in YP Galactose (2%) (a) 24 h; (b) 48 h; (c) 72 h. Histogram bars represent the mean and error bars are SEM (N =3, n =3). Black bars show the growth of the diploid hemizygous strains with the Y allele (Y/Δ), white bars of the S allele (Δ/S) and gray bar of the wild type diploid hybrid (Y/S). The mean differences for all the shown fragments are significant at $p < 0.05$

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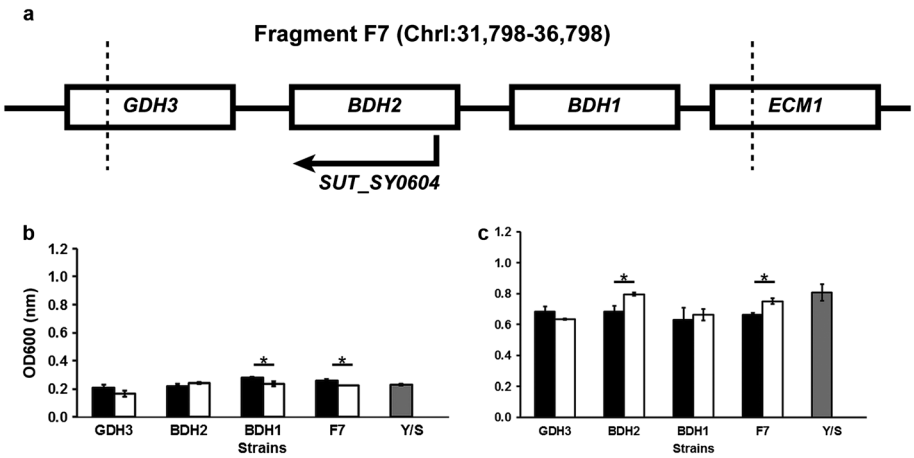

Figure 3. RHA of genes in fragment F7 in YP Ethanol (2%) (a) Deletion of F7 disrupted four genes: *GDH3*, *BDH2* (overlapping SUT, *SY0604*), *BDH1* and *ECM1*. The dashed lines define the 5 kb deletion boundaries; (b) RHA of genes of F7 at 48 h in YP Ethanol (2%); (c) RHA of genes of F7 at 72 h in YPD +1 M NaCl. Histogram bars represent the mean and error bars are SEM (N =3, n =3). Black bars show the growth of the diploid hemizygous strains with the Y allele (Y/Δ), white bars of the S allele (Δ/S) and gray bar of the wild type diploid hybrid (Y/S) diploid strain. Significant differences at $p < 0.05$ are represented as *

BDH1 identified the Y allele as the positive allele for ethanol growth (Figure 3b). *BDH1* is a well-characterized NAD-dependent butanediol dehydrogenase enzyme which reversibly converts acetoin to 2,3-butanediol, minor products of aerobic respiration [González *et al.,* 2000]. *BDH1* expression increases during the stationary phase and the diauxic shift [DeRisi *et al.,* 1997; Gasch *et al.,* 2000; González *et al.,* 2000] and the activity of Bdh1 increases on glucose deprivation [González *et al.,* 2000]. Coding sequence comparisons of *BDH1* between the S and Y strains, identified 11 SNPs of which 5 were non-synonymous. On the other hand, the RHA analysis identified *BDH2* as causative gene leading to phenotypic differences in 1 M NaCl, with the S allele imparting better growth. *bdh2*Δ in the S288c background is known to cause sensitivity to 1 M NaCl [Yoshikawa *et al.,* 2009]. In line with these results, we have found that the S allele of *BDH2* showed better growth than the Y allele in high salt stress condition (Figure 3c). Even though Bdh2 has 51% homology to Bdh1, *BDH2* is poorly characterized with no annotated function. Sequence alignment of *BDH2* identified 8 SNPs in the coding region of the gene between S and Y, of which 2 were non-synonymous.

Two fragments F22 and F23 showed allelic growth differences in galactose (Figure 2) and maltose at 24, 48 and 72 h (Table 2). Since *CCR4* was an overlapping gene between these fragments, it was tested with RHA (Figure 4a), which confirmed that variation in *CCR4* contributed to observed growth differences in galactose (Figure 4b, 4c, 4d) and maltose (data not shown). *CCR4*, which is conserved across organisms, is part of the Ccr4-NOT protein complex, a global transcript regulator [Liu *et al.,* 1998; Collart, 2003]. The complex has been implicated to regulate mRNA degradation, transcript elongation, transcription initiation and post-transcriptional modification, thus, affecting growth and stress response [reviewed in Collart, 2003; Miller and Reese, 2012]. Sequence comparison of coding region of *CCR4* in the S and Y identified 24 SNPs including a 6 bp deletion in the Y. While *ccr4*Δ strain have not been documented to show galactose sensitivity, *NOT4*, an interacting partner of *CCR4,* deletion showed impaired growth in galactose [Deluen *et al.,* 2002].

Discussion

Several traits of clinical, agricultural and evolutionary importance are quantitative in nature. However, a comprehensive identification of the causative genes and their genetic architecture (additive and non-additive interactions) is lacking. This is essential for a thorough understanding of the complex relationship between phenotypic and

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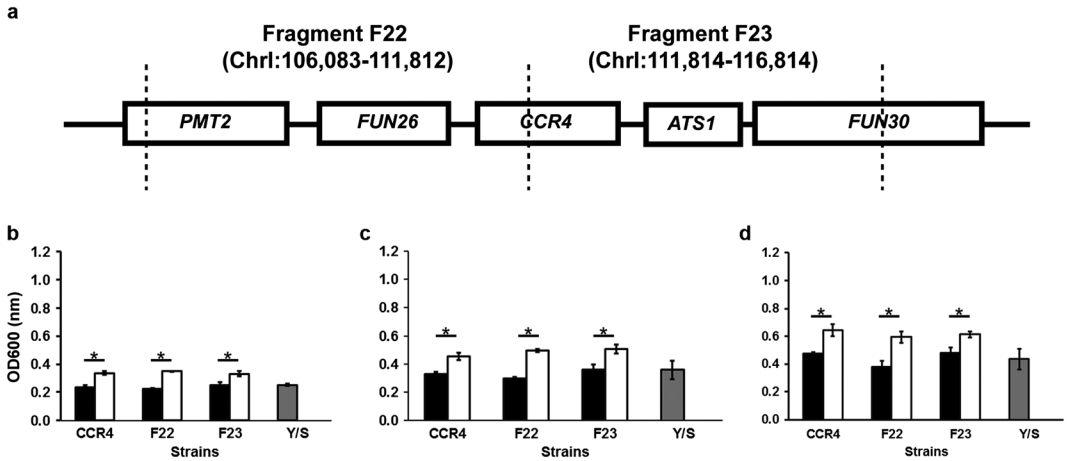

Figure 4. RHA of *CCR4* common in fragments F22-F23 in YP Galactose (2%) (a) *CCR4* is a common gene disrupted by deletion of F22 and F23. The dashed lines define the 5 kb deletion boundaries; Growth of the RHA strain of *CCR4* and the RHS strains of F22 and F23 at (b) 24 h; (c) 48 h; (d) 72 h. Histogram bars represent the mean and error bars are SEM (N =3, n =3). Black bars show growth of the diploid hemizygous strains with the Y allele (Y/Δ), white bars of the S allele (Δ/S) and gray bar of the wild type diploid hybrid (Y/S). Significant differences at $p < 0.05$ are represented as *

genetic variation. This comprehensive identification has primarily been limited by the power and resolution of detection of QTL by linkage mapping. Yeast, as a model for quantitative traits, has been extensively employed to gain fundamental understanding of quantitative traits [Swinnen *et al.,* 2012a; Liti and Louis, 2012; Fay, 2013]. RHA has been used widely for identifying the causative allelic variants for a phenotype in a mapped QTL [Steinmetz *et al.,* 2002; Deutschbauer and Davis, 2005; Sinha *et al.,* 2006, 2008; Gerke *et al.,* 2009; Swinnen *et al.,* 2012b; Yang *et al.,* 2013]. Recently, Wilkening *et al.* [2014] constructed a genome-wide single-gene RHS as a high-throughput method to identify all possible variants contributing to phenotypic variation. However, to ascertain the reason for high rate of false positives, 50 RHS strains (38 false positives and 12 random) were sequenced and a high degree (12 of 38 false positives and 4 of 12 random) of aneuploidy or chromosomal aberrations were found [Wilkening *et al.,* 2014]. This confounded the results of the RHS collection and thus, the collection was not used further by the authors [Wilkening *et al.,* 2014].

We report here the construction of a potentially genome-wide RHS collection that has advantages over the previously reported hemizygous collections [Kim *et al.,* 2012; Wilkening *et al.,* 2014]. The RHS collection reported here for the ChrI is a 5 kb genomic fragments tiled design which gives a greater genomic coverage, spanning both genes and inter-genic regions. Apart from this it reduces the total number of strain pairs, compared to the single gene collections, to be constructed from 102 to 45. Unlike the single-gene RHS collection [Wilkening *et al.,* 2014], which was in duplicate, our collection was generated in triplicate, which provided a more confident estimate of the allelic differences. Addition of a third biological replicate in this design would require approximately a total of 14,400 strains for a genome-wide collection. This is a 24% reduction compared to the genome-wide collection reported by Wilkening *et al.* [2014] with duplicates per strain. Since *de novo* construction of this RHS collection was done in the diploid parental strains that were crossed to the wild type parent of the opposite genetic background, the secondary site mutations should occur at a reduced rate. This is corroborated by more accurate and robust identification of the allelic variants on phenotyping this RHS collection. However, for a definitive proof of the absence or a lesser number of mutations one would need to sequence some of these RHS strain pairs.

Expanding this tiled RHS collection to a genome-wide scale would generate a robust high-throughput screening tool for the identification of the allelic variants contributing to the phenotypic differences. Detailed growth analysis, like growth kinetics, is expected to yield a larger number to variants, primarily

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due to the difference in regulation of growth during the exponential and the stationary phase [Bhatia *et al.,* 2014]. Since in this RHS collection one parent is a clinical isolate (YJM145 strain), this collection can be used for screening allelic variants contributing to clinically relevant traits like fungal pathogenicity and anti-fungal resistance. The phenotypic analysis of such a genome-wide RHS collection will, however, provide insights into trait variation that exists only between the two parental strains. The collection, therefore will not be able to account for the vast amount of genetic and phenotypic diversity found in the nature [Liti *et al.,* 2009]. Irrespective of this shortcoming, the power and resolution of this tool will help enumerate a comprehensive list of genes modulating a quantitative trait. Once a comprehensive list of the variants is identified, it would become possible to identify the pathway(s) central to trait variation and understand the molecular basis of variation. Since the tiled fragment deletions span both the coding and non-coding regions of the genome, it allows the possibility to determine, if and how do the non-coding regions, not overlapping with coding regions, play a role in the trait variation? With the recent evidences in the yeast showing that the non-coding transcripts have functional and regulatory roles [reviewed in Pelechano and Steinmetz, 2013], the ability of this RHS collection design provides a screening tool for testing phenotypic consequences of the non-overlapping ncRNA variation. Apart the growth phenotype, variants can be mapped for gene expression [Brem *et al.,* 2002], protein abundance [Foss *et al.,* 2007] and metabolite concentrations [Breunig *et al.,* 2014]. These phenotypes with RHS collection can provide an insight into the genes and pathways causative of the physiological variation. Thus, a genome-wide version of this RHS collection will help in bridging the phenotype to genotype gap.

Author contributions

HS conceived the project, RS constructed the collection and analyzed the data, and RS and HS wrote the paper.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web site:

Supplementary Table 1. List of ChrI fragment variants identified in the various environments at different time points, the coordinates and the putative causative genes within the fragment and their functions.
Supplementary Table 2. Fold differences in growth of all the RHS strains at 24, 48 and 72 h in all the environments tested. Significant differences ($p < 0.05$) are indicated in bold.
Supplementary File 1. List of coordinates for the RHS fragments along with the genes disrupted, the strains constructed for the RHS and the RHA and the sequence of primers used during construction.
Supplementary File 2. Raw growth values of all the 35 ChrI RHS strains in the tested 15 environments at 24, 48 and 72 h.

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