Critical Review

Genome Wide Analysis of Meiotic Recombination in Yeast: For a Few SNPs More

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Abstract

Diploid organisms undergo meiosis to produce haploid germ cells. Crossover events during meiosis promote genetic diversity and facilitate accurate chromosome segregation. The baker's yeast *Saccharomyces cerevisiae* is extensively used as a model for analysis of meiotic recombination. Conventional methods for measuring recombination events in *S. cerevisiae* have been limited by the number and density of genetic markers. Next generation sequencing (NGS)-based analysis of hybrid yeast genomes bearing thousands of heterozygous single nucleotide polymorphism (SNP) markers has revolutionized analysis of meiotic recombination. By facilitating analysis of marker segregation in the whole genome with unprecedented resolution, this method has resulted in the generation of high-resolution recombination maps in wild-type and meiotic mutants. These studies have provided novel insights into the mechanism of meiotic recombination. In this review, we discuss the methodology, challenges, insights and future prospects of using NGS-based methods for whole genome analysis of meiotic recombination. The objective is to facilitate the use of these high through-put sequencing methods for the analysis of meiotic recombination given their power to provide significant new insights into the process. © 2018 The Authors. IUBMB Life published by Wiley Periodicals, Inc. on behalf of International Union of Biochemistry and Molecular Biology, 70(8): 743–752, 2018

INTRODUCTION

All sexually reproducing organisms undergo two rounds of division (Meiosis I and Meiosis II) to produce haploid gametes from diploid progenitor cells. Homologous recombination events during Meiosis I, such as crossovers, non-crossovers and gene conversions generate genetic diversity. In addition, crossovers facilitate disjunction of homologous chromosomes during Meiosis I by promoting physical linkages between the homolog pairs that oppose the spindle generated forces pulling the homologs apart. The opposing forces provide the tension necessary for the correct alignment and disjunction of the homologous chromosomes (1). The number and spatial distribution of crossovers are tightly regulated to ensure at least one crossover per homolog pair. Segregation errors in meiosis results in aneuploidy, which is a major cause of genetic birth defects in humans (2).

The baker's yeast *Saccharomyces cerevisiae*, has been used extensively as a model organism to study meiosis. *S. cerevisiae* can undergo meiotic divisions rapidly (~12 h for SK1 strain). Further, the small genome size of *S. cerevisiae* (12 Mb) and the ease of genetic modification facilitate a wide
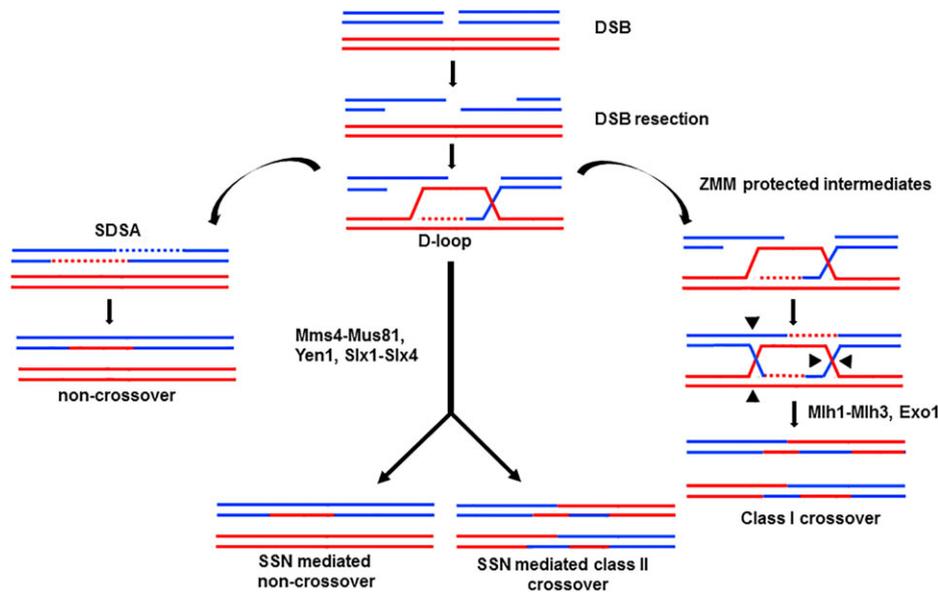
FIG 1 *Recombination outcomes of DSB repair pathways during meiosis. DSB resection and strand invasion leads to D-loop formation. Dissolution of the D-loop results in non-crossovers by SDSA mechanism. Protection of D-loops from disassembly by the ZMM proteins allow double Holliday junction formation that is acted on by Mlh1-Mlh3 and Exo1 to form the class I crossovers. Alternatively the joint molecules, may also be processed by the structure selective nucleases Mms4-Mus81, Slx1-Slx4 and Yen1 to form the class II crossovers and non-crossovers.*

array of experimental analysis. In *S. cerevisiae*, crossovers are initiated by the formation of 140–170 double strand breaks (DSBs) by a conserved type II topoisomerase Spo11 along with accessory factors (3,4). Meiotic DSB repair, preferentially using the homolog as a template results in either a crossover or non-crossover outcome (Fig. 1). During DSB repair, the invading strand may get displaced from the homolog and ligate with the opposite end of the break leading to the formation of non-crossovers by synthesis dependent strand annealing (SDSA) pathway (5). If the strand invasion is stabilized by the ZMM proteins (Zip1, Zip2, Zip3, Zip4, Mer3, Msh4, Msh5 and Spo16), it may be extended further by repair synthesis using the homolog and capture the second end of the DSB and form double Holliday junction (6). Biased resolution of these double Holliday junctions facilitated by the ZMM, STR (Sgs1, Top3, Rmi1), Exo1 and the Mlh1-Mlh3 endonuclease leads to crossovers (7–14). These class I crossovers show interference—a phenomena where the occurrence of a crossover event in a genetic interval makes it less likely for crossovers to occur in adjacent intervals. Another set of crossovers (Class II) are generated from the Holliday junction intermediates by Mms4-Mus81, Yen1, and Slx1-Slx4, the structure selective nucleases (SSNs). Resolution by the SSNs lack crossover bias, and both crossovers and non-crossovers are produced. These class II crossovers do not show interference (15,16).

Classical genetics, cytological methods, and physical analysis, have been conventionally used to characterize meiotic recombination in *S. cerevisiae*. In this review, we describe the advantages of next generation sequencing (NGS)-based methods for genome wide analysis of meiotic recombination compared to these conventional methods of recombination mapping. We also discuss issues related to the experimental and bioinformatics aspects of genome wide recombination analysis to make this comparatively new area more familiar to the researchers.

CONVENTIONAL METHODS FOR ANALYSIS OF MEIOTIC RECOMBINATION IN *S. cerevisiae*

Classical genetic methods involve the use of auxotrophic or drug markers whose segregation can be visually monitored in meiotic spores to measure crossover frequency and gene conversions. The information from segregation of markers in meiotic spore progeny gives an estimate about the recombination frequency between those markers. This method is still popular as it is cost-effective and provides a basic idea of the recombination frequency before initiating a more elaborate and expensive NGS-based approach. The ease of genetic manipulation in *S. cerevisiae* means the markers can be inserted at the desired location in the genome to estimate the recombination frequency of the locus. These estimates are often extrapolated on a whole genome scale. For example in the *S. cerevisiae* SK1 strain, a 395 kb region in chromosome XV has been modified with six auxotrophic markers that corresponds to a genetic distance of 100.9 cM in wild-type meiosis (17). To generate data from more loci, additional genetic markers were integrated into representative small, medium and large chromosomes (Chr III, VIII and VII, respectively) (15). The crossover defects in many meiotic mutants have been analyzed using

these strains (12,17–20). The major drawback of the recombination maps generated from this method is that it requires the analysis of a large population of tetrads and provides locus specific data. Since recombination rates are not constant across the *S. cerevisiae* genome, locus specific measures may not accurately represent the genome-wide recombination frequency. Another drawback is the dependence on spore viability making it difficult to analyze meiotic mutants with severe defects in viability or meiotic progression. Also only a limited number of auxotrophic or drug markers can be introduced.

Unlike classical genetic analysis, cytological methods involve immunostaining of crossover specific proteins like Zip3, Msh4/Msh5 on meiotic chromosome spreads. This method provides information on recombination frequency on a genome wide scale and is independent of the viability of the spores (13,18). The number of foci correlates with an increase or decrease in the recombination events. The drawback of this method is that one has to rely on the proportional change of the foci number, which does not give a count of the actual number of recombination events and it also provides low resolution data. Another cytological method relies on the use of fluorescent tetrads to detect recombination events (21). This method bypasses the necessity of viable tetrads. The selected homolog pairs are marked in the appropriate loci with fluorescent markers and direct visualization of the tetrads under the microscope reveals the marker segregation pattern. This method can analyze crossovers, non-crossovers, chromosome non-disjunction without dissecting numerous tetrads (21). In spite of its advantages, the method provides locus specific data.

Besides genetic and cytological methods, single locus physical assays have been developed to quantify the recombination products in *S. cerevisiae*. A well-characterized DSB hotspot (*e.g. HIS4-LEU2* on Chromosome III) is modified with restriction enzyme sites to distinguish the homologous chromosomes (11,12). The DNA from synchronized meiotic cultures are digested with the appropriate restriction enzymes. The products are analyzed by two-dimensional gel electrophoresis and probed to identify DSBs, joint molecules—both interhomolog and intersister, crossover and non-crossover products (11,22). This method circumvents the issue of spore viability and has been used to study meiotic mutants with severe viability defects (10,14–16,23–25). But this method also has the limitation of reporting data of only one locus.

In summary, classical genetic analysis, and physical assays provide recombination information from a specific locus with good resolution, whereas cytology can provide genome wide recombination data but at low resolution. With NGS-based methods, the trade off between resolution and genome coverage becomes irrelevant. But like any genetic method, recombination analysis by NGS requires the four spores to be viable, making it difficult to analyze mutants with poor spore viability or defects in meiotic progression. With advances in DNA sequencing techniques, the complete sequence of many *S. cerevisiae* strains are accessible in the *Saccharomyces* Genome Database (https://www.yeastgenome.org/). The presence of a wide variety of *S. cerevisiae* strains with sequenced genomes provides a multitude of strain combinations to generate hybrids. Sufficient density of single nucleotide polymorphisms in the hybrid strains allows us to map recombination events genome wide at high-resolution using NGS analysis as described below.

NGS-BASED ANALYSIS OF HYBRID *S. cerevisiae* STRAINS TO MAP MEIOTIC RECOMBINATION GENOME WIDE

Though high-resolution genome wide recombination analysis is now routinely performed using NGS, it started with microarray based methods by Mancera et al. (26) using *S. cerevisiae* S288c/YJM789 hybrid (Fig. 2). The YJM789 strain is a clinical isolate and 0.6% diverged from the standard laboratory S288c strain (27). DNA isolated from four viable spore tetrads of the hybrid were fragmented, fluorescently labeled and hybridized against the microarray that contains probes for both the parental SNPs at 4 bp resolution. Around 52,000 SNPs were called which were uniformly distributed throughout the genome with an average inter-marker distance of 78 bp (26). A similar study by Chen et al. called 8,000 markers between the two parental strains (S288c, YJM789) with an average distance of 1.5 kb between two consecutive markers (28). Both the studies reported almost similar number of crossovers (90.5 and 95), but non-crossovers (19) were fewer in Chen et al. (28) compared to the non-crossovers (66) from Mancera et al. (26). This is because lower SNP density affects the detection of non-crossovers but not crossovers. Microarray-based analysis of genome wide recombination data has largely given way to NGS-based methods due to falling sequencing costs as well as some of the limitations of microarrays (Fig. 2). These include (i) requirement of prior knowledge of the genome sequence of the organism to design oligo probes for hybridization. (ii) Single nucleotide resolution provided by NGS methods compared to microarray based methods where the resolution is dependent on the probe density. (iii) Potential for errors due to false hybridization in microarray based methods. Below we discuss some of the issues involved in the design of experimental and bioinformatics methods for genome wide recombination mapping in hybrid yeast using NGS.

Choice of the Appropriate Yeast Hybrid and Marker Density

The small size of the *S. cerevisiae* genome (12 Mb) and the availability of the genome sequences of various *S. cerevisiae* strains have made genome wide recombination analysis easier and cheaper compared to other organisms having complex, large genomes. The presence of a sufficient number of uniformly distributed SNP markers is a requisite for high-resolution recombination mapping. But a very high density of SNP markers may not be advantageous as the SNPs are treated as mismatches during DSB repair using the homolog. High sequence divergence may cause the mismatch repair response to reject strand invasion into the homolog. This may result in
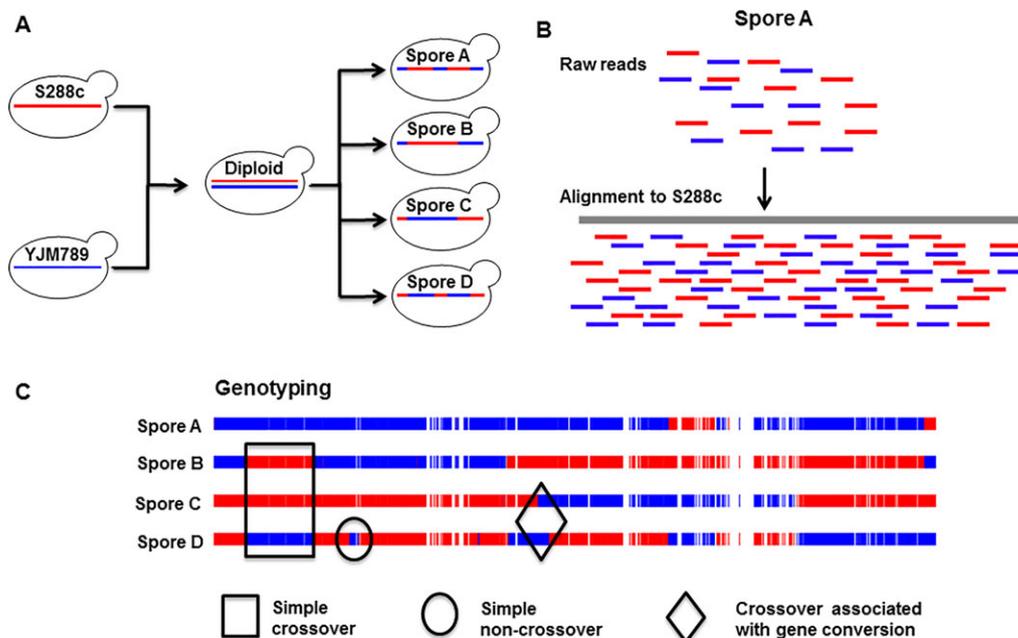
Recombination mapping using NGS analysis of hybrid yeast strains. (A) Representative cross involving the S. cerevisiae S288c and YJM789 strains to generate hybrid diploid. The diploid is sporulated and the spores are sequenced using NGS methods. (B) Alignment of whole genome sequence data from spores to a reference genome for calling variants (SNPs). (C) Recombination outcomes that may be detected from SNP segregation data in the four spores from a single tetrad. Rectangular box shows simple crossovers that can be identified by the reciprocal exchange of flanking markers in 2:2 segregation pattern. Diamond box shows crossovers accompanied by gene conversions with the segregation of markers in 1:3 or 3:1 ratio around the exchange sites. Circle shows simple non-crossovers which can be detected by the presence of 1:3 or 3:1 segregation tracts without any exchange of flanking markers. In addition minority recombination outcomes caused by events like multiple chromatid invasions or exchanges involving more than two chromatids can also be detected by marker segregation patterns containing signatures unique to the mechanism.

recombination outcomes that favor sister chromatid repair as well as complex recombination outcomes that are difficult to detect (29,30). For example, Martini et al. have shown that in a cross between *S. cerevisiae* SK1 and S288c strains (0.7% divergence), 73 crossovers are made (30). Deletion of the mismatch repair gene, *MSH2* in both the parents increased the crossovers to 92 (30). Hybrids with higher SNP density also show a significant drop in viability (Table 1), suggesting a negative correlation of spore viability with heterozygosity (30,32–34). So, choosing the appropriate hybrid is important. Hybrid choice should be determined by the evolutionary origin of the strains. The *S. cerevisiae* SK1 strain has evolved separately from all other strains used in the lab. As a result higher sequence divergence, reduced viability and recombination is observed in crosses involving SK1 with other *S. cerevisiae* strains (Table 1). On the other hand, *S. cerevisiae* strains like S288c, RM11, YJM789 etc., are of similar origin (35,36). Crosses involving these strains like S288c/YJM789 or RM11/S288c show better spore viability and have similar crossover frequency as the well-characterized isogenic SK1 strain (Table 1). An important advantage of using the S288c/YJM789 hybrid is its non-mutagenic nature. The mitotic base mutation rate of this hybrid (1.82×10^{-10} per base per division) is almost similar to that of the isogenic S288c and SK1 strains (34).

Sequencing and Phasing of SNPs

In principle, any NGS method can be used to genotype SNPs in the meiotic spores for recombination mapping. Sequencing techniques with shorter reads like Illumina (https://www.illumina.com) increase the chance of misalignment in the repeat

S. cerevisiae artificial hybrids

Hybrids	SNPs	Crossovers	Spore viability (%)
S288c/YJM789 (26, 31)	52,000	90.5	84
RM11-1a/YJM789 (32)	30,000	NA	90
S288c/RM11-1a (33)	46,000	91	85
S288c/SK1 (30)	62,000	73	70
SK1/YJM789 (34)	65,000	NA	77
SK1/RM11-1a (34)	69,000	NA	76

The approximate number of SNPs, average crossovers and spore viability of different hybrids is shown.

Quality Control (QC)

QC analysis of reads (coverage, GC content, adapter contamination, read length distribution, base distribution)

QC of aligned reads (mapping quality of reads)

Pre-processing

Read pre-processing, trimming, adapter removal, removing bases with low quality

Remove low quality reads, duplicate reads

Variant filtering (separating SNPs and In-dels, discard low quality variants)

Raw sequence reads

Alignment to reference genome

In-del realignment

Variant calling

Generating segregation file

Recombination analysis

Statistical analysis and visualization

FIG 3 *Flowchart of the bioinformatics analysis for inferring recombination events from whole genome sequence data of meiotic spores.*

sequences. So, the telomeric regions, Ty element containing sequences etc are excluded from the SNP analysis when using such technology. Sequencing technologies with longer reads like PacBio (https://www.pacb.com) or nanopore (https://nano-poretech.com) are more useful if SNPs from repeat regions need to be analyzed for recombination data. New mutations that are heterozygous can arise while the spore cultures are grown for several generations to isolate DNA. Although genotyped, these are usually ignored and not used for downstream recombination analysis. It is therefore useful to sequence the parent strains available in the lab so that new mutations in spore DNA can be identified and eliminated from the analysis or used if they are present in the parent as well.

Whole genome recombination mapping requires the knowledge of the phases of the SNP markers, that is, the concerned marker belongs to which parent. Without phasing, the segregation analysis of the SNP markers in the spores is not possible. For standard artificial hybrids like S288c/YJM789, SK1/S288c or RM11/S288c, the reference genomes of the parent strains are available. Therefore the phases of the SNP markers are known and any sequencing method, even those with short reads (*e.g.*, Illumina) is fine. But in nature, many yeast hybrids exist and to understand meiotic recombination in a natural hybrid can be of great value. But the problem of phasing of the SNP markers

arises. To overcome this problem, techniques (PacBio, nanopore), which give very long reads, are useful since SNPs frequently occurring together in a single read are likely to be in phase. With long reads (in kbs), the alignment will be easier, and all the SNP markers can be phased (37,38).

Bioinformatic Analysis of SNP Segregation and Recombination

The computational analysis of SNP segregation and recombination from Illumina short sequence reads involves multiple steps (Fig. 3).

Quality Control (QC) Analysis, Preprocessing and Coverage.

Raw sequence reads that are generated from the Illumina platform are in fastq file format which includes the quality information of the bases. It is critical to thoroughly check the base qualities and read statistics (number of reads, overall coverage, base distribution, GC content, over represented reads, adapters contamination, read duplication, etc.) for the samples that were sequenced. Low quality bases at the ends of the reads are trimmed and sequencing adapters and artifacts are removed. The minimum coverage required varies for different types of sequencing projects. For mapping recombination events, we analyze the change in a set of consecutive markers, since a crossover or conversion tract involves

TABLE 2   *Widely used open source softwares for bioinformatics analysis of NGS data*

Module and specific tool	Website link
Quality control analysis	
FastQC	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
NGS QC Toolkit	http://www.nipgr.res.in/ngsqctoolkit.html
Pre-processing of reads	
Trimmomatic	http://www.usadellab.org/cms/?page=trimmomatic
FASTX-Toolkit	http://hannonlab.cshl.edu/fastx_toolkit/
Alignment to reference genome	
Bowtie2	http://bowtie-bio.sourceforge.net/ bowtie2/ index.shtml
BWA MEM	http://bio-bwa.sourceforge.net/
Stampy	http://www.well.ox.ac.uk/project-stampy
Removal of duplicate reads	
Picard (Markduplicates)	http://broadinstitute.github.io/picard/
Samtools (rmdup)	http://samtools.sourceforge.net/
Indel realignment	
GATK (IndelRealignment)	https://software.broadinstitute.org/gatk/
Genotyping	
GATK (HaplotypeCaller/UnifiedCaller)	https://software.broadinstitute.org/gatk/
Samtools (mpileup)	http://samtools.sourceforge.net/
Recombination analysis	
ReCombine	https://sourceforge.net/projects/recombine/
Statistical analysis and visualization	
R	https://www.r-project.org/
IGV	http://software.broadinstitute.org/software/igv/

multiple markers. Therefore the probability of all the markers within a tract being genotyped incorrectly due to sequencing error is low. So each SNP coverage can be lower ($\sim 10\times$) compared to the coverage required for mutational analysis. Paired end reads are preferred over single end reads for better accuracy in mapping the reads to a reference genome and genotyping. Some of the widely used softwares to check the statistics and the quality of bases in the reads are listed in Table 2.

Read Alignment.   After QC analysis and preprocessing, the reads are aligned to the reference genome. The result of the aligned reads are in SAM (sequence alignment map) format which may be compressed to yield BAM (binary alignment map) files. BAM files contain information regarding the read location in the reference genome and also some additional information

(eg mapping quality, uniquely represented reads, duplicated reads, etc). Duplicate reads detected from the alignment should be removed as they may skew the allele frequency and lead to false genotyping. Indel realignment facilitates removal of alignment artifacts which reduce the accuracy of genotyping. If the percentage of mapped reads from the alignment is less, we may be able to call fewer SNPs. For example, if a sample contains around 8,000 SNPs (*i.e.* 1 SNP per 1.5 kb) then that sample can detect crossovers but not some gene conversions that often have a median length of 1.5 kb (28).

Genotyping and Generation of Segregation Files.   Multi-sample genotyping or variant calling is preferred. This involves calling variants across multiple samples (spores) at a given location, if, any one of the samples contains a variant in the

same location. Multi-sample variant calling reduces the computational load and is easier for a small genome like that of *S. cerevisiae*. This step returns a file containing variants called VCF (variant calling format). Custom scripts (any programming language) are used to generate segregation files by clubbing VCF files of genotyped spores as tetrads.

Recombination Analysis. For recombination analysis, the software should correctly identify and classify the recombination events from the segregation file. Mancera et al. used the ssGenotyping program to identify the crossover and non-crossover events from the microarray data (26). Anderson et al. developed ReCombine package (written in python) that can analyze recombination events from microarray or NGS data from yeast tetrads (39). This program takes processed fastq files, genotypes and generates segregation files. The CrossOver program in the ReCombine package can use the segregation file to detect crossover, non-crossover and gene conversion events and classify them into categories (39). By default, ReCombine merges adjacent crossovers or a crossover, non-crossover event if they are separated by less than 5 kb as they are likely to have initiated from the same DSB. This parameter can be adjusted. The drawback of ReCombine is that it may call discontinuous non-crossover tracts from the same initiating DSB as separate events thereby skewing the non-crossover numbers (29). To avoid this problem CrossOver may be run with 0 kb cut off to identify all the crossover and non-crossover tract changes as individual events (29). This is then used as an input for "groupEvents" program, which groups the crossover and non-crossover events, lying within 5 kb range, together as a single event (29). These modifications can be used to analyze the complexities of the non-crossover conversion tracts. So adjacent discontinuous non-crossover tracts caused by multiple strand invasions from the same initiating DSB which would have been called as two separate events in Crossover program will be now merged as a single non-crossover event using the groupEvents script (29). The "groupEvents" program enabled Oke et al. 2014 (29) to accurately characterize conversion tracts in meiotic mutants and detect complex recombination outcomes in yeast and the causal mechanisms (29). Although ReCombine makes recombination mapping in yeast easy, it can only generate segregation files with crosses involving S288c. For other yeast hybrids, the program has to be modified to align and call the SNPs from a different reference genome. Alternatively, the segregation file can be separately generated and used as input for the CrossOver module in ReCombine.

Non-detectable Recombination Events. Although NGS analysis of spores from hybrid strains can facilitate high-resolution genome wide mapping of recombination events, it is important to note that not all recombination events can be detected. The DSBs are repaired using either the homolog or the sister chromatid as a template. In wild-type meiosis, interhomolog recombination is preferred over intersister events (40,41). In many mutants like *pch2*, *mek1*, etc. the bias is affected, and the intersister events increase (42–44). NGS-based recombination analysis requires heterozygous SNPs, so only the interhomolog events are detected. The lack of markers on the sister chromatids makes it difficult to quantify inter-sister recombination events, especially in mutants where these may be elevated. As a consequence overall recombination events are under-estimated and may not correlate with DSB frequency. The Strand-seq technique was recently used to map inter-sister exchange events genome-wide during mitosis (45,46) in *S. cerevisiae*. Such methods could be further developed to possibly detect inter-sister repair events during meiosis. Yeast hybrids often have high marker densities (*e.g.* 1 SNP every 78 bp in S288c/YJM789 hybrid), which facilitates detection of all crossover events, since they affect flanking markers. But it is possible that non-crossover events that occur in between adjacent markers are not detected (26).

Statistical Analysis, Visualization and Data Management. All statistical analysis and visualization may be performed using any statistical analysis package (*e.g.* R, Matlab, etc.). The data can be also visualized with many open source softwares like UCSC genome browser, Integrated Genomics Viewer (IGV) etc. (Table 2). These provide the user an interactive analysis tool at a single base pair scale (47–49). Data management and analysis are challenging since millions of short reads amounting to terabytes of data are generated from whole genome sequencing of spores. Error free SNP calling requires the genome to be sequenced at high coverage ($10\times$ or greater). For statistical significance in the data, sufficient numbers of tetrads are sequenced to obtain a reliable count of crossover/non-crossover numbers. These generate a huge amount of sequence data even for a single experiment. Storing the raw data requires sufficient space ($\sim$2–3 GB per haploid yeast genome at $30\times$ coverage).

Correlating Data from Hybrids with Isogenic Strains

High-resolution recombination mapping in yeast is built on the use of hybrids. Apart from issues like sequence divergence and incompatibilities, the asynchrony in the meiotic kinetics of hybrids poses problems. Asynchrony does not affect recombination mapping in hybrid strains, as the tetrads are specifically selected. But other techniques like immunofluorescence analysis or ChIP sequencing requires the sporulating culture to be highly synchronous as the analysis at a particular time point should reflect the state of the majority of the cell population. So, these techniques cannot be used in hybrids to compare with the genetic recombination data obtained from NGS analysis. Instead, isogenic strains like SK1, which shows high synchrony in meiosis, are used for cytological and ChIP-sequencing studies and the data are correlated with the recombination analysis in hybrid strains. A disadvantage of this approach is the assumption that the isogenic and the hybrid strains have similar properties of meiotic recombination, which may not be the case (50). In addition, meiotic mutants may show differences in sporulation efficiency and spore viability in the hybrid compared to isogenic strains. For example, *mms4Δ* sporulates in isogenic SK1 and shows 46%–

51% viability (15,20), but it fails to sporulate in S288c/YJM789 hybrid.

NEW INSIGHTS FROM GENOME WIDE ANALYSIS OF MEIOTIC RECOMBINATION

Genome-wide fine-scale mapping of recombination using hybrid *S. cerevisiae* genomes have provided new insights into the mechanisms of genetic recombination in eukaryotes. The initial study by Mancera et al. (26) using the S288c/YJM789 hybrid, provided the first genome wide recombination map in yeast with many new mechanistic insights, such as the presence of non-crossover hotspots in the genome, interference between crossovers and non-crossovers etc. A similar study by Qi et al. using a different hybrid (S288c/RM11) described crossover, non-crossover and associated gene conversion tracts at single base resolution in wild type *S. cerevisiae* (33). Further studies by Mancera et al. (51) provided information on the genome wide distribution and prevalence of post meiotic segregation events. Similarly, genome wide analysis of various meiotic mutants have revealed novel functions of meiotic genes. For example, a role for Zip1 in suppressing crossing over at the centromere (28); the effect of diminishing recombination initiation in *spo11* hypomorphs on DSB repair outcome (52); a role for Zip3 in biased resolution of Holliday junctions into crossovers and a role for Mms4 in suppressing multiple strand invasions during DSB repair (29). Genome wide mapping of recombination in *tel1* mutants showed loss of crossover interference and a role for Tel1 in regulating DSB distribution along the chromosome (53). Genome wide recombination mapping of *msh2Δ* elucidated barriers mismatch repair poses to recombination as well as new models for recombination (30). Similarly genome wide recombination mapping in *mlh2Δ* mutants showed a role for Mlh1-Mlh2 in regulating the extent of gene conversion tracts (54). Genome wide recombination mapping in *msh4* hypomorphs that make fewer crossovers but have normal viability, supported a role for crossover distribution mechanisms in ensuring the obligate crossover (55). Genome wide recombination mapping in *pch2* mutants revealed increased crossovers and non-crossovers as well as loss of chromosome size dependent DSB formation (31). Another study in a series of *mlh3* point mutants showed a genome wide increase in non-crossovers that supports a structural role for this complex in deciding the fate of meiotic recombination intermediates (56). Furthermore, recent studies in alternate yeast models such as *Lachancea kluyveri* hybrid have shed light on the significant inter-specific variation in meiotic recombination frequency (57). Whole genome analysis of *L. kluyveri* meiotic spores revealed lower crossover frequencies compared to *S. cerevisiae*, a high proportion of non-exchange chromosomes as well as a high frequency of 4:0 conversion tracts in the hybrid indicative of the role of abortive meiosis in genome evolution (57,58). These above mentioned studies are a few examples of the new insights into meiotic recombination mechanisms using genome wide recombination mapping methods.

FUTURE PROSPECTS

Apart from yeast, genome wide analysis of meiotic recombination is also being used in other species like humans, *Arabidopsis*, maize, etc. (59–62). There are a few areas where further developments may overcome some of the limitations in using NGS analysis for mapping recombination events.

In artificial *S. cerevisiae* hybrids like S288c/YJM789 many meiotic mutants show poor viability. Use of other uncharacterized hybrids or natural hybrids may address the poor viability issue of some meiotic mutants in a hybrid context. Another approach is to develop methods for sequencing the spores directly without germinating them. With the advent of single cell sequencing technology, the spores isolated from the tetrads can be lysed and the DNA amplified from each spore can be sequenced to map recombination events. This method also has the additional advantage that it eliminates selection bias towards viable spores.

Making use of the NGS data requires multiple programs, most of which are command line based. Expertise in computational biology is necessary for the data analysis. As many biologists lack exposure in the computational field, it becomes difficult for them to analyze the whole genome data. To make the process more user-friendly, a graphical interface could be developed instead of command lines, where the user could feed the segregation files as input and obtain the crossover, non-crossover and gene conversion data. Such an advancement will make the data analysis considerably easy. Simplification of data analysis through user friendly and easy to use input output formats while maintaining accuracy need to be developed to promote wider acceptance of NGS in recombination analysis. These developments and newer NGS technologies will make whole genome recombination analysis more popular and accessible to the scientific community.

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