

# Rapid and Robust Resampling-Based Multiple-Testing Correction with Application in a Genome-Wide Expression Quantitative Trait Loci Study

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**ABSTRACT** Genome-wide expression quantitative trait loci (eQTL) studies have emerged as a powerful tool to understand the genetic basis of gene expression and complex traits. In a typical eQTL study, the huge number of genetic markers and expression traits and their complicated correlations present a challenging multiple-testing correction problem. The resampling-based test using permutation or bootstrap procedures is a standard approach to address the multiple-testing problem in eQTL studies. A brute force application of the resampling-based test to large-scale eQTL data sets is often computationally infeasible. Several computationally efficient methods have been proposed to calculate approximate resampling-based *P*-values. However, these methods rely on certain assumptions about the correlation structure of the genetic markers, which may not be valid for certain studies. We propose a novel algorithm, **rapid and exact multiple testing correction by resampling (REM)**, to address this challenge. REM calculates the exact resampling-based *P*-values in a computationally efficient manner. The computational advantage of REM lies in its strategy of pruning the search space by skipping genetic markers whose upper bounds on test statistics are small. REM does not rely on any assumption about the correlation structure of the genetic markers. It can be applied to a variety of resampling-based multiple-testing correction methods including permutation and bootstrap methods. We evaluate REM on three eQTL data sets (yeast, inbred mouse, and human rare variants) and show that it achieves accurate resampling-based *P*-value estimation with much less computational cost than existing methods. The software is available at <http://csbio.unc.edu/eQTL>.

**G**ENOME-WIDE studies of expression quantitative trait loci (eQTL) have been widely used to dissect the genetic basis of gene expression and molecular mechanisms underlying complex traits (Bochner 2003; Rockman and Kruglyak 2006; Michaelson *et al.* 2009). In a typical eQTL study, the association between each expression trait and each genetic marker [e.g., single-nucleotide polymorphism (SNP)] is assessed separately, which leads to a huge number of correlated tests. Appropriate multiple-testing correction is crucial for eQTL studies. The resampling-based test using permutation or bootstrap (Good 2005) has been widely used for multiple-

testing correction across multiple genetic markers for each phenotype (Barrett *et al.* 2005; Purcell *et al.* 2007) by simulating the null distribution using permuted or bootstrapped phenotype values (Westfall and Young 1993; Churchill and Doerge 1994; McClurg *et al.* 2007). More specifically, the phenotype values are randomly shuffled and reassigned to individuals with or without replacement (*i.e.*, bootstrap and permutation, respectively). For each resampled phenotype a whole-genome scan is performed to find the maximum test statistic among all SNPs. The corrected *P*-value is the proportion of the resampled phenotypes where the maximum test statistics are greater than the maximum test statistic in the original data. We refer to such a corrected *P*-value as the *resampling-based P-value*. The resampling-based test preserves the correlation structure of the SNPs and does not require any distribution assumption on the test statistic.

Another level of multiple-testing problem in eQTL studies is the multiple tests across tens of thousands of gene

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expression traits (Kendzioriski and Wang 2006). One standard solution is first to estimate the resampling-based  $P$ -value (of the most significant association) for each expression trait and then to determine a threshold for the corrected  $P$ -values across all expression traits by controlling the false discovery rate (FDR) (Benjamini and Hochberg 1995; Storey 2003).

Although this approach is ideal for eQTL studies, its intensive computational burden has greatly limited its practical use. For example, supposing that a corrected  $P$ -value threshold of 0.01 is needed to control the FDR, up to 100,000 permutations/bootstraps may be needed to estimate such a resampling-based  $P$ -value accurately. Consider a typical scenario where there are 500,000 SNPs and 20,000 expression traits. The total number of tests is  $500,000 \times 20,000 \times 100,000 = 10^{15}$ . A brute force implementation of the resampling-based test is clearly not practical. Computationally efficient methods are highly desirable.

To tackle the computational challenge, several methods have been proposed to approximate the resampling-based  $P$ -values. One approach is to estimate the effective number of independent tests from the eigenvalues of the correlation matrix of the SNPs (Cheverud 2001; Nyholt 2004; Li and Ji 2005; Gao *et al.* 2008; Moskvina and Schmidt 2008; Pe'er *et al.* 2008). Previous studies have shown that these methods may yield inaccurate results (Salyakina *et al.* 2005; Han *et al.* 2009). Another approach, relying on the assumption that the test statistics over multiple SNPs asymptotically follow a multivariate normal distribution (MVN), calculates the permutation  $P$ -values by directly sampling from the MVN distribution (Lin 2005; Seaman and Müller-Myhsok 2005; Conneely and Boehnke 2007; Han *et al.* 2009). Both the “effective number of independent tests” and MVN methods cannot be directly applied to genome-wide association studies when the number of SNPs is much larger than the sample size. A common strategy is to divide all the SNPs into contiguous blocks, to apply these two methods to each block of SNPs, and then to combine the results across the SNP blocks. This partition–ligation approach implicitly assumes that the SNPs between two blocks are weakly correlated. From a different perspective, an approximation method based on a geometric interpretation of permutation  $P$ -values has been proposed in Sun and Wright (2010). This method does not require any asymptotic distribution of the test statistics, but it assumes a Markov type of dependency among the markers. Another method has been developed to correct very significant  $P$ -values in disease association studies by importance sampling (Kimmel and Shamir 2006). This method is designed for binary phenotypes and cannot be directly applied to quantitative expression traits.

Despite the success of the aforementioned approximation methods, their accuracies rely on the validity of the following assumption: the nearby SNPs are correlated whereas the distant SNPs are not. This assumption may not be valid. Please refer to supporting information, [File S1](#) for further discussion on the assumptions of the existing approximation methods.

For the resampling-based test, the total computational time is equal to the average time needed to calculate each test statistic multiplied by the total number of tests. Several algorithms have been developed to speed up the exhaustive resampling-based test by calculating each test statistic more efficiently. For example, if the phenotype is binary, computational efficiency can be improved by efficient computation of contingency tables (Browning 2008) or by bit arithmetic operations (Pahl and Schafer 2010). For eQTL studies, a summation tree can be utilized to compute the test statistics incrementally (Gatti *et al.* 2009). Note that these methods are specifically designed for permutation tests and are not applicable to bootstrapping. In this article we tackle the computational challenge of the exhaustive resampling-based test from another perspective. That is, we try to reduce the number of test statistics that need to be calculated. We present an algorithm, **rapid and exact multiple testing correction by resampling (REM)**, which prunes the search space and performs tests only on a small proportion of the SNPs. This is achieved by constructing a two-layer indexing structure that groups SNPs on the basis of their genotypes. The SNPs in one group share a common upper bound on the test statistics. Actual tests are performed only in the groups whose upper bounds are greater than a certain threshold. REM is *guaranteed* to find the exact resampling-based  $P$ -values. It can be applied to a variety of resampling-based methods, including permutation (Westfall and Young 1993; Churchill and Doerge 1994) and bootstrap (McClurg *et al.* 2007). We evaluate the performance of REM on yeast segregants, mouse inbred strains, and human rare variants data. The results demonstrate that REM not only returns the exact resampling-based  $P$ -values, but also runs orders of magnitude faster than alternative methods.

## Materials and Methods

### Problem formulation

We consider binary genotype data. The binary genotype may appear in haploid organisms, such as yeast, inbred diploid organisms, such as inbred mice, or rare variants where the genotype of a homozygous rare allele is unlikely to occur and is collapsed with a heterozygous genotype if it does occur. Without loss of generality, we denote the common and rare genotypes by 0 and 1, respectively.

The permutation test based on the maximum test statistic across all SNPs was originally described in Westfall and Young (1993) and applied to a genome scan in Churchill and Doerge (1994). This approach preserves the correlation structure of the SNPs and requires a much smaller number of permutations than the procedures that first compute point-wise  $P$ -values and then apply adjustments (Nettleton and Doerge 2000). The weighted bootstrap approach (McClurg *et al.* 2007) that accounts for population structure has also been proposed. We collectively refer to the permutation or bootstrap  $P$ -values as resampling-based  $P$ -values.

Let  $\{X_1, \dots, X_N\}$  be the SNPs and  $\{Y_1, \dots, Y_M\}$  be the gene expression traits. Let  $\mathcal{T}(X_n, Y_m)$  denote the test statistic between  $X_n$  ( $1 \leq n \leq N$ ) and  $Y_m$  ( $1 \leq m \leq M$ ). Let  $\mathcal{T}_{Y_m}$  be the maximum test statistic for  $Y_m$ . Suppose that we resample the phenotype  $K$  times. For a resampled phenotype  $Y_m^k$ , we denote its maximum test statistic as  $\mathcal{T}_{Y_m^k}$ . For  $k = 1, \dots, K$ , let

$$A_k = \begin{cases} 1 & \text{if } \mathcal{T}_{Y_m} < \mathcal{T}_{Y_m^k} \\ 0 & \text{if } \mathcal{T}_{Y_m} \geq \mathcal{T}_{Y_m^k} \end{cases}$$

The resampling-based  $P$ -value of  $Y_m$  is defined as

$$P_{\text{res}}(Y_m) = \frac{\sum_{k=1}^K A_k + 1}{K + 1}.$$

To calculate the resampling-based  $P$ -value of expression trait  $Y_m$ , the *computational problem* is to determine, for every  $Y_m^k$  ( $1 \leq k \leq K$ ), whether its maximum test statistic  $\mathcal{T}_{Y_m^k}$  is larger than  $\mathcal{T}_{Y_m}$ . A brute force solution to this problem is that, for every  $Y_m^k$ , we perform a complete scan of all SNPs to find the maximum test statistic and compare it to  $\mathcal{T}_{Y_m}$ . This approach has been adopted in most existing exhaustive methods that calculate the exact resampling-based  $P$ -values. Next, we discuss how our algorithm, REM, can efficiently compute the solution without calculating the test statistics for all SNPs.

### General idea of the proposed method

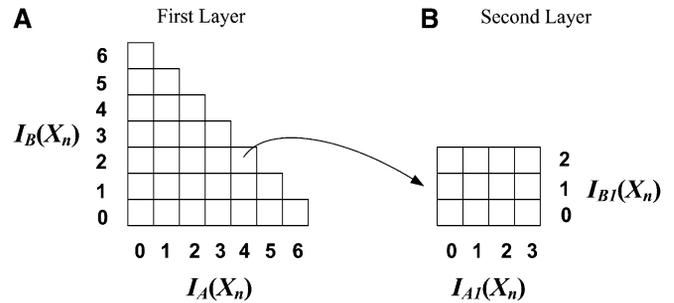
The key idea of the proposed algorithm is as follows. We partition SNPs into different groups on the basis of their allele frequencies. For each group of SNPs, we can find an upper bound on the test statistics. If the upper bound is lower than a certain threshold, there is no need to calculate the test statistics for this group of SNPs. The actual tests are performed only for the groups of SNPs whose upper bounds are larger than the threshold.

The groups of SNPs are organized into a two-layer indexing structure. Utilizing indexing structures for efficient computation has been a longstanding research focus of the database management and theoretical computation research communities. The idea of indexing SNPs and exploring the upper bound on test statistics was originally investigated in epistasis detection in a genome-wide association study (Zhang *et al.* 2009, 2010). In this article we apply this general methodology to the problem of multiple-testing correction in eQTL studies.

Next we first introduce the two-layer indexing structure that groups SNPs on the basis of allele frequencies. Then we discuss how to use this indexing structure to obtain the upper bound on the test statistics for each group of SNPs and thus dramatically reduce the number of test statistics to be calculated.

### The indexing structure

**First layer:** Suppose that there are  $S$  individuals  $\{I_1, I_2, \dots, I_S\}$  in the study. We partition the individuals into two subsets:  $I_A = \{I_1, I_2, \dots, I_{\lfloor S/2 \rfloor}\}$  and  $I_B = \{I_{\lfloor S/2 \rfloor + 1}, I_{\lfloor S/2 \rfloor + 2}, \dots, I_S\}$ . Note



**Figure 1** The two-layer indexing structure for a study of 12 individuals. (A) The first layer indexing structure. (B) The second layer indexing structure for a particular entry (4, 2) in the first layer indexing structure.

that the partition of individuals is random and only done once for the entire data set.

For a SNP  $X_n$ , we use  $|I_A(X_n)|$  and  $|I_B(X_n)|$  to denote the numbers of rarer genotype (*i.e.*, the number of 1's) in partitions  $I_A$  and  $I_B$ , respectively. We can group the SNPs by their  $(|I_A(X_n)|, |I_B(X_n)|)$  values and index them in a two-dimensional (2D) array. The SNPs with the same  $(|I_A(X_n)|, |I_B(X_n)|)$  values will fall into the same entry in the array.

For example, suppose that there are  $S = 12$  individuals in our study. We can partition them into two subsets, each having 6 individuals. Figure 1A shows the first layer of the 2D indexing structure. The SNPs having the same  $(|I_A(X_n)|, |I_B(X_n)|)$  value fall into the same entry in the 2D array. In this example, the possible values of  $|I_A(X_n)|$  and  $|I_B(X_n)|$  are  $\{0, 1, 2, \dots, 6\}$ . Recall that 1 denotes the rarer genotype, and thus the number of 1's in any SNP is no greater than  $\lfloor S/2 \rfloor$ ; *i.e.*,  $|I_A(X_n)| + |I_B(X_n)| \leq \lfloor S/2 \rfloor$ . Therefore, the indexing structure has entries only below the diagonal, as shown in Figure 1A.

Later we show that the SNPs in the same entry [*i.e.*, with the same  $(|I_A(X_n)|, |I_B(X_n)|)$  value] share a common upper bound on their test statistics.

**Second layer:** Applying a similar idea, we can build a second layer indexing structure for each entry in the first layer. For a group of SNPs in the same entry in the first layer, we further partition individuals in  $I_A$  into two subsets:  $I_{A_1} = \{I_1, I_2, \dots, I_{\lfloor S/4 \rfloor}\}$  and  $I_{A_2} = \{I_{\lfloor S/4 \rfloor + 1}, I_{\lfloor S/4 \rfloor + 2}, \dots, I_{\lfloor S/2 \rfloor}\}$ . Similarly, we partition  $I_B$  into two subsets  $I_{B_1}$  and  $I_{B_2}$  of similar sizes. Note that these partitions are random and done only once for the entire data set. For any SNP  $X_n$ , we use  $|I_{A_1}(X_n)|$ ,  $|I_{A_2}(X_n)|$ ,  $|I_{B_1}(X_n)|$ , and  $|I_{B_2}(X_n)|$  to denote the number of 1's of  $X_n$  in  $I_{A_1}$ ,  $I_{A_2}$ ,  $I_{B_1}$ , and  $I_{B_2}$ , respectively. The group of SNPs in the same entry in the first layer is further partitioned by its  $(|I_{A_1}(X_n)|, |I_{B_1}(X_n)|)$  values. The SNPs with the same  $(|I_{A_1}(X_n)|, |I_{B_1}(X_n)|)$  values fall in the same entry in the second layer indexing structure.

Following the previous example where there are 12 individuals, its first layer indexing structure is shown in Figure 1A. For a particular first layer entry  $(|I_A(X_n)|, |I_B(X_n)|) = (4, 2)$ , its second layer indexing structure is shown in Figure 1B. It is easy to see that the maximum value that  $|I_{A_1}(X_n)|$  can take is  $\min\{|I_A(X_n)|, \lfloor S/4 \rfloor\}$ , and the

maximum value that  $|I_{B_1}(X_n)|$  can take is  $\min\{|I_B(X_n)|, \lceil S/4 \rceil\}$ . This is reflected in the following example: the maximum value of  $|I_{A_1}(X_n)|$  is  $\min\{4, 3\} = 3$ , and the maximum value of  $|I_{B_1}(X_n)|$  is  $\min\{2, 3\} = 2$ .

Similar to the first layer, the SNPs in the same entry in the second layer also share a common upper bound on their test statistics. We will show that this bound is tighter than (or at least as tight as) the bound derived from the first layer.

### Upper bound on the test statistics

In this subsection, we give a common upper bound on the test statistics for the SNPs in the same entry in the indexing structure. An intuitive explanation is as follows. For SNP  $X_n$  and a resampled phenotype  $Y_m^k$ , let  $\bar{Y}_1$  represent the sum of the phenotype values of the individuals with rarer genotypes (*i.e.*, when  $X_n = 1$ ). It can be shown that many statistics are convex functions of  $\bar{Y}_1$  (see File S1 for details). If we can determine the range of  $\bar{Y}_1$ , we can easily derive an upper bound of the statistics. It can be shown that the SNPs in the same entry have the same range for  $\bar{Y}_1$  and hence share a common upper bound.

Next, we first discuss how to obtain the upper bound for a SNP group in the first layer, and then we show that tighter bounds can be achieved for the groups in the second layer.

**First layer:** Recall that when building the first layer indexing structure, we partition the individuals into two subsets,  $I_A$  and  $I_B$ . The indexing structure groups SNPs on the basis of their  $(|I_A(X_n)|, |I_B(X_n)|)$  values, *i.e.*, the numbers of rarer genotypes in the two subsets of individuals. For a resampled phenotype vector  $Y_m^k$ , we use  $Y_m^k(I_A) = \{y_{1,S/2_1}^k, y_{2,S/2_1}^k, \dots, y_{|I_A(X_n)|, S/2_1}^k\}$  and  $Y_m^k(I_B) = \{y_{1, \lfloor S/2_1 \rfloor + 1}^k, y_{2, \lfloor S/2_1 \rfloor + 2}^k, \dots, y_{|I_B(X_n)|, S}^k\}$  to denote the phenotype values of the individuals in  $I_A$  and  $I_B$ , respectively.

Let  $y_{A(1)}^k \leq y_{A(2)}^k \leq \dots \leq y_{A(|I_A|)}^k$  represent the ordered phenotype values in  $Y_m^k(I_A)$  and  $y_{B(1)}^k \leq y_{B(2)}^k \leq \dots \leq y_{B(|I_B|)}^k$  represent the ordered values in  $Y_m^k(I_B)$ . For any SNP  $X_n$  in the first layer entry  $(|I_A(X_n)|, |I_B(X_n)|)$ , we have that

$$\underbrace{\sum_{i=1}^{|I_A(X_n)|} y_{A(i)}^k + \sum_{i=1}^{|I_B(X_n)|} y_{B(i)}^k}_{R_L} \leq \bar{Y}_1, \quad (1)$$

where  $\sum_{i=1}^{|I_A(X_n)|} y_{A(i)}^k$  is the sum of the smallest  $|I_A(X_n)|$  phenotype values of individuals in  $I_A$ , and  $\sum_{i=1}^{|I_B(X_n)|} y_{B(i)}^k$  is the sum of the smallest  $|I_B(X_n)|$  phenotype values of individuals in  $I_B$ . Recall that  $|I_A(X_n)| + |I_B(X_n)|$  is equal to the number of rarer genotypes and  $\bar{Y}_1$  is the sum of the phenotype values of individuals with rarer alleles. Clearly, the inequality holds. We use  $R_L$  to represent the left-hand side of inequality (1).

Similarly we have that

$$\bar{Y}_1 \leq \underbrace{\sum_{i=|I_A| - |I_A(X_n)| + 1}^{|I_A|} y_{A(i)}^k + \sum_{i=|I_B| - |I_B(X_n)| + 1}^{|I_B|} y_{B(i)}^k}_{R_U}. \quad (2)$$

We use  $R_U$  to represent the right-hand side of inequality (2). For a given resampled phenotype vector  $Y_m^k$ , inequalities (1) and (2) give us the range of  $\bar{Y}_1$ . That is, for any SNP  $X_n$  in

the entry  $(|I_A(X_n)|, |I_B(X_n)|)$  of the first layer indexing structure, we have that  $\bar{Y}_1 \in [R_L, R_U]$ .

The maximum value of any convex function is attained at the boundary of its convex domain (Boyd and Vandenberghe 2004). Therefore, for the SNPs in the entry  $(|I_A(X_n)|, |I_B(X_n)|)$ , the upper bound on test statistics is attained when  $\bar{Y}_1 = R_L$  or  $\bar{Y}_1 = R_U$ .

**Second layer:** Similarly, we can obtain an upper bound on the test statistics for the SNPs in an entry of the second layer. We partition the values in the resampled phenotype vector  $Y_m^k$  into four groups, each across individuals in  $I_{A_1}$ ,  $I_{A_2}$ ,  $I_{B_1}$ , and  $I_{B_2}$ , respectively. Let  $\{y_{A_1(i)}^k\}$ ,  $\{y_{A_2(i)}^k\}$ ,  $\{y_{B_1(i)}^k\}$ , and  $\{y_{B_2(i)}^k\}$  represent the ordered phenotype values in the four groups. We have the following two inequalities:

$$\underbrace{\sum_{i=1}^{|I_{A_1}(X_n)|} y_{A_1(i)}^k + \sum_{i=1}^{|I_{A_2}(X_n)|} y_{A_2(i)}^k + \sum_{i=1}^{|I_{B_1}(X_n)|} y_{B_1(i)}^k + \sum_{i=1}^{|I_{B_2}(X_n)|} y_{B_2(i)}^k}_{R'_L} \leq \bar{Y}_1, \quad (3)$$

$$\begin{aligned} \bar{Y}_1 \leq & \sum_{i=|I_{A_1}| - |I_{A_1}(X_n)| + 1}^{|I_{A_1}|} y_{A_1(i)}^k + \sum_{i=|I_{A_2}| - |I_{A_2}(X_n)| + 1}^{|I_{A_2}|} y_{A_2(i)}^k \\ & + \sum_{i=|I_{B_1}| - |I_{B_1}(X_n)| + 1}^{|I_{B_1}|} y_{B_1(i)}^k + \sum_{i=|I_{B_2}| - |I_{B_2}(X_n)| + 1}^{|I_{B_2}|} y_{B_2(i)}^k. \end{aligned} \quad (4)$$

Letting  $R'_L$  ( $R'_U$ ) be the left (right)-hand side of inequality (3) [inequality (4)], then  $\bar{Y}_1 \in [R'_L, R'_U]$ . The upper bound on the test statistics is attained when  $\bar{Y}_1 = R'_L$  or  $\bar{Y}_1 = R'_U$ . It is easy to show that  $R_L \leq R'_L$  and  $R'_U \leq R_U$ . Therefore, we can get a tighter (or at least an equally tight) upper bound by utilizing the second layer indexing.

### The REM algorithm

Given the SNPs and gene expression traits, REM returns the significant traits whose resampling-based  $P$ -values are no greater than a user-specified threshold  $P_t$ . If  $P_t = 1$ , REM returns the resampling-based  $P$ -values for all expression traits. The number of permutations/bootstraps is an input parameter provided by the user. The pseudocode of the algorithm is outlined in File S1.

For every phenotype  $Y_m$ , REM first scans all SNPs to find the maximum statistic  $\mathcal{T}_{Y_m}$ . Then REM calculates a variable *count*, which records the number of resampled phenotypes whose maximum test statistics are greater than  $\mathcal{T}_{Y_m}$ , as follows. For each  $Y_m^k$ , REM first checks the entries in the first layer of the indexing structure. It goes to the second layer only if the upper bound of a first layer entry is greater than  $\mathcal{T}_{Y_m}$ . If the upper bound of the second layer entry is still greater than  $\mathcal{T}_{Y_m}$ , REM will perform actual tests on the SNPs in the second layer entry; otherwise this group of SNPs will be skipped because their test statistics are guaranteed to be no greater than  $\mathcal{T}_{Y_m}$ .

In addition to applying the upper bound to reduce the number of SNPs to be examined, there are two other strategies to further prune the search space. One is that,

for a resampled phenotype vector  $Y_m^k$ , as long as we find any SNP whose test statistic is greater than  $T_{Y_m}$ , we know its maximum test statistic  $T_{Y_m^k}$  will also be greater than  $T_{Y_m}$ . Therefore, there is no need to scan the remaining SNPs. The other pruning strategy is based on the significance threshold  $P_c$ . For an expression trait  $Y_m$ , once we have already examined enough resampled phenotype vectors such that its corrected  $P$ -value is greater than  $P_c$ , there is no need to examine the remaining resampled phenotype vectors. The detailed analysis of the time complexity of REM is given in File S1.

REM can be applied to various resampling methods. For example, in addition to the permutation and bootstrap methods (Westfall and Young 1993; Churchill and Doerge 1994; McClurg *et al.* 2007) that preserve the correlation structure of SNPs, a resampling method that preserves the correlation structure of the expression data was proposed in Breitling *et al.* (2008). When generating resampled phenotype vectors, this approach permutes the individual labels so that each individual is assigned the genotype of another random individual, while the expression profile of this individual is unchanged. When the mapping is repeated on these permuted data, the correlation structure of gene expression is maintained. These methods differ in how they generate the resampled phenotype vectors, *i.e.*, line 4 in File S1, Algorithm S1. REM will find the exact resampling-based  $P$ -values for the resampling method adopted by the user.

Note that REM can be easily modified to identify multiple SNPs that are significantly associated with a gene expression trait. One naive approach is to apply REM to each of the top  $T$  SNPs that are significantly associated with the gene expression trait. Since we compare the test statistic of the  $t$ -th most significant association with the maximum test statistics from the resampled data, the estimate of the resampling-based  $P$ -value is conservative. However, as long as  $T$  is much smaller than the total number of SNPs, the bias of the estimate is small. A drawback of this naive approach is the computational burden increases linearly with  $T$ . An alternative, and more sophisticated approach is to directly calculate the desired percentile of the maximum test statistic. For example, suppose that the desired significance level is  $\alpha = 0.05$ , and thus we aim to estimate the 95th percentile of the maximum test statistic from 1000 permutations/bootstraps. In other words, we want to find the 50th largest maximum test statistics among the 1000 maximum test statistics. We can first carry out 50 permutations/bootstraps and record the corresponding 50 maximum test statistics without pruning. Then we carry out the following permutation/bootstraps with pruning by keeping track of the largest 50 maximum test statistics and using the value of the 50th one as the threshold for pruning.

## Results

We evaluate the accuracy and computational efficiency of REM and several representative existing methods. For approximation methods, we choose SimpleM (Gao *et al.*

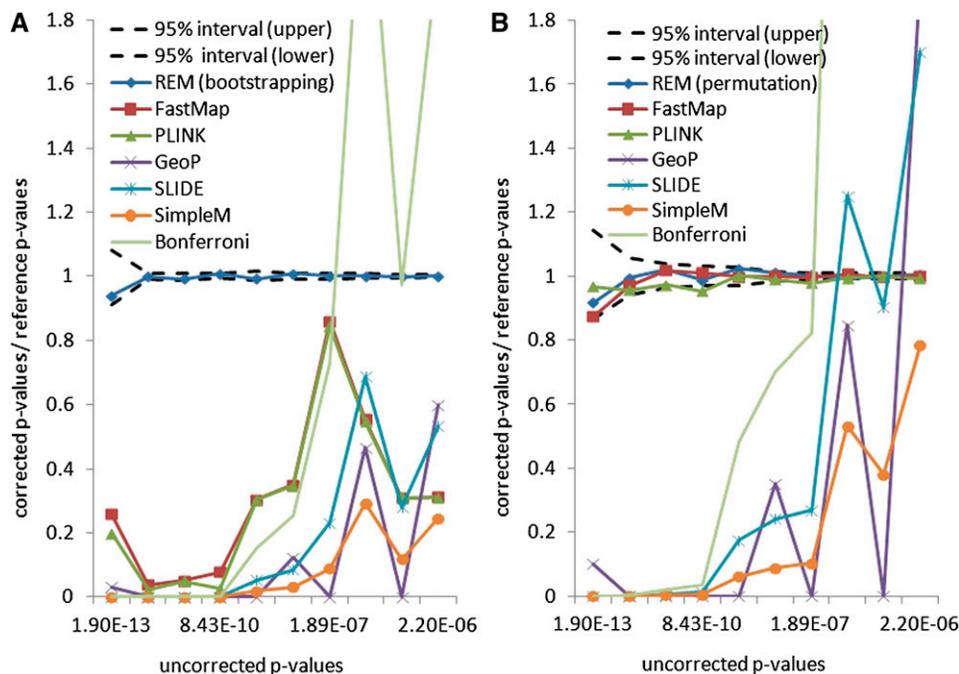
2008) to represent the approaches of estimating the number of independent tests, and SLIDE (Han *et al.* 2009) to represent the approaches using the MVN framework. These two methods have been shown to be superior to other alternatives within their classes. We also study the performance of the method in Sun and Wright (2010), which we call GeoP. For exhaustive methods, we study the performance of PLINK (Purcell *et al.* 2007) and FastMap (Gatti *et al.* 2009). Note that these two methods are designed to speed up only permutation test but not bootstrapping. Other approaches, such as the approximation method RAT (Kimmel and Shamir 2006) and exhaustive methods PRESTO (Browning 2008) and PERMORY (Pahl and Schafer 2010), are applicable only to binary phenotypes and therefore are not applicable to eQTL mapping. We evaluate the performance of the selected methods on three eQTL data sets: inbred mouse strains, yeast segregants, and human rare variants.

### Inbred mouse strains

We use the hypothalamus eQTL data of inbred mice from McClurg *et al.* (2007). There are 32 mouse strains in the data set. The total number of SNPs is 156,525. The missing values are imputed by the algorithm in Roberts *et al.* (2007). There are 36,182 probes on the gene expression array. Here we refer to the gene expression captured by each probe as an expression trait. Following the same filtering method as in Gatti *et al.* (2009), we dropped any expression trait whose expression values are all  $<200$  or whose largest difference in expression across the 32 mouse strains is less than three-fold. There are 3,672 expression traits left after filtering.

**Accuracy evaluation:** Due to the population structure of these inbred mouse strains, some strains are more similar to each other than other strains, and thus a direct application of a permutation test is not appropriate (Fei *et al.* 2006; McClurg *et al.* 2007; Churchill and Doerge 2008). We adopt the weighted bootstrap approach proposed by McClurg *et al.* (2007) in REM to account for the population structure. We studied 10 expression traits, whose uncorrected  $P$ -values range from  $1.9 \times 10^{-13}$  to  $2.2 \times 10^{-6}$ . We estimated the corrected  $P$ -values by 100 million bootstraps, treated them as the gold standard, and referred to them as the *reference P-values*. The reference  $P$ -values of these 10 expression traits vary from 0.00057 to 0.17. A method is considered accurate if its corrected  $P$ -values are close to the reference  $P$ -values.

We use 1 million weighted bootstraps to estimate the corrected  $P$ -values by REM. Because FastMap and PLINK cannot perform weighted bootstrapping, we apply FastMap and PLINK to these data using 1 million permutations to estimate the corrected  $P$ -values. The approximation methods also employ an implicit assumption that all samples are equally exchangeable, for example, in calculating the correlation matrix. For comparison purposes, we apply these approximation methods even though the exchangeability assumption is invalid in these data due to the population structure. We set the window size to be 100 and the number



**Figure 2** Accuracy evaluation of selected methods on real gene expression traits in an inbred mouse data set. (Each line represents the ratio between the corrected  $P$ -values and the reference  $P$ -values for a method. The reference  $P$ -values are obtained using 100 million resamplings. An accurate method should yield a ratio of 1. In A, the reference  $P$ -values are estimated by a weighted bootstrap method. In B, the reference  $P$ -values are estimated by a permutation test.)

of resamplings to be 1 million for SLIDE. The number of resamplings is also set to be 1 million for GeoP. SimpleM is not a resampling-based method and we use its default setting. Following a similar approach as in Han *et al.* (2009; Pahl and Schafer 2010), we construct a 95% confidence interval to cover the sampling error of REM.

Figure 2A depicts the ratios between the corrected  $P$ -values and the reference  $P$ -values for the selected methods. A method is accurate if its ratio is close to 1. As shown in Figure 2A, there is discrepancy between the bootstrap and the permutation test (as implemented in PLINK and FastMap). This reflects the effect of the population structure of the inbred mouse strains. The permutation test is anticonservative compared to the weighted bootstrap method. The approximation methods, which are designed to estimate the permutation  $P$ -values, are shown to be anticonservative compared to both the bootstrapping and the permutation test. This indicates that these approximation methods are not replacements for exhaustive permutation tests. The corrected  $P$ -values from the widely used Bonferroni correction are also shown in Figure 2A. It can be seen that the Bonferroni correction can be either conservative or anticonservative for different uncorrected  $P$ -values. Thus it is not an ideal approach for multiple-testing correction in eQTL studies.

For comparison purposes, in addition to the weighted bootstrap method, we also apply the permutation test using REM. The basic experimental setting is similar to that in the weighted bootstrap method. We estimate the reference permutation  $P$ -values of the 10 expression traits by 100 million permutations. The reference permutation  $P$ -values range from 0.00018 to 0.053. For the three exact methods, REM, FastMap, and PLINK, we use 1 million permutations to estimate the corrected  $P$ -values.

Figure 2B shows the ratio between the corrected  $P$ -values and the reference permutation  $P$ -values. The corrected  $P$ -values generated by the three exact methods fall in the confidence region of the reference  $P$ -values. In contrast, the three approximation methods, GeoP, SLIDE, and SimpleM, do not estimate the permutation  $P$ -values accurately. Thus these approximation methods cannot replace the exact permutation test. Further comparison between the exhaustive permutation test and the approximation methods will be conducted in yeast segregants and human rare variants data.

Using the genotype data of these 32 mouse inbred strains, we also study the accuracy of the selected methods for three synthetic phenotypes whose values follow standard normal, exponential, and uniform distributions. The approximation methods are shown to be anticonservative. Please see File S1 for further details.

**Computational efficiency evaluation:** We evaluate the computational efficiency of the three selected exact resampling methods, REM, FastMap, and PLINK. The approximation methods are usually very fast but do not provide accurate  $P$ -value correction. They are not considered in the computational efficiency evaluation. All experiments in this subsection are performed on a single CPU of a 2.6-GHz PC with 8 G memory running the Linux operating system.

Table 1 shows the runtime of the three methods when applied to the entire eQTL data set with 3600 phenotypes. As can be seen, with 100,000 resamplings, REM can finish within 1 day if we set the threshold to be 1. If we find only the expression traits that have significant associations, REM can finish within a few hours. FastMap and PLINK will take a much longer time.

**Table 1 Runtime of REM, FastMap, and PLINK on the entire inbred mouse hypothalamus eQTL data**

No. resamplings	REM ( $P_t = 0.01$ )	REM ( $P_t = 0.05$ )	REM ( $P_t = 1$ )	FastMap	PLINK
1,000	2.3 min	4.6 min	13 min	1.2 hr	21 days
10,000	0.3 hr	0.7 hr	2 hr	10 hr	211 days
100,000	2.8 hr	6.7 hr	0.9 day	4 days	5.8 yr
1 million	1.2 days	2.8 days	8.6 days	40 days	58 yr

The data set contains 150,000 SNPs and 3600 gene expression traits over 32 strains. The runtimes >10 days are estimated from smaller-scale experiments.

Figure 3 shows the percentage of the SNPs that are pruned by REM under different significance levels. The pruned SNPs are the ones on which we do not perform any test. If we calculate the corrected  $P$ -values for all expression traits, >80% of SNPs are pruned. In most cases, we are interested only in the traits whose corrected  $P$ -values are less than a certain threshold. At a significance level of 0.01, >97% of SNPs are pruned, which means that we need to calculate test statistics only for <3% of SNPs. This dramatically reduced search space explains the improved computational efficiency of REM. Recall that REM has three pruning strategies to reduce the search space. They are (1) pruning by the upper bound, (2) pruning by the maximum statistic (lines 13–16 in File S1, Algorithm S1), and (3) pruning by the significance threshold (lines 23–25 in File S1, Algorithm S1). Figure 3 also shows the breakdown of which pruning strategy is used to prune the search space. Strategy 2 provides 0.4–2.1% pruning ratios across different significance levels. When the significance level is set to be 1, the pruning strategy 1 alone prunes >80% of the search space. Strategy 3 plays a more important role for smaller significance levels. This is reasonable since, for smaller significance levels, more phenotypes will not be examined once we know they will not become significant. Please refer to File S1 for more results on computational efficiency evaluation.

### Yeast segregants

The original yeast data set consists of 112 yeast segregants generated from two parent strains (Brem and Kruglyak 2005; Brem *et al.* 2005). Expression levels of 6229 genes and genotypes of 2956 SNPs were measured in each of the segregants. After removing SNPs with >10% missing values and combining SNPs with the same genotype profiles, there are 1017 distinct genotype profiles.

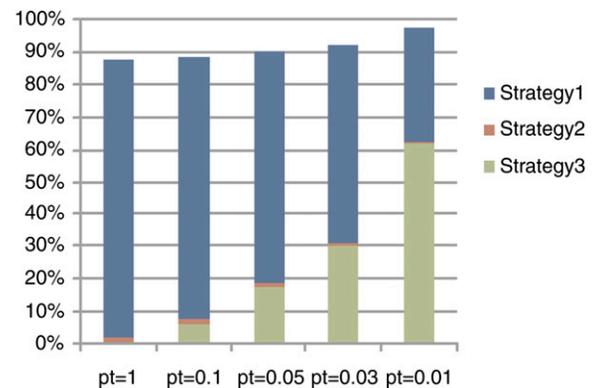
Since this data set only has a very small number of SNPs, the exact methods FastMap and REM can both finish within a few hours for 1 million permutations. To evaluate the accuracy of the selected methods, we use the same ratio measurement as in the inbred mouse data set. Figure 4 shows the ratios between the 10 corrected  $P$ -values and the reference  $P$ -values (calculated by 100 million permutations) for the selected methods. The uncorrected  $P$ -values of the gene expression traits range from  $4.8 \times 10^{-8}$  to  $9.6 \times 10^{-5}$ . After correction, the  $P$ -values range from 0.000016 to 0.046. As expected, the corrected  $P$ -values of the exact methods all fall into the confidence region. Both GeoP and SLIDE provide overall unbiased permutation  $P$ -value esti-

mates. This is because the underlying correlation structure assumption of these approximation methods is appropriate in this yeast data set, as illustrated in File S1, Figure S1.

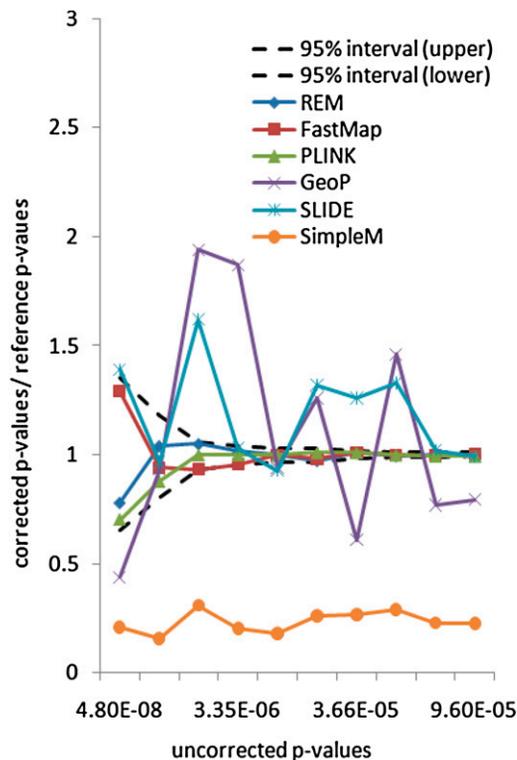
### Human rare variants

Individual rare variants provide little power for association studies because of their low minor allele frequencies. A commonly used approach is to collapse nearby rare variants and use these collapsed rare variants for association studies (Li and Leal 2008). In this study, we generated a data set of collapsed rare variants on the basis of genotype data from 65 HapMap Yoruba in Ibadan (YRI) samples (Frazer *et al.* 2007). Specifically, the genotype data of >2 million SNPs were downloaded from the HapMap Web site. We first chose the markers with minor allele frequency <0.05, and no missing values, and then collapsed them within a moving window of 50 kb that shifts 10 kb each time. The final data set includes 143,000 collapsed SNPs. We downloaded expression data of these 65 samples (measured by RNA-seq) from the Pritchard laboratory's Web site (<http://eqtl.uchicago.edu/>) (Pickrell *et al.* 2010).

Figure 5A shows the ratios between the corrected  $P$ -values and the reference  $P$ -values for the selected methods. The uncorrected  $P$ -values of the gene expression traits range from  $4.9 \times 10^{-10}$  to  $7.1 \times 10^{-7}$ . After correction, the  $P$ -values range from  $8 \times 10^{-6}$  to 0.046. The corrected  $P$ -values of the exact methods still fall into the confidence region. For the approximation methods, SLIDE and SimpleM are conservative for small  $P$ -values but become more accurate for



**Figure 3** The pruning ratio (percentage of the SNPs that are pruned without performing actual tests) of REM for different significance thresholds when using an inbred mouse data set. This also provides the breakdown of the effects of the three pruning strategies used in REM. See text for more details.



**Figure 4** Accuracy evaluation of selected methods on the yeast data set.

larger ones. GeoP estimates are overall unbiased but with large fluctuations.

Figure 5B shows the percentage of the SNPs that are pruned by REM under different significance levels. Note that, at significance level 0.01, >95% SNPs are pruned, and only <5% SNPs need to be examined for their test values. Compared to FastMap, REM speeds up the process by 20–30 times.

#### Applying REM to a large sample study through meta-analysis

The computational efficiency of REM comes from the indexing structure. For each entry in the index structure, REM performs a single computation to calculate a bound of the test statistics of all SNPs in this entry. If the number of samples is large, the number of entries in the indexing structure will also be large. This may impair the computational efficiency of the algorithm. This problem may be alleviated by meta-analysis (Munafo and Flint 2004). In contrast to direct analysis of pooled individual-level data, meta-analysis combines the summary statistics from different studies.

We apply REM to a large sample data set (with 1000 samples) through meta-analysis. The samples are partitioned into groups of equal size. For each group, we apply REM to calculate the resampling-based  $P$ -values for 1000 simulated phenotypes. For each phenotype, a combined  $P$ -value is computed by applying Fisher's method to the group  $P$ -values (Fisher 1925). The combined  $P$ -values and original

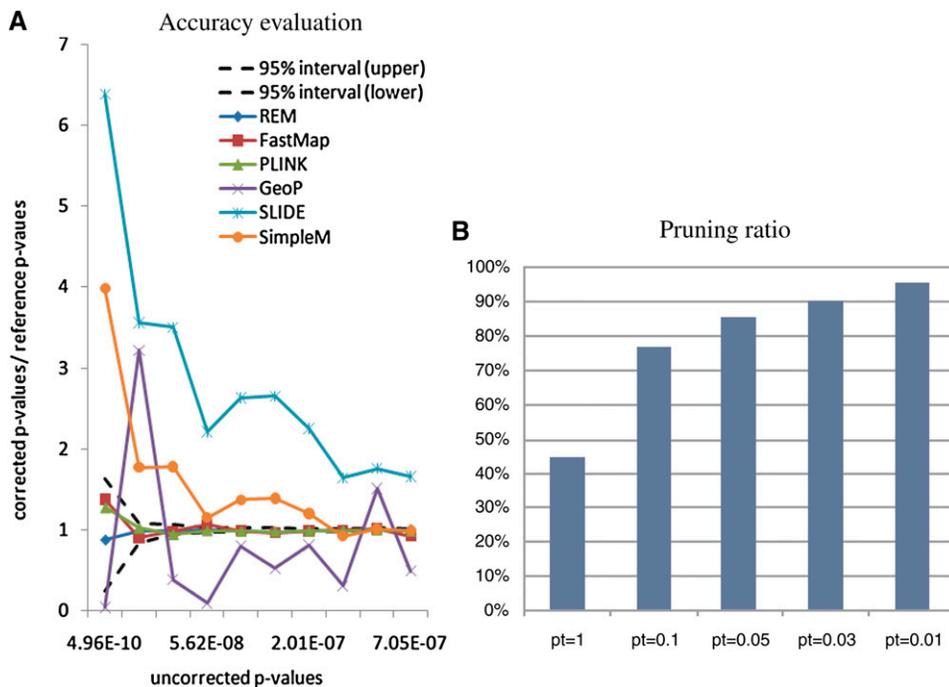
resampling-based  $P$ -values (when using all samples) are almost perfectly correlated. Specifically, the correlations are 0.99, 0.98, and 0.96 when we partition the samples into 2, 5, and 10 groups, respectively. Therefore, for large sample studies, we can apply REM to groups and combine the  $P$ -values by using meta-analysis. The combined  $P$ -values are robust estimations of the original resampling-based  $P$ -values. More detailed discussion on applying REM to a large sample study through meta-analysis can be found in File S1.

#### Discussion

The resampling-based test is widely used to address the multiple-testing correction problem in genetic association studies. Its main disadvantage is the intensive computational burden. In eQTL studies, the computational problem becomes more severe since one needs to correct the  $P$ -values for tens of thousands of gene expression traits. In this article, we present a rapid and robust algorithm, REM, that dramatically speeds up the process of the exact resampling-based test. It builds a two-layer indexing structure that groups SNPs by their genotypes. By estimating the upper bound of the test statistics for all SNPs within one group, REM prunes away most of the SNPs. Moreover, since usually we are interested only in the expression traits whose corrected  $P$ -values are less than a certain significance level, REM can further improve the computational efficiency by filtering out the insignificant expression traits in early stages. Most importantly, REM guarantees that we find the exact resampling-based  $P$ -values even if it performs the actual tests on only a small number of SNPs. REM can be applied to a wide range of resampling procedures. It provides the flexibility to the user to determine the appropriate resampling strategy for the data set under consideration. We use three eQTL data sets to evaluate the performances of several selected algorithms and demonstrate that REM produces accurate estimates of resampling-based  $P$ -values with much less computational cost than other alternatives.

We have shown that the performances of approximation methods vary for different data sets with no method being consistently superior to other methods. The approximation methods achieve higher accuracy on the yeast data set than the other two data sets since the correlation structure in the yeast data set matches the assumptions of the approximation methods. However, in inbred mice and human rare variants data sets, nearby markers do not have higher correlations and the heat maps do not show clear banding correlation structure among SNPs (File S1, Figure S1). Therefore, the correlation assumption of the approximation methods is not valid, which leads to their poor performance.

Association studies of the low-frequency/rare variants have recently attracted much research attention (Bodmer and Bonilla 2008; Manolio *et al.* 2009). With the advance of sequencing techniques, we expect in the near future that rare variants will be used in most association studies. Multiple-testing correction for rare variants association is of great research



**Figure 5** Accuracy evaluation and pruning ratio on the human rare variant data set.

interest. Since a single rare variant has little power to detect an association signal, a common approach is to collapse the rare variants at a locus so that 1/0 indicates the presence/absence of any rare variant (Li and Leal 2008; Morris and Zegginc 2010). The collapsed rare variants often do not have strong correlations among nearby loci, which violates the assumption underlying most approximation methods for permutation  $P$ -value estimation. To the best of our knowledge, this article is the first effort to address the multiple-testing correction problems of rare variant association and our REM algorithm provides an accurate and computationally efficient solution for this problem.

There is room to improve the REM algorithm. In this article, we have focused on the cases where genotype data are binary. The general principle used in REM can also be applied to the situation where one marker may have three possible genotypes, which is among our future research directions.

In summary, the REM algorithm provides an efficient solution to calculate the exact resampling-based  $P$ -values for a variety of statistical tests in eQTL studies. It has been demonstrated to be much faster than recently developed methods. The software is implemented in C++ and is publicly available at <http://csbio.unc.edu/eQTL>. The algorithm can be easily parallelized, for example, by parallelizing the computation for each gene expression trait.

## Acknowledgments

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# GENETICS

Supporting Information

<http://www.genetics.org/content/suppl/2012/01/31/genetics.111.137737.DC1>

## **Rapid and Robust Resampling-Based Multiple-Testing Correction with Application in a Genome-Wide Expression Quantitative Trait Loci Study**

Xiang Zhang, Shunping Huang, Wei Sun, and Wei Wang

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### Assumption of Existing Approximation Methods for Multiple Testing Correction

Figure S1 illustrates the correlation structures of genotype data in yeast segregants (BREM *et al.* 2005) (Figure S1(a-b)), mouse inbred strains (MCCLURG *et al.* 2007) (Figure S1(c-d)), and rare variants in human population (FRAZER *et al.* 2007) (Figure S1(e-f)). More details of these three datasets have been discussed in the Results Section. The yeast segregants data is a typical genetic dataset from a cross of inbred strains where markers within a chromosome are highly correlated (Figure S1 (a)) and form an approximate banding structure (Figure S1 (b)). Thus this dataset satisfies the assumptions needed for the approximation methods. In contrast, we do not observe such correlation structure in the data of mouse inbred strains and human rare variants. Both mouse inbred strains and human rare variants data are commonly encountered in genetic studies. The insufficiency of the approximation methods for these datasets motivates the recent development of the *exhaustive* methods that calculate the exact resampling-based  $P$ -values.

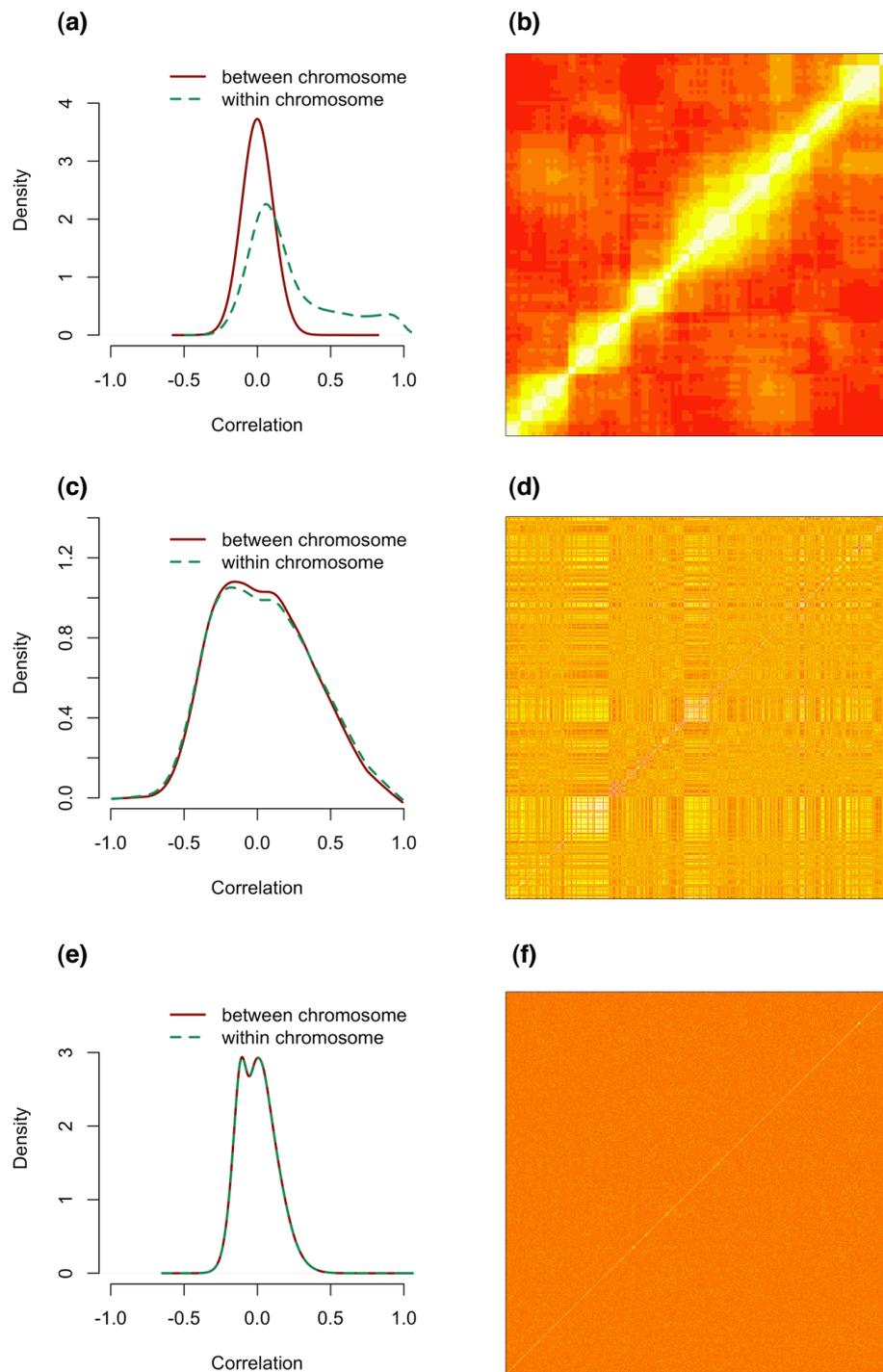


Figure S1: Difference between correlation structures in the yeast [(a) and (b)], inbred mouse [(c) and (d)], and human rare variant [(e), (f)] data sets. (a), (c), (e) compare the correlation density for marker pairs within and between chromosomes. (b), (d), and (f) are the heat maps of correlation matrices in chromosome 12 of the three data sets.

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## Convexity of Commonly Used Statistical Tests

It has been shown that most of the commonly used statistical tests in eQTL studies, such as Pearson's correlation, Student's t-test, analysis of variance (ANOVA F-test), and likelihood ratio test are equivalent for binary genotype data (GATTI *et al.* 2009). Without loss of generality, we show that the ANOVA F-test is a convex function of  $\bar{Y}_1$ . Recall that  $\bar{Y}_1$  is defined as follows: for SNP  $X_n$  and a resampled phenotype  $Y_m^k$ ,  $\bar{Y}_1$  represents the sum of the phenotype values of the individuals with rarer alleles (i.e., when  $X_n$  equals to 1).

The ANOVA F-test partitions the total sum of squares  $SS_T$  into a between-group sum of squares  $SS_B$  and a within-group sum of squares  $SS_W$ . The F-statistic is  $F = cSS_B/SS_W$ , where  $c$  is a fixed constant for a particular study. Let  $SS_T$  be the total sum of squares. We have that  $F = cSS_B/SS_W = cSS_B/(SS_T - SS_B)$ . For a given resampled phenotype vector  $Y_m^k$ , the F-statistic is a monotone function of  $SS_B$ . From now on, we will use  $SS_B$  as our test statistic. For SNP  $X_n$  and resampled phenotype vector  $Y_m^k$ ,

$$SS_B(X_n, Y_m^k) = \frac{\bar{Y}_0^2}{S_0} + \frac{\bar{Y}_1^2}{S_1} - \frac{\bar{Y}^2}{S},$$

where  $\bar{Y}_0$  and  $\bar{Y}_1$  are the sums of the phenotype values in  $Y_m^k$  when  $X_n$  equals to 0 and 1, respectively,  $S_0$  and  $S_1$  are the numbers of 0's and 1's in  $X_n$ , respectively,  $\bar{Y}$  is the sum of all phenotype values in  $Y_m^k$ , and  $S$  is the total number of individuals. Clearly,  $\bar{Y}_0 + \bar{Y}_1 = \bar{Y}$ ,  $S_0 + S_1 = S$ , and thus we can rewrite  $SS_B$  as

$$SS_B(X_n, Y_m^k) = \frac{(\bar{Y} - \bar{Y}_1)^2}{S - S_1} + \frac{\bar{Y}_1^2}{S_1} - \frac{\bar{Y}^2}{S}. \quad (1)$$

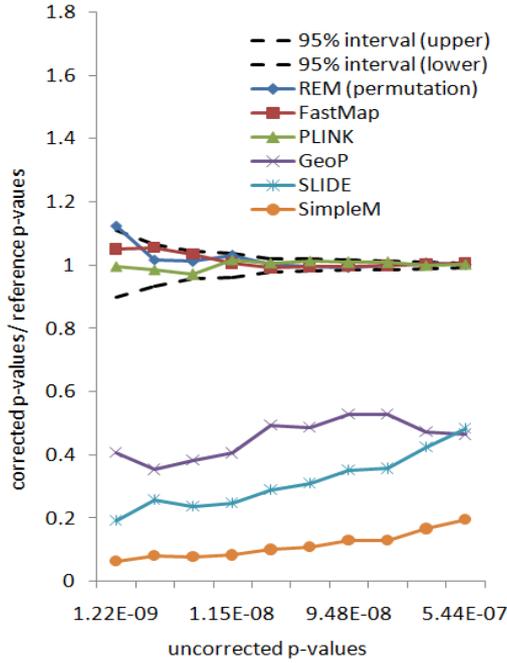
Clearly,  $SS_B(X_n, Y_m^k)$  is a convex function (more specifically a quadratic function) of  $\bar{Y}_1$ .

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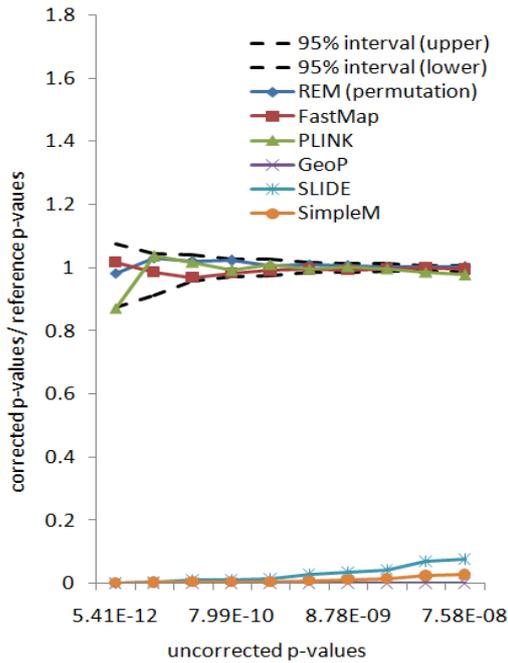
### Accuracy Evaluation using Synthetic Phenotypes

To further study the accuracy of the selected methods on the inbred mouse data set, we generate three synthetic phenotypes whose values follow standard normal, exponential, and uniform distributions. For each synthetic phenotype, we use the selected methods to correct the  $P$ -values. The uncorrected  $P$ -values ranges from  $1.2 \times 10^{-9}$  to  $5.4 \times 10^{-7}$  (normal),  $5.4 \times 10^{-12}$  to  $7.6 \times 10^{-8}$  (exponential), and  $2.7 \times 10^{-11}$  to  $2.1 \times 10^{-7}$  (uniform). After correction (by 100M permutations), the  $P$ -values range from 0.00039 to 0.052 (normal), 0.00044 to 0.053 (exponential), and 0.00026 to 0.05 (uniform). Then we apply different methods to estimate the corrected  $P$ -values.

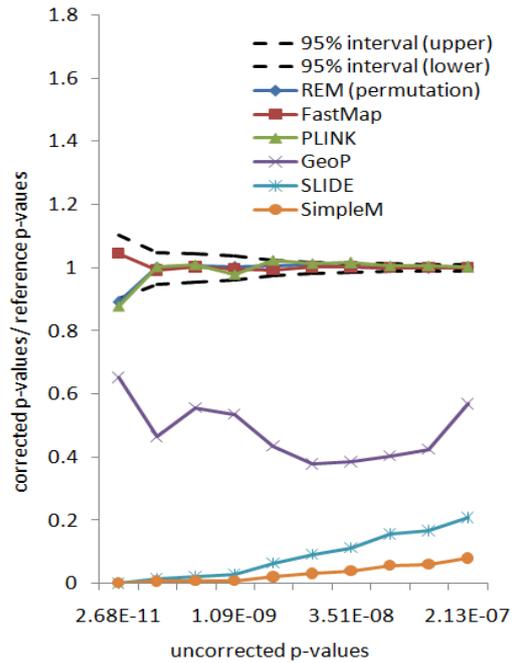
Figures S2(a), S2(b), and S2(c) show the results when using the permutation  $P$ -values as reference. From these figures, we can observe a similar trend using the three synthetic datasets to that using the real expression traits data. The approximation methods are anti-conservative. Moreover, they do not provide accurate estimation for the permutation  $P$ -values. Their performances vary for phenotypes with different distributions. GeoP does not work for exponentially distributed phenotypes (with all corrected  $P$ -values being 0), though it performs better than SLIDE and SimpleM on the other two distributions. This demonstrates that the distribution of the phenotypes plays an important role in the performances of the approximation methods.



(a) Synthetic normally distributed trait



(b) Synthetic exponentially distributed trait



(c) Synthetic uniformly distributed trait

Figure S2: Accuracy evaluation of selected methods on synthetic gene expression traits using inbred mouse data set. (Each line represents the ratio between the corrected  $P$ -values and the reference  $P$ -values for a method. The reference  $P$ -values are obtained using 100M permutations. An accurate method should yield a ratio of 1. In this figure, the reference  $P$ -values are estimated by permutation test.)

## Computational Efficiency Evaluation when Varying the Size of the Data Set

We randomly sample 1K real gene expression traits for the evaluation. Unless otherwise specified, the default experimental setting is as follows: number of SNPs = 150K, number of traits = 1K, and number of resamplings = 100K. PLINK is not computationally efficient enough for this setting. However, since its runtime is linear to the number of resamplings, we estimate its runtime for 100K resamplings by first running it with 100 resamplings, and then multiplying the runtime by 1000. We examine the runtimes of REM for three different thresholds of corrected  $P$ -values, 1, 0.05, and 0.01. When the threshold is set to be 1, REM will find the corrected  $P$ -values for all traits. Otherwise REM automatically finds the traits whose corrected  $P$ -values are less than the threshold. As shown in Figure S3, FastMap is about two orders of magnitude faster than PLINK. REM further improves the computational efficiency by about two orders of magnitude. The computational efficiency of REM is dramatically improved when the corrected  $P$ -value threshold decreases, because REM can filter out insignificant traits in a very early stage of the process.

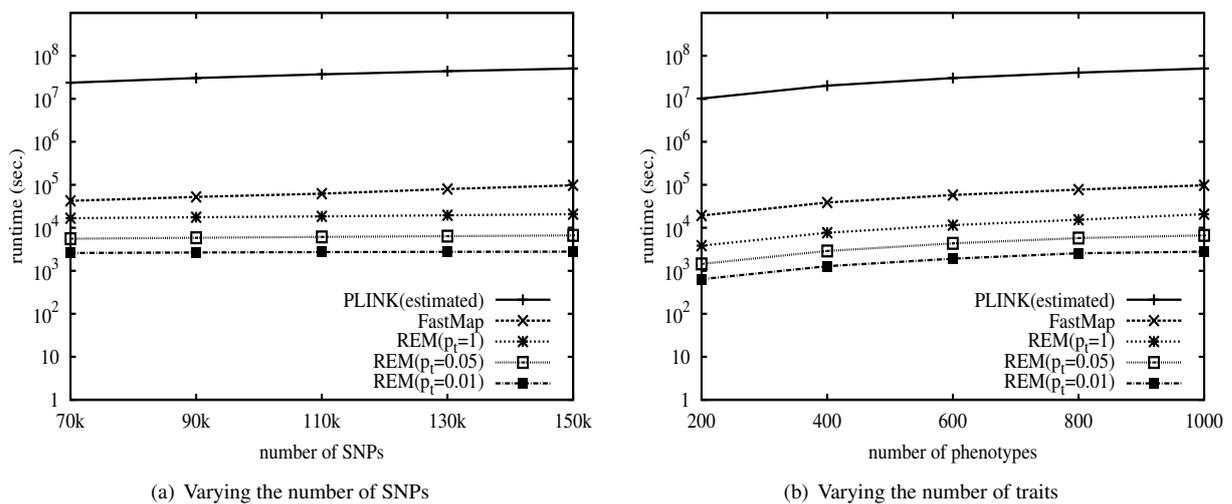


Figure S3: Efficiency evaluation of three exact methods, PLINK, FastMap, and REM, when varying the number of SNPs and the number of traits in the mouse data set. The y-axis (runtime) is in logarithmic scale. The runtime of PLINK is estimated based on small scale experiments. See text for more details.

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## Pseudo Code of the REM Algorithm

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### Algorithm S1: REM - Rapid and Exact Multiple testing correction by resampling

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**Input:** SNPs  $\{X_1, X_2, \dots, X_N\}$ , gene expression traits  $\{Y_1, Y_2, \dots, Y_M\}$ , number of resamples  $K$ , and desired resampling-based  $P$ -value threshold  $p_t$ .

**Output:** Significant gene expression traits, i.e., the ones whose resampling-based  $P$ -values are no greater than  $p_t$ .

```
1 index SNPs  $\{X_1, X_2, \dots, X_N\}$  by the two-layer indexing structure;
2 for every  $Y_m$  ( $1 \leq m \leq M$ ) do
3   scan all SNPs to find maximum statistic  $\mathcal{T}_{Y_m}$ ;
4   generate resampled phenotype vectors  $\{Y_m^1, Y_m^2, \dots, Y_m^K\}$ ;
5    $count = 0$ ;
6    $p_{res}(Y_m) = \frac{count+1}{K+1}$ ;
7   for every  $Y_m^k$  ( $1 \leq k \leq K$ ) do
8     for every  $e_{1i}$  ( $e_{1i}$  is a first layer entry) do
9       if  $ub(e_{1i}) > \mathcal{T}_{Y_m}$  then
10        for every  $e_{2j}$  ( $e_{2j}$  is a second layer entry of  $e_{1i}$ ) do
11          if  $ub(e_{2j}) > \mathcal{T}_{Y_m}$  then
12            for every  $X_n$  in entry  $e_{2j}$  do
13              if  $\mathcal{T}(X_n, Y_m^k) > \mathcal{T}_{Y_m}$  then
14                 $count = count + 1$ ;
15                 $p_{res}(Y_m) = \frac{count+1}{K+1}$ ;
16                goto line 23;
17            end
18          end
19        end
20      end
21    end
22  end
23  if  $p_{res}(Y_m) > p_t$  then
24    goto line 2;
25  end
26 end
27 return  $Y_m$  as significant;
28 end
```

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### Time Complexity of REM

Supposed that we have  $S$  individuals,  $N$  SNPs,  $M$  Phenotypes, and  $K$  Permutations/bootstraps. In Line 1 of Algorithm S1, the overall time complexity for indexing the SNPs is  $O(NS)$ . In Line 9, the total number of upper bounds in the first layer we need to check is  $(S/2)^2/2$ . The complexity of each check is  $O(1)$ . So the overall time complexity for searching the first layer is  $O(KMS^2)$ . In Line 11, in the worse case, each first layer entry has  $O(S^2)$  second layer entries. However, the total number of secondary entries cannot be larger than the total number of SNPs  $N$ . Thus, the worst case time complexity for searching the second layer is  $O(KMN)$ . Moreover, in practice, for a first layer entry, a second layer indexing is only needed when its number of SNPs is larger than the possible number of second layer entries. Only a small portion of the first layer entries will actually have the second layer indexing. The overall time complexity of REM is  $O(NS + KMS^2 + KMN)$ .

Note that the complexity analysis only provides an asymptotic description of the worst case performance of the algorithm. The actual performance of the algorithm heavily depends on the tightness of the upper bound, which has been demonstrated by extensive experimental evaluation.

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## Applying REM to Large Sample Study through Meta-Analysis

REM can be effectively applied to large sample study through meta-analysis. In a meta-analysis, the samples are partitioned into several groups. The resampling-based  $P$ -values within each group are calculated using REM. The  $P$ -values are then combined by applying Fisher's method (FISHER 1925).

We simulate data sets of large samples to demonstrate the efficacy of REM. We use SNPs in chromosome 22 of 1000 randomly selected individuals from the genome-wide association study of Schizophrenia (SHI *et al.* 2009). At each locus, the heterozygous genotype is combined with the homozygous genotype of major allele. There are 6,679 SNPs of MAF no less than 0.05. The phenotypes  $Y$  are simulated by a linear model:  $Y = Xb + \epsilon$ , where  $X$  is the genotype of a SNP,  $b$  is the coefficient, and  $\epsilon$  is the residual error. In our experiments,  $b$  varies from 0.3 to 0.7, and  $\epsilon$  follows a standard Gaussian distribution.

The square of Pearson's correlation,  $R^2$ , is used as the test statistic. We denote the maximum  $R^2$  of the original phenotype to be  $r_0^2$ . The permutation  $P$ -value across the 1000 individuals is calculated as the proportion of the permutations with maximum  $R^2$  larger than  $r_0^2$ .

For meta-analysis, we randomly partitioned the data into two groups, each of which has 500 samples. In each group, we calculated the permutation  $P$ -value as the proportion of the permutations whose maximum  $R^2$  are larger than  $f r_0^2$ , where  $f$  is a constant. We then apply Fisher's method to combine the permutation  $P$ -values of every group to obtain the meta permutation  $P$ -value.

We repeat the above simulation 1000 times and compare the permutation  $P$ -values from the whole group (the 1000 individuals) to the meta permutation  $P$ -values. Figure S4 depicts that they are highly correlated. Specifically, when the factor  $f$  equals to 2.0 (red points in the figure), the correlation is 0.99.

Meta-analysis using 5 and 10 groups are also performed. The results are similar to that of 2 groups. In particular, the correlation is 0.98 for 5 groups, and 0.96 for 10 groups.

The nearly perfect correlation between the permutation  $P$ -value (of the whole group) and the meta permutation  $P$ -value enables us to apply REM to studies with large samples effectively. Specifically, we first partition the samples into smaller groups and apply REM to get the permutation  $P$ -values for each group. We then combine these  $P$ -values by the Fisher's method to get a meta permutation  $P$ -value. Finally, we map the meta permutation  $P$ -value to the original permutation  $P$ -value following the estimated relationship (e.g., the line corresponding to factor 2 in Figure S4) between these two values. Note that the relationship between the original permutation  $P$ -value and the meta permutation  $P$ -value can be estimated by using a small number (e.g., tens) of phenotypes.

The exact value of the factor  $f$  is not essential to the success of our method. This is because, for any given factor  $f$ , we can always estimate the relationship between the meta permutation  $P$ -value and the original permutation  $P$ -value. For example, the three lines in Figure S4 correspond to three different values of  $f$ . Any one of them can be used to

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map between the two  $P$ -values. In theory, however, it is interesting to investigate whether there exists an optimal  $f$  value that gives the highest correlation between the two  $P$ -values. This is among our future research directions.

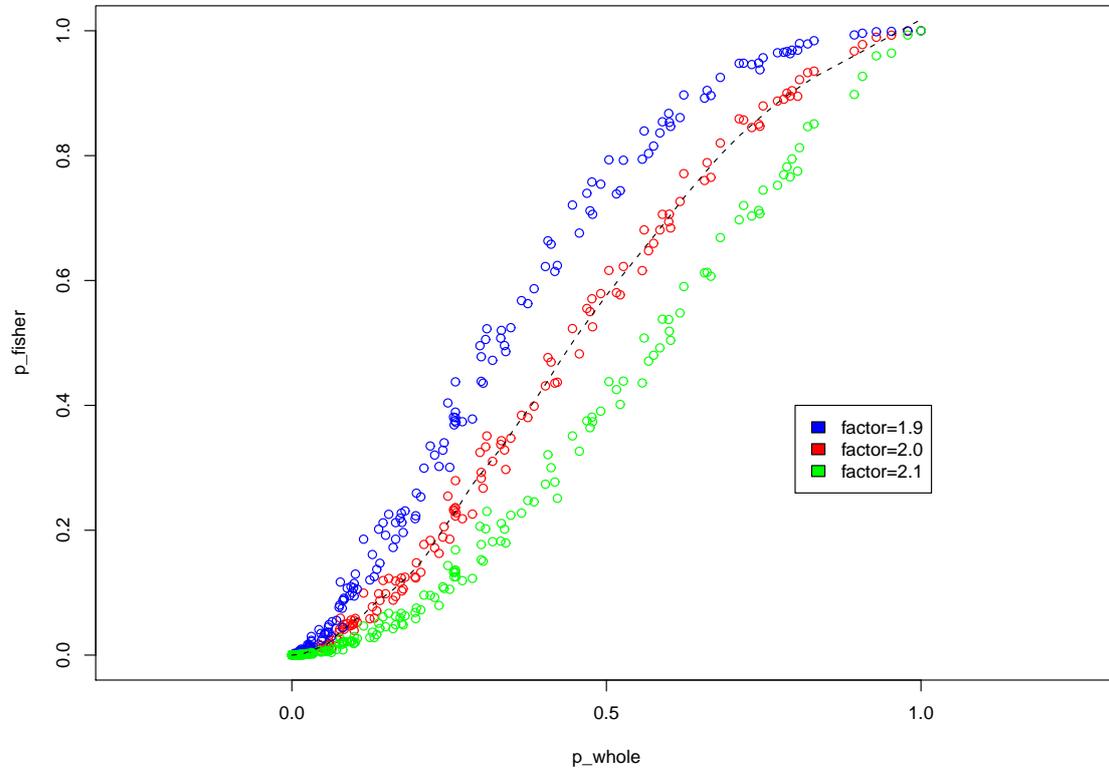


Figure S4: Relationship between the combined  $P$ -values and the original  $P$ -values

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