

Statistical tests for the intersection of independent lists of genes: sensitivity, FDR, and Type I error control

LOKI NATARAJAN, MINYA PU, KAREN MESSER¹

Division of Biostatistics and Bioinformatics

UCSD School of Medicine

Moore's UCSD Cancer Center # 0901

University of California, La Jolla, CA 92093

email: kmesser@ucsd.edu

Abstract

Public data repositories have enabled researchers to compare results across multiple genomic studies in order to replicate findings. A common approach is to first rank genes according to an hypothesis of interest within each study. Then, lists of the top-ranked genes within each study are compared across studies. Genes recaptured as highly ranked (usually above some threshold) in multiple studies are considered to be significant. However, this comparison strategy often remains informal, in that Type I error and false discovery rate (FDR) are usually uncontrolled. In this paper, we formalize an inferential strategy for this kind of list-intersection discovery test. We show how to compute a p-value associated with a ‘recaptured’ set of genes, using a closed-form Poisson approximation to the distribution of the size of the recaptured set. We investigate operating characteristics of the test as a function of the total number of studies considered, the rank threshold within each study, and the number of studies within which a gene must be recaptured to be declared significant. We investigate the trade off between FDR control and expected sensitivity (the expected proportion of true-positive genes identified as significant). We give practical guidance on how to design a bioinformatic list-intersection study with maximal expected sensitivity and prespecified control of Type I error (at the set level) and false discovery rate (at the gene level). We show how optimal choice of parameters may depend on particular alternative hypothesis which might hold. We illustrate our methods using prostate cancer gene-expression datasets from the curated Oncomine database, and discuss the effects of dependence between genes on the test.

Key words: *Concordance; Validation; Gene-ranking; Meta-analysis; Rank based methods; Gene expression analysis; Microarray; next generation sequencing; Cancer.*

1 Introduction

Given several independent genomic data sets which address a similar question, it is common to compare the lists of the top-ranked genes from each study. Genes selected as highly ranked in multiple studies may be considered validated or replicated. Curated databases of gene lists are

¹To whom correspondence should be addressed.

available which include tools for comparing lists and intersecting lists of top-ranked genes across multiple similar studies [Glez-Pena et al., 2009, Culhane et al., 2010]. The ‘correspondence at the top’ concordance statistic is an example of this approach [Irizarry et al., 2005]. Perhaps the most well known example is the study by Tomlins *et al* of gene expression in solid tumors, [Tomlins et al, 2005] which compared the top 10 genes from each of 132 cancer studies in a publicly available microarray data repository. Within each study, the genes were ranked according to a statistic scoring potential ‘fusion gene’ properties, as such fusion genes are known to be important drivers of malignancy in several hematologic cancers. Tomlins *et al* targeted a candidate gene list of 300 known cancer genes; any candidate gene which ranked among the top 10 in two or more of the studies was considered to be a potential hit. Two significant genes were found, one which ranked among the top 10 in two different studies and another in five studies. For these two genes, a fusion product was subsequently experimentally confirmed in prostate cancer and these remain the only common fusion transcripts discovered in an epithelial tumor.

In this paper, we show how to conduct such an intersection-of-lists approach to assessing significance while controlling Type I error (at the set level) and false discovery rate (at the gene level). Given N independent studies, there are two parameters which define a ‘hit’: the rank threshold, r , above which a gene must lie in each study ($r = 10$ in the Tomlins example) and the number of lists, n , among which a gene must be ranked ($n = 2$ in the Tomlins example) in order to be declared significant. Our first goal is to define an exact p-value which is easy to compute, when assessing the intersection of n lists of top-ranked genes, at rank r or above. This entails defining an appropriate test statistic and corresponding hypothesis test, which we call a List-Intersection discovery test, as this is an ‘unsupervised’ or discovery approach. We apply these ideas to the related ‘supervised’ case of an *a priori* candidate gene list which is compared against N other independent studies, as in [Tomlins et al, 2005]. Here the aim is to validate the genes appearing in the researcher’s *a priori* list with a formal test of hypothesis. Following [Irizarry et al., 2005] we call this a List-Intersection concordance test. We then develop practical guidelines for choices of r and n which maximize the expected sensitivity at a given false discovery rate (FDR), that is, which maximize the expected proportion of true-positive genes that are declared to be significant at a given FDR. We give example applications of both the discovery and concordance test using data from the Tomlins study.

To state the discovery problem more precisely, consider data from N independent (gene-expression) studies. Within each study, suppose T genes are independent and are ranked according to a statistic, and consider the list of the r top-ranked genes in each study. The set of genes which lie in the intersection of n or more of these lists, $S_n(r)$, are those genes ‘recaptured’ as significant at least n times across N independent studies. However, the degree of confidence in this validation remains to be assessed. For example, considering $N = 6$ independent studies each with 10,000 possible genes, it may be very likely that by chance alone at least 50 genes would be recaptured among the top $r = 200$ genes in $n = 2$ or more studies (as we shall demonstrate, probability 0.84), somewhat likely that 5 or more genes would be so recaptured across $n = 3$ studies (probability 0.02), and very unlikely that any one gene would be recaptured in $n = 5$ out of 6 studies just by chance (probability 0.0002). In this paper, we show how to compute these probabilities (these examples are computed in Section 2.2), how to assess the statistical significance of the recaptured set for given r and n , and how to estimate the false discovery rate within the recaptured set. The test statistic we use is $|S_n(r)|$, the size of the intersection set; in the above three examples $|S_n(r)| \geq 50, 5$ and 1 respectively. In making these computations we have assumed the genes behave independently, which is no doubt not

true in practice, and this is addressed theoretically and in simulations in the last section of the paper.

The paper is organized as follows: In Section 2 we derive the distribution of the List-Intersection test statistic under the null hypothesis and show how to compute a p-value for a gene-set and how to estimate the within-set false discovery rate (FDR). In Section 3 we derive the distribution of the List-Intersection concordance test statistic. In Section 3.2 we apply the test to data in Tomlins *et al* [Tomlins et al, 2005]. In Section 4, we discuss how to control the Type I error of the discovered set, and how to control the false discovery rate of the genes within the discovered set. We give a strategy for finding good choices of r and n . (Section 4.2). In Section 5 we give an example of how to mine a data repository for a “statistically significant” discovered gene set while controlling the Type I error at the set level and the within-set FDR. Section 6 addresses what happens if independence on genes does not hold, and then gives conclusions and future directions. Simulation studies, code, and additional proofs are in an Appendix.

2 The List-Intersection discovery test

The List-Intersection test compares the top-ranked gene lists from multiple studies in order to discover a common significant set of genes. Suppose we consider N studies, each of which investigate T genes, and that the genes within a study are ranked according to a pre-specified scoring procedure which might be fold change, a between group t-test, or might differ from study to study. Consider the list of ‘top r ’ genes within each study, and consider the set of genes, $S_n(r)$, which lie in n or more of these top-ranked lists. (We will often omit the dependence on r for convenience.) In this section we find the expected count $E_{H_0}|S_n|$ under the null hypothesis of random ranking of the genes. We show that $|S_n|$ has an approximately Poisson distribution under the assumptions the genes within a study are independent, and that $T \gg r$ and $E|S_n| \ll r$ where both T and r are large, and use this to compute a p-value.

2.1 Null distribution of $|S_n(r)|$, estimated FDR, and p-values

For an arbitrary gene g , let the Bernoulli trial $B_g = 1$ if the gene ranks among the top r genes in n or more studies, with $B_g = 0$ otherwise. Then

$$|S_n| = \sum_{g=1}^T B_g. \quad (2.1)$$

Let $P_n^0 = P(B_g = 1)$ denote the associated probability under the null hypothesis. (Note that we have suppressed the dependence of P_n^0 on r for convenience.) While the Bernoulli trials B_g are not independent even under the assumption of independent genes (given that one gene lies among the top r genes, the next gene is less likely to do so), they are identically distributed, and so it is immediate that

$$E_{H_0}|S_n| = TP_n^0. \quad (2.2)$$

To evaluate P_n^0 under the null hypothesis of random ranking among T genes in each study, index the studies from $i = 1$ to N and again consider an arbitrary gene g . Let A_i be the Bernoulli trial that counts a success if g ranks among the top r genes in study i , with $P(A_i = 1) = p_i$. Under the null hypothesis, $p_i = r/T \equiv p_0$, and the A_i are independent. Let X count the

number of successes for gene g . Then $X \sim \text{Bin}(N, p_0)$, and the probability P_n^0 that gene g is listed among the top r genes in n or more studies is given by

$$P_n^0 = P(X \geq n), \quad (2.3)$$

an easily computed binomial probability. Using (2.2) and (2.3) one may then estimate the within-set FDR by comparing the expected number of discoveries under the null hypothesis to the total number of discoveries made:

$$F\hat{D}R = E_{H_0}[|S_n|]/|S_n| \quad (2.4)$$

Under the null hypothesis of *independent* random ranking of the genes, we can derive the distribution of $|S_n|$. Note that for large T with $T \gg r$, selection of the r top-ranked genes within a study has nearly the same distribution as random sampling with replacement. If in addition $E|S_n| \ll r$, then B_g and B_h are approximately independent for any pair of genes g and h . In this case $|S_n|$ will have an approximate Binomial distribution with parameters T and P_n^0 . If in addition T is large and P_n^0 small, it follows that the distribution of $|S_n|$ is approximately Poisson with mean TP_n^0 . We consider the effects of correlation between genes in section 6.1.

2.2 Example computations using the List-Intersection statistic

Here we show how to use (2.2) and (2.3) to compute the expected number of genes recaptured just by chance, as well as the p-value of the size of the recaptured set and the estimated FDR for genes within the set. These quantities depend on the total number of studies considered, N , the depth of the top-ranked list, r , and the number of lists intersected, n . Throughout we let $T = 10,000$. We also investigate the quality of the Binomial and Poisson approximations for these examples.

- (i) As in the introduction, consider that we have ranked $T = 10,000$ genes, and that the top $r = 200$ genes are the top-ranked set. Then, under the null hypothesis of independent random ranking, $p_0 = r/T = 0.02$. From (2.3), given $N = 6$ studies to compare, the probability of seeing a given gene in the top 200 from $n = 2$ or more studies is $P_2^0 = 0.0057$. It follows from (2.2) that $|S_2(200)|$, the number of genes captured in 2 or more studies, is then approximately Poisson with mean $TP_2^0 = 57$.

To evaluate the accuracy of p-values computed from this Poisson approximation, note that two of the three key assumptions, that $T \gg r$ and P_n^0 be small, are met. However, $E|S_2|/r \approx 0.30$, so that $E|S_2|$ is not particularly small compared to r . Suppose the observed value of $|S_2(200)| = 50$. Then, from simulation under the null hypothesis of random ranking, $P(|S_2(200)| \geq 50) = 0.86$, compared to the corresponding Poisson p-value of 0.84, yielding a relative error of 2.3%. Further examples show the simulated 95th percentile of the null distribution is 68, while the Poisson approximation gives 70 (relative error, 2.9%). At 1% significance level, the relative error is 2.7% (simulated value 73; Poisson approximation 75). Thus the Poisson approximation appears to work well in this case.

- (ii) Continuing the example, if we require the genes to be recaptured in three or more studies (so that $n = 3$ rather than 2), the mean number of genes captured under the null is

only 1.53. As in the introduction, suppose the observed value of $|S_3(200)| = 5$. Under the null hypothesis of random ranking the probability that 5 or more genes would be in the intersection list is $P(|S_3(200)| \geq 5) = 0.02$, where $|S_3(200)|$ is Poisson with mean 1.53. Thus we would have seen a statistically significant event with a p-value of 0.02. The estimated within-set FDR would be $1.53 / 5$, or 31%. Note that from simulation, $P(|S_3(200)| \geq 5) = 0.018$, again demonstrating the adequacy of the Poisson approximation.

- (iii) Now suppose only $N = 4$ rather than 6 total studies are considered, and $n = 2$ as before. then the expected number of genes captured by chance falls by half, to a mean of 23 genes.

Again the relative error of the 95th and 99th percentiles of $|S_2(200)|$ from the Poisson approximation is 3% (the simulated 95th percentile of the null distribution is 31, while the Poisson approximation gives 32; the simulated 99th percentile is 34, while the Poisson approximation gives 35.)

- (iv) When $N = 6$ but the depth of the list is halved so that only the top $r = 100$ genes are considered, the mean number of genes captured by chance falls by 3/4, from 57 to 15. The relative approximation error of the Poisson distribution is 0% for the 95th percentile, and 4% for the 99th percentile.

These examples show how to use the Poisson approximation to the distribution of $S_n(r)$ to calculate p-values and FDRs. Over the range of parameters considered here, the Poisson approximation appears to be very good. Additional simulations are reported in Sections 5 and Appendix section 1.1.

3 The List-Intersection Concordance test

The concordance test evaluates whether an *a priori* candidate list of m genes, say from the researcher's new study, is significantly reproduced among the top r genes in N independent ranked lists of genes, say from other experiments or from the literature. Suppose each study investigates T genes and consider the set of genes, $C_n^m(r)$, from the *a priori* candidate list which also lie in n or more of these top-ranked lists. As before, we show that $|C_n^m|$ has an approximately Poisson distribution under the null hypothesis of independent random ranking of the genes, however with a different mean, under the assumptions that $T \gg r$ and $E|C_n^m| \ll r$ and both T and r are large.

3.1 Null distribution of $|C_n^m(r)|$

Again, index the studies from $i = 1$ to N . Consider an arbitrary gene g drawn from the *a priori* list of m genes of interest, and as before, for study i let A_i be the event that gene g is listed among the top r genes. Under the null hypothesis of random ranking among T genes in each study, $p_i = r/T \equiv p_0$, as before. As in Section 2.1, equation (2.3) gives P_n^0 , the probability under the null hypothesis that n or more of the events A_1, \dots, A_N occur simultaneously. Now consider the m genes on the *a priori* list, and let $B_g = 1$ if the g th gene ranks among the top r genes in n or more studies, with $B_g = 0$ otherwise. Under the null hypothesis $P(B_g = 1) = P_n^0$,

Table 1: **Example: expected and observed number of recaptured candidate genes, p-values and estimated FDR within the recaptured set.** Data from Tomlins (2005); $T = 10,000$ genes across $N = 132$ studies, with the top $r = 10$ genes considered from each study. The length of the *a priori* candidate gene list is $m = 300$. The recapture rate n varies from 2 to 5; $n = 2$ was the choice used in Tomlins (2005)

	$n = 2$	$n = 3$	$n = 4$	$n = 5$
$E C_n $ under null	2.5	0.11	< 0.01	< 0.01
Observed C_n	{ERBB2, ERG, ETV1, IRTA1}	ERG	ERG	ERG
Observed $ C_n $	4	1	1	1
p-value	0.25	0.006	7×10^{-6}	5×10^{-9}
Estimated FDR	0.63	0.11	< 0.01	< 0.01

and as $|C_n^m| = \sum_{g=1}^m B_g$, it is immediate that

$$E|C_n^m| = mP_n^0 \quad (3.5)$$

under the null. Further, for large T with $T \gg r$, selection of the r top-ranked genes within a study has nearly the same distribution as random sampling with replacement. If in addition $E|C_n^m| \ll r$, then B_g and B_h are approximately independent for any pair of genes g and h . In this case $|C_n^m|$ will have an approximate Binomial distribution with parameters m and P_n^0 , which in turn is approximately Poisson with mean mP_n^0 for m large and P_n^0 small.

3.2 Example test using data from Tomlins *et al*

We apply these computations to the data from Tomlins [Tomlins et al, 2005]. They considered the Cancer Gene Census [Futreal et al., 2004] published list of 300 genes known to be involved in cancer, and compared this candidate gene list across 132 studies from the Oncomine [Rhodes et al, 2007] repository of microarray data. Within each study, they ranked all genes according to a score characteristic of a fusion gene. They then looked for the occurrence of any candidate cancer genes among the 10 top-ranked genes in each study, and for each cancer gene, reported how many times it was ‘captured’ in a top-10 list. To define parameters, each microarray platform interrogated about 10,000 expressed genes. Thus we have $T = 10,000$ genes across $N = 132$ studies, with the top $r = 10$ genes considered from each study. The length of the *a priori* list is $m = 300$.

We applied (2.3) and (3.5) to find the expected number of cancer genes which appear in the intersection of n multiple lists under the null hypothesis of independent random ranking, for n ranging from 2 (the case considered by Tomlins et al) to 5. These results are given in Table 1, in the row labeled $E|C_n|$. We give the set of actual genes found by Tomlins *et al* in the intersection of n or more lists (the observed set C_n), taken from their supplementary table S1. We also record the count of cancer genes recaptured n or more times (the observed count $|C_n|$). We then compute the p-value for each value of n , computed as the probability that a Poisson variate with the given mean would lie above the observed value of $|C_n|$, and the estimated FDR within each recaptured set. Notably, the observed set of cancer genes which is in the intersection of 2 or more lists, the set considered by Tomlins *et al*, has a p-value of

0.25, indicating it is plausible that this many genes would reappear just by chance. Four genes were 'discovered' in this recaptured set, while the expected number recaptured under the null is 2.5 for an estimated FDR of $2.5/4$ or 63%. However, the p-values attached to the single gene ERG, which reappears in 5 studies, is highly significant. Both ERG and the related ETV1 were subsequently validated as fusion genes.

This example illustrates how to compute the p-value for the size of an observed set of concordant genes. However, notice that multiple p-values are presented in Table 1, corresponding to multiple choices of r and n . Unless we specify r and n in advance, we are open to charges of data snooping, that is, of tailoring the choice of r and n to the results they yield in a given data set, rendering the nominal p-values invalid. Thus, this example also highlights the need for a strategy for choosing r and n , and importantly, the need to specify the choice of r and n before the analysis is carried out. We discuss these issues in the remainder of the paper.

4 Control of Type I error and within-set FDR

For a given pre-specified choice of r and n , the List Intersection test will declare a gene set to be significant only if $|S_n(r)|$ (or $|C_n^m(r)|$) has a p-value below the stated significance level α . This procedure will strictly control the Type I error rate; that is, under the null model, the probability will be at least $1 - \alpha$ that no gene set will be declared to be significant. Given a statistically significant gene set, it remains to investigate the FDR within set, and the expected proportion of true positive genes that are captured (the expected sensitivity).

Importantly, as noted above, control of Type I error requires both n and r to be specified in advance. For example, there may be several sets $S_n(r)$ with p-values falling below any given significance level, and *post hoc* selection of one or more of these sets without correction for multiple testing would of course leave both the Type I error and the set-level FDR uncontrolled. In addition, failure to pre-specify r and n is likely to lead to data snooping, in which the chosen r and n are consciously or unconsciously tailored to yield the most "interesting" set of selected genes. Thus it remains to consider how to make good *a priori* choices of r and n . In this section, we give an example which illustrates how good choices of r and n may depend on which particular alternative hypothesis holds, and then propose a general design strategy. We leave as future work discussion of the more computationally and mathematically involved data-driven strategies to control the FDR.

4.1 Example choices of r and n : expected sensitivity and false discovery rate

Different choices of the threshold r and the recapture rate n will trade-off between an increased false discovery rate within the set $S_n(r)$ and increased power to capture any truly positive genes. For example, for fixed n , as r increases and more genes are included in the set of 'top- r ' genes, any truly significant genes ("true positives") will be more likely to be selected within each study and thus more likely to land in the intersection set $S_n(r)$. However, at the same time more null genes will be captured, thereby potentially increasing the FDR within $S_n(r)$, and possibly reducing power to call $S_n(r)$ a statistically significant set. Good choices for r and n will evidently depend on how many truly positive genes exist, as well as the effect size for each, as the latter determines the probability that a given positive gene rises to the top of the list.

Table 2: **Expected sensitivity (ESns) and FDR under different alternative hypotheses and choices of r (within-study significance threshold), and n (recapture rate).** Note: $N = 4$ independent studies; $T = 10,000$ genes measured in each study; EFP = expected # of false positives; ETP = expected # of true positives; ESns= ETP/# true positives; FDR = EFP/(EFP+ETP).

Alternative Hypothesis I: # of true positive genes = 25 each with 3-σ upregulation as compared to null genes						
Recapture-rate (n)		$r = 500$	$r = 100$	$r = 50$	$r = 25$	$r = 10$
2	EFP	128.43	3.99	0.71	0.10	0.003
	ETP	24.93	23.36	21.02	16.91	9.05
	ESns	0.99	0.93	0.84	0.68	0.36
	FDR	0.84	0.15	0.03	0.006	< 0.001
3	EFP	4.21	0.02	0.002	< 0.001	< 0.001
	ETP	23.90	17.42	12.65	7.54	2.24
	ESns	0.96	0.70	0.51	0.30	0.09
	FDR	0.15	0.001	< 0.001	< 0.001	< 0.001
4	EFP	0.05	< 0.001	< 0.001	< 0.001	< 0.001
	ETP	17.06	6.94	3.64	1.47	0.22
	ESns	0.68	0.28	0.15	0.059	0.009
	FDR	0.003	< 0.001	< 0.001	< 0.001	< 0.001
Alternative Hypothesis II: # of true positive genes = 2 each with 4-σ upregulation as compared to null genes						
Recapture-rate (n)		$r = 500$	$r = 100$	$r = 50$	$r = 25$	$r = 10$
2	EFP	139.14	5.70	1.38	0.32	0.04
	ETP	2.00	2.00	2.00	1.99	1.95
	ESns	1.00	1.00	1.00	1.00	0.98
	FDR	0.99	0.74	0.41	0.14	0.02
3	EFP	4.76	0.04	0.005	< 0.001	< 0.001
	ETP	2.00	1.97	1.93	1.85	1.65
	ESns	1.00	0.99	0.97	0.93	0.83
	FDR	0.70	0.02	0.002	< 0.001	< 0.001
4	EFP	0.06	< 0.001	< 0.001	< 0.001	< 0.001
	ETP	1.93	1.64	1.44	1.19	0.84
	ESns	0.97	0.82	0.72	0.60	0.42
	FDR	0.03	< 0.001	< 0.001	< 0.001	< 0.001

To illustrate these trade-offs, in Table 2 we compute the expected number of true discoveries and false discoveries for several choices of r and n , under two simple alternative hypothesis scenarios. We considered $N = 4$ independent studies, each investigating $T = 10,000$ genes. We assume that the statistic used to rank the genes has an approximately normal null distribution, such as a two sample t-statistic or a maximum likelihood statistic. We assume a total of tp genes are true positives, and for each such gene, the statistic is assumed to be normally distributed with mean μ and standard deviation 1. We constructed two scenarios: Alternative I had 25 true positive genes, each upregulated by 3 standard deviations as compared to the null genes, with the remaining $T - tp$ constituting the null genes. In Alternative II, we considered $tp = 2$ true positive genes, each with expression levels upregulated by 4 standard deviations as compared to the null genes. Thus Alternative I has multiple significant genes, each with moderate effect-sizes, and Alternative II has a few true hits with large effect-sizes. Under each of these illustrative alternative hypotheses, we computed the expected number of null and significant genes recaptured by the list intersection statistic. The mathematical argument for these expectations uses an argument from Feller [Feller, 1957] and is given in the Appendix (Section 2). For our two chosen scenarios, and for given r and n , Table 2 displays the FDR within the intersection gene set as well as the expected sensitivity (the expected proportion of true-positive genes that are captured). We considered recapture rates n from 2 to 4, and within-study thresholds r from 500 to 10.

For Alternative Hypothesis I, (25 true-positive genes, each with 3σ upregulation), when $n = 2$, a high expected sensitivity can be achieved by choosing r to be large. For example, $r = 500$ has an expected capture rate of 24.93 true positives out of 25 total, for an expected sensitivity of 99.7%. However this is at the cost of an FDR of over 80%, as the expected number of false positives is over 128 with a total expected set size of 153.36. Hence the pair $r = 500, n = 2$ does not appear to be a good choice here. Lowering r from 500 to 100 reduces the expected number of false positives to 4 while maintaining the expected number of true positives captured at about 23 out of 25 (92% expected sensitivity); thus $(r = 100, n = 2)$ appears to be a reasonable choice. Lowering r further achieves a lower FDR, but at the cost of lower expected sensitivity: with $n = 2$ as r decreases from 50 to 10, the expected sensitivity decreases from 84% to 36%. A better trade off would be to require a larger recapture rate with $n = 3$ while maintaining $r = 500$, as this combination maintains a sensitivity of 95.6% (ETP= 23.9 out of 25) while reducing false discoveries (EFP = 4.2 and FDR = 15%) Requiring a recapture rate of 4 out of 4 studies is too stringent for the scenario considered here. Thus either $(r = 100, n = 2)$ or $(r = 500, n = 3)$ appear to be good choices for Alternative Hypothesis I; both have expected sensitivity over 90% and FDR under 15%. Note that it may not always be possible to achieve high sensitivity and low FDR; in this case, as is evident in Figure 1 below, the number of studies N must be increased.

For Alternative Hypothesis II (2 true positive genes, each upregulated by 4σ), for a recapture rate of $n = 2$, thresholds of $r = 500, 100$ or 50 all have expected sensitivity of 100%, but also have FDR over 40%. (However, note that the *number* of false discoveries may not be prohibitive.) A cut-off of $r = 25$ or $r = 10$ gives better control of the FDR, while maintaining a high expected sensitivity. When $n = 3$, stringent thresholds such as $r = 25$ or 10 result in capture of fewer true positives, whereas setting $r = 100$ appears to be a good trade-off. Again, requiring a recapture rate of $n = 4$ reduces the expected sensitivity, so the reasonable pairs among those considered appear to be either $(r = 100, n = 3)$ or $(r = 10, n = 2)$.

We have illustrated how equations (2.3) and Appendix (2.1) can be used to calculate the expected number of true and false positives, and FDR and expected sensitivity for various

postulated hypotheses. In the next section we examine how these methods might be applied when designing a bioinformatic search to test *a priori* hypotheses of interest.

4.2 Choosing r and n to maximize sensitivity, with $\text{FDR} \leq q$

The example in Section 4.1 illustrates that the best choice of threshold r and recapture rate n will depend on the number of true positive genes, as well as the effect size for these genes. These considerations suggest how to design a list-intersection test: given an acceptable FDR q , find r and n that maximize the expected sensitivity while maintaining the gene-wise FDR $\leq q$. This can be computed for a prespecified alternative hypothesis which postulates tp true positive genes and corresponding effect-sizes as outlined below:

1. Set an acceptable FDR = q .
2. For each possible recapture rate $n = 1, 2, \dots, N$, find the maximum threshold $r(n)$ which still maintains $\text{FDR} < q$:
 - (a) For each $r = 1, \dots, T$:
 - i. compute the expected number of recaptured false positive genes $EFP(n, r) = (T - tp)P_n^0(r)$ (see Appendix equation (2.1)).
 - ii. Given tp true positive genes and their effect-sizes, calculate $ETP(n, r)$, the expected number of recaptured true positive genes. This can be obtained using Appendix equation (2.1) as $ETP(n, r) = \sum_{a=1}^{tp} P_n^a(r)$.
 - iii. Calculate $\text{FDR}(n, r)$ as $\frac{EFP(n, r)}{EFP(n, r) + ETP(n, r)}$.
 - (b) Let $r(n) = \max_{r=1}^T \{r | \text{FDR}(n, r) < q\}$.
3. For each pair $(n, r(n))$, calculate its expected sensitivity, $ETP(n, r(n))/tp$.
4. Choose the optimal pair, $(n, r(n))$, as the pair for which this expected sensitivity is maximized.

To illustrate this strategy, we again examined the two alternative scenarios discussed in Section 4.1. We set $T = 10,000$, as before and let N vary from 4 to 10 studies. We bounded the FDR by $q = 0.01$. Table 3 lists the maximum threshold $r(n)$ which satisfies the FDR bound, as obtained from step 2 of the above algorithm, for each possible recapture rate n . Note that $r(n)$ increases rapidly with increasing n . For example, under Alternative Hypothesis I, with $N = 4$ studies and $n = 2$, the maximum threshold which maintains the FDR cutoff is $r(2) = 30$, whereas if we consider intersections across all 4 studies (i.e., $n = 4$), the maximum threshold is as expected larger at 683, since null genes will be less likely to be recaptured across all studies. Note that $r(n)$ decreases as the number of studies N increases since the chance of a false positive increases with the total number of studies and hence the size of the recaptured list would need to be smaller to satisfy the pre-specified FDR.

For each given number of total studies N , Figure 1 plots the expected sensitivity, ETP/tp , against n for the optimal $r(n)$ from Table 3. Given N studies in total, the pair $(n, r(n))$ that maximizes sensitivity would be the optimal *a priori* design choice for the study. For instance, under Alternative Hypothesis I and an FDR cutoff of 0.01, with $N = 4$ total studies, the maximal expected sensitivity of $\sim 84\%$ is achieved at recapture rate $n = 3$, which from Table 3 is achieved at threshold $r(3) = 195$. The other two scenarios corresponding to $(n, r(n)) =$

Table 3: **The maximum threshold $r(n)$ that satisfies $\text{FDR} \leq 0.01$, for a given number of studies N and recapture rate n .** Two alternative scenarios, $T = 10,000$ genes. Expected sensitivity of the test at the maximum threshold is plotted in Figure 1, as a function of n .

Alternative Hypothesis I:				
25 significant genes, each upregulated by $3\text{-}\sigma$				
	Total # of Studies (N)			
Recapture rate (n)	4	6	8	10
2	30	24	20	18
3	195	126	95	77
4	683	384	270	210
5	-	869	575	434
6	-	1698	1034	755
Alternative Hypothesis II:				
2 significant genes, each upregulated by $4\text{-}\sigma$				
	Total # of Studies (N)			
Recapture rate (n)	4	6	8	10
2	7	5	4	3
3	81	48	34	27
4	373	194	133	102
5	-	513	332	247
6	-	1123	659	475

(2, 30) or (4, 683) achieve an expected sensitivity of less than 80%. Hence for Alternative I and 4 total studies, $(n = 3, r(3) = 195)$ is the optimal design choice. Note that under a given alternative, as the total number of studies N increases, the best choice of recapture rate n increases, as does the expected proportion of true positive genes recaptured (the expected sensitivity at the optimal choice of $(n, r(n))$).

The calculations in Table 3 and Figure 1 illustrate how a good choice of r and n involves maintaining control of the FDR while maximizing the chance of capturing true positive genes. The best choice of the pair $(n, r(n))$ of course depends on whether one expects many significant genes with small-moderate effect sizes similar to Alternative I, or few differentially expressed genes at large effect sizes, similar to Alternative II. For a given alternative hypothesis, our design strategy chooses the optimal combination of r and n which maximizes the expected sensitivity, while controlling the FDR at the desired level. Note that, if the expected sensitivity at the optimal pair $(n, r(n))$ is not satisfactory, then either the number of studies considered N must be increased or the desired FDR level must be relaxed.

If multiple alternatives are proposed with no clear “winner”, the above procedure can be used to choose the optimal design for several proposed alternatives. Then a Bonferroni correction could be applied, and the gene-sets that pass a Bonferroni corrected significance level would be candidates for further research. Specifically, for a given alternative and optimal design choice $(n, r(n))$, a p-value can be calculated for each $|S_n(r(n))|$ the test statistic of the observed data. This p-value might be computed using the approximate Binomial or Poisson distributions (Section 2) or via simulation. Then for m possible alternatives, and a significance level α , the gene-sets for which the corresponding p-values are less than α/m are considered “significant”. This procedure strictly controls the Type I error rate on the selected significant sets. Thus

under the null model, the probability is α or less of declaring a set of genes to be significant.

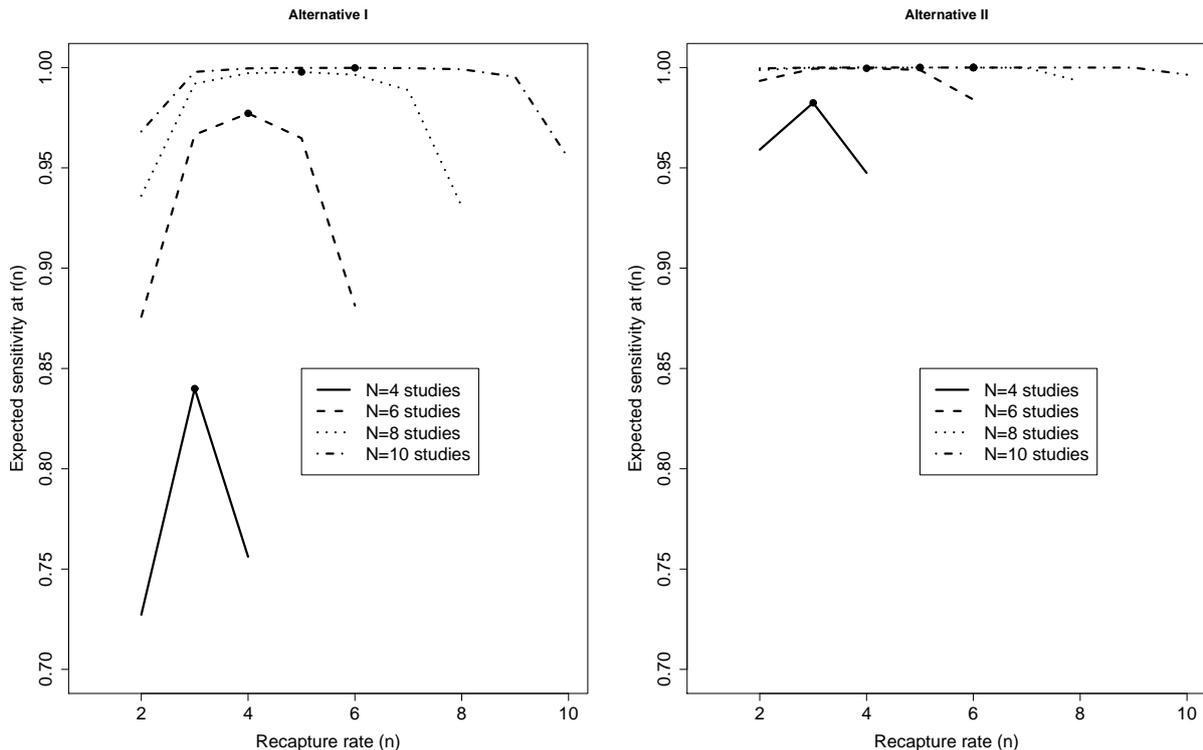


Figure 1: **Expected sensitivity at $r(n)$ versus recapture rate n , given N studies in total and $\text{FDR} \leq 0.01$, under two alternative scenarios.** For each recapture rate n , the expected sensitivity at the optimal threshold $r(n)$ is plotted. The filled circle represents the maximum expected sensitivity achievable for a given N . As expected, sensitivity increases as the total number of studies N increases. The recapture rate (n) and threshold ($r(n)$) which maximize sensitivity represent the optimal design choices which control FDR at the prescribed rate. Here # of features/genes = $T = 10,000$, and $\text{FDR} \leq 0.01$; the value of $r(n)$ is from Table 3. **Left:** Alternative I: 25 true-positive genes each with effect-size = 3σ . Optimal design for (i) $N = 4$ at $n = 3, r = 195$; (ii) $N = 6$ at $n = 4, r = 384$; (iii) $N = 8$ at $n = 5, r = 575$; (iv) $N = 10$ at $n = 6, r = 755$. **Right:** Alternative II: 2 true-positive genes each with effect-size 4σ . Optimal design for (i) $N = 4$ at $n = 3, r = 81$; (ii) $N = 6$ at $n = 4, r = 194$; (iii) $N = 8$ at $n = 5, r = 332$; (iv) $N = 10$ at $n = 6, r = 475$.

5 Example: Mining the Oncomine database for candidate fusion genes in prostate cancer

To illustrate our methods we carried out an example List-Intersection discovery study using the publicly available Oncomine database [Rhodes et al, 2007], as in the original Tomlins study [Tomlins et al, 2005]. We identified 4 suitable microarray gene expression prostate cancer studies [Dhanasekaran et al., 2004, Lapointe et al, 2004, Tiwari et al, 2003, Tomlins et al, 2006]; our selection criterion was that all use a similar cDNA microarray platform, and as is common in such studies [Tomlins et al, 2005] we assumed that $\sim 10,000$ genes would be expressed. Thus

we have $N = 4$ and $T = 10,000$. Note that here, unlike in [Tomlins et al, 2005] we are conducting a genome-wide discovery test, rather than a concordance test based on a list of candidate cancer genes. A second major difference is that Tomlins *et al* considered $N=132$ studies across all cancer types and all platforms. Of course, Tomlins *et al* used an *ad hoc* strategy to select interesting sets of genes for validation, rather than the statistically motivated use of p-values we are illustrating here. In this setting we know there to be least two true positive genes (the fusion genes identified in Tomlins, et al), and we are interested to see whether our *a priori* search strategy will find them.

We set the significance level to $\alpha = 0.05$. We next will pre-specify the pair $(n, r(n))$, in order to avoid data snooping. Thus under the null hypothesis there would be only 5% probability that the study will declare any gene set to be significant (see example 3.2 and Section 4). As discussed in Section 4.2, good choice of r and n depends on the particular alternative hypothesis postulated. Because in this somewhat artificial setting we know that fusion genes are rare and that at least two exist, Alternative Hypotheses II (see Table 2) with 2 significant genes, each with an effect-size of 4 is a reasonable choice for our study design. As in Table 2, we chose a stringent FDR cutoff of 0.01, with the rationale that then all discoveries within a significant set are likely to be true. Under these conditions, the optimal design choice is $(n = 3, r = 81)$, so that the set of genes $S_3(81)$ will be tested for statistical significance (Table 3 and Figure 1).

Next, within each of the 4 identified prostate cancer studies, we ranked the genes according to the “cancer outlier profile analysis” (COPA) procedure implemented in the Oncomine website [Rhodes et al, 2007]. This statistic measures “fusion-like” properties, and was used by Tomlins et al. [Tomlins et al, 2005]. We computed the observed test statistic $|S_3| = |S_3(81)|$ with $N = 4$ (Section 2) by counting the number of genes that were among the top 81 genes in at least 3 studies (Table 4). As seen in Table 4, the set $S_3(81)$ contained 1 “hit” . To compute the associated p-value, we obtained the expected value of $|S_3(81)|$ under the null hypothesis as $10,000P_3^0(81) = 0.021$ using equation (2.3). Then the probability that a Poisson variate with mean 0.021 will exceed 4 is 0.02, giving the p-value reported in Table 4. Thus we declare the set $S_3(81)$ to be a statistically significant set. The single gene in the set $S_3(81)$ is ERG, a gene also found by Tomlins et al. in their study, and this would be the single gene recommended for further investigation from our study. At the stringent within-set FDR, we would have confidence at about the 95% level that this was not a false positive result.

To gain additional insight, Table 4 presents p-values and FDR’s for recaptured sets over a range of thresholds r and recapture rates n . Note that only $S_3(81)$ (Table 4) is considered a discovery according to our pre-specified analysis strategy; other sets could be presented as exploratory descriptive results. For completeness we also examined the four genes corresponding to $|S_2(25)|$, as this had a highly significant p-value and a reasonable 9% FDR : these are ERG, ETV1, EST and VGLL3, of which the first two were validated as participants in a fusion gene by Tomlins et al. [Tomlins et al, 2005]. Thus by setting our FDR to the stringent level of 0.01, we accomplished the goal of identifying a significant set which contained no false discoveries, however we missed one of the truly positive genes. Since validation of such bioinformatic searches using rtPCR or other experimental techniques is expected, applying a less stringent a priori FDR may be a reasonable approach.

To investigate the adequacy of the Poisson approximation, the p-values in Table 4 were also verified by direct simulation as in Appendix Section 2.1. The p-value for the observed $|S_3(81)| = 1$ (Table 4) via direct simulation was 0.0188 compared to the Poisson approximation p-value of 0.0209. Considering the observed counts $|S_2(r)|$ in Table 4, the p-values derived from the simulated null distribution of $S_2(r)$ were 0.0775, 0.2001, 0.0602, 0.0004, and 0.0018

Table 4: **List-intersection discovery of fusion-gene candidates across $N=4$ independent prostate cancer studies.** The test statistic $|S_n(r)|$ is the observed number of genes that are ranked among the top r genes in at least n studies; p-value represents the probability of observing $|S_n(r)|$ or more genes under the null hypothesis of independent random ranking. The entry in bold corresponds to our *a priori* choice of $n = 3, r = 81$; as the p-value for this entry is less than 0.05 the corresponding gene set is declared significant.

# of studies (n)		$r = 100$	$r = 81$	$r = 50$	$r = 25$	$r = 10$
2	$ S_2(r) $	10	6	4	4	2
	p-value	0.08	0.20	0.06	0.0006	0.002
	est.FDR*	0.59	0.65	0.37	0.09	0.03
3	$ S_3(r) $	1	1	1	1	1
	p-value	0.04	0.02	0.005	0.0006	< 0.0001
	est.FDR*	0.04	0.02	0.005	0.0006	< 0.0001
4	$ S_4(r) $	0	0	0	0	0
	p-value	1	1	1	1	1

* est.FDR = estimated FDR = $E|S_n(r)|/|S_n(r)|$

respectively, for the corresponding thresholds r of 100, 81, 50, 25, and 10. Thus the Poisson approximation p-values and simulated p-values show good concordance.

6 Discussion

6.1 Dependence between genes

In this paper we have assumed independence of the genes within each study, however, in fact expression levels may be positively or negatively correlated between genes. Importantly, our strategy for study design (i.e. for choice of r and n , given in Section 4) depends only the mean of the test statistic $|S_n|$, which is unchanged under arbitrary dependence. (To see this, note that if genes g and h are correlated, (2.2) and (2.3) still hold.) However, correlation between genes will induce correlation between the Bernoulli trials in expression (2.1), and thus p-values computed under the assumption of independence under the null hypothesis may no longer be correct. How to adjust for correlation between genes in the analysis of gene expression studies is an active area of research [Efron, 2010, Benjamini and Yekutieli, 2001, Sun and Cai, 2009]. Here we give some quantitative guidance in the current setting, using simulation and by considering theoretical cases of extreme dependence. More detailed analysis will be the subject of future work.

First, it is easy to see that negative correlation between genes may be generally expected to reduce, and positive correlation to increase, p-values as compared to the independent case. This is because correlation between genes will induce correlation between the Bernoulli trials B_g in (2.1). The variance of the sum will be correspondingly decreased or increased with the mean remaining unchanged, rendering the distribution of $|S_n(r)|$ either more or less concentrated about its mean. It thus is most important to consider the effect of positive correlation between genes because this will potentially increase p-values and thus Type I error, if p-values are computed under the (incorrect) assumption of independence. The magnitude of the perturbation to

p-values clearly depends on both the number of correlated genes and the strength of their correlation, while the exact perturbation depends on the joint distribution of the correlated genes. Simulation studies reported in the Appendix (Section 1.2) show that moderate correlation (half of genes with weak correlation or a few genes with strong correlation) does not appear to appreciably affect p-values. Further support for these observations is found in the literature on models for correlated Bernoulli trials, of which [Yu and Zelterman, 2002, Gupta and Tao, 2010] give relevant examples.

Quantitative insight on the potential magnitude of a perturbation can be gained by considering the following extreme model: suppose the T genes can be partitioned into modules of size m , where two genes within a module have correlation $\rho > 0$ but any two genes in different modules are independent. In the limiting case with $\rho = 1$, it is easy to see that $|S_n(r)|$ has the same null distribution as the statistic $|m\tilde{S}_n(r/m)|$, where the distribution of $|\tilde{S}_n|$ is computed using $\tilde{T} = T/m$ independent genes, and is thus approximately Poisson with mean TP_n^0/m . It follows that under this model, $|S_n(r)|$ has unchanged mean, variance inflated by a factor of m , and that corrected probabilities can be computed using the relation

$$P(|S_n| > x) = P(|\tilde{S}_n| > x/m). \quad (6.6)$$

As the postulated within-module correlation decreases from $\rho = 1$ towards zero, the correct tail probabilities will smoothly interpolate from the correction given in (6.6) to the original values as computed in section 2.1 under independence. Thus, given correlated gene modules of postulated maximum size m , relation (6.6) might be used to give a conservative ballpark correction to computed p-values.

An example can be computed using the data in Table 4, Section 5. For example, four genes were recaptured by $S_2(25)$ (line 1 of Table 4), which had a p-value of 0.0006 under the null hypothesis of independent random ranking of genes. Suppose we wondered if correlation under the null hypothesis would be sufficient to account for the observed data. After consideration, suppose we decided there were several pairs of strongly correlated genes, so that we wanted to conservatively adjust for many correlated gene modules of size 2, so $m = 2$. As before, $p_0 = r/T = 25/10,000$ and $P_2^0 = P(X \geq 2) = 3.74 \times 10^{-5}$, so that under independence $|S_2(25)|$ is approximately Poisson with mean $TP_n^0 = 0.374$. However, under the correlated model with $\rho = 1$, $|S_2(25)|$ is distributed as $|2\tilde{S}_2(25)|$, where $|\tilde{S}_2(25)|$ is approximately Poisson with mean $TP_n^0/2 = 0.187$. The p-value adjusted for correlation would be $P(|\tilde{S}_n| \geq 4/2) = 0.015$. Thus, the maximum correlation assuming pair-wise modules would be unable to completely account for the observed data. Several simulation examples showing the effect of other correlation structures are presented in the Appendix.

6.2 Conclusions and future directions

Public repositories of genomic data continue to grow, and list- intersection approaches similar to those considered here are likely to become even more common in the future, as several repositories of curated gene lists have recently been published which include tools for comparing lists and intersecting lists of top-ranked genes across multiple similar studies [Glez-Pena et al., 2009, Culhane et al., 2010]. The primary statistical challenges for analyzing data from such repositories are controlling the number of false positive results and maintaining a valid basis for inference when combining multiple studies [Benjamini et al., 2008].

A well-established method for pooling results across multiple studies is meta-analysis. This approach is usually conducted gene-by-gene, and produces a combined p-value (or effect-

size) for each gene [Zaykin et al, 2002, Benjamini and Heller, 2008, Garrett-Mayer et al., 2008, Pyne et al, 2006]. However, under this approach it is possible that a significant gene can be declared based on a few studies which display large effects, with null effects observed in most studies, and this can lead to high false positive rates [Pyne et al, 2006]. Garrett-Mayer and others [Garrett-Mayer et al., 2008] discuss the importance of first identifying genes that are consistently measured across different microarray platforms, which is clearly a useful preliminary analysis for reducing false positives. There is evidence that rank-based approaches may be more robust and better guard against false discoveries, while maintaining adequate power, compared to more traditional methods of meta-analysis [Hong and Breitling, 2008]. Formal or informal rank-based meta analyses for combining effect sizes across multiple studies have been proposed in the applied and methodological literature [Chan et al., 2008, Jeffries et al, 2006, Deng et al., 2008, Miller and Stamatoyannopoulos, 2010].

Our approach compares within-study ranks to a common threshold, and is an effort to explore the inferential basis of the list-intersection approach. We provide exact formulas which allow examination of power and false discovery rates. Our rank-threshold method does not combine individual per-gene effect sizes, such as ranks, across multiple studies. Instead, we evaluate the entire set of genes recaptured as above a rank threshold across multiple studies. Loosely speaking, this is akin to acceptance sampling procedures, where a “lot” (i.e., the set $S_n(r)$ in our notation) may be deemed “acceptable” if the number of “defectives” (i.e., false discoveries) is below some level (defined by the FDR). However, a salient point in our setup is that many “lots” ($S_n(r)$) may be acceptable, in that they satisfy the FDR criteria. How to choose among these multiple “acceptable” gene-sets is a major focus of our work. We discuss expected sensitivity of the gene-sets, and also obtain a p-value per recaptured set, with tight control of the set-wise Type I error rate. In this sense our method is more stringent than an approach which combines gene-by gene effect sizes across studies. Our set-based method may also provide tighter control of gene-level Type I error and false discovery rates, although this is the subject of future research.

In related work [Pyne et al, 2006], a pooled p-value is calculated together with a *consensus parameter* defined as the number of studies in which a feature has to be declared significant before it is considered significantly validated across studies. Thus the consensus parameter plays a similar role as our recapture rate n . Pyne et al. [Pyne et al, 2006] describe results for different values of such consensus parameters but do not give guidelines on how to choose this parameter. Our work provides the applied practitioner with p-values and expected number of false positives under various choices for within-study significance thresholds r and recapture rates n , which could be used to guide decisions on significant “gene-sets”.

Another method, the partial conjunction hypothesis test [Benjamini and Heller, 2008], uses a p-value threshold to consider among how many studies out of N , a given gene is found to be significant at a given level, where each study addresses a different research hypothesis. For each gene g , the set of hypotheses that the gene is null in n or fewer studies is simultaneously tested, for $1 \leq n \leq N$. A general data-driven method for controlling the FDR across all genes is presented, where the number of false discoveries is defined as the number of genes which have been called significant in at least one study in which the gene was truly null. In this setting the studies may address differing alternative hypotheses, and the focus is on the situation where a gene can be truly null in some but not other studies, and where this may differ from gene to gene. Thus it is of interest to ascertain for each gene in which studies among all N considered it is truly significant. This differs from the scenario considered in the present paper, in which the studies are assumed to each test the same hypothesis. In the setting of Benjamini *et al* power

necessarily declines as the total number of studies N increases [Benjamini and Heller, 2008]. This is in contrast to our Figure 1, in which the expected sensitivity increases with the total number of studies N . Benjamini et al have the advantage, however, of controlling the false discovery rate in a data-driven manner. By contrast, we allow the user to set the number of studies n that a gene is required to be captured by, and we study how the expected true positive proportion and false discovery rates are affected by which alternative is considered to hold. Our interest is in scenarios where the alternative hypothesis is the same across studies, as is often the case in genomic studies.

In another approach to the problem, Lu et al. [Lu et al, 2008] develop a bootstrap methodology for assessing the average frequency with which significant genes will be rediscovered under independent validation. This approach is useful at the end of a study when significant gene-lists have been identified, and no external validation dataset is available. In particular, it can be used to estimate the internal stability of discovered genes, and also to compare different ranking procedures applied within the same study. Our focus is on external validation. We aim to provide a formal statistical method for evaluating genes that replicate across multiple studies. We discuss how one might *a priori* choose within-study significance thresholds (i.e., r) and cross-study recapture rates (i.e., n) to ensure (i) adequate probability of capturing true positives, and (ii) low false discovery rate within the recaptured set, when designing a bioinformatic search across multiple genomic datasets. After this search is complete, our methods can be applied to obtain p-values for observed recaptured sets, although permutation tests could also be used to obtain p-values, while the bootstrap could be used to obtain distributions of test statistics.

Our approach has some limitations. We assume that the threshold r for determining high-ranking genes is the same for all studies, as are the corresponding probabilities p_0 of selecting a null gene (used in computing p-values) and p_a of selecting a differentially expressed gene (used in computing the expected proportion of true positives for study design). These assumptions could be relaxed computationally, although the distributional calculations would lose their simple closed form solutions. Often when comparing results across studies the technology used to generate the data will be similar, in which case requiring similar parameters across studies should not pose a serious problem. In fact, this assumption is analogous to the homogeneity test in meta-analysis where only studies with similar design, populations, and measurement methods are pooled. Further, as with many methods in common use in the analysis of gene-expression data, our calculations assume that genes are independent which is unlikely to be the case in practice, as discussed in Section 6.1. As with any analysis of gene-expression data using microarrays, RNA-seq or other technologies, it is expected that results will be independently verified using different experimental methods.

In summary, in this article we describe a simple and rigorous inferential method for evaluating the consistency of results across multiple independent studies, using a combined Type I error for discovery of a significant gene set, and an estimated FDR within the gene set. We show how to choose study parameters to maximize the expected number of significant genes that will be captured. Future work will consider related approaches which are based on FDR control. The framework we describe for selecting a significant set of genes is used widely by biologists and bioinformaticians [Tomlins et al, 2005, Pyne et al, 2006, Benjamini et al., 2008, Glez-Pena et al., 2009, Culhane et al., 2010]. We hope that providing a simple computational and statistical underpinning for such studies will lead to more formal use of these methods with corresponding improved control of Type I error rates.

Funding

This work was supported by the Breast Cancer Research Foundation [Barbara Parker, PI], and the National Institute of Health (U54 HL108460).

Conflict of Interest: None declared.

References

- BENJAMINI Y. AND HELLER R. (2008). Screening for partial conjunction hypotheses. *Biometrics* **64**, 1215- 1222.
- BENJAMINI Y., HELLER R. AND YEKUTIELI D. (2008). Selective inference in complex research. *Phil Transactions Royal Society A* **367**, 4255- 4271.
- BENJAMINI, Y., AND YEKUTIELI, D. (2001), The Control of False Discovery Rate in Multiple Testing Under Dependency, *Annals of Statistics***29**, 1165-1188.
- CHAN S.K., GRIFFITH O.L., ET AL. (2008). Meta-analysis of colorectal cancer gene expression profiling studies identifies consistently reported candidate biomarkers. *Cancer Epidemiology Biomarkers & Prevention* **17**(3): 543-552.
- CULHANE AC, SCHWARZL T, ET AL (2010). GeneSigDB-a curated database of gene expression signatures.” *Nucleic Acids Research* **38**, D716-D725.
- DENG X. T., XU J., ET AL. (2008). Improving the power for detecting overlapping genes from multiple DNA microarray-derived gene lists. *BMC Bioinformatics* **9**: 8.
- DHANASEKARAN S.M., DASH A., YU N., MAINE I.P., LAXMAN B., TOMLINS S.A., CREIGHTON C.N., MENON A., RUBIN M.A. AND CHINNAIYAN .A.M. (2004). Molecular profiling of human prostate tissues: insights into gene expression patterns of prostate development during puberty. *FASEB J* **18**, 243-245.
- EDGAR R, DOMRACHEV M, LASH AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Research* **30** , 207-210. <https://www.oncomine.org/resource/login.html>, accessed 12/14/2009.
- EFRON, B., Correlated z-Values and the Accuracy of Large-Scale Statistical Estimates. *Journal of the American Statistical Association*. **105**, 1042-1055.
- FELLER W. (1957). *An Introduction to Probability Theory and Its Applications Vol. 1*. Wiley Series in Probability and Mathematical Statistics.
- FUTREAL P.A., COIN L., MARSHALL M., DOWN T., HUBBARD T., WOOSTER R., RAHMAN N., STRATTON M.R. (2004) A census of human cancer genes. *Nat Rev Cancer*. **4**(3):177-83.
- GARRETT-MAYER E, PARMIGIANI G, ZHONG XG, COPE L, GABRIELSON E. Cross-study validation and combined analysis of gene expression microarray data. *Biostatistics* **9**:333-54.
- GLEZ-PENA D, GOMEZ-LOPEZ G, PISANO DG, FDEZ-RIVEROLA, F. WhichGenes: a web-based tool for gathering, building, storing and exporting gene sets. *Biostatistics* 2008;**9**:333-54.

- GUPTA, R.C. AND TAO H., (2010) A generalized correlated binomial distribution with application in multiple testing problems. *Metrika*,. **71**,. 59-77.
- HONG F.X., BREITLING R. (2008). A comparison of meta-analysis methods for detecting differentially expressed genes in microarray experiments. *Bioinformatics* **24** (3): 374-382.
- IRIZARRY R A, WARREN D ET AL. Multiple-laboratory comparison of microarray platforms *Nature Methods* **5**: 345-349.
- JEFFRIES C.D., WARD W.O., ET AL. (2009). Discovering collectively informative descriptors from high-throughput experiments. *BMC Bioinformatics* **10**: 9.
- LAPOINTE N., LI C., HIGGINS N.P., VAN DE RIJN M., BAIR E., MONTGOMERY K., FERRARI M., EGEVAD L., RAYFORD W., BERGERHEIM U., EKMAN P., DEMARZO A.M., TIBSHIRANI R., BOTSTEIN D., BROWN P.O., BROOKS N.D. AND POLLACK N.R. (2004). Gene expression profiling identifies clinically relevant subtypes of prostate cancer. *Proceedings of the National Academy of Sciences* **101**, 811-816.
- LU X., GAMST A., XU R. (2008). RDCurve: a nonparametric method to evaluate the stability of ranking procedures. *Trans. Comput. Biolo. & Bioinf.* **138**.
- MILLER B.G., STAMATOYANNOPOULOS J.A. (2010). Integrative Meta-Analysis of Differential Gene Expression in Acute Myeloid Leukemia. *Plos One* **5**(3): 13.
- PYNE S., FUTCHER B. AND SKIENA S. (2006). Meta-analysis based on control of false discovery rate: combining yeast ChIP-chip datasets. *Bioinformatics* **22**, 2516-2522.
- ROWLEY JD. (1973). Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* **243**, (5405): 2903.
- RHODES DR, KALYANA-SUNDARAM S, MAHAVISNO V, ET AL. (2007). Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* **9**, 166-180.
- SUN, W., AND CAI, T. (2009), Large-Scale Multiple Testing Under Dependence, *Journal of the Royal Statistical Society, Ser. B* **xx** , 393424.
- TIWARI G., SAKAUE H., POLLACK N.R. AND ROTH R.A. (2003). Gene expression profiling in prostate cancer cells with Akt activation reveals Fra-1 as an Akt-inducible gene. *Molecular Cancer Research* **1**, 475-484.
- TOMLINS S.A., RHODES, D.A., PERNER, S., DHANASEKARAN, S.M., MEHRA, R., SUN, X., VARAMBALLY, S., CAO, X., TCHINDA, N., KUEFER, R. and others (2005). Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Gens in Prostate Cancer. *Science* **310**, 644-648.
- TOMLINS S.A., MEHRA R., RHODES D.R., CAO X., WANG L., DHANASEKARAN S.M., KALYANA-SUNDARAM S., WEI N.T., RUBIN M.A., PIENTA K.N., SHAH R.B. AND CHIN-NAIYAN A.M. (2006). Integrative molecular concept modeling of prostate cancer progression. *Nature Genetics* **39**, 41-51.

YU, C. AND D. ZELTERMAN,(2002) Sums of dependent Bernoulli random variables and disease clustering. *Statistics and Probability Letters*,. **57**,363-373.

ZAYKIN D.V., ZHIVOTOVSKY L.A., WESTFALL P.H. AND WEIR B.S. (2002). Truncated product method for combining P-values. *Genetic Epidemiology* **22**, 170-185.