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Contributions Y.R. conceived the iterative use of randomly picked genes to derive a proper differential analysis free of any knowledge of so-called housekeeping genes. D.D. and Y.R. did the mathematical modeling and analysis of the procedure. D.D. and Y.R. implemented the procedure to confirm empirically this analysis and derive supplementary results about the power. They performed the differential analysis on the real data.

C.H. and B.H. conceived and designed the biological experiments. C.H. performed the biological experiments. C.H. and B.H. commented the results obtained on real data.

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Abstract

Transcriptomic analysis are characterized by being not directly quantitative and only providing relative measurements of expression levels up to an unknown individual scaling factor. This difficulty is enhanced for differential expression analysis. Several methods have been proposed to circumvent this lack of knowledge by estimating the unknown individual scaling factors however, even the most used one, are suffering from being built on hardly justifiable biological hypotheses or from having weak statistical background. Only two methods withstand this analysis : one based on largest connected graph component hardly usable for large amount of expressions like in NGS, the second based on log-linear fits which unfortunately require a first step which uses one of the methods described before.

We introduce a new procedure for differential analysis in the context of transcriptomic data. It is the result of pooling together several differential analyses each based on randomly picked genes used as reference genes. It provides a differential analysis free from the estimation of the individual scaling factors or any other knowledge. Theoretical

properties are investigated both in term of FWER and power. Moreover in the context of Poisson or negative binomial modelization of the transcriptomic expressions, we derived a test with non asymptotic control of its bounds. We complete our study by some empirical simulations and apply our procedure to a real data set of hepatic miRNA expressions from a mouse model of non-alcoholic steatohepatitis (NASH), the CDAHFD model. This study on real data provides new hits with good biological explanations.

1 Introduction

Transcriptomic analysis of a tissue sample (an individual) results in the measurements inside either a fix volume or a fix weight of expressions of some given set of expressions (that for simplicity we will consider gene expressions). As the amount of molecules inside the fix analyzed quantity is not controlled, it is known that all these measurement are scaled by a single unknown factor depending of the individual at hand. In other words, transcriptomic analysis are characterized by being not directly quantitative and by only providing relative measurements of expression levels up to an unknown individual scaling factor. When some of this genes are housekeeeping genes with known expression properties, they serve as reference genes and one can use their observed relative expression levels to get a normalization (Vandesompele et al. 2002). However, in exploratory differential analysis, such reference genes cannot always be known in advance and we therefore have to compare several sets of expression levels, each set depending of the unknown scaling factor attached to the corresponding individual.

Apart from the crude normalization by the total count (Marioni et al. 2008 ; Mortazavi et al. 2008), several methods have been proposed to circumvent this issue : upper quantile (Bullard et al. 2010), trimmed mean of M values (TMM) (Mark D. Robinson and Oshlack 2010) and interindividual median count ratio accross gene (Anders and Huber 2010), which can be found in the Bioconductor packages DESeq2 (Love, Huber, and Anders 2014) and EdgeR (Mark D Robinson, McCarthy, and Smyth 2010). All these methods are based on the belief that reference genes may be identified as their expressions are expected to be less variable in the overall population and hence less variable even in presence of fluctuations of the scaling factors. This belief is neither proven nor mathematically justifiable. Counter-examples can be built for example by considering reference genes showing more variability than non-reference ones.

More recently, Li et al. (2012) proposed to use $\log$-linear fits to detect DE genes, however it also relies on a scaling factor estimation achieved by starting from the total count to selected iteratively a subset of genes associated with small values of a Poisson goodness-of-fit statistic.

Up to our knowledge, only Curis et al. (2019), have proposed an approach free of this preliminary selection of reference genes and of the estimation of the scaling factors. Having in mind that the ratio of two expressions for one individual is free from the unknown scaling factor, only expression ratios are compared. To this end, they are considered to be

vertices of a graph. Two vertices are connected if the expression ratios may be considered as equal between the two populations. The largest connected component of the graph is expected to be made of non differential genes and DE genes are expected to live outside of this largest component. Without discussing the hypotheses used to build the largest connected component, it is easily understandable that the number of vertices of this graph being the square of the number of expressions, such an approach is reserved to transcriptomic studies like qPCR where the number of expressions is small, unlike high-throuput experiments which are our interest.

In brief, actual procedures for differential analysis in such high-throuput transcriptomic experiments are build on a preliminary step, which consists in finding some non differential expressions to estimate the scaling factors. Then data are reused for testing. It is not only unsatisfactory to lack a good recipe for this first step, but also unproper and statistically worst, to do a differential analysis by having to run at first a non-differential analysis on the same data.

In view of this drawback that affects the methods in use, our proposal does not aim at finding first some non differential expressions to rescale the data. Instead, we propose an iterative framework in which a rescaling is realized at each step of the iteration from a randomly selected subset of expressions which are no longer assumed to be non differentially expressed, the differential analysis being performed on the remaining data. In this sense, our proposal relies only on the use of a differential test, which is only expected to have good type I and type II errors when the data are well scaled, without an estimation of the "real" scaling factors, which may be estimated afterwards.

In this transcriptomic context, for a given individual i and a gene j, the measure is modelized by a Poisson random variable X_{ij} with intensities λ_{ij} being the product of an individual scaling factor s_i, which only depends on the individual i, times a gene dependent expression level μ_j^A or μ_j^B with the upper script refering to the conditions A or B. To account for observed over-dispersion with respect to the Poisson model, gamma convoluted modelization of this model is also used leading to the so-called negative binomial model. This latter modelization is all the more suitable when transcriptomic expressions are measured at large genomic scale like gene expression levels pooling together reads of non-homogeneous origins over few hundreds of base-pairs, unlike miRNA or siRNA experiments which cover around 100 base-pairs and are more likely to be mimicked by simple Poisson random variables. In both modelizations, the expectation satisfies

$$\mathbb{E}(X_{ij}) = \lambda_{ij} = s_i \times \mu_j$$

with μ_j representing μ_j^A or μ_j^B.

In what follows, the expressions will be considered to be gene-expressions, however they could come from any transcriptomic experiment (RNA-seq, miRNA-seq, etc). The individuals belong to two populations or have been studied under two differents conditions A and B, providing n_A and n_B individuals under each condition. We aim to find those genes which are differentially expressed (DE) genes between the two populations. We point out that transcriptomic

studies using miRNA or siRNA are often explanatory studies made on two sub-populations of mice for example, such that they are characterized by small sample sizes, n_A and n_B being usually between 5 and 10 individuals for each subpopulation.

Our intensive iterative random procedure for detection can be summarized in more details as follows. At each step of the iteration, a random subset of genes is selected and considered to be made of reference genes, used to get a normalization. After this normalization, the non-selected genes are tested for differential behaviors. Along the iterations, the detections for each gene are pooled. After the iterations, the pooled detections are compared to the rates of potential wrong detections due to miss-picking randomly genes in the unknown set of DE genes. Our method controls the FWER for any test procedure having its level and power controled when the scaling factors are known. It is adaptive to the unknown number of genes which would be detectable, given the observations, if the scaling factors were known, assuming only that the number of DE genes is less than half of the total number of genes.

Moreover, taking advantage that our procedure behaves as if reference genes were available, we propose and study a unified testing procedure for differential analysis, adapted to our random detector for the two classical modelizations (Poisson and Negative binomial). This test derives from a procedure where scaling factors would be known and in this sense satisfies the requirements in term of type I and II errors of our random procedure. Assuming that the expressions levels are high enough, we study their properties. It is shown to be approximately a standard Gaussian and we derive non-asymptotic control for this approximation so that the test can have its level well controled at finite distance.

To complete our study, we run an intensive simulation. Finally we apply our procedure on a real data set of hepatic miRNA expressions from a mouse model of non-alcoholic steatohepatitis (NASH), the CDAHFD (choline-deficient, L-amino acid-defined, high-fat diet) model, with 4 cases of NASH with hepatic fibrosis and 4 controls without hepatic lesions.

2 Formulation of the problem

We observe counts X_{ij} for $i = 1, ..., n$ and $j = 1, ..., m$ the m expressions of n individuals belonging to two populations caracterized by index subsets A and B of sizes n_A and n_B, with $n = n_A + n_B$. The first n_A individuals belonging to groupe A.

In an homogeneous population, the expectation $\lambda_{ij} := \mathbb{E}(X_{ij})$ is assumed to be the product of a scaling factor s_i attached to the individual i together with μ_j an unknown relative quantification of gene j potentially varying with the population, $\lambda_{ij} = s_i\mu_j$. By convention, we set $\sum_{i=1}^{n} s_i = n$. With this convention, when all experiments perform the same and A and B are two independent sub-populations of the same population then $s_i = 1$ for $i = 1, \ldots, n$. In the latter case, μ_j stands for the relative expression level of gene j in this population.

When two sub-populations A and B are considered, μ_j is assumed to depend only on the experimental condition under which i is considered. In other words, $\mu_j = \mu_j^A$ if $i \in A$ and $\mu_j = \mu_j^B$ if $i \in B$.

For a given j in $\{1, ..., m\}$, we want to test whether the j-th expression is differentially expressed under experimental conditions A and B. This can be stated as a test of the following hypotheses

$$H_0^j : \text{gene } j \text{ is not DE} \quad \text{against} \quad H_1^j : \text{gene } j \text{ is DE.} \tag{1}$$

In other words, distinguishing the relative quantification of gene j in populations A and B :

$$\mathbb{E}(X_{ij}) = s_i \, \mu_j^A, \ \text{for } i \in A \qquad \text{and} \qquad \mathbb{E}(X_{ij}) = s_i \, \mu_j^B \ \text{for } i \in B, \tag{2}$$

the hypotheses defined by (1), may be formulated as

$$H_0^j : \mu_j^A = \mu_j^B \quad \text{against} \quad H_1^j : \mu_j^A \neq \mu_j^B. \tag{3}$$

A gene j satisfying H_0^j is called “invariant” (between conditions A and B) or a “reference gene”.

Definition 1 (DE η-detectable) *Let h be an increasing function and the s_i, $i = 1, ..., n$ be known. Suppose that we have at hand a symmetric test statistic T^* with distribution only depending on the parameter $h(\mu_j^A) - h(\mu_j^B)$ and which is stochastically increasing with respect to this parameter. Let us denote by $q^*(1 - \eta/2)$ an upper quantile of $T^*(X_{1j}, ..., X_{nj})$ when $\mu_j^A = \mu_j^B$. We say that gene j is "DE η-detectable" at level $\eta > 0$ if and only if the null hypothesis H_0^j is rejected at this level that is if and only if*

$$|T^*(X_{1j}, ..., X_{nj})| \geq q^*(1 - \frac{\eta}{2}). \tag{4}$$

The index set of the DE η-detectable genes is denoted $\mathcal{D}^*$.

Clearly this definition depends, for all j, on the observed sample $(X_{1j}, ..., X_{nj})$, hence all what follows including our test construction and its analysis is conditional to this observation.

The set $\mathcal{D}^*$ may be empty but it is unlikely to be large, unless the design of the experiment is irrelevant. By construction its cardinal satisfies $|\mathcal{D}^*| = d^*$ and we assume further that d^* is samller than $m/2$.

Note that the quantile $q^*(1 - \eta/2)$ does not depend on parameters μ_j^A and μ_j^B as T^* is assumed pivotal. The symmetry of T^* is only here for simplicity. Clearly being DE η-detectable depends on the choice of T^*. A bad choice could even

conduct to declare all genes to be either not DE or DE.

In the special case where the s_i, $i = 1, ..., n$ are known, we denote by p_j^* the p-value associated to the test of the gene j and the sorted p-values by $p_{(j)}^*$ such that $p_{(j)}^* \leq p_{(j+1)}^*$. In order to control the family-wise error rate (FWER) and account for multiple testing, several procedures can be applied, we focus on the Holm's step-down procedure (Holm 1979).

Procedure 1 (Holm's procedure) *The s_i, $i = 1, ..., n$ being known, let d^* be the minimal index $j \geq 0$ such that $p_{(j+1)}^* \geq \alpha/(m-j)$, the genes corresponding to $p_{(1)}^*$, ..., $p_{(d^*)}^*$ are declared differentially expressed with the convention that if $d^* = 0$ then all gene are declared invariant.*

This procedure is known to ensure that the FWER is less than α

Guide of lecture The next section introduces several notations. Section 4 is devoted to the construction of a practical statistic based on randomly picked "reference" genes when the s_i are unknown which is derived from T^*. Section 4.1 contains Theorem 1 which proves that this construction controls the FWER as soon as T^* satisfies simple conditions on the type I and II errors. Section 4.2 presents Theorem 2 and its corollary which give necessary conditions on the number of iterations to get FWER and exponential control of the power. Section 5 focuses on the construction of a satisfying test T^* when the distribution of gene expressions is assumed to follow Poisson or Negative Binomial distribution. The adjusted rejection region corresponding to 4 for a practical implementation is a consequence of Theorem 3 and its corollary. In Section 7, we study empirically the behavior of our procedure including its level and its power. Section 8 is devoted to the study of a real data set of hepatic miRNA expressions from a mouse model. The proofs of our two theorems are reported in Appendix.

3 Notations

We will further use the convention that $\bullet$ in exponent indicates the set $-A$ or $B-$ the index i is belonging to. For example $\mu_j^\bullet$ denotes μ_j^A if $i \in A$ and μ_j^B if $i \in B$.

Given two integers k and r, we consider the sampling of r subsets of indexes in $\{1, \ldots, m\}$ of size k, denoted $S_1, \ldots, S_r$. We denote by $\mathcal{S} := \{S_1, \ldots, S_r\}$. Each subset from $\mathcal{S}$ will be used to provide an estimation of the unknown s_i and later refered as a normalisation subset. Given $S \in \mathcal{S}$, the genes with index in S are used for the estimation of the scaling factors s_i. The remaining expressions with index in $\bar{S} := \{1, \ldots, m\} \setminus S$ are tested for differential behavior between the two populations.

For a given j in $\{1, \ldots, m\}$, we denote by $\mathcal{S}_j := \{S \in \mathcal{S} \,|\, j \notin S\}$ the set of subsets in $\mathcal{S}$ which do not contain j. The total

number of tests run for gene j is $r_j := |\mathcal{S}_j|$. Clearly $r_j \sim \mathcal{B}(r, \kappa)$ with

$$\kappa := P(S \in \mathcal{S}_j) = 1 - \binom{m-1}{k} \Big/ \binom{m}{k} = \frac{m-k}{m} = 1 - k/m. \tag{5}$$

If a subset S_j contains at least one gene j in $\mathcal{D}^*$ then the normalization may be wrong. We call such subset a badly selected subset or a wrong normalization subset. Note that a such subset exists if $\mathcal{D}^*$ is not empty, that is if $d^* > 0$.

The number of wrong normalization subsets not containing j is

$$B_j := \sum_{S \in \mathcal{S}_j} (S \cap \mathcal{D}^* \neq \varnothing) = |\{S \in \mathcal{S}_j, S \cap \mathcal{D}^* \neq \varnothing\}|. \tag{6}$$

Taking into account that j is DE η-detectable ($j \in \mathcal{D}^*$) or not and assuming that $d^* = d$, the random variable B_j given the observation of r_j follows a binomial distribution

$$B_j | r_j \sim \begin{cases} \mathcal{B}(r_j, \pi_d^0) & \text{if} \quad j \notin \mathcal{D}^* \\ \mathcal{B}(r_j, \pi_d^1) & \text{if} \quad j \in \mathcal{D}^* \end{cases},$$

where

$$\pi_d^0 := \begin{cases} 1 - \binom{m-d-1}{k} \Big/ \binom{m-1}{k} & \text{if} \quad 0 \leq d \leq m-k-1 \\ 1 & \text{if} \quad d \geq m-k \end{cases} \quad \text{and} \quad \pi_d^1 := \begin{cases} 1 - \binom{m-d}{k} \Big/ \binom{m-1}{k} & \text{if} \quad 0 < d \leq m-k \\ 1 & \text{if} \quad d \geq m-k+1 \end{cases} \tag{7}$$

with the convention that $\pi_0^1 = 0$. The number π_d^0 (respectively π_d^1) represents the probability that a wrong normalization subset does not contain j when the latter is invariant, (respectively DE η-detectable).

Property 1 *The numbers π_d^0 and π_d^1 satisfy $\pi_d^0 > \pi_d^1$ and the sequence π_d^0 / π_d^1 is decreasing and converges to 1 when d grows.*

4 Testing using randomly picking reference genes

When a subset S of independent reference genes is known, estimates of the unkown s_i, $i = 1, ..., n$ are

$$\hat{s}_i^S := \frac{n \sum_{j \in S} X_{ij}}{\sum_{i=1}^n \sum_{j \in S} X_{ij}}. \tag{8}$$

These estimates appear as a ratio of moment estimates. Under the Poisson assumption, it is also the maximum likehood estimators since $\log \lambda_{ij} = \log s_i + \log \mu_j$ can be written as a linear predictor of indicator variables for the individual i

and the gene j :

$$\log \lambda_{ij} = \phi_i \times I_i + \theta_j \times I_j.$$

In practice such reference genes are unknown and it is conceptually difficult to believe that they could be known when one new hypothesis is tested. This problem is all the more difficult as the s_i are unknown. Our methodology bypasses this lack of knowledge through the sampling of the subsets $S_1,\ldots,S_r$ which are used each in turn as normalization subset as if they were made of reference genes (see Section 3).

For a given normalization subset S in $\mathcal{S} = \{S_1, ..., S_r\}$, our test statistic, denoted $T_S(X_{1j}, ..., X_{nj})$, is asymptotically equivalent to $T^*(X_{1j}, ..., X_{nj})$ with the s_i replaced by $\hat{s}_i^S$. The rejection region is adapted accordingly through a non asymptotic control of the level by replacing $q^*(1-\eta/2)$ with

$$q(1-\eta/2) := (1+\sqrt{c\log n})\, q^*(1-\eta/2) \tag{9}$$

where c is a positive constant. As a consequence, for any gene with index j not in S, we can determine if the null H_0^j is rejected or not with a prescribed significance level.

4.1 Our procedure

For any gene $j = 1, ..., m$ and any normalization subset $S \in \mathcal{S}_j$, let us define the detection indicator (the indicator of the rejection of the null hypothesis) for gene j when the subset S is used for normalization by

$$\mathbb{1}_S(j) := \left(|T_S(X_{1j}, ..., X_{nj})| > q(1-\eta/2)\right). \tag{10}$$

We denote by $P_j(\cdot) = P(\cdot|S \in \mathcal{S}_j)$ the conditionnal probability with respect to the event $S \in \mathcal{S}_j$.

Using total probability formula, we decompose the detection rate

$$P_j(\mathbb{1}_S(j)=1) = P_j(\mathbb{1}_S(j)=1|S\cap\mathcal{D}^*=\varnothing)\, P_j(S\cap\mathcal{D}^*=\varnothing) + P_j(\mathbb{1}_S(j)=1|S\cap\mathcal{D}^*\neq\varnothing)\, P_j(S\cap\mathcal{D}^*\neq\varnothing). \tag{11}$$

Assuming that the definition of $q(1-\eta/2)$ given by (9) ensures that the detection rates for a good normalization subset

	$H_0^j : j$ invariant $\mu_j^A = \mu_j^B$	$H_1^j : j$ is DE η-detectable $j \in \mathcal{D}^*$
Good normalization subset $S \cap \mathcal{D} = \varnothing$	$\leq \eta$	$\geq 1 - \beta$

TABLE 1 – Assumptions on the detection rates for a gene j regarding its status when a good normalization subset is used.

satisfy Table 1, it follows that

$$P_j(\mathbb{1}_S(j) = 1) \leq \eta(1 - \pi_{d^*}^0) + \pi_{d^*}^0 \quad \text{when } j \notin \mathcal{D}^* \tag{12}$$

$$P_j(\mathbb{1}_S(j) = 1) \geq (1 - \beta)(1 - \pi_{d^*}^1) \quad \text{when } j \in \mathcal{D}^*. \tag{13}$$

where π_d^0 and π_d^1 are defined in (7).

Thanks to the use of a pivotal statistic, $P_j(\mathbb{1}_S(j) = 1 | S \cap \mathcal{D}^* = \varnothing)$ does not depend on j hence η can be chosen small even when the number of genes is large.

We consider R_j the number of detections for the gene j through its $r_j := |\mathcal{S}_j|$ associated normalizations. Let us define

$$p_j^d(\eta) := 1 - B\left(R_j; r, \kappa(\eta(1 - \pi_d^0) + \pi_d^0)\right) \tag{14}$$

where $\kappa = 1 - k/m$ (see (5)) and $B(., n, x)$ is the c.d.f. of the binomial with parameter n and x. As a consequence $p_j^d(\eta)$ appears as the p-value associated with R_j when $\mathcal{D}^*$ is of cardinality d and R_j is considered to come from a binomial with parameters r and $\kappa(\eta(1 - \pi_d^0) + \pi_d^0)$.

Noticing that r and $\kappa(\eta(1 - \pi_d^0) + \pi_d^0)$ does not depend on j the order of the p-values does not depend on η or d. As a consequence, we can order the genes accordingly to the $p_j^d(\eta)$ independently from d and η to satisfy

$$p_{(1)}^d(\eta) \leq p_{(2)}^d(\eta) \leq ... \leq p_{(m)}^d(\eta), \text{ for all } d \text{ and } \eta. \tag{15}$$

We now derive our detection procedure :

— Gene (1) is declared to be DE if and ony if $p_{(1)}^0(\eta) < \alpha/m$;

— Gene (2) is declared to be DE if and only if $p_{(2)}^1(\eta) < \alpha/(m-1)$;

— ...

— Gene (d) is declared to be DE if and only if $p_{(d)}^{d-1}(\eta) < \alpha/(m-d+1)$;

— Gene $(d+1)$ is declared to be DE if and only if $p_{(d)}^d(\eta) < \alpha/(m-d)$;

— ...

Finally, given $\eta > 0$, we define $\hat{d}$, the number of genes declared DE by our procedure, as follows :

$$\hat{d} := \begin{cases} 0 & \text{if} \quad p^0_{(1)}(\eta) \geq \alpha/m, \\ \min\left\{ d > 0,\, p^{d-1}_{(d)}(\eta) < \alpha/(m-d+1) \quad \text{and} \quad p^d_{(d+1)}(\eta) \geq \alpha/(m-d) \right\} & \text{otherwise.} \end{cases} \tag{16}$$

If $\hat{d} > 0$, the genes associated with the $\hat{d}$ smallest p-values are declared DE, otherwise all genes are declared invariant. We recall that the order of the p-values does not depend on d and η, hence the meaning of "smallest p-values" is well defined.

Clearly, our procedure will be all the more powerful that the detection rate difference $(1-\beta)(1-\pi^1_d) - (\eta(1-\pi^0_d) + \pi^0_d)$ is large when $d \leq d^*$. Our main result controls the FWER of non DE gene detection for this randomize procedure.

Theorem 1 *Assuming that the genes are independent and that the detection rates for the test defined by (10) satisfy the assumptions provided by Table 1 for any good normalization subset S, then the FWER is bounded by $\alpha + o_r(1)$ as soon as*

$$(1-\beta)(1-\pi^1_{d^*}) > \eta(1-\pi^0_{d^*}) + \pi^0_{d^*}. \tag{17}$$

The proof of Theorem 1 is reported in Appendix 9.1

4.2 Rates of Detection and Power

We now focus on the link between η and α. We first focus on the case $d^* = 0$ to derive condition on r based on a level control. Then, we study the power when $d^* > 0$.

If $d^* = 0$, the $p^0_j(\eta)$ derived from the R_j are associated with binomial $\mathcal{B}(r, \theta_0)$ with expectation $r\theta_0$ where $\theta_0 := \kappa\eta$. In this case, each normalisation subsets S is a good normalisation subset and the rate of detection satisfies $\theta_S(j) := P_j(\mathbb{1}_S(j) = 1) \leq \theta_0$ such that $P(R_j > x) \leq P\left(\mathring{R}_j > x\right)$ where $\mathring{R}_j$ is a $\mathcal{B}(r, \theta_0)$. This result is a consequence of the following lemma which provides a more general result, true for any d^*.

Lemma 1 *Denoting*

$$\theta_0 := \kappa(\eta(1-\pi^0_d) + \pi^0_d) \quad \textit{and} \quad \theta_1 := \kappa(1-\beta)(1-\pi^1_d),$$

under assumption provided by Table 1, if $j_0 \notin \mathcal{D}^*$ then $R_{j_0} \leq \mathring{R}_{j_0}$ where $\mathring{R}_{j_0} \sim \mathcal{B}(r, \theta_0)$. Similarly, if $j_1 \in \mathcal{D}^*$ then $R_{j_1} \geq \hat{R}_{j_1}$ where $\hat{R}_{j_1} \sim \mathcal{B}(r, \theta_1)$. In term of c.d.f it follows that

$$P(R_{j_0} \leq x) \geq P(\mathring{R}_{j_0} \leq x) \quad \textit{and} \quad P(R_{j_1} \leq x) \leq P(\hat{R}_{j_1} \leq x).$$

Proof of Lemma 1 For any S in $\mathcal{S}_{j_0}$, as $\mathbb{1}_S(j_0)$ is a Bernoulli with parameter

$$\theta_S(j_0) := P(\mathbb{1}_S(j_0) = 1) \leq \kappa(\eta(1-\pi_d^0) + \pi_d^0) = \theta_0, \tag{18}$$

it follows that $\mathbb{1}_S(j_0) = \mathbb{1}_{U_S \leq \theta_S(j_0)} \leq \mathbb{1}_{U_S \leq \theta_0}$ where U_S are independent uniform random variables. Consequently $R_{j_0} \leq \mathring{R}_{j_0}$ where $\mathring{R}_{j_0}$ is a Binomial $\mathcal{B}(r, \theta_0)$. Similarly for $j_1 \in \mathcal{D}^*$, for any S in $\mathcal{S}_{j_1}$, $\mathbb{1}_S(j_1) = \mathbb{1}_{U_S \leq \theta_S(j_1)} \geq \mathbb{1}_{U_S \leq \theta_1}$ with

$$\theta_S(j_1) := P(\mathbb{1}_S(j_1) = 1) \geq \kappa(1-\beta)(1-\pi_d^1) = \theta_1 \tag{19}$$

and $R_{j_1} \geq \hat{R}_{j_1}$ where $\hat{R}_{j_1}$ is a Binomial $\mathcal{B}(r, \theta_1)$. □

As $d^* = 0$, the FWER is the probability under the global null, $\bigcap_j H_0^j$, to have one R_j too large and can be upper bounded as follow

$$P\left(\exists j \in \{1, ..., m\}, R_j > r\theta_0 + r\varepsilon\right) \leq P\left(\exists j \in \{1, ..., m\}, \mathring{R}_j > r\theta_0 + r\varepsilon\right) \leq mP\left(\mathring{R}_1 > r\theta_0 + r\varepsilon\right). \tag{20}$$

Using (Massart 1990, Theorem 2),

$$P(\mathring{R}_1 - r\theta_0 > r\varepsilon) \leq \exp\left(-\frac{r\varepsilon^2}{2(\theta_0 + \varepsilon/3)(1 - \theta_0 - \varepsilon/3)}\right) \leq \exp\left(-\frac{r\varepsilon^2}{2(\theta_0 + \varepsilon/3)}\right). \tag{21}$$

This inequality is non trivial for deviations $r\varepsilon$ of order the standard deviation of $\mathring{R}_1$ which is of not smaller than $\sqrt{r\theta_0}$. To go further, we consider that the deviations statisfies $r\varepsilon = \sqrt{\theta_0}\, r^{0.5+\xi}$ with $0 < \xi < 0.5$ such that

$$\varepsilon = \sqrt{\theta_0}\, r^{\xi - 0.5}. \tag{22}$$

The latter is smaller than θ_0 as soon as $r \geq \theta_0^{1/(2\xi-1)}$. In this case (21) becomes

$$P(\mathring{R}_1 - r\theta_0 > r\varepsilon) \leq \exp\left(-\frac{3r\varepsilon^2}{8\theta_0}\right) \leq \exp\left(-\frac{3}{8}r^{2\xi}\right). \tag{23}$$

Using (20)

$$P\left(\exists j \in \{1, ..., m\}, R_j > r\theta_0 + r\varepsilon\right) \leq m \exp\left(-\frac{3}{8}r^{2\xi}\right). \tag{24}$$

Equating the right-hand side term with α, it follows that the FWER is smaller than α as soon as

$$r \geq \theta_0^{1/(2\xi-1)} \vee \left[-\frac{8}{3}\log(\frac{\alpha}{m})\right]^{1/2\xi}. \tag{25}$$

We now suppose $d^* > 0$ and assume the rate of rejections to be not smaller than θ_1 with $\theta_1 \geq \theta_0$ for the genes with index in $\mathcal{D}^*$. Given $j \in \mathcal{D}^*$, the error of second kind can be controlled as follow :

$$P\left(R_j < r\theta_0 + r\varepsilon\right) \leq P\left(\hat{R}_j < r\theta_0 + r\varepsilon\right) = P\left(\hat{R}_j - r\theta_1 < -r(\theta_1 - \theta_0 - \varepsilon)\right)$$

where $\hat{R}_j \sim \mathcal{B}(r, \theta_1)$, see Lemma 1. Again using Massart's inequality, it follows that

$$P\left(\hat{R}_j < r\theta_0 + r\varepsilon\right) \leq \exp\left(-\frac{r(\theta_1 - \theta_0 - \varepsilon)^2}{2(1 - \theta_1 + (\theta_1 - \theta_0 - \varepsilon)/3)(\theta_1 - (\theta_1 - \theta_0 - \varepsilon)/3)}\right) \tag{26}$$

$$\leq \exp\left(-\frac{r(\theta_1 - \theta_0 - \varepsilon)^2}{2(\theta_1 - (\theta_1 - \theta_0 - \varepsilon)/3)}\right) \tag{27}$$

$$\leq \exp\left(-\frac{r(\theta_1 - \theta_0 - \varepsilon)^2}{2\theta_1}\right). \tag{28}$$

The last inequality comes from assuming $\varepsilon \leq \theta_1 - \theta_0$. Assuming the stronger constraint $\varepsilon \leq (\theta_1 - \theta_0)/2$, it follows than

$$P\left(\hat{R}_j < r\theta_0 + r\varepsilon\right) \leq \exp\left(-\frac{r(\theta_1 - \theta_0)^2}{8\theta_1}\right) \leq \exp\left(-\frac{r(\theta_1 - \theta_0)^2}{8}\right). \tag{29}$$

Taking care of the multiplicity, it follows that

$$P\left(\exists j \in \mathcal{D}^*, \hat{R}_j < r\theta_0 + r\varepsilon\right) \leq d^* \exp\left(-\frac{r(\theta_1 - \theta_0)^2}{8}\right) \leq m \exp\left(-\frac{r(\theta_1 - \theta_0)^2}{8}\right). \tag{30}$$

Using (22), the constraint $\varepsilon \leq (\theta_1 - \theta_0)/2$ becomes

$$r \geq \left[\frac{2\sqrt{\theta_0}}{\theta_1 - \theta_0}\right]^{1/(0.5-\xi)}.$$

Finally (using $\xi = 0.25$) we obtain the following theorem and its corollary which control level and power of our randomized strategy :

Theorem 2 *Under the assumptions of Th. 1, our procedure is of FWER α and has its power growing exponentially fast with r –all the faster as $\theta_1 - \theta_0$ the larger– as soon as*

$$r \geq \frac{1}{\sqrt{\theta_0}} \vee \left[-\frac{8}{3}\log(\frac{\alpha}{m})\right]^2 \vee \left[\frac{2\sqrt{\theta_0}}{\theta_1 - \theta_0}\right]^4$$

with

$$\theta_1 - \theta_0 = (1 - \frac{k}{m})\left[(1-\beta)(1-\pi^1_{d^*}) - (\eta(1-\pi^0_{d^*}) + \pi^0_{d^*})\right].$$

In other words,

Corrolary 1 *Under the assumptions of Th. 1, when* $r \geq 1/\sqrt{\theta_0}$ *and*

$$\frac{(1-\beta)(1-\pi^1_{d^*}) - (\eta(1-\pi^0_{d^*}) + \pi^0_{d^*})}{\sqrt{\eta(1-\pi^0_{d^*}) + \pi^0_{d^*}}} \geq 2\frac{m}{m-k}\left(-\frac{8}{3}\log\frac{\alpha}{m}\right)^{0.5} \tag{31}$$

our procedure is of FWER α *with power growing exponentially fast as soon as* $r \geq \left[-\frac{8}{3}\log(\alpha/m)\right]^{-0.5}$.

The left-hand side in (31) appears as lower bound of the normalized difference of detection rates between DE and not-DE genes.

5 Testing procedure for Poisson and Negative binomial models

Usually, the gene expressions are modelled by negative binomials $X_{ij} \sim \mathcal{NB}(\gamma_j^\bullet, \gamma_j^\bullet/(\gamma_j^\bullet + \lambda_{ij}))$ such that $\mathbb{E}(X_{ij}) = \lambda_{ij} = s_i\mu_j^\bullet$ and $\text{var}(X_{ij}) = \lambda_{ij}(1 + \lambda_{ij}/\gamma_j^\bullet)$. In this parametrization, the gene dependent parameter $\gamma_j^\bullet$ governs the overdispersion of the count data X_{ij} with respect to the Poisson case for which $\gamma_j^\bullet = +\infty$. We will denote further $\rho_j^\bullet := \mu_j^\bullet/\gamma_j^\bullet$ which is zero for the Poisson case.

Under the assumption that $\lambda_{ij} = s_i\,\mu_j^\bullet$ is large enough, the Gaussian approximation of the negative binomial counts X_{ij} provides

$$X_{ij} \sim \mathcal{NB}(\gamma_j^\bullet, \gamma_j^\bullet/(\gamma_j^\bullet + \lambda_{ij})) \approx \mathcal{N}(s_i\,\mu_j^\bullet, s_i\,\mu_j^\bullet(1 + s_i\,\rho_j^\bullet)) \tag{32}$$

such that

$$2\sqrt{X_{ij}} \approx \mathcal{N}(2\sqrt{s_i\,\mu_j^\bullet}, 1 + s_i\,\rho_j^\bullet).$$

This Gaussian approximation is just an extension of the Poisson ($\rho_j^\bullet$) approximation obtained by variance stabilization.

Given a normalization subset S, let us consider an integer $j \notin S$ and let us specify the expression to be compared under conditions A and B.

Assuming that the Gaussian approximation for negative binomial holds, from (32), we obtain

$$\begin{aligned} U_{ij} :&= 2\sqrt{\frac{X_{ij}}{s_i}} \approx \mathcal{N}(2\sqrt{\mu_j^\bullet}, \rho_j^\bullet + 1/s_i) \\ &= 2\sqrt{\mu_j^\bullet} + \sqrt{\rho_j^\bullet + 1/s_i}\varepsilon_{ij} = 2\sqrt{\mu_j^\bullet} + V_{ij} \end{aligned} \tag{33}$$

with $\varepsilon_{ij} \sim \mathcal{N}(0,1)$ and $V_{ij} := \sqrt{\rho_j^\bullet + 1/s_i}\varepsilon_{ij}$. By construction the ε_{ij} for $i = 1,\dots,n$ are independent. We denote further $\omega_i^\bullet := (\rho_j^\bullet + 1/s_i)^{1/2}$.

Defining Σ_S^2 as

$$\Sigma_S^2 := \sum_{i=1}^{n}(\omega_i^\bullet)^2 = \sum_{i\epsilon A}(\omega_i^A)^2 + \sum_{i\epsilon B}(\omega_i^B)^2 = \frac{\rho_A}{n_A} + \frac{\rho_B}{n_B} + \frac{1}{n_A^2}\sum_{i\epsilon A}\frac{1}{s_i} + \frac{1}{n_B^2}\sum_{i\epsilon B}\frac{1}{s_i}, \tag{34}$$

we consider the hypotheses test statistic

$$\frac{\bar{U}_l^A - \bar{U}_l^B}{\Sigma_s} = 2\frac{\sqrt{\mu_j^A} - \sqrt{\mu_j^B}}{\Sigma_s} + \frac{\bar{V}_l^A - \bar{V}_l^B}{\Sigma_s}$$

which, as (33) holds, under the null hypothesis is reduced to $(\bar{V}_l^A - \bar{V}_l^B)/\Sigma_s$ and follows approximately $\mathcal{N}(0,1)$.

Since ρ_j^A, ρ_j^B and the s_i are unknown, the latter cannot be used directly as a test statistic. Therefore we consider further

$$Y_{ij} := 2\sqrt{X_{ij}/\hat{s}_i} = \sqrt{s_i/\hat{s}_i}U_{il} \tag{35}$$

where $\hat{s}_i$ is an estimator of s_i, see (8). Together with an estimator of Σ_S^2 :

$$\hat{\Sigma}_S^2 = \frac{1}{n_A(n_A-1)}\sum_{i\epsilon A}(Y_{ij} - \bar{Y}_j^A)^2 + \frac{1}{n_B(n_B-1)}\sum_{i\epsilon B}(Y_{ij} - \bar{Y}_j^B)^2 \tag{36}$$

we build our testing procedure on the following statistic

$$T := \frac{\bar{Y}_j^A - \bar{Y}_j^B}{\hat{\Sigma}_S}.$$

The justification for this construction is provided by a decomposition of T that we obtain by using algebraic computations. First, for a real vector $x_j := (x_{1j},\dots,x_{nj})^T$ from $\mathbb{R}^n$, we denote the empirical means of x_j in A and B by

$$\bar{x}_l^A := \frac{1}{n_A}\sum_{i\epsilon A}x_{ij} \quad \text{and} \quad \bar{x}_l^B := \frac{1}{n_B}\sum_{i\epsilon B}x_{ij}.$$

Then we establish the relation between the difference of means and the vector x_j

$$(\bar{x}_j^A - \bar{x}_j^B)\mathbb{1}_n = Hx_j$$

where

$$\mathbb{1}_n \coloneqq \underbrace{(1,\dots,1)}_{n \text{ times}}^T \quad \text{and} \quad H \coloneqq \begin{pmatrix} \frac{1}{n_A} J_{n_A} & -\frac{1}{n_B} J_{n_A,n_B} \\ \frac{1}{n_A} J_{n_B,n_A} & -\frac{1}{n_B} J_{n_B} \end{pmatrix}.$$

and J being the unit matrix consisting of integers equal to 1.

With respect to these notations the following decomposition of T holds

$$\begin{aligned}
T\mathbb{1}_n &= \frac{1}{\hat{\Sigma}_S} H Y_j = \frac{\Sigma_S}{\hat{\Sigma}_S}\left(\frac{1}{\Sigma_S} H(Y_j - U_j) + \frac{1}{\Sigma_S} H U_j\right) \\
&= \frac{\Sigma_S}{\hat{\Sigma}_S}\left(\frac{1}{\Sigma_S} H(\mathrm{diag}(\sqrt{s_i/\hat{s}_i})U_j - U_j) + \frac{1}{\Sigma_S} H(2\sqrt{\mu_j^\bullet} + V_j)\right) \\
&= \frac{\Sigma_S}{\hat{\Sigma}_S}\left(\frac{1}{\Sigma_S} H\mathrm{diag}(\sqrt{s_i/\hat{s}_i} - 1)U_j + \frac{2}{\Sigma_S}(\sqrt{\mu_j^A} - \sqrt{\mu_j^B}) + \frac{\bar{V}_j^A - \bar{V}_j^B}{\Sigma_S})\right) \\
&= \frac{\Sigma_S}{\hat{\Sigma}_S}\Bigg(\frac{1}{\Sigma_S} \underbrace{H\mathrm{diag}(\sqrt{s_i/\hat{s}_i} - 1)\mathrm{diag}(\sqrt{\rho_j^\bullet + 1/s_i})}_{R_1}\varepsilon_j \\
&\quad + \frac{2}{\Sigma_S} \underbrace{H\mathrm{diag}(\sqrt{s_i/\hat{s}_i} - 1)(\sqrt{\mu_j^A}\mathbb{1}_{n_A}^T, \sqrt{\mu_j^B}\mathbb{1}_{n_B}^T)^T}_{R_2} \\
&\quad + \frac{2}{\Sigma_S}(\sqrt{\mu_j^A} - \sqrt{\mu_j^B}) + \frac{\bar{V}_j^A - \bar{V}_j^B}{\Sigma_S})\Bigg) \qquad (37) \\
&= \frac{\Sigma_S}{\hat{\Sigma}_S}\left(\frac{R_1\varepsilon_j}{\Sigma_S} + 2\frac{R_2}{\Sigma_S} + \frac{2}{\Sigma_S}(\sqrt{\mu_j^A} - \sqrt{\mu_j^B}) + \frac{\bar{V}_j^A - \bar{V}_j^B}{\Sigma_S}\right). \qquad (38)
\end{aligned}$$

We recall that by construction $(\bar{V}_j^A - \bar{V}_j^B)/\Sigma_S \sim \mathcal{N}(0,1)$. Moreover the following theorem holds which controls the distribution of our test statistic with respect to the Gaussian under the null.

Theorem 3 *Assuming that* $\max_i \left|\sqrt{s_i/\hat{s}_i} - 1\right| \le 1/2$*, the following relations hold with probability larger than* $1 - 5n^{-c}$

$$\frac{\Sigma_S}{\hat{\Sigma}_S} \le (1 + \sqrt{c\log n})(1 + 2\max_i \left|\sqrt{s_i/\hat{s}_i} - 1\right|) \le (1 + \sqrt{c\log n})\left(1 + 2\left[2(1+c)(1+o(1))\frac{1 + s_{\max}\bar{\rho}_S}{\sum_{j\in S}\mu_j}\frac{\log n}{n}\right]^{1/2}\right),$$

$$\frac{\|R_1\varepsilon\|^2}{\Sigma_S^2} \le 2(1 + 2\sqrt{c\log n} + 2c\log n)(1+c)(1+o(1))\frac{1 + s_{\max}\bar{\rho}_S}{\sum_{j\in S}\mu_j}\log n,$$

$$\frac{\|R_2\|^2}{\Sigma_S^2} \le 2(1+c)(1+o(1))\left(\sqrt{\mu_j^A} + \sqrt{\mu_j^B}\right)^2 \times \frac{1 + s_{\max}\bar{\rho}_S}{\sum_{j\in S}\mu_j}(\frac{n}{n_A} \vee \frac{n}{n_B})\log n.$$

$$s_{\max} \coloneqq \max_{i=1,\dots,n} s_i \quad \textit{and} \quad \bar{\rho}_S \coloneqq \sum_{j\in S} \mu_j \rho_j \Big/ \sum_{j\in S} \mu_j. \tag{39}$$

To understand the meaning of these inequalities, one has to think that $\sum_{j\in S}\mu_j$ is intended to be large. As a consequence, $\Sigma_S/\hat{\Sigma}_S$ is of order $1+\sqrt{c\log n}$ for any $c>0$ and the bias terms $R_1\varepsilon/\Sigma_S$ and R_2/Σ_S are negligeable. Section 7 discusses strategies to ensure that $\sum_{j\in S}\mu_j$ is large.

The proof of this theorem is reported in Appendix 9.2 Let us denote Φ the cumulative distribution function of the standard Gaussian.

Corrolary 2 *Under assumption of the Theorem 3,* $T_S^*(X_{\cdot j}) \approx \frac{\Sigma_S}{\hat{\Sigma}_S} T^0(X_{\cdot j})$. *Moreover, if we consider* $q^*(1-\alpha/2) = (1+\sqrt{c\log n})\Phi^{-1}(1-\alpha/2)$, *then the test defined by the rejection region*

$$|T_S^*(X_{\cdot j})| > q^*(1-\alpha/2)$$

is of level α.

6 Implementation strategies

The technical assumption given by (17) constraints $\hat{d}$ to be not larger than a quantity Δ which depends on η, β, k and m and is given by

$$\Delta = \max_d \{d \,/\, (1-\beta)(1-\pi_d^1) > \eta(1-\pi_d^0) + \pi_d^0\}.$$

It expresses that the number of genes which can be detected is upper bounded. In order to bypass this constraint on the maximal number of possible detections, we propose an iterative implementation of our procedure which still controls the FWER at level $\alpha + o_r(1)$. It is built as follow. Starting from the m genes, the procedure is run at level $\alpha/2$. At the i-th step, if the number of detections reaches the upper bound Δ (computed at each step), then the remaining non DE-detected genes are tested using $\alpha/2^{i+1}$ instead of α. The global FWER is then controlled by $\sum_{i=1}^{\infty}\alpha/2^i \le \alpha$.

One essential term in the control provided by Theorem 3 is $\sum_{j\in S}\mu_j$ which should be large. We can imagine two implementations to ensure this property. The first one, which is the closest to our theoretical setting, consists in fixing a minimal expected intensity μ_0 per gene and select the subset made of those genes satisfying $\mu_j \ge \mu_0$. Along the iterations, the normalizing subset S is such that $\sum_{j\in S}\mu_j \ge |S|\times\mu_0$. The second consists, given a fix M_0, in growing at each iteration S until $\sum_{j\in S}\mu_j \ge M_0$.

Both strategies depend on the knowledge of the s_i however, using the estimates $\hat{s}_i$ obtained from S, it is possible to get an idea whether the size of S is large enough or not by using, for example,

$$\left(\frac{1}{n}\sum_{i=1}^{n}\sqrt{Y_{ij}/\hat{s}_i}\right)^2 \approx \mu_j \qquad \text{and} \qquad \sum_{j\in S}\left(\frac{1}{n}\sum_{i=1}^{n}\sqrt{Y_{ij}/\hat{s}_i}\right)^2 \approx \sum_{j\in S}\mu_j.$$

In the second strategy, the growth of S stops as soon as the latter left hand side term is larger than M_0.

7 Empirical study

For the empirical study, we use our iterative procedure as described in Section 6. We consider two populations having equal size $n/2$ assuming that all experiments perform perfectly ($s_i = 1$ for $i = 1, \ldots, n$) and that all genes follow a Poisson distribution ($\rho_A = \rho_B = 0$).

In population A, $\mu_j^A = \mu_0$ for all j. In population B, $\mu_j^B = \mu_j^A = \mu_0$ for $j > m_1$ and $\mu_j^B = \mu_0\varphi$ for $j \leq m_1$.

According to (37), for the differentially expressed genes ($j \leq m_1$), up to the biais terms R_1 and R_2 and up to the term $\Sigma_S/\hat{\Sigma}_S$, the expected fold change is $2(\sqrt{\mu_j^B} - \sqrt{\mu_j^A})/\Sigma_S$. As $\Sigma_S = 2/\sqrt{n}$, the test statistic T is of order $\sqrt{n\mu_0}\,|\sqrt{\varphi} - 1|$ and it should be compared to $-q_{\alpha/2m}(1 + \sqrt{c\log n})$, where $q_{\alpha/2m}$ is the quantile of order $\alpha/2m$ of the standard Gaussian.

From the relation $\sqrt{n\mu_0}\,|\sqrt{\varphi} - 1| > -q_{\alpha/2m}(1 + \sqrt{c\log n})$, we compute the lower and upper threshold values of φ to have detections with probability larger than α :

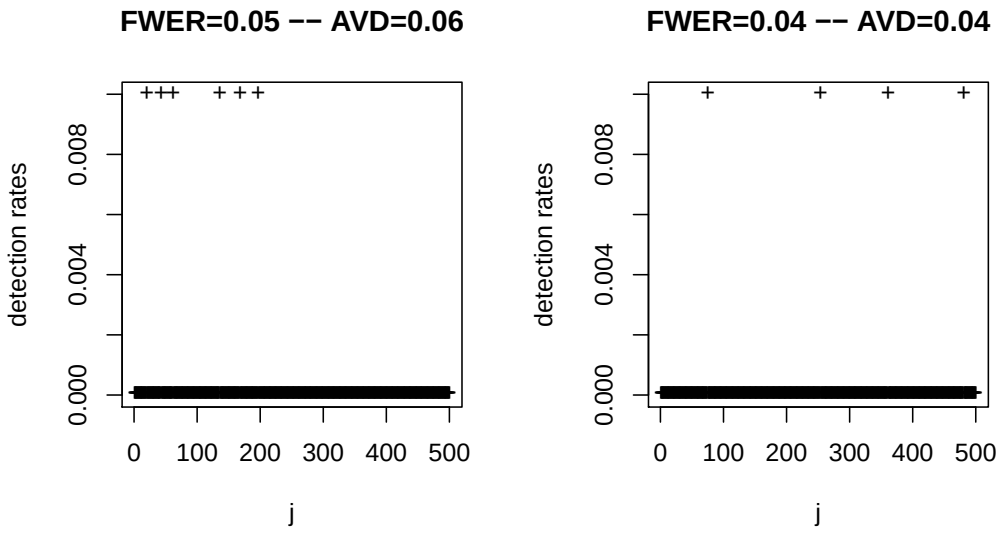
$$\varphi_{low} = \Big(1 - \frac{q_{\alpha/2m}(1 + \sqrt{c\log n})}{\sqrt{n\mu_0}}\Big)^2$$
$$\varphi_{up} = \Big(1 + \frac{q_{\alpha/2m}(1 + \sqrt{c\log n})}{\sqrt{n\mu_0}}\Big)^2.$$

In all examples, we fix $n = 12$, $m = 500$, $\mu_0 = 100$, $\eta = 5$ %, $\alpha = 5$ %, $\beta = 10$ % and tune $c = 2$ in Theorem 3 and its Corrolary 2 to ensure a proper FWER control. For each setting, 100 data samples are simulated for two populations A and B.

We start our empirical study by considering the FWER, taking $\mu_j^A = \mu_j^B = \mu_0$ for all j. Then we study empirically the power of our procedure for various values of the shift φ between population A and B for the genes j with $j = 1, ..., 225$.

7.1 Under the global null

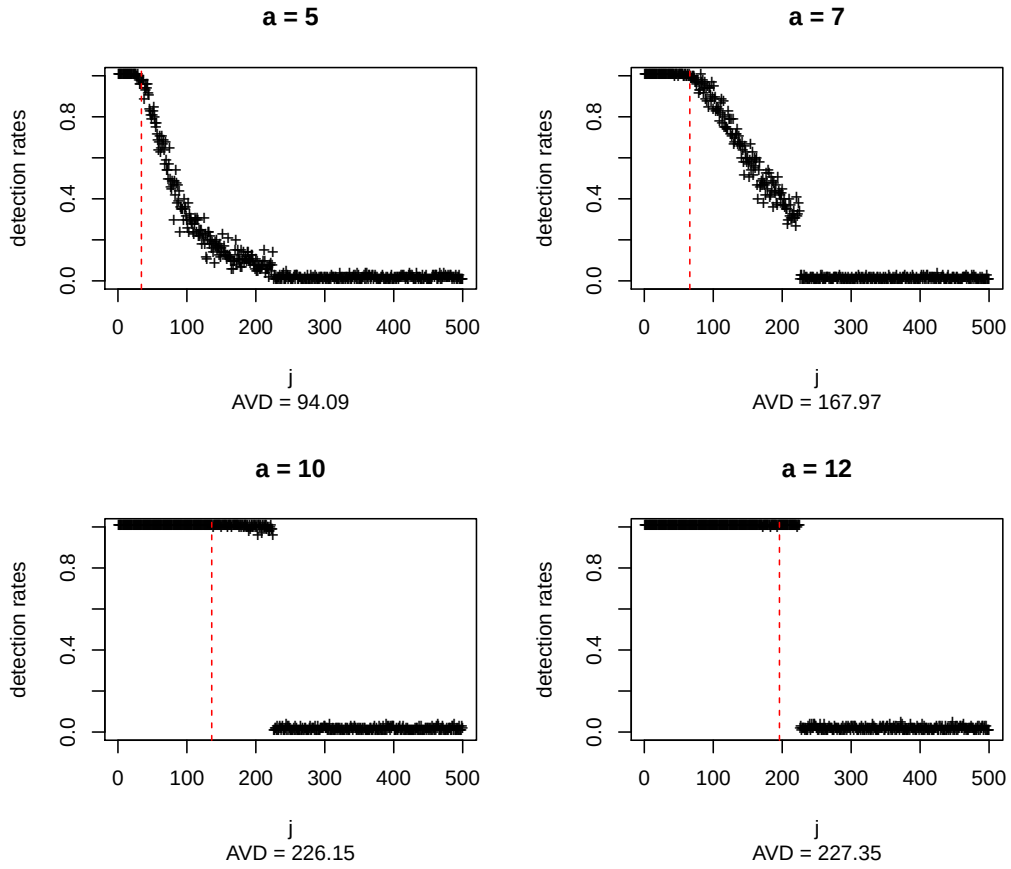
Here we assume that we are under the global null hypothesis for which $\mu_j^A = \mu_j^B = \mu_0$ for $j = 1, ..., m$ and we run 2 complete sets of simulations.



We observe on the first simulation (left graph) that the FWER is 0.05 with 0.06 average detected genes. For the second simulation (right graph) FWER and average detected genes are respectively 0.04 and 0.04.

7.2 Empirical study of the power

We now study the power of our procedure by assuming that $m_1 = 225$ and, while $\mu_J^B = \mu_j^A$ for $j > m_1$, for $j \leq m_1$, $\mu_j^B = \varphi_j \mu_j^A$ with $\phi_j := 1 + a/\sqrt{j}$ such that the relative decay $(\mu_j^B - \mu_j^A)/\mu_j^A$ varies as $a/\sqrt{j}$. Using $a = 5, 7, 10$ and 12, we let the number of expected detections vary from low to high and study the behavior of our iterative procedure when the number of potential detections is approaching $m/2$. As $15^2 = 225$, the largest relative decay is $a/15$. The detection rates through simulations are depicted in each graph. On each plot below, the red dashed line specifies j corresponding to the threshold value of φ_{up} when $\mu_1 \geq \mu_0$. We observed that, as soon as the relative decay is larger than 2/3 ($a = 10$), we achieved an almost perfect detection.



8 Real data - Mice model of NASH

We apply the iterative procedure, as described in Section 6, using the same settings as in the Section 7, on the real data set of hepatic miRNA expressions from a mouse model of non-alcoholic steatohepatitis (NASH), the CDAHFD (choline-deficient, L-amino acid-defined, high-fat diet) model, with 4 cases of NASH with hepatic fibrosis and 4 controls without hepatic lesions (Hoffmann et al. 2020). One mouse showed a strong disequilibrium of library size with respect to the others of about a third.

Expressions with less than 20 reads shown in total in the 8 mice were filtered out prior to analysis and the testing was done for 749 remaining miRNA's. For these remaining genes, if their are not differentially expressed, then $\mu_0 \approx 2.5$ (see Section 6). For all 2500 randomizations, normalization subsets were formed with 10 random reference genes.

The results achieved on this real experiments were compared to those obtained using the trimmed mean of M values (TMM) (Mark D. Robinson and Oshlack 2010).

The procedure detectes the total of 14 miRNA differentially expressed : mmu-miR-31-3p, mmu-miR-31-5p, mmu-miR-34a-5p, mmu-miR-96-5p, mmu-miR-141-3p, mmu-miR-141-5p, mmu-miR-200a-3p, mmu-miR-200b-3p, mmu-miR-

200b-5p, mmu-miR-200c-3p, mmu-miR-429-3p, mmu-miR-582-5p, mmu-miR-802-3p, mmu-miR-802-5p.

Within the mmu-miR-34 family members that are known to regulate hepatic fibrosis (Li et al. 2015 ; Jiang et al. 2017), only the major isoform mmu-miR-34a-5p whose monocistronic gene is located on chromosome 4 has been detected and identified strongly upregulated while the other major isoforms mmu-miR-34b-5p and mmu-miR-34c-5p whose polycistronic gene is located on chromosome 9 are not. All other minor microRNA isoforms mmu-miR-34a/b/c/-3p does not exhibit significant differences in the reads between the experimental groups.

The microRNA mmu-miR-96-5p is involved in both fibrosis (Chandel et al. 2018) and cancerization processes (Leung et al. 2015), showing that there are possible linkages between these different pathologies. In our experimental model of NASH we observe a clear increase in its expression, however it is also consistant with the fact that nodules were observed in the livers of mice at the time of sacrifice.

For mmu-miR-34a-5p and mmu-miR-96-5p, due to the clear increase in expression observed both our procedure and TMM behave the same. For the further miRNA, TMM clearly shows a lower sensibility.

Our differential expression analysis also identified a subset of 7 microRNAs that had significant differences between the experimental groups. These belong to the mmu-miR-200 family, which is known for its involvement in various liver diseases including NASH, fibrosis, and hepatocellular carcinoma (Murakami et al. 2011 ; Gregory et al. 2008 ; Jiang et al. 2017). The mmu-miR-200 family is divided into two different clusters : one of the clusters is located on chromosome 4, which contains mmu-miR-200a/b/429 members, the other cluster which is located on chromosome 6 includes mmu-miR-200c/141 members. Although belonging to different clusters located in different chromosomes, the major microRNA isoforms (mmu-miR-141-3p, mmu-miR-200a-3p, mmu-miR-200b-3p, mmu-miR-200c-3p, mmu-miR-429-3p) are all overexpressed in the CDAHFD model, and the same evolution is observed with the trimmed mean of M-values normalization method (TMM normalization method). On the other hand, our procedure is able to show that minor microRNA isoforms (mmu-miR-141-5p, mmu-miR-200b-5p) are also upregulated in the CDAHFD model, which is not observed with TMM.

Interestingly, mmu-miR-31-5p which was previously shown overexpressed in liver fibrosis (Hu et al. 2015), cirrhosis, and hepatocellular carcinoma (Karakatsanis et al. 2013 ; Tessitore et al. 2016) is found upregulated in the CDAHFD model. In this case our procedure is able to show that its minor microRNA counterpart mmu-miR-31-3p is also upregulated. Here again this result is not observed with TMM.

Finally, the last three microRNAs (mmu-miR-582-5p (Zhang et al. 2015), mmu-miR-802-3p, mmu-miR-802-5p (Zhen et al. 2018 ; Yang et al. 2019)) are intriguing because their known activities are not specific to the field of fibrosis, so further investigation is required to understand their involvement in the pathology. It should be emphasized that the increase in their expression involves a relatively small number of reads and are not detected by TMM.

All this biological arguments show that our method is more sensitive than those used in daily practice by biologists and offer some meaningful results.

From a statistical point-of-view, as our procedure is based on some random sampling, it depends on the seed of the random generator. To get an idea of the stability with respect to this seed, we run few times the complete procedure on our real data. On the 14 detected miRNA, only mmu-miR-96-5p was not present in the list of detected on few runs.

9 Appendix

9.1 FWER control of randomly picking reference genes

We recall that the $p_j^d(\eta)$ defined in (14) correspond to the p-values associated to the numbers of detections R_j when $d^* = d$ and the error of type I is control by η for a good normalization subset.

In order to establish that our randomized procedure respects FWER at level α, we first yield the following lemma which controls the order between DE genes and non-DE genes of the $p_j^d(\eta)$.

Lemma 2 *If the genes are independent and the following relation holds*

$$P_{j\notin\mathcal{D}^*}(\mathbb{1}_S(j)=1) \le \eta(1-\pi^0_{d^*}) + \pi^0_{d^*} < (1-\beta)(1-\pi^1_{d^*}) \le P_{j\in\mathcal{D}^*}(\mathbb{1}_S(j)=1)$$

then, for all d, with small probability the $p_j^d(\eta)$ for DE genes are larger than those of non-DE genes, that is :

$$P\left(\min_{j_0\notin\mathcal{D}^*} p^d_{j_0}(\eta) < \max_{j_1\in\mathcal{D}^*} p^d_{j_1}(\eta)\right) = o_r(1)\,.$$

Given this lemma, with large probability, the discoveries of the DE genes are first and false discoveries occurs if the number of discoveries is larger than d^*. As a consequence, with large probability, a false discovery occurs if and only if

$$p^{d^*}_{(d^*+1)}(\eta) < \frac{\alpha}{m-d^*}.$$

In other words, with large probability, a false discovery occurs if and only if one of the $m-d^*$ non-DE genes is detected at the corrected $\alpha/(m-d^*)$ level which is the ad-hoc Holm's correction to control the FWER. Finally, the FWER is controled by $\alpha + o_r(1)$.

Proof of Lemma 2 : From Lemma 1, for all $j_0 \notin \mathcal{D}^*$ and all $j_1 \in \mathcal{D}^*$, we have $\hat{R}_{j_1} \le R_{j_1}$ and $R_{j_0} \le \mathring{R}_{j_0}$ with $\mathring{R}_{j_0} \sim \mathcal{B}(r,\theta_0)$ and $\hat{R}_{j_1} \sim \mathcal{B}(r,\theta_1)$. As a consequence, if $\max_{j_0\notin\mathcal{D}^*} R_{j_0} > \min_{j_1\in\mathcal{D}^*} R_{j_1}$ then $\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > \min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1}$.

As a consequence, the following relation holds

$$P\left(\max_{j_0\notin\mathcal{D}^*} R_{j_0} > \min_{j_1\in\mathcal{D}^*} R_{j_1}\right) \leq P\left(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > \min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1}\right) \tag{40}$$

and it is enough to prove that $\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > \min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1}$ occurs with small probability when r goes to infinity. Using total probability formula, for all x

$$\begin{aligned}
P\left(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > \min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1}\right) &= P(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > \min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1} | \max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} \leq x) P(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} \leq x) \\
&\quad + P(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > \min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1} | \max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > x) P(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > x) \\
&\leq P(\min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1} \leq x) + P(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > x) \\
&= P(\min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1} \leq x) + 1 - P(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} \leq x).
\end{aligned}$$

As $|\mathcal{D}^*| = d^*$, usual derivations of the c.d.f. for the minimum and the maximum of independent variables provide

$$\begin{aligned}
P\left(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > \min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1}\right) &= 1 - [1 - P(\hat{R}_{j_1} \leq x)]^{d^*} + 1 - [P(\mathring{R}_{j_0} \leq x)]^{m-d^*} \\
&= 1 - [1 - P(\hat{R}_{j_1} \leq x)]^{d^*} + 1 - [1 - P(\mathring{R}_{j_0} > x)]^{m-d^*} \\
&\approx 1 - (1 - d^* P(\hat{R}_{j_1} \leq x)) + 1 - (1 - (m - d^*) P(\mathring{R}_{j_0} > x)) \\
&= d^* P(\hat{R}_{j_1} \leq x) + (m - d^*) P(\mathring{R}_{j_0} > x)
\end{aligned}$$

For any $0 < \xi < 1$, let $x := B^{-1}(1-\xi, r, \theta_0)$ be the $(1-\xi)$-quantile under $\mathcal{B}(r, \theta_0)$. Then $P(\mathring{R}_{j_0} > x) \leq \xi$. If $P(\hat{R}_{j_1} \leq x) \leq \xi$ also holds, that is if

$$B^{-1}(1-\xi, r, \theta_0) \leq B^{-1}(\xi, r, \theta_1), \tag{41}$$

then

$$P\left(\max_{j_0\notin\mathcal{D}^*} R_{j_0} > \min_{j_1\in\mathcal{D}^*} R_{j_1}\right) \leq P\left(\max_{j_0\notin\mathcal{D}^*} \mathring{R}_{j_0} > \min_{j_1\in\mathcal{D}^*} \hat{R}_{j_1}\right) \leq m\xi. \tag{42}$$

We now prove that ξ can be chosen satisfying $P(\hat{R}_{j_1} \leq x) \leq \xi$ if r is large enough. From the Massart'90 inequality, we obtain that $B^{-1}(\xi, r, \theta_1) \geq r(\theta_1 - \hat{\varepsilon})$ with

$$P(\hat{R}_{j_1} < r(\theta_1 - \hat{\varepsilon})) \leq \exp\left(-\frac{r\hat{\varepsilon}^2}{2(1-\theta_1+\hat{\varepsilon}/3)(\theta_1-\hat{\varepsilon}/3)}\right) \leq \exp\left(-\frac{r\hat{\varepsilon}^2}{2\theta_1}\right), \quad j_1 \in \mathcal{D}^*$$

similarly $B^{-1}(1-\xi, r, \theta_0) \leq r(\theta_0 + \mathring{\varepsilon})$ with

$$P(\mathring{R}_{j_0} > r(\theta_0 + \mathring{\varepsilon})) \leq \exp(-\frac{r\mathring{\varepsilon}^2}{2(\theta_0 + \mathring{\varepsilon}/3)(1-\theta_0 - \mathring{\varepsilon}/3)}) \leq \exp(-\frac{r\mathring{\varepsilon}^2}{2(\theta_0 + \mathring{\varepsilon}/3)}), \quad j_0 \notin \mathcal{D}^*.$$

Let us assume from now on that $\mathring{\varepsilon}$ and $\hat{\varepsilon}$ are not larger than $(\theta_1 - \theta_0)/2$, then

$$B^{-1}(1-\xi, r, \theta_0) \leq r(\theta_0 + \mathring{\varepsilon}) \leq r(\theta_1 - \hat{\varepsilon}) \leq B^{-1}(\xi, r, \theta_1).$$

Moreover $\mathring{\varepsilon}/3 \leq \theta_1 - \theta_0$ such that

$$P(\mathring{R}_{j_0} > r(\theta_0 + \mathring{\varepsilon})) \leq \exp(-\frac{r\mathring{\varepsilon}^2}{2\theta_1}), \quad j_0 \notin \mathcal{D}^*.$$

Consequently $P(\mathring{R}_{j_0} > B^{-1}(1-\xi, r, \theta_0)) \leq \xi$ and $P(\hat{R}_{j_1} \leq B^{-1}(\xi, r, \theta_1)) \leq \xi$ are satisfied as soon as $\hat{\varepsilon}$ and $\mathring{\varepsilon}$ are non smaller than $\sqrt{-2\theta_1 \ln(\xi)/r}$. The assumption that $\mathring{\varepsilon}$ and $\hat{\varepsilon}$ are not larger than $(\theta_1 - \theta_0)/2$ is then fulfilled as soon as

$$r \geq \frac{-8\theta_1 \ln \xi}{(\theta_1 - \theta_0)^2}.$$

Replacing ξ by ξ/m ends the demonstration of the lemma. □

9.2 Proof of Theorem 3

In order to derive the distribution of our test statistic, we need the following lemma and its corrolary to control the deviations of $\max_i |\sqrt{s_i/\hat{s}_i} - 1|$.

Lemma 3 *Let $\hat{s}_i$ be the estimator of s_i given by* (8)*. Assuming the Gaussian approximations hold, then if t^2 is of smaller order than $n \sum_{i\in S} \mu_j / (1 + s_{\max} \bar{\rho}_S)$, with probability larger than $1 - 4n\exp(-t^2/2)$,*

$$\left| \sqrt{\frac{s_i}{\hat{s}_i}} - 1 \right| \leq (1 + o(1)) t \sqrt{\frac{1 + s_{\max}\bar{\rho}_S}{n \sum_{j\in S} \mu_j}} \qquad \textit{for all } i = 1, \ldots, n. \tag{43}$$

Corrolary 3 *Taking $t^2 = 2(1+c)\log(n)$, assuming that $(1 + s_{\max}\bar{\rho}_S)\log(n)/n\sum_{i\in S}\mu_j$ is of smaller order than 1, we have with probabilty larger than $1 - 4n^{-c}$ for all $i = 1, ..., n$*

$$\left(\sqrt{\frac{s_i}{\hat{s}_i}} - 1 \right)^2 \leq 2(1+c)(1+o(1)) \frac{1 + s_{\max}\bar{\rho}_S}{\sum_{j\in S}\mu_j} \frac{\log n}{n}. \tag{44}$$

If $\sum_{j\in S}\mu_j \geq 1 + s_{\max}\bar{\rho}_S$, (44) is always satisfied. This condition means that the sum of the absolute levels over the

normalization set is large enough to compensate for over-dispersion.

The proof of Lemma 3 in reported in Appendix 9.3

9.2.1 Control of $\Sigma_S/\hat{\Sigma}_S$

Let us consider the operator $\mathbb{S}(\cdot)$ defined for all $x \in \mathbb{R}^n$ by $\mathbb{S}^2(x) := \|L(I-K)x\|^2$ where

$$K = \begin{pmatrix} \frac{1}{n_A} J_{n_A} & 0_{n_A,n_B} \\ 0_{n_B,n_A} & \frac{1}{n_B} J_{n_B} \end{pmatrix}$$

and L is the diagonal matrix $\text{diag}(1/\sqrt{n_A(n_A-1)},\ldots,1/\sqrt{n_A(n_A-1)},1/\sqrt{n_B(n_B-1)},\ldots,1/\sqrt{n_B(n_B-1)})$. Simple computations show that $\hat{\Sigma}_S^2 = \mathbb{S}^2(Y)$.

The following relation holds

$$\mathbb{S}^2(U) = \|L(I-K)U\|^2 = \|L(I-K)\text{diag}(1-\sqrt{s_i/\hat{s}_i})U + L(I-K)\text{diag}(\sqrt{s_i/\hat{s}_i})U\|^2$$

and using triangular inequality, as $Y_{ij} = \sqrt{s_i/\hat{s}_i}U_{ij}$, we obtain

$$\mathbb{S}(U) \leq \|L(I-K)\text{diag}(1-\sqrt{s_i/\hat{s}_i})U\| + \|L(I-K)Y\| \leq \max_i \left|\sqrt{s_i/\hat{s}_i}-1\right| \mathbb{S}(U) + \mathbb{S}(Y). \tag{45}$$

It is clear that operator $\mathbb{S}(\cdot)$ is invariant by any translation of a vector which is constant over the indexes in A and in B, which is the case for $E(U)$. As a consequence, $\mathbb{S}(U) = \|L(I-K)\text{diag}(\omega_i)\varepsilon\|$ where $\omega_i^2 = \rho_j^\bullet + 1/s_i$ satifies $U_{il} = 2\sqrt{\mu_j^\bullet} + \omega_i\varepsilon_{ij}$. We control this latter norm using Gendre (2014 Lemma 8.2).

Let us denote $A := L(I-K)\text{diag}(\omega_i)$, we now show that $\text{Tr}(AA^T) = \Sigma_S^2$ and bound the ratio $\Sigma_S^2/\mathbb{S}^2(Y)$ from above :

$$AA^T = L(I-K)\text{diag}(\omega_i)\text{diag}(\omega_i)^T(I-K)^TL^T = L(I-K)\text{diag}(\omega_i^2)(I-K)L.$$

Since $I-K$ and L are both symmetric, all the matrices in this latter product are symmetric such that we can use any permutation of them to compute the trace of this product. Hence $\text{Tr}(AA^T) = \text{Tr}(L^2(I-K)^2\text{diag}(\omega_i^2))$.

By definition $L^2 = \text{diag}(1/n_A(n_A-1),\ldots,1/n_A(n_A-1),1/n_B(n_B-1),\ldots,1/n_B(n_B-1))$ and, as K is a matrix of projection $K^2 = K$, the same holds for $I-H$ such that $(I-K)^2 = I-K$.

We write $L^2(I-K)\text{diag}(\omega_i^2)$ as a block matrix

$$L^2(I-K)\text{diag}(\omega_i^2) = \begin{pmatrix} \frac{1}{n_A(n_A-1)}(I_{n_A} - \frac{1}{n_A}J_{n_A})\text{diag}(\frac{1}{s_i}+\rho_j^A) & 0_{n_A,n_B} \\ 0_{n_B,n_A} & \frac{1}{n_A(n_A-1)}(I_{n_B} - \frac{1}{n_B}J_{n_B})\text{diag}(\frac{1}{s_i}+\rho_j^B) \end{pmatrix}$$

whose trace is $\frac{1}{n_A^2}\sum_{i\in A}(\frac{1}{s_i}+\rho_j^A) + \frac{1}{n_B^2}\sum_{i\in B}(\frac{1}{s_i}+\rho_j^B)$ which is Σ_S^2.

Clearly, for all $x \in \mathbb{R}^n$, $x^T A^T A x = \|Ax\|^2 \geq 0$, hence the matrix AA^T is positive and has all its eigenvalue non negative which are all smaller than $\text{Tr}(AA^T) = \Sigma_S^2$. It follows from Gendre (2014 Lemma 8.2) that, with probability larger than $1-e^{-x}$

$$1 - 2\sqrt{x} \leq \frac{\mathbb{S}^2(U)}{\Sigma_S^2}$$

or in other terms

$$\frac{\Sigma_S}{\mathbb{S}(U)} \leq \frac{1}{\sqrt{1-2\sqrt{x}}} \approx 1 + \sqrt{x}. \tag{46}$$

Multiplying (45) and (46) together, we get

$$\frac{\Sigma_S}{\mathbb{S}(Y)} \leq \frac{1+\sqrt{x}}{1 - \max_i \left|\sqrt{s_i/\hat{s}_i} - 1\right|}.$$

Assuming moreover that the maximum is smaller than 1/2, we can use that $1/(1-x) \leq 1+2x$ for $x \leq 1/2$, such that Lemma 1 provides

$$\frac{\Sigma_S}{\mathbb{S}(Y)} \leq (1+\sqrt{x})(1 + 2\max_i \left|\sqrt{s_i/\hat{s}_i} - 1\right|) \leq (1+\sqrt{x})\left(1 + 2\left[2(1+c)(1+o(1))\frac{1+s_{\max}\bar{\rho}_S}{\sum_{j\in S}\mu_j}\frac{\log n}{n}\right]^{1/2}\right).$$

The last inequality in the Theorem 3 comes from equating e^{-x} to n^{-c} which implies that $x = c\ln n$.

9.2.2 Control of $\|R_1\varepsilon\|^2$

One can check that

$$\begin{aligned} R_1 R_1^T &= H\text{diag}(\sqrt{s_i/\hat{s}_i} - 1)\text{diag}(\omega_i^2)\text{diag}(\sqrt{s_i/\hat{s}_i} - 1)H^T = H\text{diag}(\sqrt{s_i/\hat{s}_i} - 1)^2\text{diag}(\omega_i^2)H^T \\ &\leq \max_i(\sqrt{s_i/\hat{s}_i} - 1)^2\Sigma_S^2 J_n. \end{aligned}$$

As J_n has only one non-zero eigenvalue which is also its trace and which is equal to n, it follows using Gendre (2014 Lemma 8.2) that for any $x > 0$, with probability larger than $1 - e^{-x}$,

$$\frac{\|R_1\varepsilon\|^2}{n \max_i (\sqrt{s_i/\hat{s}_i} - 1)^2 \Sigma_S^2} \leq 1 + 2\sqrt{x} + 2x \tag{47}$$

and

$$\frac{\|R_1\varepsilon\|^2}{\Sigma_S^2} \leq 2(1 + 2\sqrt{x} + 2x)(1 + c)(1 + o(1)) \frac{1 + s_{\max}\bar{\rho}_S}{\sum_{j\in S} \mu_j} \log n$$

9.2.3 Control of $\|R_2\|^2$

Simple algebra computations show that

$$\begin{aligned}\|R_2\|^2 &= n\left(\frac{\sqrt{\mu_j^A}}{n_A}\sum_{i\in A}(\sqrt{s_i/\hat{s}_i} - 1) - \frac{\sqrt{\mu_j^B}}{n_B}\sum_{i\in B}(\sqrt{s_i/\hat{s}_i} - 1)\right)^2 \\ &\leq n(\sqrt{\mu_j^A} + \sqrt{\mu_j^B})^2\left(\frac{1}{n_A}\sum_{i\in A}(\sqrt{s_i/\hat{s}_i} - 1) + \frac{1}{n_B}\sum_{i\in B}(\sqrt{s_i/\hat{s}_i} - 1)\right)^2\end{aligned}$$

such that, using Cauchy-Schwarz inequality and $\sqrt{s_i/\hat{s}_i} - 1 = \sqrt{s_i}(\sqrt{s_i/\hat{s}_i} - 1)/\sqrt{s_i}$, we have :

$$\frac{\|R_2\|}{\sqrt{n}(\sqrt{\mu_j^A} + \sqrt{\mu_j^B})} \leq \left(\frac{1}{n_A^2}\sum_{i\in A}\frac{1}{s_i} + \frac{1}{n_B^2}\sum_{i\in B}\frac{1}{s_i}\right)^{1/2}\left(\frac{1}{n_A}\sum_{i\in A} s_i(\sqrt{s_i/\hat{s}_i} - 1)^2 + \frac{1}{n_B}\sum_{i\in B} s_i(\sqrt{s_i/\hat{s}_i} - 1)^2\right)^{1/2}.$$

It follows that

$$\begin{aligned}\frac{\|R_2\|}{\sqrt{n}(\sqrt{\mu_j^A} + \sqrt{\mu_j^B})} &\leq \Sigma_S \max_i \left|\sqrt{s_i/\hat{s}_i} - 1\right|\left(\frac{1}{n_A}\sum_{i\in A} s_i + \frac{1}{n_B}\sum_{i\in B} s_i\right)^{1/2} \\ &\leq \Sigma_S \max_i \left|\sqrt{s_i/\hat{s}_i} - 1\right| \sqrt{\max(n/n_A, n/n_B)}.\end{aligned}$$

Finally

$$\frac{\|R_2\|^2}{\Sigma_S^2} \leq 2(1 + c)(1 + o(1))\left(\sqrt{\mu_j^A} + \sqrt{\mu_j^B}\right)^2 \times \frac{1 + s_{\max}\bar{\rho}_S}{\sum_{j\in S}\mu_j}\left(\frac{n}{n_A} \vee \frac{n}{n_B}\right) \log n.$$

9.3 Proof of Lemma 3

We recall that $\hat{s}_i = nX_{i\bullet}^S \Big/ X_{\bullet\bullet}^S$ where $X_{i\bullet}^S = \sum_{j\in S} X_{ij}$ and $X_{\bullet\bullet}^S = \sum_{i=1}^n \sum_{j\in S} X_{ij}$. Under the Gaussian approximation, taking into account that $\sum_{i=1}^n s_i = n$, we have

$$X_{i\bullet}^S \approx \mathcal{N}(s_i \sum_{j\in S}\mu_j, s_i \sum_{j\in S}\mu_j + s_i^2 \sum_{j\in S}\mu_j\rho_j) \quad \text{and} \quad X_{\bullet\bullet}^S \approx \mathcal{N}(n \sum_{j\in S}\mu_j, n \sum_{j\in S}\mu_j + \sum_{i=1}^n s_i^2 \sum_{j\in S}\mu_j\rho_j).$$

Using Hoeffding's inequality applied to the two latter random variables, for all $t > 0$, the following inequalities hold

with probability larger than $1 - 4\exp(-t^2/2)$:

$$\frac{n\sum_{j\in S}\mu_j - t\sqrt{n\sum_{j\in S}\mu_j + \sum_{i=1}^n s_i^2\sum_{j\in S}\mu_j\rho_j}}{s_i\sum_{j\in S}\mu_j + t\sqrt{s_i\sum_{j\in S}\mu_j + s_i^2\sum_{j\in S}\mu_j\rho_j}} \leq \frac{X_{\bullet\bullet}^S}{X_{i\bullet}^S} \leq \frac{n\sum_{j\in S}\mu_j + t\sqrt{n\sum_{j\in S}\mu_j + \sum_{i=1}^n s_i^2\sum_{j\in S}\mu_j\rho_j}}{s_i\sum_{j\in S}\mu_j - t\sqrt{s_i\sum_{j\in S}\mu_j + s_i^2\sum_{j\in S}\mu_j\rho_j}}$$

Considering that $\sum_{i=1}^n s_i^2 \leq s_{\max}\sum_{i=1}^n s_i = n\,s_{\max}$ with $s_{\max} = \max(s_i)$, we have

$$\frac{1}{s_i}\frac{\sum_{j\in S}\mu_j - t\sqrt{\sum_{j\in S}\mu_j + s_{\max}\sum_{j\in S}\mu_j\rho_j}\Big/\sqrt{n}}{\sum_{j\in S}\mu_j + t\sqrt{\sum_{j\in S}\mu_j + s_i\sum_{j\in S}\mu_j\rho_j}\Big/\sqrt{s_i}} \leq \frac{X_{\bullet\bullet}^S}{nX_{i\bullet}^S} \leq \frac{1}{s_i}\frac{\sum_{j\in S}\mu_j + t\sqrt{\sum_{j\in S}\mu_j + s_{\max}\sum_{j\in S}\mu_j\rho_j}\Big/\sqrt{n}}{\sum_{j\in S}\mu_j - t\sqrt{\sum_{j\in S}\mu_j + s_i\sum_{j\in S}\mu_j\rho_j}\Big/\sqrt{s_i}}$$

Denoting

$$\bar{\rho}_S := \sum_{j\in S}\mu_j\rho_j \Big/ \sum_{j\in S}\mu_j$$

we have as $s_i \leq n$

$$\frac{1 - t\sqrt{1 + s_{\max}\bar{\rho}_S}\Big/\sqrt{n\sum_{j\in S}\mu_j}}{1 + t\sqrt{1 + s_{\max}\bar{\rho}_S}\Big/\sqrt{n\sum_{j\in S}\mu_j}} \leq \frac{s_i}{\hat{s}_i} \leq \frac{1 + t\sqrt{1 + s_{\max}\bar{\rho}_S}\Big/\sqrt{n\sum_{j\in S}\mu_j}}{1 - t\sqrt{1 + s_{\max}\bar{\rho}_S}\Big/\sqrt{n\sum_{j\in S}\mu_j}}$$

Using that $(1+x)^{-1} \approx 1 - x(1 + o(1))$ when x is small, we get our inequality

$$\left|\sqrt{\frac{s_i}{\hat{s}_i}} - 1\right| \leq (1 + o(1))t\sqrt{\frac{1 + s_{\max}\bar{\rho}_S}{n\sum_{j\in S}\mu_j}}$$

for t^2 being small in front of $n\sum_{i\in S}\mu_j\big/(1 + s_{\max}\bar{\rho}_S)$ in order to have the approximation valid.

□

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Data and Code Availability Transcriptomic analysis on murine sample together with the code for simulation will be made available as open data. The code implementing our procedure will be made available as an open source R software.

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