Reviewing and assessing existing meta-analysis models and tools

Adaptively Weighted (AW) Fisher's method [6]:

The adaptive weighted statistics can be derived following the step by step calculations below:

1. Suppose we have a total of G genes in a differentially expressed data across j studies. Define the observed weighted statistics as

$$u_g(w_g) = -\sum_{j=1}^{J} w_{gj} \log(P_{gj})$$

and similarly

$$u_g^{(b)}(w_g) = -\sum_{j=1}^{J} w_{gj} \log(P_{gj^{(b)}})$$

where w_g is the corresponding weight for gene g across J studies, that is, $w_g = (w_{g1}, \ldots, w_{gJ})$ and P_{gj} is the p-value of gene g in the jth study with $1 \leq g \leq G$, $1 \leq j \leq J$ and $1 \leq b \leq B$, where B is the total number of times permutation occurs in each study.

2. Estimate the p-value of the observed weighted statistics of a given gene g and weight w_g as,

$$pU\left(u_g(w_g)\right) = \frac{\sum_{b=1}^{B}\sum_{g'=1}^{G} I\{u_{g'}^{(b)}(w_g) \geq u_g(w_g)\}}{B \cdot G}$$

Likewise compute,

$$pU\left(u_g^{(b)}(w_g)\right) = \frac{\sum_{b'=1}^{B}\sum_{g'=1}^{G} I\{u_{g'}^{(b')}(w_g) \geq u_g^{(b)}(w_g)\}}{B \cdot G}$$

3. Using a) and b), calculate the optimal weight as

$$w_g^* = \arg\min_{w_g \in W} pU\left(u_g(w_g)\right),$$

and likewise

$$w_g^{(b)*} = \arg\min_{w_g \in W} pU\left(u_g^{(b)}(w_g)\right)$$

where $W = \{w \mid w_g \in \{0,1\}\}$. This optimal weight gives indication of which studies contribute to the statistical significance or differentially expressed evidence of the meta-analysis. Hence, the adaptive weighted statistics

$$S_g = pU\left(u_g(w_g^*)\right)$$

is the p-value of the minimum p-value among all possible weights. Similarly,

$$S_g^{(b)} = pU\left(u_g^{(b)}(w_g^{(b)*})\right)$$

4. Next, evaluate the corresponding p-value of the AW-statistics S_g as,

$$p\left(S_g\right) = \frac{\sum_{b=1}^{B}\sum_{g'=1}^{G} I\{S_{g'}^{(b)} \leq S_g\}}{B \cdot G}$$

5. Also, evaluate the corresponding q-value of the AW-statistics S_g as,

$$q\left(S_g\right) = \frac{\hat{\pi_0}\sum_{b=1}^{B}\sum_{g'=1}^{G} I\{S_{g'}^{(b)} \leq S_g\}}{B\sum_{g'=1}^{G} I\{S_{g'} \leq S_g\}}$$

where

$$\hat{\pi_0} = \frac{\sum_{g=1}^{G} I\{p(V_g) \in A\}}{l(A) \cdot G}$$

is the estimated proportion of null genes with $A = [0.5, 1]$ and $l(A) = 0.5$ [6, 8] where $I\{\cdot\}$ is an indicator function. Hence, the detected genes will be the list of all genes whose $q(V_g) \leq 0.05$.

For example, let's assume from four different studies, gene D is with p-values $= (1, 1, 1e^{-4}, 1)$ and gene E is with p-values $= (1e^{-2}, 1e^{-2}, 1e^{-2}, 1e^{-2})$, then the adaptively weighted Fisher's method will generate adaptive weights $w = (0, 0, 1, 0)$ for gene D indicating statistical significance only in the third study and $w = (1, 1, 1, 1)$ for gene E indicating statistical significance in all four studies.

Fixed effect model (FE) [1, 9]:

FE model assumes that all the studies under consideration shares a common/fixed true effect size. Let T_j be the effect size estimate of each study which follows a normal distribution with mean μ and variance σ^2. Also, let the weight assigned to each study, $w_j = v_j^{-1}$ be the inverse of the variance, where v_j is the within-study variance for study j in a meta-analysis of J studies. The inverse-variance-weighted effect-size estimator of the true effect size is given by

$$\overline{T} = \frac{\sum_{j=1}^{J} w_j T_j}{\sum_{j=1}^{J} w_j}.$$

The variance of $\overline{T}$ is calculated as,

$$v = \frac{1}{\sum_{j=1}^{J} w_j},$$

and its standard error,

$$SE(\overline{T}) = \sqrt{v}.$$

By default, $\overline{T}$ also follows a normal distribution and the test statistics can therefore be calculated as

$$S_{FE} = \frac{\overline{T}}{SE(\overline{T})} = \frac{\sum_{j=1}^{J} w_j T_j}{\sqrt{\sum_{j=1}^{J} w_j}}$$

which follows $\mathcal{N}(0,1)$ under the null hypothesis that there is no association. If we assumed a one-tailed test, the p-value of the association is given by

$$p = 1 - \Phi(S_{FE})$$

while for a two-tailed test,

$$p = 2[1 - \Phi(|S_{FE}|)]$$

where Φ is the standard normal cumulative distribution function. This model is mostly applied to GWAS dataset [1, 9].

Random effect model (RE) [1, 9]:

Unlike the FE model, the RE model assumes that the effect size for each study in the meta-analysis are different and the effect sizes are drawn from a normal distribution with mean μ and variance σ^2. The RE analysis approach is to decompose the observed variance into its two component parts, i.e., the within-study and between-study variance. Using the Cochran's Q test statistics to evaluate the between-study heterogeneity as explained earlier, that is,

$$\begin{aligned} Q &= \sum_{j=1}^{J} w_j (T_j - \overline{T})^2 \\ &= \sum_{j=1}^{J} w_j T_j^2 - \frac{\left[\sum_{j=1}^{J} w_j T_j\right]^2}{\sum_{j=1}^{J} w_j} \end{aligned}$$

The between-study variance

$$\tau^2 = \begin{cases} \frac{Q-df}{c}, & \text{if } Q > df \\ 0, & \text{if } Q \leq df \end{cases}$$

where

$$c = \sum w_j - \frac{\sum w_j^2}{\sum w_j}.$$

Similarly, in RE model the weight assigned to each study,

$$w_j^* = v_j^{*-1}$$

, where

$$v_j^* = v_j + \tau^2$$

with v_j and τ^2 the within-study and between-study variance respectively. Also, the inverse-variance-weighted effect-size estimator of the true effect size is given by

$$\overline{T}^* = \frac{\sum_{j=1}^{J} w_j^* T_j}{\sum_{j=1}^{J} w_j^*}.$$

The variance of $\overline{T}^*$ is calculated as,

$$v^* = \frac{1}{\sum_{j=1}^{J} w_j^*},$$

and its standard error,

$$SE(\overline{T}^*) = \sqrt{v^*}.$$

The test statistics is similarly calculated as

$$S_{RE} = \frac{\overline{T}^*}{SE(\overline{T}^*)} = \frac{\sum_{j=1}^{J} w_j^* T_j}{\sqrt{\sum_{j=1}^{J} w_j^*}}$$

and the p-value is

$$p = 1 - \Phi(S_{RE})$$

for one-tailed test and

$$p = 2[1 - \Phi(|S_{RE}|)]$$

for a two-tailed test, where Φ is the standard normal cumulative distribution function. This model is mostly applied to GWAS dataset [1, 9].

Binary effect model (BE) [1]:

BE is a new type of random effect model of meta-analysis that captures studies with or without an effect together. This model is the weighted sum of z-scores method where the *m-values*, the posterior probability that effect exists in each study of a meta-analysis, are incorporated into the weights. More likely, it assigns more weight to studies predicted to have an effect and lesser weight to the studies predicted not to have an effect. So let $z_j = \frac{T_j}{\sqrt{v_j}}$ be the z-score of the *jth* study. Just as the test statistics of FE model can be written in the form of weighted sum of z-score, that is,

$$S_{FE} = \frac{\sum_{j=1}^{J} \sqrt{w_j} z_j}{\sqrt{\sum_{j=1}^{J} w_j}}.$$

The binary effect model statistics is given as

$$S_{BE} = \frac{\sum_{j=1}^{J} m_j \sqrt{w_j} z_j}{\sqrt{\sum_{j=1}^{J} m_j^2 w_j}},$$

where the weight $\sqrt{w_j} \approx \sqrt{Np(1-p)}$ with N the sample size and p the effect size (minor allele) frequency and $\sqrt{w_j} \approx \sqrt{N}$ when the effect size is the same between studies. m_j is the corresponding m-value of study j and it is given as

$$m_j = \frac{\pi N(T_j; \mu, v_j)}{(1-\pi)N(T_j; 0, v_j) + \pi N(T_j; \mu, v_j)}$$

where π is the prior probability that each study will have an effect, i.e.,

$$\pi = P(X_j = 1)$$

where X_j is a random variable such that

$$X_j = \begin{cases} 1, & \text{if study} j \text{ has an effect} \\ 0, & \text{if if study} j \text{ does not have an effect} \end{cases}$$

and an assumption of a beta prior is made on π, that is, $\pi \sim Beta(\alpha, \beta)$ where α and β can be chosen but most likely $\alpha = 1$ and $\beta = 1$. The other terms are explained thus, if there is no effect in study j,

$$P(T_j|noeffect) = N(T_j; 0, v_j),$$

and

$$P(T_j|effect) = N(T_j; \mu, v_j),$$

if there is effect in study j. $N(\cdot)$ is the probability density function of a normal distribution and μ is the unknown true effect size. This model is mostly applied to GWAS dataset [1].

Bayesian meta-analysis:

Bayesian approach meta-analysis assumes that a given population cluster with the same ethnic group will possibly shares the same effect size , but there is difference in effect sizes among different population clusters. Assume the observed effect size of the jth study $b_j \sim N(\beta_j, s_j)$ where s_j is the corresponding standard error and β_j is the population-specific effect for the jth population cluster. Let M_0 be the null hypothesis of no association and M_1 the alternative hypothesis in a Bayesian framework. The evidence of association can be assessed by means of Bayes' factor

$$\Lambda = \frac{f(\mathbf{b}, \mathbf{s}|M_1)}{f(\mathbf{b}, \mathbf{s}|M_0)}$$

where

$$f(\mathbf{b}, \mathbf{s}|M) = \int_\theta f(\mathbf{b}, \mathbf{s}|\theta) f(\theta|M) \partial\theta$$

is the marginal likelihood of the observed effect size under the model M with θ denoting the unknown model parameter which includes the population specific effect β and hyper-parameters relating to prior distribution as discussed below. The likelihood

$$f(\mathbf{b}, \mathbf{s}|\theta) = f(\mathbf{b}, \mathbf{s}|\beta) = \prod_{j=1}^{J} f(b_j, s_j|\theta_j),$$

and

$$f(b_j, s_j|\theta_j) \propto \frac{1}{s_j} \exp\left[-\frac{(b_j - \beta_j)^2}{2s_j^2}\right]$$

However, the population specific allelic effect β is determined by assigning the populations to ethnic cluster, and this is based on the assumption that effect sizes are likely to vary among different ethnic groups. Hence,

$$f(b_j, s_j|\theta_j) = f(b_j, s_j|K, \mathbf{C}, \psi) \propto \frac{1}{s_j} \exp\left[-\frac{(b_j - \sum_{k=1}^{K} T_{jk}\psi_k)^2}{2s_j^2}\right]$$

where $\mathbf{C} = \{C_1, C_2, \ldots, C_K\}$ is the cluster center, ψ is the corresponding allelic effect and the tessellation $T_{jk} = 1$ if population P_j is assigned to the cluster with center C_k and 0, otherwise.

The prior density function $f(\theta|M_0)$, of parameters under null model M_0 is given by,

$$f(\theta|M_0) = \begin{cases} 1, & \text{if } \beta = 0 \\ 0, & \text{otherwise} \end{cases}$$

while under the alternative model, M_1,

$$f(\theta|M) \propto f(K)(N-K)!\frac{\exp(-\sigma)}{\sigma}\prod_{k=1}^{K}\exp\left(-\frac{(\psi_k-\mu)^2}{2\sigma^2}\right)$$

where

$$f(K) = \begin{cases} \frac{1}{2}, & \text{if } K = 1 \\ \frac{2^{N-1}}{2^K(2^{N-1}-1)}, & \text{otherwise} \end{cases}$$

N is the total number of different populations and the cluster allelic effect have a prior $N(\mu, \sigma)$ distribution, independent of $\mathbf{C}$, where μ has a prior uniform distribution and σ has a prior exponential distribution with expectation 1. Since the marginal likelihood $f(\mathbf{b}, \mathbf{s}|M)$ can't be evaluated directly, the joint posterior density of

$$f(\theta|\mathbf{b}, \mathbf{s}, M) \propto f(\mathbf{b}, \mathbf{s}|\theta)f(\theta|M)$$

is considered instead and it is approximated using the Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithm.

This approach performs better compared to fixed-effects and random effect meta-analysis, especially in terms of power to detect association, and localization of the causal variant, over a range of models of heterogeneity between ethnic groups and also has increased power and mapping resolution when the similarity in allelic effects between populations is well captured by their relatedness. This model is mostly applied to GWAS dataset [7].

RankProd (RP) & RankSum (RS) methods:

Suppose we have a total of G genes in a differential expression data across J replicated experiments. Let $r_{i,j}$ be the position of the i^{th} gene in the j^{th} replicate experiment in a list ordered according to fold changes (in a decreasing/increasing order if we are interested in up-regulated/down-regulated genes respectively). On one hand, considering a single replicate, it follows that under the null hypothesis (no differentially expressed genes present in the dataset), the rank of a gene in the list generated comes from a uniform distribution, that is,

$$P(r_i = g) = \frac{1}{G}$$

where $g \in \{1, \ldots, G\}$. On the other hand, while considering J replicates, it is expected that not all replicates will have same gene at the top of there list. Thus, the probability of a gene being ranked first in each replicate is $\frac{1}{G^J}$.

Hence, the rank product (RP) statistics for the i^{th} gene is defined as the geometric mean of all the rank of genes obtained in each replicate. That is,

$$RP_i = \left(\prod_{j=1}^{J} r_{i,j}\right)^{\frac{1}{J}}$$

Also, the rank sum (RS) statistics is defined as the arithmetic mean of all the ranks. That is,

$$RS_i = \frac{1}{J}\sum_{j=1}^{J} r_{i,j}$$

In the case whereby the datasets we are analysing is an unpaired dataset. For example having treatment (T) vs control (C) experiment. The RP/RS is performed using the following algorithm. Suppose we have n studies with (n_{iT}, n_{iC}) replicates where $i = 1, 2, \ldots, J$.

1. Form J pairwise comparison, i.e., $J_i = n_{iT} \times n_{iC}$ comparison and evaluate J list of pairwise ratios within each study of their fold changes, i.e., $\frac{T_{ip}}{C_{iq}}$ where $p = 1, 2, \ldots, n_{iT}$ and $q = 1, 2, \ldots, n_{iC}$.

2. Rank the ratios within each comparison with the largest $\implies$ *rank 1*, where r_{gi} is the rank of the g^{th} gene in the i^{th} comparison.

3. Determine the RP/RS for each gene as;

$$RP_g = \left(\prod_i r_{gi} \right)^{\frac{1}{J}},$$

and

$$RS_g = \frac{(\sum_i r_{gi})}{J},$$

respectively, with $J = J_1 + J_2 + \cdots + J_n$.

4. Independently permute expression value within each single array relative to gene ID, repeat step (a)-(c) and obtain the statistic $RP_g^{(b)}$

5. Repeat step (d) B times, form reference distribution with $RP_g^{(b)}$, $(b = 1, \ldots, B)$ and $(g = 1, \ldots, G)$, determine p-value and FDR associated with each gene using the formula given below:

$$p_g = \frac{\sum_b \sum_g I\left(|RP_g^{(b)}| \leq RP_g\right)}{G \cdot B}$$

$$FDR_g = \frac{\sum_b \sum_g I\left(|RP_g^{(b)}| \leq RP_g\right)}{B \sum_g I\left(|RP_g^{(b)}| \leq RP_g\right)}$$

where $I(\cdot)$ is an indicator function. This model is mostly applied to gene expression dataset [3, 4]

Heterogeneity

Heterogeneity is the difference in effect sizes between studies. This can be caused by so may factors including; *genetic factor* as a result of difference in the populations between studies, *environmental factor* due to using subjects from different regions, *design factor* which causes statistical heterogeneity when the true effect size is invariant, heterogeneity shown by collected markers as a result of difference in the linkage disequilibrium structures between studies and also heterogeneity caused by different imputation accuracies and different genotyping errors when studies use different genotyping platforms.

Once this factors has been identified in meta-analysis result, it will be easier to know what approach to take by correctly interpreting the cause of heterogeneity which may lead to a better understanding of the disease mechanism in the case of genetic or environmental factor or, designing a more effective replication study in the case of statistical heterogeneity caused by the design factor. The existence of true heterogeneity among studies can be determined using different test statistics such as, the Cochran's Q test statistics [2, 5] , that is,

$$Q = \sum_{j=1}^{J} w_j (T_j - \overline{T})^2$$

where J is the number of studies, w_j is the inverse of the within-study variance for the jth study, T_j is the effect size estimate of study j and $\overline{T} = \frac{\sum_{j=1}^{J} w_j T_j}{\sum_{j=1}^{J} w_j}$ is the inverse-variance-weighted effect-size estimator of the true effect size. Q test statistic follows a χ^2 distribution with degree of freedom $df = J - 1$ under the assumption of genetic homogeneity. Another alternative heterogeneity test statistics which were reported to be more robust than Q when considering small number of studies are; the I^2 index [2, 5] ,

$$I^2 = \frac{[Q - (J-1)]}{Q} \times 100\%$$

$$I^2 = \begin{cases} \frac{[Q-(J-1)]}{Q} \times 100\%, & \text{if } Q > df \\ 0, & \text{if } Q \leq df \end{cases}$$

the H statistics [2],

$$H = \sqrt{\frac{Q}{J-1}}$$

and the R statistics [2],

$$R = \sqrt{\frac{\sum w_j}{\sum w_j^*}} = \sqrt{\frac{\sum w_j}{\sum (w_j^{-1} + \tau^2)}}$$

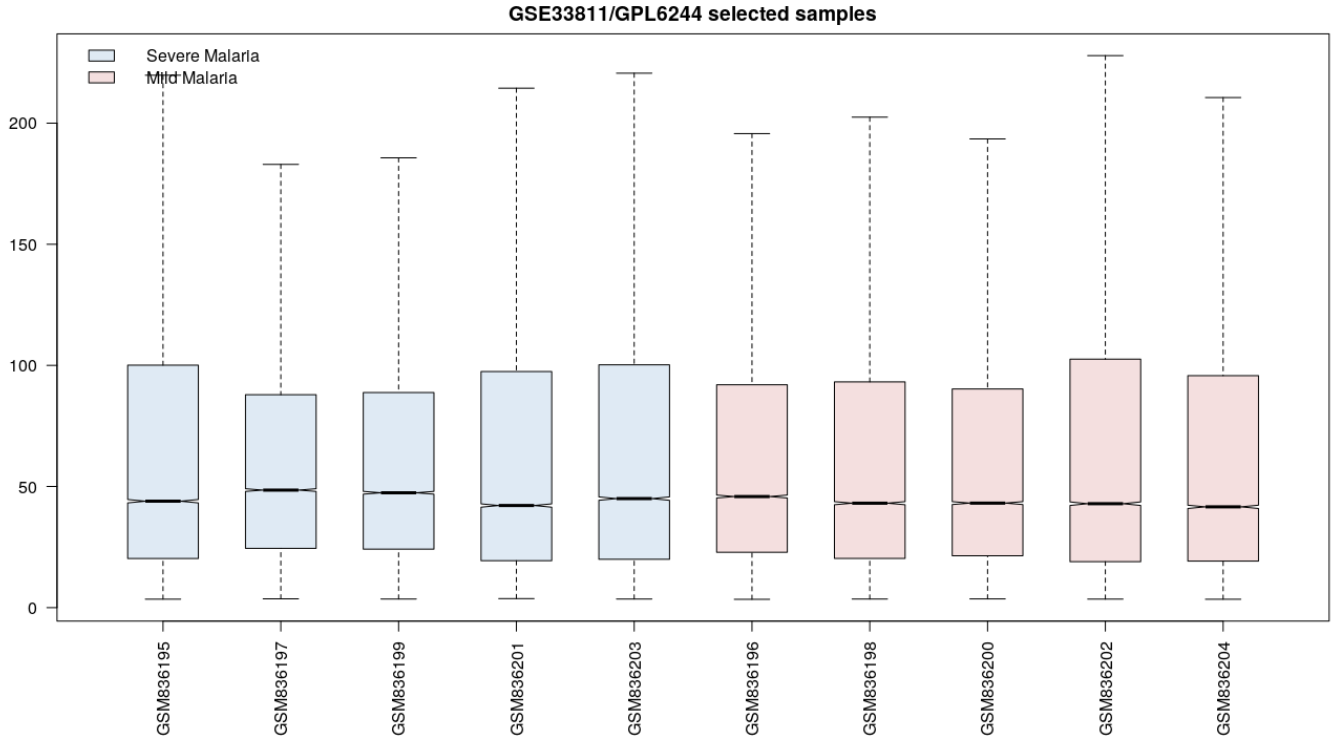
where the between-study variance

$$\tau^2 = \begin{cases} \frac{Q-df}{c}, & \text{if } Q > df \\ 0, & \text{if } Q \leq df \end{cases}$$

where

$$c = \sum w_j - \frac{\sum w_j^2}{\sum w_j}.$$

Box plot of Malaria Study


GSE33811/GPL6244 selected samples
Severe Malaria
Mild Malaria
200
150
100
50
0
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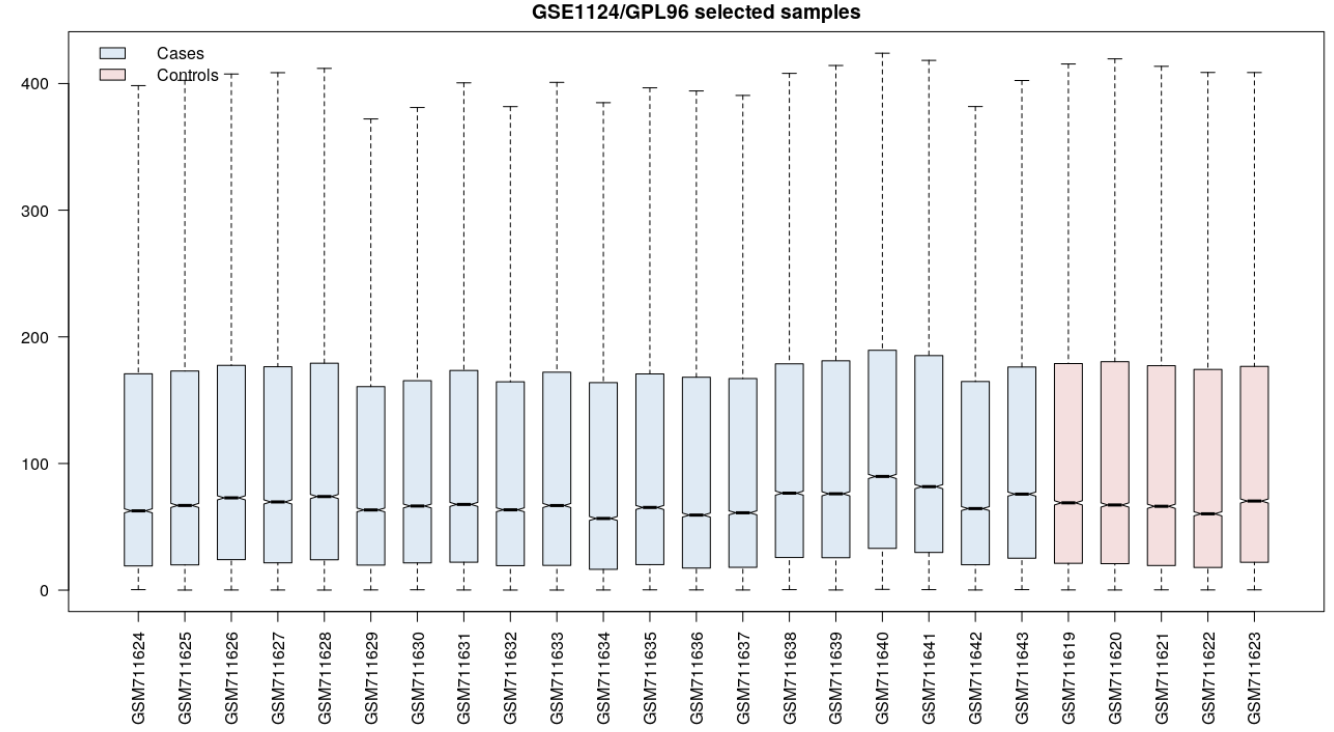
GSE1124/GPL96 selected samples
Cases
Controls
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300
200
100
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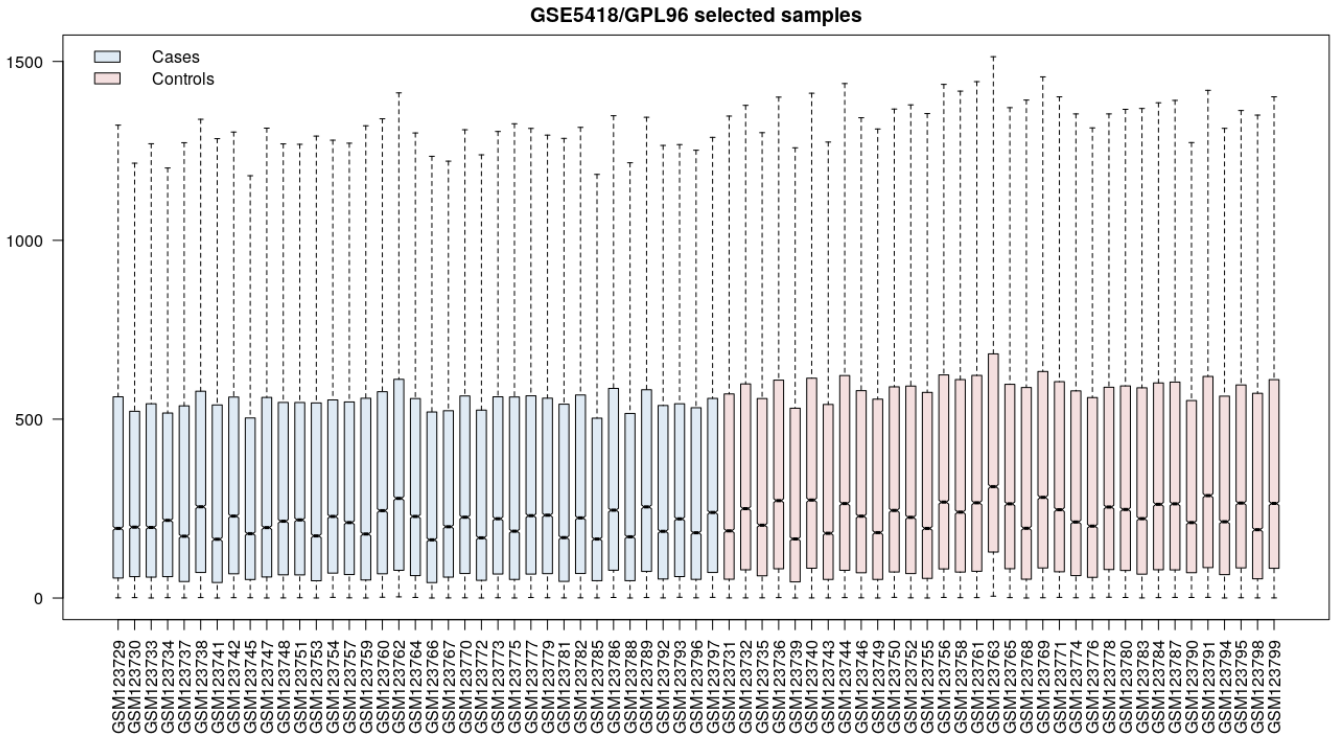
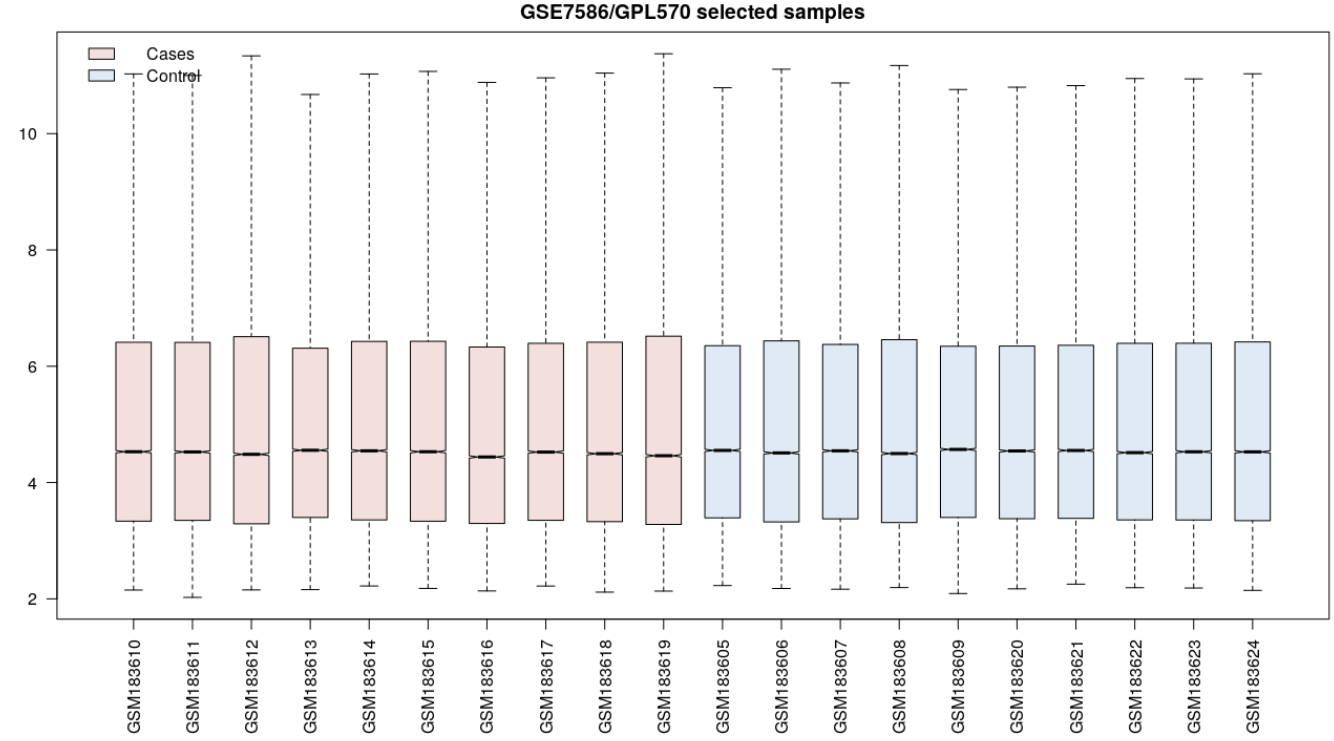
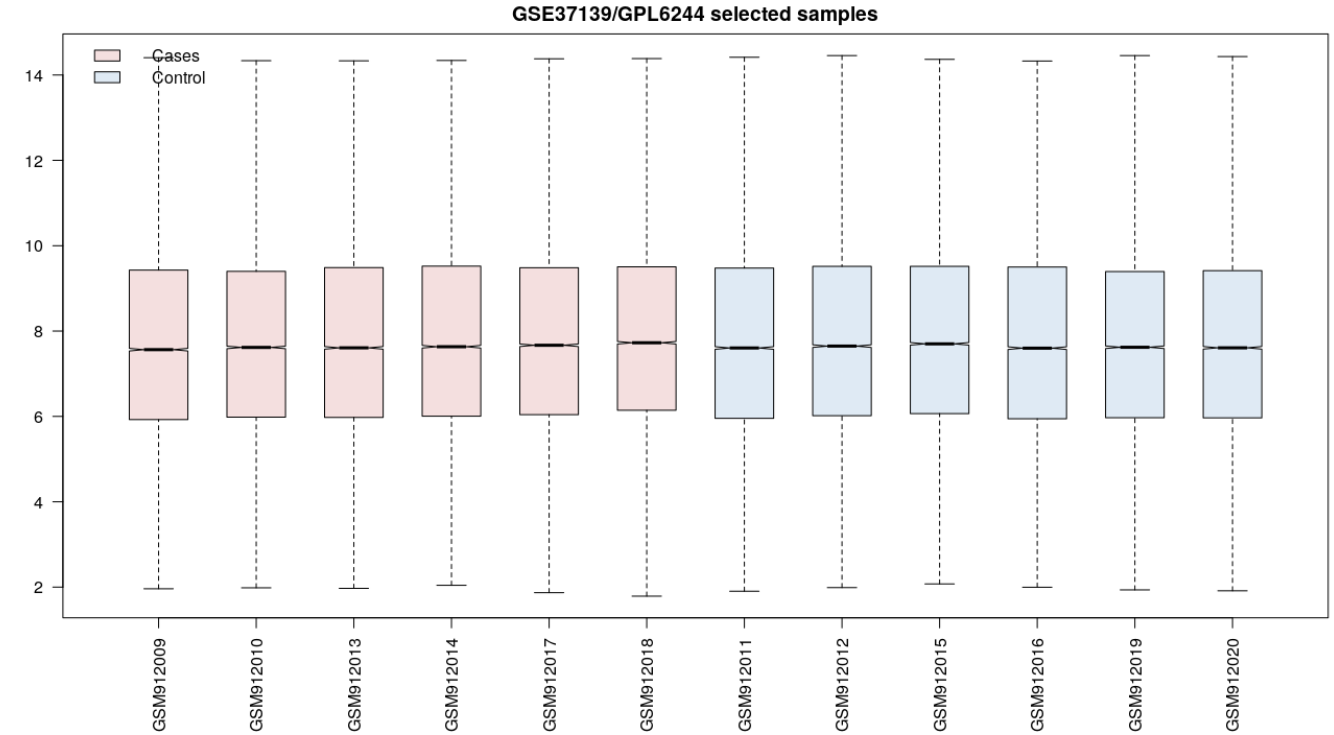




Box plot of Breast Cancer Study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Cases
Control
14
12
10
8
6
4
2
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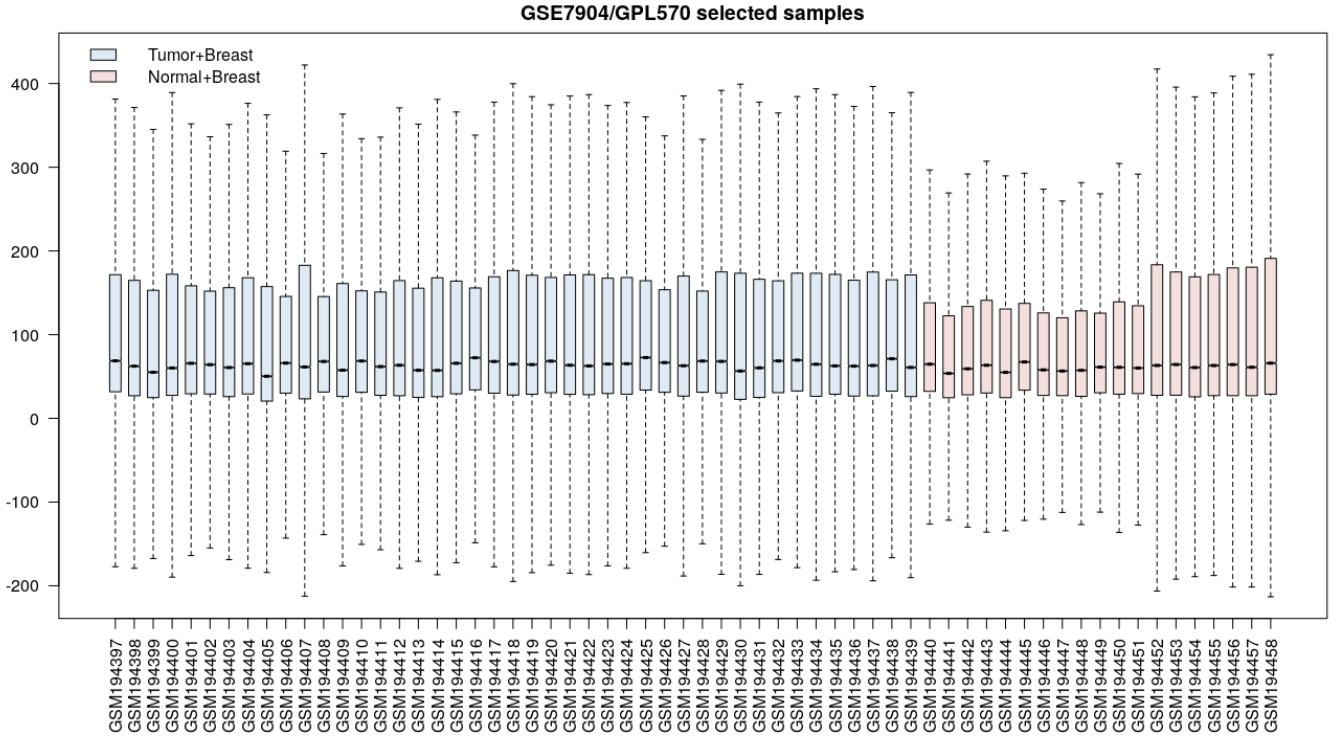
GSE7904/GPL570 selected samples
Tumor+Breast
Normal+Breast
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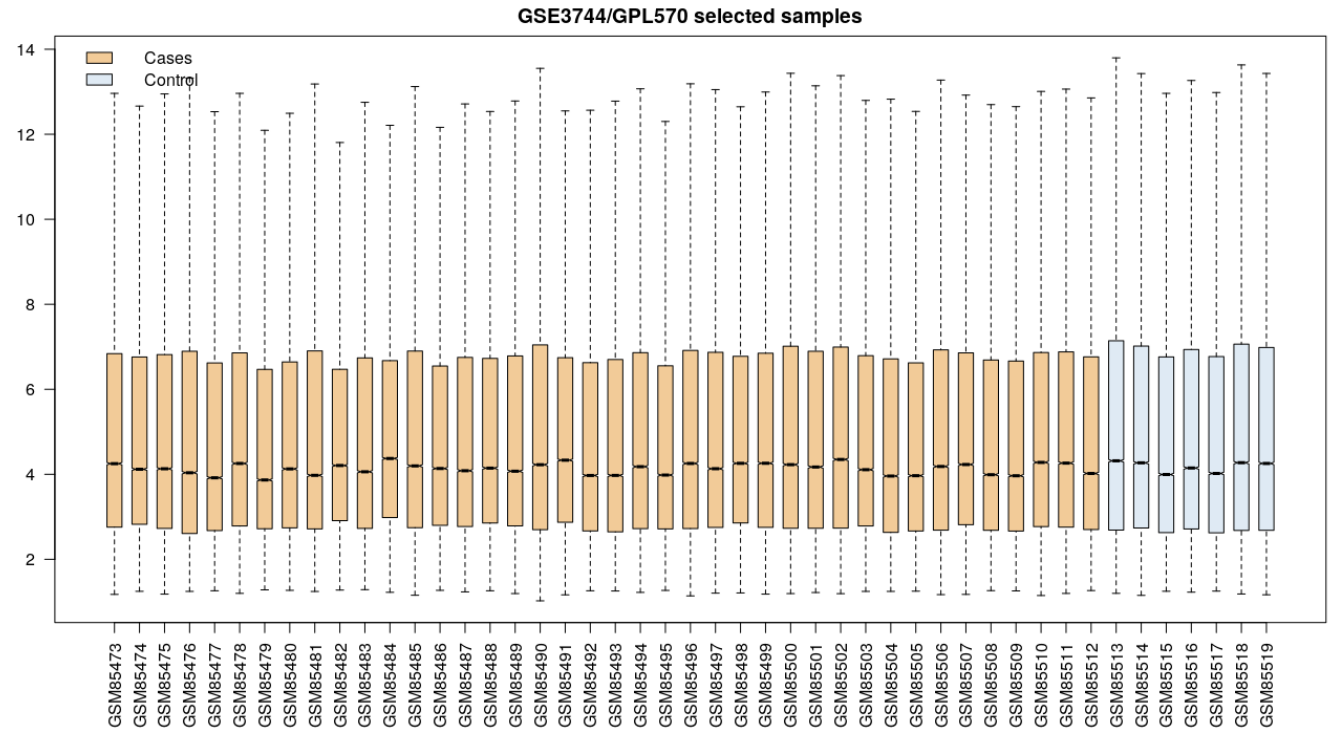
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Cases
Control
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8
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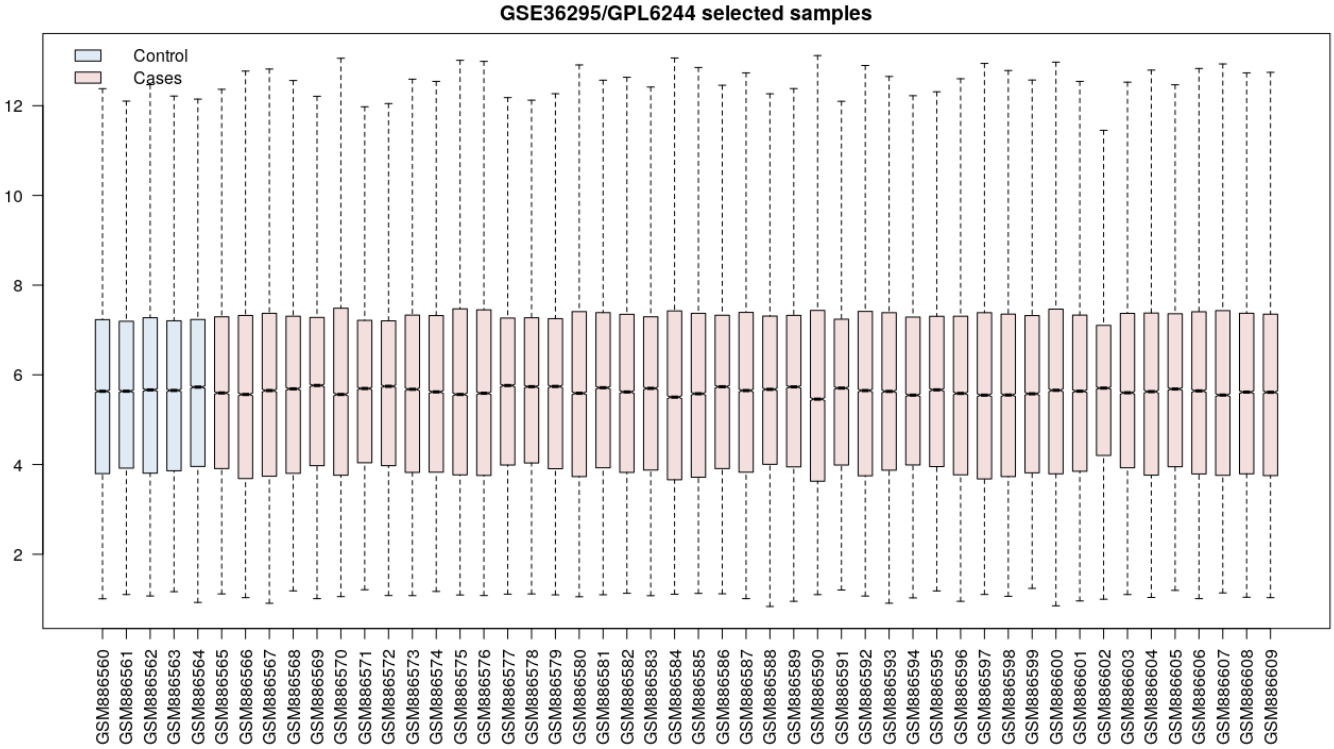
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Control
Cases
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