

## Supplementary Material

Main document: *A compositional model to assess expression changes from single-cell RNA-seq data*

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This supplement is organized to match the sectioning of the main document. In summary,

### 1. Introduction

- DEC vs EC data analysis
- R package

### 2. Modeling

- Data Structure, Sampling Model, and Parameters  
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# 1 Introduction

## 1.1 DEC vs EC

## 1.2 R package

\*\*on scDDboost, web page, etc\*\*

# 2 Modeling

## 2.1 Data Structure, Sampling Model, and Parameters

Proof of Theorem 2.

## 2.2 Method Structure and Clustering

### 2.2.1 EBSeq

Suppose we have  $K$  subtypes, let  $X_g^I = X_{g,1}^I, \dots, X_{g,S_1}^I$  denote transcripts at gene  $g$  from subtype  $I, I = 1, \dots, K$ . In the EBSeq model it assumed that counts within subtype  $I$  are distributed as Negative Binomial:  $X_{g,s}^I | r_{g,s}, q_g^I \sim NB(r_{g,s}, q_g^I)$  Where

$$P(X_{g,s}^I | r_{g,s}, q_g^I) = \binom{X_{g,s} + r_{g,s} - 1}{X_{g,s}} (1 - q_g^I)^{X_{g,s}} (q_g^I)^{r_{g,s}}$$

and  $\mu_{g,s}^I = r_{g,s}(1 - q_g^I) / q_g^I$ ;  $\sigma_{g,s}^I = r_{g,s}(1 - q_g^I) / (q_g^I)^2$ .

The EBSeq model assumed a prior distribution on  $q_g^I : q_g^I | \alpha, \beta^I \sim \text{Beta}(\alpha, \beta^I)$ . The hyperparameter  $\alpha$  is shared by all the isoforms and  $\beta^I$  is  $I_g$  specific. We further assume that  $r_{g,s} = r_{g,0} * l_s$  where  $r_{g,0}$  is an isoform specific parameter common across subtypes and  $r_{g,s}$  depends on it through the sample-specific normalization factor  $l_s$

What we are interested at those  $K$  groups comparison is the expression pattern, through EBSeq modeling we are able to obtain posterior probabilities over

$$M_{g,\pi} = \{\theta \in \Theta : \mu_{g,k} = \mu_{g,k'} \iff k, k' \in b, b \in \pi\}.$$

For any partition  $\pi$  of  $K$  elements.

For example  $K = 3$ , there are 5 expression pattern,  $P_1, P_2, \dots, P_5$

$$\begin{aligned} P1 : q_g^1 &= q_g^2 = q_g^3 \\ P2 : q_g^1 &= q_g^2 \neq q_g^3 \\ P3 : q_g^1 &\neq q_g^2 = q_g^3 \\ P4 : q_g^1 &= q_g^3 \neq q_g^2 \\ P5 : q_g^1 &\neq q_g^2 \neq q_g^3 \text{ and } q_g^1 \neq q_g^3 \end{aligned}$$

Under the assumption that two groups  $I$  and  $J$  share the same  $q_g$  we can pool the counts from the two groups by viewing them come from same distribution i.e.  $X_g^{I,J}|r_{g,s}, q_g \sim NB(r_{g,s}, q_g)$ ,  $q_g|\alpha, \beta^{I_g} \sim Beta(\alpha, \beta^{I_g})$  and obtained the prior predictive function  $f_0^{I_g}(X_g^{I,J}) = \int_0^1 P(X_g^{I,J}|r_{g,s}, q_g) * P(q_g|\alpha, \beta^{I_g}) dq_g = \left[ \prod_{s=1}^S \binom{X_{g,s} + r_{g,s} - 1}{X_{g,s}} \right] \frac{Beta(\alpha + \sum_{s=1}^S r_{g,s}, \beta^{I_g} + \sum_{s=1}^S X_{g,s})}{Beta(\alpha, \beta^{I_g})}$ . Consequently, we have prior predictive function for  $P1, \dots, P5$  as

$$\begin{aligned} P1 : q_g^1 &= q_g^2 = q_g^3 \\ P2 : q_g^1 &= q_g^2 \neq q_g^3 \\ P3 : q_g^1 &\neq q_g^2 = q_g^3 \\ P4 : q_g^1 &= q_g^3 \neq q_g^2 \\ P5 : q_g^1 &\neq q_g^2 \neq q_g^3 \text{ and } q_g^1 \neq q_g^3 \end{aligned}$$

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$$\begin{aligned} g_1^{I_g}(X_g^{1,2,3}) &= f_0^{I_g}(X_g^{1,2,3}) \\ g_2^{I_g}(X_g^{1,2,3}) &= f_0^{I_g}(X_g^{1,2}) f_0^{I_g}(X_g^3) \\ g_3^{I_g}(X_g^{1,2,3}) &= f_0^{I_g}(X_g^1) f_0^{I_g}(X_g^{2,3}) \\ g_4^{I_g}(X_g^{1,2,3}) &= f_0^{I_g}(X_g^{1,3}) f_0^{I_g}(X_g^2) \\ g_5^{I_g}(X_g^{1,2,3}) &= f_0^{I_g}(X_g^1) f_0^{I_g}(X_g^2) f_0^{I_g}(X_g^3) \end{aligned}$$

Then the marginal distribution of counts  $X_g^{1,2,3}$  is  $\sum_{k=1}^5 p_k g_k^{I_g}(X_g^{1,2,3})$ , where proportion parameters  $p_k$  satisfying  $\sum_{k=1}^5 p_k = 1$  and are estimated by EM algorithm. Thus, the posterior probability of an expression pattern  $k$  is obtained by:

$$\frac{p_k g_k^{I_g}(X_g^{1,2,3})}{\sum_{k=1}^5 p_k g_k^{I_g}(X_g^{1,2,3})}$$

## 2.2.2 modalClust

### Product Partition Model

Let  $X = (X_1, X_2, \dots, X_n)$  be  $n$  one dimension observed data, given a partition for the data  $\pi = \{S_1, \dots, S_q\}$ , where  $S_i$  are disjoint subsets of  $\{1, 2, \dots, n\}$  and  $\bigcup_{i=1}^q S_i = \{1, 2, \dots, n\}$ . The likelihood for  $X$

satisfying such partition is

$$p(X|\pi) = \prod_{i=1}^q f(X_{S_i})$$

where  $X_{S_i}$  is the vector of observations corresponding to the items of component  $S_i$ , The component likelihood  $f(X_S)$  is defined for any non-empty component  $S$  and can take any form. The partition  $\pi$  is the only parameter we are interested at. Any other parameters that may have been involved in the model have been integrated over their prior.

The prior distribution for a partition  $\pi$  is also taken as a product form. We use the partition that maximize the posterior  $p(\pi|X) \propto p(X|\pi)p(\pi)$  as the estimated clustering of  $X$ .

Dahl demonstrated by some choice of  $f$  and prior of  $\pi$ , we can reduce the time complexity of finding the MAP partition from factorial( $n$ ) to  $O(n^2)$  ?, And the crucial condition for  $f$  is that if  $X_{S_1}$  and  $X_{S_2}$  are overlapped in the sense that  $\min\{X_{S_2}\} < \max\{X_{S_1}\} < \max\{X_{S_2}\}$  or  $\min\{X_{S_1}\} < \max\{X_{S_2}\} < \max\{X_{S_1}\}$ ,  $X_{S_1^*}$  and  $X_{S_2^*}$  be the sets of swapping one pair of those overlapped terms and keep the other unchanged. Then  $f(X_{S_1})f(X_{S_2}) \leq f(X_{S_1^*})f(X_{S_2^*})$ . Under such condition, we know that possible MAP candidates must be those partition that for any two subgroups of data, all the data from subgroup1 has to be either greater or smaller than all the data from subgroup2.

In Poisson-Gamma Model we assuming:

$$\begin{aligned} X_i|\pi, \lambda &\sim \text{Poisson}(X_i|\lambda_1 \mathbf{I}\{i \in S_1\} + \dots + \lambda_q \mathbf{I}\{i \in S_q\}) \\ \pi &\sim p(\pi) \\ \lambda_j &\sim \text{Gamma}(\alpha_0, \beta_0) \end{aligned}$$

where  $p(\pi) \propto \prod_{i=1}^q \eta_0 \Gamma(|S_i|)$ . Integrate out  $\lambda$ ,  $f(X_S)$  is obtained as:

$$f(X_S) = \frac{\beta^\alpha}{(|S| + \beta)^{\sum_{i \in S} X_i + \alpha}} \frac{\Gamma(\sum_{i \in S} X_i + \alpha)}{\Gamma(\alpha)} \frac{1}{\prod_{i \in S} X_i}$$

$f(X_S)$  still satisfying the condition mentioned

*Proof.* if  $X_{S_1}$  and  $X_{S_2}$  are overlapped, without loss of generality, we assume  $\min\{X_{S_2}\} < \max\{X_{S_1}\} < \max\{X_{S_2}\}$ , and we swap  $\max\{X_{S_1}\}$  with  $\min\{X_{S_2}\}$  and keep the rest unchanged or we could also swap  $\max\{X_{S_1}\}$  with  $\max\{X_{S_2}\}$ . We denote the new set forming by swap of  $\max\{X_{S_1}\}$  with  $\min\{X_{S_2}\}$  as  $S_1^*$  and  $S_2^*$  and swap of  $\max\{X_{S_1}\}$  with  $\max\{X_{S_2}\}$  as  $S_1^{**}, S_2^{**}$  accordingly.

Then we need to show at least one of the following happens

$$f(X_{S_1^*})f(X_{S_2^*}) \geq f(X_{S_1})f(X_{S_2}) \quad (1)$$

$$f(X_{S_1^{**}})f(X_{S_2^{**}}) \geq f(X_{S_1})f(X_{S_2}) \quad (2)$$

Let  $a = \max\{X_{S_1}\}$ ,  $b = \min\{X_{S_2}\}$  and  $c = \max\{X_{S_2}\}$ .  $h_1 = \sum_{i \in S_1} X_i - a$  and  $h_2 = \sum_{i \in S_2} X_i - b$ ,  $n_1$  and

$n_2$  are the number of elements in  $S_1$  and  $S_2$ . Then

$$\begin{aligned}
f(X_{S_1^*})f(X_{S_2^*}) &\geq f(X_{S_1})f(X_{S_2}) \\
&\iff \\
\frac{\Gamma(h_1 + a + \alpha)}{(n_1 + \beta)^{h_1 + a + \alpha}} \frac{\Gamma(h_2 + b + \alpha)}{(n_2 + \beta)^{h_2 + b + \alpha}} &\leq \frac{\Gamma(h_2 + a + \alpha)}{(n_2 + \beta)^{h_2 + a + \alpha}} \frac{\Gamma(h_1 + b + \alpha)}{(n_2 + \beta)^{h_1 + b + \alpha}} \\
&\iff \\
\frac{\Gamma(h_1 + a + \alpha)}{\Gamma(h_1 + b + \alpha)} \frac{\Gamma(h_2 + b + \alpha)}{\Gamma(h_2 + a + \alpha)} &\leq \left(\frac{n_1 + \beta}{n_2 + \beta}\right)^{a-b}
\end{aligned}$$

Left hand side of above formula is  $\text{LHS}_1 = \frac{(h_1 + b + \alpha) \dots (h_1 + a - 1 + \alpha)}{(h_2 + b + \alpha) \dots (h_2 + a - 1 + \alpha)}$  by the property of Gamma function and  $X_i$  are integer.

Similarly,

$$\begin{aligned}
f(X_{S_1^{**}})f(X_{S_2^{**}}) &\geq f(X_{S_1})f(X_{S_2}) \\
&\iff \\
\frac{\Gamma(h_2 + c + \alpha)}{\Gamma(h_2 + a + \alpha)} \frac{\Gamma(h_1 + a + \alpha)}{\Gamma(h_1 + c + \alpha)} &\leq \left(\frac{n_2 + \beta}{n_1 + \beta}\right)^{c-a}
\end{aligned}$$

Left hand side of above formula is  $\text{LHS}_2 = \frac{(h_2 + a + \alpha) \dots (h_2 + c - 1 + \alpha)}{(h_1 + a + \alpha) \dots (h_1 + c - 1 + \alpha)}$

If  $h_1 \leq h_2$ , then  $\text{LHS}_1 \leq \left(\frac{h_1 + a - 1 + \alpha}{h_2 + a - 1 + \alpha}\right)^{a-b}$  and  $\text{LHS}_2 \leq \left(\frac{h_2 + c - 1 + \alpha}{h_1 + c - 1 + \alpha}\right)^{a-b}$

So if  $\frac{h_1 + a - 1 + \alpha}{h_2 + a - 1 + \alpha} \leq \frac{n_1 + \beta}{n_2 + \beta}$  then (12) holds, if  $\frac{h_2 + c - 1 + \alpha}{h_1 + c - 1 + \alpha} \leq \frac{n_1 + \beta}{n_2 + \beta}$  then (13) holds

We multiply those two inequalities, we found that  $\frac{h_1 + a - 1 + \alpha}{h_2 + a - 1 + \alpha} * \frac{h_2 + c - 1 + \alpha}{h_1 + c - 1 + \alpha} = \frac{h_1 + a - 1 + \alpha}{h_1 + c - 1 + \alpha} * \frac{h_2 + c - 1 + \alpha}{h_2 + a - 1 + \alpha} \leq 1$  as  $c > a$  and  $h_1 \leq h_2$  But  $\frac{n_1 + \beta}{n_2 + \beta} * \frac{n_1 + \beta}{n_2 + \beta} = 1$ . At least one equality holds, consequently at least one of (12) and (13) holds.

Similar proof for the case  $h_1 > h_2$ .

□

### 2.2.3 Randomized K-means

### 2.2.4 Selecting K

In order to determine the number of clusters, we consider the change of  $validity = \frac{\text{intra}}{\text{inter}}$  defined in ?, where  $\text{intra} = \frac{1}{N} \sum_{i=1}^K \sum_{x \in C_i} ||x - z_i||^2$ ,  $\text{inter} = \text{mean}(|z_i - z_j|^2), i = 1, 2, \dots, K-1, j = i+1, \dots, K$  and  $z_i$  is the center (medoids) of cluster  $i$ . **intra** is the average of distance of a point to its corresponding cluster center, which measures the compactness of clusters. We made a small change here, in original paper **inter** was defined as minimum distance between medoids, we use average instead for the purpose of getting a smoother quantity. **inter** is the average distance of two cluster centers, which measures the separation between clusters. We want to have a small intra-cluster distance and a big inter-cluster distance, consequently we want to minimize the *validity*. From empirical study, we constantly observe a monotone decreasing relation between number of clusters and *validity*. However this quantity stabilize

when  $K$  is sufficiently large. The stopping rule for searching  $K$  is when  $|validity_K - \min(validity_K)| < \epsilon$  is satisfied.

### 2.3 Double Dirichlet Mixture

On properties 1-8, and Theorem 3

On the double Dirichlet masses, using notation as in Section 2.3 we have density functions:

$$p_\pi(\phi, \psi) = q_\pi(\Phi_\pi, \Psi_\pi) \prod_{b \in \pi} [p(\tilde{\phi}_b) p(\tilde{\psi}_b)]$$

with

$$q_\pi(\Phi_\pi, \Psi_\pi) = \frac{\Gamma(\sum_{b \in \pi} \beta_b)}{\prod_{b \in \pi} \Gamma(\beta_b)} \left[ \prod_{b \in \pi} \Phi_b^{\beta_b - 1} \right] 1[\Phi_\pi = \Psi_\pi]$$

and

$$p(\tilde{\phi}_b) = \frac{\Gamma(\sum_{k \in b} \alpha_k)}{\prod_{k \in b} \Gamma(\alpha_k)} \prod_{k \in b} \tilde{\phi}_k^{\alpha_k - 1}, \quad p(\tilde{\psi}_b) = \frac{\Gamma(\sum_{k \in b} \alpha_k)}{\prod_{k \in b} \Gamma(\alpha_k)} \prod_{k \in b} \tilde{\psi}_k^{\alpha_k - 1}.$$

## 3 Numerical Experiments

### 3.1 Synthetic Data

details on splatter study

...maybe full reporting of ROC's ...

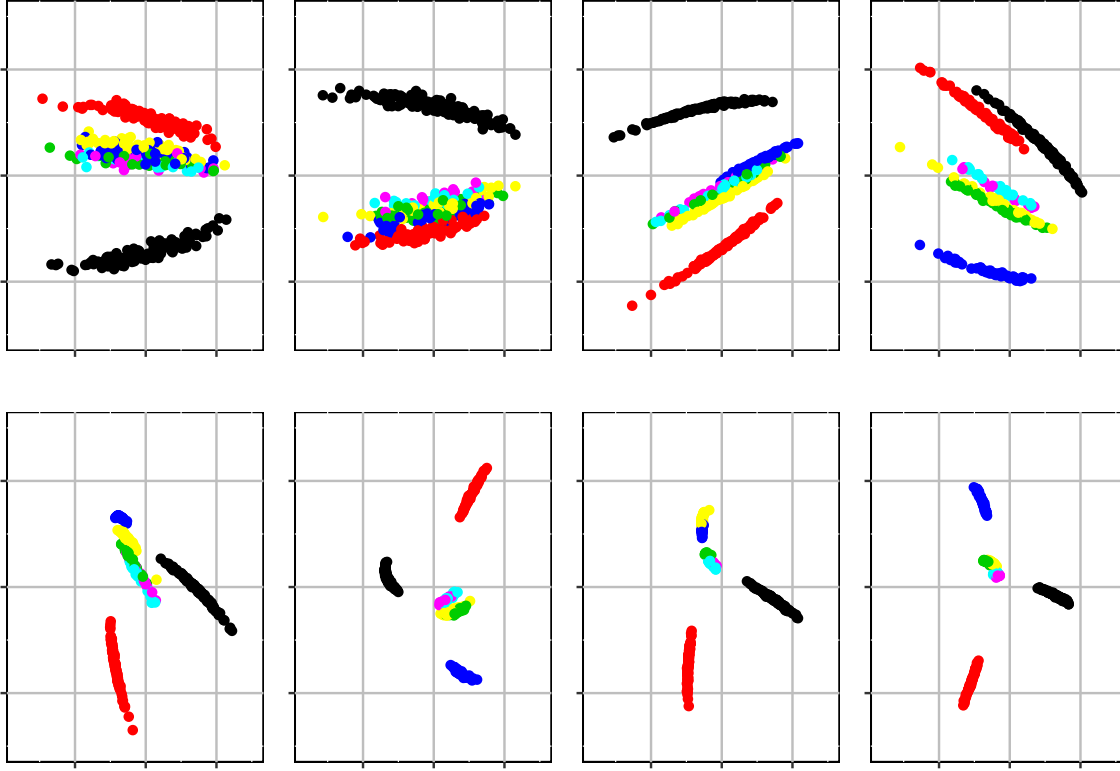
### 3.2 Empirical Study

Data sets

### 3.3 Null cases

Although bulk methods seems to be the most powerful one, we found it also has a higher false discovery rate comparing to single cell methods. We validate false discovery rate on ten null datasets from table 1. For each null dataset, we randomly split the cells from one condition into two subsets and test difference of gene expression between those subsets. Since the two subsets of cells actually came from same condition, there should not be any differential distributed genes, any positive call would be a false positive. We repeat the random split and testing for five times on each null data set. We evaluate the type I error control for the methods returning nominal p-values, by recording the fraction of genes(with a valid p-value) that are assigned a nominal p-value below 0.05 (Fig 6).

scDDboost could control FDR since we assume cells are sampled from population composed of different subtypes. Cells from one subtype are equal likely to be assigned to either one of the two subsets. Consequently, it is very likely that proportions of subtypes remain unchanged among the two subsets.



**Figure 1:** first two principal components of transcripts under different parameters for simulated data. Different parameters resulted in different degree of separation of subtypes. We have 4 different settings for hyper-parameters of simulation, each setting has 2 replicates

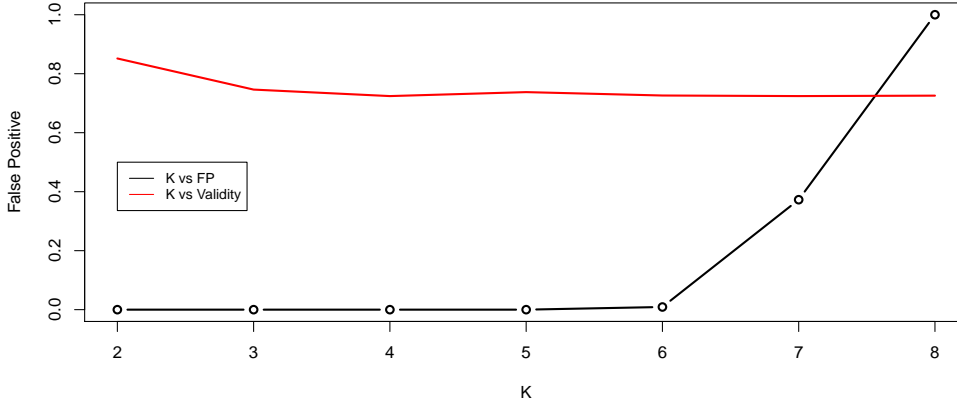
## 4 ?Other

### 4.1 Effect of $K$ on posterior probabilities

Number of subtypes  $K$  is a crucial factor controlling the accuracy of our modeling. Too small  $K$  may end up in an underfit such that cells within same subtype can still be very different, mean expression change among subtypes is incapable to capture the distribution change for some genes and consequently reducing the power of scDDBoost. Too big  $K$  may end up in an overfit such that two subtypes can be very similar, given we have fixed number of samples (cells), allowing more clusters will introduce many patterns (both for mean expression change and proportion change) to infer. Also notice the limitation of DDM model (see section 4), overestimating  $K$  in scDDBoost may lose FDR control (Fig7).

Data set	Conditions	Number of cells/condition	Organism	Ref	K
GSE52529	T0 vs T24	96,96	human	(?)	6
GSE57872	patient1 vs patient2	192,96	human	(?)	7
GSE48968-GPL13112	BMDC (2h LPS stimulation) vs 6h LPS	96,96	mouse	(?)	8
GSE60749-GPL13112	serum + LIF vs 2i + LIF	90,94	mouse	(?)	3
GSE74596	NKT1 vs NTK2	46,68	mouse	(?)	5
EMTAB2805	G1 vs G2M	95,96	mouse	(?)	7
GSE71585-GPL13112	Gad2tdTpositive vs Cux2tdTnegative	80,140	mouse	(?)	7
GSE64016	G1 vs G2	91,76	human	(?)	8
GSE79102	patient1 vs patient2	51, 89	human	?	4
GSE45719	16-cell stage blastomere vs mid blastocyst cell	50, 60	mouse	(?)	5
GSE63818	Primordial Germ Cells, develop- mental stage: 7 week gestation vs Somatic Cells, developmental stage: 7 week gestation	40,26	mouse	(?)	6
GSE75748	DEC vs EC	64, 64	human	(?)	9
GSE84465	neoplastic cells vs non-neoplastic cells	546, 664	human	(?)	9

Table 1: datasets used for comparisons of DD analysis under different methods



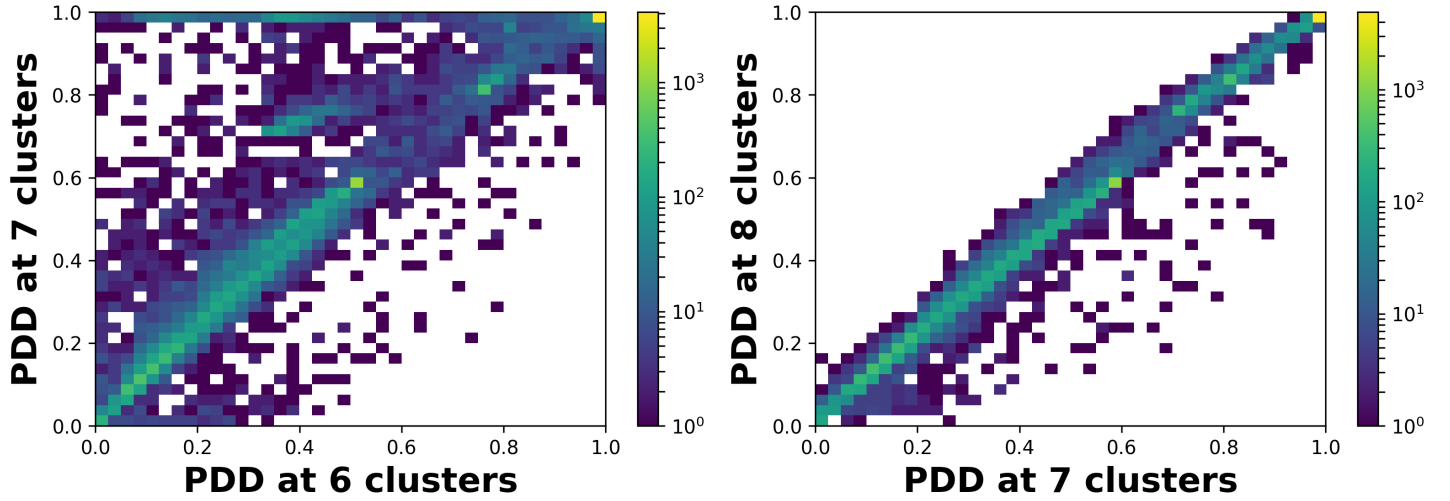
**Figure 3:** under NULL case, using dataset GSE52529, when using too big  $K$  we may completely lose FDR control (black line shows proportion of false positive identified by scDDboost under 0.05 threshold, while validity score become stable at  $K = 3$ )

\*\*\*something about change of PDD over  $K$ , even though PDD is monotone increasing but it would remain stable in the sense that  $PDD_{K+1} - PDD_K$  will have small variance over different genes\*\*\*

From our empirical experience, it would be sufficient to capture the heterogeneity underlying cells with number of clusters not greater than 9. And we generally obtain stable in validity score and PDD simultaneously (see supplementary)

We demonstrate the change of posterior probabilities of differential distribution given different number of subtypes at data GSE75748 and GSE48968. In both cases, if allowing one more subtype would result in a lot increases in posterior probabilities, which suggests that the number of subtypes is underestimated since we found more distribution differences between conditions given one more



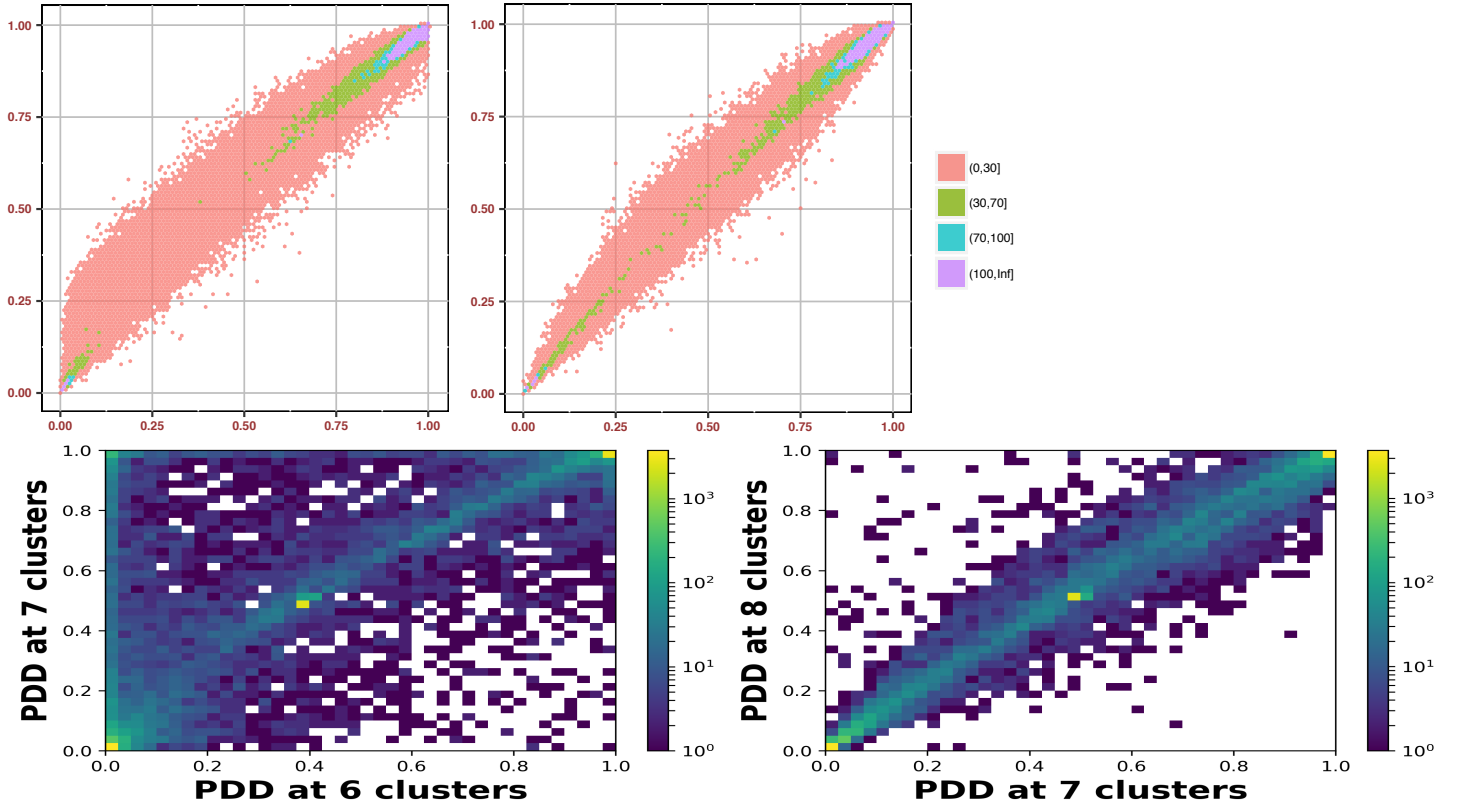


**Figure 2:** comparison of posterior probabilities of being DD among different number of subtypes, when we underestimate the number of subtypes, the difference is huge, see PDD between 6 subtypes and 7 subtypes. There is an approximate horizontal line with massive points at the top of left panel, which indicate that we underestimate lots of DD genes due to underestimate the number of subtypes. While in the case when we overestimate the number of subtypes 7 subtypes vs. 8 subtypes, though inflating PDD but the variation of difference is small, from 6 to 8 subtypes the PDD become more linear related.

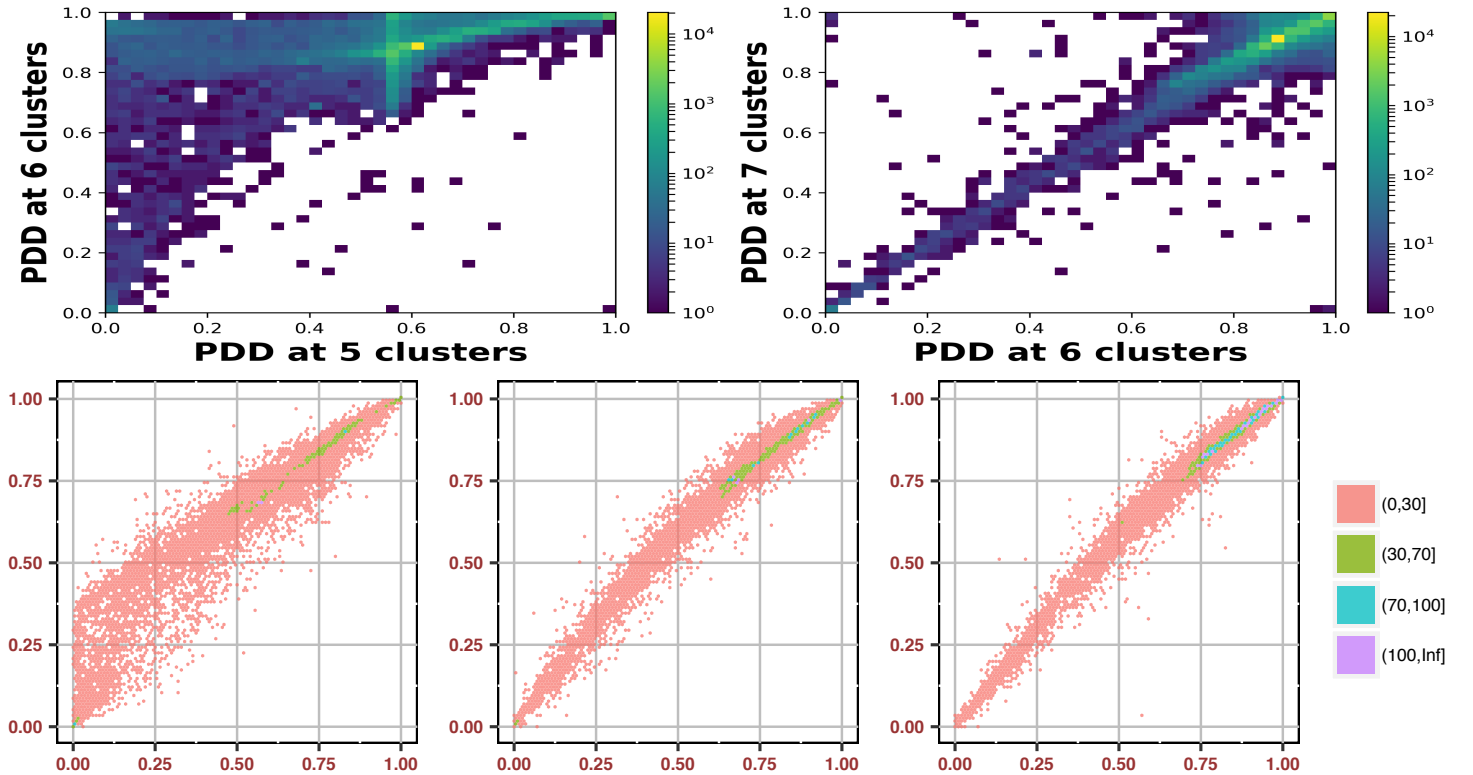
Data set	Conditions	Number of cells/condition	Organism
GSE57872null	patient1	96,96	human
GSE52529null	T0	48, 48	human
GSE48968-GPL13112null	BMDC (2h LPS stimulation)	48,48	mouse
GSE60749-GPL13112null	v6.5 mouse embryonic stem cells, culture conditions: 2i+LIF	45,45	mouse
GSE74596null	NKT1	23,23	mouse
EMTAB2805null	G1	48,48	mouse
GSE71585-GPL13112null	Gad2tdTpositive	40,40	mouse
GSE64016null	G1	46,45	human
GSE79102null	patient1	26, 25	human

Table 2: datasets used for null cases, as cells are coming from same biological condition, there should not be any differential distributed genes, any positive call is false positive

mixture component. If posterior inference is stable after increasing the number of subtypes, then we consider previous number of subtypes to be optimal.



**Figure 4:** selecting number of subtypes for data GSE57872, we observe posterior probabilities become stable at more than 6 subtypes. Since increasing number of subtypes tends to decrease sample size of each subtypes, make complicate constraints for equivalent distribution and inflate estimated PDD. We select number of subtypes to be 7



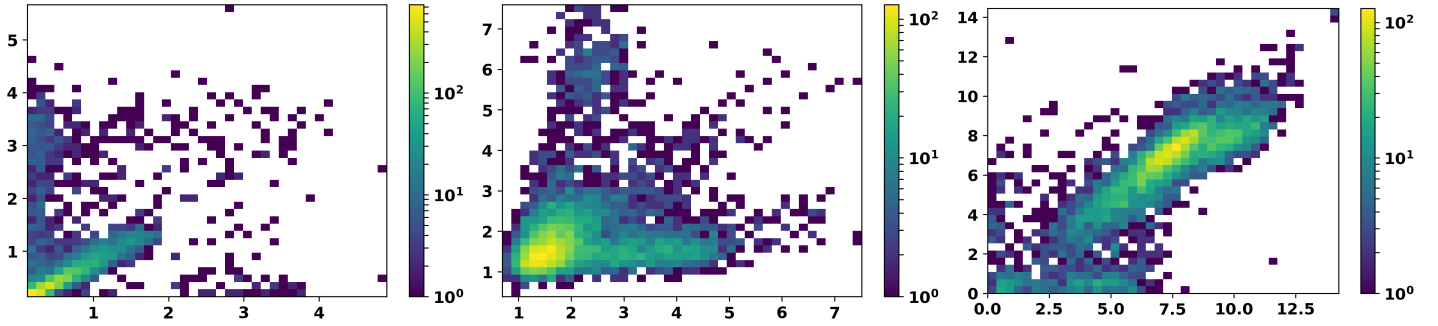
**Figure 5:** selecting number of subtypes for data GSE48968, we observe posterior probabilities become stable at more than 5 subtypes

## 4.2 Bursting parameters

\*\*\*on the method estimated p-value, update later\*\*\*

D3E(?) is a distributional method that can identify bursting parameters of transcripts. Rate of promoter activation, rate of promoter inactivation and the rate of transcription when the promoter is in the active state are estimated by D3E. We investigate DD genes identified by scDDboost and their change of those three parameters on dataset GSE71585

**Figure 6:** D3E method will estimate 3 bursting parameters probability of a gene being on (a) and off (b) and the expression rate when the gene expression is on (c), we plot the hexbin plot of probability of a gene being DD under our method v.s. the absolute value of log fold change of a, b and c across the two conditions accordingly. The log fold change is scaled by dividing the largest log fold change so that ends up in a value between 0 and 1. Here we use the GSE71585 data



**Figure 7:** 2D histogram for bursting parameters of DD genes identified by scDDboost from dataset EMTAB2805 estimated by D3E. Left panel : comparison of rate of promoter activation between two conditions, similarly, middle panel : rate of promoter inactivation and right panel: rate of transcription when the promoter is in the active state. We observe that difference between transcription rate is smaller compare to difference between the activation and inactivation rate.

We observed that DD genes identified by scDDboost tends to have similar transcription rate when the promoter is active across condition, while there are lots of variabilities in the action and inactivation rate. Estimations from D3E reveals that the major factor to drive DD genes are activation and inactivation rate (proportions of different subtypes), it make sense to consider mixture model like scDDboost.

## 5 Theoretical issues

### 5.1 Posterior consistency

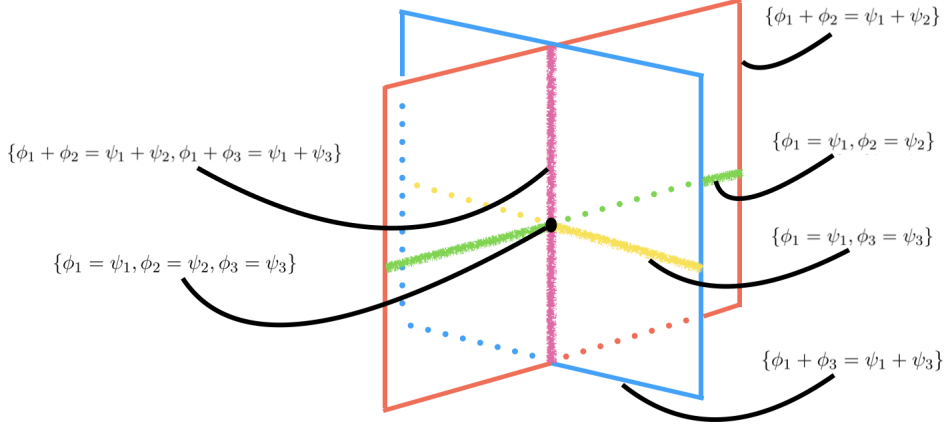
Under some parameters settings, the double dirichlet prior will have limited resolution and lead to inconsistency of posterior probabilities, which we investigate with the following asymptotic analysis.

We first give the expression of posterior probability. Since there is no information favorable of any particular  $A_\pi$ , we select discrete uniform distribution as the prior for it, then the posterior probability is

$$p(A_\pi | t^1, t^2) = c * \sum_{\pi' \text{ refines } \pi} p(t^1 | t_{\pi'}^1) p(t^2 | t_{\pi'}^2) p(t_{\pi'}^1, t_{\pi'}^2 | A_{\pi'}) \quad (3)$$

for a normalizing constant  $\frac{1}{c} = \sum_{\pi' \in \Pi} p(t^1 | t_{\pi'}^1) p(t^2 | t_{\pi'}^2) p(t_{\pi'}^1, t_{\pi'}^2 | A_{\pi'})$ .

Let  $\Omega = \{(\phi, \psi) : \sum_{i=1}^K \phi_i = \sum_{i=1}^K \psi_i = 1, \phi_i \geq 0, \psi_i \geq 0, i = 1, \dots, K\}$  be the whole space. There is a subset of  $\Omega$  we lack posterior inference. Let us first see an example:



**Figure 8:** Four subtypes of cells, simplexes of  $(\phi, \psi)$  satisfying different constraints.

In Fig 10, there are four subtypes, the rectangle with magenta boundary is a simplex  $A_{\pi_1} = \{(\phi, \psi) : \phi_1 + \phi_2 = \psi_1 + \psi_2\}$ , the rectangle with blue boundary is a simplex  $A_{\pi_2} = \{(\phi, \psi) : \phi_1 + \phi_3 = \psi_1 + \psi_3\}$ . The green line refers to  $A_{\pi_3} = \{(\phi, \psi) : \phi_1 = \psi_1, \phi_2 = \psi_2\}$ , the yellow line refers to  $A_{\pi_4} = \{(\phi, \psi) : \phi_1 = \psi_1, \phi_3 = \psi_3\}$ , the purple line refers to  $A_{\pi_5} = \{(\phi, \psi) : \phi_1 + \phi_2 = \psi_1 + \psi_2, \phi_1 + \phi_3 = \psi_1 + \psi_3\}$ , which is the intersection of  $A_{\pi_1}$  and  $A_{\pi_2}$ , and finally the black dot which is the intersection of those three lines refers to the simplex with finest partitions,  $\phi_i = \psi_i, \forall i = 1, \dots, 4$ . We lack posterior inference for  $(\phi, \psi)$  along the purple line except the black dot. While on the green line, yellow line and black dot, we have consistent posterior inference (theorem 2). To explain why some space lacking posterior inference and define such space, we define a special subset  $A_{\pi}^*$  of simplex  $A_{\pi}$ .  $A_{\pi}^* = A_{\pi} \setminus \bigcup_{\tilde{\pi} \text{ is not coarser than } \pi} A_{\tilde{\pi}}$ ,  $A_{\pi}^*$  is obtained by removing all intersection with other  $A_{\tilde{\pi}}$  (excluding those  $A_{\tilde{\pi}}$  that is superset of  $A_{\pi}$ ) from  $A_{\pi}$ . Since we removed those intersection parts. It is intuitive that  $A_{\pi}^*$  will be disjoint subsets of  $\Omega$ .

**Proposition 1.** if  $\pi_1 \neq \pi_2$ , then  $A_{\pi_1}^* \cap A_{\pi_2}^* = \emptyset$

Let  $Q = \Omega \setminus \bigcup_{\pi \in \Pi} A_{\pi}^*$ , and we have following proposition of the existence of  $Q$ .

**Proposition 2.** Let  $K$  be number of subtypes. When  $K > 3$ ,  $Q \neq \emptyset$ , when  $K \leq 3$ ,  $Q = \emptyset$

When the number of subtypes is bigger than three, we lack posterior inference on  $Q$ . To see that we can rewrite  $A_{\pi}^*$  as  $A_{\pi}^* = A_{\pi} \setminus \bigcup_{\tilde{\pi} \text{ is not coarser than } \pi} (A_{\tilde{\pi}} \cap A_{\pi})$ ,  $\tilde{\pi}$  is not coarser than  $\pi$ , which is equivalently to say  $\pi$  is not refinement of  $\tilde{\pi}$ . By property 8 in section 2,  $A_{\tilde{\pi}} \cap A_{\pi}$  is a lower dimensional subset of  $A_{\pi}$ . So  $A_{\pi} \setminus A_{\pi}^*$  is a lower dimensional subset of  $A_{\pi}$ . For posterior on  $Q$ , it degenerates to integral on a lower dimensional subset of the simplex associating with densities, which will vanish

**Proposition 3.** When  $K > 3$ ,  $p(Q|z^1, z^2) = 0$

But for  $(\phi, \psi) \in \Omega \setminus Q$ , we have consistent posterior inference.

**Theorem 1.** Let  $n = \min(n_1, n_2)$  be the smaller number of cells of two conditions and  $n_1 = O(n_2)$  namely  $\ln(\frac{n_1}{n_2}) = 0$ , and hyper parameters of DDM  $\alpha^1, \alpha^2$  be vectors of constants,  $\alpha_k^j \geq 1, \forall k, j$  and  $\beta = \alpha^1 + \alpha^2$ . Then if parameter  $(\phi, \psi) \in \Omega \setminus Q$  we have

$$p(A_\pi|y, z) \xrightarrow[n \rightarrow \infty]{a.s.} \begin{cases} 1 & \text{if } (\phi, \psi) \in A_\pi \\ 0 & \text{otherwise} \end{cases}$$

Things become more complicate when  $(\phi, \psi)$  falling into  $Q$ , we know  $p(Q|y, z)$  vanishes, but  $p(A_\pi|y, z)$  may not.

Recall  $N(\pi)$  represents number of blocks  $b$  in  $\pi$ . Let  $S = \{\pi, (\phi, \psi) \in A_\pi\}$ , which is the collection of partitions whose associated simplexes covering  $(\phi, \psi)$ . Let  $N^* = \max_{\pi \in S} N(\pi)$ , which is the max number of blocks of partitions from  $S$ . Let  $S^* = \{\pi, (\phi, \psi) \in A_\pi \text{ and } N(\pi) = N^*\}$ , which is the collection of partitions that covering  $(\phi, \psi)$  with number of blocks equal to the max number  $N^*$ .

For example, when  $K = 7$ , For a  $(\phi, \psi) \in A_{\pi_1} \cap A_{\pi_2} \cap A_{\pi_3}$ ,  $\pi_1 = \{\{1, 2, 3\}, \{4, 5, 6, 7\}\}$ ,  $\pi_2 = \{\{1, 6, 7\}, \{2, 4\}, \{3, 5\}\}$ ,  $\pi_3 = \{\{1, 2, 3, 4, 5, 6\}\}$ , and also  $(\phi, \psi)$  does not belong to any other simplex  $A_\pi$ . Then  $S = \{\pi_1, \pi_2, \pi_3\}$ ,  $N^* = 3$ ,  $S^* = \{\pi_2\}$ .

**Theorem 2.** Following the setting in theorem 1, when parameter  $(\phi, \psi) \in Q$ , and we have

$$(p(A_\pi|y, z))_{\pi \in S^*} \xrightarrow[n \rightarrow \infty]{d} (V_1, \dots, V_{N(S^*)})$$

$V_1, \dots, V_{N(S^*)}$  are random variables and  $V_1 + \dots + V_{N(S^*)} = 1$

Still using above example, in limiting case, we have  $p(A_{\pi_3}|y, z) = 1$ ,  $p(A_{\pi_2}|y, z) = 1$  and  $p(A_{\pi_1}|y, z) = 0$ . When the DE pattern is  $B_{\pi_1}$  for some genes and our estimation of  $p(A_{\pi_1}|y, z) = 0$ , we will falsely classify those genes as differential distributed.

The asymptotic properties help us gain insight of the performance of our approach, scDDboost may work poorly, when  $(\phi, \psi) \in Q$ , we may underestimate the posterior probability of true proportion change pattern, which reduce the posterior probabilities of true negative and enlarge false positive rate.

## 5.2 Random weighting

In this section, we gave an intuitive justification for consistency between bayesian framework clustering analysis and random weighting procedure. A full bayesian analysis for clustering needs to specify the density of data given the partition. Specifically, in single cell analysis we need to know the density of transcripts of genes given the partitions which requires understanding of co-expression and dependence between genes. Instead of trying to untangle the mystery behind the dependence of genes, we consider following approximation

$$P(\text{Partition}|X) \leftarrow P(\text{Partition}|D) \leftarrow P(\Delta|D) \leftarrow D/W$$

where  $D$  is the estimated distance matrix of  $X$ ,  $\Delta$  is the true distance of  $X$  and  $W$  is randomly distributed matrix of weights. We conjecture that the probability of partitions given data can be

approximated by switching conditioning on data to conditioning on the estimated distance of data. As distance matrix typically gave the geometrical structure between elements which can be used to infer how likely a partition is. In addition, partition can be obtained by distance based clustering algorithm (K-medoids) on true distance matrix  $\Delta$ . To approximate distribution  $(\Delta|D)$ , we use our random weighting procedure, namely sampling a weighting matrix  $W$  first and then do the component-wisely dividing of original distance matrix  $D$  by  $W$ .

We gave a brief justification for this approximation, suppose units  $i$  and  $j$  are merged into a common cluster if (and only if)  $d_{i,j} < c$ . Then  $P(d_{i,j}^* < c) = P(w_{i,j} > c/d_{i,j})$ ,  $w_{i,j} \sim \text{Gamma}(a, b)$ . From Bayesian perspective, given the true distance  $\Delta_{i,j}$ ,  $d_{i,j}|\Delta_{i,j} \sim \text{Gamma}(a_1, a_1/\Delta_{i,j})$ , so that the sampling mean of  $d_{i,j}$  is  $\Delta_{i,j}$ . Further, for simplicity we ignore any issues about the  $d$ 's or  $\Delta$ 's being true distances. The condition for qualifiable distance matrix is the triangle inequality among the pairwise distances, such condition would not affect our clustering results too much. But, a simple analysis might suppose that a-priori  $1/\Delta_{i,j} \sim \text{Gamma}(a_0, d_0)$ . The scaling is such that  $E(1/\Delta_{i,j}) = a_0 d_0$ . The posterior, by conjugacy, has  $1/\Delta_{i,j}|d_{i,j} \sim \text{Gamma}(a_0 + a_1, d_0 + a_1 d_{i,j})$ . Then the posterior probability that  $i$  and  $j$  should be clustered is the posterior probability that  $\Delta_{i,j} < c$ , which is  $P(\text{Gamma}((a_0 + a_1), (d_0 + a_1 d_{i,j})) > (d_0 + a_1 * d_{i,j}) / (a_0 + a_1) * 1/c)$ , parameters  $(a_0, d_0, a_1)$  are estimated from maximizing the marginal likelihood of  $d_{i,j}$ .

In order to match the posterior probability that elements  $i$  and  $j$  belongs to the same cluster through the simple bayesian analysis to random weighting, which is equivalently to match

$$P(\Delta_{i,j} < c|d_{i,j}) = P(1/\Delta_{i,j} > 1/c|d_{i,j})$$

and

$$P(d_{i,j}/w_{i,j} < c|d_{i,j}) = P(w_{i,j}/d_{i,j} > 1/c|d_{i,j})$$

yielding  $a = a_0 + a_1$  and  $b = a_1$ . Therefore, we gave a way of modeling the distribution of weights such that partition based on random generated distance  $D/W$  would approximate the partition given data based on a full bayesian framework.

## Randomized k-means

### 5.3 simulation

We random generate one-dimensional data  $X$  from a mixture of 5 normal distributions with different means and same variance. We compare clustering results between random weighting and bayesian clustering with Dirichlet process as prior in terms of posterior probabilities that two elements belong to the same class given the whole data and adjusted rand index comparing to the underlying true class label (Fig 12).

#### Proofs:

**Lemma 1.** *If  $\pi_2$  is not refinement of  $\pi_1$  then  $A_{\pi_1} \cap A_{\pi_2}$  is a lower dimensional subset of  $A_{\pi_2}$*

Proof of lemma 1

*Proof.* Let  $V$  denote the orthogonal space of  $\phi - \psi$ , when  $(\phi, \psi) \in A_{\pi_1} \cap A_{\pi_2}$ , and  $\dim(A_{\pi_1} \cap A_{\pi_2}) = \dim(\phi - \psi) + \dim(\psi) = 2K - \dim(V) - 1$ . Also let  $\pi_1 = \{b_1^1, \dots, b_s^1\}$ ,  $\pi_2 = \{b_1^2, \dots, b_t^2\}$ . The corresponding vectors are  $v_1^1, \dots, v_s^1$  and  $v_1^2, \dots, v_t^2$ . We claim there must be a  $b_i^1 \in \pi$  whose corresponding  $v_i^1$  is linear independent with  $v_1^2, \dots, v_t^2$ . If not, for every  $v_i^1$  there exists  $\alpha_1^i, \dots, \alpha_t^i$  such that

$$v_i^1 = \sum_{j=1}^t \alpha_j^i v_j^2 \quad (*)$$

If  $b_j^2 \cap b_i^1 \neq \emptyset$ , then multiply  $v_j^2$  on both sides of (\*), we obtain  $v_i^1 * v_j^2 = \alpha_j^i (v_j^2)^2$ , as  $v_j^2$  are orthogonal vectors, and  $v_i^1 * v_j^2 > 0$  implies  $\alpha_j^i > 0$ . Consider  $x = f(b_j^2 \setminus b_i^1)$ , we have  $x * v_i^1 = 0$  and we multiply  $x$  on both sides of (\*) to obtain  $\alpha_j^i v_j^2 * x = 0$ , thus  $x$  must be zero vector and  $b_j^2 \setminus b_i^1 = \emptyset$ , which implies  $b_j^2 \subset b_i^1$ . That is to say when  $b_j^2 \cap b_i^1 \neq \emptyset$ ,  $b_j^2$  must be subset of  $b_i^1$ . So  $b_i^1$  is union of some blocks in  $\pi_2$ . Which implies  $\pi_2$  is refinement of  $\pi_1$ , contradiction.

Consequently there exists  $b \in \pi_1$  with  $v(b)$  linear independent with  $v(b')$ ,  $b' \in \pi_2$ .  $\dim(V)$  is at least  $N(\pi_2) + 1$ ,  $\dim(A_{\pi_1} \cap A_{\pi_2}) < \dim(A_{\pi_2})$   $\square$

Proof of property 8

Proof. by lemma 1, it is easy to verify.  $\square$

As the density of DDM is computed by product or ratio over bunches of gamma function and gamma function is not easy to direct work on it and derive limiting theorem. To proof theorem 4 and 5, we need a crucial lemma which gave us an approximation to the gamma function, namely

**Lemma 2.** For  $x \geq 1$ ,  $\frac{x^{x-c}}{e^{-x}} \leq \Gamma(x) \leq \frac{x^{x-1/2}}{e^{-x}}$ , where  $c = 0.577215\dots$  is the Euler-Mascheroni constant.

Proof. By (?), we have  $\frac{x^{x-c}}{e^{-x}} \leq \Gamma(x) \leq \frac{x^{x-1/2}}{e^{-x}}$  for  $x > 1$  and now we added the case when  $x = 1$ ,  $\Gamma(x) = 1$  so that both sides will include the equality case.  $\square$

**Lemma 3.** For positive integer  $n$ ,  $\sqrt{2\pi n}^{n+1/2} e^{-n} \leq \Gamma(n+1) \leq e n^{n+1/2} e^{-n}$

We have another two lemmas and theorem 1 and 2 are just proportion of the lemma

**Lemma 4.** If  $(\phi, \psi) \in A_{\pi_1} \cap A_{\pi_2}$ , follow the conditions in theorem 1 then

$$\frac{\omega_{\pi_1}^{post}}{\omega_{\pi_2}^{post}} \xrightarrow[n \rightarrow \infty]{a.s.} 0 \quad \text{if } N(\pi_1) < N(\pi_2)$$

Proof. Recall  $\omega_{\pi}^{post} \propto p_{\pi}(t^1 | t_{\pi}^1, y) p_{\pi}(t^2 | t_{\pi}^2, y) p_{\pi}(t_{\pi}^1, t_{\pi}^2 | y) \omega_{\pi}$ . and  $\text{RHS} = g(\pi, \alpha, \beta, n_1, n_2) f(\pi, t^1, t^2, \alpha, \beta)$  and  $\frac{\omega_{\pi_1}^{post}}{\omega_{\pi_2}^{post}} = \frac{g(\pi_1, \alpha, \beta, n_1, n_2) f(\pi_1, t^1, t^2, \alpha, \beta)}{g(\pi_2, \alpha, \beta, n_1, n_2) f(\pi_2, t^1, t^2, \alpha, \beta)}$  where

$$g(\pi, t^1, t^2, \alpha, \beta) = \left[ \prod_{j=1}^2 \prod_{b \in \pi} \prod_{k \in b} \frac{\Gamma(\sum_{k \in b} \alpha_k^j)}{\Gamma(\alpha_k^j)} \right] \frac{\Gamma(n_1 + 1) \Gamma(n_2 + 1)}{\prod_{b \in \pi} \Gamma(\beta_b)} \frac{\Gamma(\sum_{b \in \pi} \beta_b)}{\Gamma(n_1 + n_2 + \sum_{b \in \pi} \beta_b)}$$

$$f(\pi, t^1, t^2, \alpha, \beta) = \left[ \prod_{j=1}^2 \prod_{b \in \pi} \prod_{k \in b} \frac{1}{\Gamma(t_k^j + 1)} \frac{\prod_{k \in b} \Gamma(\alpha_k^j + t_k^j)}{\Gamma(t_b^j + \sum_{k \in b} \alpha_k^j)} \right] \prod_{b \in \pi} \Gamma(\beta_b + t_b^1 + t_b^2)$$

For notation simplicity, we use the abbreviation  $g(\pi), f(\pi)$  to substitute  $g(\pi, \alpha, \beta, n_1, n_2), f(\pi, t^1, t^2, \alpha, \beta)$ . We take log on  $\frac{\omega_{\pi_1}^{post}}{\omega_{\pi_2}^{post}}$ , denote it as LR.  $\text{LR} = \ln g(\pi_1) - \ln g(\pi_2) + \ln f(\pi_1) - \ln f(\pi_2)$ . Denote  $C(\pi_1, \pi_2, \alpha, \beta) = \ln g(\pi_1) - \ln g(\pi_2)$ ,  $C(\pi_1, \pi_2, \alpha, \beta)$  does not change with sample size  $n_1, n_2$  and is a constant determined by partition  $\pi_1, \pi_2$  and hyper parameters  $\alpha, \beta$ . For further convenience of notation let  $h(x) = \ln \Gamma(x)$  and  $\gamma_b^j = \sum_{k \in b} \alpha_k^j$ . Denote  $R(\pi_1, \pi_2, t^1, t^2, \alpha, \beta) = \ln f(\pi_1) - \ln f(\pi_2)$ . And removing the common part of  $f(\pi_1)$  and  $f(\pi_2)$ , we have

$$R(\pi_1, \pi_2, t^1, t^2, \alpha, \beta) = d(\pi_1, t^1, t^2, \alpha, \beta) - d(\pi_2, t^1, t^2, \alpha, \beta)$$

where

$$d(\pi, t^1, t^2, \alpha, \beta) = \sum_{b \in \pi} h(\beta_b + t_b^1 + t_b^2) - \sum_{j=1}^2 \sum_{b \in \pi} h(t_b + \gamma_b^j)$$

Recall  $\beta_b = \gamma_b^1 + \gamma_b^2$  and from lemma 2,  $(x - c)\ln(x) - x \leq h(x) \leq (x - 1/2)\ln(x) - x$  we have

$$d(\pi, t^1, t^2, \alpha, \beta) \geq \sum_{b \in \pi} (\beta_b + t_b^1 + t_b^2 - c)\ln(\beta_b + t_b^1 + t_b^2) - \sum_{j=1}^2 \sum_{b \in \pi} (t_b^j + \gamma_b^j - 1/2)\ln(t_b^j + \gamma_b^j) \quad (4)$$

$$d(\pi, t^1, t^2, \alpha, \beta) \leq \sum_{b \in \pi} (\beta_b + t_b^1 + t_b^2 - 1/2)\ln(\beta_b + t_b^1 + t_b^2) - \sum_{j=1}^2 \sum_{b \in \pi} (t_b^j + \gamma_b^j - c)\ln(t_b^j + \gamma_b^j) \quad (5)$$

$$\begin{aligned} \text{RHS of (4)} &= \sum_b [(t_b^1 + \gamma_b^1)\ln(1 + \frac{t_b^2 + \gamma_b^2}{t_b^1 + \gamma_b^1}) + (t_b^2 + \gamma_b^2)\ln(1 + \frac{t_b^1 + \gamma_b^1}{t_b^2 + \gamma_b^2}) \\ &\quad + (1 - c)\ln(\beta_b + t_b^1 + t_b^2) - 1/2(\ln(1 + \frac{t_b^2 + \gamma_b^2}{t_b^1 + \gamma_b^1}) + \ln(1 + \frac{t_b^1 + \gamma_b^1}{t_b^2 + \gamma_b^2}))] \end{aligned}$$

By Taylor expansion at  $x = 1$ ,  $\ln(x + 1) = \ln 2 + 1/2(x - 1) - 1/8(x - 1)^2 + g(\xi)(x - 1)^3$ , where  $g(\xi)$  is the reminder term of form  $\frac{1}{3(1+\xi)^3}$  for  $0 < \xi < x$  For a fixed  $n_1, n_2$ , we have

$$\begin{aligned} \text{RHS of (4)} &= (n_1 + n_2)\ln 2 - \sum_{b \in \pi} (1/8(X_b^1 + X_b^2) \\ &\quad + g(\xi_b)(Y_b^1 + Y_b^2)) + T(\pi) \end{aligned}$$

$$\text{where } X_1 = \frac{(t_b^1 - t_b^2 + \gamma_b^1 - \gamma_b^2)^2}{t_b^1 + \gamma_b^1}, X_2 = \frac{(t_b^1 - t_b^2 + \gamma_b^1 - \gamma_b^2)^2}{t_b^2 + \gamma_b^2}, Y_1 = \frac{(t_b^1 - t_b^2 + \gamma_b^1 - \gamma_b^2)^3}{(t_b^1 + \gamma_b^1)^2}, Y_2 = \frac{(t_b^1 - t_b^2 + \gamma_b^1 - \gamma_b^2)^3}{(t_b^2 + \gamma_b^2)^2} \text{ and}$$

$$T(\pi) = \sum_{b \in \pi} [(1 - c)\ln(\beta_b + t_b^1 + t_b^2) - 1/2(\ln(1 + \frac{t_b^2 + \gamma_b^2}{t_b^1 + \gamma_b^1}) + \ln(1 + \frac{t_b^1 + \gamma_b^1}{t_b^2 + \gamma_b^2}))]$$

Similarly

$$\begin{aligned} \text{RHS of (5)} &= (n_1 + n_2)\ln 2 - \sum_{b \in \pi} (1/8(X_b^1 + X_b^2) \\ &\quad + g(\xi_b)(Y_b^1 + Y_b^2)) + U(\pi) \end{aligned}$$

$$U(\pi) = \sum_{b \in \pi} [(2c - 1/2)\ln(\beta_b + t_b^1 + t_b^2) - c(\ln(1 + \frac{t_b^2 + \gamma_b^2}{t_b^1 + \gamma_b^1}) + \ln(1 + \frac{t_b^1 + \gamma_b^1}{t_b^2 + \gamma_b^2}))]$$

Using above inequalities, we have

$$\begin{aligned} R(\pi_1, \pi_2, t^1, t^2, \alpha, \beta) &\geq T(\pi_1) - U(\pi_2) - 1/8(\sum_{b \in \pi_1} (X_b^1 + X_b^2) - \sum_{b \in \pi_2} (X_b^1 + X_b^2)) \\ &\quad - \sum_{b \in \pi_1} g(\xi_b)(Y_b^1 + Y_b^2) - \sum_{b \in \pi_2} g(\xi_b)(Y_b^1 + Y_b^2) \\ R(\pi_1, \pi_2, t^1, t^2, \alpha, \beta) &\leq U(\pi_1) - T(\pi_2) - 1/8(\sum_{b \in \pi_1} (X_b^1 + X_b^2) - \sum_{b \in \pi_2} (X_b^1 + X_b^2)) \\ &\quad + \sum_{b \in \pi_1} g(\xi_b)(Y_b^1 + Y_b^2) - \sum_{b \in \pi_2} g(\xi_b)(Y_b^1 + Y_b^2) \end{aligned}$$

By CLT we know  $X_b^1, X_b^2$  are asymptotic gamma( $\chi$ -square) distributed and  $Y_b^1, Y_b^2$  are  $o_p(1)$ ,  $g(\xi_b)$  has bounded variance and  $T(\pi_1) - U(\pi_2) = -\ln(n)$  if  $N(\pi_2) < N(\pi_1)$  so we complete the proof

□



**Lemma 5.** *If  $(\phi, \psi) \in A_{\pi_1} \cap A_{\pi_2}$ , follow the conditions in theorem 1 and further we have  $\alpha^j, j = 1, 2$  be vector of integers then*

$$\frac{\omega_{\pi_1}^{post}}{\omega_{\pi_2}^{post}} \xrightarrow[n \rightarrow \infty]{d} v \quad \text{if } N(\pi_1) = N(\pi_2)$$

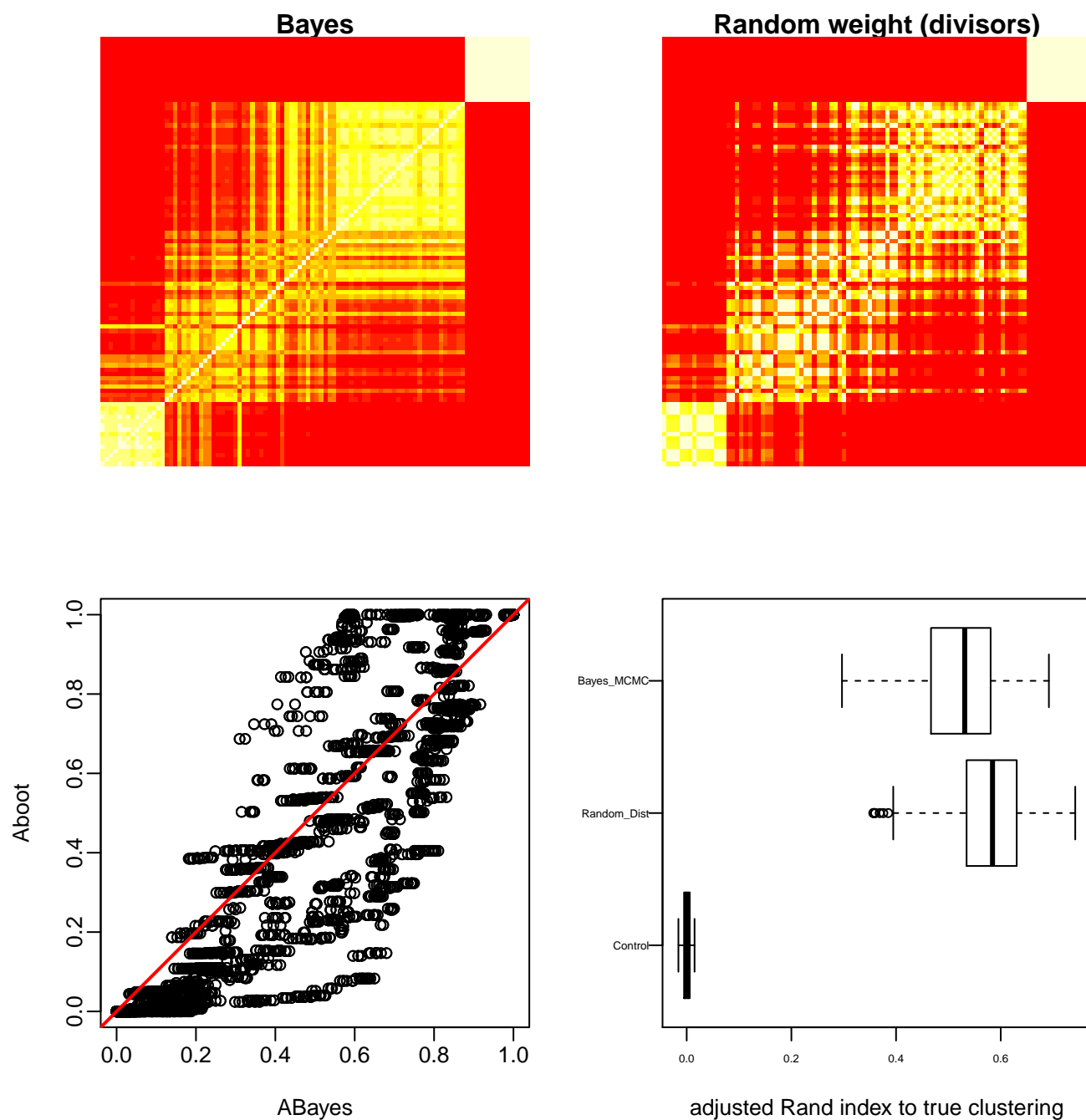
*v is a random variable*

Proof of theorem 1 and theorem 2

*Proof.* By lemma 3,  $\sum_{\pi} \omega_{\pi}^{post} = 1$  and  $P(A_{\pi}|y, z) = \sum_{\tilde{\pi} \in \Pi} \omega_{\tilde{\pi}}^{post} 1[\tilde{\pi} \text{ refines } \pi]$ . We get the results of theorem 1 and 2

1

□



**Figure 9:** comparison between random weighting scheme and bayesian clustering procedure in terms of posterior probabilities that two elements belong to the same class given the whole data and adjusted rand index comparing to the underlying true class label