R commands

Here, we list the R commands for each method that were used to analyze the data in this study. As explained in the main text, we run each method in default settings. All analyses were performed with R version 3.2.2. The data matrix and conditions are denoted as cdat and cgroup, respectively.

**ABSSeq**

The ABSSeq package, version 1.6.1, is available in Bioconductor.

```r
> library(ABSSeq)
> obj <- ABSDataSet(cdat, cgroup)
> obj <- ABSSeq(obj)
> res <- results(obj, c("Amean","Bmean","foldChange","pvalue","adj.pvalue"))
```

**DESeq**

The DESeq package, version 1.20.0, can be obtained from Bioconductor.

```r
> library(DESeq)
> cds <- newCountDataSet(cdat, cgroup)
> cds <- estimateSizeFactors(cds)
> if(length(cgroup) == 2){
>   cds <- estimateDispersions(cds, method= "blind", fitType='local', sharingMode='fit-only')
> }else{
>   cds <- estimateDispersions(cds, method= "per-condition", fitType='local')
> }
> res <- nbinomTest(cds, "condA", "condB")
```

**edgeR**

The edgeR package, version 3.10.2, can be obtained from Bioconductor.

```r
> library(edgeR)
> y <- DGEList(counts=cdat, group=cgroup)
> y <- calcNormFactors(y)
> if(2 == length(cgroup)){
>   y$common.dispersion = 0.4
> }else{
>   y <- estimateCommonDisp(y)
>   y <- estimateTagwiseDisp(y)
> }
> et <- exactTest(y)
> res <- topTags(et,n=nrow(et))
```

**DESeq2**
The DESeq2 package, version 1.8.1, can be obtained from Bioconductor.

```r
> library(DESeq2)
> ds <- DESeqDataSetFromMatrix(countData = cdat, colData = data.frame(cgroup), design = ~ cgroup)
> ds <- DESeq(ds)
> res <- results(ds)
```

**limma**

The limma package, version 3.24.15, can be obtained from Bioconductor. It has been evaluated with two different settings: Voom and QN.

Here is Voom.

```r
> library(limma)
> library(edgeR)
> design <- model.matrix(~0+cgroup)
> colnames(design) <- levels(cgroup)
> nf <- calcNormFactors(cdat)
> dat <- voom(cdat, design, plot=FALSE, lib.size=colSums(cdat) * nf)
> fit <- lmFit(dat,design)
> contrast.matrix <- makeContrasts("condB - condA", levels=design)
> fit2 <- contrasts.fit(fit, contrast.matrix)
> fit2 <- eBayes(fit2)
> res=decideTests(fit2,p.value=q.cut,lfc=lfc)
> tab <- topTable(fit2, adjust = "BH", number=nrow(fit2), sort.by='logFC')
```

Here is QN.

```r
> library(limma)
> design <- model.matrix(~0+cgroup)
> colnames(design) <- levels(cgroup)
> dat <- log2(cdat+1)
> dat <- normalizeBetweenArrays(dat,method='quantile')
> fit <- lmFit(dat,design)
> contrast.matrix <- makeContrasts("condB - condA", levels=design)
> fit2 <- contrasts.fit(fit, contrast.matrix)
> fit2 <- eBayes(fit2)
> res=decideTests(fit2,p.value=q.cut,lfc=lfc)
> tab <- topTable(fit2, adjust = "BH", number=nrow(fit2), sort.by='logFC')
```

**baySeq**

The baySeq package, version 2.2.0, can be obtained from Bioconductor.

```r
> library(baySeq)
> cl=NULL
```
EBSeq

The EBSeq package, version 1.10.0, can be obtained from Bioconductor.