The problems encountered during microarray data analysis

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UP Poznań

Październik 4, 2009

A few words about the data...

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- One microarray consists of: experimental probe - RNA sample from a patient or a healthy person and control probe - RNA isolated from cell line HL60 (a subtype of AML)
- 86 hybridization: 1-2 HL60 versus Control,3-68 HL60 versus Leukemia,69-86 HL60 versus Control

Gpr file from GenePix for AML experiment.

Block	Column	Row	Name	Flags
1	1	1	ERG-Operon	100
1	2	1	ERG-Operon	100
1	3	1	ERG-Operon	100
1	4	1	FLT3-Operon	-50
1	5	1	FLT3-Operon	-50
1	6	1	FLT3-Operon	-50
1	7	1	GAPDHS-Operon	-50
1	8	1	GAPDHS-Operon	-50
1	9	1	GAPDHS-Operon	-50

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- For flags less than the cutoff value we give weights equal 0 and 1 otherwise
- We choose cutoff=-50 to downweight bad or absent spots

Problem Number One

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How to calculate the mean intensity for each gene taking into consideration the weight of the spot?

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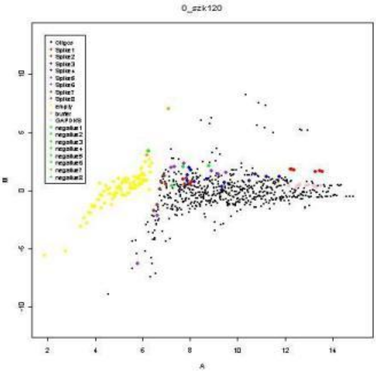
R code

```
> # MA.A - data after normalization
> Mean_intesity <- avedups(MA.A, ndups=3,
weights=MA.A$weights)
```

MA plots: before and after using avedups function



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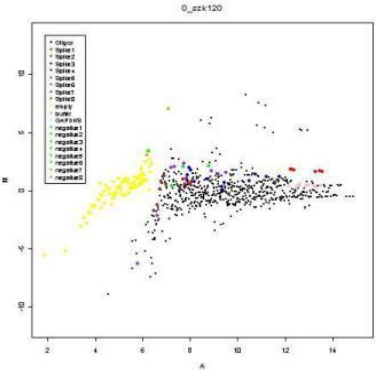

before using avedups function

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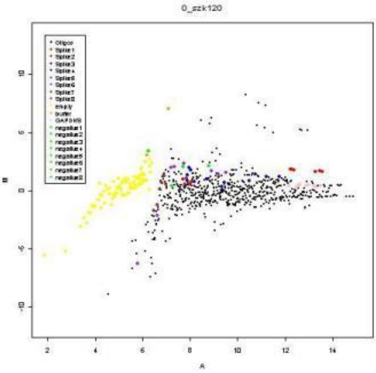

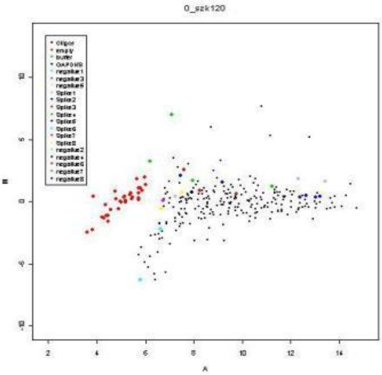

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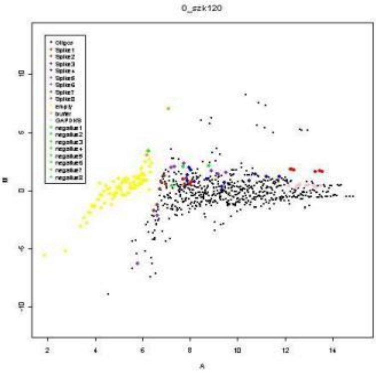

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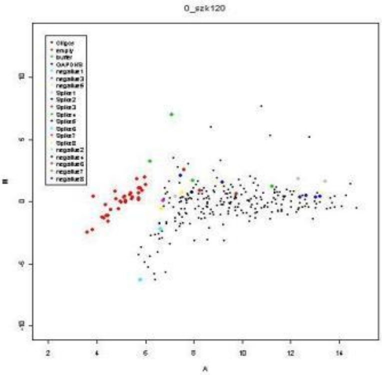

after using avedups function

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The statistics used for these calculations are:

- two sample t-statistics
- sam-statistics
- fc-statistics

DEDS

DEDS package

Yuanyuan Xiao and Yee Hwa Yang
April 21, 2009
University of California

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deds.stat.linkC(X, L, B, tests = c(”t”, ”fc”, ”sam”,”...”))

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- X: A matrix, in the case of gene expression data, rows correspond to N genes and columns to p mRNA samples

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- X: A matrix, in the case of gene expression data, rows correspond to N genes and columns to p mRNA samples
- L: A vector of integers corresponding to observation (column) class labels

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deds.stat.linkC(X, L, B, tests = c(”t”, ”fc”, ”sam”,”...”))

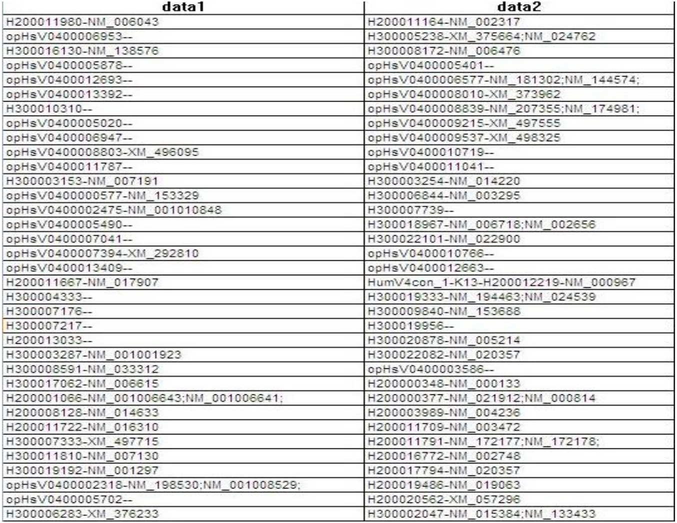
- X: A matrix, in the case of gene expression data, rows correspond to N genes and columns to p mRNA samples
- L: A vector of integers corresponding to observation (column) class labels
- B: The number of permutations

Solution II

R code

```
> library(DEDS)
> # from targets file 0-control, 1-leukemia
> L<-rep(c(0,1,0),c(2,66,18))
> data<-as.matrix(Mean_intesity)
> d <- deds.stat.linkC(data, L, B=200)
> # for the comparisons between the 3 statistics
> t_genes<-topgenes(d,number=50,Mean_intesity$genes$Name,
+ sort.by="t")
> fc_genes<-topgenes(d,number=50,Mean_intesity$genes$Name,
+ sort.by="fc")
> sam_genes<-topgenes(d,number=50,Mean_intesity$genes$Name,
+ sort.by="sam")
```

Data from DEDS...



data1	data2
H200011980-NM_006043	H200011164-NM_002317
opHsV0400006953--	H300005238-XM_375664;NM_024762
H300016130-NM_138576	H300008172-NM_006476
opHsV0400005878--	opHsV0400005401--
opHsV0400012693--	opHsV0400006577-NM_181302;NM_144574;
opHsV0400013392--	opHsV0400008010-XM_373962
H300010310--	opHsV0400008839-NM_207355;NM_174981;
opHsV0400005020--	opHsV0400009215-XM_497555
opHsV0400006947--	opHsV0400009537-XM_498325
opHsV0400008803-XM_496095	opHsV0400010719--
opHsV0400011787--	opHsV0400011041--
H300003153-NM_007191	H300003254-NM_014220
opHsV0400000577-NM_153329	H300006844-NM_003295
opHsV0400002475-NM_001010848	H300007739--
opHsV0400005490--	H300018967-NM_006718;NM_002656
opHsV0400007041--	H300022101-NM_022900
opHsV0400007394-XM_292810	opHsV0400010766--
opHsV0400013409--	opHsV0400012663--
H200011667-NM_017907	HumV4con_1-K13-H200012219-NM_000967
H300004333--	H300019333-NM_194463;NM_024539
H300007176--	H300009840-NM_153688
H300007217--	H300019956--
H200013033--	H300020878-NM_005214
H300003287-NM_001001923	H300022082-NM_020357
H300008591-NM_033312	opHsV0400003586--
H300017062-NM_006615	H200000348-NM_000133
H200001066-NM_001006643;NM_001006641;	H200000377-NM_021912;NM_000814
H200008128-NM_014633	H200003989-NM_004236
H200011722-NM_016310	H200011709-NM_003472
H300007333-XM_497715	H200011791-NM_172177;NM_172178;
H300011810-NM_007130	H200016772-NM_002748
H300019192-NM_001297	H200017794-NM_020357
opHsV0400002318-NM_198530;NM_001008529;	H200019486-NM_019063
opHsV0400005702--	H200020562-XM_057296
H300006283-XM_376233	H300002047-NM_015384;NM_133433

Venn diagram

R code

```
> w<-c(data1,data2)
+ hm<-duplicated(w)
```

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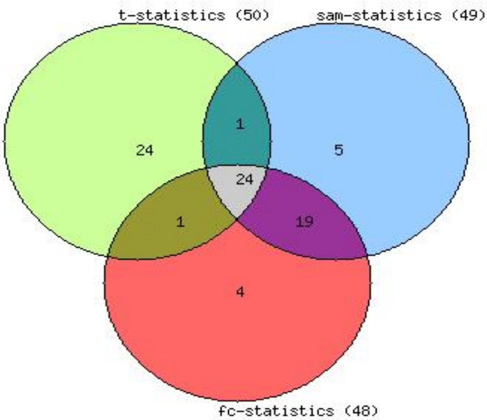
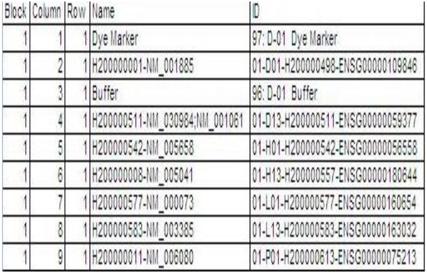


Another example



Block	Column	Row	Name	ID
1	1	1	Dye Marker	97: D-01 Dye Marker
1	2	1	H200000001-NM_001885	01-D01-H200000498-ENSG00000109846
1	3	1	Buffer	96: D-01 Buffer
1	4	1	H200000511-NM_030984;NM_001061	01-D13-H200000511-ENSG00000059377
1	5	1	H200000542-NM_005658	01-H01-H200000542-ENSG00000056558
1	6	1	H200000008-NM_005041	01-H13-H200000557-ENSG00000180644
1	7	1	H200000577-NM_000073	01-L01-H200000577-ENSG00000160654
1	8	1	H200000583-NM_003385	01-L13-H200000583-ENSG00000163032
1	9	1	H200000011-NM_006080	01-P01-H200000613-ENSG00000075213

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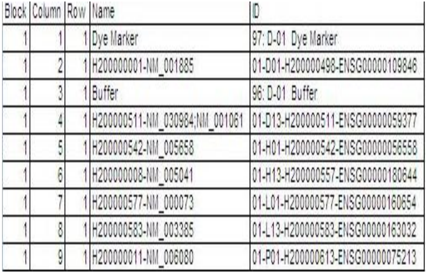

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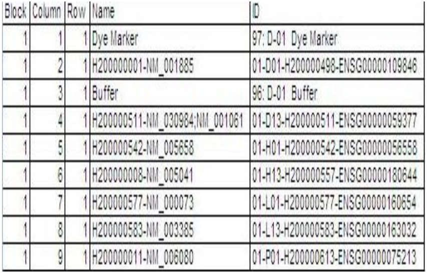
GPR data

Another example



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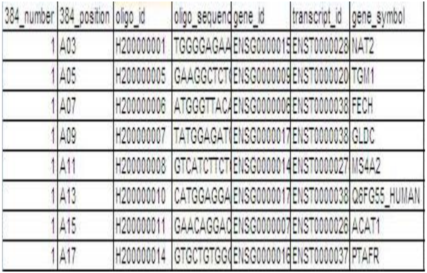
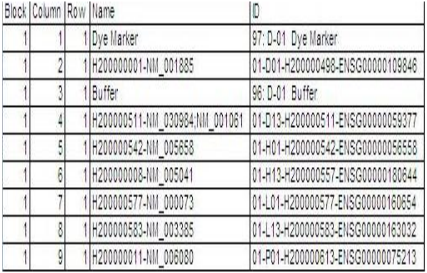


384_number	384_position	oligo_id	oligo_sequenc	gene_id	transcript_id	gene_symbol
1	A03	H200000001	TGGGGAGAA	ENSG0000015	ENST0000028	NAT2
1	A05	H200000005	GAAGGCTCT	ENSG0000009	ENST0000020	TGM1
1	A07	H200000006	ATGGGTTACA	ENSG0000006	ENST0000038	FECH
1	A09	H200000007	TATGGAGAT	ENSG0000017	ENST0000038	GLDC
1	A11	H200000008	GTCATCTTCT	ENSG0000014	ENST0000027	MS4A2
1	A13	H200000010	CATGGAGGA	ENSG0000017	ENST0000038	Q6FG55_HUMAN
1	A15	H200000011	GAACAGGAC	ENSG0000007	ENST0000026	ACAT1
1	A17	H200000014	GTGCTGTGG	ENSG0000016	ENST0000037	PTAFR

GPR data

Another example



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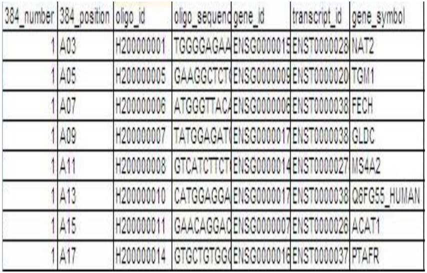

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1	A07	H200000006	ATGGGTTAC	ENSG0000006	ENST0000038	FECH
1	A09	H200000007	TATGGAGAT	ENSG0000017	ENST0000038	GLDC
1	A11	H200000008	GTCATCTTCT	ENSG0000014	ENST0000027	MS4A2
1	A13	H200000010	CATGGAGGA	ENSG0000017	ENST0000038	Q6FG55_HUMAN
1	A15	H200000011	GAACAGGA	ENSG0000007	ENST0000026	ACAT1
1	A17	H200000014	GTGCTGTGG	ENSG0000016	ENST0000037	PTAFR

GPR data

GAL data

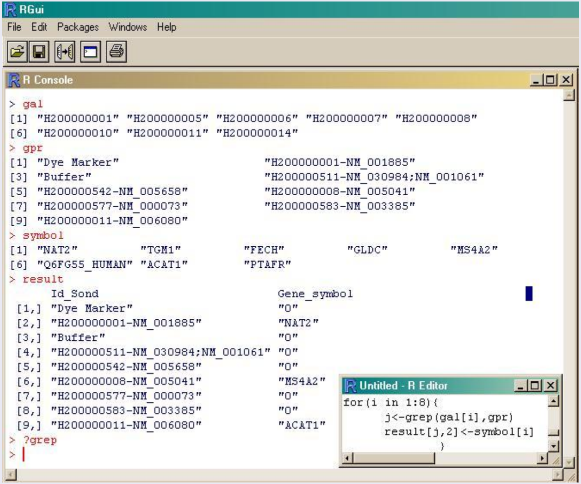
Solution III

R code

```
> gal<-read.table("gal.csv",dec=",", sep=";")
> gpr<-read.table("gpr.csv",dec=",", sep=";")
> gal<-gal[,3]
> gal<-as.character(gal)
> gpr<-gpr[,4]
> gpr<-as.character(gpr)
> symbol<-gal[,9]
> symbol<-as.character(symbol)
> result<-matrix(0,length(gpr),2)
> result[,1]<-gpr
> colnames(result)<-c("Sonda","Gen_symbol")
```

Data after grep function

Finally we obtain id sond in the first column and the gene symbol in the second



```
> gal
[1] "H200000001" "H200000005" "H200000006" "H200000007" "H200000008"
[6] "H200000010" "H200000011" "H200000014"
> gpr
[1] "Dye Marker"                    "H200000001-NM_001885"
[3] "Buffer"                        "H200000511-NM_030984;NM_001061"
[5] "H200000542-NM_005658"          "H200000008-NM_005041"
[7] "H200000577-NM_000073"          "H200000583-NM_003385"
[9] "H200000011-NM_006080"
> symbol
[1] "NAT2"        "TGM1"        "FECH"        "GLDC"        "MS4A2"
[6] "Q6FG55_HUMAN" "ACAT1"       "PTAFR"
> result
      Id_Sond                          Gene_symbol
 [1,] "Dye Marker"                     "0"
 [2,] "H200000001-NM_001885"           "NAT2"
 [3,] "Buffer"                         "0"
 [4,] "H200000511-NM_030984;NM_001061" "0"
 [5,] "H200000542-NM_005658"           "0"
 [6,] "H200000008-NM_005041"           "MS4A2"
 [7,] "H200000577-NM_000073"           "0"
 [8,] "H200000583-NM_003385"           "0"
 [9,] "H200000011-NM_006080"           "ACAT1"
> ?grep
>
```

```
for(i in 1:8){
      j<-grep(gal[i],gpr)
      result[j,2]<-symbol[i]
            }
```

:-)