Acute Respiratory Distress Syndrome is a TH17 and Treg immune disease

By Wan-Jiung Hu
Postdoctorate
Genomics Research Center
Academia Sinica
No 128 Academia Road section2
Nangang 115, Taipei, Taiwan

Previous Institutes:

Department of Pediatrics
Taipei Municipal Chung-Hsin Hospital

Department of Internal Medicine
Taipei Tzu-Chi Buddhist Hospital (Medical Center)

Department of Neurology
Taipei Mackay Memorial Hospital (Medical Center)

Graduate Institute of Immunology
National Taiwan University College of Medicine

Department of International Health (Vaccine science track)
Johns Hopkins University Bloomberg School of Public Health

Abstract

Acute Respiratory Distress Syndrome (ARDS) is a very severe syndrome leading to respiratory failure and subsequent mortality. Sepsis is the leading cause of acute respiratory distress syndrome. Thus, extracellular bacteria play an important role in the pathophysiology of ARDS. Overactivated neutrophils are the major effector cells in ARDS. Thus, extracellular bacteria triggered TH17 host immunity with neutrophil activation counts for the etiology of ARDS. Here, I use microarray analysis to describe TH17 related cytokine up-regulation in whole blood of ARDS patients. In addition, TGF-β secreting Treg cells play important roles in lung fibrosis. Thus, ARDS is actually a TH17 and Treg immune disorder.

About the author

Wan-Jiung Hu is a MD PhD. His former name is Wan-Chung Hu. His MD degree was awarded from National Taiwan University. His PhD degree was awarded from Vaccine science track of Department of International Health of Johns Hopkins University. His PhD thesis was using microarray to identify the host immunological pathway after malaria infection. His first first-author paper: “Common and divergent immune response signaling pathways discovered in peripheral blood mononuclear cell gene expression patterns in presymptomatic and clinically apparent malaria” is published in Infection and Immunity in 2006 October. Thus, he first proposed the THαβ immunity which is host immunity against viruses. A subsequent paper in 2008 called it TH9 immunity. However, TH9 immunity is not a good name since IL-9 is a TH2 cytokine. He was trained as a neurology resident in Department of Neurology of Taipei Mackay Memorial Hospital of Taiwan. Currently, he is doing postdoc research in Genomic Research Center of Academia Sinica, Taiwan. His current research topic is cancer immunotherapy. Besides, he is doing functional genomics studies. The author would like to publish this manuscript. If journal editors are interested in this paper, please feel free to contact me.

Introduction

Acute respiratory distress syndrome (ARDS) is a severe cause of respiratory failure. Despite of current treatment, the mortality rate is very high. We still don’t have successful management strategies to deal with ARDS. Most important of all, we still don’t know the exact pathophysiology of ARDS. Sepsis or bacteremia is the leading cause of ARDS. Besides, neutrophil activation is reported in many studies of lung of ARDS patients. Thus, extracellular bacteria induced TH17 immunity overactivation should be the etiology of ARDS. Here, I use a microarray analysis to study immune-related gene profiles in peripheral leukocytes of ARDS patients. I found several TH17 related effector molecules are activated in ARDS. That supports that ARDS is a TH17 dominant inflammatory disease.

Material and Methods

Microarray dataset

According to Dr. J. A. Howrylak’s research in Physiol Genomics 2009, he collected total RNA from whole blood in sepsis and ARDS patients. He tried to find out

molecular signature of ARDS compared to sepsis patients. His dataset is available in Gene Expression Omnibus (GEO) www.ncbi.nlm.nih.gov/geo (assession number GSE 10474). The second dataset is from GSE20189 of Gene Expression Omnibus. This dataset was collected by Dr. Melissa Rotunno in Cancer Prevention Research 2011. Molecular signature of early stage of lung adenocarcinoma was studied by microarray. I use the healthy control whole blood RNA from this dataset to compare the ARDS patients. In this study, I perform further analysis to study peripheral leukocyte gene expression profiles of ARDS compared to those of healthy controls.

Statistical analysis

Affymetrix HG-U133A 2.0 genechip was used in both samples. RMA express software(UC Berkeley, Board Institute) is used to do normalization and to rule out the outliners of the above dataset. I rule out the potential outliners of samples due to the following criteria:

1. Remove samples which have strong deviation in NUSE plot
2. Remove samples which have broad spectrum in RLE value plot
3. Remove samples which have strong deviation in RLE-NUSE mutiplot
4. Remove samples which exceed 99% line in RLE-NUSE T2 plot

RT-PCR confirmation

Dr. J. A. Howrylak performed real time PCR for selected transcripts (cip1, kip2) by using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA). In the second dataset, Dr. Melissa Rotunno also performed qRT-PCR test to validate the microarray results. RNA quantity and quality was determined by using RNA 600 LabChip-Aligent 2100 Bioanalyzer. RNA purification was done by the reagents from Qiagen Inc. All real-time PCRs were conducted by using an ABI Prism 7000 Sequence Detection System with the designed primers and probes for target genes and an internal control gene-GAPDH. This confirms that their microarray results are convincing compared to RT-PCR results.

Results

RMA analysis of whole blood from healthy normal control

The RMA analysis was performed for RNA samples from whole blood of healthy control of the lung adenocarcinoma dataset. Raw boxplot, NUSE plot, RLE value plot,

RLE-NUSE multiplot, and RLE-NUSE T2 plot were generated. Then, sample was included and excluded by using these graphs(Figure 1A, 1B, 1C, 1D, 1E)

RMA analysis of whole blood from acute lung injury patients

The RMA analysis was performed for RNA samples from whole blood of healthy control of the ARDS dataset. Raw boxplot, NUSE plot, RLE value plot, RLE-NUSE multiplot, and RLE-NUSE T2 plot were generated. Then, sample was included and excluded by using these graphs(Figure 2A, 2B, 2C, 2D, 2E)

Discussion

Acute respiratory distress syndrome (ARDS) is a very severe respiratory complication. Sepsis is the major risk factor of ARDS. Sepsis is the uncontrolled bacteremia by extracellular bacteria infection. In addition, PMNs overactivation is very important in the pathogenesis of ARDS. Thus, extracellular bacteria induced TH17 immunity with neutrophil activation should be the key in the pathophysiology of ARDS.

According to Harrison's internal medicine, the time course of ARDS can be divided into three stages. First, the exudative phase. In this phase, injured alveolar capillary endothelium and type I pneumocytes cause the loss of tight alveolar barrier. Thus, edema fluid rich in protein accumulate in the interstitial alveolar spaces. It has been reported that cytokines (IL-1, IL-6, and TNF-α) and chemokines (IL-8, and leukotriene B4) are present in lung in this phase. A great numbers of neutrophils traffic into the pulmonary interstitium and alveoli. Alveolar edema predominantly leads to diminished aeration and atelectasis. Hyaline membranes start to develop. Then, intrapulmonary shunting and hypoxemia develop. The situation is even worse with microvascular occlusion which leads to increasing dead space and pulmonary hypertension. The exudative phase encompasses the first seven days of disease after exposure to a precipitating ARDS risk factor such as sepsis, aspiration pneumonia, bacteria pneumonia, pulmonary contusion, near drowning, toxic inhalation injury, severe trauma, burns, multiple transfusions, drug overdose, pancreatitis, and post-cardiopulmonary bypass.

Second, proliferative phase. This phase usually lasts from day 7 to day 21. Although many patients could recover during this stage, some patients develop progressive lung injury and early change of pulmonary fibrosis. Histologically, this phase is the

initiation of lung repair, organization of alveolar exudates, and a shift from a neutrophil to a lymphocyte dominant pulmonary infiltrate. There is a proliferation of type II pneumocytes which can synthesize new pulmonary surfactants. They can also differentiate into type I pneumocytes. In addition, there is beginning of type III procollagen peptide presence which is the marker of pulmonary fibrosis.

Third, fibrotic phase. Although many patients with ARDS recover lung function three weeks after the initial lung injury, some enter a fibrotic phase that may require long term support on mechanical ventilators. Histologically, the alveolar edema and inflammatory exudates in early phases are converted to extensive alveolar duct and interstitial fibrosis. Intimal fibroproliferation in the pulmonary microvascular system leads to progressive vascular occlusion and pulmonary hypertension.

Here, I propose a detail pathogenesis to explain the three stages of ARDS. In the first exudative stage, neutrophils are attracted to lung due to chemotaxic agents such as IL-8. During sepsis, bacterial infection in pulmonary tissue can trigger pulmonary epithelial cells, pulmonary endothelial cells, pulmonary fibroblast, and alveolar macrophage to be activated. Toll-like receptors 1,2,4,5 as well as heat shock proteins (HSP60, HSP70,HSP90) are key molecules to trigger TH17 host immunity. Heat sock proteins are important stress proteins in situation such as burn, trauma, hemorrhagic shock, near drowning or acute pancreatitis. Thus, TH17 related cytokines such as IL-17, IL-1, TNF-α, and IL-6 as well as TH-17 related chemokines such as IL-8 and other CXCL group chemokines will be triggered. TH17 cytokines will start to activate TH17 immunity including activating PMN effector function and drive T helper cells to TH17 CD4 T cells. It will also activate B cells to produce IgA, IgM, and IgG2 for immunity against extracellular bacteria. The cytokine storm during ARDS now explained. It is worth noting that both innate immunity and adaptive immunity including neutrophils and lymphocytes are triggered. Thus, antibody against bacteria as well as autoantibody can be generated. The most common autoantibody found in ARDS is IL-8 autoantibody. IL-8 is the main chemoattractant in pulmonary tissue. It was first identified in lung giant cell lines. The immune-complex of IL-8 and IL-8 autoantibody can further recruit and activate neutrophils. IL-8 autoantibody is also related to the prognosis of ARDS. In other conditions inducing ARDS such as trauma, burn, pancreatitis, or hemorrhagic shock, the presence of autoantibody can cause the sustain of ARDS disease progress. Besides, IL-8 has high affinity to bind to the heparin sulfate and chondroitin sulfate enriched lung tissue. And, IL-8 retention in pulmonary tissue can further recruit neutrophils to lung. It can explain why IL-8 secreted from distant site such as pancreas during acute pancreatitis can cause

ARDS.

Bacterial infection is the most common risk factor of ARDS. However, certain pathogens other than bacteria also are risks for developing ARDS. Plasmodium falciparum malarial infection can also cause the complication of ARDS. The reason for this is that Plasmodium falciparum can activate heat shock proteins to trigger TH17 immunity to cause ARDS.(author's paper in press) SARS-CoV and H1N1 Avian flu virus can also down-regulate normal anti-viral interferon-α/β and up-regulate TH17 immunity to trigger ARDS. (author's paper in press: Viral Immunology) Thus, the above phenomonons suggest that TH17 inflammation is the key to the pathogenesis of ARDS. If different pathogens lead to a common pathway of TH17 immunity, they will cause the same consequence of ARDS. It is also seen in burn, trauma, or pancreatitis when TH17 autoimmunity is also activated.

In the second proliferative stage, lymphocytes replace neutrophils and become the dominant population in ARDS. These lymphocytes are TH17 lymphocytes and subsequent Treg lymphocytes. TH17 helper cells can secrete TH17 cytokines such as IL-17, IL-1, IL-6, and TNF-α to continue the inflammatory process. However, once the bacterial antigen during sepsis is cleared. Toll-like receptor signaling is stopped, and no further proinflammatory cytokines such as IL-6 is synthesized. In addition, no further IL-8 is synthesized. IL-8 is the autoantigen for generating IL-8 autoantibody in ARDS. If there is no further IL-8 antoantigen, IL-8 autoantibody producing B cells will stop to proliferate. In TH17 immunity, both TGF-β and IL-6 are two important triggering cytokines. If there is no longer IL-6 signaling, only TGF-β is generated. IL-6 is the key factor to regulate the balance between Treg cells and TH17 cells. If there is enough IL-6, Treg cells will become TH17 cells. If there is not enough IL-6, TGF-β secreting Treg cells will be maintained. Thus, in the third fibrosis stage, TGF-β secreting Treg cells are the dominant effector cells in ARDS. TGF-β is a very strong fibrosis promoting agent. TGF-β will promote the synthesis of multiple collagen genes. Thus, overproduction of TGF-β in lung tissue will cause pulmonary fibrosis. TGF-β caused fibrosis is usually a process for repairing cavity after bacterial infection locus such as abscess. This mechanism can solve many controversial studies before. Several studies found that TLR4 and heat shock proteins can aggravate ARDS. However, another studies found that TLR4 or heat shock protein can protect from pulmonary fibrosis after acute lung injury. It is because TLR and heat shock signaling can maintain the activation of proinflammatory cytokines such as IL-6. Thus, no solely TGF-β overproduction happens for lung fibrosis. Thus, TH17 and Treg inflammatory process can fully explain the pathogenesis of ARDS. After knowing the complete

pathophysiology of acute respiratory distress syndrome, we can develop better treatment strategies to managing this highly detrimental disease.

References

Howrylak J.A. et al. Discovery of the gene signature for acute lung injury in patients with sepsis. Physiol Genomics 37,133-139 (2009)

Rotunno M et al. A gene expression signature from peripheral whole blood for stage 1 lung adenocarcinoma. Cancer Prevention Research 4,1599-1608 (2011)

Figure legends

Figure 1. RMA express plot for selecting samples in normal healthy controls.
1-A NUSE boxplot for normal control
1-B RLE boxplot for normal control
1-C RLE-NUSE multiplot for normal control
1-D RLE-NUSE T2 plot for normal control
1-E Raw data Boxpolt for normal control

Figure 2. RMA express plot for selecting samples in ARDS patients.
2-A NUSE boxplot for ARDS patients
2-B RLE boxplot for ARDS patients
2-C RLE-NUSE multiplot for ARDS patients
2-D RLE-NUSE T2 plot for ARDS patients
2-E Raw data Boxplot for ARDS patients

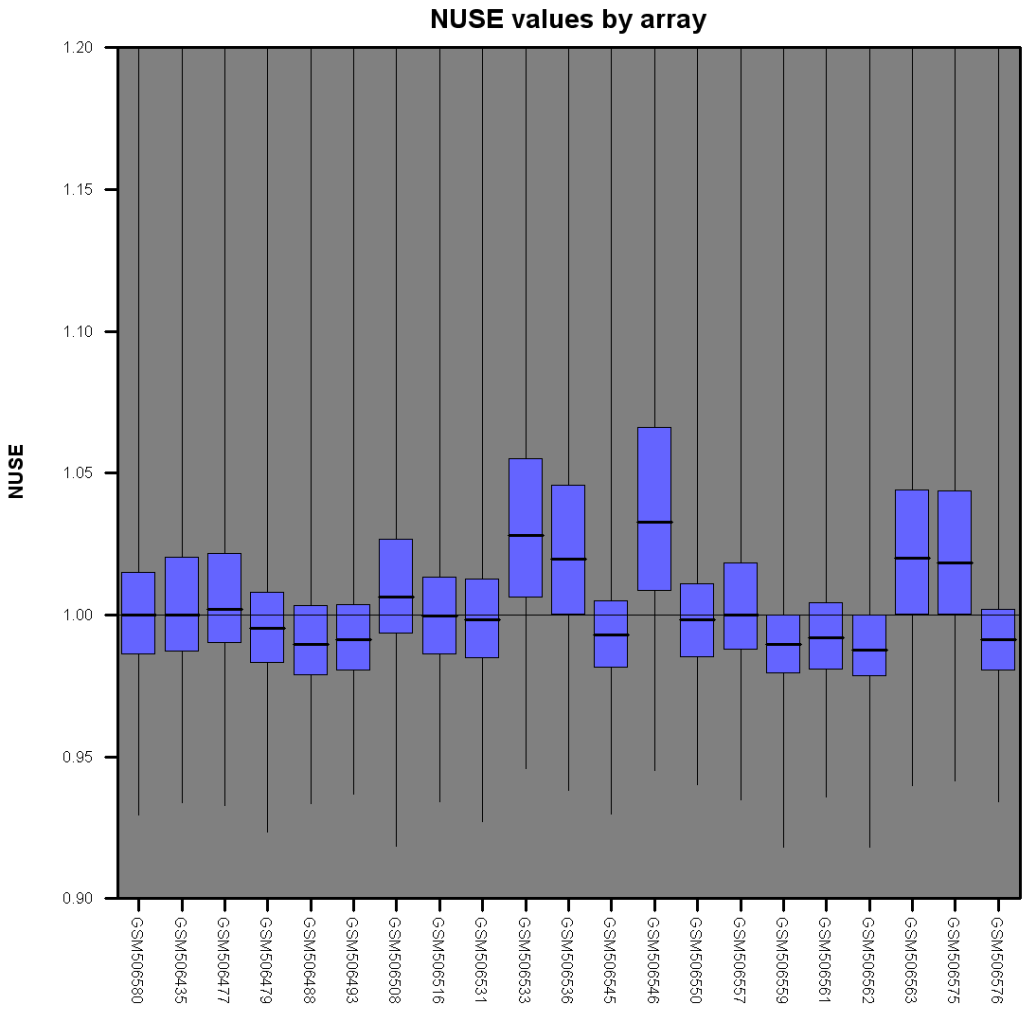
NUSE values by array
NUSE
1.20
1.15
1.10
1.05
1.00
0.95
0.90
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-A

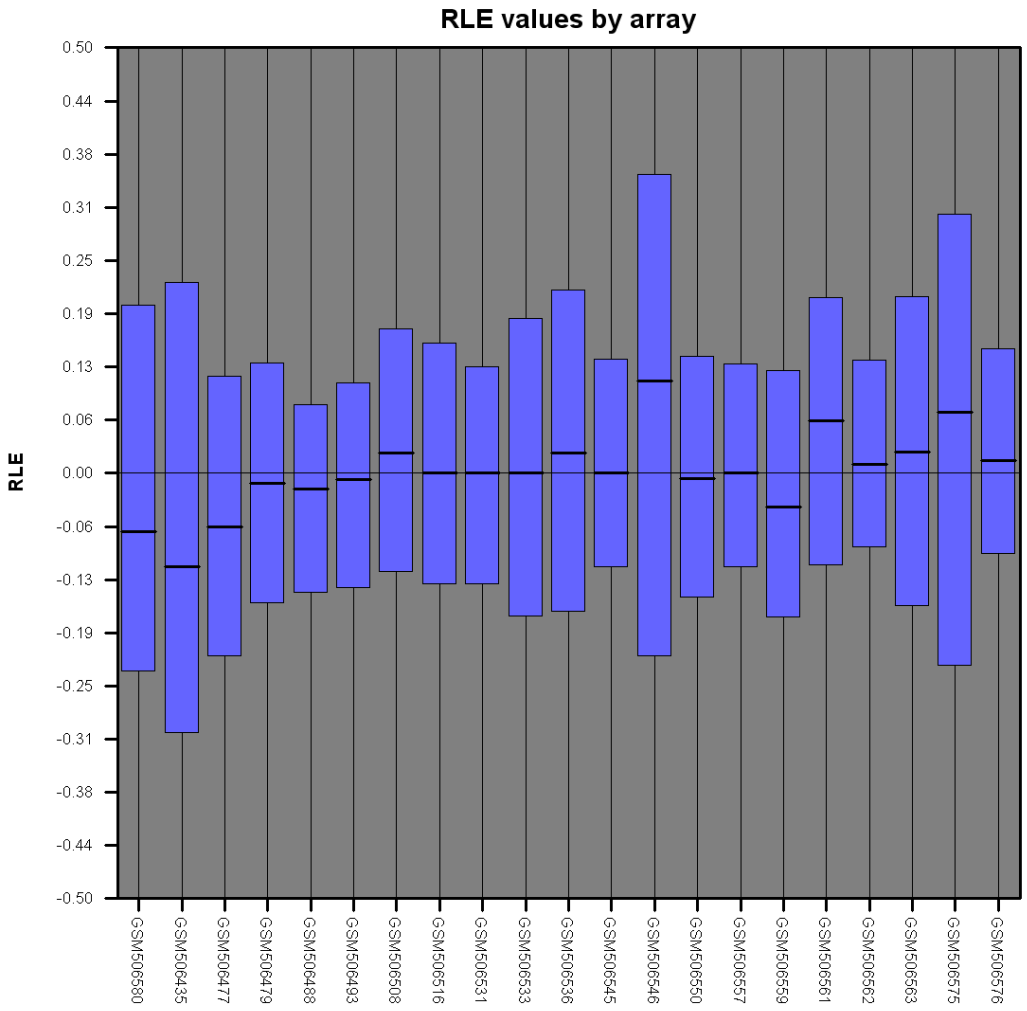
RLE values by array
RLE
0.50
0.44
0.38
0.31
0.25
0.19
0.13
0.06
0.00
-0.06
-0.13
-0.19
-0.25
-0.31
-0.38
-0.44
-0.50
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-B

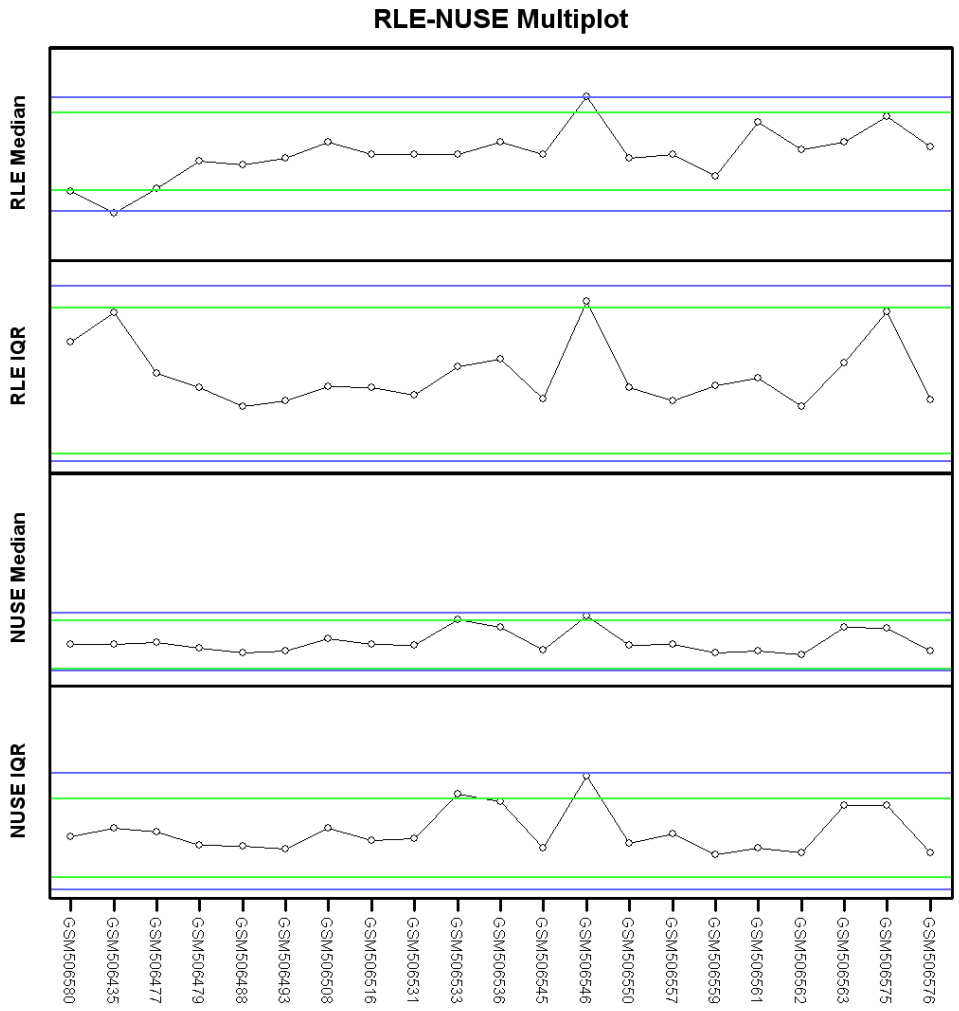
RLE-NUSE Multiplot
RLE Median
RLE IQR
NUSE Median
NUSE IQR
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-C

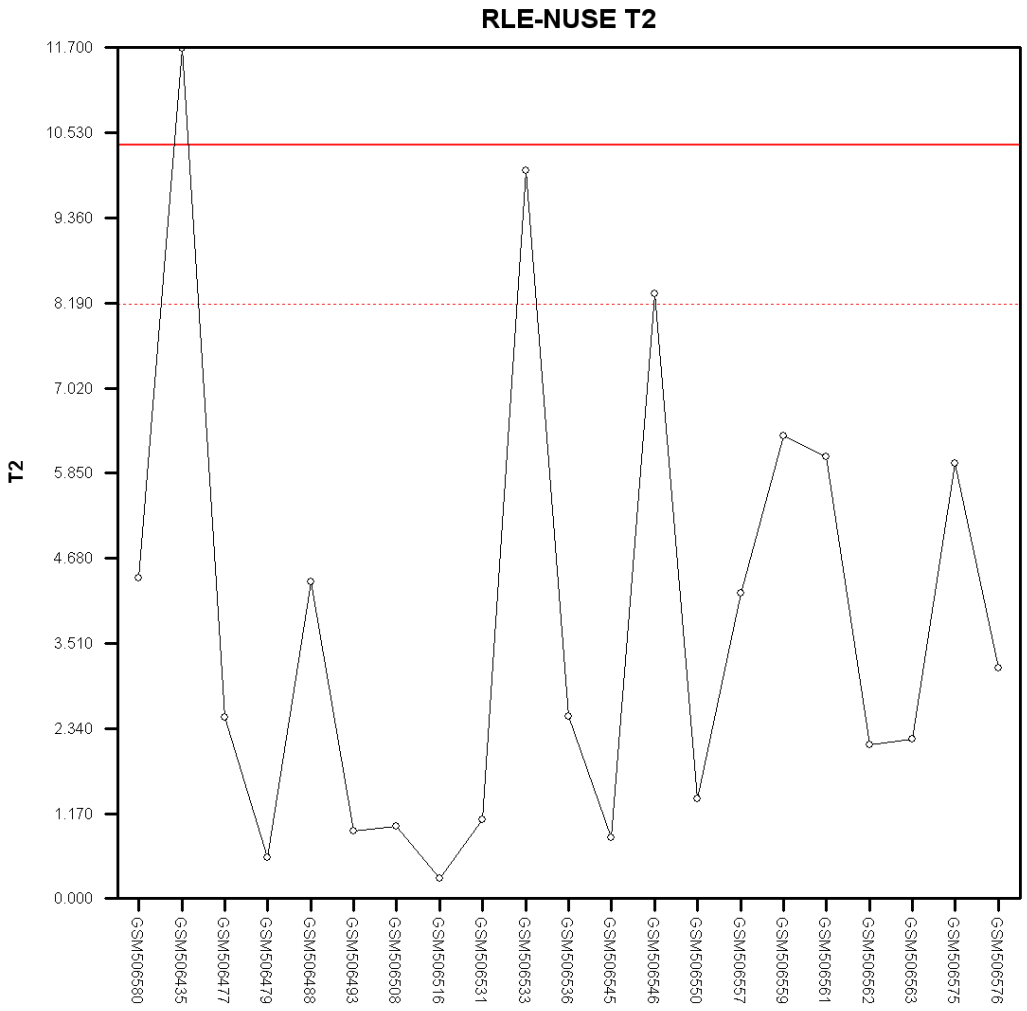
RLE-NUSE T2
T2
11.700
10.530
9.360
8.190
7.020
5.850
4.680
3.510
2.340
1.170
0.000
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-D

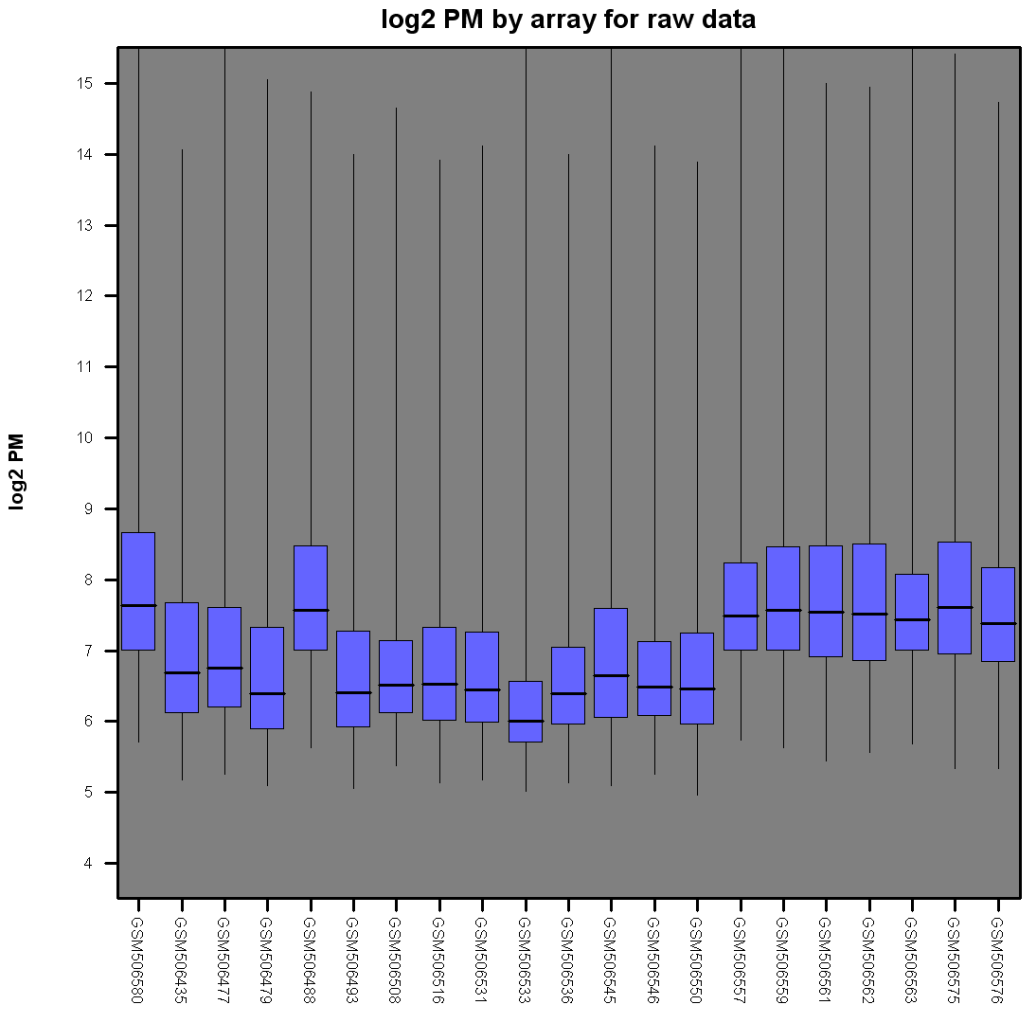
log2 PM by array for raw data
log2 PM
15
14
13
12
11
10
9
8
7
6
5
4
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-E

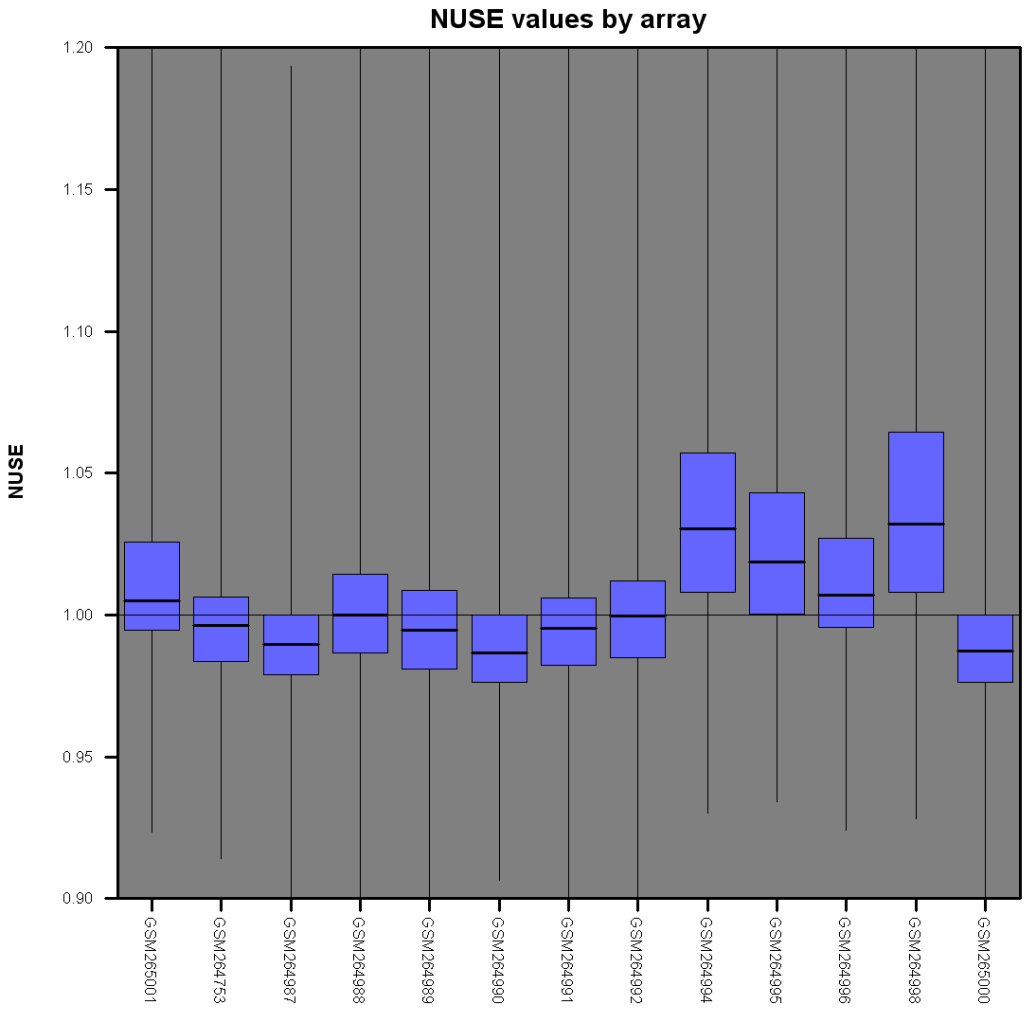
NUSE values by array
NUSE
1.20
1.15
1.10
1.05
1.00
0.95
0.90
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-A

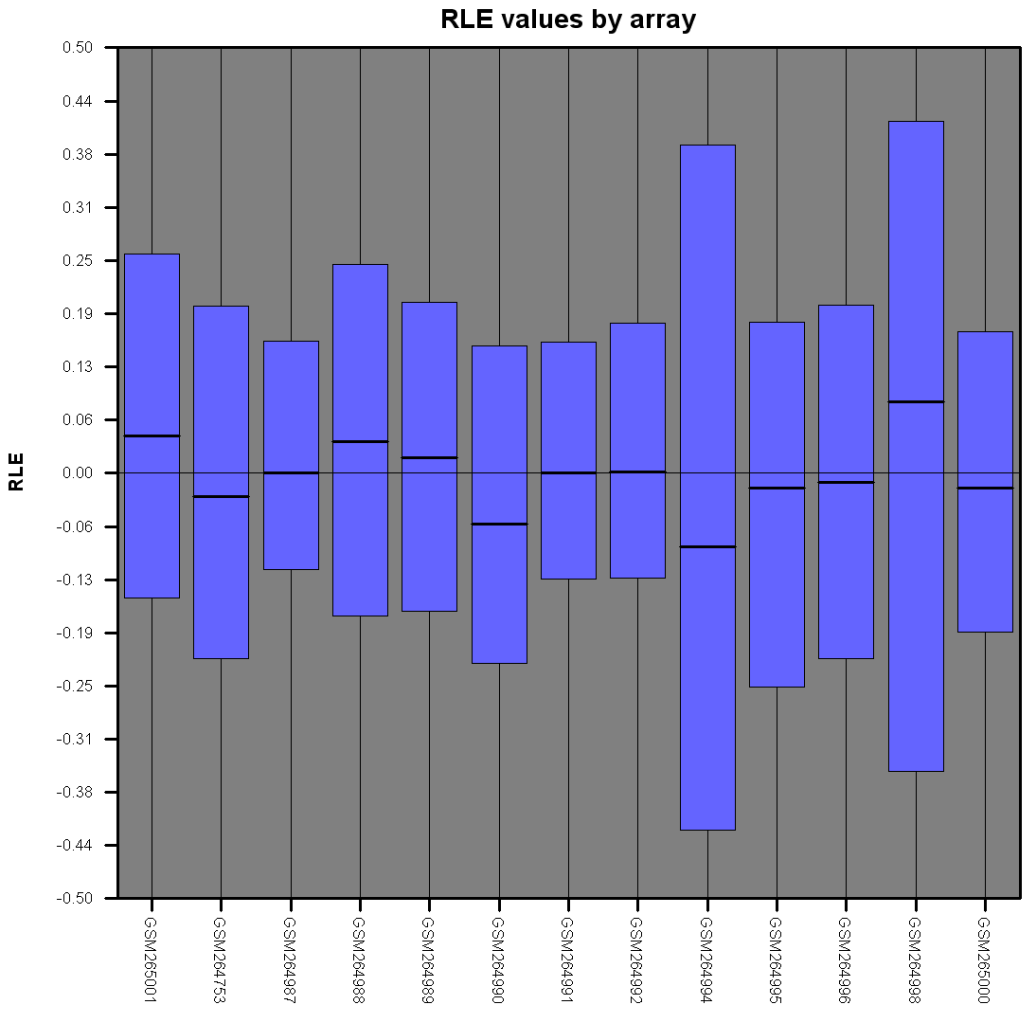
RLE values by array
RLE
0.50
0.44
0.38
0.31
0.25
0.19
0.13
0.06
0.00
-0.06
-0.13
-0.19
-0.25
-0.31
-0.38
-0.44
-0.50
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-B

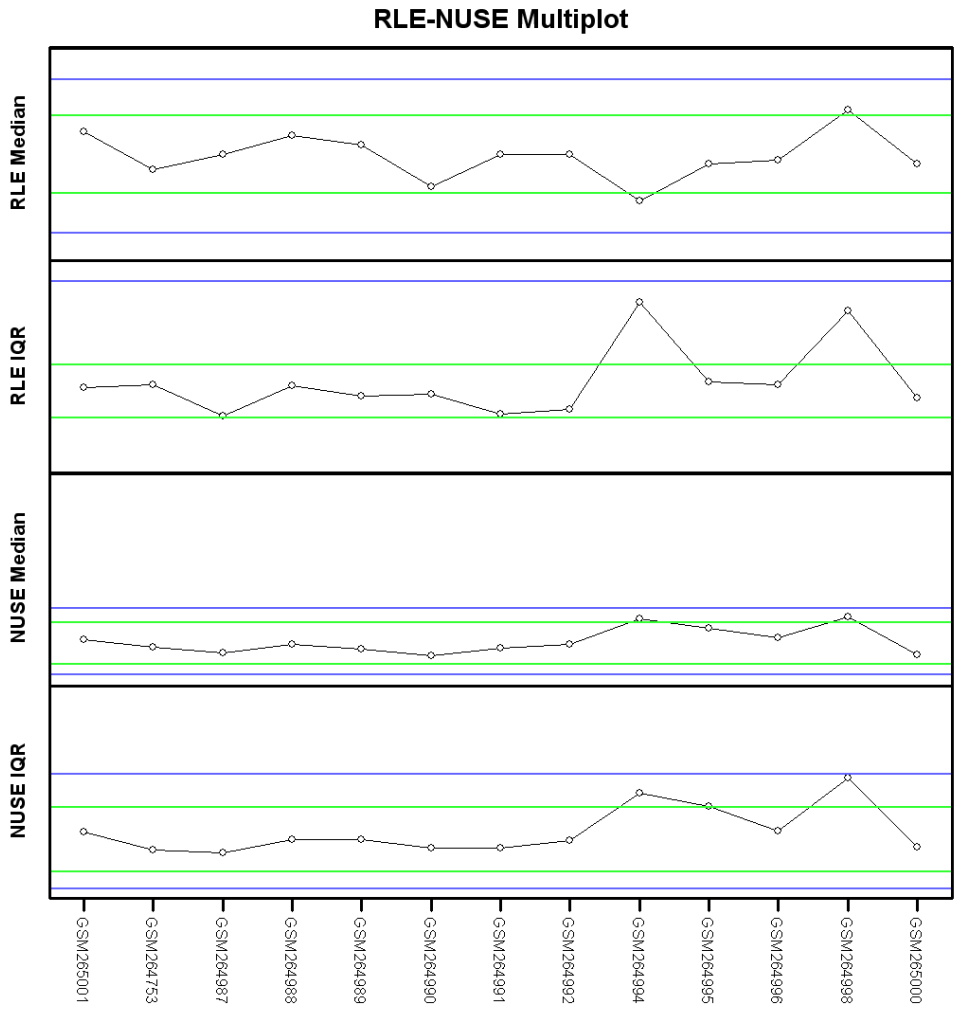
RLE-NUSE Multiplot
RLE Median
RLE IQR
NUSE Median
NUSE IQR
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-C

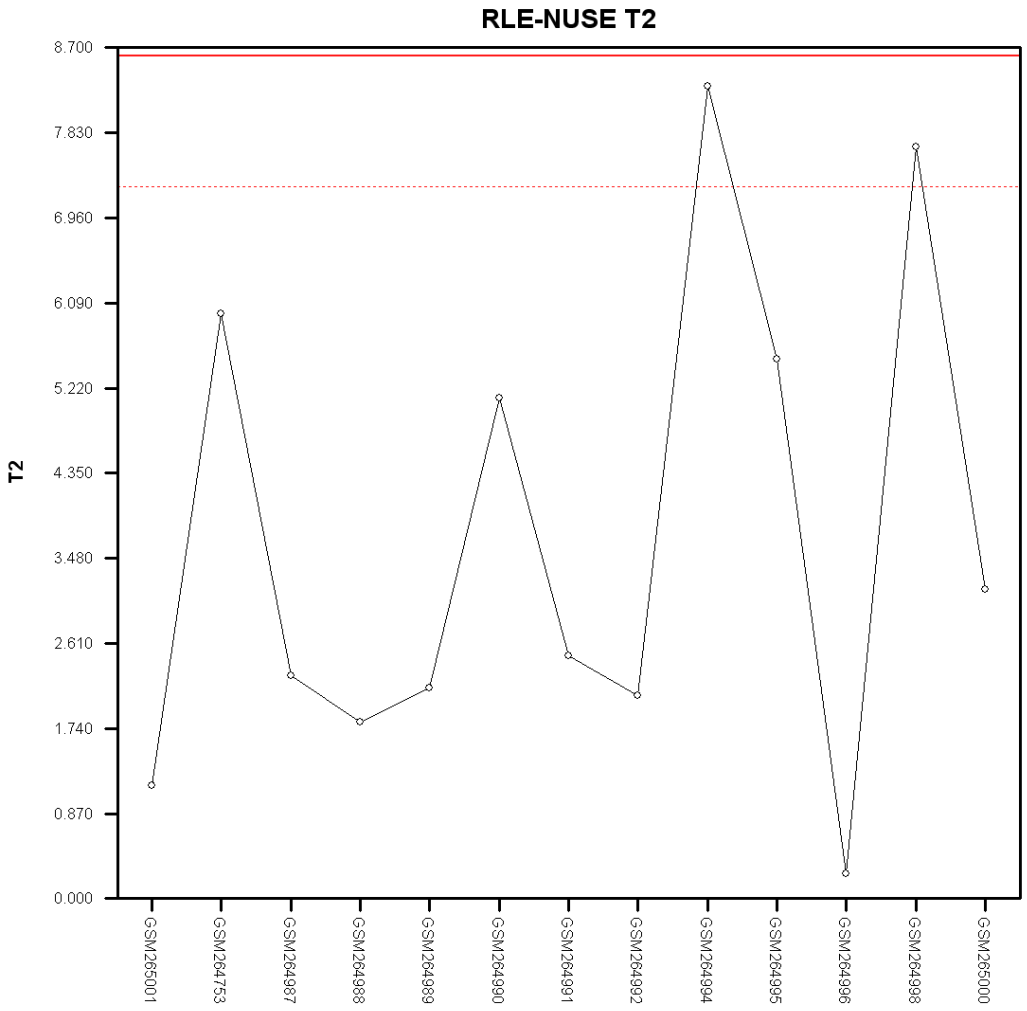
RLE-NUSE T2
T2
8.700
7.830
6.960
6.090
5.220
4.350
3.480
2.610
1.740
0.870
0.000
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-D

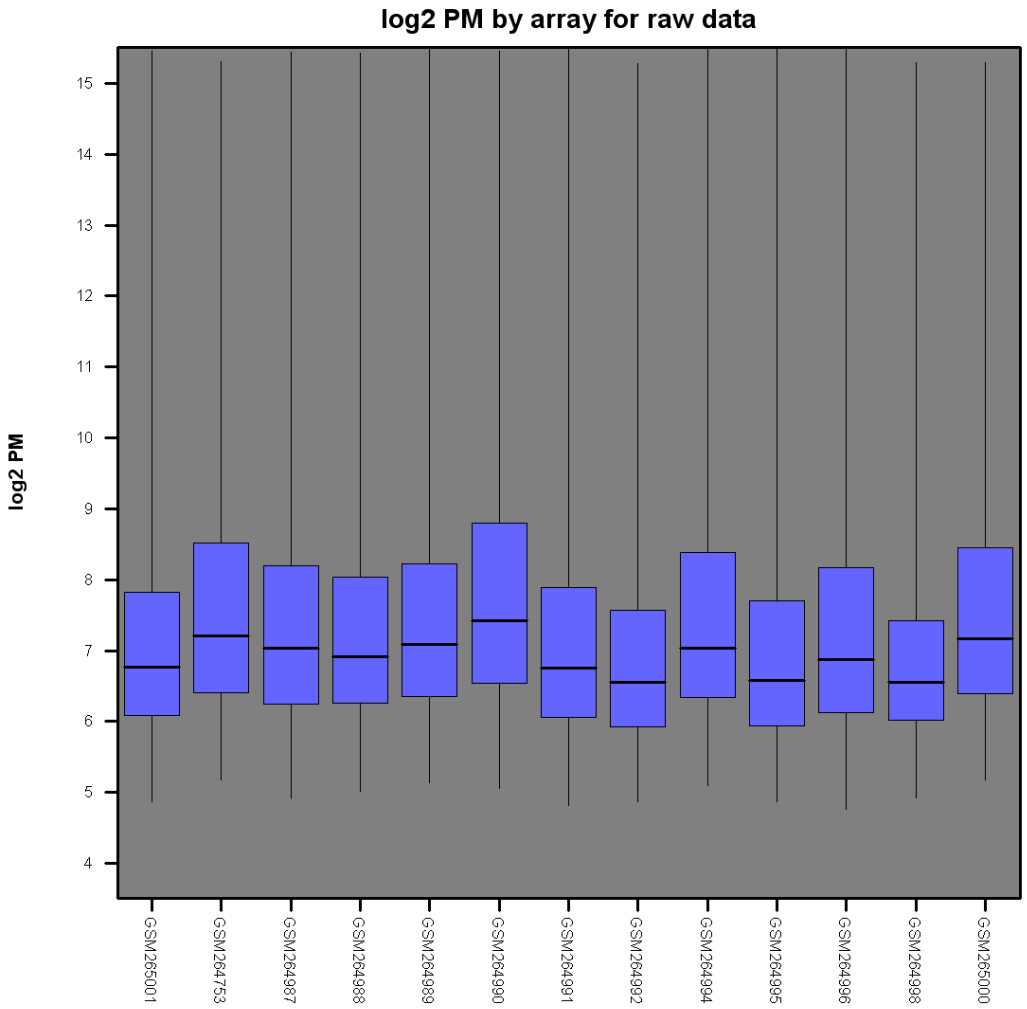
log2 PM by array for raw data
log2 PM
15
14
13
12
11
10
9
8
7
6
5
4
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-E