**ISSN 0974-3618 (Print)**
**0974-360X (Online)**

**www.rjptonline.org**

***RESEARCH ARTICLE***

# Computational studies on Differential gene Expression in Malaria Microarray Dataset

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## ABSTRACT:
Malaria is considered to be one of the deadliest diseases among various parasitic diseases. Over the past ten decades, *Plasmodium falciparum* developed high drug resistance towards the existing standard drug quinine. The drug quinine is one of the natural alkaloids derived from the bark of the cinchona tree and for many centuries, it has been used as the anti-malarial drug. One of the main reasons for the development of drug-resistance in the parasite was that the drug quinine may lose its ability to inhibit the function of parasite genes which was responsible for causing 90% of the malarial disease to humans. In the present study, an in silico analysis was performed over malarial genes of *Plasmodium falciparum* using GEO (Gene Expression Omnibus) Databases. GEO2R tool is used to compare two or more set of experimental sample present in GEO Datasets, in order the recognize genes which show signs of the drug resistance towards the prescribed drug. Thus in the present study, the malaria micro-array datasets with the standard drug quinine were analyzed using bioinformatics tools and databases. The results retrieved from the databases predicted the genes which exhibit the drug resistance towards the standard drug quinine. Thus in silico study might provide novel clues in identifying the drug-resistant malarial genes of parasite *Plasmodium falciparum*.

**KEYWORDS:** Malaria, Drug Quinine, GEO Datasets, GEO2R

## INTRODUCTION:
Malaria is one of the life-threatening fever caused by parasitic protozoans[1]. The parasite invades the red blood cells (RBC) and is transmitted by mosquitoes in many tropical, subtropical regions which affect both humans and animals[2,3]. Five types of Plasmodium parasites specifically infect humans. They are *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi*.

The *Plasmodium knowlesi* has been identified recently[4]. Among the five different parasites, only *Plasmodium falciparum* was responsible for causing 90% of the malarial infection and the infection is transmitted through the female Anopheles mosquito[5-7]. P.falciparum malaria is a malignant disease. It has the highest complication rates and mortality[8, 9]. Once the parasite enters into the host's bloodstream and infects the liver cells and red blood cells (RBC). Multiple infections of red blood cells are more common in P. falciparum than in other species[10-12].

Malaria remains a disease of global health importance with 3.3 billion people in 97 countries at risk, leading to an estimated 200 million cases and around 600,000 deaths (WHO, 2015)[13]. In 2016 overall 216 million cases of malaria occurred around the world and 445,000 people died, mostly children in the region of Africa[14]. In every year about 1,700 malaria cases were diagnosed in USA[15,16]. In India, 6% of global malaria cases and 7% of deaths were reported in the year 2016 according to a report released by the World Health Organization

Received on 02.08.2019 Modified on 09.09.2019
Accepted on 31.10.2019 
*Research J. Pharm. and Tech 2020; 13(3): 1368-1376.*
**DOI: 10.5958/0974-360X.2020.00252.8**

(WHO)[17]. Globally 4,45,000 deaths were reported due to malarial infection in 2016 and in that count; about 80% of the malarial deaths were observed in 15 countries, namely, India and 14 countries in Sub-Saharan Africa[18]. The WHO figures and suggests that India is unlikely to reduce its case burden beyond 40% by 2020[19].

A recent survey on the malarial proportion revealed that among 46% cases, 70% of populations are affected with *Plasmodium falciparum*[20] and 47% of death cases are registered[21,22]. Though malaria is preventable and treatable in India, during recent decades the curable rate has been gradually decreased and now it has become one of the major public health problems[23, 24]. The anti-malarial drug quinine remains a drug for almost 400 years in treating the malarial disease[25-27]. The drug quinine inhibits the function of the malarial genes by both biological[28] and physiological process[29,30]. Due to the prolonged existence of the drug, the malarial genes of parasite *Plasmodium falciparum* developed drug resistance capacity even to overcome the high dose of the quinine[31-33]. The present study is carried out to identify the genes which enhance the malarial infection even after the treatment of the standard drug quinine.

The bioinformatics tools GEO(Gene Expression Omnibus)2R was used in this study to identify DEGs (Differential gene expressions) based on the statistical analysis of adj.p.value, P-value, log FC value and False Discovery Rate (FDR) value. In contrast to the experimental study, the *in silico* study predict the genes which exhibit the enhancing activity even after the ingestion of the standard drug[34].

## MATERIALS AND METHODS:

**Geo profiles**:

The GEO Profiles database stores gene expression profiles which were derived from curate GEO Datasets. Each gene profile is represented as a chart format which displays the expression level of one gene across all samples within a data set. The experimental value of the genes was provided in bars along the bottom of the charts, by analyzing the experimental values genes which are differentially expressed across different experimental conditions were identified. GEO profiles have various types of internal links and external links that connect genes which exhibit similar behavior.

**GEO Datasets:**

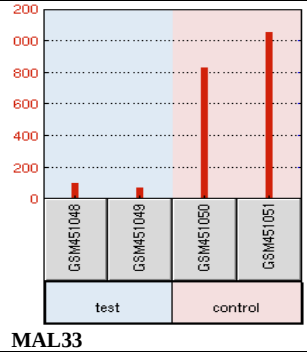
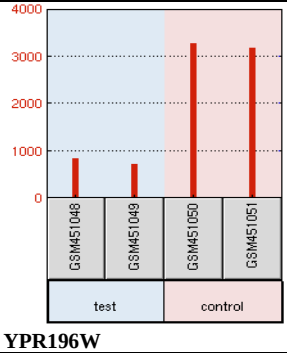
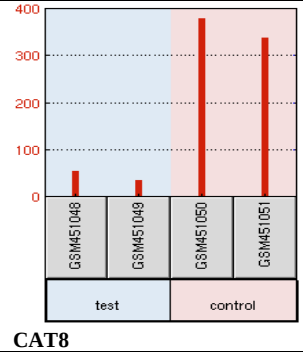
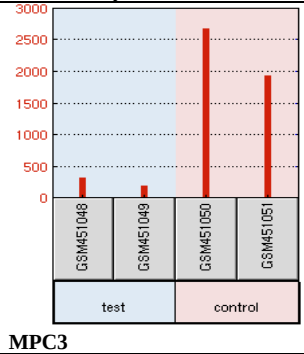
The GEO datasets contain microarray blood samples, next-generation sequencing and other forms of high-throughput functional genomic data. The database stores the curate gene expression dataset, original series, and platform records in the GEO depository. GEO dataset records contain additional database including cluster tools and differential gene expression (DEG) queries. About 90% of the data available in GEO database are collected from gene expression studies through a broad range of investigation in biological properties which including disease development, ecology, evolution, immunity, toxicology, and metabolism.

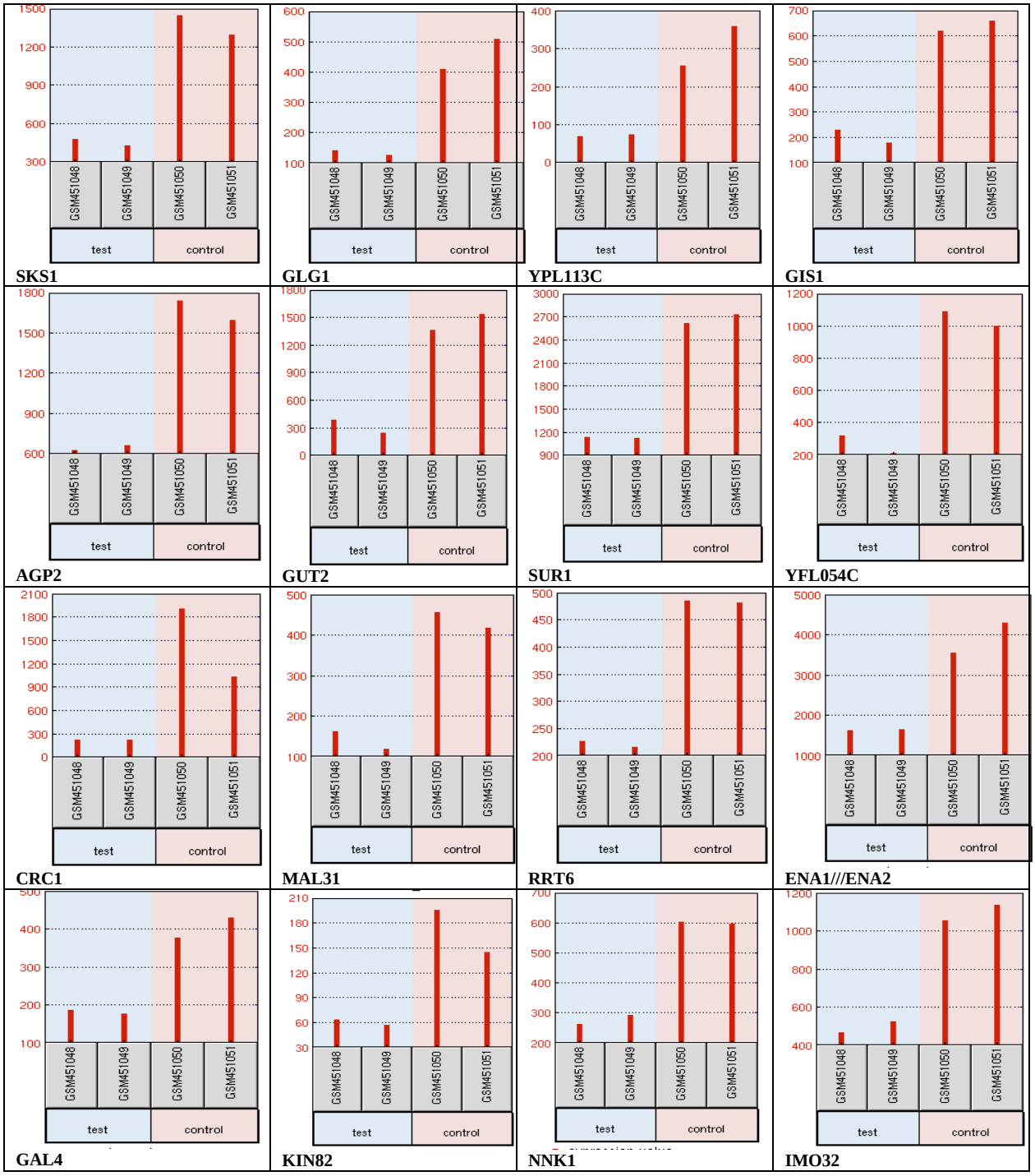
**GEO2R:**

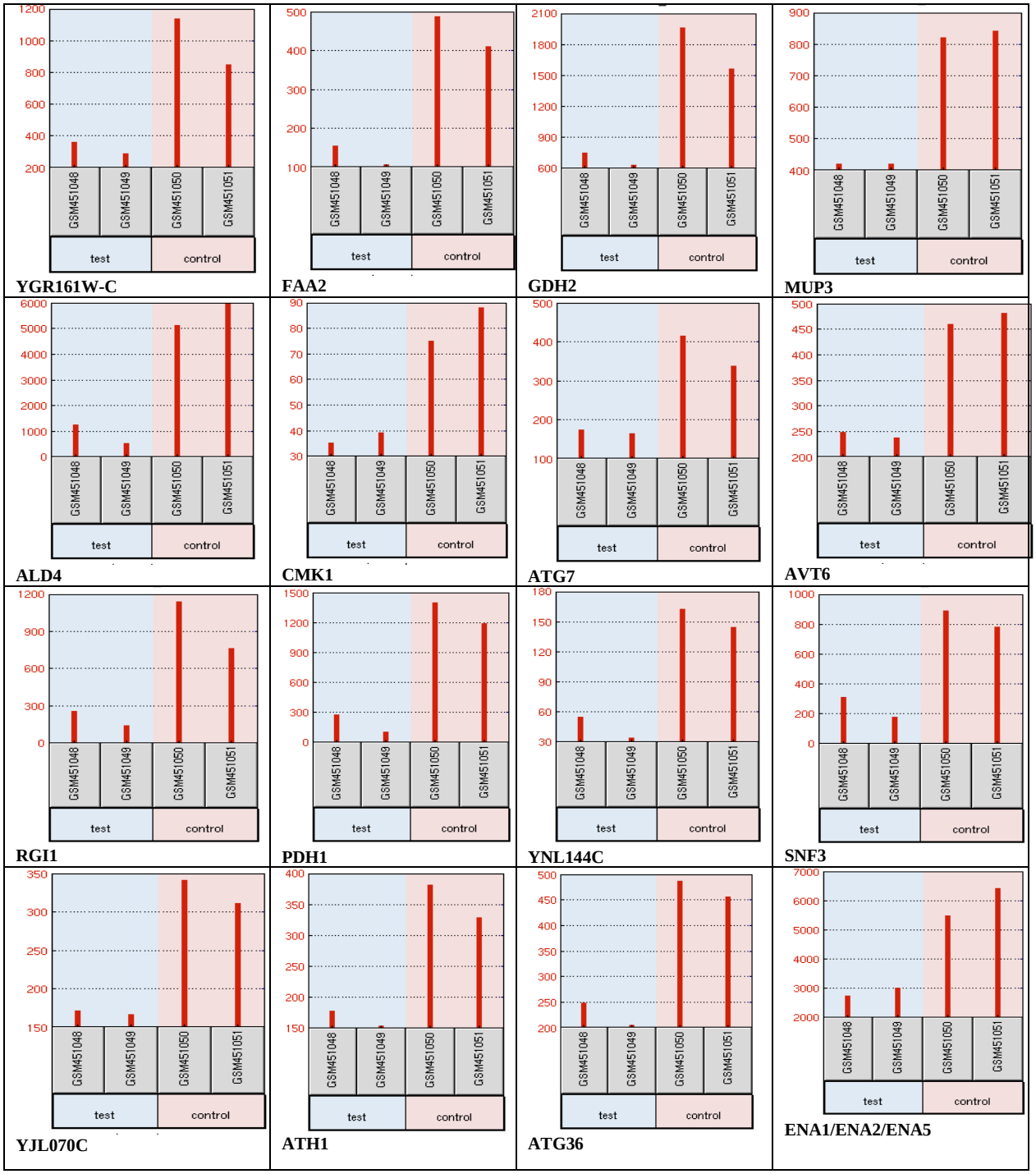
GEO2R database compares two or more group of microarray samples in a GEO Series to identify genes that are differentially expressed across experimental conditions. Results predicted by the database are presented in the form of table and list of genes order in the table based its significant properties[35]. In GEO2R database, there are 5 steps to be followed for analyzing the data. They are as follows:

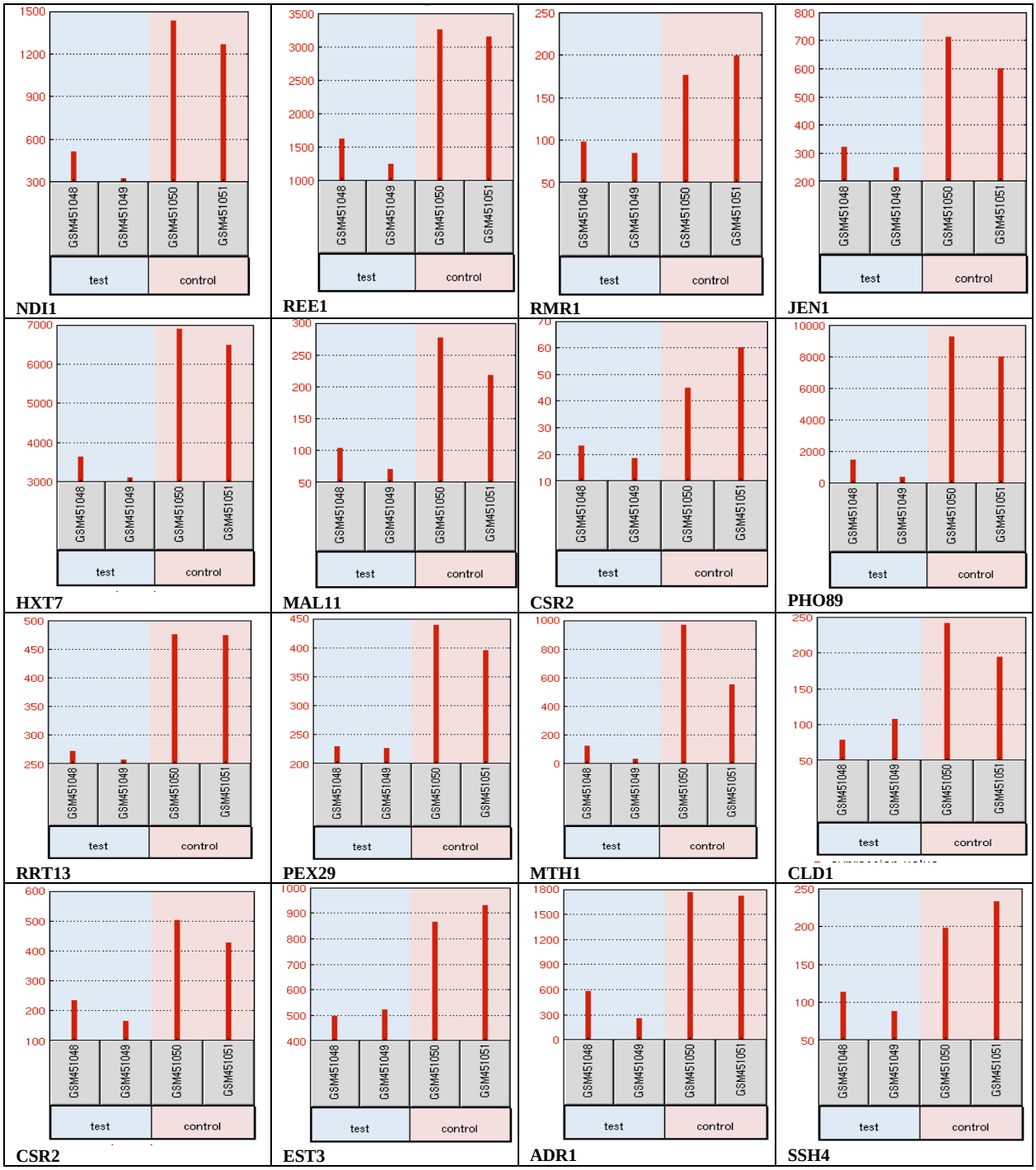
1. Selection of experiment from the GEO Profile (Quinine effect on *Saccharomyces cerevisiae*).
2. Define sample groups (GSE18037)
3. Analyze the dataset with GEO2R database
4. Assign samples to groups (Test and Control); Test sample (GSM451048 and GSM1049) & Control sample (GSM451050 and GSM451051)
5. Interpret the GEO result with the profile graph.

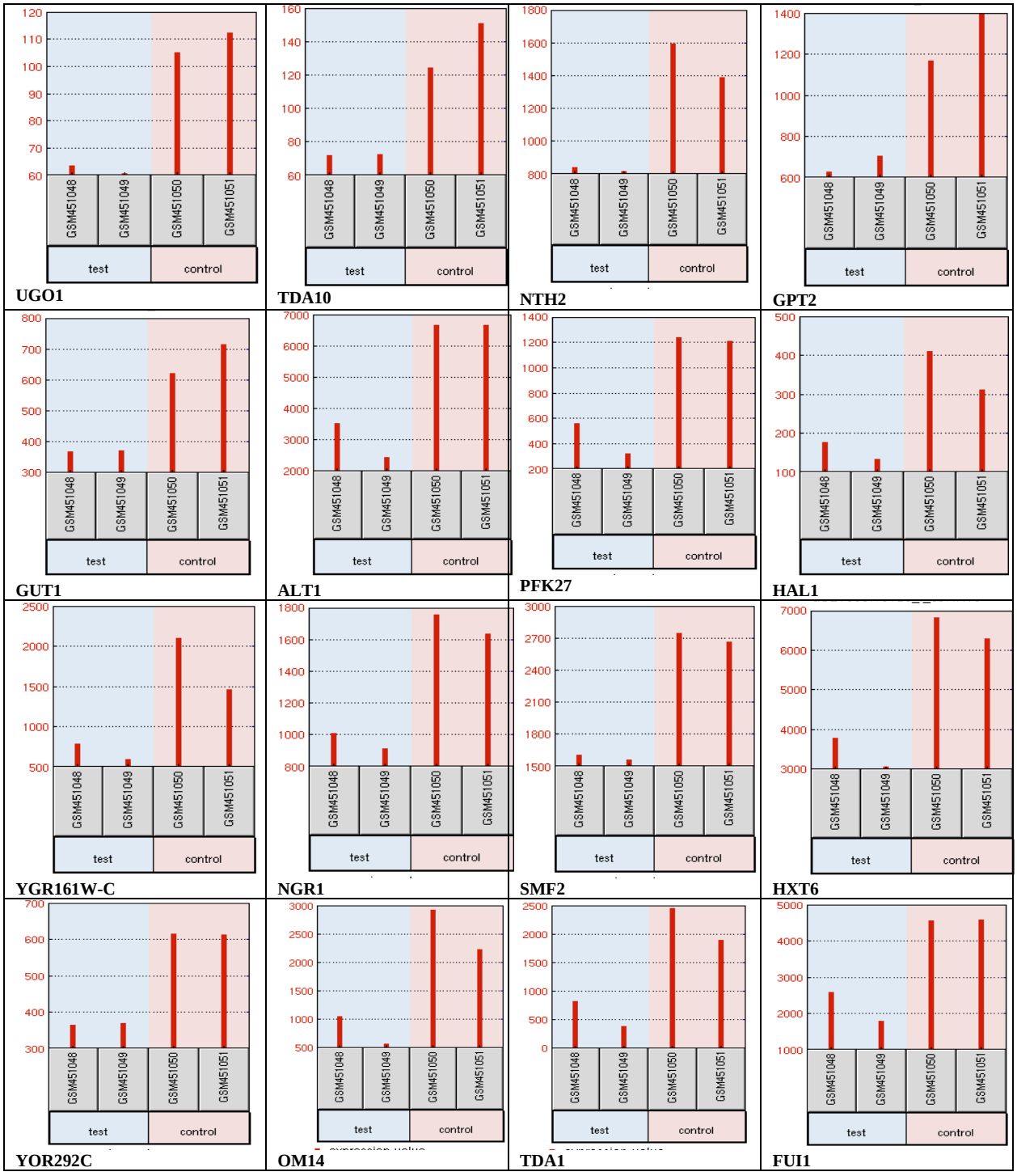
## RESULTS AND DISCUSSION:

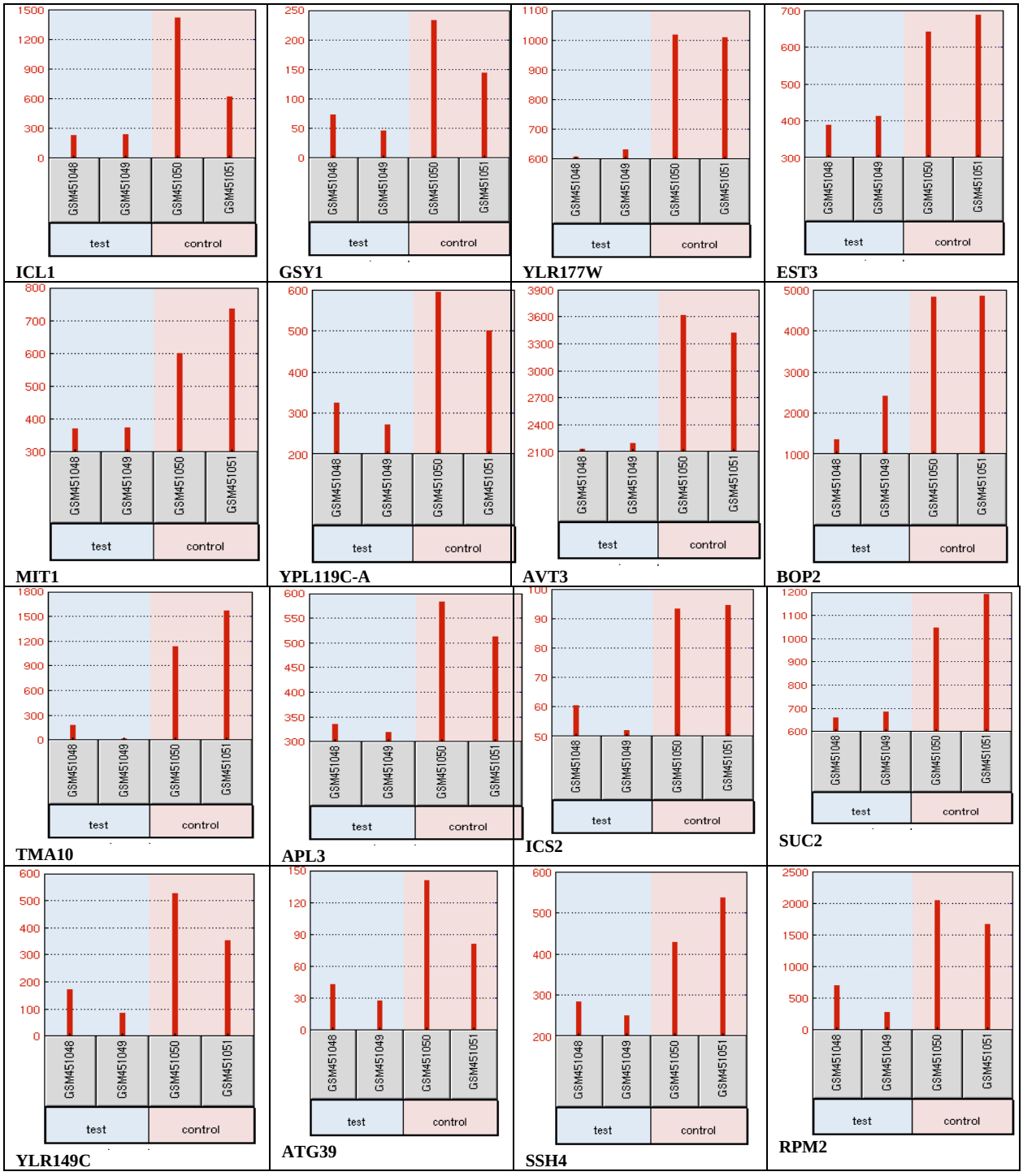
| Description of the gene and its Profile Graph | Description of the gene and its Profile Graph | Description of the gene and its Profile Graph | Description of the gene and its Profile Graph |
|---|---|---|---|
|  |  |  |  |
| **MAL33** | **YPR196W** | **CAT8** | **MPC3** |

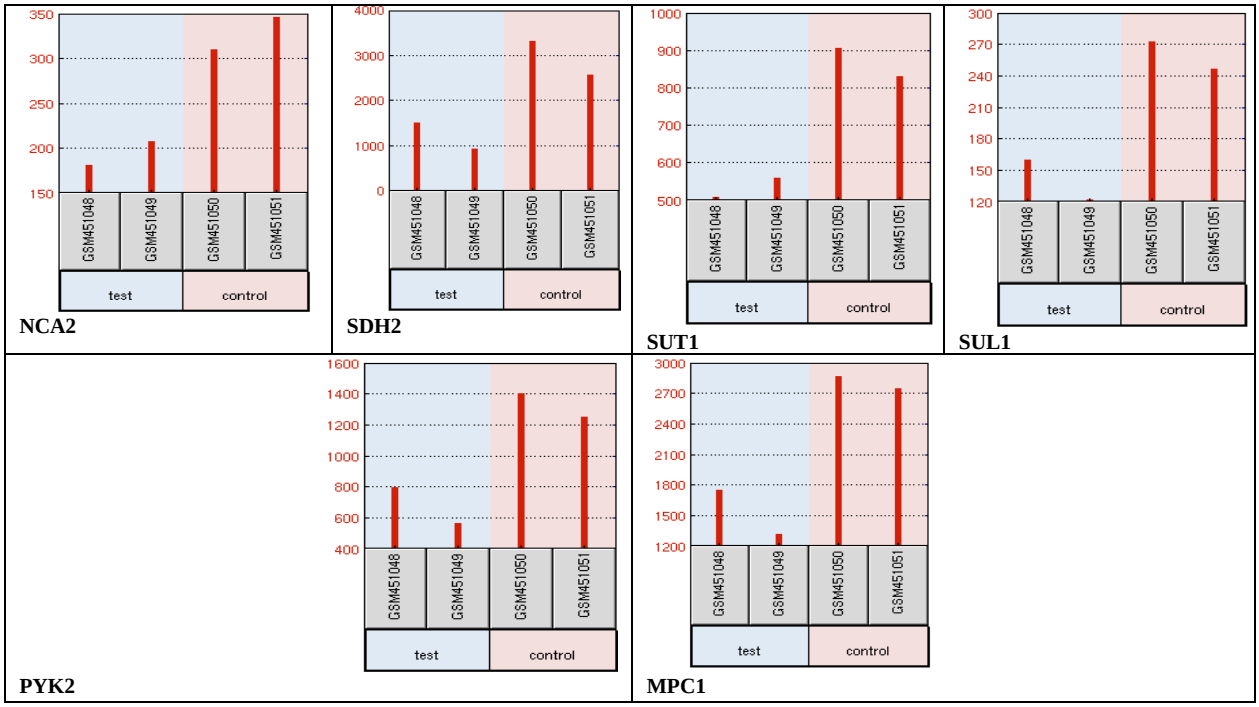

SKS1
GLG1
YPL113C
GIS1
AGP2
GUT2
SUR1
YFL054C
CRC1
MAL31
RRT6
ENA1///ENA2
GAL4
KIN82
NNK1
IMO32
GSM451048
GSM451049
GSM451050
GSM451051
test
control


YGR161W-C
FAA2
GDH2
MUP3
ALD4
CMK1
ATG7
AVT6
RGI1
PDH1
YNL144C
SNF3
YJL070C
ATH1
ATG36
ENA1/ENA2/ENA5
GSM451048
GSM451049
GSM451050
GSM451051
test
control


NDI1
REE1
RMR1
JEN1
HXT7
MAL11
CSR2
PHO89
RRT13
PEX29
MTH1
CLD1
CSR2
EST3
ADR1
SSH4
GSM451048
GSM451049
GSM451050
GSM451051
test
control


UGO1
TDA10
NTH2
GPT2
GUT1
ALT1
PFK27
HAL1
YGR161W-C
NGR1
SMF2
HXT6
YOR292C
OM14
TDA1
FUI1
GSM451048
GSM451049
GSM451050
GSM451051
test
control


ICL1
GSY1
YLR177W
EST3
MIT1
YPL119C-A
AVT3
BOP2
TMA10
APL3
ICS2
SUC2
YLR149C
ATG39
SSH4
RPM2
GSM451048
GSM451049
GSM451050
GSM451051
test
control




The GEO2R tool predicted DEG profile graphs for the malarial dataset GSE18037 patient samples (GSM451048, GSM451049, GSM451050, GSM451051) treated by the drug quinine. On analyzing the graphs further[36], it was predicted that the drug quinine showed good inhibitory activity towards 201 malarial genes and drug quinine showed poor inhibitory activity towards 49 genes, they are as follows : HXT7, MAL11, CSR2, PHO89, RRT13, PEX29, MTH1, CLD1, CSR2, EST3, ADR1, SSH4, UGO1, TDA10, NTH2, GPT2, GUT1, ALT1, PFK27, HAL1, YGR161W-C, NGR1, SMF2, HXT6, OM14, TDA1, FUI1, ICL1, GSY1, YLR177W, EST3, MIT1, YPL119C-A, AVT3, BOP2, TMA10, APL3, ICS2, SUC2, YLR149C, ATG39, SSH4, RPM2, NCA2, SDH2, SUT1, SUL1, PYK2, MPC1. From the results of GEO2R tool, it was revealed that the above 49 genes showed upregulation towards the standard drug quinine and it also confirmed that the quinine does not overcome the mechanical and physiological action of the genes in malarial patients. The results obtained in this current study would be useful in the further designing and development of novel anti-malarial drug compounds.

## CONCLUSION:

Malaria dataset of GSE18037 was analyzed by GEO2R to identify genes differentially expressed across investigational conditions. The result of the current study revealed that the 49 genes were responsible for causing malaria even after the intake of standard drug quinine in the primary stage. It also confirms that the malarial drug quinine does not exhibit any inhibitory activity towards those 49 genes and rather the drug enhances functions of those genes. Thus the current *in silico* analysis would pay the way for the design and development of novel anti-malarial drug molecules.

## CONFLICT OF INTEREST:

The authors declare they have no competing interests.

## ACKNOWLEDGMENT:

We acknowledge the Vels Institute of Science, Technology and Advanced Studies (VISTAS) for providing us with the required infrastructure and support system needed.

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