

The CCBR microarray pipeline

Shiny App

Quick Start

Running the Microarray App locally

```
library(shiny)  
runApp("MicroArrayPipeline")
```

App Interface

CCBR Microarray analysis workflow

(For Affymetrix human and mouse data)

Step1- Provide input

Project ID:

Select CEL files

6 files

Upload complete

Choose phenotype file

Phenotype.txt

Upload complete

Choose contrast file

Contrast.txt

Upload complete

P-value threshold for DEGs

FC threshold for DEGs

P-value threshold for pathways

Step2- click on **start** to load/normalize data

1

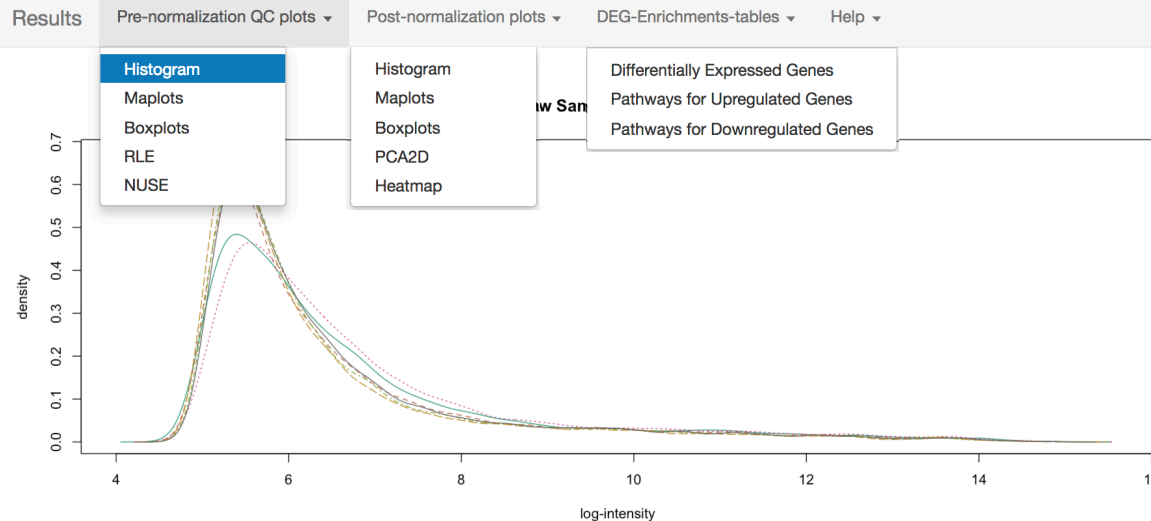
2

3

Step4- select contrast and enrichment analysis thresholds

Choose contrast to show

Step5- generate Html report and download results



Step 3- access all menu options

App Input


- Mandatory input
 - Project id
 - Affymetrix Raw data (cel files)
 - Phenotype data file
 - List of contrasts file

Supported platforms


- Current supported human platforms:
 - *GeneChip™ Human Genome U133 Plus 2.0 Array*
 - *GeneChip™ Human Gene 2.0 ST Array*
 - *Clariom™ S Assay HT, human*
 - *Clariom™ S Assay, human*
- Current supported mouse platforms:
 - *GeneChip™ Mouse Gene 2.0 ST Array*
 - *Clariom™ S Assay HT, mouse*
 - *Clariom™ S Assay, mouse*

Phenotype file

Mandatory Field names



SampleName	SampleID	SampleGroup	GroupNumber
b1.CEL	b1	gb	1
b2.CEL	b2	gb	1
b3.CEL	b3	gb	1
s1.CEL	s1	gs	2
s2.CEL	s2	gs	2
s3.CEL	s3	gs	2
...



Only one factor
(you can simulate multifactor variable)

Contrast file

Group1	Vs. group2
gs	gb
...	...

App Output

- Plots/reports
 - Raw data QC plots:
 - Histogram, MAplot, boxplot
 - RLE: Relative Log Expression
 - NUSE: Normalized Unscaled Standard Error
 - Post-normalization RMA data QC plots
 - Histogram, MAplot, boxplot
 - PCA (2D)
 - Heatmap (samples clustering)
 - Html report
- Interactive tables
 - Differentially Expressed Genes, Pathways
- Downloadable files
 - Differentially Expressed Genes and Pathways results files
 - Html report

DEMO: use case

ORIGINAL ARTICLE

Gene expression profiling identifies *FYN* as an important molecule in tamoxifen resistance and a predictor of early recurrence in patients treated with endocrine therapy

D Elias¹, H Vever¹, A-V Lænkholm², MF Gjerstorff¹, CW Yde³, AE Lykkesfeldt³ and HJ Ditzel^{1,4}

To elucidate the molecular mechanisms of tamoxifen resistance in breast cancer, we performed gene array analyses and identified 366 genes with altered expression in four unique tamoxifen-resistant (TamR) cell lines vs the parental tamoxifen-sensitive MCF-7/S0.5 cell line. Most of these genes were functionally linked to cell proliferation, death and control of gene expression, and include *FYN*, *PRKCA*, *ITPR1*, *DPYD*, *DACH1*, *LYN*, *GBP1* and *PRLR*. Treatment with *FYN*-specific small interfering RNA or a SRC family kinase inhibitor reduced cell growth of TamR cell lines while exerting no significant effect on MCF-7/S0.5 cells. Moreover, overexpression of *FYN* in parental tamoxifen-sensitive MCF-7/S0.5 cells resulted in reduced sensitivity to tamoxifen treatment, whereas knockdown of *FYN* in the *FYN*-overexpressing MCF-7/S0.5 cells restored sensitivity to tamoxifen, demonstrating growth- and survival-promoting function of *FYN* in MCF-7 cells. *FYN* knockdown in TamR cells led to reduced phosphorylation of 14-3-3 and Cdc25A, suggesting that *FYN*, by activation of important cell cycle-associated proteins, may overcome the anti-proliferative effects of tamoxifen. Evaluation of the subcellular localization of *FYN* in primary breast tumors from two cohorts of endocrine-treated ER+ breast cancer patients, one with advanced disease ($N=47$) and the other with early disease ($N=76$), showed that in the former, plasma membrane-associated *FYN* expression strongly correlated with longer progression-free survival ($P < 0.0002$). Similarly, in early breast cancer patients, membrane-associated expression of *FYN* in the primary breast tumor was significantly associated with increased metastasis-free ($P < 0.04$) and overall ($P < 0.004$) survival independent of tumor size, grade or lymph node status. Our results indicate that *FYN* has an important role in tamoxifen resistance, and its subcellular localization in breast tumor cells may be an important novel biomarker of response to endocrine therapy in breast cancer.

Oncogene (2015) **34**, 1919–1927; doi:10.1038/onc.2014.138; published online 2 June 2014

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67916>

Comparing Resistant vs Sensitive: Phenotype and contrast files

SampleName	SampleID	SampleGroup
GSM1658401_TamR1_replicate1.CEL.gz	TamR1_r1	TamR
GSM1658402_TamR1_replicate2.CEL.gz	TamR1_r2	TamR
GSM1658403_TamR1_replicate3.CEL.gz	TamR1_r3	TamR
GSM1658404_TamR4_replicate1.CEL.gz	TamR4_r1	TamR
GSM1658405_TamR4_replicate2.CEL.gz	TamR4_r2	TamR
GSM1658406_TamR4_replicate3.CEL.gz	TamR4_r3	TamR
GSM1658407_TamR7_replicate1.CEL.gz	TamR7_r1	TamR
GSM1658408_TamR7_replicate2.CEL.gz	TamR7_r2	TamR
GSM1658409_TamR8_replicate1.CEL.gz	TamR8_r1	TamR
GSM1658410_TamR8_replicate2.CEL.gz	TamR8_r2	TamR
GSM1658411_Tam_sensitive_MCF7_replicate1.CEL.gz	TamS_r1	TamS
GSM1658412_Tam_sensitive_MCF7_replicate2.CEL.gz	TamS_r2	TamS
GSM1658413_Tam_sensitive_MCF7_replicate3.CEL.gz	TamS_r3	TamS
GSM1658414_Tam_sensitive_MCF7_replicate4.CEL.gz	TamS_r4	TamS
GSM1658415_Tam_sensitive_MCF7_replicate5.CEL.gz	TamS_r5	TamS
GSM1658416_Tam_sensitive_MCF7_replicate6.CEL.gz	TamS_r6	TamS
GSM1658417_Tam_sensitive_MCF7_replicate7.CEL.gz	TamS_r7	TamS
GSM1658418_Tam_sensitive_MCF7_replicate8.CEL.gz	TamS_r8	TamS

group1	vs.group2
TamR	TamS

Run: Comparing All TamR vs. TamS

CCBR Microarray analysis workflow

(For Affymetrix human and mouse data)

Project ID:

ccbr

Select CEL files

Browse...

18 files

Upload complete

Choose phenotype file

Browse...

Phenotype.txt

Upload complete

Choose contrast file

Browse...

Contrast.txt

Upload complete

P-value threshold for DEGs

0.05

FC threshold for DEGs

1.5

Choose contrast to show

1

P-value threshold for pathways

0.05

Start

Generate Report

Download Report

Download Tables

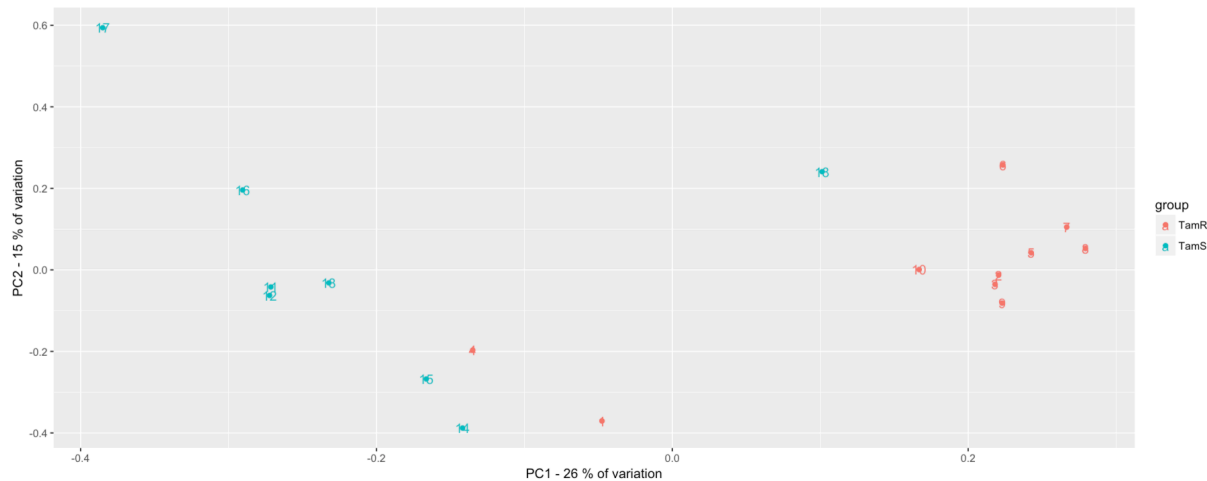
Results

Pre-normalization QC plots ▾

Post-normalization plots ▾

DEG-Enrichments-tables ▾

Help ▾



DEG: Comparing All TamR vs. TamS

FILTER

	P-value<0.05	+ abs(FC)>=3	+ abs(FC)>=4
TamR vs. TamS	20,302	542	222

SEARCH

Show entries

Search:

contrast: TamR-TamS

	SYMBOL	probsetID	ACCNUM	DESC	FC	P.Value	adj.P.Val	AveExpr	t	B
19510	FYN	210105_s_at	M14333	FYN proto-oncogene, Src family tyrosine kinase	3.04075606957281	8.574950e-08	2.221969e-05	6.63906412241529	8.31550957893765	8.20120638011214
13848	EFS	204400_at	NM_005864	embryonal Fyn-associated substrate	-2.26084948884447	3.981983e-06	2.973638e-04	5.52745931956857	-6.36016968781933	4.43630703498231
25326	FYN	216033_s_at	S74774	FYN proto-oncogene, Src family tyrosine kinase	1.51991108953597	4.584397e-04	6.018054e-03	5.15821116227609	4.21485524565363	-0.235116437888612
45174	FRK	235924_at	N73742	fyn related Src family tyrosine kinase	1.68272981374658	1.735699e-02	6.562431e-02	7.13108538812293	2.60315537413466	-3.71863600654487

Showing 1 to 4 of 4 entries (filtered from 6,132 total entries)

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Pathways: Comparing All TamR vs. TamS

Show 10 entries

Search: cancer

Pathways for the top 500 Upregulated Genes: TamR-TamS

	path_id	source	description	type	pval	fdr	ratio	gene.list	nb.hits	nb.genes.path	nb.user.genes	tot.back.genes
120	hsa05205	KEGG	Proteoglycans in cancer	pathway	0.000667793	0.101653541	0.040777258	CTSL FAS ITPR1 NRAS PAK1 PIK3R...	12	203	361	19688
121	ko05205	KEGG	Proteoglycans in cancer	pathway	0.000667793	0.101653541	0.040777258	CTSL FAS ITPR1 NRAS PAK1 PIK3R...	12	203	361	19688
377	ko05223	KEGG	Non-small cell lung cancer	pathway	0.005756547	0.279762113	0.067870854	NRAS PIK3R3 PLCG2 PRKCA STAT3	5	58	361	19688
378	hsa05223	KEGG	Non-small cell lung cancer	pathway	0.005756547	0.279762113	0.067870854	NRAS PIK3R3 PLCG2 PRKCA STAT3	5	58	361	19688
810	hsa05200	KEGG	Pathways in cancer	pathway	0.02353953	0.535277282	0.017106996	AR CCDC6 EPAS1 FAS FGFR2 JUP N...	14	395	361	19688
1030	ko05212	KEGG	Pancreatic cancer	pathway	0.035033227	0.622253621	0.044163958	PIK3R3 SMAD3 STAT1 STAT3	4	64	361	19688
1032	hsa05212	KEGG	Pancreatic cancer	pathway	0.035033227	0.622253621	0.044163958	PIK3R3 SMAD3 STAT1 STAT3	4	64	361	19688

Showing 1 to 7 of 7 entries (filtered from 1,255 total entries)

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