

The CCBR microarray pipeline

Shiny App

Quick Start

Running locally the Microarray App

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

App Interface

CCBR Microarray analysis workflow

(For Affymetrix human and mouse data)

Step1- Provide input

Project ID:

Select CEL files
 no files selected

Choose phenotype file
 no file selected

Choose contrast file
 no file selected

Which contrast to show

KEGG/GO Enrichment Pvalue threshold

KEGG/GO Enrichment logFC threshold

Start

Generate Report

Download Report

Download Tables

Step2- click on start to load/normalize data

Step5- generate Html report and download results

Step4- select contrast and enrichment analysis thresholds

Step 3- access all menu options

Microarray

Results

Pre-normalization QC plots ▾

Post-normalization plots ▾

DEG-Enrichments-tables ▾

Help ▾

Histogram
Maplots
Boxplots
RLE
NUSE

Histogram
Maplots
Boxplots
PCA
Heatmap


DEG
KEGG
GO

App Input


- Mandatory input
 - Project id
 - Affymetrix Raw data (cel files)
 - *Current supported platforms: Human 133 plus 2 and Mouse gene 2.0 st*
 - Phenotype data file
 - List of contrasts file

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 per group, boxplot
 - RLE: Relative Log Expression
 - NUSE: Normalized Unscaled Standard Error
 - Post-normalization RMA data QC plots
 - Histogram, MAplot per group, boxplot
 - PCA (2D)
 - Heatmap (samples clustering)
 - Html report
- Interactive tables
 - DEG, KEGG and GO
- Downloadable files
 - DEG, KEGG and GO 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

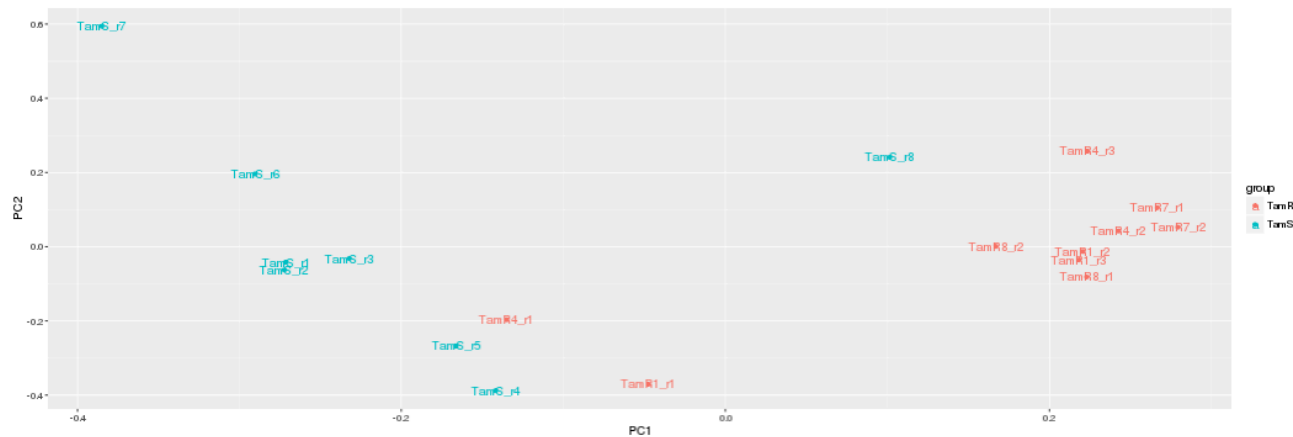
Run: Comparing All TamR vs. TamS

CCBR Microarray analysis workflow

(For Affymetrix human and mouse data)

Project ID: <input type="text" value="micro2"/>	Select CEL files <input type="button" value="Browse..."/> 18 files selected. <div>Upload complete</div>	Choose phenotype file <input type="button" value="Browse..."/> PhenotypeGSE67916_v2.txt <div>Upload complete</div>	Choose contrast file <input type="button" value="Browse..."/> contrastsGSE67916_v2.txt <div>Upload complete</div>	KEGG/GO Enrichment Pvalue threshold <input type="text" value="0.05"/>
Which contrast to show <input type="text" value="1"/>				KEGG/GO Enrichment logFC threshold <input type="text" value="1.5"/>
<div>Start</div> <div>Generate Report</div> <div> Download Report</div> <div> Download Tables</div>				

Microarray Results Pre-normalization QC plots Post-normalization plots DEG-Enrichments-tables Help



DEG: Comparing All TamR vs. TamS

	adjPvalue<0.05	+ abs(logFC)>=1.5	+ abs(logFC)>=2
TamR vs. TamS	12684	633	221

Show entries

Search:

contrast: TamR-TamS

	probsetID	logFC	AveExpr	t	P.Value	adj.P.Val	B	ACCNUM	SYMBOL	DESC
22397	213093_at	1.64832000	6.786033	6.4469632	3.321973e-06	2.639954e-04	4.614902	AI471375	PRKCA	protein kinase C alpha
24490	215195_at	1.51564416	7.366115	9.4630326	1.137850e-08	4.749006e-06	10.157990	AF035594	PRKCA	protein kinase C alpha
16370	206923_at	1.24447796	5.345408	9.7350904	7.216863e-09	3.461245e-06	10.595798	NM_002737	PRKCA	protein kinase C alpha

contrast: TamR-TamS

	probsetID	logFC	AveExpr	t	P.Value	adj.P.Val	B	ACCNUM	SYMBOL	DESC
19510	210105_s_at	1.6044300876	6.639064	8.315509579	8.574950e-08	2.221969e-05	8.2012064	M14333	FYN	FYN proto-oncogene, Src family tyrosine kinase

KEGG: Comparing All TamR vs. TamS

Project ID: <input type="text" value="micro2"/>	Select CEL files <input type="button" value="Browse..."/> 18 files selected. <div>Upload complete</div>	Choose phenotype file <input type="button" value="Browse..."/> PhenotypeGSE67916_v2.txt <div>Upload complete</div>	Choose contrast file <input type="button" value="Browse..."/> contrastsGSE67916_v2.txt <div>Upload complete</div>	KEGG/GO Enrichment Pvalue threshold <input type="text" value="0.05"/>
Which contrast to show <input type="text" value="1"/>				KEGG/GO Enrichment logFC threshold <input type="text" value="1.5"/>
<div>Start</div> <div>Generate Report</div> <div>Download Report</div> <div>Download Tables</div>				

Microarray Results Pre-normalization QC plots - Post-normalization plots - DEG-Enrichments-tables - Help -

Show 10 entries

Search:

KEGG for contrast: TamR-TamS

SigGeneNumInGroup	SigGeneNum	GeneNumInGroup	GeneNumInDB	Pvalue	Gene	db.type	db.name	Bonferroni	BH
8	83	44	2217	2.27081540865773e-05	CTNNA1 CXCL12 EZR PIK3R1 PIK3R3 PRKCA ROCK2 VAV3	has04670	Leukocyte transendothelial migration	0.00165769524832014	0.00165769524832014
7	83	41	2217	9.59327557760328e-05	FYN KRAS LYN PIK3R1 PIK3R3 PRKCA VAV3	has04664	Fc epsilon RI signaling pathway	0.00700309117165039	0.0035015455858252
11	83	97	2217	0.000181664774468718	FYN HLA-B HLA-C KRAS NFAT5 PIK3R1 PIK3R3 PPP3R1 PTPN11 TNFSF10 VAV3	has04650	Natural killer cell mediated cytotoxicity	0.0132615285362164	0.00410261543049598
7	83	46	2217	0.000224800845506629	CDC42 KRAS NFAT5 PIK3R1 PIK3R3 PPP3R1 PRKCA	has04370	VEGF signalling pathway	0.0164104617219839	0.00410261543049598
6	83	39	2217	0.000465233839805225	CDC42 EPAS1 KRAS PIK3R1 PIK3R3 PTPN11	has05211	Renal cell carcinoma	0.0339620703057814	0.00679241406115628
7	83	58	2217	0.00114403289836334	ACTB CDC42 CTNNA1 EPB41L2 KRAS PRKCA ZAK	has04530	Tight junction	0.0835144015805238	0.0122501358575442
12	83	135	2217	0.00117467056168232	ACTB ARHGEF7 CDC42 FGFR2 FGFR4 GNA13 ITGB5 KRAS PIK3R1 PIK3R3 ROCK2 VAV3	has04810	Regulation of actin cytoskeleton	0.0857509510028094	0.0122501358575442
3	83	15	2217	0.0018193412747634	LSS SQLE TM7SF2	has00100	Biosynthesis of steroids	0.132811913057728	0.016601489132216
4	83	28	2217	0.00324733229064828	JAK1 KRAS STAT1 STAT3	has05212	Pancreatic cancer	0.237055257217324	0.0248021136250714
6	83	54	2217	0.00339754981165362	FYN KRAS PIK3R1 PIK3R3 PPP3R1 VAV3	has04660	T cell receptor signaling pathway	0.248021136250714	0.0248021136250714

Showing 1 to 10 of 73 entries

Previous **1** 2 3 4 5 ... 8 Next

GO: Comparing All TamR vs. TamS

132	404	4216	18501	1.59543272675232e-06	STAT1 STAT3 TPR EZR CDC6 PIK3R3 ENC1 CASK USO1 STX16 MBTPS1 ADAM9 SNAP23 MTMR1 BANF1 CPNE3 KYNU CLIC3 SPAG9 NFS1 ROCK2 ISG15 CLINT1 KIAA0513 ACTR2 HIPK3 MBNL2 DLC1 NCOA2 AGR2 EXOC5 NFAT5 MAP3K2 STIP1 IFI44L SYNE2 FBXW11 SLC7A8 CLIC4 TMOD3 PI15 TUBD1 GULP1 GDE1 ERAP1 ZAK GDAP1 ANLN XAF1 SAMD9 PSPC1 PPP1R9A NBPF1 SCYL2 NXT2 LXN TAOK1 PLEKHA2 EXOC4 RTP4 CLEC7A TNKS2 FRMD5 RERG UHMK1 AMOTL1 ALDH1L2 PAPD4 SYNPO2 HIPK1 RBM24 YTHDF3	GO:0005737	Component: cytoplasm	0.00156671493767078	0.000783357468835389
67	404	1782	18501	3.52933762633763e-06	ACTA2 ACTB RHOB ATP2A3 ATP6V1A ATRX BMPR1B BUB1 CDC42 DACH1 DDX3X DHX9 DYNC1H1 EIF5 EPHA4 EPRS ERBB4 FGFR2 FGFR4 FYN GBP1 GBP3 GEM GNAS HSPA4 HSP90AB1 HSPD1 IGF1R JAK1 KRAS LYN MCM4 SEPT2 PRKCA ABCD3 RAP2A SKIL TAP2 TPR CASK ABCC3 PRPF4B PAPSS2 ROCK2 ABCG1 ACTR2 HIPK3 GNA13 MAP3K2 RAB31 DDX42 CSTF2T GIMAP2 TUBD1 TUBE1 ZAK PSPC1 DDX60 ATP10D TAOK1 RNF213 CPEB4 RERG UHMK1 CMPK2 HIPK1 RBM24	GO:000166	Function: nucleotide binding	0.00346580954906355	0.00115526984968785
3	404	7	18501	7.44326484549945e-06	SNAP23 SCAMP1 EXOC5	GO:0006892	Process: post-Golgi vesicle- mediated transport	0.00730928607828046	0.00150719817202435
16	404	221	18501	7.67412511214028e-06	DDX3X FYN HLA-B HLA-C HSPD1 SP110 LYN CD46 MDM2 PIK3R1 STAT1 STAT3 BANF1 CLDN1 ISG15 WWP1	GO:0044419	Process: interspecies interaction between organisms	0.00753599086012175	0.00150719817202435