

## ELMER v2

An R/Bioconductor package to reconstruct gene regulatory networks from DNA methylation and transcriptome profiles

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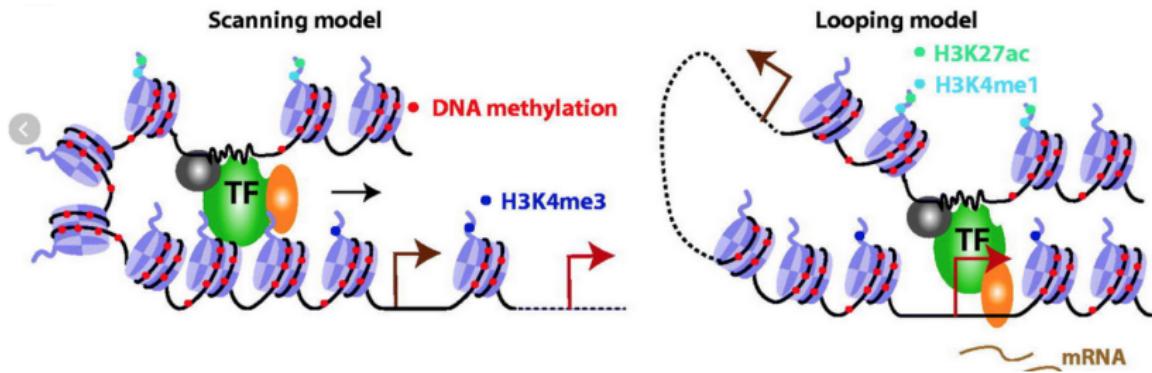




# Overview

- ① Introduction
- ② Objectives
- ③ Methods: Algorithms and tools
- ④ Analysis
- ⑤ Conclusion

## Enhancer-mediated gene regulation



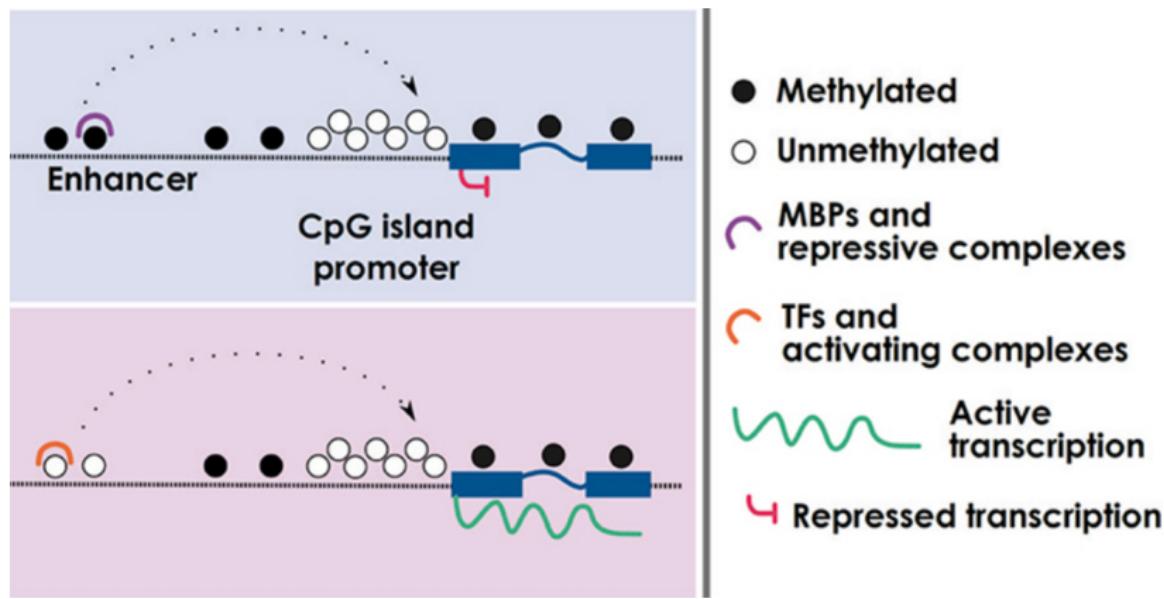
Source: Yao et al. *Genome Biology* (2015)



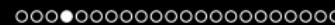
# Enhancer-mediated gene regulation

- 73% of the tested distal elements do not link to the nearest gene (Sanyal et al., 2012)
- 40% of the enhancers involved in loops do not interact with the TSS of the nearest gene (Li et al., 2012),
- one-third of the distal interactions were not directed to the promoter of the nearest gene (Mifsud et al., 2015),
- 85% of tumor-specific enhancers that could be linked to the expression of a nearby gene skipped the nearest gene (Yao et al., 2015).

# Enhancer-mediated gene regulation



Source: Carrio et al. Frontiers in aging neuroscience (2015)

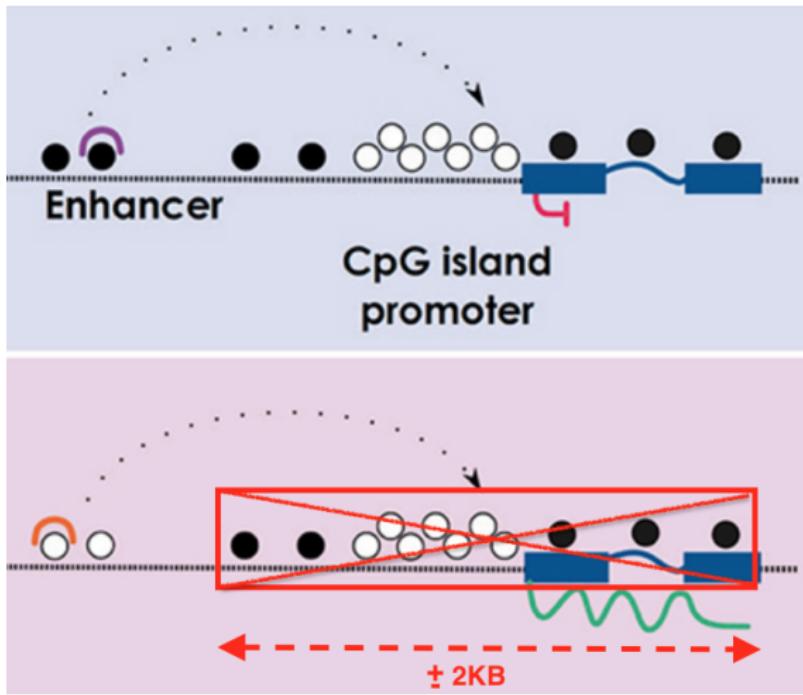


# Algorithm

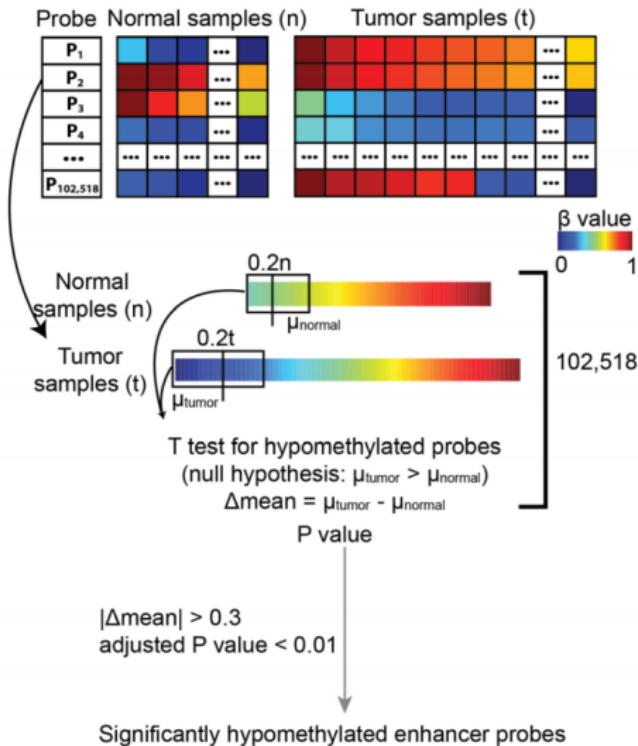
## Steps

- ① Identify distal probes on HM450K/EPIC.
- ② Identify distal probes with significantly different DNA methylation level in group 1 compared to group 2.
- ③ Identify putative target genes for differentially methylated distal enhancer probes.
- ④ Identify enriched motifs for the distal probes which are significantly differentially methylated and linked to a putative target gene.
- ⑤ Identify regulatory TFs whose expression associate with DNA methylation at motifs.

## Step 1: Identify distal probes

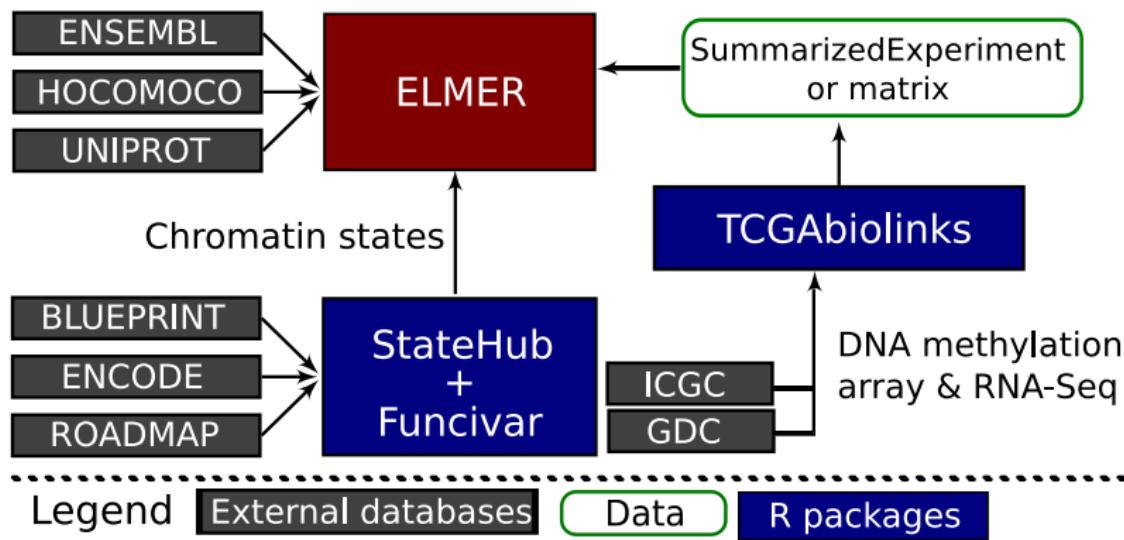


## Step 2: Differentially methylated distal probes



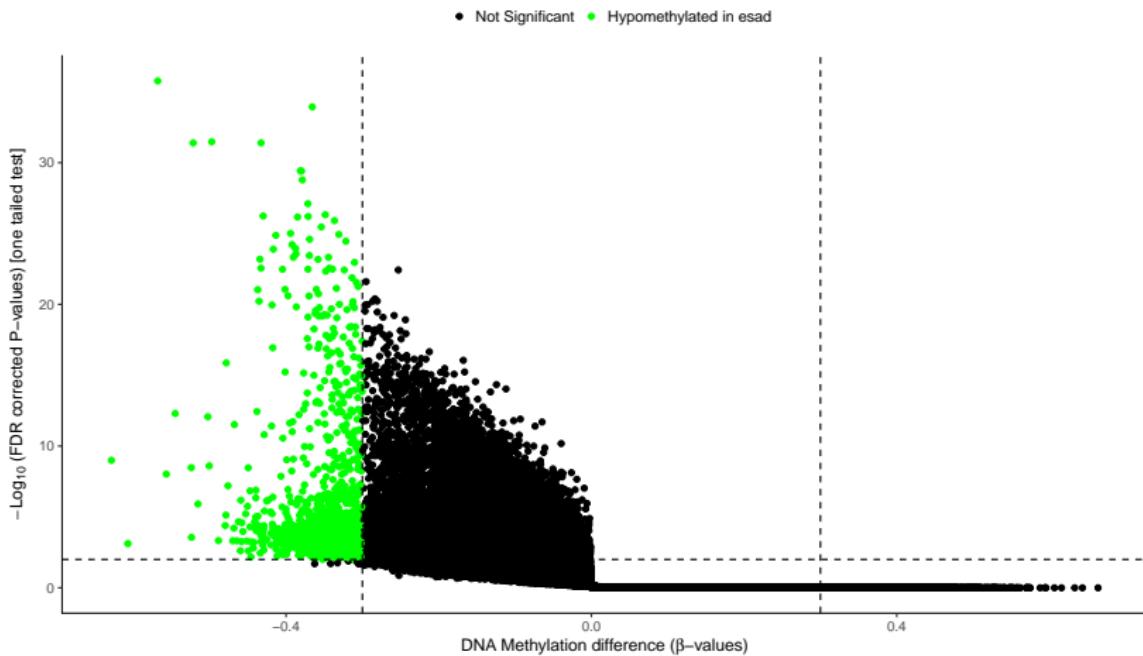
Source: Yao et al. *Genome Biology* (2015)

## ELMER v.2 interactions

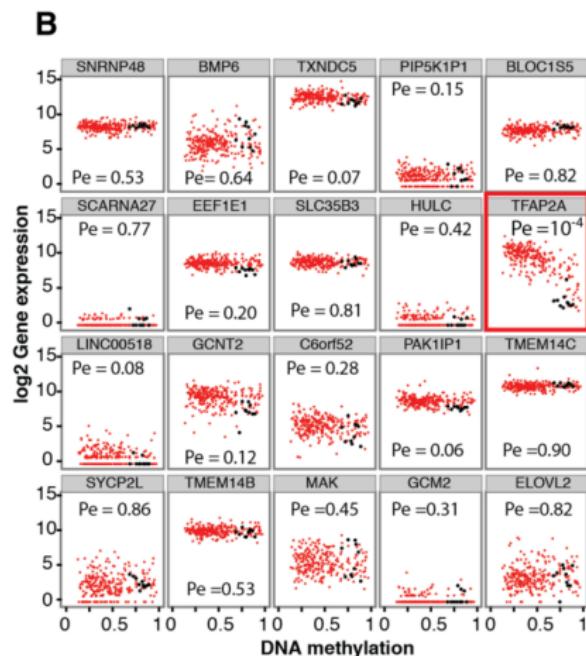
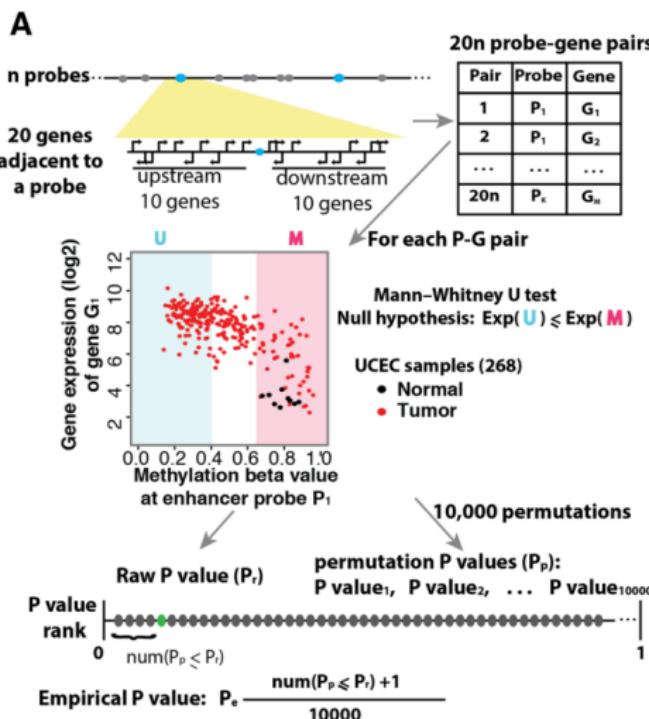


## Step 2: Differentially methylated distal probes

### Volcano plot – Probes hypomethylated in esad vs normal



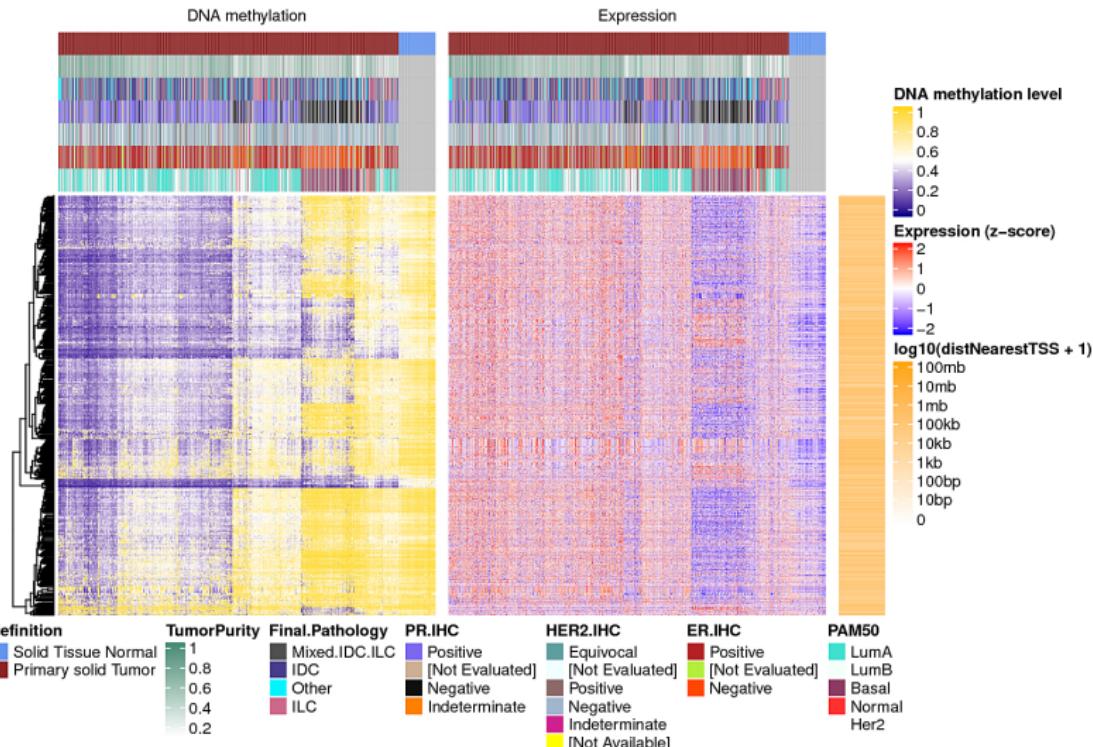
### Step 3: Identification of putative target gene(s)



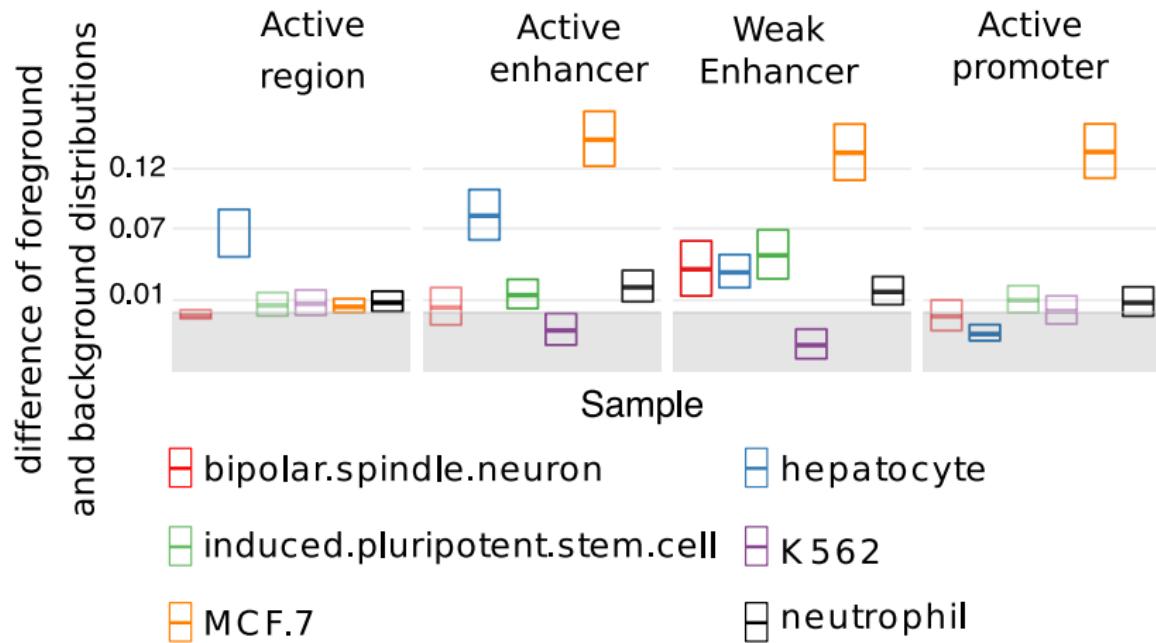
Source: Yao et al. Genome Biology (2015)

### Step 3: Probe-target gene pairs inferred

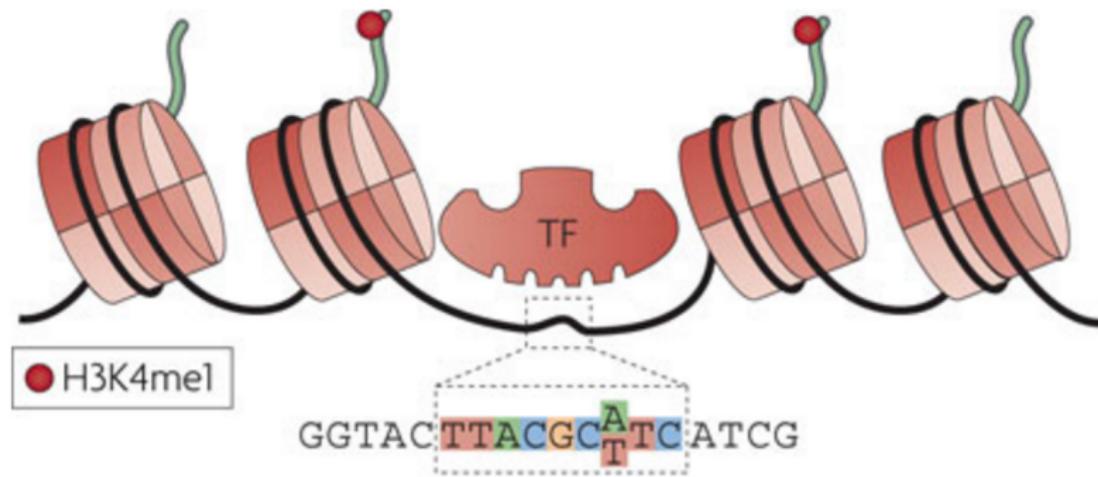
## Correspondence between probe DNA methylation and distal gene expression



# Chromatin state enrichment analysis



## Step 4: Motif enrichment analysis



Nature Reviews | Genetics

Hawkins RD, et al. Next-generation genomics: an integrative approach. Nature Reviews Genetics (2010)

# Step 4: TF motifs source

HOCOMOCO Home Human TFs Mouse TFs Tools Downloads Help

Switch to CORE collection Reset Select Columns Get CSV HUMAN\_mono\_motifs.tsv PWMS for HUMAN transcription factors (full)

Model	LOGO	Transcription factor	Quality	TF family	TF subfamily
ASCL1_HUMAN.H11MO.0.A		ASCL1 (GeneCards)	A	MyoD / ASC-related factors[1.2.2]	Achaete-Scute-like factors[1.2.2.2]
ASCL2_HUMAN.H11MO.0.D		ASCL2 (GeneCards)	D	MyoD / ASC-related factors[1.2.2]	Achaete-Scute-like factors[1.2.2.2]
AHR_HUMAN.H11MO.0.B		AHR (GeneCards)	B	PAS domain factors[1.2.5]	Ahr-like factors[1.2.5.1]
EPAS1_HUMAN.H11MO.0.B		EPAS1 (GeneCards)	B	PAS domain factors[1.2.5]	Ahr-like factors[1.2.5.1]
HIF1A_HUMAN.H11MO.0.C		HIF1A (GeneCards)	C	PAS domain factors[1.2.5]	Ahr-like factors[1.2.5.1]
AIRE_HUMAN.H11MO.0.C		AIRE (GeneCards)	C	AIRE[5.3.1]	AIRE[5.3.1.0.1]
ALX1_HUMAN.H11MO.0.B		ALX1 (GeneCards)	B	Paired-related HD factors[3.1.3]	ALX[3.1.3.1]
ALX3_HUMAN.H11MO.0.D		ALX3 (GeneCards)	D	Paired-related HD factors[3.1.3]	ALX[3.1.3.1]
ALX4_HUMAN.H11MO.0.D		ALX4 (GeneCards)	D	Paired-related HD factors[3.1.3]	ALX[3.1.3.1]
AP2A_HUMAN.H11MO.0.A		TFAP2A (GeneCards)	A	AP-2[1.3.1]	AP-2alpha[1.3.1.0.1]
AP2B_HUMAN.H11MO.0.B		TFAP2B (GeneCards)	B	AP-2[1.3.1]	AP-2beta[1.3.1.0.2]
AP2D_HUMAN.H11MO.0.D		TFAP2D (GeneCards)	D	AP-2[1.3.1]	AP-2delta[1.3.1.0.4]
AP2C_HUMAN.H11MO.0.A		TFAP2C (GeneCards)	A	AP-2[1.3.1]	AP-2gamma[1.3.1.0.3]

HOCOMOCO v11 (<http://hocomoco11.autosome.ru/human/mono?full=true>), Accessed: 25-12-2017



## Step 4: Motif enrichment analysis

### Objective

Evaluate the enrichment of transcription factors in certain genomic regions.

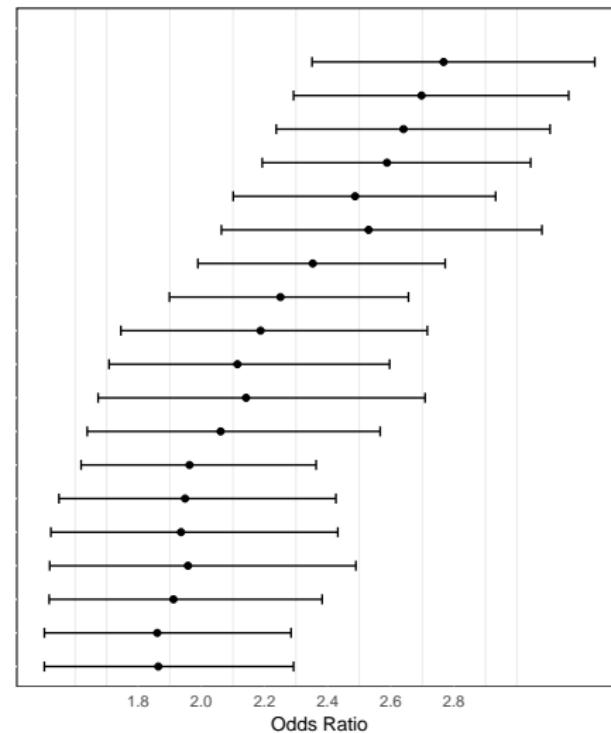
- ① Perform motif matching of transcription factors in probes regions (window  $\pm 250\text{bp}$ ). Performed using HOMER (Hypergeometric Optimization of Motif EnRichment) with HOCOMOCO motifs.
- ② Evaluate which transcription factors are more likely to occur in those regions than in background regions using Fisher's exact test with FDR correction.

### Fisher's exact test

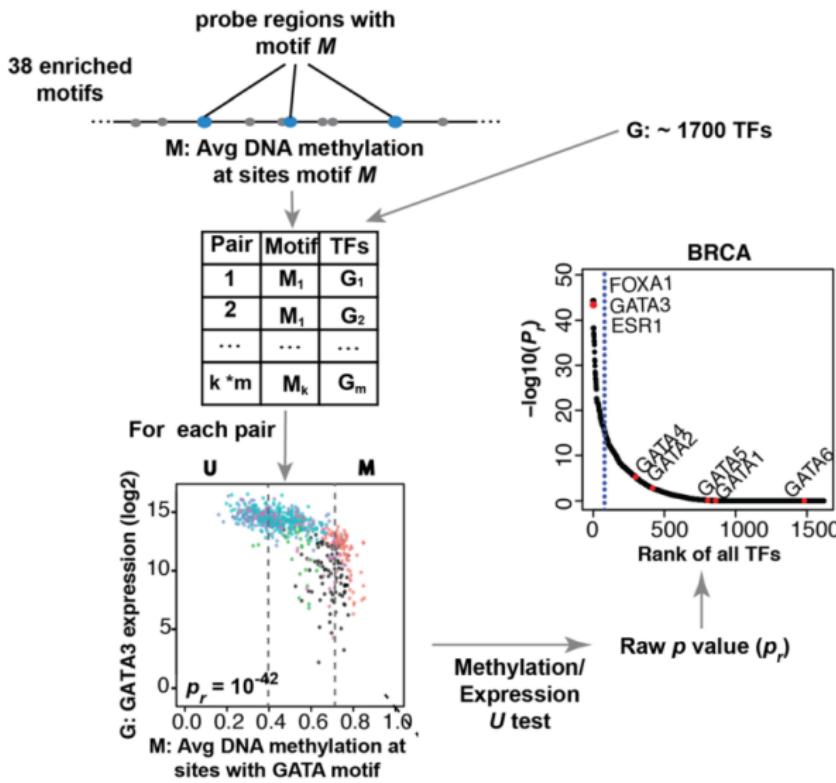
- a: nb of input regions with a match for TF motif.
- b: nb of input regions with no match for TF motif.
- c: nb of background regions with a match for TF motif.
- d: nb of background regions with no match for TF motif.

## Step 4: Motif enrichment analysis

Motif	Odds ratio (95% CI)	# probes (% of paired)
FOSL2	2.77 (2.35–3.25)	201 (0.23%)
FOSL1	2.7 (2.29–3.16)	202 (0.23%)
FOSB	2.64 (2.24–3.1)	193 (0.22%)
FOS	2.59 (2.19–3.04)	193 (0.22%)
JUN	2.49 (2.1–2.93)	184 (0.21%)
BATF	2.53 (2.06–3.08)	118 (0.13%)
JUND	2.35 (1.99–2.77)	186 (0.21%)
JUNB	2.25 (1.9–2.66)	181 (0.2%)
HXB13	2.19 (1.74–2.72)	94 (0.11%)
PIT1	2.11 (1.71–2.6)	106 (0.12%)
PRRX1	2.14 (1.67–2.71)	78 (0.09%)
CDX1	2.06 (1.64–2.57)	91 (0.1%)
LMX1A	1.96 (1.62–2.36)	134 (0.15%)
BATF	1.95 (1.55–2.43)	91 (0.1%)
NKX32	1.94 (1.52–2.43)	83 (0.09%)
HME1	1.96 (1.52–2.49)	74 (0.08%)
IRX3	1.91 (1.52–2.38)	90 (0.1%)
PO4F3	1.86 (1.5–2.28)	106 (0.12%)
PO4F1	1.86 (1.5–2.29)	104 (0.12%)



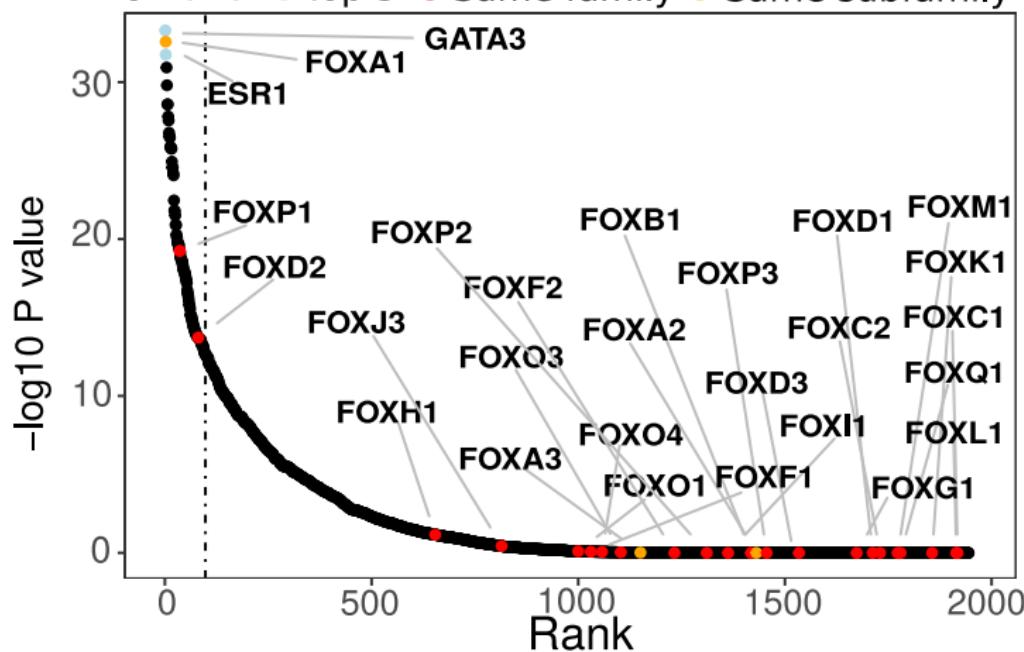
# Step 5: Identification of master regulator TF

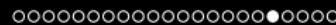


Source: Yao et al. Genome Biology (2015).

# TFs ranking for the FOXA3 motif

TF classification (Motif: FOXA3)  
Probes hypomethylated in LumB vs Basal  
● None ● Top 3 ● Same family ● Same subfamily





# BRCA supervised analysis: Candidate MRs

TF	LUMB (vs basal)	Basal (vs LumB)	Basal (vs HER2)	HER2 (vs Basal)
AR	x			
EMX1	x			
ESR1	x			
GLI1	x			
HOXB1	x			
LMX1B	x			
NR2E3	x			
RARA	x			
ZNF467	x			
FOXA1	x			x
FOXP1	x			x
GATA3	x			x
HOXB3				x
HOXC10				x
BCL11A		x	x	
CEBPB		x	x	
E2F3		x	x	
ETV6		x	x	
KLF5		x	x	
SOX8		x	x	
SOX9		x	x	
SOX11		x		
ZIC1		x	x	

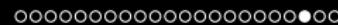
# Step 5: Master Regulator TF table

motif	OR	top.potential.TF.family	pvalue.TF.family	top.potential.TF.subfamily	pvalue.TF.subfamily
All		All	All	All	All
HXB13_HUMAN.H11MO.0.A	2.19	HOXB7	6.39e-7	HOXA13	0.00000105
CDX1_HUMAN.H11MO.0.C	2.06	HOXB7	6.39e-7	CDX2	8.20e-7
HXD9_HUMAN.H11MO.0.D	1.98	HOXB7	6.39e-7	HOXA13	0.00000105
PDX1_HUMAN.H11MO.1.A	1.89	HOXB7	6.39e-7	PDX1	0.0000355
HXC11_HUMAN.H11MO.0.D	1.84	HOXB7	6.39e-7	HOXA13	0.00000105
HXB6_HUMAN.H11MO.0.D	1.84	HOXB7	6.39e-7	HOXB7	6.39e-7
HXD8_HUMAN.H11MO.0.D	1.84	HOXB7	6.39e-7	HOXC8	0.00000134
CDX2_HUMAN.H11MO.0.A	1.83	HOXB7	6.39e-7	CDX2	8.20e-7
HXD12_HUMAN.H11MO.0.D	1.77	HOXB7	6.39e-7	HOXA13	0.00000105
HXC9_HUMAN.H11MO.0.C	1.74	HOXB7	6.39e-7	HOXA13	0.00000105

Showing 1 to 10 of 31 entries

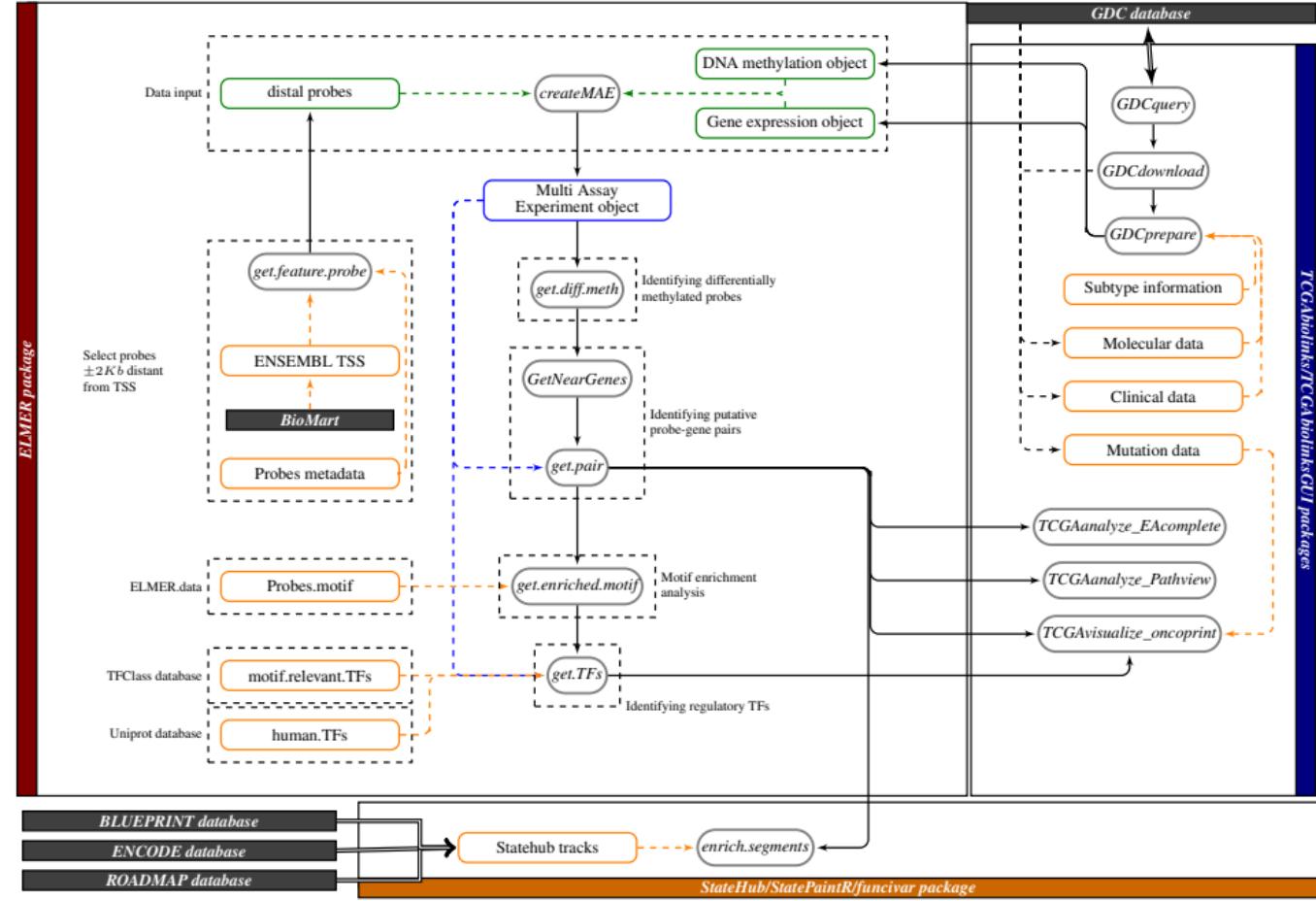
Previous

1 2 3 4 Next



## Main differences between ELMER old version (v.1) and the new version (v.2)

Features	ELMER Version 1	ELMER Version 2
Primary data structure	mee object (custom data structure)	MAE object (Bioconductor data structure)
Auxiliary data	Manually created	Programmatically created
Number of human TFs	1,982	2,014 (UniProt database)
Number of TF motifs	91	771 (HOCOMOCO v11 database)
TF classification	78 families	82 families and 331 subfamilies (TFClass database, HOCOMOCO)
Analysis performed	Normal vs tumor samples	Group 1 vs group 2
Statistical grouping	Unsupervised only	Unsupervised or supervised using labeled groups
TCGA data source	The Cancer Genome Atlas (TCGA)	The NCI's Genomic Data Commons (GDC)
Genome of reference	GRCh37 (hg19)	GRCh37 (hg19)/GRCh38 (hg38)
DNA methylation platforms	HM450	EPIC and HM450
Graphical User Interface (GUI)	None	TCGAbiolinksGUI
Automatic report	None	HTML summarizing results
Annotations	None	StateHub



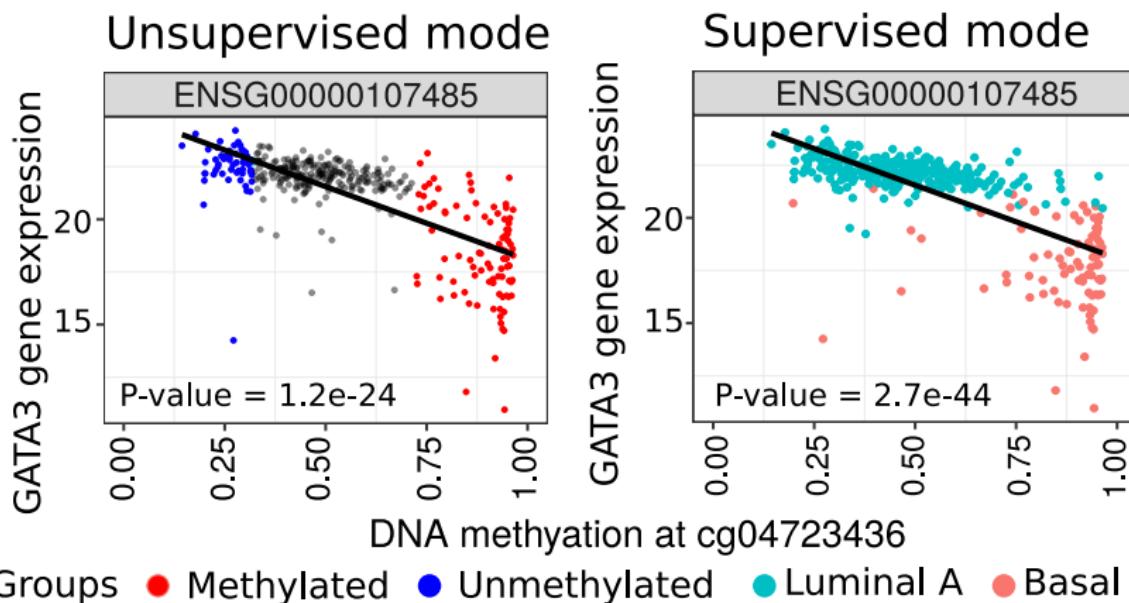
Groups  $U$  and  $M$  definition in (un)supervised mode

Figure: A: *unsupervised mode*; when `minSubgroupFrac` argument is set to 40%, the methylated group is defined as the highest quintile and the unmethylated group as the lowest quintile; B: *supervised mode*; methylated and unmethylated group are defined as one of the known molecular subtypes.



# Case study: TCGA Breast Invasive Carcinoma (BRCA)

Table: Summary of the available samples in TCGA for BRCA

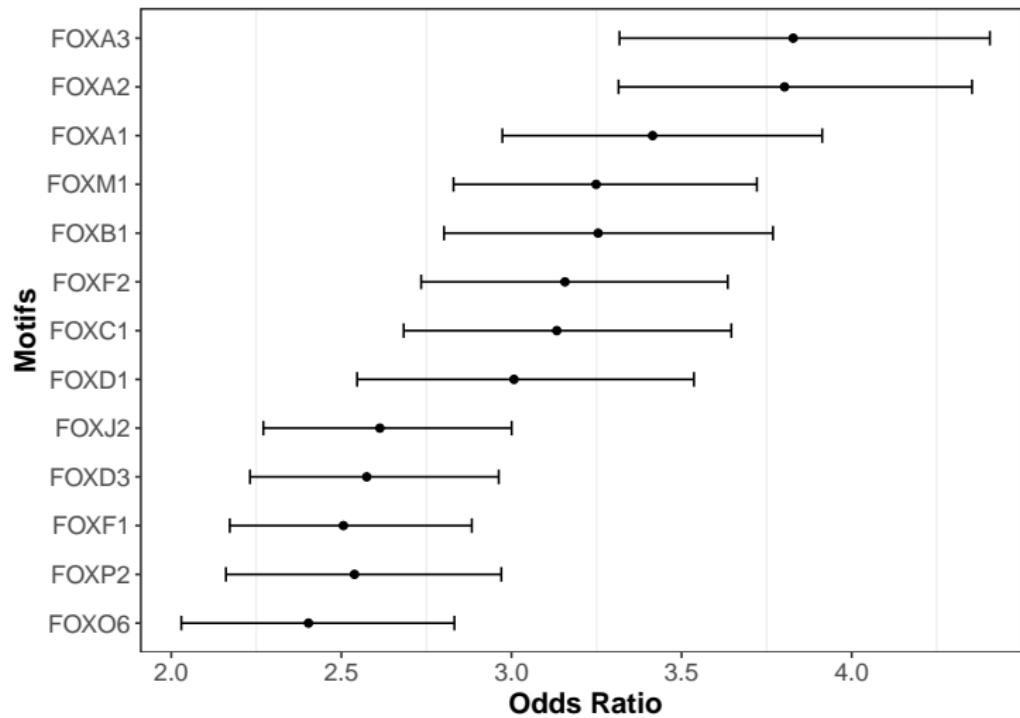
Group	Samples w/ DNA methylation (450K)	Samples w/ gene expression (FPKM-UQ)	Samples w/ both
Primary solid Tumor	791	1102	778
Solid Tissue Normal	96	113	83

Table: Results supervised mode

Inferred gene-probe pairs	2167
Enriched motifs	312
Master Regulator TF	17

# Top enriched motifs

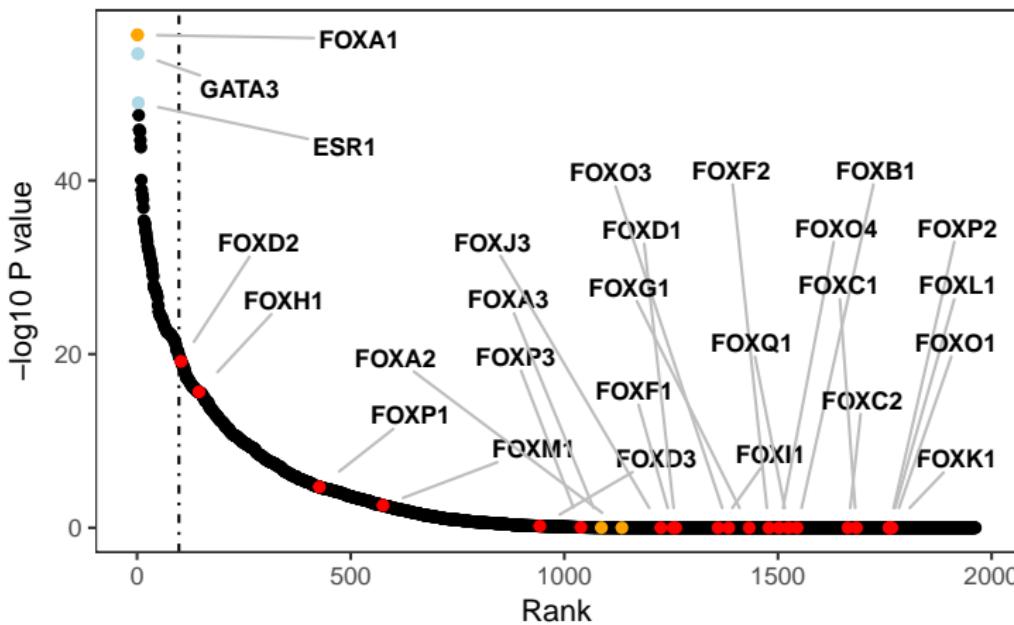
Probes hypomethylated in Primary solid Tumor vs Solid Tissue Normal





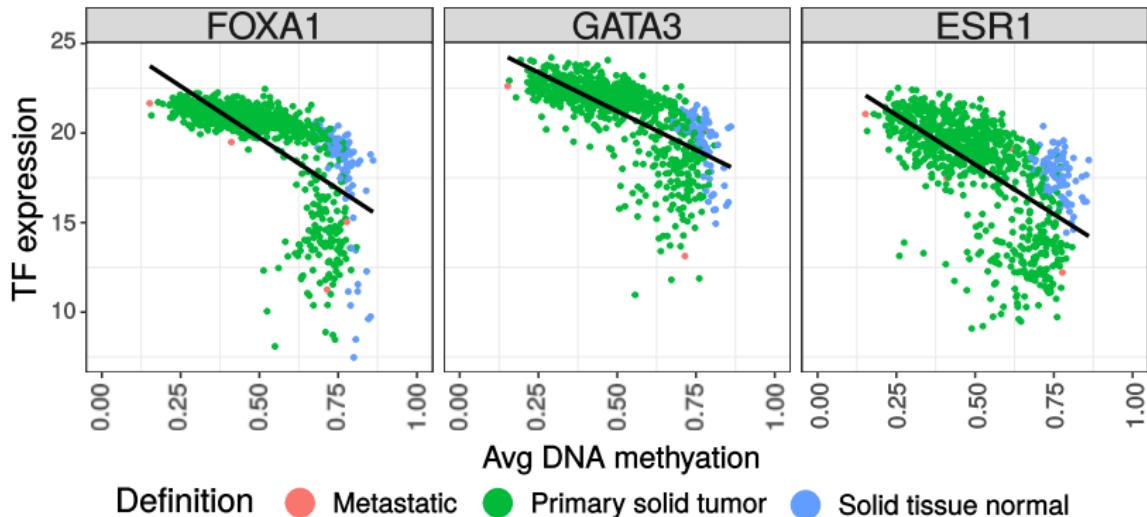
## Motif: FOXA3

TF classification • None • Same family • Same subfamily • Top 3





## TF vs avg DNA methylation





# Candidate master regulator TF

RESEARCH ARTICLE | OPEN ACCESS

## Expression of FOXA1 and GATA-3 in breast cancer: the prognostic significance in hormone receptor-negative tumours

André Albergaria, Joana Paredes, Bárbara Sousa, Fernanda Milanezi, Vítor Carneiro, Joana Bastos, Sandra Costa, Daniella Vieira, Nair Lopes, Eric W Lam, Nuno Lunet and Fernando Schmitt 

Breast Cancer Research 2009 11:R40 | DOI: 10.1186/bcr2327 | © Albergaria et al.; licensee BioMed Central Ltd. 2009

Received: 5 January 2009 | Accepted: 23 June 2009 | Published: 23 June 2009

Article | [OPEN](#)

## Retinoic acid receptor alpha is associated with tamoxifen resistance in breast cancer

Henrik J. Johansson, Betzabe C. Sanchez, Filip Mundt, Jenny Forshed, Aniko Kovacs, Elena Panizza, Lina Hultin-Rosenberg, Bo Lundgren, Ulf Martens, Gyöngyvér Máthé, Zohar Yakhini, Khalil Helou, Kamilla Krawiec, Lena Kanter, Anders Hjerpe, Olle Stål, Barbro K. Linderholm & Janne Lehtio 

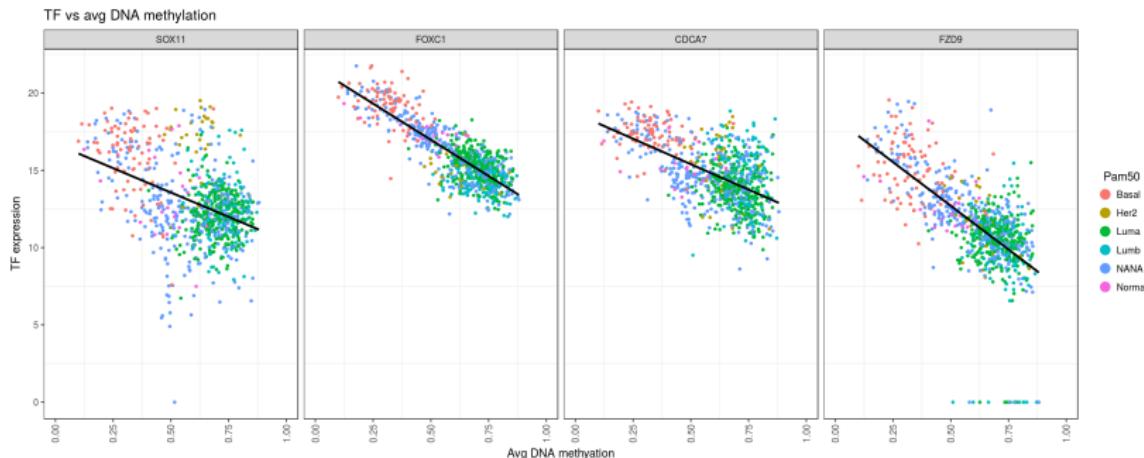


Figure: SOX11 and top3 TF expression vs avg DNA methylation of paired enriched probes for SOX10 - Probes hypermethylated in LumA vs Basal



# Master regulator TF: molecular known subtypes

**The SOX11 transcription factor is a critical regulator of basal-like breast cancer growth, invasion, and basal-like gene expression**

Jonathan H. Shepherd<sup>1,3</sup>, Ivan P. Uray<sup>3</sup>, Abhijit Mazumdar<sup>3</sup>, Anna Tsimelzon<sup>2</sup>, Michelle Savage<sup>3</sup>, Susan G. Hilsenbeck<sup>2</sup>, Powel H. Brown<sup>1,3</sup>

**FOXA1 repression is associated with loss of BRCA1 and increased promoter methylation and chromatin silencing in breast cancer**

C Gong,<sup>1,2,6</sup> K Fujino,<sup>1,3,6</sup> L J Monteiro,<sup>1</sup> A R Gomes,<sup>1</sup> R Drost,<sup>4</sup> H Davidson-Smith,<sup>5</sup> S Takeda,<sup>3</sup> U S Khoo,<sup>2</sup> J Jonkers,<sup>4</sup> D Sproul,<sup>5</sup> and E W-F Lam<sup>1,\*</sup>

negative breast cancer cell lines to regain hormonal sensitivity.<sup>41</sup> In addition to promoting mammary luminal phenotype, FOXA1 might also have a more direct role in repressing the basal breast cancer phenotype. It has been shown that FOXA1 also inhibits the transcription of basal-type associated genes such as CD58, ANXA1, JAG1 and SOX9, whereas the loss of FOXA1 leads to the derepression of these basal genes.<sup>13</sup> These findings together highlight a critical role of FOXA1 in maintaining the luminal and

**GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland.**

Kouros-Mehr H<sup>1</sup>, Slorach EM, Sternlicht MD, Werb Z.

Author information

**GATA3 acts upstream of FOXA1 in mediating ESR1 binding by shaping enhancer accessibility**

## Next steps: TF knockdown

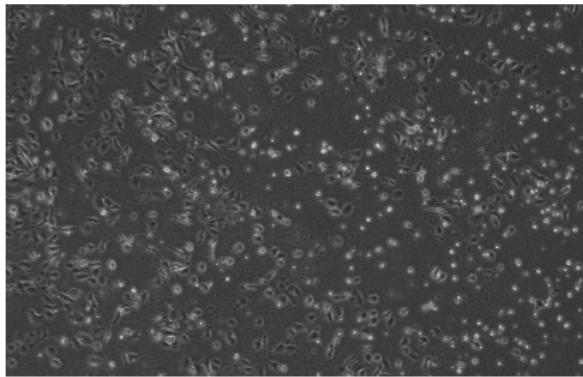
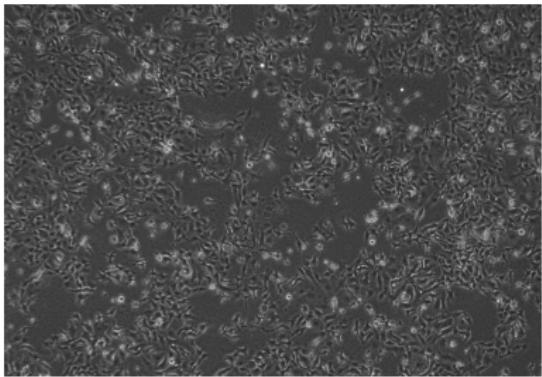


Figure: Candidate master regulator Transcription Factors (TF) knockdown in the SKGT4 human esophageal adenocarcinoma cell line. Figure produced by Dr. Dechen Lin.