

ELMER v2

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

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Houtan Noushmehr, De-Chen Lin, Benjamin P Berman

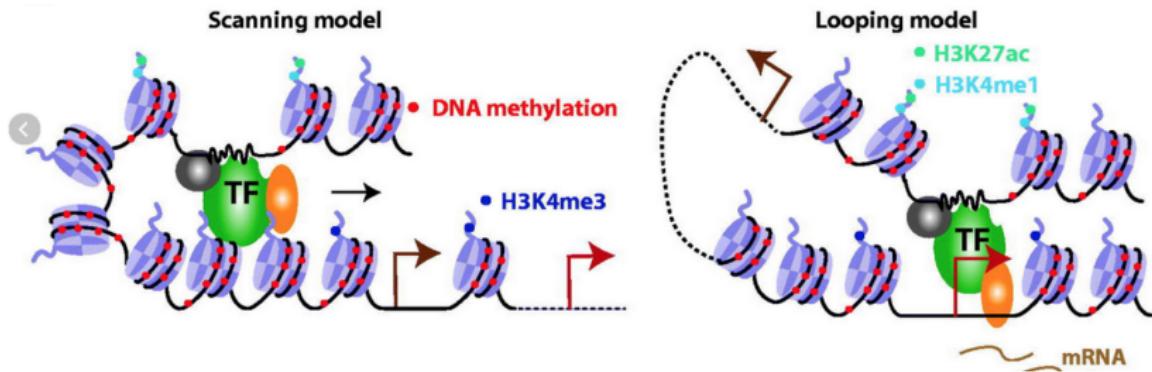
2018-04-20



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# Enhancer-mediated gene regulation

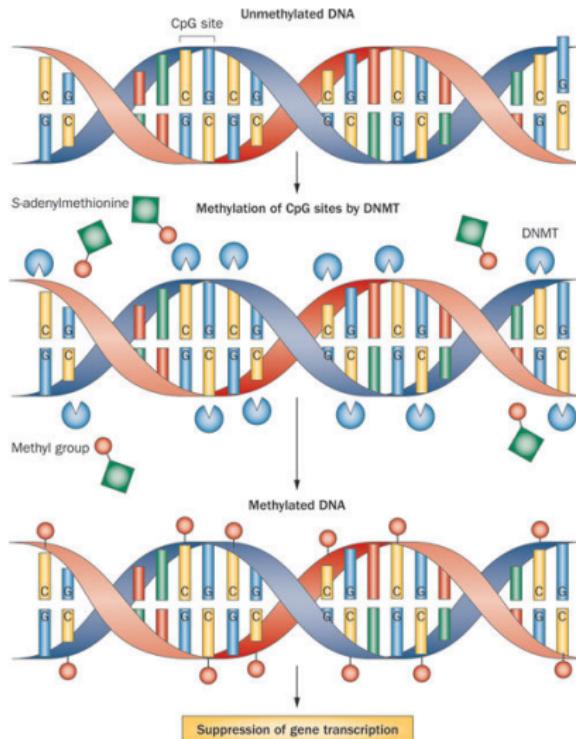


Source: Yao et al. Genome Biology (2015)

## Enhancer-mediated gene regulation

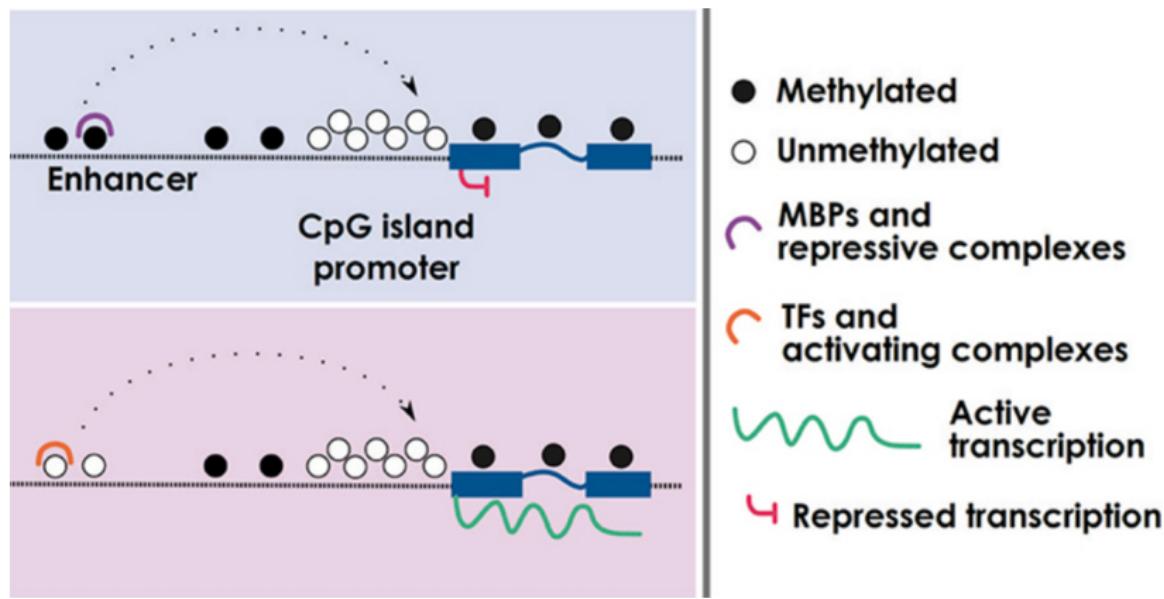
- 73% of the tested distal elements do not link to the nearest gene (Sanyal et al., 2012)
  - 40% of the enhancers involved in loops do not interact with the TSS of the nearest gene (Li et al., 2012),
  - one-third of the distal interactions were not directed to the promoter of the nearest gene (Mifsud 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).

# Epigenetics alterations - DNA methylation



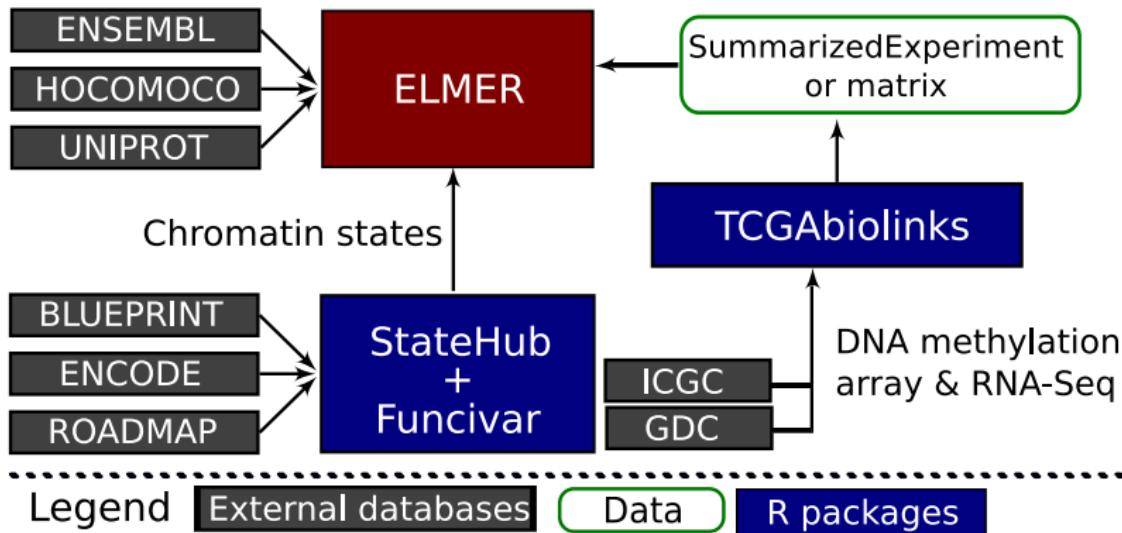
Source: Koch MW, et al. Nat Rev Neurol. (2013)

# Enhancer-mediated gene regulation



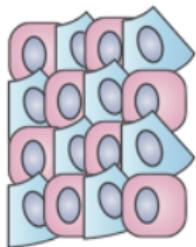
Source: Carrio et al. Frontiers in aging neuroscience (2015)

ELMER v.2: Enhancer Linking by Methylation/Expression Relationship



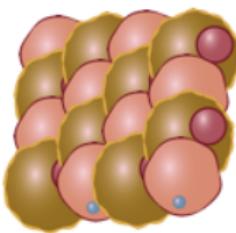
ELMER modes - Dealing with different molecular subtypes

## Unsupervised mode



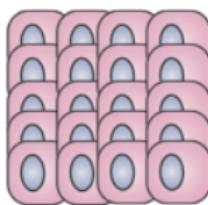
## Molecular subtype A, B

vs



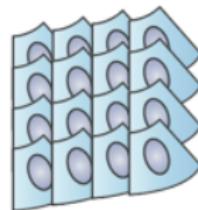
## Molecular subtype C, D

## Supervised mode



## Molecular subtype A

vs



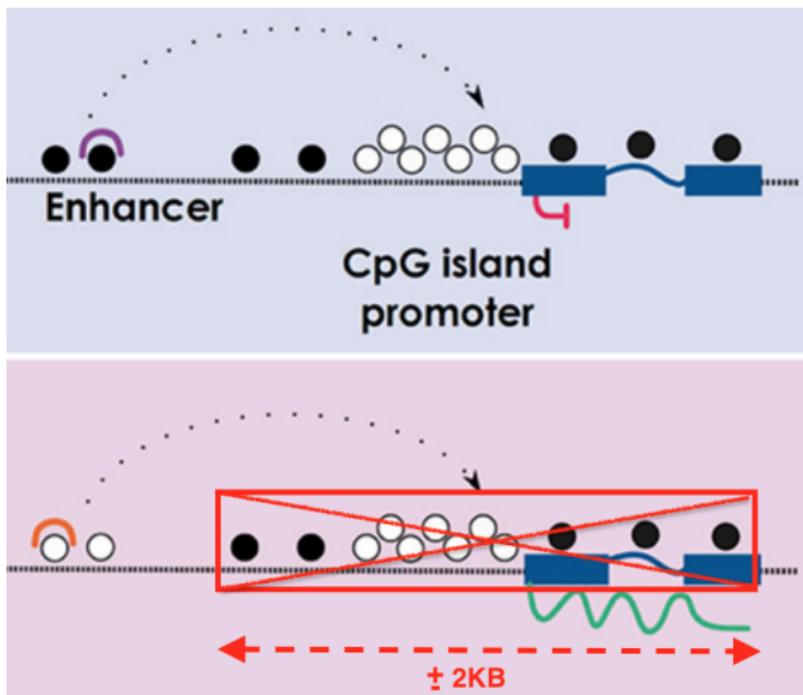
## Molecular subtype B

## Algorithm

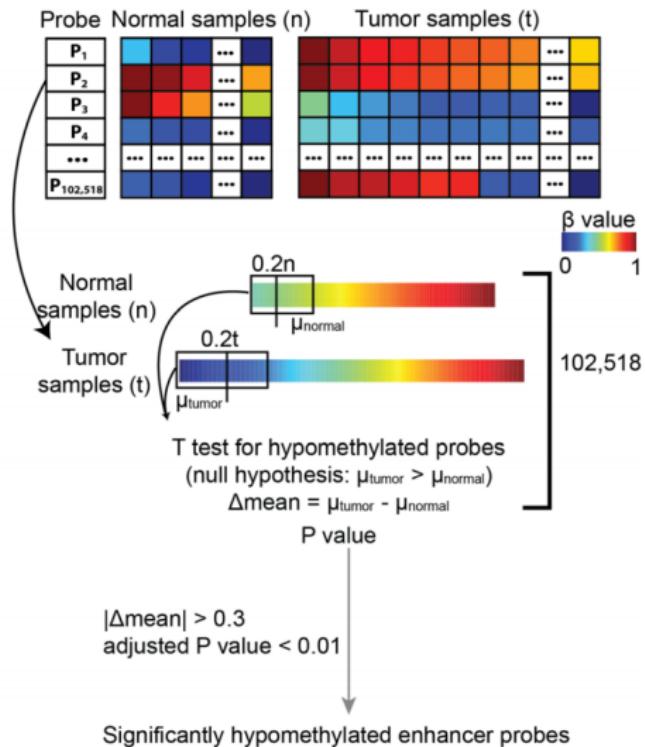
## Steps

- 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

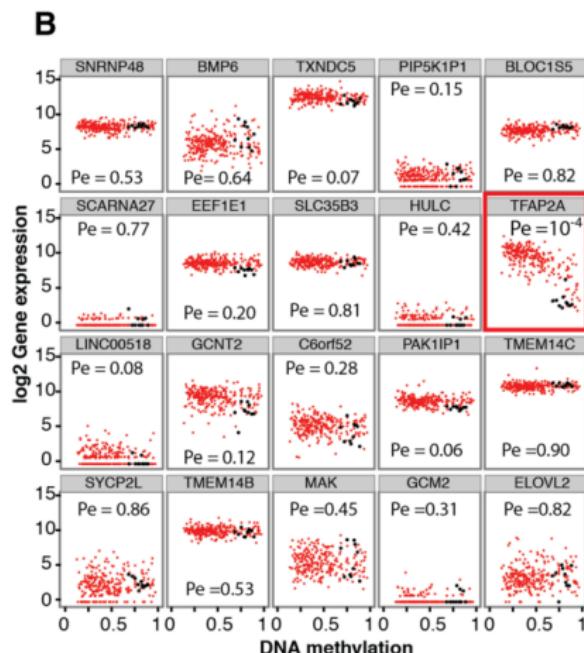
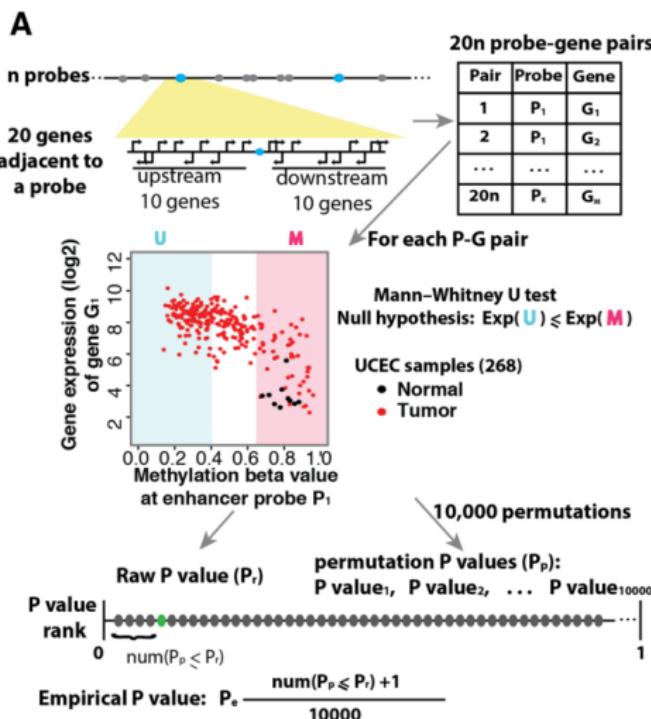


## Step 2: Differentially methylated distal probes



Source: Yao et al. Genome Biology (2015)

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



Source: Yao et al. Genome Biology (2015)

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

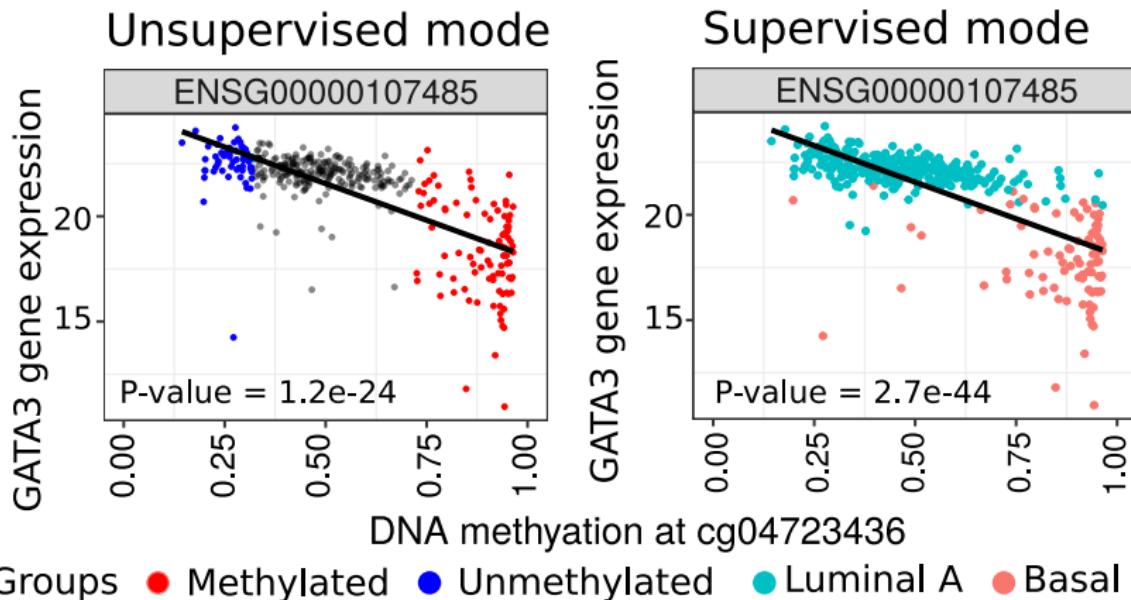
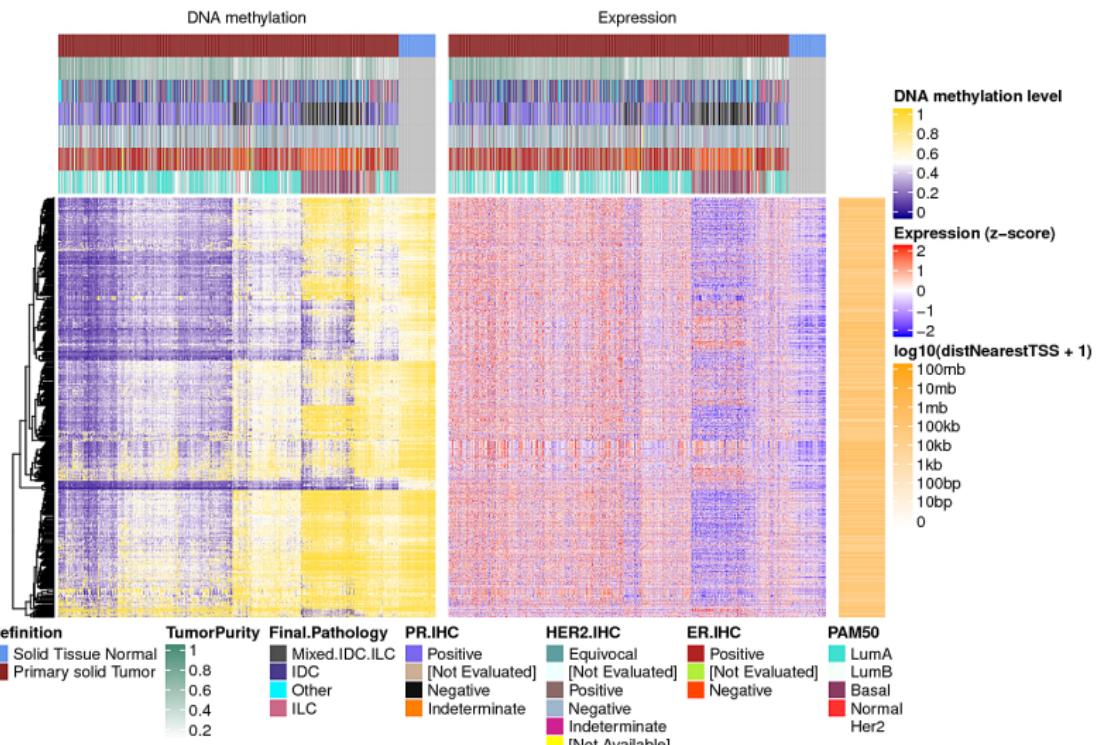


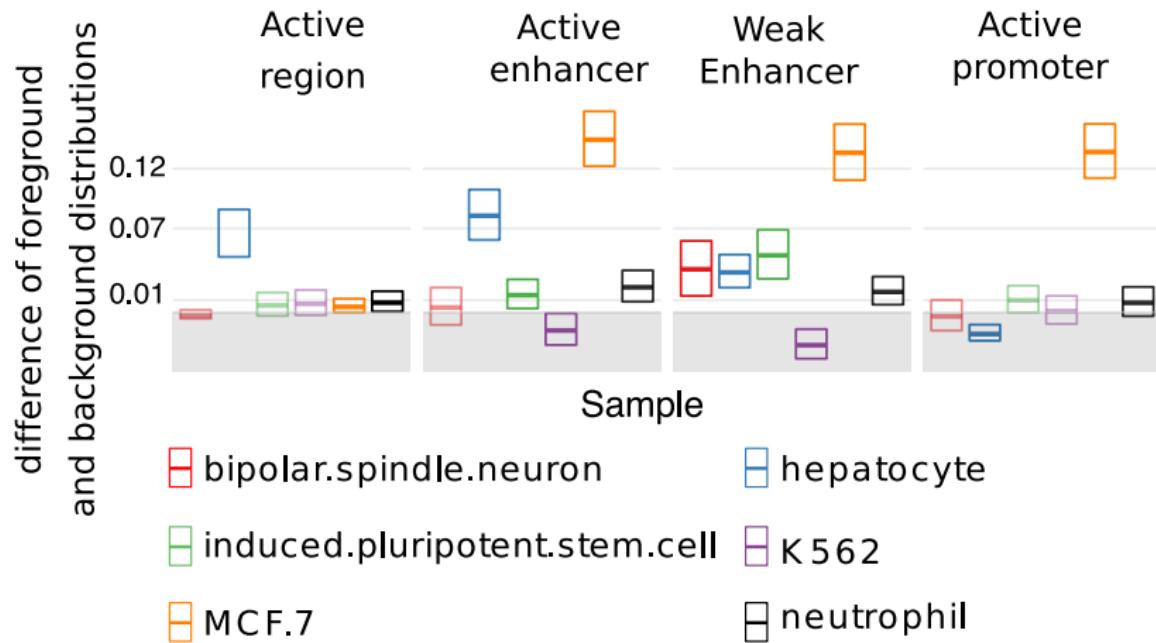
Figure: A: *unsupervised* mode; when minSubgroupFrac argument is set to 40%, the methylated group is defined as the highest quintile and the unmethylated group as the lowest quintile; B: *supervised* mode; methylated and unmethylated group are defined as one of the known molecular subtypes.

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

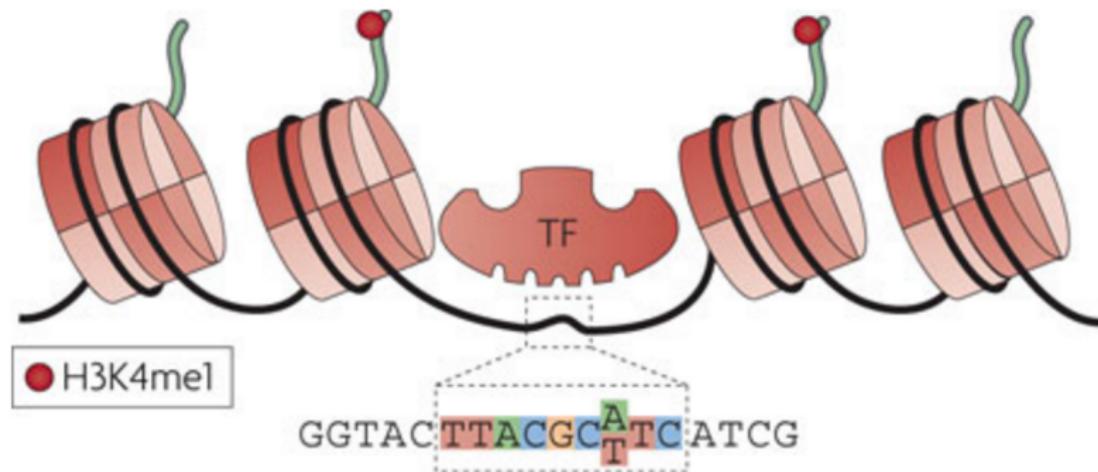
## Correspondence between probe DNA methylation and distal gene expression



# 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 Search: Human

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

Model	LOGO	Transcription factor	TF family	TF subfamily
		FOX		
FOXA1_HUMAN.H11MO.0.A	TgTTTAC...	FOXA1 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXA[3.3.1.1]
FOXA2_HUMAN.H11MO.0.A	TgTTTAC...	FOXA2 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXA[3.3.1.1]
FOXA3_HUMAN.H11MO.0.B	TgTTTAC...	FOXA3 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXA[3.3.1.1]
FOXB1_HUMAN.H11MO.0.D	AA TATTACATA	FOXB1 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXB[3.3.1.2]
FOXC1_HUMAN.H11MO.0.C	TGTTTACCTA	FOXC1 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXC[3.3.1.3]
FOXC2_HUMAN.H11MO.0.D	ATGTTTGAG	FOXC2 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXC[3.3.1.3]
FOXD1_HUMAN.H11MO.0.D	TgTTTAC...	FOXD1 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXD[3.3.1.4]
FOXD2_HUMAN.H11MO.0.D	ATAAATATT	FOXD2 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXD[3.3.1.4]
FOXD3_HUMAN.H11MO.0.D	TGTTT	FOXD3 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXD[3.3.1.4]

HOCOMOCO v11 (<http://hocomoco11.autosome.ru/human/mono?full=true>), Accessed: 26-12-2017

## Step 4: Motif enrichment analysis

### Objective

Evaluate the enrichment of transcription factors in certain genomic regions.

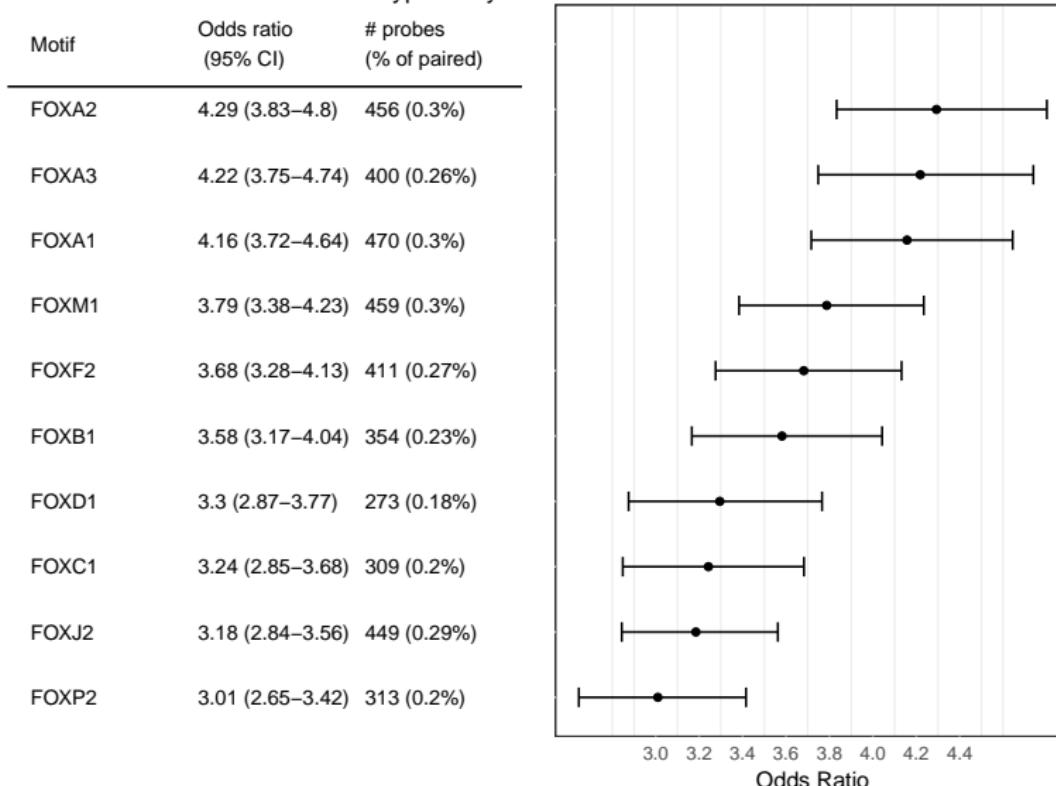
- ① Perform motif matching of transcription factors in probes regions (window  $\pm 250\text{bp}$ ). Performed using HOMER (Hypergeometric Optimization of Motif EnRichment) with HOCOMOCO motifs.
- ② Evaluate which transcription factors are more likely to occur in those regions than in background regions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

### Fisher's exact test

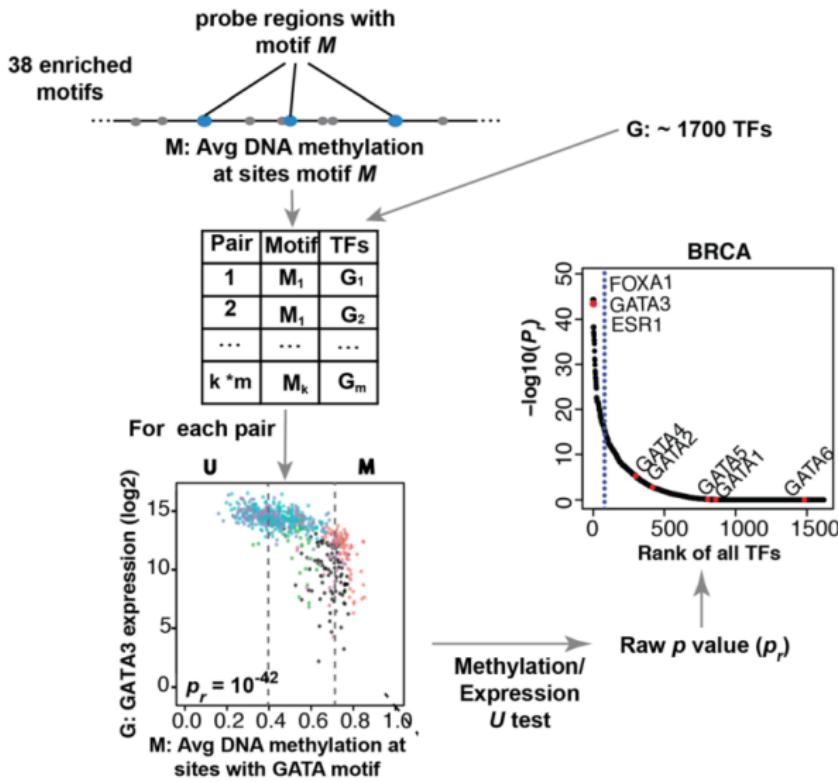
- a: nb of input regions with a match for TF motif.
- b: nb of input regions with no match for TF motif.
- c: nb of background regions with a match for TF motif.
- d: nb of background regions with no match for TF motif.

## Step 4: Motif enrichment analysis

Probes hypomethylated in LumB vs Basal



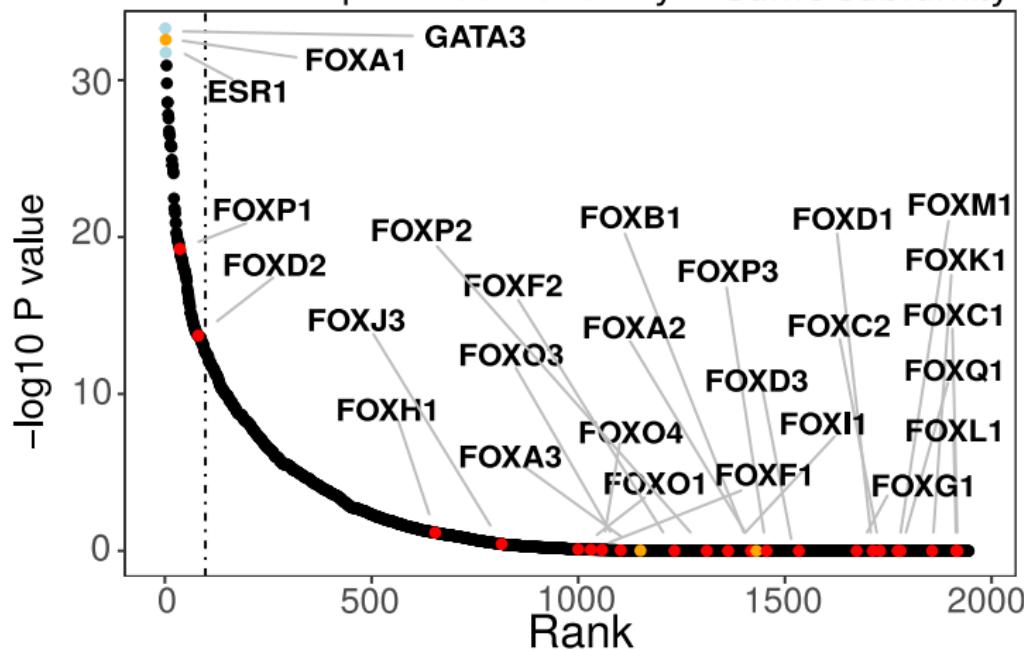
## Step 5: Identification of master regulator TF



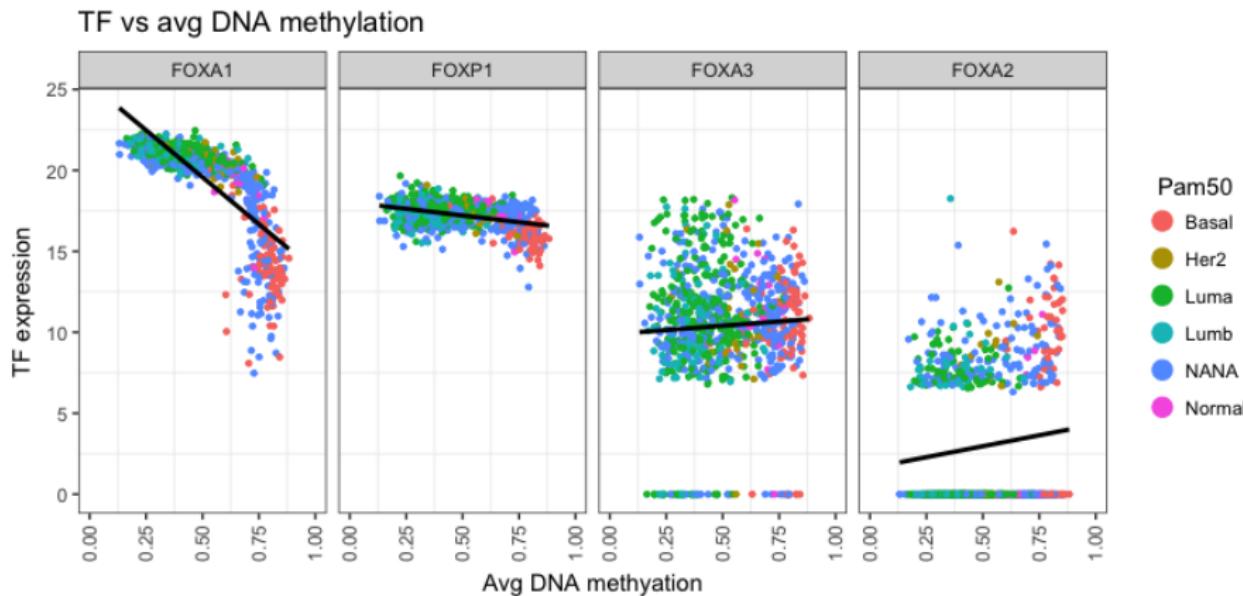
Source: Yao et al. Genome Biology (2015).

# TFs ranking for the *FOXA2* motif

TF classification (Motif: FOXA2)  
 Probes hypomethylated in LumB vs Basal  
 ● None ● Top 3 ● Same family ● Same subfamily



# TFs ranking for the *FOXA2* motif



# Step 5: Master Regulator TF table

motif	OR	potential.TF.family	pvalue
All	All	All	All
FOXA2_HUMAN.H11MO.0.A	4.29	FOXA1	3.29e-33
FOXA2_HUMAN.H11MO.0.A	4.29	FOXD2	1.94e-14
FOXA2_HUMAN.H11MO.0.A	4.29	FOXP1	5.85e-20
FOXA3_HUMAN.H11MO.0.B	4.22	FOXA1	3.29e-33
FOXA3_HUMAN.H11MO.0.B	4.22	FOXD2	1.94e-14
FOXA3_HUMAN.H11MO.0.B	4.22	FOXP1	5.85e-20
FOXA1_HUMAN.H11MO.0.A	4.16	FOXA1	3.29e-33
FOXA1_HUMAN.H11MO.0.A	4.16	FOXD2	1.94e-14
FOXA1_HUMAN.H11MO.0.A	4.16	FOXP1	5.85e-20
FOXM1_HUMAN.H11MO.0.A	3.79	FOXA1	3.29e-33

Showing 1 to 10 of 256 entries

Previous 1 2 3 4 5 ... 26 Next

## Case study: TCGA Breast Invasive Carcinoma (BRCA)

Table: Summary of the available samples in TCGA for BRCA

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

Table: Number of samples of the molecular subtypes of breast cancer

Molecular subtype	Number of samples
Basal	85
Her2	34
LumA	288
LumB	117
Normal-like	22

	Unsupervised	Supervised			
TF	Tumor (vs Normal)	LumB (vs basal)	Basal (vs LumB)	Basal (vs HER2)	HER2 (vs Basal)
<i>AR</i>	X	X			
<i>EMX1</i>	X	X			
<i>ESR1</i>	X	X			
<i>GATA3</i>	X	X			
<i>LMX1B</i>	X	X			
<i>NR2E3</i>	X	X			
<i>PBX1</i>	X	X			
<i>RARA</i>	X	X			
<i>ZNF467</i>	X	X			
<i>FOXA1</i>	X	X			X
<i>FOXP1</i>		X			X
<i>HOXB3</i>					X
<i>HOXC10</i>					X
<i>HOXC11</i>					X
<i>BCL11A</i>			X	X	
<i>E2F3</i>			X	X	
<i>ETV6</i>			X	X	
<i>KLF5</i>			X	X	
<i>SOX8</i>			X	X	
<i>SOX9</i>			X	X	
<i>SOX11</i>			X		
<i>ZIC1</i>			X	X	

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>, Powell 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

## Acknowledgments

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University of São Paulo

- Houtan Noushmehr

