

ELMER v2

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

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RECOMB-CBB

2018-04-20



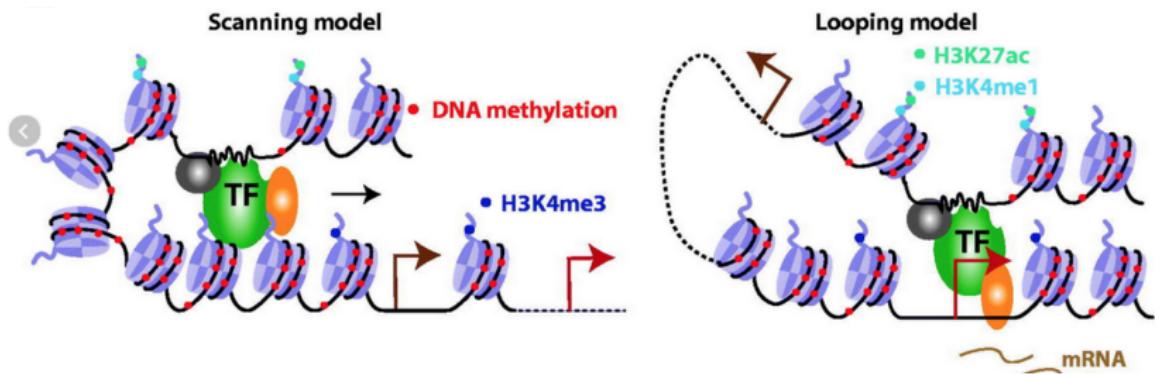
CEDARS-SINAI®



Overview

- 1 Introduction
 - 2 Methods: ELMER algorithm
 - 3 Case of study: TCGA-BRCA
 - 4 Conclusion

Enhancer-mediated gene regulation

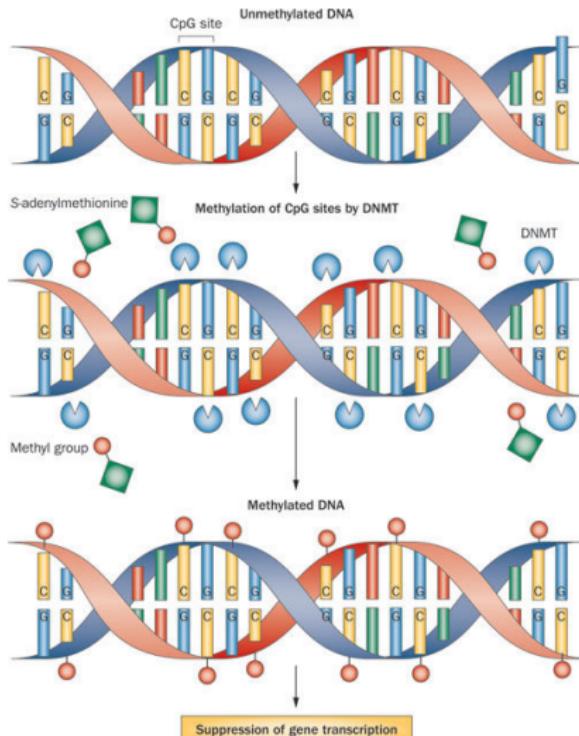


Source: Yao et al. Genome Biology (2015)

Enhancer-mediated gene regulation

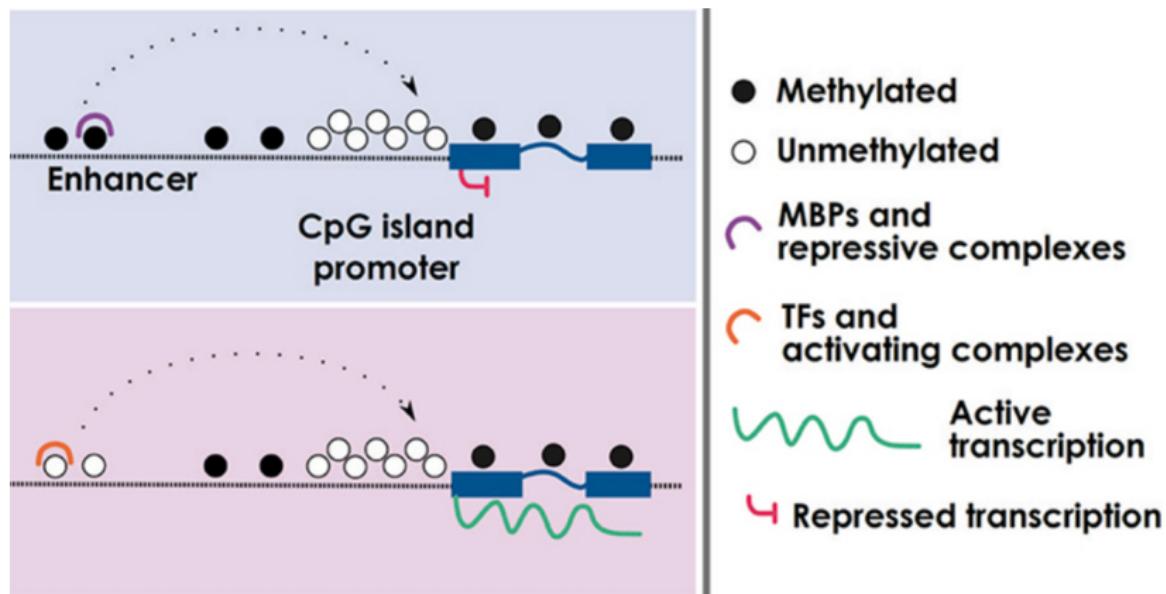
- 73% of the tested distal elements do not link to the nearest gene (Sanyal et al., 2012)
 - 40% of the enhancers involved in loops do not interact with the TSS of the nearest gene (Li et al., 2012),
 - one-third of the distal interactions were not directed to the promoter of the nearest gene (Mifsud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

Epigenetics alterations - DNA methylation



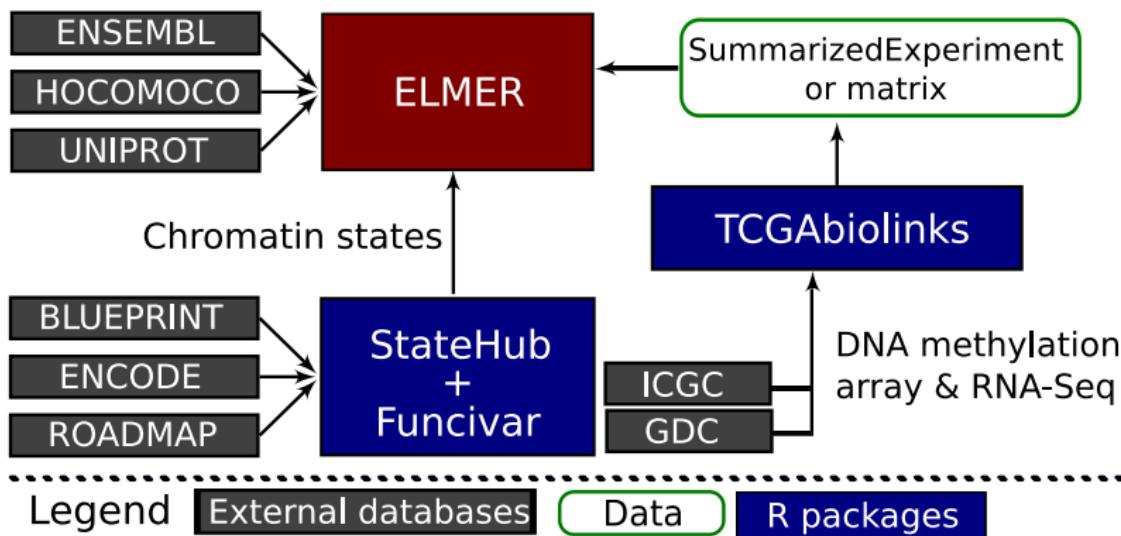
Source: Koch MW, et al. Nat Rev Neurol. (2013)

Enhancer-mediated gene regulation



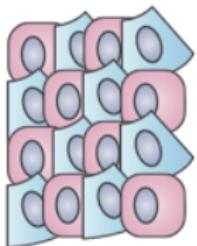
Source: Carrio et al. Frontiers in aging neuroscience (2015)

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



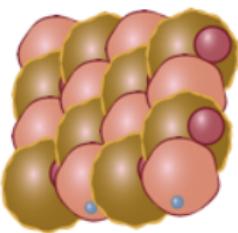
ELMER modes - Dealing with different molecular subtypes

Unsupervised mode



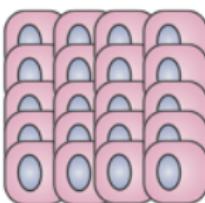
Molecular subtype A, B

vs



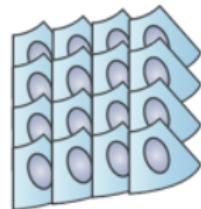
Molecular subtype C, D

Supervised mode



Molecular subtype A

vs



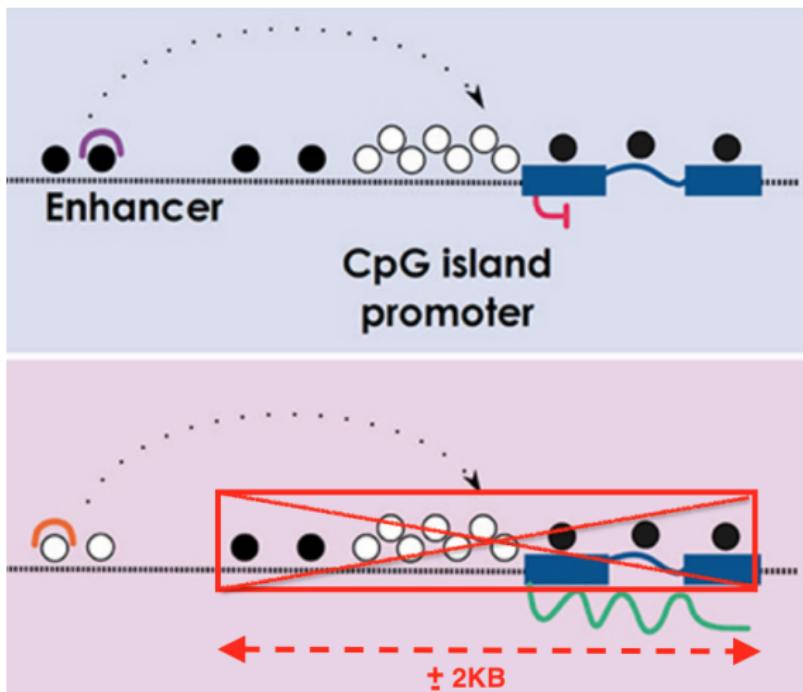
Molecular subtype B

Algorithm

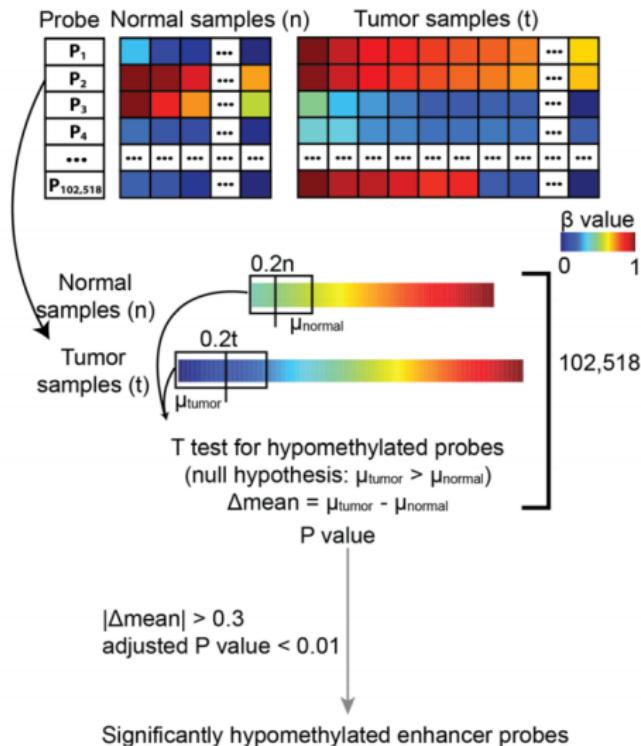
Steps

- 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

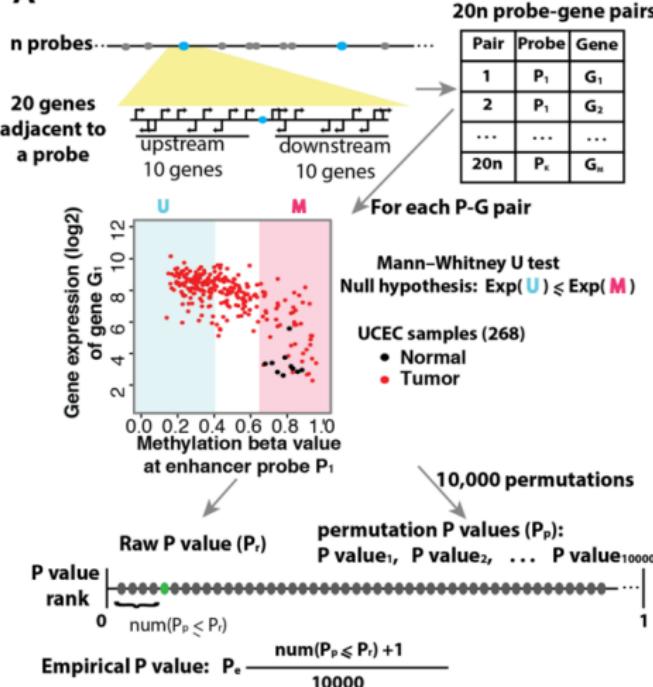
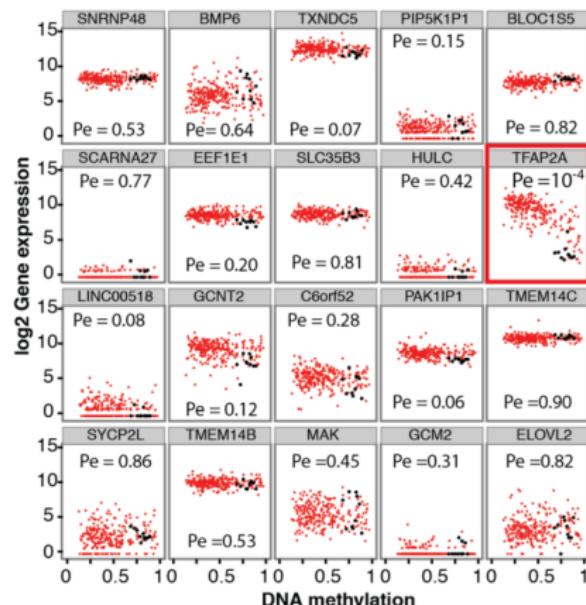


Step 2: Differentially methylated distal probes



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

Step 3: Identification of putative target gene(s)

A**B**

Source: Yao et al. Genome Biology (2015)

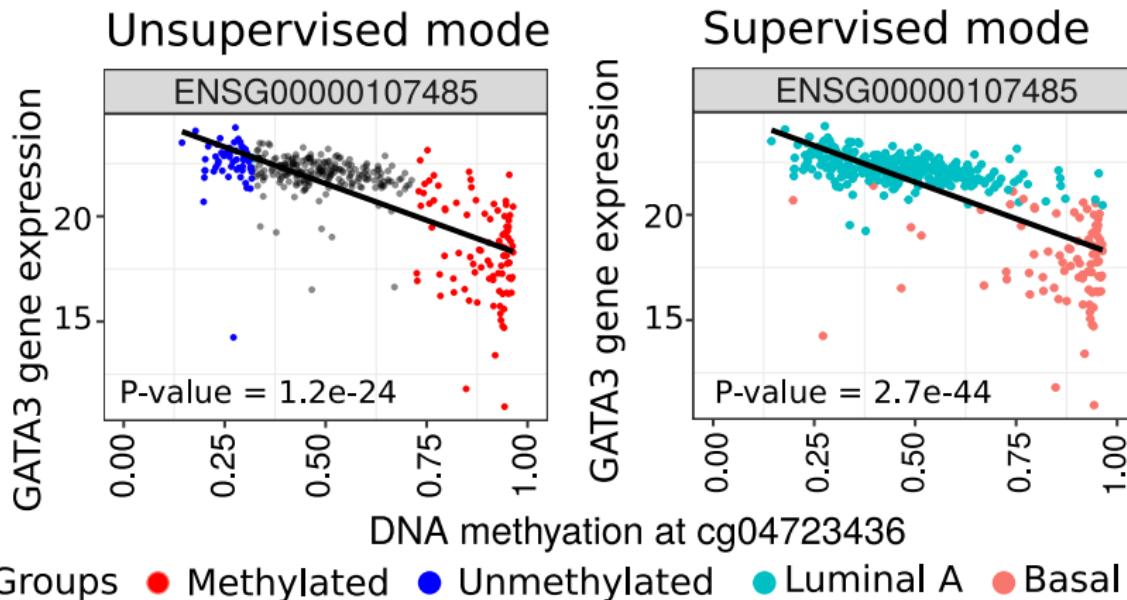
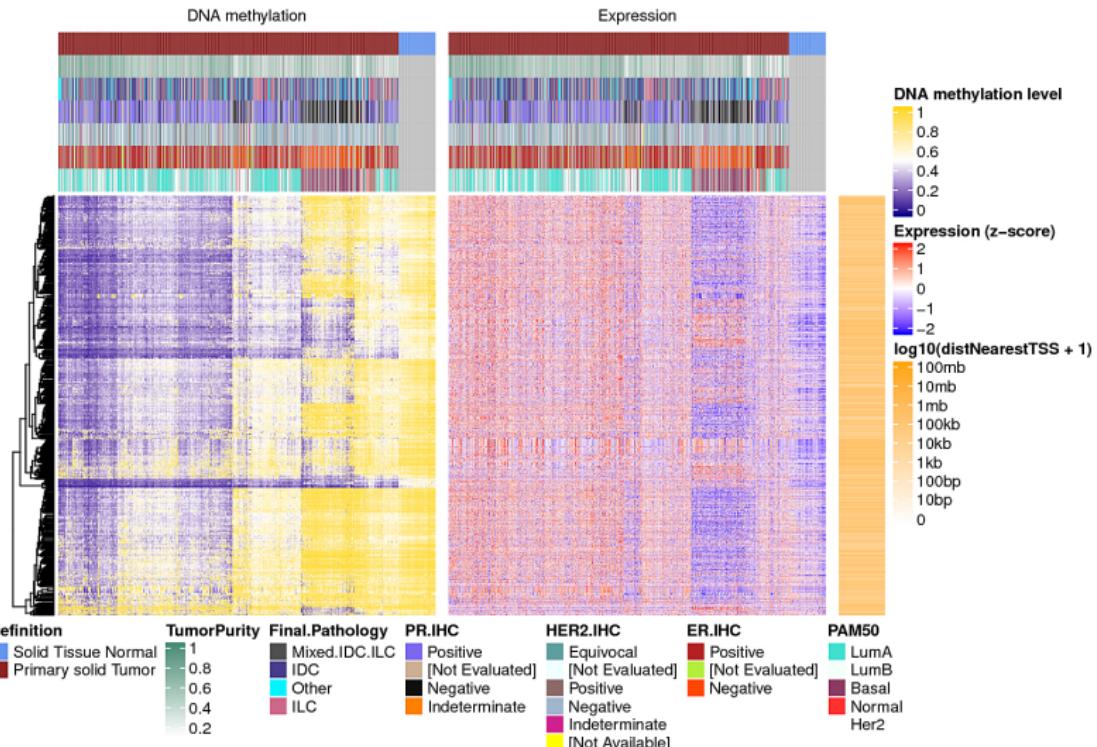
Groups *U* and *M* definition in (*un*)supervised mode

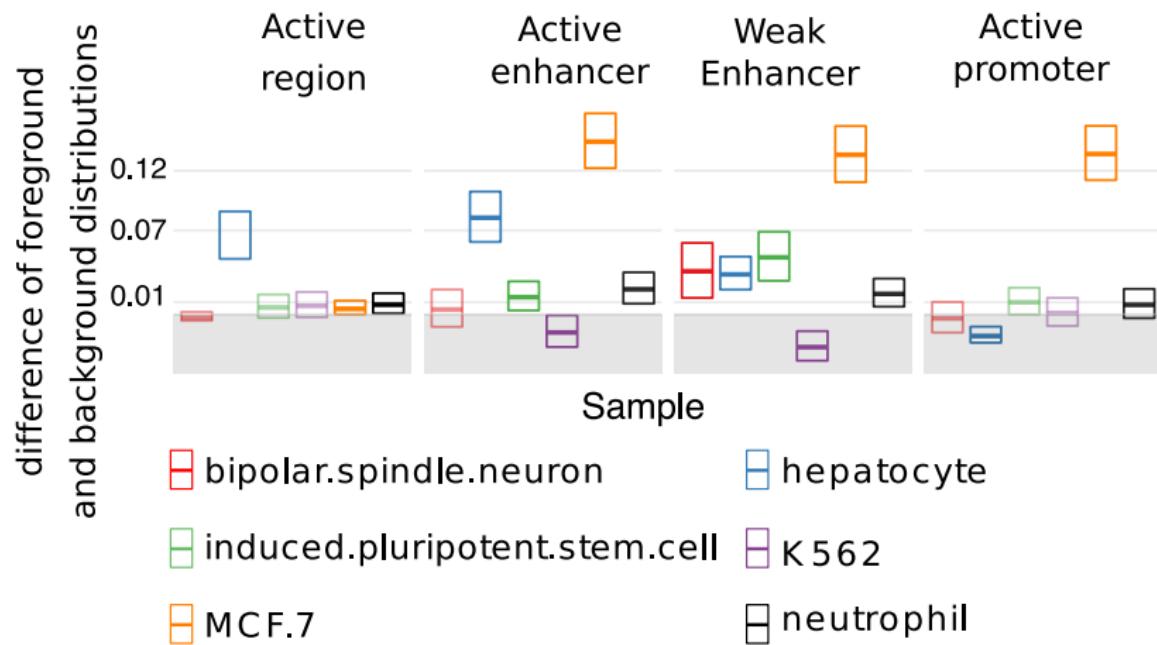
Figure: A: *unsupervised* mode; when `minSubgroupFrac` argument is set to 40%, the methylated group is defined as the highest quintile and the unmethylated group as the lowest quintile; B: *supervised* mode; methylated and unmethylated group are defined as one of the known molecular subtypes.

Step 3: Probe-target gene pairs inferred

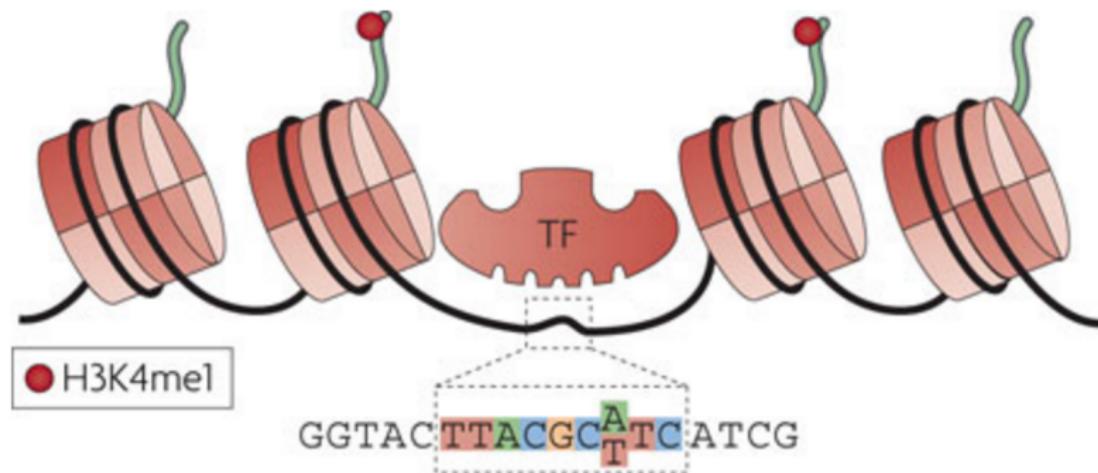
Correspondence between probe DNA methylation and distal gene expression



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	T _g T _T T _T A _C _T _A _T _A	FOXA1 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXA[3.3.1.1]
FOXA2_HUMAN.H11MO.0.A	T _g T _T T _T A _C _T _A _T _A	FOXA2 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXA[3.3.1.1]
FOXA3_HUMAN.H11MO.0.B	T _g T _T T _T A _C _T _A _T _A	FOXA3 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXA[3.3.1.1]
FOXB1_HUMAN.H11MO.0.D	~A _A T _T T _T A _C A _T A _T	FOXB1 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXB[3.3.1.2]
FOXC1_HUMAN.H11MO.0.C	T _G T _T T _T A _C T _A _T _A	FOXC1 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXC[3.3.1.3]
FOXC2_HUMAN.H11MO.0.D	~T _T G _T _T _T G _A _G	FOXC2 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXC[3.3.1.3]
FOXD1_HUMAN.H11MO.0.D	~T _T G _T T _T A _C _T _A	FOXD1 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXD[3.3.1.4]
FOXD2_HUMAN.H11MO.0.D	~G _T A _A T _T T _T _T _A _T _A	FOXD2 (GeneCards)	Forkhead box (FOX) factors[3.3.1]	FOXD[3.3.1.4]
FOXD3_HUMAN.H11MO.0.D	T _G T _T T _T _C _T _A	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.

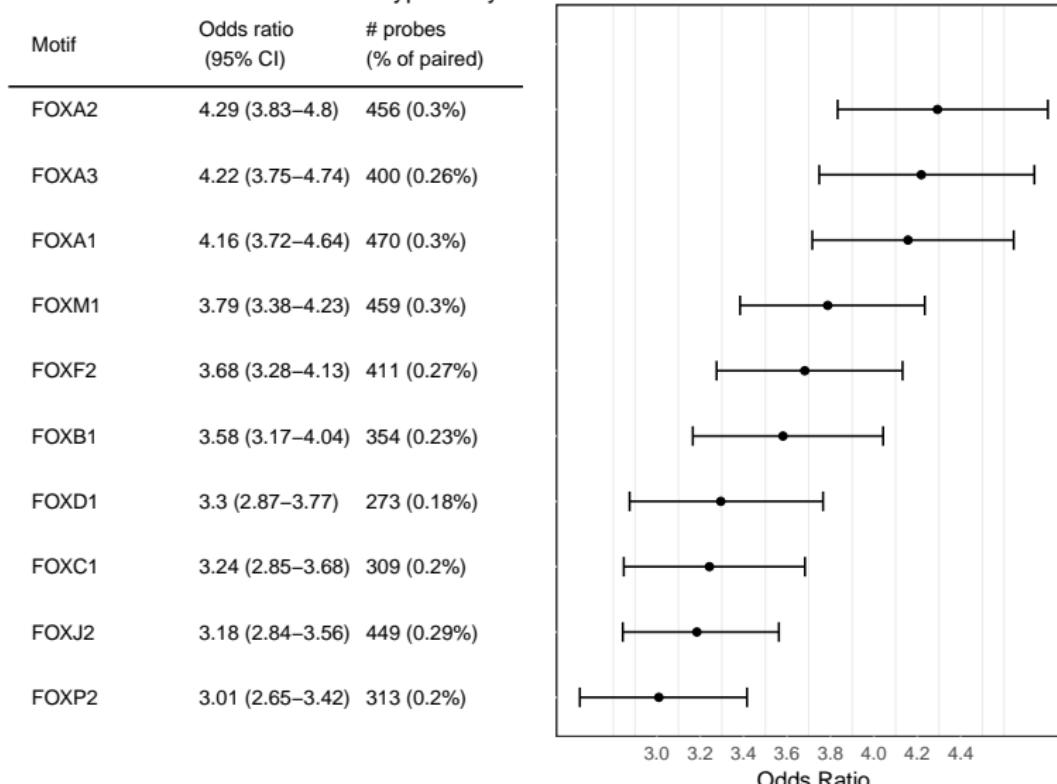
- ① Perform motif matching of transcription factors in probes regions (window $\pm 250\text{bp}$). Performed using HOMER (Hypergeometric Optimization of Motif EnRichment) with HOCOMOCO motifs.
- ② Evaluate which transcription factors are more likely to occur in those regions than in background regions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

Fisher's exact test

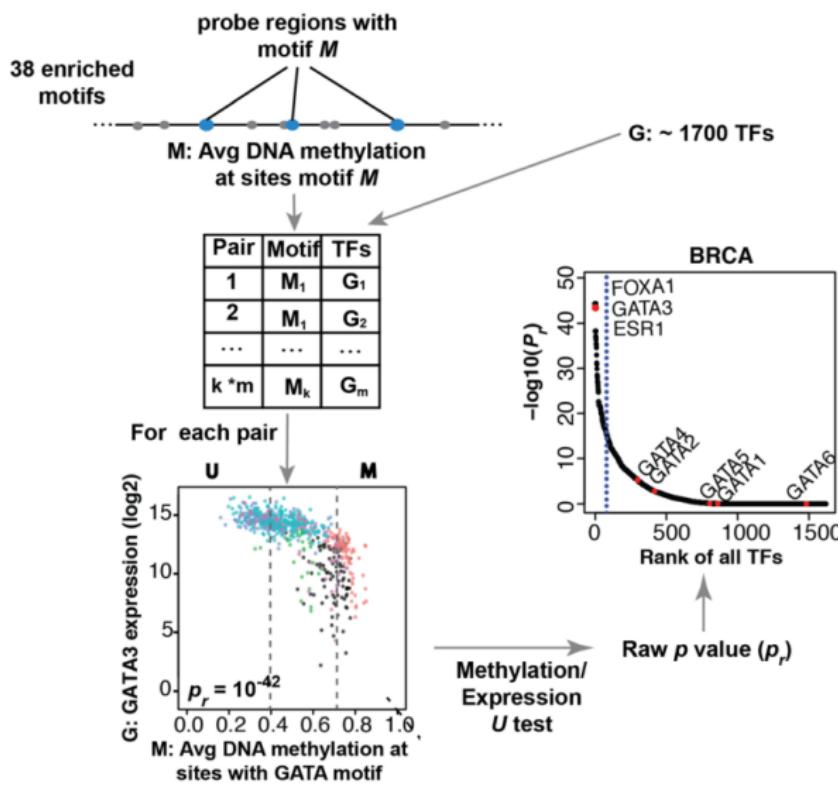
- a: nb of input regions with a match for TF motif.
- b: nb of input regions with no match for TF motif.
- c: nb of background regions with a match for TF motif.
- d: nb of background regions with no match for TF motif.

Step 4: Motif enrichment analysis

Probes hypomethylated in LumB vs Basal



Step 5: Identification of master regulator TF



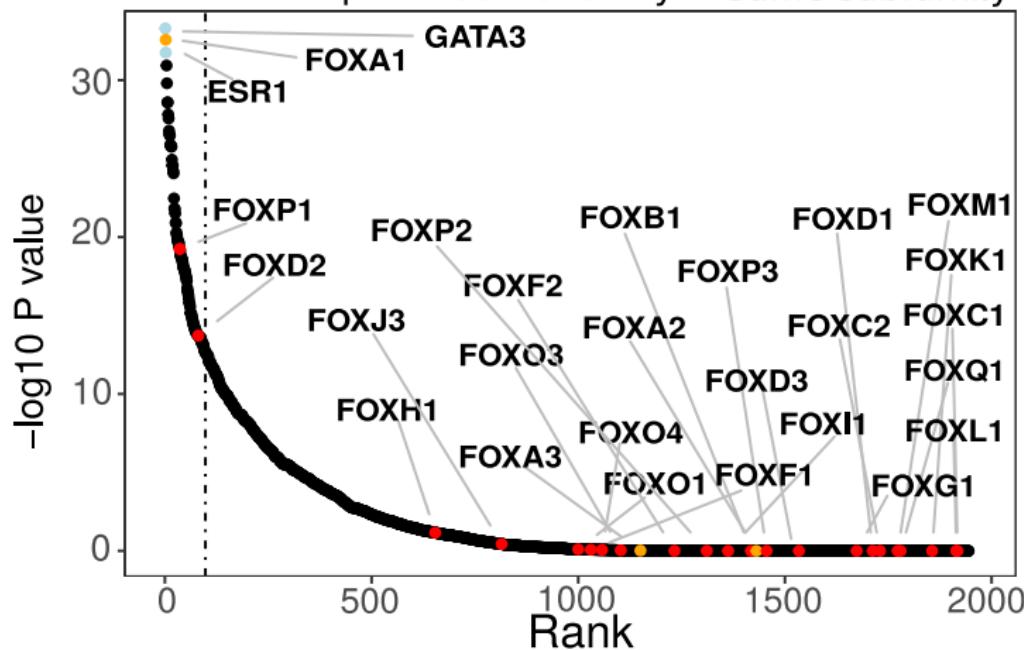
Source: Yao et al. Genome Biology (2015).

TFs ranking for the *FOXA3* motif

TF classification (Motif: FOXA2)

Probes hypomethylated in LumB vs Basal

- None
- Top 3
- Same family
- Same subfamily



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

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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^{1,3}, Ivan P. Uray³, Abhijit Mazumdar³, Anna Tsimelzon², Michelle Savage³, Susan G. Hilsenbeck², Powell H. Brown^{1,3}

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

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

negative breast cancer cell lines to regain hormonal sensitivity.⁴¹ 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.¹³ 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 H¹, Slorach EM, Sternlicht MD, Werb Z.

Author information

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

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