TITLE

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Contents

TITLE

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Abstract

1 Words

Introduction

FOXA1 Expression and ERα+ Breast Cancer

tRNAs and Gene Expression

Materials & Methods

Acquisition of Public ChIP-seq Datasets

ChIP-seq was performed on genetically modified MCF7L cells (*insertion, using a lentiviral cDNA delivery system to express Dox-inducible FOXA1*)[1]. Datasets were deposited into the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA)[2] under accession no. PRJNA512997 (Table 1). Using "Genetic Manipulation Tools" within the Galaxy[3] environment (v 23.0.rc1), SRAs were converted to FastQ files. FastQ files were then aligned to the human genome assembly GRCh37 (hg19) using Bowtie2 (v 2.5.0)[4] to output BAM files.

Table 1. Publicly available ChIP-seq SRA files aquired from the NCBI SRA database (accession no. PRJNA512997).

Experiment	SRA	Factor	Tissue	Assembly
PRJNA512997	SRR8393424	FOXA1	MCF-7LP	GRCh37 (Hg19)
	SRR8393425			
	SRR8393426			
	SRR8393427	H3K27ac		
	SRR8393428			
	SRR8393431	None (input)		
	SRR8393432			

EaSeq for Chip-seq Peak Quantification

BAM files were uploaded into EaSeq (v1.111) as "Datasets" using the standard settings for Chip-seq data. GRCh37 (hg19) tRNA sequences (n = 606) were downloaded as a "Geneset" from the UCSC Table Browser[5], (available at https://genome.ucsc.edu). High-confidence tRNAs (n = 416) identified in the GtRNAdb[6] were extracted as a "Regionset".

Signal peak intensities surrounding tRNAs were quantified using the EaSeq "quantify" tool. Here the default settings "Normalize to reads per million" and "Normalize counts to DNA-fragments" were left checked. The default setting "Normalise to a signal of 1000 bp" was unchecked. The window size was offset ±500bp from the start of each tRNA gene. Outputs are referred to as "Q-values".

To quantify upstream and downstream signals, the "quantify" tool was used with adjusted window sizes. The upstream region was defined as 500 bp preceding and the first nucleotide of tRNA loci. Thus, the start position was offset to 0 bp, and the end position was offset to -500 bp. The downstream region constitutes the 500 bp region beginning with the first nucleotide of tRNA gene body. The start position was offset to 1 bp, and the end position was offset to 500 bp.

Following quantification, tRNA binding events were arranged in ascending order -DOX Q-value and visualised as heatmaps. Data was also visualised with "average", and "overlay" EaSeq tools.

EaSeq[7] is avaiable at http://easeq.net.

Motif Analysis

Multiple EM for Motif Elicitation ChIP (MEME) Suite

Statistics

Statistical tests and graphs were generated with R[8] (v 4.2.3), R Studio[9] (v 2023.03.0.386) and the tidyverse[10] package.

Results

FOXA1 and H3k27ac Binding Events

Background

-

Why? What?

To investigate the impact of FOXA1 on tRNA enhancers in ER+ MCF-7 cells, public ChIP-Seq datasets from Fu et al. (2019)[1] were interrogated. In this paper, a doxycycline (Dox) inducible OE system was used to achieve FOXA1 OE akin to tamoxifen-resistant (TamR) MCF-7 cells[1].

Heatmaps

Quantification of 416 high-confidence tRNAs revealed that both FOXA1 and H3k27ac interact with the majority of tRNA genes, relative to ±10kb flanking regions. Whereas input reads generated minimal peak enrichment (Supplementary Figures). Upon FOXA1 OE, average FOXA1 binding at tRNAs increases tRNA genes. On the other hand, H3K27ac binding decreases.

Average Tracks

This was confirmed by average signal intensity tracks 4/1.

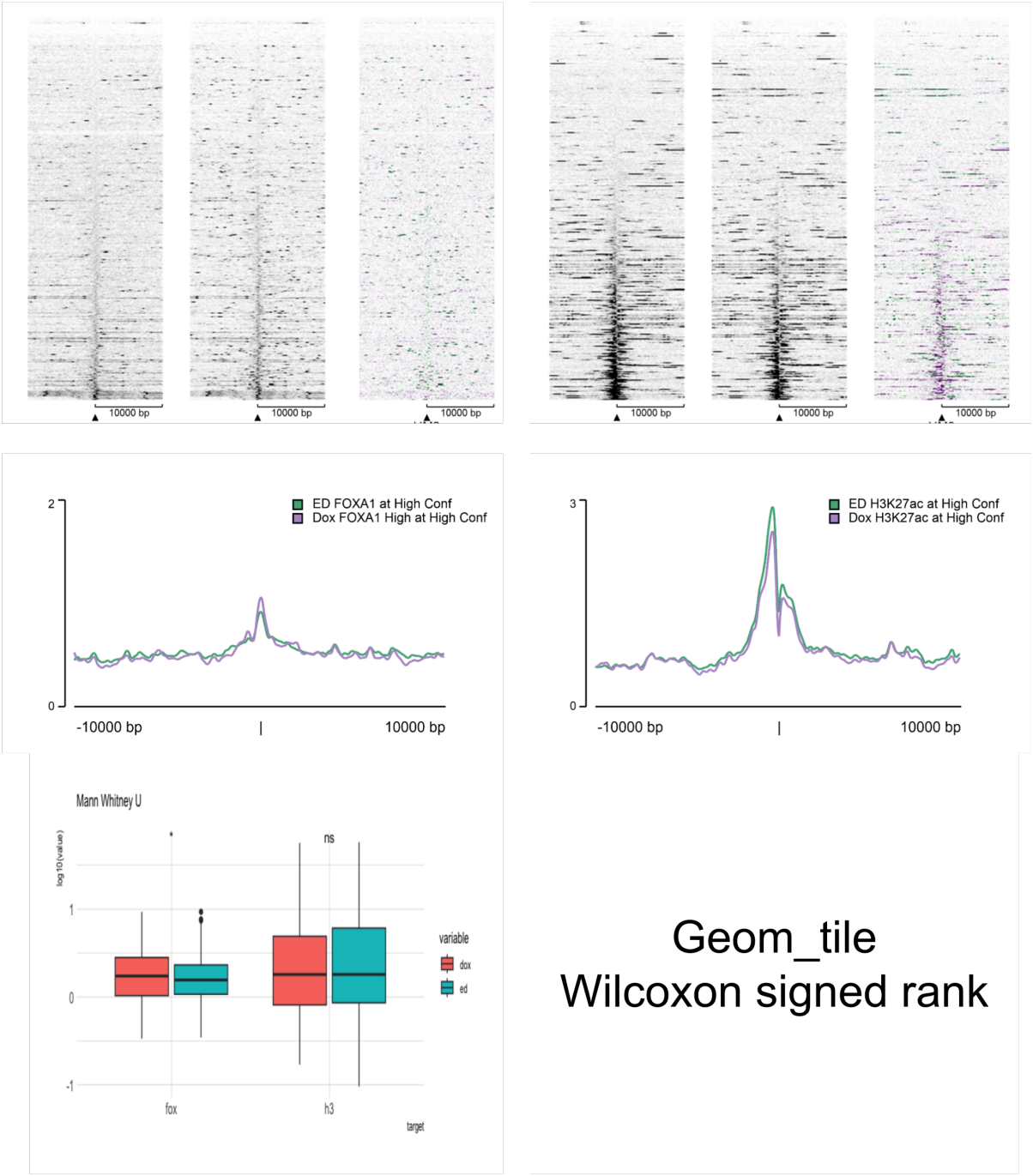


Figure 1. (B) Ratiometric heatmaps of the log2 ratio between the binding of FOXA1 or H3k27ac with endogenous FOXA1 expression vs. the binding of FOXA1 or H3k27ac with FOXA1 OE.

Suggests

Boxplots

Why?

What?

Mann Whitney U to compare means

Boxplot

Volcano

X2 = x number of significantly upregulated and downregulated genes

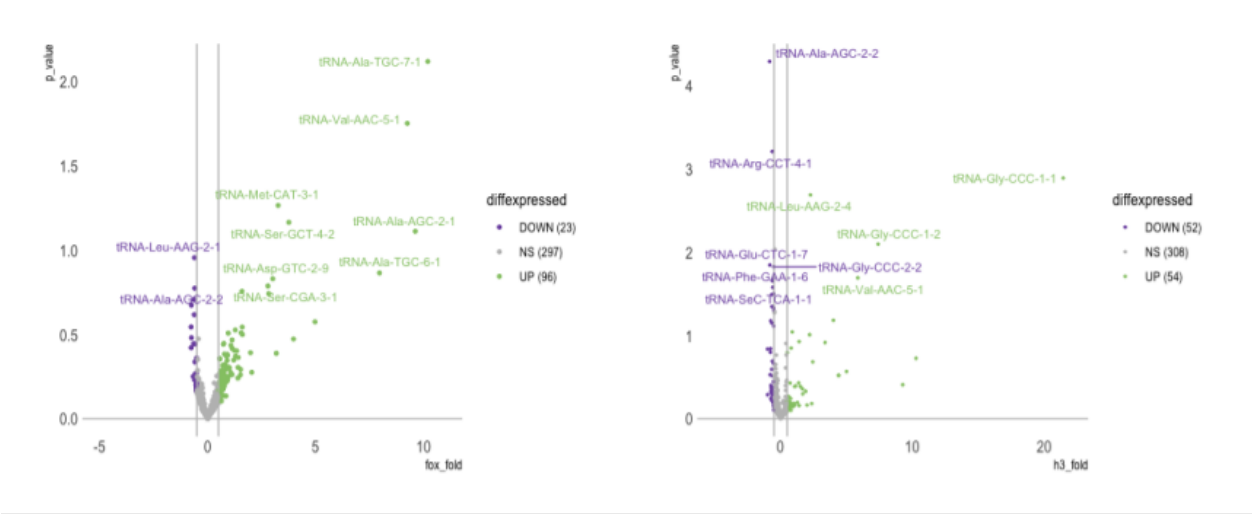
more Up than down (FOX)

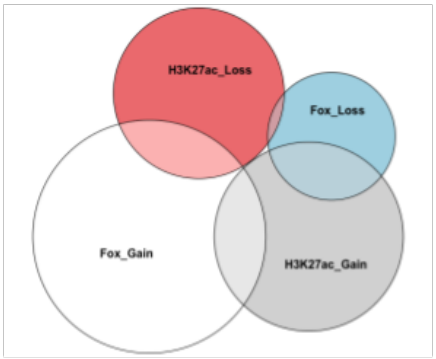
about the same for H3

Localisation of FOXA1 at individual tRNA genes in MCF-7 cells

Table 2. .

Group	Function
ALOXE	Insulator Function[11,12]
Ebersole	Insulator Function[12,13]
HES7	
Per1	
TMEM107	Insulator Function[11,12]
Arg-CCG	Implicated in Cancer Progression[14]
Glu-TTC	Implicated in Cancer Progression[14]
iMET	Proliferation of Breast Cancer
Met	iMet Control
SeC	Involved in REDOX[15]





Distinct and shared
Fill Tracks

Figure 2. (B) Ratiometric heatmaps of the log2 ratio between the binding of FOXA1 or H3k27ac with endogenous FOXA1 expression vs. the binding of FOXA1 or H3k27ac with FOXA1 OE.

Discussion

- not compared mcf-7 to treatment responsive cells

Future

- FOXA1 alone not efficient to increase activity
  - p300
- FOXA1 moves nucleosomes to make other TF acessible
- Loses fox = weak binding?
- Dynamic and stable marks
- pertubations
- ATAC-seq

574 Words

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