

TITLE

by Natasha Louise Hopkins

Master of Biology (Honours), Molecular Cell Biology
University of York, UK

Project Director
Prof. Robert J White

Examination Date
17 April, 2023

Word Count
Abstract:
Main:



Contents

1	Introduction	1
1.1	FOXA1 Expression and ER+ Breast Cancer	1
1.2	tRNAs and Gene Expression	1
2	Materials & Methods	1
2.1	MCF7L ChIP-seq Data from NCBI	1
2.2	EaSeq for the Quantification of FOXA1 and H3K27ac at tRNA genes	1
2.3	Motif Analysis	2
2.4	Statistics	2
3	Results	2
3.1	Localisation of FOXA1 at tRNA genes in MCF-7 cells	2
3.2	Co-localisation of FOXA1 with enhancer marks at tRNA genes	2
4	Discussion	2
4.1	Future	2
	References	3

TITLE

Natasha L. Hopkins

Abstract

1 Words

1 Introduction

1.1 FOXA1 Expression and ER+ Breast Cancer

1.2 tRNAs and Gene Expression

2 Materials & Methods

2.1 MCF7L ChIP-seq Data from NCBI

ChIP-seq was performed on MCF7L cells expressing Dox-inducible FOXA1, established using a lentiviral cDNA delivery system^{1,2}. Datasets were deposited into the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession no. PRJNA512997³ (Table 1). SRAs were downloaded by Galaxy (v23.0.rc1)⁴ and converted to FastQ files and then into BAM files using ‘Genetic Manipulation Tools’.

Table 1. Publicly available ChIP-seq SRA files acquired 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			

2.2 EaSeq for the Quantification of FOXA1 and H3K27ac at tRNA genes

BAM files were uploaded into EaSeq (v1.111)⁵ as ‘Datasets’ using the standard settings for Chip-seq data. The reference genome hg19 was downloaded as a ‘Geneset’ from the UCSC Table Browser⁶. High-confidence tRNAs identified in the GtRNAdb⁷ were extracted as a ‘Regionset’. Signal peak intensities surrounding tRNAs

were quantified using the 'Quantify' tool. Here, the default settings were left checked, with the exception of 'Normalise to a signal of'. The window size was offset 500bp from the start of each gene. Outputs are referred to as 'Q-values'.

- sorted by q value
- heatmap, filltrack, ect

2.3 Motif Analysis

2.4 Statistics

3 Results

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

Table 2

Table 2. .

X.Group.	Function
ALOXE	Insulator Function ⁸
Ebersole	
HES7	
Perl	
TMEM107	
Arg-CCG	Implicated in Cancer
Glu-TTC	
iMET	Proliferation of Breast Cancer
Met	iMet Control
SeC	Involved in REDOX

3.2 Co-localisation of FOXA1 with enhancer marks at tRNA genes

4 Discussion

4.1 Future

- FOXA1 alone not efficient to increase activity
 - p300

- FOXA1 moves nucleosomes to make other TF accessible
- Loses fox = weak binding?
- Dynamic and stable marks
- perturbations
- ATAC-seq

246 Words

References

- 1 Fu X, Jeselsohn R, Pereira R, Hollingsworth EF, Creighton CJ, Li F *et al.* FOXA1 overexpression mediates endocrine resistance by altering the ER transcriptome and IL-8 expression in ER-positive breast cancer. *Proceedings of the National Academy of Sciences* 2016; **113**. doi:10.1073/pnas.1612835113.
- 2 Fu X, Pereira R, De Angelis C, Veeraraghavan J, Nanda S, Qin L *et al.* FOXA1 upregulation promotes enhancer and transcriptional reprogramming in endocrine-resistant breast cancer. *Proceedings of the National Academy of Sciences* 2019; **116**: 26823–26834.
- 3 Leinonen R, Sugawara H, Shumway M. [The Sequence Read Archive](#). *Nucleic Acids Research* 2010; **39**: D19–D21.
- 4 Afgan E, Nekrutenko A, Grüning BA, Blankenberg D, Goecks J, Schatz MC *et al.* [The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2022 update](#). *Nucleic Acids Research* 2022; **50**: W345–W351.
- 5 Lerdrup M, Johansen JV, Agrawal-Singh S, Hansen K. [An interactive environment for agile analysis and visualization of ChIP-sequencing data](#). *Nature Structural & Molecular Biology* 2016; **23**: 349–357.
- 6 Karolchik D. [The UCSC Table Browser data retrieval tool](#). *Nucleic Acids Research* 2004; **32**: 493D–496.
- 7 Chan PP, Lowe TM. GtRNAdb 2.0: an expanded database of transfer RNA genes identified in complete and draft genomes. *Nucleic Acids Research* 2015; **44**: D184–D189.
- 8 Raab JR, Chiu J, Zhu J, Katzman S, Kurukuti S, Wade PA *et al.* [Human tRNA genes function as chromatin insulators](#). *The EMBO Journal* 2011; **31**: 330–350.