Use Case 15 [optional]: Exploration of Estrogen Receptor alpha (ERa) Network in Breast Cancer Cells

> Epigenome Informatics Workshop Bioinformatics Research Laboratory

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SPARK and GREAT tools References

Nielsen, C., Younesy, H., O'Geen, H., Xu, X., Jackson, A., Milosavljevic, A., Wang, T., Costello, J., Hirst, M., Farnham, P., et al. (2012). Spark: a navigational paradigm for genomic data exploration. Genome Research 22, 2262–2269.

McLean, C., Bristor, D., Hiller, M., Clarke, S., Schaar, B., Lowe, C., Wenger, A., and Bejerano, G. (2010). GREAT improves functional interpretation of cisregulatory regions. Nature Biotechnology 28, 495–501.

The data for this use case was obtained from the following references

Kittler, R., Zhou, J., Hua, S., Ma, L., Liu, Y., Pendleton, E., Cheng, C., Gerstein, M., and White, K. (2013). A Comprehensive Nuclear Receptor Network for Breast Cancer Cells. Cell Reports.

Hua, S., Kittler, R., and White, K. (2009). Genomic antagonism between retinoic acid and estrogen signaling in breast cancer. Cell 137, 1259–1271.

Use Case 15 [optional]: Exploration of Estrogen Receptor alpha (ERa) Network in Breast Cancer Cells

Background:

In breast cancer, estrogen receptor alpha (ERa) is known to play a major role in carcinogenesis and cancer progression. It drives proliferation in about 60-70% of breast cancer patients. Therefore, ERa is one of the main targets in breast cancer therapy. However, recently presence and importance of other nuclear receptors in breast cancer had been shown. Proper understanding of several additional nuclear receptors role in breast cancer may provide better diagnostic markers or novel targets for therapy.

Previous work mapping ERa binding sites genome-wide revealed that FoxA1 is required for ERa binding. However, mapping of retinoic acid receptor (RAR) nuclear receptors showed widespread antagonistic interaction of liganded RARs with ERa in the regulation of breast cancer-associated genes.

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Model for the Antagonistic Regulation of Target Genes by RAR and ERa



FoxA1 has been implicated in recruitment of ERa to distal sites. Hua et al showed that FoxA1 similarly required for RAR recruitment to genomic binding sites. Many of the genomic regions bound by RAR overlap with ERa. Majority of the overlap regions (71%) have shared binding regions of ERa and RARs. Transcriptional analysis indicate that RARs and ERa tend to exhibit antagonistic effects on the transcription of target genes. Based on the known functions of their target genes in breast cancer, ERa and RARs appear to be 'yin and yang' for the genetic regulation of proliferation and survival that are promoted by ERa and inhibited by RARs.

Comprehensive Nuclear Receptors profiling in Breast Cancer Cells

Kittler et al, Cell 2013 - Performed ChIP-chip profiling of various factors in MCF-7 cells (Breast Cancer Cells)

- 24 Nuclear receptors
- 6 chromatin states
- 14 breast-cancer-associated transcription factors

This dataset is already uploaded in Genboree for you.

Using SPARK tool in Genboree, we would like to demonstrate, how such dataset can be used to determine genes that are regulated by ERa and RARs antagonistic interaction.

SPARK analysis determines antagonistic regions shared by ERa and RARs

ERa binding sites



Genes regulated by antagonistic interaction between ERa and RARs are involved in pathways associated with Breast Cancer progression

GREAT analysis is used to associate cis-regulatory elements to gene sets Following are Pathway Commons GO terms enriched by these gene sets

	Pathway Commons GO terms			-log10(Binomial p value)					
	0	5		10	15	20	25		
	AP-1 transcription factor network							29.44	
	Integrin-linked kinase signaling							28.91	
	CDC42 signaling events							5.33	
	Regulation of CDC42 activity							5.24	
	Validated nuclear estrogen receptor alpha network					_	24.50		
C-MYB transcription factor network					15.5				
ALK1 pathway					14.96				
Syndecan-4-mediated signaling events					14.77				
Regulation of nuclear beta catenin signaling and target gene transcription					14.67				
	Canonical Wnt signaling pathway				14.20				
	Regulation of nuclear SMAD2/3 signaling				13.78				
	Regulation of cytoplasmic and nuclear SMAD2/3 signaling				13.78				
	TGF-beta receptor signaling				13.78				
	ALK1 signaling events				13.59				
	Noncanonical Wnt signaling pathway				.2.89 2.52				
	Validated targets of C-MYC transcriptional repression E-cadherin signaling events				.04				
	E-cadherin signaling in the nascent adherens junction				.04 .92				
Stabi	lization and expansion of the E-cadherin adherens junction				.92				
Wnt signaling network				11.				6	
	Whit signaling network			1 1.					

Genes associated by antagonistic interaction are regulated by ERa and required for breast cancer progression

MSigDB Perturbation

-log10(Binomial p value)

Genes down-regulated in TMX2-28 cells (breast cancer) which do not express ESR1 [Gene ID=2099]) compared to the parental MCF7 cells which do.	57.17
Genes down-regulated in basal subtype of breast cancer samles.	44.46
he 'group 1 set' of genes associated with acquired endocrine therapy resistance in breast tumors expressing ESR1 and ERBB2 [Gene ID=2099, 2064].	44.30
Genes up-regulated in luminal-like breast cancer cell lines compared to the mesenchymal-like ones.	38.99
group 4 set' of genes associated with acquired endocrine therapy resistance in breast tumors expressing ESR1 but not ERBB2 [Gene ID=2099, 2064].	35.40
Genes down-regulated in breast cancer tumors (formed by MCF-7 xenografts) resistant to tamoxifen [PubChem=5376].	31.98
Genes up-regulated in luminal-like breast cancer cell lines compared to the basal-like ones.	31.83
Genes changed in breast cancer samples according to the ESR1 [Gene ID=2099] status: ER positive vs ER negative tumors.	27.53
Genes within amplicon 17q21-q25 identified in a copy number alterations study of 191 breast tumor samples.	26.31
gulated genes from the optimal set of 550 markers discriminating breast cancer samples by ESR1 [Gene ID=2099] expression: ER(+) vs ER(-) tumors.	25.66

Antagonistic regions between ERa and RARs do regulate genes and pathways that are involved in breast cancer progression



Use case instructions for this is similar to use case example of RAD21 in ESCs. Datasets required for the analysis are located as follows-



Nuclear Receptor binding site score data are located in Epigenome ToolSet Demo Input Data > Databases > Binding Sites Demo > Tracks > Class: Nuclear Receptor Binding Site Data

Nuclear Receptor binding site/region of interests (ROIs) are located in ROI Repository > Databases > ROI Repository - hg19 > Tracks > **Class:NR BindingSites** 9