*Ontoscope:* Determining “identity-defining” transcription factors for various cell types

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**1 Introduction**

This document offers an overview of the R package *Ontoscope*, which uses publicly available expression and protein-protein interaction data to computationally determine a list of transcription factors able to facilitate conversion from one cell type to another. Following determination of this transcription factor list, it is also designed to assign confidence scores to each factor based on literature search and validation against published experimental data and/or other computational prediction software.

The *Ontoscope* package is designed to independently validate the findings of Owen Rackham and colleagues’ *Mogrify* ([www.mogrify.net](http://www.mogrify.net))1. Its workflow is based on the published *Mogrify* protocol, with modifications including the usage of updated transcription factor definitions and the inclusion of different regulatory networks (specifically, the TRRUST2 and Regnet3 protein-protein interaction datasets).

This vignette contains an overview of the package workflow, defining initialization parameters and walking through each submodule with examples and usage instructions provided.

**2 Processing overview**

*Ontoscope* determines transcription factors required for conversion based on calculation of network- and expression-based influence scores for differentially expressed factors in the target cell line as compared to the source. Target and source cells are identified by FANTOM consortium cell line IDs, and are restricted to those cell types for which FANTOM gene expression data from CAGE-seq is available.

Calculation involves six primary phases:

1. *Import of expression data:* The first step is to define source and target cell lines, reading in raw FANTOM expression count data for each cell line. Transcription factors are defined from published literature and databases, and all gene names are normalized to HGNC IDs in order to ensure accuracy of comparison between cell lines and datasets. Download of FANTOM count data is automatic upon input of cell line IDs, and does not require further input from the user.
2. *Calculation of background:* Something
3. *Differential expression-based influence score assignment:* Something
4. *Interaction-based influence score assignment:* Something
5. *Integration and binning:* Something
6. *Visualization and validation:* The TFs required for cell conversion can be visualized using a heat map. Further, a literature based confidence score can be used to validate the TFs required for any cell conversion.

**3 Submodules and workflow**

This section outlines examples and functions underlying the usage of *Ontoscope* to generate lists of transcription factors for conversion.

Users should begin by defining cells of origin and desired target cell type as follows:

> sourcecell <- “eye”

> target <- “fibroblast”

Here, a retinal to fibroblast cell conversion has been used as an example. It is important to define both keywords and FANTOM IDs for desired cell types, as while FANTOM IDs will be used for the actual Ontoscope workflow, keyword definitions are important for visualization and literature validation.

Next, users should select their desired FANTOM IDs for source and target cells by sourcing and running the **fantom\_import** submodule for both source and target cell types. Output for the source cell search is as follows:

> source(“./fantom\_import/fantom\_main.R”)

> fantomSearch(sourcecell)

V1 FANTOM.5.Ontology.ID FANTOM.5.Access.Number

1375 eye - muscle inferior rectus, donor1 FF:10272-104E2 1381

1376 eye - muscle lateral, donor2 FF:10298-104H1 1382

1377 eye - muscle medial, donor2 FF:10299-104H2 1383

1378 eye - muscle superior, donor2 FF:10297-104G9 1384

1379 eye - vitreous humor, donor1 FF:10268-104D7 1385

1380 eye, fetal, donor1 FF:10054-101G9 1386

From the list, the desired specific subtype (ie: lateral eye muscle, vitreous humor, fetal eye) of cell can be selected, and the FANTOM Ontology ID (FF:ID) noted. Here, we have chosen to convert inferior rectus eye muscle to cardiac fibroblast. FF:IDs may then be defined as follows:

> sourceFF <- “FF:10272-104E2”

> targetFF <- “FF:11268-116G8”

Following this, simply running runOntoscope.R will process the entire workflow for you, outputting a list of top transcription factors required for conversion with confidence rankings based on literature as well as heatmap and interaction network visualizations. The runOntoscope.R package also contains built-in functionality to cross-validate the transcription factor lists generated by Ontoscope against published conversion lists from either literature or the MOGRIFY package.

**3.1 Normalization**

Prior to beginning the workflow proper, it is important to first normalize all gene identifiers in order to allow comparison between gene lists and data from different sources. Ontoscope takes as input four main sources of data: gene expression data from FANTOM5, and protein-protein interaction and regulatory network data from STRING4, TRRUST, and REGNET. While FANTOM5 and TRRUST datasets contain HGNC identifiers for genes, STRING primarily identifies genes based on Ensembl IDs, and must be normalized to HGNC symbols for comparison purposes. Furthermore, while REGNET uses HGNC identifiers for its gene interactions, these IDs are four years old at the time of publication of this vignette, and may be outdated.

In order to normalize STRING interactions, the **normalizeWeave.R** submodule is sourced, using the R Bioconductor package biomaRt to create a new datafile from the base STRING data table with approximately 8.5 million protein-protein interactions. In order to avoid extremely lengthy processing times, STRING Ensembl protein IDs are placed in a new data frame as row names, following which a new vector of HGNC symbols is created with biomaRt. The updated file, **curatedOutput.Rdata**, is then available for usage in downstream applications.

Should Regnet files require updated HGNC symbol assignments, Entrez IDs can be retrieved from Regnet files and transcribed into HGNC symbols as well by altering the following lines in **normalizeRegnet.R** :

> IDmap <- data.frame(entrezgene=IDmap, HGNC=””, stringsAsFactors=FALSE)

> BMmap <- getBM(filters = “entrezgene”,

attributes = c(“entrezgene”, output),

values = IDmap$entrezgene,

mart = ensembl)

> colnames(BMmap) <- c(“entrezgene”, output)

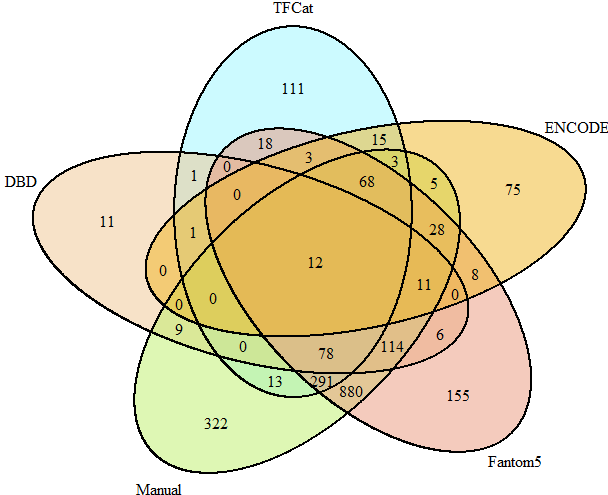
All input datafiles will now have been normalized to identify genes by HGNC symbols.

Finally, transcription factors must be defined prior to performing analysis. Sourcing the **normalizeTF.R** subscript will automatically compile a list of transcription factors common among at least two out of the five following datasets:

1. The TFCat database
2. The DBD (DNA-binding domain) database
3. A manually assembled list of transcription factors with annotations5
4. The FANTOM5 transcription factor dataset
5. ENCODE transcription factor data derived from their ChIP-seq antibody list

The choice of how many lists to use as a cutoff was based on variations in overlap between the lists **(Figure 1)**, and may be easily modified through subsetting of the MergedList table generated by this submodule, which lists all transcription factors and whether or not they are present in each dataset, via modification of the following line:

> TFList <- unique(MergedList[!MergedList$Count==”1”,])$Gene.Symbol



**Figure 1: Varying degrees of overlap between human transcription factor datasets.** Venn diagram depicting large overlap between the manually curated and Fantom5 lists, while other datasets possess less unique and overlapping transcription factors. Generated by the VennDiagrams package: draw.quintuple.venn()

Note that all transcription factors have been normalized to HGNC symbols where appropriate. The normalize submodule allows for easy replacement or update of lists through the replacement of their .csv datafiles with updated data.

**3.2 FANTOM expression data import**

Expression profiles for genes across available cell lines have been derived from cap analysis of gene expression (CAGE) sequencing by the FANTOM5 consortium. The FANTOM import submodule allows for the import and processing of raw read counts from this sequencing data, accepting several different possible search terms as inputs and allowing for import of normalized read counts as an alternative. For the purposes of the Ontoscope workflow, the **fantom\_main.R** submodule has already been sourced. Furthermore, as we perform our own normalization and background derivations further downstream, we must focus on importing raw read counts rather than normalized expression data.

The **runOntoscope.R** script first searches the FANTOM5 database for matching FF IDs and downloads the raw expression counts for each as follows:

> FFVect <- c(sourceFF, targetFF)

> fantomOntology(FFVect)

[1] "Sample\_DB Loaded!"

Returning RAW COUNTS

MATCHED: 2 of 2

2 Search Result(s) Were Found. Loading...

Loading Results from Fantom Access Number 1381 ( 1 / 2 ) ...

Results from Fantom Access Number 1381 Loaded!

Loading Results from Fantom Access Number 377 ( 2 / 2 ) ...

Results from Fantom Access Number 377 Loaded!

All results have been loaded into fantomResults

fantomResults is a large, subsettable list of dataframes containing genetic annotations, peak numbers, gene names, and HGNC, Uniprot, and Entrez Gene IDs for every sample, with one dataframe per sample. These results can be summarized in one dataframe, with normalized HGNC symbol gene names and raw expression counts for each gene, through the fantomSummarize command:

> fantomSummarize(5)

Preparing the Genes

Summarizing:eye - muscle inferior rectus, donor1.CNhs13444.10272-104E2

Summarizing:Fibroblast - Cardiac, donor1.CNhs12498.11268-116G8

Filtering Relevant Results. This step takes awhile ...

Preparing Normalized Gene Names ...

All Genes Normalized!

Fixing Duplicates ...

Applying Threshold ...

Your results have been summarized in: fantomCounts!

The bracketed number indicates the minimum threshold for read counts in each gene. Genes with read counts less than the threshold number (here, 5) will not be included in the summarized fantomCounts

file, which is now ready for downstream processing.

**3.3 Background derivation from cell ontology**

**3.4 Protein and transcription factor network analysis**

3.4.1 STRING

3.4.2 TRRUST

3.4.3 Regnet

**3.5 Differential gene expression analysis**

**3.6 Integration of network- and expression-based influence scores**

**3.7 Selection of transcription factors needed for conversion**

**4 Post-processing and analysis**

**4.1 Visualization**

To visualize the TF expression in the source, target and background cells, heat maps can be used. This visualization is based on the TRRUST network. The TRRUST network can aid one to visualize which genes a particular TF affects and/or which TFs a particular set of genes interact with.

The **fantomCounts** file with counts for the source, target and background cells is required to run this module. After importing and processing the **fantomTFs** files for heat map generation, a **ConversionList** of TFs is generated.

Next the heat map is generated and exported as follows:

> png(filename=”Transcription Factor Expression Across Human Cell Lines.png”, width = 1000, height = 1000, res = 300)

heatmap(as.matrix(fantomTFs[ConversionList,]),margins=c(7,5))

dev.off()

Here, a heat map is generated for each TF of interest.

**4.2 Literature-based confidence score**

This module provides literature evidence for the transcriptions factors (TFs) required for conversion between a *source* and a *target* cell. The script performs a collection of abstracts for the source and the target cell hits. For this script to run, the user must have generated a list of candidate TFs and defined the source and target cells.

> TFList <- c(“TP53”, TP63”, “TP73”, “MYC”)

sourcecell <- “brain”

target <- “fibroblast”

Each TF is ranked based on key parameters that evaluate the abstracts that mention that TF. If there are no abstracts for a given TF, then it is assigned the value “not applicable” and given the lowest rank. For the TFs with available abstracts, they are assessed based on the following parameters: (i) number of hits for the TF and the cell origin (i.e., brain), (ii) ratio of target cell hits to source cell hits, (iii) the total number of available abstracts for each TF and (iv) the percentage of abstracts mentioning the target cell out of the total number of abstracts for that TF.

Of these parameters, the ratio of the target to source cell hits determines the association of the TF with the target cell in comparison to the source cell. This ratio is used to rank the TFs with a higher ratio corresponding to a higher rank. If two TFs have the same rank, then the total number of abstracts available for that TF is assessed with more abstracts conferring a higher rank for that TF.

To appropriately *weight* these ratios, a confidence score is assigned. This score is assigned as follows:

* A TF with less than 44 abstract hits is assigned an *NA*, since the bottom 20 % of TFs have less than 44 hits.
* A TF with a target to source cell hit less than 1 is assigned a confidence score of 0, since there is no literature evidence showing that this TF is needed for a conversion.
* The remaining TFs are assigned a confidence score based on the percentage of hits for the TF divided by the percentage of hits for the TF “AR” (androgen receptor), which has the highest number of hits. The upper cut off is set at 12.1 % since 4390 of the total number of abstracts for AR (36 191) mention the associated cell type, “prostate”.
* A TF with a confidence score greater than 1 is set to 1.

A sample output table for the TFs listed above is as follows:

|  |  |  |
| --- | --- | --- |
| **TFList** | **RatioVals** | **Confidence Score** |
| TP63 | 4.1 | 0.25 |
| MYC | 2.7 | 0.63 |
| TP53 | 0.9 | 0.00 |
| TP73 | 0.2 | 0.00 |

Thus, based on these results, TP63 and MYC both have a high target to source cell hits ratio but because there are more abstract hits for MYC, there is more confidence in MYC involved in this cell conversion than TP63.

**4.3 Validation from published experimental data**

**5 Acknowledgments**

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**6 Session Info**

> sessionInfo(package=NULL)

(The output of the above command needs to be filled in by Dr. Steipe after integrating all the code and running it).

**7 References**

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