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

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Compiled: April 27, 2016

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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:* Something

**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)

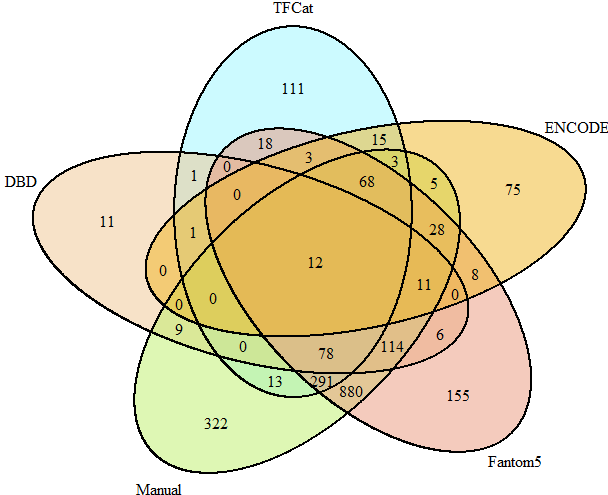
All input datafiles will now have been normalized to identify genes by HGNC symbols.

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

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**3.5 Differential gene expression analysis**

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**4 Post-processing and analysis**

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**4.3 Validation from published experimental data**

**5 Acknowledgments**

This package was developed as part of the BCB420/JTB2020 bioinformatics class as part of the Department of Biochemistry at the University of Toronto, Canada, with the support of Dr. Boris Steipe. We wish to acknowledge all co-authors of *Ontoscope* package modules, who have equally contributed to the development of this project: Julian Mazzitelli, Dmitry Horodetsky, Christopher Li, Kartikay Chadha, Fupan Yao, Shivani Kamdar, Dan Litovitz, Howard Wu, Anam Qudrat, Jialun Tom Chen, Ryoga Li, Eugenia Barkova, Burton Mendonca, Phil Fradkin, and Boris Steipe.

**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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