

Gene Annotation

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FinalGeneSetAnnotation.R

This script has the following functions:

0. `annotate.final.geneset.round1`

Input: `integrated_tse_ara.txt`

It reads the main integrated gene file from `integrated_tse_ara.txt`

Keeps genes with either their ara score is > 106 or their tse score is > 49

Column **genefun** is added to the genefile table and filled in the following order:

1. genes with same tse and ara identity are assign the same identity
2. genes with different identity, pseudo|truncated genes, genes with un assigned identity and genes with letter N in their sequence are shown as #

Column **note** is added and fill it in the following order:

1. genes with the same ara and tse marked as "T"
2. genes with unassigned identity by both tse and ara are set as "UnAssigned"
3. genes with letter N in their sequence are set as "ContainsN"
4. genes with unmatched identity between ara and tse are set as "Undet"

these top 4 cases had no overlap!

I kept track of the remained number of genes at each step and wrote it as comments.

1. `genome.nuc.composition`:

Input: `GenomeNucComposition.txt` and **output of function** `annotate.final.geneset.round1`

The complete script for calculating the nucleotide composition is in `FinalGeneSetAnnotation.R`. Reading 46 genomes and calculating the compositions is a bit time consuming, so I have already done that part in function `genome.nuc.composition()` in the main R script and saved the table as `GenomeNucComposition.txt`. Here, in function `genome.nuc.composition2()` I only read file `GenomeNucComposition.txt`, and marge it with a new column called `genecounts` which I calculate from the output of function `annotate.final.geneset.round1()`. In the end the file is written in latex format.

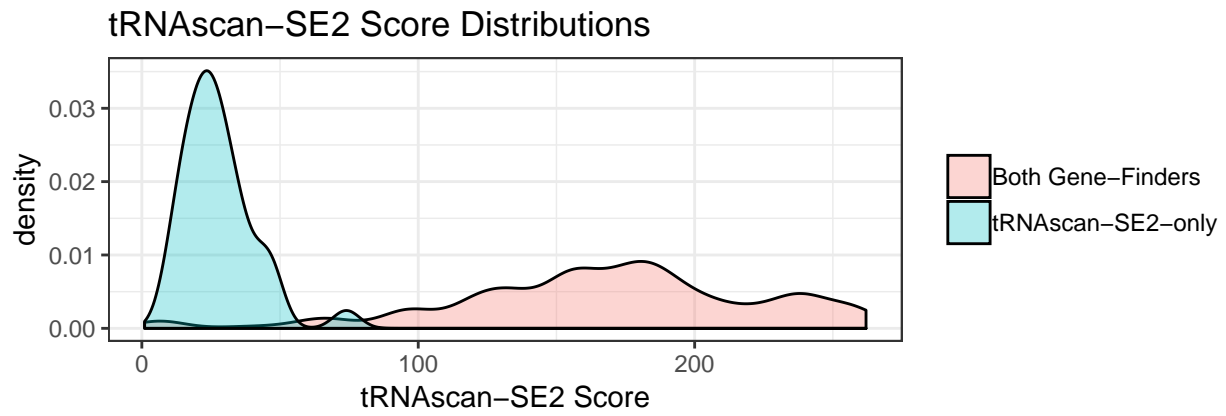
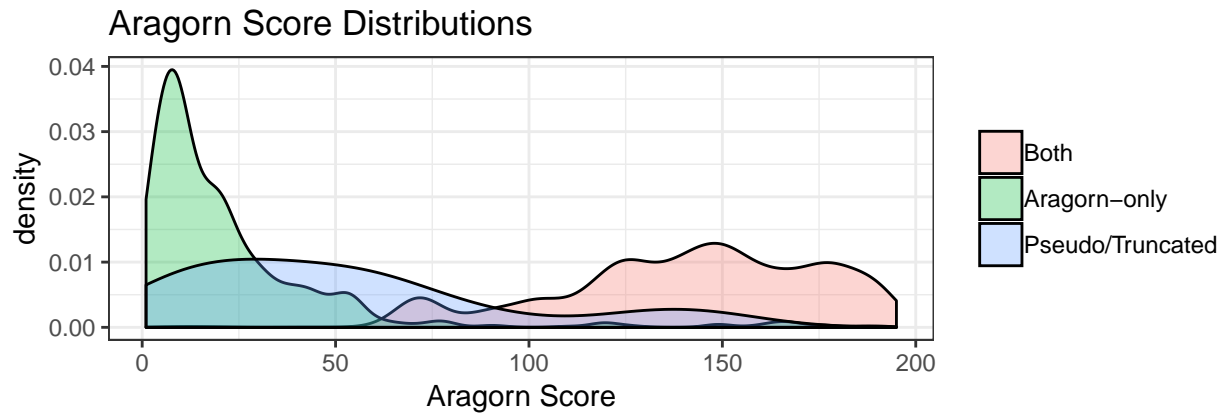
| ## | organism_shortname | Aperc | Tperc | Cperc | Gperc | GCp | genecounts |
|----|----------------------------------|-------|-------|-------|-------|-----|------------|
| ## | TrangeliSC58 | 24 | 23 | 27 | 26 | 53 | 6 |
| ## | TcruziCLBrener | 23 | 23 | 27 | 27 | 53 | 18 |
| ## | TcruziDm28c | 25 | 25 | 26 | 25 | 50 | 51 |
| ## | TcruzimarinkelleiB7 | 22 | 22 | 23 | 23 | 45 | 57 |
| ## | TcruziCLBrenerEsmeraldo-like | 20 | 20 | 20 | 20 | 40 | 57 |
| ## | TcruziCLBrenerNon-Esmeraldo-like | 21 | 21 | 22 | 22 | 43 | 57 |
| ## | PconfusumCUL13 | 18 | 18 | 28 | 28 | 57 | 61 |
| ## | TbruceigambienseDAL972 | 26 | 26 | 24 | 24 | 47 | 64 |
| ## | LamazonensisMHOMBR71973M2269 | 20 | 20 | 30 | 30 | 59 | 66 |
| ## | TevansiSTIB805 | 27 | 27 | 23 | 23 | 47 | 67 |

| | | | | | | | |
|----|----------------------------|----|----|----|----|----|-----|
| ## | TbruceiLister427 | 26 | 26 | 22 | 23 | 45 | 67 |
| ## | TcruziSylvioX10-1 | 24 | 23 | 25 | 25 | 50 | 69 |
| ## | BayalaiB08-376 | 22 | 22 | 27 | 27 | 55 | 69 |
| ## | TcruziSylvioX10-1-2012 | 24 | 24 | 26 | 26 | 51 | 72 |
| ## | TcongolenseIL3000 | 21 | 21 | 20 | 20 | 40 | 72 |
| ## | TbruceiTREU927 | 27 | 27 | 23 | 23 | 45 | 73 |
| ## | TcruziJRcl4 | 24 | 23 | 26 | 24 | 50 | 74 |
| ## | TcruziEsmeraldo | 23 | 22 | 24 | 23 | 47 | 74 |
| ## | LpanamensisMHOMPA94PSC1 | 21 | 21 | 28 | 28 | 56 | 74 |
| ## | LtarentolaeParrotTarII | 21 | 21 | 27 | 27 | 55 | 79 |
| ## | LspMARLEM2494 | 20 | 20 | 30 | 29 | 59 | 80 |
| ## | LgerbilliLEM452 | 20 | 20 | 30 | 29 | 59 | 81 |
| ## | LmajorSD75.1 | 20 | 20 | 30 | 30 | 59 | 82 |
| ## | LenriettiiLEM3045 | 20 | 20 | 29 | 29 | 59 | 82 |
| ## | TvivaxY486 | 21 | 21 | 23 | 23 | 46 | 82 |
| ## | LbraziliensisMHOMBR75M2904 | 21 | 21 | 29 | 29 | 58 | 83 |
| ## | LaethiopicaL147 | 19 | 20 | 30 | 29 | 59 | 83 |
| ## | LdonovaniBHU1220 | 19 | 20 | 29 | 29 | 57 | 84 |
| ## | LinfantumJPCM5 | 20 | 20 | 30 | 30 | 60 | 84 |
| ## | LmajorFriedlin | 20 | 20 | 30 | 30 | 60 | 84 |
| ## | LmexicanaMHOMGT2001U1103 | 20 | 20 | 30 | 30 | 60 | 84 |
| ## | LmajorLV39c5 | 20 | 20 | 29 | 29 | 59 | 84 |
| ## | LarabicaLEM1108 | 20 | 20 | 29 | 29 | 58 | 85 |
| ## | LdonovaniBPK282A1 | 19 | 20 | 29 | 29 | 57 | 85 |
| ## | LturanicaLEM423 | 19 | 20 | 30 | 29 | 59 | 86 |
| ## | LbraziliensisMHOMBR75M2903 | 19 | 20 | 27 | 27 | 53 | 86 |
| ## | LtropicalL590 | 19 | 19 | 29 | 28 | 57 | 87 |
| ## | LpanamensisMHOMCOL81L13 | 21 | 21 | 29 | 28 | 57 | 88 |
| ## | LseymouriATCC30220 | 22 | 22 | 28 | 28 | 55 | 94 |
| ## | TgrayiANR4 | 23 | 23 | 27 | 27 | 54 | 95 |
| ## | TcruzicruziDm28c | 24 | 24 | 26 | 26 | 52 | 97 |
| ## | EmonterogeiiLV88 | 23 | 23 | 26 | 26 | 52 | 104 |
| ## | LpyrrhocorisH10 | 21 | 22 | 28 | 28 | 56 | 104 |
| ## | CfasciculataCfC1 | 21 | 22 | 29 | 28 | 57 | 105 |
| ## | TcruziTulac12 | 22 | 21 | 23 | 23 | 46 | 121 |
| ## | TtheileriEdinburgh | 26 | 26 | 17 | 17 | 35 | 159 |

2. Score.visualization

Input: Input: integrated_tse_ara.txt

This functions read the main integrated gene file integrated_tse_ara.txt and shows the distribution of gene scores



3. create.summary.table

Input: output of function `annotate.final.geneset.round1()`

Read the annotated gene file (output of function `annotate.final.geneset.round1()`) and Creates a summary table of genes after the score filtering.

Last three columns are each for one gene set. Sets are defined as: a) Intersection Of two gene finders. Two genes are considered same gene if their coordinate overlaps at least one base. Displacement of overlapped genes between ARA and TSE does not pass 4bp. b) Union of two gene finders. c) Genes found by only ARA. Genes marked as # include: pseudo|truncated genes (6 genes), genes with different predicted identity by two genefinders(23 genes), genes with unassigned identity|anticodon by any of genefinders (2 genes), and genes with letter N in their sequence(we had 4 of these genes). we also had 1 genes preicted by only TSE labeled as # which is not shown in this table as a sepearte column, however it is considered in the union set.

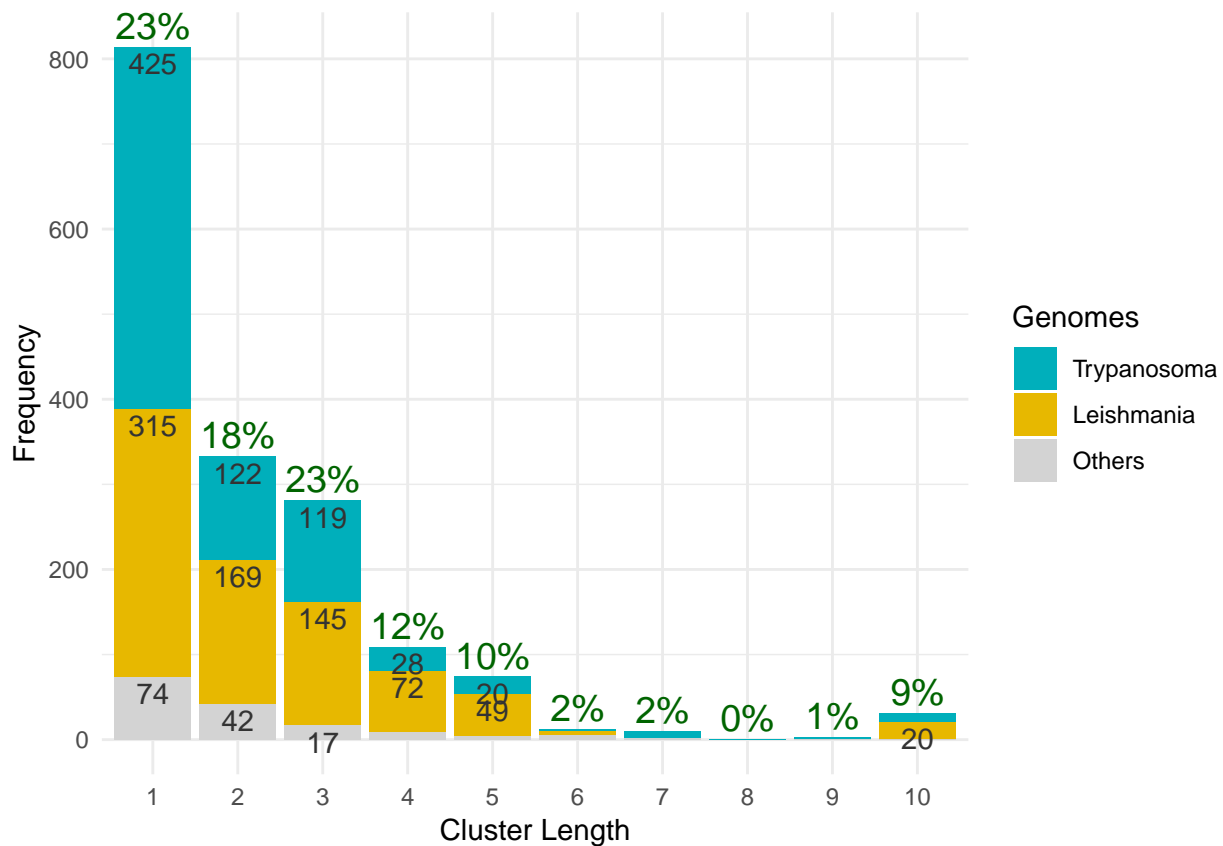
| ## | Annotation | Intersection | ARAonly | Union |
|----|-----------------|--------------|---------|-------|
| ## | #tRNA | 3579 | 36 | 3616 |
| ## | #N/#G | 74 | 98 | 75 |
| ## | Min Gene Length | 68 | 71 | 68 |
| ## | Max Gene Length | 89 | 206 | 206 |
| ## | %intron | 2 | 28 | 3 |
| ## | %G | 32 | 33 | 32 |
| ## | %C | 26 | 26 | 26 |
| ## | %T | 23 | 23 | 23 |
| ## | %A | 19 | 18 | 19 |
| ## | A | 210 | 2 | 212 |
| ## | C | 64 | 1 | 65 |
| ## | D | 105 | 1 | 106 |
| ## | E | 160 | 1 | 161 |
| ## | F | 104 | 2 | 106 |
| ## | G | 228 | 3 | 231 |
| ## | H | 80 | 4 | 84 |
| ## | I | 171 | 1 | 172 |
| ## | K | 183 | 1 | 184 |
| ## | L | 335 | 6 | 341 |
| ## | M | 97 | 0 | 97 |
| ## | N | 125 | 0 | 125 |
| ## | P | 200 | 0 | 200 |
| ## | Q | 161 | 0 | 161 |
| ## | R | 348 | 2 | 350 |
| ## | S | 228 | 7 | 235 |
| ## | T | 218 | 4 | 222 |
| ## | V | 236 | 0 | 236 |
| ## | W | 52 | 1 | 53 |
| ## | X | 76 | 0 | 76 |
| ## | Y | 88 | 0 | 88 |
| ## | Z | 76 | 0 | 76 |
| ## | # | 34 | 0 | 35 |

4. clustersize.dist.visualize

Input: output of function `annotate.final.geneset.round1()`

Read the annotated gene file (output of function `annotate.final.geneset.round1()`) and visualizes the Cluster size distribution for three categories of TryTryp genomes. Labels in green on top of each bar show the percentage of total number of genes as cluster of a specific length. Each color refers to one category of TriTryp genomes. Numbers within each color section of the bar shows the counts of clusters with a specific length.

```
## Scale for 'fill' is already present. Adding another scale for 'fill',  
## which will replace the existing scale.
```



5. prepare.tsfm.input

Input: output of function `annotate.final.geneset.round1()`

Output: `tsfm_input_geneset.txt`

Read the annotated gene file, removes **genes with ambiguity** marked as # (35 genes), genes with function **Secs** (76 genes) and genes from two genomes genes of genomes **TrangeliSC58** and **TcruziCLBrenner** and writes the selected genes in file `tsfm_input_geneset.txt` (17 genes). `tsfm_input_geneset.txt` file should be used for further alignment for the purpose of creating CIFs.

We have **3478** genes left in this file to be aligned

The gene set `tsfm_input_geneset.txt` is passed to the script `TriTrypAlignment.R` to be aligned with Human tRNA genes.

TriTrypAlignment.R

Alignment Steps:

1. Genes from `tsfm_input_geneset.txt` are read and functional classes are added at the end of the geneID
2. Variable arms are removed based on reported secondary structure from genefinders
3. Gene introns are removed based in the secondary sctructure
4. the result is merged with the Human tRNA genes (the headers for Homo genes are also updated) and the result is saved in file `coveainput.fasta`.
5. `coveainput.fasta` is aligned to the Eukaryote model using `covea`
6. the result (`Aligned_TriTryp_Homo.covea`) is edited based on the fallowing criteria in order:
 - a. sites that have more than 98
 - b. sequences with more than 3 gaps are removed
 - c. sequences with two or more gaps next to eachother are removed
7. the alignment result is saved as fasta file in `Aligned_TriTryp_Homo.fasta`, with secondary structure saved as `Aligned_TriTryp_Homo_structfile.txt`

SplitAlignedGenes.R

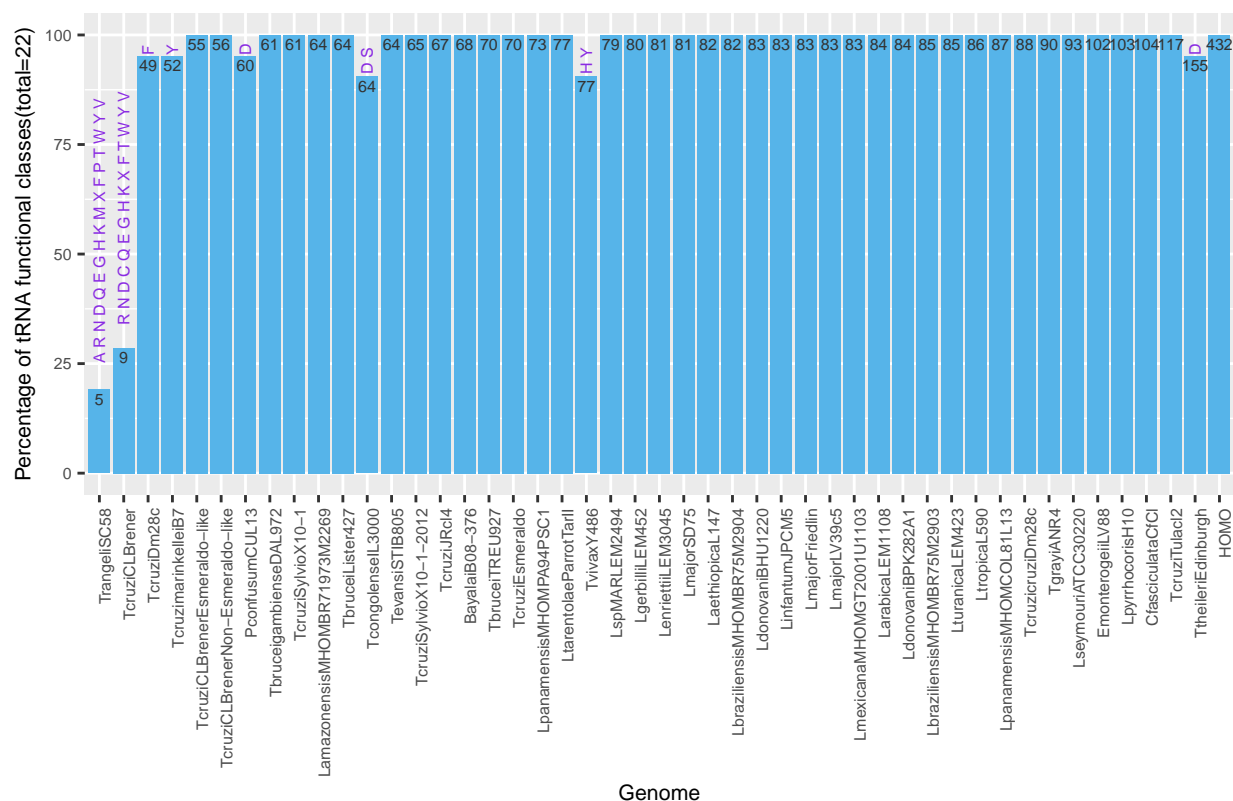
The alignment Result `Aligned_TriTryp_Homo.fasta` will be passed to this script to be splitted either by genome, or clusters of genomes.

The result fasta file for each genome is saved as a file in `tsfm/input` folder.

The missing functional class for each genome or cluster of genomes is visualized as a bar plot.

(*****This last plot is not updated yet!*****)

Percentage of 22 tRNA functional classes covered by each cluster



Fasta gene files will be splitted based on tRNA functional class by running script splitFuncClass.sh.