Gene Annotation

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FinalGeneSetAnnotation.R

This script has the fallowing functions:

0. annotate.final.geneset.round1
Input: **tsfm_input_geneset.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 fallowing order:

1. genes with same tse and ara identity are assign the same idenitity
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 fallowing 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 caclulating 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.

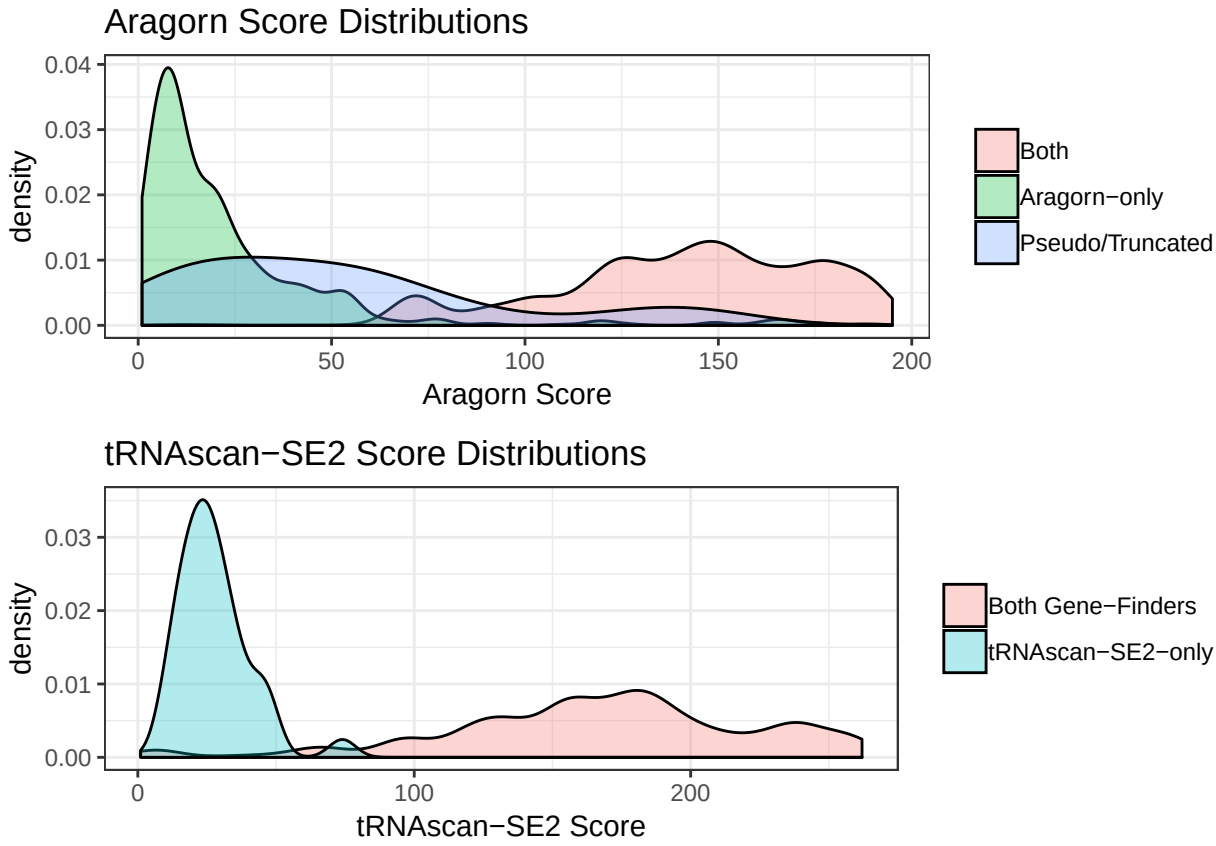
```
##                organism_shortname Aperc Tperc Cperc Gperc GCp genecounts
##                     TrangeliSC58    24    23    27    26  53          6
##                   TcruziCLBrener    23    23    27    27  53         18
##                      TcruziDm28c    25    25    26    25  50         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
##                    LtropicaL590      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
##                  CfasciculataCfCl     21    22    29    28  57        105
##                     TcruziTulacl2     22    21    23    23  46        121
##                  TtheileriEdinburgh     26    26    17    17  35        159
```

2. Score.visualization

Input: **integrated_tse_ara.txt**

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


Aragorn Score Distributions
density
0.04
0.03
0.02
0.01
0.00
0
50
100
150
200
Aragorn Score
Both
Aragorn−only
Pseudo/Truncated
tRNAscan−SE2 Score Distributions
density
0.03
0.02
0.01
0.00
0
100
200
tRNAscan−SE2 Score
Both Gene−Finders
tRNAscan−SE2−only

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 seperate column, however it is considered in the union set.

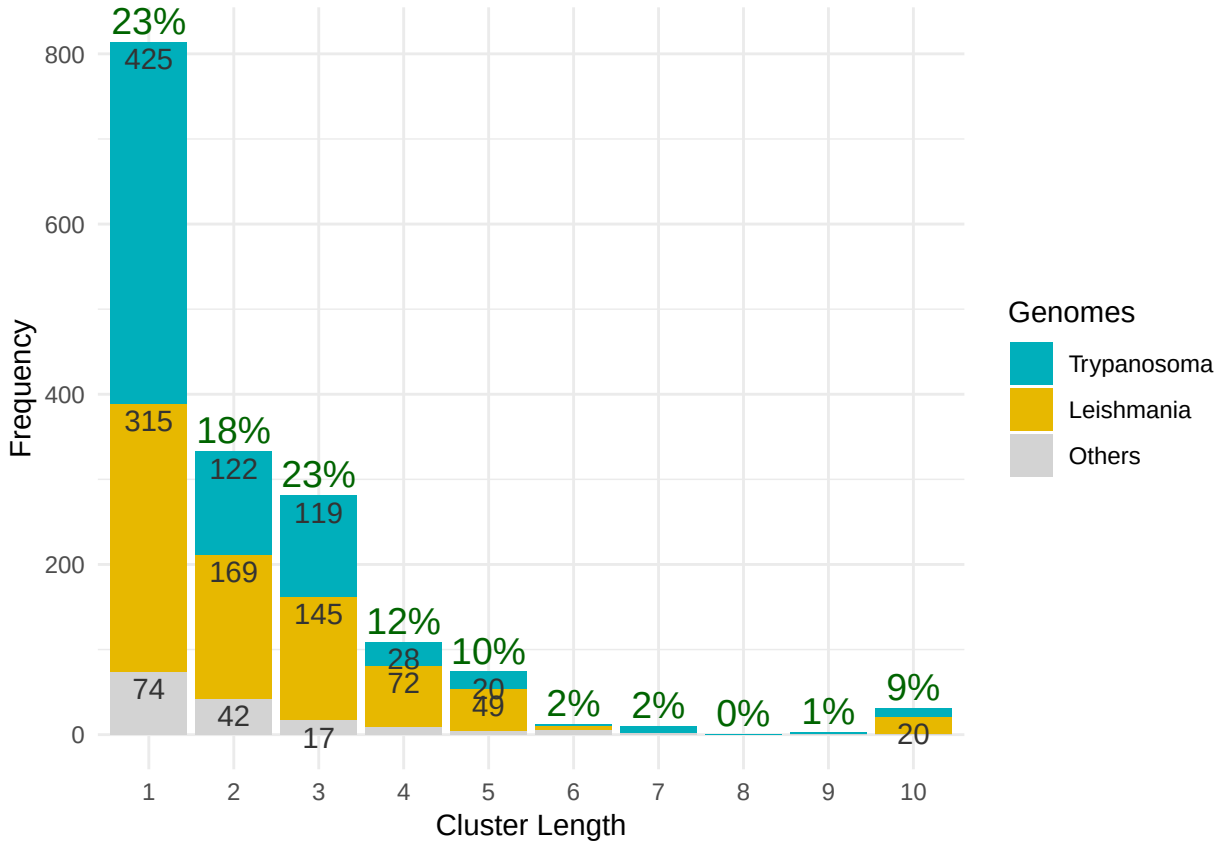
```
##       Annotation Intersection ARAonly Union
##            #tRNA         3579      36  3616
##            #N/#G           74      98    75
##  Min Gene Length           68      71    68
##  Max Gene Length           89     206   206
##          %intron            2      28     3
##               %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.
```



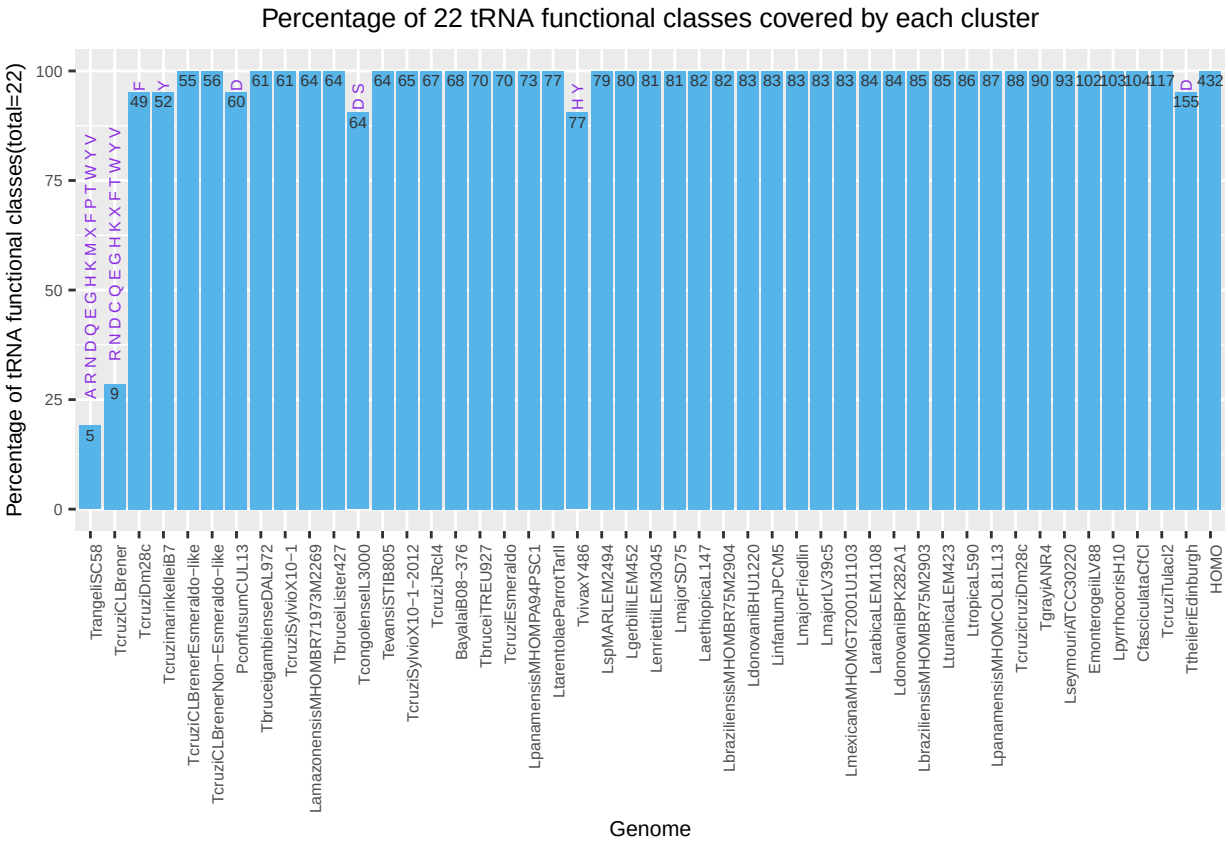
5. prepare.tsfm.input
Input: output of function annotate.final.geneset.round1()
Output: **tsfm_input_geneset.txt**
Read the annotated gene file, removes **genes with ambiguty** marked as # (35 genes), genes with function **Sec**s (76 genes) and genes from two genomes genes of genomes **TrangeliSC58** and **TcruziCLBrener** 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!**********)



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