Gene Annotation

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FinalGeneSetAnnotation.R

This script has the fallowing 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 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**
output: output is saved in latex format in this file **GenomeNucComposition__Latex.txt**

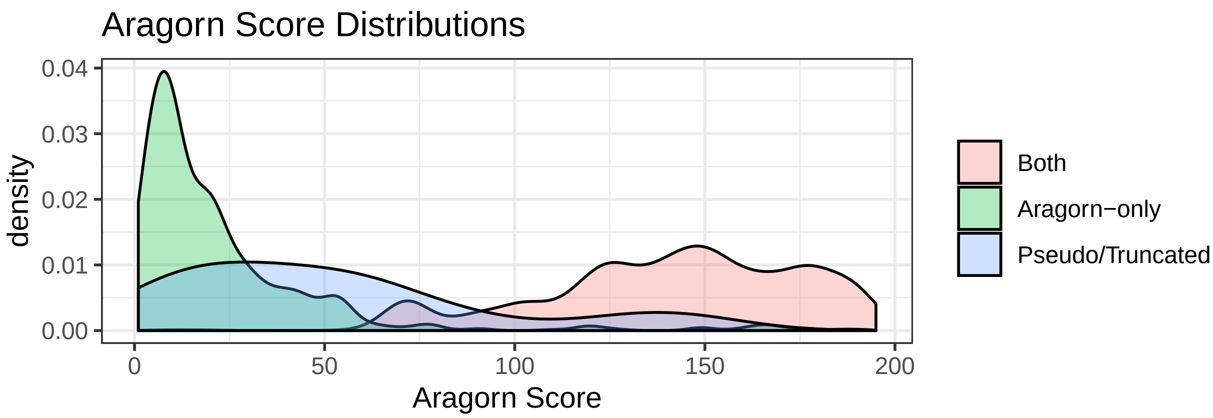
The complete script for calculating the nucleotide composition is in FinalGeneSetAnnotation.R. Reading 46 genomes and caclulating the compositions is a bit time consuming, so I have already done that part in function genome.nuc.composition() in the main R script and saved the table as GenomeNucComposition.txt. 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.

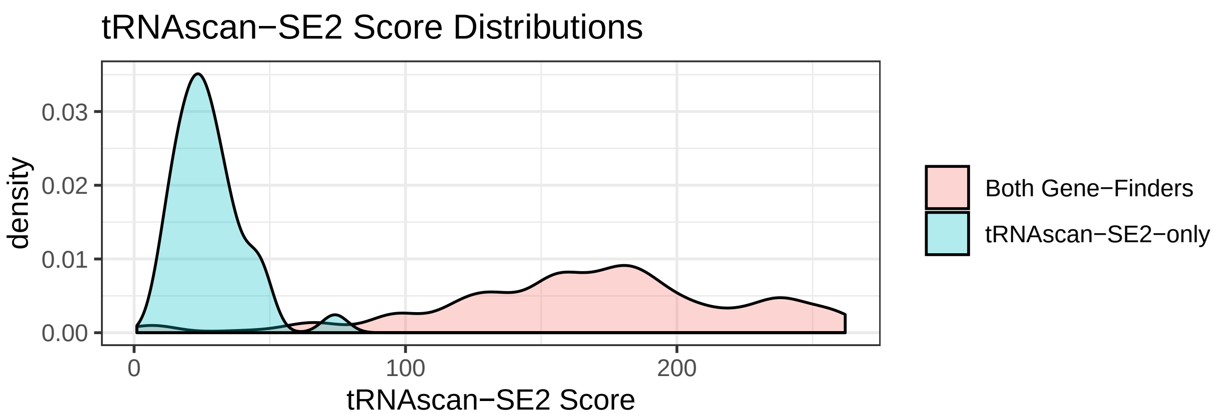
```
##                organism_shortname Aperc Tperc Cperc Gperc GCp genecounts
##                       TrangeliSC58    24    23    27    26  53          6
##                     TcruziCLBrener    23    23    27    27  53         18
##                        TcruziDm28c    25    25    26    25  50         51
##      TcruziCLBrenerEsmeraldo-like    20    20    20    20  40         57
##  TcruziCLBrenerNon-Esmeraldo-like    21    21    22    22  43         57
##                TcruzimarinkelleiB7    22    22    23    23  45         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
##              TcongolenseIL3000   21   21   20   20  40          72
##         TcruziSylvioX10-1-2012   24   24   26   26  51          72
##                 TbruceiTREU927   27   27   23   23  45          73
##          LpanamensisMHOMPA94PSC1 21   21   28   28  56          74
##                    TcruziJRcl4   24   23   26   24  50          74
##                TcruziEsmeraldo   23   22   24   23  47          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
##              LdonovaniBPK282A1   19   20   29   29  57          85
##                LarabicaLEM1108   20   20   29   29  58          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
##               LpyrrhocorisH10    21   22   28   28  56         104
##             EmonterogeiiLV88     23   23   26   26  52         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: **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
0.04
0.03
0.02
0.01
0.00
density
0
50
100
150
200
Aragorn Score
Both
Aragorn−only
Pseudo/Truncated


tRNAscan−SE2 Score Distributions
0.03
0.02
0.01
0.00
density
0
100
200
tRNAscan−SE2 Score
Both Gene−Finders
tRNAscan−SE2−only

3. create.summary.table
Input: output of function annotate.final.geneset.round1()
output: output is saved in latex format in this file **SummaryTable__Latex.txt**

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.

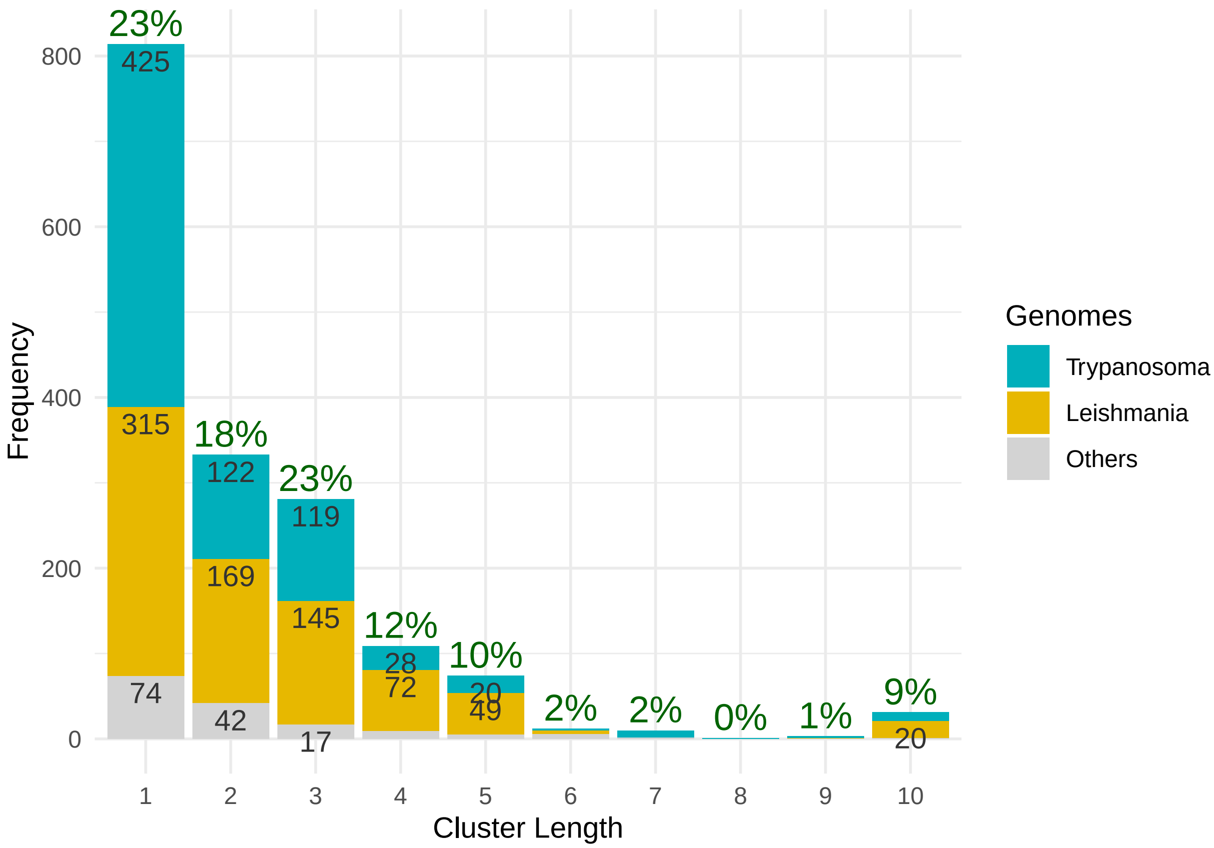
```
##       Annotation Intersection ARAonly Union
##            #tRNA         3579      36  3616
##            #N/#G           74      98    75
##  Min Gene Length           68      71    68
##  Max Gene Length           89     206   206
##          %intron            2      28     3
##            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

6. write.tsfm.input.in.fasta()
This function will write the tsfm__input__geneset.txt file into a fasta format: **final__CIF__geneset.fasta**

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.

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