

CONTENTS

1	Preparing Data	2
1.1	tRNA gene annotation	2
1.1.1	tRNA gene prediction	2
1.1.2	Initiator tRNA prediction	2
1.2	Summary of predicted TryTryp tRNA genes	3
2	TryTryp Classification	5

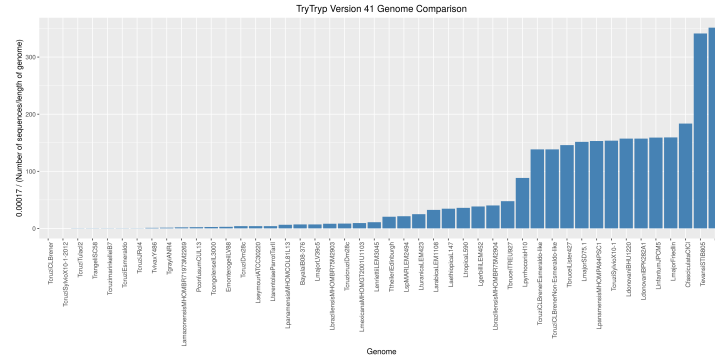


Figure 1: Comparing genomes based on formula $\left(\frac{f(x)}{\max(f(x))}\right)^{-1}$. $f(x)$ = Number of sequences in genome divided by the length of genome. The length of the bars shows how good the genomes are sequenced.

PREPARING DATA

TryTryp genome data. From tritrypdb website, we downloaded the version 41 of 46 TryTryp genomes released on 2018-12-05. Genomes are compared based on number of sequence fragments relative to their length as shown in figure 1. All the results, scripts for TryTryp version 41 can be found [here](#).

tRNA gene annotation

tRNA gene prediction

In order to annotate tRNA genes for the sequenced TryTryp genomes, we used two computational methods for tRNA prediction, tRNAscan-SE and Aragorn. We integrated the result of both genefinders by keeping the union of tRNA gene predictions generated by tRNAscan-SE v2.0 using default options (Lowe and Eddy 1997) and Aragorn v1.2.38 using options -i116 -t -br -seq -w -e -l -d (Laslett and Canback 2004). Genes with overlapped coordinate were considered as one gene. However, the identity and exact coordinate of both genefinders we saved separately to be analysed later.

Initiator tRNA prediction

We predicted the initiator tRNAs for the genes with anticodon 'CAT' from intersection of both tRNAscan (TSE) and Aragorn (ARA) Based on Conserved positions of initiators in Eukarya from the study by CHRISTIAN MARCK and HENRI GROSJEAN. Based on this study we have the following criteria for initiators:

1. In all eukaryotic tDNA-iMet, positions 11–24 are occupied by C-G, However, eukaryotic elongators also prefer C-G at these positions.
2. Initiator tDNAs from Eukarya use A54 and A60. Some eukaryotic elongators also use either A54 or A60 but none (with only one exception) uses both
3. Initiator tDNA-iMet (CAT) from all domains display the GGG sequence (Mandal et al+, 1996) or, very seldom, the AGG sequence at positions 29 to 31, pairing with the complementary CCC or CCT sequences at positions 39 to 41

4. Another domain-specific feature in all eukaryotic initiators is the systematic nonoccupancy of all optional positions of the D-loop (17, 17a, 20a, and 20b) whereas in elongators, only position 17a is always unoccupied.
5. At position 20, A is strictly conserved in all eukaryotic initiators

To investigate all these features we clustered CAT tRNA genes using Levenshtein (edit) distance between gene sequences and Ward.D2 method to measure the dissimilarity between each two clusters. We ended up with three clusters. Table 1 investigates each of these features in each column. from this table we see that only tRNA genes in cluster 1 have almost all the conserved features for eukaryotic initiators. So, we marked these genes as initiators represented with letter X in our gene file.

Table 1: Table of CAT clusters to show how many tRNA genes in each cluster satisfy each feature

Clusters	# tRNAs	11-24(C-G)	54-60(A-A)(T-T)	1-72(A-T)	29-31(GGG)	39-41(CCC/CCT)	# posisInDloop	20A	distanceRange
Cluster1	76	76	76	76	76	76	7	75	0-6
Cluster2	95	95	2	0	95	95	8	0	0-8
Cluster3	2	2	2	0	0	0	8/9	0	0-22

Summary of predicted TryTryp tRNA genes

To investigate and compare tRNA genes predicted by two gene finders TSE and ARA, we made four sets of genes. Set one, TSE and ARA intersection, Set two, TSE and ARA union, Set three, genes found by ARA and Set four, genes found by TSE . for intersection set, we dismissed genes which had different identity by ARA and TSE. for union set, we picked TSE identity over ARA. Table 2 shows a summary of these four sets. Further, to compare the coordinates of genes annotated by ARA and TSE we made a heatmap shown in figure 2. We see from this figure that the coordinates of same genes annotated by ARA and TSE do not always match. We Analysed the reason for each set of displacement in this figure as follow:

1. Genes with 0 displacement in both ends: These genes have same identity for both TSE and ARA except for 33 genes with Anticodon loop of more than 8bp. Also, both ARA and TSE reported genes up to base 73.
2. Genes with 0 displacement at 5 prime end and 1 displacement at 3 prime end: Identity of these genes matches between ARA and TSE. They all have anticodon loop of length 7. The reason for the displacement in 3 prime end is that ARA reports up to position 74, however, TSE reports only up to position 73.
3. Genes with one base displacement at both ends: In this case ARA reports one extra base at both ends which is because these two bases pair together and in most cases this makes the Amino Acid arm one base longer than what TSE reports. Although in a few cases, the AminoAcid arm will stay 7bp, but we see insertions in this arm.
4. Genes with two base displacement at 3 prime end and one at 5 prime end: In this case ARA is reporting 2 extra bases at one end and 1 for the other end which usually leads to a longer AminoAcid arm. Also, the extra 2 bases reported by ARA at 3 prime end are mostly 'ac'.
5. Genes with two base displacement at 3 prime end and 0 displacement at 5 prime end: In this case TSE reports up to position 73 as always,

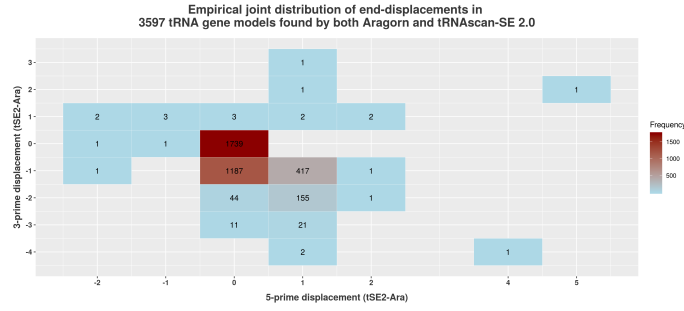


Figure 2: Empirical joint distribution of end-displacements in ? tRNA gene models found by both Aragorn and tRNAscan-SE 2.0.

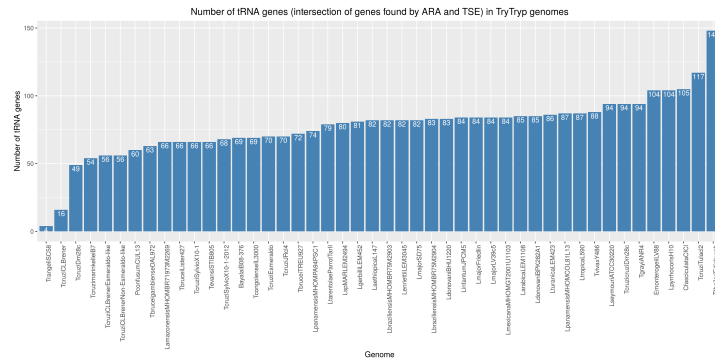


Figure 3: Number of genes annotated by both TSE and ARA for each TryTrypt genome.

but ARA is reporting genes up to position 75. the last three bases of ARA are mainly 'acc'

- Genes with three base displacement at 3 prime end and 0 displacement at 5 prime end. In this case ARA is reporting 3 extra bases at 3 prime end and these three bases are 'cc'.

We expect our genefinders to annotate tRNA genes of all 22 functional classes for each genome. To investigate this we visualized the number of genes annotated for each genome in Figure 3 and tRNA functional classes annotated by both TSE and ARA for each genome in Figure 4.

Table 2: summary of the predicted genes by TSE and ARA. We marked pseudo genes as \$, initiators as X, stop as #, sup as "?", sec as Z and pyl as O

Geneset	# RNA	# nucleotides	N/T	gene length	%A	%C	%G	%U	%Initiator	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	X	Y	Z	#	O	
TSE	3914	270155	74.46	50-164	31.09	28.11	22.22	18.68	2.616	214	64	105	163	110	234	80	179	130	338	108	126	201	162	350	238	210	241	52	94	76	78	28	3	0
ARA	4347	372939	85.70	70-215	32.44	26.90	22.87	17.57	15.377	257	86	124	193	125	339	120	213	194	393	101	153	228	175	420	362	248	282	60	90	76	82	0	2	4
UNION	4381	377734	86.22	50-215	32.81	26.66	22.87	17.65	14.699	259	86	119	194	130	344	120	220	197	380	112	143	229	175	421	359	249	282	57	108	76	82	28	3	2
INTERSECTION	3986	285180	79.44	68-89	32.21	28.13	22.22	18.64	2.330	215	64	105	165	109	239	80	172	137	338	97	125	200	168	349	230	238	241	52	78	76	6	0	0	

from figure 3 we see that few of the 22 tRNA classes are not annotated for all the genomes. To improve the annotation we included 33 genes annotated by both gene finders, with mismatched identities. you can see a summary of these genes in table 3. To determind the identity of these genes we built a structural alignment of all the TryTrypt tRNA genes from our intersection set and 33 genes (We picked TSE reported sequences over ARA for alignment, because TSE reports genes up to position 73, however ARA can report a few bases after 73 as we see in figure 2. We used removed introns and other nucleotides in non-conserved positions, and variable arms prior to the alignment. We used covea v2.4.2 (Sean Eddy 1994) for the structural alignment and edited the alignment by removing sites with more than 99%

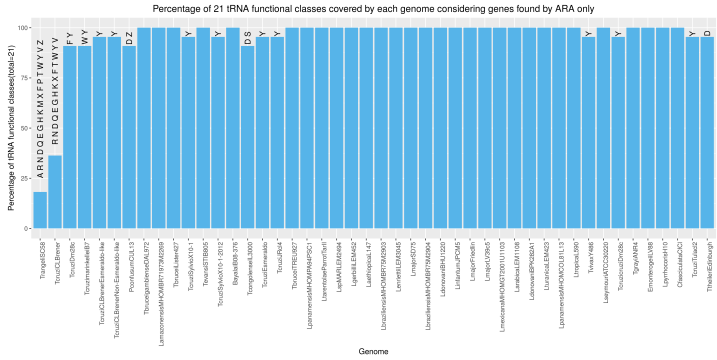


Figure 4: Percentage of 22 tRNA types annotated by both TSE and ARA for each TryTryp genomes. The label on top of each bar shows which tRNA classes are not annotated for the genome.

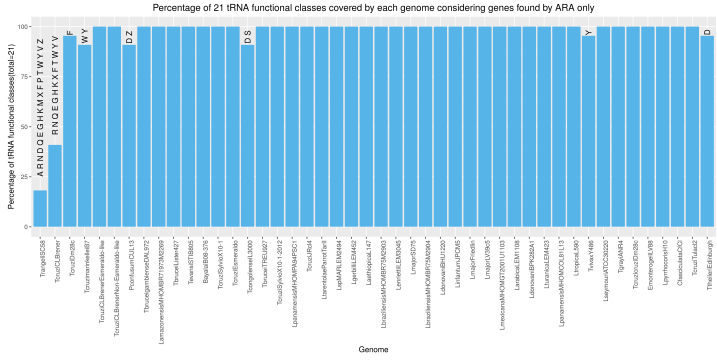


Figure 5: Percentage of 22 tRNA types annotated by both TSE and ARA for each TryTryp genomes after including 32 genes to intersection set. The label on top of each bar shows which tRNA classes are not annotated for the genome.

gap, genes with more than 8 gaps in their aligned sequence, and genes with letter N in their sequence.). Later, using only the intersection aligned genes we made a profile covariance model for 22 functional classes. We calculated the score of 33 genes for each of these 22 models. For each gene, we compared the score of two models made for the identities reported by TSE and ARA and picked the one with higher score. you can see the result in this [file](#). We were able to include 32 of these genes to our intersection set which improved the annotation of genomes as you can see in figure 5

Table 3: 33 genes annotated by both TSE and ARA with mismatched identity

ARA\TSE	D\I	L\?	L\E	L\M	N\Y	O\I	S\I	W\G
Number of genes	5	3	1	9	11	2	1	3

TRITRYP TRNA MODEL ALIGNMENT

To explore the evolution of TriTryp gene models, we first need to structurally align them. To do this we preformed the following pipeline:

- 1
- 2
- 3
- 4

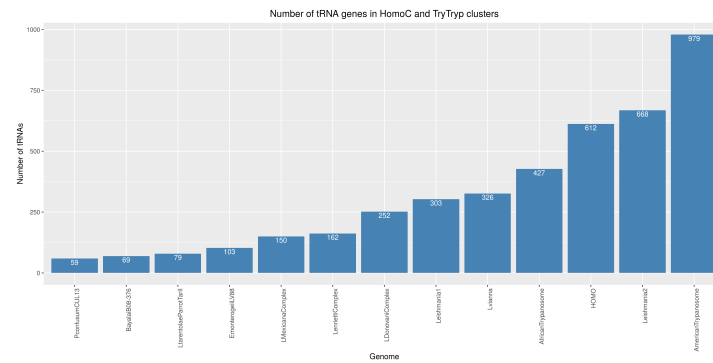


Figure 6: Number of genes annotated by both TSE and ARA for each TryTryp cluster.

TRYTRYP CLASSIFICATION

We visualized differences in tRNA identity determinants between TryTryp and Human, and across TryTryp genomes, using four different Logos:

- 1 Function Logos to estimate the potential identity determinants for each genome
- 2 Information Difference logos (ID logos), to show the evolutionary gain or loss of functional information between Human and TryTryp genomes
- 3 KullbackLeibler divergence Difference logos (KLD logos) to show changes in the functional associations of features between Human and TryTryp genomes
- 4 Using Three Logos mentioned above, we made bubble plots to show gains and shifts in functions of tRNAs in Trypanosomea contrasted against human tRNAs.

Using phylogenetic trees of *Trypanosoma* from these works [1–4], we grouped TryTryp genomes as table 4. figure 6 shows a summary of the number of tRNA genes for each cluster and figure 7 shows the tRNA functional classes not annotated for clusters of genome. we excluded genome PconfusumCUL13 from the study, until we find a well sequenced version of this genome for which we can annotate all 22 functional classes of tRNAs. The Logo data for TryTryp genomes and Human can be found [here](#). you can find the bubble plots for each cluster [here](#). we have 11 pages and each page has 21 models for all tRNA classes in a cluster.

Table 4: Classification of TryTryp genomes. genomes not mentioned here are clustered as one genome.

Leontitis Complex	African Trypanosome	American Trypanosome	Leishmania 1	Leishmania 2	LD New World Complex	Emberizans Complex	Luambia
		1. TrypanINt4					
		2. TrypanGLCyl					
		3. TrypanCLBosser					
		4. TrypanCLBosserEmberizans-like					
	1. ThreegenusambrosiaeDM.075	5. TrypanCLBosserNon-Emberizans-like			1. LeishpoxFriedlin		
	2. TrypanLdang07	6. TrypanLdang07			2. LeishpoxV395		
1. LepMARLEMA094		7. TrypanLdang07			3. LeishpoxD25		
2. LeontitisMCM045		8. TrypanLdang07	1. ClasciculaeCCL1	4. UnusanaeCLM043	1. Ldneww010BUB.120	1. Lamanensis MKRMBR7957/M049	1. Unusanaensis MKRMBR7957/M049
		9. TrypanSTB005	2. LeishmontATCC-9300	5. LdnewwCLM043	2. Ldneww010BUB.120	2. Lamanensis MKRMBR7957/M049	2. Unusanaensis MKRMBR7957/M049
		10. TrypanSTB005	3. TrypanLdang07	6. LdnewwCLM043	3. LeishmontATCC-9300		
		11. TrypanSTB005	4. TrypanLdang07	7. LeishmontATCC-9300			
		12. TrypanSTB005	5. TrypanLdang07	8. LeishmontATCC-9300			
		13. TrypanSTB005	6. TrypanLdang07	9. LeishmontATCC-9300			
		14. TrypanSTB005	7. TrypanLdang07	10. LeishmontATCC-9300			
		15. TrypanSTB005	8. TrypanLdang07	11. LeishmontATCC-9300			
		16. TrypanSTB005	9. TrypanLdang07	12. LeishmontATCC-9300			
		17. TrypanSTB005	10. TrypanLdang07	13. LeishmontATCC-9300			
		18. TrypanSTB005	11. TrypanLdang07	14. LeishmontATCC-9300			
		19. TrypanSTB005	12. TrypanLdang07	15. LeishmontATCC-9300			
		20. TrypanSTB005	13. TrypanLdang07	16. LeishmontATCC-9300			
		21. TrypanSTB005	14. TrypanLdang07	17. LeishmontATCC-9300			
		22. TrypanSTB005	15. TrypanLdang07	18. LeishmontATCC-9300			
		23. TrypanSTB005	16. TrypanLdang07	19. LeishmontATCC-9300			
		24. TrypanSTB005	17. TrypanLdang07	20. LeishmontATCC-9300			
		25. TrypanSTB005	18. TrypanLdang07	21. LeishmontATCC-9300			
		26. TrypanSTB005	19. TrypanLdang07	22. LeishmontATCC-9300			
		27. TrypanSTB005	20. TrypanLdang07	23. LeishmontATCC-9300			
		28. TrypanSTB005	21. TrypanLdang07	24. LeishmontATCC-9300			
		29. TrypanSTB005	22. TrypanLdang07	25. LeishmontATCC-9300			
		30. TrypanSTB005	23. TrypanLdang07	26. LeishmontATCC-9300			
		31. TrypanSTB005	24. TrypanLdang07	27. LeishmontATCC-9300			
		32. TrypanSTB005	25. TrypanLdang07	28. LeishmontATCC-9300			
		33. TrypanSTB005	26. TrypanLdang07	29. LeishmontATCC-9300			
		34. TrypanSTB005	27. TrypanLdang07	30. LeishmontATCC-9300			
		35. TrypanSTB005	28. TrypanLdang07	31. LeishmontATCC-9300			
		36. TrypanSTB005	29. TrypanLdang07	32. LeishmontATCC-9300			
		37. TrypanSTB005	30. TrypanLdang07	33. LeishmontATCC-9300			
		38. TrypanSTB005	31. TrypanLdang07	34. LeishmontATCC-9300			
		39. TrypanSTB005	32. TrypanLdang07	35. LeishmontATCC-9300			
		40. TrypanSTB005	33. TrypanLdang07	36. LeishmontATCC-9300			
		41. TrypanSTB005	34. TrypanLdang07	37. LeishmontATCC-9300			
		42. TrypanSTB005	35. TrypanLdang07	38. LeishmontATCC-9300			
		43. TrypanSTB005	36. TrypanLdang07	39. LeishmontATCC-9300			
		44. TrypanSTB005	37. TrypanLdang07	40. LeishmontATCC-9300			
		45. TrypanSTB005	38. TrypanLdang07	41. LeishmontATCC-9300			
		46. TrypanSTB005	39. TrypanLdang07	42. LeishmontATCC-9300			
		47. TrypanSTB005	40. TrypanLdang07	43. LeishmontATCC-9300			
		48. TrypanSTB005	41. TrypanLdang07	44. LeishmontATCC-9300			
		49. TrypanSTB005	42. TrypanLdang07	45. LeishmontATCC-9300			
		50. TrypanSTB005	43. TrypanLdang07	46. LeishmontATCC-9300			
		51. TrypanSTB005	44. TrypanLdang07	47. LeishmontATCC-9300			
		52. TrypanSTB005	45. TrypanLdang07	48. LeishmontATCC-9300			
		53. TrypanSTB005	46. TrypanLdang07	49. LeishmontATCC-9300			
		54. TrypanSTB005	47. TrypanLdang07	50. LeishmontATCC-9300			
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		57. TrypanSTB005	50. TrypanLdang07	53. LeishmontATCC-9300			
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		59. TrypanSTB005	52. TrypanLdang07	55. LeishmontATCC-9300			
		60. TrypanSTB005	53. TrypanLdang07	56. LeishmontATCC-9300			
		61. TrypanSTB005	54. TrypanLdang07	57. LeishmontATCC-9300			
		62. TrypanSTB005	55. TrypanLdang07	58. LeishmontATCC-9300			
		63. TrypanSTB005	56. TrypanLdang07	59. LeishmontATCC-9300			
		64. TrypanSTB005	57. TrypanLdang07	60. LeishmontATCC-9300			
		65. TrypanSTB005	58. TrypanLdang07	61. LeishmontATCC-9300			
		66. TrypanSTB005	59. TrypanLdang07	62. LeishmontATCC-9300			
		67. TrypanSTB005	60. TrypanLdang07	63. LeishmontATCC-9300			
		68. TrypanSTB005	61. TrypanLdang07	64. LeishmontATCC-9300			
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		70. TrypanSTB005	63. TrypanLdang07	66. LeishmontATCC-9300			
		71. TrypanSTB005	64. TrypanLdang07	67. LeishmontATCC-9300			
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		148. TrypanSTB005	141. TrypanLdang07	144. LeishmontATCC-9300			
		149. TrypanSTB005	142. TrypanLdang07	145. LeishmontATCC-9300			
		150. TrypanSTB005	143. TrypanLdang07	146. LeishmontATCC-9300			
		151. TrypanSTB005	144. TrypanLdang07	147. LeishmontATCC-9300			
		152. TrypanSTB005	145. TrypanLdang07	148. LeishmontATCC-9300			
		153. TrypanSTB005	146. TrypanLdang07	149. LeishmontATCC-9300			
		154. TrypanSTB005	147. TrypanLdang07	150. LeishmontATCC-9300			
		155. TrypanSTB005	148. TrypanLdang07	151. LeishmontATCC-9300			
		156. TrypanSTB005	149. TrypanLdang07	152. LeishmontATCC-9300			
		157. TrypanSTB005	150. TrypanLdang07	153. LeishmontATCC-9300			
		158. TrypanSTB005	151. TrypanLdang07	154. LeishmontATCC-9300			
		159. TrypanSTB005	152. TrypanLdang07	155. LeishmontATCC-9300			
		160. TrypanSTB005	153. TrypanLdang07	156. LeishmontATCC-9300			
		161. TrypanSTB005	154. TrypanLdang07	157. LeishmontATCC-9300			
		162. TrypanSTB005	155. TrypanLdang07	158. LeishmontATCC-9300			
		163. TrypanSTB005	156. TrypanLdang07	159. LeishmontATCC-9300			
		164. TrypanSTB005	157. TrypanLdang07	160. LeishmontATCC-9300			
		165. TrypanSTB005	158. TrypanLdang07	161. LeishmontATCC-9300			
		166. TrypanSTB005	159. TrypanLdang07	162. LeishmontATCC-9300			
		167. TrypanSTB005	160. TrypanLdang07	163. LeishmontATCC-9300			
		168. TrypanSTB005	161. TrypanLdang07	164. LeishmontATCC-9300			

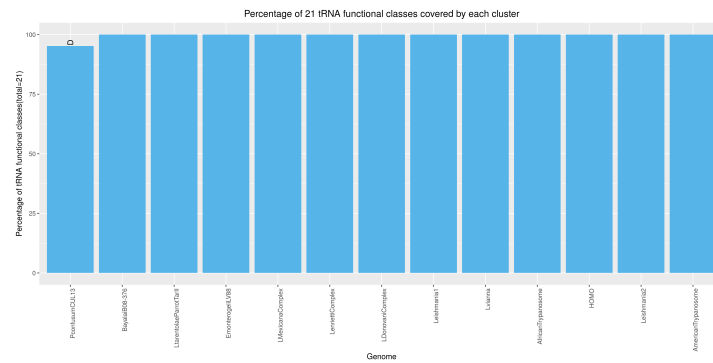


Figure 7: Percentage of 22 tRNA types annotated by both TSE and ARA for each TryTryp cluster. The label on top of each bar shows which tRNA classes are not annotated for the genome.

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