## A1163 GC content features GH

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# 1 GC content of various features of A1163 annotation Aspergillus fumigatusa1163.ASM15014v1.53.gff3

```
[1]: # Import libraries
import pandas as pd
import numpy as np
import scipy.stats
```

## 2 get number of genes

cat Aspergillus\_fumigatusa<br/>1163. ASM15014v1.53.gff3|awk '\$3~ "gene" {print \$0}'|wc -l<br/>10109 (10106 gffcompare)

## 2.1 GC content genome 49.54%

in bash: cat Aspergillus\_fumigatusa1163.ASM15014v1.dna.toplevel.fa | grep -v ">" | awk 'BEGIN{a=0; c=0; g=0; t=0;} {a+=gsub("A",""); c+=gsub("C",""); g+=gsub("G",""); t+=gsub("T","");} END{print a,c,g,t}'

7346850 7202298 7214161 7333693 49.546%

```
[2]: a=7346850
c=7202298
g=7214161
t=7333693
(c+g)/(a+c+g+t)*100
```

#### [2]: 49.54620067043333

#### 2.2 Sequenced genome size - 29097002

#### 2.2.1 GC content CDS - 53.91%

in bash: cat Aspergillus\_fumigatusa1163. ASM15014v1.53.gff3|awk '\$3~ "CDS" {print \$0}'>A1163\_CDS.gff3

conda activate aligners

bedtools nuc -fi Aspergillus\_fumigatusa<br/>1163. ASM15014v1.dna.toplevel.fa -bed A1163\_CDS.gff<br/>3 > AF1163\_CDS\_GC.bed

conda deactivate

```
[3]: # read GC files (Had to check which columns were which)

df=pd.read_csv("AF1163_CDS_GC.bed",comment="#", sep="\t",header=None)

# don't include anything with more than 5 ns

df=df[df.iloc[:,15]<5]

df["GC"]=df.iloc[:,13]+ df.iloc[:,12]

df["ACGT"]= df.iloc[:,11]+df.iloc[:,12]+df.iloc[:,13]+df.iloc[:,14]

sum(df.GC)/sum(df.ACGT)
```

[3]: 0.5391493140194445

```
[4]: ## percent sequenced genome coding 50.01% sum(df.ACGT)/28809969
```

[4]: 0.5001624611258693

```
[5]: df=pd.read_csv("A1163_mRNA.GC.bed",comment="#", header=None,sep="\t")

df=df[df.iloc[:,10]<5]
df["GC"]=df.iloc[:,12]+ df.iloc[:,13]
df["ACGT"]= df.iloc[:,11]+df.iloc[:,12]+df.iloc[:,13]+df.iloc[:,14]
sum(df.GC)/sum(df.ACGT)</pre>
```

[5]: 0.5319151239202807

#### 2.3 GC content mobile elements - from annotation 33.66%

in bash: cat Aspergillus\_fumigatusa1163.ASM15014v1.53.gff3|grep ena\_mobile\_element> a1163\_mobile.gff3 conda activate aligners bedtools nuc fi Aspergillus\_fumigatusa1163.ASM15014v1.dna.toplevel.fa -bed a1163\_mobile.gff3 > a1163 mobile.gc.bed conda deactivate

```
[6]: df=pd.read_csv("a1163_mobile.gc.bed",comment="#", header=None,sep="\t")
# don't include anything with more than 5 ns
df=df[df.iloc[:,15]<5]
df["GC"]=df.iloc[:,13]+ df.iloc[:,12]
df["ACGT"]= df.iloc[:,11]+df.iloc[:,12]+df.iloc[:,13]+df.iloc[:,14]
sum(df.GC)/sum(df.ACGT)</pre>
```

[6]: 0.3366425454899046

## 2.4 size of assembly gaps -106411

in bash: cat Aspergillus\_fumigatusa1163.ASM15014v1.53.gff3|grep ena\_assembly\_gap >a1163 aasembly gaps.gff3

```
[7]: #size of assembly gaps
df=pd.read_csv("a1163_aasembly_gaps.gff3",comment="#", header=None,sep="\t")
sum(df.iloc[:,4]-df.iloc[:,3])
```

[7]: 106411

## 2.5 GC content of feature that are not coding not including mobile elements or assembly gaps

in bash:

bedtools intersect -a Aspergillus\_fumigatusa<br/>1163. ASM15014v1.53.gff3 -b A1163\_CDS.gff3 -v > A1163\_transcribed\_nc.bed

bedtools intersect -a A1163\_transcribed\_nc.bed -b a1163\_mobile.gff3 -v > A1163\_transcribed\_nc\_no\_mob.bed

bedtools intersect -a A1163\_transcribed\_nc\_no\_mob.bed -b a1163\_aasembly\_gaps.gff3 -v > A1163\_transcribed\_nc\_no\_mob\_no\_ass.bed

 $\#And\ now\ get\ GC\ content\ bedtools\ nuc\ -fi\ Aspergillus\_fumigatusa1163. ASM15014v1. dna. toplevel. fa\\-bed\ A1163\_transcribed\_nc\_no\_mob\_no\_ass. bed\ >\ A1163\_transcribed\_nc\_no\_mob\_no\_ass. GC. bed$ 

```
[8]: df=pd.read_csv("A1163_transcribed_nc_no_mob_no_ass.GC.bed",comment="#",__
     →header=None,sep="\t")
     # don't include anything with more than 5 ns
     df=df[df.iloc[:,15]<5]</pre>
     df["GC"]=df.iloc[:,13]+ df.iloc[:,12]
     df["ACGT"] = df.iloc[:,11]+df.iloc[:,12]+df.iloc[:,13]+df.iloc[:,14]
     df ["GCP"] = df ["GC"] / df ["ACGT"]
                      "chr",
     df.columns= [
                                   "ena",
                                               "feature",
                                                               "start",
                                                                             "end", ⊔
           "x",
                     "strand",
                                   7,
                                            "info",
                               11.
                                      12,
                                                      14.
                                                               15. 16.
                       10.
                                               13,
                                                                                17.
              'GC', 'ACGT', 'GCP']
     df_tRNA=df[df.iloc[:,2].apply(lambda x: "tRNA"in x)]
     df 5prime=df[df.iloc[:,2].apply(lambda x: 'five prime UTR'in x)]
     df_3prime=df[df.iloc[:,2].apply(lambda x: 'three_prime_UTR'in x)]
     df_ncexon=df[df.iloc[:,2].apply(lambda x: 'exon'in x)]
     df pseudogene=df[df.iloc[:,2].apply(lambda x: 'pseudogene'in x)]
     df_pseudogenic_transcript=df[df.iloc[:,2].apply(lambda x:_
      ⇔'pseudogenic_transcript'in x)]
     df_ncRNA_gene=df[df.iloc[:,2].apply(lambda x: 'ncRNA_gene'in x)]
```

```
2.6 5' (#10) 3' (#8) > 30 nt GC-content 47.98\%, 47.19\% respectively
 [9]: len(df_3prime)
 [9]: 28
[10]:
     len(df_5prime)
[10]: 32
[11]: sum(df_5prime.GC)/sum(df_5prime.ACGT)
[11]: 0.47980259550356424
[12]: len(df_5prime[df_5prime.ACGT>30])
[12]: 10
[13]: \(\sum(\df_5\prime[\df_5\prime.ACGT>30].GC))/(\sum(\df_5\prime[\df_5\prime.ACGT>30].ACGT))
[13]: 0.4793958605258251
[14]: | (sum(df_3prime[df_3prime.ACGT>30].GC))/(sum(df_3prime[df_3prime.ACGT>30].ACGT))
[14]: 0.4719321148825065
[15]: len(df_3prime[df_3prime.ACGT>30])
[15]: 8
          GC content tRNAs 55.45% #175
[16]: sum(df_tRNA.GC)/sum(df_tRNA.ACGT)
[16]: 0.5544775598520276
[17]: len(df_tRNA.GC)
[17]: 175
```

#### 2.8 INTRON GC 46.53% and sizes, 80.92 mean, sem (0.64)

in bash: ### extract splice sites HISAT2 (https://github.com/DaehwanKimLab/hisat2/blob/master/hisat2\_extract\_python hisat2\_extract\_splice\_sites.py Aspergillus\_fumigatusa1163.ASM15014v1.53.gtf > A1163\_splice\_sites.bed

#### 2.9 get intron gc content

conda activate aligners bed<br/>tools nuc -fi Aspergillus\_fumigatusa1163. ASM15014v1.<br/>dna.toplevel.fa -bed A1163\_splice\_sites.bed > A1163\_splice\_GC.<br/>bed conda deactivate

```
[18]: df=pd.read_csv("A1163_splice_GC.bed",comment="#", header=None,sep="\t")
    df=df[df.iloc[:,10]<5]
    df["GC"]=df.iloc[:,7]+ df.iloc[:,8]
    df["ACGT"]= df.iloc[:,6]+df.iloc[:,7]+df.iloc[:,8]+df.iloc[:,9]
    sum(df.GC)/sum(df.ACGT)</pre>
```

#### [18]: 0.4652782349941584

```
[19]: df=df[df.iloc[:,10]<5]
    df["GC"]=df.iloc[:,7]+ df.iloc[:,8]
    df["ACGT"]= df.iloc[:,6]+df.iloc[:,7]+df.iloc[:,8]+df.iloc[:,9]
    sum(df.GC)/sum(df.ACGT)</pre>
```

#### [19]: 0.4652782349941584

```
[20]: np.mean(df.iloc[:,2]-df.iloc[:,1])
```

#### [20]: 80.91664055155124

```
[21]: scipy.stats.sem(df.iloc[:,2]-df.iloc[:,1])
```

#### [21]: 0.6369252116687439

## 2.10 Get Intergenic region in bash - without assembly gaps and transposons = 45.5%

In bash: ### make genome -mobile elements - assembly and subtract genes ### first make a genome as a bed file conda activate GFF\_utils gff2bed <a1163\_mobile.gff3>a1163\_mobile.bed gff2bed <a1163\_assembly\_gaps.gff3>a1163\_assembly\_gaps.bed conda deactivate

#### 2.10.1 from top of gff3 file get info on chromosomes and make into a bed file

cat for A1163\_genome.txt|awk 'BEGIN { OFS="" $^{\circ}$  } {print \$2"" 0""\$4}'> A1163\_chromSizes.bed

### 2.10.2 need to sort the gene side

```
cat Aspergillus_fumigatusa<br/>1163.ASM15014v1.53.gff3|awk '$3~ "gene" {print $0}'>A1163_gene.gff3
```

#### 2.10.3 need to make this in to a bed file

conda activate GFF utils gff2bed < A1163 gene.gff3>A1163 gene.bed conda deactivate

#### 2.11 now need to sort the bed file

#sort the bed file sort -V -k1,1 -k2,2 A1163\_gene.bed >A1163\_gene.sorted.bed conda activate aligners ### now can subtract to get intergenic regions bedtools subtract -a A1163\_chromSizes.bed -b A1163\_gene.sorted.bed > A1163\_intergenic\_regions.bed

#### 2.11.1 now subtract mobile elements and assembly gaps

```
\label{lem:bed-subtract-allow} bed tools \ subtract -a \ A1163\_intergenic\_regions.bed -b \ a1163\_intergenic\_no\_transposons.bed \\ bed tools \ subtract \ -a \ A1163\_intergenic\_no\_transposons.bed \ -b \\ a1163\_aasembly\_gaps.bed > A1163\_intergenic\_no\_transposons\_no\_ass\_gap.bed
```

#### 2.11.2 now get gc content

conda activate aligners bed<br/>tools nuc -fi Aspergillus\_fumigatusa 1163.ASM15014v1.dna.toplevel.fa -bed<br/> A1163\_intergenic\_no\_transposons\_no\_ass\_gap.bed > a<br/>1163\_intergenic.gc.bed

conda deactivate

```
[22]: df=pd.read_csv("a1163_intergenic.gc.bed",comment="#", header=None,sep="\t")
# don't include anything with more than 5 ns
df=df[df.iloc[:,9]<5]
df["GC"]=df.iloc[:,6]+ df.iloc[:,7]
df["ACGT"]= df.iloc[:,5]+df.iloc[:,6]+df.iloc[:,7]+df.iloc[:,8]
sum(df.GC)/sum(df.ACGT)</pre>
```

[22]: 0.45525616162995786