基因预测和基因结构分析

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>
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> MarkdownNotes/软件第5次作业.md at main · Bluuur/MarkdownNotes (github.com)
> 生物信息学原理/软件第5次作业.md · blur/MarkdownNotes - 码云 - 开源中国 (gitee.com)

实验目的

1. 掌握常用基因从头预测软件的使用和结果解读
2. 熟悉文件格式 GFF3 的基本信息
3. 熟悉至少一种基因组浏览器的使用
4. 了解基因结构和非编码基因预测等分析

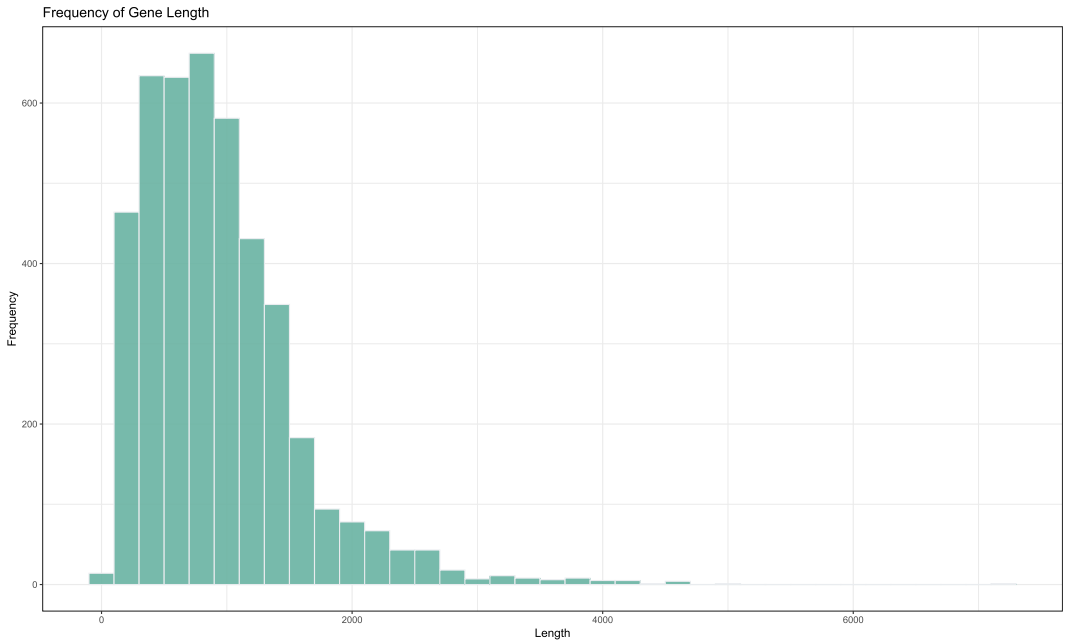
实验内容

1. 使用 Prodigal 对上节课组装的大肠杆菌的序列（其中 1 Mb 序列，已提供，见文件 `ecoli.hifi.fa`）进行基因预测，统计预测得到的基因的长度分布，并用直方图进行可视化。（预测结果、统计结果、直方图展示）
   - 预测结果

```
(base) [uu01@localhost prodigal]$ ls -lh
total 27M
-rw-r--r--. 1 uu01 WBJIAO 4.5M Nov 30 21:08 ecoli.hifi.fa
-rw-r--r--. 1 uu01 WBJIAO  753 Nov 30 21:04 prodigal.log
-rw-r--r--. 1 uu01 WBJIAO 4.5M Nov 30 21:04 ref.cds
-rw-r--r--. 1 uu01 WBJIAO 980K Nov 30 21:04 ref.gff
-rw-r--r--. 1 uu01 WBJIAO 1.9M Nov 30 21:04 ref.pep
-rw-r--r--. 1 uu01 WBJIAO  15M Nov 30 21:04 ref.stat
```

   - 统计结果并作图

```
> ref.gff <- read.table(file.choose(), quote = "#")
> ggplot(data = ref.gff, aes(x = V5 - V4 + 1)) +
+     geom_histogram(binwidth = 200, fill = "#69b3a2",
+                    color = "#e9ecef", alpha = 0.9) +
+     theme_bw() +
+     labs(x = "Length", y = "Frequency", title = "Frequency of Gene
Length")
```



2. 从给定的拟南芥基因组（TAIR10 版本）的某段序列（二号染色体 7.5 - 8.5 Mb, 文件名：`Ath.1mb.fa`），完成以下任务：

    1. 使用 GenScan、Augustus、GlimmerHMM 等软件（至少两种软件）预测该序列所包含的蛋白质编码基因。统计不同软件组装得到的基因、exon、CDS 的数量和长度分布等信息，并选用适当的图表将结果进行展示。

        - 预测结果

```
(base) [uu01@localhost augustus]$ ls -lh
total 664K
lrwxrwxrwx. 1 uu01 WBJIAO   33 Dec  5 14:54 Ath.1mb.fa -> /home/uu01/data/part05/Ath.1mb.fa
-rw-r--r--. 1 uu01 WBJIAO 554K Dec  5 14:58 augustus.out
-rw-r--r--. 1 uu01 WBJIAO    0 Dec  5 14:56 log.txt
-rw-r--r--. 1 uu01 WBJIAO 107K Dec  5 15:02 seq.fa
(base) [uu01@localhost glimmerhmm]$ ls -lh
total 160K
lrwxrwxrwx. 1 uu01 WBJIAO   33 Dec  5 15:04 Ath.1mb.fa -> /home/uu01/data/part05/Ath.1mb.fa
-rw-r--r--. 1 uu01 WBJIAO   16 Dec  5 15:10 log.txt
-rw-r--r--. 1 uu01 WBJIAO 154K Dec  5 15:10 ref.gff
```

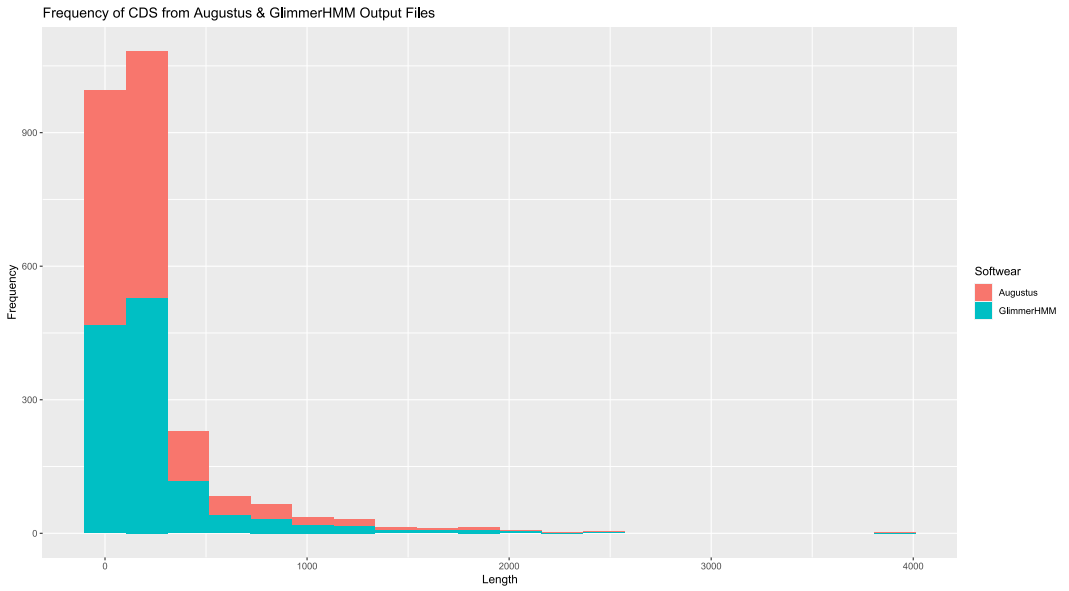
        - 作图：

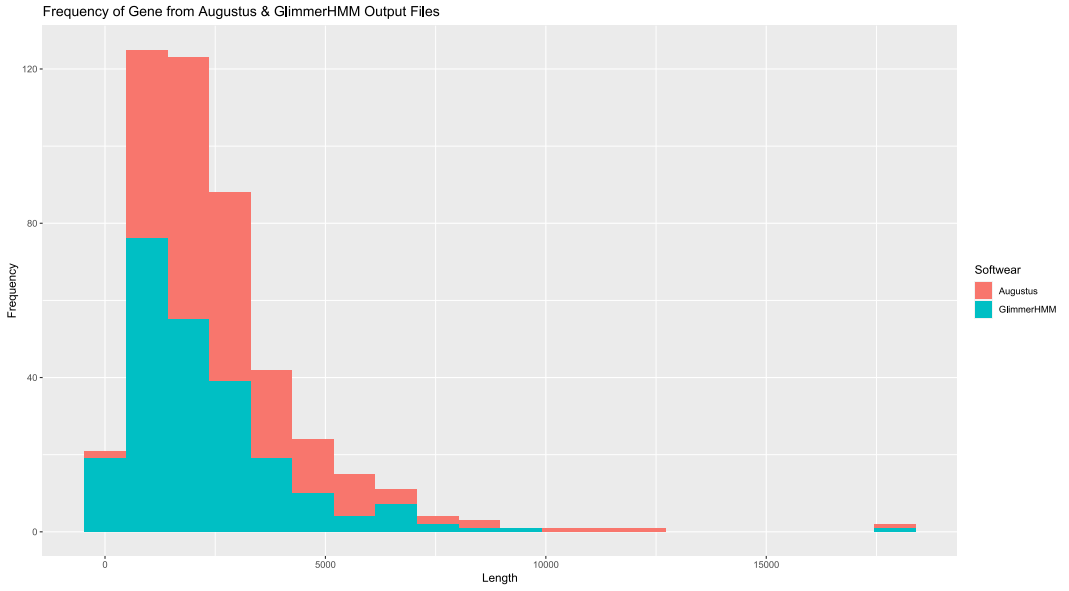
```
# 读取文件
> Augustus <- read.table(file.choose(), quote = "#", fill = 1)
> Glimmerhmm <- read.table(file.choose(), quote = "#")

# 整理数据
> glimmerHMMCDS <- Glimmerhmm[which(Glimmerhmm[, 3] == "CDS"), 3:5]
> glimmerHMMGene <- Glimmerhmm[which(Glimmerhmm[, 3] == "mRNA"),
3:5]
> augustusCDS <- Augustus[which(Augustus[, 3] == "CDS"), 3:5]
> augustusGene <- Augustus[which(Augustus[, 3] == "gene"), 3:5]
> augustusExon <- Augustus[which(Augustus[, 3] == "exon"), 3:5]
> augustusCDS[, 1] <- "Augustus"
> augustusExon[, 1] <- "Augustus"
> augustusGene[, 1] <- "Augustus"
> glimmerHMMCDS[, 1] <- "GlimmerHMM"
> glimmerHMMGene[, 1] <- "GlimmerHMM"
> CDSData <- rbind(augustusCDS, glimmerHMMCDS)
> geneData <- rbind(augustusGene, glimmerHMMGene)
> colnames(CDSData) <- c("Softwear", "Start", "End")
> colnames(geneData) <- c("Softwear", "Start", "End")

# 作图
```

```
> ggplot(data = geneData, mapping = aes(x = End - Start +  1, fill = Softwear)) +
+     geom_histogram(bins = 20) +
+     labs(x = "Length", y = "Frequency", title = "Frequency of Gene from Augustus & GlimmerHMM Output Files")
> ggplot(data = CDSData, mapping = aes(x = End - Start +  1, fill = Softwear)) +
+     geom_histogram(bins = 20) +
+     labs(x = "Length", y = "Frequency", title = "Frequency of CDS from Augustus & GlimmerHMM Output Files")
```





2. 从 TAIR10 网站下载该区间的基因注释信息（已下载到服务器，见文件 `tair10.ch2_7.5-8.5Mb.genes.gff3`），作为标准参考集，试评估第 1 题中使用的不同软件预测结果的准确率和灵敏度等。（可以考虑从基因、exon、CDS三个水平上进行比较，比如：对于某个基因，augustus 预测的跟 tair10 的基因重叠区域超过各自注释区间的 90%，则认为两者一致，其他 exon、CDS 的比较，可采用相同标准）

■

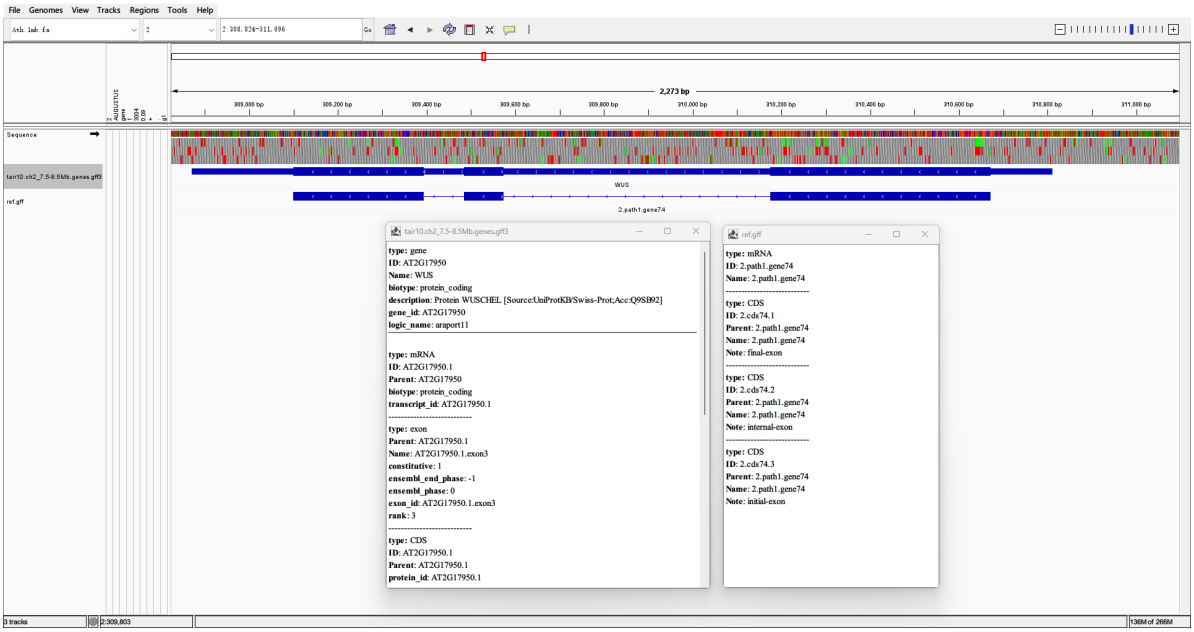
```
> agtCDSMatchRate
[1] 0.867515
> agtExonMatchRate
[1] 0.6848617
> agtGeneMatchRate
[1] 0.5131579
> glmCDSMatchRate
[1] 0.8698795
> glmGeneMatchRate
[1] 0.1367521
```

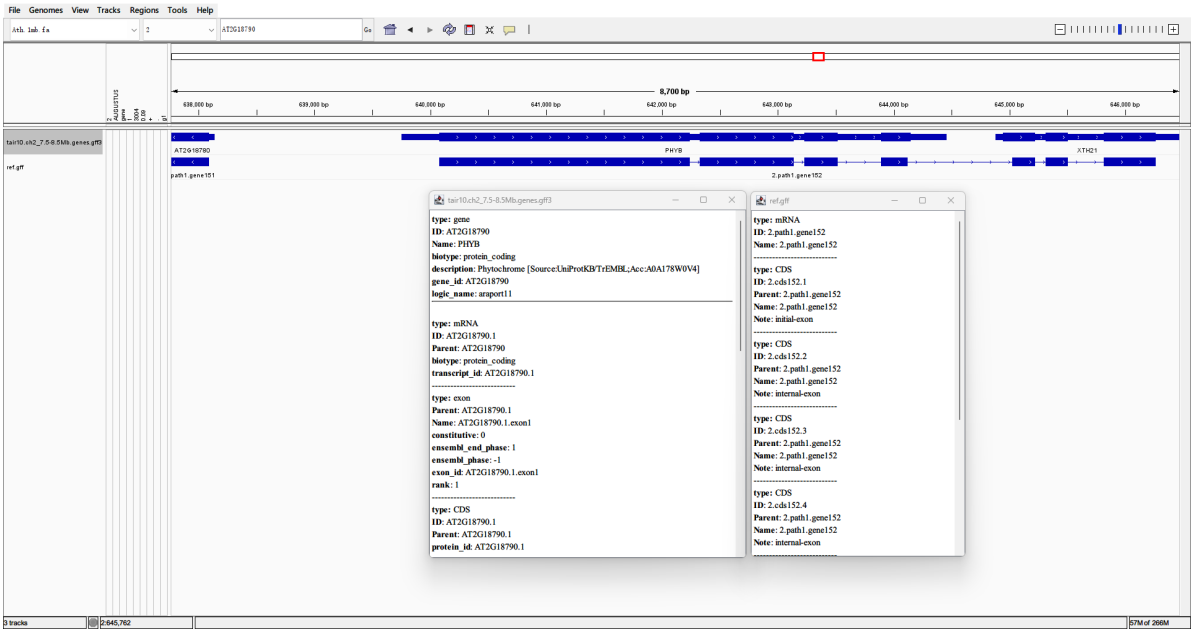
- 代码实现

```
> matchGlmGene <- 0
> matchGlmCDS <- 0
> matchAgtGene <- 0
> matchAgtExon <- 0
> matchAgtCSD <- 0
> for (i in 1:nrow(augustusCDS)) {
+     for (j in 1:nrow(refCDS)) {
+         overlap <- intersect(augustusCDS$Start[i]:augustusCDS$End[i], refCDS$Start[j]:refCDS$End[j])
+         agtMatchRate <- length(overlap) / augustusCDS$Length[i]
+         refMatchRate <- length(overlap) / refCDS$Length[j]
+         if (agtMatchRate >= 0.9 && refMatchRate >= 0.9) {
+             matchAgtCSD = matchAgtCSD + 1
+         }
+     }
+ }
> for (i in 1:nrow(augustusExon)) {
+     for (j in 1:nrow(refExon)) {
+         overlap <- intersect(augustusExon$Start[i]:augustusExon$End[i], refExon$Start[j]:refExon$End[j])
+         agtMatchRate <- length(overlap) / augustusExon$Length[i]
+         refMatchRate <- length(overlap) / refExon$Length[j]
+         if (agtMatchRate >= 0.9 && refMatchRate >= 0.9) {
+             matchAgtExon = matchAgtExon + 1
+         }
+     }
+ }
> for (i in 1:nrow(augustusGene)) {
+     for (j in 1:nrow(refGene)) {
+         overlap <- intersect(augustusGene$Start[i]:augustusGene$End[i], refGene$Start[j]:refGene$End[j])
+         agtMatchRate <- length(overlap) / augustusGene$Length[i]
+         refMatchRate <- length(overlap) / refGene$Length[j]
+         if (agtMatchRate >= 0.9 && refMatchRate >= 0.9) {
```

```
+             matchAgtGene = matchAgtGene + 1
+         }
+     }
+ }
> for (i in 1:nrow(glimmerHMMCDS)) {
+     for (j in 1:nrow(refCDS)) {
+         overlap <-
intersect(glimmerHMMCDS$Start[i]:glimmerHMMCDS$End[i],
refCDS$Start[j]:refCDS$End[j])
+         glmMatchRate <- length(overlap) /
glimmerHMMCDS$Length[i]
+         refMatchRate <- length(overlap) / refCDS$Length[j]
+         if (glmMatchRate >= 0.9 && refMatchRate >= 0.9) {
+             matchGlmCDS = matchGlmCDS + 1
+         }
+     }
+ }
> for (i in 1:nrow(glimmerHMMGene)) {
+     for (j in 1:nrow(refGene)) {
+         overlap <-
intersect(glimmerHMMGene$Start[i]:glimmerHMMGene$End[i],
refGene$Start[j]:refGene$End[j])
+         glmMatchRate <- length(overlap) /
glimmerHMMGene$Length[i]
+         refMatchRate <- length(overlap) / refGene$Length[j]
+         if (glmMatchRate >= 0.9 && refMatchRate >= 0.9) {
+             matchGlmGene = matchGlmGene + 1
+         }
+     }
+ }
> agtCDSMatchRate <- matchAgtCDS / length(augustusCDS$Length)
> agtExonMatchRate <- matchAgtExon /
length(augustusExon$Length)
> agtGeneMatchRate <- matchAgtGene /
length(augustusGene$Length)
> glmCDSMatchRate <- matchGlmCDS / length(glimmerHMMCDS$Length)
> glmGeneMatchRate <- matchGlmGene /
length(glimmerHMMGene$Length)
> agtCDSMatchRate
[1] 0.867515
> agtExonMatchRate
[1] 0.6848617
> agtGeneMatchRate
[1] 0.5131579
> glmCDSMatchRate
[1] 0.8698795
> glmGeneMatchRate
[1] 0.1367521
```

3. 试列举 1-2 个基因的在不同软件和 TAIR10 中的注释差异情况（结合 IGV 展示不同软件的注释结果）。可参考的基因：WUS、PHYTOCHROME B （gff3 文件中 ID 号 `AT2G17950`、`AT2G18790`）





讨论

在这次实验中主要学习了几种基因预测和基因结构分析软件，并回顾了 R 语言作图相关操作。