R for bioinformatics, data wrangler part 2

HUST Bioinformatics course series

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section 1: TOC

前情提要

tidyr

- gather()
- spread()
- 没有仔细讲的
- unite()
- separate()

dplyr

- select()
- filter()
- mutate()
- summarise()
- arrange()
- group_by() ...

前情提要, cont.

factors (部分)

- 概念
- 应用（特别是作图）

本次提要

1. 3 个生信任务的 R 解决方案
2. factors 的更多应用 (forcats)
3. pipe

section 2: contents

生信任务 1：network analysis and visualisation (dplyr & some plot packages)

protein-protein interaction data

why PPI is important?

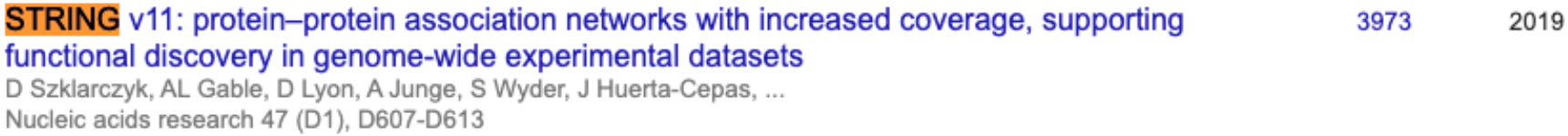
1. most of the time, protein functions together with other proteins (interactions)
2. interacting partners tend to have similar functions (guilty by association, can be used in gene annotation)

STRING database

1. contains >2 billion interactions for 24.6 million proteins in 5090 organisms (as of May 2021; ver 11.0b)
2. contains:

- physical interaction
- genetic interactions
- gene co-occurrence (text-mining)
- transfers through orthologous relationships

STRING is one of the most cited resources


STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets 3973 2019
D Szklarczyk, AL Gable, D Lyon, A Junge, S Wyder, J Huerta-Cepas, ...
Nucleic acids research 47 (D1), D607-D613

screenshot took in May 2021

more tools at Peer Bork's group:
`https://scholar.google.com/citations?hl=en&user=M6Etr6oAAAAJ&view_op=list_works&sortby=pubdate`

a typical STRING plot

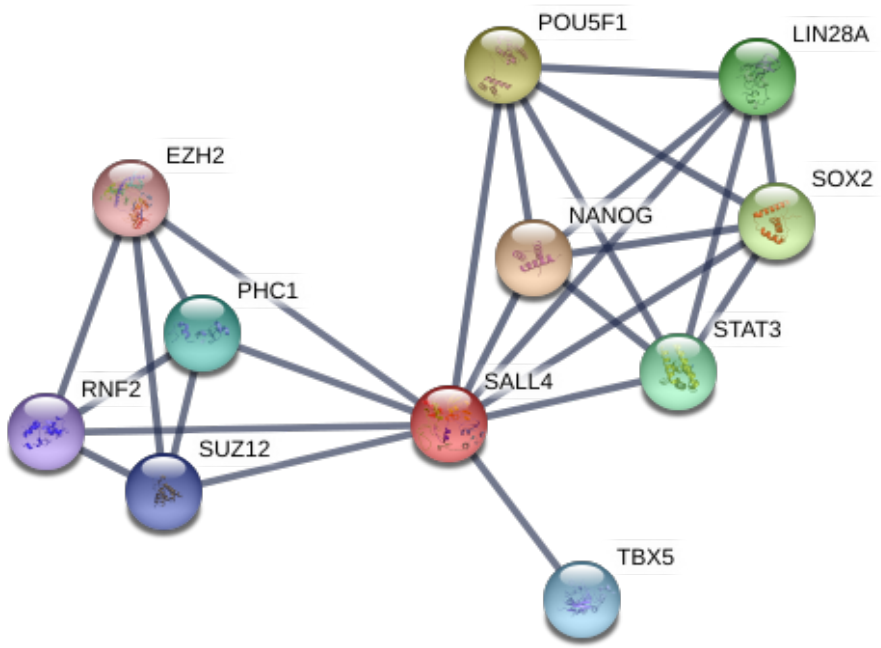


Figure 1: interacting partners of SALL4

note to previous plot

note this plot was generated using the following parameters:

- use SALL4 in human as query
- show only the top ten connectivity partners
- only connectivity score >= 900 (or 0.9) were shown

tasks of task 1

1. get human PPI data
2. limit the interactions to those with scores >= 900 (or 0.9)
3. find SALL4 and its top ten interaction parteners
4. visualize the PPI network
5. calculate connectivities of sall4 and its top ten parteners in the dataset

问题

生物学意义何在 ??

download human ppi data from STRING

go to : `https://string-db.org/cgi/download.pl`

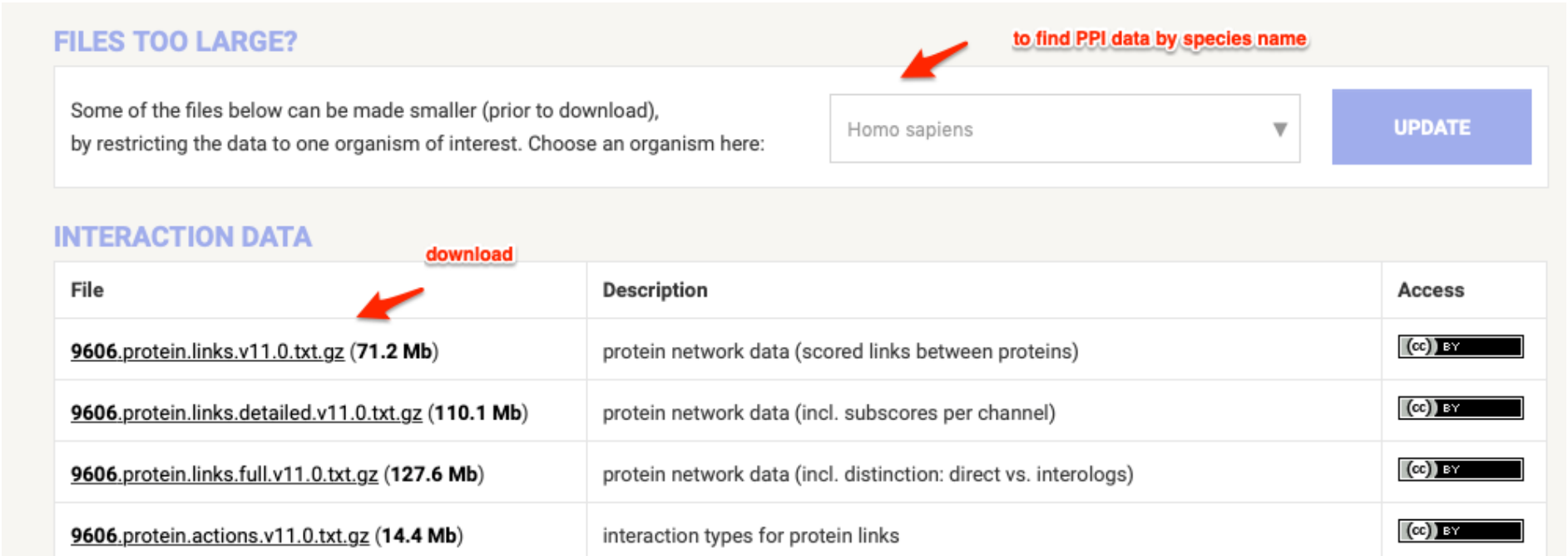


Figure 2: Download human PPI data from STRING

load and load the human PPI data

```
library(tidyverse);
```

```
## read_csv 也能处理压缩文件!!!
ppi <- read_delim( file = "data/talk06/ppi900.txt.gz", col_names = T,
                   delim =  "\t", quote = "" );

## 查看一下数据 --
ppi %>% filter( gene1 == "SALL4" ) %>% do( head(.,  n = 10 ) );
```

```
## # A tibble: 10 x 3
##    gene1 gene2   score
##    <chr> <chr>   <dbl>
##  1 SALL4 POLR2E    900
##  2 SALL4 POLR2C    900
##  3 SALL4 POLR2I    900
##  4 SALL4 NANOG     992
##  5 SALL4 SALL1     923
##  6 SALL4 ZSCAN10   912
##  7 SALL4 LIN28A    957
##  8 SALL4 POU5F1    986
##  9 SALL4 SMAD2     906
## 10 SALL4 EPAS1     900
```

start to process the data

```
## get top 10 interacting partners of SALL4 by interaction score ...
toppart <- ppi %>% filter( gene1 == "SALL4" ) %>%
  arrange( desc( score ) ) %>% slice( 1:10 );

## get the interaction network consisting the top genes --
genes <- unique( c( "SALL4", toppart$gene2 ) );
netdata <- ppi %>% filter( gene1 %in% genes & gene2 %in% genes );
nrow(netdata);
```

```
## [1] 80
```

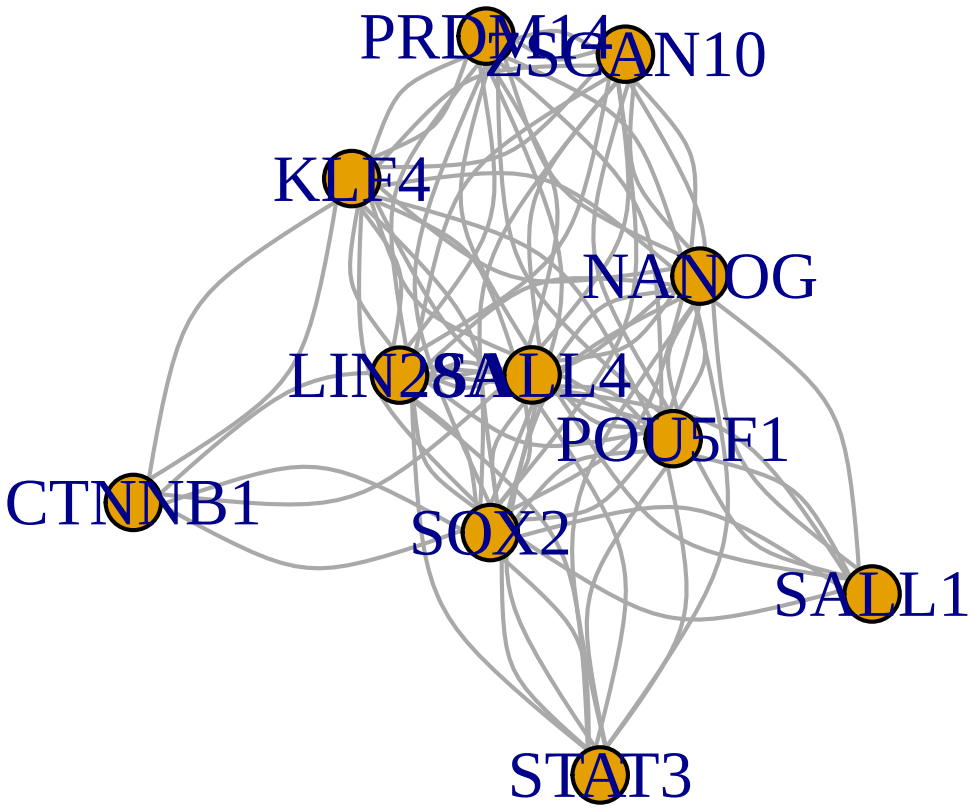
load the igraph package

```
## -- to make sure the installation will only run once ...
if (!require("igraph")){
  chooseCRANmirror();
  install.packages("igraph");
}

library( igraph );
```

visualise the network ...

```
netnet <- graph_from_data_frame( netdata, directed = FALSE );
plot(netnet);
```



图的问题

- very ugly
- two lines (redundancy) between every two nodes

redundancy among data

```
netdata %>% filter( gene1 %in% c("SALL4", "NANOG") & gene2 %in% c("SALL4", "NANOG") );
```

```
## # A tibble: 2 x 3
##   gene1 gene2 score
##   <chr> <chr> <dbl>
## 1 NANOG SALL4   992
## 2 SALL4 NANOG   992
```

how to remove redundancy?

```
## create a new column, sort the two gene names, and concatenate them ...
testdata <-
  netdata %>% filter( gene1 %in% c("SALL4", "NANOG") & gene2 %in% c("SALL4", "NANOG") ) %>%
  mutate( group  =
            if_else( gene1 > gene2,
                     str_c( gene1, gene2, sep = "-" ),
                     str_c( gene2, gene1, sep = "-" ) ) );

testdata;
```

```
## # A tibble: 2 x 4
##   gene1 gene2 score group
##   <chr> <chr> <dbl> <chr>
## 1 NANOG SALL4   992 SALL4-NANOG
## 2 SALL4 NANOG   992 SALL4-NANOG
```

Note `str_c` is from the `stringr` package!!

remove redundancy!

```
testdata %>% group_by( group ) %>% slice( 1 );
```

```
## # A tibble: 1 x 4
## # Groups:   group [1]
##   gene1 gene2 score group
##   <chr> <chr> <dbl> <chr>
## 1 NANOG SALL4   992 SALL4-NANOG
```

remove redundancy, cont.

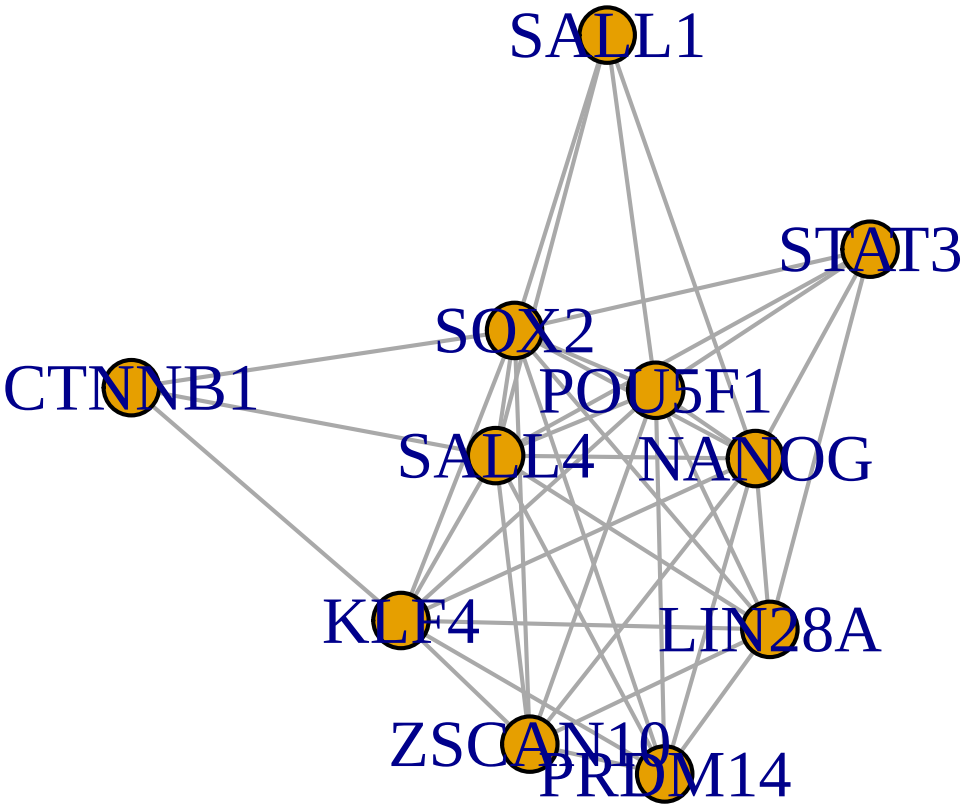
```
netdata.nr <-
  netdata %>%
  mutate( group  =
            if_else( gene1 > gene2,
                     str_c( gene1, gene2, sep = "-" ),
                     str_c( gene2, gene1, sep = "-" ) ) ) %>%
  group_by( group ) %>% slice( 1 );

nrow(netdata.nr);
```

```
## [1] 40
```

plot the non-redundant data

```
netnet.nr <- graph_from_data_frame( netdata.nr, directed = FALSE );
plot(netnet.nr);
```



redundant 的数据可以用来计算 degree

```
net.stats <-
  netdata %>% group_by( gene1 ) %>% summarise( degree = n() ) %>%
  arrange( desc (degree) );
net.stats;
```

```
## # A tibble: 11 x 2
##    gene1   degree
##    <chr>    <int>
##  1 SALL4       10
##  2 SOX2        10
##  3 NANOG        9
##  4 POU5F1       9
##  5 KLF4         8
##  6 LIN28A       8
##  7 PRDM14       7
##  8 ZSCAN10      7
##  9 STAT3        5
## 10 SALL1        4
## 11 CTNNB1       3
```

继续美化 network

```
## node 大小由 degree 决定
## 查看网络中基因名存储的顺序
V(netnet.nr)$name;
```

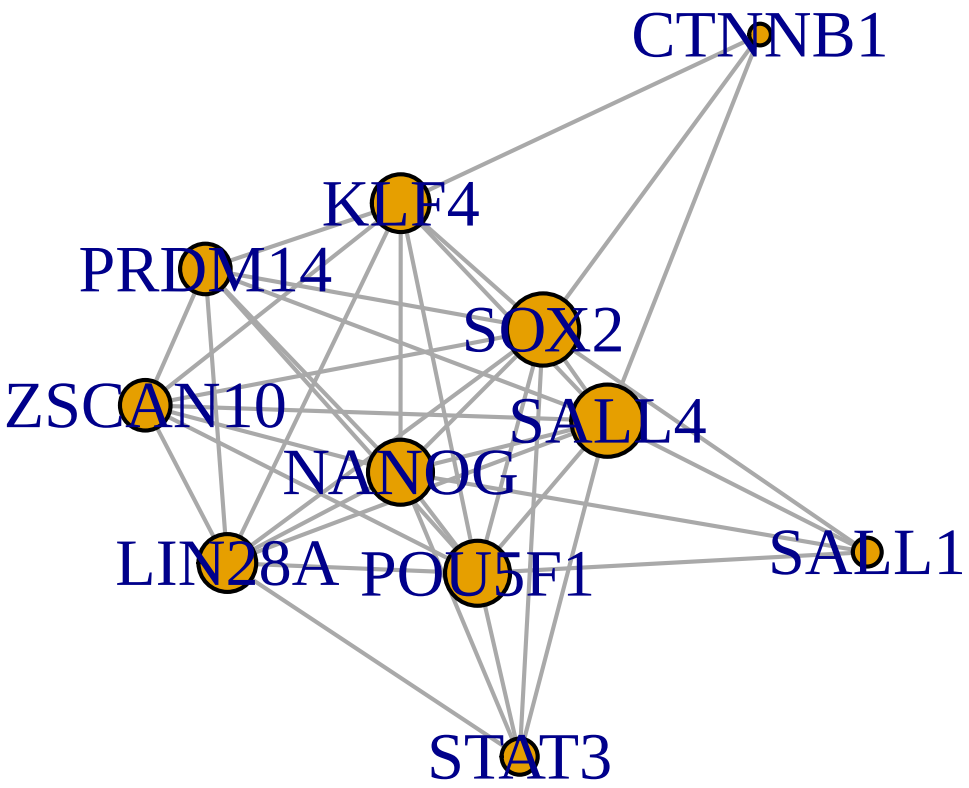
```
##  [1] "KLF4"    "LIN28A"  "POU5F1"  "PRDM14"  "SALL1"   "CTNNB1"  "NANOG"
##  [8] "SOX2"    "STAT3"   "ZSCAN10" "SALL4"
```

```
## 获取它们相对应的 degree ...
net.stats[match( V(netnet.nr)$name , net.stats$gene1 ),  ];
```

```
## # A tibble: 11 x 2
##    gene1   degree
##    <chr>    <int>
##  1 KLF4         8
##  2 LIN28A       8
##  3 POU5F1       9
##  4 PRDM14       7
##  5 SALL1        4
##  6 CTNNB1       3
##  7 NANOG        9
##  8 SOX2        10
##  9 STAT3        5
## 10 ZSCAN10      7
## 11 SALL4       10
```

继续美化 network, cont.

```
vertex_attr(netnet.nr, "size") <-
  net.stats$degree[match( V(netnet.nr)$name , net.stats$gene1 ) ] * 2;
plot( netnet.nr );
```



更多美化

详见：

1. igraph introduction
2. a comprehensive network visualization tutorial in R

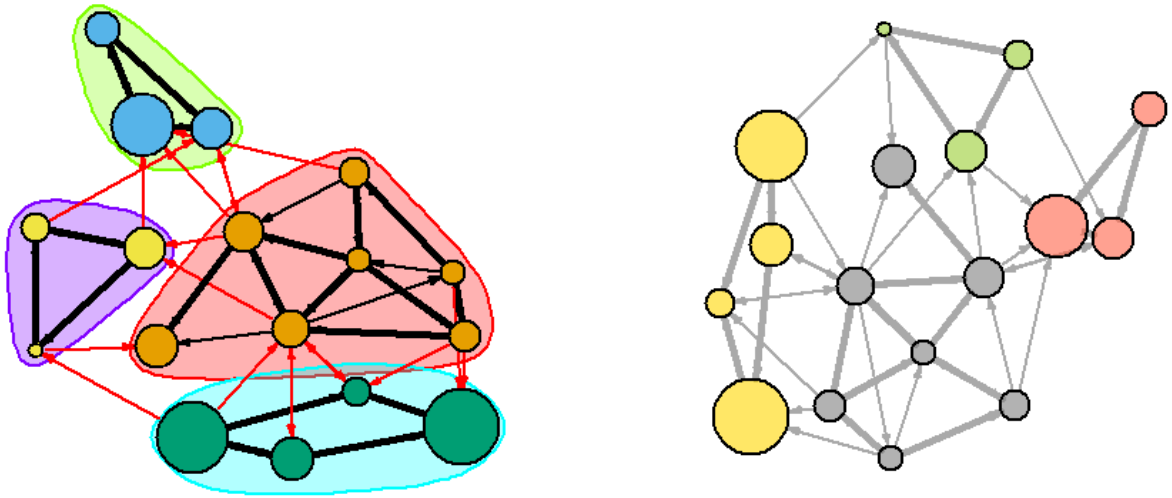


Figure 3: Example final outcomes

other network visualisation packages

1. ggnet
2. interactive networkD3 R package
3. plotly.js
4. D3

生信任务 2：宏基因基因数据展示的小应用（forcats）

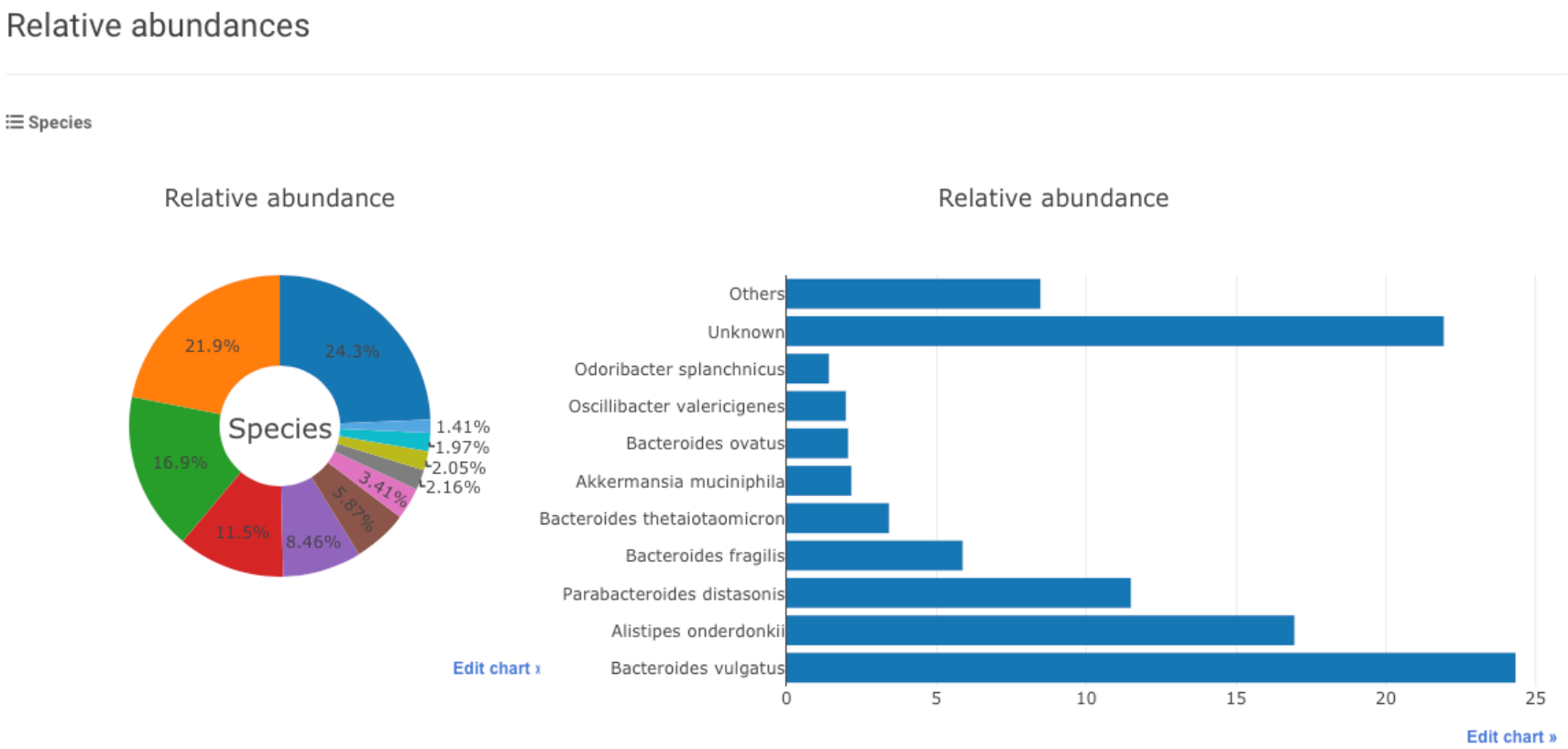


Figure 4: 肠道物种丰度示意图

Find more at the GMrepo database.

什么是宏基因组？

Metagenomics is the study of genetic material recovered directly from environmental samples.

research contents

- mostly prokaryotes
- unicellular eukaryotes
- viruses

techniques

- 16S (universially conserved gene in prokaryotes)
- whole genome sequencing (WGS or metagenomics)

what can metagenomics do?

- identify new species
- reveal species kinds and abundances
- relate species changes to human health and disease

biomes

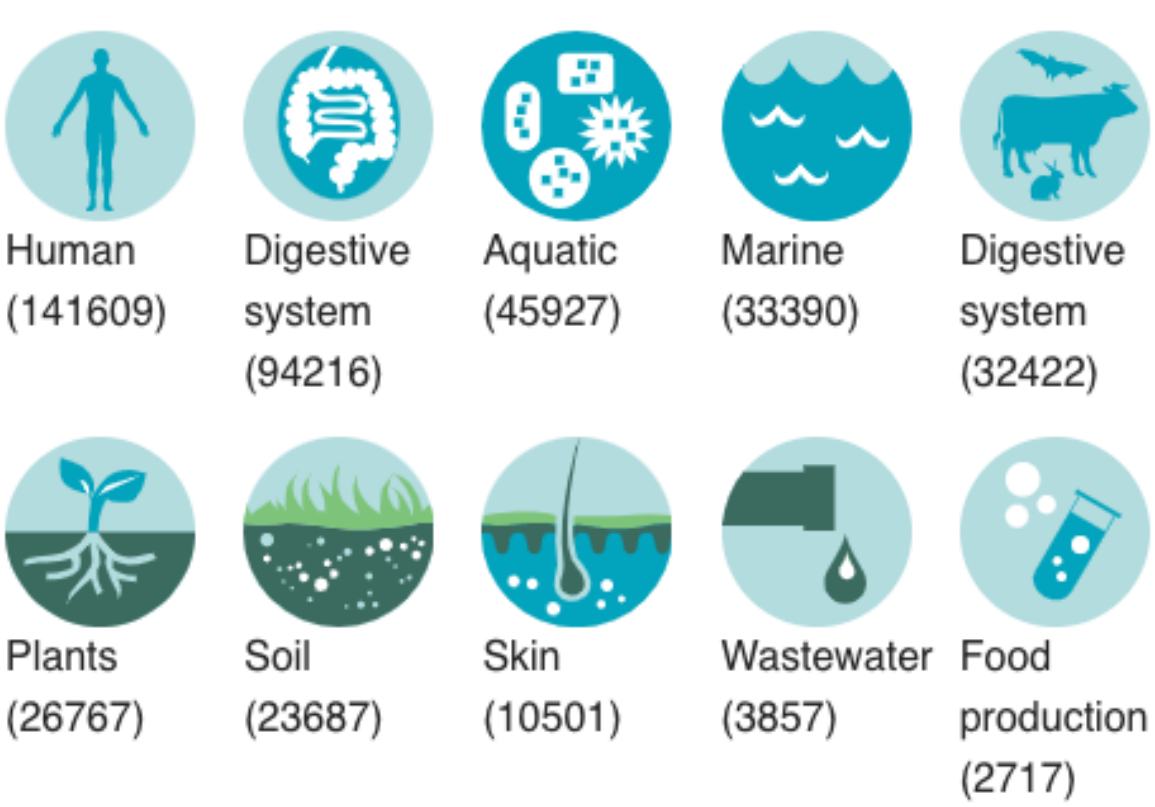


Figure 5: Biomes

Screenshot taken from the `EBI MGnify database` on `Aug 4, 2021`;

enviromental microbiome

- soil
- ocean

Figure 6: The tara oceans expedition

host associated microbiomes

- human body sites
- animals & plants

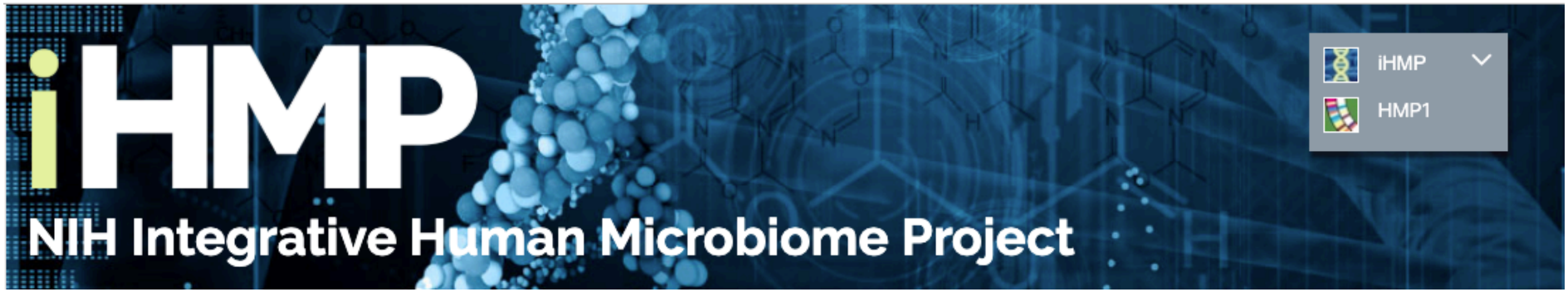


Figure 7: NIH human microbiome project

why human gut microbiota is important?

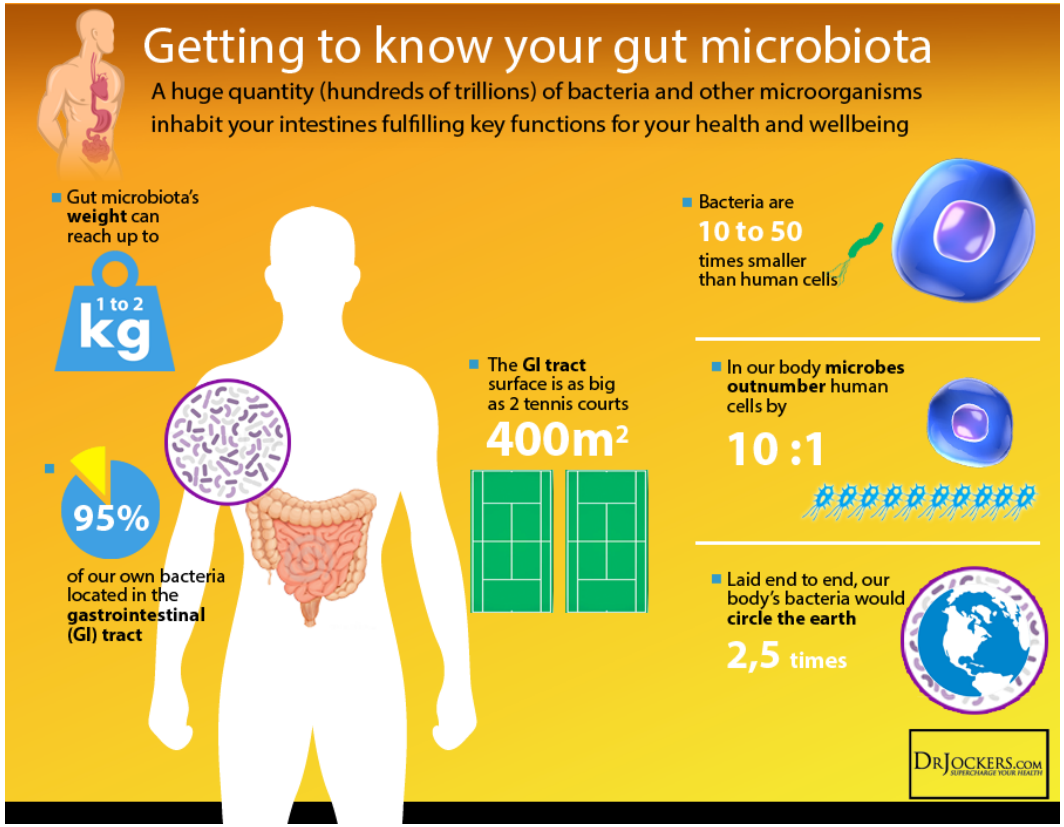


Figure 8: human gut microbita

tasks of human gut microbiota analysis

- identify new species
- find good, bad and commensal microbes
- link microbial variations to human health
- mechanisms
- modulation, intervention and regulation

typical human gut microbiome data

Species abundances

```
abu <-
  read_delim(
    file = "data/talk06/relative_abundance_for_RUN_ERR1072629_taxonlevel_species.txt",
    delim = "\t", quote = "", comment = "#");
```

```
##
## -- Column specification --------------------------------------------------------
## cols(
##   ncbi_taxon_id = col_double(),
##   relative_abundance = col_double(),
##   scientific_name = col_character()
## )
```

```
nrow(abu);
```

```
## [1] 122
```

Species abundances, cont.

```
abu %>% arrange( desc( relative_abundance ) ) %>% do( head(., n = 10) );
```

```
## # A tibble: 10 x 3
##    ncbi_taxon_id relative_abundance scientific_name
##            <dbl>              <dbl> <chr>
##  1           821              24.3  Bacteroides vulgatus
##  2            -1              21.9  Unknown
##  3        328813              16.9  Alistipes onderdonkii
##  4           823              11.5  Parabacteroides distasonis
##  5           817               5.87 Bacteroides fragilis
##  6           818               3.41 Bacteroides thetaiotaomicron
##  7        239935               2.16 Akkermansia muciniphila
##  8         28116               2.05 Bacteroides ovatus
##  9        351091               1.97 Oscillibacter valericigenes
## 10         28118               1.41 Odoribacter splanchnicus
```

相对丰度作图要求

1. 按丰度从高到低排序
2. 只取前 10 个 species (保留 10 行)
3. 将后面的丰度累加在一起，汇总为 “Others” 分类

数据处理

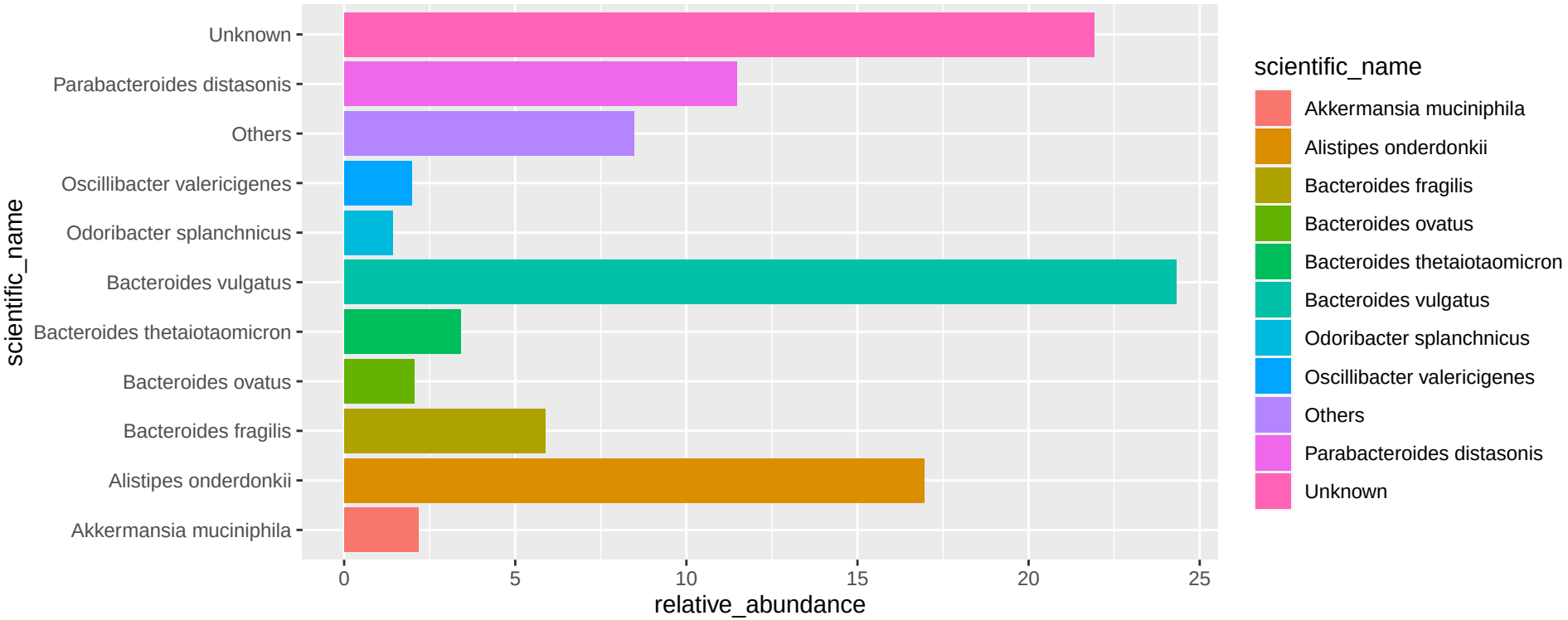
```
library( tidytidbits );
abu.dat <-
  abu %>% arrange( desc( relative_abundance ) ) %>%
    lump_rows( scientific_name, relative_abundance, n = 10, other_level = "Others" );

head(abu.dat, n = 11);
```

```
## # A tibble: 11 x 3
##    ncbi_taxon_id relative_abundance scientific_name
##            <dbl>              <dbl> <chr>
##  1           821               24.3  Bacteroides vulgatus
##  2            -1               21.9  Unknown
##  3        328813               16.9  Alistipes onderdonkii
##  4           823               11.5  Parabacteroides distasonis
##  5           817                5.87 Bacteroides fragilis
##  6           818                3.41 Bacteroides thetaiotaomicron
##  7        239935                2.16 Akkermansia muciniphila
##  8         28116                2.05 Bacteroides ovatus
##  9        351091                1.97 Oscillibacter valericigenes
## 10         28118                1.41 Odoribacter splanchnicus
## 11      31199992                8.46 Others
```

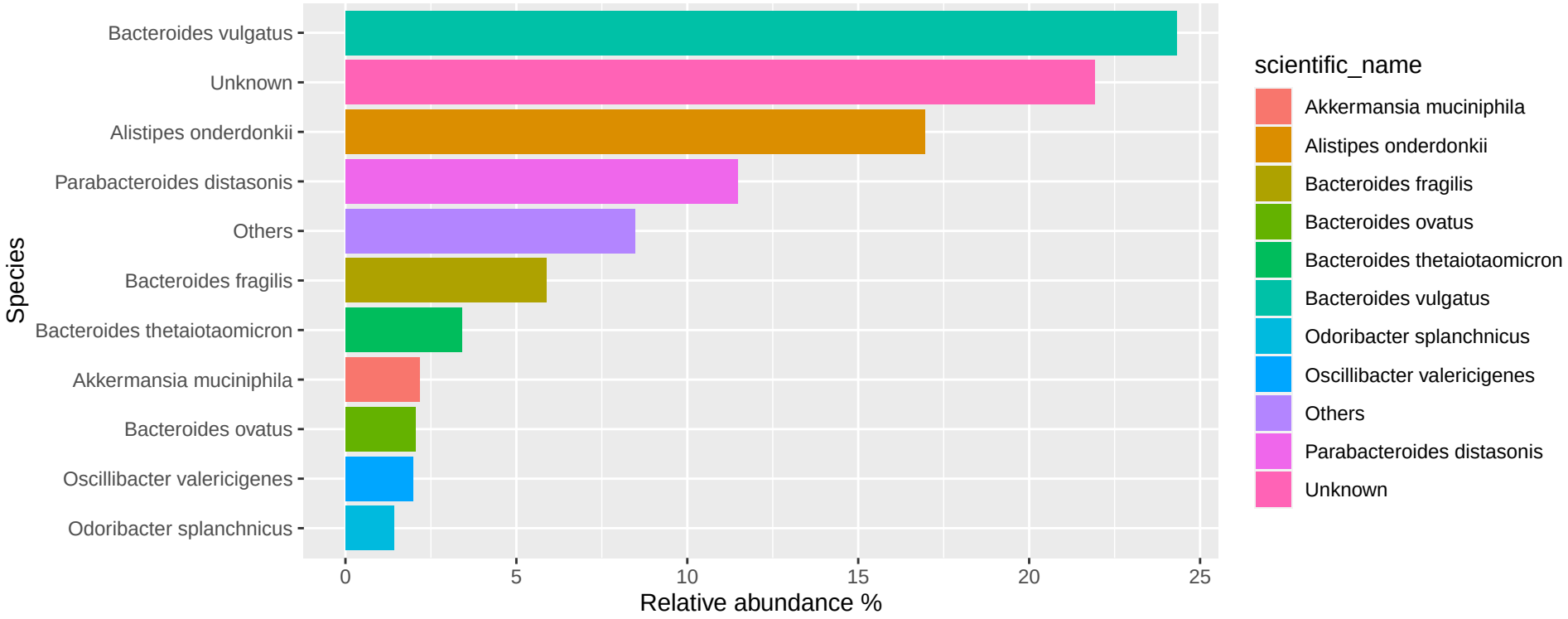
尝试作图

```
ggplot(abu.dat, aes(x = scientific_name, y = relative_abundance, fill = scientific_name ) ) +
  geom_bar( stat = "identity" ) +
  coord_flip()
```



调整排列顺序

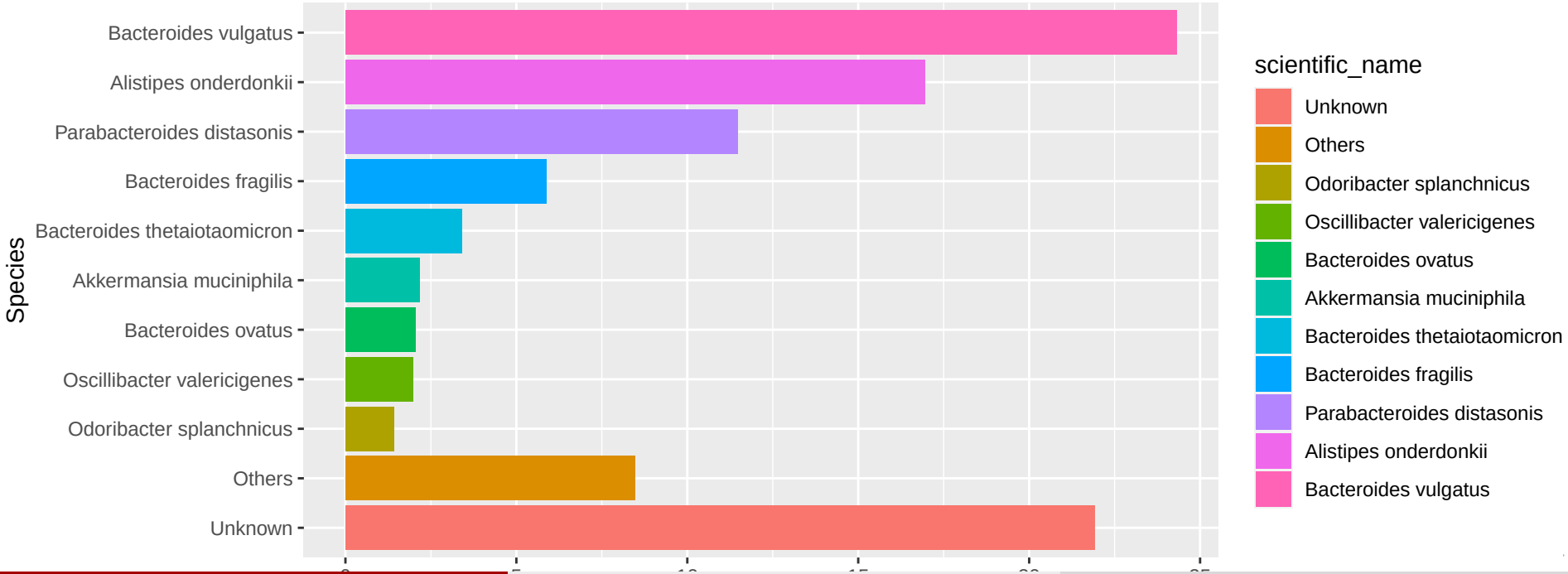
```
## 用 forcat 包的 fct_reorder 函数; 注意它 3 个参数的意义!!!
ggplot(abu.dat, aes(x = fct_reorder( scientific_name, relative_abundance, .desc = F),
                    y = relative_abundance, fill = scientific_name ) ) +
  geom_bar( stat = "identity" ) +
  coord_flip() + xlab("Species") + ylab( "Relative abundance %" )
```



把 Others 和 Unknown 放在最后

注意 fct_reorder 的用法:

```
##
abu.dat$scientific_name <-
  fct_relevel( fct_reorder( abu.dat$scientific_name, abu.dat$relative_abundance, .desc = F),
               "Unknown",  "Others" );
ggplot(abu.dat, aes(x = scientific_name,
                    y = relative_abundance, fill = scientific_name ) ) +
  geom_bar( stat = "identity" ) +
  coord_flip() + xlab("Species") + ylab( "Relative abundance %" )
```



更多 forcats 的应用 ...

see here: https://cran.r-project.org/web/packages/forcats/vignettes/forcats.html

更多应用将会在作图（ggplot2）时讲到。

生信任务 3：整合基因表达、甲基化、突变数据 (dplyr::join)

在生信分析中，常需要将多个来源的数据整合在一个表格中，以方便后续分析。

```
meth <- read_delim( file = "data/talk06/methylation_data.txt.gz",
                    delim = "\t", quote = "", col_names = T);
```

```
##
## -- Column specification --------------------------------------------------------
## cols(
##   gene = col_character(),
##   site = col_character(),
##   methylation_score = col_double()
## )
```

```
head(meth, n = 3);
```

```
## # A tibble: 3 x 3
##   gene  site    methylation_score
##   <chr> <chr>               <dbl>
## 1 A1BG  1stExon             0.799
## 2 A1BG  5UTR                0.799
## 3 A1BG  Body                0.811
```

表达数据

```
expr <- read_delim( file = "data/talk06/expression_data.txt.gz",
                    delim = "\t", quote = "", col_names = T );
```

```
##
## -- Column specification --------------------------------------------------------
## cols(
##   gene = col_character(),
##   rkpm = col_double()
## )
```

```
head( expr, n = 5);
```

```
## # A tibble: 5 x 2
##   gene      rkpm
##   <chr>    <dbl>
## 1 5S_RRNA      0
## 2 7SK          0
## 3 A1BG         1
## 4 A1BGAS1      4
## 5 A1CF         0
```

整合后的结果应该是什么 ??

gene	TSS200	TSS1500	UTR	body	expression
gene1	0.1	0.2	NA	0.8	100
gene2	0.12	0.32	NA	0.9	18

...

第一种方法，使用 spread

先合并，再 spread 用 bind_rows 合并两个 tibble 时，列名需要一致

```
meth2 <- meth %>% select( gene, group=site, value=methylation_score );
head(meth2, n=2);
```

```
## # A tibble: 2 x 3
##   gene  group   value
##   <chr> <chr>   <dbl>
## 1 A1BG  1stExon 0.799
## 2 A1BG  5UTR    0.799
```

```
expr2 <- expr %>% mutate( group = "rkpm" ) %>%
  select( gene, group, value=rkpm ) %>%
  group_by( gene ) %>% slice( 1 );
head(expr2, n=2);
```

```
## # A tibble: 2 x 3
## # Groups:   gene [2]
##   gene    group value
##   <chr>   <chr> <dbl>
## 1 5S_RRNA rkpm      0
## 2 7SK     rkpm      0
```

注： gene name 与 group 组合必须是唯一的。即：基因 A 只能有一个表达量值。

合并 & spread

```
comb <- bind_rows( meth2, expr2 );
comb.wide <- comb %>% spread( group, value );

head(comb.wide);
```

```
## # A tibble: 6 x 8
##   gene    `1stExon` `3UTR` `5UTR`     Body  rkpm TSS1500 TSS200
##   <chr>       <dbl>  <dbl>  <dbl>    <dbl> <dbl>   <dbl>  <dbl>
## 1 5S_RRNA    NA       NA     NA     NA            0  NA     NA
## 2 7SK        NA       NA     NA     NA            0  NA     NA
## 3 A1BG        0.799   NA      0.799  0.811        1   0.852  0.850
## 4 A1BGAS1    NA       NA     NA     NA            4  NA     NA
## 5 A1CF        0.0946  NA      0.118  0.00798      0   0.110 NA
## 6 A2M         0.899    0.776 NA      0.716        1   0.654 NA
```

方法二：使用 join ...

首先对 methylation 数据进行处理

```
meth3 <-
  meth %>% spread( site, methylation_score );

head(meth3);
```

```
## # A tibble: 6 x 7
##   gene   `1stExon` `3UTR` `5UTR`    Body TSS1500 TSS200
##   <chr>      <dbl>  <dbl>  <dbl>   <dbl>   <dbl>  <dbl>
## 1 A1BG       0.799  NA       0.799 0.811     0.852  0.850
## 2 A1CF       0.0946 NA       0.118 0.00798   0.110 NA
## 3 A2M        0.899   0.776 NA     0.716     0.654 NA
## 4 A2ML1      0.859   0.469  0.859 0.678     0.441 NA
## 5 A4GALT    NA       0.814  0.844 0.821     0.890  0.513
## 6 A4GNT      0.680  NA       0.680 0.538     0.402 NA
```

dplyr::join

```
comb2 <- left_join( meth3, expr2, by = "gene" ) %>% select( -group );
head(comb2);
```

```
## # A tibble: 6 x 8
##   gene   `1stExon` `3UTR` `5UTR`    Body TSS1500 TSS200 value
##   <chr>      <dbl>  <dbl>  <dbl>   <dbl>   <dbl>  <dbl> <dbl>
## 1 A1BG       0.799  NA       0.799 0.811     0.852  0.850     1
## 2 A1CF       0.0946 NA       0.118 0.00798   0.110 NA         0
## 3 A2M        0.899   0.776 NA     0.716     0.654 NA         1
## 4 A2ML1      0.859   0.469  0.859 0.678     0.441 NA         0
## 5 A4GALT    NA       0.814  0.844 0.821     0.890  0.513     0
## 6 A4GNT      0.680  NA       0.680 0.538     0.402 NA         0
```

注意 left_join 的语法

join 详解

- left_join(): return all rows from x, and all columns from x and y. Rows in x with no match in y will have NA values in the new columns. If there are multiple matches between x and y, all combinations of the matches are returned.
- inner_join()
- right_join()
- full_join()

更多请见：`https://dplyr.tidyverse.org/reference/join.html`

section 3: pipe

什么是 pipe ？

- pipe 就是 %>%
- it comes from the `magrittr` package by **Stefan Milton Bache**
- Packages in the tidyverse load %>% for you automatically, so you don't usually load magrittr explicitly.
- **实质是中间值的传递**

示例:

```
## 比如: 这段代码可以合并为:
comb <- bind_rows( meth2, expr2 );
comb.wide <- comb %>% spread( group, value );

## -- 新代码 ...
bind_rows( meth2, expr2 ) %>%
  spread( group, value );
```

```
## # A tibble: 46,225 x 8
##    gene     `1stExon` `3UTR` `5UTR`     Body  rkpm TSS1500 TSS200
##    <chr>        <dbl>  <dbl>  <dbl>    <dbl> <dbl>   <dbl>  <dbl>
##  1 5S_RRNA     NA       NA     NA     NA            0  NA     NA
##  2 7SK         NA       NA     NA     NA            0  NA     NA
##  3 A1BG         0.799   NA      0.799  0.811        1   0.852  0.850
##  4 A1BGAS1     NA       NA     NA     NA            4  NA     NA
```

是否所有函数都支持 pipe ？

是的。

通常需要用 . 指代传递来的数据，并以参数的形式赋予下游函数:

```
ppi %>% filter( gene1 == "SALL4" ) %>% do( head(.,  n = 4 ) );
```

```
## # A tibble: 4 x 3
##   gene1 gene2  score
##   <chr> <chr>  <dbl>
## 1 SALL4 POLR2E   900
## 2 SALL4 POLR2C   900
## 3 SALL4 POLR2I   900
## 4 SALL4 NANOG    992
```

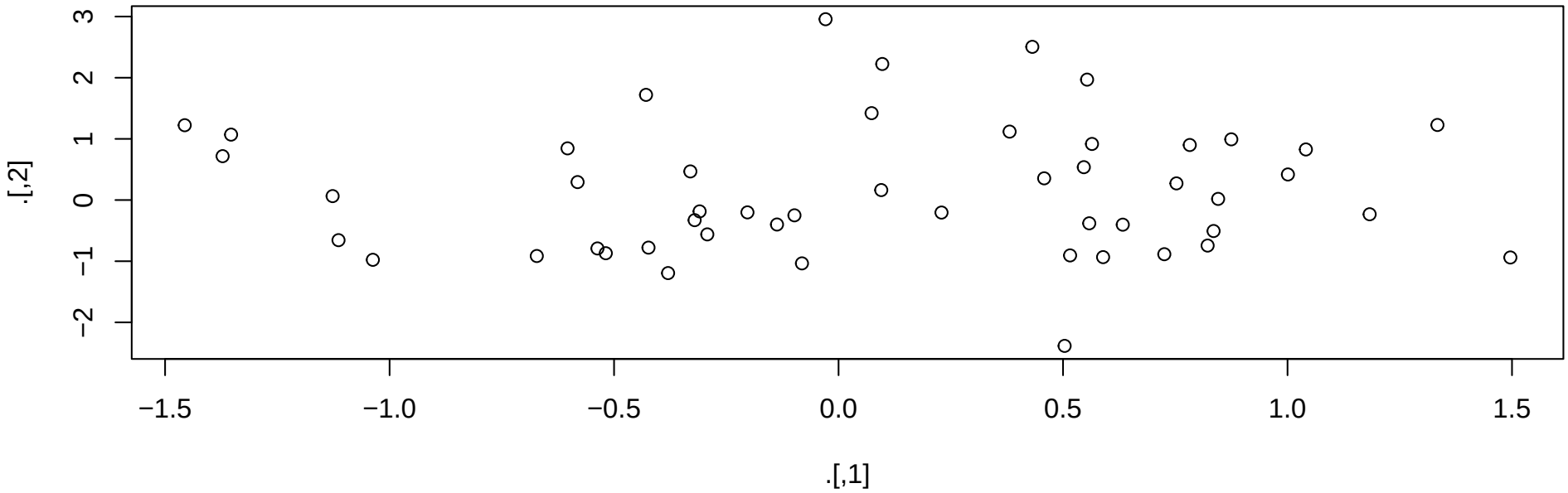
```
## 也可以写为
ppi %>% filter( gene1 == "SALL4" ) %>% head(.,  n = 4 );
```

```
## # A tibble: 4 x 3
##   gene1 gene2  score
##   <chr> <chr>  <dbl>
## 1 SALL4 POLR2E   900
## 2 SALL4 POLR2C   900
## 3 SALL4 POLR2I   900
## 4 SALL4 NANOG    992
```

其它形式的 pipe

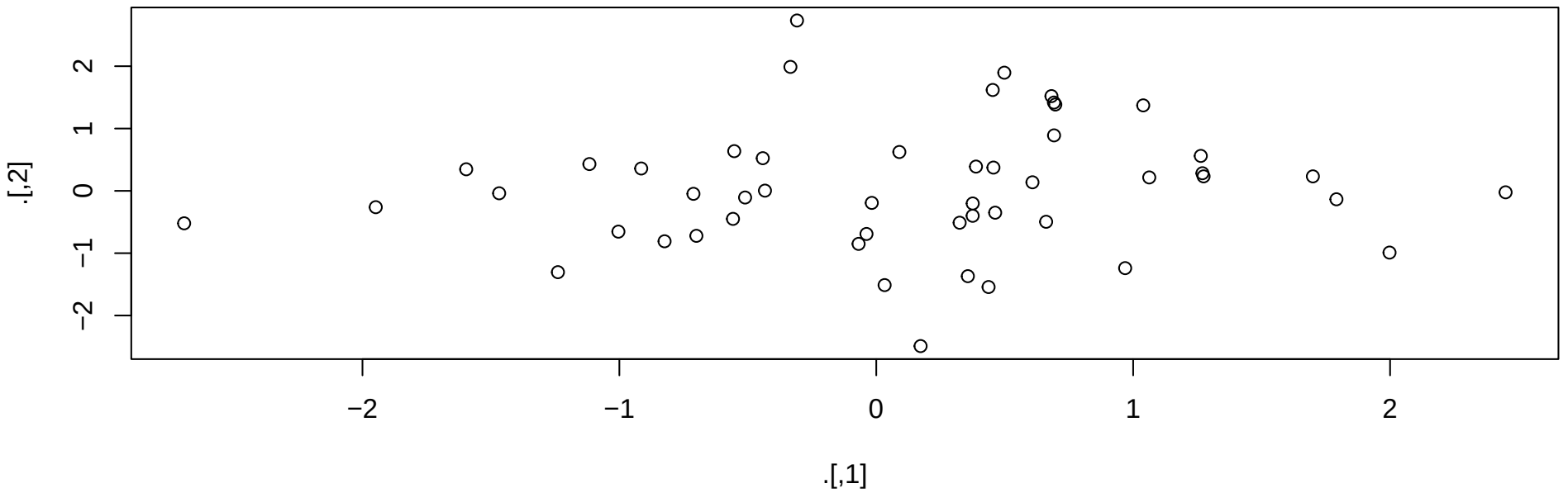
%T>%：返回上游的值（???）

```
library(magrittr)
## 示例：res1 是空值 ...
res1 <-
  rnorm(100) %>%
    matrix(ncol = 2) %>%
    plot();
```



%T>%: 返回上游值 (left-side values)

```
## 示例: res2 是 matrix() 内容 ...
res2 <-
  rnorm(100) %>%
    matrix(ncol = 2) %T>%
    plot();
```



%T>%: 返回上游值 (left-side values), cont.

```
head(res2);
```

```
##              [,1]       [,2]
## [1,]  0.96879759 -1.2398801
## [2,] -0.01740683 -0.1932372
## [3,] -1.23919452 -1.3045170
## [4,] -0.55765986 -0.4495766
## [5,] -0.55285251  0.6367222
## [6,]  0.32460850 -0.5111303
```

%$% : attach ???

```
attach( mtcars ); ## note the warning message ...
```

```
## The following object is masked from package:ggplot2:
##
##     mpg
```

```
cor.test( cyl, mpg ); ## 汽缸数与燃油效率
```

```
##
##  Pearson's product-moment correlation
##
## data:  cyl and mpg
## t = -8.9197, df = 30, p-value = 6.113e-10
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.9257694 -0.7163171
## sample estimates:
##       cor
## -0.852162
```

%$% : attach ??? , cont.

```
detach( mtcars );
with( mtcars, cor.test( cyl, mpg ) );
```

```
##
##  Pearson's product-moment correlation
##
## data:  cyl and mpg
## t = -8.9197, df = 30, p-value = 6.113e-10
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.9257694 -0.7163171
## sample estimates:
##       cor
## -0.852162
```

%$% : attach ??? , cont.

```
mtcars %$%
  cor.test( cyl, mpg );
```

```
##
##  Pearson's product-moment correlation
##
## data:  cyl and mpg
## t = -8.9197, df = 30, p-value = 6.113e-10
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.9257694 -0.7163171
## sample estimates:
##       cor
## -0.852162
```

其它 pipe 及注意事项

```
## 双向 pipe
mtcars %<>% transform(cyl = cyl * 2);
```

注

- pipe 的使用可以使思路更清晰
- 因此，尽量使用 %>% （方向明确），而不使用其它方向不明确的 pipe

section 4: Exercise & home work

练习 & 作业

- Exercises and homework 目录下 talk06-homework.Rmd 文件;
- 完成时间：见钉群的要求

小结

今次提要

1. 3 个生信任务的 R 解决方案
2. factors 的更多应用 (forcats)
3. pipe

下次预告

- Strings and regular expression

important

- all codes are available at Github:
  `https://github.com/evolgeniusteam/R-for-bioinformatics`