

R implementation

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1 Loaded functions:

↑ Project started July 10 2015

```
#source ("~/media/Data/Dropbox/humanR/01funcs.R")
rm(list=ls())
```

2 Load packages.

```
pkgs <- c('xlsx','caret','leaps','glmnet','lattice','latticeExtra',
         'ggplot2', 'dplyr', 'tidyR')
lapply(pkgs, require, character.only = TRUE)
```

3 1 Quality controls and preprocessing

4 Many different options are available while trimming reads.

- 5 • Nature of PCR adapters (trueSeq2 or trueSeq3)
- 6 • Sliding window while reading contigs
- 7 • Crop less than a desired read length
- 8 • Minimum length for reads
- 9 • Trailing while removing the ends of reads with low quality

10 Different trimming options were tested. Iterations were run on combination of the trimming options. The
11 plot shows the number of reads remaining after trimming using different adapters and a combination of
12 trimming parameters (shapes). The *default* parameters includes clipping low quality segments and size
13 of reads. The *slide* option include frameshifting while filtering out low quality reads and the defaults.
14 The *crop* option includes trimming the end of the reads and the defaults. The *slcrop* option includes
15 sliding and cropping and the defaults. The *default2* parameters rely on using different adapters for paired
16 end sequencing. The *slide2* option include frameshifting with the default parameters and different set of
17 adapters.

```
trim <- read.xlsx("./data/Classeur1.xlsx", header = T, sheetName = "Feuille1")
trim <- trim[1:3, ]
key.variety <- list(space = "right",
                      text = list(colnames(trim[, -c(1:2)])),
                      points = list(pch = c(15:18, 25, 4)))

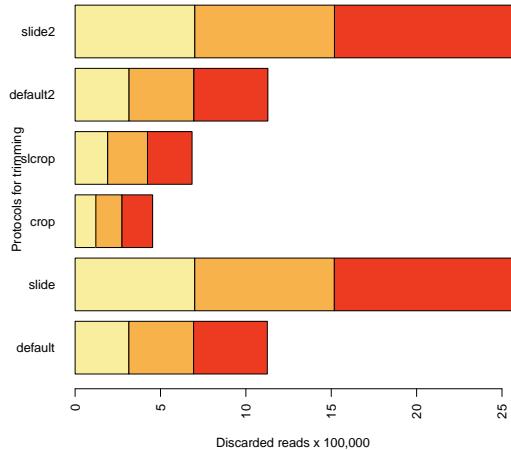
dotplot(c(trim$Total-trim$default)/100000 +
        c(trim$Total-trim$slide)/100000 +
        c(trim$Total-trim$crop)/100000 +
        c(trim$Total-trim$slcrop)/100000 +
        c(trim$Total-trim$default2)/100000 +
        c(trim$Total-trim$slide2)/100000
        ~ trim$Sample,
        data = trim,
        type = 'o',
        pch = c(15:18, 25, 4),
        key = key.variety,
        lty = 1, cex = 1.5,
        xlab = 'Nodule samples',
        ylab = 'Discarded reads x 100,000')
```

18

19 Another way to visualize the discarded reads. A1 = yellow, A2 = orange, A3 = red.

```
custom.colors <- c(col1 = "#ffeda0", col2 = "#feb24c", col3 = "#f03b20")

barplot(as.matrix((trim$Total-trim[, -c(1:2)]) / 100000),
       horiz = TRUE,
       col = custom.colors,
       xlab = 'Discarded reads x 100,000',
       ylab = 'Protocols for trimming',
       las = 2)
```



20

2 Mapping reads to reference

Two sets of reads were mapped to 3 different assembled references. First batch from the trimmed reads with the default parameters (adapters clipping, trailing, and minimum length) and TrueSeq3 adapters. Second batch were also trimmed with default settings but using TrueSeq2 adapters. With the second batch of adapters, more reads were trimmed and discarded. This analysis will try to show the regression of mapped reads to the length of each reference.

[†]The references v017 and genome v21 are test references, while genome v015 is the one used in the published work.

```
ref.genome1 <- read.table("./data/refGenome/A1.htseq.counts.txt")
ref.genome2 <- read.table("./data/refGenome/A2.htseq.counts.txt")
ref.genome3 <- read.table("./data/refGenome/A3.htseq.counts.txt")
ref.genome4 <- read.table("./data/refGenome/A1-4.htseq.counts.txt")
ref.genome5 <- read.table("./data/refGenome/A2-4.htseq.counts.txt")
ref.genome6 <- read.table("./data/refGenome/A3-4.htseq.counts.txt")
```

27 Merge by reference position all the mapped reads from different trimming options.

```

ref.genome <- data.frame(
  A1 = ref.genome1[-c(556:560), 2],
  A2 = ref.genome2[-c(556:560), 2],
  A3 = ref.genome3[-c(556:560), 2],
  A1.4 = ref.genome4[-c(556:560), 2],
  A2.4 = ref.genome5[-c(556:560), 2],
  A3.4 = ref.genome6[-c(556:560), 2],
  contigs = ref.genome1[-c(556:560), 1])
dim(ref.genome)
[1] 555    7

genome <- read.table("./data/QPX_Genome_v017.gff3")
genome1 <- data.frame(contigs= genome[,1], length = genome$V5)

```

28 Merge by reference position the length and number of mapped reads.

```

ref.genome.mix <- merge(genome1, ref.genome)
head(ref.genome.mix)

  contigs length   A1   A2   A3 A1.4 A2.4 A3.4
1 QPX_v017_contig_1007 15433 117 197 249 117 197 249
2 QPX_v017_contig_1020 12397 123 164 171 123 164 171
3 QPX_v017_contig_1021 18562 335 487 596 335 488 596
4 QPX_v017_contig_1023 19919 116 198 331 116 198 331
5 QPX_v017_contig_103 10989 71 111 107 71 111 105
6 QPX_v017_contig_1034 10178 196 289 655 196 289 655

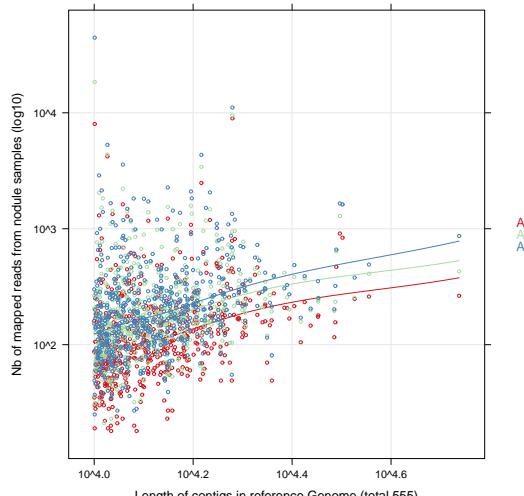
```

29 Correlation between read length and number of mapped reads on the (testing) genome of QPX with the
30 remaining reads from the default trimming with TrueSeq3 adapters.

```

custom.colors <- c('#d7191c', '#abdd4', '#2b83ba')
key.variety <- list(space = "right",
                      text = list(colnames(ref.genome.mix[, 3:5])),
                      col = custom.colors)
xyplot(A1 + A2 + A3 ~ length,
       data = ref.genome.mix,
       xlab = 'Length of contigs in reference Genome (total 555)',
       ylab = 'Nb of mapped reads from nodule samples (log10)',
       col = custom.colors,
       cex = 0.5,
       type = c("g", "p", "smooth"),
       scales = list(log = 10),
       key = key.variety)

```



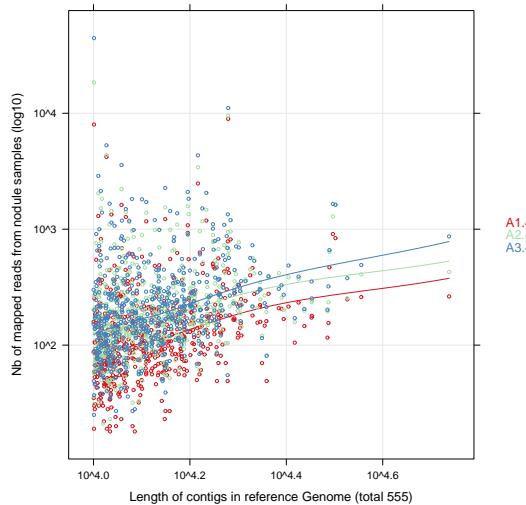
[†] Data are from trimming with TrueSeq3 (selected) and TrueSeq2 (testing). We only plot TrueSeq3.

31
32 Regression between reads and length of contigs in reference genome with (testing) adapters TrueSeq2
33 under default trimming settings.

```

custom.colors <- c('#d7191c', '#abddaa4', '#2b83ba')
key.variety <- list(space = "right",
                      text = list(colnames(ref.genome.mix[, 6:8])),
                      col = custom.colors)
xyplot(A1.4 + A2.4 + A3.4 ~ length,
#                               alpha = .5,
  data = ref.genome.mix,
  xlab = 'Length of contigs in reference Genome (total 555)',
  ylab = 'Nb of mapped reads from nodule samples (log10)',
  col = custom.colors,
  cex = 0.5,
  type = c("g", "p", "smooth"),
  scales = list(log = 10),
  key = key.variety)

```



34
35 Previously we regressed the number of mapped reads over the reference genome (v21) of QPX. Now its
36 time to do the same thing over the reference transcriptome of QPX (v17). Both references belong the
37 Steve Roberts.

```

ref.transcriptome1 <- read.table("./data/refTranscriptome/A1.htseq.counts.txt")
ref.transcriptome2 <- read.table("./data/refTranscriptome/A2.htseq.counts.txt")
ref.transcriptome3 <- read.table("./data/refTranscriptome/A3.htseq.counts.txt")
ref.transcriptome4 <- read.table("./data/refTranscriptome/A1-4.htseq.counts.txt")
ref.transcriptome5 <- read.table("./data/refTranscriptome/A2-4.htseq.counts.txt")
ref.transcriptome6 <- read.table("./data/refTranscriptome/A3-4.htseq.counts.txt")
dim(ref.transcriptome1)

[1] 11779      2

tail(ref.transcriptome1)

          V1      V2
11774 QPX_transcriptome_v2_Contig_9_3     6
11775           __no_feature      0
11776           __ambiguous    568
11777           __too_low_aQual 147407
11778           __not_aligned 29746511
11779           __alignment_not_unique      0

```

38 Merge by the position on the reference transcriptome all the mapped reads.

```
ref.transcriptome <- data.frame(
```

[†] Data is from trimming with TrueSeq3 (selected) and TrueSeq2 (testing). We only plot TruSeq3

```

A1 = ref.transcriptome1[-c(11775:11779), 2],
A2 = ref.transcriptome2[-c(11775:11779), 2],
A3 = ref.transcriptome3[-c(11775:11779), 2],
A1.4 = ref.transcriptome4[-c(11775:11779), 2],
A2.4 = ref.transcriptome5[-c(11775:11779), 2],
A3.4 = ref.transcriptome6[-c(11775:11779), 2],
contigs = ref.transcriptome1[-c(11775:11779), 1])
dim(ref.transcriptome)

[1] 11774      7

head(ref.transcriptome)

  A1 A2 A3 A1.4 A2.4 A3.4           contigs
1  0  2  0    0    2    0 QPX_transcriptome_v2_Contig_10002_1
2  0  0  0    0    0    0 QPX_transcriptome_v2_Contig_10002_2
3  2  2  7    2    2    7 QPX_transcriptome_v2_Contig_1000_1
4  1  0  0    1    0    0 QPX_transcriptome_v2_Contig_1000_2
5  0  0  0    0    0    0 QPX_transcriptome_v2_Contig_1000_3
6 58 72 99   59   72  100 QPX_transcriptome_v2_Contig_1000_4

transcriptome <- read.table("./data/QPX_transcriptome_v2orf.gff3")
transcriptome1 <- data.frame(contigs= transcriptome[,1], length = transcriptome$V5)

```

39 Merge by the position on the reference transcriptome the length and number of the mapped reads.

```

ref.transcriptome.mix <- merge(transcriptome1, ref.transcriptome)
head(ref.transcriptome.mix)

  contigs length  A1  A2  A3 A1.4 A2.4
1 QPX_transcriptome_v2_Contig_10_1    345    0    0    0    0    0
2 QPX_transcriptome_v2_Contig_10_2   1416   222   253  469  222  253
3 QPX_transcriptome_v2_Contig_10_3    234    0    0    0    0    0
4 QPX_transcriptome_v2_Contig_10_4    201    0    0    0    0    0
5 QPX_transcriptome_v2_Contig_1000_1   201    2    2    7    2    2
6 QPX_transcriptome_v2_Contig_1000_2   258    1    0    0    1    0
A3.4
1    0
2   469
3    0
4    0
5    7
6    0

```

40 Plot the correlation between read length and number of mapped reads on the transcriptome of QPX with the remaining reads from the default trimming with TrueSeq3 adapters.

```

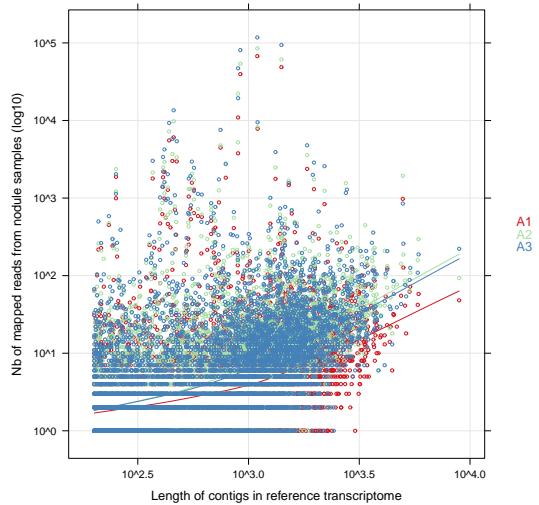
custom.colors <- c('#d7191c', '#abdda4', '#2b83ba')
key.variety <- list(space = "right",
                      text = list(colnames(ref.transcriptome.mix[, 3:5])),
                      col = custom.colors)
xyplot(A1 + A2 + A3 ~ length,
       data = ref.transcriptome.mix,
       xlab = 'Length of contigs in reference transcriptome',
       ylab = 'Nb of mapped reads from nodule samples (log10)',
       col = custom.colors,
       cex = 0.5,
       type = c("g", "p", "smooth"),
       scales = list(log = 10),
       key = key.variety)

```

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2.1 Concentration of contigs in different libraries

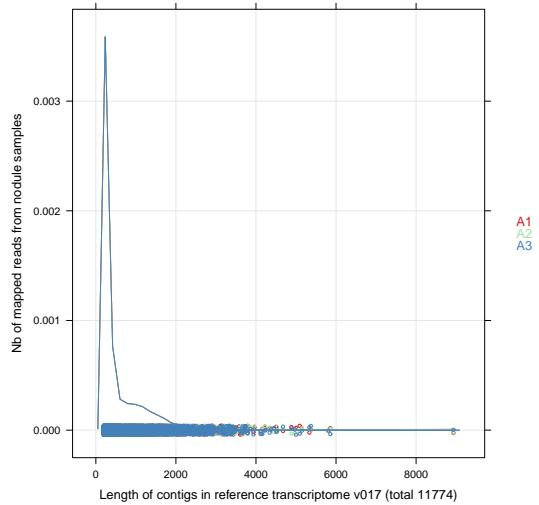
Density plot between reference transcriptome and assembled contigs. The plot shows a high concentration of contigs under 2000 base pair. Trimming parameters are of default with TrueSeq3 adapters.



```
custom.colors <- c('#d7191c', '#abdda4', '#2b83ba')
key.variety <- list(space = "right",
                      text = list(colnames(ref.genome.mix[, 3:5])),
                      col = custom.colors)
densityplot(A1 + A2 + A3 ~ length,
            data = ref.transcriptome.mix,
            #           alpha = .7,
            xlab = 'Length of contigs in reference transcriptome v017 (total 11774)',
            ylab = 'Nb of mapped reads from nodule samples',
            col = custom.colors,
            cex = 0.5,
            type = c("g", "p", "smooth"),
            #           scales = list(log = 10),
            key = key.variety)
```

47
48

Plot correlation same as above but with TrueSeq2 adapters with default parameters.

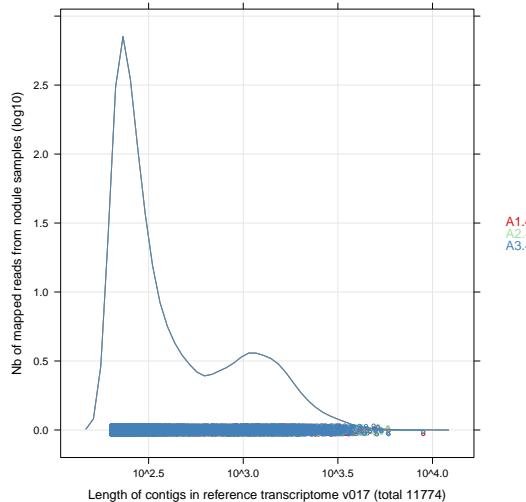


```

key.variety <- list(space = "right",
                     text = list(colnames(ref.genome.mix[, 6:8])),
                     col = custom.colors)
densityplot(A1.4 + A2.4 + A3.4 ~ length,
            data = ref.transcriptome.mix,
#             alpha = .9,
            xlab = 'Length of contigs in reference transcriptome v017 (total 11774)',
            ylab = 'Nb of mapped reads from nodule samples (log10)',
            col = custom.colors,
            cex = 0.5,
            type = c("g", "p", "smooth"),
            scales = list(log = 10),
            key = key.variety)

Warning in densityplot.formula(A1.4 + A2.4 + A3.4 ~ length, data = ref.transcriptome.mix,
: Can't have log Y-scale

```



49
50 **2.2 Testing contig length and mapping with different assembled references**
51 Load the number of mapped reads to the MMETSP0098 transcriptome before discarding duplicates.

```

ref.dupA1R3 <- read.table("./data/refMME98/A1.htseq.counts.txt")
ref.dupA2R3 <- read.table("./data/refMME98/A2.htseq.counts.txt")
ref.dupA3R3 <- read.table("./data/refMME98/A3.htseq.counts.txt")

```

52 Merge all mapped reads to MMETSP0098 reference transcriptome before discarding duplicates (ie, raw counts).

```

ref.mme98 <- data.frame(A1 = ref.dupA1R3$V2,
                         A2 = ref.dupA2R3$V2,
                         A3 = ref.dupA3R3$V2,
                         contigs = ref.dupA1R3$V1)

```

54 Add the length values to each contig mapped to MMETsp0098. But first remove extra rows.

```

nr <- nrow(ref.mme98)
ref.mme98 <- ref.mme98[1:(nr-5), ]
tail(ref.mme98)

      A1  A2  A3          contigs
15484  0  0  0 MMETSP0098-20131031|9992
15485 12  6 17 MMETSP0098-20131031|9993
15486  0  0  0 MMETSP0098-20131031|9995
15487  3  3  3 MMETSP0098-20131031|9996
15488  0  0  0 MMETSP0098-20131031|9998
15489  2  9  5 MMETSP0098-20131031|9999

```

↑ MMETSP is a project for sequencing different strains of QPX. MMETSP0098 and MMETPS00992 were used here. Both strains come from New York and Virginia respectively. Official project can be found [here](#).

55 Load the number of mapped reads to the MMETSP0099_2 transcriptome before discarding duplicates.

```
ref.dupA1R4 <- read.table("./data/refMME992/A1.htseq.counts.txt")
ref.dupA2R4 <- read.table("./data/refMME992/A2.htseq.counts.txt")
ref.dupA3R4 <- read.table("./data/refMME992/A3.htseq.counts.txt")
```

56 Merge all mapped reads to MMETSP0099_2 reference transcriptome before discarding duplicates (ie, raw counts).

```
ref.mme992 <- data.frame(A1 = ref.dupA1R4$V2,
                           A2 = ref.dupA2R4$V2,
                           A3 = ref.dupA3R4$V2,
                           contigs = ref.dupA1R4$V1)
```

58 Add the length values to each contig mapped to MMETsp0099_2. But first remove extra rows.

```
nr <- nrow(ref.mme992)
ref.mme992 <- ref.mme992[1:(nr-5), ]
tail(ref.mme992)

      A1  A2  A3          contigs
11762  0  0  0 MMETSP0099_2-20121227|9994
11763  0  8  9 MMETSP0099_2-20121227|9995
11764  0  0  0 MMETSP0099_2-20121227|9996
11765  0  0  1 MMETSP0099_2-20121227|9997
11766  0  0  0 MMETSP0099_2-20121227|9998
11767  0  0  0 MMETSP0099_2-20121227|9999
```

59 Load the number of mapped reads to SR v015 genome before discarding duplicates.

```
ref.dupA1R5 <- read.table("./data/refGenomV015/A1.htseq.counts.txt")
ref.dupA2R5 <- read.table("./data/refGenomV015/A2.htseq.counts.txt")
ref.dupA3R5 <- read.table("./data/refGenomV015/A3.htseq.counts.txt")
```

60 Merge all mapped reads to SR v015 reference genome before discarding duplicates (ie, raw counts).

```
ref.genomv015 <- data.frame(A1 = ref.dupA1R5$V2,
                               A2 = ref.dupA2R5$V2,
                               A3 = ref.dupA3R5$V2,
                               contigs = ref.dupA1R5$V1)
```

61 Add the length values to each contig mapped to SR v015 reference genome. But first remove extra rows.

```
nr <- nrow(ref.genomv015)
ref.genomv015 <- ref.genomv015[1:(nr-5), ]
tail(ref.genomv015)

      A1  A2  A3          contigs
21275  0  1  2 QPX_v015_contig_9994
21276  0  0  0 QPX_v015_contig_9995
21277  1  5  3 QPX_v015_contig_9996
21278  1  2  2 QPX_v015_contig_9997
21279  3  1  2 QPX_v015_contig_9998
21280  9  5 21 QPX_v015_contig_9999
```

63 After aligning the reads to a reference duplicates must be removed. Testing was done with different reference transcriptomes and genomes to assess the strength of the parameters used for removing the duplicate reads and reducing bias for better coverage.

64 First load the sample reads mapped to reference genome (without duplication) of Steve Roberts.

```
nodupA1R1 <- read.table("./data/nodupR1/A1.htseq.nodup.counts.txt")
nodupA2R1 <- read.table("./data/nodupR1/A2.htseq.nodup.counts.txt")
nodupA3R1 <- read.table("./data/nodupR1/A3.htseq.nodup.counts.txt")
```

67 Second load the sample reads mapped to reference transcriptome (withtout duplication) of Steve Roberts.

68

```
nodupA1R2 <- read.table("./data/nodupR2/A1.htseq.nodup.counts.txt")
nodupA2R2 <- read.table("./data/nodupR2/A2.htseq.nodup.counts.txt")
nodupA3R2 <- read.table("./data/nodupR2/A3.htseq.nodup.counts.txt")
```

69 Third load the sample reads mapped to reference transcriptome (without duplication) of MMESTO0098.

```
nodupA1R3 <- read.table("./data/nodupR3/A1.htseq.counts.nodup.txt")
nodupA2R3 <- read.table("./data/nodupR3/A2.htseq.counts.nodup.txt")
nodupA3R3 <- read.table("./data/nodupR3/A3.htseq.counts.nodup.txt")
```

70 Forth load of sample reads mapped to reference transcriptome MMETSP0099_2.

```
nodupA1R4 <- read.table("./data/nodupR4/A1.htseq.counts.nodup.txt")
nodupA2R4 <- read.table("./data/nodupR4/A2.htseq.counts.nodup.txt")
nodupA3R4 <- read.table("./data/nodupR4/A3.htseq.counts.nodup.txt")
```

71 Forth load of sample reads mapped to reference SR genome v015 with approximately 21,000 contigs.

```
nodupA1R5 <- read.table("./data/nodupR5/A1.htseq.counts.nodup.txt")
nodupA2R5 <- read.table("./data/nodupR5/A2.htseq.counts.nodup.txt")
nodupA3R5 <- read.table("./data/nodupR5/A3.htseq.counts.nodup.txt")
```

72 Merge mapped reads relative to the following references.

- 73 • R1 = genome of QPX (steve roberts, 555 contigs)
- 74 • R2 = transcriptome of QPX (steve roberts)
- 75 • R3 = transcriptome of QPX MMETSP0098, New York strain
- 76 • R4 = transcriptome of QPX MMETSP0099_2, Virginia strain
- 77 • R5 = genome of QPX (steve roberts v015, approx. 21,000 contigs)

```
allR1 <- data.frame(A1n = nodupA1R1$V2,
                      A2n = nodupA2R1$V2,
                      A3n = nodupA3R1$V2,
                      reference = rep("genomSRv017", nrow(nodupA1R1)),
                      contigs = nodupA1R1$V1)
allR1 <- allR1[1:555, ]

allR2 <- data.frame(A1n = nodupA1R2$V2,
                      A2n = nodupA2R2$V2,
                      A3n = nodupA3R2$V2,
                      reference = rep("trxSRv022", nrow(nodupA1R2)),
                      contigs = nodupA1R2$V1)
allR2 <- allR2[1:11774, ]

allR3 <- data.frame(A1n = nodupA1R3$V2,
                      A2n = nodupA2R3$V2,
                      A3n = nodupA3R3$V2,
                      reference = rep("trxMME98", nrow(nodupA1R3)),
                      contigs = nodupA1R3$V1)
allR3 <- allR3[1:15489, ]

allR4 <- data.frame(A1n = nodupA1R4$V2,
                      A2n = nodupA2R4$V2,
                      A3n = nodupA3R4$V2,
                      reference = rep("trxMME992", nrow(nodupA1R4)),
                      contigs = nodupA1R4$V1)
allR4 <- allR4[1:c(nrow(nodupA1R4))-5], 

allR5 <- data.frame(A1n = nodupA1R5$V2,
                      A2n = nodupA2R5$V2,
                      A3n = nodupA3R5$V2,
                      reference = rep("genomSRv015", nrow(nodupA1R5)),
                      contigs = nodupA1R5$V1)
allR5 <- allR5[1:c(nrow(nodupA1R5))-5],
```

78 Put before /after duplicates removal in one dataset for genome of Steve Roberts.

```
genomeSR <- merge(ref.genome.mix[, 1:5], allR1)
head(genomeSR)

  contigs length A1 A2 A3 A1n A2n A3n  reference
1 QPX_v017_contig_1007 15433 117 197 249 109 191 240 genomSRv017
2 QPX_v017_contig_1020 12397 123 164 171 118 157 159 genomSRv017
3 QPX_v017_contig_1021 18562 335 487 596 319 460 568 genomSRv017
4 QPX_v017_contig_1023 19919 116 198 331 109 196 326 genomSRv017
5 QPX_v017_contig_103 10989 71 111 107 68 106 104 genomSRv017
6 QPX_v017_contig_1034 10178 196 289 655 185 279 592 genomSRv017

rownames(genomeSR) <- genomeSR$contigs
genomeSR <- t(genomeSR[, -c(1, 9)])
genomeSR[, 1:3]

  QPX_v017_contig_1007 QPX_v017_contig_1020 QPX_v017_contig_1021
length          15433           12397          18562
A1              117             123            335
A2              197             164            487
A3              249             596            596
A1n             109             118            319
A2n             191             157            460
A3n             240             159            568

genomeSR <- data.frame(genomeSR,
                         y = c(2, rep(0, 3), rep(1, 3)))
```

79 Put the before /after duplicates removal in one dataset for transcriptome of SR.

```
transcriptomeSR <- merge(ref.transcriptome.mix[, 1:5], allR2)
head(transcriptomeSR)

  contigs length A1 A2 A3 A1n A2n A3n  reference
1 QPX_transcriptome_v2_Contig_10_1    345   0   0   0   0   0   0
2 QPX_transcriptome_v2_Contig_10_2   1416  222  253  469  202  238  409
3 QPX_transcriptome_v2_Contig_10_3    234   0   0   0   0   0   0
4 QPX_transcriptome_v2_Contig_10_4    201   0   0   0   0   0   0
5 QPX_transcriptome_v2_Contig_1000_1   201   2   2   7   2   2   7
6 QPX_transcriptome_v2_Contig_1000_2   258   1   0   0   1   0   0
reference
1 trxSRv022
2 trxSRv022
3 trxSRv022
4 trxSRv022
5 trxSRv022
6 trxSRv022
```

80 Present difference for each sample mapped to the references. First merge all samples before /after duplicates were removed.

```
allRefs <- rbind(allR1, allR2, allR3, allR4, allR5)
```

```

dim(allRefs)
[1] 60865      5

summary(allRefs$reference)
genomSRv017    trxSRv022    trxMME98    trxMME992  genomSRv015
                555        11774       15489       11767       21280

allRefs.raw <- rbind(ref.genome.mix[, 3:5],
                      ref.transcriptome.mix[, 3:5],
                      ref.mme98[, 1:3],
                      ref.mme992[, 1:3],
                      ref.genomv015[, 1:3])
dim(allRefs.raw)
[1] 60865      3

allDF <- cbind(allRefs, allRefs.raw)
allDF[sample(1:20000, 5), ]

   A1n A2n A3n reference                               contigs A1 A2
559     1   0   0 trxSRv022 QPX_transcriptome_v2_Contig_1000_2  0   0
17259    5  13  11 trxMME98           MMETSP0098-20131031|15673  5  13
4971     0   0   0 trxSRv022 QPX_transcriptome_v2_Contig_3035_4  0   0
9008     0   0   0 trxSRv022 QPX_transcriptome_v2_Contig_5719_3  0   0
9904     0   0   0 trxSRv022 QPX_transcriptome_v2_Contig_6569_1  0   0

   A3
559     0
17259   11
4971     0
9008     0
9904     0

```

82 Plot the difference before and after duplicates were discarded. The number of mapped reads to the
 83 reference contigs is descriptive for any bias in contig assembly. For example in the case of SR genome
 84 v017, more than 20 % of A1, A2, A3 reads align to a small set of contigs. The best distribution is a
 85 constant one.

86 Even though we did not plot length of contigs, the analyzes above demonstrate that length is linearly
 87 correlated to the number of mapped reads. Therefore, peaks indicate a specific preference that reads
 88 have to map to an assembled reference.

```
custom.colors <- c(col1 = "#762a83",
```

[†] A high resolution version of the plot below can be found in the Supplemental Information

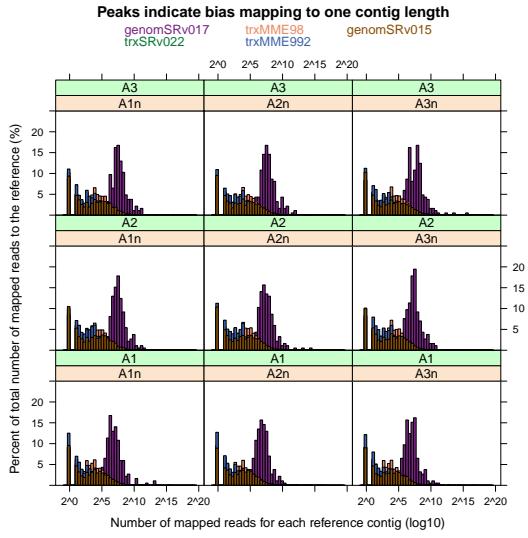
```

        col2 = "#1b7837",
        col3 = "#ef8a62",
        col4 = "#2166ac",
        col5 = "#8c510a",
        col6 = "#e6ab02")

histogram( ~ A1 + A2 + A3 | c('A1n', 'A2n', 'A3n'),
  data = allDF,
  nint = 50,
  scales = list(log = 2),
  type = "p",
  ylim = c(0,25),
  groups = allDF$reference,
  panel = function(...) panel.superpose(...,
    panel.groups = panel.histogram,
    col = custom.colors,
    alpha = 1),
  auto.key=list(columns=3,
    rectangles = FALSE,
    col = custom.colors),
  main = 'Peaks indicate bias mapping to one contig length',
  ylab = 'Percent of total number of mapped reads to the reference (%)',
  xlab = 'Number of mapped reads for each reference contig (log10)'
)

Warning in histogram.formula(~A1 + A2 + A3 | c("A1n", "A2n", "A3n"), data = allDF,
: Can't have log Y-scale
Warning in pmax('c("A1n", "A2n", "A3n")' = c(FALSE, FALSE, FALSE, FALSE, : an
argument will be fractionally recycled
Warning in id & (if (is.shingle(var)) ((var >= levels(var)[[levels[i]]][1]) &
: longer object length is not a multiple of shorter object length
Warning in pmax('c("A1n", "A2n", "A3n")' = c(FALSE, FALSE, FALSE, FALSE, : an
argument will be fractionally recycled
Warning in id & (if (is.shingle(var)) ((var >= levels(var)[[levels[i]]][1]) &
: longer object length is not a multiple of shorter object length
Warning in pmax('c("A1n", "A2n", "A3n")' = c(FALSE, FALSE, FALSE, FALSE, : an
argument will be fractionally recycled
Warning in id & (if (is.shingle(var)) ((var >= levels(var)[[levels[i]]][1]) &
: longer object length is not a multiple of shorter object length
Warning in pmax('c("A1n", "A2n", "A3n")' = c(FALSE, FALSE, FALSE, FALSE, : an
argument will be fractionally recycled
Warning in id & (if (is.shingle(var)) ((var >= levels(var)[[levels[i]]][1]) &
: longer object length is not a multiple of shorter object length
Warning in pmax('c("A1n", "A2n", "A3n")' = c(FALSE, FALSE, FALSE, FALSE, : an
argument will be fractionally recycled
Warning in id & (if (is.shingle(var)) ((var >= levels(var)[[levels[i]]][1]) &
: longer object length is not a multiple of shorter object length
Warning in pmax('c("A1n", "A2n", "A3n")' = c(FALSE, FALSE, FALSE, FALSE, : an
argument will be fractionally recycled
Warning in id & (if (is.shingle(var)) ((var >= levels(var)[[levels[i]]][1]) &
: longer object length is not a multiple of shorter object length
Warning in pmax('c("A1n", "A2n", "A3n")' = c(FALSE, FALSE, FALSE, FALSE, : an
argument will be fractionally recycled
Warning in id & (if (is.shingle(var)) ((var >= levels(var)[[levels[i]]][1]) &
: longer object length is not a multiple of shorter object length
Warning in pmax('c("A1n", "A2n", "A3n")' = c(FALSE, FALSE, FALSE, FALSE, : an
argument will be fractionally recycled
Warning in id & (if (is.shingle(var)) ((var >= levels(var)[[levels[i]]][1]) &
: longer object length is not a multiple of shorter object length

```



89

3 Extracting QPX reads

91 Load file with read counts per sample.

- A samples as nodule tissue
 - B samples as non nodule diseased tissue
 - C samples as non nodule non diseased tissue

95 Raw reads have been trimmed, mapped to reference genome (Steve Roberts v15 with 21280 contigs),
96 sorted by position on the genome, and cleaned from duplicated reads.

↑ Hypothetically these raw reads includes specific QPX reads

```

reads.counts <- read.xlsx("./data/mappedNodules.xlsx", sheetIndex = 1)
reads.counts$fill <- gl(3, 3, 9, labels = c("a", "b", "c"))
ggplot(reads.counts,
       aes(x = sample,
            y = raw.reads,
            fill = fill)) +
  coord_flip() +
  theme_bw() +
  geom_bar(stat = "identity") +
  geom_text(aes(x = sample,
                y = raw.reads,
                ymax = raw.reads,
                label = raw.reads,
                size = 2,
                hjust = 1.3)) +
  scale_fill_hue(c = 40, l = 60) +
  labs(x = "Number of raw sequenced reads",
       y = "Sequenced replicated samples")

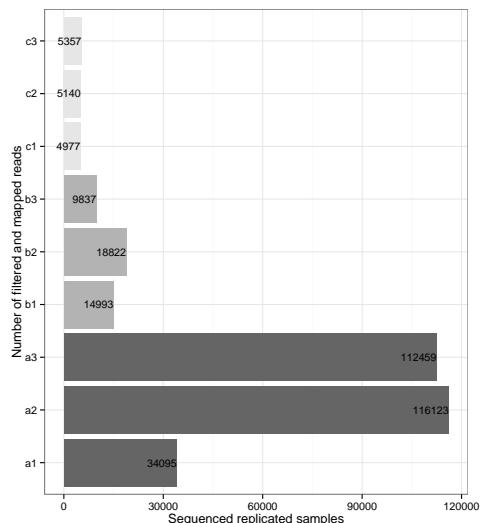
```

97

98 Number of reads that mapped to the reference genome of QPX.

↑ These reads are probably those of QPX's

```
ggplot(reads.counts,
  aes(x = sample,
      y = mapped.reads,
      fill = fill)) +
  geom_bar(stat = "identity") +
  geom_text(aes(x = sample,
                y = mapped.reads,
                ymax = mapped.reads,
                label = mapped.reads,
                hjust = 1,
                size = 2)) +
  coord_flip() +
  theme_bw() +
  scale_fill_grey(start = .4, end = .9) +
  labs(x = "Number of filtered and mapped reads",
       y = "Sequenced replicated samples")
```



99

100 Mapped reads to the QPX reference are then assembled into contigs (ie, the reads showing in the chart above).

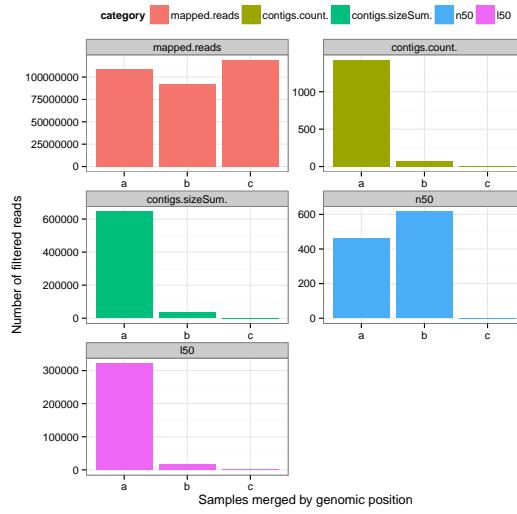
↑ These contigs must be specific transcripts to QPX

```
reads.counts <- read.xlsx("./data/mappedNodules.xlsx", sheetIndex = 2)
```

```

reads.counts <- gather(reads.counts, "category", "counts", 3:7)
ggplot(reads.counts,
  aes(x = sample,
    y = counts,
    fill = category)) +
  geom_bar(stat = "identity") +
  theme_bw() +
  facet_wrap( ~ category,
    ncol = 2,
    scales = "free") +
  theme(legend.position = "top") +
  labs(x = "Samples merged by genomic position",
    y = "Number of filtered reads")

```



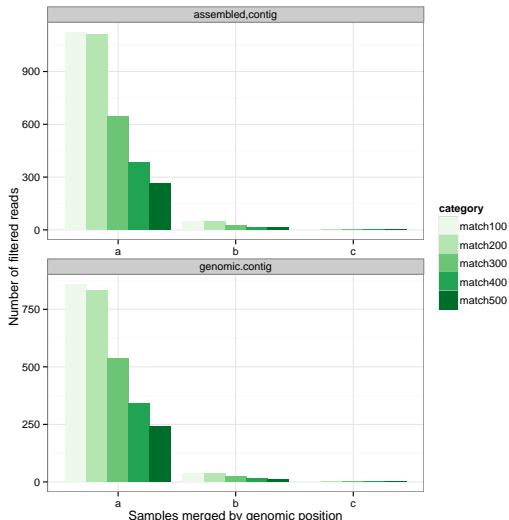
102
103 All contigs were then aligned to the reference genome QPX. The chart shows the number of contigs (both
104 genomic and mRNA sequenced) that align with an increasing length of 100>200>300>400>500.

↳ Helps discard misassembled
contigs or non QPX ones

```

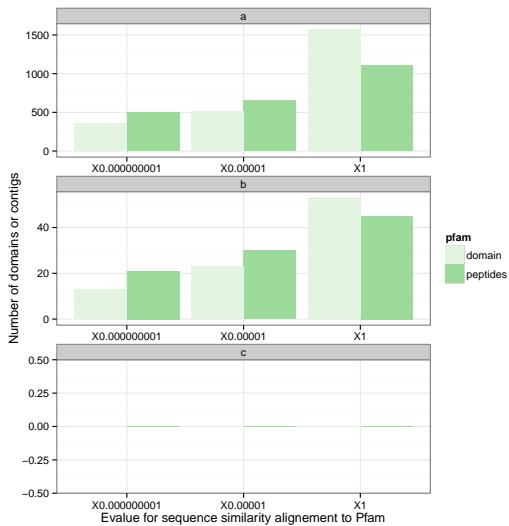
reads.counts <- read.xlsx("./data/mappedNodules.xlsx", sheetIndex = 4)
reads.counts <- gather(reads.counts, "category", "count", 3:7)
ggplot(reads.counts,
  aes(x = sample,
    y = count,
    fill = category)) +
  theme_bw() +
  geom_bar(stat = "identity",
    position = "dodge") +
  facet_wrap( ~ blat, ncol = 1, scale = "free") +
  scale_fill_manual(values = brewer.pal(5, "Greens")) +
  labs(x = "Samples merged by genomic position",
    y = "Number of filtered reads")

```



105
106 All contigs were then translated to peptides in 6 frames. Each peptide was then aligned to the whole
107 PFAM library (v28, date: Jul 14 2015).

```
reads.counts <- read.xlsx("./data/mappedNodules.xlsx", sheetIndex = 3)
reads.counts <- gather(reads.counts, "category", "count", 3:5)
ggplot(reads.counts,
  aes(x = category,
      y = count,
      fill = pfam)) +
  geom_bar(stat = "identity",
            position = "dodge") +
  theme_bw() +
  facet_wrap(~ sample, ncol = 1,
            scales = "free") +
  scale_fill_manual(values = brewer.pal(3, "Greens")) +
  labs(x = "Evalue for sequence similarity alignment to Pfam",
       y = "Number of domains or contigs")
```



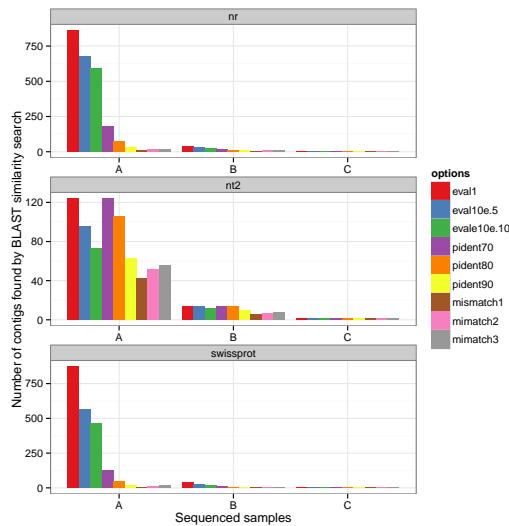
108
109 **3.1 Evaluating identified QPX contigs with sequence similarities**
110 BLAST is used at this step to align the contigs found to non redundant (NR), nucleotide (NT), and swis-
111 sprot databases. Contigs were translated into peptide sequencing with EMBOSS transeq in all 6 possible
112 frames. All contigs were mapped to genome v15 of S. Roberts. Top hit sequences were filtered either
113 with an evalue score, the percentage of identity between query and target sequences, and the number of
114 mismatches found in the aligned region.

```
blast <- read.xlsx("./data/blast.xlsx", sheetIndex = 1)
```

```

blast <- gather(blast[, -c(13:14)], "options", "counts", 4:12)
ggplot(blast,
  aes(x = sample,
      y = counts,
      fill = options)) +
  geom_bar(stat = "identity",
            position = "dodge") +
  theme_bw() +
#  geom_text(aes(x = sample,
#                 y = counts,
#                 ymax = counts,
#                 label = counts,
#                 size = 1,
#                 hjust = .5),
#            position = position_dodge(width = 1)) +
  facet_wrap(~ ncbi, ncol = 1,
            scales = 'free') +
  labs(x = "Sequenced samples",
       y = "Number of contigs found by BLAST similarity search") +
  scale_fill_brewer(type = "qual",
                    palette = 6)

```



115
 116 Sequences from A, B, C samples are also aligned to 10 other databases. We used interpro accession
 117 numbers to get GO-terms too. We show below the number of contigs if we filter them only by e-value.
 118 The no-filter label represent the total number of sequences per database.

```
interpro <- read.xlsx("./data/interpro.xlsx", sheetIndex = 1)
```

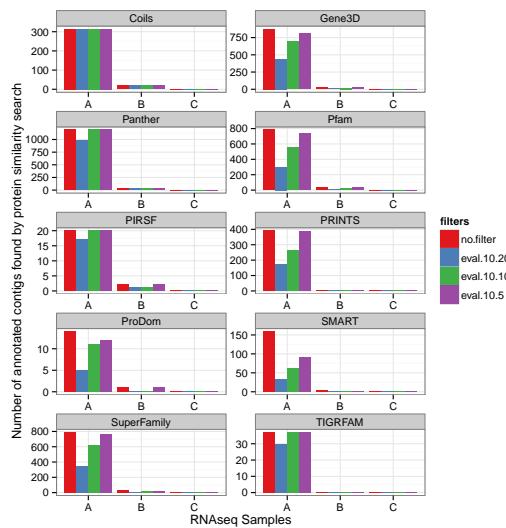
```

interpro <- gather(interpro, "filters", "value", 2:5)
head(interpro)

  database sample   filters value
1  ProDom      A no.filter    14
2  PIRSF       A no.filter   20
3 TIGRFAM      A no.filter   37
4   SMART       A no.filter 159
5   Coils       A no.filter 314
6  PRINTS       A no.filter 391

ggplot(interpro,
  aes(x = sample,
      y = value,
      fill = filters)) +
  geom_bar(stat = "identity",
            position = "dodge") +
  theme_bw() +
  facet_wrap(~ database,
             ncol = 2,
             scales = "free") +
  labs(x = "RNAseq Samples",
       y = "Number of annotated contigs found by protein similarity search") +
  scale_fill_brewer(type = "qual",
                    palette = 6)

```



119

120 We show here the number of contigs annotated by database and filtered by both evalue and the length of
121 the similarity between query and target sequences.

```
interpro <- read.xlsx("./data/interpro.xlsx", sheetIndex = 2)
```

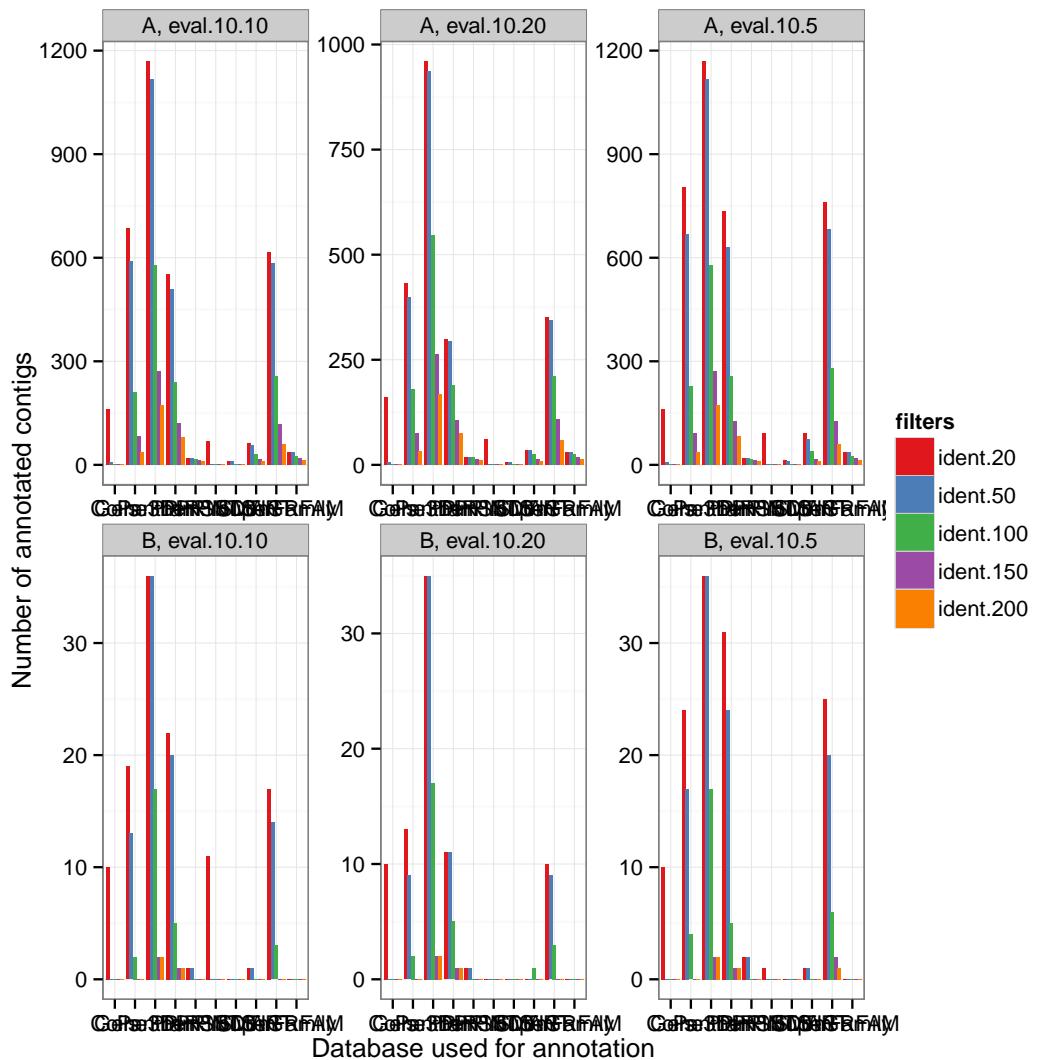
```

interpro <- gather(interpro, "filters", "value", 3:7)
head(interpro)

  database      option sample filters value
1  ProDom eval.10.20      A ident.20     5
2  PIRSF eval.10.20      A ident.20    17
3 TIGRFAM eval.10.20      A ident.20    30
4   SMART eval.10.20      A ident.20    34
5   Coils eval.10.20      A ident.20   161
6 PRINTS eval.10.20      A ident.20    61

ggplot(interpro,
       aes(x = database,
            y = value,
            fill = filters)) +
  theme_bw() +
  geom_bar(stat = "identity",
            position = "dodge") +
  facet_wrap(~ sample + option,
             ncol = 3,
             scales = "free") +
  labs(x = "Database used for annotation",
       y = "Number of annotated contigs") +
  scale_fill_brewer(type = "qual",
                    palette = 6)

```



123 **4 System Information**

124 The version number of R and packages loaded for generating the vignette were:

```
## save(list=ls(pattern=".*/.*"), file="PD.Rdata")
sessionInfo()

R version 3.2.1 (2015-06-18)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows 8 x64 (build 9200)

locale:
[1] LC_COLLATE=English_United States.1252
[2] LC_CTYPE=English_United States.1252
[3] LC_MONETARY=English_United States.1252
[4] LC_NUMERIC=C
[5] LC_TIME=English_United States.1252

attached base packages:
[1] stats      graphics   grDevices utils      datasets   methods
[7] base

other attached packages:
[1] tidyverse_0.2.0    dplyr_0.4.2       latticeExtra_0.6-26
[4] RColorBrewer_1.1-2 glmnet_2.0-2      foreach_1.4.2
[7] Matrix_1.2-1     leaps_2.9        caret_6.0-47
[10] ggplot2_1.0.1    lattice_0.20-31  xlsx_0.5.7
[13] xlsxjars_0.6.1   rJava_0.9-6      knitr_1.10.5
[16] RevoUtilsMath_3.2.1

loaded via a namespace (and not attached):
[1] Rcpp_0.11.6          formatR_1.2       nloptr_1.0.4
[4] plyr_1.8.3           highr_0.5        iterators_1.0.7
[7] tools_3.2.1          digest_0.6.8     lme4_1.1-8
[10] evaluate_0.7         nlme_3.1-120    gtable_0.1.2
[13] mgcv_1.8-6           DBI_0.3.1       parallel_3.2.1
[16] brglm_0.5-9          SparseM_1.6     proto_0.3-10
[19] BradleyTerry2_1.0-6 stringr_1.0.0  gtools_3.5.0
[22] grid_3.2.1           nnet_7.3-9      R6_2.0.1
[25] minqa_1.2.4          reshape2_1.4.1  car_2.0-25
[28] magrittr_1.5          scales_0.2.5    codetools_0.2-11
[31] MASS_7.3-40           splines_3.2.1   assertthat_0.1
[34] pbkrtest_0.4-2        colorspace_1.2-6 labeling_0.3
[37] quantreg_5.11         stringi_0.5-5  lazyeval_0.1.10
[40] munsell_0.4.2
```