

R implementation

Sleiman Bassim, PhD

February 4, 2016

1 Loaded functions:

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

↑ Project started July 10
2015

2 Load packages.

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

3 1 Quality controls and preprocessing

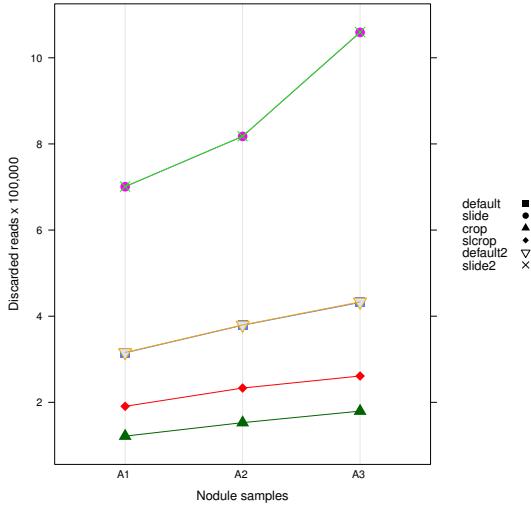
4 Many different options are available while trimming reads.

- 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
11 options. The plot shows the number of reads remaining after trimming using different adapters
12 and a combination of trimming parameters (shapes). The *default* parameters includes clipping
13 low quality segments and size of reads. The *slide* option include frameshiftting while filtering
14 out low quality reads and the defaults. The *crop* option includes trimming the end of the
15 reads and the defaults. The *slcrop* option includes sliding and cropping and the defaults.
16 The *default2* parameters rely on using different adapters for paired end sequencing. The
17 *slide2* option include frameshiftting with the default parameters and different set of adapters.
18

```
trim <- read.xlsx("./data/Classeur1.xlsx", header = T, sheetName = "Feuill1")
trim <- trim[1:3, ]
key.variety <- list(space = "right",
                      text = list(colnames(trim[, -c(1:2)])),
                      points = list(pch = c(15:18, 25, 4)))

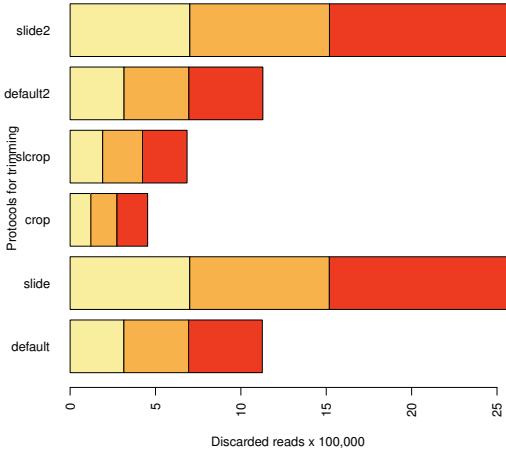
dotplot(c(trim$Total-trim$default)/100000 +
        c(trim$Total-trim$slide)/100000 +
        c(trim$Total-trim$crop)/100000 +
        c(trim$Total-trim$slcrop)/100000 +
        c(trim$Total-trim$default2)/100000 +
        c(trim$Total-trim$slide2)/100000
        ~ trim$Sample,
        data = trim,
        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')
```



19
20 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)
```



21
22 2 Mapping reads to reference
23 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). The references v017 and genome v21 are test
24 and TrueSeq3 adapters. Second batch were also trimmed with default settings but using TrueSeq3 adapters. With the second batch of adapters, more reads were trimmed and discarded. This v015 is the one used in analysis will try to show the regression of mapped reads to the length of each reference.
25
26
27
28

```
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")
```

29 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)

```

30 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

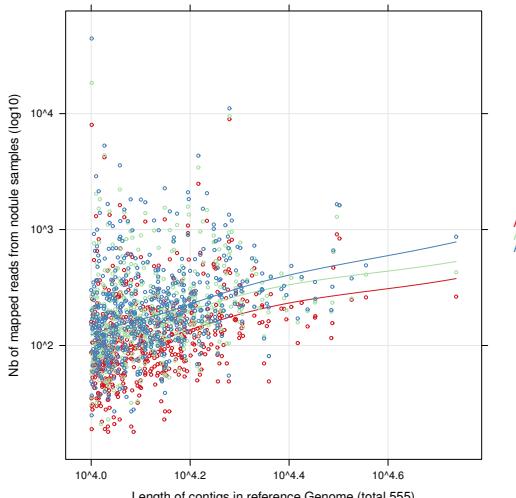
```

31 Correlation between read length and number of mapped reads on the (testing) genome of QPX
 32 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.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)

```

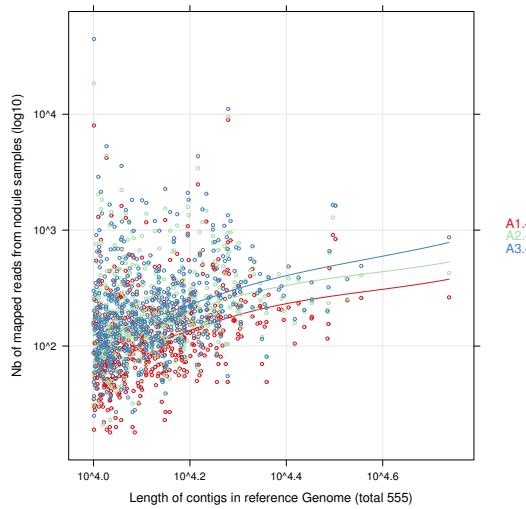


33
 34 Regression between reads and length of contigs in reference genome with (testing) adapters
 35 TrueSeq2 under default trimming settings.

```

custom.colors <- c('#d7191c', '#abdda4', '#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)

```



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

```

40 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)

```

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

```

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

  contigs length A1 A2 A3 A1.4 A2.4 A3.4
1 QPX_transcriptome_v2_Contig_1000_1    201  2  2  7    2    2    7
2 QPX_transcriptome_v2_Contig_1000_2    258  1  0  0    1    0    0
3 QPX_transcriptome_v2_Contig_10002_1   477  0  2  0    0    2    0
4 QPX_transcriptome_v2_Contig_10002_2   264  0  0  0    0    0    0
5 QPX_transcriptome_v2_Contig_1000_3    321  0  0  0    0    0    0
6 QPX_transcriptome_v2_Contig_1000_4   1473 58 72 99   59   72  100

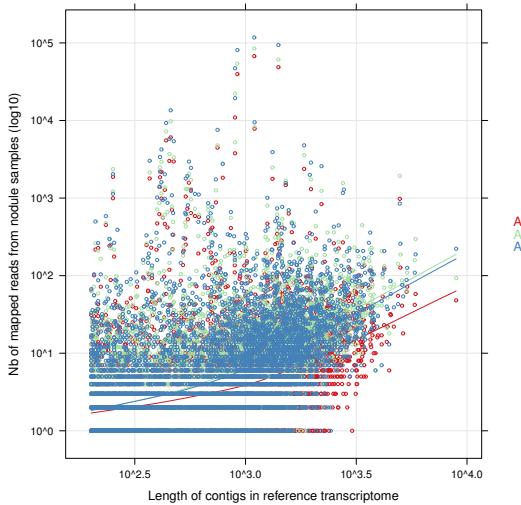
```

43 Plot the correlation between read length and number of mapped reads on the transcriptome
 44 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)

```

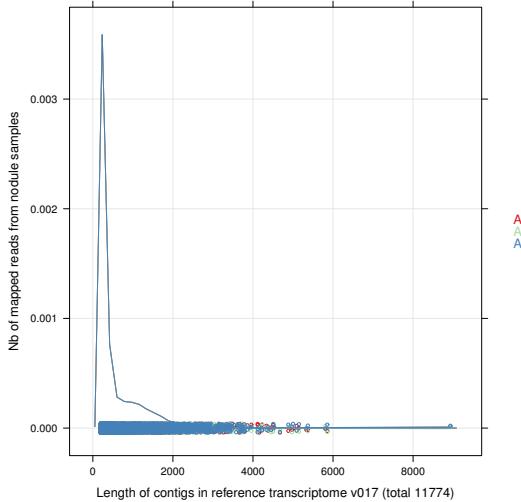


45

46 2.1 Concentration of contigs in different libraries

47 Density plot between reference transcriptome and assembled contigs. The plot shows a high
 48 concentration of contigs under 2000 base pair. Trimming parameters are of default with TrueSeq3
 49 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)
```



50

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

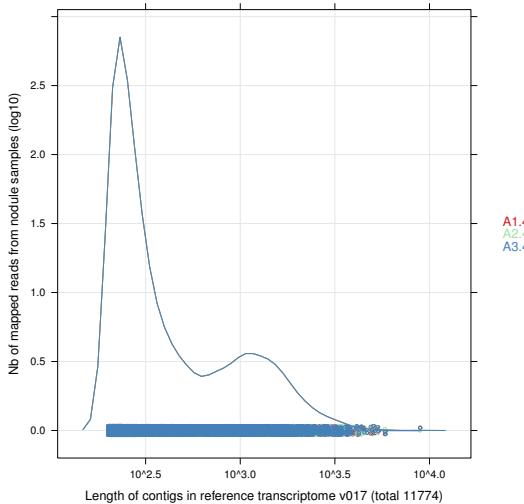
```
custom.colors <- c('#d7191c', '#abdda4', '#2b83ba')
```

```

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

```



52
53 2.2 Testing contig length and mapping with different assembled references
54 Load the number of mapped reads to the MMETSP0098 transcriptome before discarding duplicates
55

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")

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

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

58 Add the length values to each contig mapped to MMETsp0098. But first remove extra rows.
59

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 MMETPS0092 were used here. Both strains come from New York and Virginia respectively. Official project can be found [here](#).

```

60 Load the number of mapped reads to the MMETSP0099_2 transcriptome before discarding duplicates.
61

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")

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

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

64 Add the length values to each contig mapped to MMETSP0099_2. But first remove extra rows.
65

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

66 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")

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

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

69 Add the length values to each contig mapped to SR v015 reference genome. But first remove
70 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

71 After aligning the reads to a reference duplicates must be removed. Testing was done with
72 different reference transcriptomes and genomes to assess the strength of the parameters used
73 for removing the duplicate reads and reducing bias for better coverage.
74 First load the sample reads mapped to reference genome (without duplication) of Steve Roberts.
75
```

```

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")

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

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")

78 Third load the sample reads mapped to reference transcriptome (without duplication) of MMEST00098.
79

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")

80 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")

81 Forth load of sample reads mapped to reference SR genome v015 with approximately 21,000 contigs.
82

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")

83 Merge mapped reads relative to the followingf references.
84     • R1 = genome of QPX (steve roberts, 555 contigs)
85     • R2 = transcriptome of QPX (steve roberts)
86     • R3 = transcriptome of QPX MMETSP0098, New York strain
87     • R4 = transcriptome of QPX MMETSP0099_2, Virginia strain
88     • 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, ]

```

89 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                  171              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)))

```

90 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
1 QPX_transcriptome_v2_Contig_1000_1    201  2  2  7   2   2   7
2 QPX_transcriptome_v2_Contig_1000_2    258  1  0  0   1   0   0
3 QPX_transcriptome_v2_Contig_10002_1   477  0  2  0   0   2   0
4 QPX_transcriptome_v2_Contig_10002_2   264  0  0  0   0   0   0
5 QPX_transcriptome_v2_Contig_1000_3    321  0  0  0   0   0   0
6 QPX_transcriptome_v2_Contig_1000_4   1473 58 72 99  52  69  94
reference
1 trxSRv022
2 trxSRv022
3 trxSRv022
4 trxSRv022
5 trxSRv022
6 trxSRv022

#1 Present difference for each sample mapped to the references. First merge all samples before
#2 /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
6661     0   0   5 trxSRv022 QPX_transcriptome_v2_Contig_3982_3  5   9
4447     0   0   0 trxSRv022 QPX_transcriptome_v2_Contig_2747_2  0   0
16993    2   3   8 trxMME98           MMETSP0098-20131031|15407  2   4
11051    2   1   2 trxSRv022 QPX_transcriptome_v2_Contig_7840_3 11  11
1950     1   1   0 trxSRv022 QPX_transcriptome_v2_Contig_1456_6  1   1
      A3
6661     3
4447     0
16993    8
11051    8
1950     0

#3 Plot the difference before and after duplicates were discarded. The number of mapped reads
#4 to the reference contigs is descriptive for any bias in contig assembly. For example in
#5 the case of SR genome v017, more than 20 % of A1, A2, A3 reads align to a small set of contigs.
#6 The best distribution is a constant one.
#7 Even though we did not plot length of contigs, the analyzes above demonstrate that length
#8 is linearly correlated to the number of mapped reads. Therefore, peaks indicate a specific
#9 preference that reads 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

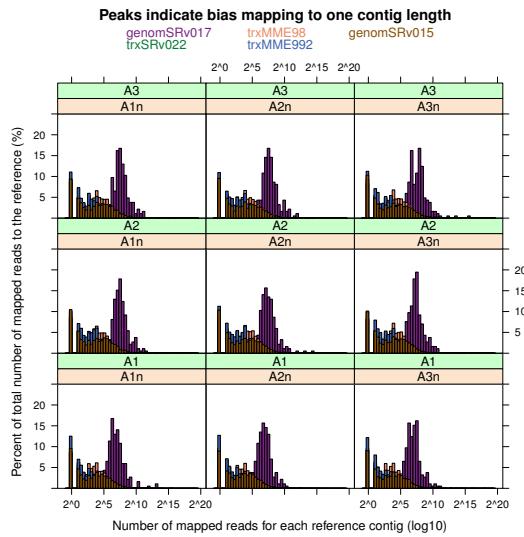
```

        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
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

```



100

101 3 Extracting QPX reads

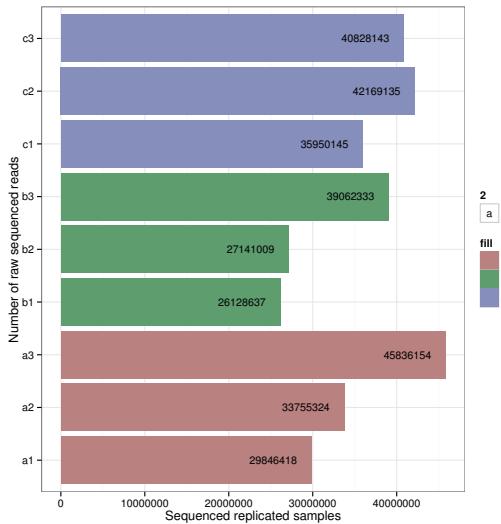
102 Load file with read counts per sample.

- 103 • A samples as nodule tissue
- 104 • B samples as non nodule diseased tissue
- 105 • C samples as non nodule non diseased tissue

106 Raw reads have been trimmed, mapped to reference genome (Steve Roberts v15 with 21280 contigs),
107 sorted by position on the genome, and cleaned from duplicated 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")
```

[†] Hypothetically these raw reads includes specific QPX reads

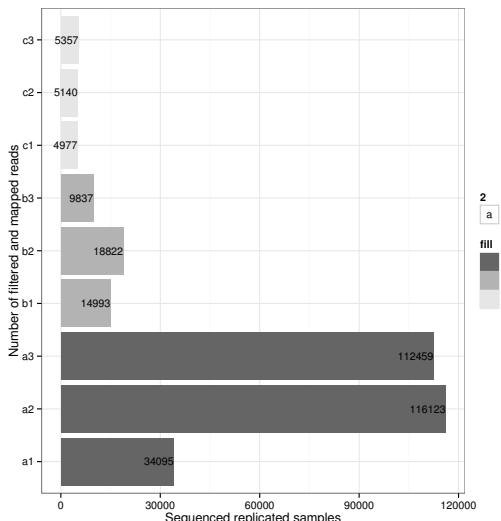


108

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

↑ These reads are probably those of QPXs'

```
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")
```



110

111 3.1 QPX genes assembled without hosts genes

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

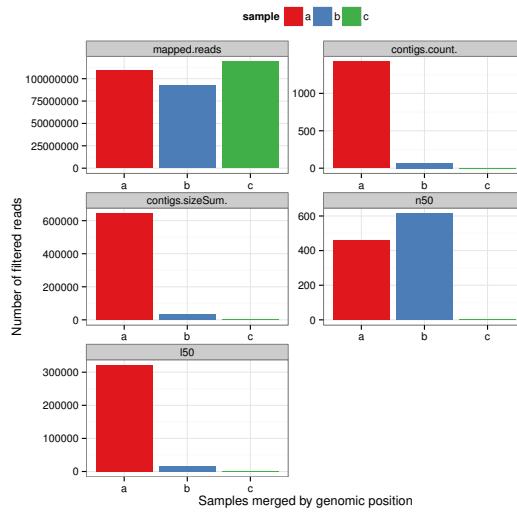
↑ These contigs must be specific transcripts to QPX

```
read.xlsx("./data/mappedNodules.xlsx", sheetIndex = 2) %>%
```

```

gather("category", "counts", 3:7) %>%
  ggplot(aes(x = sample,
             y = counts,
             fill = sample)) +
  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") +
  scale_fill_brewer(type = "qual", palette = 6)

```



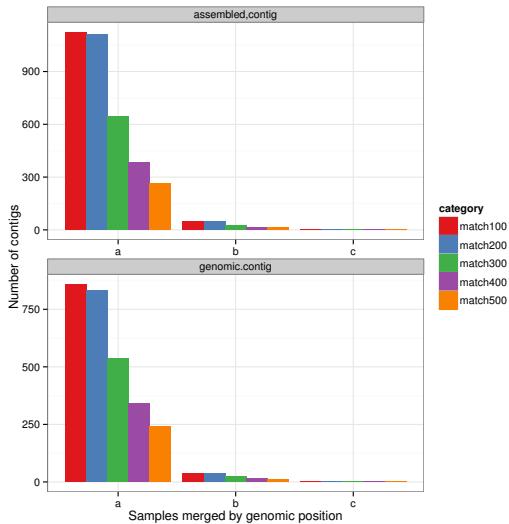
114
115 All contigs were then aligned to the reference genome QPX. The chart shows the number of
116 contigs that align with an increasing length of 100>200>300>400>500.

↳ Helps discard
misassembled contigs or
non QPX ones

```

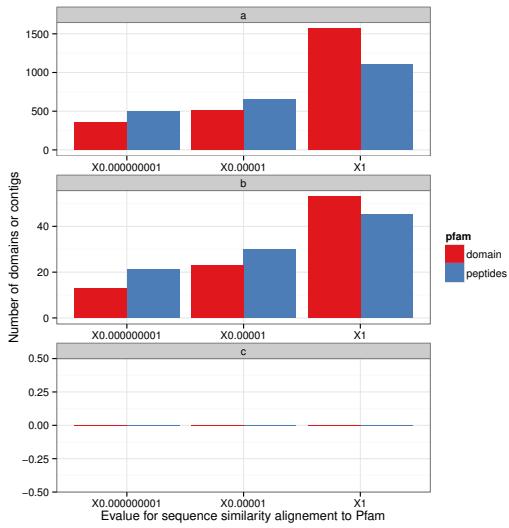
read.xlsx("./data/mappedNodules.xlsx", sheetIndex = 4) %>%
  gather("category", "count", 3:7) %>%
  ggplot(aes(x = sample,
             y = count,
             fill = category)) +
  theme_bw() +
  geom_bar(stat = "identity",
           position = "dodge") +
  facet_wrap(~ blat, ncol = 1, scale = "free") +
  # scale_fill_manual(values = brewer.pal(5, "Greens")) +
  labs(x = "Samples merged by genomic position",
       y = "Number of contigs") +
  scale_fill_brewer(type = "qual", palette = 6)

```



```
117
118 All contigs were then translated to peptides in 6 frames. Each peptide was then aligned
119 to the whole PFAM library (v28, date: Jul 14 2015).
```

```
read.xlsx("./data/mappedNodules.xlsx", sheetIndex = 3) %>%
  gather("category", "count", 3:5) %>%
  ggplot(aes(x = category,
             y = count,
             fill = pfam)) +
  geom_bar(stat = "identity",
            position = "dodge") +
  theme_bw() +
  facet_wrap(~ sample, ncol = 1,
             scales = "free") +
  labs(x = "Evaluate for sequence similarity alignment to Pfam",
       y = "Number of domains or contigs") +
  scale_fill_brewer(type = "qual", palette = 6)
```



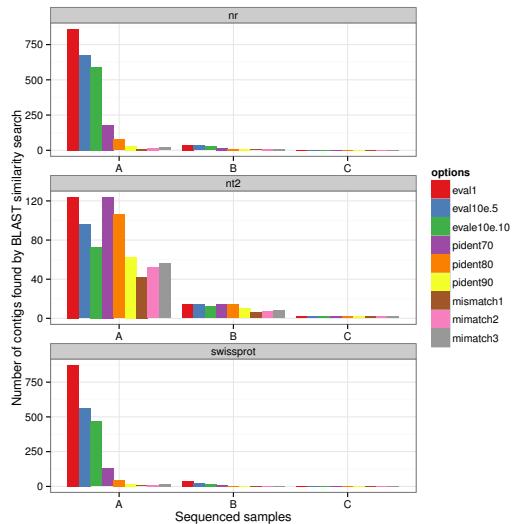
```
120
121 3.2 Evaluating identified QPX contigs with sequence similarities
122 BLAST is used at this step to align the contigs found to non redundant (NR),
123 and swissprot databases. Contigs were translated into peptide sequencing with EMBOSS transeq
124 in all 6 possible frames. All contigs were mapped to genome v15 of S. Roberts. Top hit
125 sequences were filtered either with an evaluate score, the percentage of identity between query
126 and target sequences, and the number of mismatches found in the aligned region.
```

```
read.xlsx("./data/blast.xlsx", sheetIndex = 1) %>%
```

```

gather("options", "counts", 4:12) %>%
  ggplot(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)

```



```

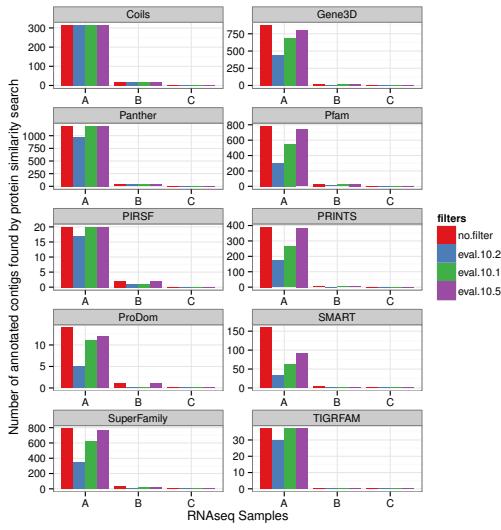
127
128 Sequences from A, B, C samples are also aligned to 10 other databases. We used interpro
129 accession numbers to get GO-terms too. We show below the number of contigs if we filter
130 them only by e-value. The no-filter label represent the total number of sequences per database.
131

```

```

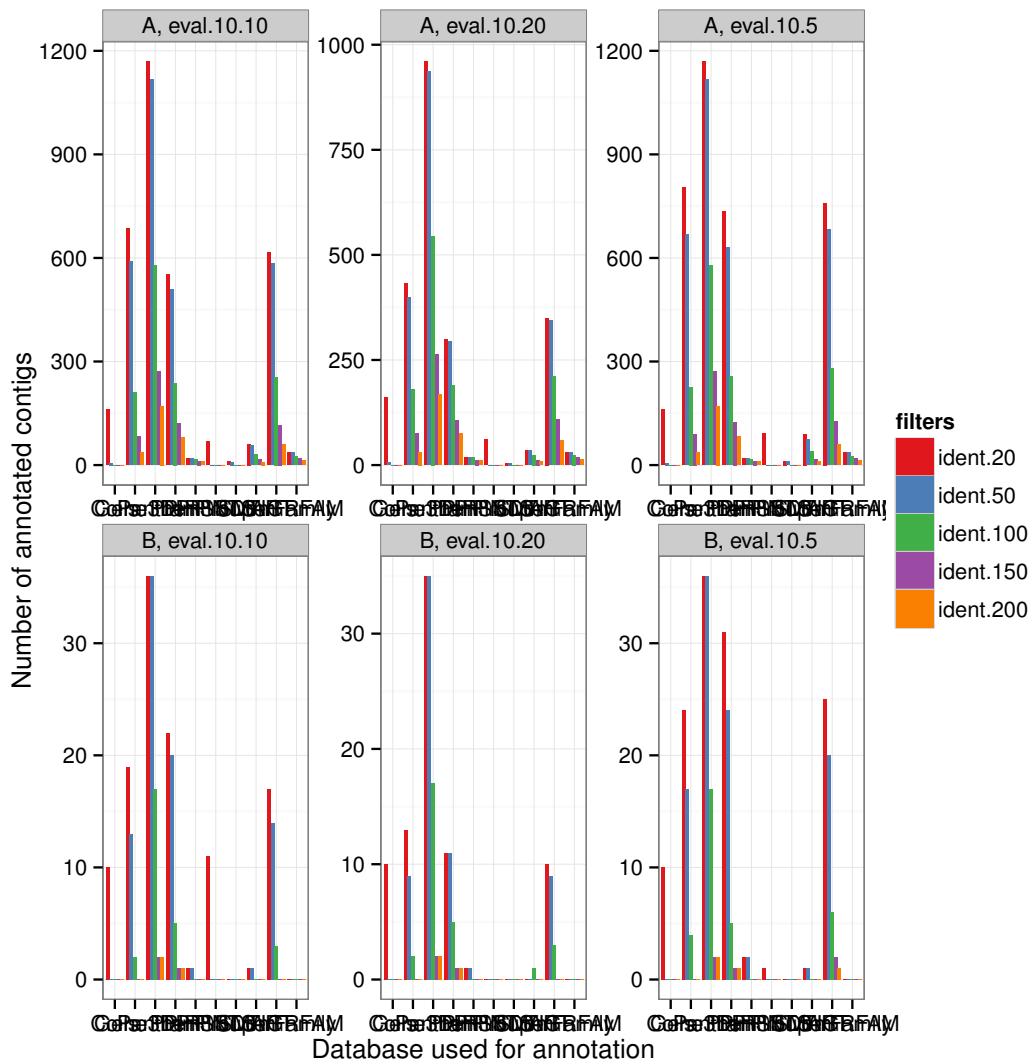
read.xlsx("./data/interpro.xlsx", sheetIndex = 1) %>%
  gather("filters", "value", 2:5) %>%
  ggplot(aes(x = sample,
             y = value,
             fill = filters)) +
  geom_bar(stat = "identity",
            position = "dodge") +
  theme_bw() +
  facet_wrap(~ database,
             ncol = 2,
             scales = "free") +
  labs(x = "RNAseq Samples",
       y = "Number of annotated contigs found by protein similarity search") +
  scale_fill_brewer(type = "qual",
                    palette = 6)

```



132
 133 We show here the number of contigs annotated by database and filtered by both evalue and
 134 the length of the similarity between query and target sequences.

```
read.xlsx("./data/interpro.xlsx", sheetIndex = 2) %>%
  gather("filters", "value", 3:7) %>%
  ggplot(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)
```



135

136 4 Removing clam genes from QPX inferred genes

137 For each sequenced we assembled a list of genes. A-list theoretically contains QPX genes,
 138 B- and C- contain mostly clam genes. Confidence in the A-list is only valid if clam genes
 139 were discarded from, and QPX only genes remained.

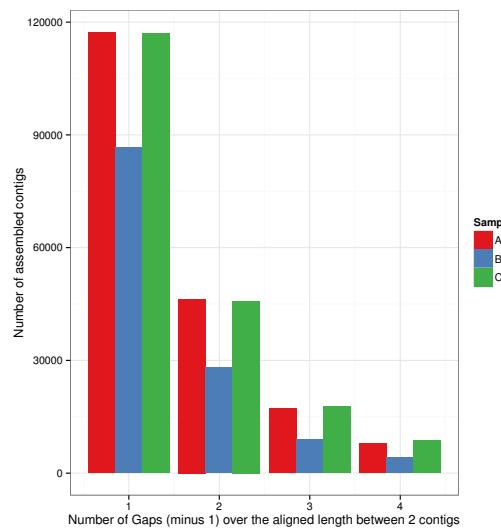
140 The same strategy of contig assembly is carried out for clam. A, B, C raw reads are mapped
 141 to clam reference transcriptome. Only the mapped reads are used to assemble 3 lists of hypothetically
 142 clam genes. There is many genes so HMMER is used to select the ones containing significant
 143 protein domains. The selected genes are mapped to the clam reference and only the genes
 144 that map without any gap (on the aligned length) are kept.

```
gapsA <- read.table("./data/A.e10.blat.gaps.clam.txt")
```

```

gapsB <- read.table("./data/B.e10.blat.gaps.clam.txt")
gapsC <- read.table("./data/C.e10.blat.gaps.clam.txt")
df <- full_join(gapsA, gapsB, by="V2") %>%
  full_join(gapsC, by="V2")
colnames(df) <- c("A", "Gaps", "B", "C")
df[, c(2,1,3,4)] %>%
  gather("Sample", "Count", 2:4) %>%
  filter(Gaps<5) %>%
  ggplot(aes(x = Gaps,
             y = Count,
             fill = Sample)) +
  theme_bw() +
  geom_bar(stat = "identity",
            position = "dodge") +
  labs(x = "Number of Gaps (minus 1) over the aligned length between 2 contigs",
       y = "Number of assembled contigs") +
  scale_fill_brewer(type = "qual", palette = 6)

```



- 145
 146 Get the number of clam contigs from A, B, and C that align to the clam reference.
 147 • A contig can map entirely to a reference.
 148 • A contig can align to 2 regions of a reference with 1 gap in between
 149 • A contig can align to n regions of a reference with $n-1$ gaps in between

150 The blocksize in the chart below is the length of the aligned region between contig and reference
 151 in case there is no gap (ie., 1) or there is 4 gaps (ie., 5).

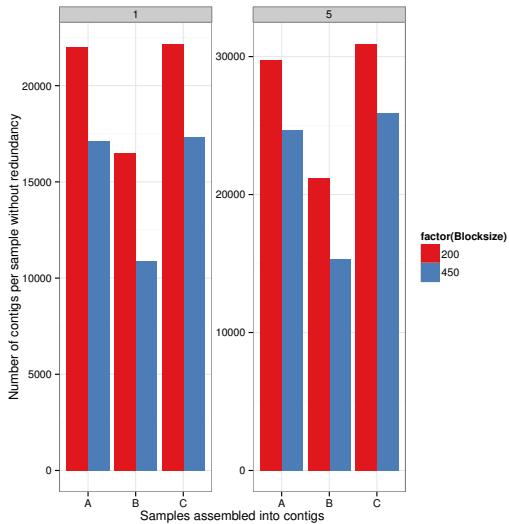
↑ One sample contig can map to several reference contigs. The latter are mainly length-isofroms of 1 gene.

↑ We chose gaps=5 and blocksize=450

```

read.table("./data/gaps.blocksize.clam.txt", header = T) %>%
  ggplot(aes(x = Sample,
             y = Count,
             fill = factor(Blocksize))) +
  theme_bw() +
  geom_bar(stat = "identity",
            position = "dodge") +
  facet_wrap(~ Gaps,
             ncol = 2,
             scales = "free") +
  labs(x = "Samples assembled into contigs",
       y = "Number of contigs per sample without redundancy") +
  scale_fill_brewer(type = "qual", palette = 6)

```



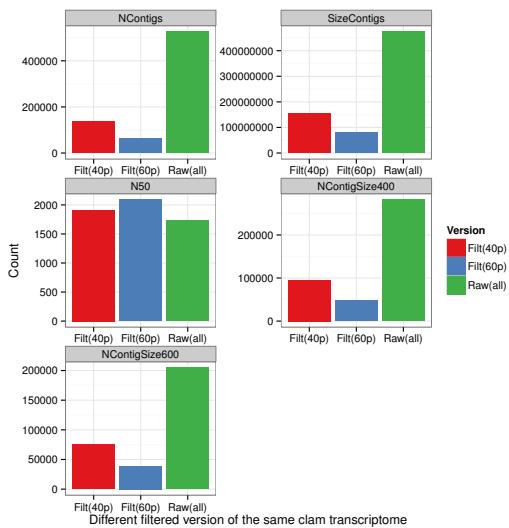
152

153 4.1 Preprocessing the clam transcriptome

154 A, B, and C reads were merged and used to assemble one transcriptome (previous study, not ↗ We used the
 155 here). The raw output transcriptome done in Trinity was then reduced by 40% and 60% using transcriptome reduced by
 156 different strategy of quality controls (not here). Transcriptome size, N50, and contig length ↗ 40% only
 157 are shown below for each transcriptome.

```
read.table("./data/clam.filtered.trxome.txt", header = T) %>%
  gather("Category", "Count", 2:6) %>%
  ggplot(aes(x = Version,
             y = Count,
             fill = Version)) +
  theme_bw() +
  geom_bar(stat = "identity",
           position = "dodge") +
  facet_wrap(~ Category,
             ncol = 2,
             scales = "free") +
  labs(x = "Different filtered version of the same clam transcriptome",
       y = "Count") +
  scale_fill_brewer(type = "qual", palette = 6)
```

↗ N50 is the mean length
 of contigs assembled from
 50% of sequenced
 nucleotides



158

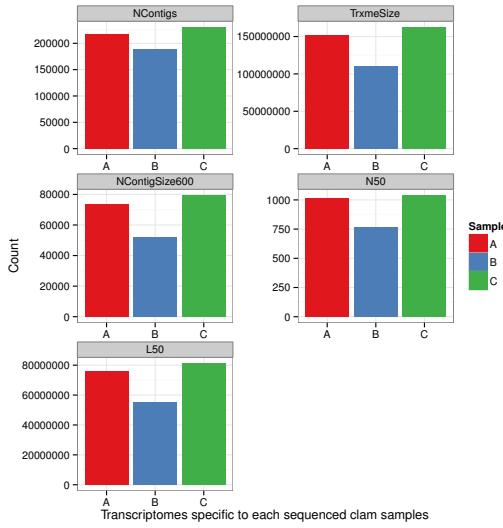
159 From the selected transcriptome (40%) a guided Trinity assembly is done for each of sample
 160 which generated 3 separate clam transcriptomes. These 3 raw transcriptomes have lots of
 161 contigs, many of which can be discarded.

```
read.table("./data/clam.trxome.txt", header = T) %>%
```

```

gather("Category", "Count", 2:6) %>%
  ggplot(aes(x = Sample,
             y = Count,
             fill = Sample)) +
  theme_bw() +
  geom_bar(stat = "identity",
            position = "dodge") +
  facet_wrap(~ Category,
             ncol = 2,
             scales = "free") +
  labs(x = "Transcriptomes specific to each sequenced clam samples",
       y = "Count") +
  scale_fill_brewer(type = "qual", palette = 6)

```



- 162
- 163 5 QPX genes remaining after cleanup
 164 Raw reads of A, B, C samples are mapped to QPX reference genome. The true mapped reads are
 165 then assembled into contigs with Trinity genome guided approach. Number of genes resulted
 166 for each sample are shown in Section 3.1. These genes are then processed to select 932,
 167 44, 0 genes/isoforms for QPX in sample A, B, and C respectively.
- 168 1. Remove redundant clam genes common with A, B, C clam assemblies
 169 2. Remove PFAM domains with an e-value higher than 10^{-5}
 170 3. Remove non-mapped contigs to QPX reference genome that have more than 4 gaps, less than
 171 100 in contig size, and less than 250 in blocksize (if gaps exist)

- 172 6 System Information
 173 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-unknown-linux-gnu (64-bit)
Running under: elementary OS Luna

locale:
[1] LC_CTYPE=en_US.UTF-8          LC_NUMERIC=C
[3] LC_TIME=en_US.UTF-8          LC_COLLATE=en_US.UTF-8
[5] LC_MONETARY=en_US.UTF-8       LC_MESSAGES=en_US.UTF-8
[7] LC_PAPER=en_US.UTF-8         LC_NAME=en_US.UTF-8
[9] LC_ADDRESS=en_US.UTF-8        LC_TELEPHONE=en_US.UTF-8
[11] LC_MEASUREMENT=en_US.UTF-8    LC_IDENTIFICATION=en_US.UTF-8

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_3.1-121    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_7.3-10     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_7.3-41         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

```