

The ribosomal and RNA (if available) alignment files are specified.

```
> ribofiles <- paste(datadir,  
+                   "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")  
> rnafiles <- paste(datadir,  
+                   "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")
```

The aligned ribosomal (and RNA) data can be read in using the `readRibodata` function. The columns can be specified as a parameter of the `readRibodata` function if the data in the alignment files are differently arranged.

```
> riboDat <- readRibodata(ribofiles, replicates = c("WT", "WT", "M", "M"))
```

The alignments can be assigned to frames relative to the coding coordinates with the `frameCounting` function.

```
> fCs <- frameCounting(riboDat, fastaCDS)
```

The predominant reading frame, relative to coding start, can be estimated from the frame calling (or from a set of coordinates and alignment data) for each n-mer. The weighting describes the proportion of n-mers fitting with the most likely frameshift. The reading frame can also be readily visualised using the `plotFS` function.

```
> fS <- readingFrame(rC = fCs); fS  
[[1]]  
      26  27  28  29  30  
0      712 10579 2228 1175 227  
1      865  696 2095  531 358  
2      316  3318 7987 1919 947  
frame.ML 1    0    2    2    2  
  
[[2]]  
      26  27  28  29  30  
0      698 8485  597 160  73  
1      715  537 1012 128 123  
2      286 2663 3644 238 148  
frame.ML 1    0    2    2    2  
  
[[3]]  
      26  27  28  29  30  
0      542 5066  266 188  91  
1      435  268  447  98  99  
2      215 1467 1429 171 184  
frame.ML 0    0    2    0    2  
  
[[4]]  
      26  27  28  29  30  
0      1177 12129  622 201  74  
1      1379  586  961 117 126  
2      407  3690 3554 218 135  
frame.ML 1    0    2    2    2  
  
> plotFS(fS)
```

These can be filtered on the mean number of hits and unique hits within replicate groups to give plausible candidates for coding. Filtering can be limited to given lengths and frames, which may be inferred from the output of the `readingFrame` function.

```
> ffCs <- filterHits(fCs, lengths = c(27, 28), frames = list(1, 0),
+                   hitMean = 50, unqhitMean = 10, fS = fS)
```

We can plot the total alignment at the 5' and 3' ends of coding sequences using the `plotCDS` function. The frames are colour coded; frame-0 is red, frame-1 is green, frame-2 is blue.

```
> plotCDS(coordinates = ffCs@CDS, riboDat = riboDat, lengths = 27)
```

Note the frameshift for 28-mers.

```
> plotCDS(coordinates = ffCs@CDS, riboDat = riboDat, lengths = 28)
```

We can plot the alignment over an individual transcript sequence using the `plotTranscript` function. Observe that one CDS (on the right) contains the 27s in the same phase as the CDS (they are both red) while the putative CDSes to the left are not in phase with the aligned reads, suggesting either a sequence error in the transcript or a misalignment. The coverage of RNA sequenced reads is shown as a black curve (axis on the right).

```
> plotTranscript("CUFF.37930.1", coordinates = ffCs@CDS,
+               riboData = riboDat, length = 27, cap = 200)
```

NULL

We can extract the counts from a `riboCoding` object using the `sliceCounts` function

```
> riboCounts <- sliceCounts(ffCs, lengths = c(27, 28), frames = list(0, 2))
```

Counts for RNA-sequencing can be extracted using from the `riboData` object and the coding coordinates using the `rnaCounts` function. This is a relatively crude counting function, and alternatives have been widely described in the literature on mRNA-Seq.

```
> rnaCounts <- rnaCounts(riboDat, ffCs@CDS)
```

These data may be used in an analysis of differential translation through comparison with the RNA-seq data. See the description of a beta-binomial analysis in the `baySeq` vignettes for further details.

```
> library(baySeq)
> pD <- new("countData", replicates = ffCs@replicates,
+         data = list(riboCounts, rnaCounts),
+         groups = list(NDT = c(1,1,1,1), DT = c("WT", "WT", "M", "M")),
+         annotation = as.data.frame(ffCs@CDS),
+         densityFunction = bbDensity)
> libsizes(pD) <- getLibsizes(pD)
> pD <- getPriors(pD, cl = NULL)
> pD <- getLikelihoods(pD, cl = NULL)
.
> topCounts(pD, "DT", normaliseData = TRUE)
      seqnames start end width strand frame startCodon stopCodon context
```

4	Cre17.g723750.t1.3	271	471	201	*	0	ATG	TGA	GGAATGG
3	CUFF.9661.1	424	582	159	*	0	ATG	TGA	ACAATGG
1	CUFF.43721.1	6	161	156	*	2	ATG	TAA	GCAATGC
2	CUFF.9523.1	78	1040	963	*	2	ATG	TAA	AAAATGG
	minus3 plus1	WT.1	WT.2	M.1	M.2	likes	DT	FDR.DT	
4	G	G	11:11	1:1	2:2	1:1	0.05882353	M=WT	0.9411765
3	A	G	1:1	1:1	1:1	0:0	0.05882352	M=WT	0.9411765
1	G	C	5:5	4:4	3:3	0:0	0.05882350	M=WT	0.9411765
2	A	G	841:841	592:592	298:298	721:721	0.05882193	M=WT	0.9411769
	FWER.DT								
4	0.9411765								
3	0.9965398								
1	0.9997965								
2	0.9999880								

```

[[1]]
      26  27  28  29  30
0     712 10579 2228 1175 227
1     865  696 2095  531 358
2     316 3318 7987 1919 947
frame.ML 1  0  2  2  2

```

```

[[2]]
      26  27  28  29  30
0     698 8485  597 160  73
1     715  537 1012 128 123
2     286 2663 3644 238 148
frame.ML 1  0  2  2  2

```

```

[[3]]
      26  27  28  29  30
0     542 5066  266 188  91
1     435  268  447  98  99
2     215 1467 1429 171 184
frame.ML 0  0  2  0  2

```

```

[[4]]
      26  27  28  29  30
0    1177 12129  622 201  74
1    1379  586  961 117 126
2     407 3690 3554 218 135
frame.ML 1  0  2  2  2

```

1

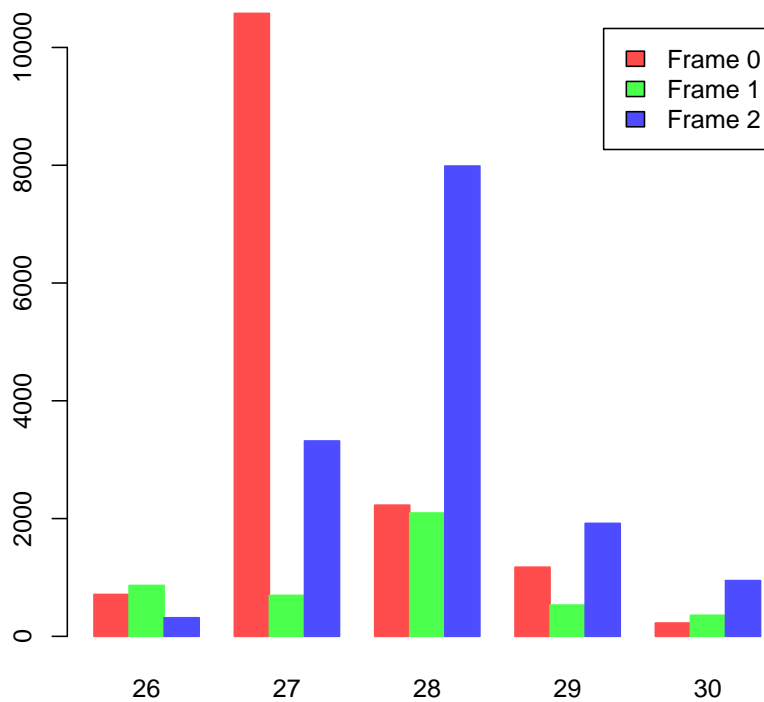


Figure 1: Number of n-mers in each frame relative to coding start
 27-mers are predominantly in frame-1, while 28-mers are chiefly in frame-0.

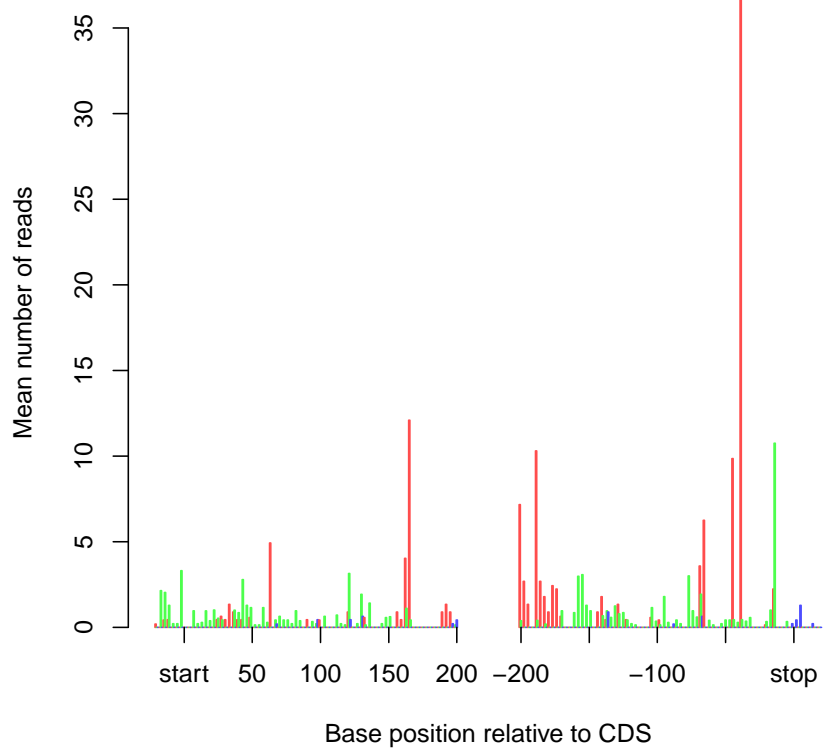


Figure 2: Average alignment of 27-mers to 5' and 3' ends of coding sequences

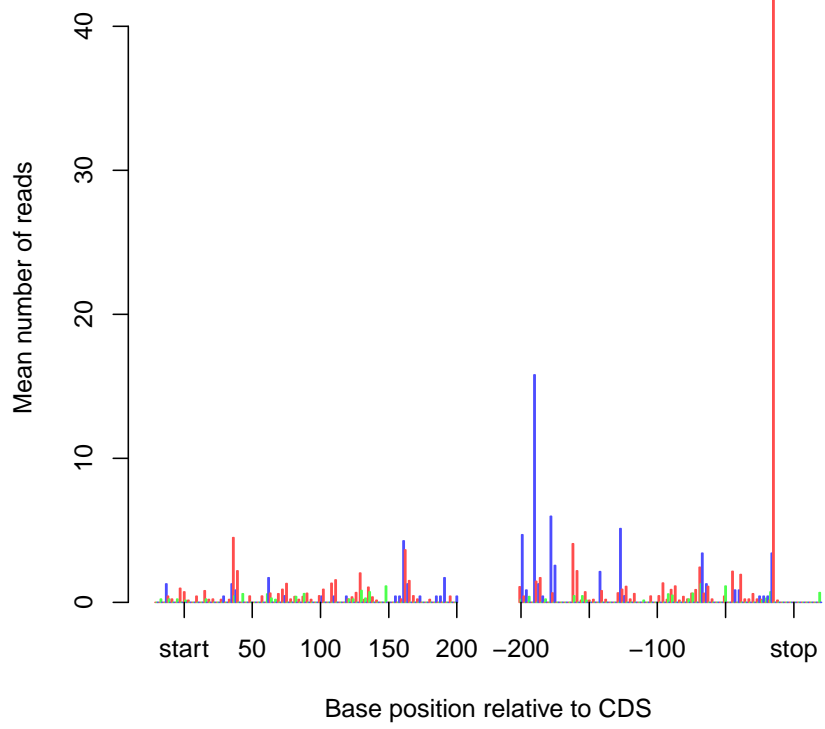


Figure 3: Average alignment of 28-mers to 5' and 3' ends of coding sequences

NULL

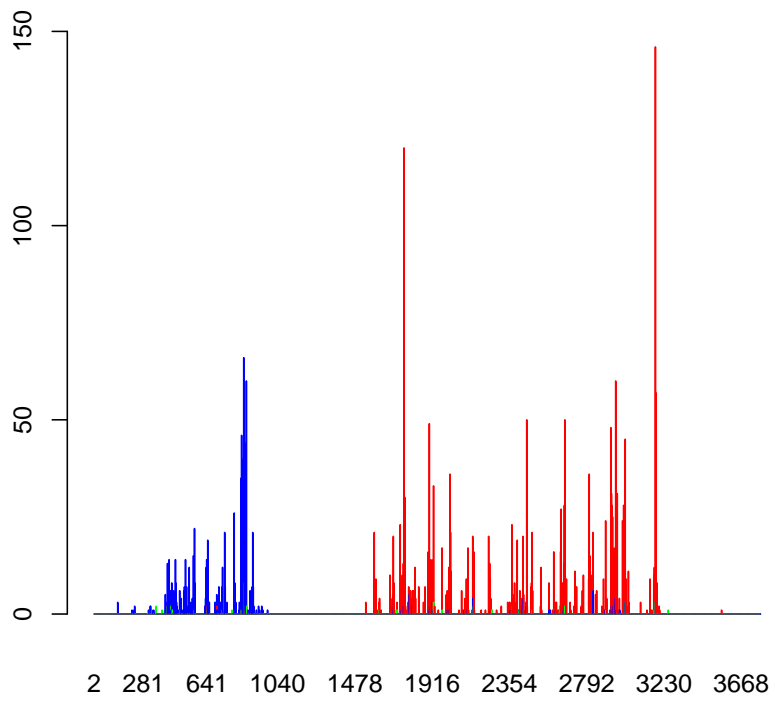


Figure 4: Alignment to individual transcript

Session Info

```
> sessionInfo()

R version 4.0.0 (2020-04-24)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 18.04.4 LTS

Matrix products: default
BLAS: /home/biobuild/bbs-3.11-bioc/R/lib/libRblas.so
LAPACK: /home/biobuild/bbs-3.11-bioc/R/lib/libRlapack.so

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

attached base packages:
[1] parallel stats4 stats graphics grDevices utils datasets
[8] methods base

other attached packages:
[1] baySeq_2.22.0      riboSeqR_1.22.0    abind_1.4-5
[4] GenomicRanges_1.40.0 GenomeInfoDb_1.24.0 IRanges_2.22.0
[7] S4Vectors_0.26.0   BiocGenerics_0.34.0

loaded via a namespace (and not attached):
 [1] Rcpp_1.0.4.6      edgeR_3.30.0      knitr_1.28
 [4] XVector_0.28.0    zlibbioc_1.34.0    BiocParallel_1.22.0
 [7] lattice_0.20-41   rlang_0.4.5       tools_4.0.0
[10] grid_4.0.0        xfun_0.13         seqLogo_1.54.0
[13] htmltools_0.4.0   yaml_2.2.1        digest_0.6.25
[16] crayon_1.3.4      GenomeInfoDbData_1.2.3 BiocManager_1.30.10
[19] bitops_1.0-6      RCurl_1.98-1.2    evaluate_0.14
[22] rmarkdown_2.1     limma_3.44.0      compiler_4.0.0
[25] Rsamtools_2.4.0   Biostrings_2.56.0 locfit_1.5-9.4
[28] BiocStyle_2.16.0
```

References

- [1] BY Chung and TJ Hardcastle and JD Jones and N Irigoyen and AE Firth and DC Baulcombe and I Brierley *The use of duplex-specific nuclease in ribosome profiling and a user-friendly software package for Ribo-seq data analysis*. RNA (2015).