

riboSeqR

Thomas J. Hardcastle, Betty Y.W. Chung

April 24, 2017

Introduction

Ribosome profiling extracts those parts of a coding sequence currently bound by a ribosome (and thus, are likely to be undergoing translation). Ribosomes typically cover between 20-30 bases of the mRNA (dependant on conformational changes) and move along the mRNA three bases at a time. Sequenced reads of a given length are thus likely to lie predominantly in a single frame relative to the start codon of the coding sequence. This package presents a set of methods for parsing ribosomal profiling data from multiple samples and aligned to coding sequences, inferring frameshifts, and plotting the average and transcript-specific behaviour of these data. Methods are also provided for extracting the data in a suitable form for differential translation analysis. For a fuller description of these methods and further examples of their use, see Chung & Hardcastle *et al* (2015) [1].

Getting Data

riboSeqR currently reads alignment data from BAM files, or from flat text files, although BAM format is recommended.

Workflow Example

Begin by loading the riboSeqR library.

```
> library(riboSeqR)
```

Identify the data directory for the example data.

```
> datadir <- system.file("extdata", package = "riboSeqR")
```

The `fastaCDS` function can be used to guess at potential coding sequences from a (possibly compressed; see `base::file`) fasta file containing mRNA transcripts (note; do not use this on a genome!). These can also be loaded into a `GRanges` object from an annotation file.

```
> chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
> fastaCDS <- findCDS(fastaFile = chlamyFasta,
+                   startCodon = c("ATG"),
+                   stopCodon = c("TAG", "TAA", "TGA"))
```

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) <- getLiblesizes(pD)
> pD <- getPriors(pD, cl = NULL)
```

```
Nelder-Mead direct search function minimizer
function value for initial parameters = 4.847551
Scaled convergence tolerance is 4.84755e-50
Stepsize computed as 0.050000
```

BUILD	4	5.247653	4.847551
EXTENSION	6	4.900713	3.982480
LO-REDUCTION	8	4.869122	3.982480
HI-REDUCTION	10	4.847551	3.982480
HI-REDUCTION	12	4.624786	3.982480
HI-REDUCTION	14	4.571306	3.982480
HI-REDUCTION	16	4.449566	3.982480
REFLECTION	18	4.399546	3.933923
HI-REDUCTION	20	4.286933	3.933923
HI-REDUCTION	22	4.227781	3.933923
HI-REDUCTION	24	4.151207	3.933923
HI-REDUCTION	26	4.120819	3.933923
HI-REDUCTION	28	4.074530	3.933923
HI-REDUCTION	30	4.055103	3.933923
HI-REDUCTION	32	4.028065	3.933923
HI-REDUCTION	34	4.014904	3.933923
HI-REDUCTION	36	3.999103	3.933923
HI-REDUCTION	38	3.990226	3.933923
HI-REDUCTION	40	3.982480	3.933923
LO-REDUCTION	42	3.980862	3.933923
LO-REDUCTION	44	3.974975	3.933923
REFLECTION	46	3.966510	3.933463
HI-REDUCTION	48	3.949603	3.933463
REFLECTION	50	3.937705	3.916470
REFLECTION	52	3.933923	3.915330
LO-REDUCTION	54	3.933463	3.915330
HI-REDUCTION	56	3.924966	3.915330
HI-REDUCTION	58	3.920784	3.915330
REFLECTION	60	3.920359	3.912554
HI-REDUCTION	62	3.916880	3.912554
LO-REDUCTION	64	3.916470	3.912554
HI-REDUCTION	66	3.915330	3.912554
REFLECTION	68	3.915025	3.912248
HI-REDUCTION	70	3.913813	3.912248
HI-REDUCTION	72	3.913751	3.912248

REFLECTION	74	3.913210	3.911792
HI-REDUCTION	76	3.912570	3.911792
REFLECTION	78	3.912554	3.911441
HI-REDUCTION	80	3.912248	3.911441
HI-REDUCTION	82	3.912058	3.911441
HI-REDUCTION	84	3.911844	3.911441
REFLECTION	86	3.911817	3.911421
LO-REDUCTION	88	3.911792	3.911421
HI-REDUCTION	90	3.911614	3.911421
HI-REDUCTION	92	3.911528	3.911421
REFLECTION	94	3.911522	3.911361
HI-REDUCTION	96	3.911448	3.911361
LO-REDUCTION	98	3.911441	3.911361
HI-REDUCTION	100	3.911421	3.911361
REFLECTION	102	3.911412	3.911350
HI-REDUCTION	104	3.911393	3.911350
LO-REDUCTION	106	3.911387	3.911350
REFLECTION	108	3.911366	3.911336
HI-REDUCTION	110	3.911361	3.911336
HI-REDUCTION	112	3.911357	3.911336
HI-REDUCTION	114	3.911353	3.911336
HI-REDUCTION	116	3.911351	3.911336
LO-REDUCTION	118	3.911350	3.911336
LO-REDUCTION	120	3.911349	3.911336
HI-REDUCTION	122	3.911342	3.911336
REFLECTION	124	3.911337	3.911330
HI-REDUCTION	126	3.911337	3.911330
HI-REDUCTION	128	3.911336	3.911330
REFLECTION	130	3.911335	3.911329
HI-REDUCTION	132	3.911334	3.911329
REFLECTION	134	3.911333	3.911329
HI-REDUCTION	136	3.911331	3.911329
HI-REDUCTION	138	3.911330	3.911329
EXTENSION	140	3.911330	3.911327
HI-REDUCTION	142	3.911329	3.911327
LO-REDUCTION	144	3.911329	3.911327
REFLECTION	146	3.911329	3.911327
EXTENSION	148	3.911328	3.911325
HI-REDUCTION	150	3.911327	3.911325
LO-REDUCTION	152	3.911327	3.911325
REFLECTION	154	3.911327	3.911325
EXTENSION	156	3.911326	3.911321
HI-REDUCTION	158	3.911325	3.911321
LO-REDUCTION	160	3.911325	3.911321
HI-REDUCTION	162	3.911325	3.911321
EXTENSION	164	3.911324	3.911318
EXTENSION	166	3.911322	3.911314
EXTENSION	168	3.911321	3.911309
EXTENSION	170	3.911318	3.911300
EXTENSION	172	3.911314	3.911287
EXTENSION	174	3.911309	3.911268
EXTENSION	176	3.911300	3.911241
EXTENSION	178	3.911287	3.911197
EXTENSION	180	3.911268	3.911159
EXTENSION	182	3.911241	3.911096
EXTENSION	184	3.911197	3.911030
LO-REDUCTION	186	3.911159	3.911030
REFLECTION	188	3.911096	3.911025

LO-REDUCTION	190	3.911067	3.911025
EXTENSION	192	3.911030	3.910995
HI-REDUCTION	194	3.911029	3.910995
EXTENSION	196	3.911025	3.910964
EXTENSION	198	3.911013	3.910935
LO-REDUCTION	200	3.910995	3.910935
EXTENSION	202	3.910968	3.910880
EXTENSION	204	3.910964	3.910840
EXTENSION	206	3.910935	3.910765
EXTENSION	208	3.910880	3.910588
EXTENSION	210	3.910840	3.910381
LO-REDUCTION	212	3.910765	3.910381
EXTENSION	214	3.910588	3.909826
EXTENSION	216	3.910434	3.909458
EXTENSION	218	3.910381	3.908886
EXTENSION	220	3.909826	3.907417
EXTENSION	222	3.909458	3.906007
EXTENSION	224	3.908886	3.903592
EXTENSION	226	3.907417	3.899757
EXTENSION	228	3.906007	3.894970
EXTENSION	230	3.903592	3.887634
EXTENSION	232	3.899757	3.879151
EXTENSION	234	3.894970	3.871010
EXTENSION	236	3.887634	3.866672
LO-REDUCTION	238	3.879151	3.866370
LO-REDUCTION	240	3.871010	3.866370
HI-REDUCTION	242	3.867974	3.866370
LO-REDUCTION	244	3.867263	3.866370
LO-REDUCTION	246	3.866777	3.866370
HI-REDUCTION	248	3.866672	3.866370
HI-REDUCTION	250	3.866476	3.866370
HI-REDUCTION	252	3.866411	3.866370
HI-REDUCTION	254	3.866390	3.866349
HI-REDUCTION	256	3.866371	3.866349
HI-REDUCTION	258	3.866370	3.866347
HI-REDUCTION	260	3.866353	3.866347
HI-REDUCTION	262	3.866349	3.866344
HI-REDUCTION	264	3.866348	3.866343
HI-REDUCTION	266	3.866347	3.866343
LO-REDUCTION	268	3.866344	3.866343
REFLECTION	270	3.866343	3.866341
LO-REDUCTION	272	3.866343	3.866341
HI-REDUCTION	274	3.866343	3.866341
LO-REDUCTION	276	3.866341	3.866341
REFLECTION	278	3.866341	3.866341
HI-REDUCTION	280	3.866341	3.866341
REFLECTION	282	3.866341	3.866341
REFLECTION	284	3.866341	3.866341
EXTENSION	286	3.866341	3.866340
LO-REDUCTION	288	3.866341	3.866340
EXTENSION	290	3.866341	3.866340
EXTENSION	292	3.866340	3.866339
LO-REDUCTION	294	3.866340	3.866339
EXTENSION	296	3.866340	3.866339
LO-REDUCTION	298	3.866340	3.866339
EXTENSION	300	3.866339	3.866336
LO-REDUCTION	302	3.866339	3.866336
EXTENSION	304	3.866339	3.866336

EXTENSION	306	3.866337	3.866332
EXTENSION	308	3.866336	3.866329
LO-REDUCTION	310	3.866336	3.866329
EXTENSION	312	3.866332	3.866320
EXTENSION	314	3.866329	3.866314
EXTENSION	316	3.866329	3.866302
EXTENSION	318	3.866320	3.866285
EXTENSION	320	3.866314	3.866259
EXTENSION	322	3.866302	3.866225
EXTENSION	324	3.866285	3.866209
EXTENSION	326	3.866259	3.866119
LO-REDUCTION	328	3.866225	3.866119
LO-REDUCTION	330	3.866209	3.866119
EXTENSION	332	3.866154	3.866044
EXTENSION	334	3.866153	3.865987
EXTENSION	336	3.866119	3.865801
LO-REDUCTION	338	3.866044	3.865801
EXTENSION	340	3.865987	3.865546
EXTENSION	342	3.865833	3.865192
LO-REDUCTION	344	3.865801	3.865192
EXTENSION	346	3.865546	3.864435
EXTENSION	348	3.865193	3.863698
HI-REDUCTION	350	3.865192	3.863698
REFLECTION	352	3.864772	3.863461
HI-REDUCTION	354	3.864435	3.863461
HI-REDUCTION	356	3.864291	3.863461
HI-REDUCTION	358	3.864112	3.863461
REFLECTION	360	3.864011	3.863304
HI-REDUCTION	362	3.863738	3.863304
HI-REDUCTION	364	3.863698	3.863304
EXTENSION	366	3.863604	3.863175
HI-REDUCTION	368	3.863461	3.863175
HI-REDUCTION	370	3.863431	3.863175
HI-REDUCTION	372	3.863362	3.863175
HI-REDUCTION	374	3.863335	3.863175
HI-REDUCTION	376	3.863304	3.863175
HI-REDUCTION	378	3.863298	3.863175
HI-REDUCTION	380	3.863277	3.863175
HI-REDUCTION	382	3.863254	3.863175
REFLECTION	384	3.863247	3.863155
LO-REDUCTION	386	3.863240	3.863155
HI-REDUCTION	388	3.863196	3.863155
HI-REDUCTION	390	3.863180	3.863155
LO-REDUCTION	392	3.863179	3.863155
HI-REDUCTION	394	3.863175	3.863155
HI-REDUCTION	396	3.863169	3.863155
HI-REDUCTION	398	3.863164	3.863155
LO-REDUCTION	400	3.863164	3.863155
HI-REDUCTION	402	3.863160	3.863155
LO-REDUCTION	404	3.863160	3.863155
REFLECTION	406	3.863159	3.863154
HI-REDUCTION	408	3.863157	3.863154
HI-REDUCTION	410	3.863156	3.863154
EXTENSION	412	3.863156	3.863152
REFLECTION	414	3.863155	3.863151
HI-REDUCTION	416	3.863154	3.863151
LO-REDUCTION	418	3.863153	3.863151
EXTENSION	420	3.863152	3.863148

LO-REDUCTION	422	3.863152	3.863148
HI-REDUCTION	424	3.863151	3.863148
REFLECTION	426	3.863150	3.863148
HI-REDUCTION	428	3.863149	3.863148
HI-REDUCTION	430	3.863149	3.863148
EXTENSION	432	3.863149	3.863148
LO-REDUCTION	434	3.863148	3.863148
LO-REDUCTION	436	3.863148	3.863148
REFLECTION	438	3.863148	3.863147
LO-REDUCTION	440	3.863148	3.863147
HI-REDUCTION	442	3.863148	3.863147
EXTENSION	444	3.863148	3.863147
EXTENSION	446	3.863147	3.863146
LO-REDUCTION	448	3.863147	3.863146
EXTENSION	450	3.863147	3.863144
EXTENSION	452	3.863146	3.863143
EXTENSION	454	3.863146	3.863141
EXTENSION	456	3.863144	3.863139
EXTENSION	458	3.863143	3.863135
EXTENSION	460	3.863141	3.863131
LO-REDUCTION	462	3.863139	3.863131
EXTENSION	464	3.863135	3.863127
LO-REDUCTION	466	3.863135	3.863127
EXTENSION	468	3.863131	3.863121
LO-REDUCTION	470	3.863130	3.863121
EXTENSION	472	3.863127	3.863119
LO-REDUCTION	474	3.863126	3.863119
EXTENSION	476	3.863121	3.863112
LO-REDUCTION	478	3.863121	3.863112
LO-REDUCTION	480	3.863119	3.863112
LO-REDUCTION	482	3.863118	3.863112
EXTENSION	484	3.863113	3.863102
LO-REDUCTION	486	3.863113	3.863102
EXTENSION	488	3.863112	3.863092
EXTENSION	490	3.863104	3.863083
EXTENSION	492	3.863102	3.863064
EXTENSION	494	3.863092	3.863029
LO-REDUCTION	496	3.863083	3.863029
EXTENSION	498	3.863064	3.862967
LO-REDUCTION	500	3.863045	3.862967
EXTENSION	502	3.863029	3.862886
EXTENSION	504	3.862968	3.862769
LO-REDUCTION	506	3.862967	3.862769
EXTENSION	508	3.862886	3.862535
EXTENSION	510	3.862783	3.862367
EXTENSION	512	3.862769	3.862219
EXTENSION	514	3.862535	3.861943
LO-REDUCTION	516	3.862367	3.861943
LO-REDUCTION	518	3.862219	3.861943
LO-REDUCTION	520	3.862017	3.861943
LO-REDUCTION	522	3.861985	3.861941
LO-REDUCTION	524	3.861979	3.861941
LO-REDUCTION	526	3.861948	3.861939
REFLECTION	528	3.861943	3.861935
HI-REDUCTION	530	3.861941	3.861935
HI-REDUCTION	532	3.861939	3.861934
LO-REDUCTION	534	3.861935	3.861934
HI-REDUCTION	536	3.861935	3.861933

HI-REDUCTION	538	3.861934	3.861933
LO-REDUCTION	540	3.861934	3.861933
LO-REDUCTION	542	3.861934	3.861933
HI-REDUCTION	544	3.861933	3.861933
EXTENSION	546	3.861933	3.861933
LO-REDUCTION	548	3.861933	3.861933
EXTENSION	550	3.861933	3.861932
EXTENSION	552	3.861933	3.861932
EXTENSION	554	3.861933	3.861931
EXTENSION	556	3.861932	3.861930
LO-REDUCTION	558	3.861932	3.861930
EXTENSION	560	3.861931	3.861927
EXTENSION	562	3.861930	3.861924
LO-REDUCTION	564	3.861930	3.861924
EXTENSION	566	3.861927	3.861918
LO-REDUCTION	568	3.861926	3.861918
EXTENSION	570	3.861924	3.861908
EXTENSION	572	3.861918	3.861902
EXTENSION	574	3.861918	3.861898
REFLECTION	576	3.861908	3.861887
HI-REDUCTION	578	3.861902	3.861887
LO-REDUCTION	580	3.861900	3.861887
HI-REDUCTION	582	3.861898	3.861887
REFLECTION	584	3.861895	3.861886
HI-REDUCTION	586	3.861893	3.861886
REFLECTION	588	3.861892	3.861885
HI-REDUCTION	590	3.861889	3.861885
HI-REDUCTION	592	3.861887	3.861885
EXTENSION	594	3.861887	3.861884
EXTENSION	596	3.861886	3.861881
LO-REDUCTION	598	3.861885	3.861881
LO-REDUCTION	600	3.861884	3.861881
REFLECTION	602	3.861883	3.861880
REFLECTION	604	3.861882	3.861880
REFLECTION	606	3.861881	3.861878
HI-REDUCTION	608	3.861880	3.861878
HI-REDUCTION	610	3.861880	3.861878
HI-REDUCTION	612	3.861880	3.861878
HI-REDUCTION	614	3.861880	3.861878
LO-REDUCTION	616	3.861879	3.861878
EXTENSION	618	3.861879	3.861877
EXTENSION	620	3.861878	3.861876
HI-REDUCTION	622	3.861878	3.861876
LO-REDUCTION	624	3.861877	3.861876
HI-REDUCTION	626	3.861877	3.861876
LO-REDUCTION	628	3.861877	3.861876
LO-REDUCTION	630	3.861876	3.861876
REFLECTION	632	3.861876	3.861876
HI-REDUCTION	634	3.861876	3.861876
HI-REDUCTION	636	3.861876	3.861876
HI-REDUCTION	638	3.861876	3.861876
LO-REDUCTION	640	3.861876	3.861876
HI-REDUCTION	642	3.861876	3.861876
LO-REDUCTION	644	3.861876	3.861876
HI-REDUCTION	646	3.861876	3.861876
REFLECTION	648	3.861876	3.861876
SHRINK	653	3.861876	3.861876
LO-REDUCTION	655	3.861876	3.861876

REFLECTION	657	3.861876	3.861876
SHRINK	662	3.861876	3.861876
REFLECTION	664	3.861876	3.861875
HI-REDUCTION	666	3.861876	3.861875
SHRINK	671	3.861876	3.861875
LO-REDUCTION	673	3.861876	3.861875
HI-REDUCTION	675	3.861876	3.861875
SHRINK	680	3.861876	3.861875
SHRINK	685	3.861876	3.861875
LO-REDUCTION	687	3.861876	3.861875
SHRINK	692	3.861876	3.861875
LO-REDUCTION	694	3.861876	3.861875
SHRINK	699	3.861876	3.861875
LO-REDUCTION	701	3.861876	3.861875
HI-REDUCTION	703	3.861876	3.861875
LO-REDUCTION	705	3.861876	3.861875
SHRINK	710	3.861878	3.861875
LO-REDUCTION	712	3.861876	3.861875
LO-REDUCTION	714	3.861876	3.861874
LO-REDUCTION	716	3.861875	3.861874
SHRINK	721	3.861876	3.861874
LO-REDUCTION	723	3.861876	3.861874
SHRINK	728	3.861876	3.861874
LO-REDUCTION	730	3.861875	3.861874
SHRINK	735	3.861876	3.861874
LO-REDUCTION	737	3.861876	3.861874
LO-REDUCTION	739	3.861875	3.861874
SHRINK	744	3.861876	3.861874
LO-REDUCTION	746	3.861876	3.861874
LO-REDUCTION	748	3.861876	3.861874
SHRINK	753	3.861876	3.861874
LO-REDUCTION	755	3.861876	3.861874
HI-REDUCTION	757	3.861876	3.861874
HI-REDUCTION	759	3.861875	3.861874
SHRINK	764	3.861877	3.861874
LO-REDUCTION	766	3.861876	3.861874
SHRINK	771	3.861876	3.861874
LO-REDUCTION	773	3.861876	3.861874
LO-REDUCTION	775	3.861876	3.861874
LO-REDUCTION	777	3.861875	3.861874
SHRINK	782	3.861876	3.861874
HI-REDUCTION	784	3.861876	3.861874
LO-REDUCTION	786	3.861876	3.861874
HI-REDUCTION	788	3.861875	3.861874
HI-REDUCTION	790	3.861875	3.861874
SHRINK	795	3.861876	3.861874
HI-REDUCTION	797	3.861876	3.861874
HI-REDUCTION	799	3.861875	3.861874
SHRINK	804	3.861877	3.861874
REFLECTION	806	3.861876	3.861873
LO-REDUCTION	808	3.861875	3.861873
SHRINK	813	3.861876	3.861873
SHRINK	818	3.861876	3.861873
SHRINK	823	3.861876	3.861873
SHRINK	828	3.861877	3.861873
LO-REDUCTION	830	3.861876	3.861873
SHRINK	835	3.861876	3.861873
SHRINK	840	3.861876	3.861873

LO-REDUCTION	842	3.861876	3.861873
LO-REDUCTION	844	3.861876	3.861873
LO-REDUCTION	846	3.861875	3.861873
SHRINK	851	3.861877	3.861873
LO-REDUCTION	853	3.861876	3.861873
LO-REDUCTION	855	3.861876	3.861873
SHRINK	860	3.861876	3.861873
SHRINK	865	3.861876	3.861873
LO-REDUCTION	867	3.861875	3.861873
SHRINK	872	3.861876	3.861873
LO-REDUCTION	874	3.861876	3.861873
HI-REDUCTION	876	3.861876	3.861873
LO-REDUCTION	878	3.861875	3.861873
SHRINK	883	3.861876	3.861873
SHRINK	888	3.861876	3.861873
LO-REDUCTION	890	3.861876	3.861873
LO-REDUCTION	892	3.861876	3.861873
LO-REDUCTION	894	3.861875	3.861873
SHRINK	899	3.861876	3.861873
HI-REDUCTION	901	3.861876	3.861873
LO-REDUCTION	903	3.861875	3.861873
HI-REDUCTION	905	3.861874	3.861873
SHRINK	910	3.861876	3.861873
SHRINK	915	3.861875	3.861873
SHRINK	920	3.861876	3.861873
SHRINK	925	3.861876	3.861873
LO-REDUCTION	927	3.861876	3.861873
HI-REDUCTION	929	3.861875	3.861873
SHRINK	934	3.861876	3.861873
LO-REDUCTION	936	3.861876	3.861873
LO-REDUCTION	938	3.861876	3.861873
HI-REDUCTION	940	3.861875	3.861873
SHRINK	945	3.861876	3.861873
LO-REDUCTION	947	3.861876	3.861873
LO-REDUCTION	949	3.861875	3.861873
SHRINK	954	3.861876	3.861873
LO-REDUCTION	956	3.861875	3.861873
SHRINK	961	3.861876	3.861873
LO-REDUCTION	963	3.861876	3.861873
LO-REDUCTION	965	3.861876	3.861873
LO-REDUCTION	967	3.861876	3.861873
SHRINK	972	3.861876	3.861873
LO-REDUCTION	974	3.861876	3.861873
HI-REDUCTION	976	3.861876	3.861873
LO-REDUCTION	978	3.861875	3.861873
HI-REDUCTION	980	3.861875	3.861873
SHRINK	985	3.861876	3.861873
SHRINK	990	3.861876	3.861873
LO-REDUCTION	992	3.861876	3.861873
SHRINK	997	3.861875	3.861873
SHRINK	1002	3.861876	3.861873
LO-REDUCTION	1004	3.861876	3.861873
SHRINK	1009	3.861876	3.861873
SHRINK	1014	3.861876	3.861873
HI-REDUCTION	1016	3.861876	3.861873
SHRINK	1021	3.861876	3.861873
LO-REDUCTION	1023	3.861876	3.861873
SHRINK	1028	3.861876	3.861873

```

LO-REDUCTION    1030 3.861875 3.861873
SHRINK          1035 3.861874 3.861873
SHRINK          1040 3.861876 3.861873
LO-REDUCTION    1042 3.861876 3.861873
LO-REDUCTION    1044 3.861875 3.861873
SHRINK          1049 3.861876 3.861873
Exiting from Nelder Mead minimizer
  1051 function evaluations used
  Nelder-Mead direct search function minimizer
function value for initial parameters = 24.805091
  Scaled convergence tolerance is 2.48051e-49
Stepsize computed as 0.050000
BUILD           4 25.788737 24.805091
REFLECTION      6 24.905453 23.446387
LO-REDUCTION    8 24.905083 23.446387
EXTENSION      10 24.805091 21.077181
HI-REDUCTION   12 24.120749 21.077181
LO-REDUCTION   14 24.076975 21.077181
HI-REDUCTION   16 23.446387 21.077181
LO-REDUCTION   18 23.358639 21.077181
HI-REDUCTION   20 22.600773 21.077181
HI-REDUCTION   22 22.523364 21.077181
REFLECTION     24 22.144453 20.812016
HI-REDUCTION   26 21.626889 20.812016
LO-REDUCTION   28 21.602122 20.812016
REFLECTION     30 21.208933 20.519666
EXTENSION     32 21.077181 19.766486
HI-REDUCTION   34 20.812016 19.766486
EXTENSION     36 20.605883 18.572115
HI-REDUCTION   38 20.519666 18.572115
HI-REDUCTION   40 20.080953 18.572115
LO-REDUCTION   42 19.966354 18.572115
HI-REDUCTION   44 19.766486 18.572115
LO-REDUCTION   46 19.424483 18.572115
REFLECTION     48 19.026399 18.377630
REFLECTION     50 18.747542 17.809286
HI-REDUCTION   52 18.572115 17.809286
HI-REDUCTION   54 18.377630 17.809286
HI-REDUCTION   56 18.308800 17.809286
REFLECTION     58 18.239541 17.771307
HI-REDUCTION   60 18.060618 17.771307
REFLECTION     62 17.974074 17.486335
HI-REDUCTION   64 17.809286 17.486335
LO-REDUCTION   66 17.771307 17.486335
LO-REDUCTION   68 17.747252 17.486335
REFLECTION     70 17.643406 17.346431
LO-REDUCTION   72 17.563058 17.346431
HI-REDUCTION   74 17.486335 17.346431
EXTENSION     76 17.470146 17.251457
REFLECTION     78 17.452388 17.242974
EXTENSION     80 17.346431 17.109549
EXTENSION     82 17.251457 16.721648
HI-REDUCTION   84 17.242974 16.721648
LO-REDUCTION   86 17.109549 16.721648
EXTENSION     88 17.102627 16.647456
EXTENSION     90 16.875426 16.030212
LO-REDUCTION   92 16.721648 16.030212
EXTENSION     94 16.647456 15.792412

```

REFLECTION	96	16.108597	15.274100
LO-REDUCTION	98	16.030212	15.274100
LO-REDUCTION	100	15.792412	15.274100
HI-REDUCTION	102	15.588970	15.274100
LO-REDUCTION	104	15.564982	15.274100
EXTENSION	106	15.487884	15.048455
HI-REDUCTION	108	15.323745	15.048455
HI-REDUCTION	110	15.301289	15.048455
LO-REDUCTION	112	15.274100	15.048455
LO-REDUCTION	114	15.238786	15.048455
LO-REDUCTION	116	15.130204	15.048455
REFLECTION	118	15.129923	15.019594
HI-REDUCTION	120	15.079956	15.019594
REFLECTION	122	15.070175	15.014786
HI-REDUCTION	124	15.048455	15.014786
HI-REDUCTION	126	15.045089	15.014786
HI-REDUCTION	128	15.034457	15.014786
HI-REDUCTION	130	15.031634	15.014786
HI-REDUCTION	132	15.025966	15.014786
REFLECTION	134	15.023741	15.004860
HI-REDUCTION	136	15.019594	15.004860
HI-REDUCTION	138	15.016304	15.004860
HI-REDUCTION	140	15.014786	15.004860
HI-REDUCTION	142	15.012435	15.004860
REFLECTION	144	15.012302	15.004749
HI-REDUCTION	146	15.010433	15.004749
HI-REDUCTION	148	15.008982	15.004749
HI-REDUCTION	150	15.007871	15.004749
HI-REDUCTION	152	15.007326	15.004749
HI-REDUCTION	154	15.006662	15.004749
HI-REDUCTION	156	15.006359	15.004749
HI-REDUCTION	158	15.005968	15.004749
HI-REDUCTION	160	15.005770	15.004749
HI-REDUCTION	162	15.005539	15.004749
HI-REDUCTION	164	15.005406	15.004749
HI-REDUCTION	166	15.005268	15.004749
HI-REDUCTION	168	15.005180	15.004749
REFLECTION	170	15.005096	15.004617
HI-REDUCTION	172	15.004917	15.004617
HI-REDUCTION	174	15.004860	15.004617
LO-REDUCTION	176	15.004827	15.004617
EXTENSION	178	15.004749	15.004353
REFLECTION	180	15.004620	15.004299
HI-REDUCTION	182	15.004617	15.004299
EXTENSION	184	15.004518	15.004078
EXTENSION	186	15.004353	15.003777
EXTENSION	188	15.004299	15.003571
LO-REDUCTION	190	15.004078	15.003571
EXTENSION	192	15.003777	15.003285
LO-REDUCTION	194	15.003700	15.003285
EXTENSION	196	15.003571	15.003070
LO-REDUCTION	198	15.003431	15.003070
LO-REDUCTION	200	15.003287	15.003070
EXTENSION	202	15.003285	15.002830
LO-REDUCTION	204	15.003146	15.002830
EXTENSION	206	15.003070	15.002549
HI-REDUCTION	208	15.002875	15.002549
REFLECTION	210	15.002868	15.002488

LO-REDUCTION	212	15.002830	15.002488
HI-REDUCTION	214	15.002684	15.002488
HI-REDUCTION	216	15.002660	15.002488
LO-REDUCTION	218	15.002613	15.002488
REFLECTION	220	15.002549	15.002409
HI-REDUCTION	222	15.002512	15.002409
LO-REDUCTION	224	15.002488	15.002409
REFLECTION	226	15.002488	15.002394
HI-REDUCTION	228	15.002445	15.002394
HI-REDUCTION	230	15.002423	15.002394
REFLECTION	232	15.002419	15.002380
LO-REDUCTION	234	15.002409	15.002380
HI-REDUCTION	236	15.002399	15.002380
HI-REDUCTION	238	15.002398	15.002380
LO-REDUCTION	240	15.002394	15.002380
LO-REDUCTION	242	15.002394	15.002380
LO-REDUCTION	244	15.002388	15.002380
EXTENSION	246	15.002382	15.002369
HI-REDUCTION	248	15.002380	15.002369
LO-REDUCTION	250	15.002380	15.002369
REFLECTION	252	15.002378	15.002367
EXTENSION	254	15.002370	15.002351
LO-REDUCTION	256	15.002369	15.002351
LO-REDUCTION	258	15.002367	15.002351
HI-REDUCTION	260	15.002361	15.002351
HI-REDUCTION	262	15.002360	15.002351
HI-REDUCTION	264	15.002359	15.002351
LO-REDUCTION	266	15.002358	15.002351
SHRINK	271	15.002359	15.002351
EXTENSION	273	15.002354	15.002341
LO-REDUCTION	275	15.002354	15.002341
EXTENSION	277	15.002351	15.002337
EXTENSION	279	15.002343	15.002323
LO-REDUCTION	281	15.002341	15.002323
LO-REDUCTION	283	15.002337	15.002323
EXTENSION	285	15.002328	15.002315
EXTENSION	287	15.002327	15.002302
EXTENSION	289	15.002323	15.002286
LO-REDUCTION	291	15.002315	15.002286
EXTENSION	293	15.002302	15.002241
LO-REDUCTION	295	15.002292	15.002241
EXTENSION	297	15.002286	15.002198
EXTENSION	299	15.002259	15.002120
LO-REDUCTION	301	15.002241	15.002120
EXTENSION	303	15.002198	15.001952
EXTENSION	305	15.002125	15.001798
LO-REDUCTION	307	15.002120	15.001798
EXTENSION	309	15.001952	15.001326
LO-REDUCTION	311	15.001818	15.001326
EXTENSION	313	15.001798	15.000870
EXTENSION	315	15.001360	15.000026
LO-REDUCTION	317	15.001326	15.000026
EXTENSION	319	15.000870	14.998570
EXTENSION	321	15.000141	14.997345
EXTENSION	323	15.000026	14.996243
EXTENSION	325	14.998570	14.994357
LO-REDUCTION	327	14.997345	14.994357
LO-REDUCTION	329	14.996243	14.994295

LO-REDUCTION	331	14.994396	14.994228
REFLECTION	333	14.994357	14.994133
HI-REDUCTION	335	14.994295	14.994133
LO-REDUCTION	337	14.994228	14.994133
LO-REDUCTION	339	14.994210	14.994133
LO-REDUCTION	341	14.994155	14.994133
HI-REDUCTION	343	14.994142	14.994132
REFLECTION	345	14.994136	14.994130
HI-REDUCTION	347	14.994133	14.994129
LO-REDUCTION	349	14.994132	14.994129
REFLECTION	351	14.994130	14.994127
LO-REDUCTION	353	14.994129	14.994127
HI-REDUCTION	355	14.994129	14.994127
LO-REDUCTION	357	14.994128	14.994127
HI-REDUCTION	359	14.994127	14.994127
HI-REDUCTION	361	14.994127	14.994127
LO-REDUCTION	363	14.994127	14.994127
EXTENSION	365	14.994127	14.994127
EXTENSION	367	14.994127	14.994126
LO-REDUCTION	369	14.994127	14.994126
EXTENSION	371	14.994127	14.994126
EXTENSION	373	14.994126	14.994125
EXTENSION	375	14.994126	14.994124
EXTENSION	377	14.994126	14.994124
LO-REDUCTION	379	14.994125	14.994124
EXTENSION	381	14.994124	14.994122
EXTENSION	383	14.994124	14.994122
HI-REDUCTION	385	14.994124	14.994122
EXTENSION	387	14.994123	14.994119
LO-REDUCTION	389	14.994122	14.994119
LO-REDUCTION	391	14.994122	14.994119
EXTENSION	393	14.994120	14.994116
EXTENSION	395	14.994119	14.994111
LO-REDUCTION	397	14.994119	14.994111
EXTENSION	399	14.994116	14.994107
EXTENSION	401	14.994111	14.994093
LO-REDUCTION	403	14.994111	14.994093
EXTENSION	405	14.994107	14.994092
EXTENSION	407	14.994097	14.994058
LO-REDUCTION	409	14.994093	14.994058
LO-REDUCTION	411	14.994092	14.994058
EXTENSION	413	14.994065	14.994003
LO-REDUCTION	415	14.994061	14.994003
LO-REDUCTION	417	14.994058	14.994003
EXTENSION	419	14.994028	14.993951
EXTENSION	421	14.994010	14.993908
EXTENSION	423	14.994003	14.993822
LO-REDUCTION	425	14.993951	14.993822
EXTENSION	427	14.993908	14.993703
EXTENSION	429	14.993853	14.993628
EXTENSION	431	14.993822	14.993398
LO-REDUCTION	433	14.993703	14.993398
EXTENSION	435	14.993628	14.993237
EXTENSION	437	14.993479	14.992983
EXTENSION	439	14.993398	14.992474
HI-REDUCTION	441	14.993237	14.992474
LO-REDUCTION	443	14.993060	14.992474
HI-REDUCTION	445	14.992983	14.992474

EXTENSION	447	14.992828	14.992071
REFLECTION	449	14.992551	14.991898
EXTENSION	451	14.992474	14.991299
LO-REDUCTION	453	14.992071	14.991299
HI-REDUCTION	455	14.991898	14.991299
REFLECTION	457	14.991764	14.991222
EXTENSION	459	14.991387	14.990617
LO-REDUCTION	461	14.991299	14.990617
LO-REDUCTION	463	14.991222	14.990617
REFLECTION	465	14.990769	14.990225
EXTENSION	467	14.990658	14.989888
HI-REDUCTION	469	14.990617	14.989888
EXTENSION	471	14.990407	14.989611
EXTENSION	473	14.990225	14.988911
LO-REDUCTION	475	14.989888	14.988911
LO-REDUCTION	477	14.989611	14.988911
REFLECTION	479	14.989073	14.988411
LO-REDUCTION	481	14.989040	14.988411
HI-REDUCTION	483	14.988911	14.988411
LO-REDUCTION	485	14.988806	14.988411
HI-REDUCTION	487	14.988654	14.988411
LO-REDUCTION	489	14.988628	14.988411
REFLECTION	491	14.988519	14.988394
HI-REDUCTION	493	14.988508	14.988394
HI-REDUCTION	495	14.988462	14.988394
HI-REDUCTION	497	14.988445	14.988394
HI-REDUCTION	499	14.988428	14.988394
REFLECTION	501	14.988416	14.988345
HI-REDUCTION	503	14.988411	14.988345
HI-REDUCTION	505	14.988394	14.988345
HI-REDUCTION	507	14.988389	14.988345
HI-REDUCTION	509	14.988377	14.988345
LO-REDUCTION	511	14.988375	14.988345
LO-REDUCTION	513	14.988373	14.988345
HI-REDUCTION	515	14.988362	14.988345
REFLECTION	517	14.988361	14.988337
HI-REDUCTION	519	14.988351	14.988337
HI-REDUCTION	521	14.988346	14.988337
REFLECTION	523	14.988346	14.988336
LO-REDUCTION	525	14.988345	14.988336
HI-REDUCTION	527	14.988341	14.988336
HI-REDUCTION	529	14.988340	14.988336
LO-REDUCTION	531	14.988339	14.988336
HI-REDUCTION	533	14.988338	14.988336
REFLECTION	535	14.988338	14.988336
LO-REDUCTION	537	14.988337	14.988336
REFLECTION	539	14.988336	14.988336
SHRINK	544	14.988337	14.988335
LO-REDUCTION	546	14.988336	14.988335
SHRINK	551	14.988337	14.988335
REFLECTION	553	14.988336	14.988334
SHRINK	558	14.988337	14.988334
LO-REDUCTION	560	14.988336	14.988334
SHRINK	565	14.988336	14.988334
SHRINK	570	14.988337	14.988334
HI-REDUCTION	572	14.988337	14.988334
HI-REDUCTION	574	14.988336	14.988334
SHRINK	579	14.988338	14.988334

LO-REDUCTION	581	14.988337	14.988334
HI-REDUCTION	583	14.988337	14.988334
LO-REDUCTION	585	14.988336	14.988334
HI-REDUCTION	587	14.988336	14.988334
SHRINK	592	14.988338	14.988334
LO-REDUCTION	594	14.988337	14.988334
SHRINK	599	14.988337	14.988334
HI-REDUCTION	601	14.988337	14.988334
HI-REDUCTION	603	14.988336	14.988334
SHRINK	608	14.988337	14.988334
LO-REDUCTION	610	14.988335	14.988334
SHRINK	615	14.988337	14.988334
LO-REDUCTION	617	14.988336	14.988334
SHRINK	622	14.988338	14.988334
LO-REDUCTION	624	14.988336	14.988334
SHRINK	629	14.988337	14.988334
HI-REDUCTION	631	14.988337	14.988334
HI-REDUCTION	633	14.988337	14.988334
LO-REDUCTION	635	14.988336	14.988334
HI-REDUCTION	637	14.988336	14.988334
HI-REDUCTION	639	14.988335	14.988334
SHRINK	644	14.988338	14.988334
LO-REDUCTION	646	14.988336	14.988334
SHRINK	651	14.988337	14.988334
LO-REDUCTION	653	14.988337	14.988334
LO-REDUCTION	655	14.988336	14.988334
SHRINK	660	14.988337	14.988334
LO-REDUCTION	662	14.988337	14.988334
SHRINK	667	14.988338	14.988334
LO-REDUCTION	669	14.988337	14.988334
HI-REDUCTION	671	14.988336	14.988334
SHRINK	676	14.988338	14.988334
LO-REDUCTION	678	14.988337	14.988334
LO-REDUCTION	680	14.988337	14.988334
LO-REDUCTION	682	14.988336	14.988334
SHRINK	687	14.988337	14.988334
HI-REDUCTION	689	14.988336	14.988334
LO-REDUCTION	691	14.988336	14.988334
HI-REDUCTION	693	14.988336	14.988334
SHRINK	698	14.988336	14.988334
SHRINK	703	14.988338	14.988334
HI-REDUCTION	705	14.988337	14.988334
LO-REDUCTION	707	14.988337	14.988334
LO-REDUCTION	709	14.988337	14.988334
LO-REDUCTION	711	14.988336	14.988334
SHRINK	716	14.988336	14.988334
SHRINK	721	14.988338	14.988334
LO-REDUCTION	723	14.988335	14.988334
SHRINK	728	14.988337	14.988334
LO-REDUCTION	730	14.988337	14.988334
LO-REDUCTION	732	14.988336	14.988334
SHRINK	737	14.988337	14.988334
LO-REDUCTION	739	14.988336	14.988334
LO-REDUCTION	741	14.988335	14.988334
SHRINK	746	14.988338	14.988334
LO-REDUCTION	748	14.988336	14.988334
HI-REDUCTION	750	14.988336	14.988334
SHRINK	755	14.988337	14.988334

LO-REDUCTION	757	14.988336	14.988334
HI-REDUCTION	759	14.988336	14.988334
HI-REDUCTION	761	14.988336	14.988334
HI-REDUCTION	763	14.988335	14.988334
SHRINK	768	14.988338	14.988334
LO-REDUCTION	770	14.988336	14.988334
LO-REDUCTION	772	14.988336	14.988334
SHRINK	777	14.988337	14.988334
LO-REDUCTION	779	14.988336	14.988334
SHRINK	784	14.988338	14.988334
LO-REDUCTION	786	14.988337	14.988334
HI-REDUCTION	788	14.988336	14.988334
LO-REDUCTION	790	14.988336	14.988334
LO-REDUCTION	792	14.988336	14.988334
SHRINK	797	14.988338	14.988334
LO-REDUCTION	799	14.988337	14.988334
LO-REDUCTION	801	14.988335	14.988334
SHRINK	806	14.988337	14.988334
SHRINK	811	14.988337	14.988334
LO-REDUCTION	813	14.988337	14.988334
HI-REDUCTION	815	14.988337	14.988334
LO-REDUCTION	817	14.988336	14.988334
HI-REDUCTION	819	14.988336	14.988334
LO-REDUCTION	821	14.988336	14.988334
LO-REDUCTION	823	14.988335	14.988334
SHRINK	828	14.988337	14.988334
LO-REDUCTION	830	14.988336	14.988334
LO-REDUCTION	832	14.988335	14.988334
SHRINK	837	14.988337	14.988334
SHRINK	842	14.988337	14.988334
HI-REDUCTION	844	14.988335	14.988334
SHRINK	849	14.988337	14.988334
LO-REDUCTION	851	14.988336	14.988334
LO-REDUCTION	853	14.988335	14.988334
SHRINK	858	14.988337	14.988334
SHRINK	863	14.988336	14.988334
HI-REDUCTION	865	14.988335	14.988334

Exiting from Nelder Mead minimizer

867 function evaluations used

Nelder-Mead direct search function minimizer

function value for initial parameters = 2.395523

Scaled convergence tolerance is 2.39552e-50

Stepsize computed as 0.050000

BUILD	4	2.566998	2.395523
EXTENSION	6	2.415624	2.109593
LO-REDUCTION	8	2.405573	2.109593
HI-REDUCTION	10	2.395523	2.109593
HI-REDUCTION	12	2.312510	2.109593
HI-REDUCTION	14	2.292622	2.109593
HI-REDUCTION	16	2.251857	2.109593
REFLECTION	18	2.235022	2.100643
HI-REDUCTION	20	2.201307	2.100643
HI-REDUCTION	22	2.181863	2.100643
HI-REDUCTION	24	2.160422	2.100643
HI-REDUCTION	26	2.150724	2.100643
HI-REDUCTION	28	2.138163	2.100643
HI-REDUCTION	30	2.132223	2.100643
HI-REDUCTION	32	2.124970	2.100643

HI-REDUCTION	34	2.121095	2.100643
HI-REDUCTION	36	2.116862	2.100643
HI-REDUCTION	38	2.114317	2.100643
HI-REDUCTION	40	2.111801	2.100643
HI-REDUCTION	42	2.110143	2.100643
HI-REDUCTION	44	2.109593	2.100643
LO-REDUCTION	46	2.108621	2.100643
LO-REDUCTION	48	2.107551	2.100643
LO-REDUCTION	50	2.106087	2.100643
REFLECTION	52	2.101836	2.096425
LO-REDUCTION	54	2.101480	2.096425
HI-REDUCTION	56	2.100643	2.096425
HI-REDUCTION	58	2.099751	2.096425
HI-REDUCTION	60	2.099186	2.096425
HI-REDUCTION	62	2.098669	2.096425
HI-REDUCTION	64	2.098299	2.096425
REFLECTION	66	2.097984	2.096104
HI-REDUCTION	68	2.097565	2.096104
LO-REDUCTION	70	2.097276	2.096104
HI-REDUCTION	72	2.096689	2.096104
REFLECTION	74	2.096425	2.095789
HI-REDUCTION	76	2.096304	2.095789
HI-REDUCTION	78	2.096197	2.095789
HI-REDUCTION	80	2.096104	2.095789
HI-REDUCTION	82	2.096101	2.095789
REFLECTION	84	2.096064	2.095779
HI-REDUCTION	86	2.095981	2.095779
REFLECTION	88	2.095946	2.095721
HI-REDUCTION	90	2.095842	2.095721
HI-REDUCTION	92	2.095793	2.095721
REFLECTION	94	2.095789	2.095697
HI-REDUCTION	96	2.095779	2.095697
HI-REDUCTION	98	2.095749	2.095697
HI-REDUCTION	100	2.095735	2.095697
LO-REDUCTION	102	2.095731	2.095697
HI-REDUCTION	104	2.095721	2.095697
LO-REDUCTION	106	2.095720	2.095697
HI-REDUCTION	108	2.095716	2.095697
HI-REDUCTION	110	2.095712	2.095697
HI-REDUCTION	112	2.095708	2.095697
HI-REDUCTION	114	2.095707	2.095697
HI-REDUCTION	116	2.095705	2.095697
LO-REDUCTION	118	2.095704	2.095697
HI-REDUCTION	120	2.095701	2.095697
HI-REDUCTION	122	2.095701	2.095697
HI-REDUCTION	124	2.095700	2.095697
HI-REDUCTION	126	2.095700	2.095697
HI-REDUCTION	128	2.095699	2.095697
REFLECTION	130	2.095699	2.095697
HI-REDUCTION	132	2.095698	2.095697
HI-REDUCTION	134	2.095698	2.095697
LO-REDUCTION	136	2.095697	2.095697
EXTENSION	138	2.095697	2.095695
REFLECTION	140	2.095697	2.095695
HI-REDUCTION	142	2.095697	2.095695
EXTENSION	144	2.095696	2.095695
EXTENSION	146	2.095695	2.095694
LO-REDUCTION	148	2.095695	2.095694

HI-REDUCTION	150	2.095695	2.095694
HI-REDUCTION	152	2.095694	2.095694
LO-REDUCTION	154	2.095694	2.095694
EXTENSION	156	2.095694	2.095693
REFLECTION	158	2.095694	2.095693
HI-REDUCTION	160	2.095694	2.095693
HI-REDUCTION	162	2.095693	2.095693
HI-REDUCTION	164	2.095693	2.095693
HI-REDUCTION	166	2.095693	2.095693
REFLECTION	168	2.095693	2.095693
HI-REDUCTION	170	2.095693	2.095693
LO-REDUCTION	172	2.095693	2.095693
HI-REDUCTION	174	2.095693	2.095693
HI-REDUCTION	176	2.095693	2.095693
HI-REDUCTION	178	2.095693	2.095693
HI-REDUCTION	180	2.095693	2.095693
HI-REDUCTION	182	2.095693	2.095693
REFLECTION	184	2.095693	2.095693
HI-REDUCTION	186	2.095693	2.095693
HI-REDUCTION	188	2.095693	2.095693
HI-REDUCTION	190	2.095693	2.095693
SHRINK	195	2.095693	2.095693
LO-REDUCTION	197	2.095693	2.095693
LO-REDUCTION	199	2.095693	2.095693
HI-REDUCTION	201	2.095693	2.095693

Exiting from Nelder Mead minimizer

203 function evaluations used

Nelder-Mead direct search function minimizer

function value for initial parameters = 5.190928

Scaled convergence tolerance is 5.19093e-50

Stepsize computed as 0.050000

BUILD	4	5.587631	5.190928
EXTENSION	6	5.237953	4.300551
LO-REDUCTION	8	5.217746	4.300551
HI-REDUCTION	10	5.190928	4.300551
HI-REDUCTION	12	4.973567	4.300551
HI-REDUCTION	14	4.917962	4.300551
HI-REDUCTION	16	4.796876	4.300551
REFLECTION	18	4.745162	4.230417
HI-REDUCTION	20	4.629238	4.230417
HI-REDUCTION	22	4.565806	4.230417
HI-REDUCTION	24	4.482257	4.230417
HI-REDUCTION	26	4.449166	4.230417
HI-REDUCTION	28	4.396682	4.230417
HI-REDUCTION	30	4.375279	4.230417
HI-REDUCTION	32	4.343970	4.230417
HI-REDUCTION	34	4.329283	4.230417
HI-REDUCTION	36	4.310826	4.230417
HI-REDUCTION	38	4.300809	4.230417
HI-REDUCTION	40	4.300551	4.230417
LO-REDUCTION	42	4.289855	4.230417
LO-REDUCTION	44	4.283156	4.230417
REFLECTION	46	4.269761	4.227038
HI-REDUCTION	48	4.248776	4.227038
REFLECTION	50	4.234143	4.206784
REFLECTION	52	4.230417	4.204785
LO-REDUCTION	54	4.227038	4.204785
HI-REDUCTION	56	4.216565	4.204785

HI-REDUCTION	58	4.211397	4.204785
REFLECTION	60	4.210185	4.201202
HI-REDUCTION	62	4.206784	4.201202
LO-REDUCTION	64	4.206310	4.201202
HI-REDUCTION	66	4.204785	4.201202
LO-REDUCTION	68	4.204516	4.201202
HI-REDUCTION	70	4.203273	4.201202
EXTENSION	72	4.203088	4.200517
HI-REDUCTION	74	4.201797	4.200517
HI-REDUCTION	76	4.201606	4.200517
HI-REDUCTION	78	4.201202	4.200517
EXTENSION	80	4.201181	4.199571
HI-REDUCTION	82	4.200822	4.199571
HI-REDUCTION	84	4.200621	4.199571
HI-REDUCTION	86	4.200517	4.199571
HI-REDUCTION	88	4.200379	4.199571
LO-REDUCTION	90	4.200296	4.199571
HI-REDUCTION	92	4.200134	4.199571
HI-REDUCTION	94	4.200004	4.199571
HI-REDUCTION	96	4.199892	4.199571
HI-REDUCTION	98	4.199826	4.199571
HI-REDUCTION	100	4.199757	4.199571
HI-REDUCTION	102	4.199716	4.199571
HI-REDUCTION	104	4.199674	4.199571
HI-REDUCTION	106	4.199649	4.199571
REFLECTION	108	4.199623	4.199481
HI-REDUCTION	110	4.199586	4.199481
HI-REDUCTION	112	4.199571	4.199481
HI-REDUCTION	114	4.199570	4.199481
HI-REDUCTION	116	4.199542	4.199481
LO-REDUCTION	118	4.199539	4.199481
HI-REDUCTION	120	4.199537	4.199481
HI-REDUCTION	122	4.199517	4.199481
HI-REDUCTION	124	4.199514	4.199481
HI-REDUCTION	126	4.199505	4.199481
HI-REDUCTION	128	4.199502	4.199481
HI-REDUCTION	130	4.199498	4.199481
HI-REDUCTION	132	4.199496	4.199481
REFLECTION	134	4.199493	4.199479
HI-REDUCTION	136	4.199488	4.199479
HI-REDUCTION	138	4.199488	4.199479
HI-REDUCTION	140	4.199485	4.199479
HI-REDUCTION	142	4.199485	4.199479
HI-REDUCTION	144	4.199484	4.199479
HI-REDUCTION	146	4.199483	4.199479
HI-REDUCTION	148	4.199482	4.199479
LO-REDUCTION	150	4.199482	4.199479
HI-REDUCTION	152	4.199481	4.199479
HI-REDUCTION	154	4.199481	4.199479
HI-REDUCTION	156	4.199481	4.199479
HI-REDUCTION	158	4.199480	4.199479
HI-REDUCTION	160	4.199480	4.199479
HI-REDUCTION	162	4.199480	4.199479
REFLECTION	164	4.199480	4.199479
HI-REDUCTION	166	4.199479	4.199479
HI-REDUCTION	168	4.199479	4.199479
REFLECTION	170	4.199479	4.199479
HI-REDUCTION	172	4.199479	4.199479

HI-REDUCTION	174	4.199479	4.199479
HI-REDUCTION	176	4.199479	4.199479
HI-REDUCTION	178	4.199479	4.199479
LO-REDUCTION	180	4.199479	4.199479
SHRINK	185	4.199479	4.199479
LO-REDUCTION	187	4.199479	4.199479
REFLECTION	189	4.199479	4.199479
LO-REDUCTION	191	4.199479	4.199479
REFLECTION	193	4.199479	4.199478
HI-REDUCTION	195	4.199479	4.199478
SHRINK	200	4.199479	4.199478
LO-REDUCTION	202	4.199479	4.199478
LO-REDUCTION	204	4.199479	4.199478
SHRINK	209	4.199479	4.199478
LO-REDUCTION	211	4.199479	4.199478
SHRINK	216	4.199479	4.199478
SHRINK	221	4.199479	4.199478
LO-REDUCTION	223	4.199479	4.199478
HI-REDUCTION	225	4.199479	4.199478
SHRINK	230	4.199479	4.199478
LO-REDUCTION	232	4.199479	4.199478
SHRINK	237	4.199479	4.199478
LO-REDUCTION	239	4.199479	4.199478
SHRINK	244	4.199479	4.199478
LO-REDUCTION	246	4.199479	4.199478
LO-REDUCTION	248	4.199479	4.199478
LO-REDUCTION	250	4.199479	4.199478
SHRINK	255	4.199479	4.199478
HI-REDUCTION	257	4.199479	4.199478
SHRINK	262	4.199479	4.199478
LO-REDUCTION	264	4.199479	4.199478
LO-REDUCTION	266	4.199479	4.199478
LO-REDUCTION	268	4.199479	4.199478
LO-REDUCTION	270	4.199479	4.199478
SHRINK	275	4.199479	4.199478
SHRINK	280	4.199479	4.199478
LO-REDUCTION	282	4.199479	4.199478
SHRINK	287	4.199479	4.199478
LO-REDUCTION	289	4.199479	4.199478
SHRINK	294	4.199479	4.199478
HI-REDUCTION	296	4.199479	4.199478
HI-REDUCTION	298	4.199479	4.199478
SHRINK	303	4.199479	4.199478
HI-REDUCTION	305	4.199479	4.199478
SHRINK	310	4.199479	4.199478
LO-REDUCTION	312	4.199479	4.199478
HI-REDUCTION	314	4.199479	4.199478
LO-REDUCTION	316	4.199479	4.199478
SHRINK	321	4.199479	4.199478
LO-REDUCTION	323	4.199479	4.199478
LO-REDUCTION	325	4.199479	4.199478
HI-REDUCTION	327	4.199479	4.199478
HI-REDUCTION	329	4.199479	4.199478
SHRINK	334	4.199479	4.199478
HI-REDUCTION	336	4.199479	4.199478
SHRINK	341	4.199479	4.199478
LO-REDUCTION	343	4.199479	4.199478
HI-REDUCTION	345	4.199479	4.199478

LO-REDUCTION	347	4.199479	4.199478
SHRINK	352	4.199479	4.199478
LO-REDUCTION	354	4.199479	4.199478
SHRINK	359	4.199479	4.199478
SHRINK	364	4.199479	4.199478
LO-REDUCTION	366	4.199479	4.199478
LO-REDUCTION	368	4.199479	4.199478
SHRINK	373	4.199479	4.199478
LO-REDUCTION	375	4.199479	4.199478
SHRINK	380	4.199479	4.199478
LO-REDUCTION	382	4.199479	4.199478
SHRINK	387	4.199479	4.199478
LO-REDUCTION	389	4.199479	4.199478
HI-REDUCTION	391	4.199479	4.199478
LO-REDUCTION	393	4.199479	4.199478
LO-REDUCTION	395	4.199479	4.199478
SHRINK	400	4.199479	4.199478
LO-REDUCTION	402	4.199479	4.199478
HI-REDUCTION	404	4.199479	4.199478
SHRINK	409	4.199479	4.199478
HI-REDUCTION	411	4.199479	4.199478
LO-REDUCTION	413	4.199479	4.199478
SHRINK	418	4.199479	4.199478
HI-REDUCTION	420	4.199479	4.199478
LO-REDUCTION	422	4.199479	4.199478
SHRINK	427	4.199479	4.199478
LO-REDUCTION	429	4.199479	4.199478
SHRINK	434	4.199479	4.199478
LO-REDUCTION	436	4.199479	4.199478
SHRINK	441	4.199479	4.199478
HI-REDUCTION	443	4.199479	4.199478
HI-REDUCTION	445	4.199479	4.199478
HI-REDUCTION	447	4.199479	4.199478
SHRINK	452	4.199479	4.199478
LO-REDUCTION	454	4.199478	4.199478
SHRINK	459	4.199479	4.199478
LO-REDUCTION	461	4.199479	4.199478
HI-REDUCTION	463	4.199479	4.199478
HI-REDUCTION	465	4.199479	4.199478
SHRINK	470	4.199479	4.199478
HI-REDUCTION	472	4.199479	4.199478
SHRINK	477	4.199479	4.199478
HI-REDUCTION	479	4.199479	4.199478
LO-REDUCTION	481	4.199479	4.199478
HI-REDUCTION	483	4.199478	4.199478
SHRINK	488	4.199479	4.199478
HI-REDUCTION	490	4.199479	4.199478
HI-REDUCTION	492	4.199479	4.199478
LO-REDUCTION	494	4.199478	4.199478
SHRINK	499	4.199479	4.199478
HI-REDUCTION	501	4.199479	4.199478
HI-REDUCTION	503	4.199479	4.199478
HI-REDUCTION	505	4.199479	4.199478
SHRINK	510	4.199479	4.199478
LO-REDUCTION	512	4.199479	4.199478
SHRINK	517	4.199479	4.199478
LO-REDUCTION	519	4.199479	4.199478
SHRINK	524	4.199479	4.199478

```

LO-REDUCTION      526 4.199479 4.199478
SHRINK            531 4.199479 4.199478
HI-REDUCTION     533 4.199479 4.199478
LO-REDUCTION     535 4.199478 4.199478
SHRINK           540 4.199479 4.199478
HI-REDUCTION     542 4.199479 4.199478
LO-REDUCTION     544 4.199479 4.199478
SHRINK           549 4.199479 4.199478
Polytope size measure not decreased in shrink
Exiting from Nelder Mead minimizer
  551 function evaluations used

> pD <- getLikelihoods(pD, cl = NULL)
.

> topCounts(pD, "DT", normaliseData = TRUE)

      seqnames start  end width strand frame startCodon stopCodon context minus3
1 Cre17.g723750.t1.3  271 471  201   *    0      ATG      TGA GGAATGG    G
2      CUFF.9661.1   424 582  159   *    0      ATG      TGA ACAATGG    A
3      CUFF.43721.1     6 161  156   *    2      ATG      TAA GCAATGC    G
4      CUFF.9523.1    78 1040  963   *    2      ATG      TAA AAAATGG    A
plus1  WT.1  WT.2  M.1  M.2 Likelihood ordering  FDR.DT  FWER.DT
1  G  3:3  1:1  2:2  5:5 0.05882353  M=WT 0.9411765 0.9411765
2  G  0:0  1:1  1:1  0:0 0.05882352  M=WT 0.9411765 0.9965398
3  C  1:1  3:3  2:2  0:0 0.05882350  M=WT 0.9411765 0.9997965
4  G 246:246 491:491 247:247 3586:3586 0.05882193  M=WT 0.9411769 0.9999880

```

Session Info

```

> sessionInfo()

R version 3.4.0 (2017-04-21)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 16.04.2 LTS

Matrix products: default
BLAS: /home/biocbuild/bbs-3.5-bioc/R/lib/libRblas.so
LAPACK: /home/biocbuild/bbs-3.5-bioc/R/lib/libRlapack.so

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

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

other attached packages:
[1] baySeq_2.10.0      riboSeqR_1.10.0      abind_1.4-5          GenomicRanges_1.28.0
[5] GenomeInfoDb_1.12.0 IRanges_2.10.0       S4Vectors_0.14.0    BiocGenerics_0.22.0

loaded via a namespace (and not attached):
[1] Rcpp_0.12.10      edgeR_3.18.0        knitr_1.15.1
[4] XVector_0.16.0    magrittr_1.5        zlibbioc_1.22.0
[7] BiocParallel_1.10.0 lattice_0.20-35     stringr_1.2.0

```

[10] tools_3.4.0	grid_3.4.0	seqLogo_1.42.0
[13] htmltools_0.3.5	yaml_2.1.14	rprojroot_1.2
[16] digest_0.6.12	GenomeInfoDbData_0.99.0	bitops_1.0-6
[19] RCurl_1.95-4.8	evaluate_0.10	rmarkdown_1.4
[22] limma_3.32.0	stringi_1.1.5	compiler_3.4.0
[25] Biostrings_2.44.0	backports_1.0.5	Rsamtools_1.28.0
[28] locfit_1.5-9.1	BiocStyle_2.4.0	

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).


```

[[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

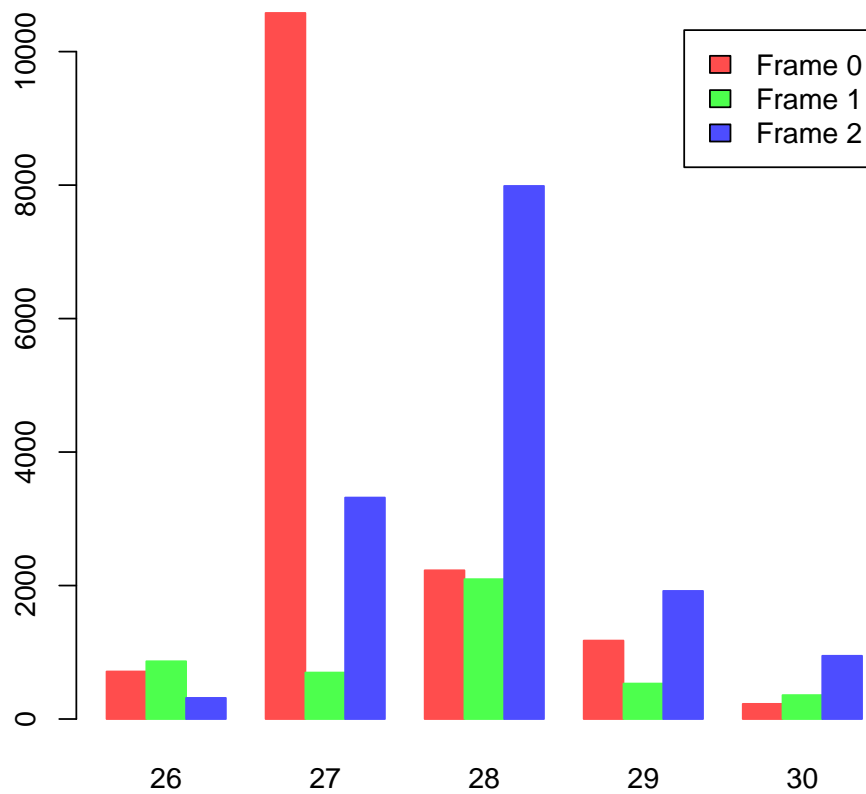


Figure 1: Number of n-mers in each frame relative to coding start. 27-mers are predominantly in frame-1, while 28-mers are chiefly in frame-0.

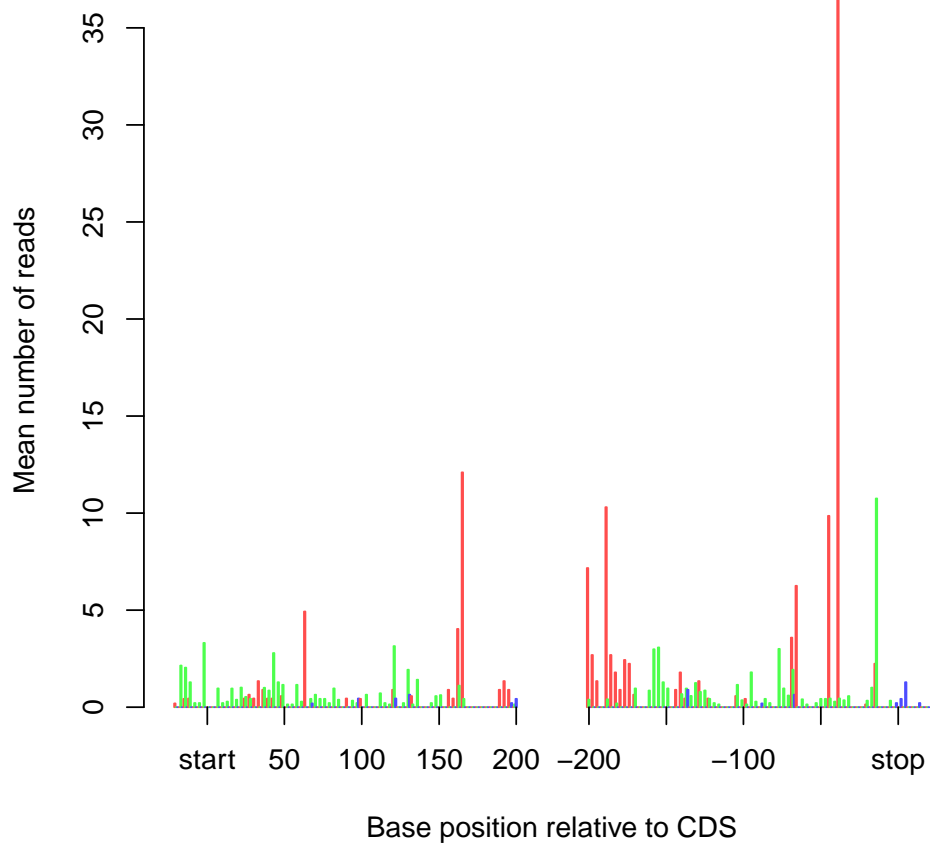


Figure 2: Average alignment of 27-mers to 5' and 3' ends of coding sequences.

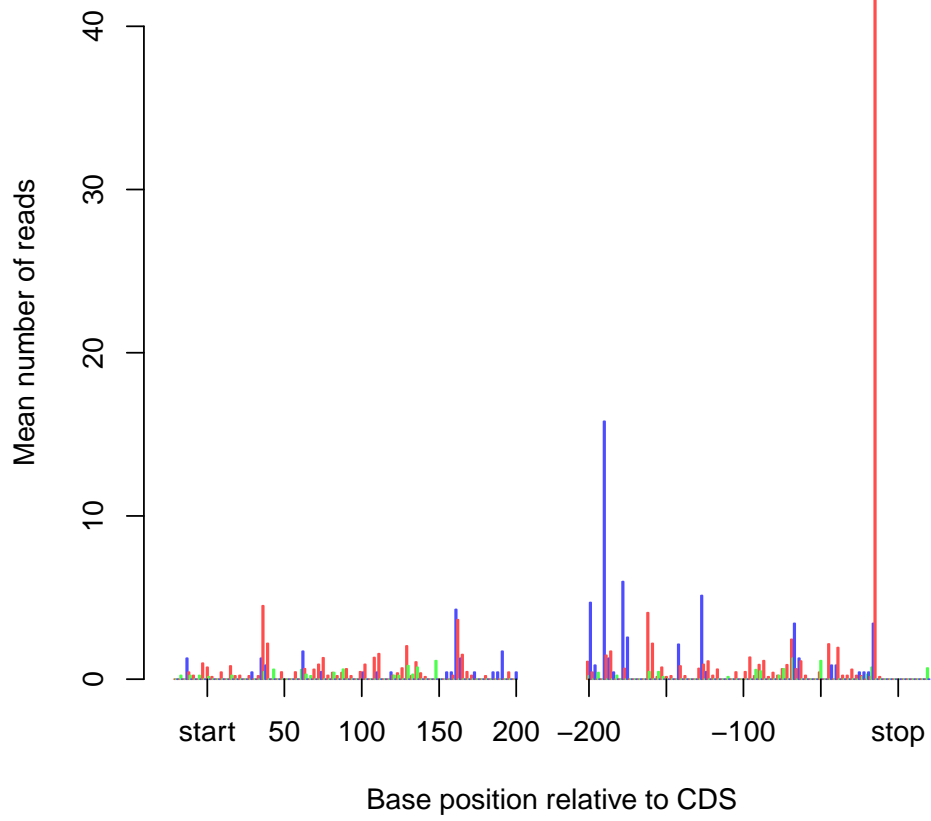


Figure 3: Average alignment of 28-mers to 5' and 3' ends of coding sequences.

NULL

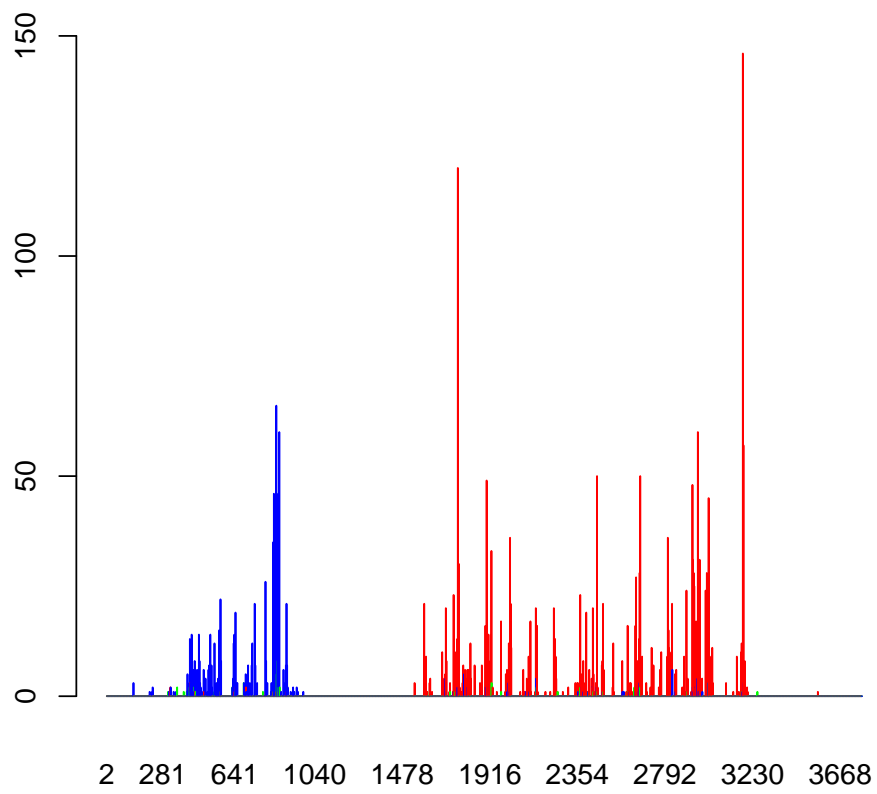
chlamy236_plus_deNovo_plusOnly_Index17 :: CUFF.37930.1

Figure 4: Alignment to individual transcript.