Quality and Index Swapping Analysis

Adrian Bubie

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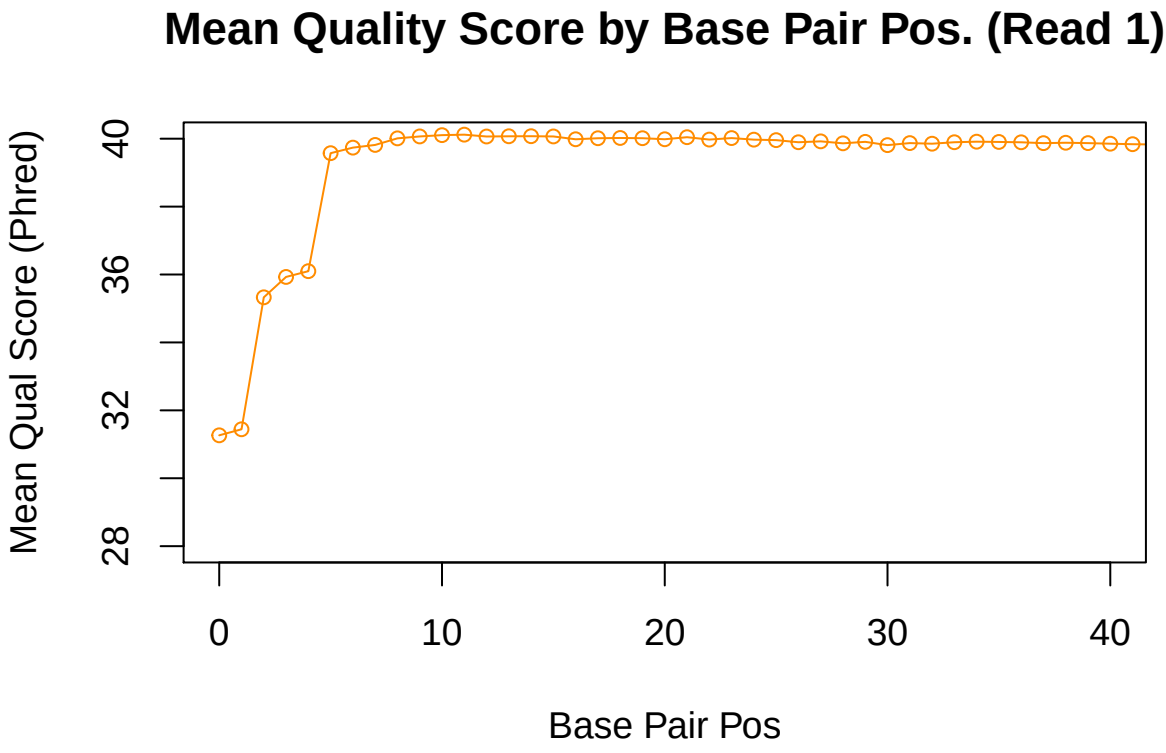
Goals: Determine if index swapping occurred in our Hiseq4000 class sequencing samples.

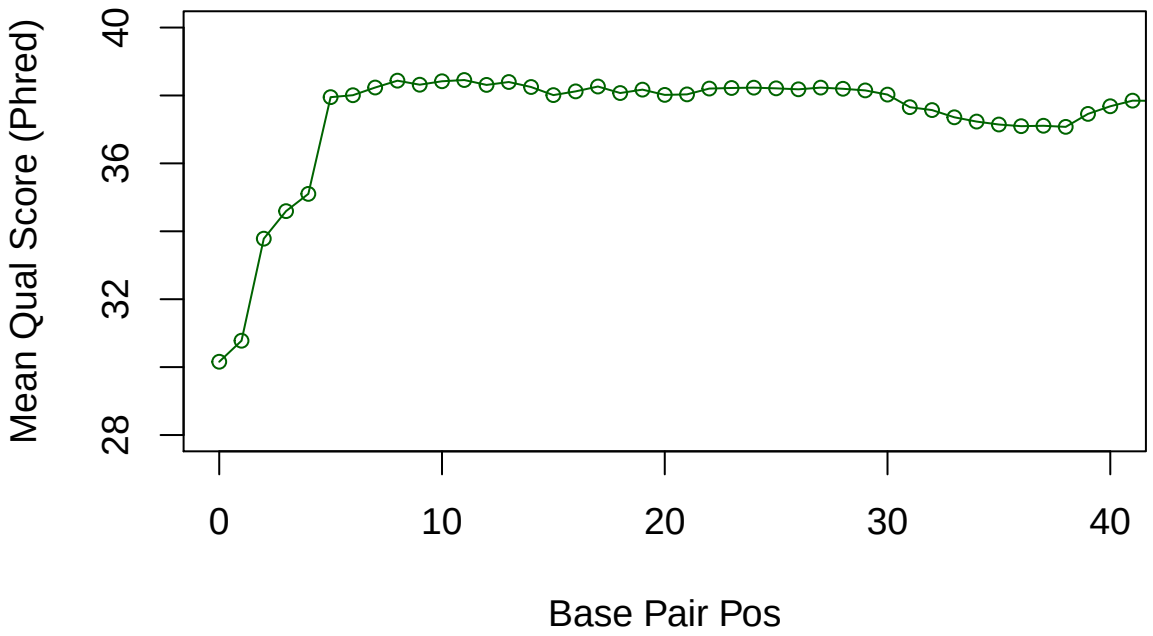
De-multiplex samples to create 48 FASTQ files that contain acceptable index pairs (read1 and read2) and two files of undetermined files that contain unacceptable index pairs, low quality, or undetermined (read1 and read2).

1. (Please see the associated python script, "Qual_Mean_Calc.py" for reference on how the files used to plot here were created).

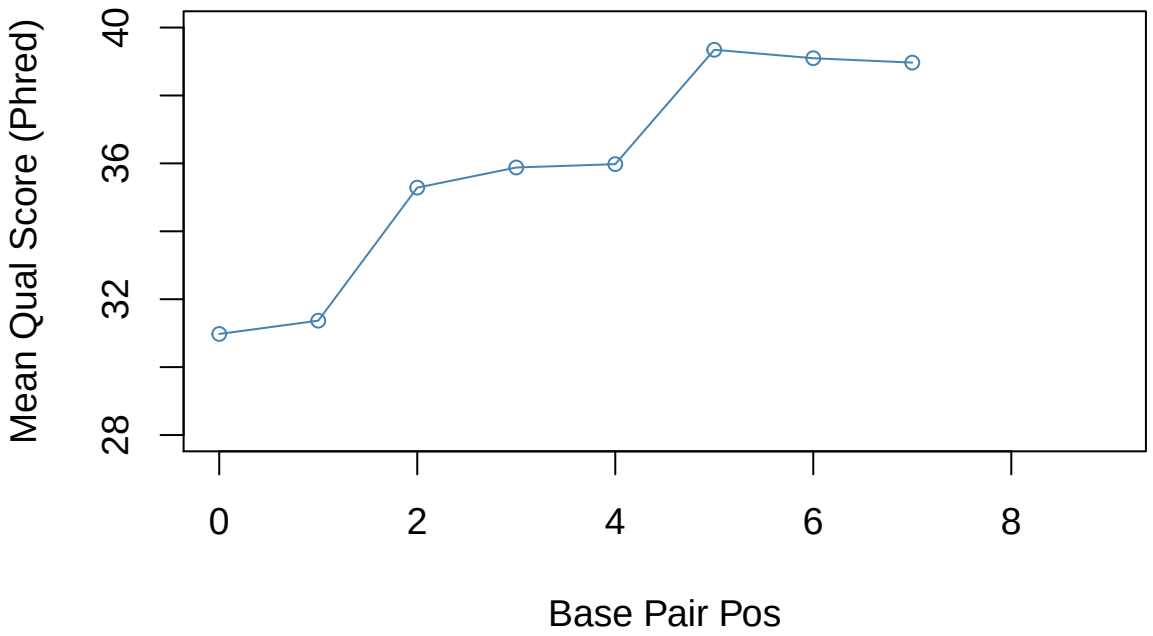
Quality plots per basepair position and distribution of average quality score per read, for each of our 4 input files (read1, read2, index1, index2):

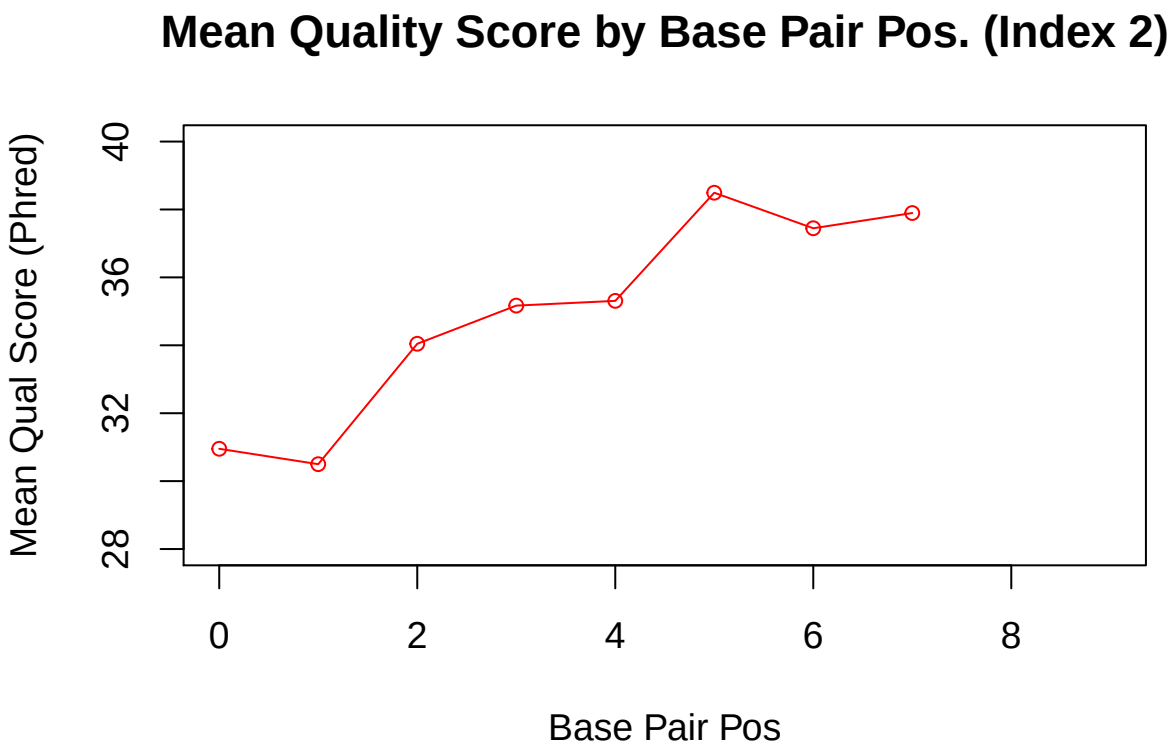


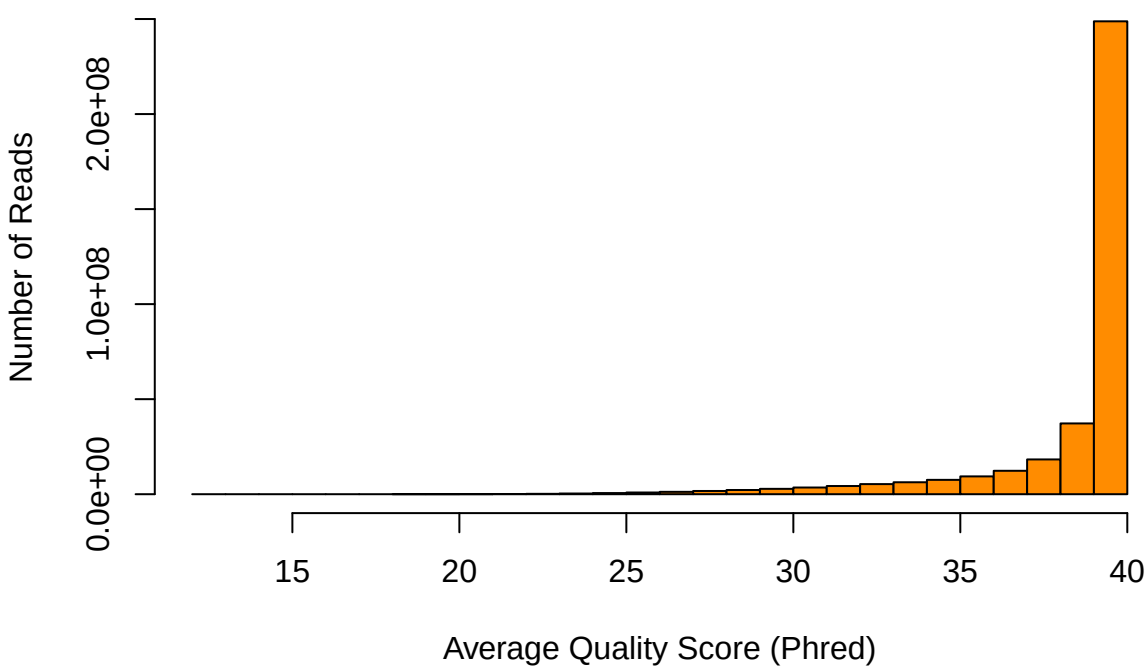
Mean Quality Score by Base Pair Pos. (Read 2)

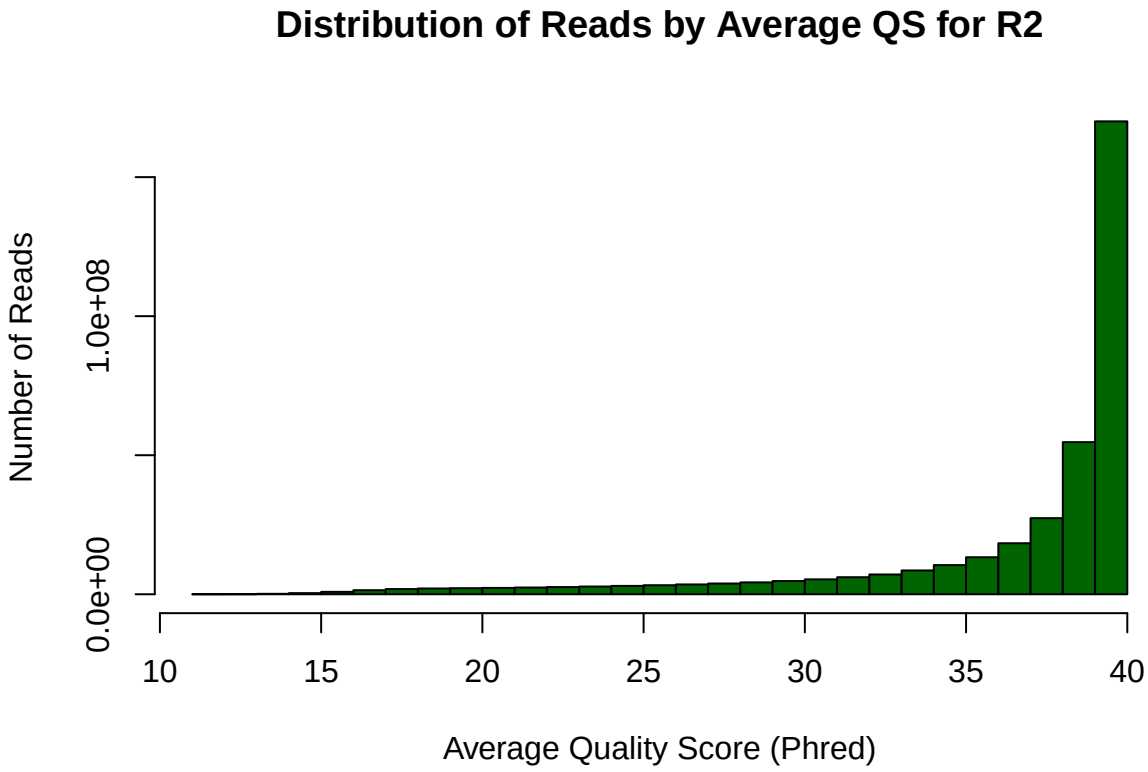


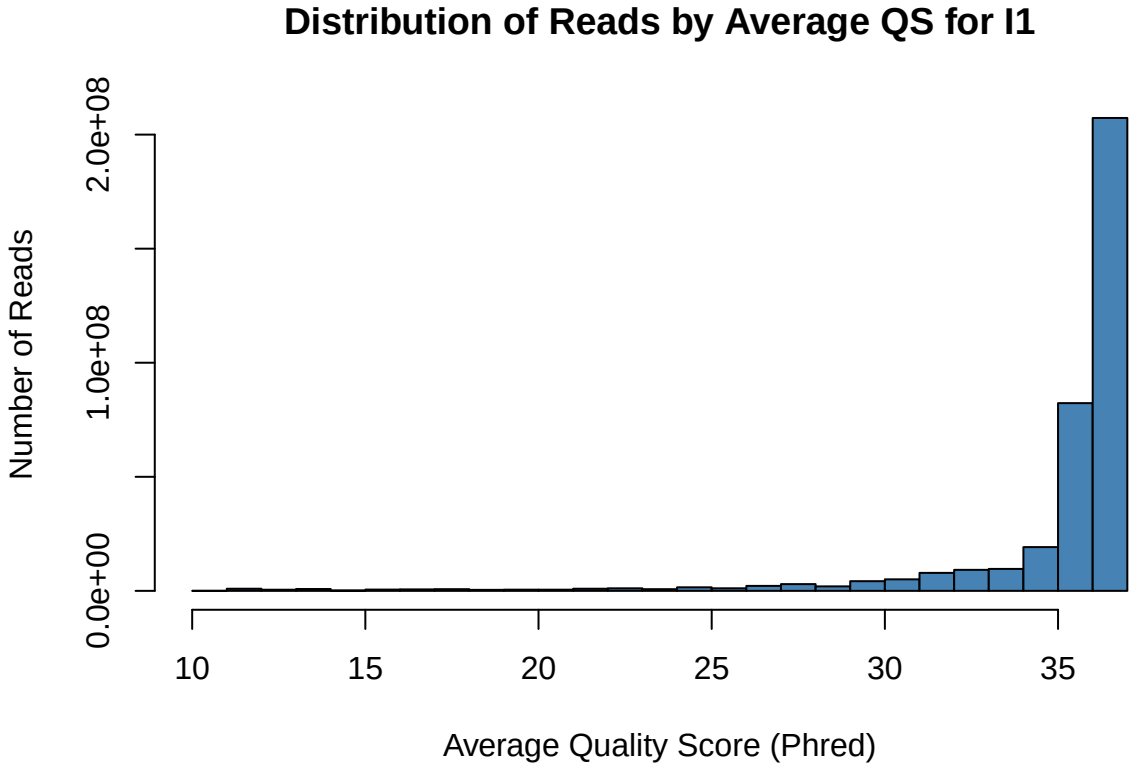
Mean Quality Score by Base Pair Pos. (Index 1)

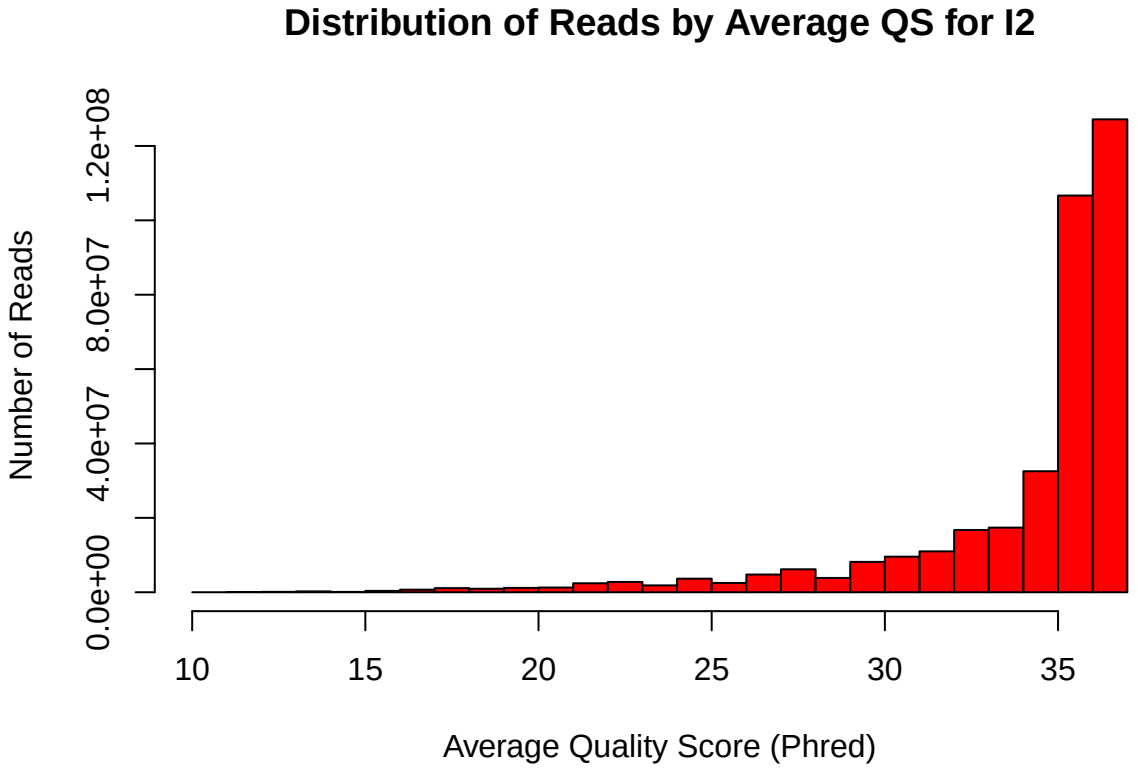



Mean Quality Score by Base Pair Pos. (Index 2)
Mean Qual Score (Phred)
28
32
36
40
0
2
4
6
8
Base Pair Pos


Distribution of Reads by Average QS for R1
Number of Reads
0.0e+00
1.0e+08
2.0e+08
15
20
25
30
35
40
Average Quality Score (Phred)


Distribution of Reads by Average QS for R2
Number of Reads
0.0e+00
1.0e+08
10
15
20
25
30
35
40
Average Quality Score (Phred)


Distribution of Reads by Average QS for I1
Number of Reads
0.0e+00
1.0e+08
2.0e+08
10
15
20
25
30
35
Average Quality Score (Phred)



From these distributions, we can most likely set a quality cutoff of 35 for the index pairs and quality cutoff of 37 for our paired reads, and retain about 3/4 (about 250 million) of our reads, while improving the quality of our reads overall.

We also know that the illumina system will sometimes record bases as 'N' for 'any' when it is not certain what base to call for a particular read. We can count the number of indexes that contain an 'N' in the sequence using the following UNIX command:

```
>$ for FILE in /projects/bgmp/2017_sequencing/*.fastq; do counts=$(awk 'NR%4==2' $FILE |
grep "N" | wc -l); echo $FILE $counts; done
/projects/bgmp/2017_sequencing/1294_S1_L008_R1_001.fastq 2602560
/projects/bgmp/2017_sequencing/1294_S1_L008_R2_001.fastq 3976613
/projects/bgmp/2017_sequencing/1294_S1_L008_R3_001.fastq 3328051
/projects/bgmp/2017_sequencing/1294_S1_L008_R4_001.fastq 3591851
```

Our Indexes are contained in the 'R2' and 'R3' files; so we have 3976613 and 3328051 reads with 'N's for our index 1 and index 2, respectively (a total of 7304664 reads across both indexes)

Overall, the read distributions show that most of our data clustered pretty high on the quality scale. Illumina defines Phred 30 as 99.9% accuracy for the basecall, and <90% of our reads have an average score of 30 or above. However, there is significant disparity between our Index read scores and our insert read qualities. Index scores appear to be overall of lower quality on average than our insert reads' qscore; additionally, we see in the graph of quality by basepair that the bases at the beginning of each index/read almost always have a average quality score lower than those of the bases further into the read. This indicates that the bases read at the beginning of the sequence (near the oligo linkage on the flowcell) are more difficult for the sequencer to resolve properly. Notice also that vast majority of the Indexes that contain an 'N' base (for unresolved or 'any' base) have that 'N' at the starting position of the index. This provides further evidence that the sequencer's resolution for bases starts poor but gets much better as synthesis continues into the insert.

2. (Please see the associated python script, "Qual_Index_Swp.py" for reference and indication of how reads were binned by index pairs). Based on analysis of the index reads above, the reads were filtered by an index average quality cut-off Phred score of 35. This means that reads that had an *both* indexes

with an average quality score of 35 or above were retained and all other reads were removed.

a. There are a total of 363246735 reads in the original Sequencing output. After filtering by index quality, a total of 245598505 reads were retained, representing a retention rate of just over 67.6%. The read counts can be broken down by index pairs (libraries), as seen below:

```
##       Index1   Index2 Read_Count
## 1   TACCGGAT TACCGGAT   51581889
## 2   TCTTCGAC TCTTCGAC   31203016
## 3   CTCTGGAT CTCTGGAT   25933763
## 4   CTAGCTCA CTAGCTCA   13661286
## 5   TGTTCCGT TGTTCCGT   12247310
## 6   AGAGTCCA AGAGTCCA    8234276
## 7   TAGCCATG TAGCCATG    7951357
## 8   TATGGCAC TATGGCAC    7880872
## 9   TCGAGAGT TCGAGAGT    7791551
## 10  ATCATGCG ATCATGCG    7448228
## 11  GTCCTAAG GTCCTAAG    6658709
## 12  AACAGCGA AACAGCGA    6606498
## 13  AGGATAGC AGGATAGC    6571536
## 14  ACGATCAG ACGATCAG    6234505
## 15  GTAGCGTA GTAGCGTA    6033486
## 16  ATCGTGGT ATCGTGGT    5031584
## 17  GATCAAGG GATCAAGG    4900440
## 18  GCTACTCT GCTACTCT    4720342
## 19  CGATCGAT CGATCGAT    4398491
## 20  TCGGATTC TCGGATTC    3149983
## 21  CGGTAATC CGGTAATC    3097661
## 22  GATCTTGC GATCTTGC    2861868
## 23  TCGACAAG TCGACAAG    2786134
## 24  CACTTCAC CACTTCAC    2765861
## 25  TATGGCAC TGTTCCGT      63153
## 26  TGTTCCGT TATGGCAC      61181
## 27  CTAGCTCA TCGACAAG       9926
## 28  GATCAAGG TCTTCGAC       9552
## 29  TCGACAAG ATCATGCG       5896
## 30  CTCTGGAT TACCGGAT       5821
## 31  TACCGGAT CTCTGGAT       5796
## 32  TACCGGAT TCTTCGAC       5626
## 33  GTCCTAAG TATGGCAC       4869
## 34  TCTTCGAC TACCGGAT       4550
## 35  CGGTAATC TACCGGAT       3861
## 36  TCTTCGAC ATCGTGGT       3083
## 37  CTCTGGAT TCTTCGAC       2776
## 38  TCTTCGAC CTCTGGAT       2747
## 39  TACCGGAT TGTTCCGT       2339
## 40  CACTTCAC TAGCCATG       2198
## 41  TATGGCAC TACCGGAT       2105
## 42  TGTTCCGT TACCGGAT       2021
## 43  CTAGCTCA CTCTGGAT       2004
## 44  TACCGGAT CTAGCTCA       1975
## 45  TATGGCAC TCTTCGAC       1885
## 46  CTAGCTCA TACCGGAT       1846
## 47  TACCGGAT TAGCCATG       1826
## 48  TACCGGAT TATGGCAC       1813
```

```
## 49  TACCGGAT TCGAGAGT       1632
## 50  TACCGGAT CGGTAATC       1622
## 51  CTCTGGAT GCTACTCT       1471
## 52  TCTTCGAC TGTTCCGT       1413
## 53  TCGAGAGT TACCGGAT       1410
## 54  CTCTGGAT CTAGCTCA       1381
## 55  TGTTCCGT TCTTCGAC       1260
## 56  TCTTCGAC TATGGCAC       1250
## 57  TCTTCGAC TCGAGAGT       1170
## 58  TCTTCGAC CTAGCTCA       1144
## 59  CTCTGGAT TCGAGAGT       1134
## 60  TACCGGAT AGAGTCCA       1123
## 61  CTCTGGAT TGTTCCGT       1116
## 62  CTAGCTCA ATCATGCG       1103
## 63  TCTTCGAC GATCAAGG       1079
## 64  TACCGGAT AGGATAGC       1063
## 65  TACCGGAT ACGATCAG       1041
## 66  CTAGCTCA TCTTCGAC       1014
## 67  AACAGCGA TACCGGAT       1011
## 68  CTAGCTCA GCTACTCT       1010
## 69  TAGCCATG TACCGGAT       1002
## 70  AACAGCGA TCTTCGAC        998
## 71  TACCGGAT GTCCTAAG        992
## 72  TACCGGAT ATCATGCG        982
## 73  TACCGGAT ATCGTGGT        973
## 74  CGATCGAT TACCGGAT        972
## 75  CTCTGGAT AGAGTCCA        969
## 76  GTAGCGTA GCTACTCT        953
## 77  AGAGTCCA TACCGGAT        949
## 78  ATCATGCG TACCGGAT        932
## 79  TACCGGAT CGATCGAT        920
## 80  CTCTGGAT CGATCGAT        913
## 81  GTCCTAAG TACCGGAT        898
## 82  ACGATCAG TACCGGAT        888
## 83  TACCGGAT AACAGCGA        878
## 84  TCTTCGAC AGGATAGC        873
## 85  TGTTCCGT CTCTGGAT        871
## 86  TACCGGAT GTAGCGTA        857
## 87  TACCGGAT GATCAAGG        826
## 88  ATCGTGGT TACCGGAT        814
## 89  TCGAGAGT CTCTGGAT        796
## 90  GTAGCGTA TACCGGAT        791
## 91  CTCTGGAT TATGGCAC        760
## 92  TACCGGAT GCTACTCT        741
## 93  ATCATGCG TCTTCGAC        736
## 94  TCGAGAGT TCTTCGAC        724
## 95  TCTTCGAC ACGATCAG        722
## 96  TATGGCAC CTCTGGAT        720
## 97  GCTACTCT TACCGGAT        712
## 98  TCTTCGAC TAGCCATG        701
## 99  TACCGGAT TCGGATTC        677
## 100 ATCGTGGT TCTTCGAC        662
## 101 AGAGTCCA GTAGCGTA        660
## 102 TCTTCGAC TCGGATTC        651
```

```
## 103 AGGATAGC TACCGGAT        645
## 104 TCTTCGAC AGAGTCCA        623
## 105 TCTTCGAC ATCATGCG        617
## 106 AGGATAGC TCTTCGAC        615
## 107 CTAGCTCA GTAGCGTA        608
## 108 TACCGGAT CACTTCAC        590
## 109 TATGGCAC TCGGATTC        585
## 110 TCGGATTC AACAGCGA        584
## 111 ATCATGCG CTCTGGAT        582
## 112 TCTTCGAC CGATCGAT        573
## 113 TCTTCGAC GTCCTAAG        573
## 114 CGATCGAT CTCTGGAT        567
## 115 GATCAAGG TACCGGAT        567
## 116 CTCTGGAT ACGATCAG        566
## 117 CTAGCTCA TCGAGAGT        564
## 118 CTCTGGAT TAGCCATG        563
## 119 CTCTGGAT GTCCTAAG        553
## 120 TCTTCGAC GTAGCGTA        551
## 121 AGAGTCCA TCTTCGAC        548
## 122 CTCTGGAT ATCATGCG        547
## 123 CGATCGAT TCTTCGAC        537
## 124 ATCGTGGT CTCTGGAT        534
## 125 TGTTCCGT TCGGATTC        533
## 126 CTCTGGAT GTAGCGTA        529
## 127 AGAGTCCA TCGAGAGT        527
## 128 TCTTCGAC CGGTAATC        525
## 129 TCTTCGAC GCTACTCT        525
## 130 TACCGGAT GATCTTGC        519
## 131 GTCCTAAG TCTTCGAC        516
## 132 GCTACTCT CTCTGGAT        514
## 133 AACAGCGA CTCTGGAT        511
## 134 TAGCCATG TCTTCGAC        507
## 135 TCTTCGAC GATCTTGC        499
## 136 AGGATAGC GTAGCGTA        495
## 137 GTCCTAAG TGTTCCGT        493
## 138 TACCGGAT TCGACAAG        493
## 139 CTCTGGAT AGGATAGC        490
## 140 ACGATCAG TCTTCGAC        483
## 141 CTAGCTCA AGAGTCCA        481
## 142 GTAGCGTA TCTTCGAC        481
## 143 TATGGCAC TCGAGAGT        471
## 144 AGAGTCCA CTAGCTCA        456
## 145 GTAGCGTA ACGATCAG        456
## 146 CTCTGGAT AACAGCGA        453
## 147 GTCCTAAG CTCTGGAT        451
## 148 CTCTGGAT ATCGTGGT        445
## 149 TGTTCCGT AACAGCGA        439
## 150 CACTTCAC TACCGGAT        435
## 151 CGGTAATC TCTTCGAC        435
## 152 ATCATGCG TATGGCAC        428
## 153 TAGCCATG CTCTGGAT        428
## 154 GCTACTCT TCTTCGAC        418
## 155 TGTTCCGT TCGAGAGT        418
## 156 ACGATCAG CTCTGGAT        415
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```
## 157 CACTTCAC TCTTCGAC        410
## 158 CTCTGGAT CGGTAATC        410
## 159 AGAGTCCA CTCTGGAT        409
## 160 TCGGATTC TACCGGAT        404
## 161 CTAGCTCA TGTTCCGT        401
## 162 TCTTCGAC AACAGCGA        400
## 163 TCTTCGAC TCGACAAG        398
## 164 TATGGCAC GTCCTAAG        395
## 165 TCGAGAGT TGTTCCGT        394
## 166 CTCTGGAT TCGGATTC        391
## 167 GTAGCGTA TCGAGAGT        391
## 168 TCTTCGAC CACTTCAC        389
## 169 TGTTCCGT CTAGCTCA        388
## 170 TCGGATTC TCTTCGAC        386
## 171 ATCATGCG TAGCCATG        384
## 172 TGTTCCGT AGAGTCCA        383
## 173 ATCGTGGT GATCAAGG        380
## 174 GCTACTCT GTAGCGTA        377
## 175 AACAGCGA GATCTTGC        373
## 176 GTAGCGTA CTCTGGAT        373
## 177 ATCATGCG ACGATCAG        370
## 178 GTAGCGTA CTAGCTCA        363
## 179 CTAGCTCA TAGCCATG        356
## 180 CTCTGGAT GATCAAGG        350
## 181 TATGGCAC CTAGCTCA        346
## 182 CTAGCTCA TATGGCAC        345
## 183 CTAGCTCA ACGATCAG        338
## 184 TATGGCAC AACAGCGA        335
## 185 TCGAGAGT CTAGCTCA        330
## 186 GATCTTGC TACCGGAT        329
## 187 GATCTTGC TCTTCGAC        320
## 188 TCGACAAG TACCGGAT        320
## 189 TGTTCCGT ATCATGCG        317
## 190 AGGATAGC CTCTGGAT        310
## 191 CACTTCAC CTCTGGAT        300
## 192 ATCATGCG TGTTCCGT        298
## 193 CTCTGGAT CACTTCAC        297
## 194 ATCATGCG CTAGCTCA        294
## 195 TCGAGAGT ACGATCAG        294
## 196 AGAGTCCA TGTTCCGT        293
## 197 ATCATGCG GTCCTAAG        292
## 198 CTCTGGAT GATCTTGC        285
## 199 GCTACTCT CTAGCTCA        283
## 200 CTAGCTCA GTCCTAAG        281
## 201 TCGAGAGT GCTACTCT        276
## 202 TCGGATTC CTCTGGAT        275
## 203 TATGGCAC AGGATAGC        273
## 204 GATCAAGG CTCTGGAT        270
## 205 AGAGTCCA ACGATCAG        268
## 206 AACAGCGA CTAGCTCA        260
## 207 AGGATAGC GATCTTGC        259
## 208 ATCATGCG AGGATAGC        259
## 209 TGTTCCGT CGATCGAT        259
## 210 TGTTCCGT TAGCCATG        257
```

```
## 211 AACAGCGA ATCATGCG        256
## 212 CTCTGGAT TCGACAAG        256
## 213 GCTACTCT TGTTCCGT        256
## 214 CTAGCTCA AACAGCGA        254
## 215 CGGTAATC CTCTGGAT        246
## 216 TGTTCCGT GTAGCGTA        246
## 217 CGATCGAT TGTTCCGT        245
## 218 CTAGCTCA AGGATAGC        244
## 219 ACGATCAG TCGAGAGT        239
## 220 GTAGCGTA AGAGTCCA        238
## 221 TAGCCATG CTAGCTCA        234
## 222 TATGGCAC TAGCCATG        234
## 223 TCGACAAG TCTTCGAC        234
## 224 ACGATCAG TGTTCCGT        233
## 225 ATCATGCG AGAGTCCA        233
## 226 CTAGCTCA ATCGTGGT        232
## 227 AACAGCGA ACGATCAG        231
## 228 CTAGCTCA CGATCGAT        230
## 229 TATGGCAC ACGATCAG        230
## 230 TGTTCCGT ACGATCAG        230
## 231 AACAGCGA AGGATAGC        229
## 232 AACAGCGA TGTTCCGT        229
## 233 AGGATAGC ACGATCAG        228
## 234 GTAGCGTA TGTTCCGT        227
## 235 ACGATCAG CTAGCTCA        225
## 236 TAGCCATG TGTTCCGT        225
## 237 TCGAGAGT AGGATAGC        225
## 238 AACAGCGA AGAGTCCA        223
## 239 GTCCTAAG AACAGCGA        223
## 240 ACGATCAG ATCATGCG        220
## 241 GATCAAGG ACGATCAG        220
## 242 ACGATCAG TAGCCATG        219
## 243 ATCATGCG AACAGCGA        219
## 244 ATCATGCG GATCAAGG        219
## 245 TGTTCCGT AGGATAGC        217
## 246 AGAGTCCA AACAGCGA        216
## 247 AGGATAGC ATCATGCG        213
## 248 GATCAAGG ATCGTGGT        213
## 249 GCTACTCT AGAGTCCA        212
## 250 CTAGCTCA GATCAAGG        210
## 251 ACGATCAG GTCCTAAG        209
## 252 TATGGCAC ATCATGCG        209
## 253 AACAGCGA TATGGCAC        207
## 254 ACGATCAG AGAGTCCA        207
## 255 AACAGCGA GTAGCGTA        204
## 256 TATGGCAC CACTTCAC        203
## 257 GTCCTAAG CTAGCTCA        202
## 258 TGTTCCGT ATCGTGGT        202
## 259 AACAGCGA TCGGATTC        201
## 260 ATCGTGGT TCGAGAGT        201
## 261 TAGCCATG GTCCTAAG        200
## 262 AGGATAGC TATGGCAC        198
## 263 TCGAGAGT ATCGTGGT        198
## 264 GATCTTGC AGGATAGC        197
```

```
## 265 ACGATCAG CGATCGAT        196
## 266 AGGATAGC AGAGTCCA        196
## 267 AGGATAGC TGTTCCGT        195
## 268 ATCATGCG ATCGTGGT        195
## 269 GCTACTCT TCGAGAGT        194
## 270 TAGCCATG TATGGCAC        193
## 271 AGAGTCCA TATGGCAC        192
## 272 CTAGCTCA TCGGATTC        192
## 273 TCGACAAG CTCTGGAT        191
## 274 AGGATAGC CTAGCTCA        190
## 275 CGATCGAT CTAGCTCA        189
## 276 ATCATGCG TCGAGAGT        188
## 277 TGTTCCGT GCTACTCT        188
## 278 AGAGTCCA AGGATAGC        187
## 279 TATGGCAC AGAGTCCA        186
## 280 TCGAGAGT TATGGCAC        184
## 281 ACGATCAG AGGATAGC        183
## 282 GATCTTGC CTCTGGAT        183
## 283 AACAGCGA ATCGTGGT        182
## 284 CACTTCAC ACGATCAG        182
## 285 TGTTCCGT GATCTTGC        182
## 286 ATCGTGGT CTAGCTCA        179
## 287 GTCCTAAG TAGCCATG        179
## 288 TAGCCATG ACGATCAG        177
## 289 TCGAGAGT TCGGATTC        177
## 290 ACGATCAG TATGGCAC        176
## 291 AGAGTCCA ATCATGCG        176
## 292 GTCCTAAG GATCAAGG        174
## 293 TCGAGAGT AGAGTCCA        173
## 294 TGTTCCGT GTCCTAAG        172
## 295 AGGATAGC GCTACTCT        171
## 296 TATGGCAC GATCTTGC        170
## 297 GTAGCGTA GTCCTAAG        169
## 298 GTCCTAAG ATCATGCG        169
## 299 AACAGCGA TCGAGAGT        168
## 300 AGGATAGC TCGAGAGT        167
## 301 ACGATCAG TCGACAAG        166
## 302 CGATCGAT AGGATAGC        166
## 303 CGGTAATC ATCGTGGT        166
## 304 TAGCCATG ATCATGCG        165
## 305 CTAGCTCA CGGTAATC        163
## 306 TCGAGAGT GTAGCGTA        163
## 307 GTCCTAAG TCGAGAGT        162
## 308 TAGCCATG AGAGTCCA        161
## 309 TAGCCATG TCGAGAGT        161
## 310 ATCGTGGT TGTTCCGT        160
## 311 CACTTCAC TATGGCAC        160
## 312 TCGGATTC TATGGCAC        159
## 313 AACAGCGA CGATCGAT        158
## 314 GATCAAGG GTCCTAAG        158
## 315 GTCCTAAG TCGGATTC        158
## 316 GCTACTCT ACGATCAG        157
## 317 GTCCTAAG ACGATCAG        156
## 318 TCGAGAGT TAGCCATG        156
```

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## 319 TCGGATTC GATCTTGC        156
## 320 TCGAGAGT ATCATGCG        154
## 321 CTAGCTCA GATCTTGC        152
## 322 TAGCCATG GATCAAGG        152
## 323 TATGGCAC GATCAAGG        152
## 324 TATGGCAC GTAGCGTA        152
## 325 ATCATGCG TCGACAAG        151
## 326 TCGAGAGT CGATCGAT        151
## 327 AGAGTCCA TAGCCATG        149
## 328 GTAGCGTA TATGGCAC        148
## 329 TCGAGAGT GTCCTAAG        148
## 330 AACAGCGA GATCAAGG        147
## 331 GTCCTAAG GTAGCGTA        147
## 332 TGTTCCGT GATCAAGG        147
## 333 AACAGCGA TAGCCATG        146
## 334 CGGTAATC TAGCCATG        146
## 335 ATCGTGGT ACGATCAG        144
## 336 TATGGCAC CGGTAATC        143
## 337 GTAGCGTA AACAGCGA        142
## 338 TATGGCAC ATCGTGGT        142
## 339 TCGACAAG TCGAGAGT        142
## 340 AACAGCGA GTCCTAAG        140
## 341 AGGATAGC AACAGCGA        140
## 342 ATCGTGGT ATCATGCG        140
## 343 GTAGCGTA ATCATGCG        140
## 344 ATCGTGGT TATGGCAC        139
## 345 TCGGATTC CTAGCTCA        139
## 346 ATCATGCG GCTACTCT        138
## 347 GATCAAGG TGTTCCGT        138
## 348 AGAGTCCA ATCGTGGT        137
## 349 CGATCGAT TCGAGAGT        137
## 350 TCGAGAGT AACAGCGA        137
## 351 ACGATCAG GCTACTCT        136
## 352 ATCGTGGT AGAGTCCA        135
## 353 CACTTCAC CTAGCTCA        135
## 354 GATCAAGG TAGCCATG        135
## 355 GTCCTAAG AGAGTCCA        135
## 356 AGGATAGC CACTTCAC        134
## 357 ATCATGCG GTAGCGTA        134
## 358 TAGCCATG AGGATAGC        134
## 359 GTAGCGTA AGGATAGC        133
## 360 AGAGTCCA GCTACTCT        131
## 361 CGATCGAT ACGATCAG        131
## 362 TCGAGAGT TCGACAAG        131
## 363 TCGGATTC CGATCGAT        130
## 364 ACGATCAG GTAGCGTA        129
## 365 ATCGTGGT AGGATAGC        128
## 366 GTAGCGTA ATCGTGGT        127
## 367 ACGATCAG GATCAAGG        126
## 368 GCTACTCT TATGGCAC        125
## 369 GTCCTAAG CGATCGAT        125
## 370 AGGATAGC CGATCGAT        124
## 371 AGAGTCCA GTCCTAAG        123
## 372 GATCTTGC TATGGCAC        121
```

```
## 373 TCGACAAG TAGCCATG        121
## 374 AGAGTCCA CGATCGAT        120
## 375 CACTTCAC TGTTCCGT        120
## 376 GTCCTAAG AGGATAGC        120
## 377 TAGCCATG GTAGCGTA        120
## 378 TATGGCAC GCTACTCT        119
## 379 TGTTCCGT CACTTCAC        118
## 380 ACGATCAG ATCGTGGT        117
## 381 CGATCGAT AGAGTCCA        117
## 382 CGATCGAT TAGCCATG        117
## 383 CTAGCTCA CACTTCAC        117
## 384 GATCAAGG CTAGCTCA        117
## 385 GCTACTCT GTCCTAAG        117
## 386 TCGACAAG ACGATCAG        117
## 387 ACGATCAG AACAGCGA        116
## 388 AGGATAGC TAGCCATG        116
## 389 TAGCCATG CACTTCAC        115
## 390 CGGTAATC CTAGCTCA        114
## 391 GTCCTAAG GCTACTCT        114
## 392 AGGATAGC TCGGATTC        113
## 393 GCTACTCT ATCATGCG        113
## 394 TATGGCAC CGATCGAT        113
## 395 ATCGTGGT GTAGCGTA        112
## 396 GATCTTGC CGATCGAT        112
## 397 TCGACAAG GTCCTAAG        112
## 398 TCGAGAGT CGGTAATC        112
## 399 GTAGCGTA TAGCCATG        111
## 400 TGTTCCGT TCGACAAG        111
## 401 ATCATGCG GATCTTGC        110
## 402 CACTTCAC CGGTAATC        109
## 403 GATCAAGG GTAGCGTA        109
## 404 TCGAGAGT GATCAAGG        109
## 405 GATCAAGG TCGAGAGT        108
## 406 ATCATGCG CGATCGAT        107
## 407 GCTACTCT TAGCCATG        107
## 408 TATGGCAC TCGACAAG        107
## 409 TCGGATTC AGGATAGC        107
## 410 GATCAAGG ATCATGCG        106
## 411 TGTTCCGT CGGTAATC        104
## 412 CGGTAATC AGGATAGC        103
## 413 GCTACTCT AACAGCGA        103
## 414 TCGGATTC TGTTCCGT        103
## 415 AGGATAGC CGGTAATC        101
## 416 ATCGTGGT CGATCGAT        101
## 417 TCGACAAG CTAGCTCA        101
## 418 GCTACTCT AGGATAGC        100
## 419 CACTTCAC AGGATAGC         99
## 420 GATCTTGC CTAGCTCA         99
## 421 GTAGCGTA GATCAAGG         99
## 422 AACAGCGA GCTACTCT         98
## 423 CGATCGAT CGGTAATC         98
## 424 TAGCCATG TCGACAAG         98
## 425 TAGCCATG AACAGCGA         97
## 426 ACGATCAG CGGTAATC         96
```

```
## 427 ATCGTGGT TAGCCATG         96
## 428 CGATCGAT TATGGCAC         96
## 429 CGGTAATC TGTTCCGT         96
## 430 GATCAAGG AGGATAGC         95
## 431 GATCAAGG TATGGCAC         95
## 432 GATCTTGC ATCATGCG         95
## 433 GTCCTAAG ATCGTGGT         95
## 434 GTCCTAAG GATCTTGC         95
## 435 CGATCGAT ATCATGCG         93
## 436 CGATCGAT GCTACTCT         92
## 437 GTCCTAAG TCGACAAG         92
## 438 AGGATAGC ATCGTGGT         91
## 439 CGATCGAT GTAGCGTA         91
## 440 CGATCGAT GTCCTAAG         91
## 441 AGAGTCCA TCGGATTC         89
## 442 ATCGTGGT GCTACTCT         89
## 443 GCTACTCT CGATCGAT         89
## 444 GTAGCGTA CGATCGAT         89
## 445 TCGGATTC TCGAGAGT         89
## 446 GATCAAGG GATCTTGC         87
## 447 AGAGTCCA GATCAAGG         86
## 448 AGGATAGC GTCCTAAG         85
## 449 GATCAAGG AGAGTCCA         85
## 450 GCTACTCT ATCGTGGT         85
## 451 CACTTCAC CGATCGAT         84
## 452 GCTACTCT GATCAAGG         84
## 453 ATCGTGGT AACAGCGA         83
## 454 CGGTAATC TATGGCAC         83
## 455 TAGCCATG ATCGTGGT         83
## 456 GATCTTGC AACAGCGA         82
## 457 GATCTTGC AGAGTCCA         82
## 458 CGGTAATC CGATCGAT         81
## 459 GCTACTCT GATCTTGC         81
## 460 TCGACAAG TGTTCCGT         81
## 461 GATCAAGG CGATCGAT         80
## 462 ATCATGCG CACTTCAC         79
## 463 ATCATGCG CGGTAATC         79
## 464 GATCTTGC GTCCTAAG         79
## 465 GATCTTGC TGTTCCGT         78
## 466 TCGAGAGT GATCTTGC         78
## 467 TCGGATTC ACGATCAG         78
## 468 ACGATCAG CACTTCAC         77
## 469 ATCATGCG TCGGATTC         77
## 470 ATCGTGGT CGGTAATC         77
## 471 AGAGTCCA CGGTAATC         76
## 472 GATCAAGG GCTACTCT         76
## 473 GATCTTGC GTAGCGTA         76
## 474 TAGCCATG TCGGATTC         76
## 475 TCGGATTC AGAGTCCA         76
## 476 ATCGTGGT GTCCTAAG         75
## 477 CACTTCAC TCGGATTC         75
## 478 CGATCGAT ATCGTGGT         75
## 479 TCGACAAG AGGATAGC         75
## 480 GATCAAGG AACAGCGA         74
```

```
## 481 CGGTAATC TCGAGAGT         73
## 482 GATCTTGC GATCAAGG         72
## 483 AGAGTCCA GATCTTGC         71
## 484 CACTTCAC AGAGTCCA         71
## 485 CGGTAATC CACTTCAC         70
## 486 GTCCTAAG CGGTAATC         70
## 487 TAGCCATG GATCTTGC         70
## 488 AGGATAGC GATCAAGG         69
## 489 AACAGCGA CGGTAATC         68
## 490 CGATCGAT GATCAAGG         68
## 491 GATCTTGC TCGAGAGT         68
## 492 CGATCGAT AACAGCGA         67
## 493 ACGATCAG GATCTTGC         66
## 494 GATCTTGC ACGATCAG         66
## 495 GTAGCGTA GATCTTGC         66
## 496 TCGGATTC GTCCTAAG         66
## 497 CACTTCAC ATCATGCG         65
## 498 GTAGCGTA CGGTAATC         65
## 499 GTAGCGTA TCGGATTC         65
## 500 TCGGATTC ATCATGCG         65
## 501 ACGATCAG TCGGATTC         64
## 502 ATCGTGGT TCGGATTC         64
## 503 CGGTAATC TCGGATTC         64
## 504 TAGCCATG CGATCGAT         64
## 505 AGAGTCCA CACTTCAC         63
## 506 CGATCGAT TCGGATTC         63
## 507 AACAGCGA CACTTCAC         62
## 508 CACTTCAC TCGAGAGT         62
## 509 CGATCGAT GATCTTGC         62
## 510 GCTACTCT TCGGATTC         62
## 511 TAGCCATG GCTACTCT         62
## 512 TCGGATTC CGGTAATC         62
## 513 AACAGCGA TCGACAAG         61
## 514 CGGTAATC GATCAAGG         61
## 515 GATCTTGC GCTACTCT         61
## 516 TCGACAAG TATGGCAC         61
## 517 CGGTAATC GTAGCGTA         59
## 518 TAGCCATG CGGTAATC         59
## 519 ATCGTGGT GATCTTGC         57
## 520 CACTTCAC GTAGCGTA         57
## 521 GATCAAGG TCGACAAG         57
## 522 CGATCGAT CACTTCAC         56
## 523 CGGTAATC ATCATGCG         56
## 524 GATCTTGC TAGCCATG         56
## 525 GCTACTCT CGGTAATC         56
## 526 AGAGTCCA TCGACAAG         55
## 527 CGATCGAT TCGACAAG         55
## 528 TCGACAAG AGAGTCCA         55
## 529 TCGGATTC TAGCCATG         54
## 530 ATCGTGGT TCGACAAG         53
## 531 CGGTAATC GATCTTGC         53
## 532 GATCTTGC CGGTAATC         53
## 533 AGGATAGC TCGACAAG         52
## 534 TCGACAAG GATCAAGG         52
```

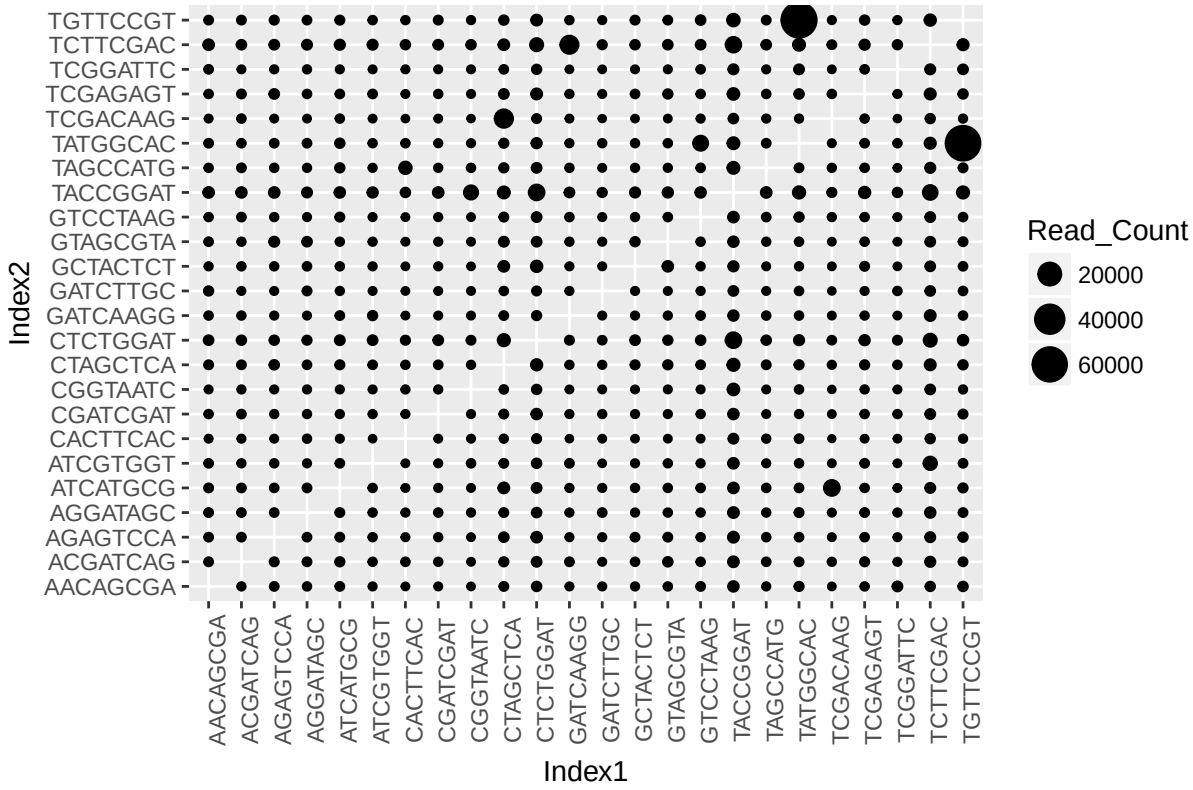
```
## 535 CGGTAATC ACGATCAG         50
## 536 TCGAGAGT CACTTCAC         49
## 537 CACTTCAC ATCGTGGT         48
## 538 CACTTCAC GATCAAGG         48
## 539 CGGTAATC AGAGTCCA         48
## 540 TCGGATTC GATCAAGG         48
## 541 GTCCTAAG CACTTCAC         47
## 542 TCGACAAG CGATCGAT         47
## 543 TCGACAAG GTAGCGTA         47
## 544 TCGGATTC ATCGTGGT         47
## 545 CACTTCAC GTCCTAAG         46
## 546 CGGTAATC GTCCTAAG         46
## 547 GATCAAGG CGGTAATC         46
## 548 GTAGCGTA CACTTCAC         46
## 549 CACTTCAC AACAGCGA         45
## 550 GATCTTGC CACTTCAC         45
## 551 TCGACAAG TCGGATTC         45
## 552 TCGGATTC CACTTCAC         45
## 553 GTAGCGTA TCGACAAG         44
## 554 TCGACAAG ATCGTGGT         44
## 555 TCGACAAG GCTACTCT         44
## 556 TCGGATTC GTAGCGTA         43
## 557 CACTTCAC GATCTTGC         41
## 558 CGGTAATC AACAGCGA         41
## 559 TCGACAAG AACAGCGA         41
## 560 TCGGATTC GCTACTCT         40
## 561 CACTTCAC GCTACTCT         38
## 562 CGGTAATC GCTACTCT         37
## 563 GATCAAGG TCGGATTC         37
## 564 GCTACTCT CACTTCAC         37
## 565 GATCTTGC TCGGATTC         36
## 566 GATCTTGC ATCGTGGT         33
## 567 GCTACTCT TCGACAAG         33
## 568 ATCGTGGT CACTTCAC         29
## 569 CACTTCAC TCGACAAG         27
## 570 CGGTAATC TCGACAAG         26
## 571 TCGGATTC TCGACAAG         25
## 572 GATCAAGG CACTTCAC         24
## 573 TCGACAAG GATCTTGC         23
## 574 TCGACAAG CGGTAATC         22
## 575 GATCTTGC TCGACAAG         19
## 576 TCGACAAG CACTTCAC         14
## 577  Unknown  Unknown    5495481
```

The first 24 lines give the number of retained reads for each of our expected (matching) index pairs. In total, these account for 239750646 of the total 245598505; 97.6% of the retained reads. (Note: I've indcluded the Sorted_Index_counts.txt file with the breakdown of reads by index pair with this document, since the output is slightly unwieldy here.)

b. There are a total of 352378 reads that have swapped indexes. Note that this does not include the counts of reads that contained indexes that did not match to any expected library (our "Unknown" counts)
c. We can visualize the read counts per swapped index-pairs by plotting the first index against the second index in a dotplot and a heat map (Note: to keep the heatmap from oversaturation for the outlier read counts, all read counts have been log corrected such that the heatmap represents the

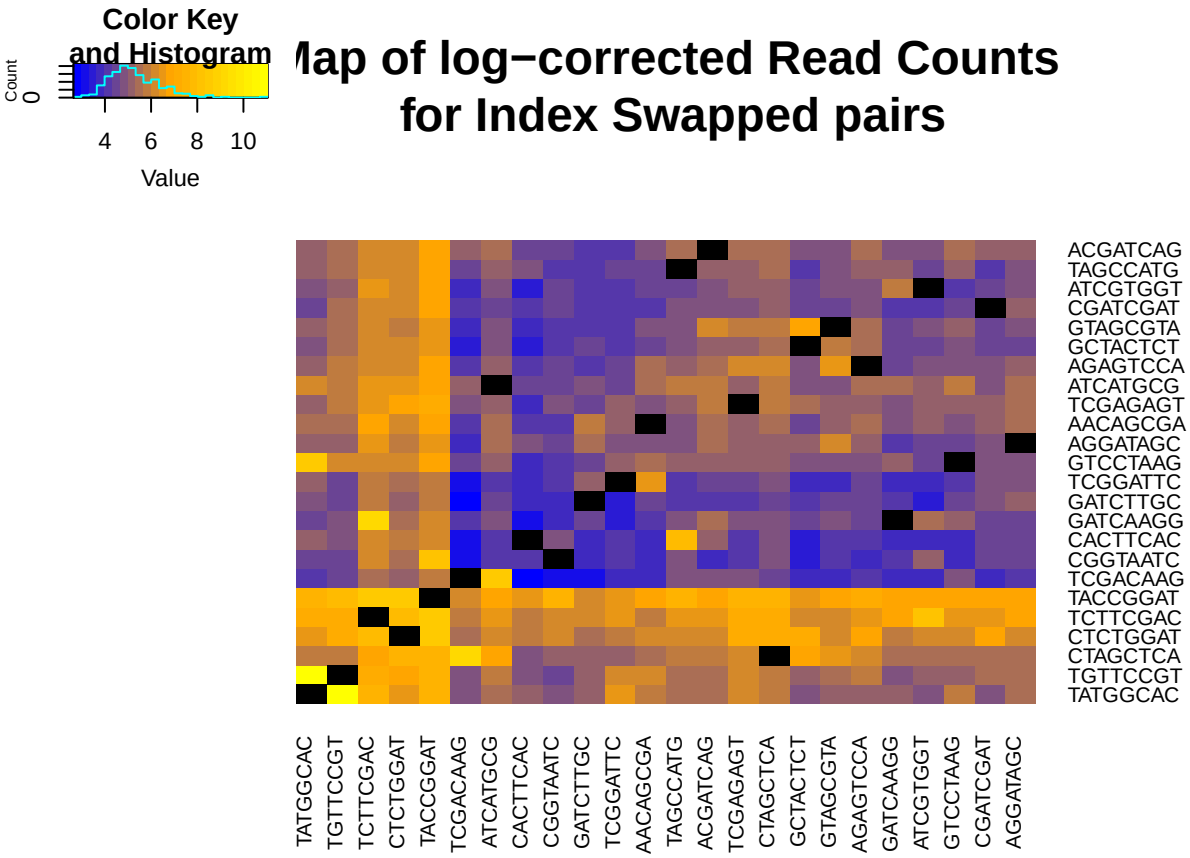
distribution of log(Read_Counts)):

```
## Warning: package 'ggplot2' was built under R version 3.3.2
```




```
##
## Attaching package: 'gplots'

## The following object is masked from 'package:stats':
##
##     lowess
```



These two visualizations illustrate that certain index swaps is much more likely to occur. The TATGGCAC (C4) index had the highest counts among the swapped index pairs, with TCTTCGAC (C10) and CTCTGGAT (B3) having very high representation as well. This makes sense, as these were also the libraries that had shared the largest proportion of the total read counts; however, the converse does not appear to be true, with the lowest represented libraries (CACTTCAC, TCGACAAG, and GATCTTGC) not appearing to be represented that much lower than some of the libraries with 2x or more reads. This could indicate a potential "read representation limit" below which index swapping does not become significantly more common.

3. No data to report – read files were not created due to file limit constraints.