Spike Coverage

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September 12, 2016

Background

We need to determine the appropriate covereage of TCR sequences that will enable us to detect unique CDR3 regions that exist at low concentrations within the sample. We would like to achieve 30-50x covereage, on average, for each unique sequence in our reaction mixture. The resulting coverage is influenced both by the amount of TCR sequence in the reaction mixture, as well as the amplification efficiencies of the different primers.

To help determine the appropriate concentration of DNA required for a sample, we're going to use the amplification of synthetic templates (spikes) to determine the relative efficiencies of the primers. We will use data from three different batches: DNA160609LC, DNA160708LC, and DNA160803LC. For the first two batches, we will only use samples that contain spike sequence only (samples 1-20 and 1-10, respectively). The third batch is a normal sequencing data set containing TCR sequence as well as spike sequence.

Process

Our input files contain one row for each sample, a few summary columns, and then one column for each spike (there are 260 total). Each cell is the count for that particular spike in that particular sample. Files need to be subset to select appropriate samples. For each batch of samples, we want to see the distribution of mean and median counts across spikes, as well as the two lowest spike values and their labels, for each sample. The latter can also be summarized per batch.

First we can look at the summaries of mean and median counts among our samples, both with raw data and graphically. These are the mean or median for all samples for each of the 260 spikes. So for example with DNA160609LC, the 20 values of the first spike (V1J1-1 or DM_1) are averaged, and again for the second spike, etc. Finally, the distribution of the mean counts for each of the 260 spikes is summarized.

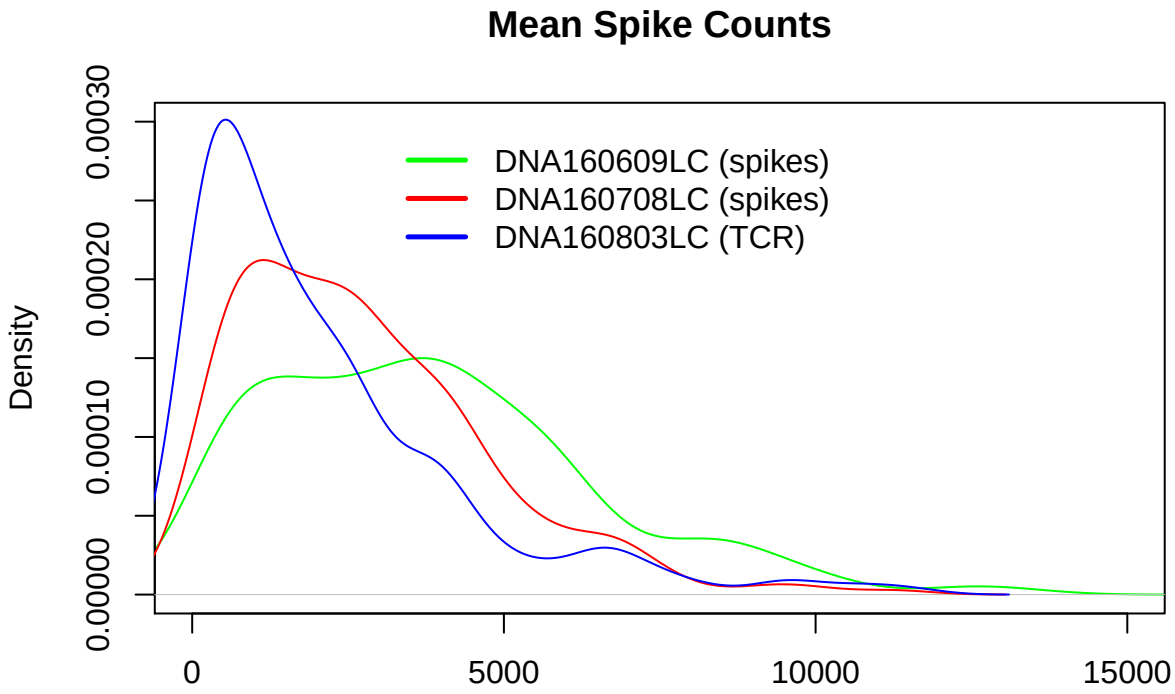
```
## Mean
```

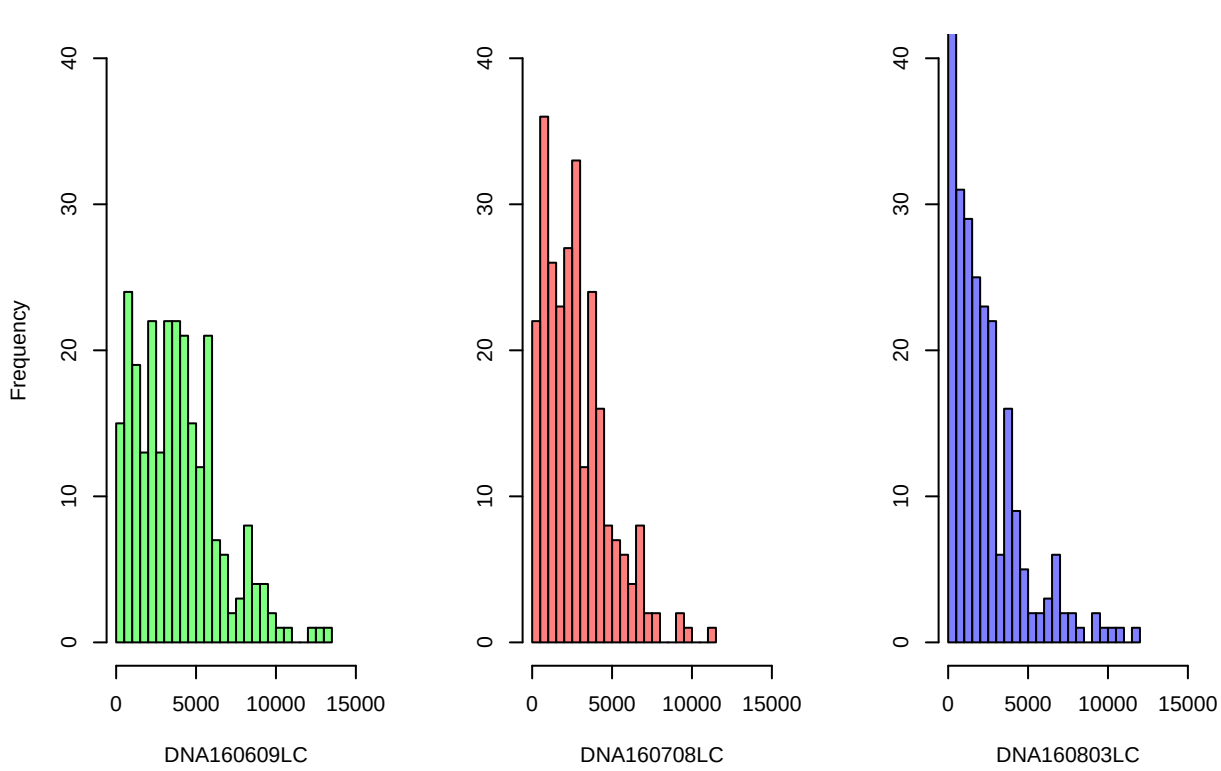
	DNA160609LC	DNA160708LC	DNA160803LC
Min.	140	130.1	20.86
1st Qu.	1864	1156.0	432.30
Median	3519	2447.0	1497.00
Mean	3800	2746.0	2115.00
3rd Qu.	5340	3901.0	2781.00
Max.	13320	11270.0	11550.00

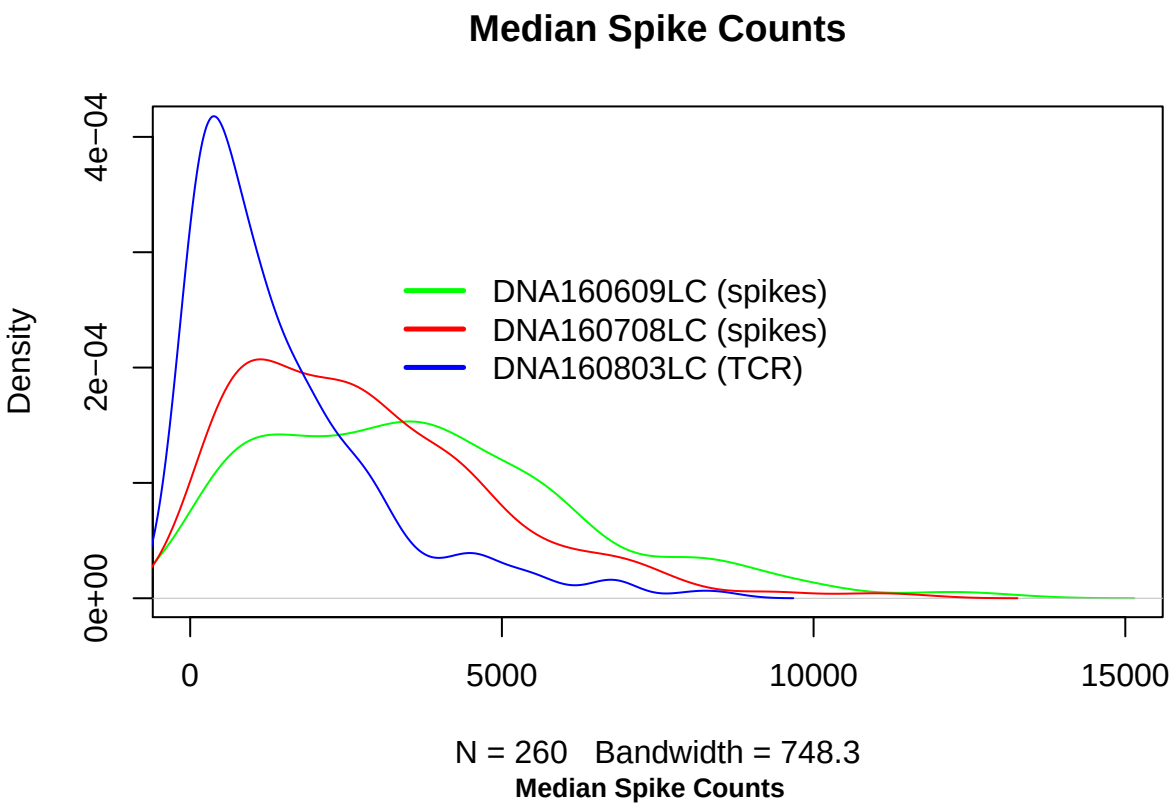
```
## Median
```

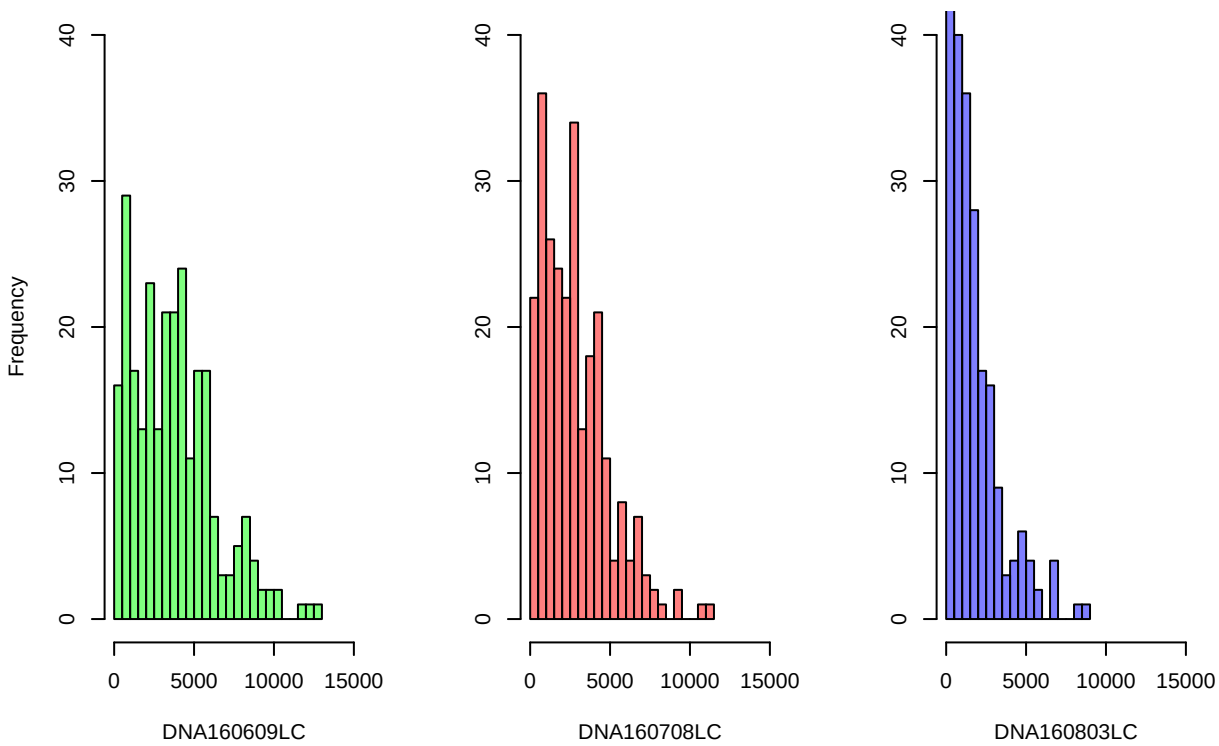
	DNA160609LC	DNA160708LC	DNA160803LC
Min.	122.5	137.5	3.5
1st Qu.	1767.0	1154.0	298.8
Median	3425.0	2479.0	1017.0
Mean	3690.0	2810.0	1486.0

	DNA160609LC	DNA160708LC	DNA160803LC
3rd Qu.	5238.0	4000.0	2050.0
Max.	12900.0	11430.0	8514.0









The spread is relatively large, about two orders of magnitude between maximum and minimum.

We are also interested in the lowest coverage spikes, as these will determine which CDR3s we would theoretically be able to observe. Here we have the names and counts of the two lowest spikes for each sample in our three batches.

```
## $DNA160609LC
##
##
## Min.ID     Min   Second.ID   Second   Max.ID         Max
## ---------  ----  ----------  -------  -------------  ------
## V26-J2-3   92    V16-J2-2    99       V12-1-2-J1-2   8663
## V26-J2-3   136   V16-J2-2    149      V12-1-2-J1-2   13576
## V26-J2-3   248   V16-J2-2    252      V12-1-2-J1-2   24018
## V26-J2-3   152   V20-J2-2    163      V12-1-2-J1-2   12548
## V26-J2-3   85    V16-J2-2    99       V12-1-2-J1-2   9764
## V20-J2-2   65    V26-J2-3    82       V12-1-2-J1-2   6048
## V26-J2-3   114   V5-J2-4     141      V12-1-2-J1-2   11603
## V5-J2-4    72    V16-J2-2    80       V12-1-2-J1-2   8589
## V26-J2-3   77    V5-J2-5     88       V12-1-2-J1-2   7337
## V26-J2-3   113   V16-J2-2    122      V12-1-2-J1-2   11451
## V26-J2-3   86    V20-J2-2    96       V12-1-2-J1-2   8942
## V20-J2-2   188   V26-J2-3    213      V12-1-2-J1-2   15233
## V26-J2-3   153   V5-J2-4     187      V12-1-2-J1-2   14627
## V26-J2-3   119   V16-J2-2    135      V1-J1-2        9971
## V16-J2-2   123   V26-J2-3    123      V12-1-2-J1-2   13249
## V26-J2-3   191   V20-J2-2    227      V12-1-2-J1-2   17947
## V20-J2-2   162   V26-J2-3    162      V12-1-2-J1-2   13843
## V26-J2-3   213   V20-J2-2    233      V12-1-2-J1-2   18044
## V26-J2-3   239   V16-J2-2    303      V12-1-2-J1-2   23294
## V26-J2-3   122   V5-J2-4     171      V12-1-2-J1-2   17804
##
## $DNA160708LC
##
##
## Min.ID     Min   Second.ID   Second   Max.ID         Max
## ---------  ----  ----------  -------  -------------  ------
## V20-J2-2   94    V26-J2-3    121      V12-1-2-J1-2   9291
## V20-J2-2   140   V26-J2-3    161      V12-1-2-J1-2   12199
## V20-J2-2   105   V5-J2-4     108      V12-1-2-J1-2   9325
## V20-J2-2   108   V26-J2-3    112      V1-J1-2        10731
## V20-J2-2   97    V26-J2-3    120      V12-1-2-J1-2   8300
## V20-J2-2   144   V26-J2-3    145      V12-1-2-J1-2   12739
## V20-J2-2   170   V26-J2-3    179      V12-1-2-J1-2   14362
## V20-J2-2   135   V26-J2-3    135      V12-1-2-J1-2   10508
## V26-J2-3   153   V20-J2-2    167      V12-1-2-J1-2   12269
## V20-J2-2   141   V26-J2-3    170      V12-1-2-J1-2   13034
##
## $DNA160803LC
##
##
## Min.ID     Min   Second.ID      Second   Max.ID         Max
## ---------  ----  -------------  -------  -------------  ------
## V5-J2-5    0     V20-J2-2       0        V12-1-2-J1-2   8509
## V20-J2-2   5     V26-J2-3       13       V1-J1-2        41254
## V5-J2-5    5     V17-J1-3       8        V1-J1-2        11996
## V20-J2-2   10    V5-J2-4        11       V1-J1-2        37304
## V5-J2-4    9     V5-J2-5        16       V1-J1-2        20577
## V3-J2-4    0     V4-J1-3        0        V1-J1-7        6668
## V26-J2-3   4     V5-J2-5        7        V1-J1-2        18022
```

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## V20-J2-2   34    V26-J2-3      36      V1-J1-2        18679
## V20-J2-2   151   V5-J2-4       162     V12-1-2-J1-2   35660
## V5-J2-5    79    V5-J2-4       106     V24-J2-3       25329
## V26-J2-1   0     V30-J1-3      0       V1-J1-7        9491
## V20-J2-2   0     V20-J1-6      1       V1-J1-2        20450
## V20-J2-2   11    V5-J2-5       23      V12-1-2-J1-2   50695
## V2-J1-3    0     V4-J2-1       0       V1-J1-2        4925
## V17-J1-3   0     V20-J1-7      0       V24-J2-3       9323
## V20-J2-2   51    V26-J2-3      130     V12-1-2-J1-2   65337
## V5-J2-4    19    V26-J2-3      27      V12-1-2-J1-2   23387
## V20-J2-2   0     V5-J2-4       1       V14-J1-2       11543
## V5-J2-5    14    V16-J2-2      15      V13-1-J2-3     25073
## V2-J1-3    0     V16-J2-2      0       V1-J1-2        12162
## V5-J2-5    0     V20-J1-7      0       V12-1-2-J1-2   3077
## V5-J2-5    10    V20-J2-2      11      V12-1-2-J1-2   22375
## V5-J2-4    3     V26-J2-3      3       V1-J1-2        14655
## V5-J2-5    0     V5-J2-4       1       V1-J1-2        10382
## V5-J2-4    1     V20-J2-2      2       V1-J1-2        9330
## V5-J2-4    1     V5-J2-5       2       V1-J1-2        6309
## V5-J2-5    0     V5-J2-4       1       V1-J1-2        7406
## V5-J2-4    14    V5-J2-5       14      V13-1-J2-3     19744
## V5-J2-4    0     V16-J2-2      0       V1-J1-2        8511
## V5-J2-5    3     V5-J2-4       4       V12-1-2-J1-2   17706
## V2-J1-3    0     V5-J1-4       0       V14-J1-2       5049
## V17-J1-3   0     V20-J2-2      0       V1-J1-2        10942
## V20-J1-3   0     V26-J2-3      0       V12-1-2-J1-2   10101
## V5-J2-4    0     V26-J2-3      0       V14-J1-2       12195
## V2-J1-3    0     V4-J2-1       0       V1-J1-2        4847
## V4-J2-4    0     V5-J2-4       0       V1-J1-2        5414
## V20-J2-2   6     V4-J2-4       30      V14-J1-2       14677
## V3-J2-4    0     V3-J2-5       0       V13-1-J2-3     3978
## V20-J1-3   0     V20-J1-6      0       V1-J1-2        11223
## V1-J2-2    0     V3-J1-3       0       V23-J2-1       4040
## V5-J2-5    1     V20-J2-3      1       V14-J1-2       8012
## V5-J2-4    0     V20-J2-2      0       V14-J1-2       11329
## V2-J1-3    0     V3-J2-4       0       V1-J2-3        4463
## V30-J1-3   1     V5-J2-5       4       V14-J1-2       14923
## V4-J2-4    0     V26-J2-3      0       V1-J1-2        19528
## V5-J2-5    24    V20-J2-2      34      V1-J1-2        23626
## V20-J1-7   0     V26-J2-3      0       V14-J1-2       21156
## V26-J2-3   0     V26-J1-7      3       V14-J1-2       11063
## V17-J2-4   0     V26-J2-3      5       V1-J1-2        19172
## V26-J2-3   3     V5-J2-4       6       V13-1-J2-3     9237
## V5-J2-4    0     V20-J1-6      0       V1-J2-3        7038
## V2-J1-3    0     V3-J1-3       0       V1-J1-7        1175
## V3-J2-4    0     V4-J1-3       0       V1-J2-3        5257
## V5-J2-4    5     V26-J2-3      6       V14-J1-2       20558
## V20-J2-2   0     V26-J2-3      0       V1-J1-2        9984
## V5-J2-5    19    V5-J2-4       24      V1-J1-7        13725
## V5-J2-4    1     V26-J2-3      1       V1-J1-7        5799
## V5-J2-4    0     V16-J1-3      0       V1-J2-3        4873
## V20-J2-2   5     V26-J2-3      5       V19-J2-7       5073
## V2-J1-3    0     V4-J1-3       0       V1-J1-2        2469
## V4-J2-4    0     V17-J1-3      0       V12-1-2-J1-2   4428
```

```
## V5-J2-4    13    V20-J2-2       43       V1-J1-2        11525
## V26-J2-3   0     V5-J2-4        1        V14-J1-2       10166
## V20-J2-4   0     V20-J2-1       1        V14-J1-2       6849
## V5-J2-5    2     V20-J2-2       11       V12-1-2-J1-2   6649
## V5-J2-4    0     V26-J2-4       0        V1-J1-7        7823
## V16-J2-2   0     V20-J2-2       0        V1-J1-7        3767
## V5-J2-5    11    V26-J2-3       13       V24-J2-3       12305
## V4-J2-4    0     V4-J2-5        0        V14-J1-2       3626
## V5-J2-4    9     V26-J2-3       37       V12-1-2-J1-2   24156
## V5-J2-4    3     V5-J2-5        4        V12-1-2-J1-2   6873
## V5-J2-5    0     V26-J2-4       0        V24-J2-3       6958
## V26-J2-3   1     V5-J2-5        4        V1-J1-2        8371
## V5-J2-4    1     V20-J1-7       1        V23-J1-2       9247
## V26-J2-3   14    V5-J2-5        15       V14-J1-2       19789
## V5-J2-5    9     V26-J2-3       10       V12-1-2-J1-2   17019
## V26-J2-3   1     V30-J1-3       2        V1-J1-2        21562
## V5-J2-4    9     V5-J2-5        18       V1-J1-2        19366
## V5-J2-5    8     V5-J2-4        13       V13-1-J2-3     17160
## V4-J2-4    0     V5-J2-4        0        V12-1-2-J1-2   809
## V5-J2-4    0     V12-1-2-J1-7   0        V12-1-2-J1-2   1296
## V20-J2-2   52    V26-J2-3       70       V12-1-2-J1-2   16237
## V4-J1-3    0     V5-J1-5        0        V12-1-2-J1-2   1858
## V3-J1-3    0     V4-J2-4        0        V1-J1-2        3855
## V4-J2-1    0     V26-J1-3       0        V13-1-J2-3     6635
## V20-J1-7   0     V26-J2-3       1        V1-J1-2        7331
## V5-J2-4    2     V20-J2-2       13       V12-1-2-J1-2   7523
## V3-J2-5    0     V4-J2-4        0        V1-J1-5        10404
## V5-J2-4    5     V19-J1-4       19       V12-1-2-J1-2   4999
## V4-J2-1    0     V30-J1-3       0        V12-1-2-J1-2   3220
## V20-J2-2   40    V5-J2-4        71       V12-1-2-J1-2   25314
## V5-J2-4    10    V20-J2-2       23       V12-1-2-J1-2   9815
```

We can also count the occurrences of each spike:

```
## $DNA160609LC
## $DNA160609LC[[1]]
##     Min.ID freq
## 1 V16-J2-2    1
## 2 V20-J2-2    3
## 3 V26-J2-3   15
## 4  V5-J2-4    1
##
## $DNA160609LC[[2]]
##   Second.ID freq
## 1  V16-J2-2    8
## 2  V20-J2-2    4
## 3  V26-J2-3    4
## 4   V5-J2-4    3
## 5   V5-J2-5    1
##
##
## $DNA160708LC
## $DNA160708LC[[1]]
##     Min.ID freq
```

```
## 1 V20-J2-2    9
## 2 V26-J2-3    1
##
## $DNA160708LC[[2]]
##   Second.ID freq
## 1  V20-J2-2    1
## 2  V26-J2-3    8
## 3   V5-J2-4    1
##
##
## $DNA160803LC
## $DNA160803LC[[1]]
##      Min.ID freq
## 1   V1-J2-2    1
## 2  V16-J2-2    1
## 3  V17-J1-3    2
## 4  V17-J2-4    1
## 5   V2-J1-3    7
## 6  V20-J1-3    2
## 7  V20-J1-7    2
## 8  V20-J2-2   13
## 9  V20-J2-4    1
## 10 V26-J2-1    1
## 11 V26-J2-3    7
## 12  V3-J1-3    1
## 13  V3-J2-4    3
## 14  V3-J2-5    1
## 15 V30-J1-3    1
## 16  V4-J1-3    1
## 17  V4-J2-1    2
## 18  V4-J2-4    5
## 19  V5-J2-4   23
## 20  V5-J2-5   17
##
## $DNA160803LC[[2]]
##       Second.ID freq
## 1  V12-1-2-J1-7    1
## 2      V16-J1-3    1
## 3      V16-J2-2    3
## 4      V17-J1-3    2
## 5      V19-J1-4    1
## 6      V20-J1-6    3
## 7      V20-J1-7    3
## 8      V20-J2-1    1
## 9      V20-J2-2   11
## 10     V20-J2-3    1
## 11     V26-J1-3    1
## 12     V26-J1-7    1
## 13     V26-J2-3   19
## 14     V26-J2-4    2
## 15      V3-J1-3    2
## 16      V3-J2-4    1
## 17      V3-J2-5    1
## 18     V30-J1-3    3
```

```
## 19      V4-J1-3    3
## 20      V4-J2-1    2
## 21      V4-J2-4    3
## 22      V4-J2-5    1
## 23      V5-J1-4    1
## 24      V5-J1-5    1
## 25      V5-J2-4   14
## 26      V5-J2-5   10
```