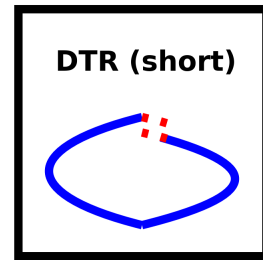
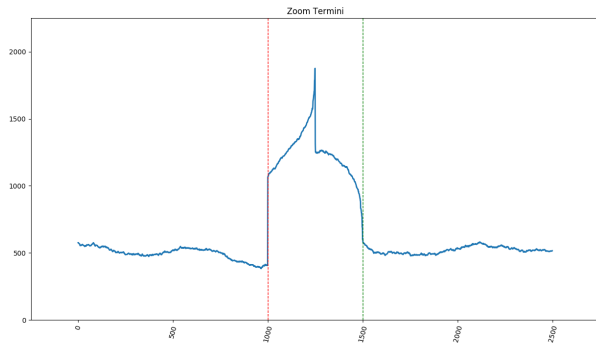


# 174phage PhageTerm Analysis



## PhageTerm Method

| Ends      | Left (red) | Right (green) | Permuted | Orientation | Class       | Type |
|-----------|------------|---------------|----------|-------------|-------------|------|
| Redundant | 6380       | 6878          | No       | NA          | DTR (short) | T7   |

\*Direct Terminal Repeats: 499 bp

| Strand | Location | T    | pvalue    | T (Start. Pos. Cov. / Whole Cov.) |
|--------|----------|------|-----------|-----------------------------------|
| +      | 6380     | 0.78 | 1.12e-185 |                                   |
|        | 10188    | 0.04 | 3.47e-01  |                                   |
|        | 6378     | 0.04 | 7.79e-01  |                                   |
|        | 18656    | 0.03 | 1.00e+00  |                                   |
|        | 22101    | 0.03 | 1.00e+00  |                                   |
| -      | 6878     | 0.60 | 4.22e-20  |                                   |
|        | 34666    | 0.04 | 3.84e-01  |                                   |
|        | 42162    | 0.03 | 2.59e-01  |                                   |
|        | 26827    | 0.03 | 3.84e-01  |                                   |
|        | 20040    | 0.03 | 1.00e+00  |                                   |

## Li's Method

| Packaging | Termini | Forward         | Reverse         | Orientation |
|-----------|---------|-----------------|-----------------|-------------|
| COS       | Fixed   | Obvious Termini | Obvious Termini | Forward     |

\*Direct Terminal Repeats: 499 bp

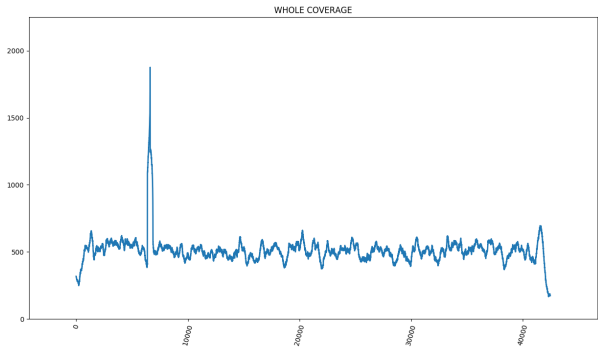
| Strand | Location | SPC | R    | SPC |
|--------|----------|-----|------|-----|
| +      | 6380     | 660 | 73.0 |     |
|        | 10188    | 9   | -    |     |
|        | 33521    | 8   | -    |     |
|        | 20176    | 8   | -    |     |
|        | 6556     | 8   | -    |     |
| -      | 6878     | 277 | 16.0 |     |
|        | 6866     | 17  | -    |     |
|        | 6856     | 16  | -    |     |
|        | 6862     | 15  | -    |     |
|        | 6871     | 13  | -    |     |

Analysis Methodology

PhageTerm software uses raw reads of a phage sequenced with a sequencing technology using random fragmentation and its genomic reference sequence to determine the termini position. The process starts with the alignment of NGS reads to the phage genome in order to calculate the starting position coverage (SPC), where a hit is given only to the position of the first base in a successfully aligned read (the alignment algorithm uses the lenght of the seed (default: 20) for mapping and does not accept gap or mismatch to speed up the process). Then the program apply 2 distinct scoring methods: i) a statistical approach based on the Gamma law; and ii) a method derived from LI and al. 2014 paper.

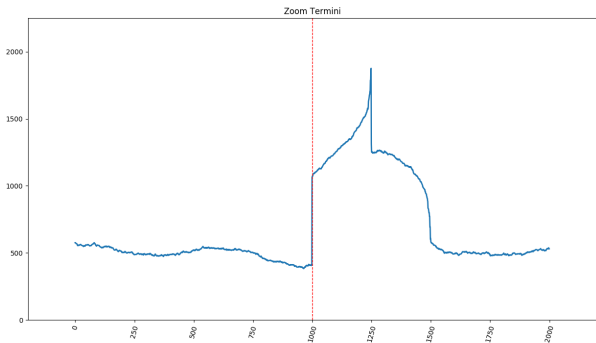
General set-up and mapping informations

|                  |          |
|------------------|----------|
| Phage Genome     | 42457 bp |
| Sequencing Reads | 1182814  |
| Mapping Reads    | 88 %     |
| OPTIONS          |          |
| Mapping Seed     | 20       |
| Surrounding      | 20       |
| Host Analysis    | No       |

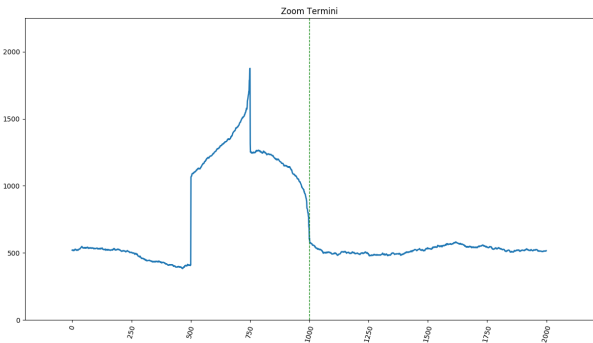


Highest peak of each side coverage graphics

Whole Coverage Zoom (Left)



Whole Coverage Zoom (Right)



General controls information

|                             |        |   |
|-----------------------------|--------|---|
| Whole genome coverage       | 253    | OK  |
| Weak genome coverage        | 0.0 %  | OK  |
| Insert mean size            | 519    | Mean insert estimated from paired-end reads |
| Reads lost during alignment | 11.6 % | OK  |

i) PhageTerm method

Reads are mapped on the reference to determine the starting position coverage (SPC) as well as the coverage (COV) in each orientation. These values are then used to compute the variable  $T = SPC / COV$ . The average value of  $T$  at positions along the genome that are not termini is expected to be  $1/F$ , where  $F$  is the average fragment size. For the termini that depends of the packaging mode. Cos Phages: no reads should start before the terminus and therefore  $X=1$ . DTR phages: for  $N$  phages present in the sample, there should be  $N$  fragments that start at the terminus and  $N$  fragments that cover the edge of the repeat on the other side of the genome as a results  $T$  is expected to be 0.5. Pac phages: for  $N$  phages in the sample, there should be  $N/C$  fragments starting at the pac site, where  $C$  is the number of phage genome copies per concatemer. In the same sample  $N$  fragments should cover the pac site position,  $T$  is expected to be  $(N/C)/(N+N/C) = 1/(1+C)$ . To assess whether the number of reads starting at a given position along the genome can be considered a significant outlier, PhageTerm first segments the genome according to coverage using a regression tree. A gamma distribution is then fitted to SPC for each segment and an adjusted  $p$ -value is computed for each position. Finally if several significant peaks are detected within a small sequence window (default: 20bp), their  $T$  values are merged.

|                                    |       |   |
|------------------------------------|-------|---|
| Nearby Termini (Forward / Reverse) | 0 / 4 | Peaks localized 20 bases around the maximum |
|------------------------------------|-------|---|

ii) Li's method

The second approach is based on the calculation and interpretation of three specific ratios  $R1$ ,  $R2$  and  $R3$  as suggested in a previous publication from Li et al. 2014. The first ratio, is calculated as follow: the highest starting frequency found on either the forward or reverse strands is divided by the average starting frequency,  $R1 = (\text{highest frequency} / \text{average frequency})$ . Li's et al. have proposed three possible interpretation of the  $R1$  ratio. First, if  $R1 < 30$ , the phage genome does not have any termini, and is either circular or completely permuted and terminally redundant. The second interpretation for  $R1$  is when  $30 \leq R1 \leq 100$ , suggesting the presence of preferred termini with terminal redundancy and apparition of partially circular permutations. At last if  $R1 > 100$  that is an indication that at least one fixed termini is present with terminase recognizing a specific site. The two other ratios are  $R2$  and  $R3$  and the calculation is done in a similar manner.  $R2$  is calculated using the highest two frequencies ( $T1-F$  and  $T2-F$ ) found on the forward strand and  $R3$  is calculated using the highest two frequencies ( $T1-R$  and  $T2-R$ ) found on the reverse strand. To calculate these two ratios, we divide the highest frequency by the second highest frequency  $T2$ . So  $R2 = (T1-F / T2-F)$  and  $R3 = (T1-R / T2-R)$ . These two ratios are used to analyze termini characteristics on each strand taken individually. Li et al. suggested two possible interpretations for  $R2$  and  $R3$  ratios combine to  $R1$ . When  $R1 < 30$  and  $R2 < 3$ , we either have no obvious termini on the forward strand, or we have multiple preferred termini on the forward strand, if  $30 \leq R1 \leq 100$ . If  $R2 > 3$ , it is suggested that there is an obvious unique termini on the forward strand. The same reasoning is applicable for the result of  $R3$ . Combining the results for ratios found with this approach, it is possible to make the first prediction for the viral packaging mode of the analyzed phage. A unique obvious termini present at both ends (both  $R2$  and  $R3 > 3$ ) reveals the presence of a COS mode of packaging. The headful mode of packaging PAC is concluded when we have a single obvious termini only on one strand. A whole coverage around 500X is needed for this method to be reliable.

|                                      |       |   |
|--------------------------------------|-------|---|
| Nearby Termini (Forward / Reverse)   | 1 / 4 | Peaks localized 20 bases around the maximum                                       |
| R1 - highest freq./average freq.     | 641   | At least one fixed termini is present with terminase recognizing a specific site. |
| R2 Forw - highest freq./second freq. | 73    | Unique termini on the forward strand.   |
| R3 Rev - highest freq./second freq.  | 16    | Unique termini on the reverse strand.   |

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Garneau, Depardieu, Fortier, Bikard and Monot. PhageTerm: Determining Bacteriophage Termini and Packaging using NGS data.

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