

## Supplementary Materials

Table S1. Genomic information of *Alteromonas* strains used in this study

| Strain  | #Accession        | Isolated from                | Depth                    | Reference                |
|---|-------------------|------------------------------|--------------------------|--------------------------|
| <i>Alteromonas macleodii</i> ATCC 27126 <sup>T</sup>  | NA                | Hawaii, Pacific Ocean Oahu   | Surface seawaters        | [1]                      |
| <i>Alteromonas marina</i> SW-47 <sup>T</sup>          | NA                | Eastern Sea, Korea           | Surface seawaters        | [2]                      |
| <i>Alteromonas stellipolaris</i> ANT 69a <sup>T</sup> | CP013926          | Antarctica                   | Surface seawaters        | [3]                      |
| <i>Alteromonas macleodii</i> AD45                     | CP003874          | Mediterranean Sea            | Surface seawaters        | [4]                      |
| <i>Alteromonas tagae</i> AT1 <sup>T</sup>             | NA                | Er-Jen River estuary, Tainan | Surface estuarine waters | [5]                      |
| <i>Alteromonas macleodii</i> MCCC 1K00172             | NA                | South China Sea              | Surface seawaters        | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K00560             | NA                | Eastern Pacific Ocean        | Surface seawaters        | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> AD037                    | NZ_JWLY01000001.1 | Port Dickson, Malaysia       | Surface seawaters        | Unpublished <sup>b</sup> |
| <i>Alteromonas macleodii</i> BSH94-8                  | NA                | Black Sea Karadag            | Surface seawaters        | [6]                      |
| <i>Alteromonas macleodii</i> AD006                    | NZ_JWLX01000001.1 | Port Dickson, Malaysia       | Surface seawaters        | [6]                      |
| <i>Alteromonas macleodii</i> MCCC 1K01332             | NA                | East Pacific Ocean           | Surface seawaters        | Unpublished <sup>a</sup> |
| <i>Alteromonas confluentis</i> DSSK2-12 <sup>T</sup>  | NZ_MDHN01000001.1 | Jeju Island, South Korea     | Surface seawaters        | [7]                      |
| <i>Alteromonas mediterranea</i> EC615                 | CP004846          | English Channel              | Surface seawaters        | [8]                      |
| <i>Alteromonas macleodii</i> MCCC 1K00460             | NA                | Western Pacific Ocean        | Surface seawaters        | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01839             | NA                | Western Pacific Ocean        | Surface seawaters        | Unpublished <sup>a</sup> |

Table S1 | Continued

| Strain  | #Accession        | Isolated from                               | Depth                       | Reference                |
|---|-------------------|---|-----------------------------|--------------------------|
| <i>Alteromonas macleodii</i> MCCC 1K01358       | NA                | Eastern Pacific Ocean                       | Surface seawaters           | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K00811       | NA                | South China Sea                             | Surface seawaters           | Unpublished <sup>a</sup> |
| <i>Alteromonas australica</i> H 17 <sup>T</sup> | CP008849          | Port Phillip Bay, Tasman Sea, Pacific Ocean | Surface seawaters           | [9]                      |
| <i>Alteromonas addita</i> R10SW13 <sup>T</sup>  | CP014322          | Chazhma Bay, Sea of Japan, Pacific Ocean    | Surface seawaters           | [10]                     |
| <i>Alteromonas lipolytica</i> JW12 <sup>T</sup> | NZ_MJIC01000001.1 | Arabian Sea, Indian Ocean                   | Surface seawaters           | [11]                     |
| <i>Alteromonas macleodii</i> BS11               | CP003845          | Black Sea Karadag                           | Surface seawaters           | [8]                      |
| <i>Alteromonas mediterranea</i> MED64           | CP004848          | Aegean Sea, Mediterranean                   | Surface seawaters           | [8]                      |
| <i>Alteromonas macleodii</i> BS7                | NA                | Black Sea Karadag                           | Surface seawaters           | [8]                      |
| <i>Alteromonas macleodii</i> EC673              | CP003844          | English Channel                             | Surface seawaters           | [8]                      |
| <i>Alteromonas alba</i> 190 <sup>T</sup>        | NZ_PVNP01000003.1 | Western Pacific                             | Surface seawaters           | [12]                     |
| <i>Alteromonas macleodii</i> BSH84-3            | NA                | Black Sea Karadag                           | Surface seawaters           | [6]                      |
| <i>Alteromonas macleodii</i> BS8                | NA                | Black Sea Karadag                           | Surface seawaters           | [8]                      |
| <i>Alteromonas simiduii</i> AS1 <sup>T</sup>    | NA                | Er-Jen River estuary, Tainan                | Surface estuarine waters    | [5]                      |
| <i>Alteromonas macleodii</i> MCCC 1K01842       | NA                | Western Pacific Ocean                       | Subsurface seawaters (75 m) | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01832       | NA                | Western Pacific Ocean                       | Subsurface seawaters (30 m) | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01840       | NA                | Western Pacific Ocean                       | Subsurface seawaters (30 m) | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01294       | NA                | Western Pacific Ocean                       | Subsurface seawaters (75 m) | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01823       | NA                | Western Pacific Ocean                       | Subsurface seawaters (75 m) | Unpublished <sup>a</sup> |

Table S1 | Continued

| Strain  | #Accession | Isolated from                              | Depth                        | Reference                |
|---|------------|--|------------------------------|--------------------------|
| <i>Alteromonas macleodii</i> A16(2794)          | NA         | South China Sea                            | Subsurface seawaters (75 m)  | Unpublished <sup>c</sup> |
| <i>Alteromonas macleodii</i> A14(2783)          | NA         | South China Sea                            | Subsurface seawaters (75 m)  | Unpublished <sup>c</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01274       | NA         | Western Pacific Ocean                      | Subsurface seawaters (100 m) | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01826       | NA         | Western Pacific Ocean                      | Subsurface seawaters (100 m) | Unpublished <sup>a</sup> |
| <i>Alteromonas mediterranea</i> DE <sup>T</sup> | CP001103   | Adriatic Sea, Urania Basin                 | Deep seawaters (1000 m)      | [6,13]                   |
| <i>Alteromonas macleodii</i> MCCC 1A04487       | NA         | Northwestern Pacific Ocean                 | Deep seawaters (2700 m)      | Unpublished <sup>a</sup> |
| <i>Alteromonas mediterranea</i> DE1             | CP003917   | Adriatic Sea, Urania Basin                 | Deep seawaters (1000 m)      | [6]                      |
| <i>Alteromonas macleodii</i> MCCC 1A07993       | NA         | Southern Atlantic Ocean                    | Deep seawaters (2147 m)      | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1A09262       | NA         | Southern Atlantic Ocean                    | Deep seawaters (3047 m)      | Unpublished <sup>a</sup> |
| <i>Alteromonas mediterranea</i> UM7             | CP004853   | Ionian Sea, Uranian Basin Western of Crete | Deep seawaters (3475 m)      | [14]                     |
| <i>Alteromonas mediterranea</i> UM8             | CP013928   | Ionian Sea, Uranian Basin Western of Crete | Deep seawaters (3475 m)      | [14]                     |
| <i>Alteromonas macleodii</i> MCCC 1A00323       | NA         | Atlantic Ocean                             | Deep seawaters (3542 m)      | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K02087       | NA         | South China Sea                            | Deep seawaters (1700 m)      | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K00565       | NA         | Eastern Pacific Ocean                      | Deep seawaters (5098 m)      | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K00800       | NA         | Eastern Pacific Ocean                      | Deep seawaters (1000 m)      | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1A02046       | NA         | Indian Ocean                               | Deep seawaters (2391 m)      | Unpublished <sup>a</sup> |
| <i>Alteromonas mediterranea</i> UM4b            | CP004855   | Ionian Sea, Uranian Basin Western of Crete | Deep seawaters (3455 m)      | [14]                     |
| <i>Alteromonas mediterranea</i> U4              | CP004849   | Ionian Sea, Uranian Basin Western of Crete | Deep seawaters (3475 m)      | [14]                     |

Table S1 | Continued

| Strain  | #Accession        | Isolated from                                  | Depth                   | Reference                |
|---|-------------------|--|-------------------------|--------------------------|
| <i>Alteromonas mediterranea</i> U7                    | CP004851          | Ionian Sea, Uranian Basin Western of Crete     | Deep seawaters (3500 m) | [14]                     |
| <i>Alteromonas mediterranea</i> U8                    | CP004852          | Ionian Sea, Uranian Basin Western of Crete     | Deep seawaters (3500 m) | [14]                     |
| <i>Alteromonas macleodii</i> U12                      | NA                | Ionian Sea, Uranian Basin Western of Crete     | Deep seawaters (3500 m) | [14]                     |
| <i>Alteromonas macleodii</i> A25                      | NA                | South China Sea                                | Deep seawaters (4058 m) | Unpublished <sup>c</sup> |
| <i>Alteromonas macleodii</i> A27                      | NA                | South China Sea                                | Deep seawaters (4058 m) | Unpublished <sup>c</sup> |
| <i>Alteromonas</i> sp. MCCC 1A07988                   | NA                | Southern Atlantic Ocean                        | Deep seawaters (5610 m) | Unpublished <sup>a</sup> |
| <i>Alteromonas gracilis</i> 9a2 <sup>T</sup>          | NZ_PVNO01000001.1 | Pacific Ocean(mannose, galactose and glucose.) | Sediment (6310 m)       | [15]                     |
| <i>Alteromonas</i> sp. MCCC 1A09157                   | NA                | Southern Atlantic Ocean                        | Sediment                | Unpublished <sup>a</sup> |
| <i>Alteromonas naphthalenivorans</i> SN2 <sup>T</sup> | CP002339          | Taeon, South Korea                             | Sediment (tidal-flat)   | [16]                     |
| <i>Alteromonas macleodii</i> MCCC 1K02779             | NA                | Atlantic Ocean                                 | Sediment (2577 m)       | Unpublished <sup>a</sup> |
| <i>Alteromonas</i> sp. MCCC 1A09130                   | NA                | Southern Atlantic Ocean                        | Sediment                | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K02456             | NA                | Northwestern Indian Ocean                      | Sediment (1818 m)       | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K02451             | NA                | Northwestern Indian Ocean                      | Sediment (2009 m)       | Unpublished <sup>a</sup> |
| <i>Alteromonas</i> sp. MCCC 1A08050                   | NA                | Southern Atlantic Ocean                        | Sediment (2481 m)       | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K02444             | NA                | Northwestern Indian Ocean                      | Sediment (2540 m)       | Unpublished <sup>a</sup> |
| <i>Alteromonas pelagimontana</i> 5.12 <sup>T</sup>    | CP052766          | Indian Ocean                                   | Sediment (2681 m)       | [17]                     |
| <i>Alteromonas macleodii</i> MCCC 1K01703             | NA                | Atlantic Ocean                                 | Sediment (2781m)        | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01716             | NA                | Atlantic Ocean                                 | Sediment (2781 m)       | Unpublished <sup>a</sup> |

Table S1 | Continued

| Strain   | #Accession         | Isolated from                    | Depth                          | Reference                |
|--|--------------------|----------------------------------|--------------------------------|--------------------------|
| <i>Alteromonas litorea</i> TF-22 <sup>T</sup>        | NA                 | Korea, Yellow Sea                | Sediment (Intertidal)          | [18]                     |
| <i>Alteromonas aestuarii</i> JDTF-113                | NZ_QRHA01000001.1  | Jindo, South Korea               | Sediment (tidal-flat)          | [19]                     |
| <i>Alteromonas</i> sp. EZ55                          | NZ_CABDXN010000001 | Tropical Pacific Ocean           | Prochlorococcus culture (20 m) | [20]                     |
| <i>Alteromonas macleodii</i> MCCC 1F01223            | NA                 | Xiamen, China                    | Algae culture                  | Unpublished <sup>a</sup> |
| <i>Alteromonas hispanica</i> F-32 <sup>T</sup>       | JAAAWP010000001.1  | Fuente de Piedra, southern Spain | Hypersaline water              | [21]                     |
| <i>Alteromonas genovensis</i> LMG 24078 <sup>T</sup> | NZ_JAAAWO010000001 | Genoa, Italy                     | Biofilm                        | [22]                     |
| <i>Alteromonas macleodii</i> MCCC 1K02452            | NA                 | Northwestern Indian Ocean        | Olivine (3042 m)               | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K00767            | NA                 | Eastern Pacific Ocean            | Seawaters (500 m)              | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> MCCC 1K01276            | NA                 | Western Pacific Ocean            | Seawaters (300 m)              | Unpublished <sup>a</sup> |
| <i>Alteromonas macleodii</i> AS7                     | NA                 | Andaman Sea                      | NA                             | Unpublished <sup>b</sup> |

a: Strains were purchased from the Marine Culture Collection of China (MCCC, China)

b: Strains were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany)

c: Strains were isolated from our laboratory.

Table S2. vB\_AmeP-R8W (R8W) genome annotation

| Genes No. | Start | End  | Length | Stand | GC%   | Putative function            | Functional categories      |
|-----------|-------|------|--------|-------|-------|------------------------------|----------------------------|
| 1         | 156   | 788  | 633    | +     | 0.382 | hypothetical protein         |                            |
| 2         | 802   | 969  | 168    | +     | 0.363 | hypothetical protein         |                            |
| 3         | 1020  | 1247 | 228    | +     | 0.333 | hypothetical protein         |                            |
| 4         | 1249  | 1512 | 264    | +     | 0.337 | hypothetical protein         |                            |
| 5         | 1608  | 3734 | 2127   | +     | 0.414 | DNA-dependent RNA polymerase | Transcription              |
| 6         | 3778  | 4320 | 543    | +     | 0.418 | hypothetical protein         |                            |
| 7         | 4335  | 4874 | 540    | +     | 0.433 | hypothetical protein         |                            |
| 8         | 4814  | 5011 | 198    | +     | 0.449 | hypothetical protein         |                            |
| 9         | 4998  | 5210 | 213    | +     | 0.352 | hypothetical protein         |                            |
| 10        | 5197  | 5376 | 180    | +     | 0.433 | hypothetical protein         |                            |
| 11        | 5373  | 5597 | 225    | +     | 0.364 | amidohydrolase               | lysis                      |
| 12        | 5711  | 6151 | 441    | +     | 0.469 | hypothetical protein         |                            |
| 13        | 6264  | 6434 | 171    | +     | 0.427 | hypothetical protein         |                            |
| 14        | 6379  | 7158 | 780    | +     | 0.421 | DNA primase                  | DNA replication and repair |
| 15        | 7149  | 7514 | 366    | +     | 0.393 | hypothetical protein         |                            |
| 16        | 7644  | 7850 | 207    | +     | 0.386 | hypothetical protein         |                            |
| 17        | 7956  | 9203 | 1248   | +     | 0.387 | DnaB-like helicase           | DNA replication and repair |

Table S2 | Continued

| Genes No. | Start | End   | Length | Stand | GC%   | Putative function                       | Functional categories      |
|-----------|-------|-------|--------|-------|-------|---|----------------------------|
| 18        | 9203  | 9787  | 585    | +     | 0.388 | hypothetical protein                    |                            |
| 19        | 9780  | 10451 | 672    | +     | 0.409 | thymidylate synthase                    | auxiliary metabolism       |
| 20        | 10459 | 10917 | 459    | +     | 0.394 | HNH endonuclease                        | DNA replication and repair |
| 21        | 10920 | 11930 | 1011   | +     | 0.394 | ribonucleotide reductase, small chain   | nucleotides metabolism     |
| 22        | 11906 | 13597 | 1692   | +     | 0.418 | ribonucleotide reductase, barrel domain | nucleotides metabolism     |
| 23        | 13588 | 14016 | 429    | +     | 0.378 | HNH endonuclease                        | DNA replication and repair |
| 24        | 14009 | 14449 | 441    | +     | 0.379 | NTP-Ppase                               | auxiliary metabolism       |
| 25        | 14954 | 17266 | 2313   | +     | 0.405 | DNA polymerase                          | DNA replication and repair |
| 26        | 17266 | 17394 | 129    | +     | 0.349 | hypothetical protein                    |                            |
| 27        | 17394 | 18275 | 882    | +     | 0.409 | hypothetical protein                    |                            |
| 28        | 18355 | 19353 | 999    | +     | 0.395 | 5'-3' exonuclease                       | DNA replication and repair |
| 29        | 19328 | 19546 | 219    | +     | 0.397 | hypothetical protein                    |                            |
| 30        | 19536 | 19667 | 132    | +     | 0.311 | hypothetical protein                    |                            |
| 31        | 19664 | 20539 | 876    | +     | 0.4   | DNA ligase                              | DNA replication and repair |
| 32        | 20532 | 20918 | 393    | +     | 0.413 | recombination endonuclease VII          | DNA replication and repair |
| 33        | 20915 | 23305 | 2391   | +     | 0.409 | DNA-dependent RNA polymerase            | Transcription              |
| 34        | 23332 | 23802 | 471    | +     | 0.395 | short tail fiber protein                | structural genes           |
| 35        | 23778 | 24026 | 249    | +     | 0.394 | hypothetical protein                    |                            |

Table S2 | Continued

| Genes No. | Start | End   | Length | Stand | GC%   | Putative function             | Functional categories |
|-----------|-------|-------|--------|-------|-------|-------------------------------|-----------------------|
| 36        | 24035 | 25573 | 1539   | +     | 0.406 | head to tail connector        | structural genes      |
| 37        | 25576 | 26445 | 870    | +     | 0.413 | capsid assembly protein       | structural genes      |
| 38        | 26473 | 27576 | 1104   | +     | 0.448 | putative major capsid protein | structural genes      |
| 39        | 27687 | 28277 | 591    | +     | 0.393 | tail tubular protein A        | structural genes      |
| 40        | 28286 | 30640 | 2355   | +     | 0.403 | tail tubular protein B        | structural genes      |
| 41        | 30640 | 31182 | 543    | +     | 0.411 | hypothetical protein          |                       |
| 42        | 31184 | 33466 | 2283   | +     | 0.407 | internal virion protein       | structural genes      |
| 43        | 33475 | 33957 | 483    | +     | 0.387 | endolysin                     | lysis                 |
| 44        | 33972 | 39428 | 5457   | +     | 0.405 | internal virion protein       | structural genes      |
| 45        | 39430 | 43908 | 4479   | +     | 0.421 | long tail fiber protein       | structural genes      |
| 46        | 43919 | 44128 | 210    | +     | 0.405 | class II holin                | lysis                 |
| 47        | 44142 | 44438 | 297    | +     | 0.374 | hypothetical protein          |                       |
| 48        | 44435 | 44896 | 462    | +     | 0.39  | PhoB                          | auxiliary metabolism  |
| 49        | 44893 | 45285 | 393    | +     | 0.399 | hypothetical protein          |                       |
| 50        | 45285 | 45602 | 318    | +     | 0.403 | terminase small subunit       | packaging genes       |
| 51        | 45602 | 47656 | 2055   | +     | 0.412 | terminase large subunit       | packaging genes       |
| 52        | 47653 | 48066 | 414    | +     | 0.43  | hypothetical protein          |                       |
| 53        | 48066 | 48380 | 315    | +     | 0.419 | hypothetical protein          |                       |



Table S3. The genetic comparison of the phage vB\_AmeP-R8W (R8W), vB\_AspP-H4/4 (H4) and prokaryotic dsDNA virus (TS) (similarity >70%).

| R8W genes No. | Start | End   | Length | Putative function                       | Functional categories      |
|---------------|-------|-------|--------|---|----------------------------|
| 17            | 7956  | 9203  | 1248   | DnaB-like helicase                      | DNA replication and repair |
| 19            | 9780  | 10451 | 672    | thymidylate synthase                    | auxiliary metabolism       |
| 21            | 10920 | 11930 | 1011   | ribonucleotide reductase, small chain   | nucleotides metabolism     |
| 22            | 11906 | 13597 | 1692   | ribonucleotide reductase, barrel domain | nucleotides metabolism     |
| 24            | 14009 | 14449 | 441    | NTP-Ppase                               | auxiliary metabolism       |
| 25            | 14954 | 17266 | 2313   | DNA polymerase                          | DNA replication and repair |
| 27            | 17394 | 18275 | 882    | hypothetical protein                    |                            |
| 32            | 20532 | 20918 | 393    | recombination endonuclease VII          | DNA replication and repair |
| 33            | 20915 | 23305 | 2391   | DNA-dependent RNA polymerase            | transcription              |
| 34            | 23332 | 23802 | 471    | short tail fiber protein                | structural genes           |
| 35            | 23778 | 24026 | 249    | hypothetical protein                    |                            |
| 36            | 24035 | 25573 | 1539   | head to tail connector                  | structural genes           |
| 38            | 26473 | 27576 | 1104   | putative major capsid protein           | structural genes           |
| 43            | 33475 | 33957 | 483    | endolysin                               | lysis                      |
| 51            | 45602 | 47656 | 2055   | terminase large subunit                 | packaging genes            |
| 52            | 47653 | 48066 | 414    | hypothetical protein                    |                            |
| 53            | 48066 | 48380 | 315    | hypothetical protein                    |                            |

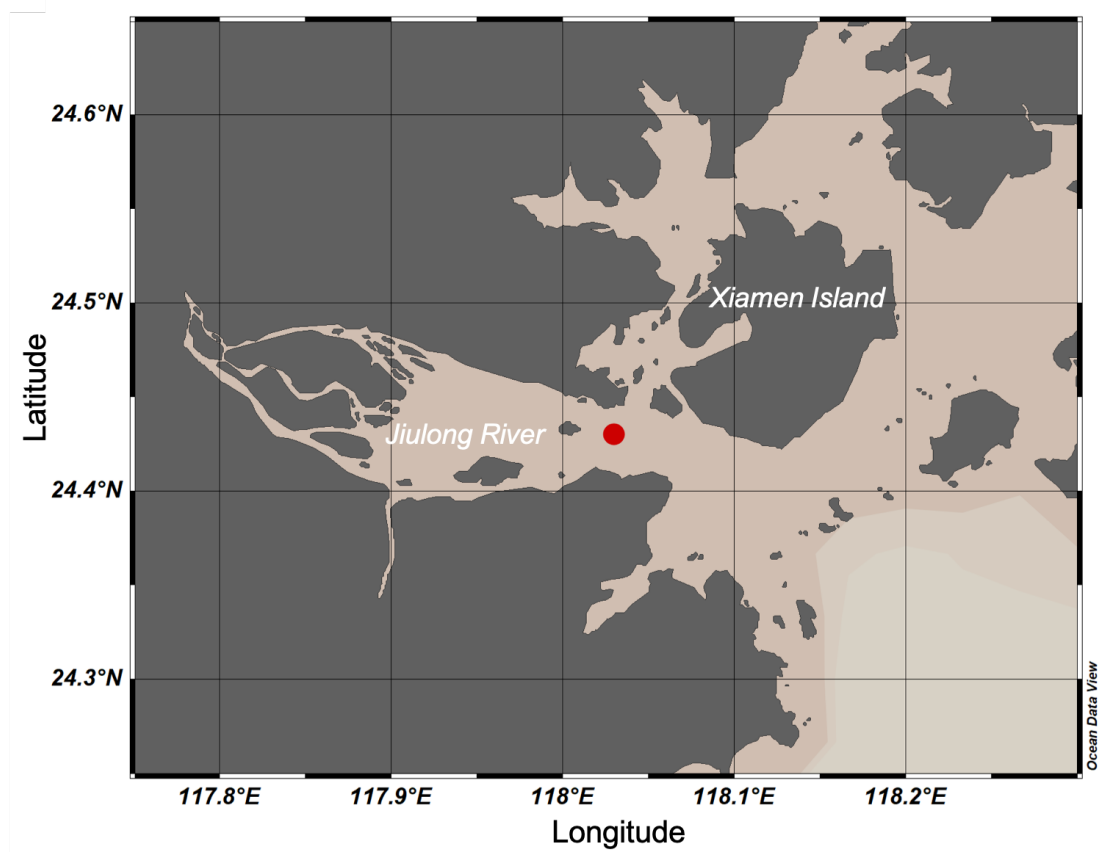


Figure S1. Station map indicating location where vB\_AmeP-R8W (R8W) was isolated.

## References

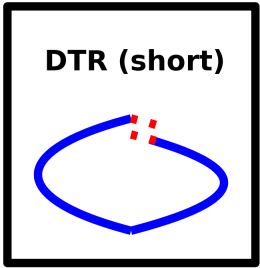
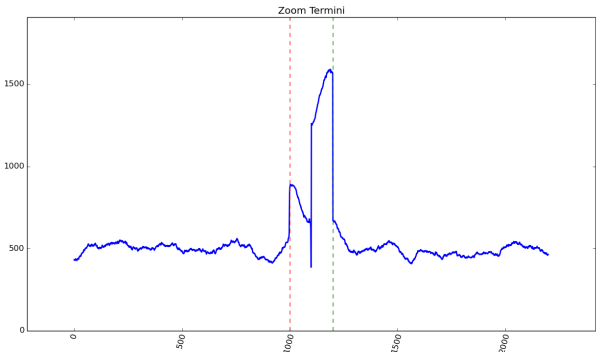
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Supplementary Data 1. Results of R8W PhageTerm Analysis



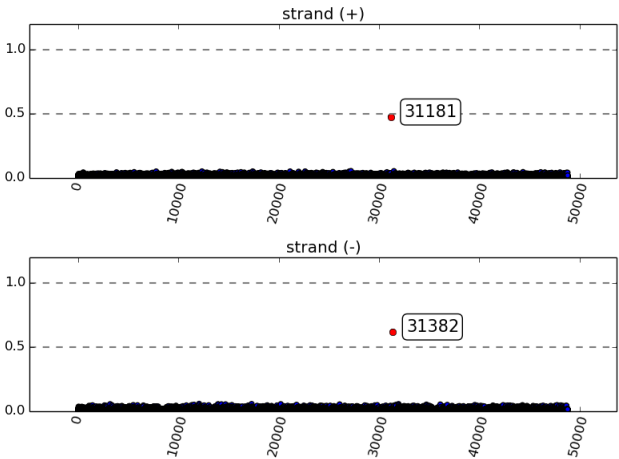
PhageTerm Method

| Ends      | Left (red) | Right (green) | Permuted | Orientation | Class       | Type |
|-----------|------------|---------------|----------|-------------|-------------|------|
| Redundant | 31181      | 31382         | No       | NA          | DTR (short) | T7   |

\*Direct Terminal Repeats: 202 bp

| Strand | Location | T    | pvalue    |
|--------|----------|------|-----------|
| +      | 31181    | 0.48 | 6.63e-55  |
|        | 27210    | 0.05 | 1.00e+00  |
|        | 31495    | 0.05 | 1.00e+00  |
|        | 27057    | 0.05 | 1.00e+00  |
|        | 12345    | 0.05 | 8.19e-01  |
| -      | 31382    | 0.62 | 9.00e-141 |
|        | 12063    | 0.06 | 1.00e+00  |
|        | 17302    | 0.05 | 1.00e+00  |
|        | 31956    | 0.05 | 1.00e+00  |
|        | 3190     | 0.05 | 1.00e+00  |

T (Start. Pos. Cov. / Whole Cov.)



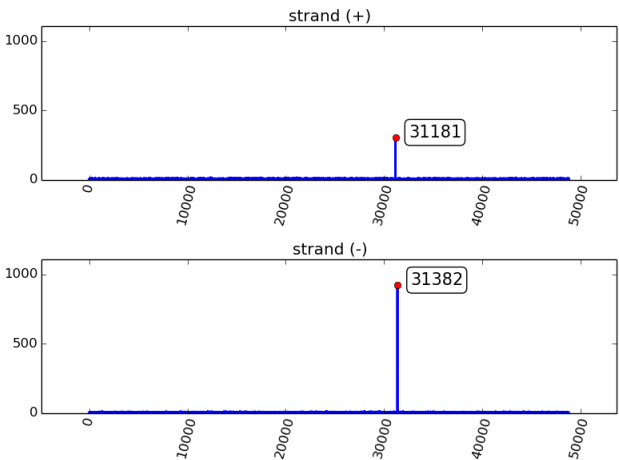
Li's Method

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

\*Direct Terminal Repeats: 202 bp

| Strand | Location | SPC | R    |
|--------|----------|-----|------|
| +      | 31181    | 306 | 18.0 |
|        | 12345    | 17  | -    |
|        | 21138    | 16  | -    |
|        | 43422    | 15  | -    |
|        | 17408    | 15  | -    |
| -      | 31382    | 924 | 66.0 |
|        | 28012    | 14  | -    |
|        | 31956    | 13  | -    |
|        | 31410    | 13  | -    |
|        | 12063    | 13  | -    |

SPC

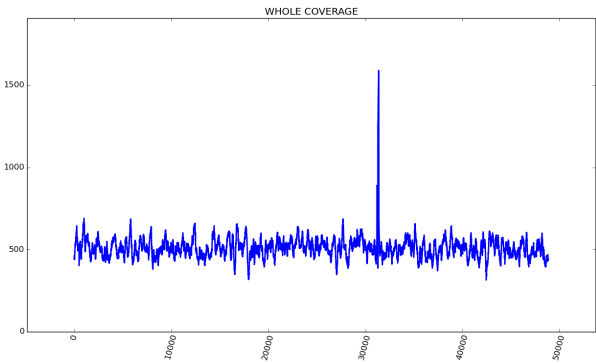


Analysis Methodology

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.

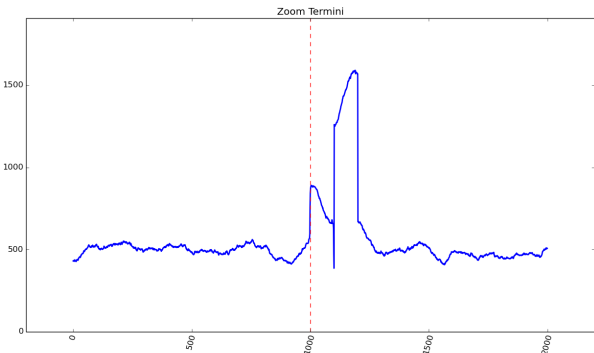
General set-up and mapping informations

|                  |          |
|------------------|----------|
| Phage Genome     | 48825 bp |
| Sequencing Reads | 1023457  |
| Mapping Reads    | 95 %     |
| OPTIONS          |          |
| Mapping Seed     | 20       |
| Surrounding      | 20       |
| Host Analysis    | No       |

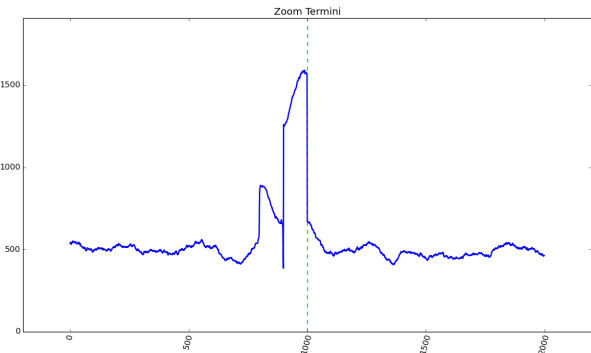


Highest peak of each side coverage graphics

Whole Coverage Zoom (Left)



Whole Coverage Zoom (Right)



General controls information

|                             |       |    |
|-----------------------------|-------|----|
| Whole genome coverage       | 252   | OK |
| Weak genome coverage        | 0.0 % | OK |
| Reads lost during alignment | 4.9 % | 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) | 2 / 0 | 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)   | 3 / 1 | Peaks localized 20 bases around the maximum                                       |
| R1 - highest freq./average freq.     | 361   | At least one fixed termini is present with terminase recognizing a specific site. |
| R2 Forw - highest freq./second freq. | 18    | Unique termini on the forward strand.   |
| R3 Rev - highest freq./second freq.  | 66    | Unique termini on the reverse strand.   |

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