

## Supplementary materials

### Isolation, characterization and genome engineering of a lytic *Pseudomonas aeruginosa* phage

Xiaomei Cong,<sup>1</sup> Shuang Zhao,<sup>1</sup> Qing Zhang,<sup>2</sup> Shuo Liu,<sup>1</sup> Youming Zhang<sup>1\*</sup> and Fu Yan<sup>1\*</sup>

<sup>1</sup> *State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, China*

<sup>2</sup> *Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Jinan 250000, China*

\* Corresponding author: zhangyouming@sdu.edu.cn and fuyan@sdu.edu.cn

**Table S1.** Primers used in this study.

Primer	Sequence (5'→3')
PpY1 repeat-F	CCCGACTCCGACACGCCAC
PpY1 Frag1-R	ACGCGGCGTGTTTACTGCGGCGCGTGCCTTACGCTTAGCCTCTGC AACTTGGTCGGCA
PpY1 Frag2-F	TGCCGACCAAGTTGCAGAGGCTAAGCGTAAGGCACGCGCCGCAGT AAACCACGCCGCGT
PpY1 Frag2-R	TGTCGGATGAAGATTTAAGGCCGGAGTAGGGAAGGGTCGTCAGGC GTTTCGAGGCGTGCG
PpY1 Frag3-F	CGCACGCCTCGAACGCCTGACGACCCTTCCCTACTCCGGCCTTAAA TCTTCATCCGACA
PpY1 Frag3-R	GATGCACTTGCTCCTATGTGGGTGACATAATCCGCCTCAGCAGAAG AACGCCCAGGAGT
PpY1 Frag4-F	ACTCCTGGGCGTTCTTCTGCTGAGGCGGATTATGTCACCCACATAG GAGCAAGTGCATC
PpY1 Frag4-R	CCAAGCTCTTGGAAGCACGTTGCGATTCCGCCATCAGTTGCCTCC TTAGGCGTGGCG
PpY1 Frag5-F	CGCCACGCCTAAAGGAGGCAACTGATGGCGGAATCGCAACGTGCT TCCCAAGAGCTTGG
PpY1 Frag5-R	CGAATCAGGGTGTAACGTCCCTTTACTTCGGTCTTAAACATTTTCA GCTCCGCCTGTA
PpY1 Frag6-F	TACAGGCGGAGCTGAAAAATGTTTAAGACCGAAGTAAAGGGACGT TACACCCTGATTCTG
PpY1 repeat-R	CCAGCCACTCCCGCAACCAC
KOgp01-Frag1-1-R	AGTTCATTACACGTGGCATCGTGCAACCTCCTACTAGGTCTGTCT CTTGAAT
KOgp01-Frag1-2-F	ATTCAAGAGACAGACCTAGTAGGAGGTTGCACGATGCCACGTGTG AATGAACT
KOgp02-Frag1-1-R	TTGGAGATTGCGTTGGTCATTACAAACCCTTCCCTTGCGA
KOgp02-Frag1-2-F	TCGCAAGGGAAGGGTTTGTAATGACCAACGCAATCTCCAA
KOgp03-Frag1-1-R	TAGCAGGCGTTTCGACTTGAGGGTATGTACCTCAGATTGAT
KOgp03-Frag1-2-F	ATCAATCTGAGGTACATACCCTCAAGTCGAACGCCTGCTA
KOgp04-Frag1-1-R	GGCGATCCAGAAGTCCATGGCGTGTTTCTCCAGTTGTCAT
KOgp04-Frag1-2-F	ATGACAACTGGAGAAACACGCCATGGACTTCTGGATCGCC
KOgp05-Frag1-1-R	CGCCAGGGCCATGACTTGAGGGTCACTCCCCCTTCGCCTG
KOgp05-Frag1-2-F	CAGGCGAAGGGGGAGTGACCCTCAAGTCATGGCCCTGGCG
KOgp06-Frag1-1-R	TAGGGATCACGCGAGGACATCGTGCAACCTCCTGGTTTAGTTCTCG CTTGTGA
KOgp06-Frag1-2-F	TCACAAGCGAGAACTAAACCAGGAGGTTGCACGATGTCCTCGCGT GATCCCTA
KOgp07-Frag1-1-R	GCTATGATGAGCCACTTCATCGTGCAACCTCCTTCACGCGAGGACA TGGTACG
KOgp07-Frag1-2-F	CGTACCATGTCCTCGCGTGAAGGAGGTTGCACGATGAAGTGGCTC ATCATAGC

KOgp08-Frag1-1-R	ACGACGTAGGTAGGATTCATCGTGCAACCTCCTTCATTTACGGGTGCTAGCT
KOgp08-Frag1-2-F	AGTACCGACCCGTAAATGAAGGAGGTTGCACGATGAATCCTACCTACGTCGT
KOgp09-Frag1-1-R	TACACGGTACGGACGGTCATCGTGCAACCTCCTTCATATGCGCTTGCTCCAGT
KOgp09-Frag1-2-F	ACTGGAGCAAGCGCATATGAAGGAGGTTGCACGATGACCGTCCGTACCGTGTA
KOgp10-Frag1-1-R	AGGATCAGTGCATCGGACATCGTGCAACCTCCTTTACTCACCTCCTTCACGA
KOgp10-Frag1-2-F	TCGTGAAGGAGGGTGAGTAAAGGAGGTTGCACGATGTCCGATGCACTGATCCT
KOgp11-Frag1-1-R	TGGGTATGGGTACGGATCATCGTGCAACCTCCTTCAGTCGTCTCCTTGGACGA
KOgp11-Frag1-2-F	TCGTCCAAGGAGACGACTGAAGGAGGTTGCACGATGATCCGTACCATAACCA
KOgp12-Frag1-1-R	CGCACCAGCCTTCGCCTTGGCTCAGTTCCTCGTCGATTTG
KOgp12-Frag1-2-F	CAAATCGACGAGGAAGTGAAGCAAGGCTGGTGCG
KOgp13-Frag1-R	GGGCCTATTACGTGCGGGCCGCTGTAGCTCCTCAGTTAGG
KOgp13-Frag2-F	CCTAACTGAGGAGCTACAGCGGCCCGCACGTAATAGGCCC
KOgp14-Frag1-R	TAAAGGTCTTTGCACTGCATGGTGACTCCTCCTATGGTAT
KOgp14-Frag2-F	ATACCATAGGAGGAGTCACCATGCAGTGCAAAGACCTTTA
KOgp16-Frag1-R	TTTCGCGATAAGCCATGTCATCGTGCAACCTCCTTCTAAATCCCTCAATTTG
KOgp16-Frag2-F	CAAATTGAGGGATTAGAAAGGAGGTTGCACGATGACATGGCTTATCGCGAA
KOgp17-Frag1-R	TCCTGAAGATGCCAGGTCATCGTGCAACCTCCTCACCTCACTCATGATCGGGT
KOgp17-Frag2-F	ACCCGATCATGAGTGAGGTGAGGAGGTTGCACGATGACCTGGCATCTTCAGGA
KOp18-Frag1-R	GAGTCTCGGCGCAGTGCCATCGTGCAACCTCCTTCATGCGGGTGCCGTAGGCG
KOp18-Frag2-F	CGCCTACGGCACCCGCATGAAGGAGGTTGCACGATGGCACTGCGCGAGACTC
KOp21-Frag1-R	ACCACGTCGCGCTTGCTCATTAGCTTGATGATCTACAC
KOp21-Frag2-F	GTGTAGATCATGCAAGCTAAATGAGCAAGCGCGACGTGGT
KOp23-Frag2-1-R	TCCTCGGTGGGCATTCTCATTCATACCTTCACTCCAGGGT
KOp23-Frag2-2-F	ACCCTGGAGTGAAGGTATGAATGAGAATGCCACCGAGGA
KOp24-Frag2-1-R	AGGATTCGGATAGTCGTCATTTCATACTTCGACTCCTACCT
KOp24-Frag2-2-F	AGGTAGGAGTCGAAGTATGAATGACGACTATCCGAATCCT
KOp26-Frag2-R	GCCGGAGTAGGGAAGGGTCGTCCTGAATCACCTCCATAT
KOp26-Frag3-F	ATATGGAGGTGATTCACTGACGACCCTTCCCTACTCCGGC
KOp27-Frag2-R	TCTTCCGATGGCAGGCGCATGCGTGGTCTCTCTCGTGTGC
KOp27-Frag3-F	CGACACGAGAGAGACCACGCATGCGCCTGCCATCGGAAGA

KOgp31-Frag3-1-R	GGTACACCAACAGATTTTACTCATGCTACCTCCTTAGTTGCTCA
KOgp31-Frag3-2-F	TGAGCAACTAAGGAGGTAGCATGAGTAAAATCTGTTGGTGTACC
KOgp32-Frag3-2-F	GGGAGAAAGGCAATGAGTAAAGGAGGTTGCACGATGGACCTG ATACAGCAGCA
KOgp32-Frag3-1-R	TGCTGCTGTATCAGGTCCATCGTGCAACCTCCTTTACTCATTGCC TTTCTCCC
KPgp34-Frag3-R	CGCCAGATGGACTTGTTTCATCGTGCAACCTCCTCTATCATATCTCCT ATGCTG
KOgp34-Frag4-F	CAGCATAGGAGATATGATAGAGGAGGTTGCACGATGAACAAGTCCA TCTGGCG
KOgp42-Frag4-R	TCCGCCATCAGTTGCCTCCTTCACACCCTCCTGTAGGTTT
KOgp42-Frag5-F	AAACCTACAGGAGGGTGTGAAGGAGGCAACTGATGGCGGA
KOgp55-Frag6-2-F	CCTTGAATCAAGGAGATTGAGCTTCAGCCCGTCCAACCTG
KOgp56-Frag6-1-R	GCTCGGCTTGAGCTACTCGAGGTTTCAGTTCCTTGTGTTAT
KOgp56-Frag6-2-F	ATAACACAAGGAACTGAACCTCGAGTAGCTCAAGCCGAGC
PpY1-KOgp57-Frag1 -2-F	TACGCATCCTGGCGGGAGTGCCTCTGTGCCTCCTGTGGTG
PpY1-KOgp57-Frag1 -1-R	CACCACAGGAGGCACAGAGGCACTCCCGCCAGGATGCGTA
KOgp01-chkL-F	ATTGAACAAGGCCAGGCAGA
KOgp02-chkR-R	ATGGCTTCGTCCAGTTCCTT
KOgp04-chkR-R	CCACAACATGCGCAGTTCAT
KOgp05-chkL-F	AGCTTCAAACAACGCCTGCA
KOgp06-chkR-R	GGTCGGGTTATGGCTTCAAT
KOgp07-chkL-F	ATGGCTGGATCAACGCTGAA
KOgp10-chkR-R	CGCGATCAGGATCAGTGCAT
KOgp11-chkL-F	AAGGCGAGTACCTCAAACCT
KOgp12-chkR-R	AGCTCCTCAGTTAGGTGGTT
KOgp13-chkL-F	AGCCTTCCACCGTGGCCATT
KOgp14-chkR-R	GTACGCTGAACATGTCCGAT
KOgp16-chkL-F	ATTACATGGCGCAGGACCTG
KOgp18-chkR-R	TGGCCGACTGCCAGGGATTG
KOgp21-chkL-F	AAATCCACCTGGGCCGTCTC
KOgp21-chkR-R	CGCGATGATGCGATCCTCCA
KOgp23-chkL-F	AACTATGGACGCATCGCCGA
KOgp23-chkR-R	GGTCAGTTGGTACAGGGAGA
KOgp24-chkR-R	CGAACATGGCATTGTGAGCT
KOgp26-chkL-F	ACATCATGGCCGACGCTGCC
KOgp26-chkR-R	CCGGTGGAGGTTTCGGACAT
KOgp41-chkL-F	AGAGGACTACGAAGGATTCT
KOgp42-chkR-R	GGTCAACCTGCTGGTTTCGT
KOgp56-chkR-R	CCAGTGGAGCACCCGACTAT
genta-KORM-F1	GCACACGTCAACTGTGGCTTGCCACTAAACATATTTGAATGAAGAG TTGAAGCGCTGATAGAAGGCACGAACCCAGTTGAC

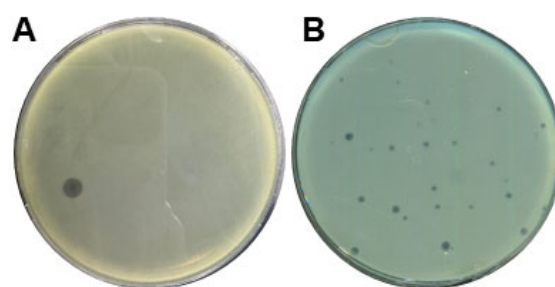
---

genta-KORM-F2	TAATGGGCACGAATGGCCGGAGCGTATTTTCCCTATCAAGGCATCC CATGGCCACGCCCCGCACACGTCAACTGTGGCTT
genta-KORM-4-R1	GCCGTTCTGGCTTGGAATAATTTTCGCAGCACTGGAAACCAGCCCA ACAGTGTTCCTCAGACCGTTCGTATAGCATACATTATAC
genta-KORM-4-R2	GCTTCCATGCGCTGAACACAATCCTCGAAATGACGGTCAAGAAAA TCGTCGAACTCCTCCGCCGTTCTGGCTTGGAATAA

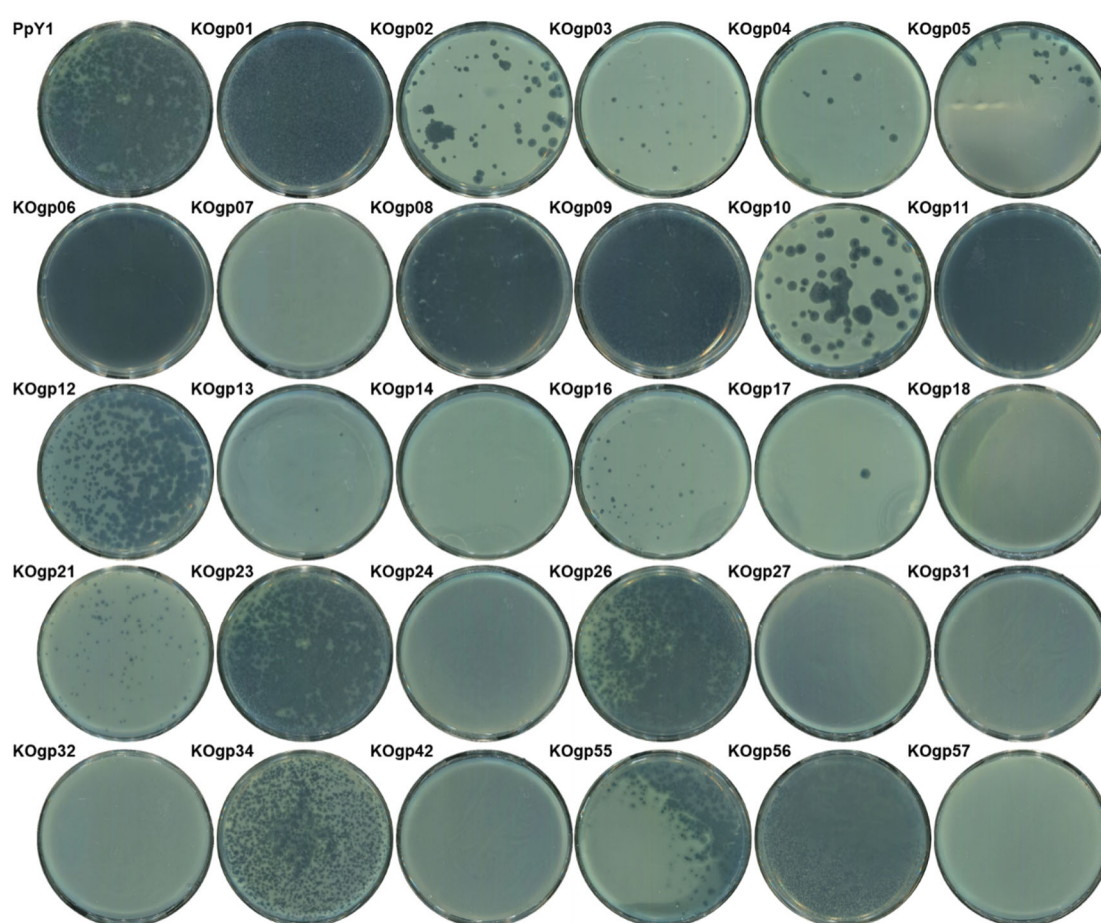
**Table S2.** Plasmids constructed in this study.

Plasmids	Characteristics	Sources	Genome rebooting
pCAP01-PpY1-KOgp01	pCAP01 carrying PpY1 genome deleted <i>gp01</i>	This study	+
pCAP01-PpY1-KOgp02	pCAP01 carrying PpY1 genome deleted <i>gp02</i>	This study	+
pCAP01-PpY1-KOgp03	pCAP01 carrying PpY1 genome deleted <i>gp03</i>	This study	+
pCAP01-PpY1-KOgp04	pCAP01 carrying PpY1 genome deleted <i>gp04</i>	This study	+
pCAP01-PpY1-KOgp05	pCAP01 carrying PpY1 genome deleted <i>gp05</i>	This study	+
pCAP01-PpY1-KOgp06	pCAP01 carrying PpY1 genome deleted <i>gp06</i>	This study	+
pCAP01-PpY1-KOgp07	pCAP01 carrying PpY1 genome deleted <i>gp07</i>	This study	—
pCAP01-PpY1-KOgp08	pCAP01 carrying PpY1 genome deleted <i>gp08</i>	This study	+
pCAP01-PpY1-KOgp09	pCAP01 carrying PpY1 genome deleted <i>gp09</i>	This study	+
pCAP01-PpY1-KOgp10	pCAP01 carrying PpY1 genome deleted <i>gp10</i>	This study	+
pCAP01-PpY1-KOgp11	pCAP01 carrying PpY1 genome deleted <i>gp11</i>	This study	+
pCAP01-PpY1-KOgp12	pCAP01 carrying PpY1 genome deleted <i>gp12</i>	This study	+
pCAP01-PpY1-KOgp13	pCAP01 carrying PpY1 genome deleted <i>gp13</i>	This study	+
pCAP01-PpY1-KOgp14	pCAP01 carrying PpY1 genome deleted <i>gp14</i>	This study	+
pCAP01-PpY1-KOgp16	pCAP01 carrying PpY1 genome deleted <i>gp16</i>	This study	+
pCAP01-PpY1-KOgp17	pCAP01 carrying PpY1 genome deleted <i>gp17</i>	This study	+
pCAP01-PpY1-KOgp18	pCAP01 carrying PpY1 genome deleted <i>gp18</i>	This study	—

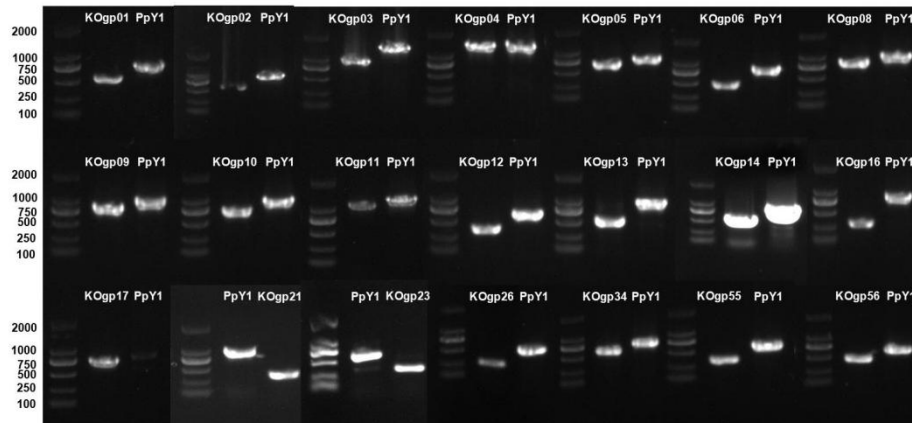
pCAP01-PpY1-KOgp21	pCAP01 carrying PpY1 genome deleted <i>gp21</i>	This study	+
pCAP01-PpY1-KOgp23	pCAP01 carrying PpY1 genome deleted <i>gp23</i>	This study	+
pCAP01-PpY1-KOgp24	pCAP01 carrying PpY1 genome deleted <i>gp24</i>	This study	—
pCAP01-PpY1-KOgp26	pCAP01 carrying PpY1 genome deleted <i>gp26</i>	This study	+
pCAP01-PpY1-KOgp27	pCAP01 carrying PpY1 genome deleted <i>gp27</i>	This study	—
pCAP01-PpY1-KOgp31	pCAP01 carrying PpY1 genome deleted <i>gp31</i>	This study	—
pCAP01-PpY1-KOgp32	pCAP01 carrying PpY1 genome deleted <i>gp32</i>	This study	—
pCAP01-PpY1-KOgp34	pCAP01 carrying PpY1 genome deleted <i>gp34</i>	This study	+
pCAP01-PpY1-KOgp42	pCAP01 carrying PpY1 genome deleted <i>gp42</i>	This study	—
pCAP01-PpY1-KOgp55	pCAP01 carrying PpY1 genome deleted <i>gp55</i>	This study	+
pCAP01-PpY1-KOgp56	pCAP01 carrying PpY1 genome deleted <i>gp56</i>	This study	+
pCAP01-PpY1-KOgp57	pCAP01 carrying PpY1 genome deleted <i>gp57</i>	This study	—



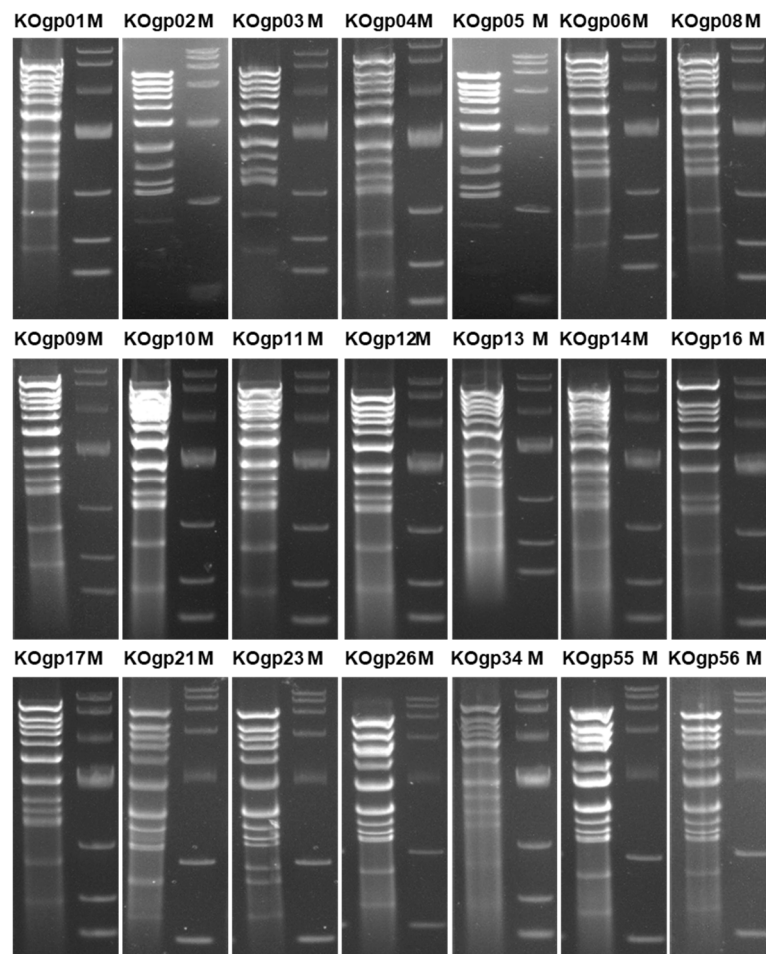
**Figure S1** Plaques of PpY1-KOgp03 generated by transferring pCAP01- PpY1-KOgp03 into PAO1 (A) and PAO1-KORM (B).



**Figure S2** Plaques of phage PpY1 and mutant phages generated by transferring assembled genomes into PAO1-KORM.

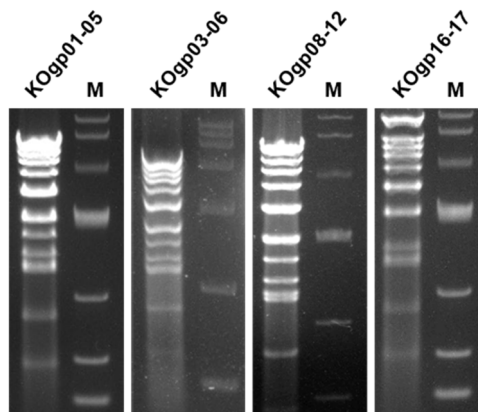


**Figure S3** Verification of mutant phage genomes by PCR. The genome of PpY1 was set as control. The amplified fragments were further validated by Sanger-sequencing.



**Figure S4** Restriction analysis of the genomes of mutant phages. The genomic DNA were digested by *EcoR* V. M, DNA marker.





**Figure S5** Restriction analysis of genome reduced phages. The genomic DNA were digested by *EcoR* V. M, DNA marker.