**Supporting Information**

# Ecological processes drive the effects of host resistance diversity on pathogen spread and susceptible host fitness

Jack Common1\*, David Walker-Sünderhauf2, Stineke van Houte1, Edze R. Westra1\*

1ESI and CEC, Biosciences, University of Exeter, Cornwall Campus, Penryn TR10 9EZ, UK

2European Centre for Environment and Human Health, University of Exeter Medical School, ESI, Cornwall Campus, Penryn TR10 9FE, UK

\* Corresponding author

Email: [jc860@exeter.ac.uk](mailto:jc860@exeter.ac.uk)

Email: [E.R.Westra@exeter.ac.uk](mailto:E.R.Westra@exeter.ac.uk)

**DATA ACCESSIBILITY**: All raw data and R code associated with this this study are publicly available at github.com/JackCommon/Common\_etal\_2019\_host\_diversity

# Supplementary Materials & Methods

## Library of BIMs and escape phages

11 *P. aeruginosa* PA14BIMs that were known to have a single CRISPR2 spacer were selected from the collection of clones used in van Houte *et al.* (2016). The additional 13 BIMs were generated by evolving *P. aeruginosa* PA14 in the presence of DMS3vir. 6ml of M9 minimal media (supplemented with 0.2% glucose; M9m) was inoculated with approximately 106 colony-forming units (cfu) of WT *P. aeruginosa* and 104 plaque-forming units (pfu) of phage in glass vials. After 24hrs, samples from the infection were plated on LB agar. Potential CRISPR clones were identified through phenotypic and PCR analyses as described previously (Westra 2015; van Houte 2016). CRISPR amplicon sequencing (SourceBioscience, UK) confirmed that each spacer carried by a BIM was unique, so that all clones used in downstream experiments carried a different spacer. Spacer sequences were mapped against the DMS3vir genome (Genbank accession: NC\_008717.1) using Geneious v9.1.8 (Kearse *et al.*, 2012) to confirm that spacers did not target overlapping regions of the phage genome. See Table S1 in Supporting Information the spacer sequences of each BIM.

To generate 24 phage clones that could infect each BIM (escape phage), 15ml LB was inoculated with approximately 106 cfu of a single BIM and approximately 106 pfu DMS3vir. We also added approximately 106 of *P. aeruginosa* PA14 *csy3::lacZ* to provide a pool of sensitive hosts on which phage could replicate and hence supply novel escape mutations. Phage extracted from these amplifications were plaque-purified to ensure a monoclonal phage stock. Each escape phage was challenged against the entire BIM library to check for a one-to-one infection match. A successful infection was defined if a clear lysis zone was visible in the top lawn of the target BIM.

*Generating labelled BIMs*

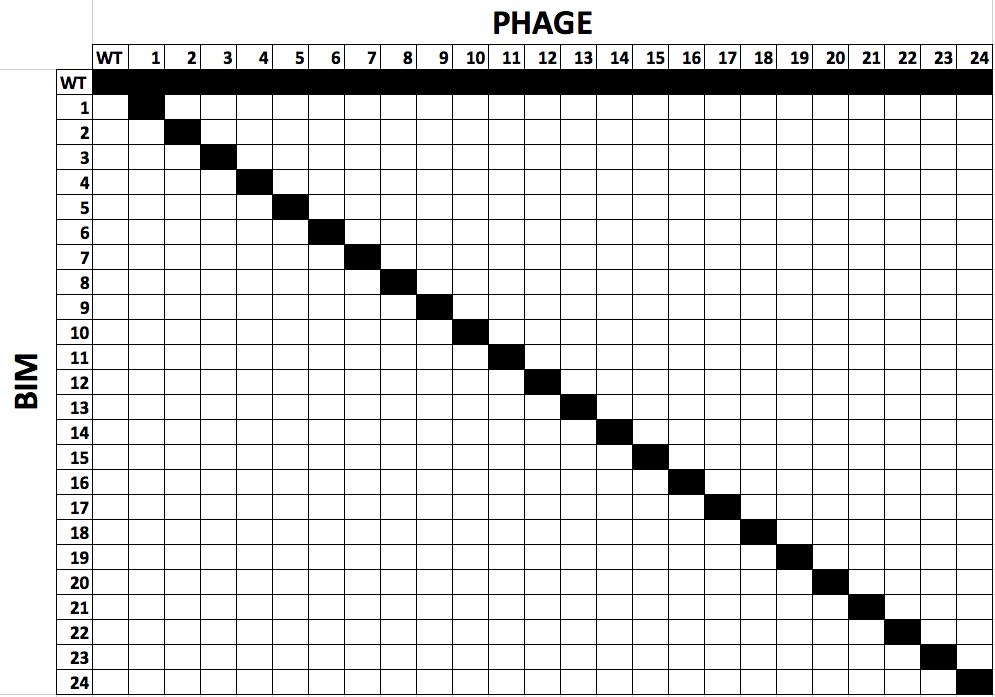
The BIMs chosen for transformation were such that a single clone could be monitored in each of the 3-clone mixtures (that is, BIMs 1, 4, 7, 10, 13, 19, and 22; see Table S1), which enabled us to measure relative frequency and fitness of a labelled BIM through time by performing a blue:white screen when plating on LB agar supplemented with 40g/ml X-gal.

All cloning reactions to generate the labelled BIMs were carried out according to manufacturers’ instructions unless stated otherwise. Restriction enzymes, Antarctic phosphatase, and T4 DNA ligase were purchased from NEB; HF versions were used if available. Strains, primers, and plasmids used for molecular work are outlined in Table S2. We used the synthetic mini-Tn5 transposon vector pBAMD1-6 (Martínez-García *et al.*, 2014) to deliver the *lacZ* gene to target BIMs. pBAMD1-6 is a non-replicative vector in *P. aeruginosa* encoding a Tn5 transposase, which allows for insertion of a gentamicin resistance gene (GmR) as well as any cargo genes into the bacterial chromosome. To introduce *lacZ* as a cargo gene, we amplified it from PA14 *csy3::lacZ* using primers lacZ\_amp\_fw and lacZ\_amp\_rv (Table S2) using Phusion High-Fidelity Polymerase (ThermoFisher). The PCR product was cleaned up (QIAgen PCR cleanup kit) and sub-cloned into pMA-RQ\_Cas (Walker-Sünderhauf, unpublished) using NcoI-HF and KpnI-HF to generate a construct in which *lacZ* gene expression is driven by a constitutive β-lactamase promoter P3 (Genbank accession: J01749, region 4156..4233). Using standard molecular cloning protocols and restriction enzymes HindIII-HF and KpnI-HF, this promoter and the downstream *lacZ* gene was inserted into pBAMD1-6 to generate pBAM1(Gm)\_lacZ. pBAM1(Gm)\_lacZ was transferred into *E. coli* MFD*pir* by electroporation.

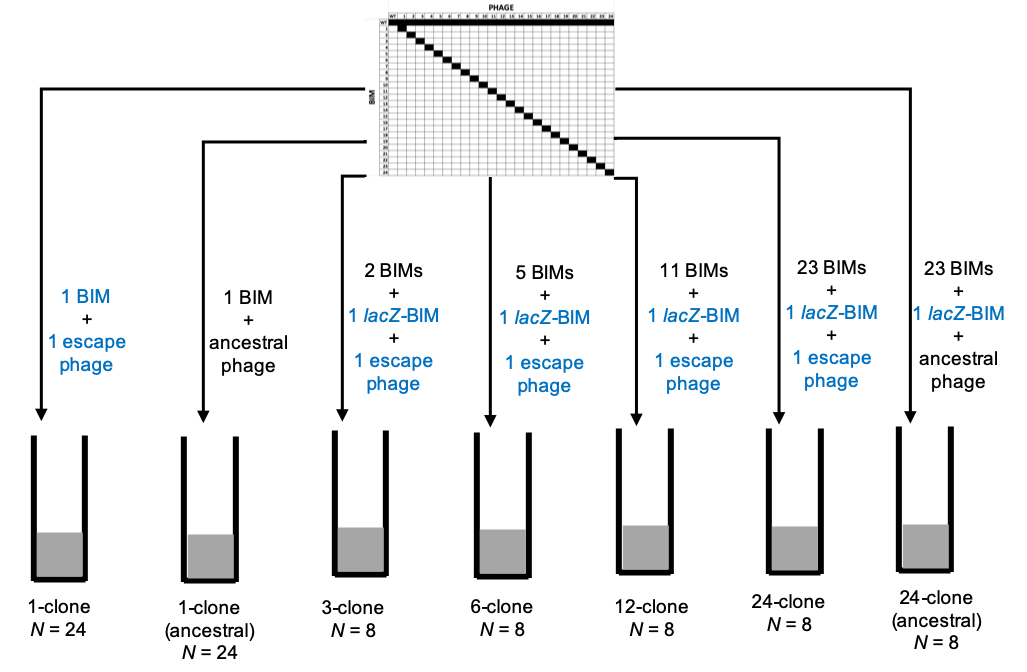
Tn5 insertions of the recipient BIMs were carried out by conjugative pBAM1(Gm)\_lacZ delivery. *E. coli MFD*pir + pBAM1(Gm)\_lacZ was used as donor and grown overnight in 5ml LB + 0.3mM diaminopimelic acid (DAP) + 30 µg/ml gentamicin at 37˚C, 180 rpm. Recipient BIMs were grown overnight in 5ml LB at 37˚C, 180rpm. 10ml of fresh media was inoculated from these overnight cultures, and grown at 37˚C and 250rpm until OD600 ~ 0.6, then pelleted and washed twice in 1x M9 salts, and resuspended in 1ml 1 x M9 salts. 600µl of donors were mixed with 200µl recipients, pelleted, and resuspended to a volume of 100µl. The entire donor-recipient mixture was pipetted onto sterile 0.2µm microfiber glass filters (Whatman) on LB agar + 0.3mM DAP plates and incubated for 2 days at 37˚C. To recover cells, filters were placed into 2.5ml LB and vortexed. 100µl of recovered cells were plated onto LB agar + 30 µg/ml gentamicin + 40µg/ml X-gal + 0.1mM IPTG plates and incubated at 37˚C for 2 days to select for BIMs with Tn5 insertions in their genome (absence of DAP selects against the donor strain).

Because Tn5 inserts at random positions in the *P. aeruginosa* genome, this may affect fitness. We therefore sampled three blue colonies of the transformants and conducted 24hr competition experiments against their untransformed counterpart to verify their fitness was unaffected. The relative fitness of the transformed BIM was calculated as described previously (*W*n = [(fraction strain A at tn) \* (1 – (fraction stain A at t0)) ] / [(fraction strain A at t0) \* (1 – (fraction strain A at tn)])(Westra *et al.*, 2015). If Tn5 insertion disrupted the CRISPR-Cas system, the transformed BIM would regain susceptibility to ancestral DMS3vir. We therefore checked for this by spotting ancestral DMS3vir on a top lawn of the transformed BIM. If no clear lysis zone was visible on the top lawn, we determined that the CRISPR-Cas system was functional.

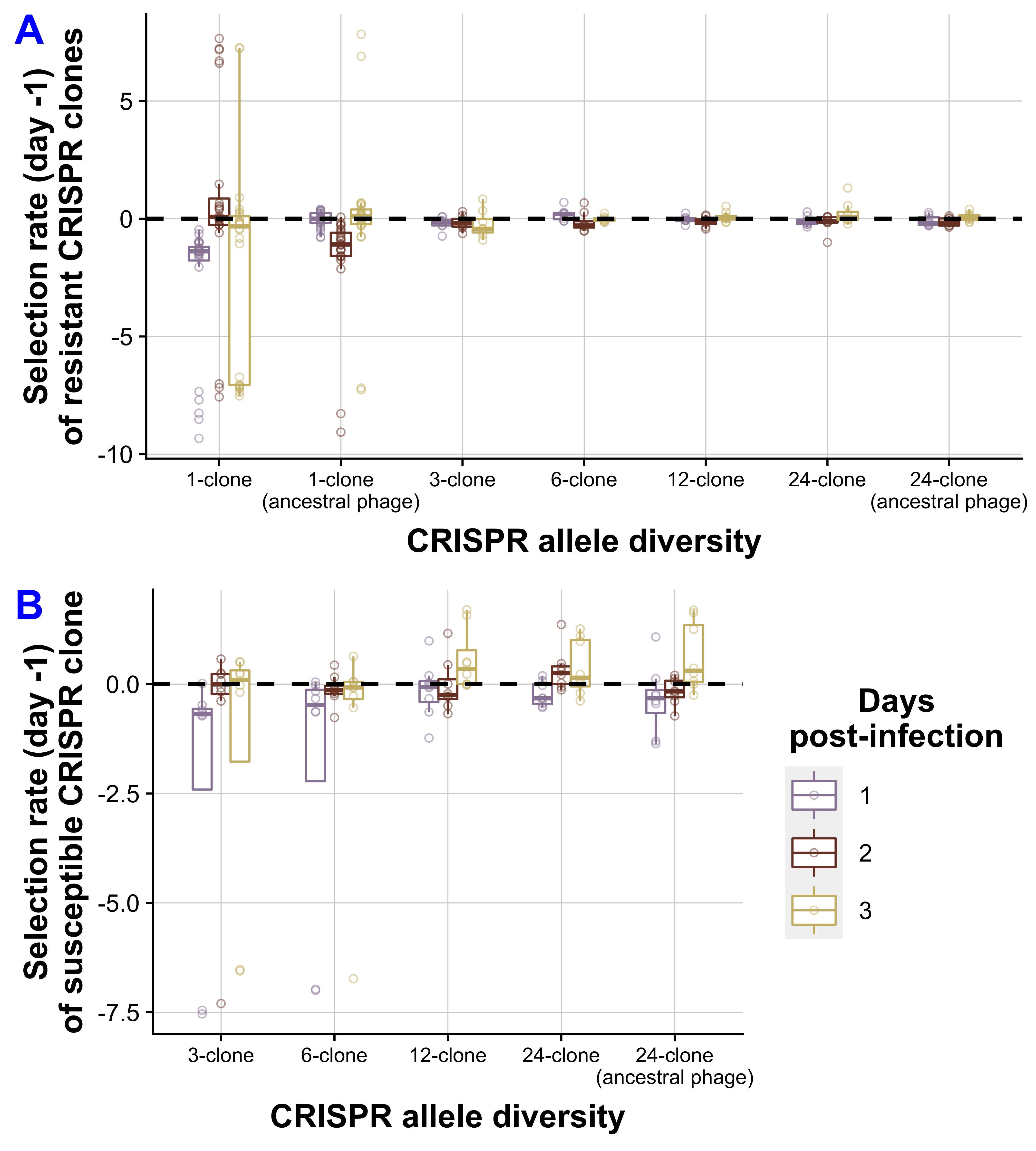
## **Supplementary Figures**



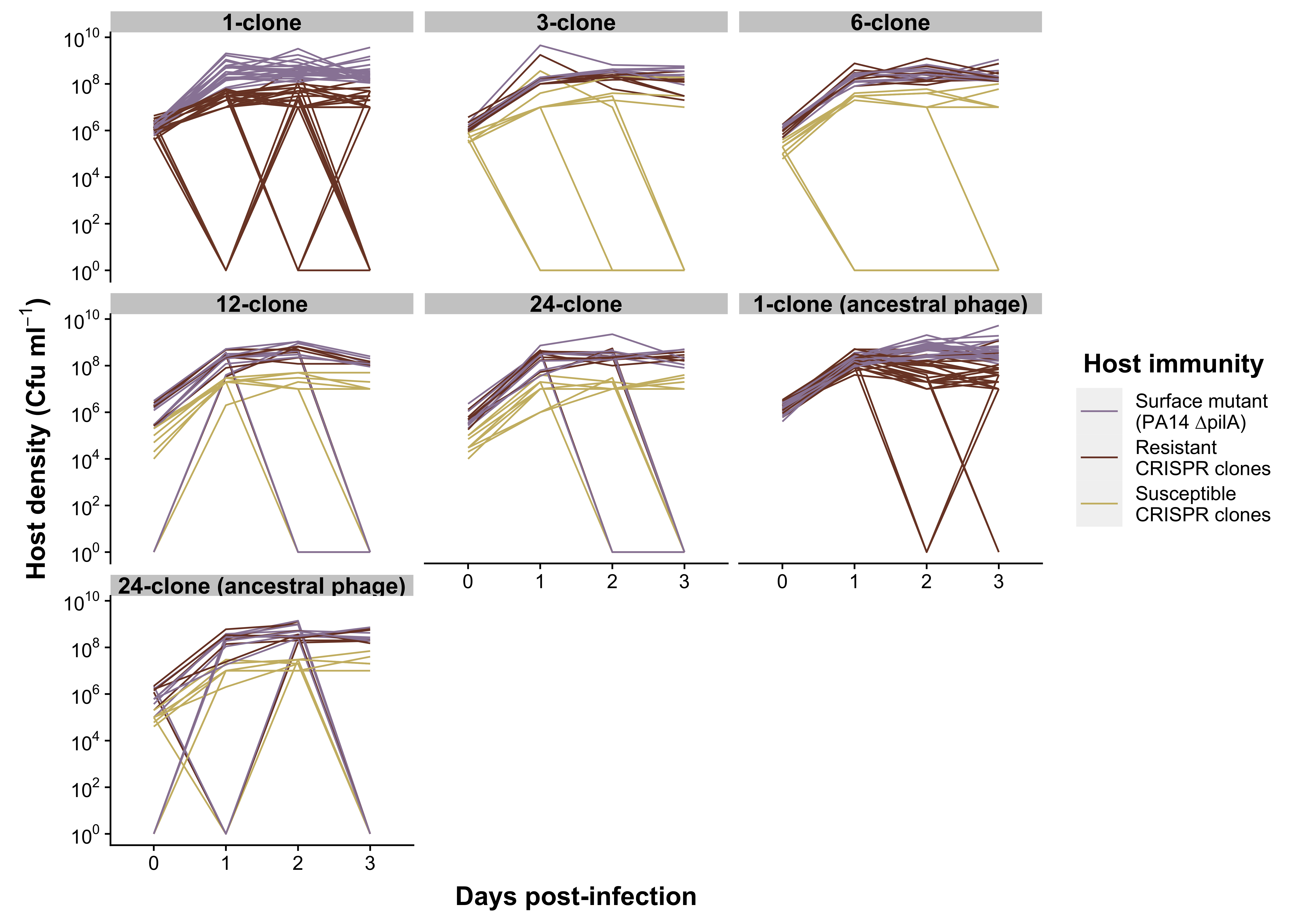
**Figure S1** Infectivity matrix of the library of BIMs and escape phages. The identity of BIMs and escape phage (1-24) are shown in the first row and column, respectively. Black squares represent infectivity, white indicates no infectivity. Infectivity of the wild-type DMS3vir is shown, as well as the infectivity of each escape phage on wild-type *P. aeruginosa* PA14.



**Figure S2** Design of the co-culture diversity experiment. The library of BIMs and escape phage is shown at the top (that is, Figure S1). For each CRISPR allele diversity treatment, the relevant numbers of BIMs and escape phage were taken from the library and used to start the experiment, as shown by the arrows. The number of BIMs is shown next to the arrows. Blue text indicates that the BIM was engineered to carry a *lacZ* reporter gene, and that it was susceptible to the escape phage. Black text indicates that the BIMs were resistant to the phage. The number of replicates (*N*) is given underneath each treatment.



**Figure S3** Selection rate of **A** resistant CRISPR clones relative to a surface mutant (which does not encode CRISPR-based immunity) and **B** susceptible CRISPR clones relative to the resistant CRISPR clones in each CRISPR allele diversity treatment (x-axis) at 1-3 days post-infection (colours). Selection rate is the natural log of the relative change in density of one competitor against another. The dotted line at zero indicates no difference in density change i.e. both are equally fit. Boxplots show the median, 25th and 75th percentile, and the interquartile range. Residuals are shown as points. In **B** the 1-clone treatments, both with infective and ancestral phage, have been excluded because the whole CRISPR population was susceptible and can therefore be found in **A**.



**Figure S4** Population dynamics of the bacterial host population at different levels of CRISPR allele diversity in the host population (panels). Lines show the density expressed as colony-forming units (cfu) ml-1 in individual replicates at each day post-infection (x-axis). Line colours indicate host immunity: *P. aeruginosa* PA14 *∆pilA* surface mutant, which is completely resistant to phage; resistant CRISPR clones from the BIM library, which are resistant phage using the CRISPR-Cas immune system; and susceptible CRISPR clones, which are susceptible to the pre-evolved phage.

# Supplementary Tables

**Table S1** Sequence of spacers in the CRISPR2 locus of each of the 24 bacteriophage-insensitive mutants (BIMs) used in the co-culture experiment. Clones transformed to carry a lacZ reporter gene using pBAM1(Gm)\_lacZ are highlighted in blue.

|  |  |
| --- | --- |
| **BIM** | **Spacer sequence** |
| 1 | ATTTCAGTCCTTCCTGATCGCGTAGAGCCAAG |
| 2 | CATCTTCCCGCTCGATGGCGGTCAGCGTGCGC |
| 3 | CGCGTGAATGGCCCGGCGCTGAGCTGCGCTAT |
| 4 | AAGGGCATCAACCTGGCCGAAGGCGGCGCGCC |
| 5 | CGGTCGAACACGCCCTTATAGCGCTTCAGGCC |
| 6 | GATGTTCATCGCTGCCGGGCAGCGCGACATAC |
| 7 | AAACAGCGTCATGTCCAGGAGCTGCCGCTCGC |
| 8 | ACGGCAAGTTGAGTCTGGCCCTGGATGCTGAC |
| 9 | CCGGAAGTCCCGGCCGGTGTAGACGAGATAAA |
| 10 | GGCTCGACCAGGCGGCCCAGGGCGGCGTCGAT |
| 11 | TCAGGACCCCGACCAGATGGCGGCCGAAATGT |
| 12 | CGCCTGGAGACCCTGAAGGCCAATACCGAAAA |
| 13 | CCGAACGCATANANGGCGCANGGCACAGGGGT |
| 14 | ATGGGGATTCAGAGCTACGGCGATACCGCCCT |
| 15 | GAAATCGGCACCGCCACGAACCACCAGAACCT |
| 16 | GTCCAGCAGGATGCCGGCATCATCAACGAAAT |
| 17 | GGCAACGATCCCCACGAGCGGCTTTGGCACCT |
| 18 | CTCAACTCCGGCGCCGAAGACGTGATTGTCGA |
| 19 | GCGGGATCGCGGAGATAGCAGCTACGCTCGTA |
| 20 | ACTTTCACGACGACCCAGAAGCGTCGGCCGTT |
| 21 | GCGGCAGGAGCGGCAGCGGGCGGCGGCAGTT |
| 22 | GCGATCAGNTGCGGCCAATCCGTGGACTGGGT |
| 23 | GATGGCGTCAAACTCGGCCTCCAGGCGCAGCG |
| 24 | AACCTCGCGCAGTCGTTGTCCAGCGGCATCAT |

**Table S2** Plasmids, Primers, and Strains used for molecular cloning work.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plasmids** | | | | |
| **Plasmid** | **Reference** | | **Accession Number** | **Culture conditions** |
| pBAMD1-6 | (Martinez-Garcia *et al.* 2014) | | KM403115 | 100 μg/mL Ampicillin, 50 μg/mL Gentamicin, 0.3 mM diaminopimelic acid. Needs a *pir* strain to replicate. |
| pBAM1(Gm)\_lacZ | This study | |  |
| **Primers** | | | | |
| **Primer** | **Sequence (5’ 🡪 3’)** | | | **Usage** |
| lacZ\_amp\_fw | TTACCATGGATGATTACGGAT TCACTGGCCGTCGT | | | Amplification of *lacZ* from PA14 *csy3:lacZ*. Adds NcoI and KpnI restriction sites onto amplicon. |
| lacZ\_amp\_rv | CAGGTACCTTATTTTTGACAC CAGACCAACTGGTAATGGT | | |
| **Bacterial Strains** | | | | |
| **Strain** | | **Reference/Supplier** | | **Usage** |
| *Pseudonomas aeruginosa* PA14 *csy3::lacZ* | | Zegans *et al.* 2009 | | Template for *lacZ* amplification. |
| *Escherichia coli* DH5α | | NEB | | Subcloning of *lacZ* behind P3 promoter. |
| *E. coli* CC18λpir | | NEB | | Cloning of promoter + *lacZ* onto pBAM1(Gm) |
| *E. coli* MFD*pir* | | (Ferrieres *et al.* 2010) | | Donor strain for pBAM1(Gm)\_lacZ delivery |
| *P. aeruginosa* PA14 BIMs | | This study; Table S1 | | Recipients for pBAM1(Gm)\_lacZ delivery |

**Supplementary References**

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., . . . Duran, C. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28*(12), 1647-1649.

Martínez-García, E., Aparicio, T., de Lorenzo, V., & Nikel, P. I. (2014). New transposon tools tailored for metabolic engineering of Gram-negative microbial cell factories. *Frontiers in Bioengineering and Biotechnology, 2*, 46.

van Houte, S., Ekroth, A. K. E., Broniewski, J. M., Chabas, H., Ashby, B., Bondy-Denomy, J., . . . Westra, E. R. (2016). The diversity-generating benefits of a prokaryotic adaptive immune system. *Nature, 532*(7599), 385-388. Retrieved from http://dx.doi.org/10.1038/nature17436. doi:10.1038/nature17436

Westra, E. R., van Houte, S., Oyesiku-Blakemore, S., Makin, B., Broniewski, Jenny M., Best, A., . . . Buckling, A. (2015). Parasite Exposure Drives Selective Evolution of Constitutive versus Inducible Defense. *Current Biology, 25*(8), 1043-1049. Retrieved from http://dx.doi.org/10.1016/j.cub.2015.01.065. doi:10.1016/j.cub.2015.01.065

**Table S3** Primers used to amplify and sequence the protospacers of interest of phage that were shown to have undergone host shift (lost infectivity to the original clone and could only infect a new clone) from the phenotypic assay. The first column indicates if the protospacer was the original pre-evolved or the new protospacer, with the identity of the protospacer shown in brackets. The primer sequence and binding direction are shown, and if the primer was used for PCR or sequencing reactions.

|  |  |  |  |
| --- | --- | --- | --- |
| **Phage** | **Sequence (5’- 3’)** | **Bind direction** | **Primer** |
| Original (7) | CCTGGACCTTCGCGCCGGAC | F | PCR |
| GAGGTGAGGTCTTCGCTTTC | R |
| GTCGCACGGAATGTTCAGCGAG | R | Sequencing |
| Original (13) | TCTGGCCAGGCGCTCACAAACAA | F | PCR |
| GAGCGGCTTTGGCACCTGGAAC | R |
| CCAAGTGTCGCTGCCGATCA | R | Sequencing |
| New (10) | AGCTGTCCACTGCGCTGGAC | F | PCR |
| CCGGAACAGATGATCCCGTT | R |
| AATGTCAGCGCGGCGGTTGC | R | Sequencing |
| New (21) | CAGCGGCATCATGGGGCTGTTTG | F | PCR |
| AGGTACTGAAGTTTTTGGAGGG | R |
| CCGCTGCTATCCAGACGGCC | F | Sequencing |

**Table S4** Protospacer sequences of evolved phage clones which showed host shift according to the phenotypic assay from replicate 3 of the 24-clone treatment at 1 day post-infection (dpi). The CRISPR-targeted protospacer and PAM sequences of the ancestral (WT) phage and of the pre-evolved phage are shown. Numbers 1-12 are separate phage isolates. The second column indicates if the protospacer was the original pre-evolved or the new protospacer, with the identity of the protospacer shown in brackets. Protospacer-adjacent motif (PAM) and protospacer sequences are shown separately. SNPs and deletions are highlighted in red.

|  |  |  |  |
| --- | --- | --- | --- |
| **24-clone, replicate 3, 1 dpi** | | | |
| Phage | Protospacer | PAM sequence | Protospacer sequence |
| WT DMSvir | Original (7) | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| Pre-evolved protospacer 7 | **A**G | CGCTCGCCGTCGAGGACCTGTACTGCGACAAC |
| 1 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 2 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 3 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 4 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 5 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 6 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 7 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 8 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 9 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 10 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 11 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| 12 | GG | CGCTCGCCGTCGAGGACCTGTACTGCGACAAA |
| WT DMSvir | New (10) | GG | TAGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| Pre-evolved protospacer 7 | GG | TAGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| Pre-evolved protospacer 10 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 1 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 2 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 3 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 4 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 5 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 6 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 7 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 8 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 9 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 10 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 11 | GG | **C**AGCTGCGGCGGGACCCGGCGGACCAGCTCGG |
| 12 | GG | **C**ANCTGCGGCGGGACCCGGCGGACCAGCTCGG |

**Table S5** Protospacer sequences of evolved phage clones which showed host shift according to the phenotypic assay from replicate 5 of the 24-clone treatment at 2 days post-infection (dpi). The CRISPR-targeted protospacer and PAM sequences of the ancestral (WT) phage and of the pre-evolved phage are shown. Numbers 1-8 are separate phage isolates. The second column indicates if the protospacer was the original pre-evolved or the new protospacer, with the identity of the protospacer shown in brackets. Protospacer-adjacent motif (PAM) and protospacer sequences are shown separately. SNPs and deletions are highlighted in red.

|  |  |  |  |
| --- | --- | --- | --- |
| **24-clone, replicate 5, 2 dpi** | | | |
| Phage | Protospacer | PAM sequence | Protospacer sequence |
| WT DMSvir | Original (13) | GG | TGGGGACACGGGACGCGGTAGATACGCAAGCC |
| Pre-evolved protospacer 13 | G**A** | TGGGGACACGGGACGCGGTAGATACGCAAGCC |
| 1 | GG | TGGGGACACGGGACGCGGTAGATACGCAAGCC |
| 2 | GG | TGGGGACACGGGACGCGGTAGATACGCAAGCC |
| 3 | GG | TGGGGACACGGGACGCGGTAGATACGCAAGCC |
| 4 | GG | TGGGGACACGGGACGCGGTAGATACGCAAGCC |
| 5 | GG | TGGGGACACGGGACGCGGTAGATACGCAAGCC |
| 6 | GG | TGGGGACACGGGACNCGGTAGATACGC**T**AGCC |
| 7 | GG | TGGGGACACGGGACGCGGTAGATACGCAAGCN |
| 8 | GG | TGGGGACACGGGACGNGGTAGATACGCAAGCC |
| WT DMSvir | New (21) | GG | TGCGGCAGGAGCGGCAGCGGGCGGCGGCAGTT |
| Pre-evolved protospacer 13 | GG | TGCGGCAGGAGCGGCAGCGGGCGGCGGCAGTT |
| Pre-evolved protospacer 21 | GG | TGCGGCAG**--------------------**CGGGCGGNGGCAGTT |
| 1 | GG | T**--------------------**GCGGCAGCGGGCGGCGGCAGTT |
| 2 | GG | T**--------------------**GCGGCAGCGGGCGGCGGCAGTT |
| 3 | GG | T**--------------------**GCGGCAGCGGGCGGCGGCAGTT |
| 4 | GG | T**--------------------**GCGGCAGCGGGCGGCGGCAGTT |
| 5 | GG | T**--------------------**GCGGCAGCGGGCGGCGGCAGTT |
| 6 | GG | T**--------------------**GCGGCAGCGGGCGGCGGCAGTT |
| 7 | GG | T**--------------------**GCGGCAGCGGGCGGCGGCAGTT |
| 8 | GG | T**--------------------**GCGGCAGCGGGCGGCGGCAGTT |