High-throughput mapping of host factors involved in ssRNA bacteriophage infecting pathways

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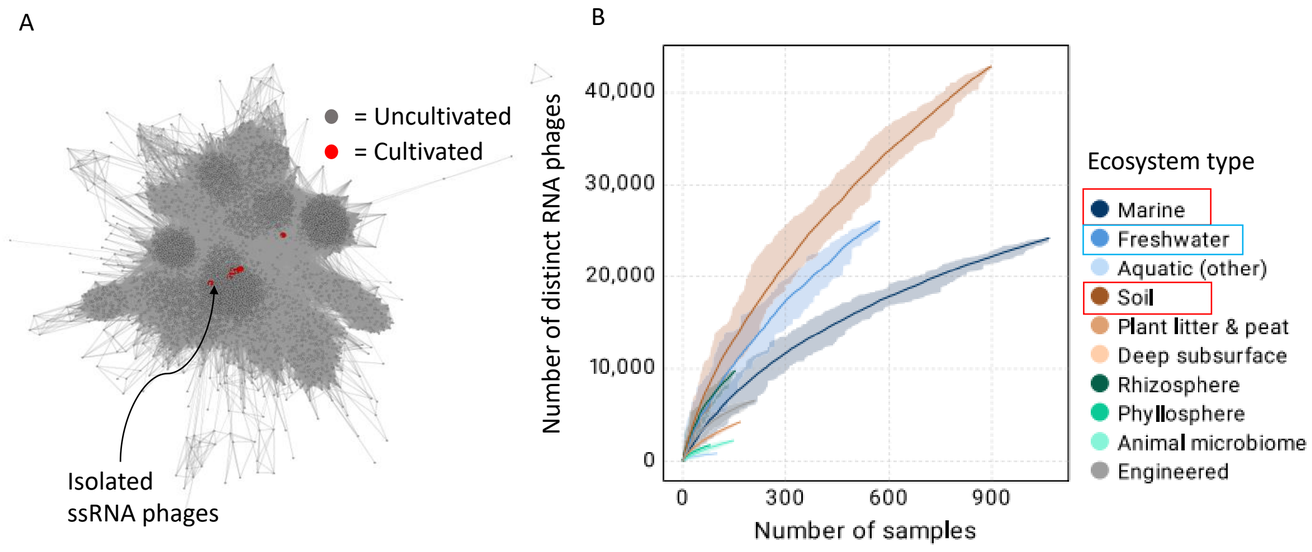
Abstract

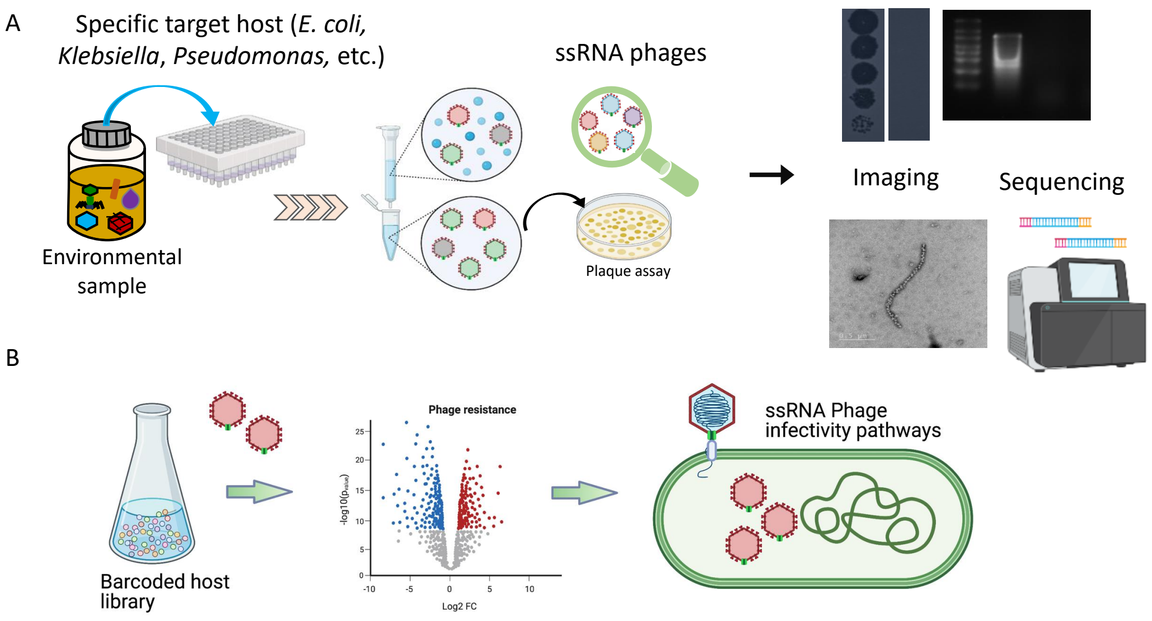
Recent studies on metatranscriptome revealed the actual ubiquity and diversity of non-dsDNA viral particles are largely understudied, and in particular single strand RNA (ssRNA) phages are especially prominent in soil ecosystems (1-6). Despite their importance, shotgun RNA sequencing efforts only provide limited and partial information about these novel ssRNA phages, and especially fail to associate these phages with their host bacteria, infectivity pathways, mechanism of host cell take-over and host lysis. For example, though ssRNA phages have been known to bind conjugative pili elements for infectivity, the knowledge and genetic basis of their infection and resistance has been limited to a couple of canonical phages (7). These early studies primarily isolated phage-resistant host mutants and characterized them using classical genetic approaches. With the recent discovery of tens of thousands of novel ssRNA phages there is a need for high-throughput technologies to characterize ssRNA phage-host interactions. Here we adopt recently developed two high-throughput genetic technologies (8), Random barcode transposon site sequencing (RB-TnSeq) and Dual-barcoded shotgun expression library sequencing (Dub-seq) to a model *E. coli* strain for discovering host factors, and gene dosage barriers crucial in ssRNA phage infection and bacterial resistance. We resourced a collection of ssRNA phages including three model ssRNA phages, MS2, Fr, and Qβ to map genetic landscape important in phage infection. Using genome-wide loss-of-function (LOF) and gain-of-function (GOF) genetic technologies, we are able to confirm the importance of conjugative pili elements as well as uncover other host factors playing an important role in ssRNA phage infective pathways. To the best of our knowledge, this is the first such report to systematically characterize non-dsDNA phage-host interactions and opens up an avenue to extend it to the other *E. coli* strains and non-*E. coli* phages.

RNA phages as under-estimated players in ecosystem



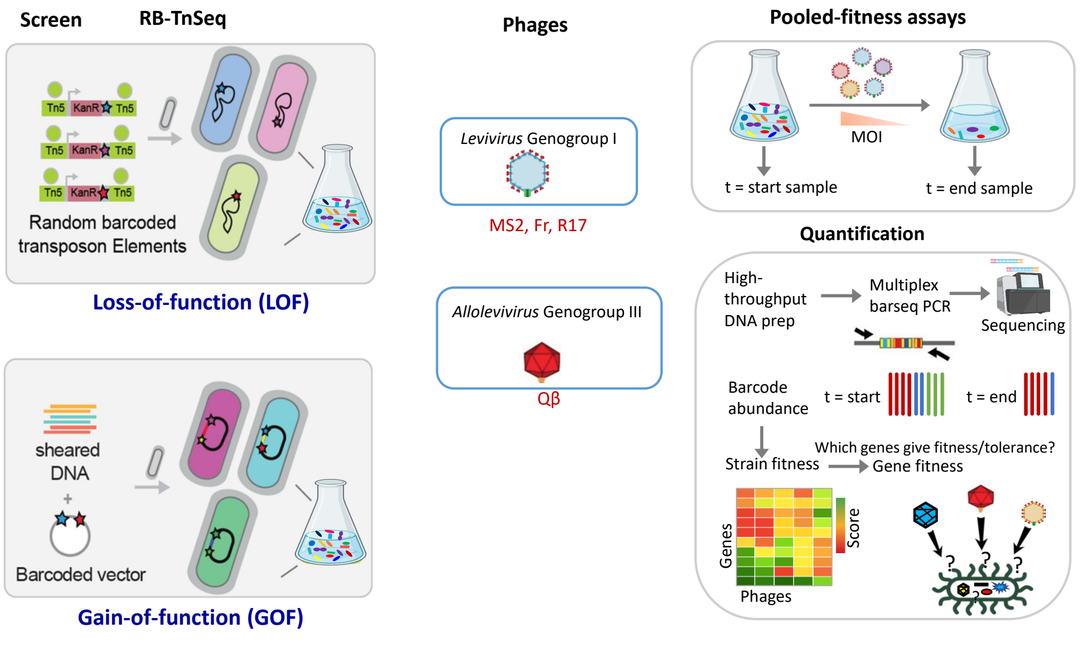
Ubiquity and diversity of ssRNA phages in the RNA Virus Metatranscriptome (RVMT) study (6). (A) Preliminary analysis of global genome diversity of Levi-like ssRNA phages from publicly available metatranscriptomes. The genome diversity represented as a network indicates that the few ssRNA phages isolated so far are highlighted as red dots, while gray genomes are all uncultivated. (B) Rarefaction curves of leviviruses distinguished by different colors based on the environment types.

Key goals: Non-model Phage isolations and study of phage-host interactions



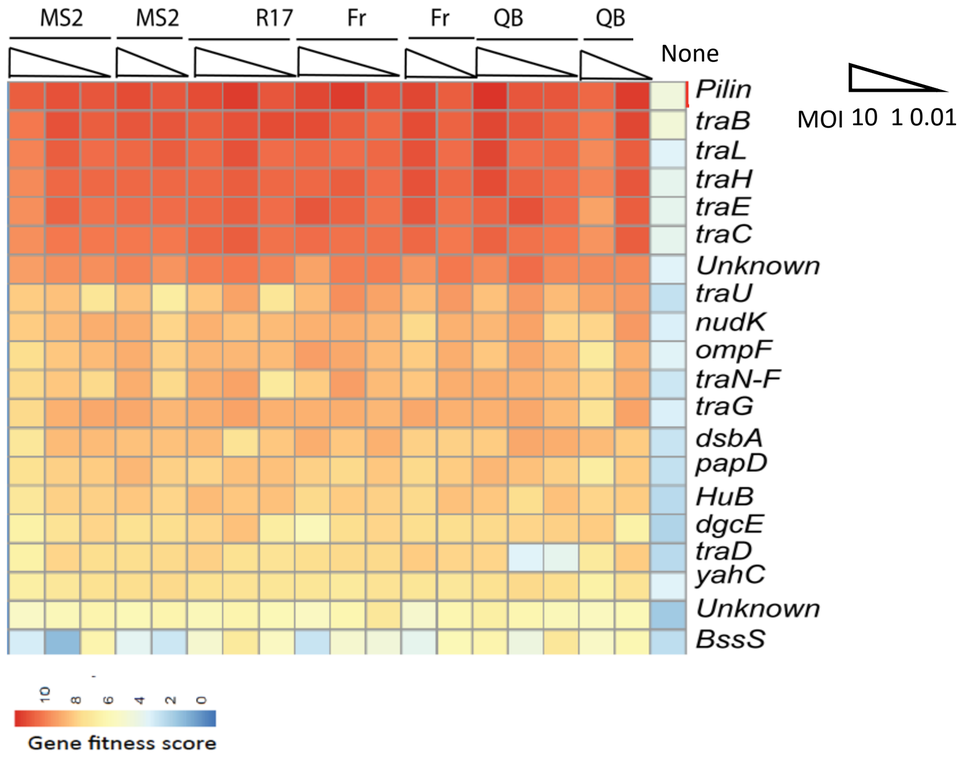
Systematic study of ssRNA-host interactions (A) Schematics of ssRNA phage enrichment and isolations. A key goal of this project is to develop a standardized method to isolate non-*E. coli* phages (ongoing work). (B) Applying high-throughput genetic screens to uncover host factors important in ssRNA phage infection and study ssRNA phage infection modes and resistance phenotypes (current poster).

High-throughput genetic screening platforms for phage-host interaction



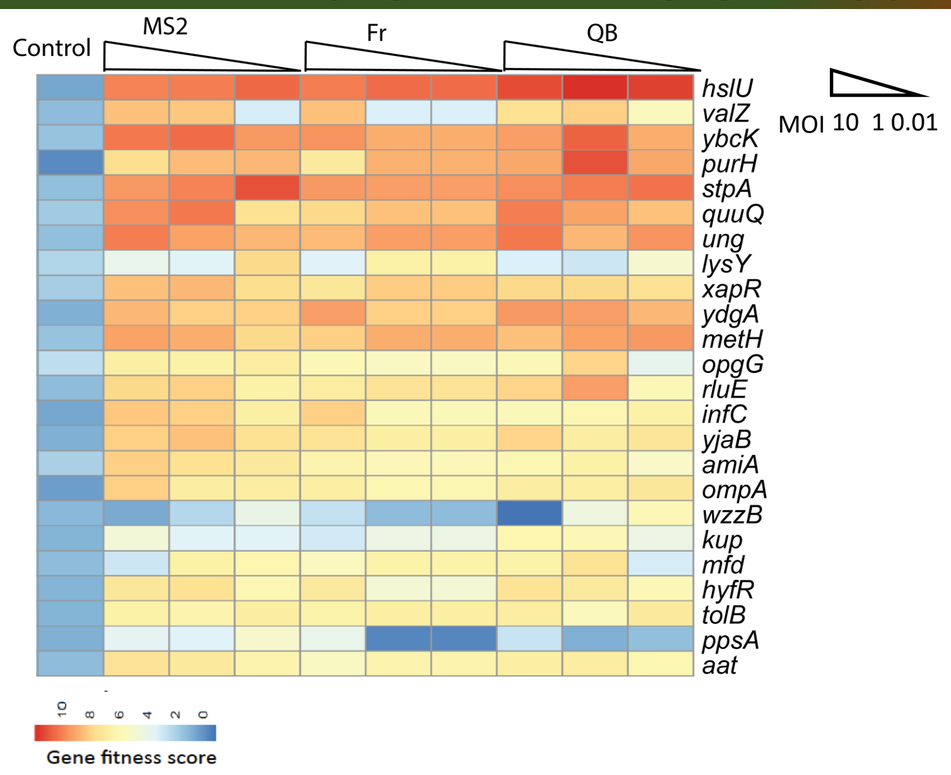
Overview of high-throughput RB-TnSeq and Dub-seq genome-wide screens. Previously developed barcoded LOF technology (RB-TnSeq) and a GOF technology (Dub-seq) in *E. coli* strain were used to screen for host factors important in phage infection and resistance (8). We analyzed 4 diverse ssRNA phages (MS2, Fr, R17 and Qβ) for RB-TnSeq and 3 ssRNA (MS2, Fr and Qβ) phages for Dub-seq, belonging to genogroup I and III *Leviviruses* and performed pooled fitness screens in planktonic format. Disruption or overexpression of certain genes provide fitness to host in the presence of phages, and we monitor these changes by quantifying the abundance of the DNA barcode associated with each strain. The individual strain abundances are then converted to gene fitness scores (normalized log2 change in the abundance of mutants in that gene). Dub-seq, dual-barcoded shotgun expression library sequencing; MOI, multiplicity of infection; RB-TnSeq, random barcode transposon site sequencing.

RB-TnSeq identifies known receptors and host factors for all 4 ssRNA phages



Heatmap of *E. coli* C3000 RB-TnSeq library data for 4 ssRNA phages at different MOI. Top genes with high-confidence effects and a gene fitness score of ≥ 6.1 in at least one phage assay are shown. The mapped protein names and functions that encode known receptors or predicted proteins for unknown genes are given on the right side of the chart. All ssRNA phages showed high fitness score for pilin related phage receptors including some new or hypothetical proteins. Competitive growth experiment was performed using two biological replicate (n = 2) including 2 technical replicate for each phage with RB-TnSeq library.

Mapping genetic determinants of phage resistance using high-throughput GOF



Genome-wide Dub-seq screens for 3 ssRNA phages at different MOIs. Overexpression or higher dosage of these genes interferes with the phage infectivity cycle and imparts fitness benefits to the host. Only genes with high-confidence effects and gene fitness score of ≥ 2.3 in at least one phage assay are shown.

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Acknowledgments

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