The Time is Now for Bacteriophage Research

Sequencing Approach Enables More In-depth Phageome Investigation

Bacteriophages (phages) are the most abundant and diverse biological entities on earth, approximately 10 to 100 times the number of bacteria. Phages are viruses that infect bacteria. They infect susceptible bacterial hosts and contribute to their reproduction (short-term) or evolution (long-term) by multiplying within host cells or integrating the phage genome into the bacterial genome through transduction as prophages. The majority of isolated phages (>95%) found to date consist of a linear double-stranded DNA (dsDNA) genome packaged in a protein capsid, and a small percentage of phages found have a tailless capsid with a dsDNA or single-stranded DNA (ssDNA) or RNA genome.

Sequencing in Phage Research

Sequencing technologies, genomics, and bioinformatics are rapid and effective tools to study phage genomics. In addition, further analysis, including transcription, translation, regulation, and modification, are also emerging techniques that are extremely helpful in exploring the life cycle, specificity, lytic ability, and lysogenicity of phages. Today, a large amount of genomic data has been integrated after being generated at high speed, which provides a strong foundation for the use of phages in personalized medicines and scientific research.

Phage Whole Genome Sequencing

An extensive amount of metagenomic data indicates that phages are the most diverse yet still not accurately identified components of the human micro-ecosystem. Whole-genome sequencing and functional gene annotation of individual phages can help to explore the genetic information and functional characteristics, thus revealing the great potential of phages in practical applications, e.g., environmental remediation and disease treatment. Therefore, whole genome sequencing of phages helps gain insight into phages and their interactions with the host or environment and may provide a scientific basis for phage classification to study their co-evolutionary history with hosts.

The genome of the phage is sequenced as a single isolated phage using the short-read or long-read sequencing techniques. Meanwhile, next-generation sequencing technologies are the most commonly used sequencing solutions for phage genomes, achieving high-throughput outcomes. Most phages obtained so far were unique and no replicate was found in existing databases. The use of long-read sequencing technologies such as Oxford Nanopore Technology (ONT) and PacBio SMRT offers additional advantages, including sufficient identification of DNA modifications, fast turnaround times, *etc.*, to obtain complete genome directly, reducing workflow for assembly and bioinformatic analysis.

Metagenomics

With continuous advancements, including the introduction of both short-read and long-read sequencing, metagenomic sequencing has overcome the inefficiency and insufficient sequencing depth of the traditional shotgun method to an optimum strategy for research. In turn, metagenomic sequencing allows for a better understanding of non-culturable microorganisms, as well as community structures and diversities.

Although the genome of a bacterium or a virus is very small, obtaining the whole genome of a single phage is relatively easy. The environment in which phage exists may be an ecology of thousands of bacteria and microorganisms together. Whether an external microbiome in soil or air or the gut microbiome of the body, phage exists as a concept of the metagenome.

The metagenomic strategy allows the sequencing and study of DNA extracted directly from environmental samples. The majority of phage genomes discovered by this approach are unknown, exposing an overwhelming diversity of phage genomes to current research. Meanwhile, viral metagenomic focus on the exploration of population structure, gene functional activity, viral-host collaborative relationship, and linkages with the environment, providing a strong basis for understanding the diversity and abundance of bacteriophages.

Furthermore, due to the great potential of phages in disease treatment, metagenomic sequencing of phages isolated from the natural environment not only clarify the safety of potential therapeutics but also reveal hidden carriages for drug resistance genes through genomic sequence analysis.

Applications

The highly specific interaction between phages and their hosts allows them to recognize and lyse target bacteria in a way that is safe for humans and animals and avoid ecological dysregulation. Currently, most phage research is focused on prevalent or model strains. Thus, the continuous improvement of genetic information for phageome will not only expand insights into the biosphere but also facilitate the translation of phage biology discoveries into valuable tools.

Gut Phageome Research

In recent years, the human phageome, especially the intestinal phageome, is emerging as a hot topic in microecological research. The resident phagosome group in the gut has many beneficial effects on human health and may even help the body to defend against pathogens. Scientists have proposed that an increased rate of prophage-induced activation or an increased proportion of lytic phages in the gut would lead to a dysregulated phage population and that this dysregulation is associated with many diseases. Studies have also identified that some phages can be used as biomarkers or targets to study digestive system diseases, such as enteritis and intestinal cancer. Thus, elucidating the structure and function of the phage community in the gut microbiota is essential for a more systematic understanding of the human microbiome and its potential applications to human health-related research or industry.

Phage Therapy

Since antibacterial drugs have broad-spectrum antibacterial ability and can kill other non-pathogenic bacteria in the body in addition to pathogenic bacteria, long-term use of antibacterial drugs can cause dysbiosis in the body and may also cause opportunistic infections. Phages are specific to infect host bacteria, which is said that they could infect specific pathogenic bacteria but not normal flora of the body. Therefore, phage therapy is an attractive alternative therapy that may stop superbug infections. The safety of phage therapy has been recognized by numerous studies, which have shown not to cause significant side effects. Meanwhile, genomic data from various phages and bacterial strains can be used as a basis for the rapid development of phage therapy-related research, including the study of targeting and regulation of microflora by phages and their intervention in infectious and non-infectious diseases. Nevertheless, genes encoding antibacterial enzymes can also be identified for potential drug development against drug-resistant bacteria.

Workflow of Phage Genome Sequencing

Step 1: Isolation of DNA/RNA from purified phage samples or nucleic acids directly from samples (for metagenome sequencing).

Step 2: Preparation of genomic libraries
The choice of library preparation method will be based on several factors, including the amount of input genomic DNA (especially for long-read sequencing), the number of genomes to be sequenced, and the estimated cost.

Step 3: Sequencing
Illumina short-read sequencing or Nanopore long-read sequencing.

Step 4: Genome assembly and annotation with our in-house analysis software suite
Quality control, genome assembly, assembly validation, genome annotation.

Step 5: Bioinformatics analysis
Strategies include phylogenetic tree construction, phylogenetic analysis, comparative genomic analysis, phage morphology identification, one-step growth curve, phage thermal and pH stability analysis, phage adsorption and lysis analysis, host range identification analysis, *etc.*

CD Genomics, an advanced sequencing service provider equipped with extensive technologies and knowledge in phageome, is now ready to provide comprehensive Phage Genome Sequencing and Metagenomics Services. Based on short-read and long-read sequencing platforms, as well as our in-house analysis software suite, we're capable of facilitating your phageome research from high-throughput sequencing files, thus sufficiently achieving genome assembly, annotation, and additional analysis.

Contact CD Genomics for more inspiration and service content.

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