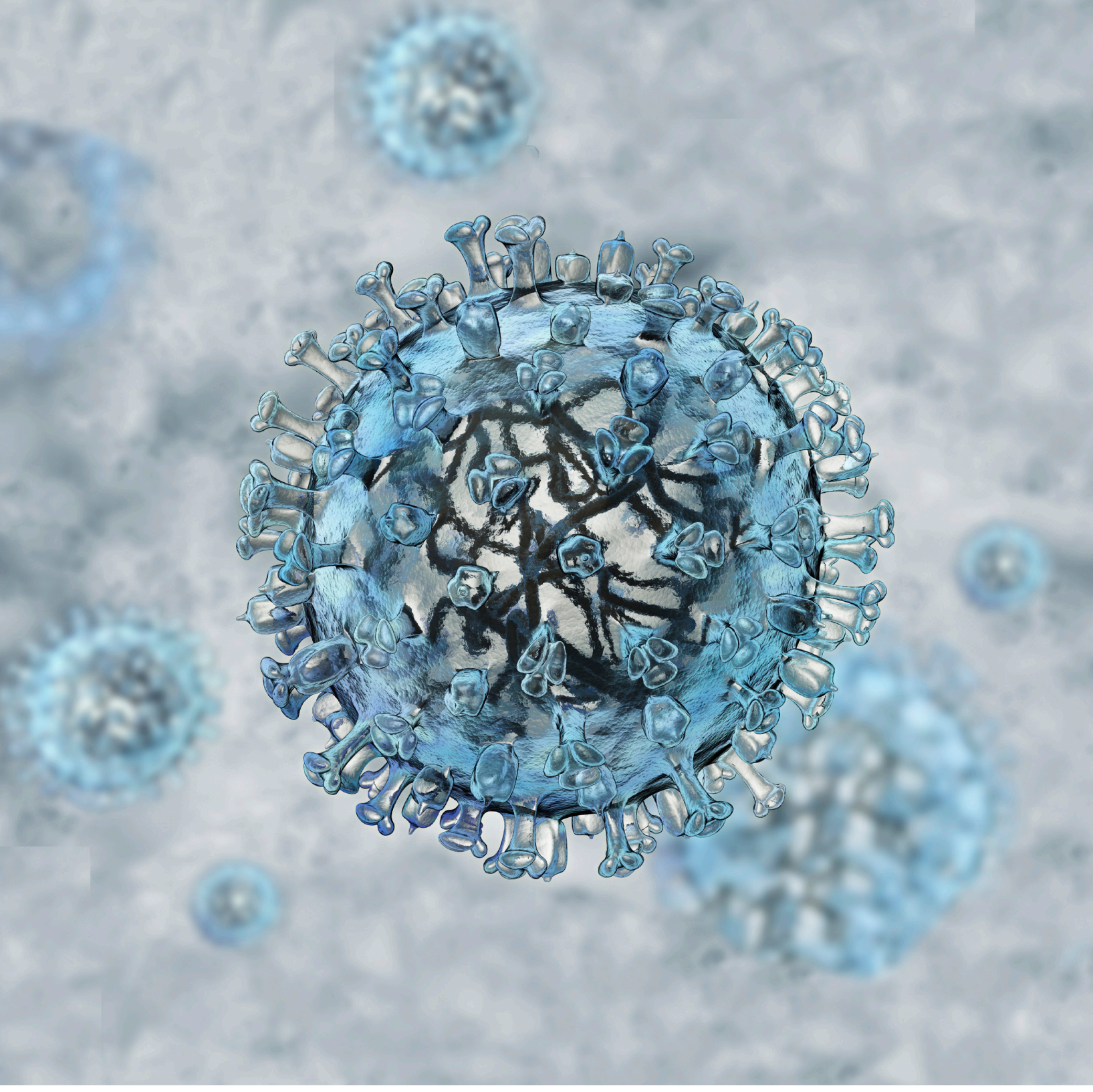

Viral Detection and Research

A review of publications featuring Illumina® Technology

TABLE OF CONTENTS

INTRODUCTION

Next-generation sequencing has developed into a powerful tool that can be used to detect, identify and quantify novel viruses in one step[1]. It is proving to be a sensitive method for detecting putative infectious agents associated with human tissues and viral transcripts can be detected at frequencies lower than 1 in 1,000,000[2]. One of the fortunate consequences of deep sequencing is the coincidental sequencing of viral DNA or RNA, which has led to the discovery of an increasing number of new viruses[3]. This comes at a time when the globalization of travel and trade, as well as climate change and its effects on vector distribution, are facilitating the emergence and reemergence of zoonoses[4].

…there are a minimum of 320,000 mammalian viruses awaiting discovery. Anthony S. J. et al. 2013

Reviews

Anthony S. J., Epstein J. H., Murray K. A., Navarrete-Macias I., Zambrana-Torrelio C. M., et al. (2013) A strategy to estimate unknown viral diversity in mammals. MBio 4:

Chiu C. Y. (2013) Viral pathogen discovery. Curr Opin Microbiol 16: 468-478

Colson P., Fancello L., Gimenez G., Armougom F., Desnues C., et al. (2013) Evidence of the megavirome in humans. J Clin Virol 57: 191-200

Lipkin W. I. and Firth C. (2013) Viral surveillance and discovery. Curr Opin Virol 3: 199-204

Lipkin W. I. (2013) The changing face of pathogen discovery and surveillance. Nat Rev Microbiol 11: 133-141

Malboeuf C. M., Yang X., Charlebois P., Qu J., Berlin A. M., et al. (2013) Complete viral RNA genome sequencing of ultra-low copy samples by sequence-independent amplification. Nucleic Acids Res 41: e13

Mokili J. L., Rohwer F. and Dutilh B. E. (2012) Metagenomics and future perspectives in virus discovery. Curr Opin Virol 2: 63-77

Wylie K. M., Weinstock G. M. and Storch G. A. (2013) Virome genomics: a tool for defining the human virome. Curr Opin Microbiol 16: 479-484

[1] Dunowska M., Biggs P. J., Zheng T. and Perrott M. R. (2012) Identification of a novel nidovirus associated with a neurological disease of the Australian brushtail possum (Trichosurus vulpecula). Vet Microbiol 156: 418-424

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Examples of Viral Pathogens Identified Using Illumina Technology[5]

Name	Technology	Disease Association	Ref.
2009 pandemic influenza A(H1N1)	Genome Analyzer$_{IIx}$	Febrile illness	[6] [7]
TMAdV (titi monkey adenovirus)	Genome Analyzer$_{IIx,}$ 73 bp paired-end reads	Pneumonia (titi monkeys)	[8]
BASV (Bas-Congo virus), a rhabdovirus	HiSeq 2000	Acute hemorrhagic fever	[9]
MWPyV/HPy10/MXPyV (MW polyomavirus)	HiSeq 2000 75 bp paired-end reads	Diarrhea	[10]
HPyV9 (human polyomavirus 9)	HiSeq 2000 100 bp paired-end reads	Diarrhea	[11]
Human enterovirus 109	Genome Analyzer$_{II}$	Acute respiratory illness	[12]
TDAV (Theiler's disease-associated virus), a novel pegivirus	HiSeq 2000 100 bp paired-end reads	Hepatitis (horses)	[13]
Canine bocavirus 3	MiSeq	Hemorrhagic diarrhea and vasculitis (dog)	[14]
Snake arenaviruses	HiSeq 100 bp paired-end reads	Inclusion body disease (snakes)	[15]
SAdV-C (simian adenovirus C)	HiSeq 2000 100 bp paired-end reads	Pneumonia (baboons) Acute respiratory illness (humans)	[16]
Chiropteran poxvirus and a novel adenovirus	Genome Analyzer$_{II}$ for 76bp paired-end reads	Asymptomatic carriers (bats)	[17]
A novel nidovirus, most closely related to the *Arteriviridae*	Genome Analyzer$_{IIx}$	Fatal neurological disease (Australian possum)	[18]
Novel hepacivirus, guereza hepacivirus	MiSeq with Nextera DNA sample preparation kit	Asymptomatic carriers, black-and-white colobus (Colobus guereza)	[19]
A novel gamma-2 herpesvirus	Genome Analyzer$_{IIx}$	Spontaneous Inflammatory Demyelinating Disease (Japanese macaque)	[20]

[5] Chiu C. Y. (2013) Viral pathogen discovery. Curr Opin Microbiol 16: 468-478

[6] Greninger A. L., Chen E. C., Sittler T., Scheinerman A., Roubinian N., et al. (2010) A metagenomic analysis of pandemic influenza A (2009 H1N1) infection in patients from North America. PLoS ONE 5: e13381

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[12] Yozwiak N. L., Skewes-Cox P., Gordon A., Saborio S., Kuan G., et al. (2010) Human enterovirus 109: a novel interspecies recombinant enterovirus isolated from a case of acute pediatric respiratory illness in Nicaragua. J Virol 84: 9047-9058

[13] Chandriani S., Skewes-Cox P., Zhong W., Ganem D. E., Divers T. J., et al. (2013) Identification of a previously undescribed divergent virus from the Flaviviridae family in an outbreak of equine serum hepatitis. Proc Natl Acad Sci U S A 110: E1407-1415

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[17] Baker K. S., Leggett R. M., Bexfield N. H., Alston M., Daly G., et al. (2013) Metagenomic study of the viruses of African straw-coloured fruit bats: detection of a chiropteran poxvirus and isolation of a novel adenovirus. Virology 441: 95-106

[18] Dunowska M., Biggs P. J., Zheng T. and Perrott M. R. (2012) Identification of a novel nidovirus associated with a neurological disease of the Australian brushtail possum (Trichosurus vulpecula). Vet Microbiol 156: 418-424

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[20] Estep R. D., Hansen S. G., Rogers K. S., Axthelm M. K. and Wong S. W. (2013) Genomic characterization of Japanese macaque rhadinovirus, a novel herpesvirus isolated from a nonhuman primate with a spontaneous inflammatory demyelinating disease. J Virol 87: 512-523

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Baker K. S., Leggett R. M., Bexfield N. H., Alston M., Daly G., et al. (2013) Metagenomic study of the viruses of African straw-coloured fruit bats: detection of a chiropteran poxvirus and isolation of a novel adenovirus. Virology 441: 95-106

Emerging viruses—such as SARS coronavirus, hantaviruses and henipaviruses—have wildlife reservoirs. To investigate the virus load on a bat species living close to human habitat, the authors isolated and sequenced virus DNA from *Eidolon helvum*. A great abundance and diversity of novel viruses were found, including novel herpes and papillomaviruses and a novel chiropteran poxvirus. The display of a variety of mammalian viruses makes the bat species a potential reservoir of viruses that may be a public health threat. This study demonstrates the ability of next-generation sequencing to detect novel viruses.
Illumina technology: Genome Analyzer$_{II}$ for 76 bp paired-end reads

Flaherty P., Natsoulis G., Muralidharan O., Winters M., Buenrostro J., et al. (2012) Ultrasensitive detection of rare mutations using next-generation targeted resequencing. Nucleic Acids Res 40: e2

The authors demonstrate that they can robustly detect mutations at 0.1% fractional representation. This represents accurate detection of one mutant per every 1,000 wild-type alleles. The method for detecting rare variants compares the baseline error rate from multiple reference replicates to the sample error rate at each position. To demonstrate the utility of the method, they analyzed nine clinical samples of H1N1 influenza A and detected an oseltamivir (antiviral therapy) resistance mutation in the H1N1 neuraminidase gene in 0.18% of the samples.
Illumina Technology: Genome Analyzer$_{IIx}$

Han Y., Zhang Y., Mei Y., Wang Y., Liu T., et al. (2013) Analysis of hepatitis B virus genotyping and drug resistance gene mutations based on massively parallel sequencing. J Virol Methods 193: 341-347

Total hepatitis B virus (HBV) DNA from 395 patients treated with single or multiple antibiotics was sequenced using the HiSeq2000 sequencer. The experiment was repeated three times and the results demonstrated the high reproducibility of the HiSeq platform. The results were also validated using PCR sequencing. The authors conclude the HiSeq system has high sensitivity, high fidelity, high throughput and automation, making it a useful method for HBV testing and genotyping.
Illumina Techology: HiSeq 2000

Law J., Jovel J., Patterson J., Ford G., O'Keefe S., et al. (2013) Identification of hepatotropic viruses from plasma using deep sequencing: a next generation diagnostic tool. PLoS ONE 8: e60595

This study presents a sequencing assay for the reliable identification of viruses in plasma. The protocol enriches viral particles from plasma filtrates with subsequent creation of RNA and DNA libraries for sequencing. The assay was tested using plasma from patients with chronic hepatitis B, chronic hepatitis C and autoimmune hepatitis. Patients without liver disease constituted the control group. Hepatitis viruses were readily detected at high coverage in hepatitis patients, and only a limited number of sequences resembling other viruses were found.
Illumina Technology: Genome Analyzer$_{IIx}$

Fancello L., Raoult D. and Desnues C. (2012) Computational tools for viral metagenomics and their application in clinical research. Virology 434: 162-174

Kato S. E., Chahal J. S. and Flint S. J. (2012) Reduced infectivity of adenovirus type 5 particles and degradation of entering viral genomes associated with incomplete processing of the preterminal protein. J Virol 86: 13554-13565

Killip M. J., Young D. F., Gatherer D., Ross C. S., Short J. A., et al. (2013) Deep sequencing analysis of defective genomes of parainfluenza virus 5 and their role in interferon induction. J Virol 87: 4798-4807

Koh Y., Wu X., Ferris A. L., Matreyek K. A., Smith S. J., et al. (2013) Differential effects of human immunodeficiency virus type 1 capsid and cellular factors nucleoporin 153 and LEDGF/p75 on the efficiency and specificity of viral DNA integration. J Virol 87: 648-658

Santini S., Jeudy S., Bartoli J., Poirot O., Lescot M., et al. (2013) Genome of Phaeocystis globosa virus PgV-16T highlights the common ancestry of the largest known DNA viruses infecting eukaryotes. Proc Natl Acad Sci U S A 110: 10800-10805

DNA VIRUSES

Routine sequencing of DNA viruses has produced a large number of viral genomes that highlight the remarkable variability of viruses. The differences between the genomes of laboratory strains and clinical isolates of the same virus can be substantial, underscoring the need to routinely sequence clinical isolates[21].

…60–99% of the sequences generated in different viral metagenomic studies are not homologous to known viruses. Mokili et al. 2012

References

Chiu C. Y., Yagi S., Lu X., Yu G., Chen E. C., et al. (2013) A novel adenovirus species associated with an acute respiratory outbreak in a baboon colony and evidence of coincident human infection. MBio 4: e00084

Sequencing provides a sensitive and highly informative diagnostic tool for analyzing outbreaks of infectious diseases. In 1997 captive baboons at a research facility in Texas suffered an outbreak of acute respiratory illness. Using clinical samples from one sick baboon and three asymptomatic baboons, whole-genome-sequencing revealed a novel adenovirus species. The specificity and resolution of Illumina sequencing allowed the tracing of viral origins to a recombinant, nonpathogenic viral strain and another unknown adenovirus. This comprehensive study includes a comparison of adenovirus known from other vertebrate hosts, including human.
Illumina technology: HiSeq 2000 for 100 bp paired-end sequencing

Conway C., Chalkley R., High A., Maclennan K., Berri S., et al. (2012) Next-generation sequencing for simultaneous determination of human papillomavirus load, subtype, and associated genomic copy number changes in tumors. J Mol Diagn 14: 104-111

This study uses next-generation sequencing to investigate viral infection in 44 head and neck tumor types from formalin-fixed paraffin-embedded (FFPE) samples. The authors were able to detect human papillomavirus (HPV) subtypes that would not have been detected by traditional methods. They then used eight cell lines to show that this approach could be applied to various tumors and viruses.
Illumina technology: Genome Analyzer with 76 bp reads

Colson P., Fancello L., Gimenez G., Armougom F., Desnues C., et al. (2013) Evidence of the megavirome in humans. J Clin Virol 57: 191-200

Minot S., Grunberg S., Wu G. D., Lewis J. D. and Bushman F. D. (2012) Hypervariable loci in the human gut virome. Proc Natl Acad Sci U S A 109: 3962-3966

[21] Szpara M. L., Parsons L. and Enquist L. W. (2010) Sequence variability in clinical and laboratory isolates of herpes simplex virus 1 reveals new mutations. J Virol 84: 5303-5313

RNA VIRUSES

The high mutation rate in RNA viruses arises from error-prone polymerases and limited RNA proofreading functions[22]. This low replication fidelity results in RNA virus populations that have been described as quasispecies: a cloud, or assemblage, of wild-type (WT) and mutant genomes that exist at a mutation-selection equilibrium[23]. Recent studies have shown that virus diversity is essential for adaptive evolution and the capacity to cause disease[24].

References

Blasdell K. R., Voysey R., Bulach D., Joubert D. A., Tesh R. B., et al. (2012) Kotonkan and Obodhiang viruses: African ephemeroviruses with large and complex genomes. Virology 425: 143-153

This study describes the complete sequences of the Obodhiang virus (OBOV) and Kotonkan virus (KOTV) genomes. Genetic and serological data indicate that KOTV and OBOV should be classified as new species in the genus Ephemerovirus. This is an example of using sequencing to identify a new RNA virus species.
Illumina Technology: Genome Analyzer with 75 bp paired-end reads

Chandriani S., Skewes-Cox P., Zhong W., Ganem D. E., Divers T. J., et al. (2013) Identification of a previously undescribed divergent virus from the Flaviviridae family in an outbreak of equine serum hepatitis. Proc Natl Acad Sci U S A 110: E1407-1415

This study applied RNA sequencing to identify a previously unknown Flaviviridae virus causing equine serum hepatitis. The authors named the virus "Theiler's disease-associated virus" (TDAV). In the outbreak studied, TDAV was detectable in all affected animals, suggesting it to be the causative virus for Theiler's disease although the authors could not rule out the potential presence of other infectious agents at low levels.
Illumina Technology: HiSeq 2000

RNA sequencing was used to identify a previously unknown Flaviviridae virus causing equine serum hepatitis[25].

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Depew J., Zhou B., McCorrison J. M., Wentworth D. E., Purushe J., et al. (2013) Sequencing viral genomes from a single isolated plaque. Virol J 10: 181

Sequencing of viruses and bacteriophages is usually preceded by production of viral stock or specific purification and amplification to obtain sufficient quantities of genomic material. This study presents a novel method of Sequence-Independent Single Primer Amplification (SISPA) to allow sequencing from as little as 10 pg of DNA template.
Illumina Technology: HiSeq 2000

Rutvisuttinunt W., Chinnawirotpisan P., Simasathien S., Shrestha S. K., Yoon I. K., et al. (2013) Simultaneous and complete genome sequencing of influenza A and B with high coverage by Illumina MiSeq Platform. J Virol Methods 193: 394-404

Active characterization of influenza viruses is essential for better preparation against possible pandemic events. In order to obtain comprehensive characterization of the influenza genome and identify emerging strains, this study applied next-generation-sequencing on Illumina MiSeq for multiplex sequencing of six virus isolates from clinical specimens collected in Thailand and Nepal. The analysis characterized three seasonal influenza A H3N2 strains, one 2009 pandemic influenza H1N1 strain and two influenza B strains.
Illumina Technology: MiSeq

Al Rwahnih M., Dolja V. V., Daubert S., Koonin E. V. and Rowhani A. (2012) Genomic and biological analysis of Grapevine leafroll-associated virus 7 reveals a possible new genus within the family Closteroviridae. Virus Res 163: 302-309

Bronkhorst A. W., van Cleef K. W., Vodovar N., Ince I. A., Blanc H., et al. (2012) The DNA virus Invertebrate iridescent virus 6 is a target of the Drosophila RNAi machinery. Proc Natl Acad Sci U S A 109: E3604-3613

Dunowska M., Biggs P. J., Zheng T. and Perrott M. R. (2012) Identification of a novel nidovirus associated with a neurological disease of the Australian brushtail possum (Trichosurus vulpecula). Vet Microbiol 156: 418-424

Hwang Y. T., Kalischuk M., Fusaro A. F., Waterhouse P. M. and Kawchuk L. (2013) Small RNA sequencing of Potato leafroll virus-infected plants reveals an additional subgenomic RNA encoding a sequence-specific RNA-binding protein. Virology 438: 61-69

Morita M., Kuba K., Ichikawa A., Nakayama M., Katahira J., et al. (2013) The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell 153: 112-125

Perera O. P., Snodgrass G. L., Allen K. C., Jackson R. E., Becnel J. J., et al. (2012) The complete genome sequence of a single-stranded RNA virus from the tarnished plant bug, Lygus lineolaris (Palisot de Beauvois). J Invertebr Pathol 109: 11-19

Roy A., Choudhary N., Guillermo L. M., Shao J., Govindarajulu A., et al. (2013) A novel virus of the genus Cilevirus causing symptoms similar to citrus leprosis. Phytopathology 103: 488-500

VIRUS mRNA

Sequencing viral mRNAs can provide a wealth of information about the activity of viruses as well as their mechanisms of action[26-27]. This information can then be used to annotate the viral genome. Until the advent of next-generation sequencing it was very difficult and laborious to sequence viruses. Lack of knowledge about viral sequence functions represents a large gap in current understanding of microbiological population dynamics[28].

Viral mRNA constitutes a surprisingly large portion of the total RNA in HIV-infected CD4+ T cells (in this study, nearly 40% by 24 hours after infection)…
Law G. L. et al. 2013

Review

Law G. L., Korth M. J., Benecke A. G. and Katze M. G. (2013) Systems virology: host-directed approaches to viral pathogenesis and drug targeting. Nat Rev Microbiol 11: 455-466

References

Lee A. S., Burdeinick-Kerr R. and Whelan S. P. (2013) A ribosome-specialized translation initiation pathway is required for cap-dependent translation of vesicular stomatitis virus mRNAs. Proc Natl Acad Sci U S A 110: 324-329

This study presents the discovery of a transcript-specific translation initiation mechanism that is mediated by the ribosome. The mechanism was found through study of vesicular stomatitis virus (VSV), which requires a protein from the large ribosomal subunit (rpL40) for its translation. Using deep sequencing, the authors further uncovered a subset of cellular transcripts that were selectively sensitive to rpL40 depletion, suggesting that this is an endogenous translation pathway.
Illumina technology: Genome Analyzer$_{II}$ for mRNA-Seq

Lusic M., Marini B., Ali H., Lucic B., Luzzati R., et al. (2013) Proximity to PML nuclear bodies regulates HIV-1 latency in CD4+ T cells. Cell Host Microbe 13: 665-677

Gene localization to specialized nuclear compartments is a mechanism for regulating gene expression. In studying the mechanisms mediating human immunodeficiency virus type 1 (HIV-1) latency, the authors discovered that silenced, but transcriptionally competent, HIV-1 proviruses reside in close proximity to promyelocytic leukemia (PML) protein. PML binds to the latent HIV-1 promoter and inhibits gene expression.
Illumina technology: mRNA-Seq

[26] Jiang X., Jiang H., Li C., Wang S., Mi Z., et al. (2011) Sequence characteristics of T4-like bacteriophage IME08 benome termini revealed by high throughput sequencing. Virol J 8: 194
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Morita M., Kuba K., Ichikawa A., Nakayama M., Katahira J., et al. (2013) The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell 153: 112-125

Despite immunization programs, influenza A viruses are a major cause of morbidity and mortality throughout the world. Several lipid-derived products have presented promising anti-inflammatory functions. In this study the lipid mediator protectin PD1 was studied to identify the anti-inflammatory mechanism. Using RNA sequencing, the authors found that PD1 disrupt the influenza virus replication via the RNA export machinery. This finding suggests that endogenous lipid mediators have potential as anti-inflammatory agents against influenza A infection.
Illumina technology: HiSeq 2000 for RNA-binding proteins (RIP) and RNA-Seq

Murakami R., Suetsugu Y., Kobayashi T. and Nakashima N. (2013) The genome sequence and transmission of an iflavirus from the brown planthopper, *Nilaparvata lugens*. Virus Res 176: 179-187

The brown planthopper is one of the most important pests of rice plants because of the damage it causes to the plant, directly as well as through transmission of the rice ragged stunt virus and rice grassy stunt virus. In this study a previously unknown iflavirus was identified in a laboratory colony of the brown planthopper. The virus and its host were characterized by sequencing, and a test of transmission showed that the virus can be transmitted horizontally.
Illumina technology: HiSeq 2000 for mRNA-Seq

Neller M. A., Burrows J. M., Rist M. J., Miles J. J. and Burrows S. R. (2013) High frequency of herpesvirus-specific clonotypes in the human T cell repertoire can remain stable over decades with minimal turnover. J Virol 87: 697-700

Ramasubramanyan S., Kanhere A., Osborn K., Flower K., Jenner R. G., et al. (2012) Genome-wide analyses of Zta binding to the Epstein-Barr virus genome reveals interactions in both early and late lytic cycles and an epigenetic switch leading to an altered binding profile. J Virol 86: 12494-12502

Rossetto C. C., Tarrant-Elorza M., Verma S., Purushothaman P. and Pari G. S. (2013) Regulation of viral and cellular gene expression by Kaposi's sarcoma-associated herpesvirus polyadenylated nuclear RNA. J Virol 87: 5540-5553

Schnettler E., Ratinier M., Watson M., Shaw A. E., McFarlane M., et al. (2013) RNA interference targets arbovirus replication in Culicoides cells. J Virol 87: 2441-2454

Wilkie G. S., Davison A. J., Watson M., Kerr K., Sanderson S., et al. (2013) Complete genome sequences of elephant endotheliotropic herpesviruses 1A and 1B determined directly from fatal cases. J Virol 87: 6700-6712

Wu L., Zhou P., Ge X., Wang L. F., Baker M. L., et al. (2013) Deep RNA sequencing reveals complex transcriptional landscape of a bat adenovirus. J Virol 87: 503-511

VIRUS SMALL RNAS (MIRNAS) AND HOST-PATHOGEN INTERACTIONS

Small RNAs play a key role in the host-pathogen interaction during virus infections[29]. Micro-RNAs (miRNAs) are a class of small noncoding RNAs involved in post-transcriptional regulation in organisms ranging from plants to higher mammals[30]. Both RNA and DNA viruses use miRNAs for host and viral gene regulation[31]. Viral metagenomics is expanding the current knowledge of virus-host interactions by uncovering genes that manipulate their hosts in unexpected ways[32].

Review

Celsi F., Catamo E., Kleiner G., Tricarico P. M., Vuch J., et al. (2013) HLA-G/C, miRNAs, and their role in HIV infection and replication. Biomed Res Int 2013: 693643

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Etebari K., Hussain M. and Asgari S. (2013) Identification of microRNAs from *Plutella xylostella* larvae associated with parasitization by *Diadegma semiclausum*. Insect Biochem Mol Biol 43: 309-318

miRNAs play important roles in many biological processes and show differential expression under changing conditions, such as development, immune challenge and stress. This study investigated miRNA expression in the Diamondback moth *Plutella xylostella* and compared the profile to expression under parasitization by *Diadegma semiclausum*. Virus-like particles and polydnaviruses (PDVs) were coinjected during ovideposition and may play significant roles at various time points after parasitization. Differential expression of host cellular miRNAs in response to parasitism was examined by making small RNA libraries from parasitized and naive larvae of *P. xylostella*. In the highly expressed miR-281*, the extended dynamic range of RNA-Seq made it possible to identify expression changes that were difficult to see in Northern blots. The identified responsive miRNAs provide insights into the insect immune response to parasitism.
Illumina technology: Genome Analyzer$_{IIx}$ with the Illumina TruSeq Small RNA Preparation Kit and 36 bp reads

Diamondback moth Plutella xylostella

[29] Gausson V. and Saleh M. C. (2011) Viral small RNA cloning and sequencing. Methods Mol Biol 721: 107-122
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[31] Whisnant A. W., Bogerd H. P., Flores O., Ho P., Powers J. G., et al. (2013) In-depth analysis of the interaction of HIV-1 with cellular microRNA biogenesis and effector mechanisms. MBio 4: e000193
[32] Rosario K. and Breitbart M. (2011) Exploring the viral world through metagenomics. Curr Opin Virol 1: 289-297

Goic B., Vodovar N., Mondotte J. A., Monot C., Frangeul L., et al. (2013) RNA-mediated interference and reverse transcription control the persistence of RNA viruses in the insect model *Drosophila*. Nat Immunol 14: 396-403

Viral infections can be either transient (possibly lethal to the organism) or persistent. In the latter case the host immune system controls the virus, but does not eliminate it. This study examines the mechanisms of persistent viral infections by using the Flock horse virus (FHV) infection of *Drosophila melanogaster* as a model system. Small RNA was sequenced to investigate the role of RNA-mediated interference pathways. The authors found virus-retrotransposon DNA chimeras produced transcripts that were processed by the RNAi machinery which, in turn, inhibited viral replication.
Illumina technology: HiSeq 2000 for small RNA-Seq and genomic DNA at 54 bp paired-end reads

Drosophila melanogaster as a model system for flock horse virus (FHV) infection[33]

Hwang Y. T., Kalischuk M., Fusaro A. F., Waterhouse P. M. and Kawchuk L. (2013) Small RNA sequencing of Potato leafroll virus-infected plants reveals an additional subgenomic RNA encoding a sequence-specific RNA-binding protein. Virology 438: 61-69

The transcription machinery of the potato leafroll virus (PLRV) creates subgenomic RNAs (sgRNA) for expression of 3'-proximal genes. Using small RNA (sRNA) sequencing, this study mapped the viral coverage of PLRV-derived sRNAs from virus-infected plants. This is the first sgRNA identified in a virus from the genus *Polerovirus* further deepening the understanding of the viral genome.
Illumina Technology: Genome Analyzer$_{IIx}$

Vereide D. T., Seto E., Chiu Y. F., Hayes M., Tagawa T., et al. (2013) Epstein-Barr virus maintains lymphomas via its miRNAs. Oncogene

Epstein-Barr virus (EBV) targets quiescent cells and drives them to proliferate. This mechanism expands the pool of virus-infected cells, but it may also make the virus oncogenic. In this RNA sequencing study, miRNAs from EBV were shown to both sustain Burkitts lymphoma in the absence of other viral oncogenes and promote the transformation of primary B lymphocytes.
Illumina technology: Genome Analyzer$_{IIx}$ for mRNA and RISC-immunoprecipitated mRNA

Whisnant A. W., Bogerd H. P., Flores O., Ho P., Powers J. G., et al. (2013) In-depth analysis of the interaction of HIV-1 with cellular microRNA biogenesis and effector mechanisms. MBio 4: e000193

The question of how HIV-1 interfaces with cellular miRNA biogenesis and effector mechanisms has been highly controversial. In this paper, the authors used deep sequencing of small RNAs in two different infected cell lines and two types of primary human cells to unequivocally demonstrate that HIV-1 does not encode any viral miRNAs.
Illumina technology: HiSeq 2000 for RNA-Seq of RISC-bound miRNAs using TruSeq Small RNA Kit

[33] Goic B., Vodovar N., Mondotte J. A., Monot C., Frangeul L., et al. (2013) RNA-mediated interference and reverse transcription control the persistence of RNA viruses in the insect model Drosophila. Nat Immunol 14: 396-403

Flores O., Nakayama S., Whisnant A. W., Javanbakht H., Cullen B. R., et al. (2013) Mutational inactivation of herpes simplex virus 1 microRNAs identifies viral mRNA targets and reveals phenotypic effects in culture. J Virol 87: 6589-6603

Gunasekharan V. and Laimins L. A. (2013) Human papillomaviruses modulate microRNA 145 expression to directly control genome amplification. J Virol 87: 6037-6043

Guo X. K., Zhang Q., Gao L., Li N., Chen X. X., et al. (2013) Increasing expression of microRNA 181 inhibits porcine reproductive and respiratory syndrome virus replication and has implications for controlling virus infection. J Virol 87: 1159-1171

Jayachandran B., Hussain M. and Asgari S. (2012) RNA interference as a cellular defense mechanism against the DNA virus baculovirus. J Virol 86: 13729-13734

Kakumani P. K., Ponia S. S., S R. K., Sood V., Chinnappan M., et al. (2013) Role of RNA Interference (RNAi) in Dengue Virus Replication and Identification of NS4B as an RNAi Suppressor. J Virol 87: 8870-8883

Leger P., Lara E., Jagla B., Sismeiro O., Mansuroglu Z., et al. (2013) Dicer-2- and Piwi-mediated RNA interference in Rift Valley fever virus-infected mosquito cells. J Virol 87: 1631-1648

Lin X., Li X., Liang D. and Lan K. (2012) MicroRNAs and unusual small RNAs discovered in Kaposi's sarcoma-associated herpesvirus virions. J Virol 86: 12717-12730

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Quax T. E., Voet M., Sismeiro O., Dillies M. A., Jagla B., et al. (2013) Massive Activation of Archaeal Defense Genes during Viral Infection. J Virol 87: 8419-8428

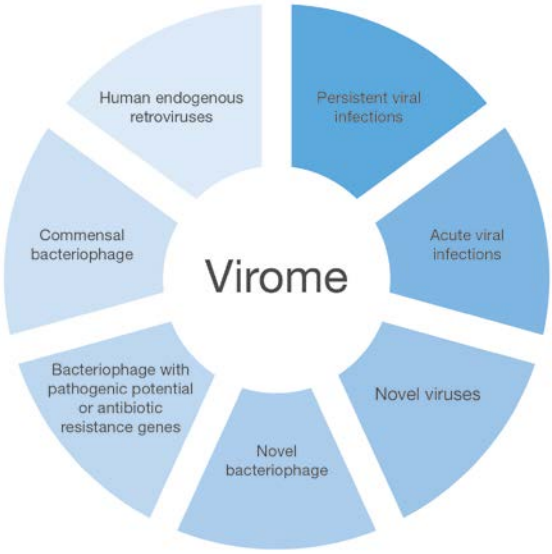
Rosewick N., Momont M., Durkin K., Takeda H., Caiment F., et al. (2013) Deep sequencing reveals abundant noncanonical retroviral microRNAs in B-cell leukemia/lymphoma. Proc Natl Acad Sci U S A 110: 2306-2311

Schnettler E., Ratinier M., Watson M., Shaw A. E., McFarlane M., et al. (2013) RNA interference targets arbovirus replication in Culicoides cells. J Virol 87: 2441-2454

Stik G., Dambrine G., Pfeffer S. and Rasschaert D. (2013) The oncogenic microRNA OncomiR-21 overexpressed during Marek's disease lymphomagenesis is transactivated by the viral oncoprotein Meq. J Virol 87: 80-93

HUMAN VIROME

The human virome is the collection of all viruses that are found in or on humans, including both eukaryotic and prokaryotic viruses. Eukaryotic viruses have an important impact on human health, ranging from mild, self-limited acute or chronic infections to those with serious or fatal consequences. Prokaryotic viruses can also influence human health by affecting the structure and function of bacterial communities that make up the human microbiome[34].



Components of the human virome that can be characterized by metagenomic sequencing[35]

Reviews

Lipkin W. I. and Firth C. (2013) Viral surveillance and discovery. Curr Opin Virol 3: 199-204

Wylie K. M., Weinstock G. M. and Storch G. A. (2013) Virome genomics: a tool for defining the human virome. Curr Opin Microbiol 16: 479-484

Moon C. and Stappenbeck T. S. (2012) Viral interactions with the host and microbiota in the intestine. Curr Opin Immunol 24: 405-410

Rho M., Wu Y. W., Tang H., Doak T. G. and Ye Y. (2012) Diverse CRISPRs evolving in human microbiomes. PLoS Genet 8: e1002441

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HUMAN VIRAL PATHOGENS

In addition to improving the detection of disease-causing viruses, genomic methods have highlighted the prevalence of viruses in healthy individuals. For example, two groups from the family *Picornaviridae* are common on mucosal surfaces: rhinoviruses and gastrointestinal enteroviruses. In contrast to a "one-pathogen–one-disease" model, a more complex model of the human virome suggests that people are almost continually exposed to viruses, which may, or may not, cause symptoms. In this context the virome is an important component of the environment that can interact with host genetic traits to contribute to the pathogenesis of complex diseases[36].

Reviews

Chiu C. Y. (2013) Viral pathogen discovery. Curr Opin Microbiol 16: 468-478

Minot S., Bryson A., Chehoud C., Wu G. D., Lewis J. D., et al. (2013) Rapid evolution of the human gut virome. Proc Natl Acad Sci U S A 110: 12450-12455

Reyes A., Semenkovich N. P., Whiteson K., Rohwer F. and Gordon J. I. (2012) Going viral: next-generation sequencing applied to phage populations in the human gut. Nat Rev Microbiol 10: 607-617

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Grard G., Fair J. N., Lee D., Slikas E., Steffen I., et al. (2012) A novel rhabdovirus associated with acute hemorrhagic fever in central Africa. PLoS Pathog 8: e1002924

Deep sequencing on an illumina HiSeq system was used to discover a novel rhabdovirus (Bas-Congo virus, or BASV) associated with a 2009 outbreak of three human cases of acute hemorrhagic fever in the Democratic Republic of Congo. BASV was detected in an acute serum sample from the lone survivor, and was subsequently de novo assembled using 140 million sequence reads. Antibodies against the virus were found in an asymptomatic nurse caring for one of the three patients, suggesting a potential human-to-human mode of transmission for the virus.
Illumina Technology: HiSeq 2000 for 100 bp paired-end reads

Kugelman J. R., Lee M. S., Rossi C. A., McCarthy S. E., Radoshitzky S. R., et al. (2012) Ebola virus genome plasticity as a marker of its passaging history: a comparison of in vitro passaging to non-human primate infection. PLoS ONE 7: e50316

The development of medical countermeasures (MCM) for filoviruses is a high priority for biodefense. In this study, the mutability of the Ebola virus (EBOV) genome was studied in both cell culture and in macaques exposed to a controlled infection. The study concluded that EBOV evolves into genomically different but defined subpopulations depending on whether they are administered to animals or cell culture. This finding has important implications for the use of animal versus cell culture models to study infectious diseases.
Illumina Technology: cBOT and Genome Analyzer$_{IIx}$ for 76 bp paired-end reads

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Kriesel J. D., Hobbs M. R., Jones B. B., Milash B., Nagra R. M., et al. (2012) Deep sequencing for the detection of virus-like sequences in the brains of patients with multiple sclerosis: detection of GBV-C in human brain. PLoS ONE 7: e31886

This study shows the feasibility of deep sequencing for the detection of occult viral infections in the brains of deceased persons with multiple sclerosis (MS). The deep sequencing analysis in this study was based on the early Illumina Genome Analyzer$_{II}$ technology, and was limited by read length (36 bp) and sequencing from only a single end of the library inserts. The investigators believe that further improvements in sequencing technologies, such as longer reads and perhaps paired-end strategies, will significantly simplify the bioinformatics.
Illumina Technology: Genome Analyzer$_{II}$

Malboeuf C. M., Yang X., Charlebois P., Qu J., Berlin A. M., et al. (2013) Complete viral RNA genome sequencing of ultra-low copy samples by sequence-independent amplification. Nucleic Acids Res 41: e13

Traditional viral detection methods rely on prior knowledge of sequence or antigens. This study presents sequence-independent viral RNA amplification and subsequent detection using Illumina MiSeq sequencing. The method presented is capable of generating almost full-length viral genomes from clinical samples with low amounts of viral RNA.
Illumina Technology: HiSeq 2000 with 101 bp paired-end reads

Han Y., Zhang Y., Mei Y., Wang Y., Liu T., et al. (2013) Analysis of hepatitis B virus genotyping and drug resistance gene mutations based on massively parallel sequencing. J Virol Methods 193: 341-347

Total hepatitis B virus (HBV) DNA from 395 patients treated with single or multiple antibiotics was sequenced using a HiSeq2000 system, and results were validated using PCR sequencing. The experiment was repeated three times and the results demonstrated the high reproducibility of the HiSeq platform. The authors conclude the HiSeq system has high sensitivity, high fidelity, high throughput and automation, making it a favorable method for HBV testing and genotyping.
Illumina Technology: HiSeq 2000

Grard G., Fair J. N., Lee D., Slikas E., Steffen I., et al. (2012) A novel rhabdovirus associated with acute hemorrhagic fever in central Africa. PLoS Pathog 8: e1002924

Janovitz T., Klein I. A., Oliveira T., Mukherjee P., Nussenzweig M. C., et al. (2013) High-throughput sequencing reveals principles of adeno-associated virus serotype 2 integration. J Virol 87: 8559-8568

Khoury J. D., Tannir N. M., Williams M. D., Chen Y., Yao H., et al. (2013) Landscape of DNA Virus Associations across Human Malignant Cancers: Analysis of 3,775 Cases Using RNA-Seq. J Virol 87: 8916-8926

Lin Z., Wang X., Strong M. J., Concha M., Baddoo M., et al. (2013) Whole-genome sequencing of the Akata and Mutu Epstein-Barr virus strains. J Virol 87: 1172-1182

Sanchez-Sampedro L., Gomez C. E., Mejias-Perez E., Perez-Jimenez E., Oliveros J. C., et al. (2013) Attenuated and replication-competent vaccinia virus strains M65 and M101 with distinct biology and immunogenicity as potential vaccine candidates against pathogens. J Virol 87: 6955-6974

Stern A., Mick E., Tirosh I., Sagy O. and Sorek R. (2012) CRISPR targeting reveals a reservoir of common phages associated with the human gut microbiome. Genome Res 22: 1985-1994

Yang H., Zhu J., Li H., Xiao L., Wang J., et al. (2012) Full genome sequence of bluetongue virus serotype 4 from China. J Virol 86: 13122-13123

Yu G., Greninger A. L., Isa P., Phan T. G., Martinez M. A., et al. (2012) Discovery of a novel polyomavirus in acute diarrheal samples from children. PLoS ONE 7: e49449

ANIMAL VIRUSES

Viruses are important pathogens of livestock. They cause economically important diseases, such as foot-and-mouth disease and bluetongue[37]. With intensification of trade, livestock are increasingly exposed to viruses that can cause severe animal diseases [38].

Reviews

Lipkin W. I. (2013) The changing face of pathogen discovery and surveillance. Nat Rev Microbiol 11: 133-141

Lipkin W. I. and Firth C. (2013) Viral surveillance and discovery. Curr Opin Virol 3: 199-204

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Biek R., O'Hare A., Wright D., Mallon T., McCormick C., et al. (2012) Whole genome sequencing reveals local transmission patterns of *Mycobacterium bovis* in sympatric cattle and badger populations. PLoS Pathog 8: e1003008

Bovine tuberculosis (bTB) outbreaks in cattle are costly and detailed understanding of transmission principles are needed to manage this disease. The bTB may be carried both by cattle and by badgers, complicating the analysis of epidemiology. In this study a geographically close sampling of bTB from five herds and samples from four badgers were combined to form a characterization of bTB development and spread. The individual bTB isolates were identified by regions of variable-number tandem repeats (VNTRs) in the *Mycobacterium bovis* genome. Single-nucleotide polymorphisms (SNPs) were found to be consistent for isolates sampled within short geographical distances.
Illumina Technology: Genome Analyzer$_{IIx}$ for 70 bp paired-end reads

Bovine tuberculosis (bTB) may be carried both by cattle and by badgers, complicating the analysis of epidemiology[39].

Blasdell K. R., Voysey R., Bulach D. M., Trinidad L., Tesh R. B., et al. (2012) Malakal virus from Africa and Kimberley virus from Australia are geographic variants of a widely distributed ephemerovirus. Virology 433: 236-244

Kimberley virus (KIMV) and Malakal virus (MALV) were first isolated in Australia and Sudan respectively. In this study the two virus genomes were characterized by Illumina sequencing and compared with respect to their genome organization and expression profiles. The high level of amino acid identity and similar expression profiles indicate that KIMV and MALV are geographic variants of the same ephemerovirus.
Illumina Technology: Genome Analyzer$_{IIx}$ for 101 bp paired-end reads with greater than 1,000-fold coverage

[37] Rao P. P., Reddy Y. N., Ganesh K., Nair S. G., Niranjan V., et al. (2013) Deep sequencing as a method of typing bluetongue virus isolates. J Virol Methods 193: 314-319

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Blasdell K. R., Voysey R., Bulach D., Joubert D. A., Tesh R. B., et al. (2012) Kotonkan and Obodhiang viruses: African ephemeroviruses with large and complex genomes. Virology 425: 143-153

KOTV and OBOV are rhabdoviruses that were isolated from arthropods in Africa and formerly classified as lyssaviruses. Both viruses have been shown to cross-react with rabies and rabies-related viruses, but their pathogenicity is not well understood. This study presents the complete genome sequences of KOTV and OBOV along with their expression profiles and includes an analysis of their phylogenetic relationships to other rhabdoviruses. The genetic and serological data indicate that both viruses should be classified as a new species in the genus *Ephemerovirus*.

Illumina Technology: Genome Analyzer$_{IIx}$ for 101 bp paired-end reads

Vasilakis N., Widen S., Mayer S. V., Seymour R., Wood T. G., et al. (2013) Niakha virus: A novel member of the family *Rhabdoviridae* isolated from phlebotomine sandflies in Senegal. Virology

This study characterizes the genome of the Niakha virus (NIAV), a previously uncharacterized rhabdovirus isolated from sandflies in Senegal. The viral RNA was sequenced using an Illumina HiSeq system, and assembled and compared to other rhabdoviral genomes. The phylogenetic analysis resolved the NIAV virus as phylogenetically distinct from the eight currently recognized *Rhabdoviridae* genera.
Illumina technology: HiSeq 1000 with 50 bp paired-end reads

Bodewes R., van der Giessen J., Haagmans B. L., Osterhaus A. D. and Smits S. L. (2013) Identification of multiple novel viruses, including a parvovirus and a hepevirus, in feces of red foxes. J Virol 87: 7758-7764

Fan W. L., Ng C. S., Chen C. F., Lu M. Y., Chen Y. H., et al. (2013) Genome-wide patterns of genetic variation in two domestic chickens. Genome Biol Evol 5: 1376-1392, Ramasubramanyan S., Kanhere A., Osborn K., Flower K., Jenner R. G., et al. (2012) Genome-wide analyses of Zta binding to the Epstein-Barr virus genome reveals interactions in both early and late lytic cycles and an epigenetic switch leading to an altered binding profile. J Virol 86: 12494-12502

Lauck M., Sibley S. D., Hyeroba D., Tumukunde A., Weny G., et al. (2013) Exceptional simian hemorrhagic fever virus diversity in a wild African primate community. J Virol 87: 688-691

Leon A. J., Banner D., Xu L., Ran L., Peng Z., et al. (2013) Sequencing, annotation, and characterization of the influenza ferret infectome. J Virol 87: 1957-1966

Ramasubramanyan S., Kanhere A., Osborn K., Flower K., Jenner R. G., et al. (2012) Genome-wide analyses of Zta binding to the Epstein-Barr virus genome reveals interactions in both early and late lytic cycles and an epigenetic switch leading to an altered binding profile. J Virol 86: 12494-12502

Rao P. P., Reddy Y. N., Ganesh K., Nair S. G., Niranjan V., et al. (2013) Deep sequencing as a method of typing bluetongue virus isolates. J Virol Methods 193: 314-319

Schnettler E., Ratinier M., Watson M., Shaw A. E., McFarlane M., et al. (2013) RNA interference targets arbovirus replication in Culicoides cells. J Virol 87: 2441-2454

Squire M. M., Carter G. P., Mackin K. E., Chakravorty A., Noren T., et al. (2013) Novel molecular type of Clostridium difficile in neonatal pigs, Western Australia. Emerg Infect Dis 19: 790-792

Subramaniam S., Johnston J., Preeyanon L., Brown C. T., Kung H. J., et al. (2013) Integrated Analyses of Genome-Wide DNA Occupancy and Expression Profiling Identify Key Genes and Pathways Involved in Cellular Transformation by a Marek's Disease Virus Oncoprotein, Meq. J Virol 87: 9016-9029

Wilkie G. S., Davison A. J., Watson M., Kerr K., Sanderson S., et al. (2013) Complete genome sequences of elephant endotheliotropic herpesviruses 1A and 1B determined directly from fatal cases. J Virol 87: 6700-6712

PLANT VIRAL PATHOGENS

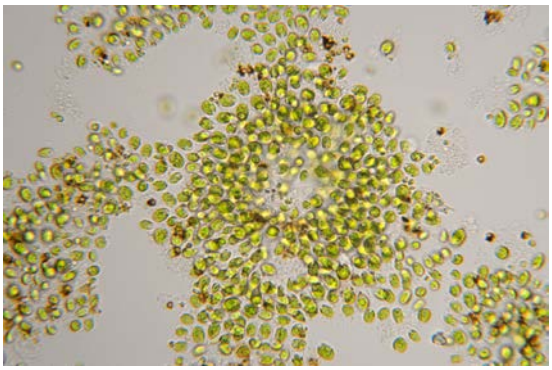
Plants have a well-defined defense mechanism against invasive nucleic acids, such as viral transcripts[40]. The silencing pathway is quite sophisticated, but the distinct steps and nature of effector complexes vary among—and even within—species[41,42].

Bronkhorst A. W., van Cleef K. W., Vodovar N., Ince I. A., Blanc H., et al. (2012) The DNA virus Invertebrate iridescent virus 6 is a target of the *Drosophila* RNAi machinery. Proc Natl Acad Sci U S A 109: E3604-3613

RNA viruses in insects are targets of an RNA interference (RNAi)-based antiviral immune response. This study investigated the role of RNAi in DNA virus infection using *Drosophila melanogaster* infected with invertebrate iridescent virus 6 (IIV-6) as a model. To investigate whether dsRNA is processed into viral small interfering RNAs (vsiRNAs), small RNAs were sequenced using an Illumina Genome Analyzer. The data indicate that abundant vsiRNAs were produced in an RNAi pathway–dependent manner, indicating that RNAi provides an antiviral defense system against dsDNA viruses.
Illumina Technology: Genome Analyzer$_{IIx}$ for small-RNA libraries

Rowe J. M., Dunigan D. D., Blanc G., Gurnon J. R., Xia Y., et al. (2013) Evaluation of higher plant virus resistance genes in the green alga, *Chlorella variabilis* NC64A, during the early phase of infection with *Paramecium bursaria* chlorella virus-1. Virology 442: 101-113

With growing industrial interest in algae, as well as their critical roles in aquatic systems, the need to understand the effects of algal pathogens is increasing. In a model algal host-virus system, Illumina RNA sequencing was applied to determine expression of genes homologous to those involved in RNA silencing and virus response in higher plants. This method detected 325 of 375 defined homologs that were expressed in healthy as well as infected algae cells, suggesting that RNA silencing may be utilized by algae as a response to virus infection.
Illumina Technology: Genome Analyzer$_{IIx}$ for RNA-Seq with 51 bp reads



Green alga, *Chlorella variabilis*, is used as a model system for plant virus resistance[43].

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[41] Alvarado V. and Scholthof H. B. (2009) Plant responses against invasive nucleic acids: RNA silencing and its suppression by plant viral pathogens. Semin Cell Dev Biol 20: 1032-1040
[42] Maree H. J., Almeida R. P., Bester R., Chooi K. M., Cohen D., et al. (2013) Grapevine leafroll-associated virus 3. Front Microbiol 4: 82
[43] Rowe J. M., Dunigan D. D., Blanc G., Gurnon J. R., Xia Y., et al. (2013) Evaluation of higher plant virus resistance genes in the green alga, Chlorella variabilis NC64A, during the early phase of infection with Paramecium bursaria chlorella virus-1. Virology 442: 101-113

Zhang Z., Zhang P., Li W., Zhang J., Huang F., et al. (2013) De novo transcriptome sequencing in Frankliniella occidentalis to identify genes involved in plant virus transmission and insecticide resistance. Genomics 101: 296-305

The western flower thrips (WFT) is an insect that causes worldwide agricultural damage, both directly by feeding and indirectly by vectoring Tospoviruses, such as tomato spotted wilt virus (TSWV). In this study the transcriptome of WFT and the differential gene expression of WFT in response to TSWV infection were characterized using an Illumina HiSeq system for RNA sequencing. The authors found that TSWV can regulate cellular processes and immune response, suggesting a mechanism that does not result in detrimental effects for its vector host, WFT.
Illumina technology: HiSeq 2000 for RNA-Seq

Hwang Y. T., Kalischuk M., Fusaro A. F., Waterhouse P. M. and Kawchuk L. (2013) Small RNA sequencing of Potato leafroll virus-infected plants reveals an additional subgenomic RNA encoding a sequence-specific RNA-binding protein. Virology 438: 61-69

Loconsole G., Onelge N., Potere O., Giampetruzzi A., Bozan O., et al. (2012) Identification and characterization of citrus yellow vein clearing virus, a putative new member of the genus Mandarivirus. Phytopathology 102: 1168-1175

Maree H. J., Almeida R. P., Bester R., Chooi K. M., Cohen D., et al. (2013) Grapevine leafroll-associated virus 3. Front Microbiol 4: 82

Roy A., Choudhary N., Guillermo L. M., Shao J., Govindarajulu A., et al. (2013) A novel virus of the genus Cilevirus causing symptoms similar to citrus leprosis. Phytopathology 103: 488-500

Roy A., Stone A., Otero-Colina G., Wei G., Choudhary N., et al. (2013) Genome assembly of citrus leprosis virus nuclear type reveals a close association with orchid fleck virus. Genome Announc 1:

INSECT VIRAL PATHOGENS

A large number of insect viruses with small RNA genomes and morphological resemblance to vertebrate picornaviruses have been characterized. While some of these viruses may cause only latent infections without significant adverse effects on the host, others may cause debilitating or lethal infections in the host[44].

References

Perera O. P., Snodgrass G. L., Allen K. C., Jackson R. E., Becnel J. J., et al. (2012) The complete genome sequence of a single-stranded RNA virus from the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). J Invertebr Pathol 109: 11-19

The authors generated a complete genome sequence of a single-stranded RNA virus (LyLV-1) by sequencing cDNA prepared from infected insects. High similarity to the honey bee sacbrood virus (SBV) genome, and similarities in the genome organization and amino acid sequence with the viruses of the family *Iflaviridae*, suggested that LyLV-1 was a novel member of this family.
Illumina Technology: Genome Analyzer$_{II}$ 36 bp reads

Murakami R., Suetsugu Y., Kobayashi T. and Nakashima N. (2013) The genome sequence and transmission of an iflavirus from the brown planthopper, *Nilaparvata lugens*. Virus Res 176: 179-187

The brown planthopper is one of the most important pests of rice plants because of the damage it causes to the plant, directly as well as through transmission of the rice ragged stunt virus and rice grassy stunt virus. In this study, a previously unknown iflavirus was identified in a laboratory colony of the brown planthopper. The virus and its host were characterized by sequencing, and a test of transmission showed that the virus can be transmitted horizontally.
Illumina technology: HiSeq 2000 for mRNA-Seq

Bronkhorst A. W., van Cleef K. W., Vodovar N., Ince I. A., Blanc H., et al. (2012) The DNA virus Invertebrate iridescent virus 6 is a target of the Drosophila RNAi machinery. Proc Natl Acad Sci U S A 109: E3604-3613

RNA viruses in insects are targets of an RNA interference (RNAi)-based antiviral immune response. This study investigated the role of RNAi in DNA virus infection using *Drosophila melanogaster* infected with invertebrate iridescent virus 6 (IIV-6) as a model. To investigate whether dsRNA is processed into viral small interfering RNAs (vsiRNAs), small RNAs were sequenced using an Illumina Genome Analyzer. The data indicate that abundant vsiRNAs were produced in a RNAi pathway–dependent manner, indicating that RNAi provides an antiviral defense system against dsDNA viruses.
Illumina Technology: Genome Analyzer$_{IIx}$ for small-RNA libraries

Etebari K., Hussain M. and Asgari S. (2013) Identification of microRNAs from Plutella xylostella larvae associated with parasitization by Diadegma semiclausum. Insect Biochem Mol Biol 43: 309-318

Goic B., Vodovar N., Mondotte J. A., Monot C., Frangeul L., et al. (2013) RNA-mediated interference and reverse transcription control the persistence of RNA viruses in the insect model Drosophila. Nat Immunol 14: 396-403

Vasilakis N., Forrester N. L., Palacios G., Nasar F., Savji N., et al. (2013) Negevirus: a proposed new taxon of insect-specific viruses with wide geographic distribution. J Virol 87: 2475-2488

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BACTERIOPHAGES

Bacteriophages (phages) are viruses that infect bacteria and play a prominent role in shaping microbial populations[45]. The genetic diversity of the population is very high, and it appears that phages have been actively evolving for billions of years. Frequent horizontal genetic exchange results in pervasive mosaicism in their architectures and the emergence of novel bacterial pathogens. For example, the shiga toxin–carrying lambdoid prophage of pathogenic *E. coli* serotype O104:H4 was responsible for the recent outbreak in Germany[46]. Conversely, the current crisis with antibiotic-resistant bacteria has renewed interest in phage therapy and biocontrol approaches in infection control[47]. With the advent of next-generation sequencing, the coming years of phage genome exploration promise to be especially revealing[48].

Bacteriophages represent an absolute majority of all organisms in the biosphere.
Hatfull et al. 2011

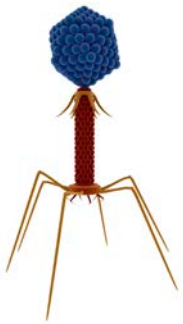

Illustration of a bacteriophage

The scale and impact of bacteriophage distribution can be dramatic. For example, about half of the primary production in the world's oceans is carried out by two cyanobacterial clades *Prochlorococcus* and *Synechococcus*. It is estimated that 40–50% of cyanobacteria are infected by cyanophages that kill 10–50% of their hosts daily. This drives rapid diversification as the bacteria develop resistance and also make dissolved carbon available as the bacterial cells lyse.

The constant threat of phage predation has led to an evolutionary arms race whereby a broad range of bacterial immunity mechanisms result in the evolution of diverse phage immune evasion strategies[49]. A primary defense strategy that eubacteria and archaea mobilize against foreign nucleic acids is based on clustered regularly

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interspaced short palindromic repeats (CRISPR) loci. These loci, together with CRISPR–associated (Cas) genes, form the CRISPR/Cas adaptive immune system[50,51,52].

Reviews

Bondy-Denomy J., Pawluk A., Maxwell K. L. and Davidson A. R. (2013) Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system. Nature 493: 429-432

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Wang J., Qi J., Zhao H., He S., Zhang Y., et al. (2013) Metagenomic sequencing reveals microbiota and its functional potential associated with periodontal disease. Sci Rep 3: 1843

The oral microbiome may have significant impact on oral health. In this study 16 samples were collected from dental swabs and plaques to characterize the microbiome composition and variation. Approximately 0.16% of the reads were assigned to phages, such as *Actinomyces* and *Streptococcus* phages. Each group of samples contained multiple distinct *Actinomyces* phages
Illumina technology: HiSeq 2000 and cBOT for 100bp paired-end reads

Rho M., Wu Y. W., Tang H., Doak T. G. and Ye Y. (2012) Diverse CRISPRs evolving in human microbiomes. PLoS Genet 8: e1002441

CRISPR and Cas genes provide acquired resistance against viruses and conjugative plasmids for most archaeal and many bacterial genomes. The distribution and diversity of known CRISPRs in human microbiomes was studied based on datasets from the Human Microbiome Project. The detailed characterization of CRISPR loci may be applied to tracing rare species and the virus exposure of individuals.
Illumina technology: Human Microbiome Illumina WGS Reads (HMIGWS) Build 1.0

McCallin S., Alam Sarker S., Barretto C., Sultana S., Berger B., et al. (2013) Safety analysis of a Russian phage cocktail: From MetaGenomic analysis to oral application in healthy human subjects. Virology 443: 187-196

Bacteriophage therapy (phage therapy) is an alternative to antibiotic treatment for bacterial infections. Bacteriophages are viruses that infect bacteria, lysing their bacterial host cell. Pharmaceutical companies in Russia produce over-the-counter phage products as liquids or pills to treat infections. This study examined the virus content of one of these products using Illumina sequencing. The analysis revealed 18 distinct phage types and no undesired genes were found in the sequences.
Illumina technology: HiSeq 2000 for 100 bp paired-end reads

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VACCINES

Viruses exist as heterogeneous and complex populations comprising similar but nonidentical genomes. Next-generation sequencing can be used to characterize the population, including rare members, with a very high degree of accuracy[54]. The immune response of the host can also be measured, as well as the T cell response and memory[55,56]. A deeper understanding of the host-pathogen response promises to greatly improve the speed and success of vaccine development.

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Glass E. J., Baxter R., Leach R. J. and Jann O. C. (2012) Genes controlling vaccine responses and disease resistance to respiratory viral pathogens in cattle. Vet Immunol Immunopathol 148: 90-99

Farm animals remain at risk of endemic, exotic and newly emerging viruses. Control measures include development of effective vaccines, as well as selective breeding for animals that are less susceptible to disease and/or have a good response to vaccination. This review describes the various approaches applied in practice today and explains why identifying relevant phenotypes for both infectious disease resistance and vaccine response is not straightforward. As one positive development, the authors mention the finer-resolution genotyping obtainable with the Illumina Bovine 50 BeadChip to guide genotype selection.
Illumina technology: Bovine 50 BeadChip

Farm animals remain at risk of endemic, exotic and newly emerging viruses[57]

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SYMBIOSIS

The evolution of intimate symbiosis requires the coordination of genome content and gene expression between the distinct partner genomes. This coordination allows the fusion of each organism's capabilities into a single integrated metabolism. Three-way symbioses have been well described in macro- and micro-ecosystems. For example, a symbiotic bacterium that inhabits the pea aphid protects the aphid from a wasp that can otherwise lay eggs in the aphid haemocoel. This protection is conferred by a phage-encoded toxin expressed by the bacterium[58].

References

Table 2 | Characteristics of bacteria, microbial eukaryotes and viruses in the human microbiome

Characteristic	Bacteria	Viruses	Eukaryotic microbes
Genome size	0.5–10 megabases	1–1,000 kilobases	10–50 megabases
Number of taxa in the human microbiome	At least thousands	Unknown, but could be as many as bacteria	Unknown, but may be fewer than bacteria
Relative abundances	Highly variable	Highly variable	Unknown
Targeted detection methods	Sequencing of genes such as 5S and 16S rRNA	No universal method for genes, but virus-specific polymerase chain reaction assays for some	Sequencing of 18S rRNA gene Spacer region in rRNA
Shotgun approach to analyses	Alignment to reference genomes or database comparison	Database comparison	Alignment to reference genomes or database comparison
Subspecies or strain diversity	Modest sequence variation Horizontal gene transfer also contributes	High sequence variation	Unknown

Adapted from Weinstock G. M. (2012)[59]

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GLOSSARY OF TERMS AND ABBREVIATIONS

archaea	Single-celled microorganisms with no cell nucleus or any other membrane-bound organelles within their cells
BASV	Bas-Congo virus, a rhabdovirus
bTB	Bovine tuberculosis
Cas	CRISPR-associated
CRISPR	Clustered regularly interspaced short palindromic repeats
EBOV	Ebola virus
EBV	Epstein-Barr virus
HBV	Hepatitis B Virus
FFPE	Formalin-fixed paraffin-embedded
FHV	Flock horse virus
HPV	Human papillomavirus
IIV	Invertebrate iridescent virus
KIMV	Kimberley virus
KOTV	Kotonkan viruses
MALV	Malakal virus
MCM	medical countermeasures
NIAV	Niakha virus
OBOV	Obodhiang viruses
PDV	Polydnaviruses
PLRV	Potato leafroll virus
PML	Promyelocytic leukemia
PyV	Polyomavirus
quasispecies	a cloud or assemblage of wild-type (WT) and mutant genomes that exist at a mutation-selection equilibrium.
SAdV	Simian adenovirus
SBV	Sacbrood virus
SISPA	Sequence-independent single primer amplification
SNP	Single-nucleotide polymorphism
TDAV	Theiler's disease-associated virus
TMAdV	Titi Monkey adenovirus
TSWV	Tomato spotted wilt virus
virome	The sum of all viruses living in the tissues of the host or infecting organisms in the microbiome. These viruses may be further divided into viruses that infect members of each of the three domains of life (e.g., bacterial virome, bacterial phages, or the eukaryotic virome).
VNTR	Variable-number tandem repeats
VSV	Vesicular stomatitis virus
vsiRNA	Viral small interfering RNA
WFT	Western flower thrips
WT	wild-type
zoonoses	An infectious disease that is transmitted between species

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