Integrating genomics into the public health architecture

Effective monitoring, prevention, and mitigation of infectious diseases using NGS

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Introduction

The increasing threat of infectious diseases

The modern world is witnessing an alarming increase in the threat posed by infectious diseases. Globalization and increased international travel facilitate the rapid spread of infectious agents across borders. Additionally, expanding urbanization and increasing population density create ideal conditions for the transmission of infectious agents. Moreover, the emergence of drug-resistant strains of bacteria and viruses poses a significant challenge in the treatment and control of infections. Finally, factors that bring humans into closer contact with animal reservoirs of infectious diseases, such as climate change, deforestation, and high-intensity farming, can increase the risk of animal-to-human transmission.[1-3]

The impact of infectious disease outbreaks on human health and the global economy

Infectious diseases have a profound impact on human health. While disease-associated symptoms vary, many infections disrupt daily life, impart physical suffering, and lead to death (**Table 1**). Beyond the individual, the burden of infectious diseases affects families, communities, and societies at large, as represented by the global economic burden of infectious diseases (**Table 1**). Infectious diseases impact the economy both directly and indirectly. Directly, infectious diseases can result in substantial healthcare costs to individuals (who must cover the costs of hospitalizations, medications, and diagnostic tests) and government agencies (who must invest in infectious disease research, prevention, and containment measures). Indirectly, infectious diseases can disrupt international trade and tourism, affect industries dependent on healthy individuals, and reduce workplace productivity.[4-13] Therefore, combating infectious diseases and implementing effective prevention and control measures is crucial for safeguarding human health and promoting well-being on a global scale.

Table 1: The public health and economic impact of the five most deadly infectious diseases

Infectious disease	Annual deaths worldwide	Economic impact (USD)[c]
Tuberculosis	1.6 million[4,b]	$.58T[5]
HIV/AIDS	630 thousand[6,a]	$37B[7]
Malaria	619 thousand[8,b]	$12B[9]
COVID-19	264 thousand[10,a]	$4.3T[11]
Cholera	95 thousand [12,c]	$2.4B[13,d]

a. In 2022.
b. In 2021.
c. Estimated annual number.
d. Across 44 countries in Africa.

To effectively address the increasing threat of infectious diseases to mitigate their effects on human health and the economy, it is crucial to prioritize investments in bolstering public health surveillance to swiftly detect, respond to, and mitigate the impact of infectious diseases.[12-17]

Infectious disease surveillance: objectives and strategies

Infectious disease surveillance is a major subgroup of public health surveillance that focuses on the monitoring of new, re-emerging, and circulating diseases. In general, the data and information gathered through infectious disease surveillance is disseminated from "reporters" to ministries of health, public health personnel, government leaders, and the public. The information is then used to combat health threats by guiding public health policy, programs, and interventions to ultimately help achieve health equity within communities.[14,16]

The goals of infectious disease surveillance are (1) to understand the epidemiology of a disease, (2) to monitor disease trends, and (3) to identify outbreaks and emerging pathogens (**Figure 1**).[17]

Figure 1: Goals of infectious disease surveillance

The first goal of infectious disease surveillance is to understand the epidemiology of a disease, such as its incidence, distribution, and behavior within a population. Ultimately, epidemiological insights function to inform public health interventions, such as vaccination strategies and drug administration policies.[17] For example, analyzing the epidemiology of prevalent seasonal flu strains enables public health authorities to identify the specific strains to target when formulating annual vaccinations. This approach facilitates the development of an optimal vaccination strategy to curb the transmission of the most prevalent or pathogenic strains of influenza.[18] Furthermore, conducting epidemiological assessments of circulating pathogens that carry antimicrobial resistance (AMR) mechanisms allows public health officials to gain valuable insights into evolving pathogens, which may adjust recommendations for the most efficacious antimicrobial treatment regimens.[19]

The second goal of infectious disease surveillance is to monitor disease trends to better understand ongoing outbreaks and track the efficacy of public health interventions. For example, ongoing surveillance of incidence, prevalence, hospitalizations, and deaths prior to and following the implementation of a vaccination strategy can provide valuable data on the effectiveness of the vaccines. This surveillance data can be used to adjust and improve ongoing vaccination strategies (if needed) and to advocate for continuing program support.[20]

The final goal of infectious disease surveillance is to identify outbreaks and emerging infections to prevent widespread transmission and protect the public.[17] Emerging infections are those that have recently appeared or significantly increased within a population. These infections can be caused by previously undetected or unknown infectious agents (often arising from a zoonotic reservoir), known agents that have spread to new, immunologically naive populations, or familiar agents that have accumulated mutations, resulting in more pathogenic or transmissible variants. Early detection of outbreaks and emerging infections is essential to mount a rapid public health response and intervention strategy.[21]

Beyond COVID: lessons learned from the pandemic

The COVID-19 pandemic showcased the effectiveness of infectious disease surveillance in rapidly characterizing a novel infectious agent, monitoring the spread of a global disease, and facilitating research that ultimately led to an effective vaccine in record time.

However, the pandemic also highlighted the overreliance of global public health surveillance systems on reacting to active outbreaks, rather than on preemptive strengthening of existing public health surveillance systems at the regional, country-wide, and global levels. Bolstering surveillance systems, training public health professionals, and ensuring adequate access to advancing technologies are essential steps for implementing an effective surveillance strategy that can better prevent and respond to future health threats. Overall, the COVID-19 pandemic served as a valuable lesson that emphasizes the need for continuous improvement and innovation in public health surveillance.[22-24]

> *Genomic surveillance is transforming public health action by providing a deeper understanding of pathogens, their evolution, and circulation.*
>
> **-World Heath Organization[24]**

Unlocking the power of the genome for enhanced infectious disease surveillance

Next-generation sequencing (NGS) for the next generation of surveillance

A powerful tool for strengthening infectious disease surveillance is the genomic interrogation of pathogens using NGS (**Figure 2**). Integrating NGS into existing infectious disease surveillance programs can increase the speed of outbreak detection, deepen the molecular understanding of pathogen behavior, and enable the development of novel therapeutics. NGS can rapidly sequence the genomes of infectious agents, allowing for a molecular-level understanding of pathogen behavior, evolution, and circulation within global populations (**Figure 2**). By empowering public health professionals with the ability to decode pathogens, more informed decisions can be made to keep the public safe.[24]

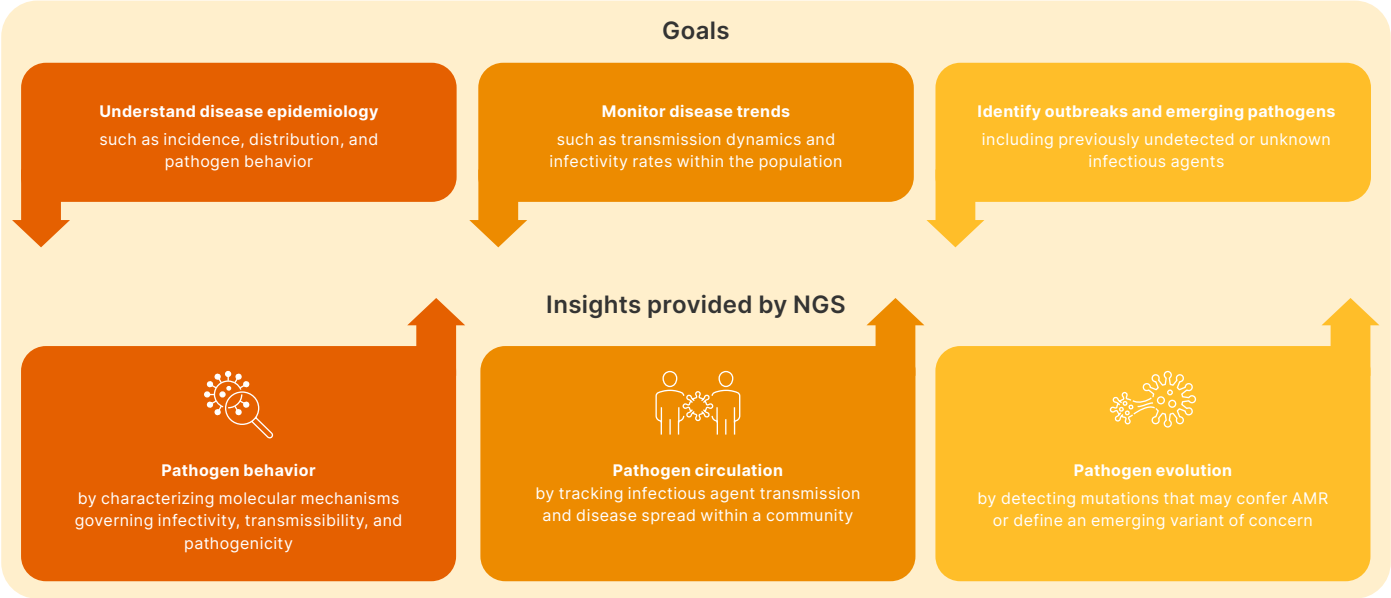
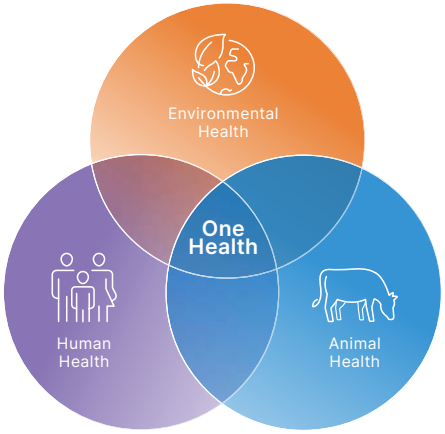


Figure 2: Infectious disease insights provided by NGS[24,25]

The One Health approach



NGS-based infectious disease surveillance has already supported several public health initiatives, including One Health, an interconnected approach that promotes human health by ensuring the health of animals and plants within the shared environment. One Health has implemented NGS to monitor threats to both humans and animals, including mobile genetic elements encoding antimicrobial resistance (AMR), genetic mutations associated with emerging strains of infectious agents, and genomic surveillance of environmental samples, such as wastewater. NGS has excelled in supporting the One Health approach in efforts to maintain public health.[26]

Harnessing the power of genomics: empowering a shift from reactive response to pandemic preparedness

Leveraging genomics to strengthen public health surveillance can advance the progress of outbreak and pandemic preparedness for infectious diseases. Genomic insights powered by NGS methods uphold three key pillars of pandemic preparedness: early detection, outbreak response, and clinical research (**Figure 3**).

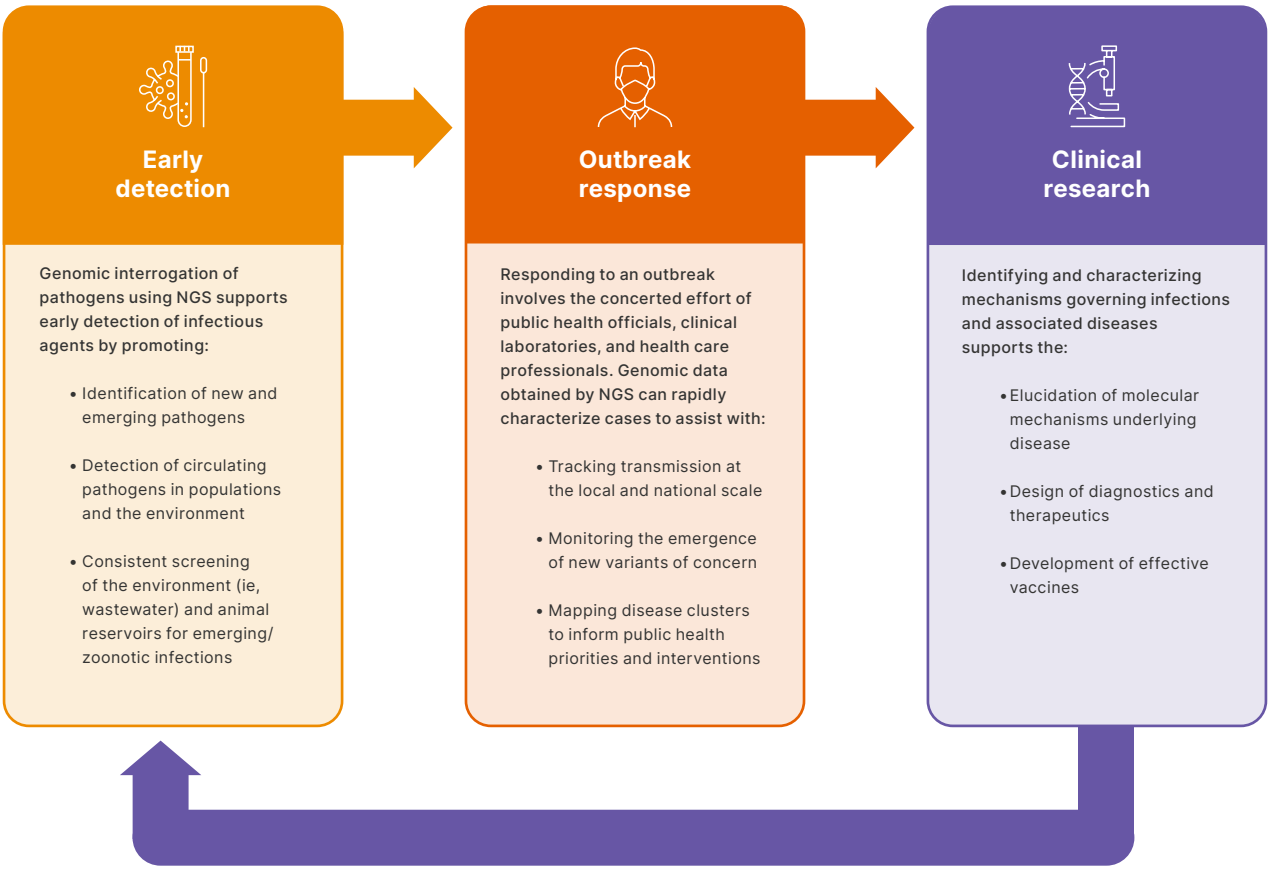


Figure 3: How NGS upholds three pillars of pandemic preparedness

By bolstering these three pillars, NGS-powered surveillance supports a robust feedback loop whereby the data generated through genomic epidemiology and research is used to drive better public health policies and interventions that prevent future outbreaks.

Key considerations for advancing the widespread adoption of NGS-powered surveillance

To address obstacles preventing widespread implementation of NGS into the public health architecture, the World Health Organization (WHO) has outlined five objectives for ensuring success[24]:

1. Improving access to tools for better geographic representation
2. Strengthening the workforce to deliver surveillance data at speed, scale, and quality
3. Enhancing data sharing and utility for streamlined local to global public health decision-making and action
4. Maximizing connectivity for timely value-add in the broader surveillance architecture
5. Maintaining a readiness posture for emergencies

Together, these objectives seek to (1) advance access to NGS technology, sequencing infrastructure, and hands-on sequencing and bioinformatics training, (2) establish data sharing standards, agreements, and principles, and (3) facilitate information sharing, collaborations, and a commitment to strengthening the process.

Ultimately, these changes can only be made by encouraging policymakers to advocate for, and raise awareness of, the value of NGS in the public health surveillance space.[24]

Funding for genomics-based infectious disease surveillance initiatives

Funding for projects focused on the research or implementation of NGS-powered infectious disease surveillance is primarily provided by international organizations, national government agencies, and private institutions. International organizations, such as the World Health Organization, the United Nations, and the European Commission, all finance programs to bolster infectious disease surveillance and response.[27-29] Moreover, national government agencies, such as the United States's National Institutes of Health, the United Kingdom's Department of Health and Social Care (DHSC), and the Japan International Cooperation Agency (JICA), offer grants that fund internal research programs and external academic laboratories.[30–32]

For example, the Fleming Fund, sponsored by DHSC, supports 25 countries across Africa and Asia in their efforts to tackle antimicrobial resistance.[31] Finally, the private sector also plays a pivotal role in allocating funds to national health agencies and research institutions. For example, the Global Fund program invests billions of dollars a year to mitigate worldwide HIV, TB, and malaria infections.[33] Moreover, the Bill and Melinda Gates Foundation funds various global research and development initiatives that seek to tackle global infectious diseases.[34] The collective support from these various funding sources enables ongoing research, technological advancements, and collaborative surveillance to safeguard worldwide public health.

Real-world applications of NGS technology

Successful integration of NGS-powered surveillance in Bangladesh[35,36]

Dr. Senjuti Saha, the Director at the Child Health Research Foundation (CHRF), has successfully fostered the growth of a genomics-based, NGS-powered surveillance program in Bangladesh. Founded in 1989, CHRF's early work to understand and improve local public health was performed when culture-based methods were the only available tool for distinguishing pathogens and detecting AMR. Their pathogen surveillance program began in 1993, with scientists still relying on the same culture-based methods. In 2002, conventional PCR tests were introduced to detect specific AMR genes in Haemophilus influenzae type b (HiB) from culture-negative samples. This effort led to the advocacy for, and implementation of, an HiB vaccine program in Bangladesh in 2009. By 2016, CHRF had replaced conventional PCR analysis with qPCR analysis to detect AMR genes in culture-negative samples.

Dr. Saha began working with CHRF in 2016, and in 2017, Dr. Saha's lab began implementing NGS-powered infectious disease surveillance. However, without sequencing instrumentation, samples had to be shipped to collaborators in resource-rich settings. Through this model, the lab identified the first ceftriaxone-resistant genome of Salmonella typhi in Bangladesh. However, given the slow timeframe to ship samples and receive results, the lab was limited in surveillance capacities. Finally, Dr. Saha's lab obtained its first sequencing system in 2018, allowing NGS analysis of various pathogens, including the first COVID-19 specimen, to be sequenced in Bangladesh. Since 2018, her lab has inspired other labs across Bangladesh to begin using NGS, leading to the sequencing of over 6,000 genomes and the establishment of NGS training programs across 30+ labs (**Figure 4**).

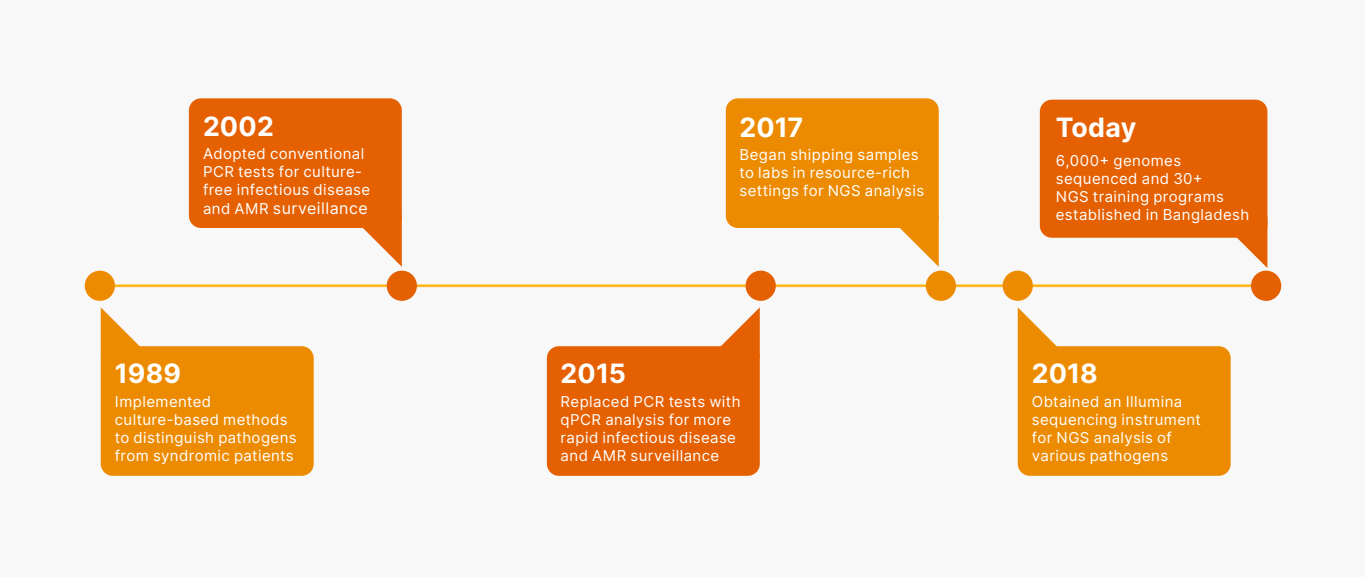


Figure 4: Steps taken in Bangladesh to integrate genomics with an aim to contribute to infectious disease surveillance and improve local public health

Dr. Saha's advocacy for and commitment to the decentralization of NGS technology and expertise have led to remarkable NGS capacity building in Bangladesh. This success serves as an exemplary illustration of the benefits that can be reaped when samples are sequenced and analyzed locally and sustainably.

Common NGS methods for infectious disease surveillance

Genomic surveillance of infectious diseases relies on multiple NGS methodologies, each with their own associated benefits and challenges. These NGS methods include shotgun DNA/RNA sequencing (metagenomics/metatranscriptomics), whole-genome sequencing (WGS) of isolates, hybridization capture, and amplicon sequencing (**Figure 5**). Culture-free NGS methods include shotgun DNA/RNA sequencing, hybridization capture, and amplicon.

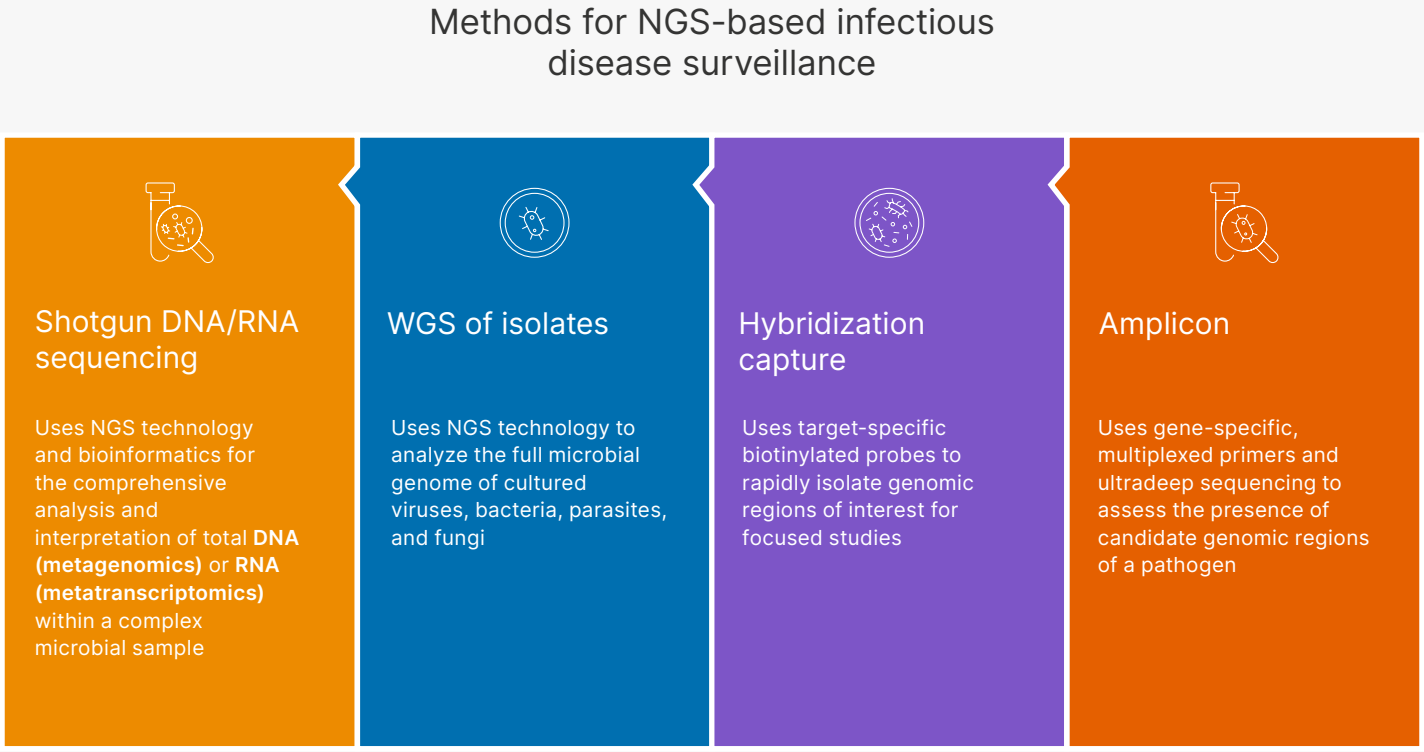


Figure 5: Methods for NGS-based infectious disease surveillance

Case studies: Early detection

Detecting circulating pathogens and AMR

Comparative analysis of drug-resistant *Mycobacterium tuberculosis (Mtb)* from sputum samples

NGS directly from uncultured sputum samples is a valuable tool with the potential to replace phenotypic DST for drug resistant tuberculosis for rapid resistance and DST.

- **Conducted by:** Microbiology Section, Department of Laboratory Medicine, P.D. Hinduja National Hospital and Medical Research Centre (Mumbai, India)
- **Funded by:** Indian Council of Medical Research (India), National Institutes of Health (United States), National Institute of Allergy and Infectious Diseases (United States)
- **NGS methodology used:** Amplicon sequencing
- **Sequencing platform:** iSeq™ 100 System
- **Description:** Rapid detection of drug-resistant mutations in *Mtb* strains is essential to combat their spread; however, traditional detection relies on time-consuming culture-based phenotypic tests or small-panel molecular tests. Researchers at the Hinduja National Hospital and Medical Centre in India compared these traditional approaches to an NGS-powered amplicon-based test. This amplicon-based test detected drug resistance with a faster turnaround time than culture-based phenotypic tests (median 3 vs. 21 days, respectively) and detected more drug resistance-associated loci than traditional molecular tests.[37]

A scalable model for NGS-based surveillance for influenza-like illness in the community

Hybridization capture NGS is a powerful tool for passive infectious disease surveillance of various human respiratory viruses.

- **Conducted by:** Aegis Sciences Corporation (Nashville, TN, USA)
- **Funded by:** Bill & Melinda Gates Foundation
- **NGS methodology used:** Hybridization capture
- **Sequencing platform:** NextSeq™ 550 System
- **Description:** Researchers at Illumina partnered with Aegis Sciences Corporation to evaluate a model for community-based surveillance of influenza-like illness using hybridization capture NGS. Upper respiratory samples collected from across the United States were analyzed using the Illumina Respiratory Pathogen ID/AMR Panel (RPIP). Results from this study showed that though symptomatic, not just one pathogen was circulating in the broad community, but rather, multiple pathogens were presenting with common symptoms. Overall, this study positioned hybridization capture NGS as a powerful tool for passive infectious disease surveillance of various human respiratory viruses.[38]

Enhanced detection system for healthcare-associated transmission of infection

WGS surveillance combined with electronic health record machine learning has the potential to save costs and enhance patient safety.

- **Conducted by:** Microbial Genomic Epidemiology Laboratory, Center for Genomic Epidemiology, University of Pittsburgh (Pittsburgh, PA, USA)
- **Funded by:** National Institutes of Health (United States)
- **NGS methodology used:** WGS of isolates
- **Sequencing platform:** NextSeq 500 System
- **Description:** Healthcare-associated infections (HAIs) are unanticipated infections that develop during a patient's hospital or long-term care stay. Current methods for detecting outbreaks of HAIs in hospitals are outdated and insufficient for disease prevention. Researchers at the Center for Genomic Epidemiology at the University of Pittsburgh developed an enhanced detection system for healthcare-associated transmission of HAIs. This system combined WGS and a machine-learning algorithm trained on electronic health records from HAI patients at the University of Pittsburgh Medical Center (UPMC). By combining these two technologies, researchers were able to identify routes of transmission during active HAI outbreaks.[39]

Characterizing new and emerging pathogens

Identification of the SARS-CoV-2 virus

Sequencing was used to identify the novel coronavirus causing COVID-19 (SARS-CoV-2) early in the outbreak.

- **Conducted by:** Shanghai Public Health Clinical Center, The Central Hospital of Wuhan
- **Funded by:** Center for Disease Control and Prevention (China), National Institute for Communicable Disease Control and Prevention (China)
- **NGS methodology used:** Metatranscriptomic RNA sequencing
- **Sequencing platform:** MiniSeq™ System
- **Description:** After molecular tests ruled out other potential etiological agents, NGS analysis of a bronchoalveolar lavage fluid sample isolated from a patient in Wuhan, China suffering from severe respiratory disease identified a novel coronavirus in December 2019. This virus was originally named "WH-Human 1" coronavirus after initial phylogenetic analysis revealed the virus was most closely related to a group of SARS-like coronaviruses previously identified in Chinese bats. Rapid identification of this causative agent, later named SARS-CoV-2, marked the start of the COVID-19 pandemic.[40]

Screening animal reservoirs

Antimicrobial resistance monitoring on Italian swine farms

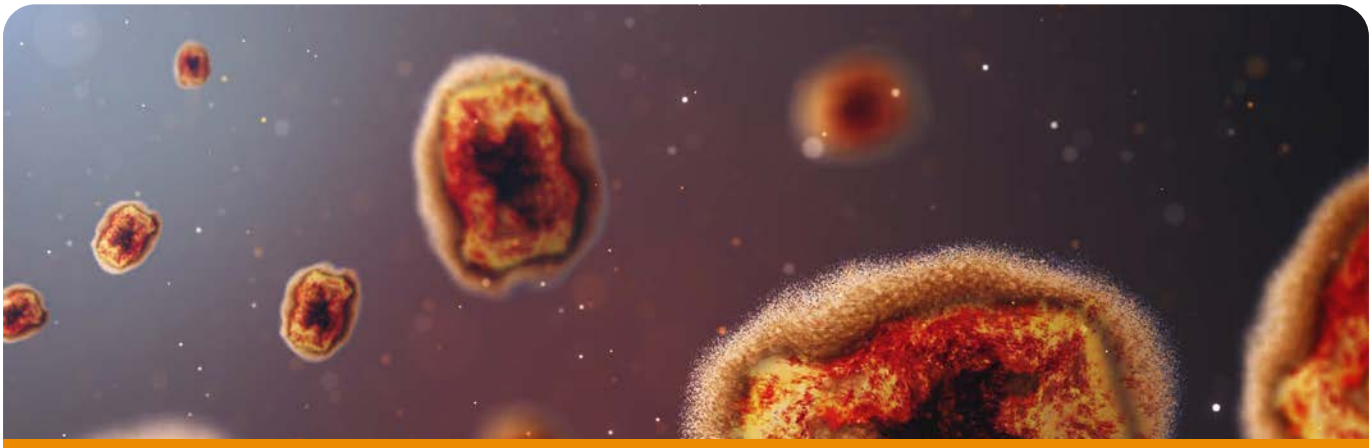
WGS surveillance of E. coli strains present at the farm level will be important to identify new or human-related clones that can disseminate in the swine production chain.

- **Conducted by:** Department of Veterinary Sciences, University of Turin (Grugliasco, Italy)
- **Funded by:** Ministry of Education (Italy)
- **NGS methodology used:** WGS of isolates
- **Sequencing platform:** MiSeq™ System
- **Description:** As part of a routine animal surveillance program for AMR emergence, WGS was used to analyze commensal *E. coli* genomes isolated from 30 pigs across four different pigsties in northern Italy. Results found that all 30 strains of commensal *E. coli* harbored mutations conferring multidrug resistance (MDR) or the production of extended-spectrum β-lactamases (ESBL), which renders several antimicrobials ineffective. The alarming discovery that pigs could serve as a reservoir for MDR and ESBL-producing *E. coli* has sparked concern, as these bacteria could potentially spread to humans via the food chain or direct contact with animals. This study prompted growing advocacy for the continued genomic surveillance of *E. coli* strains in pigs.[41]

Hendra virus surveillance in Australian horses for the detection of novel variants

This study exemplifies the power of NGS as a genomic surveillance method and highlights the need for updated routine surveillance to uphold the One Health initiative to protect the interconnected health of animals and humans.

- **Conducted by:** University of Sydney School of Veterinary Science and Institute for Infectious Diseases (Sydney, NSW, Australia)
- **Funded by:** Department of Agriculture (Australia), Water and the Environment Biosecurity Innovation Program (Australia), National Institute of Allergy and Infectious Diseases (United States), National Institutes of Health (United States)
- **NGS methodology used:** Metatranscriptomic sequencing
- **Sequencing platform:** NextSeq 500 System
- **Description:** Genomic surveillance for the highly pathogenic zoonotic Hendra virus (HeV), which naturally infects bats, has identified several spillover events during which HeV was transmitted from bats to horses. Although horse-to-human transmission of HeV is rare (only seven human cases have been reported), the fatality rate in humans is high at 57%.[42] In September 2015, an Australian horse exhibiting HeV symptoms was humanely euthanized and tested for HeV. However, routine quantitative RT-PCR and ELISA testing did not identify HeV genomic material or proteins, respectively. Therefore, researchers performed deep NGS of the sample and identified a novel variant of HeV that evaded detection by routine sentinel surveillance. This study exemplifies the power of NGS as a genomic surveillance method and highlights the need for updated routine surveillance to uphold the One Health initiative to protect the interconnected health of animals and humans.[26,43]



Early detection of mpox virus (MPXV)

In May 2022, an outbreak of mpox (formerly called “monkeypox”) unfolded across the world, driven by in-country transmission events within previously unaffected countries. On July 23, 2022, WHO declared the outbreak a public health emergency of international concern. In June 2022, metagenomic analysis enabled the rapid reconstruction of the mpox outbreak genome.[44] By September 2022, a total of 1,619 MPXV genomic sequences had been reported by country-specific researchers and public health surveillance groups. Analysis of this large sequence database identified that all documented sequences belonged to the same clade (clade IIb) except for four cases from the Democratic Republic of the Congo. Further analysis revealed the clade IIb MPXV sequences clustered with two isolated outbreaks of MPXV in the UK in 2018 and 2019.[45] These observations obtained through NGS analysis provided early insights into differences in regional trends, risk factors, demographics, and clinical characteristics of MPXV infection.

Case studies: Outbreak response

Transmission tracking

Genomic investigation of two concurrent outbreaks of *Salmonella Enteritidis* in South Africa

The use of WGS analysis of *Salmonella Enteritidis* to complement outbreak investigations is increasing globally, as public health investigators begin to understand its application in epidemiological case linking and source attribution.

- **Conducted by:** Centre for Enteric Diseases, National Institute for Communicable Diseases (Johannesburg, South Africa)
- **Supported by:** Centres for Disease Control and Prevention (Africa), Centre for Enteric Diseases (South Africa), National Institute for Communicable Diseases (South Africa)
- **Sequencing platform:** MiSeq System
- **Description:** PulseNet is a network of national and local laboratories dedicated to NGS analysis of the causative agents of foodborne illness outbreaks to identify the source of the infection. When the network identifies a cluster of patients infected with the same causative agent, national health agencies are notified to initiate rapid interventions, such as recalling or removing contaminated food products.[46] In 2018, the PulseNet principle led to the genomic investigation of two concurrent outbreaks of *Salmonella Enteritidis* in South Africa—one in children at a daycare center ("Outbreak A") and one in adults who all ate at the same restaurant ("Outbreak B"). WGS analysis of bacterial isolates from patients affected by the outbreaks revealed that contaminated eggs from the restaurant were the likely source of the *Salmonella Enteritidis* that caused Outbreak B. Moreover, comparative analysis of the genomes of the *Salmonella Enteritidis* strains that caused Outbreak A and Outbreak B revealed high genetic similarity, suggesting a high probability of epidemiological relatedness. This finding indicated the likelihood of a larger contamination problem with the same clone of *Salmonella Enteritidis* within the wider chicken and egg-producing industry of South Africa.[47]

Swiss public health measures to reduce SARS-CoV-2 transmission using genome data

Data from genomic surveillance helps public health officials understand transmission dynamics and guide implementation of public health policies.

- **Conducted by:** Swiss SARS-CoV-2 Sequencing Consortium (Switzerland)
- **Funded by:** Swiss National Science Foundation (Switzerland)
- **NGS methodology used:** Amplicon (WGS)
- **Sequencing platform:** MiSeq and NovaSeq™ 6000 Systems
- **Description:** During the COVID-19 pandemic, the Swiss public health system sequenced over 11,000 SARS-CoV-2 genomes in 2020 alone, the sixth-largest genomic surveillance effort globally at the time. Genomic data derived from these sequences were used to reconstruct introductions of the virus to Switzerland to estimate the effectiveness of the country's strict border closures. Analysis of this data revealed that strict border closures in 2020 resulted in an 86–98% reduction in virus introductions to Switzerland from neighboring countries, supporting the value of genomic surveillance as a measure to understand transmission dynamics.[48]

Monitoring the emergence of variants

Genomic surveillance of lumpy skin disease virus from an outbreak in India

This study highlights the importance of using genomic surveillance to characterize circulating strains of infectious agents to detect outbreaks and formulate effective interventions for disease control.

- **Conducted by:** CSIR Institute of Genomics and Integrative Biology (Delhi, India)
- **Funded by:** Indian Council of Scientific & Industrial Research (India)
- **NGS methodology used:** Amplicon (WGS)
- **Sequencing platform:** NovaSeq 6000 System
- **Description:** An outbreak of lumpy skin disease virus (LSDV) was reported in cattle in mid-2022 in India, which spread across seven states and resulted in the death of over 80,000 cattle over a three month period. WGS analysis of six viral isolates revealed a total of 177 unique variations, 47 of which were identified as novel mutations, suggesting the rapid emergence of novel variants that formed a distinct genetic lineage, prompting research into the mechanism of transmission. This study highlights the importance of using genomic surveillance to characterize circulating strains of infectious agents to detect outbreaks and formulate effective interventions for disease control.[49]

A genomics solution for HIV surveillance in low-income settings

This high-resolution characterization of transmission events, powered by NGS, has the potential to enhance the effectiveness of targeted responses and precise public health interventions during local HIV outbreaks.

- **Conducted by:** Wellcome Centre for Human Genetics and Li Ka Shing Centre for Health Information & Discovery, University of Oxford (Oxford, United Kingdom)
- **Funded by:** National Institute of Allergy and Infectious Diseases, US President's Emergency Plan for AIDS Relief, National Institute of Mental Health, National Institute on Drug Abuse (United States), Bill and Melinda Gates Foundation
- **NGS methodology used:** Amplicon (WGS)
- **Sequencing platform:** MiSeq System
- **Description:** The development of a high-throughput NGS-based platform made possible the analysis of HIV genomes derived from residual blood samples initially collected for routine CD4 cell count testing. This breakthrough enabled 10 urban clinics in Zambia to screen for drug-resistant HIV variants in a rapid, efficient, and cost-effective manner. Using this system to analyze viruses obtained from transmission pairs revealed that certain drug-resistant HIV subpopulations were transmitted to the recipient, while others were not transmitted, providing insight into the communicability of certain HIV variants. This high-resolution characterization of transmission events, powered by NGS, has the potential to enhance the effectiveness of targeted responses and precise public health interventions during local HIV outbreaks.[50]

Surveillance of endemic Crimean-Congo hemmorrhagic fever virus (CCHFV) in Pakistan

The results emphasize a need for the establishment of NGS facilities in areas of Pakistan where disease burden is high.

- **Conducted by:** National Institute of Health (Pakistan)
- **Funded by:** National Institute of Health (Pakistan)
- **NGS methodology used:** Shotgun RNA sequencing (WGS)
- **Sequencing platform:** MiSeq System
- **Description:** Local surveillance of circulating strains of CCHFV, a tick-borne viral zoonotic disease associated with fatal hemorrhagic fever in humans, revealed the emergence of a reassorted strain that had not been previously detected in Pakistan. Resultant sequencing of the complete genomes of circulating strains using shotgun RNA sequencing revealed a wide breadth of genetic diversity, with local CCHFV harboring mutations associated with strains from other regions in Asia and from Africa. The detection of reassorted viruses highlighted the importance of using NGS to detect reassortment events earlier and to understand the origin, prevalence, and evolution of highly mutated strains. The results also emphasize a need for the establishment of NGS facilities in areas of Pakistan where disease burden is high.[51]

Pathogenwatch: A community-driven resource for genomic epidemiology and antimicrobial resistance prediction of *Neisseria gonorrhoeae*

Community-driven tools can provide easy access to and analysis of genomic and epidemiological data to enhance public health surveillance.

- **Funded by:** Centre for Disease Prevention and Control (Europe), National Institutes of Health (United States), National Science Foundation
- **NGS methodology used:** WGS of isolates
- **Sequencing platform:** MiSeq System
- **Description:** A rise in AMR to last-line antibiotics has recently been detected in certain strains of *Neisseria gonorrhoeae*, the causative agent of the sexually transmitted infection gonorrhea. Pathogenwatch is a community-steered, web-based bioinformatics powered platform that compiles public genomic sequences of *N. gonorrhoeae*, obtained by WGS, to track AMR emergence.[52] In one study, researchers used Pathogenwatch to identify an emerging lineage of azithromycin-resistant *N. gonorrheoeae*, supporting the utility of this platform for tracking and combatting AMR variants.[53]

Outbreak response for mpox

After the MPXV strains responsible for the 2022 international outbreak of mpox were identified and characterized, public health officials implemented outbreak response initiatives to track emerging variants of concern. Several public health databases emerged to improve data sharing, including the Monkeypox Virus resource (MPoxVR), the mpoxSprectum dashboard, and the MpoxRadar dashboard. Each of these programs compiled genomic surveillance data obtained from NGS analysis of infected patient samples towards the shared goal of tracking transmissions and combatting disease spread.[45,54]

In addition to variant surveillance, public health agencies initiated surveillance programs to screen potential animal reservoirs of MPXV in order to minimize zoonotic transmission of mpox. Though the natural animal reservoir for MPXV is unknown, previous studies have found that more than 50 species of mammals can be infected with MPXV, suggesting a high likelihood of interspecies transmission.[55]

Case studies: Clinical research

Understanding disease mechanisms

Investigating the mutational frequency of latent tuberculosis infection (LTBI) in human patients

This data supported the urgency of treating LTBI early– as many anti-TB drugs work only on actively replicating TB– and highlighted the need for alternative strategies for anti-TB drug development.

- **Conducted by:** Rutgers-New Jersey Medical School
- **Funded by:** National Institutes of Health, National Institute of Allergy and Infectious Diseases (United States)
- **NGS methodology used:** WGS of cultured isolates
- **Sequencing platform:** HiSeq™ 2500 System
- **Description:** Patients infected with the *Mtb* bacterium can develop either active tuberculosis (TB) infection or latent TB infection. Even though 25% of TB-infected patients develop LTBI, little is known about the physiology underlying its reawakening to cause active disease. To assess the mutation rate and generation times of latent *Mtb*, strains isolated from latently infected household contacts of active TB patients were sequenced using NGS throughout latency. By measuring the number of accumulated single nucleotide polymorphisms (SNPs), researchers were able to conclude that mutation rates of *Mtb* remain high up to two years into latency and then drop off, indicating a transition into a quiescent state. This data supported the urgency of treating LTBI early — as many anti-TB drugs work only on actively replicating TB — and highlighted the need for alternative strategies for anti-TB drug development.[56]

Identification of complex DNA structures that mediate antimalarial resistance mutations in the human malaria parasite

Whole genome sequencing of wildtype and CNV-containing clones of *Plasmodium* reveal high likelihood of DNA hairpin formation near CNVs in antimalarial-resistant strains of *Plasmodium*.

- **Conducted by:** University of Virginia Health System
- **Funded by:** National Institutes of Health (United States)
- **NGS methodology used:** Leveraged *P. falciparum* WGS data
- **Sequencing platform:** MiSeq and NovaSeq™ 6000 Systems
- **Description:** Antimalarial resistance, conferred by copy number variations (CNVs) that facilitate the overexpression of drug targets to bypass antimalarials, is a major obstacle in the eradication of the human malaria parasite *Plasmoidum falciparum*. Prior research had identified long A/T nucleotide tracks flanking these resistance-associated CNVs, which are known to be associated with double-stranded breaks (DSBs) in DNA that lead to error-prone repair underlying CNV generation. However, A/T tracks alone are unlikely to trigger DSBs without another signal, such as a DNA hairpin. Therefore, researchers hypothesized that DNA hairpins in the *Plasmodium* genome may be trigger sites for DSBs that ultimately lead to CNV formation. Whole genome sequencing of wildtype and CNV-containing clones of *Plasmodium* was carried out and results were analyzed to reveal high likelihood of DNA hairpin formation near CNVs in antimalarial-resistant strains of *Plasmodium*. Futher investigation into these mechanisms may reveal targetable DNA repair pathways that could block adaptation to antimalarial drugs.[57]

Designing diagnostics, vaccines, and therapeutics

Determining factors of transmission and evolution of SARS-CoV-2 in populations at risk

Collaborative science and open sharing of results must continue to ensure that variants are rapidly identified and characterized to determine their impact on transmission, infection, replication, and immune evasion.

- **Funded by:** National Institutes of Health, National Institute of Allergy and Infectious Diseases (United States)
- **NGS methodology used:** Leveraged SARS-CoV-2 WGS data shared in public databases
- **Description:** Researchers across several academic and government labs collaborated to identify and characterize emerging variants of SARS-CoV-2 from NGS data obtained through the NIH-funded "SARS-CoV-2 Assessment of Viral Evolution (SAVE)" program. The SAVE program is divided into three working groups: (1) early detection and analysis; (2) *in vitro* analysis, and (3) *in vivo* analysis. The early detection and analysis group used NGS-powered genomic surveillance to track emerging variants, identify mutations in the viral spike protein, and rank variants by degree of concern. The ranked variants were then passed onto the *in vitro* group for functional characterization using various *in vitro* assays. Variants with distinct functional characteristics were then used by the *in vivo* group for vaccine and transmission analyses. Variants of highest concern were reported to the SARS-CoV-2 Interagency Group (SIG) for the development of public health policy, countermeasures, and development of diagnostics. Importantly, the SAVE group rapidly responded to the emergence of the highly transmissible Omicron variant of SARs-CoV-2 by (1) isolating, propagating, and distributing viral stocks, (2) performing binding and neutralization assays, and (3) evaluating viral infection dynamics across various animal models. Ultimately, this data was shared with government agencies to promote effective interventions.[58]

Moderna's COVID mRNA vaccine

Genomic sequencing enabled the development of an mRNA vaccine to a novel pathogen in under a year.

- **Funded by:** Department of Defense (United States), Moderna
- **Description:** Since its inception in 2010, the United States-based biotech company Moderna has been developing mRNA vaccine technology for a myriad of therapeutic areas. After the first novel coronavirus sequence was made public at the beginning of the COVID-19 pandemic in January 2020, Moderna rapidly shifted its mRNA bioplatform towards the development of an mRNA COVID vaccine. Within a month, Moderna delivered the first doses of its COVID-19 vaccine to the United States National Institutes of Health for testing on 30,000 volunteers. On December 18, 2020, just 10 months after the first doses were developed, the United States Food and Drug Administration (FDA) authorized the vaccine for emergency public use.[59]

Clinical research for mpox

The 2022 outbreak of MPXV stimulated research to uncover the mechanism of MPXV pathogenesis and immunity, towards the development of antivirals and vaccines. Analysis of MPXV sequences obtained through metagenomics-powered surveillance strategies identified that the 2022 outbreak MPXV strain harbored 50 SNPs compared to a 2018-2019 outbreak strain. When considering the predicted rate of MPXV evolution would only account for 1–2 substitutions per genome per year, these 50 mutations suggested accelerated evolution of the 2022 outbreak strain. Researchers hypothesized this hypermutation signature might be mediated by the apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (APOBEC3) enzyme, which causes the same distinct mutational bias observed in the 2022 outbreak strain. While this hypothesis has not yet been verified experimentally, this genomic-based hypothesis is likely to drive additional research.[44]

Illumina NGS solutions for infectious disease surveillance

The NGS workflow

Across NGS methods, the standard workflow entails the (1) isolation of genetic material, (2) preparation of sequencing-ready libraries, (3) sequencing of libraries, and (4) analysis and interpretation of sequencing data (**Figure 6**).

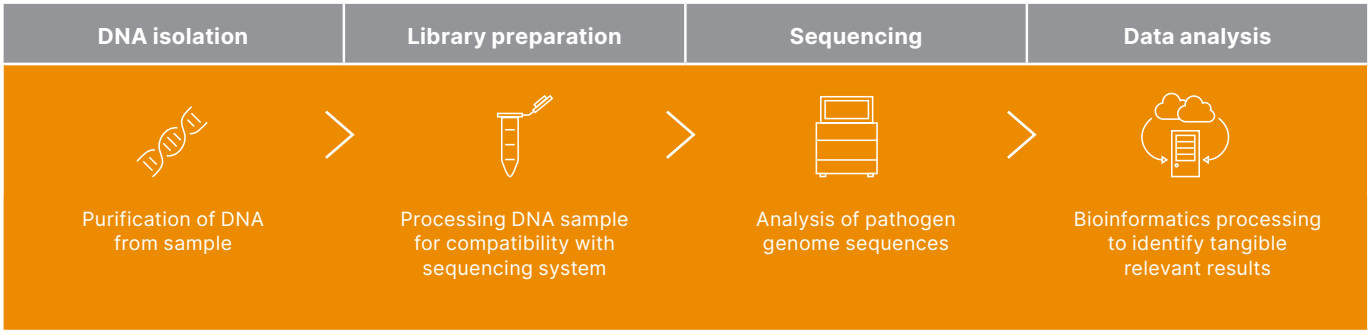


Figure 6: Standard workflow for Illumina NGS

Depending on the pathogen(s) of interest, sample type, and the data requirements needed for surveillance, different NGS workflows and solutions can be utilized. The selection of the optimal workflow should be determined by what needs to be achieved. Is it the analysis of a single known pathogen from culture or a primary sample? Is it the sequencing of multiple pathogens deemed a high priority by a national suveillance program? Or is it the hypothesis-free discovery of unknown or unculturable pathogens? Single genome, multi-pathogen, and discovery workflows can enable the full array of infectious disease surveillance applications.

For each NGS workflow, Illumina offers powerful library preparation, sequencers, and corresponding data analysis applications. Any of the Illumina sequencing systems can be used for any of the approaches described herein; however, some sequencing systems are better suited for certain methods and applications, as indicated in the following sections.

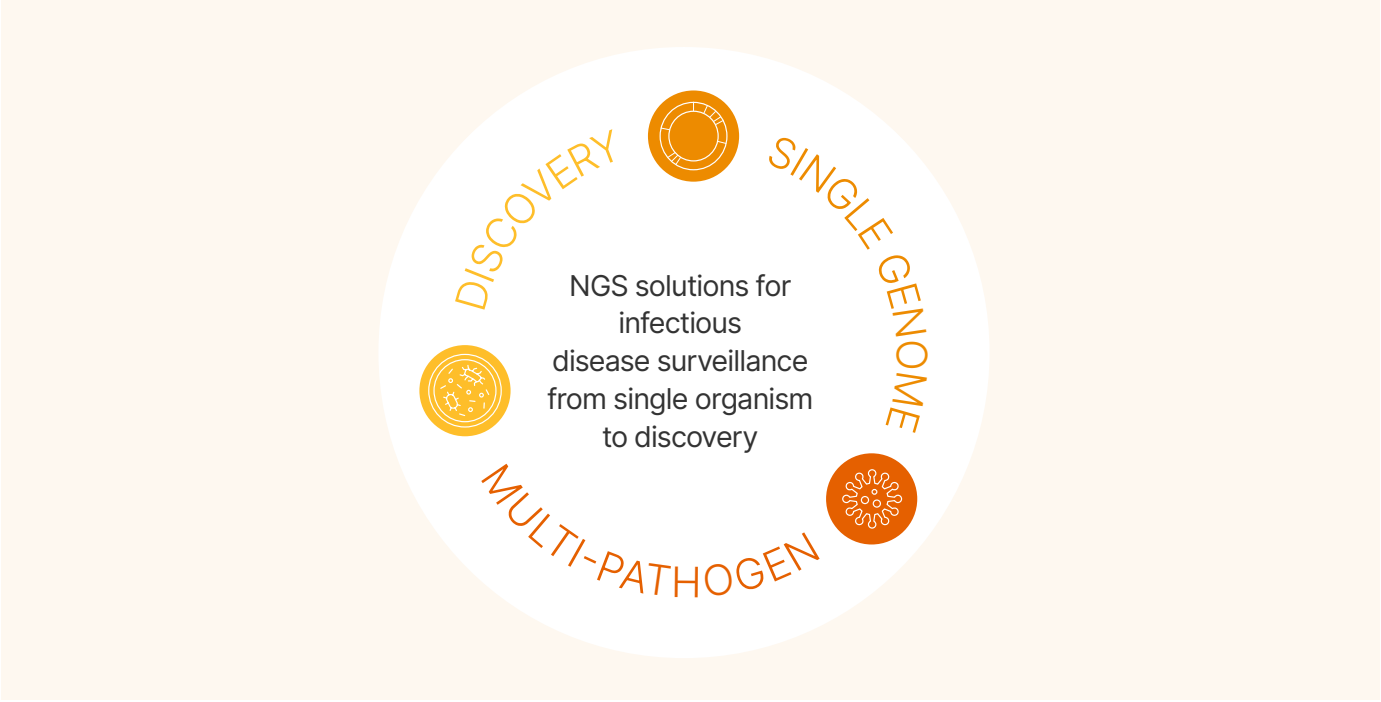


Figure 7: NGS workflows and solutions can be matched to support surveillance goals, including single genome, multi-pathogen, and discovery workflows

Single-genome analysis

Single-genome sequencing is best utilized in situations when the researcher knows what they are looking for. Whether the pathogen is from an isolated microbial colony on a culture plate or a primary sample is being surveilled for a determined pathogen of interest, the common thread is that a single, known genome is being analyzed.

Amplicon sequencing and whole-genome sequencing of isolates are the primary methods for single-genome analysis. Amplicon sequencing uses gene-specific primers to enrich known targets for ultra deep sequencing. This method does not require cultured isolates and can be used to detect specific, predefined genes associated with microbial identity, AMR mutations, or variants of concern. WGS of isolates refers to the culture of a specific, known microbe to reveal its genomic sequence, providing a comprehensive view of the genes underlying its biology and physiology.

Table 2: Solutions supporting single genome analysis

Product	What it does	Method	Recommended sequencers	Recommended analysis apps
Illumina DNA Prep	Whole-genome sequencing of cultured microbial isolates	Whole-genome sequencing of isolates	iSeq 100 System, MiniSeq System, MiSeq Series, NextSeq 550 Series, NextSeq 1000, and NextSeq 2000 Systems	SRST2 App, SPAdes Genome Assembler App
Illumina and GenoScreen Deeplex Myc-TB Combo Kit	Targeted sequencing for identification of MTBC, 15 AMR markers, and >100 non-TB mycobacteria	Amplicon sequencing (tNGS)		Deeplex Web App
COVIDSeq Assay and COVIDSeq Test	Full genome sequencing of SARS-CoV-2	Amplicon sequencing (WGS)		COVID Lineage App, DRAGEN™ Targeted Microbial App
Illumina Microbial Amplicon Prep–Influenza A/B (IMAP-Flu)	Full genome sequencing of Influenza A & B	Amplicon sequencing (WGS)		DRAGEN Targeted Microbial App
Illumina Microbial Amplicon Prep (IMAP)	Open sequencing solution for infectious disease surveillance and microbiology research	Amplicon sequencing		DRAGEN Targeted Microbial App

Multi-pathogen analysis

For instances where certain pathogens are suspected to be present, or for surveillance of multiple known pathogens from a primary sample, hybrid capture enrichment solutions are ideal. Hybridization capture uses target-specific probes to rapidly isolate and enrich genomic regions of interest from primary samples (e.g., sputum, urine, wastewater, etc.) for subsequent sequencing. This NGS method does not require cultured isolates and can be used to detect specific microbes and certain AMR mutations.

Table 3: Solutions supporting multi-pathogen analysis

Product	What it does	Method	Recommended sequencers	Recommended analysis apps
Viral Surveillance Panel (VSP)	Culture-free whole genome characterization of the most critical viral public health threats	Hybrid-capture enrichment	MiniSeq System, MiSeq Series, NextSeq 550 Series, NextSeq 1000 and NextSeq 2000 Systems	DRAGEN Targeted Microbial App
Pan Coronavirus Panel (Pan-CoV)	Culture-free whole genome characterization of known and closely related novel coronaviruses			DRAGEN Targeted Microbial App
Respiratory Virus Enrichment Kit (RVEK)	Culture-free whole genome characterization of common respiratory viruses			DRAGEN Targeted Microbial App
Respiratory Pathogen ID/ AMR Panel (RPIP)	Culture-free identification and quantitation of organisms associated with lower respiratory tract infections			Explify RPIP App
Urinary Pathogen ID/AMR Panel (UPIP)	Culture-free identification and quantification of both common and underrecognized uropathogens that can cause urinary tract infections and sexually transmitted infections			Explify UPIP App

Discovery of pathogens

A useful approach for truly unbiased analysis of a primary sample, shotgun DNA/RNA sequencing (metagenomic or metatranscriptomic) analysis of the genetic material present in complex microbial communities, such as environmental samples (e.g., wastewater), human microbiomes, and primary samples (e.g., sputum, lower respiratory aspirates), can reveal the species-level composition of the community without any prior knowledge.

Table 4: Solutions supporting the discovery of pathogens

Product	What it does	Method	Recommended sequencers	Recommended analysis apps
Illumina DNA Prep	Comprehensive sequencing of all genes in all organisms present in a given complex sample	Shotgun DNA sequencing (metagenomics)	NextSeq 550 Series, NextSeq 1000 and NextSeq 2000 Systems, NovaSeq 6000 Series, and NovaSeq X Series	DRAGEN Metagenomics Pipeline
Illumina Stranded Total RNA Prep with Ribo-Zero Plus Microbiome	Fast, efficient depletion of undesirable host and pan-bacterial rRNA from complex microbial samples for metatranscriptomics applications	Shotgun RNA sequencing (metatranscriptomics)		Microbiome Metatranscriptomics App

Technical support and training programs

Illumina offers a wide range of educational, support, and training programs to facilitate the globalization of NGS-based public health surveillance tools.

Global education programs
Providing STEM education tools to bring the powerful field of genomics into local classrooms

Comprehensive global product support services and supply chain
Tailored service plans for accessing Illumina expertise through global customer support teams, manufacturing locations, and regional supply chains

Online webinars and training videos
Streamlined how-to videos catered to inexperienced NGS users

With Illumina as a trusted partner, a team of experts is available for support 24/7 globally.

Illumina is committed to promoting global health

Illumina supports global public health surveillance initiatives through state-of-the-art library preparation kits, sequencing systems, and robust bioinformatics solutions.

Beyond product offerings, Illumina offers a wide range of educational resources, support, and training programs to facilitate the globalization of NGS-powered public health surveillance to:

- Strengthen health systems and enhance equitable access to NGS technology
- Decentralize hands-on sequencing and bioinformatics training
- Build public health surveillance capabilities through innovative NGS solutions and education

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