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RECENT ADVANCES FOR CANCER AND INFECTIOUS DISEASE DIAGNOSTIC: SEQUENCING

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ABSTRACT

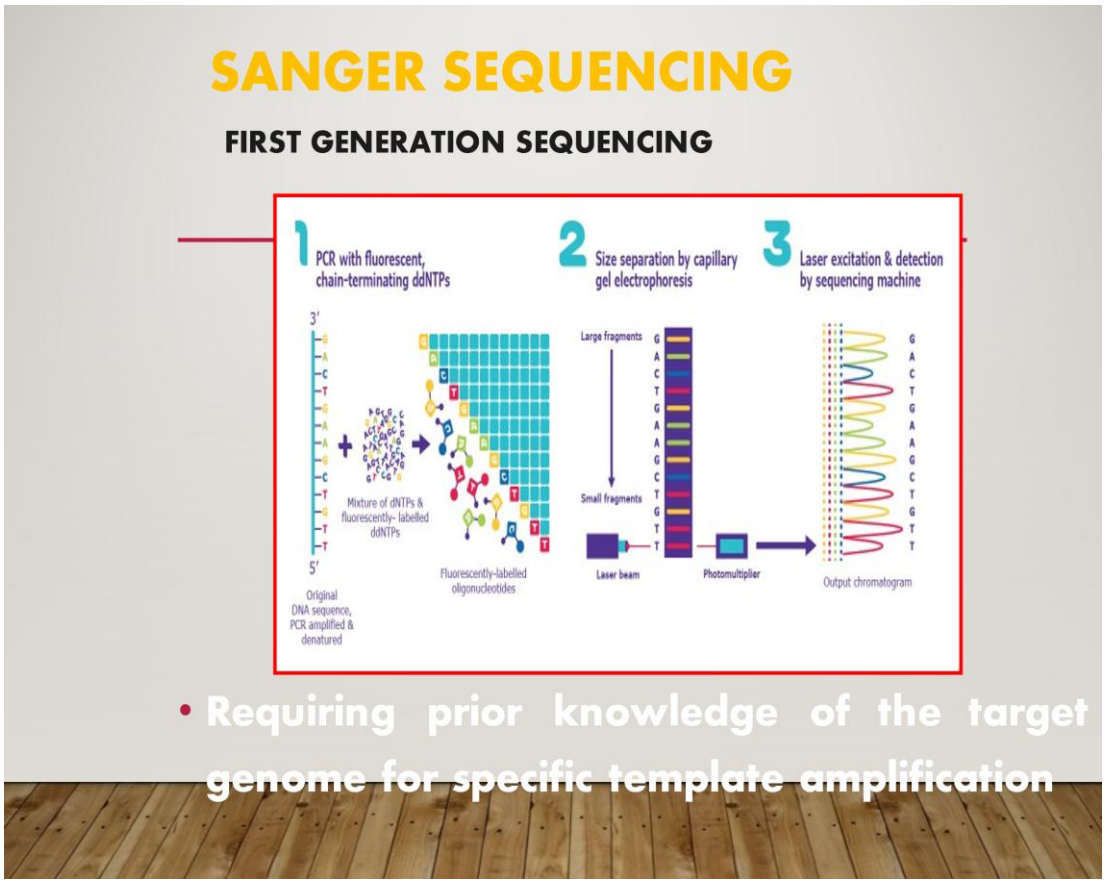
Next-generation sequencing (NGS) also referred to as deep, high- throughput or massively parallel sequencing, is a powerful new tool that can be used for complex diagnosis and intensive monitoring of infectious disease in veterinary medicine. NGS technologies are also being increasingly used to study the aetiology, genomics, evolution and epidemiology of infectious disease, as well as host -pathogen interactions, host immune responses, Complexity of infection biology, including responses to antimicrobial treatments and vaccination. NGS approaches can function as important tools for studying disease outbreaks by identifying and following transmission routes, thereby facilitating the identification of outbreak origins in zoonotic diseases. The topic covers the era of sequencing, steps in next gene sequencing, mainly various application of NGS in disease and cancer diagnosis, novel pathogen discovery, genome characterization and viral diversity, viral metagenomics, host pathogen interaction, chromatin immunoprecipitation and transcriptomics. Since the introduction of NGS platforms, continuous developments and improvements have resulted in update instruments with increases capacity to generate sequence data. Consequently, there has been a corresponding dramatic decrease in the cost per base. In Veterinary complex diseases such as post weaning multisystemic wasting syndrome, investigations of infections and diseases in cattle and sheep included characterizing and determining the phylogeny of new bluetongue virus variants ; determining host viral population diversity ; and detecting unknown and emerging new pathogens most notably the Schmallenberg virus characterisation and phylogenetic analysis of new variants of various viruses; the detection of unknown and unexpected pathogens; investigation of poultry microbiota; host pathogen interactions; in detecting and identifying some of the newly emerging variants of infectious agents have been studied using NGS.

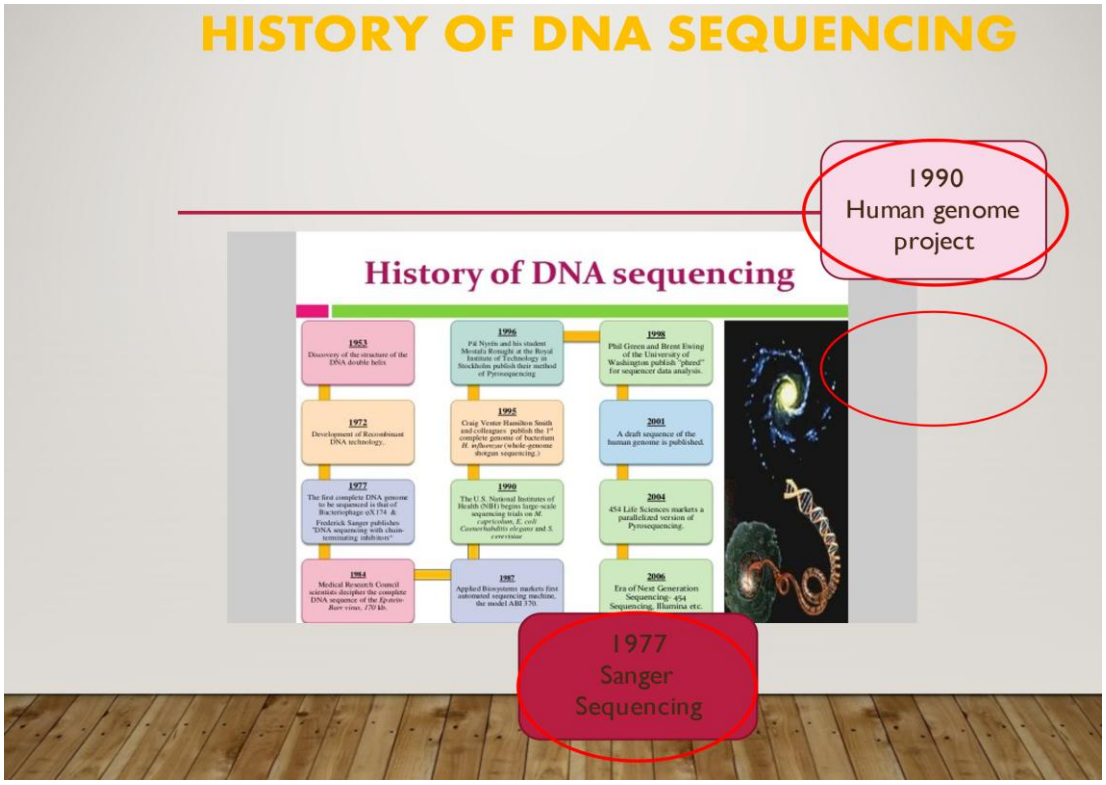
INTRODUCTION

Genetic characterization of infectious agents plays a central role in the diagnosis, monitoring, and control of infectious diseases. The development of rapid DNA sequencing methods based on the selective incorporation of chain-terminating dideoxynucleosides later termed "first-generation sequencing technologies" and the polymerase chain reaction (PCR) DNA amplification technologies has paved the way for the study of biological and evolutionary processes at the molecular level. Such technologies have been extensively applied to the diagnosis and molecular epidemiology of infectious diseases of livestock and become important tools for targeted research on host-pathogen interactions. This paradigm shift in the scale of DNA sequence data has revolutionized the way biological and evolutionary processes can be studied at the molecular level, enabling genome projects previously restricted to high profile model organisms and human pathogens to target pathogens of lesser economic and medical significance. Such advancements are now being increasingly applied to veterinary medicine. As a result, the increasing availability of these technologies combined with the rapid development of applied tools and protocols has provided a diverse array of applications for use in genomics and transcriptomics and even routine diagnostics. Over the last five years, NGS has been used as an extremely important tool in the tracing of transmission, genome characterization, and outbreak management of both viral and bacterial diseases.

Next-generation sequencing platforms provide unprecedented throughput, generating hundreds of gigabases of data in a single experiment. Although the initial capital investment and cost per experiment remain high, the price per information unit (nucleotide) has been

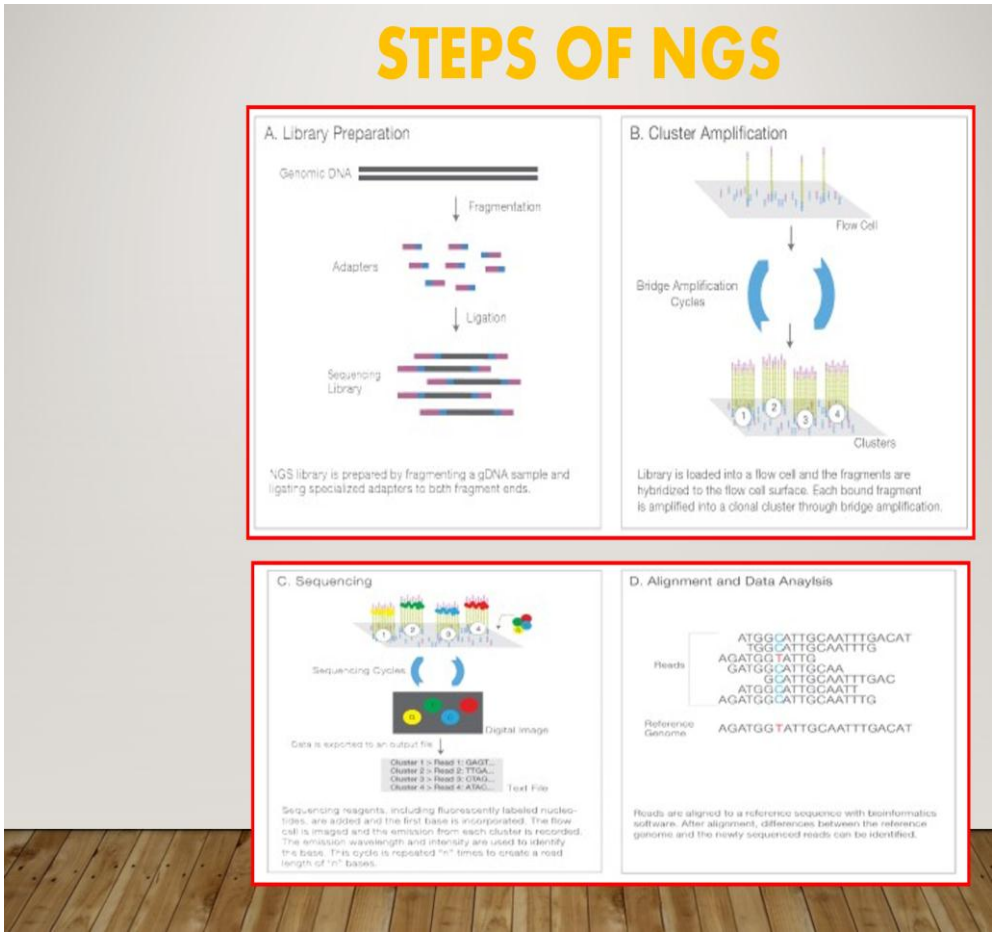
dramatically reduced in comparison with first generation sequencing. NGS technologies that are becoming commonplace in many laboratories, with an emphasis on the applications that have the potential to significantly impact on diagnosis, prevention, and control of infectious diseases in animals.





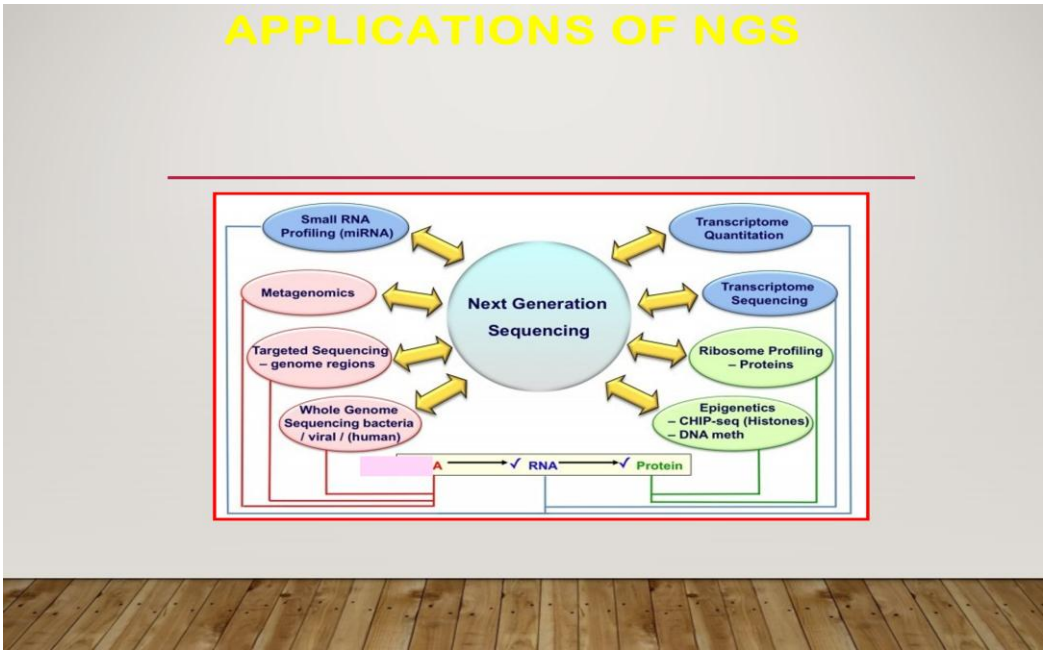
NGS - Next generation sequencing

- NGS is massively parallel sequencing of clonally amplified templates on a solid surface
- Next-generation sequencing platforms provide unprecedented throughput, generating hundreds of gigabases of data in a single experiment.
- Allow unbiased sequencing without prior knowledge of the complete DNA content in a sample while retaining the flexibility..



Commercially available NGS Technologies

Platform	Chemistry	Read Length	Run Time	Gb/Run	Advantage	Disadvantage
454 GS Junior (Roche)	Pyro-sequencing	500	8 hrs.	0.04	Long Read Length	High error rate in homopolymer
454 GS FLX+ (Roche)	Pyro-sequencing	700	23 hrs.	0.7	Long Read Length	High error rate in homopolymer
HiSeq (Illumina)	Reversible Terminator	2*100	2 days (rapid mode)	120 (rapid mode)	High-throughput / cost	Short reads Long run time (normal mode)
SOLiD (Life)	Ligation	85	8 days	150	Low Error Rate	Short reads Long run time
Ion Proton (Life)	Proton Detection	200	2 hrs.	100	Short Run times	New*
PacBio RS	Real-time Sequencing	3000 (up to 15,000)	20 min	3	No PCR Longest Read Length	High Error Rate



Application of NGS to Animal Infectious Disease

NGS technology is now being increasingly applied to study the etiology, genomics, evolution, and epidemiology of animal infectious diseases as well as host-pathogen interactions. NGS platforms have been instrumental in the completion of large animal genomes and the documentation of genomic variation. Available livestock genomes now include bovine, pig, sheep, equine, and avian which provide an important source of knowledge for understanding food production and animal interaction with infectious pathogens. The high variability and large size of the mitochondrial genome (mtDNA) of *eukaryotic parasites* have been recently explored using NGS (reviewed by mtDNA sequences proved very informative in epidemiological studies but also include comparative mtDNA sequencing of parasites with low and high zoonotic potential Examples include the characterization of the transcriptome from *Eimeria* sp. from chicken and *Taenia* sp. from sheep. In addition, RNA-Seq data have been used to predict potential drug targets and to identify key genes involved in anthelmintic resistance involve the inherent variability of many viral genomes due to replication machinery lacking efficient proofreading mechanisms. This, combined with a short generation time and high replication rate results in a complex mix of differing genomes (a "swarm" of closely related viruses) within a single host that are often termed as " *quasispecies* ,"

To study the etiology, genomics, evolution, and epidemiology of animal infectious diseases as well as host-pathogen interactions.

Cattle and sheep

- Characterizing and determining the phylogeny of new bluetongue virus variants
- Detecting unknown and emerging new pathogens, most notably the Schmallenberg virus

Swine

- Detection and identification various infectious agents involved in complex diseases such as post-weaning multisystemic wasting syndrome
- Detection of infection agents in mixed infections in enteric disease complexes
- Characterization porcine microbiota (bacterial and viral populations)

Improved Detection and Characterisation of Infectious Agents and Early Diagnosis

- NGS approaches have been approved for use in routine diagnostics to monitor the genomic diversity of AIV, early emergences and transmission of these viruses from waterfowl to domestic poultry.
- To study of genomic recombination, which has an important role in bacterial and viral evolution.
- Characterization of viral quasi species.

Genome Characterization and Virus Diversity

- Genomic sequences of viral strains can be used to identify the molecular determinants that underpin important phenotypic traits such as virulence and pathogenicity.
- Prediction of novel genes and viral features that are important for viral replication or pathogenesis.
- To study molecular epidemiology

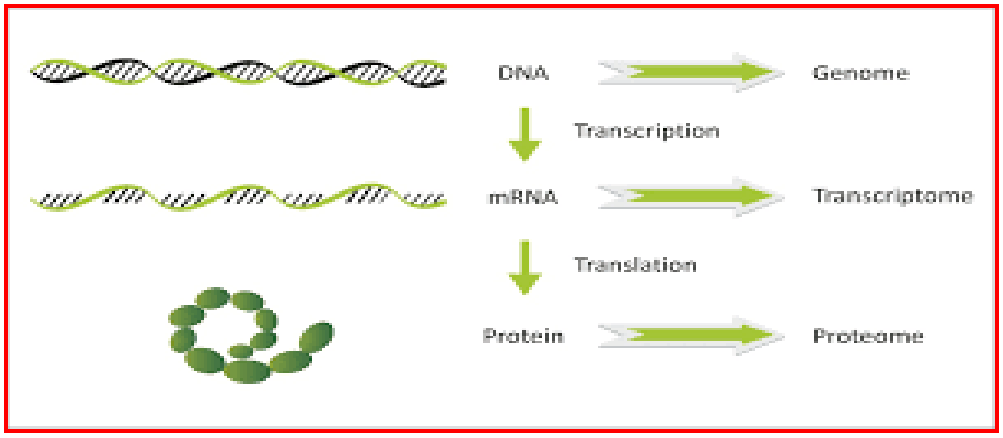
Virus discovery and viral metagenomics

The introduction of culture-independent NGS technologies, termed as viral metagenomics (community genomics), made powerful diagnostic technology to investigate the complete viral genetic populations of a sample. Metagenomics have the capacity to detect viruses either as single agents or as syndromes to identify the etiologic agents. Honkavuori *et al.* (2008) discovered two strains of a novel Borna viruses in psittacine birds with proventriculus dilatation syndrome (PDS)

characterised by gastrointestinal dysfunction and neurological signs. Metagenomic NGS work flows also have the potential use for quality control of biological products and vaccines.

Transcriptomics and virus-host interaction

Introduction of NGS technologies revolutionized transcriptome profiling, an approach referred to as RNA sequencing (RNA-seq). NGS offers an opportunity for detailed examination of transcriptomics at both host and pathogen level during an infection, thereby, elucidating the mechanisms of disease and pinpointing the functional pathways involved in the host response to infection. Huang *et al*. (2013) performed deep transcriptome analysis responsive to AIV using the lung of control ducks and ducks infected with highly pathogenic and weakly pathogenic H5N1 virus. The analysis revealed that β-defensin and butyrophilin-like gene repertoires were involved in host immune response. ChIP coupled with high-throughput sequencing (ChIP-Seq) has been applied to quantitatively analysed binding sites of DNA associated proteins across the entire genome.



Use of HTS in the field during outbreaks – 'on-site' applications

Reliable on-site diagnosis helps health authorities to rapidly identify the infectious agents and apply specific control measures. A prompt, early diagnosis and rapid implementation of specific control measures can stop outbreaks or prevent their uncontrolled spread. Therefore, on-site HTS has medical and economic benefits, and can improve socio-economic conditions and 'quality of life'.

Small RNA Profiling (Mirna)-Ngs

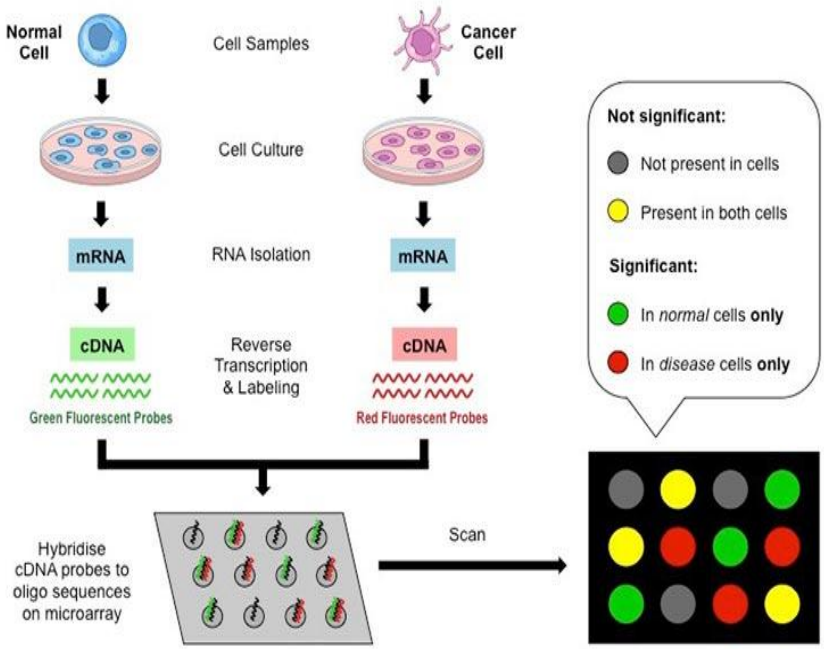
Micro-RNA are class of small noncoding RNA species that are known to have role in various biological process.

Surveillance of Disease Outbreaks and Transmission Routes

NGS-based comparative whole-genome analysis formed part of the diagnostic process used to investigate the sporadic outbreaks Ex. Outbreak of avian influenza virus (AIV) H5N8 in Europe during 2014–2015.Phylogenetic analysis revealed a close resemblance strain from South Korea and Japan. Suggesting a transmission pattern that involved migratory wild birds from Asia, possibly via overlapping flyways and common breeding sites in Siberia

Application of NGS in Clinical Oncology

With the development and improvement of new sequencing technology, next generation sequencing (NGS) has been applied increasingly in cancer genomics research over the past decade. More recently, NGS has been adopted in clinical oncology to advance personalized treatment of cancer. NGS is used to identify novel and rare cancer mutations, detect familial cancer mutation carriers, and provide molecular rationale for appropriate targeted therapy compared to traditional sequencing, NGS holds many advantages, such as the ability to fully sequence all types of mutations for a large number of genes (hundreds to thousands) in a single test at a relatively low cost. However, significant challenges, particularly with respect to the requirement for simpler assays, more flexible throughput, shorter turnaround time, and most importantly, easier data analysis and interpretation, will have to be overcome to translate NGS to the bedside of cancer patients. Overall, continuous dedication to apply NGS in clinical oncology practice will enable us to be one step closer to personalized medicine.



Strengths of Next Generation Sequencing

- Abnormalities of entire genome
- (substitution, deletion,insertion, duplications)
- Using less DNA
- Less costly, fast

Limitations of Next generation sequencing

- Sophisticated bioinformatics system, fast data processing, large data storage is required.
- Lack of computational resources , staffing to analyze and interpret

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