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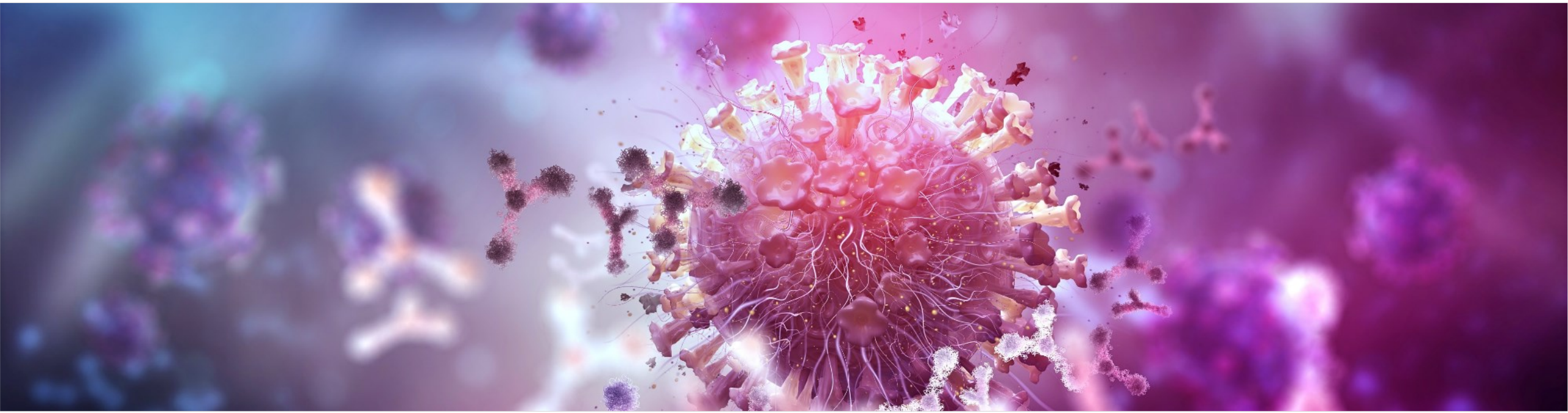

The Future of Biosafety Testing – NGS the new tool

ViruSure Workshop, Vienna, September 2022

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VIRUSURE
Quality is no coincidence

Presentation overview

- Current testing strategies
- Comparison with NGS
- Overview on NGS workflow
- Applications of NGS
- Ongoing topics at ViruSure

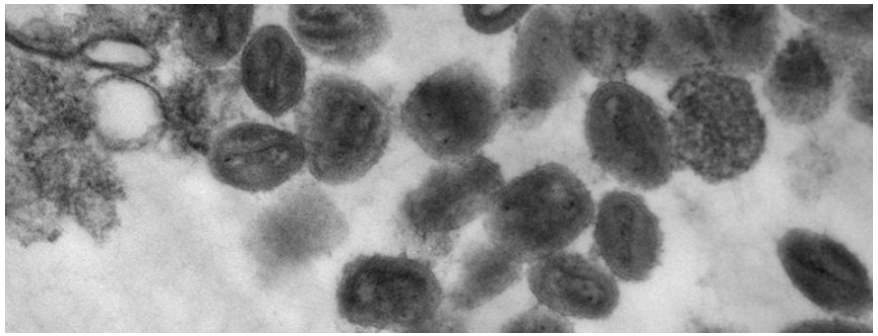
Current testing strategies (1)

- To minimize the risk of viral contamination in biological products currently a large variety of testing methods is applied

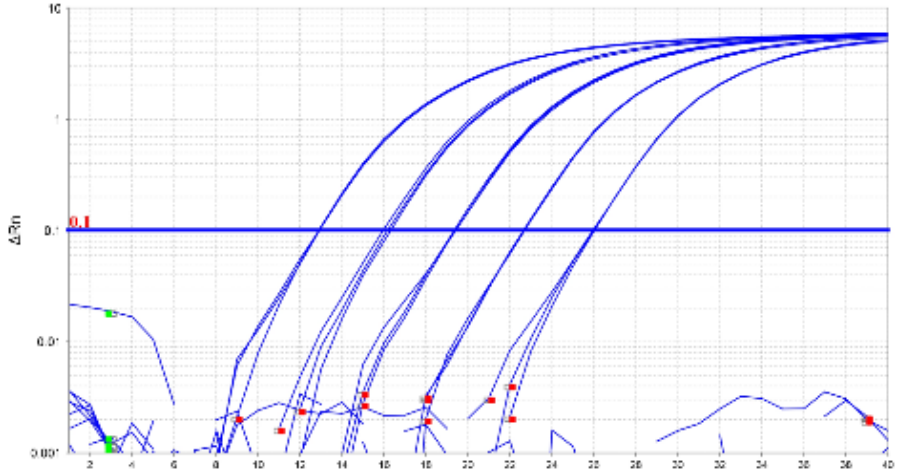
- These include:
  - In-vivo tests
    - Inoculate animals → Check health status over time/pathology upon death
    - Inoculate hen‘s eggs → test for hemagglutinating/hemadsorbing agents on red blood cells
    - Antibody Production Assays (MAP/HAP) → After inoculation check for produced antibodies against viruses
  - In-vitro tests (cell culture based)
    - General in vitro adventitious agent tests to screen for infectious viruses → inoculate cell lines and check for CPE
    - 9CFR to screen for specific infectious viruses → perform immunofluorescence antibody staining with virus specifc antibodies

Current testing strategies (2)

- Screens for Retroviruses
  - Cell culture based in vitro assays (PG-4)
  - Transmission electron microscopy
  - Reverse transcriptase acticity (PERT)



- Real-time PCR Assays



Pros/Cons of testing portfolio

Parameter	In vivo AAT	MAP/HAP	In vitro AAT	9 CFR	Retroviruses (PG-4)	TEM	RT activity	qPCR
Detection of unknown virus	✓	-	✓	-	✓	✓	✓	-
Detection of replicating virus	✓	✓	✓	✓	✓	-	-	-
Identification of virus	-	✓	-	✓	-	-	-	✓
Specificity of Detection	unspecific	specific	unspecific	specific	unspecific	unspecific	unspecific	specific
Sensitivity	+	+	+	+	+	-	+++	+++

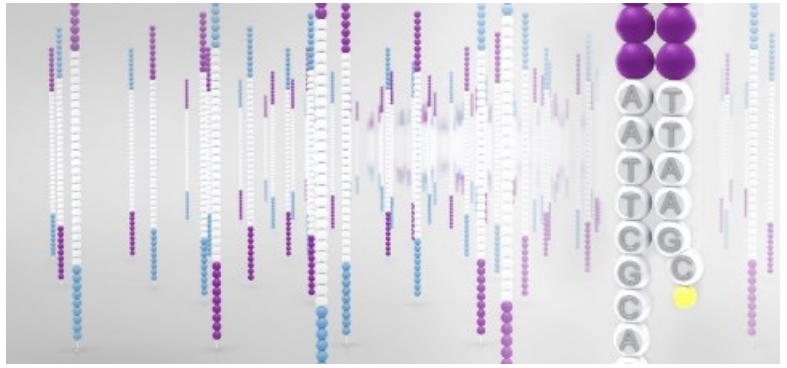
- Combined they provide a good set-up to minimize the risk of adventitious viral contaminations
- Very time consuming process to get to the final results

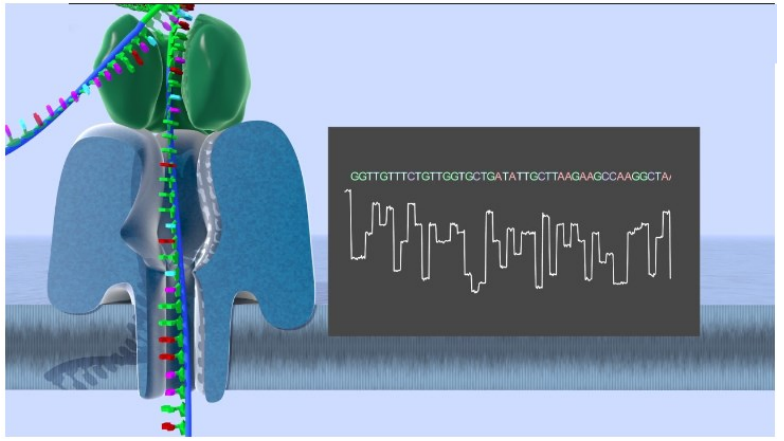
NGS as a new tool

- Due to the nature of Next Generation Sequencing (also High Throuhput Sequencing or Massively Parallel Sequencing) combines the advantages of multiple of the current assays

- Two main technologies with different principles
  - Short read sequencing = Sequencing by Synthesis approach
  - Long read sequencing = Sequencing through a pore

→ both techniques capable of capturing and sequencing all of the nucleic acids present in a sample, without any prior sequence knowledge





* Images from Illumina and Oxford Nanopore Technologies

Comparison of NGS to current testing tools

Parameter	NGS
Detection of unknown virus	✓
Detection of replicating virus	✓/ -
Identification of virus	✓
Breadth of Detection	Very high (unspecific)
Sensitivity	+++

- NGS is a non-targeted approach, therefore theoretically all contaminations can be detected identify
- The sensitivity of NGS is very high, approaching detection limits of qPCR assays.
- With the right set-up/approach it is possible to tell whether the detected virus is replicating or not
  - This could also make it possible to use NGS as a replacement for MAP/HAP testing to reduce the need for in-vivo testing
- Potential problems with NGS:
  - „Sensitivity“
    (every sequence is detected, also inactivated virus from reagents/media/etc.)
  - Database curation
    (used database needs to be well curated→ if viruses are missing or overrepresented this might lead to missed or false positive hits)

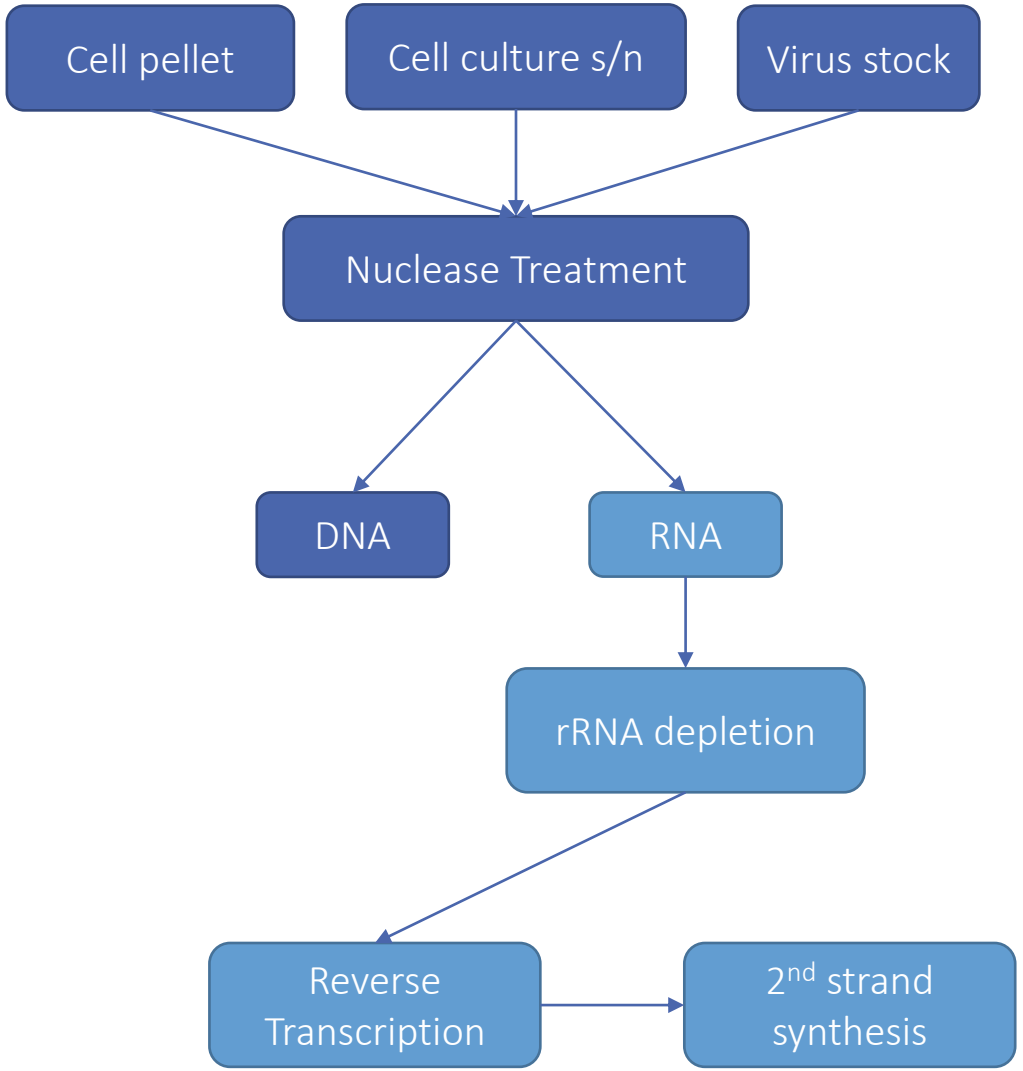
Principle of NGS

- Workflow can be roughly divided into three steps
  - Sample processing
    - Nucleic acid extraction
    - Library preparation to modify the extracted sample and bring it into a sequencable format
  - Sequencing
    - Data generation process
  - Data evaluation
    - Comparison of sequencing data with reference sequence/database

→ For each of these steps a variety of options is available and choices have to be made carefully based on the type of the sample and the goal of the experiment

Example virus detection – Sample processing

- What is the underlying sample type?
- Do we want to remove free DNA/RNA?
- Which extraction method do we need?
- Do we need additional steps for RNA?



Example virus detection – Library Preparation and Sequencing

- Which kit do we need for library preparation?

- Do we need an additional PCR step to amplify our nucleic acids?
  - Amount of DNA in the sample?
  - Could I loose sensitivity for certain virus type due to a PCR bias?

- How much data do we need to generate to answer our question?
  - Amount of background DNA?
    e.g. cell pellet/virus stock

Example virus detection – Data Evaluation

- Classification of reads
  - Do we use all reads for classification?

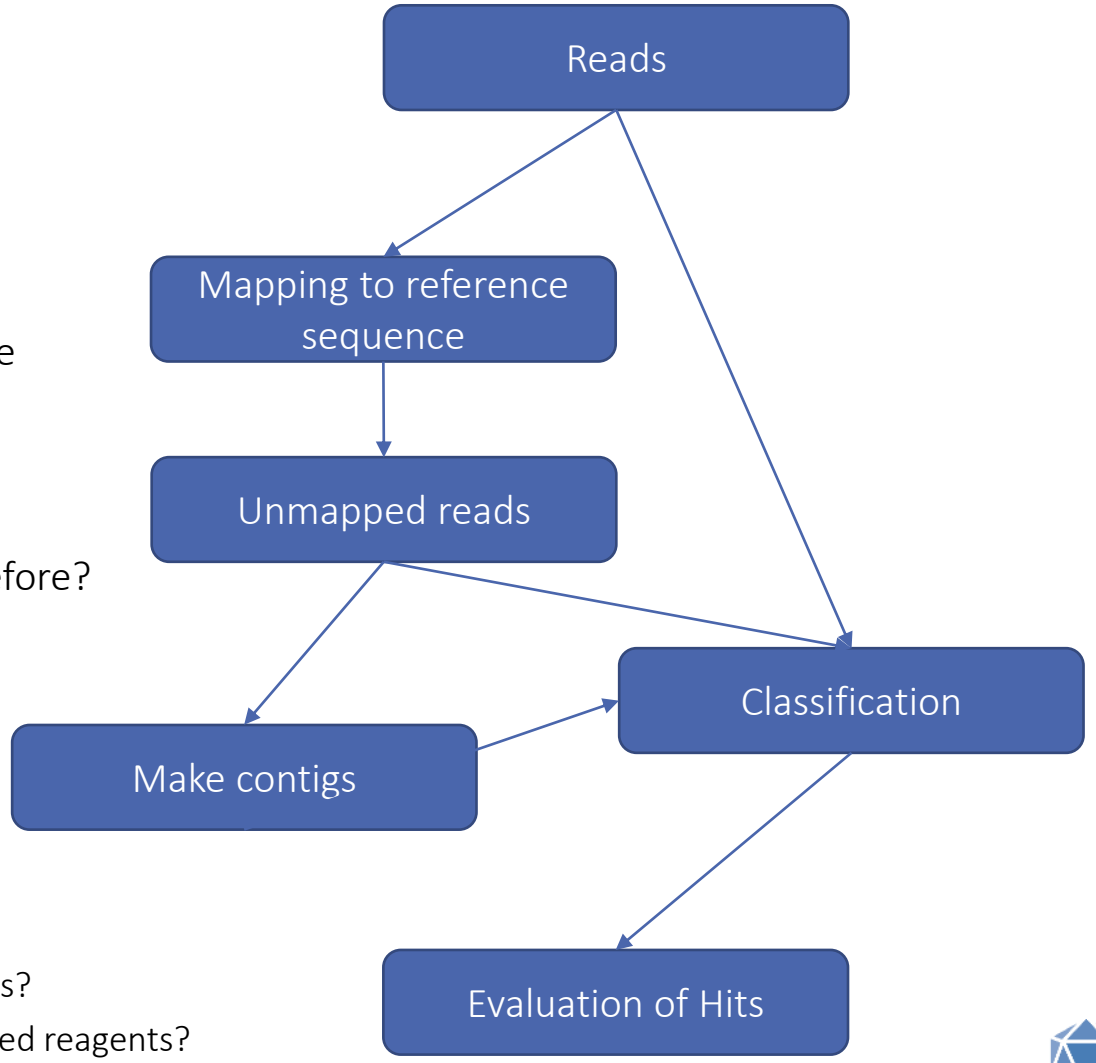
→Huge data set might take very long

  - Do we map my data to a reference sequence first and use the unmapped reads only?

→ miss probably on viruses that have integrated into host genome

  - Is it necessary to make contigs from my reads to generate longer sequences?

→ Which technology have we used? Did we fragment our DNA before?



- Evaluation of hits
  - What is a true hit?
  - How do I follow-up possible hits?
  - Definition of OOS procedures
    - Infectivity assays to determine whether it is an actual live virus?
    - Run controls to evaluate if the potential hit is derived from used reagents?

Applications – Adventitous Agent Testing

- By combining the right sample preparation methods, sequencing approach and bioinformatic tools, NGS is an ideal method for the characterization of biological products like viral vaccines, raw materials, cell lines and final drug products

- NGS is particularly well-suited for adventitious agent testing
  - By sequencing the sample and compare the results to a reference database all known potential contaminating agents can be identified
  - Can be used for adventitious virus detection, and also for sterility and mycoplasma testing
  - Not only known, but also unknown contaminating agents can be detected, by checking sequence homologies with known agents/sequence

Applications – Virus product safety and characterisation

- Applications for virus products
  - Viral vaccines – virus designed to prevent replication and induce an immune response
  - Oncolytic virotherapy – viruses that selectively target tumor cells and trigger an antitumor immune response
  - Viral gene therapy – viruses that deliver therapeutic genes to cells with genetic malfunctions
  - Viral immunotherapy – viruses that introduce specific antigens to a patient‘s immune system

- NGS for identity testing and variant detection
  - Classical approach is sanger sequencing
  - NGS provides an advantage, when genomes/areas that need to be sequenced get very large
  - Can provide better resolution, when regions are difficult to sequence (e.g. secondary structures)

Applications – Genetic Stability

- Resequencing of Genetic Inserts
  - Does the inserted gene still look like it is supposed to be looking?
- Gene copy number determination
  - How often did my gene of interest insert into the host genome?
- Gene expression analysis
  - Is my gene of interest expressed efficiently?

Current Status at ViruSure

- Obtained PromethION from Oxford Nanopore Technologies to generate larger data sets and enhance through-put to offer NGS as a service
  - Set-up GLP/GMP compliant infrastructure and workflows
  - Optimisation of protocols/bioinformatics pipelines

- Participation in Advanced Virus Detection Technologies Interest Group (AVDTIG)
  - PDA led interest group to establish NGS for biosafety testing
  - Variety of members including regulators and industry leaders
  - ViruSure joined in 2019 to stay at the top of the development and contribute with our expertise in biosafety testing
  - Participation in spiking study #4 to evaluate virus detection capabilities of long-read NGS technologies

Summary

- NGS is a tool that combines several advantages of the current pool of testing methods
  - Identification of contaminants without prior sequence knowledge
  - Very high sensitivity
  - Shorter turnaround times then the current testing portfolio

- It can be used for a variety of applications
  - Adventitious virus detection
  - Sterility/Mycoplasma testing
  - Identity testing
  - Genetic stability
  - Every task where nucleic acids can be exploited as an analyte

→ Due to the large number of options available and the enormous amount data generated, the set-up has to be planned very carefully to obtain the ideal results to answer the underlying research question

Thank you for your attention

Questions?