Detection of Adventitious Agents

Scalable high-throughput adventitious virus detection with unprecedented sensitivity and specificity

Biomanufacturing is a rapidly expanding industry, with the production of safe and effective biologics being paramount to ensuring the health and well-being of patients. However, one major obstacle that biomanufacturing faces is the unintentional introduction of adventitious viruses into the production process. These viruses can pose a significant threat to patients if not promptly detected and eliminated. For instance, they may trigger undesired immune response, rendering the biopharmaceutical product less effective or even harmful to use. Therefore, it is crucial to implement rigorous measures to minimize the risk of introducing unwanted viruses into the production process. This involves utilizing robust screening and purification methods, as well as employing stringent quality control measures along the manufacturing pipelines.
Next generation sequencing (NGS) is a tool capable of detecting adventitious viruses in a variety of samples: it can be used to test the production environment, raw materials, and final products, thereby ensuring safety, quality, and efficacy of the released biologics.

The use of NGS offers several advantages over traditional molecular methods:

1. High Sensitivity
NGS allows for the detection of ultra-low levels of viral particles, which may be missed by traditional methods.

2. Multiplexing
NGS can simultaneously detect multiple viruses in a single reaction, reducing the time and resources required. Importantly, multiplexing also allows for testing large number of viruses with only a small aliquot of a sample.

3. Broad Viral Diversity Detection
NGS can detect a wide range of viruses, both known and novel strains, which allows the identification of new or emerging viruses in a timely manner.

4. Superior Specificity
Utilizing NGS methods minimizes the risk of false-positive results.

5. Superior Accuracy
NGS provides high resolution information on the genetic diversity and evolution of viruses, which allows improved accuracy while identifying and classifying the viruses.

6. Rapid Turnaround
NGS can generate large amounts of sequencing data in a relatively short period of time, providing faster results in comparison to traditional methods that can take several days or even weeks per test.

7. Cost-Effective
NGS is becoming increasingly cost-effective, making it more accessible for large-scale or routine viral detection in various settings, such as public health surveillance, clinical diagnosis, and food and water testing.

Two NGS-based solutions are utilized in the detection of viral contaminants: agnostic and targeted. With agnostic approaches, all genetic material present in a sample is processed, and no prior knowledge of viral sequence is required. This approach allows for unbiased detection of any viruses that are present in a sample, including discovery of unknown or novel viruses. Alternatively, targeted NGS approaches use primers or probes to specifically enrich for viral nucleic acids. This method is typically more cost-effective, has increased sensitivity and requires less computational resources compared to agnostic approaches. Ultimately, the choice between agnostic and targeted NGS approaches will depend on the specific aim of the testing.

Here, we present validation of our hybridization-based target enrichment solution for viral detection in biologics, capable of screening for > 3,000 different viruses, including > 15,000 viral strains, in a single reaction. This workflow can detect viruses of all nucleic acid genome types: single-stranded RNA, double-stranded RNA, single-stranded DNA, and double-stranded DNA.

Method

Our viral pathogen detection process utilizes the highly sensitive Twist Comprehensive Viral Research Panel, which employs capture enrichment to detect a wide range of viruses. This method is effective even in samples with high levels of background nucleic acids from sources such as producer cell lines. Initially, cDNA synthesis is performed on the total nucleic acid (TNA). Next, library preparation and hybridization-capture enrich the sample for viral nucleic acid. To reduce sequencing artifacts and PCR amplification bias that may occur during library prep, we include unique molecular identifiers (UMIs) in the workflow. The library is then sequenced and analyzed using a proprietary data analysis pipeline that compares sequencing reads to a database. The pipeline assesses the similarity of the sequencing data to the virus genome (% identity) and the completeness of the viral genome (% genome covered) to identify any viruses present in the sample.

To determine the sensitivity and specificity of our viral detection workflow we performed a thorough experimental validation using 8 different spike-in controls including ssRNA, dsRNA and dsDNA viruses. As vehicle, we used either ctDNA or whole-cell nucleic acid (NA) extract, the latter containing ~10-15x as much RNA as DNA. Multiple virus combinations were tested to demonstrate sensitivity and specificity in more complex scenarios (Table 1). To determine the sensitivity, we performed testing using a dilution series using a ssRNA virus (Table 2).

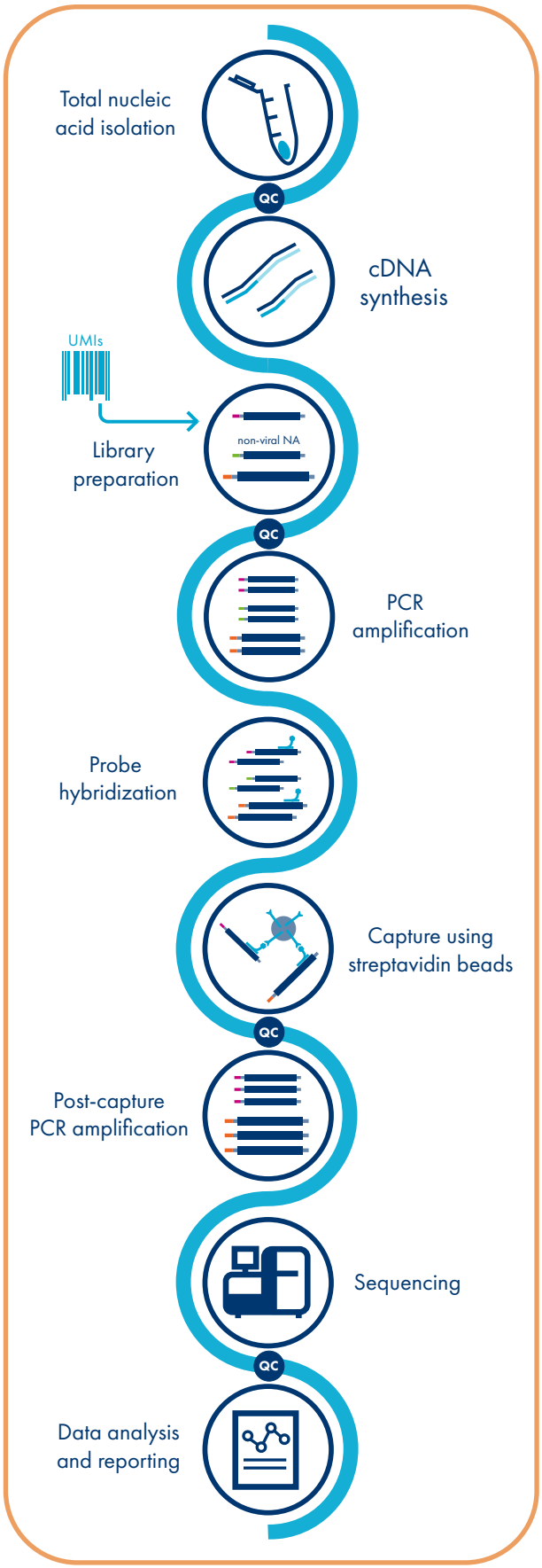

Figure 1: Schematic overview of the viral pathogen detection workflow.

Results

Sensitivity & specificity

Sample	Virus	Genome type	Copies/ reaction	Viral NA	Identity (%)	Genome covered (%)
Influenza A virus	Influenza A virus	ssRNA	500	10%	95.96%	82.65%
SARS-CoV-2	SARS-CoV-2	ssRNA	500	10%	99.85%	97.51%
Flurona mix	Influenza A virus	ssRNA	25,000	10%	96.50%	98.72%
	SARS-CoV-2	ssRNA	500	0.2%	99.81%	97.55%
Flurona mix	Influenza A virus	ssRNA	500	0.2%	96.53%	98.56%
	SARS-CoV-2	ssRNA	25,000	10%	99.87%	97.64%
Virome mix	Mastadeno virus F	dsDNA		16.67%	99.88%	99.82%
	Herpesvirus 5	dsDNA		16.67%	99.86%	97.09%
	Respiratory Syncytial virus	ssRNA		16.67%	96.44%	67.71%
	Influenza B virus	ssRNA		16.67%	98.96%	100%
	Orthoreo virus 3	dsDNA		16.67%	98.27%	98.02%
	Zika virus	ssRNA		16.67%	88.31%	88.40%

Table 1: Specificity of GenomeScan's viral detection workflow

Here, we demonstrate that our comprehensive viral panel reliably detects a wide range of viruses, also in samples with multiple viral species present, without, most importantly, any false-positive findings. Strikingly, as little as 500 viral genome copies were confidently and reproducibly detected in whole-cell NA extract. This equates to the proportion of viral NA being 1 billionth of the total NA content in the reaction (1 viral NA molecule for every 1 × 109 superscript molecules). Moreover, resulting coverage on the viral genome is directly proportional to the amount of viral genome copies spiked in, indicating reproducible capture efficiency. Altogether, these results illustrate the exceptionally high sensitivity and specificity of the workflow.

	Genome Identity (%)		Genome Covered (%)	
Sample	Replicate 1	Replicate 2	Replicate 1	Replicate 2
5000 copies	87.89%		96.33%	
2500 copies	94.33%	87.38%	96.05%	96.67%
1000 copies	83.04%	83.70%	96.54%	96.71%
500 copies	82.96%	73.37%	96.40%	96.83%

Table 2: Detection of Influenza A virus in whole-cell NA

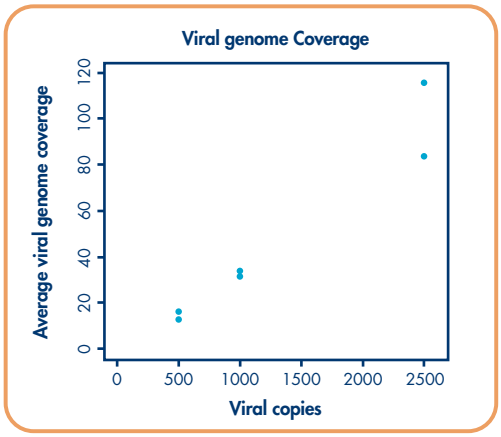

Figure 2: Resulting viral genome coverage is directly proportional to input.

The results presented herein were generated in collaboration with the department of Medical Microbiology, Leiden University Center for Infectious Diseases, Leiden University Medical Center[1].

[1] Mourik, K. et al:
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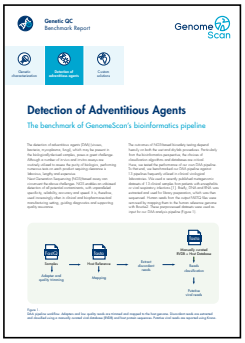

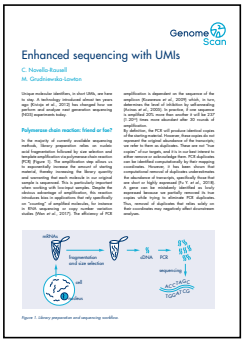

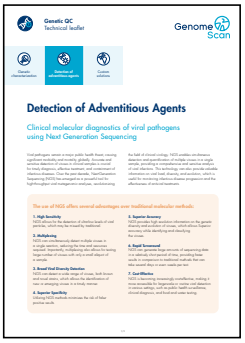

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