ASFV Best Practice

Introduction

African swine fever (ASF) is a highly contagious hemorrhagic disease caused by ASF virus (ASFV). The high morbidity and mortality of the disease has a severe impact on the global swine industry. Despite the development of anti-ASFV drugs is in need, currently there is no effective treatments or vaccines commercially available. Studies in understanding the viral etiology is halted by limited knowledge of the whole genome and proteins of the virus. The whole genome sequencing (WGS) of ASFV can contribute to understanding of the transcripts and proteins associated with the viral virulence, thereby enabling precise treatment, tracing the outbreak and transmission routes, etc. Celemics has successfully performed WGS of ASFV with ASF-infected swine blood sample. Celemics proprietary Target Enrichment method provided significantly higher number of viral specific reads than conventional WGS method by 58-fold, leading to higher coverage depth and thereby accurate analysis.

Result

One of the major challenges of screening pathogens with NGS is detecting the small fraction of viral nucleic acids among the host DNA. Screening from blood sample is particularly challenging because it is inevitable that the ASFV is contaminated by host genomic DNA in the blood sample. An alternative includes increasing the sequencing output for an accurate assay, but it is accompanied by higher sequencing costs and reduced efficiency. Obtaining sufficient amount of viral nucleic acids from the minimal amount of NGS cycles should be established for achieving accurate result with lower sequencing costs.

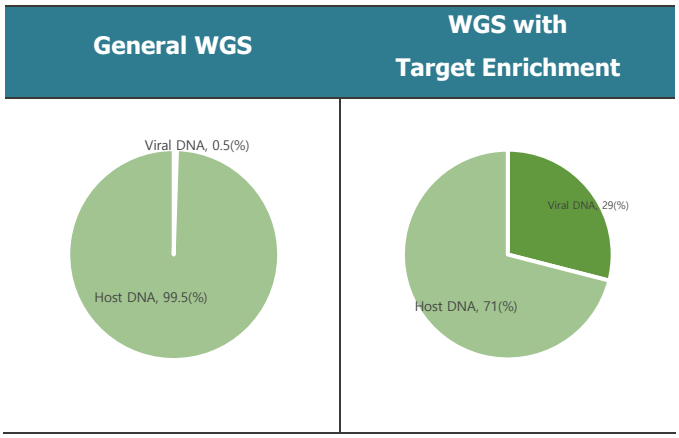

Fig 1. Comparison of on-target ratio between general WGS and WGS with target enrichment

The challenges mentioned above are overcome by the target enrichment method developed by Celemics. There are two key factors for a successful target enrichment based NGS. First, hybridization probe design technology is crucial for effectively sequencing the target genes. Second, blocking oligonucleotides should be species-specifically designed because the repetitive sequences differ by species. Celemics provides efficient target enrichment techniques with the elaborate design technologies of the probes and blocking oligonucleotides. Based on these two major technologies, we have developed and

established the target enrichment NGS method for successful detection of ASFV.

The pilot test was performed in order to evaluate the performance of the panel. The result shows that 29% of ASFV specific reads from the total reads were successfully captured (Figure 1). Compared to the number of ASFV specific reads from conventional WGS which yielded 0.5% capture, the number of reads from Celemics target enrichment based NGS increased by 58-fold. Obtaining a vast amount of data with a single NGS analysis allows for sequencing the full-length viral genome with low costs and achieving high sequencing depth for accurate analysis (Table 2).

Sequencing the whole genome of ASFV requires sufficient read depth in particular. Due to the genetic stability of the virus, there's only minimal genetic difference between strains. Also, performing NGS with blood sample is considered more challenging due to its lower viral load compared to spleen tissue sample or culture supernatant of infected cells. Regardless of such barriers, we have successfully sequenced the 190,584 bp long genome of ASFV and identified 26 ASFV strains with a single NGS operation supported by Celemics proprietary probes, block oligonucleotides and optimized reagents (ref. Georgia 2007/1).

	General NGS	NGS with target enrichment	
		Before PCR duplicate removal	After PCR duplicate removal
Mean depth	53.86	2,870.65	767.83
Mapped reads (bp)	39,261	3,625,393	1,039,018
Length (bp)	190,584	190,584	190,584
Percentage of reads (%)	0.5	29.0	

Table 1. Compared reference mapping of two NGS method (Mapping to reference; Georgia 2007/1 (LR 743116.1)

In total, we have obtained 1,039,018 reads (non-PCR duplicate reads) which is a 26-fold more reads compared to that of conventional WGS. And we have achieved mean depth of 767.8, which is 14 times of what is required for accurately distinguishing the low-level true variants from sequencing errors when performing conventional WGS (Table 1).

The exceptional capture efficiency of Celemics target enrichment method enables sequencing analysis with higher accuracy and lower costs. Since the target enrichment method is also applicable to a myriad of other viruses and microorganisms in various different samples, it is expected to contribute greatly to the research and clinical studies on virulence analysis and drug development.

Conclusion

The NGS-based Target Enrichment Kit, the main focus of Celemics, is supported by proprietary technologies in generating hybridization probe and blocking oligonucleotides, as well as optimizing reagents. Due to our elaborate optimization technology, Celemics Target Enrichment Kit is compatible with NGS platforms from Illumina and Ion Torrent. The performance of each Celemics product has been evaluated through robust validation tests and from over 10 countries, researchers have been purchasing our products optimized for research purposes and various cancer types such as solid cancer, hereditary cancer, circulating tumor DNA, and cell line QC.

Celemics has developed the target enrichment kit for WGS of ASFV from the experience of designing and manufacturing over 500 customized panels for customers. Through in-house validation, we have confirmed the high capture performance of the panel against the viral genome, subsequently providing credible analysis results. In addition, 26 ASFV strains known to date were accurately identified with a single NGS operation. Celemics target enrichment technology is also applicable to various panels for detecting other viruses.