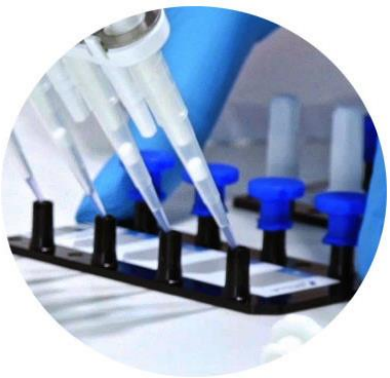
Detection of African swine fever virus ASFV using Stilla Naica digital PCR method

African swine fever (ASF, Africa Swine Fever) is an acute, highly contagious disease caused by the African swine fever virus. Due to the complexity of the etiology and epidemiology of the disease, and the lack of effective vaccines for epidemic prevention, the World Organisation for Animal Health (OIE) listed it as a real-time notification of animal epidemics, which is classified as a type of animal epidemic in China.

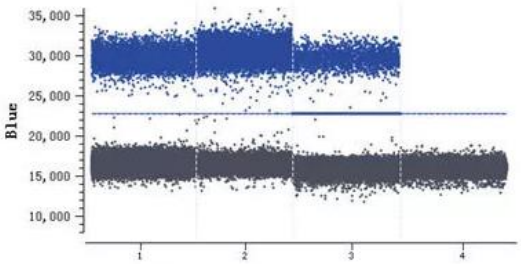
According to the recommendations of the Food and Agriculture Organization of the United Nations, the main laboratory tests for ASFV (African swine fever virus) are divided into ASFV virus detection and ASFV antibody detection.

ASFV virus detection is divided into three types: gene detection, virus isolation and antigen detection. The gene detection method is polymerase chain reaction (PCR). In recent years, fluorescence quantitative PCR has been widely used. For cases of acute, acute or subacute ASF infection, qPCR has become the preferred detection technology. The virus isolation and antigen detection methods are direct fluorescent antibody method and enzyme-linked immunosorbent assay (ELISA).



Digital PCR is the third generation PCR technology after common PCR and qPCR (real time PCR). Compared with qPCR, digital PCR has higher sensitivity and no standard. Independent of the Ct value, it can effectively overcome the influence of PCR inhibitors, can detect ASFV virus sensitively. Cycloud biotechnology develops digital PCR detection methods for African swine fever virus, which is detected early and effectively prevented.

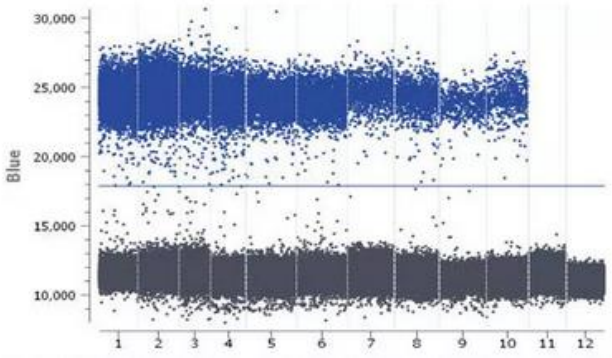
Sample Detection



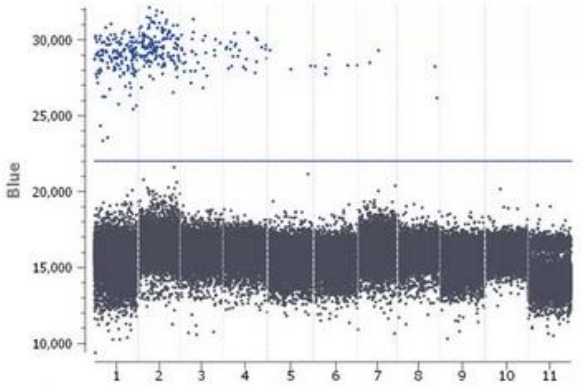
1-3: Positive sample 4: Negative sample

Using the probe method, digital PCR was performed on real samples, and the results were consistent with other verification methods.

Sensitivity



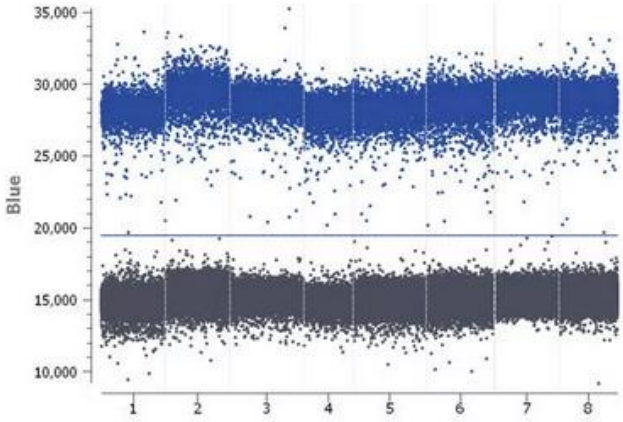
1-10: 2 times dilution (5 concentrations), 2 replicates per concentration , 11: Negative sample , 12: Blank control



1-10: 5 times dilution (5 concentrations), 2 replicates per concentration , 11: Negative sample

The minimum detection limit for digital PCR detection of African swine fever developed by Cycloud Biotechnology is 0.13 cp/ul (the true sample test value without pre-amplification step). At the same time, the performance is consistent at high concentration and medium concentration, and the performance is stable.

Repeatability



Repeat the results of eight experiments at the same concentration

The digital PCR detection method for African swine fever developed by Cycloud Biotechnology was repeated for the same concentration of samples, and the CV value was less than 4.5%.

The detection of African swine fever is now qualitatively detected by real-time quantitative PCR, and it is judged whether or not the virus is present and cannot be accurately quantified. And it requires pre-amplification to improve detection sensitivity. Cycloud Biotechnology's digital PCR detection method for African swine fever requires no standard required, no pre-amplification step, directly gives the absolute content of virus in the sample to be tested, and has higher specificity and sensitivity. Realize the early treatment and early isolation of the first line of the pig farm to achieve precise prevention and control and reduce losses. Specificity and ultra-sensitive detection in frozen meat, meat products,

and other deep processed foods.

The Naica Crystal microdrop digital PCR system developed by France's Stilla Technologies company uses innovative microfluidic chips as the only consumable. A 2D array of 25,000-30000 droplets was formed and PCR amplification experiments were performed in a single layer tiling manner. After the reaction is completed, the droplets can be imaged by three fluorescent channels. Thereby obtaining accurate nucleic acid quantity, the experimental results can be obtained in two and a half hours.