Virology 509 (2017) 159–166

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Use of Sequence-Independent, Single-Primer-Amplification (SISPA) for rapid detection, identification, and characterization of avian RNA viruses

Klaudia Chrzastek[1], Dong-hun Lee[1], Diane Smith, Poonam Sharma, David L. Suarez, Mary Pantin-Jackwood, Darrell R. Kapczynski*

US National Poultry Research Center, Agricultural Research Service, US Department of Agriculture, 934 College Station Road, Athens, GA 30605, USA

ARTICLE INFO

Keywords:
Random amplification
Sequence-independent single-primer amplification
Next generation sequencing
Avian influenza virus
Infectious bronchitis virus
Newcastle disease virus
RNA viruses
Poultry

ABSTRACT

Current technologies with next generation sequencing have revolutionized metagenomics analysis of clinical samples. To achieve the non-selective amplification and recovery of low abundance genetic sequences, a simplified Sequence-Independent, Single-Primer Amplification (SISPA) technique in combination with MiSeq platform was applied to target negative- and positive-sense single-stranded RNA viral sequences. This method allowed successful sequence assembly of full or near full length avian influenza virus (AIV), infectious bronchitis virus (IBV), and Newcastle disease virus (NDV) viral genome. Moreover, SISPA analysis applied to unknown clinical cases of mixed viral infections produced genome assemblies comprising 98% NDV and 99% of IBV genomes. Complete or near complete virus genome sequence was obtained with titers at or above $10^{4.5}$ EID_{50}/ml (50% embryo infectious dose), and virus identification could be detected with titers at or above 10^{3} EID_{50}/ml. Taken together, these studies demonstrate a simple template enrichment protocol for rapid detection and accurate characterization of avian RNA viruses.

1. Introduction

RNA viruses are a large genetically-diverse group of infectious agents whose evolutionary diversity is driven by adaptability to its host (Sanjuán et al., 2010). Characterizing the genomes by sequence-based methods is the most widely used approach to determine RNA virus diversity and thereby determine the relationships between isolates within the population. Furthermore, to fully understand the mechanisms of viral adaptation and evolution, and to perform molecular diagnosis of emerging and re-emerging viral infections there is an increasing need to produce full length viral genomes sequences.

Utilization of next generation sequencing (NGS) in molecular epidemiological analysis of outbreak strains facilitates the rapid and accurate identification of etiologic agents and can be used to provide information on the origin of viruses (Gilchrist et al., 2015). In the absence of targeted sequencing protocols, as well as during outbreak situations, the random amplification of purified nucleic acids in combination with NGS can be a useful tool for monitoring viral evolution or diagnosis. Although most molecular detection methods are unable to detect mixed viral infections, NGS allows for both target dependent and target independent sequencing of viral genomes and provides an opportunity to detect the genome of multiple viruses simultaneously (Thorburn et al., 2015). However, the samples used in such studies are often available in limited quantities of virus.

Sequence-Independent, Single-Primer Amplification (SISPA) is one of the random priming methods develop by Reyes and Kim (1991) that allows enrichment of the viral genome in only a few steps (Djikeng et al., 2008). There have been several modifications of the SISPA method since its first implementation, including random-PCR (rPCR) (Froussard, 1993). This combines first-step reverse transcription followed by denaturation, annealing and amplification by using random hexamers tagged with a known sequence which is then used as a primer binding extension sequence. It was developed to yield double-stranded cDNA in sufficient abundance for cloning and then sequencing (Froussard, 1993; Zou et al., 2003). DePew et al. (2013) further modified the SISPA protocol to identify cultivable viruses from a single plaque using 454 and HiSeq Illumina platform without the need of a cloning step.

Previous studies have combined random priming approaches with NGS for identification of a novel mink astrovirus (Blomström et al., 2010), the partial sequencing of a novel paramyxovirus in penguins (Miller et al., 2010b), influenza viruses (Afonso, 2007), metagenomics analysis of Dengue virus infected mosquitoes (Bishop-Lilly et al., 2010), and viruses in human stool samples (Victoria et al., 2009).

* Corresponding author.
E-mail address: darrell.kapczynski@ars.usda.gov (D.R. Kapczynski).
[1] KC and DHL contributed equally to this article.

http://dx.doi.org/10.1016/j.virol.2017.06.019
Received 24 April 2017; Received in revised form 14 June 2017; Accepted 15 June 2017

Moreover, the NGS approach has recently been applied for viral genome sequencing of archived serum samples from the oldest HIV-1 positive group genomes, resulting in identification of “Patient 0” (Worobey et al., 2016).

Single-stranded positive-sense RNA (ssRNA+) and negative sense RNA (ssRNA) viruses represent a large group of avian viral agents, including West Nile virus (WNV), avian metapneumovirus (aMPV), infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and avian influenza virus (AIV) (Swayne, 2013). Among RNA viruses, AIV of *Orthomyxoviridae* family and NDV of *Paramyxoviridae* family are considered as the most devastating poultry diseases owing to their highly pathogenic forms as well as worldwide distribution and economic implications (Swayne, 2008; Miller et al., 2010a; Alexander et al., 2012). IBV of the *Coronaviridae* family also causes significant morbidity and economic losses to the poultry industry affecting primarily the respiratory tract, and can demonstrate a wide range of tissues tropism, including the renal and reproductive systems (Bande et al., 2016). Combining target independent genome amplification with the high sensitivity of NGS provides the opportunity not only for detection of these viral pathogens, but can also simultaneously provide complete genomic sequences to allow further genetic characterization. Nonetheless, the feasibility of applying these techniques to diagnosis and research of pathogens requires further evaluation and optimization study.

A simplified, sequence-independent technique of directional amplification in combination with NGS was used in this study. The protocol was tested against purified virus stocks and clinical samples. Viral genome sequences were determined from both negative- and positive-sense single stranded RNA viruses. The limit of detection necessary for virus identification using SISPA–NGS and metagenomics approaches was also determined.

2. Material and methods

2.1. Viruses

Viruses used in these studies included two highly pathogenic AIV, A/duck/Vietnam/NCVD-672/2011(H5N1) (Dk/Vn), and A/turkey/Minnesota/15–12582-1/2015(H5N2) (Tk/Mn), two NDV isolates, A/duck/Vn/Long bien/78/02 (Dk/Vn/NDV) and Lasota B1 vaccine strain (LaS), and a single IBV strain, Ark99. The IBV (Ark99 strain) was kindly provided by Jackwood (University of Georgia, Athens, USA). All viruses were propagated in 9–11 day of embryonating specific pathogen free chicken eggs. Following three days of growth, the allantoic fluid was harvested for RNA extraction. Clinical samples, consisting of oral swabs, were collected from flocks of chickens in Jordan. The samples were kept under BSL3 facility in −80 °C until used. The SISPA-NGS method was applied to determine viral presence in clinical samples. Furthermore, clinical samples were used for comparison to purified virus stocks.

2.2. RNA isolation

Total RNA extraction from pure virus stocks and clinical samples was performed using RNeasy Mini Kit (QIAGEN, Valencia, USA) according to manufacturer's instruction. Optimal quality of the extracted RNA was verified by obtaining the OD_{260}/OD_{280} values using NanoDrop (Thermo Scientific, Wilmington, USA).

2.3. Viral RNA quantification

For AIV, quantitative real time RT-PCR (qRRT-PCR) was performed as previously described (Kapczynski et al., 2013; Spackman et al., 2002). Briefly, qRRT-PCR reactions targeting the influenza virus M gene was conducted using AgPath-ID one-step RT-PCR Kit (Ambion, Austin, TX) and the ABI 7500 Fast Real-Time PCR system (Applied Biosystem, Calsbad, CA). The RT step conditions for reactions were 10 min at 45 °C and 95 °C for 10 min. The cycling conditions were 45 cycles of 15 s, 95 °C; 45 s, 60 °C. For virus quantification, a standard curve was established with RNA extracted from dilutions of the same titrated stock of the viruses. Ct (cycle threshold) values of each viral dilution were plotted against viral titers. The resulting standard curve had a high correlation coefficient ($r^2 > 0.99$), and it was used to convert Ct values to EID_{50}/ml.

For NDV, qRRT-PCR targeting the NDV M gene was conducted as described previously (Wise et al., 2004). The RT step was 30 min at 50 °C, followed by 15 min at 95 °C. The cycling conditions consisted of 40 cycles of 10 s of denaturation at 94 °C, 30 s of annealing at 52 °C, and extension at 72 °C for 10 s. For virus quantification, a standard curve was established with RNA extracted from dilutions of the titrated stock of the virus qRRT-PCR was conducted using AgPath-ID one-step RT-PCR Kit (Ambion) and the ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Calsbad, CA).

For IBV, qRRT-PCR for IBV detection was performed as described previously (Callison et al., 2006). The reaction was conducted at 50 °C for 30 min; 95 °C for 15 min; 40 cycles of 94 °C for 1 s followed by 60 °C for 60 s. For virus quantification, a standard curve was established with RNA extracted from dilutions of the titrated stock of the virus. RRT-PCR was conducted using AgPath-ID one-step RT-PCR Kit (Ambion) and the ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Calsbad, CA).

2.4. Random priming (RP)-mediated SISPA

As shown in Fig. 1, first-strand cDNA was synthesized in a 20-µl reaction mixture with 5 µl of viral nucleic acids from each sample, 100

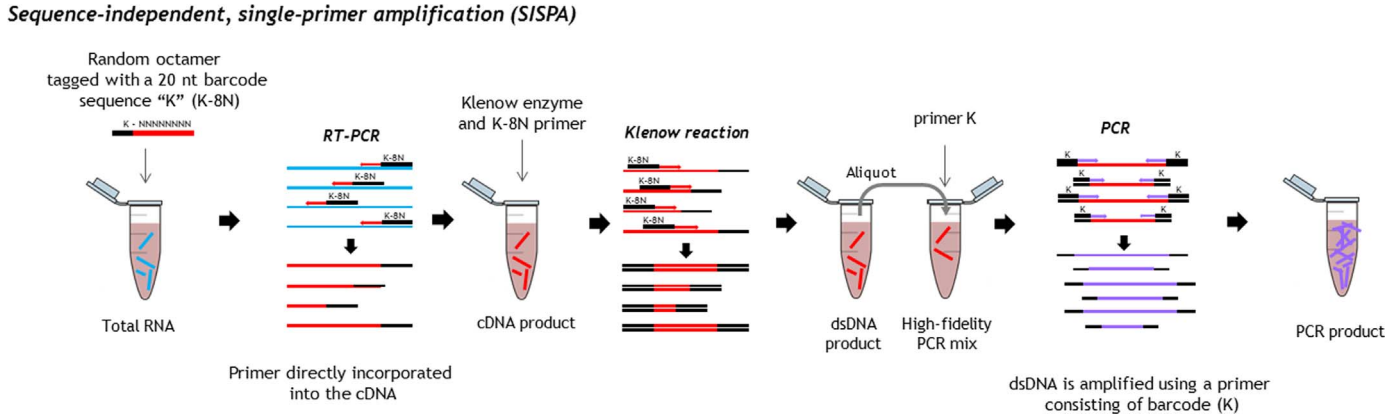

Fig. 1. Overview of the SISPA strategy.