Veterinary Research Communications, 30 (2006) 191–204

Detection of Foot and Mouth Disease and Porcine Reproductive and Respiratory Syndrome Viral Genes Using Microarray Chip

Y.-C. Liu[1]*, G.S. Huang[2] , M.-C. Wu[1], M.-Y. Hong[2] and K.-P. Hsiung[3]
[1]Department of Chemical Engineering, National Chung Hsing University, Taichung 402, Taiwan; [2]Institute of Nanotechnology, National Chiao Tung University, Taiwan; [3]R&D Division, Taiwan Unison Biotech Inc., Chunan, Taiwan, Republic of China
**Correspondence: E-mail@: ycliu@dragon.nchu.edu.tw*

Liu, Y.-C., Huang, G.S., Wu, M.-C., Hong, M.-Y. and Hsiung, K.-P., 2006. Detection of foot and mouth disease and porcine reproductive and respiratory syndrome viral genes using microarray chips. *Veterinary Research Communications*, **30(2)**, 191–204

ABSTRACT

Two viral pathogens, namely, porcine reproductive and respiratory syndrome virus (PRRSV) and foot and mouth disease virus (FMDV), were selected as models for multiple pathogen detection in a cDNA microarray. Two signature regions selected from ORF2 (around 500 bp) and ORF5 (around 600 bp) of PRRVS (America serotype), and one signature region from structural genes VP1 (around 500 bp) of FMDV type O were designed and spotted on a nylon membrane. For PCR sensitivity study, the cloned FMDV–VP1 template could be diluted to near one copy and its PCR product was still detectable in gel electrophoresis. In the microarray detection, the labelling FMDV probes (3 mg/ml) could be diluted 320 times and still maintained a visible colour when hybridized with the chip. Using the mixing primers, the microarray chip demonstrated rapid and accurate detection of the specific genes. To our knowledge, this preliminary study is the first example reported applying the long signature sequences to the multiple pathogen detection in cDNA microarray.

Keywords: cDNA microarray, gene chip, foot and mouth disease virus, porcine reproductive and respiratory syndrome virus

Abbreviations: DTT, dithiothreitol; FMDV, foot and mouth disease virus; GE, gel electrophoresis; HPLC, high-performance liquid chromatography; PCR, polymerase chain reaction; PRIT, Pig Research Institute Taiwan; PRRSV, porcine reproductive and respiratory syndrome virus, RT-PCR, reverse transcription polymerase chain reaction; VP, viral protein

INTRODUCTION

Veterinary disease control strategies have become increasingly difficult to implement owing to the rapid growth of international trade. Quarantine and inspection of imported animals are essential to prevent exotic diseases from entering a country. Both FMDV and PRRSV prevailed in Taiwan years ago, believed to be transfected from animals and/or animal products from imported sources. Thus, a quick and specific diagnostic approach is highly desirable since the consequences and potential economic losses from leaky inspection of quarantine are enormous. Reverse

transcription polymerase chain reaction (RT-PCR) and immunological diagnosis (Chenard *et al.*, 2003; Clavijo *et al.*, 2003; Reid *et al.*, 2003; Saiz *et al.*, 2003; Barlic-Maganja *et al.*, 2004) are frequently adopted to examine plants or meat imports. However, these approaches are always tedious and time consuming when applyed to multiple viral pathogens. Consequently, an alternative methodology, capable of quick and accurate detection of the viral pathogens, is required.

Since the early 1990s, cDNA microarrays capable of integrating thousands of cDNAs on a solid matrix for hybridization to their respective cDNA targets have been developed to maturity (Schena *et al.*, 1995; Debouck and Goodfellow, 1999; Ekins and Chu, 1999; Musarrat and Hashsham, 2003; t'Hoen *et al.*, 2003; Ku *et al.*, 2004; Parrish *et al.*, 2004; Rouse *et al.*, 2004). This cDNA microarray technology provides an efficient method for quantitatively detecting numerous genes in parallel (Schena *et al.*, 1995; Lee and Lee, 2000; Lockhart and Winzeler, 2000) and has attracted considerable attention from the biomedical research community (Schena *et al.*, 1995; Gerhold *et al.*, 1999; Young, 2000). It is therefore expected to become a revolutionary method with which to rapidly (within 4–12 h) and accurately diagnose infectious diseases (Debouck and Goodfellow, 1999; Gerhold *et al.*, 1999; Hacia, 1999; Young, 2000). In our laboratory, a system for manufacturing human cDNA microarrays on a nylon membrane has been successfully established and has been applied in cardiovascular research (Huang *et al.*, 2000). In this microarray system, genomic gene expression was colorimetrically measured in parallel.

In this preliminary work, cDNA microarray technology was implemented to evaluate the feasibility of detecting multiple pathogen genes. The microarray chip used for quarantine purpose should be designed to accurately detect various pathogen genes in a single reaction. The sensitivity and specificity of detection using cDNA microarrays, or DNA chips, is heavily reliant on the length of the DNA template bound to the solid support. Oligonucleotides with length below 20 nucleotides are frequently employed for detecting sequences with a single nucleotide variation. However, this type of chip suffers from reduced binding affinity and only works under strictly defined conditions owing to the inhomogeneity of melting temperatures among the spotted oligonucleotides. The use of longer DNA fragments may markedly improve the affinity and broaden the range of working conditions. However, in hybridizing conditions of lower stringency, homologous DNA sequences may create false-positive signals, resulting in lower detection specificity (Sambrook *et al.*, 1989). This implies that when the length of the DNA templates increases, the detection sensitivity generally increases while specificity decreases. An effective microarray chip must detect possible pathogenic genes with maximum sensitivity, thus saving precious inspection time. Meanwhile, ambiguous readings can be reconfirmed by adopting the follow-up immunology-based methodologies. Based on this concept, DNA fragments with lengths of around 500 base pairs are used.

Foot and mouth disease virus (FMDV) (Rasmussen *et al.*, 2003; Saiz *et al.*, 2003; Zhang and Alexandersen, 2003; Barlic-Maganja *et al.*, 2004; Carpenter *et al.*, 2004; Moonen *et al.*, 2004; Reid *et al.*, 2004) and porcine reproductive and respiratory syndrome virus (PRRSV) (Yahara *et al.*, 2002; Drolet *et al.*, 2003; Key *et al.*, 2003; Kleiboeker, 2003; Batista *et al.*, 2004) were selected as model viruses for examination

of the microarray technology to check whether it could be used to identify these two important viral pathogens of pigs exotic to Taiwan. To prevent the binding of homologous but not pathogenic sequences, sequences with common functions were avoided and the signature regions of genes specific for the pathogens were carefully selected. Two signature regions of ORF2 and ORF5 from PRRSV (America serotype) (Allende *et al.*, 1999), and one homologous region of the VP1 gene from FMDV type O (Makoff *et al.*, 1982; Tsai *et al.*, 2000) were selected from GenBank. PCR primers (Table I) against the signature regions of the genes were designed to amplify respectively ORF2 and ORF5 fragments from PRRSV viral RNA and VP1 fragment from cloned FMDV DNA fragment. A simple cDNA microarray chip is thus designed to make rapid distinction between the infective viruses. The manufacturing process is described herein with regard to the ability to detect PRRSV and FMDV.

TABLE I
Sequences of PCR primers for amplification of viral DNA

ORF2	Forward	5′ TGAAATGGGGTCCGTGCAAAGCCTT 3′
	Reverse	5′ CTACAAAACCTGCGCACTACAGGGT 3′
ORF5	Forward	5′ GTTGGGGAACTGCTTGACCGTGGGC 3′
	Reverse	5′ TTCCAGCGGAACGATGGGGTCGTCC 3′
VP1	Forward	5′ CCACCTCTGCGGGTGAGTCTGCGGA 3′
	Reverse	5′ TGTGGCACCCGCAAAACAGCTTCTG 3′

MATERIALS AND METHODS

Chemicals

Molecular size markers for gel electrophoresis (GE), agarose, ethidium bromide and diethyl pyrocarbonate were purchased from Sigma Chemical Co. (St Louis, MO, USA); biotin-16-dUTP was purchased from Roche (Mannheim, Germany); and superscript II RNase H reverse transcriptase was purchased from Gibco BRL (Rockville, MD, USA). All other reagents were chemical reagent grade from Merck (Darmstadt, Germany). Finally, the organic solvents were of HPLC grade.

PRRSV and cloned FMDV gene

The PRRSV (America serotype)-infected porcine serum was obtained from the inspection centre of the Pig Research Institute Taiwan (PRIT). The PRRS viral RNA was extracted using a routine phenol extraction procedure. Meanwhile, the cloned VP1

gene of FMDV was kindly donated by Dr S.J.T. Mao at PRIT. Candidates for the signature sequence of each gene were compared and selected from GenBank. Two different signature regions, ORF2 (around 500 bp) and ORF5 (around 600 bp) were chosen from envelope protein of PRRSV (Chueh *et al.*, 1998), while a homologous signature region (around 500 bp) of the VP1 gene from FMDV type O was selected (Tsai *et al.*, 2000). These candidate sequences showed no homology to other genes and were selected for the subsequent experiments.

Amplification of the target gene sequence

PRRS viral RNA was extracted from infected porcine serum using phenol extraction, and the extracted viral RNA was annealed with 6 μmol/L random hexamer in a total volume of 50 μl. Subsequently, cDNA synthesis was performed in a 100 μl mixture containing 0.5 mmol/L each of dATP, dCTP, dGTP and dTTP (Boehringer Mannheim, Germany), 10 mmol/L DTT (Gibco-BRL, Gaithersburg, MD, USA), 0.5 units/μl human placental ribonuclease inhibitor (HT Biotechnology Ltd, Cambridge, UK), and 200 units of Superscript RT II (Gibco-BRL, Gaithersburg). The mixture was incubated at 42°C for 90 min and then heated to 90°C for 5 min to terminate the reaction and used as the reverse transcription (RT) mixture. The viral sequences were amplified with polymerase chain reaction (PCR) from the RT mixture with specific primers (Table I), and PCR amplifications were performed in total volume of 100 μl, containing 10 mmol/L Tris-HCl, pH 8.8, 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.1% Triton X-100, 200 μmol/L dNTP, 0.2 μmol/L of each primer, and 0.5 units of *Taq* polymerase (Viogen, Taiwan). The following thermal cycles were used: 10 min at 94°C, followed by 25 cycles of 30 s at 94°C, 30 s at 57°C, and 4 min at 72°C, and finally an extension period of 10 min at 72°C. For the FMDV VP1 signature gene, the same PCR amplification process was performed using cloned VP1 gene as a template, and the PCR products were then examined by agarose GE to quantify the amplified fragments. Final DNA concentration was adjusted to 3 mg/ml by evaporation at 95°C and resuspended in 10% glycerol–0.01% bromophenol blue before spotting on the nylon membrane.

Spotting the target gene sequence on the membrane

Spotting was carried out in a DNA Arrayer 3 (Wittech Co., Taiwan) onto a positively charged nylon membrane (Boehringer Mannheim, Germany) with a density of 1600 spots/cm^2. The arrayer dispensed 100 μm diameter spots on the nylon membrane at a pitch of 225 μm, and each spot contained about 10 ng of DNA. Meanwhile, the ORF2 and ORF5 DNA fragments were spotted at concentrations of 3 mg/ml and 0.5 mg/ml, while VP1 was dispensed at single concentration of 3 mg/ml. All the ORF2, ORF5 and VP1 DNA fragments were spotted on the same membrane.

Manufacture of the labelling probes for PRRSV and FMDV

PRRSV RT mixture and FMDV VP1 fragment were used as templates for PCR reaction respectively, and the PCR reactions were performed in a total volume of 100 μl, containing 10 mmol/L Tris-HCl, pH 8.8, 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.1% Triton X-100, 200 μmol/L dNTP, 0.2 μmol/L of each primer, 0.1 μmol/L biotin-16-dUTP and 0.5 units of *Taq* polymerase (Viogen, Taiwan). The following thermal cycles were used: 10 min at 94°C, followed by 25 cycles of 30 s at 94°C, 30 s at 57°C, and 4 min at 72°C, and finally an extension period of 10 min at 72°C. The biotin labelling PCR products were examined by agarose GE to quantify the amplified fragments and the final concentration was adjusted to 3 mg/ml before use.

Hybridization and image analysis

The membrane containing spotted cDNA was pre-hybridized in 5 ml 1× hybridization buffer containing 5× SSC, 0.1% SDS, 1% BM blocking buffer (Boehringer Mannheim, Germany), and 10 μg/ml denatured salmon sperm DNA, at 60°C for 1 h. The probe was mixed with 2 μl of 10 μg/μl poly(dA)10, 2 μl of 10 μg/μl human Cot-1 DNA (Gibco BRL) and 40 μl of 2× hybridization buffer to obtain a final volume of 80 μl, and the probe mixture was then denatured at 95°C for 5 min and cooled on ice. The membrane was annealed using a probe mixture in a hybridization chamber, then incubated for 5 min at 95°C and then for 12–16 h at 63°C. The membrane was washed twice with 5 ml of 2× SSC (0.1% SDS) for 5 min at room temperature, followed by three 15 min washes with 5 ml of 0.1× SSC (0.1% SDS) at 58°C. The membrane was blocked with 5 ml of 1% BM blocking reagent containing 2% dextran sulphate at room temperature for 1 h, and this was followed by incubation with a 5 ml mixture containing 700× diluted streptavidin-β-galactosidase (1.38 U/ml, enzyme activity) (Gibco BRL), 4% polyethylene glycol 8000 (Sigma), and 0.3% BSA in TBS buffer (10 mmol/L Tris, pH 7.4, 150 mmol/L NaCl) at room temperature for 1 h. The membrane then underwent three 5 min washings with TBS buffer and was treated with 5 ml X-gal substrate containing 1.2 mmol/L X-gal, 1 mmol/L $MgCl_2$, 3 mmol/L $K_3Fe(CN)_6$ and 3 mmol/L $K_4Fe(CN)_6$ in TBS buffer for 45 min at 37°C with gentle shaking, followed by a mini-Q water wash and air drying. Finally, a colour image was generated using a UMAX PowerLook 3000 flatbed scanner at a resolution of 3048 dpi and processed using ScanAlyze (Michael Eisen, Stanford University).

RESULTS

Manufacture of the microarray chip

The signature regions of ORF2 and ORF5 from PRRSV and VP1 from FMDV were amplified via PCR and the products were examined using GE. The result is shown in Figure 1. Tailing was observed on the band of lane D in Figure 1, which might derive

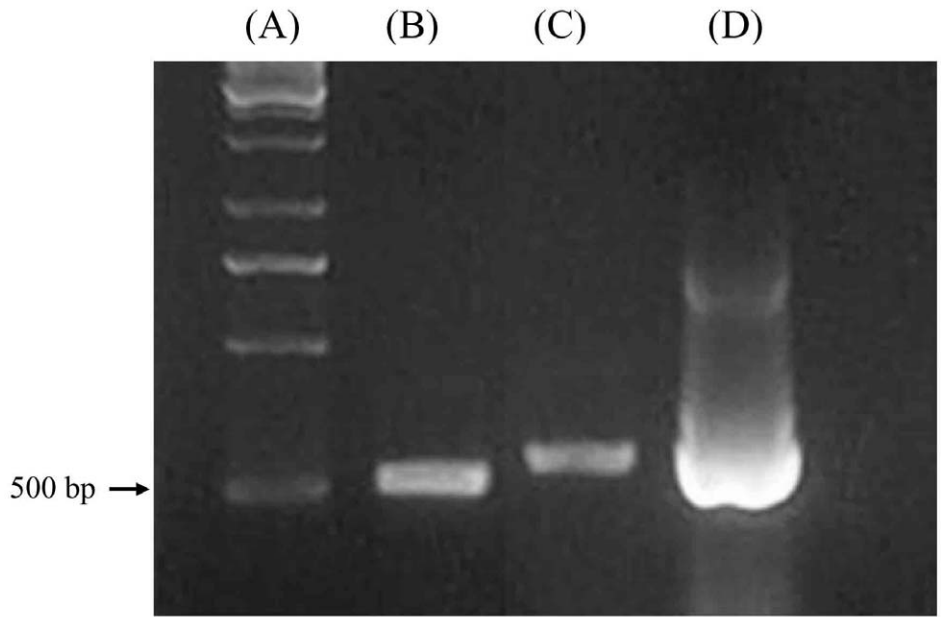

Figure 1. Gel electrophoresis of the PCR products, signature regions of (B) ORF2, (C) ORF5, and (D) signature region of VP1, manufactured using designed primer sets listed in Table I. (A) Gene markers

from either the impurity of mRNA in the extraction process or the overamplification during the PCR reaction; however, the location of the major band showed clearly. All the amplified fragments were purified, concentrated, quantified and spotted using a robotic mechanism on a positively charged nylon membrane.

Detection of viral genes using the microarray chip

To manufacture the probes, PCR was conducted for PRRSV and FMDV respectively, except that biotin-16-dUTP was incorporated during the PCR reaction. The individual PCR labelling product was hybridized with the microarray chip and the colours were developed. Accurate identification for ORF2, ORF5 fragments from PRRSV and VP1 fragment from FMDV was obtained, as shown in Figure 2. On the other hand, for PRRSV, it could be observed that the spots with high concentration (upper spots) developed more colour intensity than did low concentration spots. Generally, higher colour intensity is more sensitive for visible detection purposes, but the amounts of spotted DNA should be further optimized.

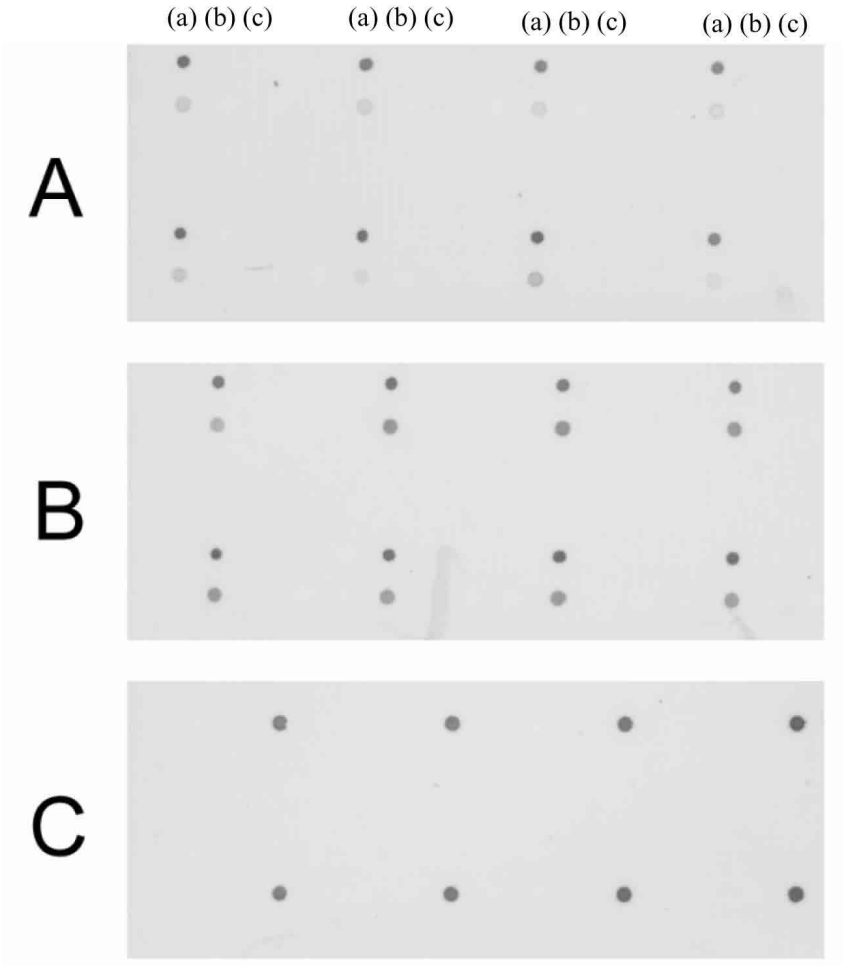

Figure 2. Colorimetric detection of ORF2, ORF5 and VP1 fragments using a microarray chip. PRRSV labelling probes were made from PRRSV RT mixture with primer pairs against ORF2 and ORF5, respectively. FMDV labelling probe was made directly from cloned FMDV gene using primer pairs against VP1. Biotin-16-dUTP was incorporated in each labelling PCR. The individual PCR product was hybridized to the microarray chip, followed by colour development. Colour spots shown in the figure indicate positions of (A) ORF2, (B) ORF5 and (C) VP1 fragments. In each microarray chip, for ORF2 and ORF5 DNA fragments, two spots at concentrations of 3 mg/ml (upper) and 0.5 mg/ml (lower) were applied on lines (a) and (b), respectively, while for the VP1 DNA fragment, a concentration of 3 mg/ml was applied on line (c)

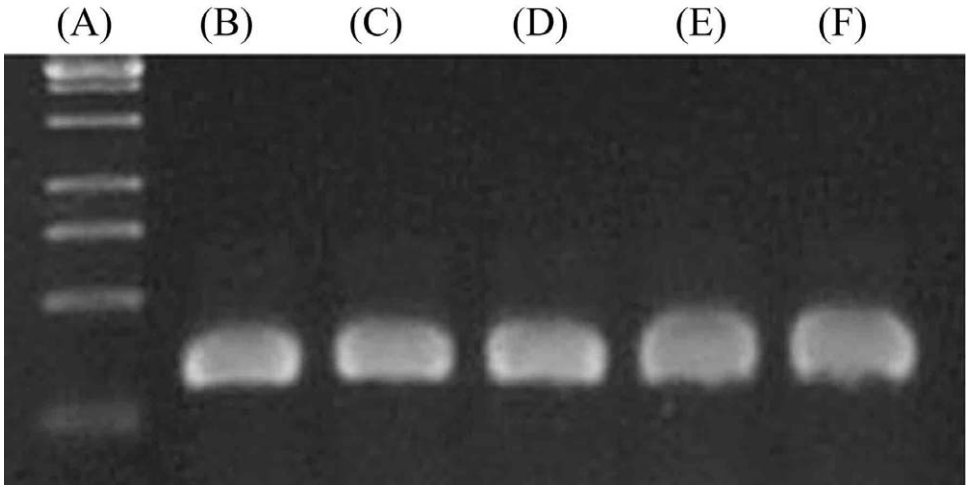

Figure 3. Gel electrophoresis of the PCR products amplified from variously diluted templates (i.e. cloned FMDV DNA). The FMDV DNA fragment was diluted to (B) 1, (C) 10, (D) 100, (E) 1000 and (F) 10 000 copy numbers in individual PCR reaction. (A) Gene markers

Detection sensitivity

For exploring PCR amplification sensitivity, the originally cloned FMDV VP1 gene (about 10 000 copies) was diluted 10, 100, 1000 and 10 000 times respectively and then used as the templates for PCR reaction with biotin-16-dUTP incorporated. After that, the PCR products (FMDV probes) were detected in GE. Figure 3 shows that, even the PCR template was diluted to copy number 1, a developed band around 500 bp was still clearly visible. For studying the microarray chip sensitivity, the manufactured FMDV labelling probe with a concentration of 3 mg/ml was diluted 160, 320, 640 and 1280-fold respectively and then hybridized with the chip. From the results displayed in Figure 4, the developed colour in the chip was visible when using this detection protocol with a 320-fold dilution.

Mixing primer detection

The PRRSV infected serum was reverse-transcribed to cDNA as the RT mixture. Four types of primer compositions for amplification of, respectively ORF2+ORF5+VP1, ORF2, ORF5 and VP1 fragments were used for probes manufacture via PCR with the PRRSV RT mixture as a template. Following the PCR labelling amplification, the probes were made and examined in GE. As displayed in Figure 5, probes produced following PRRSV primers (ORF2 or ORF5) gave the expected visible bands.

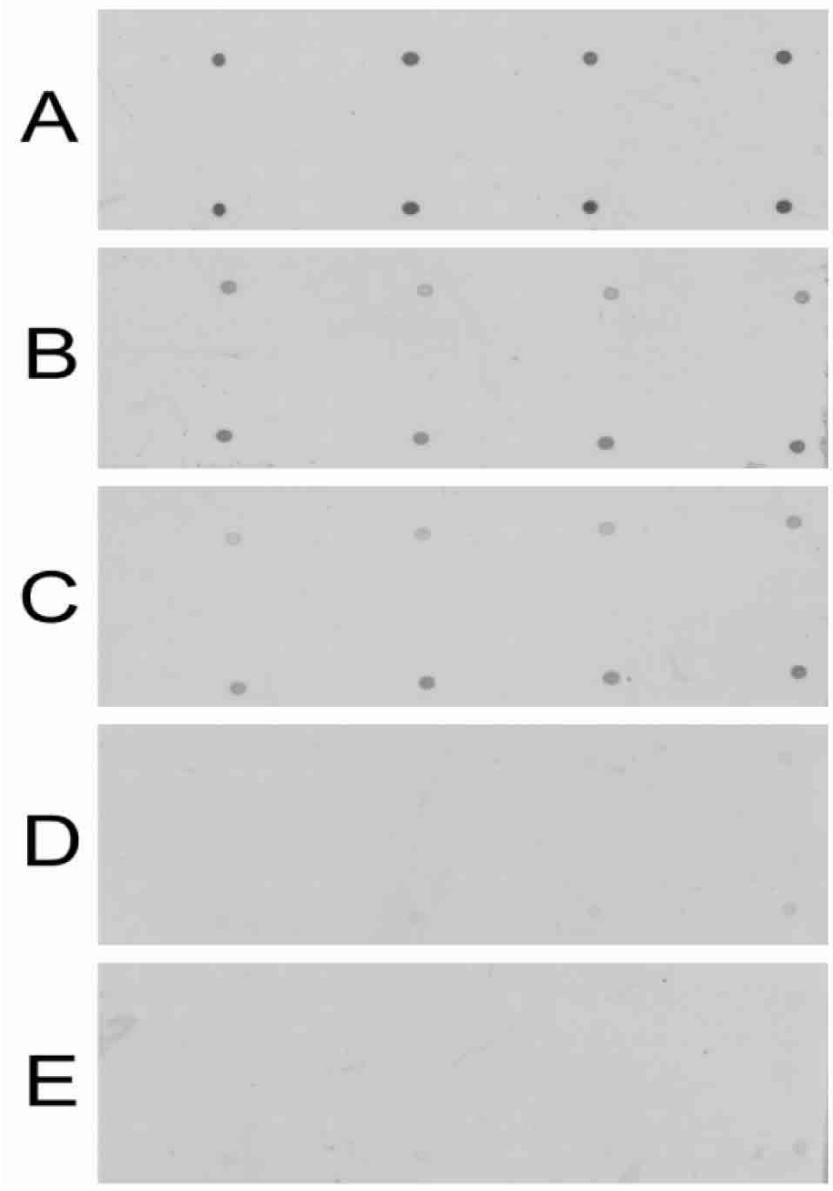

Figure 4. Effect of the dilution times of FMDV labelling probes (3 mg/ml) when detected in the microarray chip. The probes were diluted (A) to original concentration, (B) 160-fold, (C) 320-fold, (D) 640-fold and (E) 1280-fold and hybridized with the microarray

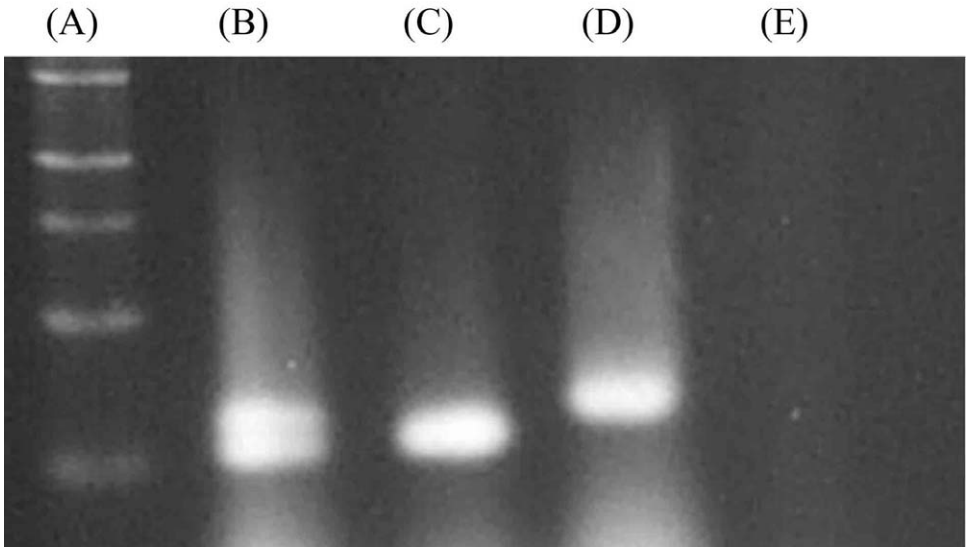

Figure 5. Gel electrophoresis of the probes of PRRSV using different primer pairs. The probes of PRRSV were made with (B) primer mixtures of ORF2, ORF5 and VP1, (C) primer pairs of ORF2, (D) ORF5 and (E) VP1. (A) Gene markers

Furthermore, by hybridizing these probes with the prepared microarray chip, followed by colour development, the specific PRRSV spots were revealed, whereas no colour was detected in the FMDV spot, as shown in Figure 6. It is also noted that some random tiny spots were observed in Figures 4D, 4E and Figures 6A, 6D, which might derive from the inappropriate blocking of the membranes; however, these spots were either too small or not in the true spots' locations, and therefore, seldom interfere with the examination results. The result indicated that various pathogen sequences could be amplified by a primer cocktail comprising primer pairs specific to individual signature regions of the exotic pathogen genes. Meanwhile, the specifically amplified sequences could be further examined and identified by hybridization to the microarray chip containing sequences from their corresponding pathogens. This approach allows the colour response at the specific positions to identify a specific pathogen.

DISCUSSION

Complementary DNA microarray technology provides an efficient method of quantitatively expressing numerous genes, in a comparative, parallel manner. In this work, the application of cDNA microarray technology to detect multiple viral genes was exemplified. Only a limited number of viral genes and a handful of viral samples

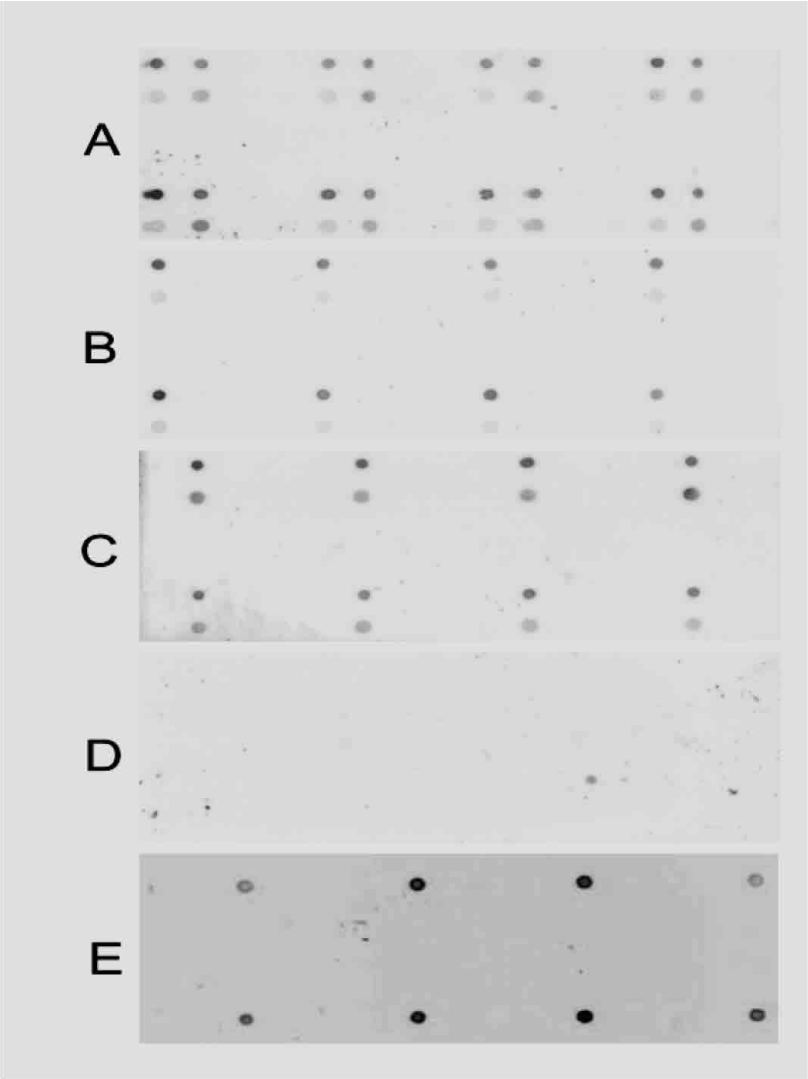

Figure 6. Detection of PRRSV in the microarray chip with different primer pairs. The labelling probes were made with (A) primer mixture of ORF2, ORF5 and VP1, (B) primer pairs of ORF2, (C) ORF5 or (D) VP1 to detect PRRSV in the microarray. (E) shows the locations of visible spots of the VP1 gene when hybridized with VP1 labelling probes

were incorporated in the current model, owing to the limited ability to handle infectious diseases. The strategy developed herein of using multiple PCR primer pairs to amplify specific viral sequences and to detect various pathogens could potentially be useful in molecular quarantine diagnosis. Presumably, by careful primer choice and gradually adding primers to the final cocktail, this approach could also be achievable when more genes are included. In the veterinary area, this microarray approach could provide a rapid pre-screening function, which might dramatically reduce the numbers of tests requiring further confirmation of the infection using the traditional immunological testing. This duplex microarray technology with long DNA fragment probes was proved to be useful in the detection of animal viral genes and may be applicable in multiplex viral diagnosis of veterinary diseases where the animal diseases share common symptoms, such as sneezing, coughing, rhinorrhoea, etc. The microarray chip, given its enormous screening power, is worth developing for quarantine and inspection uses.

ACKNOWLEDGMENTS

The authors thank the National Science Council of the Republic of China for financially supporting this research under contract nos. NSC89-2313-B-059-001 and NSC89-2313-B-059-007. Dr Konan Peck is appreciated for his generous support during the establishment of cDNA microarray instrumentation. This work was also supported in part by Department of Agriculture under contract DOA-89-KG-6-3-C-53.

REFERENCES

Allende, R., Lewis, T.L., Lu, Z., Rock, D.L., Kutish, G.F., Ali, A., Doster, A.R. and Osorio, F.A., 1999. North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. *Journal of General Virology*, **80**, 307–315

Barlic-Maganja, D., Grom, J., Toplak, I. and Hostnik, P., 2004. Detection of foot and mouth disease virus by RT-PCR and microplate hydridization assay using inactivated viral antigens. *Veterinary Research Communications*, **28**, 149–158

Batista, L., Dee, S.A., Rossow, K.D., Polson, D.D., Xiao, Z., Olin, M., Murtaugh, M.P., Molitor, T.W., Joo, H.S. and Pijoan, C., 2004. Detection of porcine reproductive and respiratory syndrome virus in pigs with low positive or negative ELISA s/p ratios. *Veterinary Record*, **154**, 25–26

Carpenter, T.E., Thurmond, M.C. and Bates, T.W., 2004. A simulation model of intraherd transmission of foot and mouth disease with reference to disease spread before and after clinical diagnosis. *Journal of Veterinary Diagnostic Investigation*, **16**, 11–16

Chenard, G., Miedema, K., Moonen, P., Schrijver, R.S. and Dekker, A., 2003. A solid-phase blocking ELISA for detection of type O foot-and-mouth disease virus antibodies suitable for mass serology. *Journal of Virological Methods*, **107**, 89–98

Chueh, L.L., Lee, K.H., Wang, F.I., Pang, V.F. and Weng, C.N., 1998. Sequence analysis of the nucleocapsid protein gene of the porcine reproductive and respiratory syndrome virus Taiwan MD-001 strain. *Advances in Experimental Medicine and Biology*, **440**, 795–799

Clavijo, A., Viera-Pereira, P.J. and Bergmann, I., 2003. Use of the reverse transcription polymerase chain reaction (RT-PCR) for the rapid diagnosis of foot and mouth disease in South America. *Veterinary Research Communications*, **27**, 63–71

Debouck, C. and Goodfellow, P.N., 1999. DNA microarrays in drug discovery and development. *Nature Genetics*, **21**, 48–50

Drolet, R., Larochelle, R., Morin, M., Delisle, B. and Magar, R., 2003. Detection rates of porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, and swine influenza virus in porcine proliferative and necrotizing pneumonia. *Veterinary Pathology*, **40**, 143–148

Ekins, R. and Chu, F.W., 1999. Microarrays: their origins and applications. *Trends in Biotechnology*, **17**, 217–218

Gerhold, D., Rushmore, T. and Caskey, C.T., 1999. DNA chips: promising toys have become powerful tools. *Trends in Biochemical Sciences*, **24**, 168–173

Hacia, H.G., 1999. Resequencing and mutational analysis using oligonucleotide microarrays. *Nature Genetics*, **21**, 42–47

Huang, G.S., Yang, S.M., Hong, M.Y., Yang, P.C. and Liu, Y.C., 2000. Differential gene expression of livers from ApoE deficient mice. *Life Sciences*, **68**, 19–28

Key, K.F., Guenette, D.K., Yoon, K.J., Halbur, P.G., Toth, T.E. and Meng, X.J., 2003. Development of a heteroduplex mobility assay to identify field isolates of porcine reproductive and respiratory syndrome virus with nucleotide sequences closely related to those of modified live-attenuated vaccines. *Journal of Clinical Microbiology*, **41**, 2433–2439

Kleiboeker, S.B., 2003. Applications of competitor RNA in diagnostic reverse transcription-PCR. *Journal of Clinical Microbiology*, **41**, 2055–2061

Ku, W.C., Lau, W.K., Tseng, Y.T., Tzeng, C.M. and Chiu, S.K., 2004. Dextran sulfate provides a quantitative and quick microarray hybridization reaction. *Biochemical and Biophysical Research Communications*, **315**, 30–37

Lee, P.S. and Lee, K.H., 2000. Genomic analysis. *Current Opinion in Biotechnology*, **11**, 171–185

Lockhart, D.J. and Winzeler, E.A., 2000. Genomics, gene expression and DNA arrays. *Nature*, **405**, 827–836

Makoff, A.J., Paynter, C.A., Rowlands, D.J. and Boothroyd, J.C., 1982. Comparison of the amino acid sequence of the major immunogen from three serotypes of foot and mouth disease virus. *Nucleic Acids Research*, **10**, 8285–8295

Moonen, P., van der Linde, E., Chenard, G. and Dekker, A., 2004. Comparable sensitivity and specificity in three commercially available ELISAs to differentiate between cattle infected with or vaccinated against foot-and-mouth disease virus. *Veterinary Microbiology*, **99**, 93–101

Musarrat, J. and Hashsham, S.A., 2003. Customized cDNA microarray for expression profiling of environmentally important genes of *Pseudomonas stutzeri* strain KC. *Teratogenesis, Carcinogenesis, and Mutagenesis*, (supplement 1), 283–294

Parrish, M.L., Wei, N., Duenwald, S., Tokiwa, G.Y., Wang, Y., Holder, D., Dai, H., Zhang, X., Wright, C., Hodor, P., Cavet, G., Phillips, R.L., Sun, B.I. and Fare, T.L., 2004. A microarray platform comparison for neuroscience applications. *Journal of Neuroscience Methods*, **132**, 57–68

Rasmussen, T.B., Uttenthal, A., de Stricker, K., Belak, S. and Storgaard, T., 2003. Development of a novel quantitative real-time RT-PCR assay for the simultaneous detection of all serotypes of foot-and-mouth disease virus. *Archives of Virology*, **148**, 2005–2021

Reid, S.M., Grierson, S.S., Ferris, N.P., Hutchings, G.H. and Alexandersen, S., 2003. Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. *Journal of Virological Methods*, **107**, 129–139

Reid, S.M., Ferris, N.P., Hutchings, G.H., King, D.P. and Alexandersen, S., 2004. Evaluation of real-time reverse transcription polymerase chain reaction assays for the detection of swine vesicular disease virus. *Journal of Virological Methods*, **116**, 169–176

Rouse, R.J., Espinoza, C.R., Niedner, R.H. and Hardiman, G., 2004. Development of a microarray assay that measures hybridization stoichiometry in moles. *BioTechniques*, **36**, 464–470

Saiz, M., De La Morena, D.B., Blanco, E., Nunez, J.I., Fernandez, R. and Sanchez-Vizcaino, J.M., 2003. Detection of foot-and-mouth disease virus from culture and clinical samples by reverse transcription-PCR coupled to restriction enzyme and sequence analysis. *Veterinary Research*, **34**, 105–117

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, New York)

Schena, M., Shalon, D., Davis, R.W. and Brown, P.O., 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, **270**, 467–470

t'Hoen, P.A., de Kort, F., van Ommen, G.J. and den Dunnen, J.T., 2003. Fluorescent labelling of cRNA for microarray applications. *Nucleic Acids Research*, **31**, 20

Tsai, C.P., Pan, C.H., Liu, M.Y., Lin, Y.L., Chen, C.M., Huang, T.S., Cheng, I.C., Jong, M.H. and Yang, P. C., 2000. Molecular epidemiological studies on foot-and-mouth disease type O Taiwan viruses from the 1997 epidemic. *Veterinary Microbiology*, **74**, 207–216

Yahara, Y., Ohkubo, Y., Kariwa, H. and Takashima, I., 2002. Evaluation of enzyme-linked immunosorbent assay (ELISA) and immunofluorescent antibody (IFA) test for the detection of porcine reproductive and respiratory syndrome virus (PRRSV) antibody in pigs from conventional farms. *The Journal of Veterinary Medical Science*, **64**, 583–588

Young, R.A., 2000. Biomedical discovery with DNA arrays. *Cell*, **102**, 9–15

Zhang, Z. and Alexandersen, S., 2003. Detection of carrier cattle and sheep persistently infected with foot-and-mouth disease virus by a rapid real-time RT-PCR assay. *Journal of Virological Methods*, **111**, 95–100

(Accepted: 21 September 2004)