Evaluation of Recombinant Foot-and-Mouth Disease 2C3AB Protein for Use as an Antigen

Jadsada Ratthanophart * , Ratchaneekorn Vitoonpong , Vara Varong and Pusayaporn Swasdison

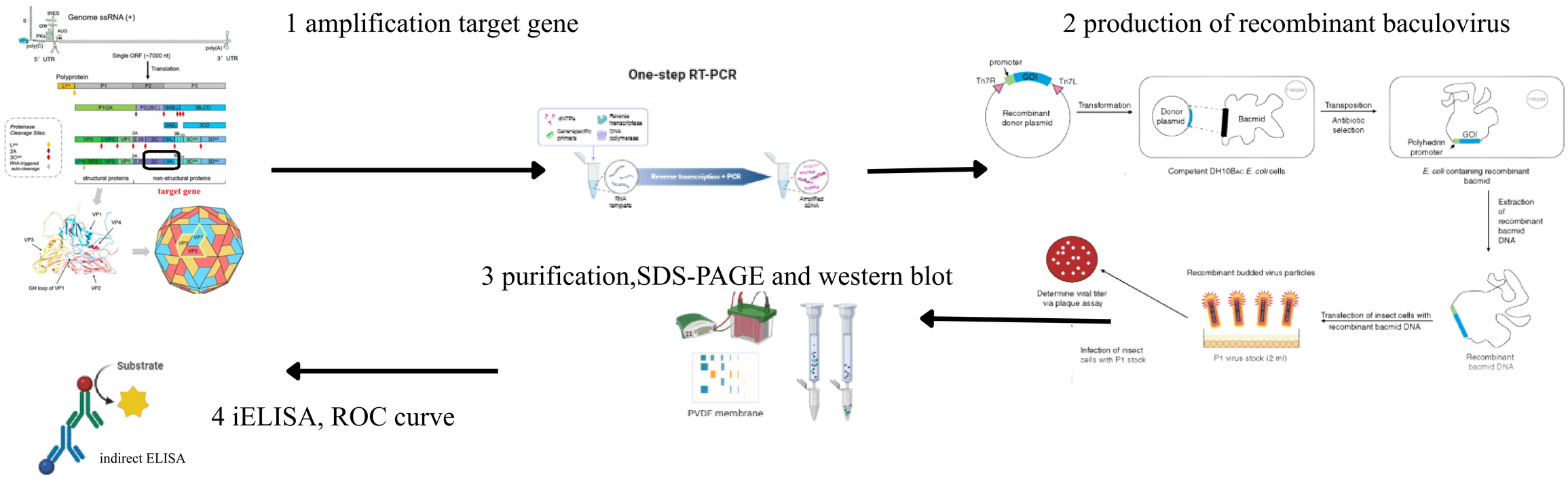
National Institute of Animal Health, Kasetklang, Chatuchak, Bangkok, 10900

***Corresponding author E-mail: jrattha@gmail.com**

Background

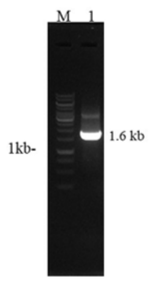
Foot-and-mouth disease (FMD) is a major threat to global livestock production. Effective surveillance requires distinguishing naturally infected animals from vaccinated populations using diagnostic markers absent in inactivated vaccines. Nonstructural proteins (NSPs) produced during natural infection serve as ideal DIVA antigens. The 2C3AB protein offers advantages over conventional 3ABC antigens, including broader epitope coverage and avoidance of processing complications, making it a promising candidate for enhanced FMD diagnostic assays. Objectives: Clone and express the 2C3AB gene from FMDV O/Mya-98 using a baculovirus system; develop an indirect ELISA using recombinant 2C3AB; and establish diagnostic performance parameters for DIVA surveillance applications.

Methods



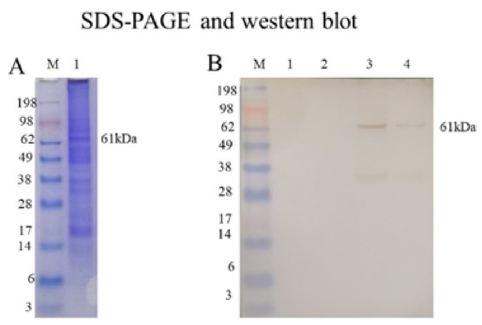
Results

1 RT-PCR successfully amplified the 2C3AB gene



PCR amplification of a 1.6 kb of 2C3AB gene from FMDV. M, molecular DNA ladder marker; 1, PCR product for target gene.

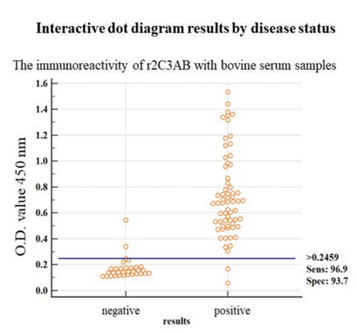
2 Recombinant r2C3AB protein expressed

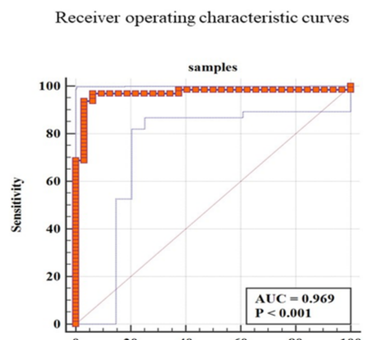


A, SDS-PAGE and B, Western blot analysis of recombinant 2C3AB expressed by baculovirus expression vector system. (A) the blot was stained by Coomassie brilliant blue and (B) the blot was probed with rabbit-anti 6xhis tag sera. Lane 1 is sf9, 2 is High Five cells. 3 and 4 is r2C3AB in 1 and 2 elution.

3 Diagnostic performance: 96.87% sensitivity, 93.73% specificity, AUC = 0.969

Optimal cutoff value: OD ≥ 0.24 (95% CI: 91.1%–99.4%)





Conclusion

The r2C3AB protein was successfully cloned and expressed using baculovirus system, demonstrating excellent diagnostic potential. The r2C3AB-based indirect ELISA achieved outstanding performance (96.87% sensitivity, 93.73% specificity, AUC = 0.969), confirming its reliability for FMDV detection in DIVA surveillance. The r2C3AB offers advantages over conventional 3ABC antigens, including avoidance of autocatalytic processing and broader epitope coverage. These findings support r2C3AB as a promising alternative diagnostic antigen for FMD serological surveillance programs

References

Mohapatra AK, Mohapatra JK, Pandey LK, et al (2014) Diagnostic potential of recombinant nonstructural protein 3B to detect antibodies induced by foot-and-mouth disease virus infection in bovines. Arch Virol. https://doi.org/10.1007/s00705-014-2089-0

Mohapatra JK, Pandey LK, Sanyal A, Pattnaik B (2011) Recombinant non-structural polyprotein 3AB-based serodiagnostic strategy for FMD surveillance in bovines irrespective of vaccination. J Virol Methods 177:184–192. https://doi.org/10.1016/j.jviromet.2011.08.006

Wang X, Zhou G, Liu Y, Yu L (2012) Establishment of indirect ELISA for detection of antibodies against FMDV with prokaryotic expressed 2C3AB as coating antigen. Zhongguo Yufang Shouyi Xuebao / Chinese J Prev Vet Med 34:543–54