CHAPTER V
CONCLUSION

Burkholderia pseudomallei is the etiology agent of an infectious disease known as melioidosis. The disease has been in humans ranges from asymptomatic to focal infection and could be life-threatening by rapid fatal septicemia. The treatment of melioidosis requires drug of choices which are ceftazidime and co-trimoxazole. An early diagnosis of the disease could decrease the fatal rate of the patients. Clinical presentation and rapid detection of the microorganism are necessary for early diagnosis of melioidosis. The conventional culture of the microbe is time-consuming and requires a full set of biochemical tests. In addition, automatic machinery for identification of such bacteria are not available in small microbiology laboratories. Therefore, a simple and rapid detection with high specificity and sensitivity of the test is required for its early diagnosis. CRISPR-Cas12a technology is an attractive tool for infectious disease diagnostic applications. This technology involves two essential components: a guide RNA to match a desired target gene, and Cas12a as an endonuclease for digestion activity.

In this study, we developed a new methodology for a rapid detection of *B. pseudomallei* using CRISPR-Cas12a technology. The T3SS is an important virulence factor of *B. pseudomallei* which T3SS gene cluster encodes a toxin delivery mechanism allowing pathogenic bacteria to inject toxic substances into the cytoplasm of the host's cells. The *B. pseudomallei* genome encodes three T3SSs which are referred to as T3SS-1, T3SS-2 and T3SS-3. T3SS-1 is present in *B. pseudomallei*, but absent in both *B. mallei* and *B. thailandensis* which are closely related Gram-nagative bacteria. Therefore, the open reading frames: *orf2* and *orf11* from T3SS-1 gene cluster that were the specific markers of *B. pseudomallei* distinguishing from another closely related species, *B. thailandensis* and *B. mallei* were selected for gRNA design in this study. The DNA of *B. pseudomallei* was extracted from the colony suspension by different methods and the boiling method without DNA precipitation gave the quickest and simplest

methodology for DNA extraction. The newly designed gRNA was shown to be specific for *orf2* and generated digested DNA fragments with larger and smaller sizes whereas the newly designed gRNA specific for *orf11* generated smaller sizes of digested DNA fragments. This indicates that the two newly designed gRNA were specific for this bacterial pathogen illustrating the endonuclease activity, therefore, they could be applied for the rapid detection of *B. pseudomallei* in clinical specimens collected from melioidosis patients. To visualize the digestion activity, a ssDNA-FQ reporter should be used for simple readouts of the target-activated CRISPR/Cas12a cleavage activity. The output fluorescence signal and its intensity are directly related to the presence and concentration of the activated Cas12a in the reaction system. In our study, we observed an increase in collateral cleavage activity of the FAM fluorophore from its quencher, according to an increased generation of fluorescence signal, in the wild-type *B. pseudomallei* strain K96243 and *B. pseudomallei* isolated from clinical samples, but not in *B. thailandensis* or control samples. Although, the finding of slightly intense *orf2* fragment of *B. thailandensis* on the agarose gel by PCR was observed, further analysis using the CRISPR/Cas12a system with ssDNA-FQ reporter demonstrated that the designed gRNA for *orf2* from T3SS-1 of *B. pseudomallei* of this study was specific for detecting *B. pseudomallei* based on fluorescence signal amplification. Furthermore, the designed gRNA for *orf11* from T3SS-1 of *B. pseudomallei* also demonstrated the specificity for detecting *B. pseudomallei* because of no fragment was found on agarose gel by PCR and the increasing of fluorescence signal was observed. These indicated that both gRNA of *orf2* and *orf11* designed in this study could specifically detect *B. pseudomallei*, but not other pathogens. As the results, these newly designed gRNA can be used for the development of a rapid diagnostic tool for melioidosis. This could facilitate a clinical microbiological laboratory in small community hospitals to detect and interpret the infection from *B. pseudomallei* more efficiently. For the further study the sensitivity of gRNA of *orf2* and *orf11* for *B. pseudomallei* detection should be determined.