THESIS TITLE : MODIFICATION OF POLYMERASE CHAIN REACTION (PCR) BASED TECHNIQUE TO DETECT HETEROZYGOUS RECESSIVE OF BOVINE LEUKOCYTE ADHESION DEFICIENCY (BLAD) IN HOLESTEIN FRESIAN CATTLE

AUTHOR :MR. UTHAI KOATDOKE

THESIS ADVISORY COMMITTEE :

..... Chairman

(Assistant Professor Dr. Suporn Katawatin)

..... Member

(Associate Professor Dr. Suchint Simaraks)

..... Member

(Assistant Professor Dr.Vanla Dittapongpitch)

ABSTRACT

Optimal conditions to amplify 58 bp target DNA based on PCR from Holestein Friesian cattle were compared in order to improve the PCR technique for BLAD detection. Optimal factors in 50 µl PCR reaction contained 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 12 piMoles of each PC 01 (3' – TCCGGAGGGCCAAGGGCTA - 5') and PC 02 (5'-GAGTAGGAGAGG TCCATCAGGTAGTACAGG-3') 3 U *Taq* DNA polymerase, and 150 ng DNA template. The PCR were performed for 40 cycles with denaturation at 94 ^{o}C for 20 seconds and annealing at 69 ^{o}C for 15 seconds. One hundred DNA template from Holestein Fresian cattle gave 58 bp target DNA from PCR technique. The target DNA was cleaned with phenol:chloroform:isoamyl alcohol (25:24:1) before digested with 5 U *Taq* I . The product of digested with *Taq* I yeilded 32 and 26 bp for all DNA samples from 100 Holestein Fresian cattle, indicated that all DNA did not have point mutation in nucleotide on gene encoding CD18 glycoprotein that caused bovine leukocyte adhesion deficiency(BLAD). It was concluded that the PCR technique with

the optimal conditions resulted from this study could be used for detecting BLAD in Holestein Fresian cattle, and all the 100 Holestein Fresian cattle studied were not the heterozygous recessive of BLAD.