

PCR

* . * . **

*
**

1.

21).

IgG

1,2),

6,17,22),

. IgG

, 가

가 .

3-6).

가 .

(heavy chain)

Gm

(light chain)

Km

PCR

7,8).

Gm

haplo -

restriction

fragment

length

type

. Gm Km

polymorphism(RFLP)

7,9).

IgG subclass

5,10-14),

2.

가^{3,15,16)},

IgG

subclass¹⁷⁻²⁰⁾

Gm

Km

1.

Actinobacillus

21)

actinomycetemcomitans

Porphyromonas

10

gingivalis

IgG subclass

DNA

ag 2 , axg 2 , agfnb 2 , axgfnb 1 ,
 agb3st 1 , abg 1 , afnb 1 10

(denaturation) 0.5 , 55
 (annealing) 0.25 , 72
 (extension) 0.5 ,
 5

2. DNA

-70
 10 1 X PBS 가 .
 4 2,000 rpm 20
 . 20ml
 (10mM Tris, pH8.0, 1mM EDTA,
 100 mM NaCl, 1 % sodium dodecyl sulfate,
 1 mg/ml proteinase K) ,
 55 12 .
 phenol 20 ml
 가 1 - 2 4
 8,000 rpm 20
 .
 2 - 3 , PCIA(25:24:1,
 phenol/ chlorform/isoamyl alcohol)
 가

7
 .
 Thermal Cycler(Perkin - Elmer
 Cetus, Norwalk, CT, USA) .

PCR Primer

KpnX(5' - CTTGCGCGCATACGCA -
 CAAC - 3')
 MCG1(5' - AACCCCTACCCTAACCC -
 CAA - 3')

3 M sodium acetate(pH
 5.5) 100 %
 가 DNA , 70 %
 .
 TE , DNA

4. DNA

PCR 10 ul 5 units
 가 12 ul
 (Gibco - BRL, Gaithersburg,
 MD, USA) 4
 1 - 2 %
 ethidium bromide

3. PCR

PCR 10 mM Tris - HCl(pH
 8.3), 50 mM KCl, 3.5mM MgCl₂, 0.01 %
 gelatin, 0.2 uM (primer), 250
 uM dinucleotide triphosphate 2.5
 units Taq (Perkin - Elmer Cetus,
 Norwalk, CT, USA) ,
 DNA 1 ug 가 100 ul
 . PCR 30

5. Souther blot analysis

plasmid HG015(Heavy Chain mu
 Probe C, HSRRB, Osaka, Japan)

HindIII BgIII
 750 bp nick translation
 [32P - aCTP]
 . Southern blot DNA
 HindIII 1 % agarose gel
 charged nylon membrane
 capillary transfer . Transfer
 UV - crosslink probe hybridize
 , X - ray film - 70 16

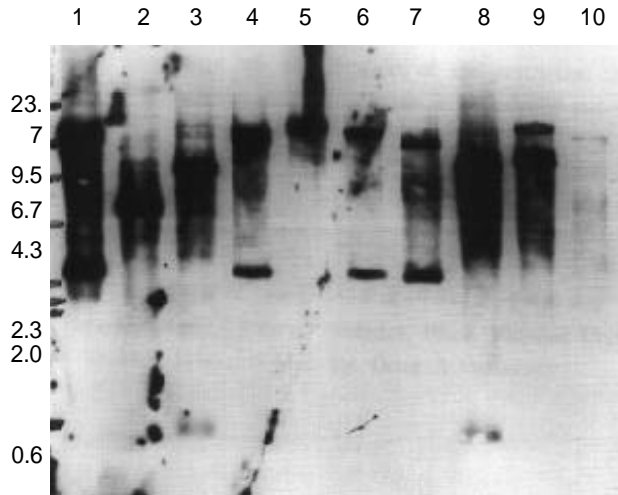


Figure 1. Restriction fragment length polymorphism (RFLP) of genomic DNA from 10 patients whose immunoglobulin(Ig) allotypes have been predetermined. Ig allotype of Lane 1 and 4 is ag, Lane 2 is agb3st, Lane 3 and 8 is agfb, Lane 5 is abg, Lane 6 and 7 is axg, Lane 9 is

III.

Figure 1

southern blot hybridization
restriction fragment length polymorphism(RFLP)
, ag, axg, agfnb,
axgfnb, agb3st, abg, afnb
DNA fragment length polymorphism
DNA band size가 ag 12.5 kb, 2.7 kb,
axg 10.1 kb, 2.7 kb, agfnb 7.0
kb, axgfnb 12.5 kb, 8.0 kb, afnb
10.0 kb, agb3st 5.1 k, abg
12.5 kb (Figure 1).

IV.

DNA primer
PCR
Southern hybridization ag ,
axg, agfnb, axgfnb, agb3st, abg, afnb 7가

가
,가
(population genetics) 가

V.

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- Abstract -

Development of PCR Technology for Identification of the Restriction Fragment Length Polymorphism(RFLP) of the Immunoglobulin Allotypes in Periodontal Patients

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Key Word: Immunoglobulin Allotype, Restriction Fragment Length Polymorphism, Polymerase Chain Reaction

The present study has been performed to develop a PCR technology to identify human immunoglobulin(Ig) allotypes with restriction fragment length polymorphism(RFLP) using a probe. Genomic DNA were amplified with PCR technology using primers from peripheral blood lymphocytes of 10 periodontal patients, whose Ig allotypes have been pre-determined by serological technique using heagglutination technique. The result indicated that the RFLP patterns could successfully differentiate the Ig allotypes, which suggests that this technology can be developed as a tool useful for population genetics studies.