Subject: Genetics in Dentistry – Practices (Spring 2011)

Name:

Date:

Interleukin 1 (IL-1)

IL-1 is a proinflammatory cytokine that is highly elevated in response to bacterial biofilms and is a potential risk factor for periodontal diseases. IL-1 family consists of three homologous proteins IL-1alpha, IL-1beta and IL-1 receptor antagonist.

The total amounts of IL- 1α and IL-1β (proinflammatrory cytokines) and the IL-1/IL-1RA tratil, have been found to correlate with alveolar bone loss in periodontitis.

The genes for IL-1 are located on the long arm of human chromosome 2 (2q13-q21). Some functional SNPs (Single Nukleotide Polymorphisms) in the IL-1 gene cluster: at position -889 (IL-1A), +3953 (IL-1B) and an 86bp VNTR (Variable Numer of Tandem Repeat) in the intron 2 of the IL-1RN polymorphism have been described and associated with cytokine production and with several immune-inflammatory diseases.

IL-1RA

1. DNA Isolation
2. Polymerase Chain Reaction = PCR
3. Electrophoresis – agarose gel

Methods:

ad1. DNA Isolation from peripheral blood

Phenol-chloroform extraction is a liquid-liquid extraction technique in molecular biology for isolating DNA, RNA and protein. Equal volumes of a phenol:chloroform mixture and an aqueous sample are mixed, forming a biphasic mixture.

1. Cell lysis
2. Removing membrane lipids by adding a detergent.
3. Removing proteins by adding a protease (optional but almost always done).
4. Extraction by phenol – chloroform
5. Precipitation the DNA with an alcohol — usually ice-cold ethanol or isopropanol.
6. Dissolution of the DNA in a buffer

ad2. PCR IL-1R VNTR

The polymerase chain reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a sequence of DNA. PCR was developted by Kary B. Mullis in 1983 (NP in 1993).

The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short oligonucleotides sequences) are complementary to the target region along with a DNA polymerase (after which the method is named). The result is 2^n (n = number of cycles) copies of the sequence of DNA.

A Variable Number Tandem Repeat (or VNTR) is a location in a genome where a short nucleotide sequence is organized as a tandem repeat.

Experiment:

Products length: 86bp

Primers:

P1 IL-1Rf CTC AGC AAC ACT CCT AT (Ta = 50,0°C…56,3°C)

P2 IL-1Rr TCC TGG TCT GCA GGT AA (Ta = 51,1°C…55,8°C)

1. use gloves and workin PCR box
2. prepare appropriate number of plastic microtubes and mark them with sample codes
3. prepare PCR mastermix by mixing aliquots shown in table (multiplied volume by number of samples + reserve)
4. mix PCR mastermix well and shortly centrifuge
5. pipette PCR mastermix into each microtube
6. pipette DNA sample into appropriate PCR microtube (don´t forget change tip for each DNA samples)
7. pipette drop of mineral oil into each microtube
8. cover lids
9. place all microtubes into thermocycler
10. run programme

PCR reaction mixture V = 25,0µl:

Master Mix (**MM**)		
solution	Per 1 sample (µl)	Per .... samples (µl)
PCR water **W**	12,5	
Buffer DYNEX **B**	2,5	
$MgCl_2$ (25mM) **M**	4,0	
Primer F **P1**	1,25	
Primer R **P2**	1,25	
dNTP **N**	0,5	
Taq polymerase ($1U\mu l^{-1}$) **P**	1,0	
23,0µl MM + 2,0µl template DNA ($50ng\mu l^{-1}$) + 1 drop of minerale oil **O**		

Samples:

Programme in thermocycler:

1. 95°C 5 minutes
2. 95°C 45 seconds step 2.- 4. – cycling 35x
3. 56°C 45 seconds
4. 72°C 45 seconds
5. 72°C 7 minutes
6. 10°C 10 minutes

ad3. Elektrophoresis in agarose gel

Gel electrophoresis is a technique used for the separation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein molecules using an electric field applied to a gel matrix. Electrophoresis is a procedure which enables the sorting of molecules based on size and charge. Ethidium bromide (EtBr) an intercalating agent is commonly used as a fluorescent tag.

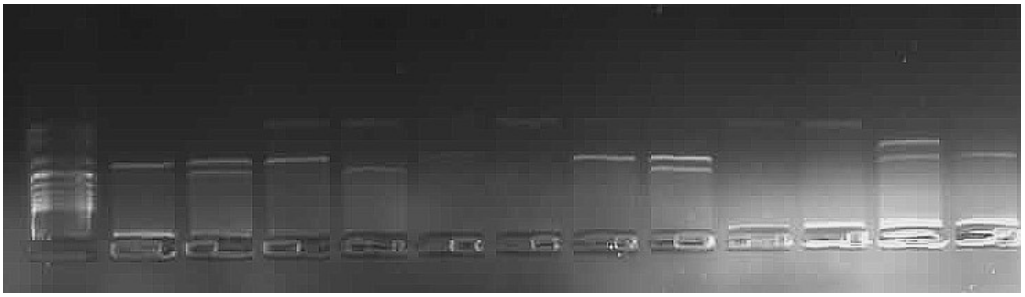
Experiment:

1. For a 3% agarose gel, weigh out 4,5g of agarose into a flask and add 150ml of 1x TBE.
2. Heat solution in a microwave or boiling water bath until agarose is completely dissolved.
3. Allow to cool in a water bath set at 50 – 55°C for 10 min.
4. Prepare gel casting tray by sealing ends of gel chamber with tape or appropriate casting system. Place appropriate number of combs in gel tray.
5. Add 15,0µl of Ethidium bromide to cooled gel and pour into gel tray. Allow to cool for 15-30 min at room temperature. Gels can also be placed in a cold space and used the following day.
6. Remove comb(s), place in electrophoresis chamber and cover with buffer (TBE as used previously).
7. Add loading buffer to samples. As a guideline, add 2,5µl of 10x Loading Buffer to a 25,0µl PCR/DNA solution.
8. Load DNA and standard (Ladder - Gene Ruler Fermentas 50bp) onto gel.
9. Electrophorese at 90V for 30 minutes.
10. Visualize DNA bands using UV lightbox or gel imaging system.

Results:

Homozygote/heterozygote	Products lenght in bp
A. 4 repeats	412
B. 2 repeats	240
C. 3 repeats	326
D. 5 repeats	498
E. 6 repeats	584

Gel:



240bp	B
326bp	C
412bp	A
498bp	D
584bp	E

	haplotypy											
st	A/A	A/D	A/B	B/D	A/B	B/B	A/A	A/D	B/B	B/D	A/C	A/A

Discussion: