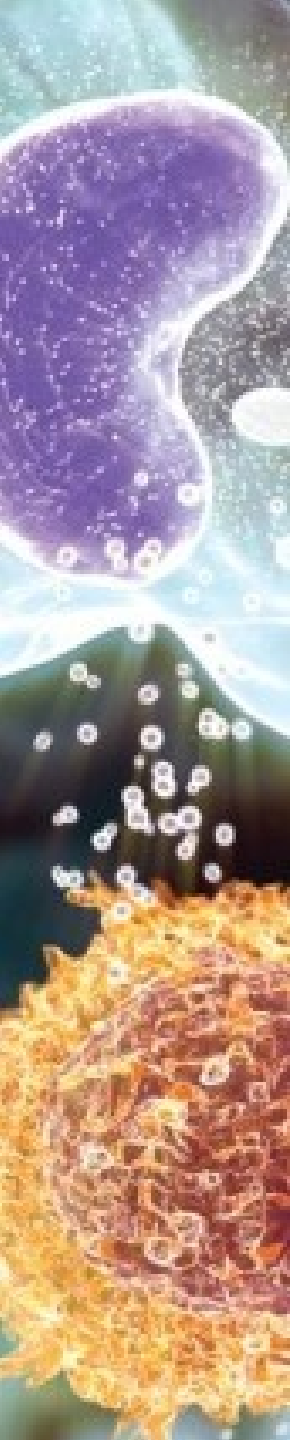
Genetics in dentistry Practices

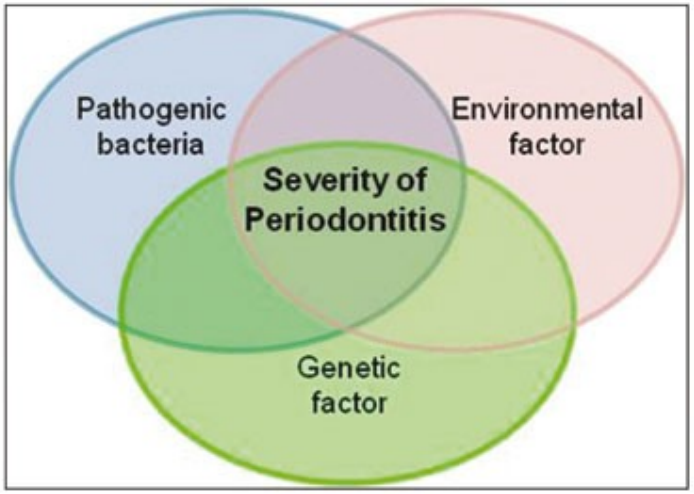
spring 2013

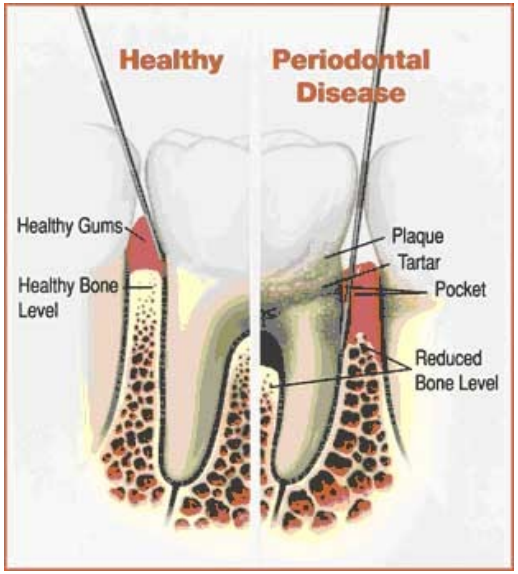
Mgr. Petra Bořilová Linhartová
peta.linhartova@gmail.com

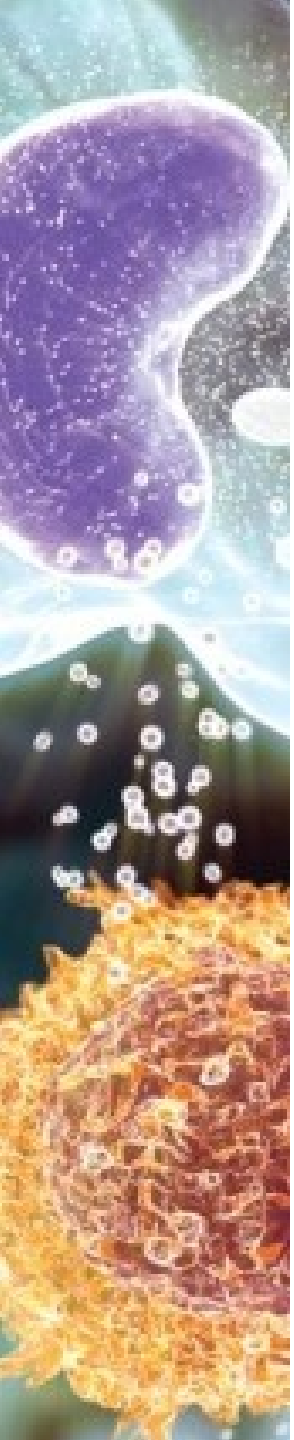


Periodontitis

- multifactorial infectious immune-inflammatory disease
- initiated by specific bacteria, predominantely gram-negative anaerobes that activate tissue mechanisms that produce series of inflammatory and immunologic changes leading to destruction of connective tissue and bone.







Periodontitis

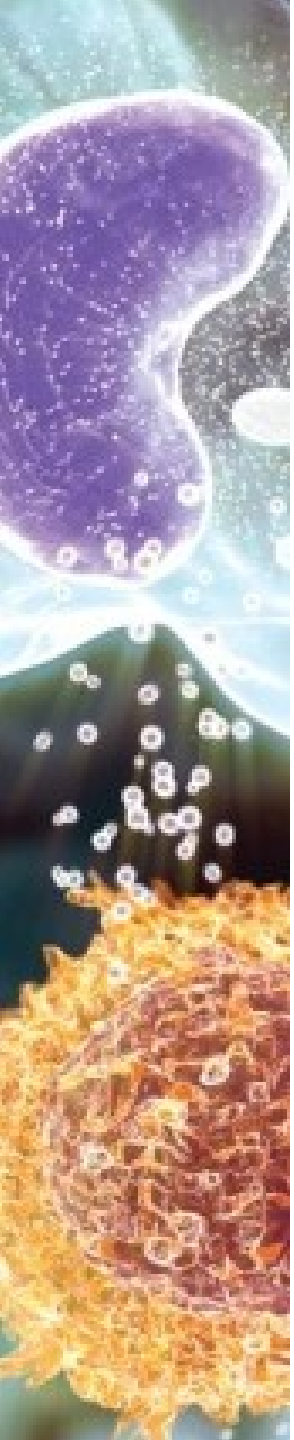
- destruction of periodont:

  - **products of bacteria**

    (toxins, enzymes, LPS,...)

  - **subtances incident by inflammation**

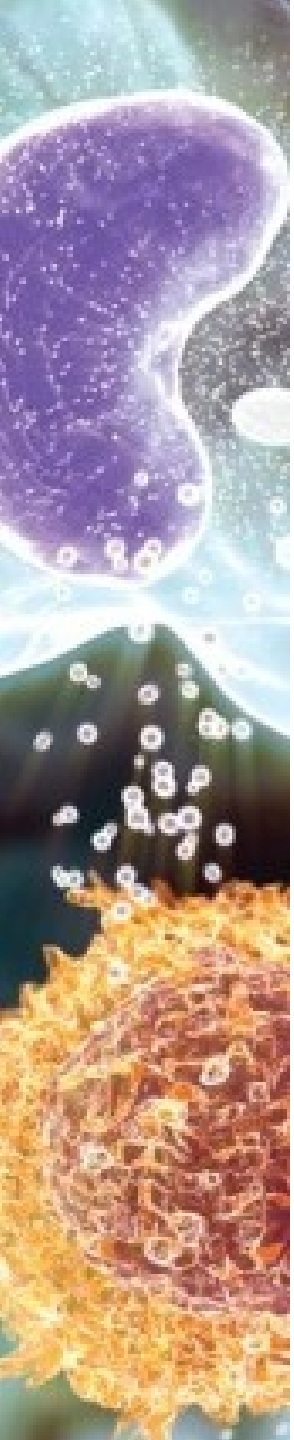
    (pro-inflammatory TNFα, **IL-1 α, β, R**)



Periodontitis

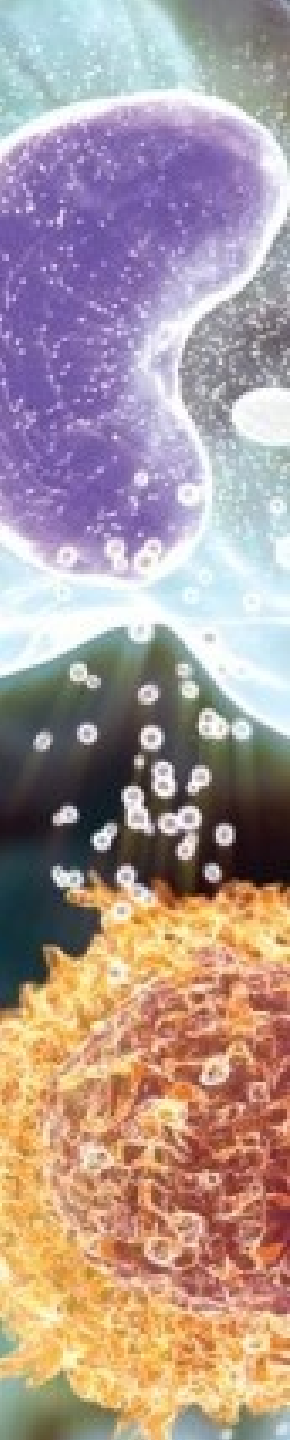
Candidate genes

- Genes for immunoregulatory factors - interleukins (IL-1, IL-4, IL-6, IL-8, IL-10, IL-18 and others)
- Metaloproteinases (MMP1, MMP3, MMP9, MMP12, and more)
- Products of bone remodelation (VDR, RAGE, SFTPD and others)

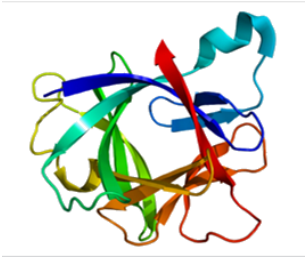


Cytokiny

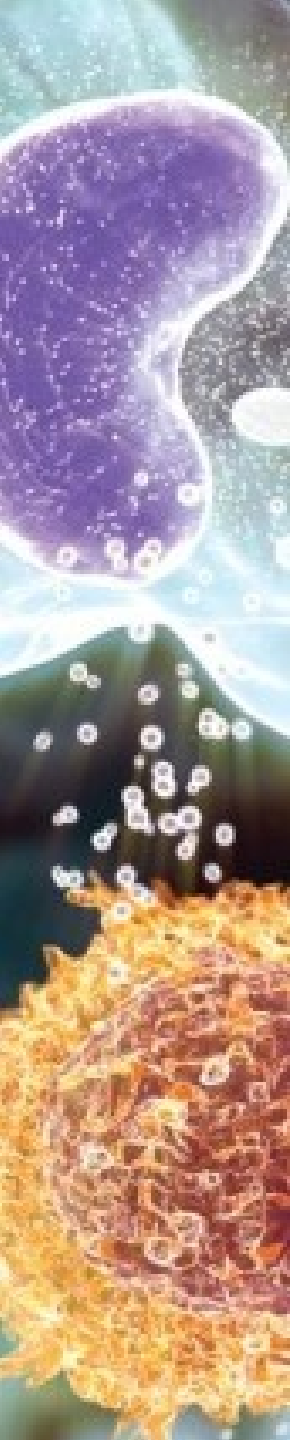
- small cell-signaling protein molecules
- secreted by numerous cells
- each cytokine has a matching cell-surface receptor
- signaling molecules used extensively in intercellular communication
- involved in control of proliferation, differentiation and function of IS cells
- involved in inflammatory processes and neuronal, hematopoietic and embryonic development of an organism
- pleiotropic



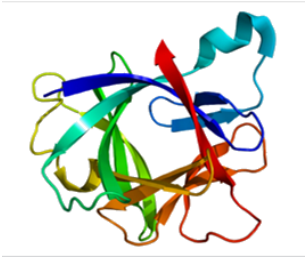
Interleukin-1



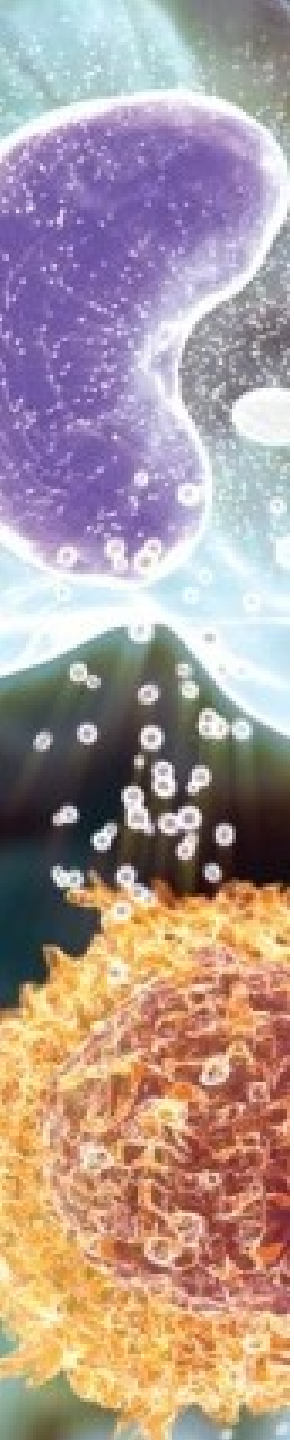
- proinflammatory cytokine
- highly elevated in response to bacterial biofilms and is a potential risk factor for periodontal diseases
- produced mainly by activated macrophages, as well as neutrophils, epithelial cells, and endothelial cells
- IL-1 family consists of three homologous proteins IL-1alpha, IL-1beta and IL-1 receptor antagonist
- stimulate bone resorption
- regulate proliferation of gingival and ligamental fibroblasts



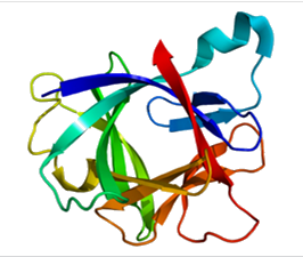
Interleukin-1



- 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.

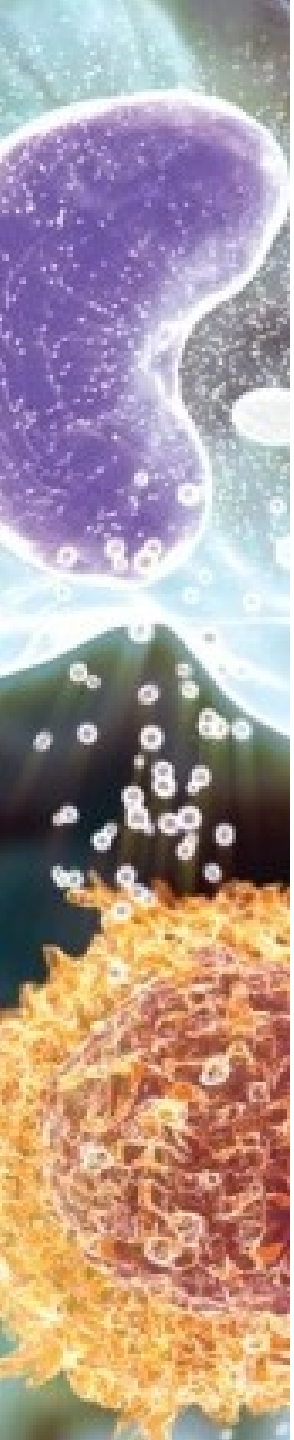


Interleukin-1



Genes of IL-1

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



Salivary biomarkers of existing periodontal disease: a cross-sectional study.

J Am Dent Assoc. 2006 Mar;137(3):322-9.
Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV.

BACKGROUND:

The authors conducted a study to determine if salivary biomarkers specific for three aspects of periodontitis--inflammation, collagen degradation and bone turnover--correlate with clinica features of periodontal disease.

METHODS:

The relationship between periodontal disease and the levels of interleukin-1 beta (IL-1beta), matrix metalloproteinase (MMP)-8, and osteoprotegerin (OPG) in whole saliva of 57 adults (28 "case" subjects with moderate-to-severe periodontal disease and 29 healthy control subjects) was examined in a case-control trial.

RESULTS:

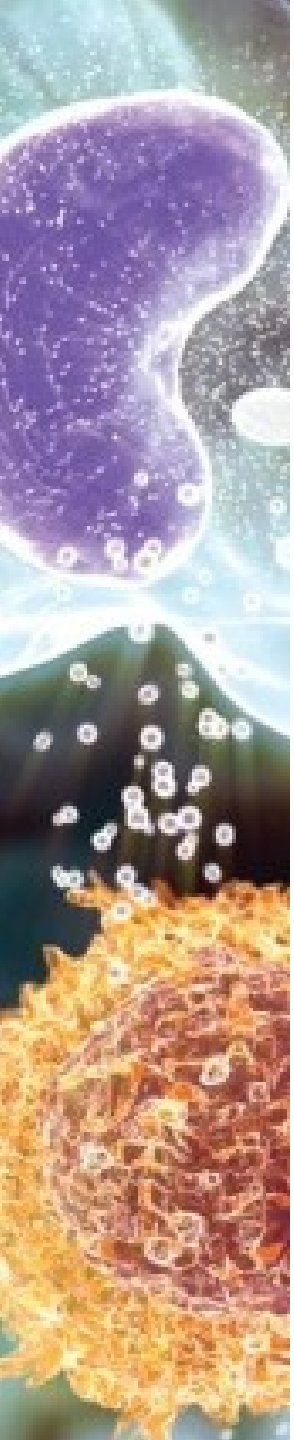
Mean levels of IL-1beta and MMP-8 in saliva were significantly higher in case subjects than in controls. Both analytes correlated with periodontal indexes, whereas, after adjustment for confounders, OPG did not. Elevated salivary levels of MMP-8 or IL-1beta (more than two standard deviations above the mean of the controls) significantly increased the risk of periodontal disease (odds ratios in the 11.3-15.4 range). Combined elevated salivary levels of MMP-8 and IL-1beta increased the risk of experiencing periodontal disease 45-fold, and elevations in all three biomarkers correlated with individual clinical parameters indicative of periodontal disease.

CONCLUSION:

Salivary levels of MMP-8 and IL-1beta appear to serve as biomarkers of periodontitis.

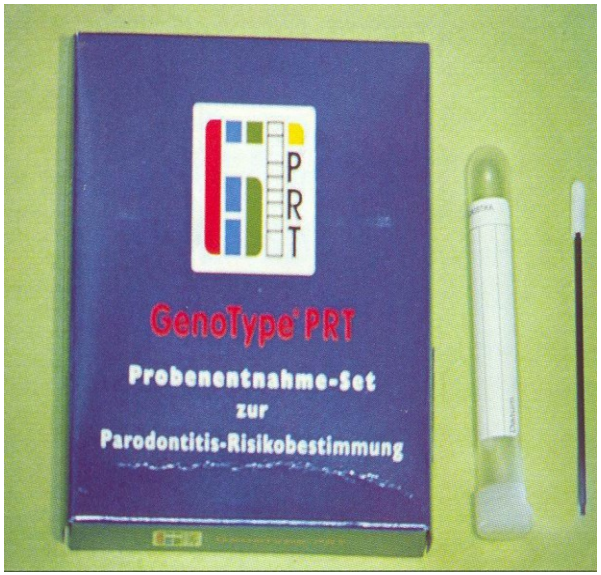
CLINICAL IMPLICATIONS:

Qualitative changes in the composition of salivary biomarkers could have significance in the diagnosis and treatment of periodontal disease.

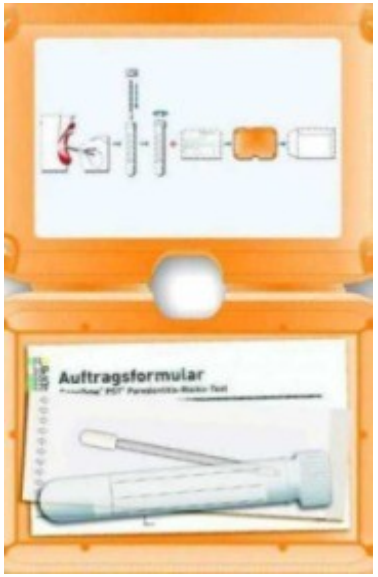


Tests

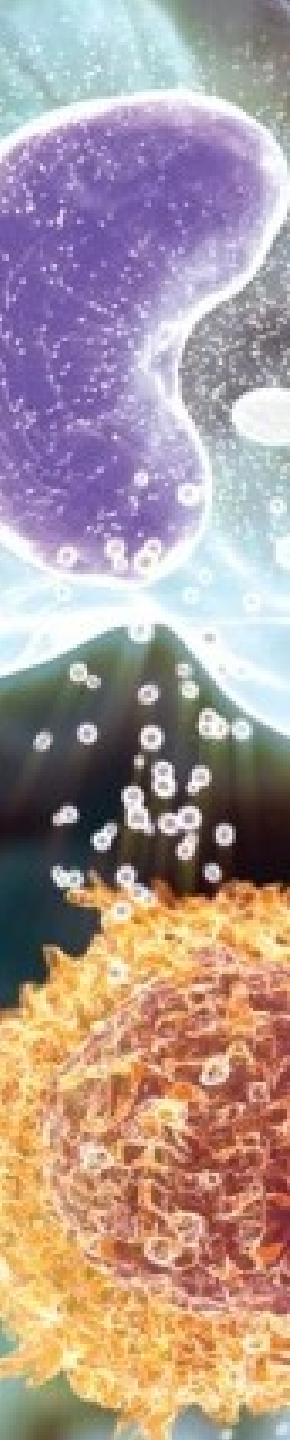
- Microbial pathogenes
- Association of IL-1 genotype with periodontitis



GenoType PST® test od HAIN Diagnostics ™



GenoType™ PST test od Dentalyse™

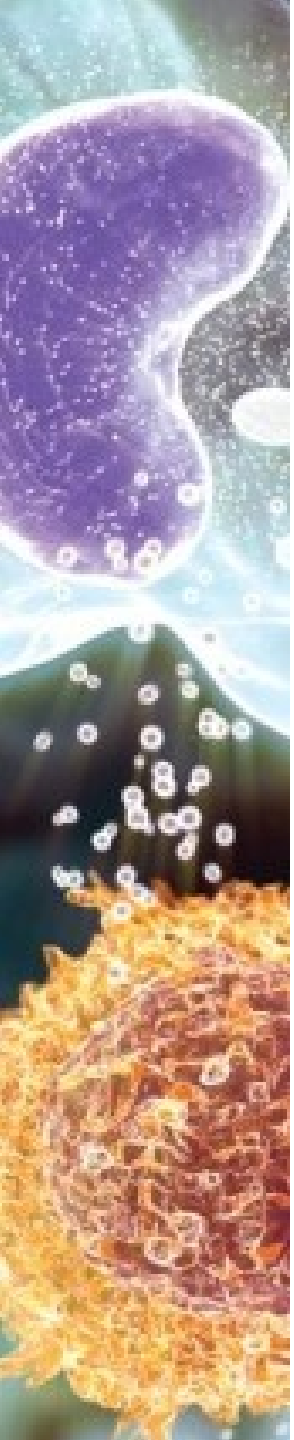


Practices

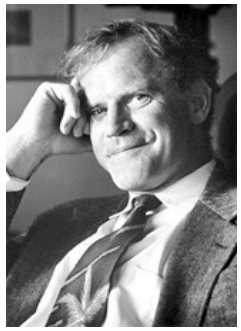
Periodontitis - DNA diagnostics of gene polymorphisms in interleukin-1 (IL-1)

- Detection of SNP in IL-1β +3953 C/T

1. Polymerase chain reaction (PCR)
2. Restriction enzyme analysis (RA)
3. Agarose gel electrophoresis (ELFO)



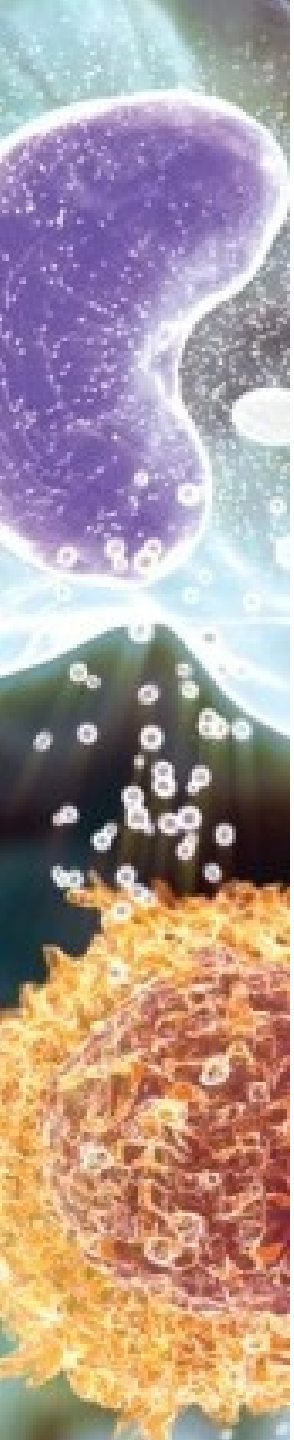
PCR



- developed by Kary B. Mullis in 1983 (1993 NP)

- used to amplify a specific region of a DNA strand
- *in vitro*
- 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

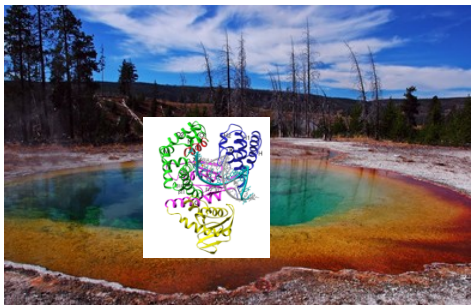
- The result is 2^n (n = number of cycles) copies of the sequence of DNA.

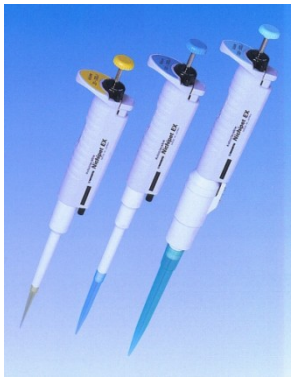


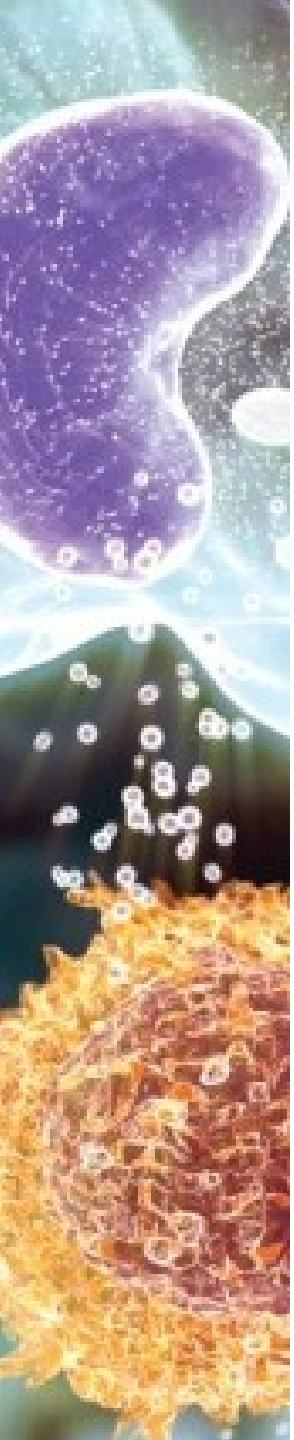
PCR

Reaction mixture

- Magnesium chloride: 0.5-2.5 mM
- Buffer: pH 8.3-8.8
- dNTPs: 20-200 μM
- Primers: 0.1-0.5 μM
- DNA Polymerase: 1-2.5 Units
- Target DNA: 50 ng μl^{-1}





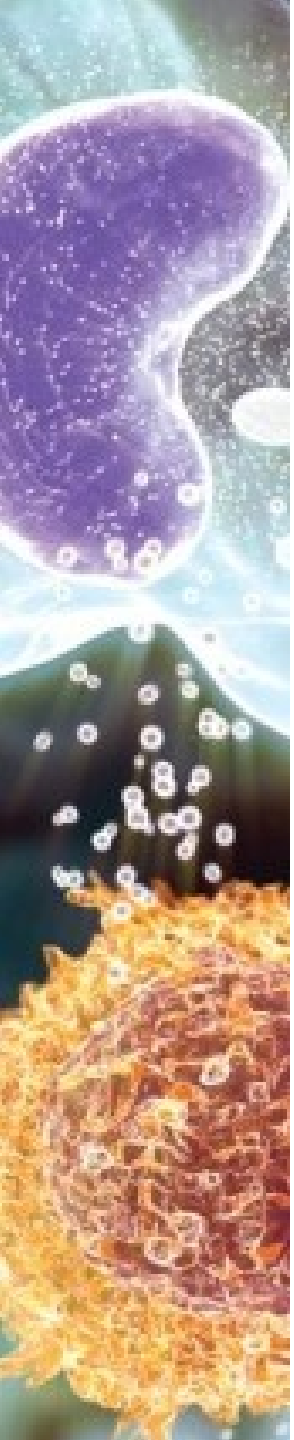


PCR

Programme in thermocycler

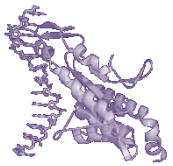
1) 95°C 2' inicial denaturation
2) 95°C 30"denaturation
3) 40-72°C 20" annealing
4) 72°C 30" elongation
5) 72°C 5' final elongation
6) 4°C 10' cooling

steps 2 – 4 repeat 30x or more



Restriction enzyme analysis

- DNA analysis method – specific digest of DNA by restriction enzymes (RE)



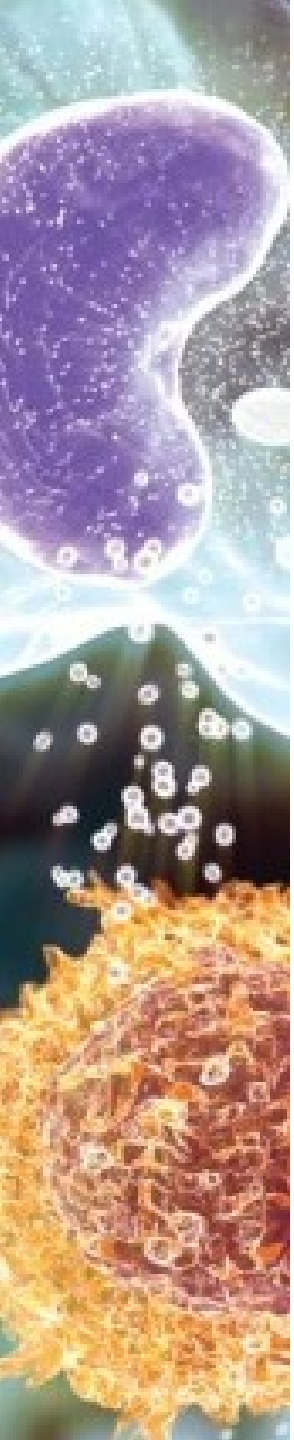
RE

- bacterial enzymes - EcoRI (*E.coli*)
- recognise of specific restriction site (palindromic)

5 -CCT G⇓AATTC AGG-3

3 -GGA CTTAA⇑G TCC-5

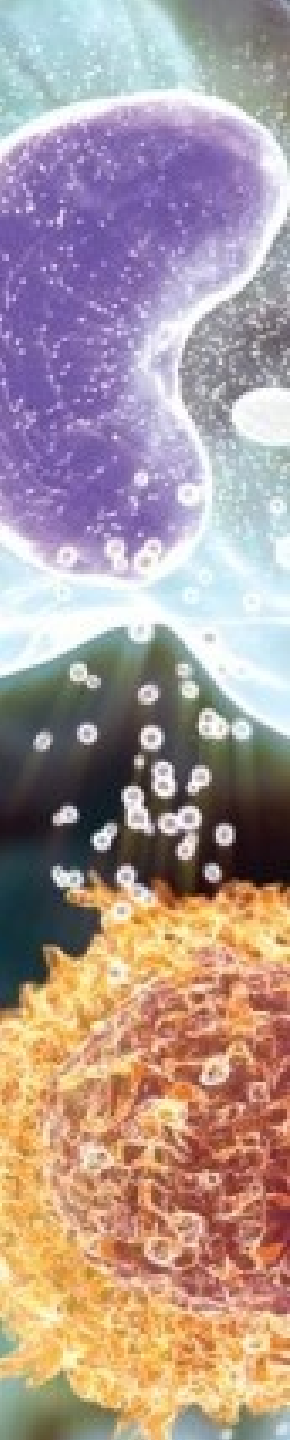
- phosphodiester bonds
- specific temperature for optimal digestion



Detection of SNP rs1143634 IL-1β +3953C/T

PCR

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



Detection of SNP rs1143634 IL-1β +3953C/T

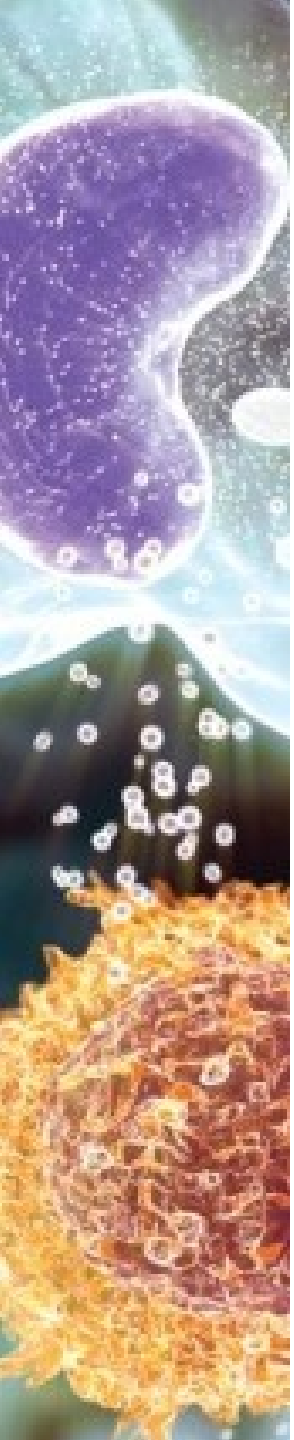
PCR – reaction mixture

Master Mix (MM)		
solution	Per 1 sample (µl)	Per… samples (µl)
PCR water	12,5	
DYNEX buffer	2,5	
$MgCl_2$ (25 mM)	4,0	
Primer F	1,25	
Primer R	1,25	
dNTPs	0,5	
Taq polymerase (1Uµl-1)	1,0	
23,0 µl MM + 2,0 µl template DNA (50 ngµl-1) + 1 drop of mineral oil per 1 sample		

Primers

IL-1BF CTC AGG TGT CCT CGA AGA AAT CAA A – forward (Ta = 58,79°C)

IL-1BR GCT TTT TTG CTG TGA GTC CCG – reverse (Ta = 58,80°C)

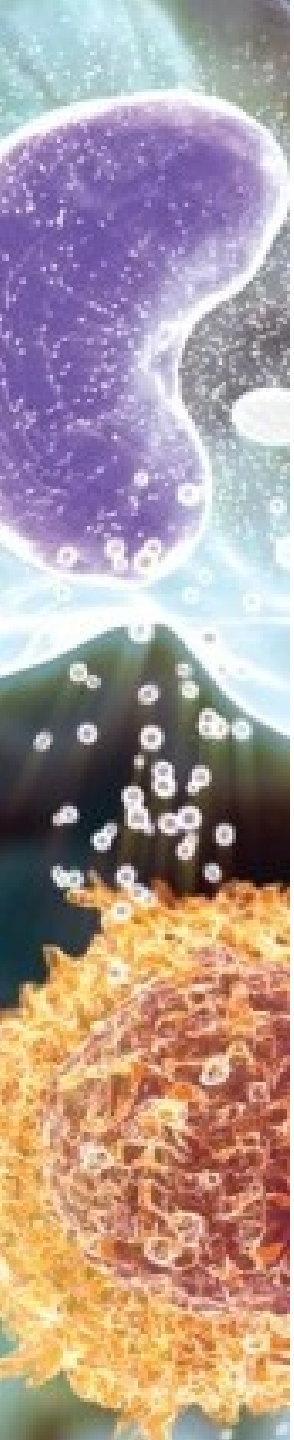


Detection of SNP rs1143634 IL-1β +3953C/T

PCR

- thermocycler Sensoquest labcycler (Schoeller)

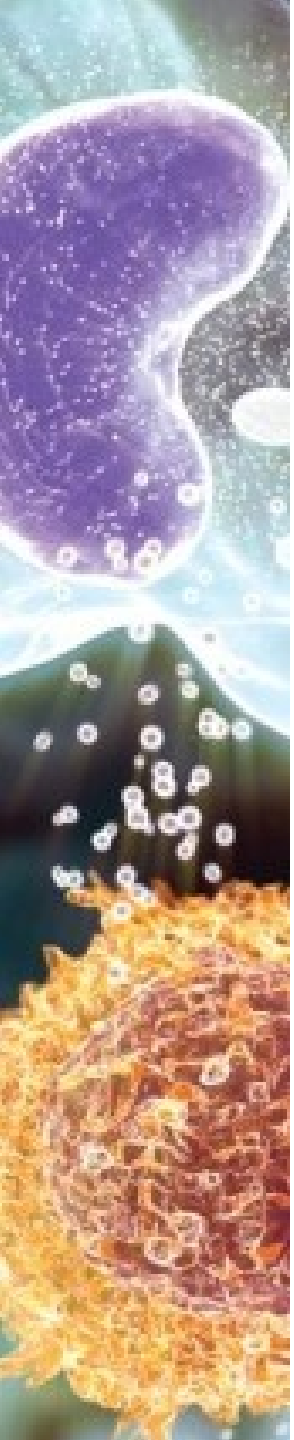
1. 95°C	5 minutes	
2. 95°C	1 minute	
3. 60°C	1 minute	
4. 72°C	1 minute	step 2.- 4. – cycling 35x
5. 72°C	7 minutes	
6. 10°C	10 minutes	



Detection of SNP rs1143634 IL-1β +3953C/T

RA

- prepare appropriate number of plastic microtubes and mark them with sample codes
- prepare RA mastermix by mixing aliquots shown in table (multiplied volume by number of samples + reserve)
- mix RA mastermix well and shortly centrifuge
- pipette RA mastermix into each microtube
- Pipette amplicon into appropriate RA microtube
- pipette drop of mineral oil into each microtube
- cover lids and place all microtubes into thermostate
- incubation for 4 hours on 65°C



Detection of SNP rs1143634 IL-1β +3953C/T

RA – reaction mixture

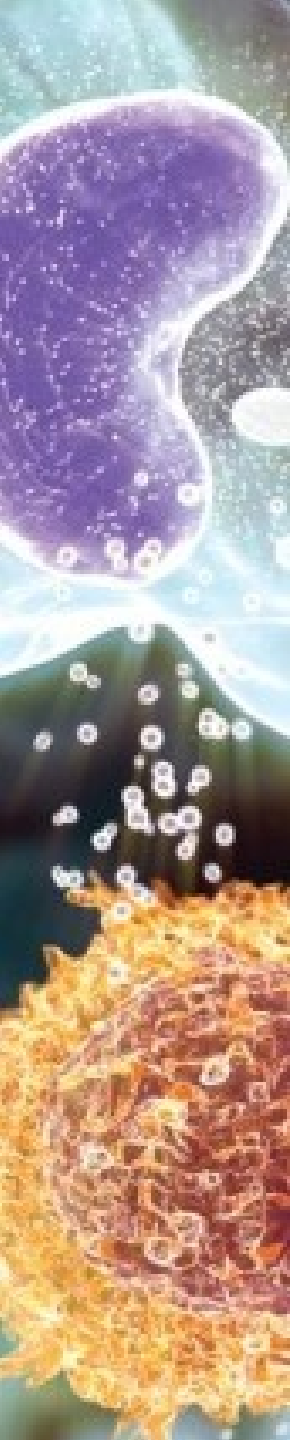
Master Mix (MM)		
solution	Per 1 sample (µl)	Per… samples (µl)
RA water	1,0	
TaqI buffer	1,7	
TaqI enzyme	0,3	
3,0 µl MM + 15,0 µl **amplicon** + 1 drop of mineral oil per 1 sample		

- TaqI enzyme

5 -T⇓CGA- 3
3 -AGC⇑T- 5

Products size

- CC 99 bp + 77 bp
- CT 176 bp + 99 bp + 77 bp
- TT 176 bp



SNP rs1143634 IL-1β +3953C/T

Sequention of IL-1beta gene

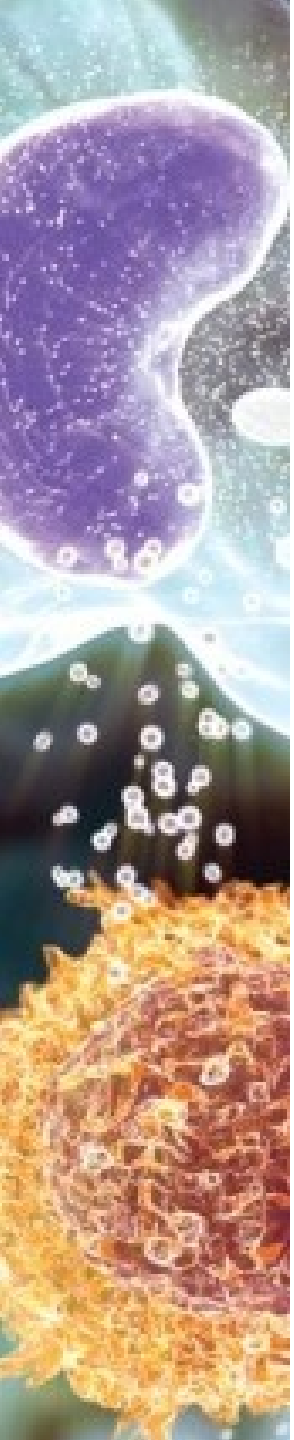
TAGTGGAAAC TATTCTTAAA GAAGATCTTG ATGGCTACTG ACATTTGCAA
CTCCCTCACT CTTTCTCAGG GGCCTTTCAC TTACATTGTC ACCAGAGGTT
CGTAACCTCC CTGTGGGCTA GTGTTATGAC CATCACCATT TTACCTAAGT
AGCTCTGTTG CTCGGCCACA GTGAGCAGTA ATAGACCTGA AGCTGGAACC
CATGTCTAAT AGTGTCAGGT CCAGTGTTCT TAGCCACCCC ACTCCCAGCT
TCATCCCTAC TGGTGTTGTC ATCAGACTTT GACCGTATAT GCTCAGGTGT
CCTCCAAGAA ATCAAATTTT GCCGCCTCGC CTCACGAGGC CTGCCCTTCT
GATTTTATAC CTAAACAACA TGTGCTCCAC ATTTCAGAAC CTATCTTCTT

Y (C/T)

GACACATGGG ATAACGAGGC TTATGTGCAC GATGCACCTG TACGATCACT
GAACTGCACG CTCCGGGACT CACAGCAAAA AAGCTTGGTG ATGTCTGGTC
CATATGAACT GAAAGCTCTC CACCTCCAGG GACAGGATAT GGAGCAACAA
GGTAAATGGA AACATCCTGG TTTCCCTGCC TGGCCTCCTG GCAGCTTGCT
AATTCTCCAT GTTTTAAACA AAGTAGAAAG TTAATTTAAG GCAAATGATC
AACACAAGTG AAAAAAAATA TTAAAAAGGA ATATACAAAC TTTGGTCCTA
GAAATGGCAC ATTTGATTGC ACTGGCCAGT GCATTTGTTA ACAGGAGTGT
GACCCTGAGA AATTAGACGG CTCAAGCACT CCCAGGACCA TGTCCACCCA

IL-1BF CTC AGG TGT CCT CGA AGA AAT CAA A

IL-1BR GCT TTT TTG CTG TGA GTC CCG



Detection of SNP rs1143634 IL-1β +3953C/T

ELFO

- technique used for the separation of DNA, RNA, or protein molecules using an electric field applied to a gel matrix
- Ethidium bromide (EtBr) an intercalating agent is commonly used as a fluorescent tag
- Fragments of linear DNA migrate through agarose gels with a mobility that is inversely proportional to the log10 of their molecular weight
- Visualization – UV lights

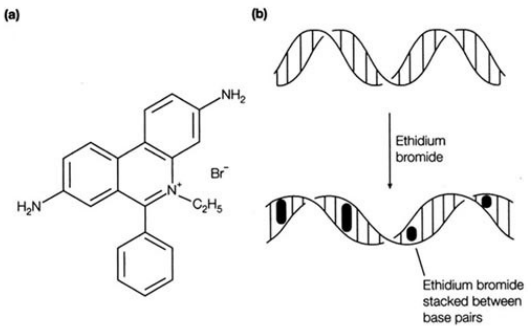
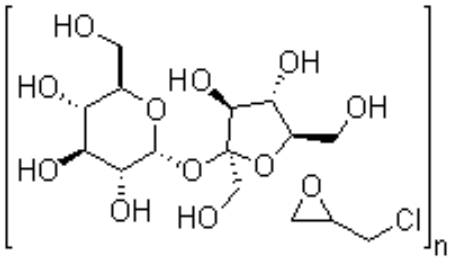


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

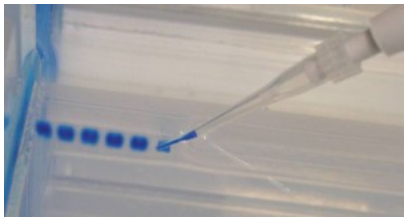
Detection of SNP rs1143634 IL-1β +3953C/T

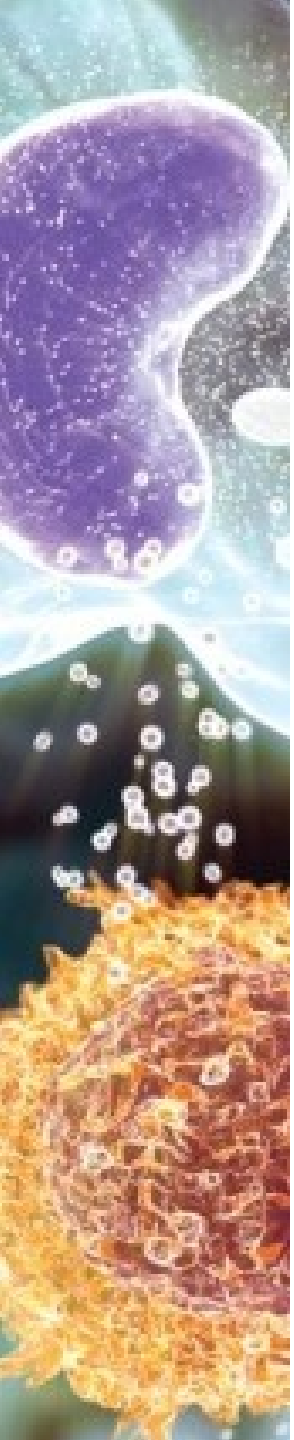
ELFO

- For a 3,0 % agarose gel, weigh out 4,5 g of agarose into a flask and add 150 ml of 1x TBE.
- Heat solution in a microwave or boiling water bath until agarose is completely dissolved.
- Allow to cool in a water bath set at 50 – 55°C for 10 min.
- Prepare gel casting tray by sealing ends of gel chamber with tape or appropriate casting system. Place appropriate number of combs in gel tray.



Ficoll



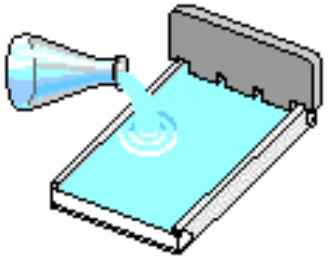


Detection of SNP rs1143634 IL-1β +3953C/T

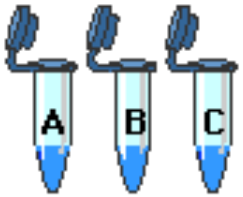
ELFO

- Add 15,0 µl of EtBr 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.
- Remove comb(s), place in electrophoresis chamber and cover with buffer (TBE as used previously).
- Add loading buffer to samples. As a guideline, add 2,0 µl of 10x Loading Buffer to a 15,0 µl PCR/DNA solution.
- Load DNA and standard (Ladder - Gene Ruler Fermentas 50bp) onto gel.
- Electrophorese at 90 V for 30 minutes.
- Visualization of DNA bands using UV lightbox or gel imaging system.

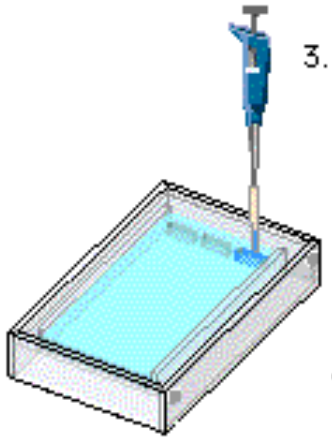
1. Make gel.



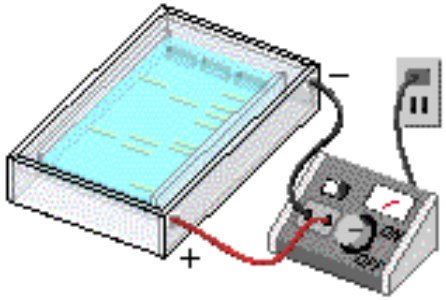
2. Obtain prepared DNA samples.



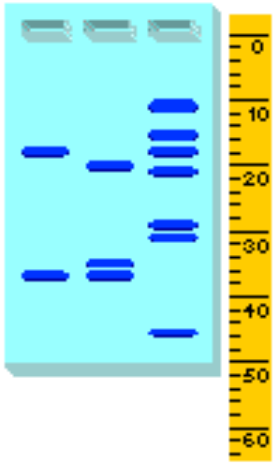
3. Load samples into gel.



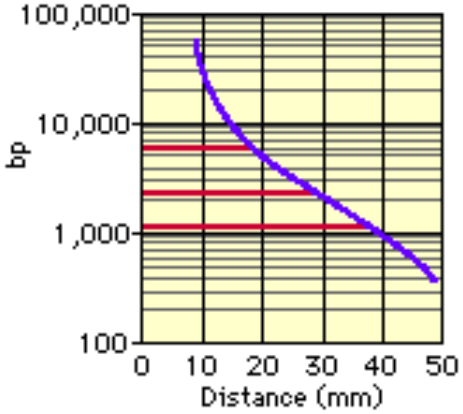
4. Separate fragments by electrophoresis.



5. Stain DNA fragments and measure distances.

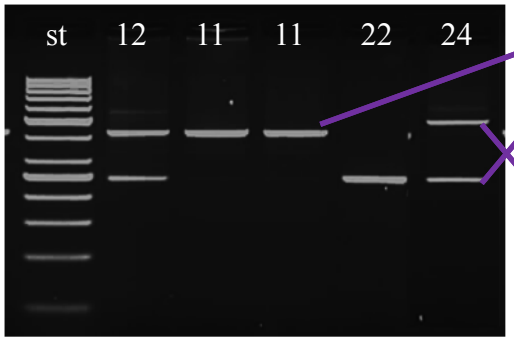


6. Prepare a standard curve. Determine fragment sizes.



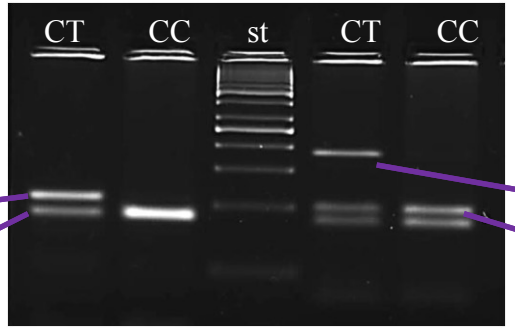
Genotype analysis results

PCR VNTR
IL-1RN
intron 2
86 bp repetice



1. 4 repetice – 412 bp
2. 2 repetice – 240 bp
3. 3 repetice – 326 bp
4. 5 repetic – 498 bp
5. 6 repetic – 584 bp

PCR RA
IL-1α -889 C/T
T 99 bp
C 16 bp + 83 bp



IL-1β +3953 C/T
exon 5
T 182 bp +12 bp
C 97 bp + 85 bp +12 bp