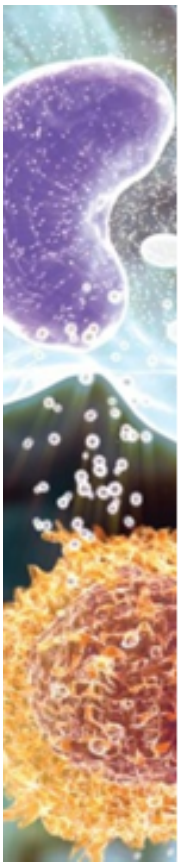
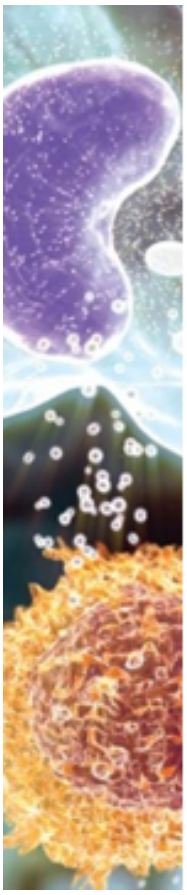


Detection of SNP rs1143634 IL-1β +3953C/T

PCR

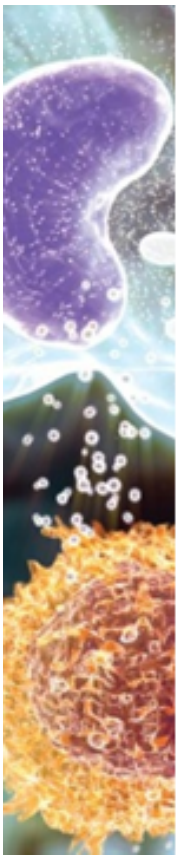
- use gloves and work in PCR box
- prepare appropriate number of plastic microtubes and mark them with sample codes
- prepare PCR mastermix by mixing aliquots shown in table (multiplied volume by number of samples + reserve)
- mix PCR mastermix well and shortly centrifuge
- pipette PCR mastermix into 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

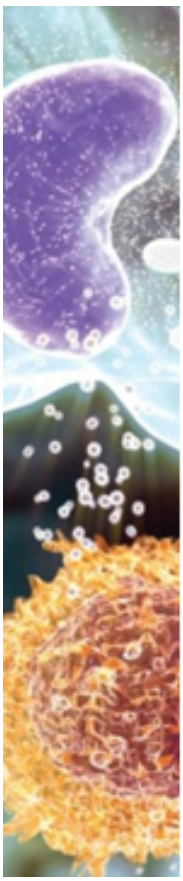
Master Mix (MM)		
solution	Per 1 sample (µl)	Per... samples (µl)
PCR water	12,5	
DYNEX buffer	2,5	
$MgCl_2$ (25 mM)	4,0	
Primer F	1,25	
Primer R	1,25	
dNTPs	0,5	
Taq polymerase (1Uµl-1)	1,0	
23,0 µl MM + 2,0 µl template DNA (50 ngµl-1) + 1 drop of mineral oil per 1 sample		



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

RA

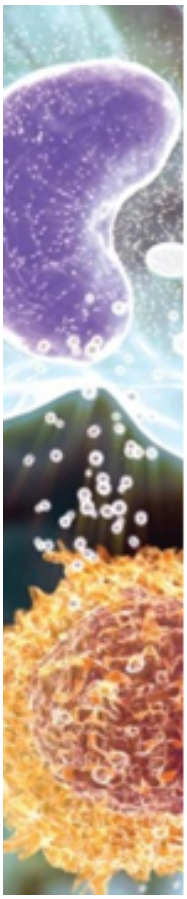
- prepare appropriate number of plastic microtubes and mark them with sample codes
- prepare RA mastermix by mixing aliquots shown in table (multiplied volume by number of samples + reserve)
- mix RA mastermix well and shortly centrifuge
- pipette RA mastermix into each microtube
- Pipette amplicon into 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 + 1drop of mineral oil per 1 sample		



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.
- 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.