Factor V Leiden PROTOCOL

STEP 1.

After you have isolated a patient's DNA, you perform a PCR. The primers used to amplify FVL gene are the following:

A' PCR

Forward primer

5' – GGAACAACACCATGATCAGAGCA – 3'

Reverse primer

TAGCCAGGAGACCTAACATGTTC

The PCR conditions for FVL are (MJ Research PTC-200 DNA Engine) :

94 °C for 5mins

30 cycles of :

- 94 °C for 30 sec
- 62 °C for 30 sec
- 72 °C for 1 min

72 °C for 5 min

8 °C indefinitely

For **50 µl** PCR reaction we use:

- 5 µl **10x PCR buffer** (500mM KCl , 200mM Tris- HCl pH 8.4, Invitrogen Platinum Taq DNA polymerase kit, Cat.10966-034)
- 1.5 µl 50mM **$MgCl_2$** (included in Invitrogen Platinum Taq DNA polymerase kit)
- 0.4 µl 100mM **dNTPs** mixture (Invitrogen Cat. 10297-018)
- 1µl of each **primer**
- 0,4 µl 5U/µl Platinum Taq **DNA polymerase** (included in Invitrogen Platinum Taq DNA polymerase kit)
- 1 µl of template **DNA**
- Up to 50 µl **distilled H_2O**

STEP 2. DIGESTION

For reaction volume of **15µl** we use :

- 3,7 µl of H_2O
- 1,5 µl of 10 x Buffer 2
- 1,5 µl of BSA

- 0,3 µl of Mnl1
- 8 µl of PCR product

Incubate in :

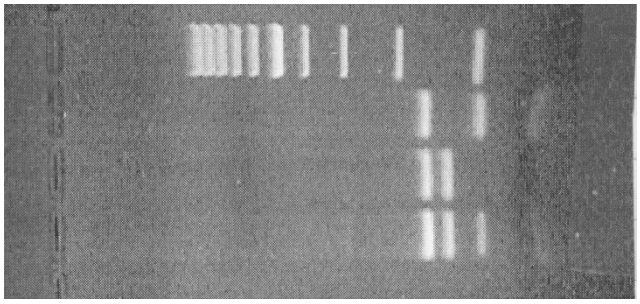
- 37 °C for 3 h
- 60 °C for 20 min

STEP 3. ANALYSIS OF RNASE CLEAVAGE PRODUCTS BY AGAROSE GEL ELECTROPHORESIS

Add 2 µl of gel loading solution (3M NaCl, 10mM Tris-HCl pH 7.5, 2mM EDTA pH 8.0, 0.25% bromophenol blue, 10µg/ml ethidium bromide, 33% glycerol) that also serves as a stop solution for the digestion process and 15 µl of the samples.

Then the product is loaded on 2% agarose gel and photographed after approximately 45 mins of electrophoresis at 120V so that each digestion fragment separates adequately from the other ones for the best visible result.

Notes : apart from the samples, we use hetero and homo controls to compare the results.



1. 100 bp DNA ladder
2. Normal sample
3. Homo control
4. Hetero control