TBC NESTED PROTOCOL

After you have isolated patient's DNA and DNA from a positive, you perform a nested PCR. The primers used to amplify are the following. For further information click HERE.

A' PCR

TJ5 primer

5' -CCG CAA AGT GTG GCT AAC-3'

TJ3 primer

5'-ATC CCC TAT CCG TAT GGT G-3'

NESTED PCR

OLI-5

5' – AAC GGC TGA TGA CCA AAC – 3'

STAN-3

5' – GTC GAG TAC GCC TTC TTG TT – 3'

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

A' PCR

94 °C for 2 min

35 cycles of:

- 94 °C for 80 sec
- 57 °C for 60 sec
- 72 °C for 70 sec

72 °C for 5 min

8 °C indefinitely

NESTED PCR

94 °C for 2 min

35 cycles of:

- 94 °C for 60 sec
- 60 °C for 45 sec
- 72 °C for 45 sec

72 °C for 5 min

8 °C indefinitely

For **50 µl** A' 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)
- 50 pmol 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**

For **50 µl** Nested 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)
- 50 pmol of each **primer**
- 0,4 µl 5U/µl Platinum Taq **DNA polymerase** (included in Invitrogen Platinum Taq DNA polymerase kit)
- 1 µl of template **PCR product** for positive control sample and up to 7 µl for samples.
- Up to 50 µl **distilled H_2O**

In a clean microcentrifuge tube add 2 µl of loading dye and 10 µl of the samples.

The product is loaded on 1.5% agarose gel and photographed after approximately 25 mins of electrophoresis at 120V so that each PCR fragment separates adequately from the other ones for the best visible result. Compare the samples' bands with the positive control band.