PROTOCOL 3. POLYMERASE CHAIN REACTION TECHNIQUE FOR AMPLIFICATION OF VIRULENCE GENES

Materials

- Eppendorf tubes (vol 1.5 ml), sterile
- Eppendorf tubes (vol 0.5 ml) thin wall, sterile
- Micropipettes and sterile tips
- Agarose
- Gloves
- Ice
- Biological cabinet
- Transilluminator
- Termocycler

Solutions

- Molecular Grade water, sterile
- 70 % Ethanol or disinfectant solution for surface cleaning.
- *Taq* polimerase buffer10X
- Magnesium cloride mM 50
- Primers (25 pm each)
- dNTPs solution (dTTP, dGTP, dCTP, dATP) (50 µM each dNTP)
- *Taq* polimerase
- Molecular Weight Marker 100 bp DNA ladder
- TBE 1X
- DNA sample

Special considerations when using PCR:

PCR can produce enough product DNA, even from a minute amount of template to be visible in a gel after electrophoresis. Carryover contamination of DNA molecules from previous amplifications can easily result in false-positive results. **The following considerations should be monitored in order to ensure both your safety and the integrity of the assay** :

- Always work in a one-way direction from area A (reagent preparation) to B (specimen preparation) to C (amplification and detection). If possible, use a biological cabinet to prepare the PCR reaction.

- If a UV box is used, you must turn off the ultravioleta light in the box before placing your arms and hands into the working area.

- Clean, dedicate area working surface must be decontaminated prior to use.

- Avoid possible aerosol contamination keeping centrifuges at a distance from areas where you are preparing master mix and controls and use pipettes with a plugged (aerosol-barrier) tip. Water baths are to be avoided; dry baths or blocks are preferable.

- Specimens must be stored separately from reagents so as not to contaminate other reagents.

- Lab coats must be worn in all areas; the coat worn in areas A and B may be the same, but the coat worn in area C must never be worn in areas A and B.

- Gloves must be worn at all times and have to be changed at each of the three areas

- Always include external and internal positive/negative controls.

Procedure

1. Place all reagents and prepare a master mix solution adding in this order: molecular biology grade water, *Taq* polymerase buffer 10 X, $MgCl_2$, primers (forward and reverse) and dNTPs solution. Finally add the enzyme and prepare as many reaction tubes as you need. A good starting reaction is 25 pmol primer, 50 µM each dNTP and 0.6 U of Taq polymerase in 25 µl final volume. Add 5µl (aprox. 25 ng) of DNA obtained by the boiling method described in protocol 2. You must also prepare positive and negative controls.

2. Place the tubes in the termocycler under the corresponding cycling conditions

When the amplification is finished run samples and controls in an agarose gel electrophoresis to make results visible.