PCR step-by-step scenario

All steps should be shooted carefully under the supervision of microbiology teacher. After all steps will be recorded properly in the order listed below, a podcast should be prepared will all necessary text and frames freezed in respect with the recommendations of the supervisor (depending on chosen methodological approach, teaching course, year of study. Please, pay attention that current scenario covers general PCR technique and can be modified for certain teaching needs.

All manipulations are carried out in a specially equipped laboratory (in a laminar flow cabinet equipped with an UV lamp), in medical overalls, and be sure to wear gloves.

1. DNA isolation

1.1. Number and position properly in the rack clean polypropylene Eppendorf tubes 1.5 ml. Fix in the protocol.
1.2. According to the corresponding optimal method chosen to isolate DNA carry out an extraction. (kit, heat lysis)
1.3. A solution of the purified DNA can be stored at -18 ...- 20 ° C for two weeks.

2. PCR performance (amplification)

2.1. Prepare and number thin-walled tubes for amplification (0.5 ml or 0.2 ml) according to the number of samples analyzed for pathogen DNA presence of. Prepare and label the tubes for the positive (marked "C+") and negative (marked "C-", “no template control”) controls.
2.2. Approximately 30 minutes before the preparation of the working amplification PCR mixture thaw reagents on an ice bath at room temperature.
2.3. Add to tubes necessary amounts of amplification reagents to the final volume of 20 μl. (determined empirically for max number of amplicons of predetermined length, to avoid non-specific products)
While the preparation of the reaction mixture, it is necessary to add all the components with individual tips.
-DH_2O
-10xPCR buffer
-2 MM deoxynucleoside triphosphate dNTPs
- 10 mM of each pair of primers
- 50 mM Mg^{2+}
- Taq-polymerase, 50 g/ml (2 AU)
2.4. After the addition of Taq-polymerase, which is added the last, the mixture must be stirred thoroughly by pipetting or vortexing (3-5 s).
2.5. Add 5 μl of the sample DNA to the appropriate tube. Mix.

2.6. Add 5 µl of the corresponding positive control DNA sample to tubes for positive control samples, and all reaction components except DNA to the test tube for the negative control sample.
2.7. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture in all tubes with 1 drop (about 25-30 L) of mineral oil.
2.8. Close the tubes and move them to a thermal cycler.
2.9. Apply the appropriate program on the 35-40 cycles. (depending on the primer chain annealing can take up to 10 minutes, the last stage about 7-10 minutes) 306 bp IS1081
1 min 94 ° C (1st cycle of 4 min) denaturation
1.5 min, 68 ° C annealing
2min 72 ° C (the 35th cycle of 10 min) extension
4 ° C ∞
BW- 6 (5` CGA CAC CGA GCA GCT TCT GGC TG 3`)
BW -7 (5` GTC GGC ACC ACG CTG GCT AGT G 3`)

3. The detection of PCR products

Separation of the amplification products by horizontal electrophoresis.
3.1. Fill the electrophoresis camera with 1xTAE buffer, prepared in distilled water by diluting 50xTAE 50 times.
3.2. Add 2.0 g of agarose to 2 ml of 50x TAE buffer and 100 ml of distilled water.
3.3. Melt the obtained mixture. Add 10 µl of a 1% ethidium bromide solution to 100 ml of agarose. Mix.
3.4. Cool the agarose to a temperature of 50-60 °C and pour into a gel casting. For agarose gel samples pockets set the comb. After solidification of the agarose carefully remove the comb from the gel and transferee gel to the electrophoresis chamber.
3.5. Add dye to the samples.
3.6. Add 10-15 µl of samples to the gel in accordance with the protocol. Add positive and negative controls and molecular weight marker. Fix the scheme.
3.7. Connect the electrophoresis chamber to the power supply and set the voltage corresponding to the electric field of 10-15 V/1 cm^2 of gel. Perform electrophoretic separation of the amplification products in the direction from the cathode (-) to the anode (+). Control of the electrophoretic separation is carried out visually by the movement of the dye band. Band dye must pass from the start 1,5-2 sm.
3.8. To visualize the results of the electrophoresis transfer gel to the UV glass transilluminator.
WARNING! Agarose gel should be contact with only in nitrile gloves. Ethidium bromide is a potential mutagen.
3.9. Analyze the results of the analysis. DNA fragments analyzed appear as red-orange luminescent bands upon irradiation with UV radiation with a wavelength of 310 nm.
(Accounting to the presence or absence of amplicons of the given size).

- the absence of orange-red color stripes strictly at the level of the positive control (PC) indicates the absence of the pathogen DNA in the sample;
-presence of the band corresponding to the electrophoretic mobility of the positive control indicates the presence of the pathogen DNA.