© 2018 Genekam Biotechnology AG
MADE IN GERMANY

MANUAL – one step

Ref. EDU04 **Expiry date: 1 year**

100 Tests (Ready to use PCR kit) **STORE AT -20°C**

Training kit

Detection of human D1S80 profile: teaching tool for biotech applications

One of the important biotechnology application is to detect the human DNA in unknown samples for police e.g. to know who is involved in the crime scene. This is being done with one simple method based on polymerase chain reaction known as PCR. This simple method is based on D1S80 locus, which is highly polymorphic because it contains large number of alleles and has no function. It is called AmpliFLP.
In this kit, the students are going to learn the followings:

- Which are the applications of PCR?
- What are primers?
- How does the DNA vary between the different persons?

The kit contains following two major components Isolation of DNA and detection of D1S80 in gel agarose based PCR: The major part of the kit is PCR kit, which contains the followings:

Tube A : Primers (Forward and backward)
Tube B: Mastermix (contains polymerase, dNTP and buffers)
Tube D1: Positive control to find whether the kit is functioning properly
Tube 2: Negative control to find whether the kit is giving nonspecific reactions

There are 3 exercises, which students should do with this kit:

1. Isolation own DNA and it will be run with this kit. Students will compare the band pattern with each other.
2. Running the kit without Tube A (without primers) ; without primers, the kit will not function.
3. To run the kit with DNA positive control to get the training how to use the PCR and which steps needed?

Exercise 1: To do this, each student should take buccal swabs. Name it and rub it against the cheeks for 20 -30seconds. After taking the sample, the swabs will be put back in plastic covering till it is used to isolate the DNA. To isolate the DNA, please proceed with isolation kit as described in the manual.
DNA isolation is done to purify the DNA as it is inside the cells, hence the isolation method will make it possible to be released from the cell and removal the other disturbing factors like proteins, polysaccharides and lipids, which may inhibit the PCR reactions.
After the isolation is complete, please proceed with PCR testing as mentioned in the manual.

Exercise 2: Please follow the manual and run 2 reactions : one without the primers (tube A; instead of tube A, user tube D2 as it is negative control and add D1 to reactions) and one with primers (tube A). Students should find that there is no band in the reaction, where the tube A is missing. To do this, follow the kit manual.

© 2018 Genekam Biotechnology AG

Exercise 3: PCR is a standard method around the world. There are many laboratories, which are using PCR kits for routine diagnostic or testing. Therefore it is necessary that student should learn how to use a PCR kit in laboratory. This kit offers all components for training. Each student can conduct one or two reactions to learn how to use the kits in routine laboratory. To do this, follow the kit manual.

Questions for students:

1. What is PCR?
2. What are nucleotides and which role do they play during the PCR?
3. What is polymerase? If user omits polymerase during PCR method, should the kit function or not?
4. What is an thermocycler? What is its cost?
5. What is D1S80 and its applications?
6. What problems do you as user face while using this kit?
7. What is gel agarose and how is it used for PCR analysis?
8. What is DNA isolation method? Why is there any need to isolate the DNA?
9. What is buccal swab sample? How can it be taken? Is it possible to make parentage testing or genetic testing with DNA isolated from buccal swab?

To conduct the exercise: Each student should take its buccal swab samples and isolate the DNA. After the isolation of DNA, PCR kit should be run on the samples to generate the bands for each student. (The kit can be run without isolation of DNA with positive control in order to train the persons about the PCR method!). Follow the part 1 and part 2 to do the practical work.

Part 1: It contains a DNA isolation kit from buccal swabs. The student will learn how to take DNA sample from mouth and how to isolate it.

© 2018 Genekam Biotechnology AG

GENEKAM DNA ISOLATION KIT:

This kit can be used to isolate the DNA from the blood samples (including blood spots on filter paper / normal paper / tissue paper / a piece of cloth), plasmids, cell lines, cell cultures, serum (plasma) as well as tissue samples. It can be used to isolate from sperms, spinal fluids, tissue, tissue pieces, mouse tail, ear punches, buccal swabs, very little bloodspots as well as bloodstained clothes and wood.

This kit is quick and effective. It does not need any expensive instruments.
Moreover all components **can be kept at room temperature**.

Components:
Solution A: It contains sodium hydroxide. Use protective clothes!
Solution B: Buffer
Solution C: Solution

Solution Z Preparation:
This solution must be made **freshly before use (very important)**. Calculate how much solution is needed for the isolations. Add 450 µl of solution C to one tube and add 50 µl of solution A to it gently (the dilution rate is 1:10; 9 parts of solution C and one part of solution

c) Isolation from buccal swab:
Preparation solution Z: This must be prepared freshly as this is very important. Calculate how much solution you need for the isolation of your probes. Add 380 ul of
Solution C gently to one tube and add to this tube 20 ul of solution A gently to tube (the dilution ratio is 1: 20 i.e. 1 part + 19 parts) . Use must use **freshly prepared solution Z as very important!**.

To do 20 isolations, take 3800 ul of solution C in a tube. To this tube, add 200 ul of solution A. Dilution ration is 1: 20.

1. Cut the top of buccal swab with clean scissor (scissor can be cleaned with distilled water and with Ethanol before use) and put in 1.5 ml reaction tube. This is very important that one scissor should used one piece per isolation per tube (clean it before new use!).

2. Add 200 µl of **freshly prepared solution Z** to your 1.5 tube containing probe.

3. Keep the tube at 88 °C for 7 minutes in heating block. During this period, vortex the tube 3-4 times.

4. Now remove the tube from heating block and add 200 µl of solution B to each tube. Add this solution in the middle of tube so that solution does not touch the walls in order to avoid loss of solution. Kindly do good vortexing for 10 seconds.

5. Add 400 ul of tube C to each tube.

6. Centrifuge the tube for 1 minute for 11 000 g.

7. Remove the buccal swab or pipette out the supernatant! Now user has supernatant containing the DNA and can be used in different applications e.g. conventional PCR, real time

© 2018 Genekam Biotechnology AG

PCR etc. 1 or 2 µl of this solution is sufficient to run the PCR, but you may need more or even less DNA according to your method. It should be stored at 4 °C or at -20 °C.

Part 2: Students will learn how to conduct a PCR test and apply for PCR analysis.

Principle and use:

© 2018 Genekam Biotechnology AG

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect D1S80 in the unknown samples.

In this exercise, one can use the kit with positive control as well as some of swabs from students, if available.

(other sources of DNA, on which this kit can be used e.g. DNA, which can be isolated from blood, skin, biopsies, tissue and any body fluid.)

Safety precautions should be taken. Always clean your hands before the test use and clean the hands after the test. Wash your face after the test, if possible. Disinfect your working place.

IMPORTANT: we added cotton or sponge in the lid of container of the kit, to avoid damage during transportation. Please remove this cotton or sponge from the lid of each container before storage.

Composition:

It contains the following **(WARNING! THAW THE TUBES SLOWLY: NEVER THAW IN HEATING BLOCK OR WITH HEAT FROM HAND):**

• Tube A (2 tubes)
• Tube B (2 tubes)
• Positive (+Ve) Control (D1) (1 tube)
• Negative (-Ve) Control (D2) (1 tube)
• Marker (tube E) (1 tube) (max 1000bp): 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000bp
• Dye (tube F) (1 tube)

Please check them before you start.

Equipment needed:

• PCR Thermocycler
• Laboratory centrifuge
• UV plateform
• UV safety goggles
• microtubes (0.2ml)
• Pipettes with and without filter (20µl, 5µl & 1µl)
• Pipettes (quality pipettes)
• Gel Agarose chamber
• Power supply
• Paper
• Pen
• Agarose (good quality)
• Staining (Ethium Bromide)
• TAE buffer 1x
• Ice
• Vortexer

Procedure:

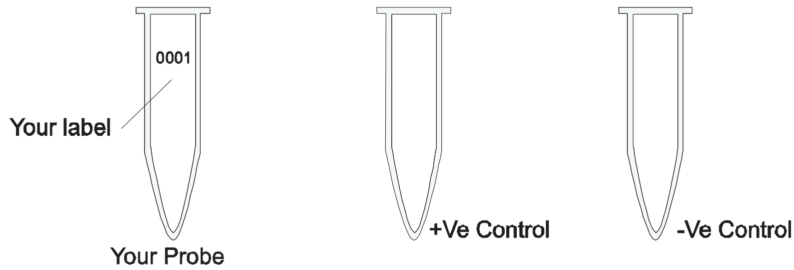
After your DNA isolation is completed. (Kindly use good quality isolation method).
Please go to PCR step

STEP A

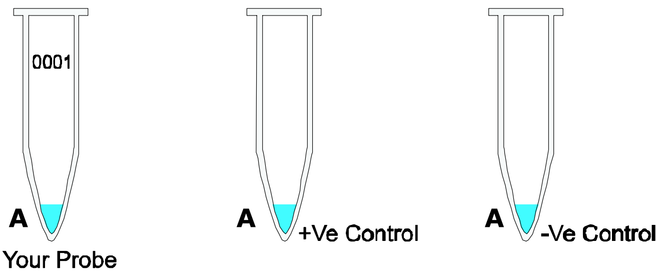
1. Kindly thaw **one tube** each: A, B, D1, D2, E and F. After thawing, kindly put the tubes on 4°C (as it is better). If the kit is not in use, store them at -20°C-.

© 2018 Genekam Biotechnology AG
MADE IN GERMANY

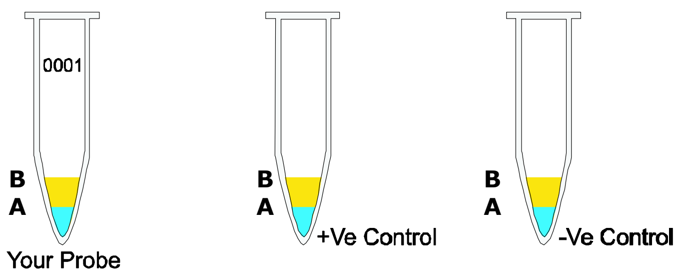
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control.



3. Thaw tube A. Add 8µl of tube A to each tube.



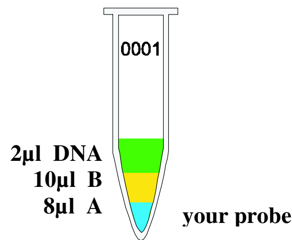
4. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.



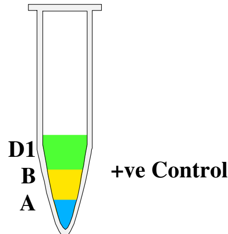
5. **TIP: you can calculate the total requirement of chemicals needed . You need 8µl A + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save time and hardware.**

6. Add 2µl of your DNA template (DNA isolated from samples) with pipette tip with filter to each microtube according to your label except +Ve and –Ve (Avoid touching the wall).
Use everytime a new pipette tip (for each sample)! Mix it.

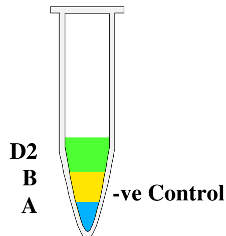
© 2018 Genekam Biotechnology AG



7. Use new pipette tip with filter. Add 2µl of +Ve (tube D1) to +Ve Control (avoid to touch the wall). Use a new pipette tip. Mix it.

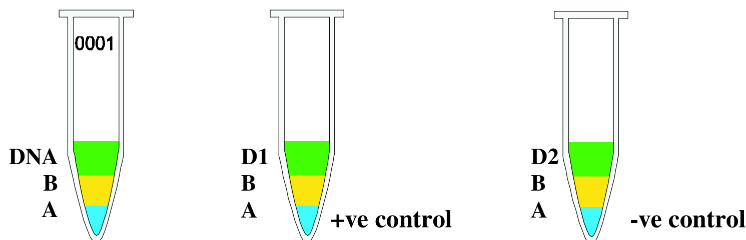


8. Use a new pipette tip. Add 2µl of –Ve (Tube D2) to –Ve Control (don't touch wall). Mix it.



9. Centrifuge all tubes for 20 sec. for 8000 rpm (this is not necessary but better). Run PCR.

10. Run the program of your thermocycler as followings: Kindly check whether you have added everything correctly as the level of the volume of each tube must be almost the same.



Now program your PCR machine as follows.

1. 240 seconds at 94°C
2. A 30 seconds at 94°C
   B 30 seconds at 54°C
   C 90 seconds at 72°C
   (steps 2A–2C: 40 cycles)
3. A 600 seconds at 72°C

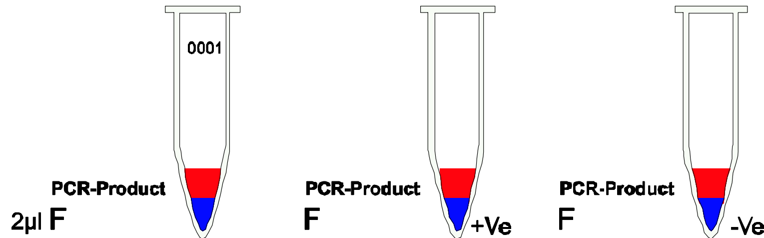
Before you start the PCR program, kindly check whether tubes are closed properly. **Microtubes must be in contact with metal block** (very important!). There should be no air or lose contact with metal block of thermocycler. Run PCR now. Please thaw tube E and F.

11. After step 10 is finished take out the microtubes.

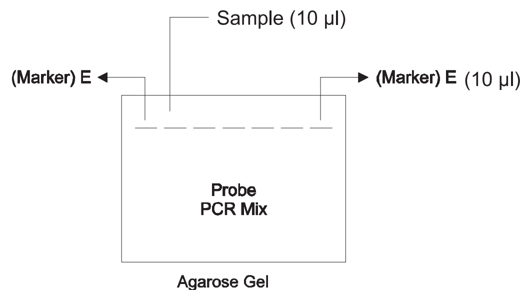
To see final bands, user can go directly to step gel electrophoresis (STEP B).

STEP B

1. Prepare the gel Agarose 2.0% in TAE (1x) buffer.
2. Let the gel dry and add this TAE (1x) buffer in gel chamber.
3. Take the tube E (marker). Make ready to use for gel electrophoresis.
4. After the PCR step is finished, now you can prepare for gel Agarose electrophoresis. Take 2µl of dye (tube F) and add to each microtube (with the same number as your PCR microtubes including +Ve & -Ve Controls) containing PCR product.



5. Add 10µl of marker (tube E: 100bp) to first and last lane of electrophoresis. (Kindly make lane plan on paper according to your probes in order to identify later and see the results).



6. Add 10µl of mix of step 4 to each lane of gel Agarose (between first and last lane). Change the pipette tip for each lane.
7. Run the gel for **60 min**. at **120 Volt**. It may vary.
8. Make staining solution ready.
9. Put the gel for 5-15 minutes staining solution (0.5µg/ml).
10. View the gel under UV light. UV light is dangerous for your eyes. Use UV goggles.
11. You must find the band in +Ve Controls and no bands in –Ve controls. (There will be bands in human samples and positive control). The students should compare the results of different samples of different persons. They must tell why the samples of different persons are showing the bands of different lengths. If the user will run a sample from same student twice, the test will show the bands at the same place.The student should tell what it means, if two different tests of the same person have the bands of same length? How can police detect the person involved in the crime?