Lot-No.

Ref. K724

100 Tests (Ready to use kit)

Expiry time: 1 year

STORE AT -20°C

RVF- RIFT VALLEY FEVER

-Only for in vitro use-
-Only for veterinary use-
-Only for research use-
-To be used by a technical person-

Principle and use:

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect RFV in nested PCR.

This kit needs RNA which can be isolated from blood, serum, mosquitoes, cell cultures, tissue and any body fluid. Kindly use good methods to isolate the RNA. Kindly take common safety laboratory precautions during working.

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.

Please use gloves during work. Proceed clean and carefully otherwise you may cause contamination problems. Do not touch other objects like pens, chairs etc. during Part 1.

Composition:

It contains the following:

- RNA Kit (HX, PF, NTP, RI, RET, DH)
- Tube A (2 tubes)
- Tube B (3 tubes)
- Tube C (2 tubes)
- positive (+ve) control (D1) (cDNA) (1 tube): **to be stored at -20°C, but to be made by user.**
- Negative (-Ve) Control (tube D2) (1 tube)
- Marker (tube E) (1 tube) (max 1000 bp): 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)
- Pipette-tips with and without filter (20µl, 5µl & 1µl)
- Pipettes (quality pipettes)
- Gel Agarose chamber
- Power supply
- Paper
- Pen
- Agarose (good quality)
- Straining (Ethium Bromide)
- TAE buffer 1x

• Ice
• Vortexer

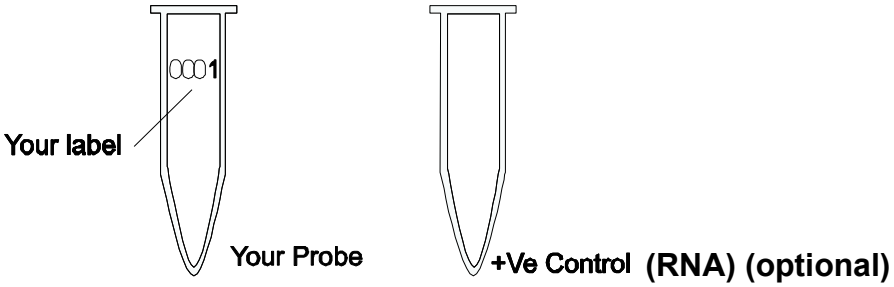
Procedure:

PART 1: Conversion of RNA into cDNA. This part should be done with our RNA.

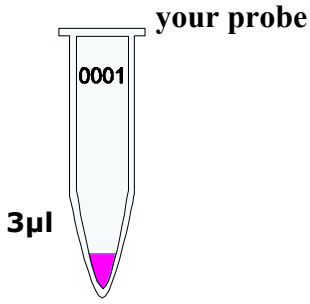
ONCE AGAIN:
VERY IMPORTANT ! PLEASE USE GLOVES ! DON'T TOUCH ANY OTHER OBJECTS, OTHERWISE THERE MAY BE RNASE CONTERMINATION DURING THIS PART.

STEP A

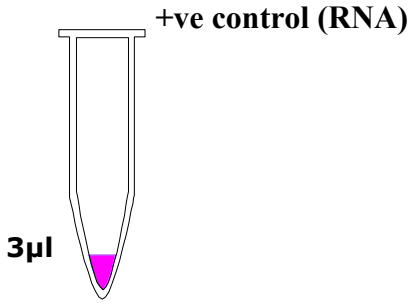
1. Mark your microtubes with a sample number and one with +Ve Control.



2. Add 3µl of your isolated RNA from your samples **(sometimes 2µl are sufficient).**



3. Add 3µl of RNA as positive control to +ve control (RNA) optional tube. This control can be made through you. It is optional, but not necessary. **For this, you can use RNA isolated from some positive sample or vaccine (live) .**



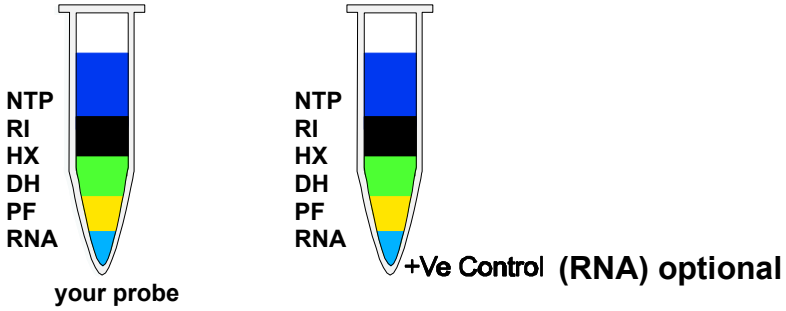
4. Add 1µl of HX (primer) and 8µl of DH (water) to each tube.

3µl of RNA
1µl of HX
8µl of DH
Total: 12µl in tube

In case you have 10 probes to test, please make a solution of 10µl HX & 80µl DH = 90µl and take out 9µl from this solution. In this way, you can save your costs.

4b. Centrifuge for a while (10-15 seconds) and incubate at 70°C for 5 minutes. Afterwards cool it down to 4°C (this can be done in the thermocycler).

6. Add: 4µl of PF (buffer)
1µl of RI (inhibitor)
2µl of NTP (dNTP mixture)
Total: 7µl in each tube

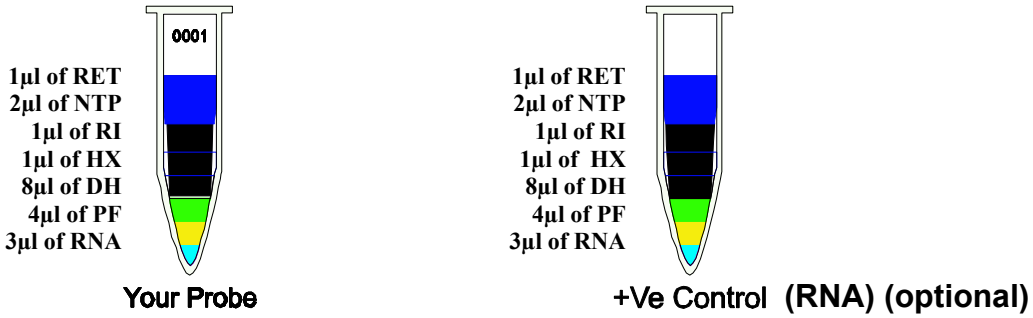


Tip: you can calculate your need for chemicals and mix them together. After that you can add 7µl to each tube.

7. Run at 25°C for 5 minutes.

8. Add 1µl of RET (Reverse-Transcriptase) to each tube.

9. Please control the level before going to the next step.



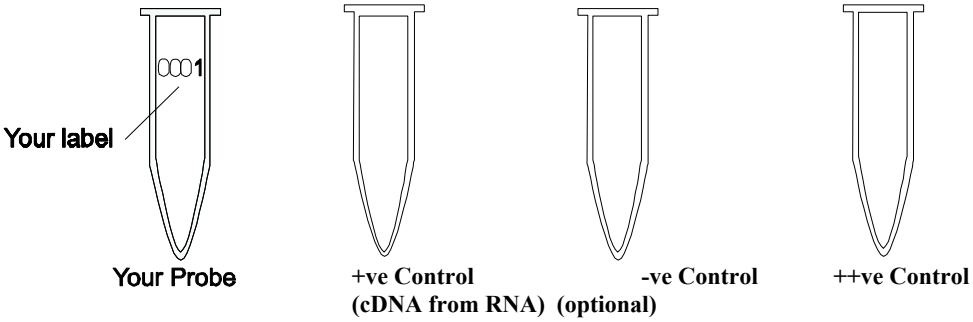
Run at: **25°C for 10 minutes**
42°C for 50 minutes
48°C for 10 minutes
70°C for 10 minutes
4°C for 5 minutes

This can be done in Thermocycler. Now you have got cDNA. Please proceed to PART 2 of the protocol (cDNA can be stored at -20°C).

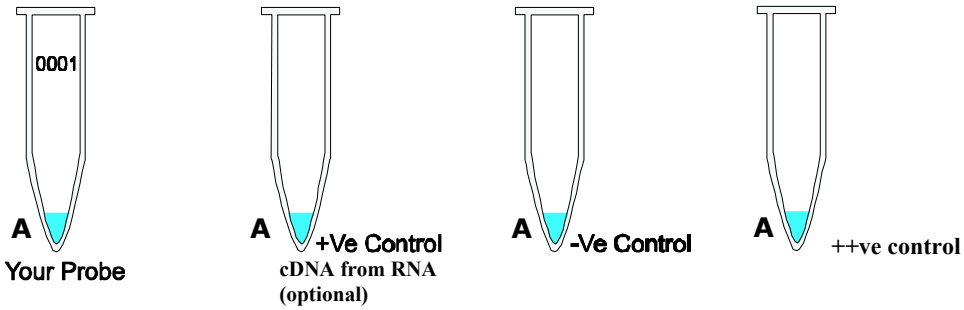
PART 2 – it is a nested PCR: STEP A

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

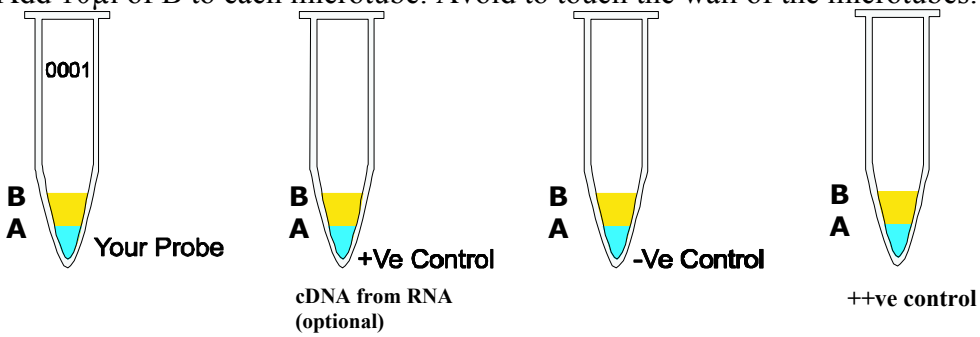
2. . Mark your microtubes with a sample number, +ve Control and –ve Control.



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

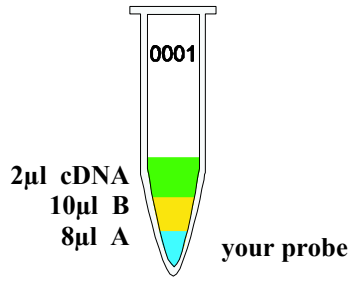


4. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.

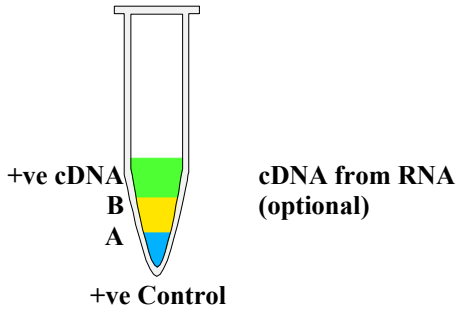


5. **TIP: you can calculate the total requirement of chemicals needed . You need 8µl A + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save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

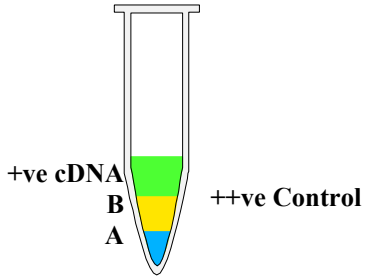
6. Add 2µl of your cDNA template with pipette tip with filter to each microtube according to your label except +Ve and –Ve (Avoid touching the wall. **Use everytime a new pipette tip** (for each sample)! Mix it gently !



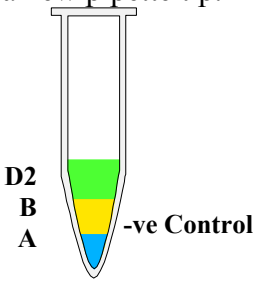
7. Use new pipette tip with filter. Add 2µl of +ve cDNA (made in Part 1) to +ve control (avoid to touch the wall). Use a new pipette tip. Mix it. This is not a necessary step.



7a. Use new pipette tip with filter. Add 2µl of cDNA from tube D1.This is positive control supplied with our kit. Mix it. **This positive control can be made by you as stated in Part 1 (point 3).**

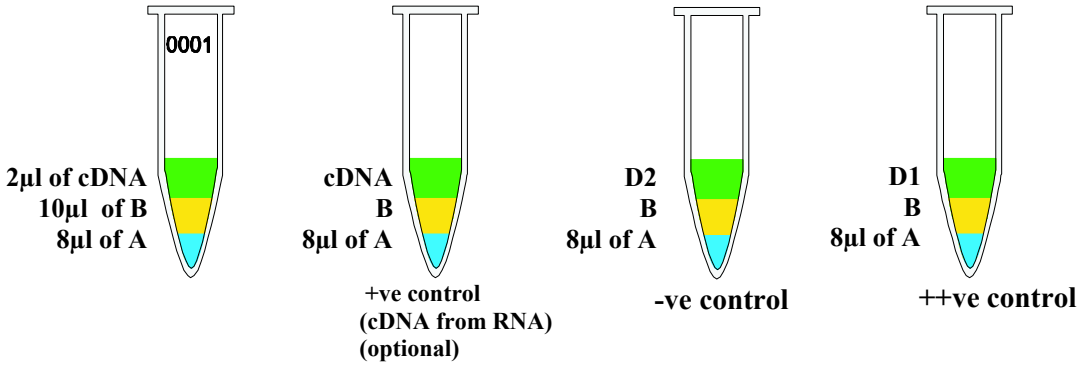


8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control . Mix it.



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

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 microtube must be almost the same.



Now program your PCR machine as follows.

A. 60 seconds at 94°C
B. 15 seconds at 94°C
15 seconds at 50°C
30 seconds at 72°C
} 35 cycles
C. 420 seconds at 72°C

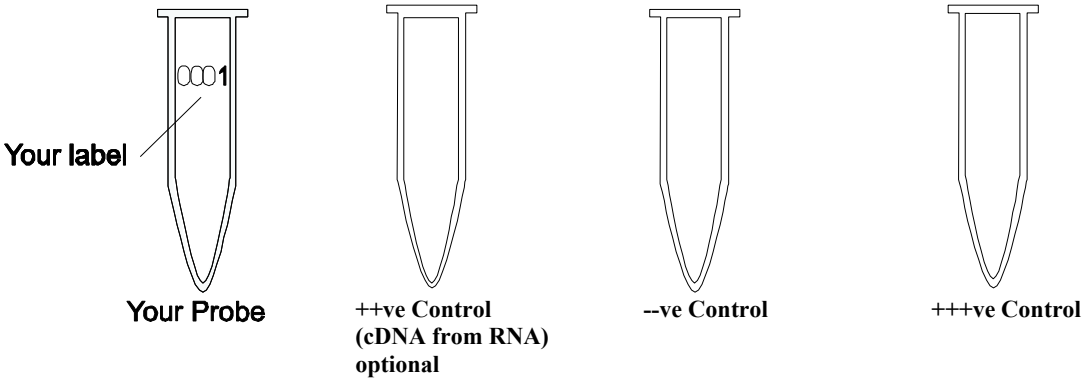
Before you start the PCR program, kindly check whether tubes are closed properly. **Microtubes must be in contact with metal block** (very important!). There should be no air or lose contact with metal block of thermocycler.

11. After step 10 is finished take out the microtubes and centrifuge for a while.

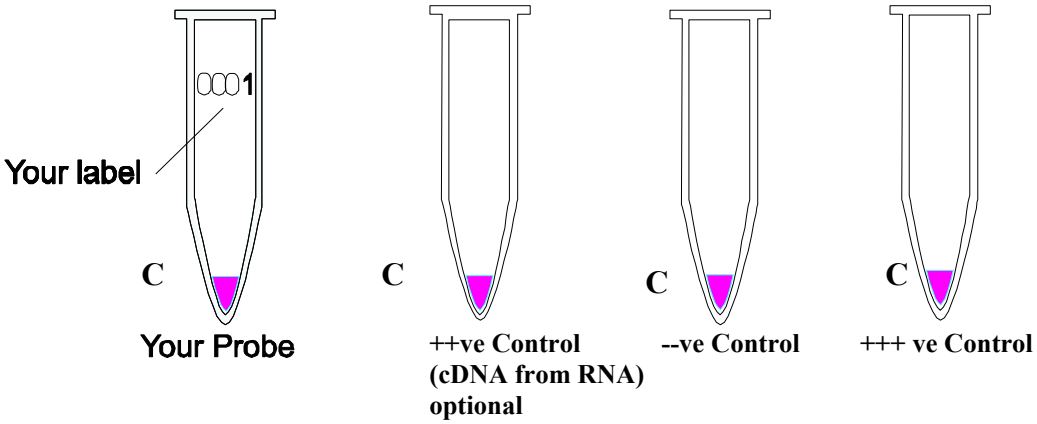
Now go to STEP B. **After this you can go to Step C (electrophoreses) to see the results of this round.**

STEP B

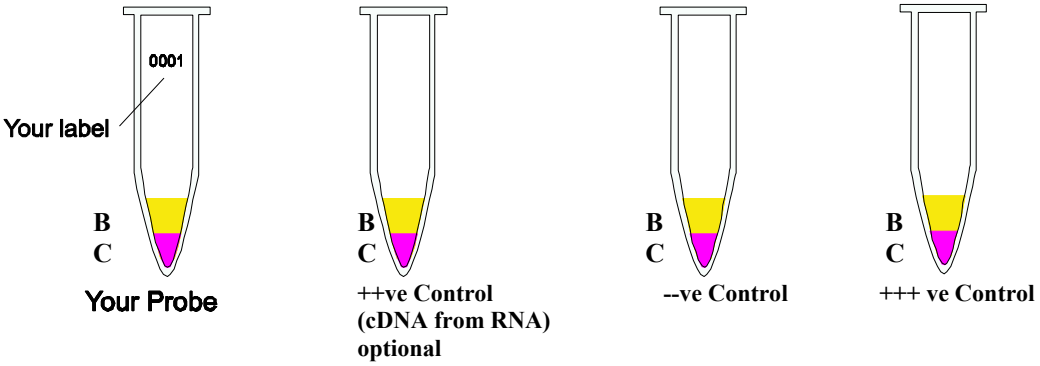
1. Mark your microtubes with a sample number, +ve Control and –ve Control.



2. Add 8µl of tube C to each tube.

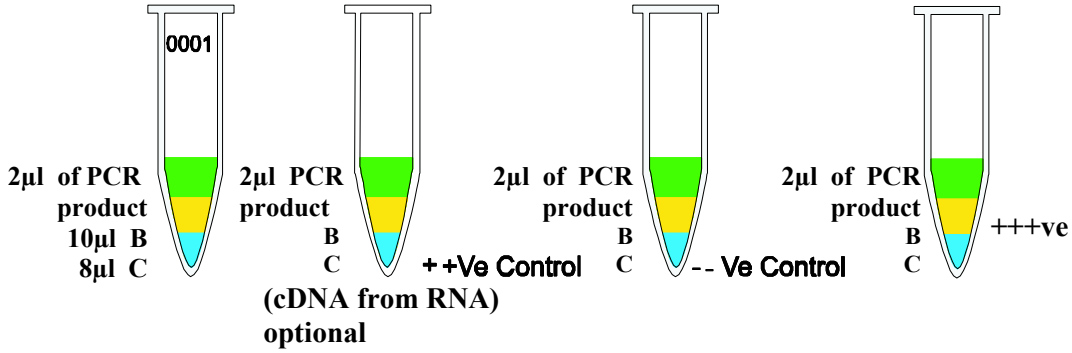


3. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.



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

5. Add 2µl of your PCR Product of step A to each tube according to your **labelling (sometimes it is sufficient to add 1µl of this).**



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

Now program your PCR machine as follows.

A. 60 seconds at 94°C
B. 15 seconds at 94°C
15 seconds at 50°C
30 seconds at 72°C
} 35 cycles
C. 420 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.

7. After step 6 is finished take out the microtubes and centrifuge for a while.

Now go to STEP C.

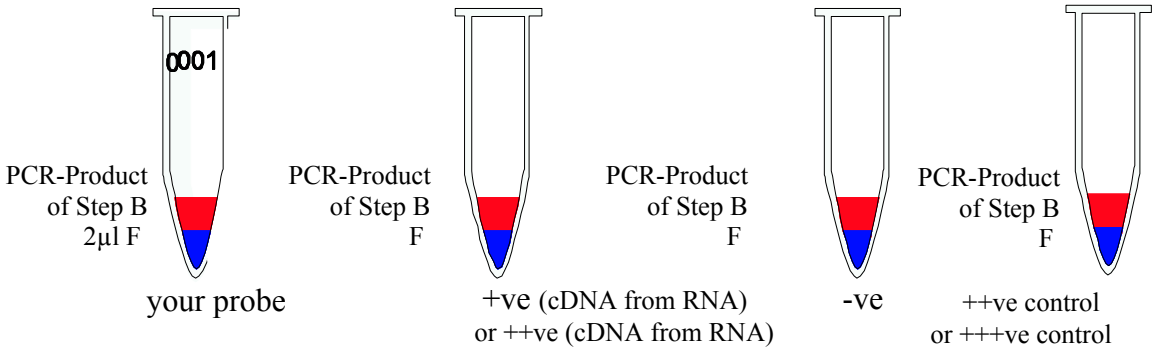
STEP C

1. Prepare the gel Agarose 1.5% in TAE (1x) buffer.

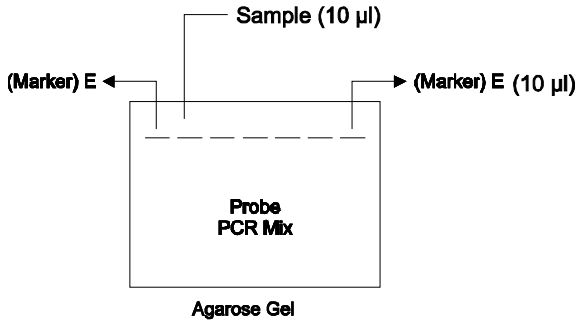
2. Let the Gel dry and add this TAE (1x) buffer in gel chamber.

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 and cDNA Controls).



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 **50 min**. at **120 Volt**.

8. Make straining solution ready.

9. Put the gel for 5-15 minutes straining 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 bands in positive control and no band in negative control.

    1st step: 551bp in positive control as well as in positive samples.
    2nd step:374bp in positive control as well as in positive samples.

TIP: gene sequencing recommended to reconfirm the positive results. Please ask for this service.

HINT:

Sometimes, when you don't find the positive band in the first step, but there is a specific band in the second step, it should be taken as positive.

Recommendation: gene sequencing is highly recommended to reconfirm the positive samples.

If you should find any mistakes, please let us know. Thank you.

Suggestion:
This manual has been written specifically for beginners, hence persons with experience in PCR must use their experience to keep each step as small as possible e.g. you should calculate the amount of the needed chemicals, before starting with testing.

Last update: 16-12-2011
v1.4

Genekam Biotechnology AG
Dammstr. 31-33
47119 Duisburg
Germany
Tel. (+49) 203 / 555858-31,-32,-33
Fax (+49) 203 / 35 82 99
anfrage@genekam.de
http://www.genekam.de