Lot No.

Ref. K031

MANUAL

Expiry date: 1 year

STORE AT -20°C

100 Tests (Ready to use PCR kit)

CANINE DISTEMPER VIRUS

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

Principle and use:

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect *Canine Distemper Virus* in nested PCR.
This kit needs RNA which can be isolated from blood, serum, tissue, respiratory swab, eye swab, respiratory fluid and any body fluid. Kindly use good methods to isolate the RNA. Kindly take common safety laboratory precautions during working. ***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.***

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:

- RNA kit (HX, PF, NTP, RI, RET, DH, DH)
- Tube A (2 tubes)
- Tube B (2 tubes)
- Tube C (2 tubes)
- positive (+ve) control (D1) (1 tube)
- 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 and pen
- Agarose (good quality)
- Staining (Ethium Bromide)
- TAE buffer 1x
- Ice
- Vortexer

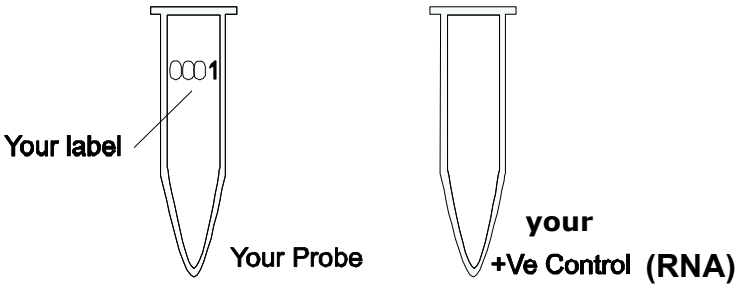
Procedure:

PART 1 – Convert RNA into cDNA. This should be done with RNA kit, which is with our kit.

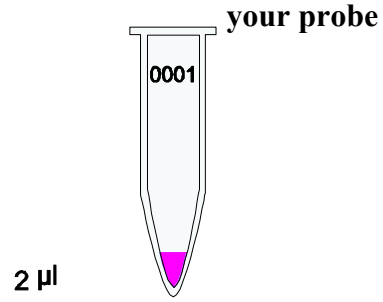
ONCE AGAIN: VERY IMPORTANT ! PLEASE USE GLOVES ! DON'T TOUCH ANY OTHER OBJECTS, OTHERWISE THERE MAY BE RNASE CONTERMINATION DURING THIS PART.

STEP A

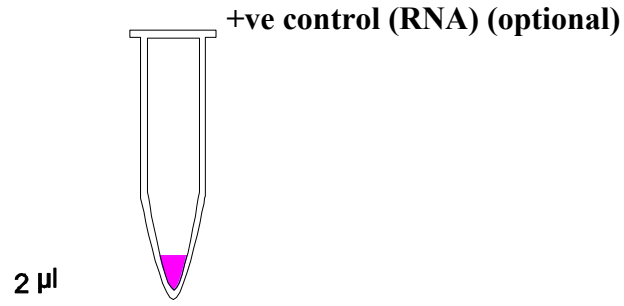
1. Mark your microtubes with a sample number and one with +Ve Control (this +ve control may be made by yourselves, if you have positive material as we supply only cDNA. **Otherwise work without this**).



2. Add 2µl of your isolated RNA from your samples.



3. Add 2µl of RNA (it can be made by you, if you have any sample of Canine Distemper Virusas positive control to +ve control tube. It is optional i.e. it is not essential. However we have added cDNA (D1) as positive control.



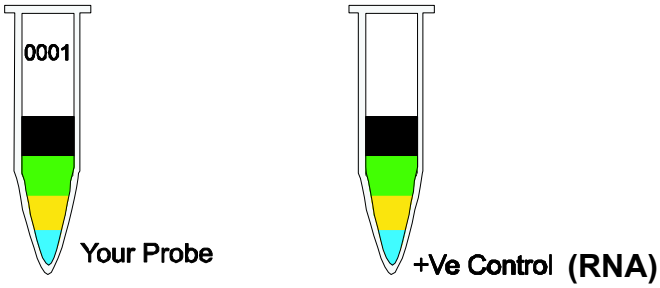
4. Add 1µl of HX (Primer) and 9µl of DH (water) to each tube.

TIP:	**RNA**	**2µl**
	HX	**1µl**
	DH	**9µl**
		12µl

In order to keep this step small, me must calculate the total requirement, e.g. for 10 probes you need 10µl of HX and 90µl of DH.

4a. 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).

5. Add: 4µl of PF (buffer)
1µl of RI (Enzyme)
2µl of NTP (dNTP-Mix) to each tube
Total: 7µl in tube

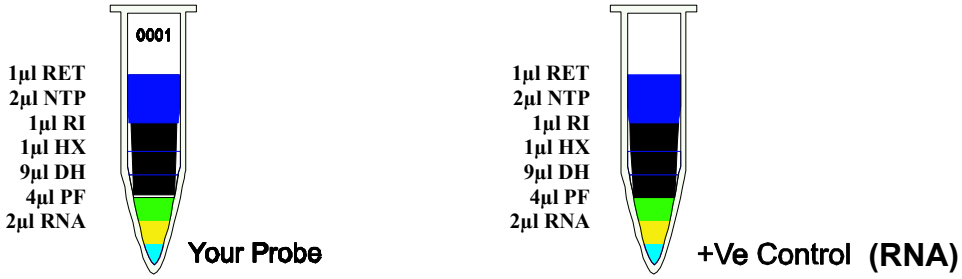


Tip: you can calculate your need for chemicals and mix them together. After that you can add 7µl to each tube, e.g. if you want to run 10 reactions, make 70 µl total (e.g. 40µl PF + 10µl RI + 20µl NTP = 70µl) . Add 7µl to each tube.

6. Run at 25°C for 5 minutes.

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

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



Run at:

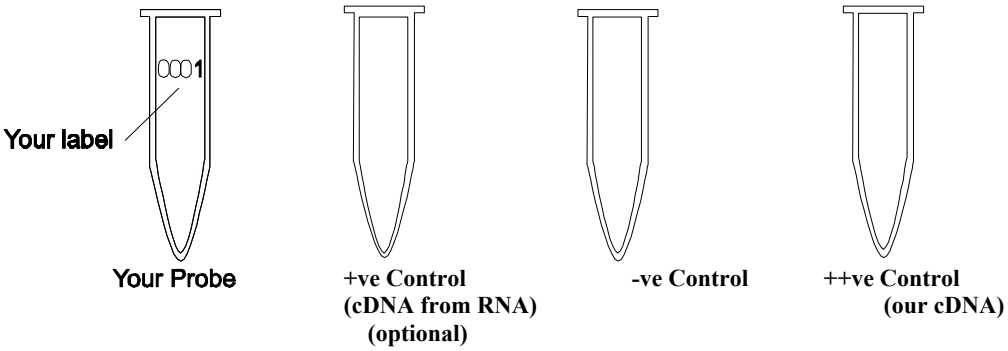
25°C for 10 minutes
42°C for 50 minutes
48°C for 10 minutes
70°C for 10 minutes
4°C for 5 minutes

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

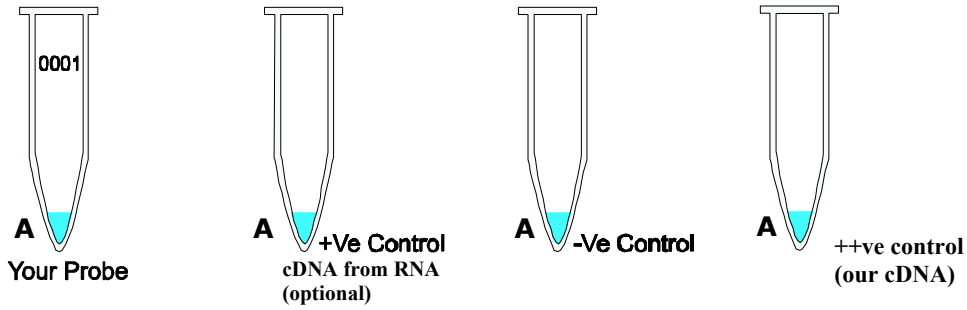
STEP A

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

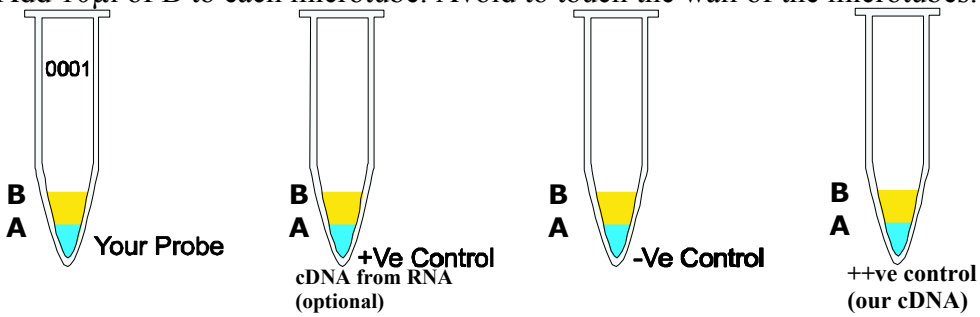
2. . Mark your microtubes with a sample number, +ve Control and –ve Control.



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

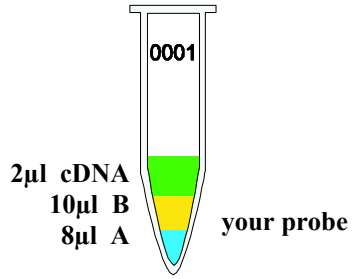


4. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.

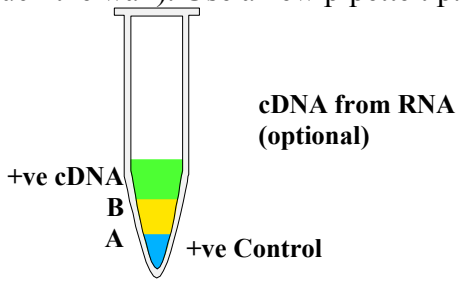


5. **TIP: you can calculate the total requirement of chemicals needed . You need 8µl A + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save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

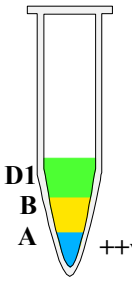
6. Add 2µl of your cDNA template (cDNA made in cDNA-synthesis, see Part I) isolated from samples) with pipette tip with filter to each microtube according to your label except +Ve and –Ve (Avoid touching the wall). **Use everytime a new pipette tip** (for each sample)!



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.

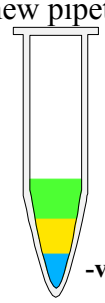


7a. Use new pipette tip with filter. Add 2µl of cDNA from tube D1.This is positive control supplied with our kit. Mix it.



++ve Control (our cDNA)

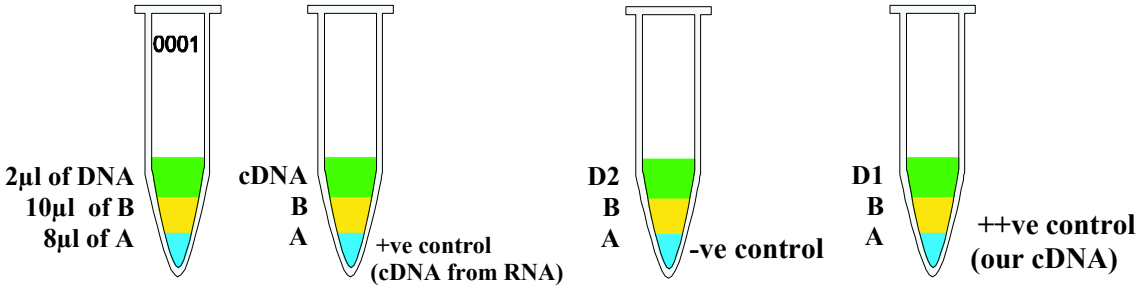
8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control . Mix it.



-ve Control

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.

1.	A.	30 seconds at 94°C	30 cycles
	B.	30 seconds at 54°C	
		60 seconds at 72°C	
2.		600 seconds at 72°C	

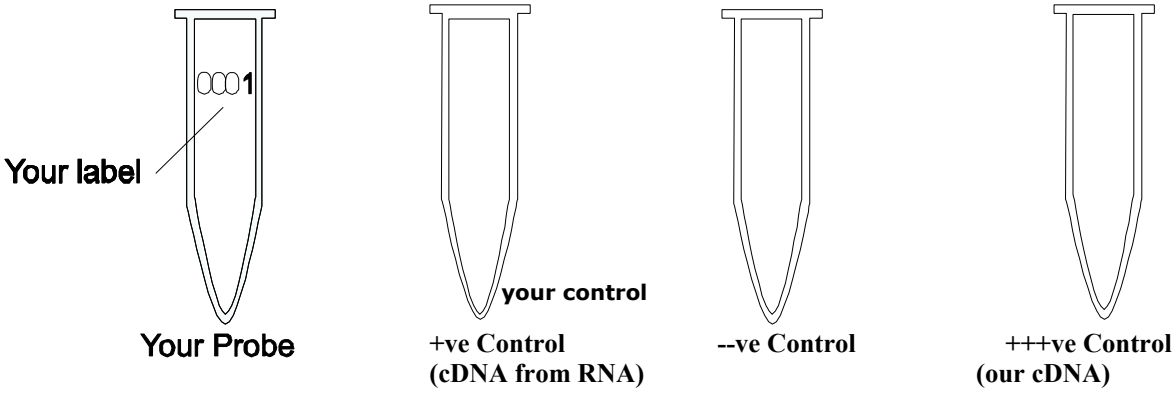
Before you start the PCR program, kindly check whether tubes are closed properly. **Microtubes must be in contact with metal block** (very important!). There should be no air or lose contact with metal block of thermocycler.

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

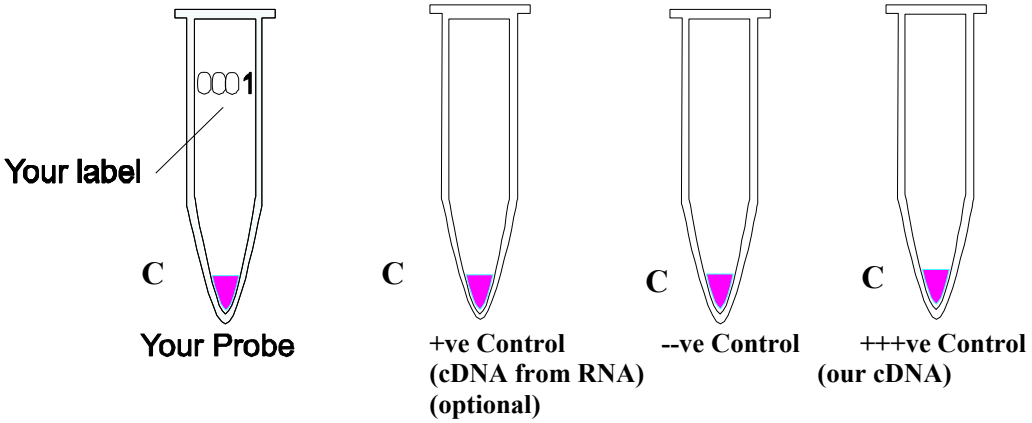
Now go to STEP B.

STEP B

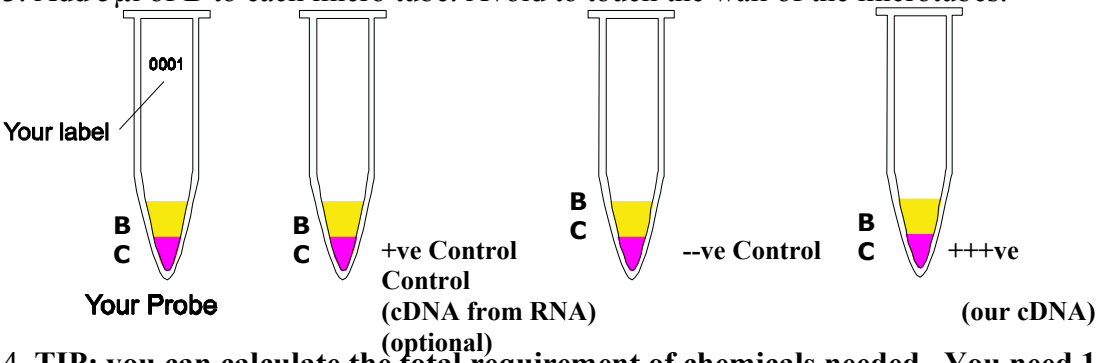
1. Mark your microtubes with a sample number, +ve Control and –ve Control.



2. Add 13µl of tube C to each tube.

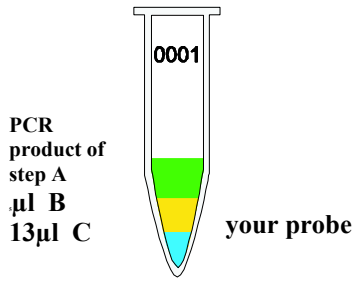


3. Add 5µl of B to each micro tube. Avoid to touch the wall of the microtubes.

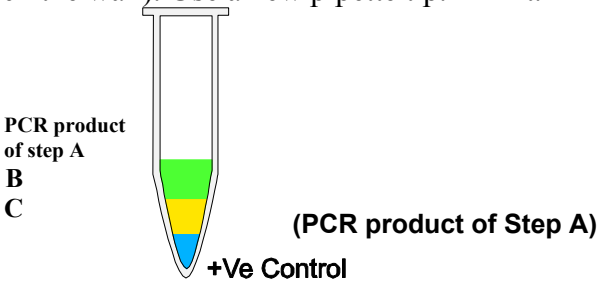


4. **TIP: you can calculate the total requirement of chemicals needed . You need 13µl C + 5µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 130µl of C + 50µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save time and hardware.**

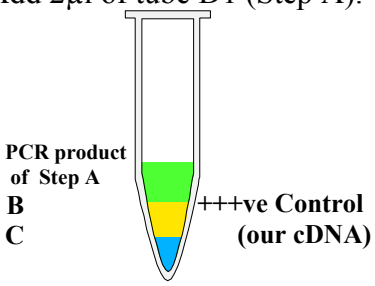
5. Add 2µl of your cDNA template (PCR product of step A) with pipette tip with filter to each micro tube according to your label except +Ve and -Ve (Avoid touching the wall).
Use every time a new pipette tip (for each sample)! Mix it.



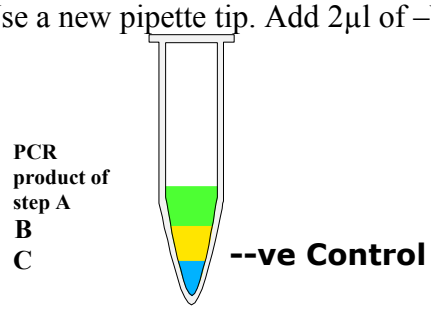
6. Use new pipette tip with filter. Add 2µl of +ve PCR product of step A to +Ve Control (avoid to touch the wall). Use a new pipette tip. Mix it.



7. Use new pipette tip with filter.
Add 2µl of tube D1 (Step A). This is the positive control supplied with our kit. Mix it.

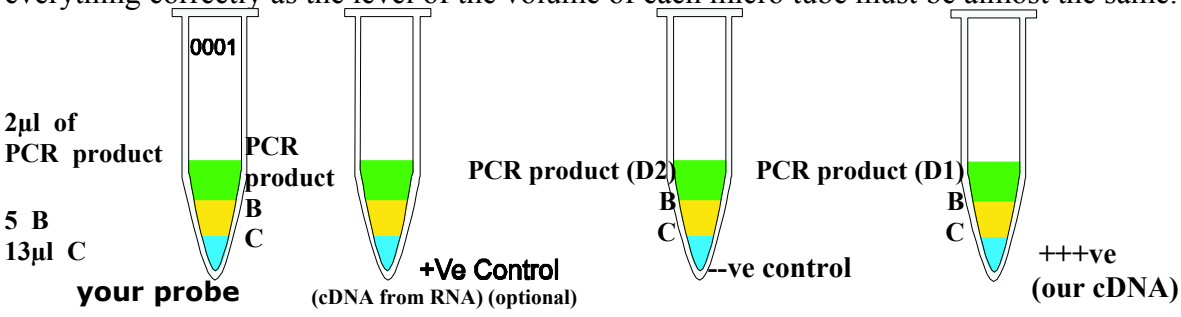


8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control (avoid the wall). Mix it.



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 micro tube must be almost the same.



Now program your PCR machine as follows:

1.	A.	30 seconds at 94°C	30 cycles
	B.	30 seconds at 54°C	
	C.	60 seconds at 72°C	
2.		600 seconds at 72°C	

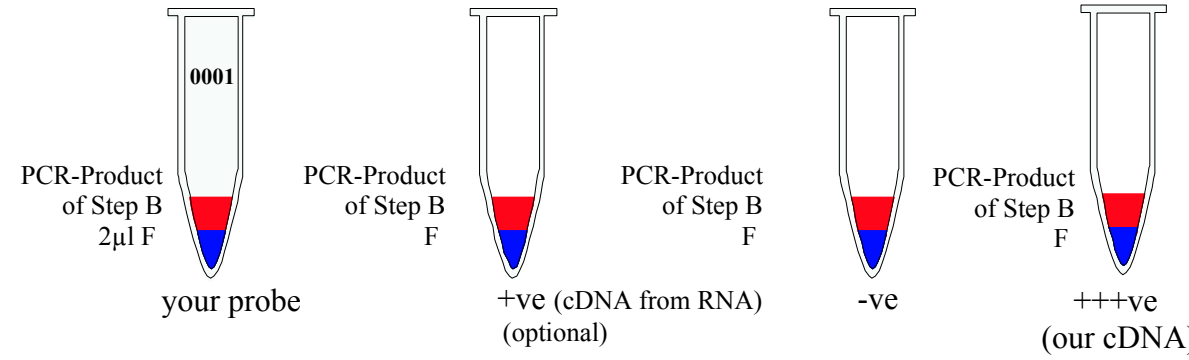
Before you start the PCR program, kindly check whether tubes are closed properly. **Microtubes must be in contact with metal block** (very important!). There should be no air or lose contact with metal block of thermocycler.

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

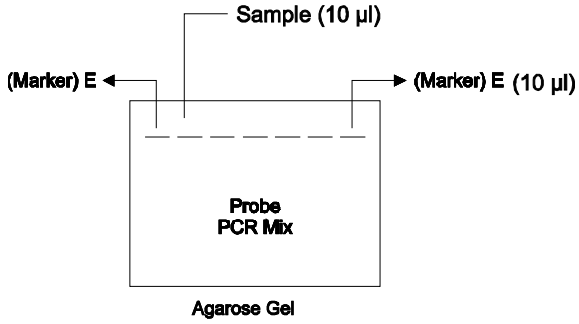
Now go to STEP C.

STEP C

1. Prepare the gel Agarose **1.5 or 2%** 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 micro tube (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 staining solution ready.
9. Put the gel for 10-30 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 bands in positive control and no band in negative control. You will see band **517bp** in positive control and positive samples for Canine Distemper Virus.

There will be no band in the first round of PCR . There will be a band in the 2nd round of PCR.

Recommendation: Gene sequencing is highly recommended to reconfirm the positive results.

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.**	**Genekam Biotechnology AG** **Dammstr. 31-33** **47119 Duisburg** **Germany** **Tel. (+49) 203 / 555858-31,-32,-33** **Fax (+49) 203 / 35 82 99**

Last update: 01-08-2011	**anfrage@genekam.de**
v1.2	**http://www.genekam.de**