Lot.
Ref. K171
100 Tests (Ready to use kit)

MANUAL – one step

Expiry date: 1 year
STORE AT -20°C

OSTRICH SEXING

-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 do *Ostrich sexing*.

This kit needs DNA which can be isolated from blood, tissue, respiratory swabs, nasal swabs, feathers, cell cultures and any body fluid. Kindly use good methods to isolate the DNA.

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:

• Tube A for Internal Control / Male (MG) (2 tubes)
• Tube C for Female Control (MS) (2 tubes)
• Tube B (2 tubes)
• Positive (+Ve) control (tube D1) for Female Control (1 tube)
• Negative (-Ve) control (tube D2) (1 tube)
• Marker (Tube E) (1 tube): 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

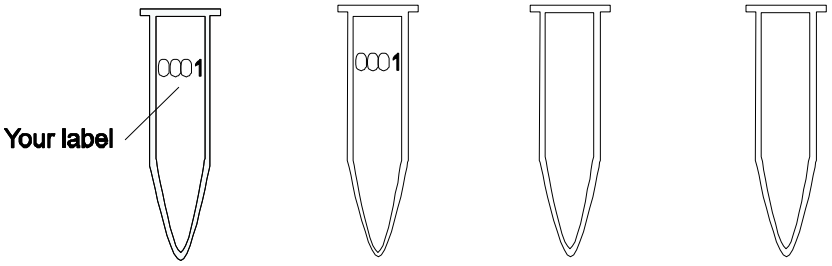
Procedure:

After your DNA isolation is completed. (Kindly use good quality isolation method).

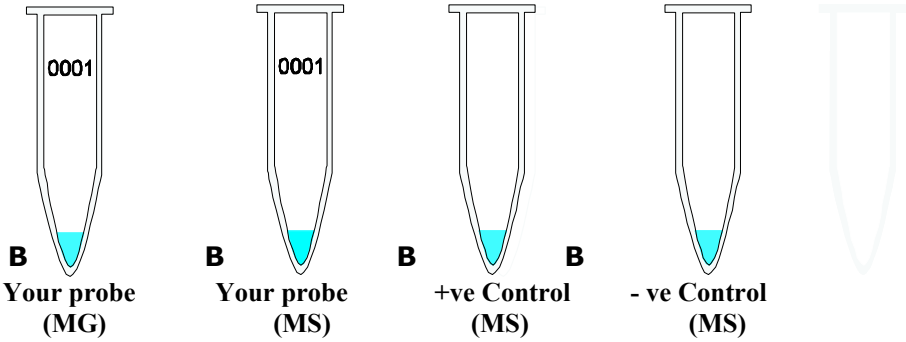
STEP A

1. Kindly thaw **one tube** each: A, B, C, D3, 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.

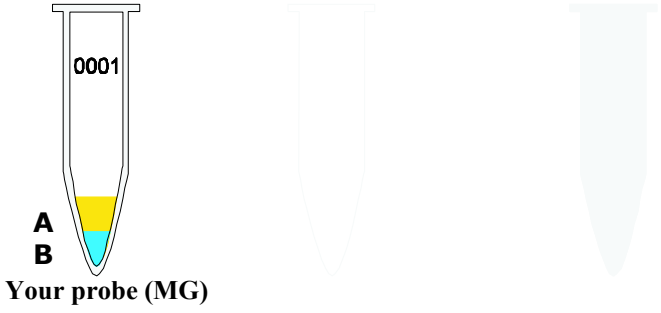
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control. You have to make two microtubes from each probe i.e. one is for MG (Internal Control / Male) and other for MS (Female).



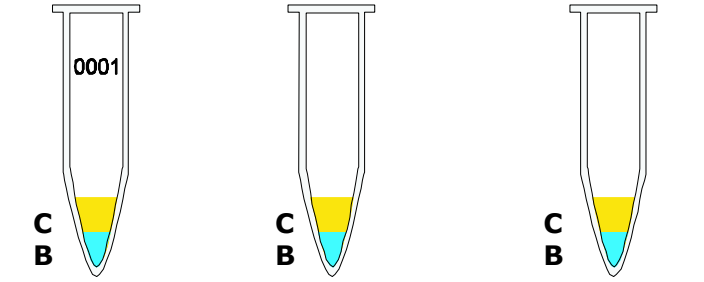
3. Add 5µl of B to each micro tube. Avoid to touch the wall of the microtubes.



4. Add 13µl of tube A to each tube with MG. These tubes will identify only Internal Control / Male.



4a. Please add 13µl of tube C to each tube for probe MS. These tubes will only identify Female.

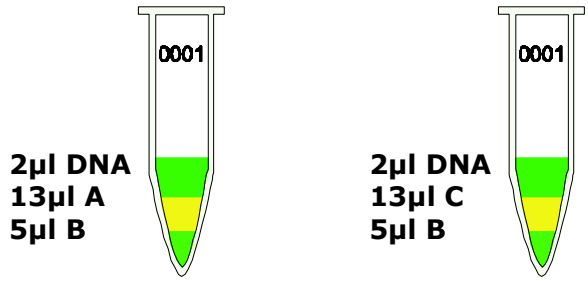


Your probe (MS) **+ve Control (MS)** **- ve Control (MS)**

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

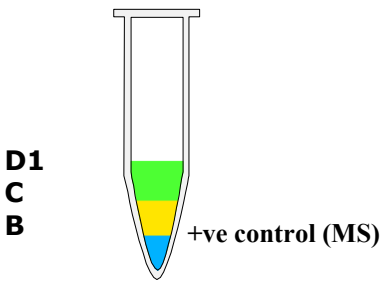
TIP: you can calculate the total requirement of chemicals needed . You need 13µl C + 5µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 130µl of C + 50µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save time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

5. Add 2µl of your DNA template (DNA isolated from samples) 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 thoroughly. **You have to add isolated DNA to each tube with sign "your probe" for MG and MS.**



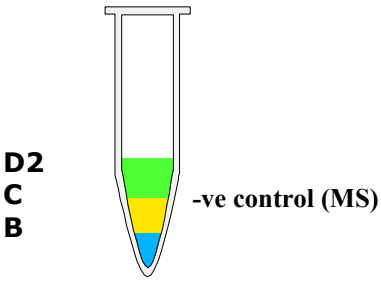
Your probe (MG) **Your probe (MS)**

6. Use new pipette tip with filter. Add 2µl of +ve (tube D1) to +ve Control (MS) (avoid to touch the wall). Use a new pipette tip. Mix it. This is only for Female.



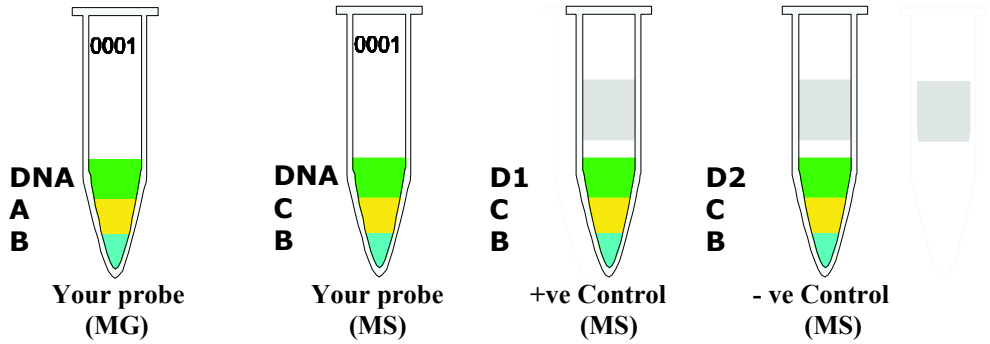
+ve control (MS)

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



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

9. 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.	120 seconds	at 95°C	
2.	60 seconds	at 94°C	27 cycles
	60 seconds	at 54°C	
	120 seconds	at 72°C	
3.	300 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.

10. After step 9 is finished take out the microtubes.

To see the results, you can go directly to step gel electrophoresis (STEP B).

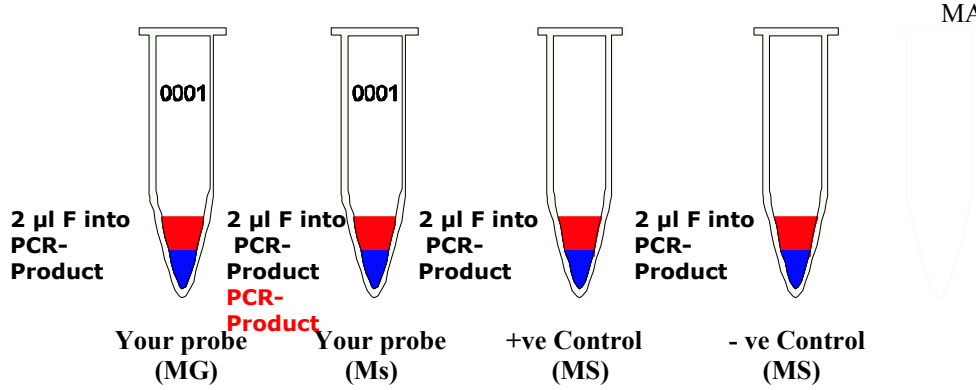
STEP B

1. Prepare the gel Agarose 2% in TAE (1x) buffer.

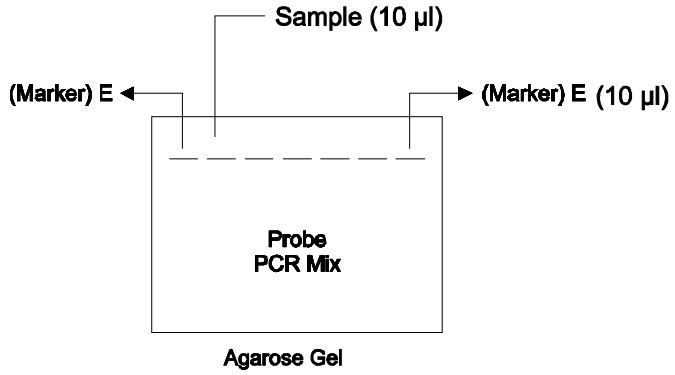
2. Let the gel dry and add this TAE (1x) buffer in gel chamber.

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 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 50 - **60min**. at **110 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 bands in +Ve Controls, positive samples and no bands in –Ve controls.
280 bp for positive samples of Internal Control and +ve control.
648 bp for positive samples of Female and +ve control.

Results Reading:

648bp + 280bp = female (both bands are specific)
280bp bands only = male (some time there are non specific bands here, but you will see 280bp clearly. Unspecific band is usually around 220bp)

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: 09-01-2012
v.1.0

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