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MADE IN GERMANY

Lot-No.

MANUAL – one step

Ref. K342 **Expiry date: 1 year**

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

IDENTIFICATIONS OF MEMBERS OF MYCOBACTERIUM TUBERCULOSIS
COMPLEX (GENOTYPING)

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

Principle and use:

This amplification kit has been manufactured by *Genekam Biotechnology AG t*o do the genotyping of *Mycobacterium tuberculosis.*

This kit needs DNA which can be isolated from PCR positive colony of this bacteria. Kindly use good methods to isolate the DNA.

It is better that you use DNA from the sample, which is positive in your PCR assay. If you have not performed such assay, you may need some times to dilute your DNA as many isolation kit can isolate the DNA from colonies very strongly, which may inhibit the assay.

It is also possible that you can use this PCR kit directly on the samples taken from the patients.

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, C, H, I, J, K, L (1 tube each)
- Tube B (3 tubes)
- Positive (+Ve) control (tube D1) (1 tube)
- Positive (+Ve) control (tube D3) (1 tube)
- Negative (-Ve) control (tube 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)
- Pipette-tips with and without filter (20µl, 5µl & 1µl)
- Pipettes (quality pipettes)
- Gel Agarose chamber
- Power supply

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• Paper and pen
• Agarose (good quality)
• Staining (Ethium Bromide)
• TAE buffer 1x
• Ice
• Vortexer

Procedure:

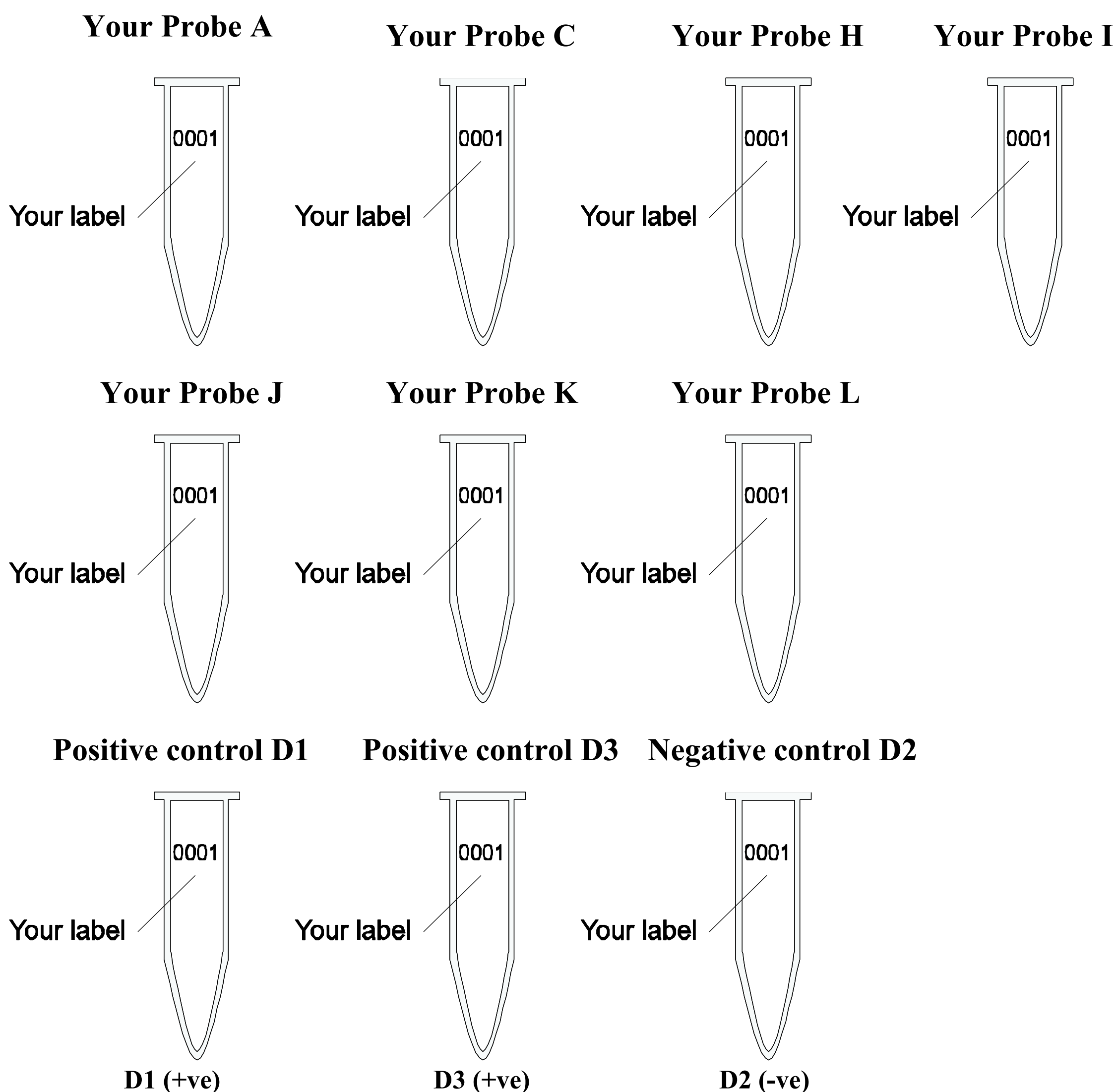
After your DNA isolation is completed. (Kindly use good quality isolation method).

STEP A

1. Kindly thaw **one tube** each: A, C, H, I, J, K, L, B, D1, D2, D3, 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 and with +Ve Control and –Ve Control. Positive and negative controls are optional (if your DNA is positive for PCR specific assay, **you may omit the positive and negative controls**. If you think that you need the controls, please run them only once for whole assay, but we have the positive and negative controls in our pictures).

Now label your Probes according to the name of tube e.g. A, C, H, I, J, K, L etc.



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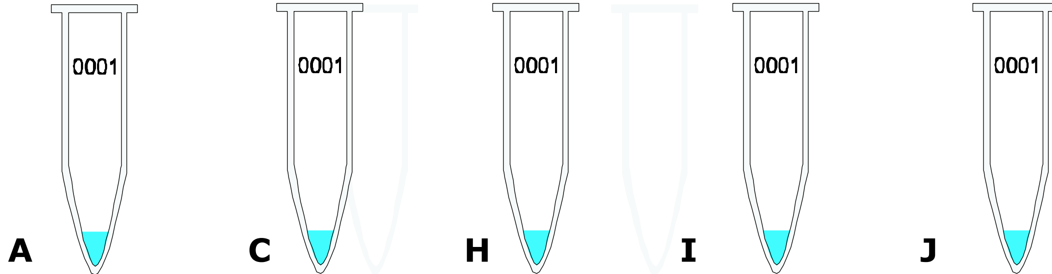
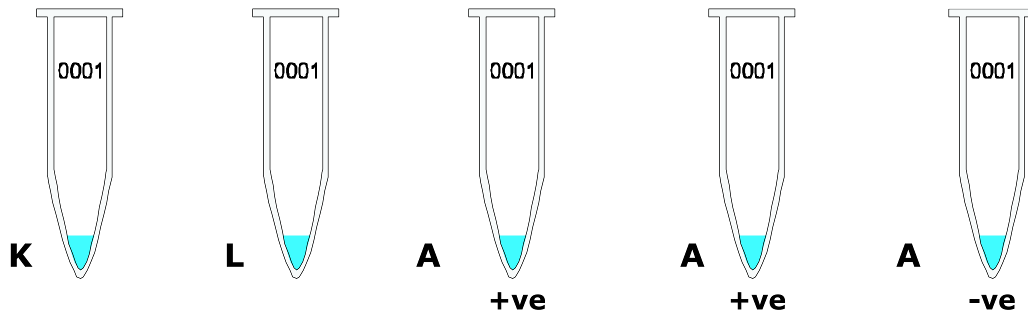
3. Add 8µl of tube A, C, H, I, J, K, L to each tube. This should be done according the number of tubes like A, C, H, I, J, K, L except positive and negative control, where you add 8ul of tube A.

Your Probe A **Your Probe C** **Your Probe H** **Your Probe I** **Your Probe J**

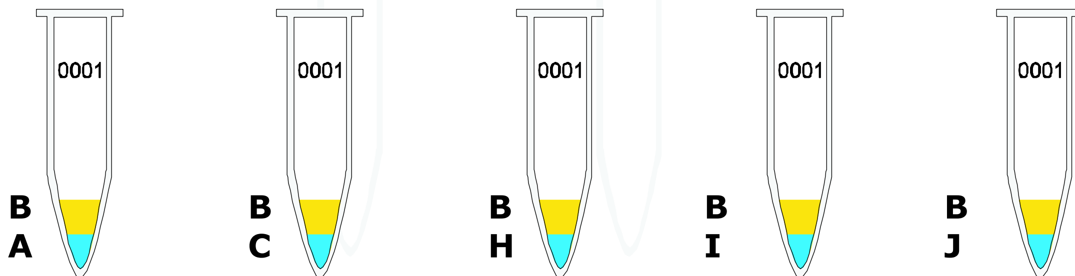


Your Probe K **Your Probe L** **Pos. control D1** **Pos. control D3** **Neg. control D2**

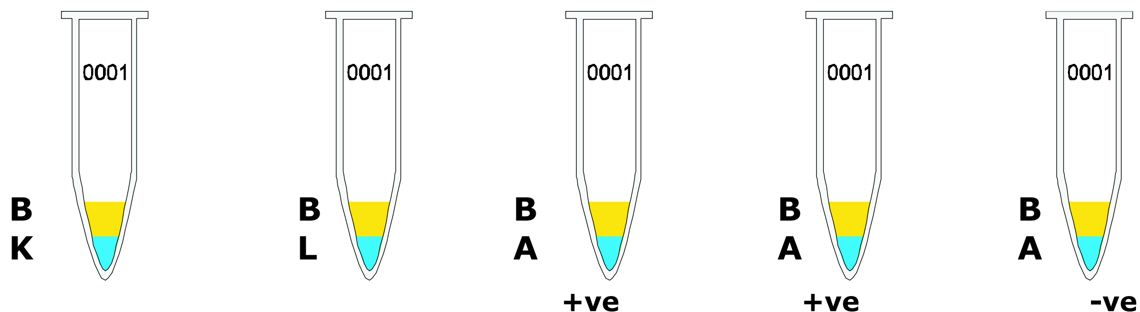


4. Add 10µl of B to each micro tube. Avoid to touch the wall of the microtubes.

Your Probe A **Your Probe C** **Your Probe H** **Your Probe I** **Your Probe J**



Your Probe K **Your Probe L** **Pos. control D1** **Pos. control D3** **Neg. control D1**



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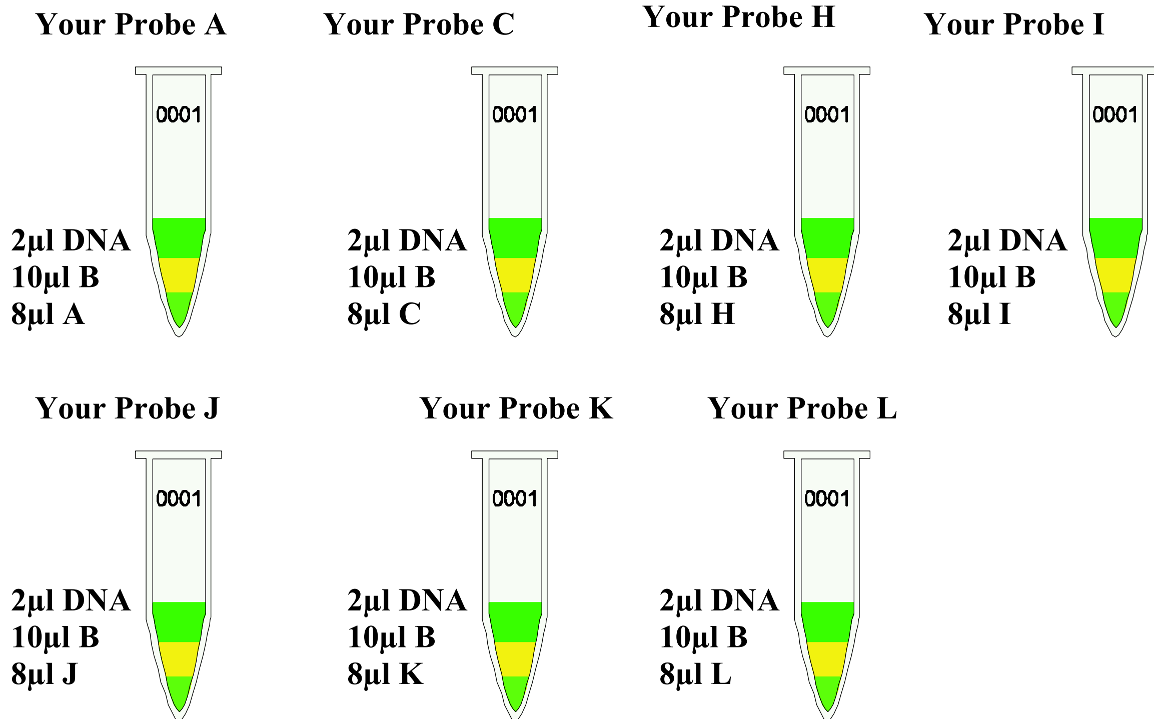
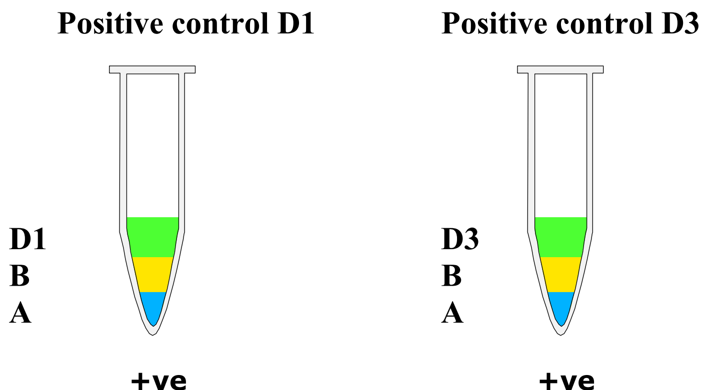
TIP: you can calculate the total requirement of chemicals needed . You need 8µl A +10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of = 180µl from which you can take 18µl and add to each tube. This way you can save time and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

5. Add 2µl of your 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 every time a new pipette tip** (for each sample)! Mix it.



6. Use new pipette tip with filter. Add 2µl of +Ve (tube D1) as well as D3 to +Ve Control (avoid to touch the wall). Use a new pipette tip. Mix it. **This is optional, not necessary.**



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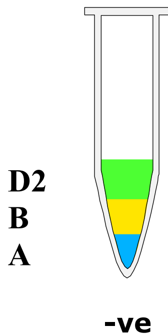
7. Use a new pipette tip. Add 2µl of –Ve (Tube D2) to –Ve Control (don't touch wall). Mix it. This is optional step. **This is optional, not necessary.**

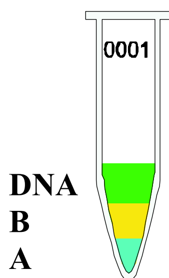
Negative control D2



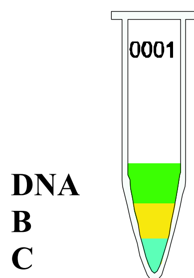
8. Centrifuge all tubes for 20 sec. for 8000 rpm (this is not necessary but it is better).

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 microtube must be almost the same.

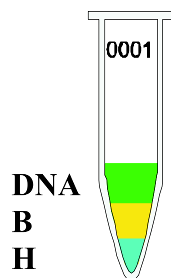
Your Probe A



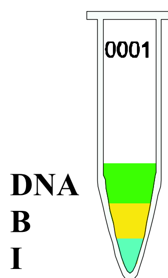
Your Probe C



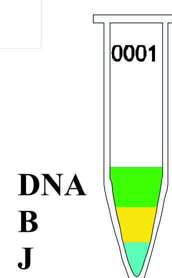
Your Probe H



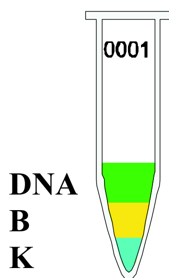
Your Probe I



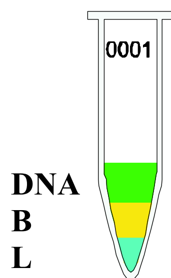
Your Probe J



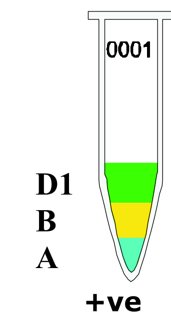
Your Probe K



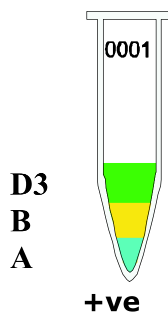
Your Probe L



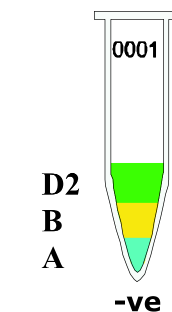
Pos. control D1



Pos. control D3



Neg. control D2



Now program your PCR machine as describe on the last page.

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.

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Program your PCR machine.

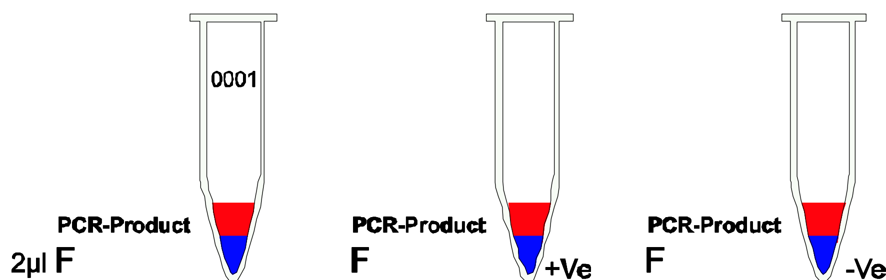
1.	300	seconds at	94°C	
2. A.	60	seconds at	94°C	35 cycles
B.	60	seconds at	60°C	
C.	60	seconds at	72°C	
3.	600	seconds at	72°C	

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

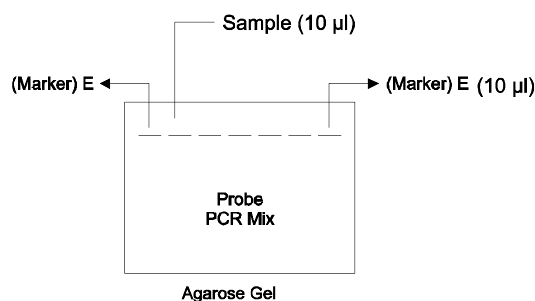
To see *Mycobacterium tuberculosis*, you can go to step gel electrophoresis (STEP B).

STEP B (Kindly do not use whole quantity of your PCR product to run the gel agarose):

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 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**. 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 samples and no bands in –Ve controls. These bands may vary.

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Results:

Tubes (Size of the band)	A (543bp)	C (786bp)	H (943bp)	I (1043bp)	J (1116bp)	K (991bp)	L (404bp)
M. tuberculosis	+	+	+	+	+	+	+
M. africum 1	+	+	+	+	-	+	+
M. africum 2	+	+	+	+	+	+	+
M. bovis	+	+	+	-	-	+	-
M. bovis BCG	+	+	+	-	-	-	-
M. caprae	+	+	+	+	-	+	-
M. microti	+	+	-	+	-	+	+
M. canettii	+	+	+	+	+	+	-
MOTT	+	-	-	-	-	-	-

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.

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