

9. Appendix

I. Abbreviations

AFB	Acid-fast bacilli
AIDS	Acquired immunodeficiency syndroms
BSA	Bovine serum albumin
CTAB	Cetyltrimethylammonium bromide
DIG	Digoxigenin-labeled
ELISA	Enzyme-linked immunosorbent assay
FR	Flanking region
HIV	Human immunodeficient virus
IS	Insertion sequence
MAIC	<i>Mycobacterium avium-intracellulare</i> complex
MB	Middlebrook Broth
NALC-NaOH	N-Acetyl-L-Cystein NaOH solution
NTM	Non-tuberculosis mycobacteria
OADC	Oleic acid, Dextrose and Catalase Supplement
PACT	Polymyxin B, Amphotericin B, Carbenicillin and Trimethoprim Supplement.

PCR	Polymerase chain reaction
PFGE	Pulse –field gel electrophoresis
RFLP	Restriction fragment length polymorphism
rRNA	ribosomal Ribonucleic acid
SSC	Sodium Chloride- Sodium Citrate Buffer
TBE	Tris-Boric acid-EDTA Buffer
TE	Tris-EDTA Buffer
ZN	Ziehl-Neelsen Stain

List of solutions and buffers

- Chloroform-Isoamyl alcohol

Dissolve 4ml isoamyl alcohol in 96ml chloroform.

- Concentrated blocking solution

Dissolve 10g of blocking reagent in 100ml washing buffer, heat at 70°C for 1 hour.

-Blocking solution

Mix 15ml concentrated blocking solution in 135ml washing buffer.

- CTAB/NaCl

Completely dissolve 10 g. CTAB and 4.1 g. NaCl, 100 ml Aqua bidest.

To dissolve CTAB, warming the mixture at 65 °C is possible.

- Detection Buffer

Dissolve 2.42 g TRIS and 1.17 g NaCl in 150 ml Aqua bidest.

Adjust pH to 9.5; fill up with Aqua bidest. to 200 ml.

- Hybridization buffer

Dissolve 0.1g Sarcosyl and 200µl of 10% SDS in 25ml of 20X SSC buffer. Fill up with Aqua bidest. to 100ml.

-Loading dye

Dissolve 6.7 g Saccharose in 8 ml Aqua bidest. on shaking plate. Dissolve 40mg Bromphenol blue and 40mg Xylene cyanol. Fill up with Aqua dest. to 10ml.

- NALC-NaOH Solution

Dissolve 500mg of NALC (N-Acetyl-L-Cystein) in 50ml 2.9% of $C_6H_5Na_3O_7 \cdot 2H_2O$ and 50ml of 4% NaOH.

- PBS-Tween 80

Dissolve 3.22g Na_2HPO_4 , 0.82g KH_2PO_4 , 7.82g NaCl and 1ml Tween 80 in 1L Aqua dest.; autoclave.

-SDS-Proteinase K solution

mix 5 µl Proteinase K (10 mg/ml) and 70 µl of 10% SDS per sample.

Mix shortly before use.

- 20X SSC Buffer

Dissolve 175.3 g NaCl and 88.2 g $C_6H_5Na_3O_7 \cdot 2H_2O$ in 800 ml Aqua bidest.

Adjust pH to 7.0; filled up with Aqua bidest to 1 L.

- 10 x TBE-Buffer

Dissolve 108 g TRIS, 55 g Boric acid and 7.5 g EDTA in 800 ml Aqua bidest

Adjust pH to 8.0-8.2; filled up with Aqua bidest to 1 L.

- 10 X TE Buffer (100 mM TRIS-HCL, 10 mM EDTA)

Dissolve 1.58 g. TRIS-HCl and 0.29g. EDTA in 100 ml Aqua bidest.

- Washing Buffer

Dissolve 11.6 g Fumaric acid, 66.7 g NaCl and 2ml Tween 20 in 800 ml Aqua bidest.

Adjust pH to 7.5; fill up with Aqua bidest. to 1L, autoclave.

III List of equipments

<u>Equipment</u>	<u>Manufacturer</u>	<u>Model</u>	<u>Country</u>
Centrifuge	Heraeus	Megafuge 1.0R	Germany
Electrophoresis	Biometra	P 25	Germany
Hybridizing oven	Biometra	OV 24	Germany
PFGE machine	Biometra	Rotaphor R 23	Germany
Shaking plate	IKA	KS 250B	Germany
Sonicator	GenProbe	T460/K	Germany
Thermocycler	Biometra	RS 232	Germany
Thermoshaker	Schuttron	TS-MG	Germany
Vortex	Genie 2™	G-560E	USA
Waterbath	GFL	1003	Germany

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