MAHIDOL UNIVERSITY
Wisdom of the Land

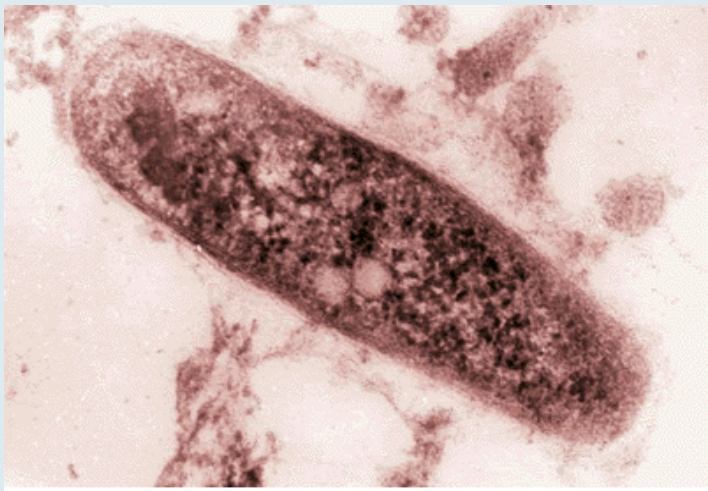
Rapid detection of MDR-TB from indoor air using modified impinger and in-house nested PCR

Pannamthip Pitaksajjakul[a], Pholsak Piyatat[b], Pongrama Ramasoota[a]

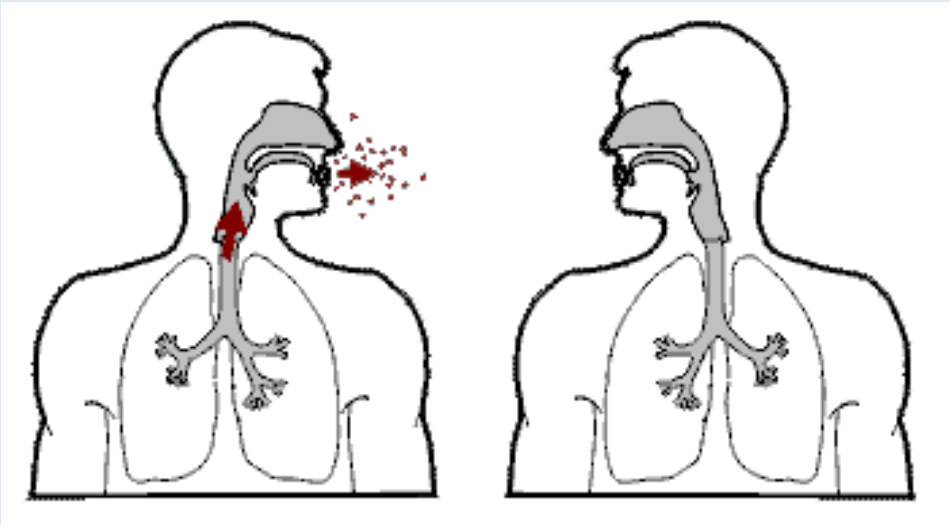
[a] Faculty of Tropical Medicine, Mahidol University, 420/6 Rajwithii road, Rachadhewee, Bangkok 10310, Thailand

[b]Alpine Filter Company (Thailand),

- Background & Rationale
- Materials & Methods
- Results
- Discussions
- Outputs & Application



Tuberculosis Bacterium



Background & Rationale

- Tuberculosis (TB) is the cause of deaths worldwide, especially the emergence of Multi drug resistant (MDR)-TB
- Airborne tubercle bacilli produced from pulmonary TB patients, as droplet nuclei remain suspended in the air for long time
- Transmission of aerosal MTB have been long time reported in many studies in different setting, such as hospitals (Schaal *et al*., 1991; Wan *et al*., 2004), Dental clinic (Bennett *et al*., 2000), and Air craft (Driver *et al*., 1994)

Background & Rationale

- In Thailand, the estimated number of TB cases was 204 cases per 100,000 people with 12,000 deaths per year (WHO, 2004)
- Report on indoor air transmission of MTB, among emergency department personnel (ED) in Bangkok between 1988 - 2002
- The TB incidence rate among ED personnel was 1,610 per 100,000 person-year (PY) (17.2 times higher than non-exposed workers)

Background & Rationale

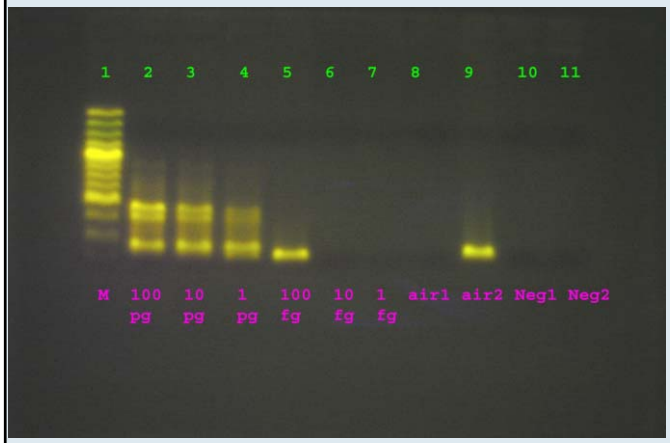
- MTB detection and diagnosis can be both conventional and advance molecular methods
- Culture-based method, which is laborious, and time-consuming (2-4 weeks), especially drug-susceptibility testing of MDR-TB took longer time
- Rapid detection of MDR-TB from indoor air is important for controlling diseases transmission and surveillance

Background & Rationale

- RIF^r-MTB can be use as marker for predicting MDR- TB (Kim *et al*, 1997).
- 95 % of RIF^r-MTB have distinct mutation in the *rpoB* gene (Telenti *et al*, 1997).
- Using high sensitivity PCR, and DNA sequencing for rapid detection of RIF^r-MTB, can reduce detection time to be only 1-2 days.

Detection of MTB in indoor air

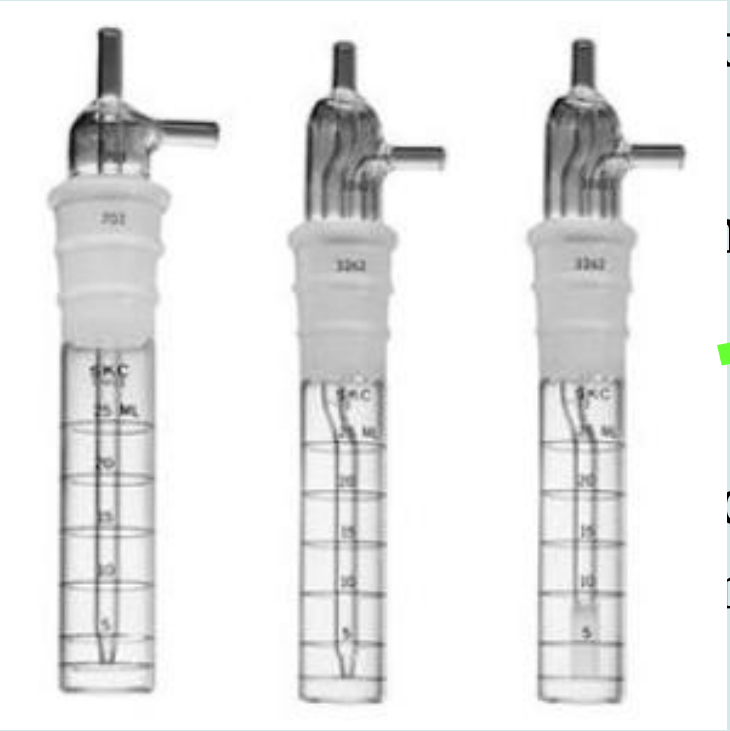
- To detect MTB from indoor air
  - Air collection
  - PCR detection

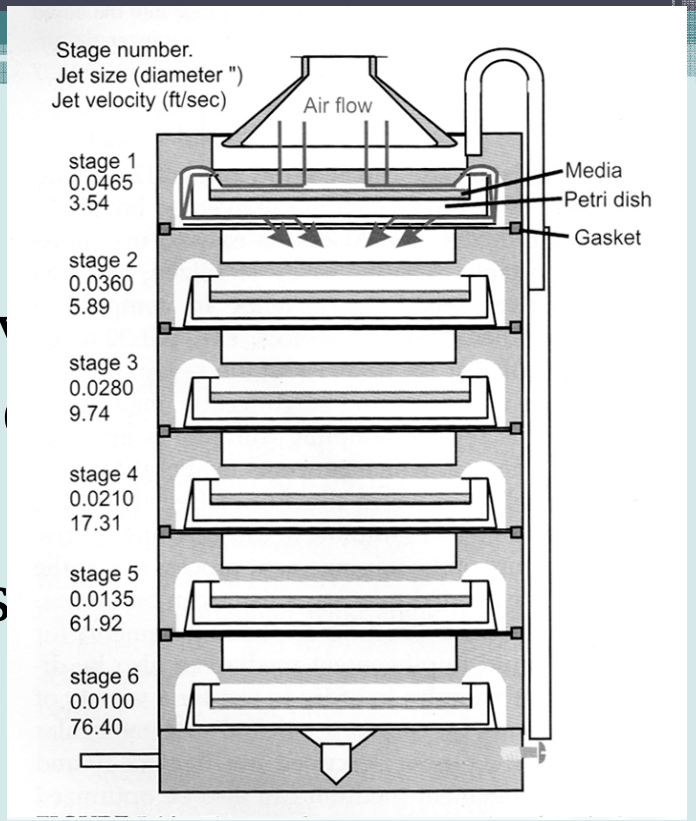


MAHIDOL UNIVERSITY
Wisdom of the Land

Background & Rationale

- For air collection, several methods hav
  tion were report
- , dehydration is
- ction, the prob
  ryin of agar p
- So, the air impinger collection was s
  are no dehydration problem, and co
  PCR experiment



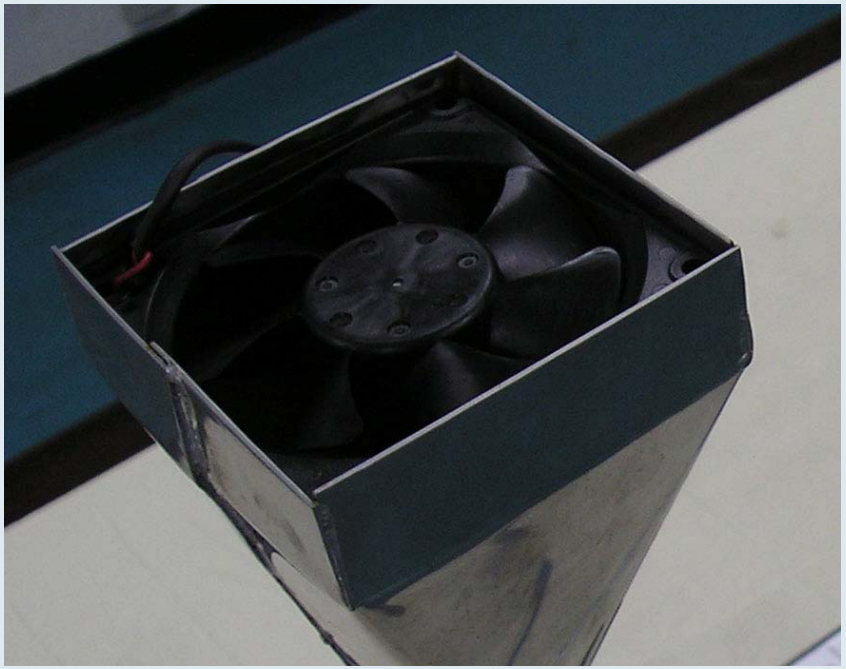


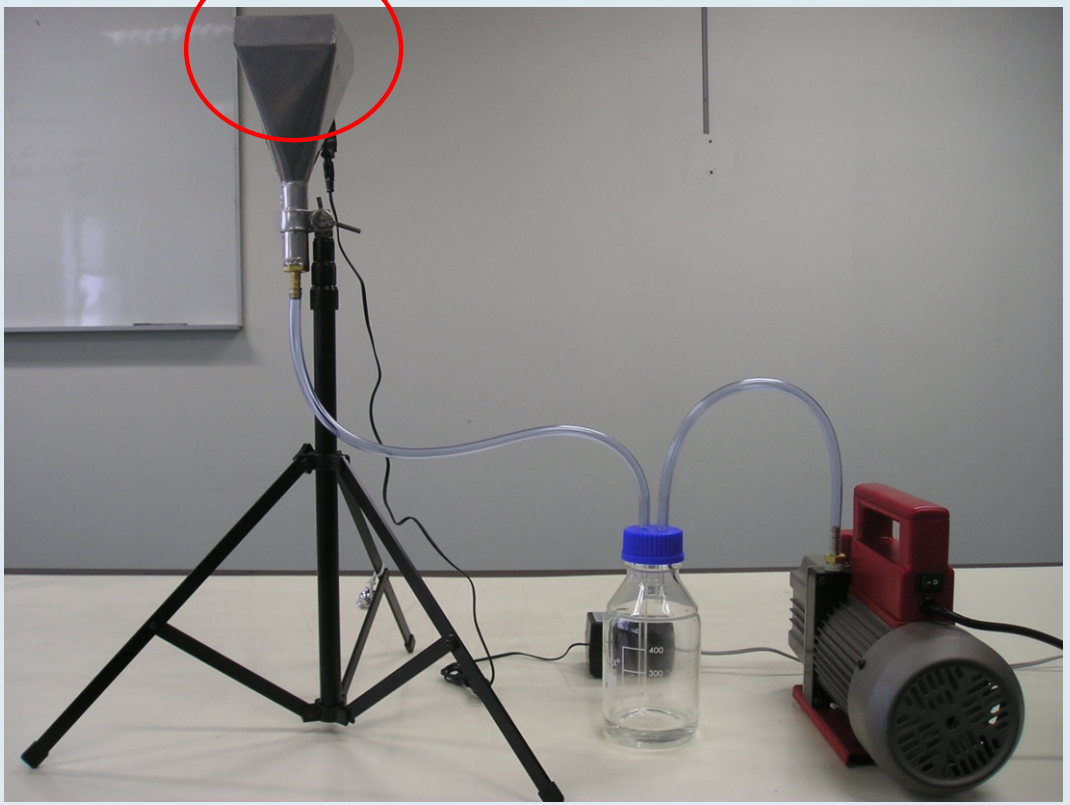
Materials & Methods

MAHIDOL UNIVERSITY
Wisdom of the Land

Air collection development

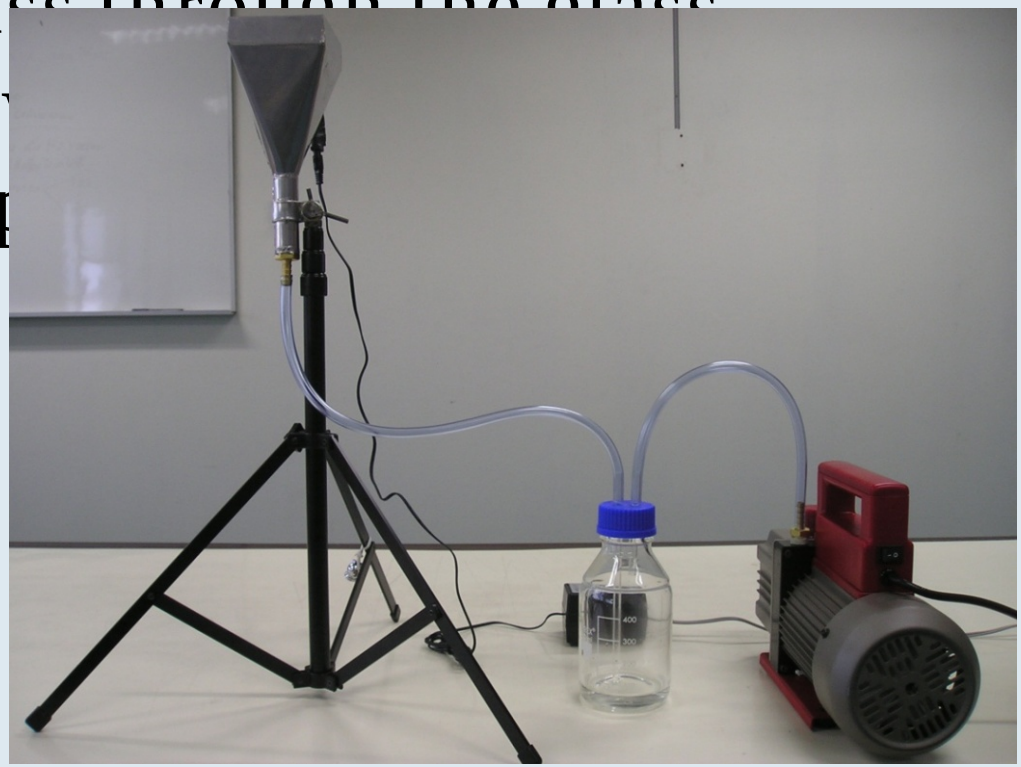
- The modified air impinger collection was developed using in-house equipment
- All equipments used in this invention was made in Thailand (10 time less cost than import product)





Air collection development

- The high flow air pump was used to pump the air at 40 L/min
- The air was collected pa
  inlet, and the organism
  solution inside glass imp
- This liquid solution will
  isolation



About Entrez
Entrez Nucleotide
Help | FAQ
Entrez Tools
Check sequence revision history
LinkOut
My NCBI (Cubby)
Related resources
BLAST
Reference sequence project
Search for Genes
Submit to GenBank
Search for full length

Found 619 nucleotide sequences. CoreNucleotide [565] EST [54]

Display Summary Show 20 Sort by Send to

All: 565 Bacteria: 508 RefSeq: 42 mRNA: 0

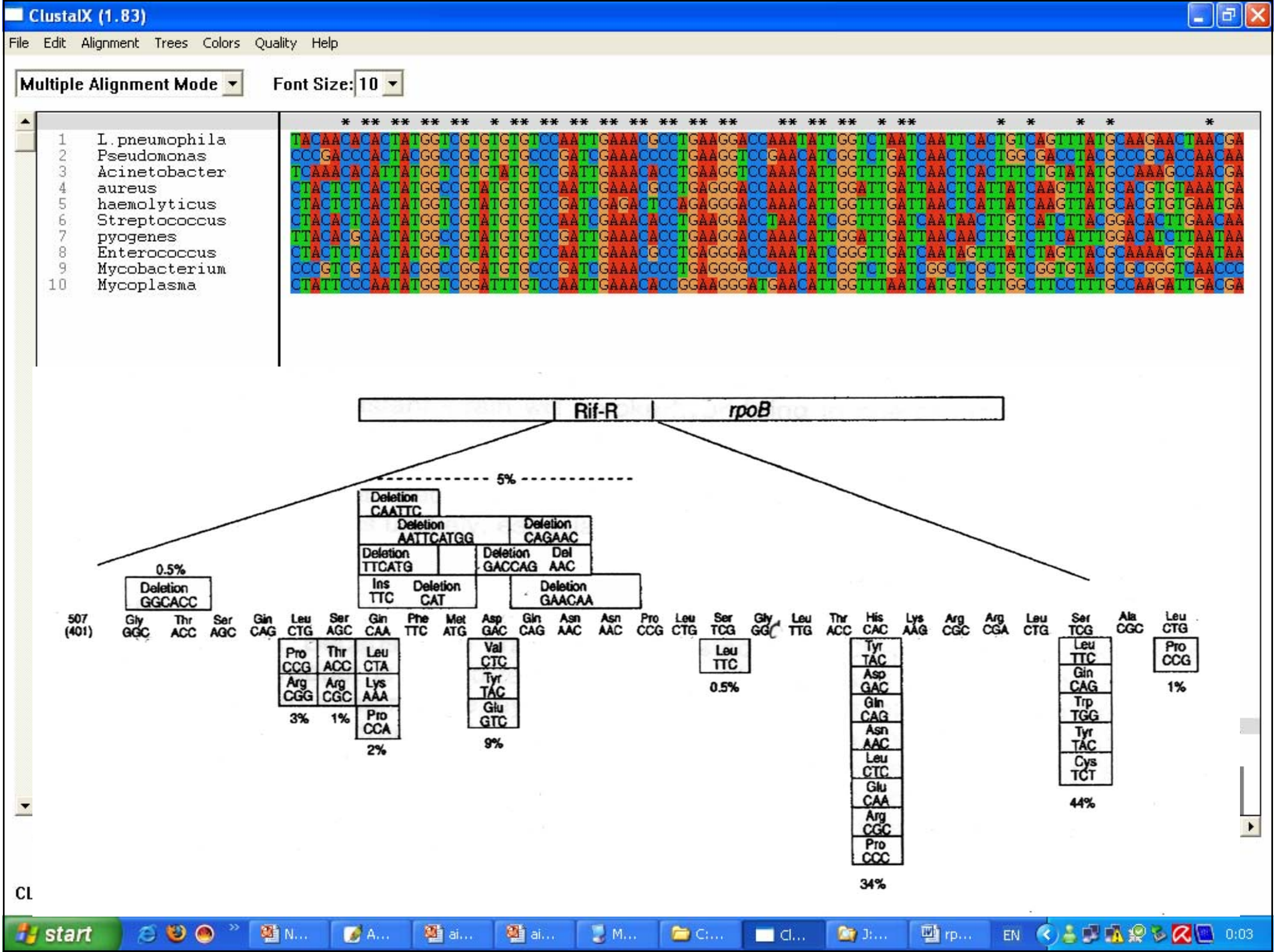
Items 1 - 20 of 565 Page 1 of 29 Next

1: NC_009565 Reports Links
Mycobacterium tuberculosis F11, complete genome
gi|148821191|ref|NC_009565.1|[148821191]

2: NC_009332 Reports Links
Streptococcus pyogenes str. Manfredo, complete genome
gi|139472888|ref|NC_009332.1|[139472888]

3: CP000143 Reports Links
Rhodobacter sphaeroides 2.4.1 chromosome 1, complete sequence
gi|77386383|gb|CP000143.1|[77386383]

4: NC_008702 Reports Links
Azoarcus sp. BH72, complete genome


ClustalX (1.83)
File Edit Alignment Trees Colors Quality Help
Multiple Alignment Mode
Font Size: 10
1 L.pneumophila
2 Pseudomonas
3 Acinetobacter
4 aureus
5 haemolyticus
6 Streptococcus
7 pyogenes
8 Enterococcus
9 Mycobacterium
10 Mycoplasma
Rif-R
rpoB
5%
Deletion CAATTC
Deletion AATTCATGG
Deletion CAGAAC
Deletion TTCATG
Deletion GACCAG
Del AAC
Ins TTC
Deletion CAT
Deletion GAACAA
0.5%
Deletion GGCACC
507 (401)
Gly GGC
Thr ACC
Ser AGC
Gln CAG
Leu CTG
Ser AGC
Gln CAA
Phe TTC
Met ATG
Asp GAC
Gln CAG
Asn AAC
Asn AAC
Pro CCG
Leu CTG
Ser TCG
Gly GGC
Leu TTG
Thr ACC
His CAC
Lys AAG
Arg CGC
Arg CGA
Leu CTG
Ser TCG
Ala CGC
Leu CTG
Pro CCG
Arg CGG
3%
Thr ACC
Arg CGC
1%
Leu CTA
Lys AAA
Pro CCA
2%
Val CTC
Tyr TAC
Glu GTC
9%
Leu TTC
0.5%
Tyr TAC
Asp GAC
Gln CAG
Asn AAC
Leu CTC
Glu CAA
Arg CGC
Pro CCC
34%
Leu TTC
Gln CAG
Trp TGG
Tyr TAC
Cys TCT
44%
Pro CCG
1%
start
0:03

PCR primer design

- By using Primer 3 software, 3 paired of primer were selected
- All primer were analyzed by Oligo-analyzer software
  - Self-priming
  - Loop forming
- The best primer were selected for further PCR optimization

The picture shows region of *rpoB* gene resequence used in this study with outer primer (rpoB-f and rpoB-r; 435 bps) shown in red and inner primer (rpoB-7 and TB8; 195 bps) shown in yellow.

PCR optimization

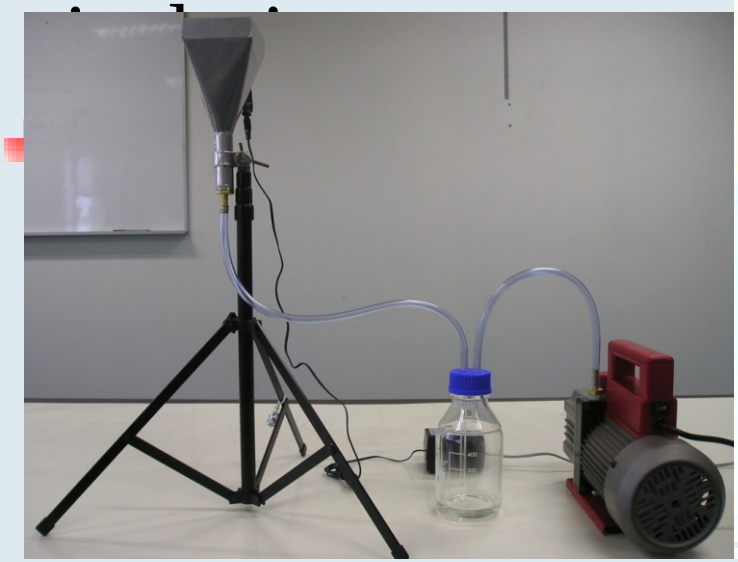
- All PCR reagents and condition were optimized
  - Annealing temperature
  - Mg^{2+}concentration
  - Primer concentration
  - dNTP concentration

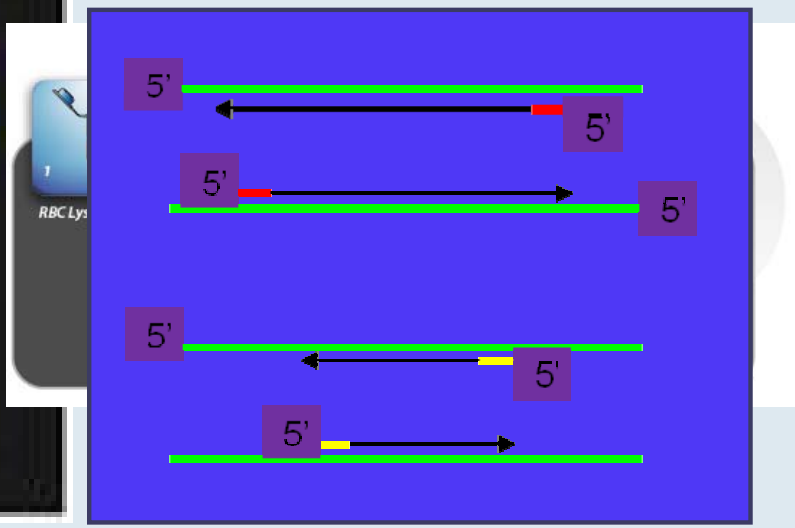
- Standard positive control were includes to determined the PCR detection limit (sensitivity)

- All other 8 airborne bacteria were tested to determined the PCR specificity (*Enterococcus spp., Psuedomonas aeruginosa, Streptococcus aureus, Enterobacter spp., Klebsiella pneumoniae, Streptococcus pyogenes, Acinetobacter spp., Legionella pneumophilla*)

Validation of Methods

- 30 samples of air collections were performed in laboratory ;spraying MTB (H37Ra) in laminar flow
- MTB containing solution was filtered through 0.45 µm polycarbonate filter
- The filter were then rinse with distilled water
- This suspension w ed for DNA





Results

PCR primer design & optimization

- Two set of primers were designed to use as outer and inner primer in nested PCR

Primer	Sequence
Outer primer	
rpoB-f	5'-TGG TCC GCT TGC ACG AGG GTC AGA-3'
rpoB-r	5'- CTC AGG GGT TTC GAT CGG GCA CAT-3'
Inner primer	
rpoB-7	5' - GAT CAC ACC GCA GAC GTT GA-3'
TB8	5' - TGC ACG TCG CGG ACC TCC A-3'

PCR condition

- The following PCR cycle were performed
  - First PCR

    95 °C, 5 mins

    95 °C, 1 min, 72 °C, min, 72 °C min, 2 cycles

    95 °C, 1 min, 71 °C, min, 72 °C min, 2 cycles

    95 °C, 1 min, 70 °C, min, 72 °C min, 2 cycles

    95 °C, 1 min, 69 °C, min, 72 °C min, 25 cycles

    72 °C, 7 mins
  - Second PCR

    95 °C, 5 mins

    95 °C, 1 min, 69 °C, min, 72 °C min, 2 cycles

    95 °C, 1 min, 68°C, min, 72 °C min, 2 cycles

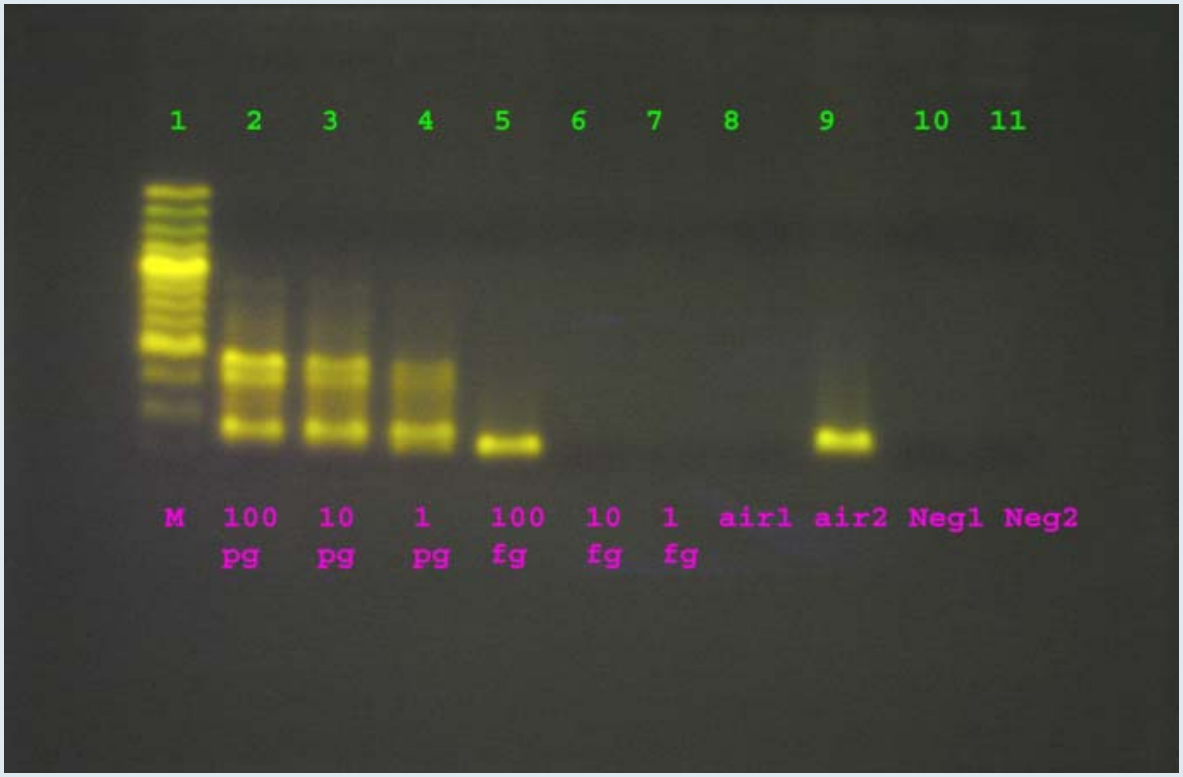
    95 °C, 1 min, 67 °C, min, 72 °C min, 2 cycles

    95 °C, 1 min, 66 °C, min, 72 °C min, 30 cycles

    72 °C, 7 mins

PCR optimization

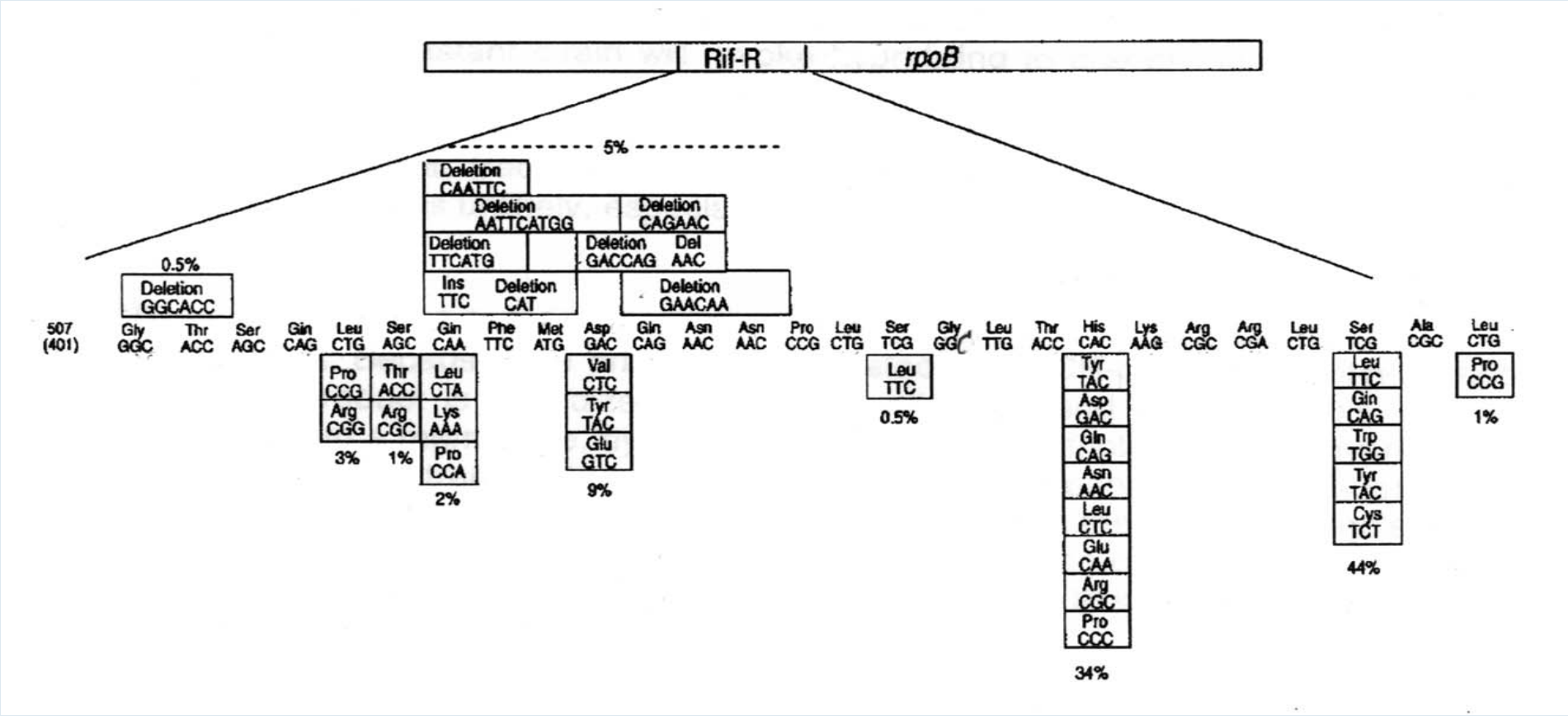
- By nested PCR, as low as 2-20 bacilli can be detected



- All other 8 airborne bacteria were not amplified

RIF^{r}-MTB detection

- After PCR, the purified PCR product can be sent for DNA-sequencing to identify mutation in hot spot region



Discussion & Conclusion

Discussion & Conclusion

- By combination of both air sampling technique using modified air impinger and in-house nested PCR, as low as 2-20 tubercle bacilli can be detected
- RIF^r-MTB also can be detected by direct DNA-sequencing of the purified PCR products
- From this development, the suspected of MTB and RIF^r-MTB contaminated in indoor air could be detected

Output

Output & Application

- Air collection & airborne bacteria and MTB detection
  - Head office, Kasikorn Bank
  - Pranungklao Hospital, Nonthaburi
  - Thonburi Hospital
  - Tesco Lotus Super Center, Ramindhra
- Detection of MTB from Patient samples
  - Tropical Medicine Hospital, Faculty of Tropical Medicine, Mahidol University

MAHIDOL UNIVERSITY
Wisdom of the Land

Output & Application

- nd

Acknowledgement

- Thailand Tropical Disease Research Programme (T2),
- National Research Council of Thailand
- Mahidol University


Thank You