Molecular diagnosis of TB and drug resistant TB: In-house development

Angkana Chaiprasert, Dr.rer.nat.
Therdsak Prammananan, Dr.rer.human.biol.
Manoon Leechawengwong, M.D.
Department of Microbiology
Faculty of Medicine Siriraj Hospital
Mahidol University

Tropical Medicine in the –omics Era: TB **October 13-14, 2008**

Objectives

- To share our past experience in

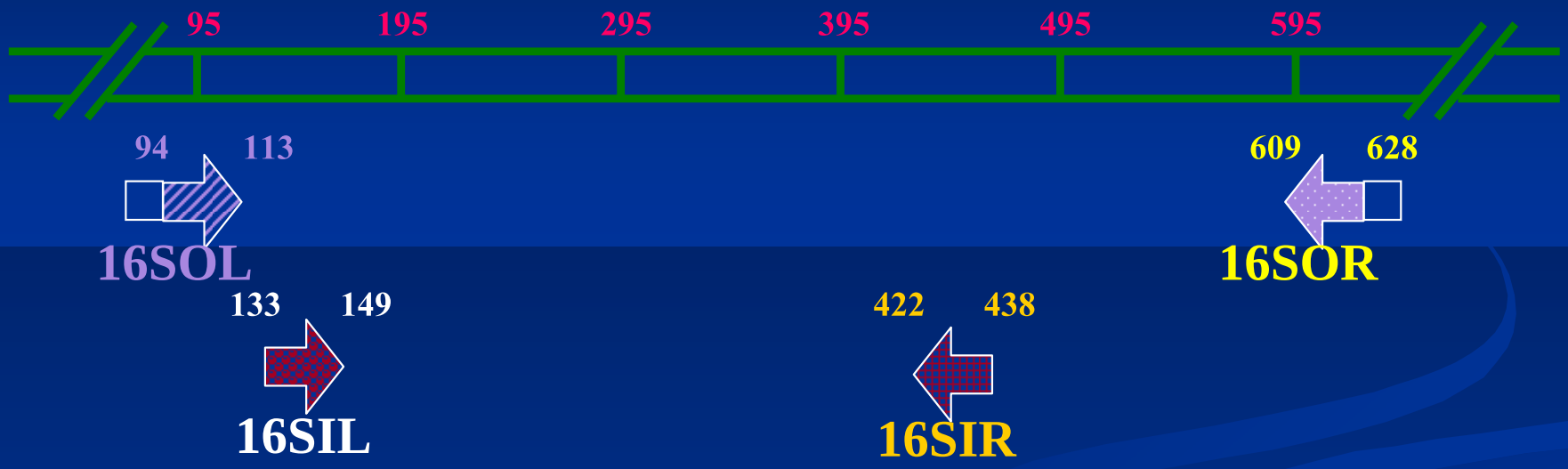
  Developing **one-tube nested PCR** for direct detection of *M. tuberculosis* complex in clinical samples

  Developing **multiplex PCR** for identification of *M. tuberculosis* and

  Developing **multiplex allele-specific (MAS) PCR** for detection of rifampicin resistant *M. tuberculosis*

One-tube nested PCR kit

M. tuberculosis 16S rDNA



PCR products

16SOL/OR

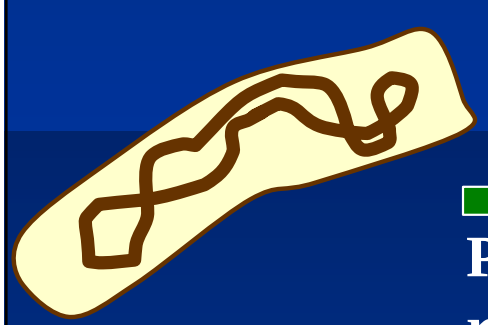
555 bp

16SIL/IR

306 bp

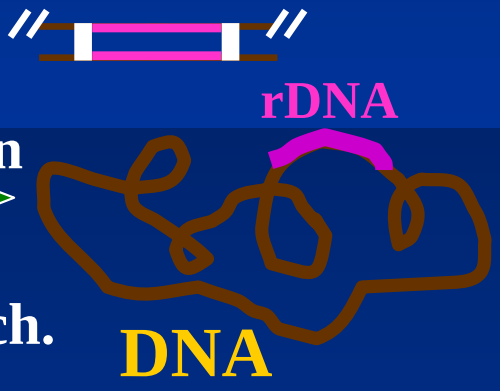
In House PCR for Rapid Detection of *Mycobacterium tuberculosis* complex

PRINCIPLE :

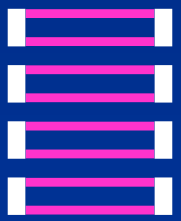


DNA extraction

Physical rupture tech.



1. **dNTP**
2. **PCR buffer**
3. ***Taq* Pol.**
4. **Primers**



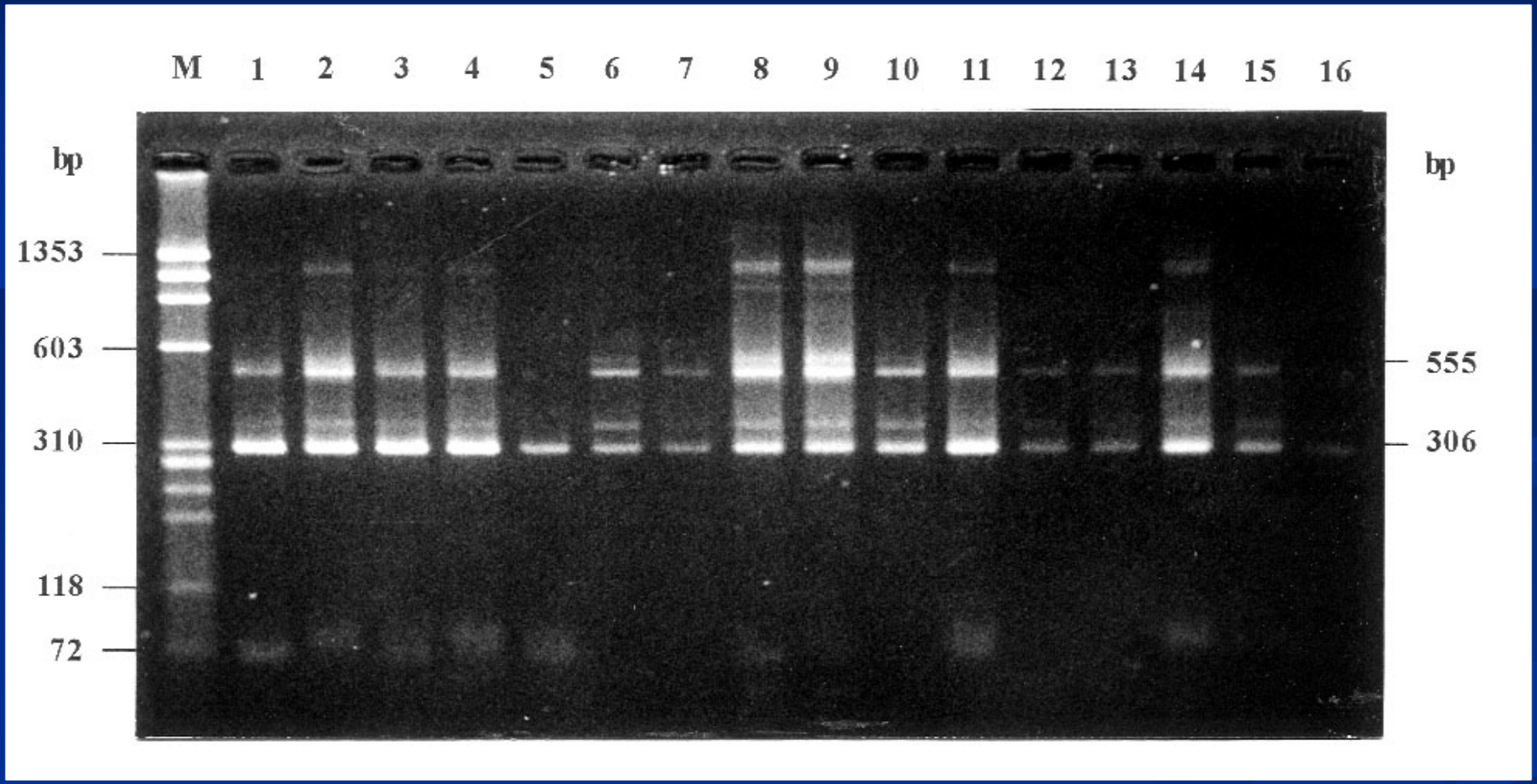
1 cycle { **Denaturation step**, **Annealing step**, **Extension step**

DNA Thermal-cycler

2^n

(n = number of cycle)

M. bovis *M. tuberculosis*



5 *M. bovis* and 11 *M. tuberculosis* isolates

DETECTION LIMIT

16S rRNA gene 100 fg
(1 cell *M.tb* → DNA ~5 femtogram)

In House PCR 16S rRNA Turnaround time 1 wk

SAMPLE	SENSITIVITY	SPECIFICITY
Sputum	91.9 %	95.0 %
Bronchoalveolar lavage	70.0 %	84.0 %
Pleural fluid	<10.0 %	>95.0 %
Cerebrospinal fluid	~20.0 %	>85.0 %

Reference :

1. **Chaiprasert A**, Prammananan T, Samerpitak K. et al. Direct detection of *M. tuberculosis* in sputum by reamplification with 16S rRNA-based primer. As Pac J Mol Biol Biotechnol 1996; 4:250-9.
2. Charoenratanakul S, **Chaiprasert A**, Yenchitsomanas P, Pattanakitsakul S, Jearanaisilavong J, Dejsomritrutai W. Clinical utility of the polymerase chain reaction in bronchoalveolar lavage in the diagnosis of smear negative pulmonary tuberculosis. Thai J Tuberc Chest Dis 1996; 17:1-9.
3. Gengvinij N, Pattanakitsakul S, Chierakul N, **Chaiprasert A**. Detection of *Mycobacterium tuberculosis* from sputum specimens using one-tube nested PCR. Southeast Asian J Trop Med Pub Health 2001; 32: 114-25.
4. Chierakul N , Anantasetagoon T , **Chaiprasert A** , Tingtoy N . Diagnostic value of gastric aspirate smear and polymerase chain reaction in smear-negative pulmonary tuberculosis. Respirology 2003;8:492-6.
5. **Srisaimanee N, Chaiprasert A, Gengvinij A, Kunakorn M, Prayoonwiwat N. Evaluation of in-house optimized semi-nested PCR and EIA for direct detection of mycobacterial DNA in CSF. Asian Pac J Aller Immunol 2002;20(4):267-77.**

Training Workshop sponsored by Biotec, Thailand

The test is used at Fact. of Medicine Siriraj and ISO 15189 accredited by Bureau of Laboratory Quality Standards MOPH

- **Retrospective analysis from 27.12.2006-12.9.2008 4,972 samples tested**
- **Smear + Culture + PCR + 266 sample**
- **PCR - 18 sample**
- **Smear - Culture + PCR + 290 sample**
- **PCR - 279 sample**
- **Smear - Culture - PCR - 4,101 sample**
- **PCR + 18 sample**

- **sensitivity for sm+ 92.6% sm- 51%**
- **specificity for all 99.6%**

Multiplex PCR kit

Molecular Identification of Mycobacteria by "home brew" kit or tests

Identification of *M. tuberculosis* and TB complex by multiplex PCR based on KS_4 and *mtp*40

Positive culture

AFB +ve

Boil in TE

20 min.

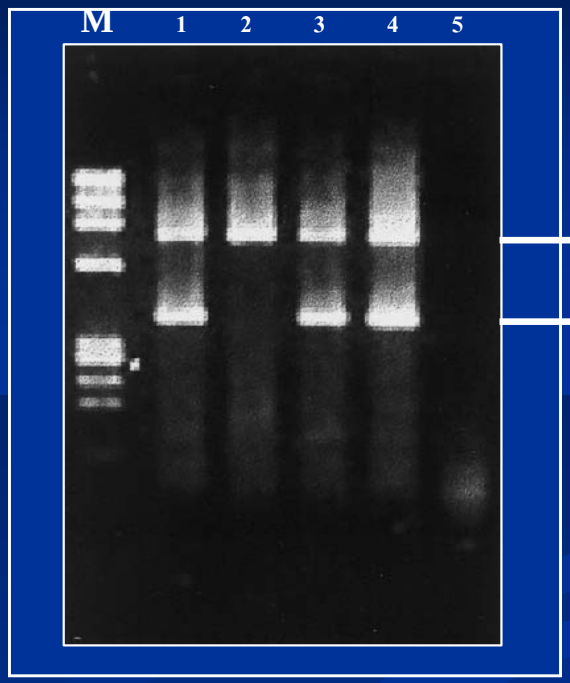
Crude DNA

multiplex PCR

Gel electrophoresis

Using In-house PCR with specific primer

- **Specific primers from: KS_4, and *mtp*$_{40}$**
- **Able to identify:**
  - ***M. tuberculosis*** **(1,3,4)**
  - ***M. tuberculosis*** **complex (2)**
  - **NTM (5)**
- **Detect DNA band by electrophoresis**

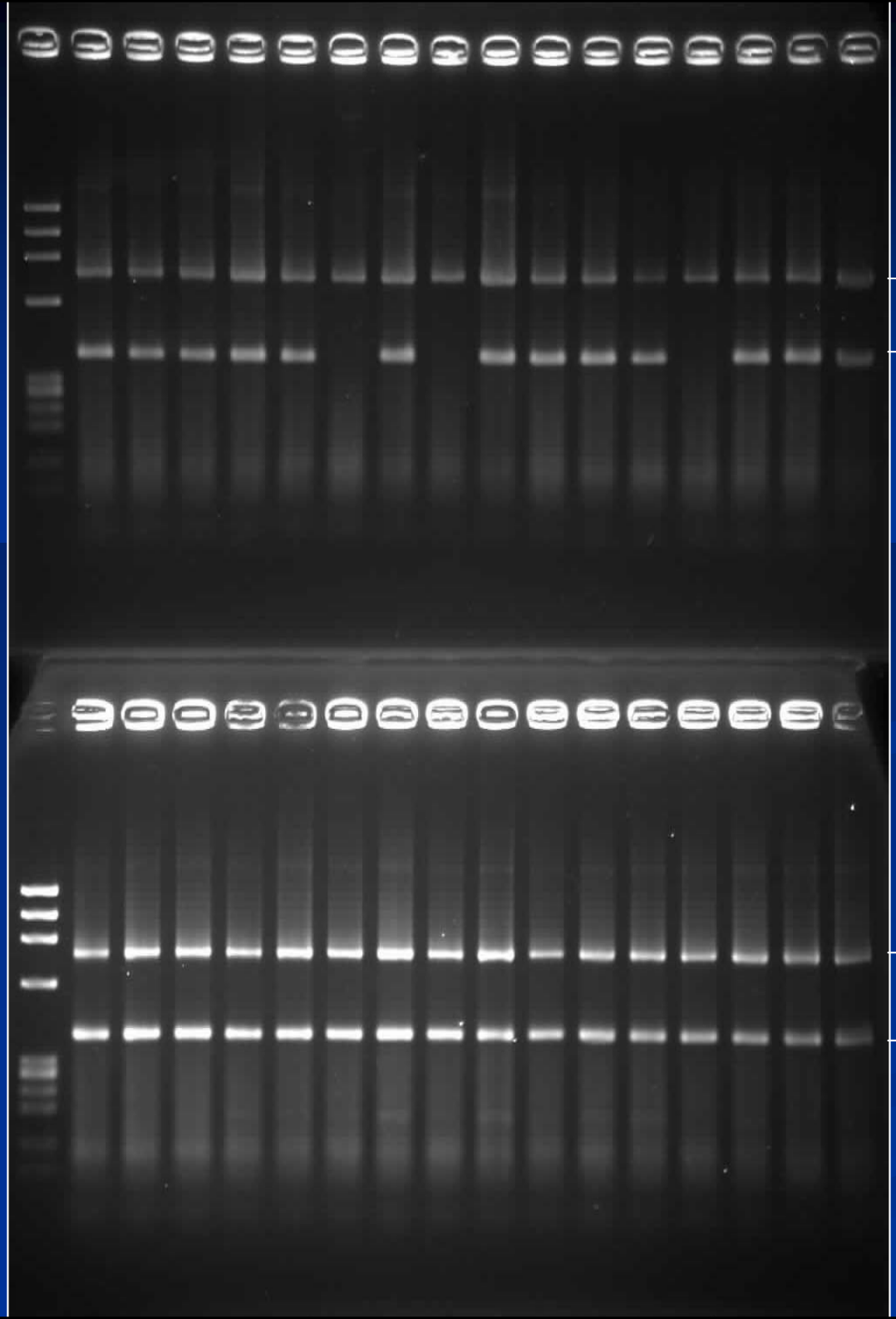


Q.C.with known species (+/- control) **TAT: 1-2 d**

Sensitivity 98%, Specificity 99%

Reference :

Chaiprasert A, Prammananan T, Tingtoy N, Na-Ubol P, Srimuang S, Samerpitak K, Rangsipanuratn W. One-tube multiplex PCR provides an alternative, inexpensive method for rapid identification of *Mycobacterium tuberculosis*. Southeast Asian J Trop Med Public Health 2006; 37: 494-501.


768 bp KS4
396 bp mtp40
768 bp KS4
396 bp mtp40

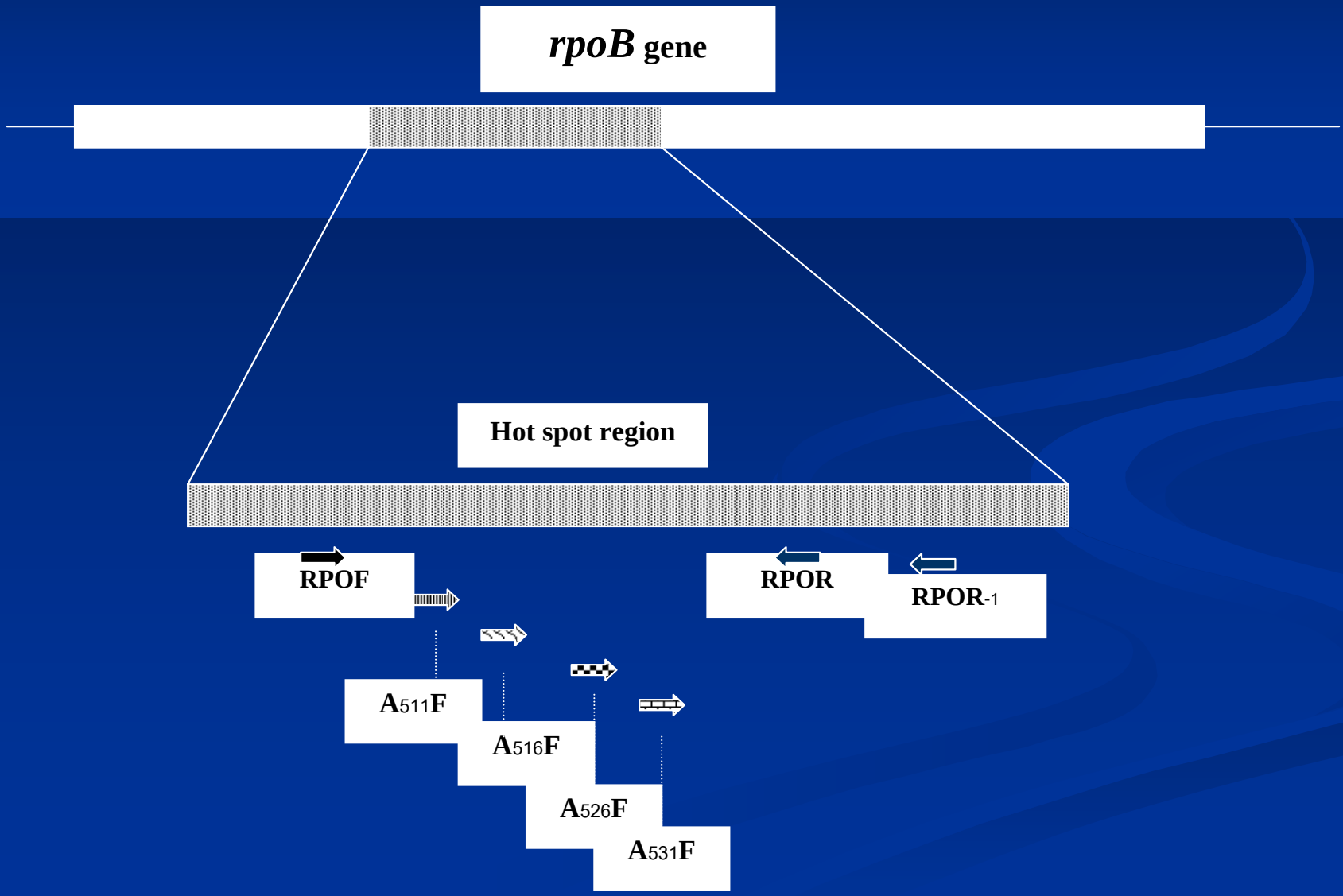
The test is used at Fact. of Medicine Siriraj and ISO 15189 accredited by Bureau of Laboratory Quality Standards MOPH

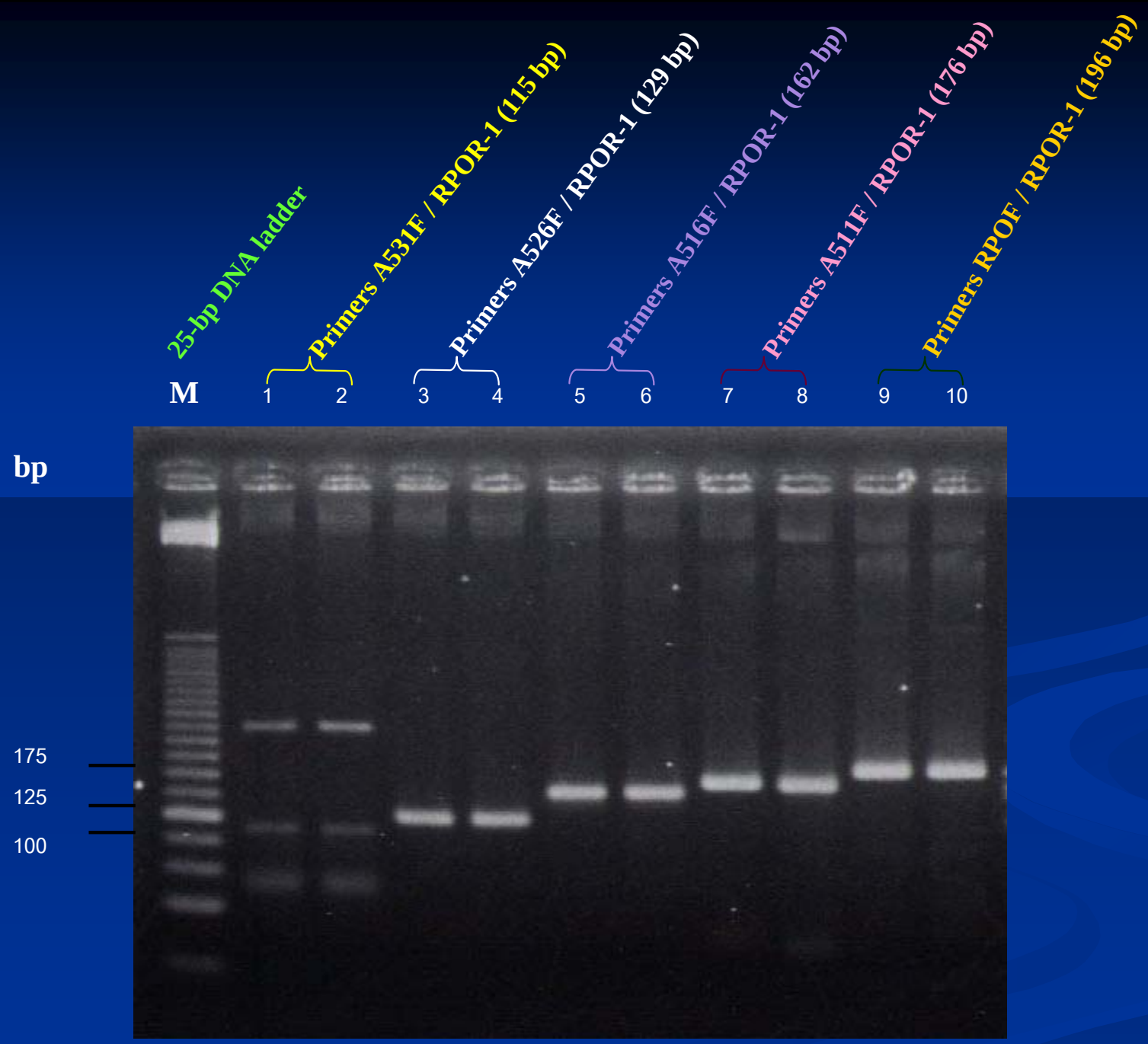
- **Retrospective analysis from 01.12.2005-01.05.2007: 3,654 LJ/M7H10 cultures tested:**
- *M. tuberculosis* 2997 isolates
- Tuberculosis complex 381 isolates
- Nontuberculous mycobacteria 276 isolates

- Turn around time (TAT) 1 working day
- Price per test (reagent cost) 1 US$

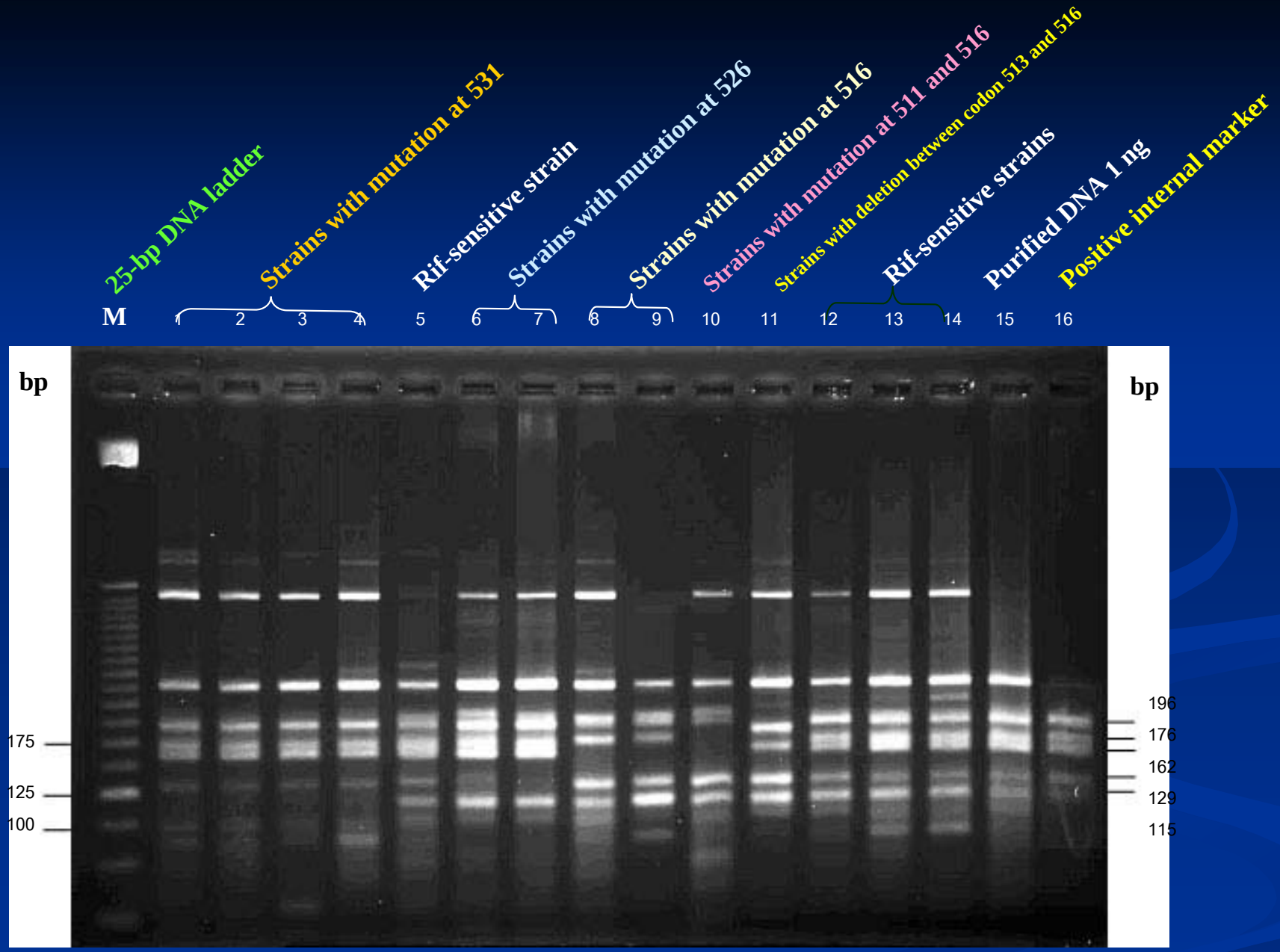
Press release in 2003

Development of a multiplex nested PCR for detection of rifampicin resistant *M. tuberculosis*





Amplified products of the simplex PCR with the primer, RPOR-1



The MAS-PCR of the strains with the known mutations

Sensitivity 94.2%, Specificity 100%, PPV 100%, NPV 95%

Acknowledgements :

National Center for Genetic Engineering and Biotechnology, Thailand

Faculty of Medicine Siriraj Hospital, Mahidol University

Drug - Resistant Tuberculosis Fund under the Patronage to the late Her Royal Highness Princess Galyani Vadhana Krom Luang Naradhiwas Rajanagarindra

Thank you for your kind attention

This is our team