SATREPS

The Project for Control of tuberculosis and glanders:

Study on Zoonotic tuberculosis

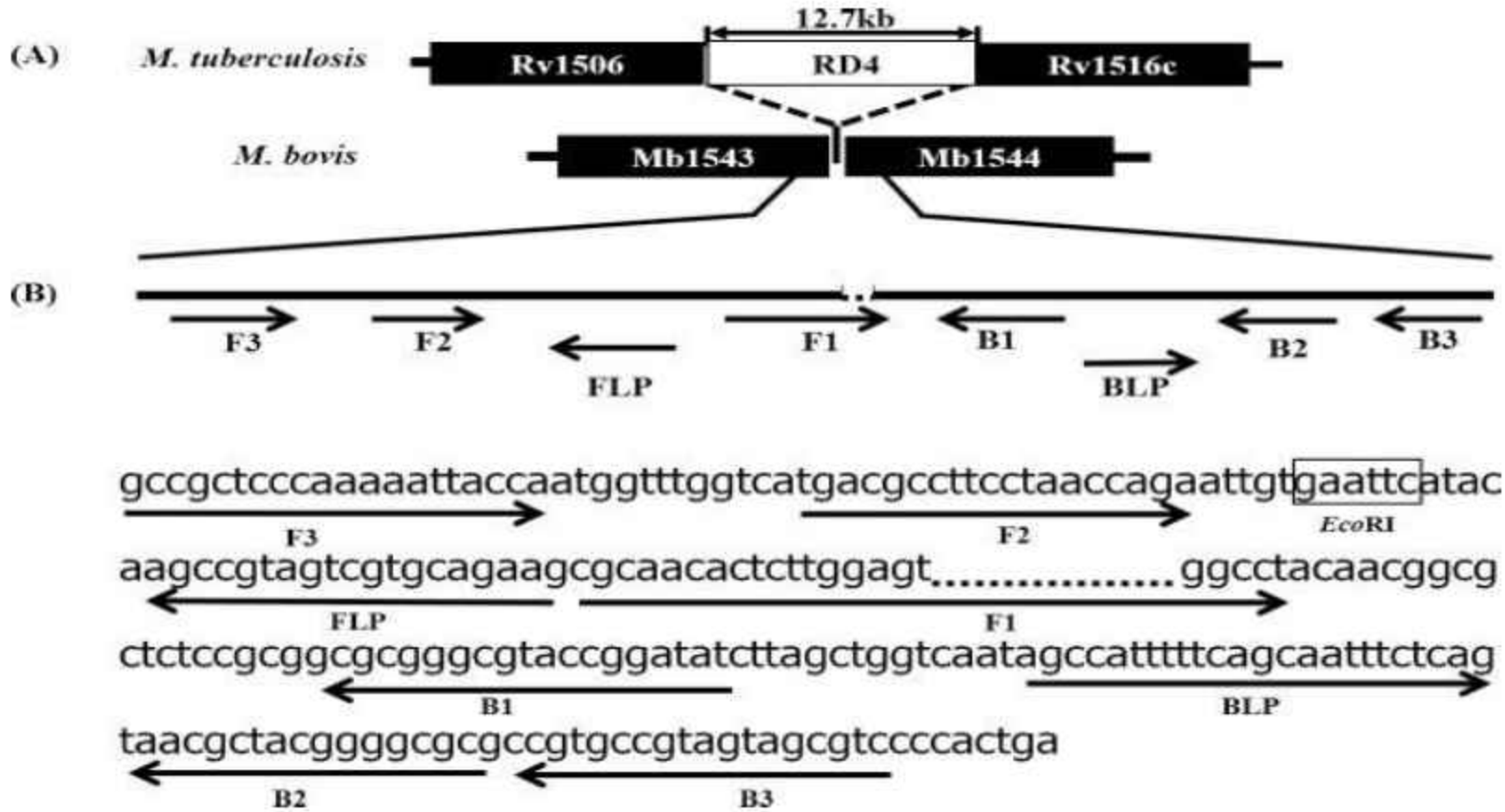
Suzuki Y, Thapa J, Nakajima C

Hokkaido University

International Institute for Zoonosis Control

Development of LAMP method method for the detection of bovine tuberculosis

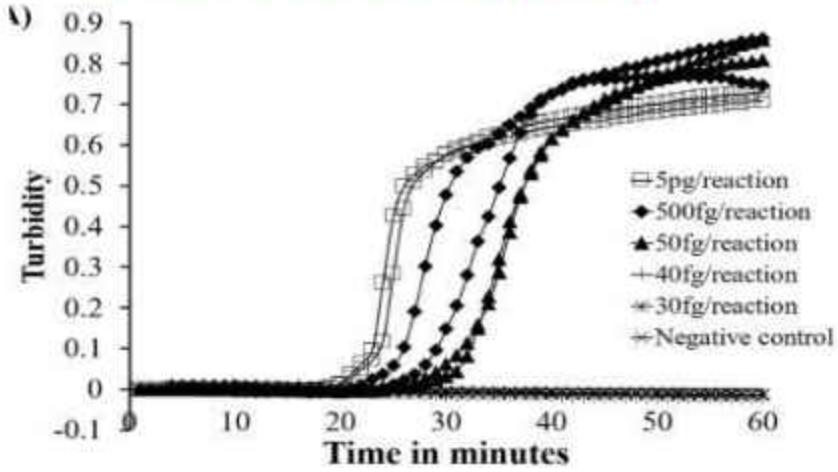
Primers used for LAMP



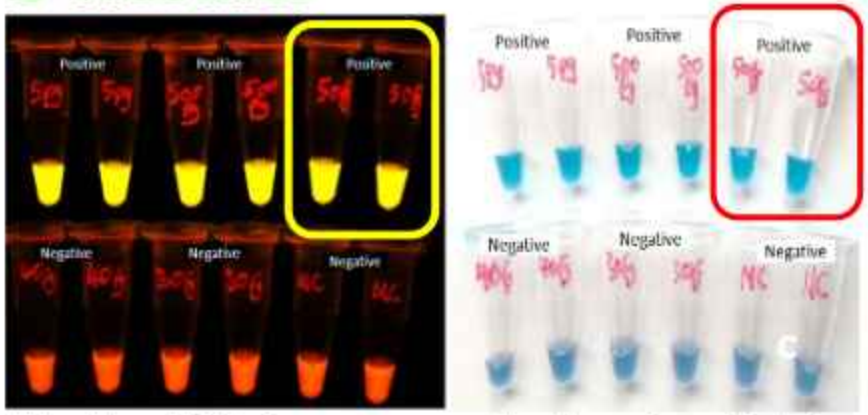
→ Primer sets were designed targeting Region of Difference 4 which is specifically deleted in *M. bovis*

Development of LAMP method method for the detection of bovine tuberculosis

A. Trend of turbidity



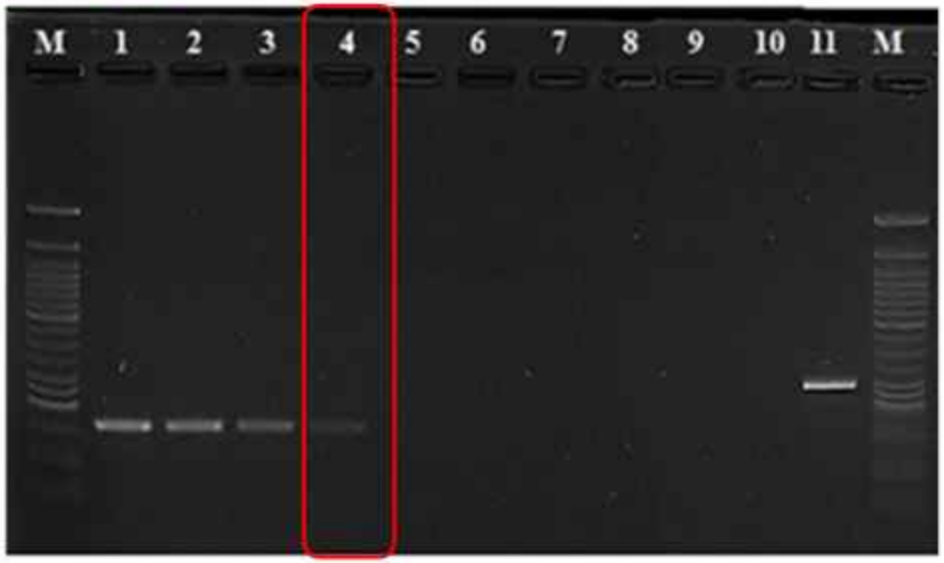
B Results



Under LED lamp

Under day light

C. PCR



Lane M, 50bp DNA marker; lanes 1 – 9, *M. bovis* BCG Tokyo 172 genomic DNA 500pg, 50pg, 20pg, 5pg, 500fg, 50fg, 40fg, 30fg, 20fg/reaction; lane 10, Negative Control; lane 11, *M. tuberculosis* H37Rv. (Bakshi et al., 2005)

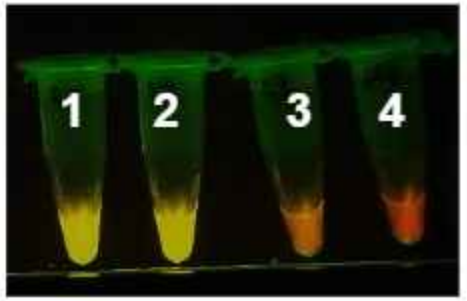
→ This method can detect up to 50 fg of DNA (equivalent to 10 *M. bovis*), which is about 100 times more sensitive than the conventional method (PCR).

Development of LAMP method method for the detection of bovine tuberculosis

Hand made

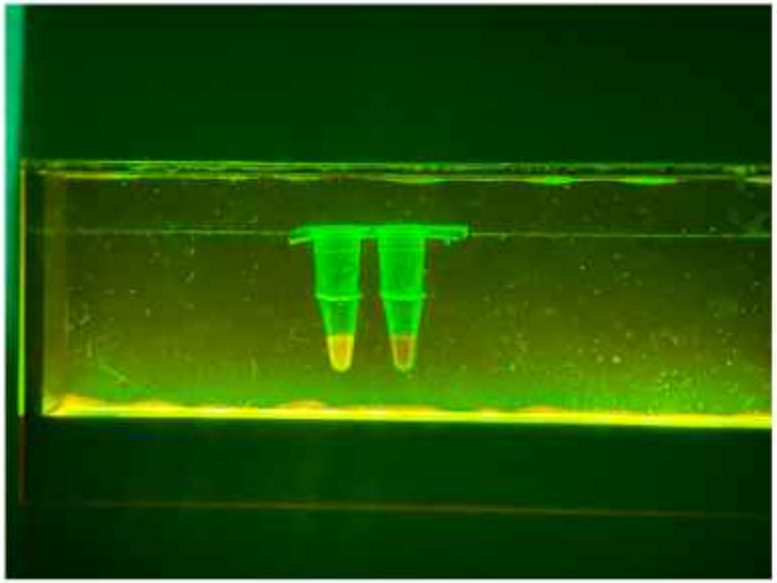
Automated

Technique for automated production of dried LAMP kit for *M. bovis* detection was transferred to two trainee from IVM at IIZC (Dec, 2023).



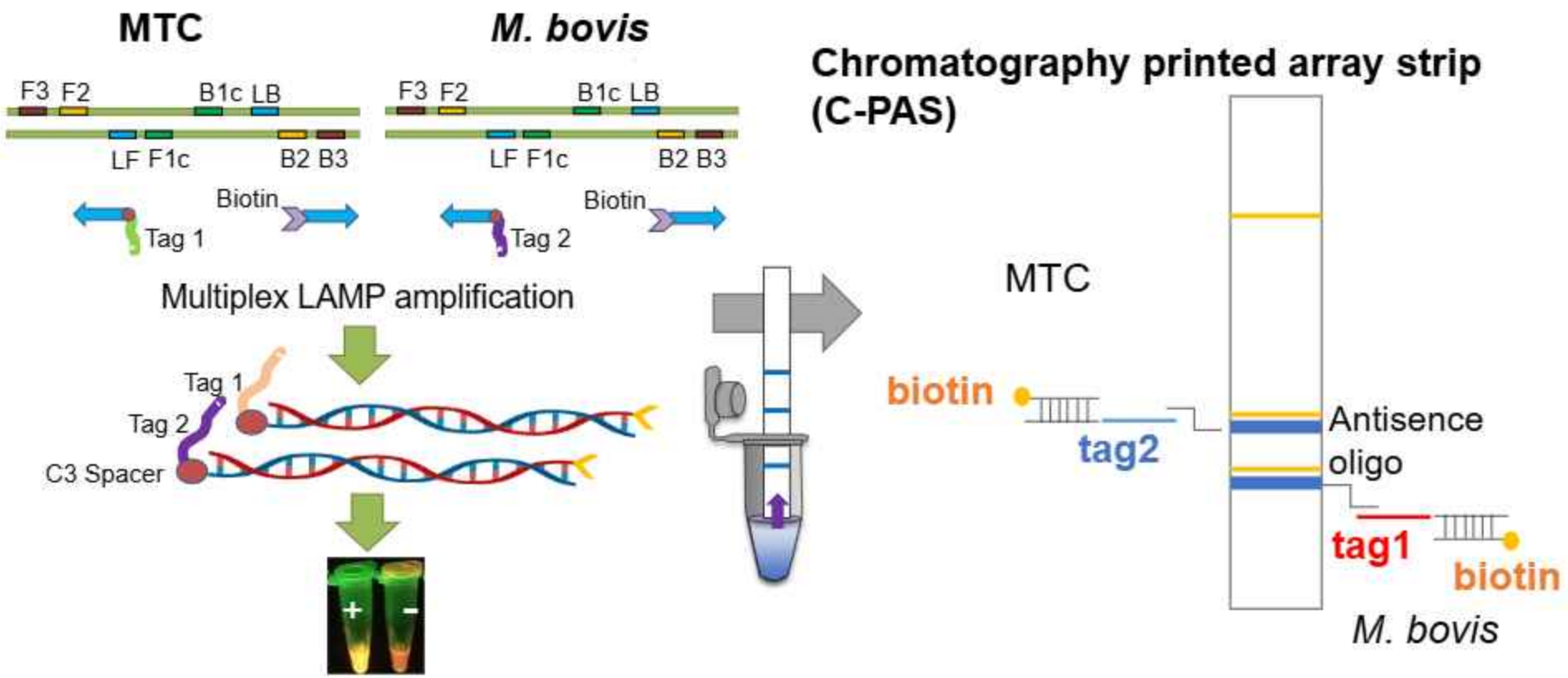
1, 2: Pos、3, 4: Neg

MTBC LAMP training



Training for the detection of *M. bovis* by dry LAMP method was held at IVM (August 8, 2023)

Development of LAMP method method for the detection of bovine tuberculosis

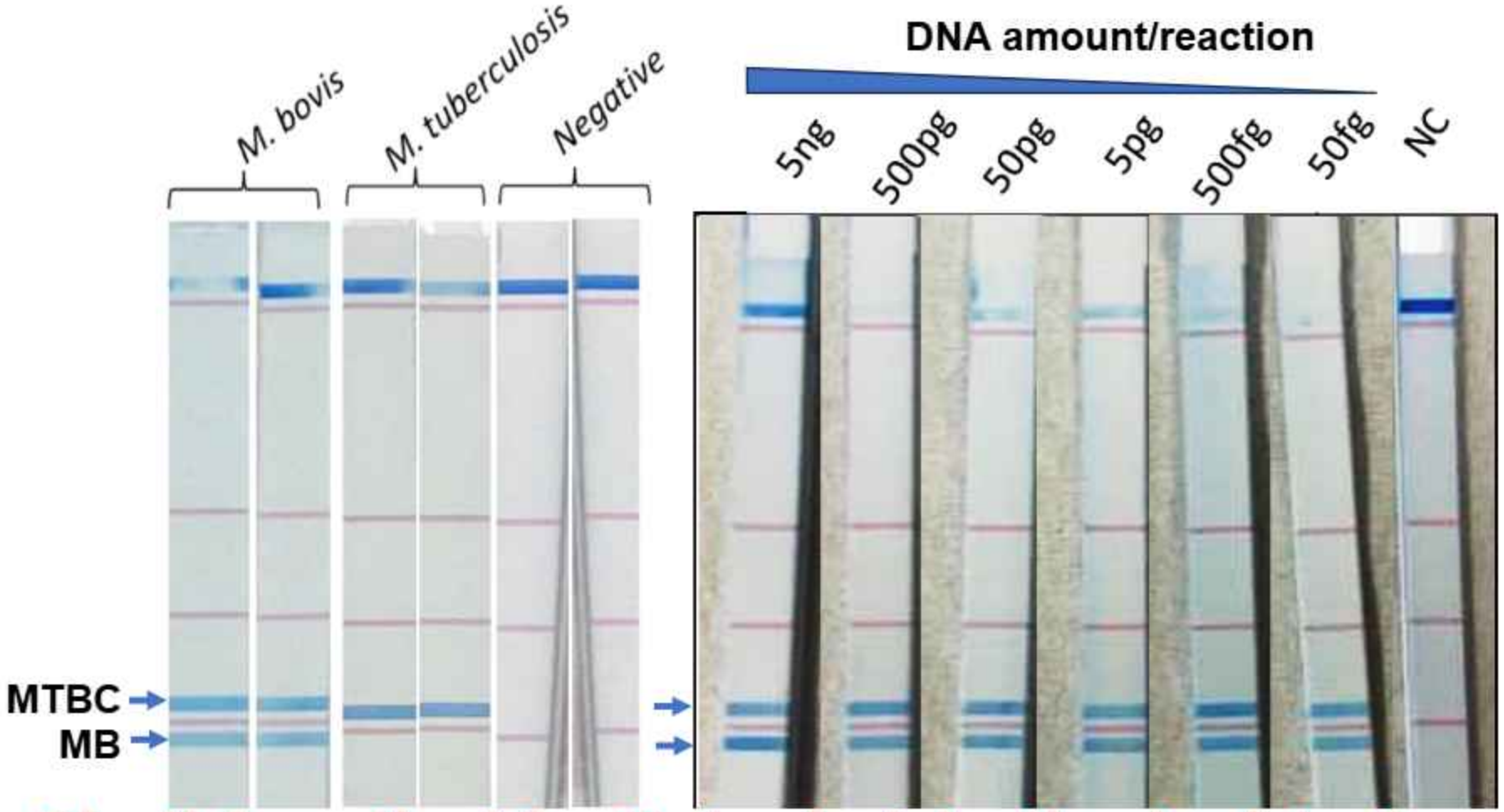


- **Multiplex amplicons indistinguishable by fluorescence**

- **Simultaneous identification of multiplexed amplicons**
- Simple with limited materials

→ **Method for the differentiation of *M. bovis* from other MTC by LAMP-DNA chromatography was designed.**

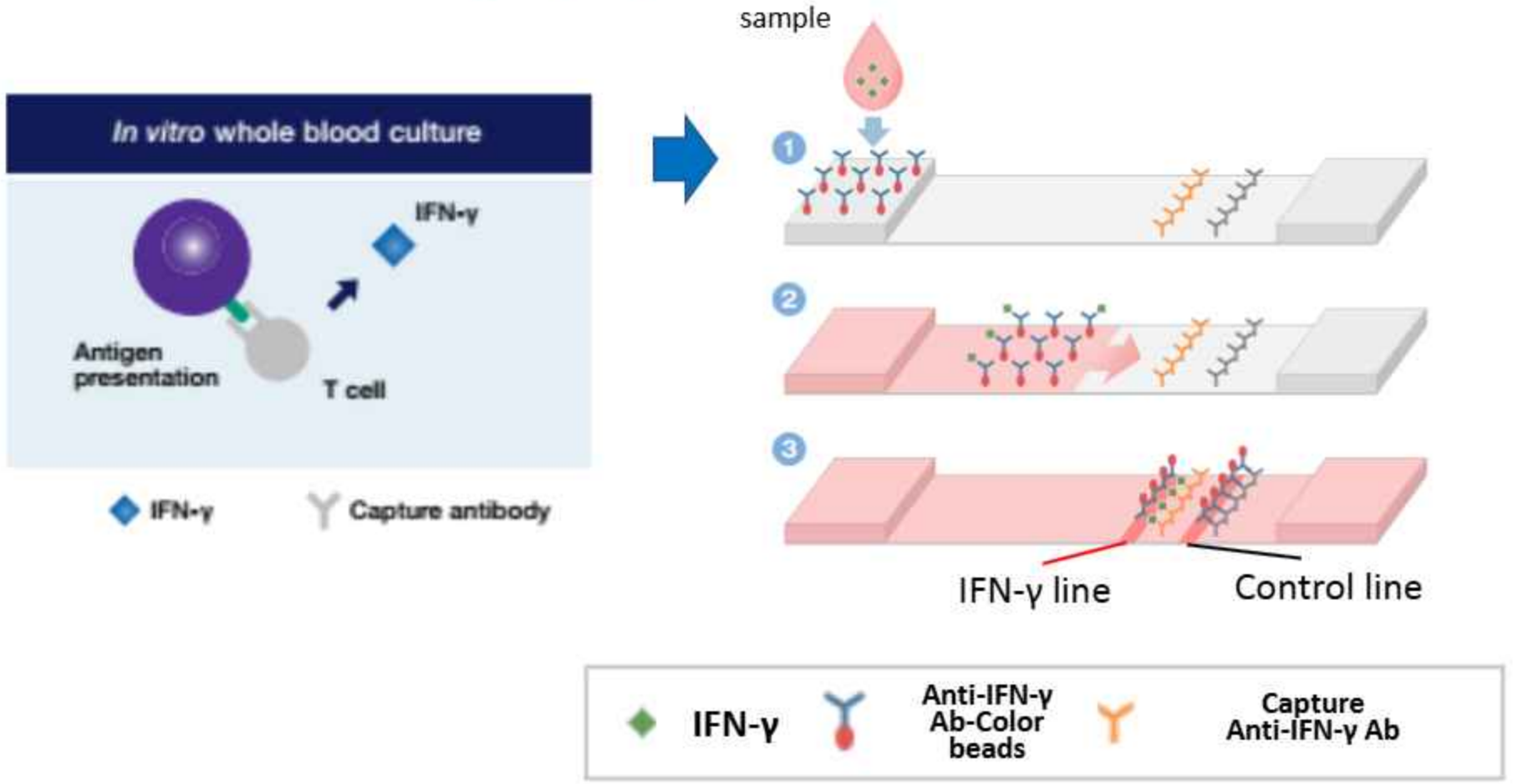
Development of LAMP method method for the detection of bovine tuberculosis



Dipstick method for *M. bovis* detection to detect 10 bacilli/reaction was successfully established and packed into contamination safe cassette.

Development of immunological methods for bovine tuberculosis diagnosis

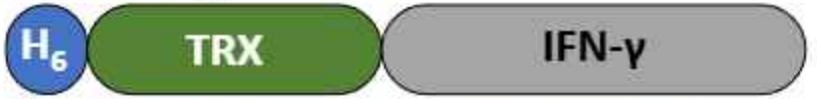
→ **Final goal is Development of semi-quantitative IFN-γ immunochromatography**



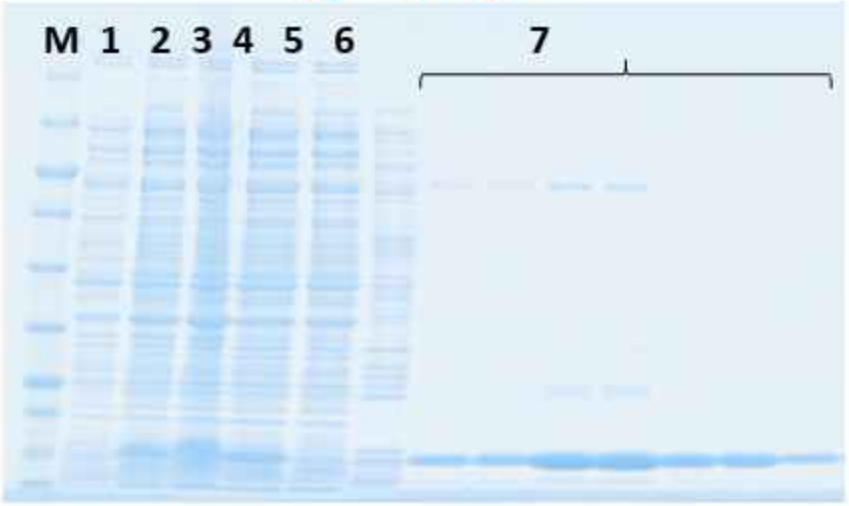
Development of immunological methods for bovine tuberculosis diagnosis

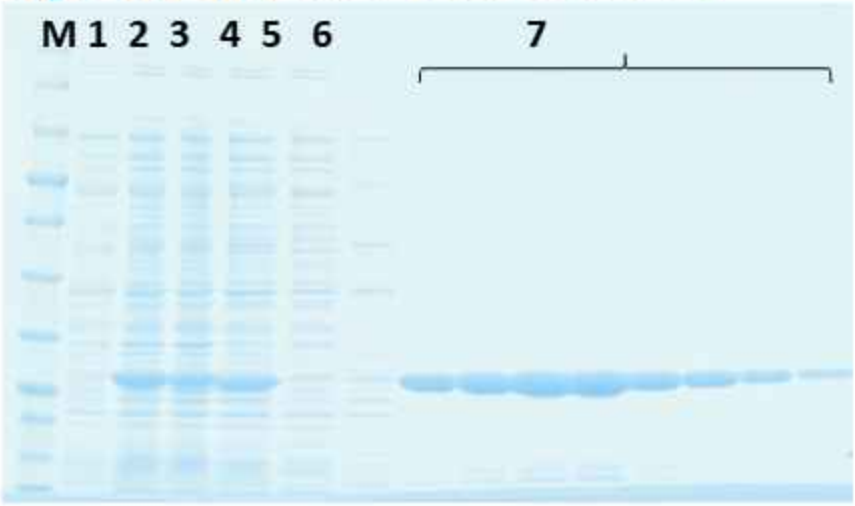
Expression and purification of recombinant IFN-γ using *E. coli* expression system

H_6 : His-tag



H_6 : His-tag, TRX : thioredoxin-tag

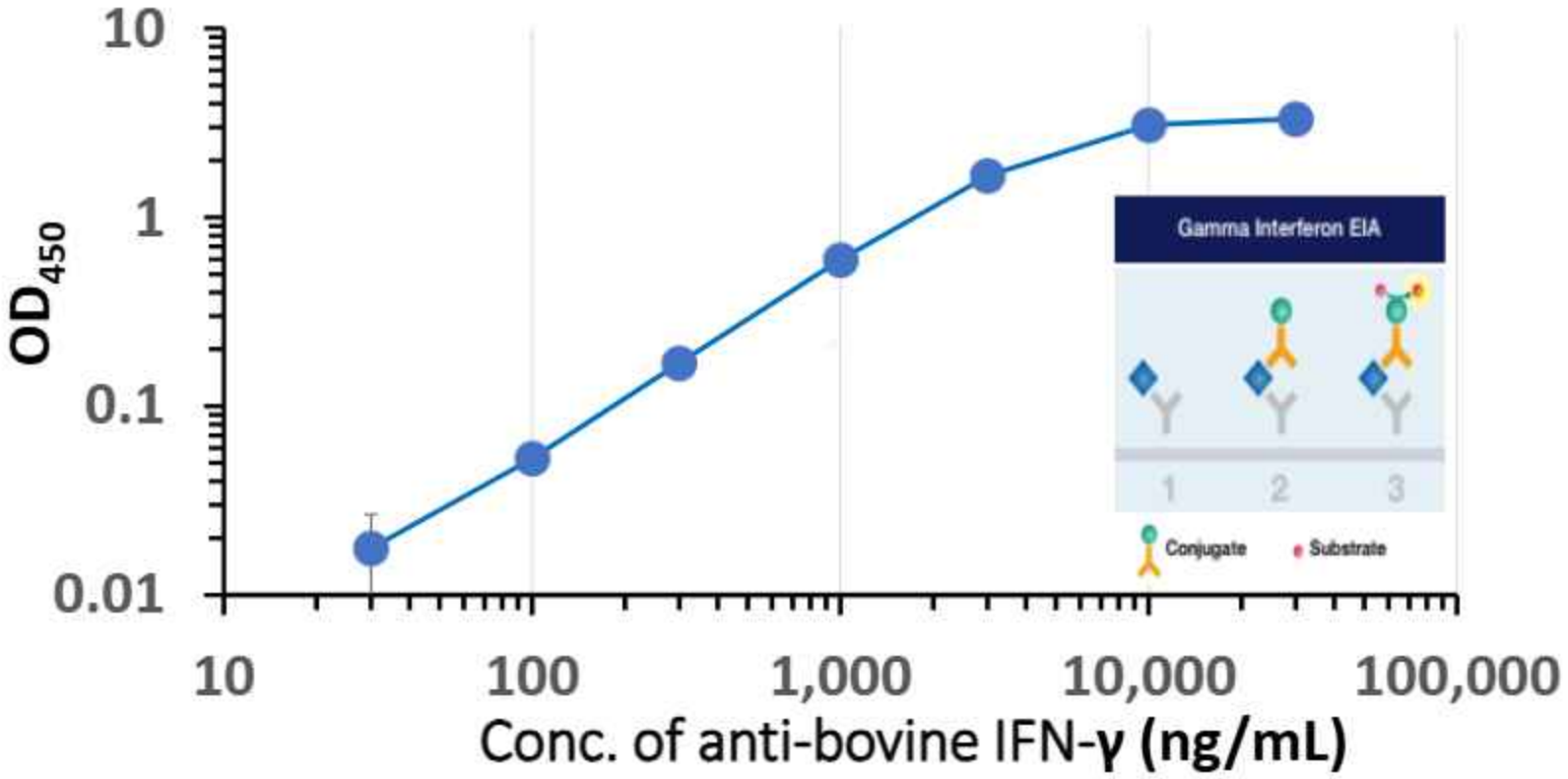




M:MW marker , 1 : IPTG(-) , 2 : IPTG(+) , 3: sup after sonication and centrifugation, 5 : pellet after sonication and centrifugation, 6 : Ni-column pass, 7 : imidasol eluates

→ We succeeded mass production and purification of two types of recombinant cattle, sheep, goat and camel IFN-γ with different tags. Preparation of antiserum is in progress.

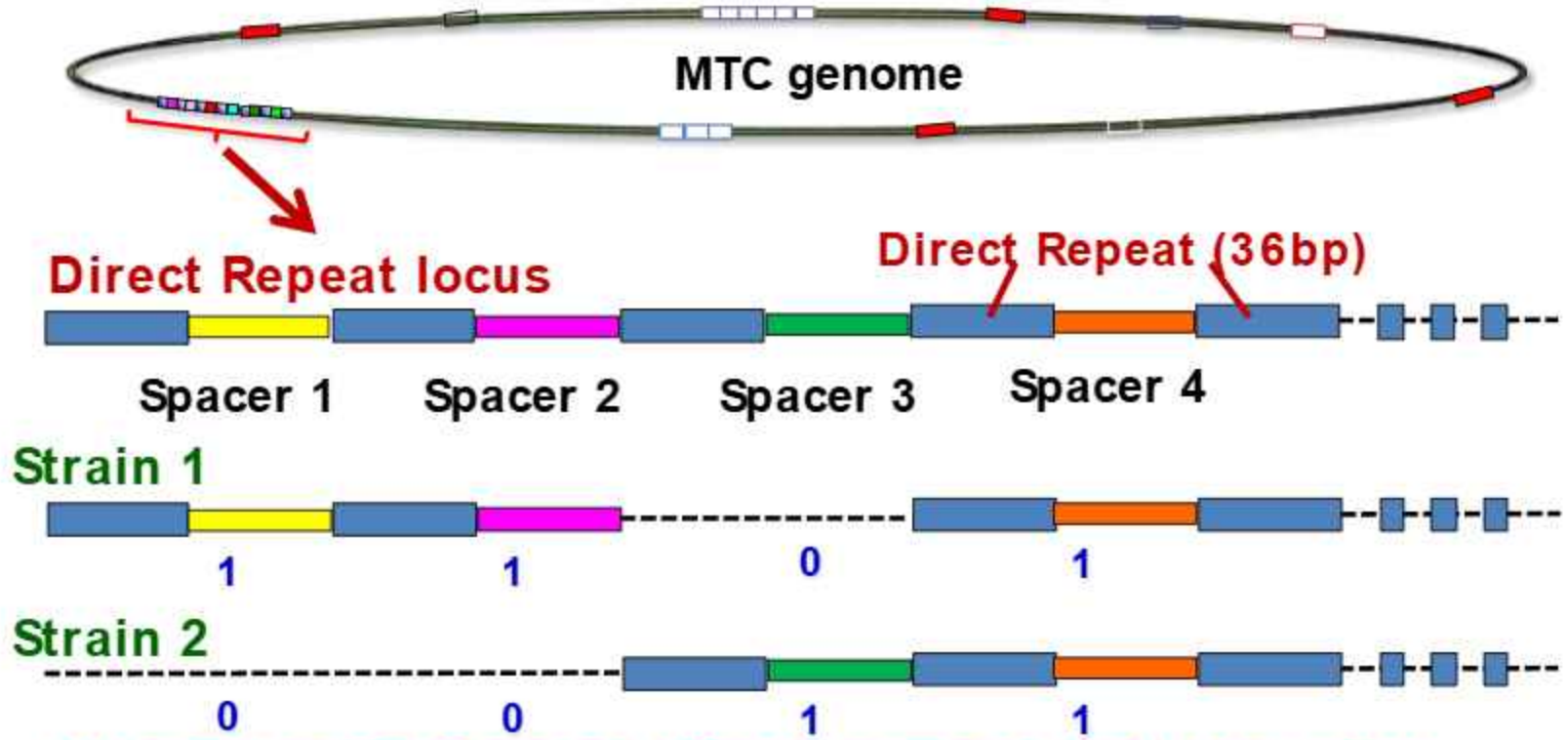
Development of immunological methods for bovine tuberculosis diagnosis



→ **The IFN-γ assay sensitive as low as 100 ng/mL by utilizing anti-cattle IFN-γ antibody affinity purified from sera of rabbit immunized by recombinant His-tagged anti-bovine-IFN-γ is ready for use.**

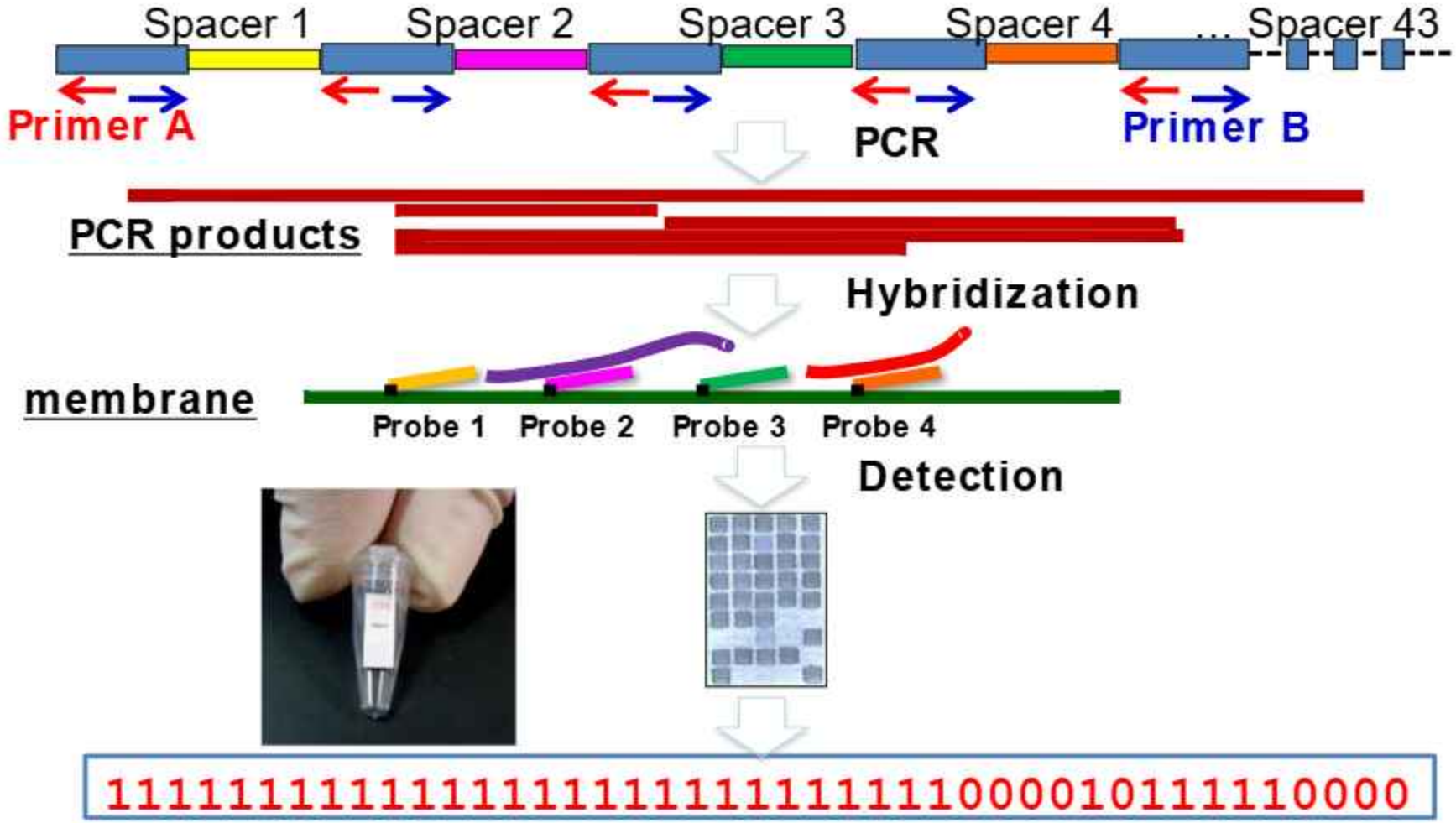
Introduction of simple and low-cost genotyping method of *M. tuberculosis* complex

Introduction of simple and low cost genotyping method of *M. tuberculosis* complex



Characterization of direct repeat loci can give digitalized genotype

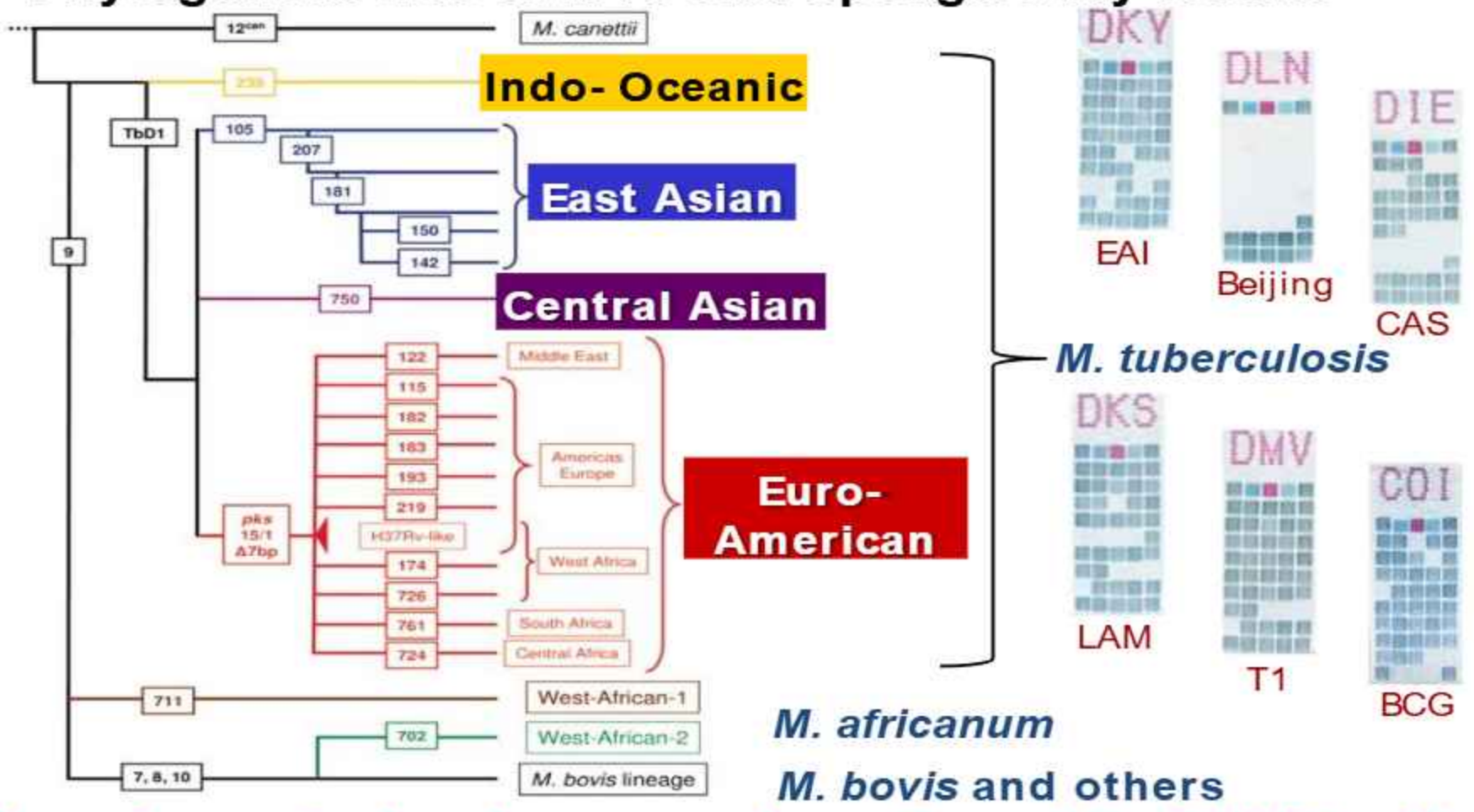
Introduction of simple and low cost genotyping method of *M. tuberculosis* complex



Digitalized genotype codes can be obtained for comparizon with global data

Introduction of simple and low cost genotyping method of *M. tuberculosis* complex

Phylogenetic tree of MTC and SpoligoArray results



SPoligo Array technology was transferred to IVM for the genotyping of Mongolian MTBC isolates

Thank you very much

Маш их баярлалаа