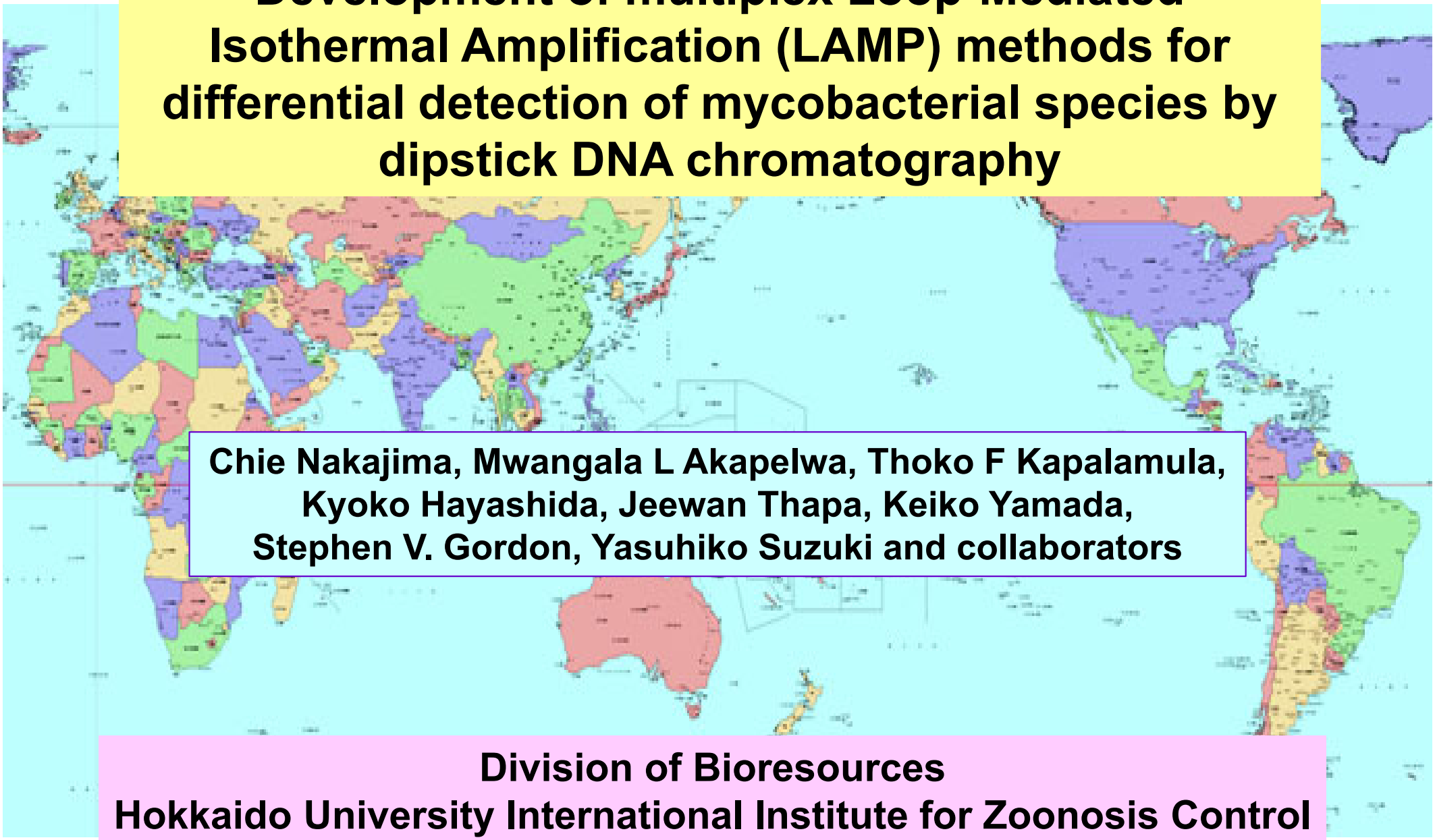
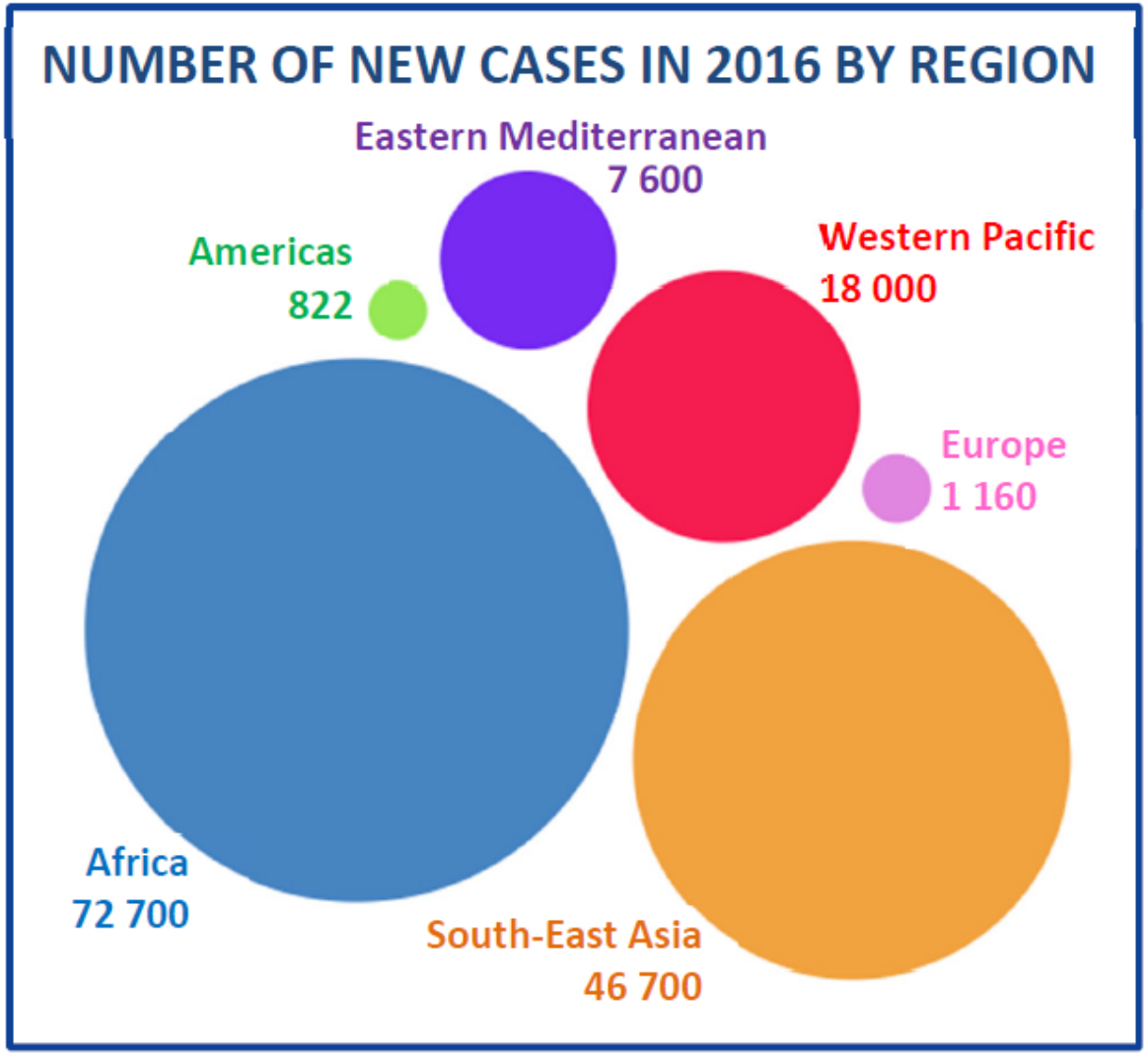
Development of multiplex Loop-Mediated Isothermal Amplification (LAMP) methods for differential detection of mycobacterial species by dipstick DNA chromatography

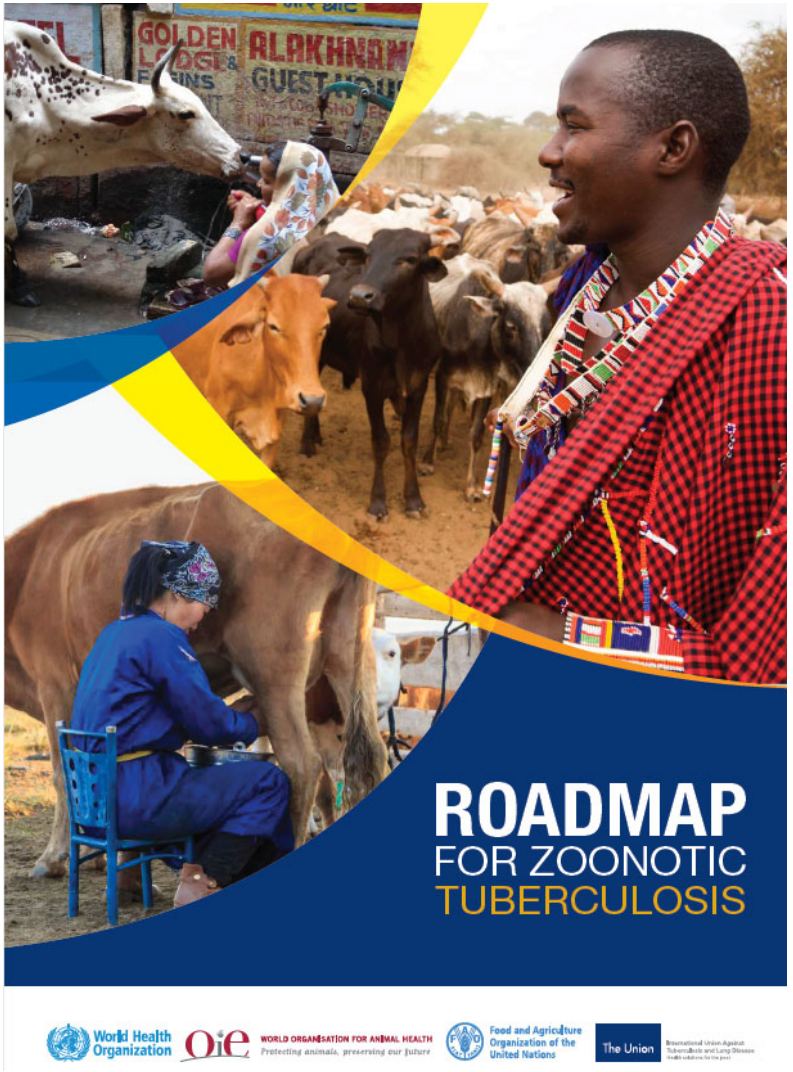


Chie Nakajima, Mwangala L Akapelwa, Thoko F Kapalamula,
Kyoko Hayashida, Jeewan Thapa, Keiko Yamada,
Stephen V. Gordon, Yasuhiko Suzuki and collaborators

Division of Bioresources
Hokkaido University International Institute for Zoonosis Control

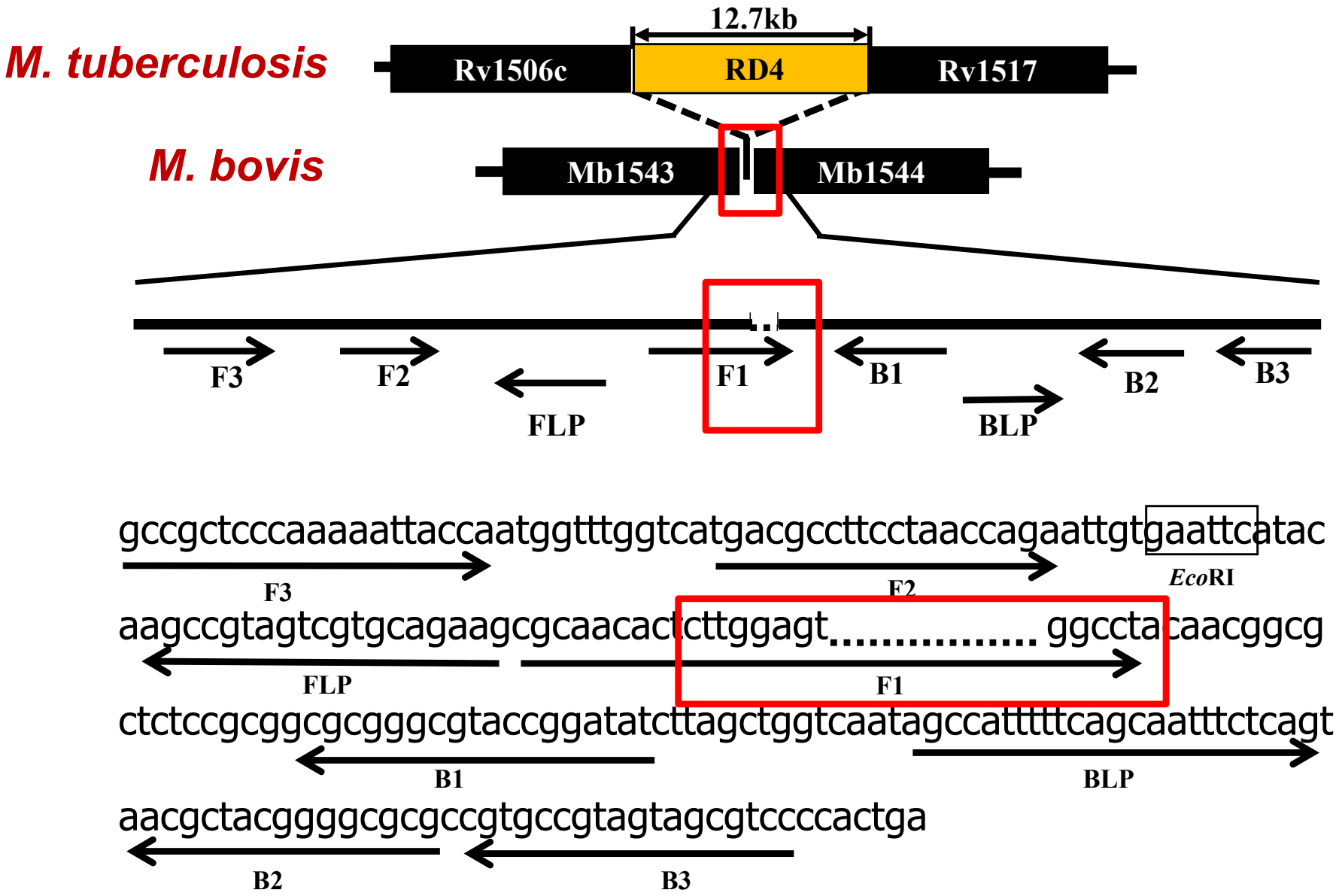
Global situation of zoonotic TB (2016, WHO)





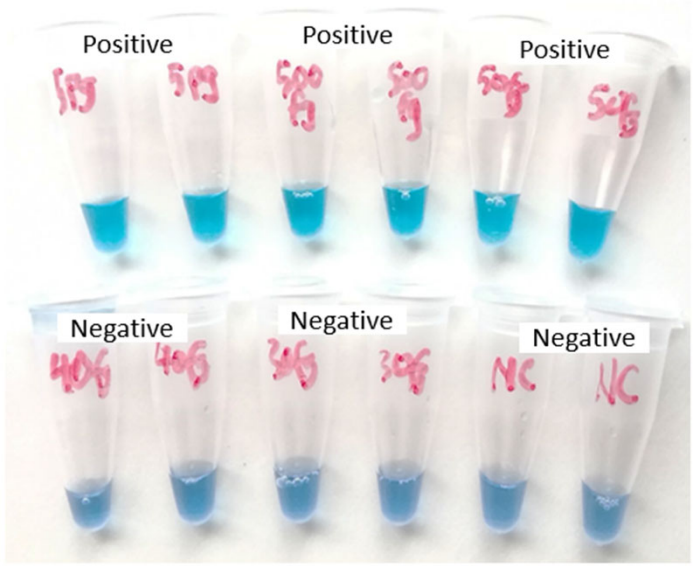
Estimated 147,000 new cases and 12,500 deaths globally due to the zoonotic TB in 2016.

RD4 LAMP for *M. bovis* specific detection

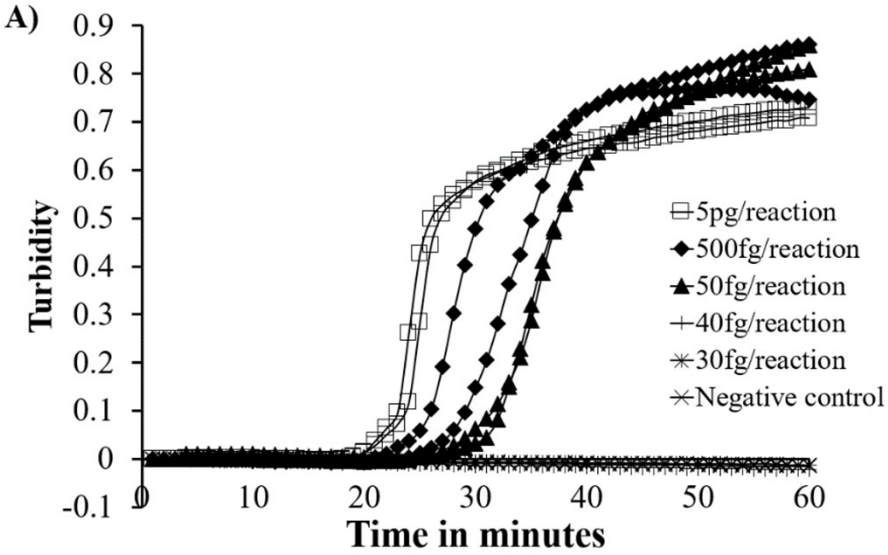


RD4 LAMP for *M. bovis* specific detection

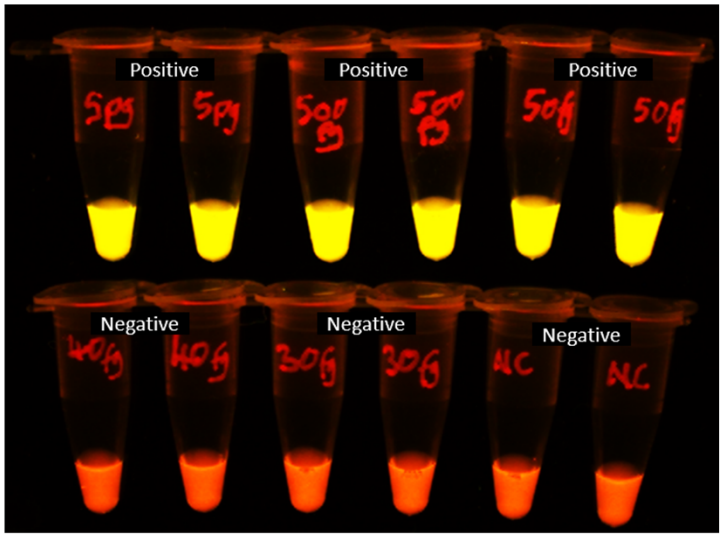
B)



A)



C)



Detection limit: 10 copies

Rapid: < 40 mins

Highly specific

65 *M. bovis*: +

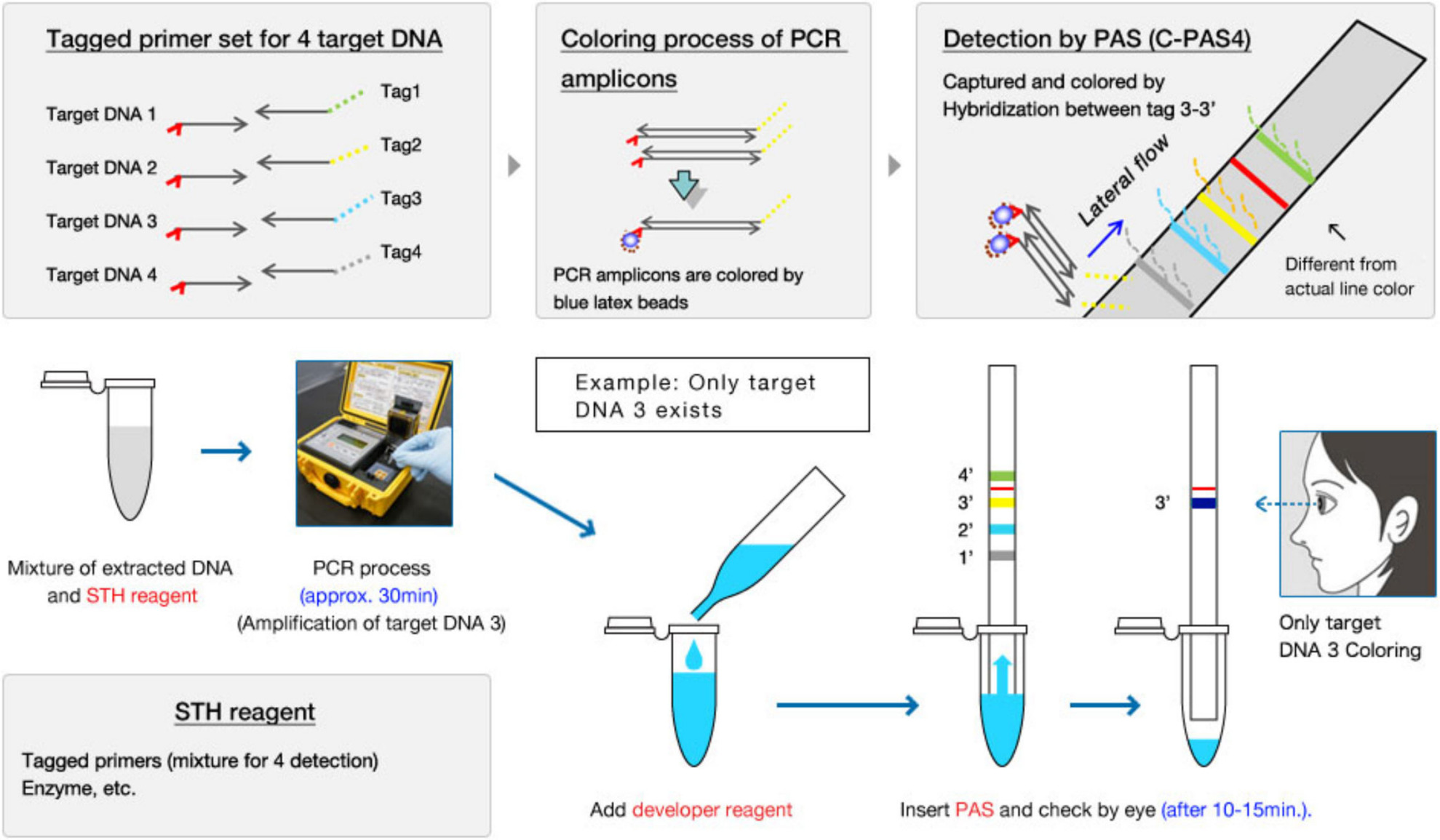
74 other bacteria: −

(including 40 MTB)

Principle of the STH-PAS technology

STH (Single-stranded Tag Hybridization) method

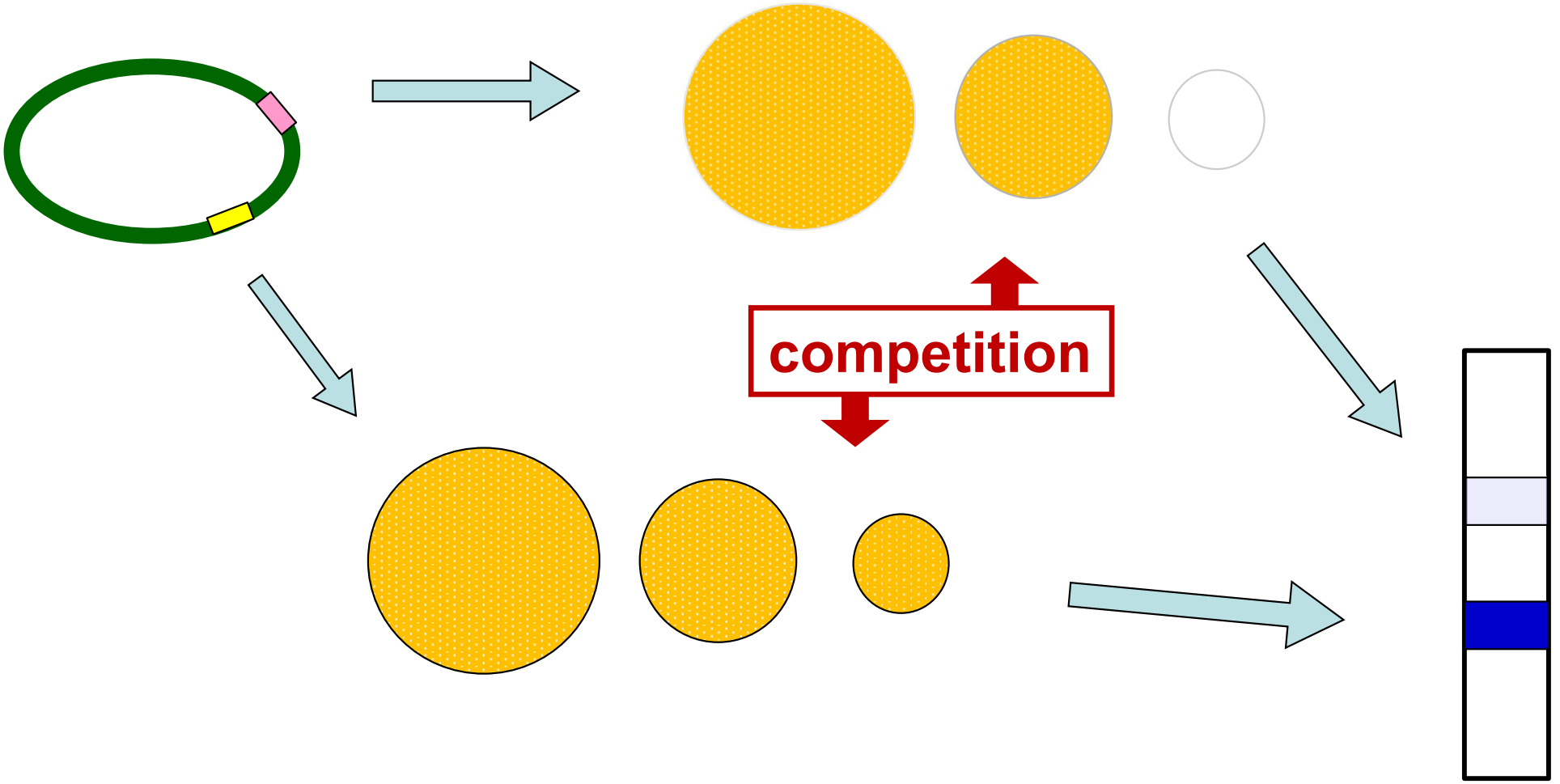
PAS(Printed-Array Strip)



TBA (Tohoku Bio Array) Co.,Ltd.

https://www.t-bioarray.com/en_contents/technology.html

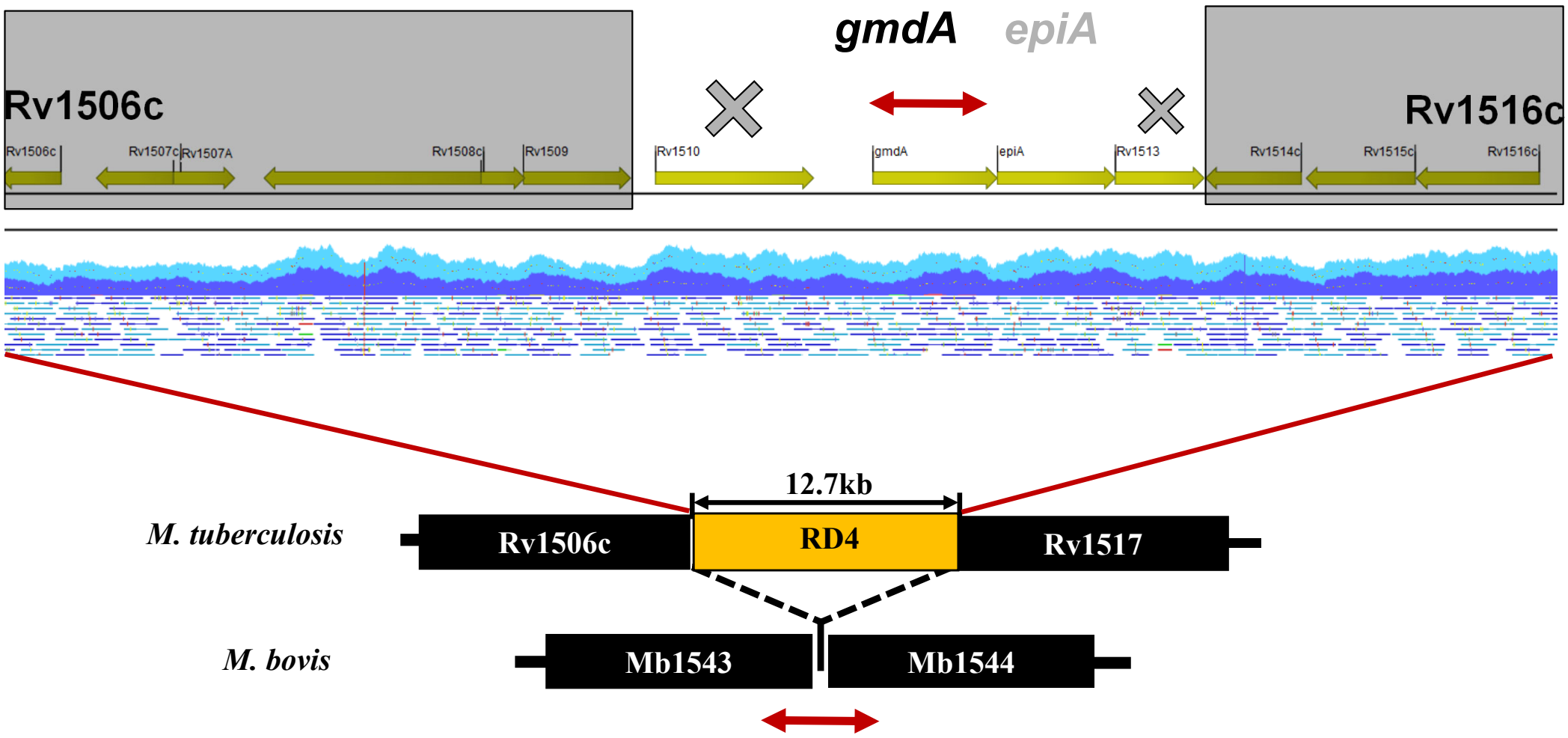
Multiple amplifications interfere with each other



To avoid intra-genomic competition, amplification targets should be limited to only one site within one genome.

Selection of the optimal gene within RD4

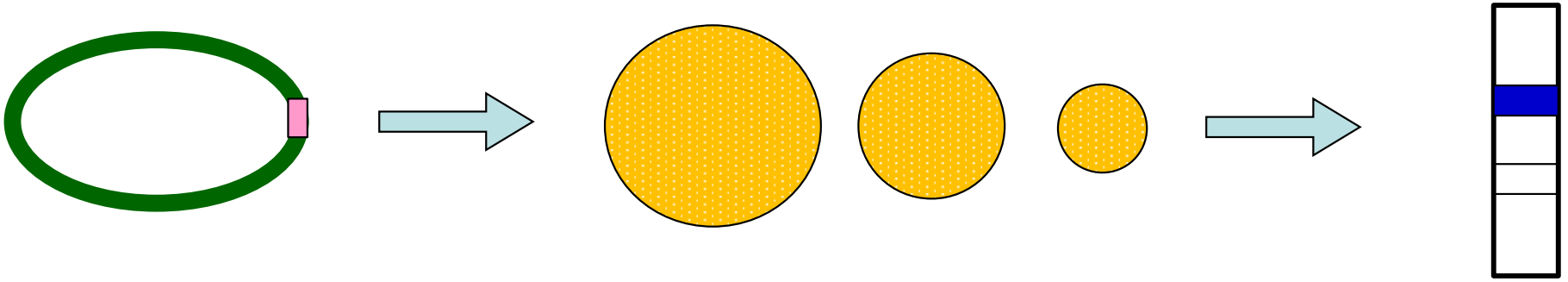
gmdA (Rv1511c) was selected as the target for MTC-LAMP



RD4 "deletion site" : target for *M. bovis* discrimination

M. bovis differentiation LAMP targeting "RD4"

MTC other than *M. bovis*: LAMP target → inside of RD4 (*gmdA*)



***M. bovis*: LAMP target → outside (deletion site) of RD4**