

***Enhelhardt Institute of Molecular Biology
OOO Biochip-IMB***

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**BIOCHIPS AS A TOOL FOR ANALYSIS OF
MYCOBACTERIA GENOMES**

12th SAC Seminar “Combating Global Infections”

September 21 - 24, 2009

Irkutsk, RF

**Engelhardt Institute of
Molecular Biology**



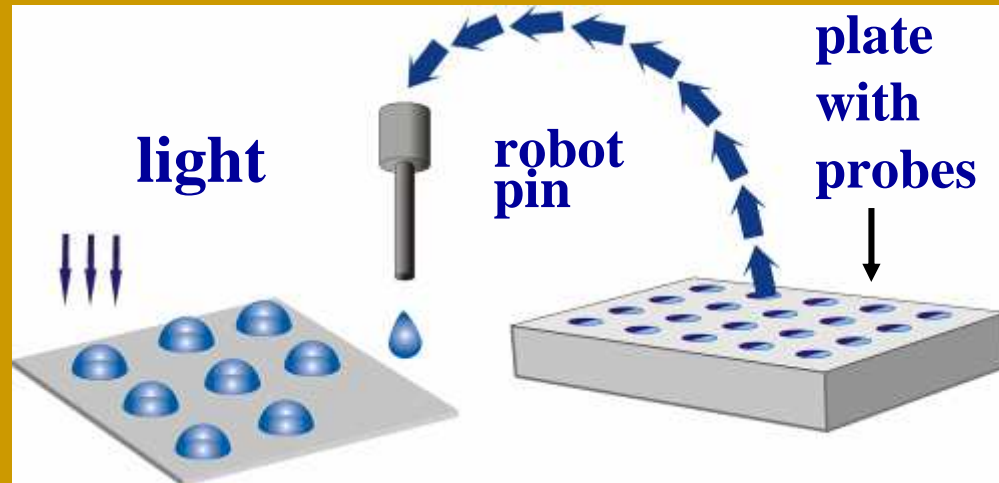
**Andrei
Mirzabekov**
(1937-2003)



Gel-based Biological Microchips (Biochip, Microarray)

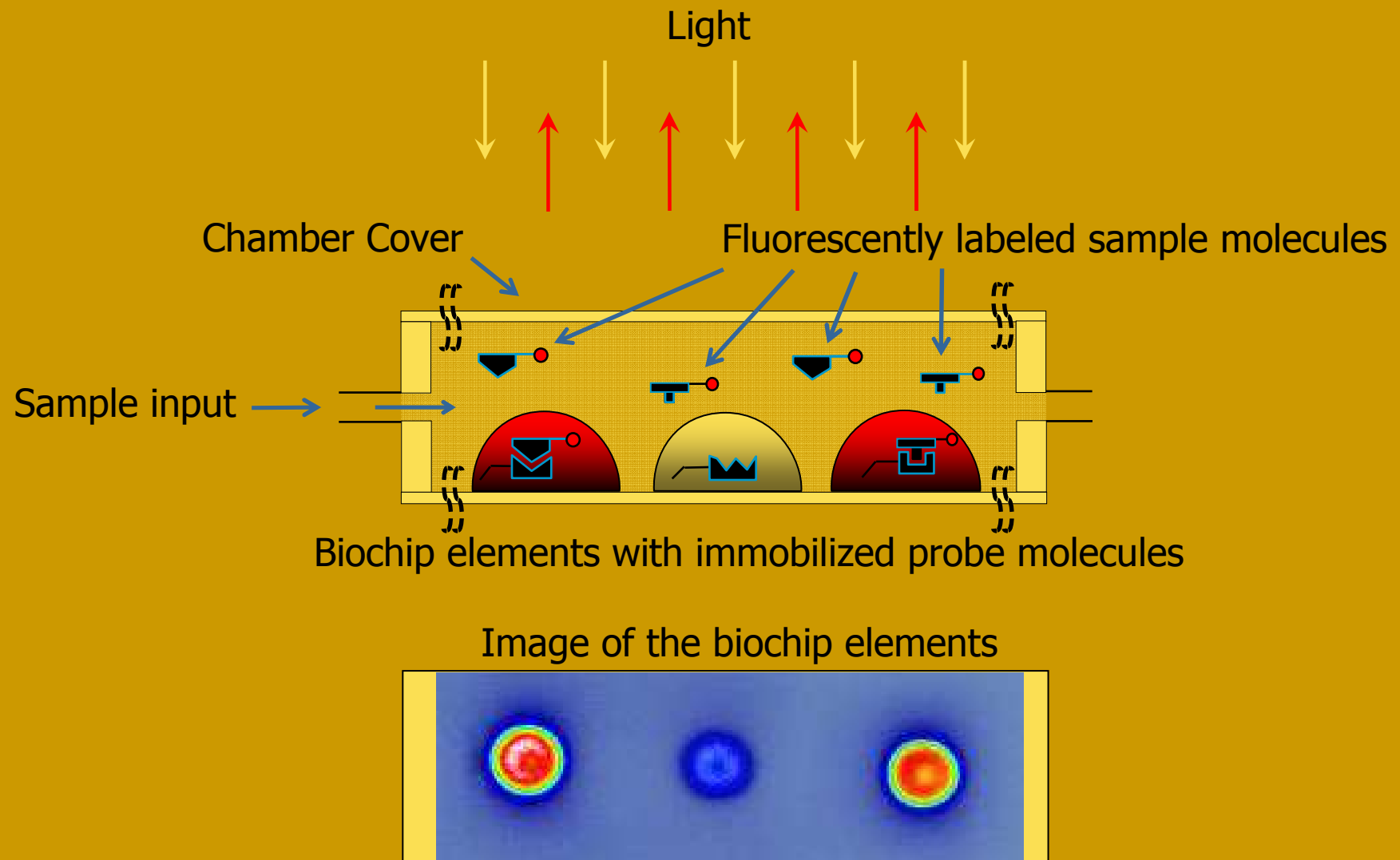


50-500 μm

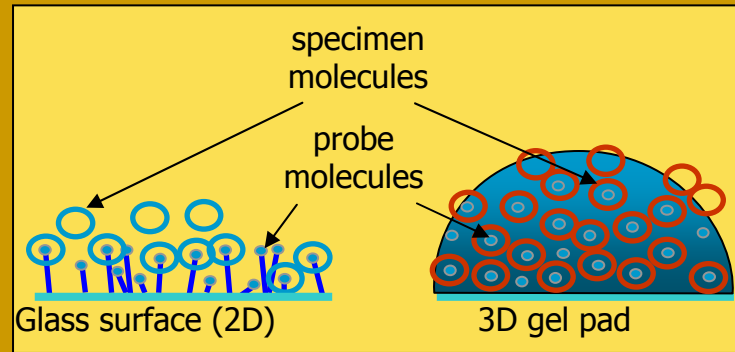


Fotopolymerization

Principle of biochip operation



Advantages of 3-dimensional BIOCHIPS



- Immobilization capacity is 1000x higher for 3D biochips which increases analysis sensitivity leading to very good discrimination level.
- There is no contact between immobilized probe molecules and support.
- Immobilized molecules retain their biological activity.
- Each cell acts as a nanotube wherein any reaction can be performed.

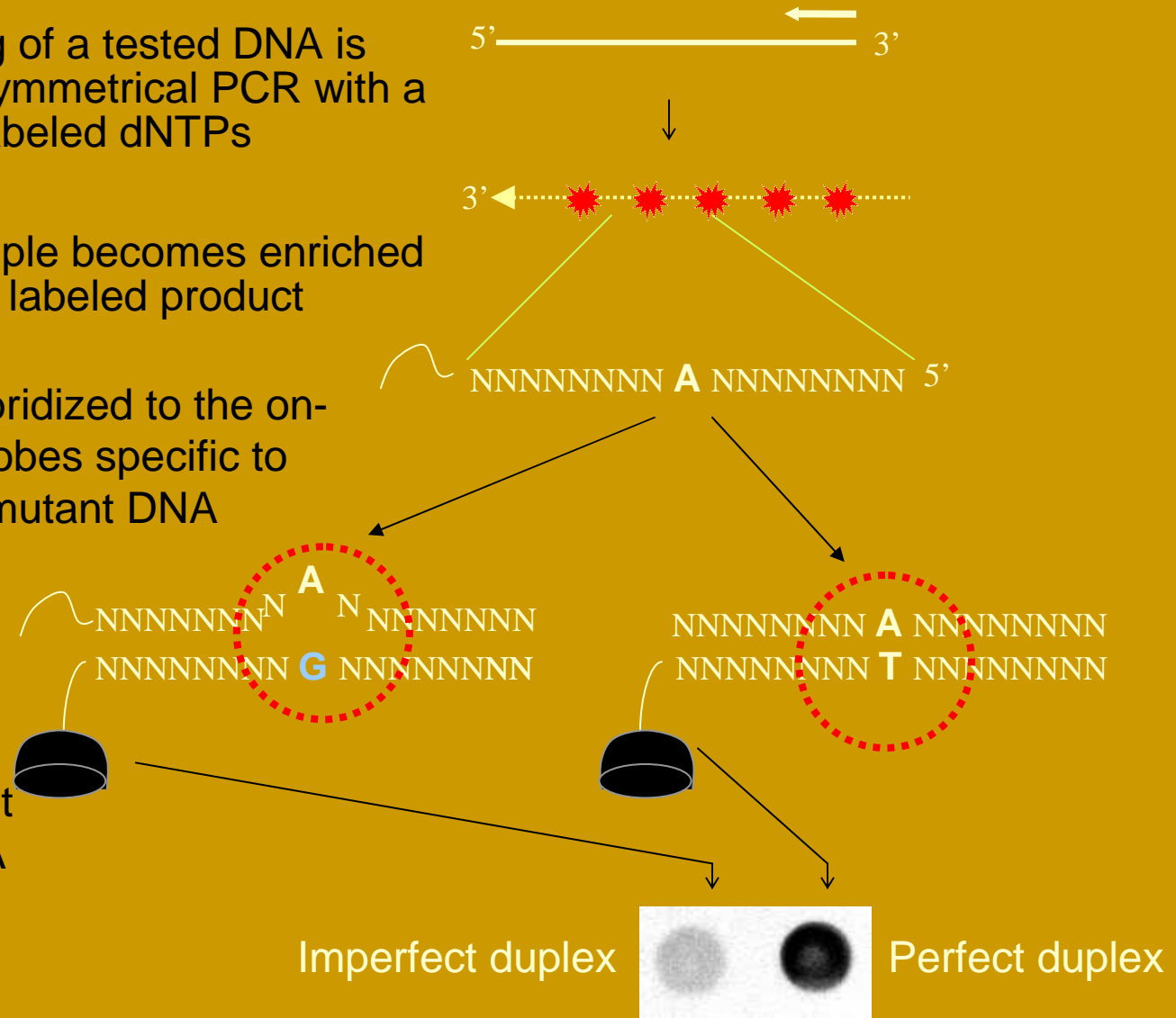
Principal Scheme of the Point Mutation Detection by Hybridization on Biochip

Fluorescent labeling of a tested DNA is performed using asymmetrical PCR with a labeled primer or labeled dNTPs

As a result, the sample becomes enriched in a single-stranded labeled product

This product is hybridized to the on-chip immobilized probes specific to both wild-type and mutant DNA

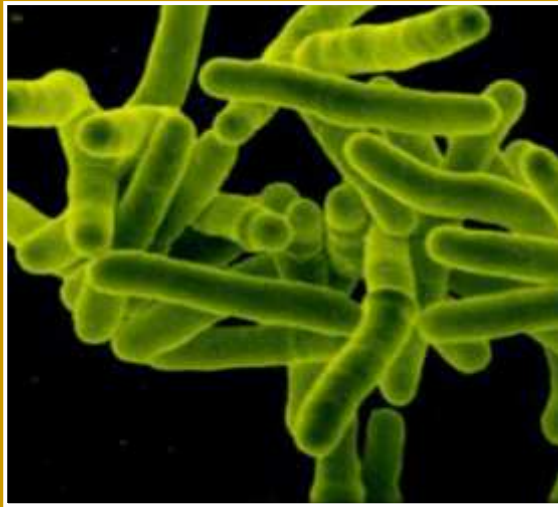
The fluorescence intensities are compared to find out whether tested DNA bears a mutation or not



Biochip Analyzer

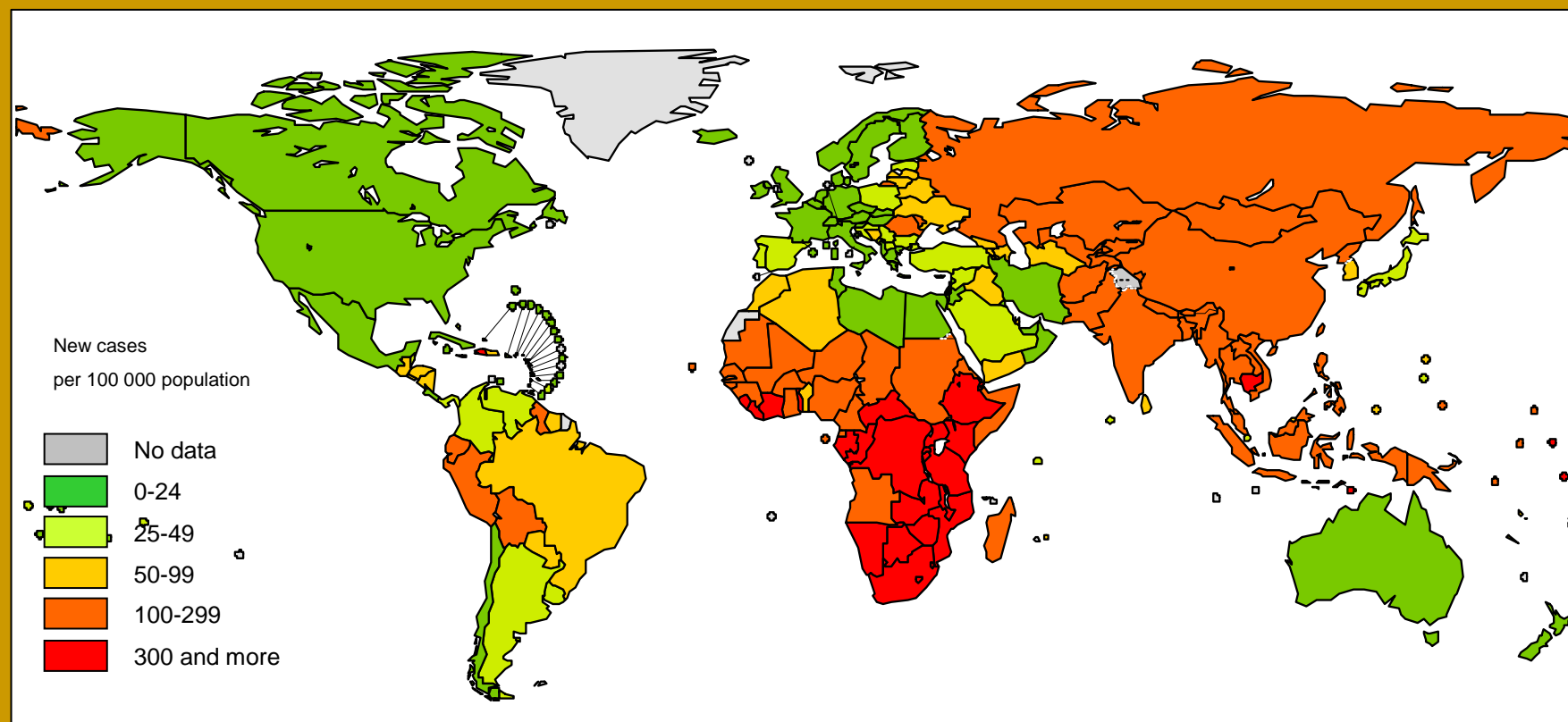


- Universal for any type of biochips produced by EIMB
- High speed and Sensitivity
- User-friendly software for medical personnel
- Certified in clinical trials

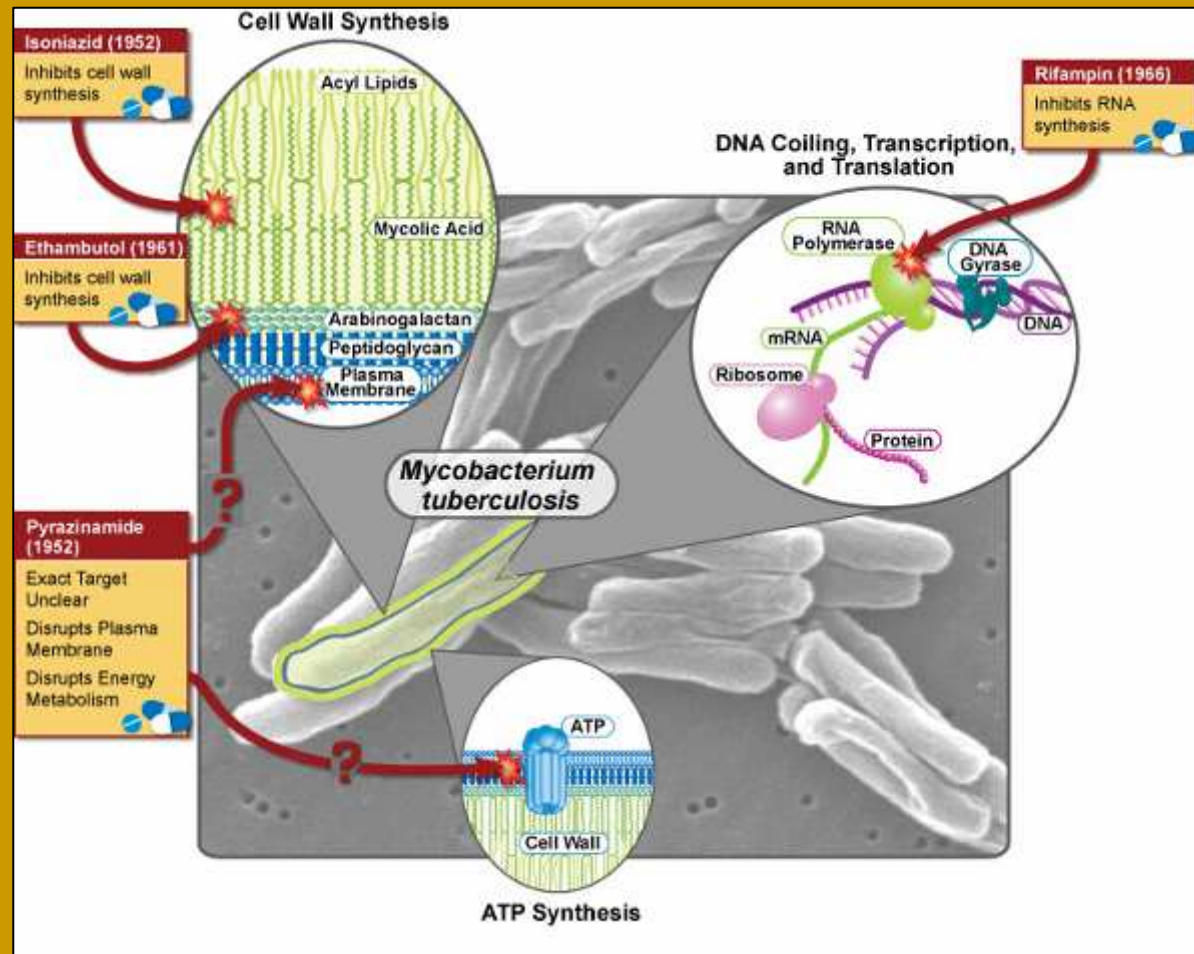


Tuberculosis

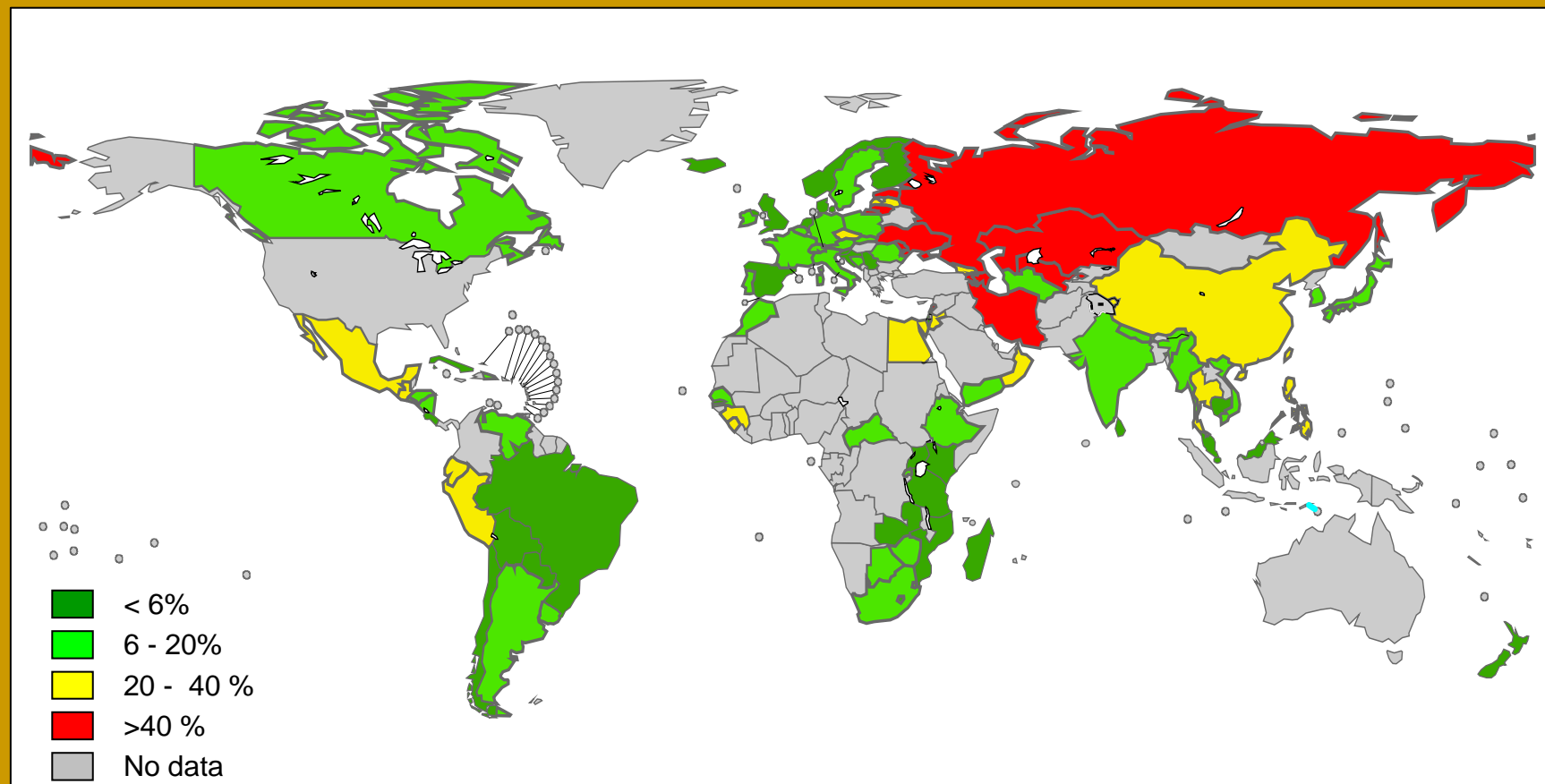
Reported TB cases in 2006



First-Line Treatment of TB



MDR in relapse cases (1994-2007)



Biochips application in TB diagnostics for fast discrimination and strain typing of multidrug-resistant tuberculosis in Russia

Lead Institute:

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences

Sub-Institution:

State Research Center of Virology and Biotechnology Vector (SRC VB VECTOR)

Duration:

Main project: April 1st, 2001 – March 31, 2003 (24 Months)

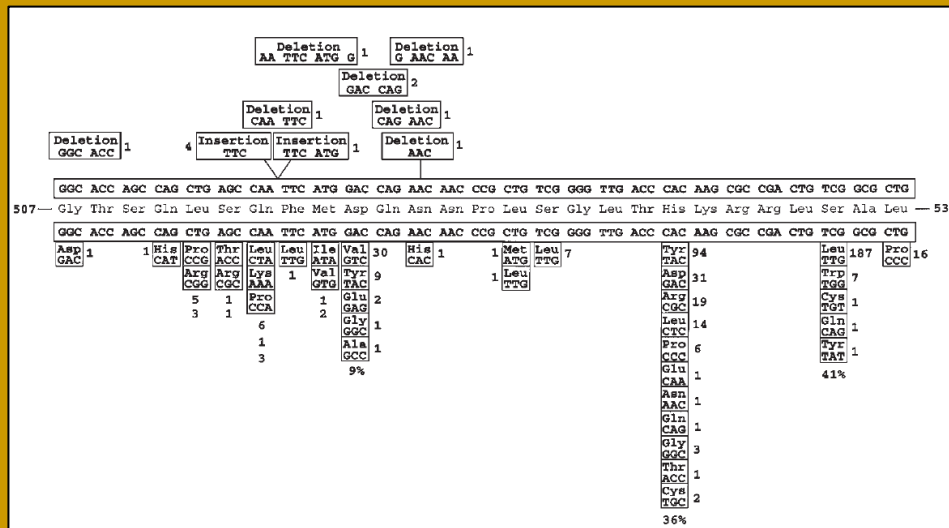
Addendum I: April 1st, 2003 – March 31, 2004 (12 Months)

Addendum II: April 1st, 2004 – July 31, 2004 (5 Months)

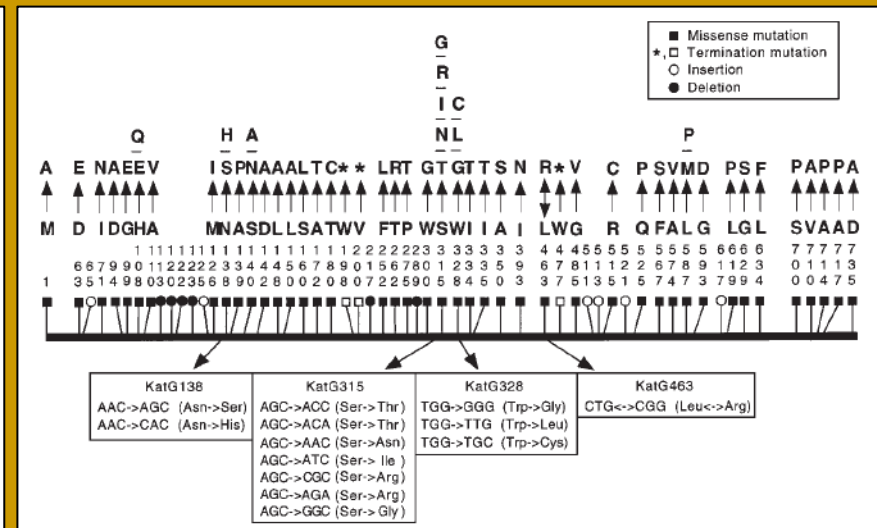
Addendum III: August 1st, 2004 – December 31, 2004 (5 months)

Mutation leading to RIF and INH resistance

rpoB



katG



TB-Biochip

M									M
	ahpC w(C10)	ahpC w(G6)	Trp 328	Ser 315	Ser 315	Leu 533	Ser 522	Met 515	WT 507
	ahpC (T10)	ahpC (A6)	Gly 328	Thr 315 ₂	Thr 315 ₁	Pro 533	Leu 522	Ile 515	Del 507
	ahpC (A10)	inhA (G16)	Leu 328	Asn 315	Leu 511	Ser 531	His 526	His 526	Asp 516
	ahpC w(G9)	inhA (T15)	Cys 328	Ile 315	Pro 511	Leu 531	Tyr 526	Asp 526	Val 516
	ahpC (A9)	inhA w(C15)	Ile 335	Arg 315 ₁	Arg 511	Trp 531	Asn 526	Leu 526	Tyr 516
0	ahpC w(C12)	inhA (G8)	Val 335	Arg 315 ₂	Arg 512	Cys 531	Arg 526	Gln 526	Gly 516
0	ahpC (T12)	inhA (A8)	inhA (T24)	Gly 315	Thr 512	Gln 531	Pro 526	Cys 526	Glu 516
M	IS6110	inhA w(T8)	inhA w(G24)	IS6110	Ser 512	Gly 513	Lys 513	Leu 513	Gln 513

Identify:

more than 95% RIF resistant forms of TB

more than 80% INH resistant forms of TB

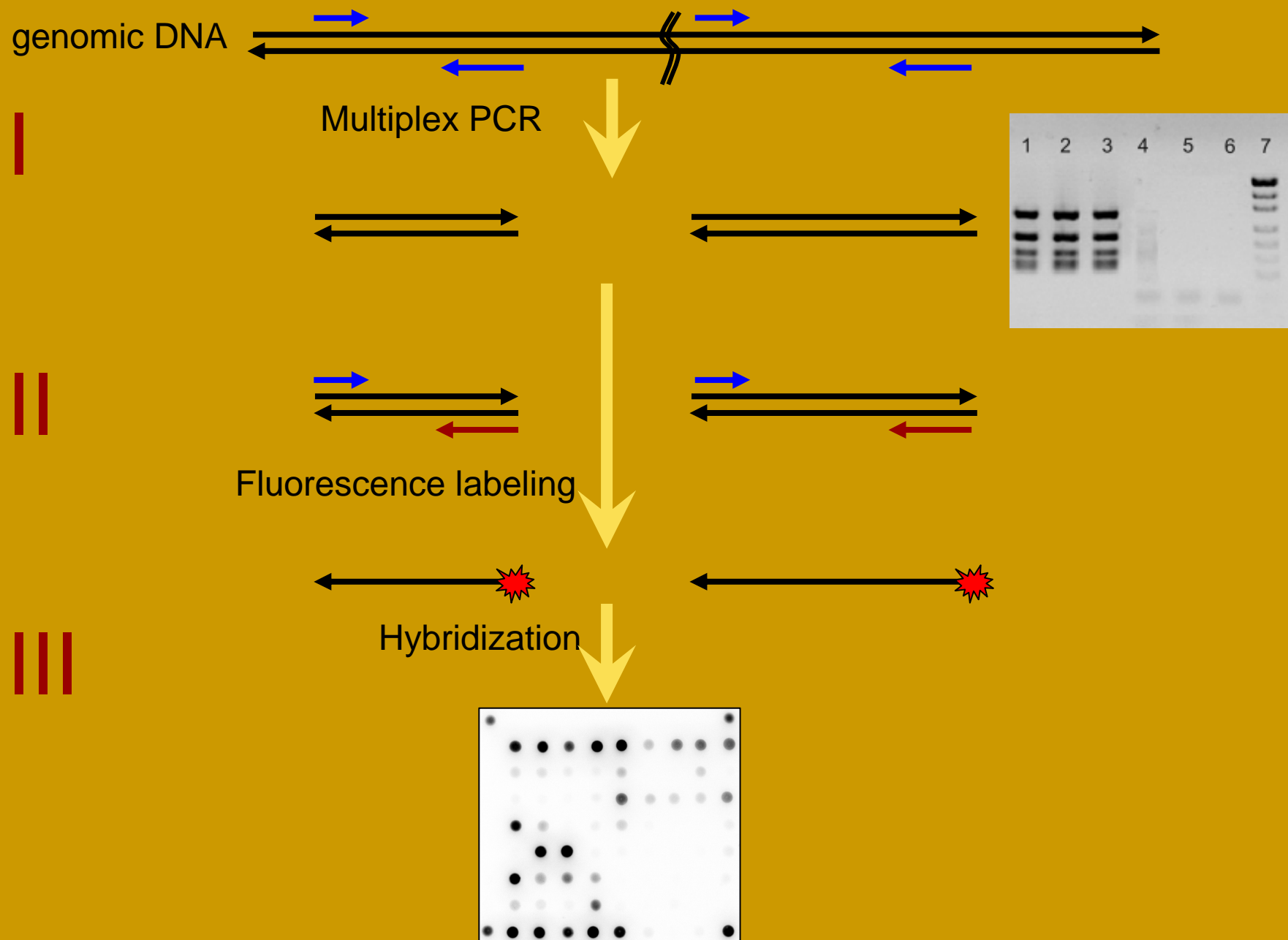
during 24 hours.

4 years of use in Russian Federation and abroad, more than 10000 analyses

Certified by Russian Ministry of Health

Регистрационное удостоверение МЗ и СР РФ № ФС 03262004/0889-04

Protocol of *M.tuberculosis* genome analysis



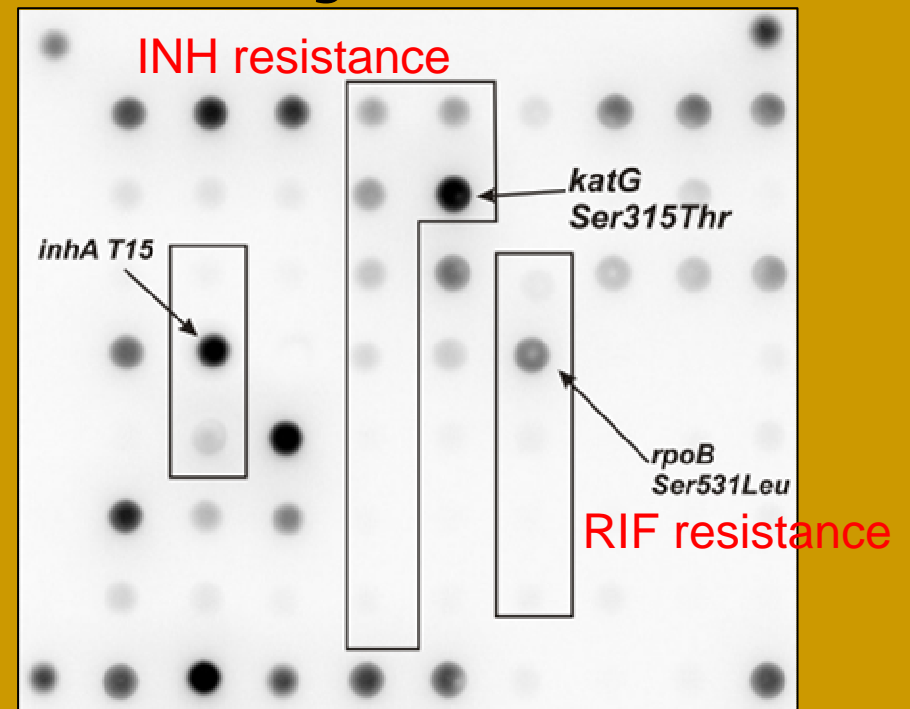
Identification of *M.tuberculosis* drug resistance

Wild Type



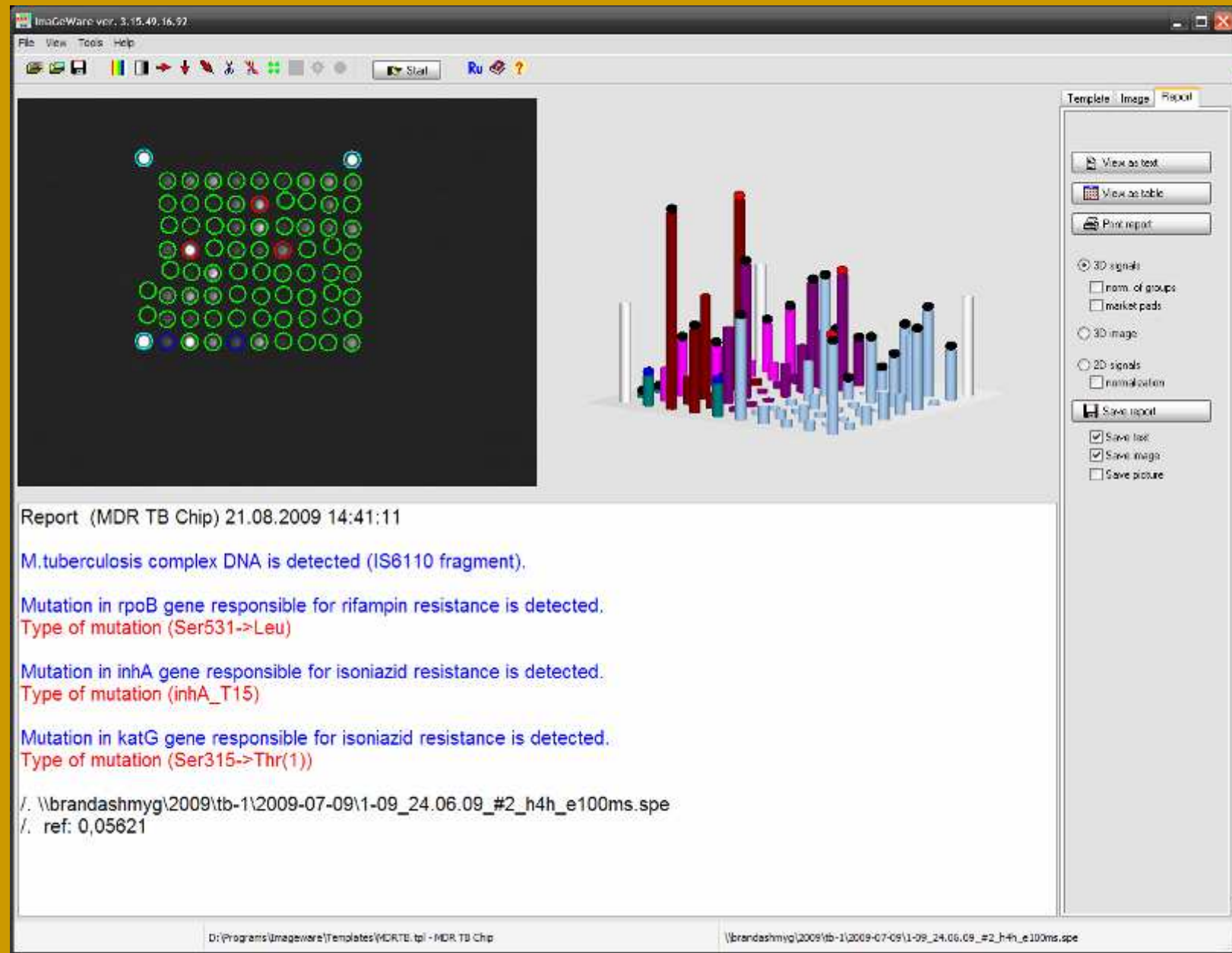
Routine therapy

Multidrug-resistant TB



Second line drugs should be used

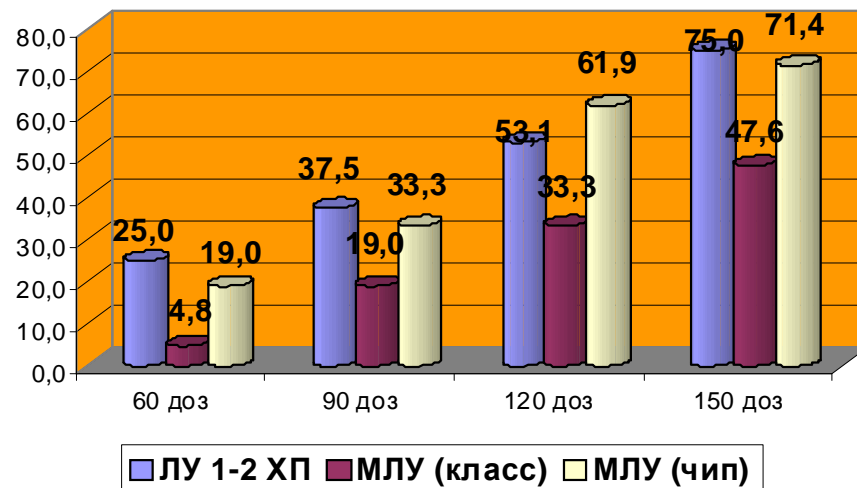
Program window with analysis results



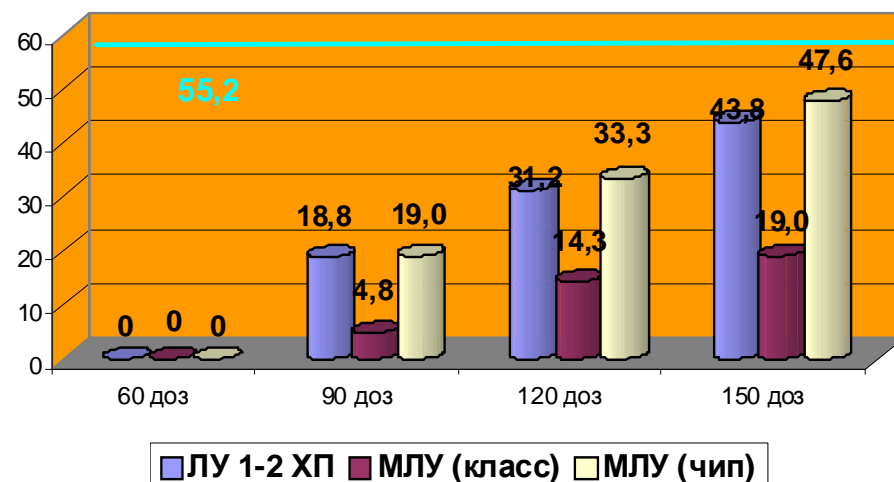
Clinical effects of TB-Biochip application

Saratov State Medical University

Прекращение бактериовыделения (%)



Закрытие полостей распада (%)



I – Resistance to 1-2 drugs of 1st line
(H,R,E,S,HS,HE)

n=32

Treating: 2-3 weeks H R Z E (S) → results of TB drug susceptibility → changing of 2 drugs to Pr+Fq

II – MDR (HR,HRS, HRE)

n=21

Curing: 2-3 weeks H R Z E (S) → results of TB drug susceptibility → Z E Pr Fq Cs (Pas)

III – MDR (HR-obtained by TB-Biochip)

n=21

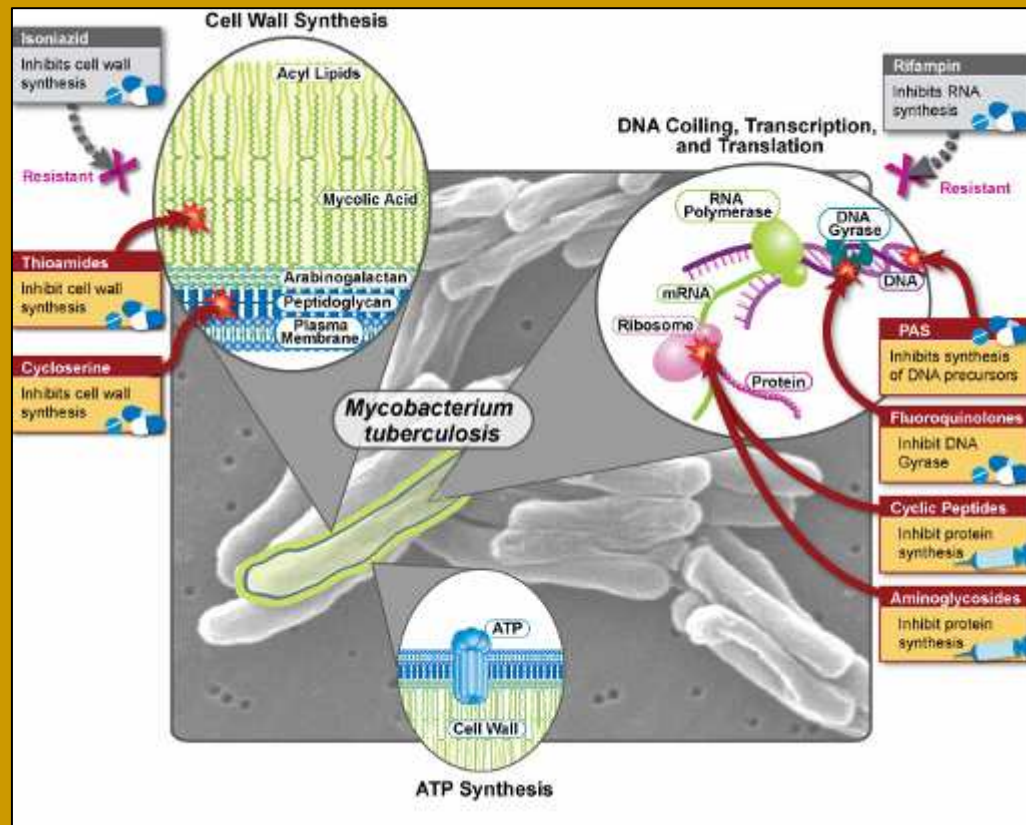
Curing: results of TB drug susceptibility **by TB-Biochip** → Z E Pr Fq Cs (Pas)

Prof. T.Morozova, 2008

Application of TB-Biochip

1	Number of analyses –	10 000
2	MBT+	9 900
	Confirmed by classical methods –	5 900
	No data –	4 000
3	RIF-resistant (TB-Biochip)	2 500
	Confirmed by classical methods –	1 500
	No data –	800
	Not confirmed* –	200
4	INH-resistant (TB-Biochip-2)	3 000
	Confirmed by classical methods –	1 800
	No data –	800
	Not confirmed* –	400

Second-Line Treatment of TB

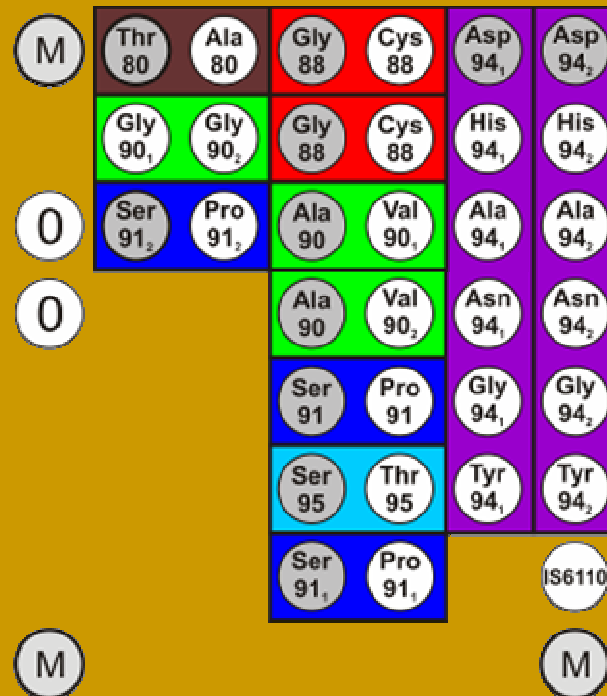


Mutation leading to FQ resistance

TB-Biochip-2 identifies mutations in QRDR region of DNA gyrase gene (*gyrA*)

80								88		90	91			94	95	
Thr								Gly		Ala	Ser			Asp	Ser	
acc	atg	ggc	aac	tac	cac	ccg	cac	ggc	gac	gcg	tcg	atc	tac	gac	agc	ctg
Ala								Cys		Val	Pro			Ala	Thr	1
gcc								tgc		gtg	ccg			gcc	acc	
																2
																3
																4
																5

TB-Biochip-2



Identifies:

More than 80% of FQ-resistant strains

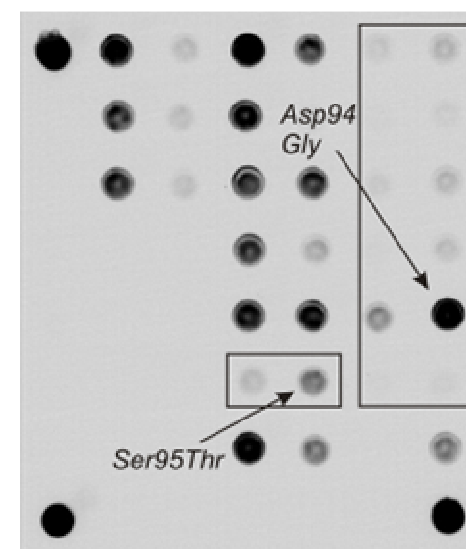
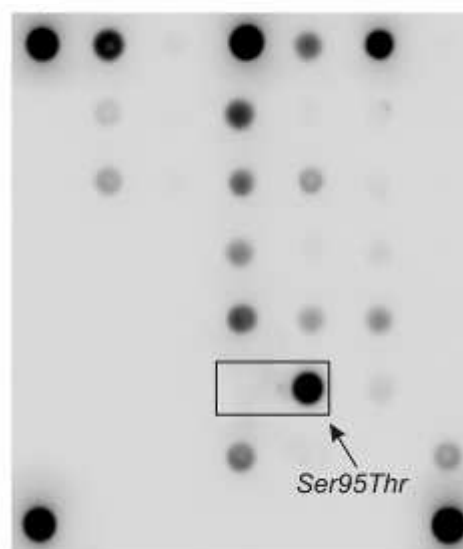
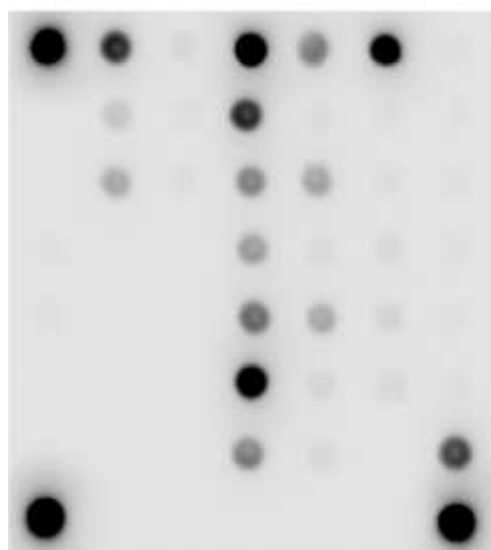
More than 3000 analyses

Certified by Russian Ministry of Health

Регистрационное удостоверение МЗ и СР РФ № ФС 01012006/3527-06

Identification of FQ-resistance of MBT

FQ-resistance



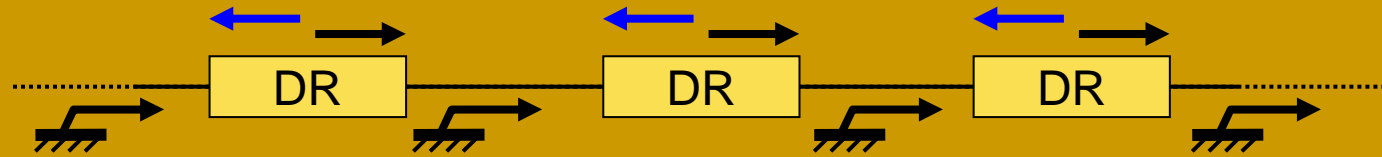
Naturally occurring
polymorphism

Application of TB-Biochip

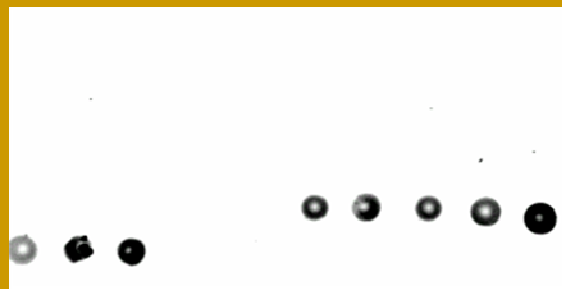
Астрахань – облтубдиспансер
Белгород – облтубдиспансер
Екатеринбург – облтубдиспансер
Екатеринбург - Уральский НИИ туберкулеза
Казань – Республиканский клинический туберкулезный диспансер Татарстана
Кемерово – облтубдиспансер
Красноярск – облтубдиспансер
Липецк – облтубдиспансер
Москва – Институт фтизиопульмонологии
Москва – МНПЦБТ
Москва – ЦНИИ туберкулеза РАМН
Москва – УФСИН (Матросская Тишина)
Нижний Новгород – облтубдиспансер
Новосибирск – НИИ туберкулеза РАМН
Ростов-на-Дону – облтубдиспансер
Санкт-Петербург – НИИ Фтизиопульмонологии РАМН
Саратов – облтубдиспансер
Тамбов – облтубдиспансер

National Center for Cardiology and Internal Medicine, Bishkek, Kyrgyzstan

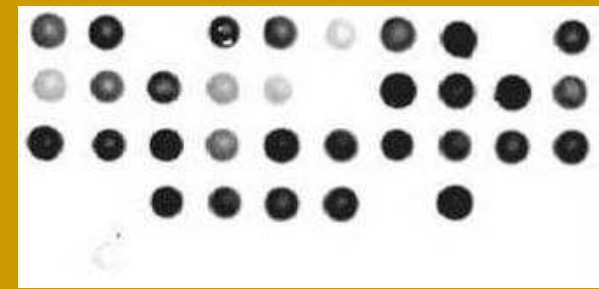
Analysis of DR-region of MTB complex (Spoligotyping)



M. tuberculosis H37Rv

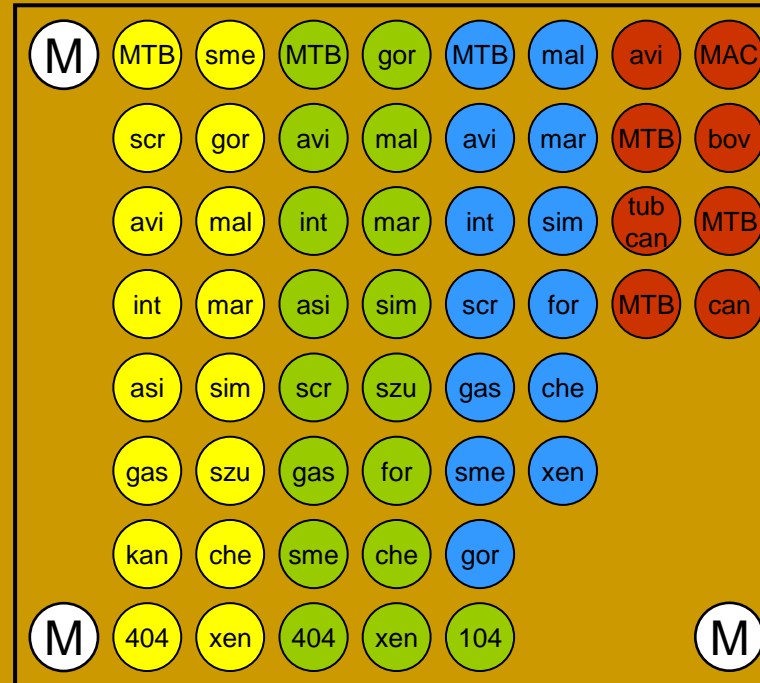
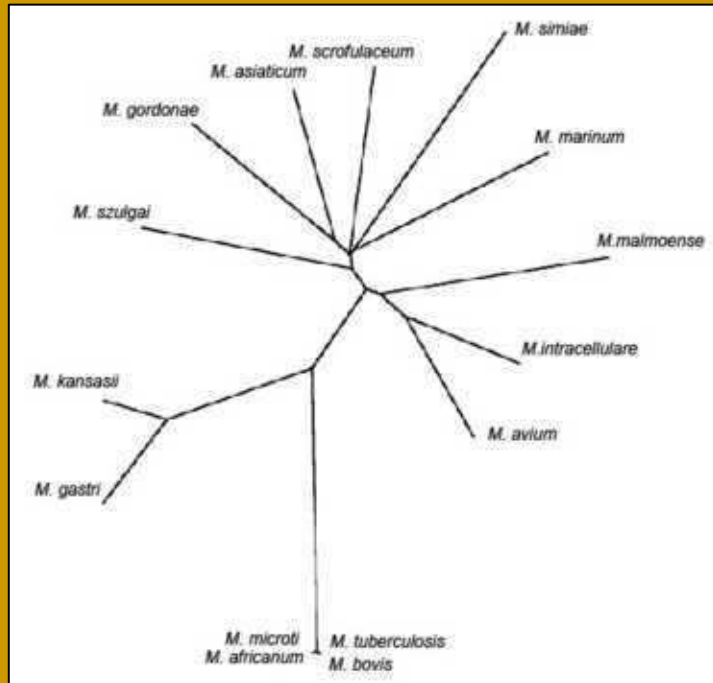


M. tuberculosis Beijing



M. bovis

Biochip for *Mycobacteria* species differentiation



M. tuberculosis



M. xenopi

Thank you for your attention!