

Molecular epidemiology of *Mycobacterium tuberculosis* in the Republic of Belarus

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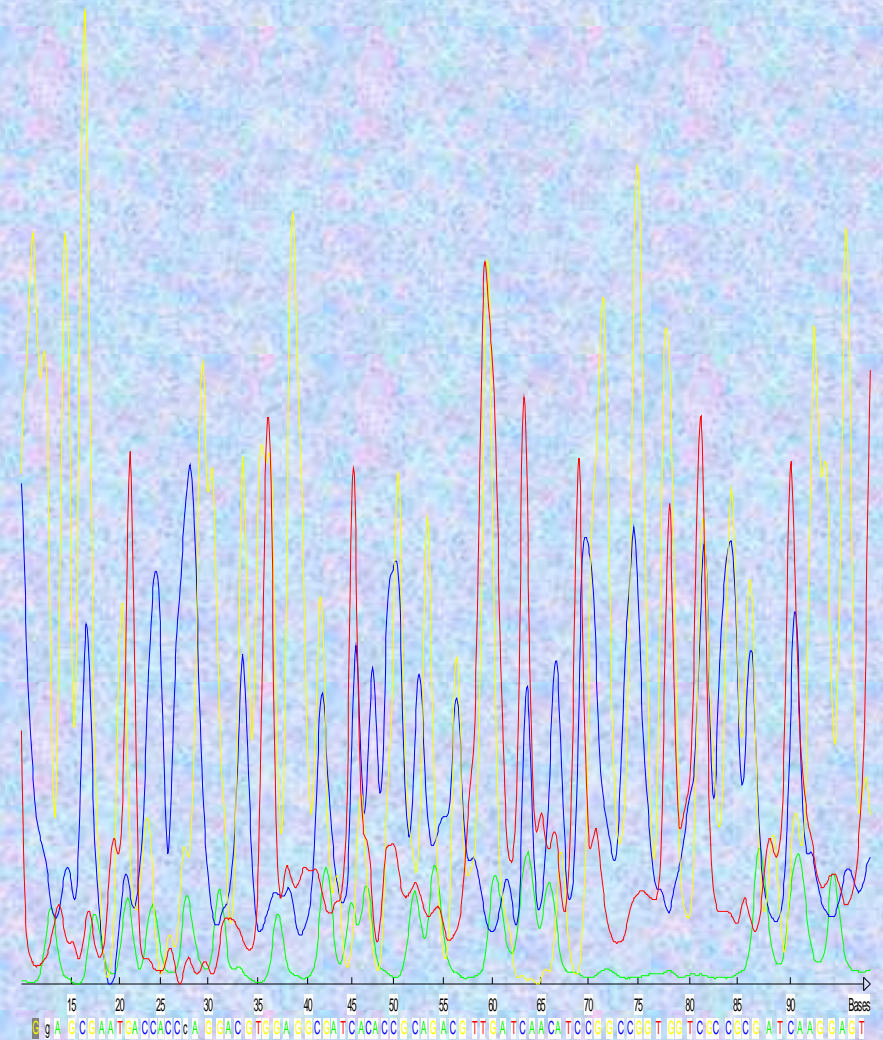
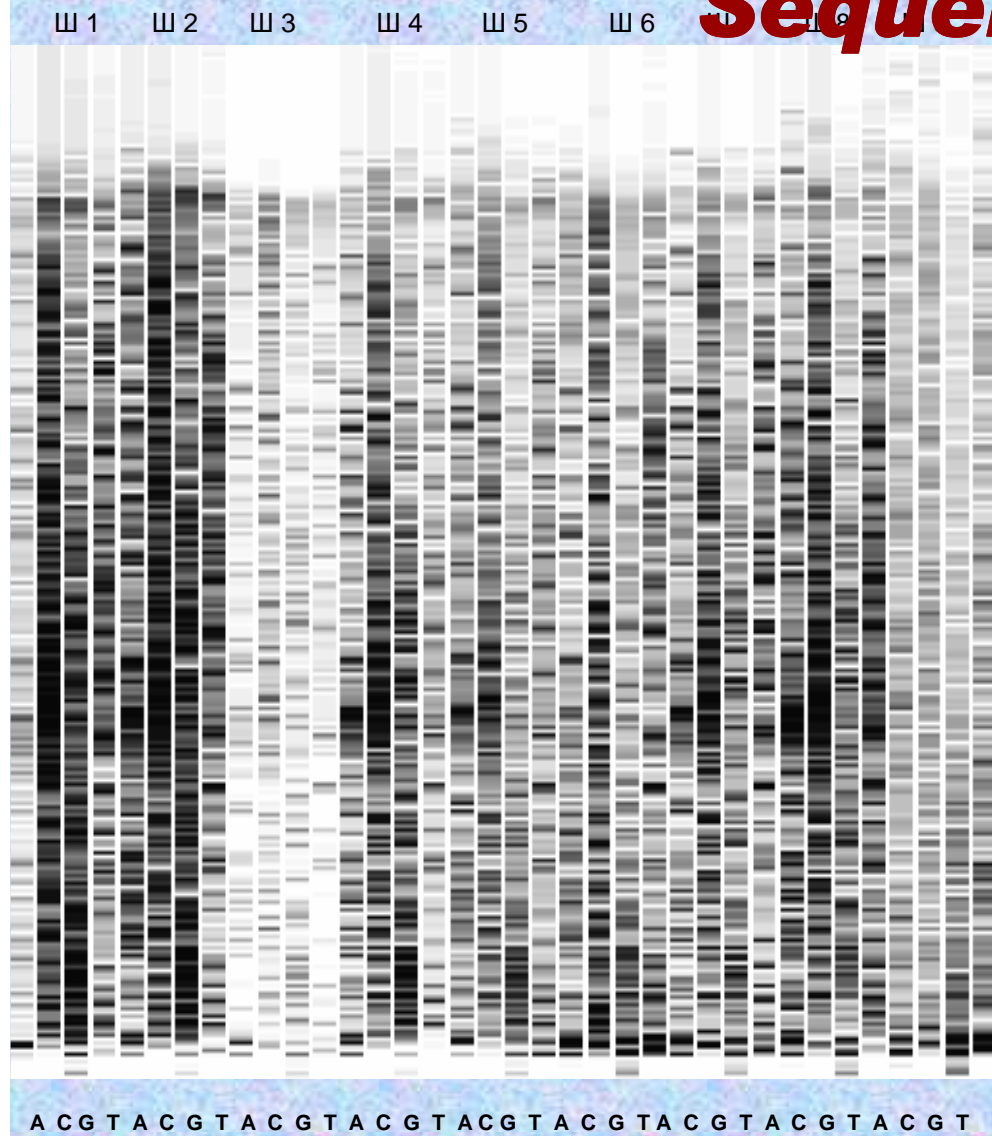
TUBERCULOSIS In THE REPUBLIC OF BELARUS

- Belarus is one of the 18 priority countries for TB control and one of the 25 MDR and XDR-TB countries in the Europe.
- In 2007, the total number of MDR-TB cases was 707 of MDR-TB and 68 of XDR-TB patients were under treatment.
- Prevalence of TB is 49 per 100 000.

Molecular Basis of Drug Resistance in *M. Tuberculosis*

- Several genes *M. tuberculosis* associated with drug resistance:
- *rpoB* – to rifampicin,
- *katG*, *inhA*, *oxyR*, *ahpC*, *kasA* – to isoniazid,
- *rpsL* – to streptomycin,
- *gyrA* – to fluoroquinolones,
- *pncA* – to pyrazinamide,
- *embB* – to ethambutol.
- Risk development of resistance makes up:
- 3.32×10^{-9} per cell division for rifampicin;
- 2.56×10^{-8} for isoniazid;
- 2.29×10^{-8} for streptomycin;
- 1.0×10^{-7} for ethambutol.
- Less 10^{-15} - development of resistance to 2 drugs

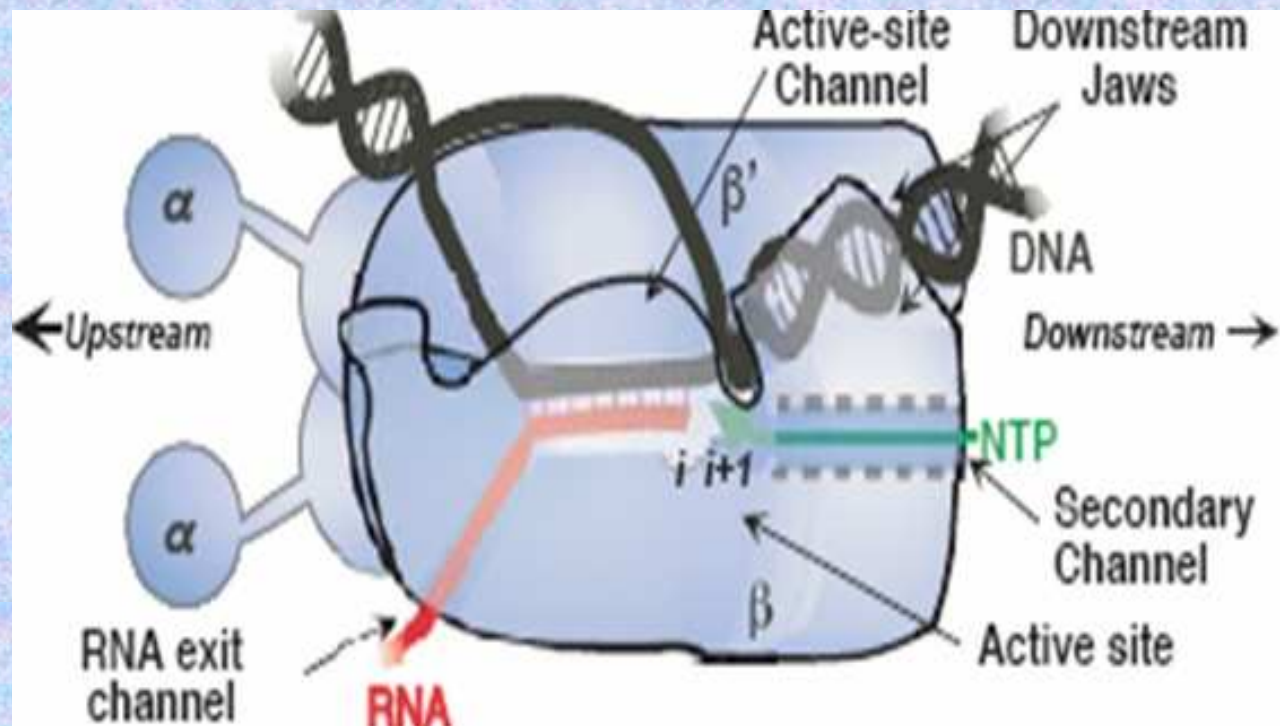
PCR method and DNA Sequencing

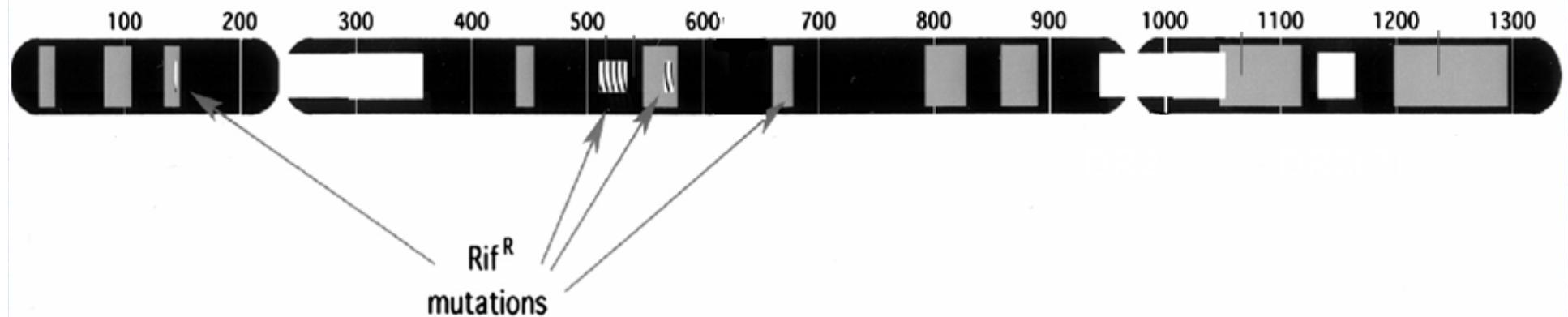


Sequencing results and sequencing gel appearance

Бактериальная РНК-полимераза

RNA polymerase catalyse synthesis of 3 types molecules - mRNA, rRNA, tRNA and consist of 4 subunits: α_2 , β , β'





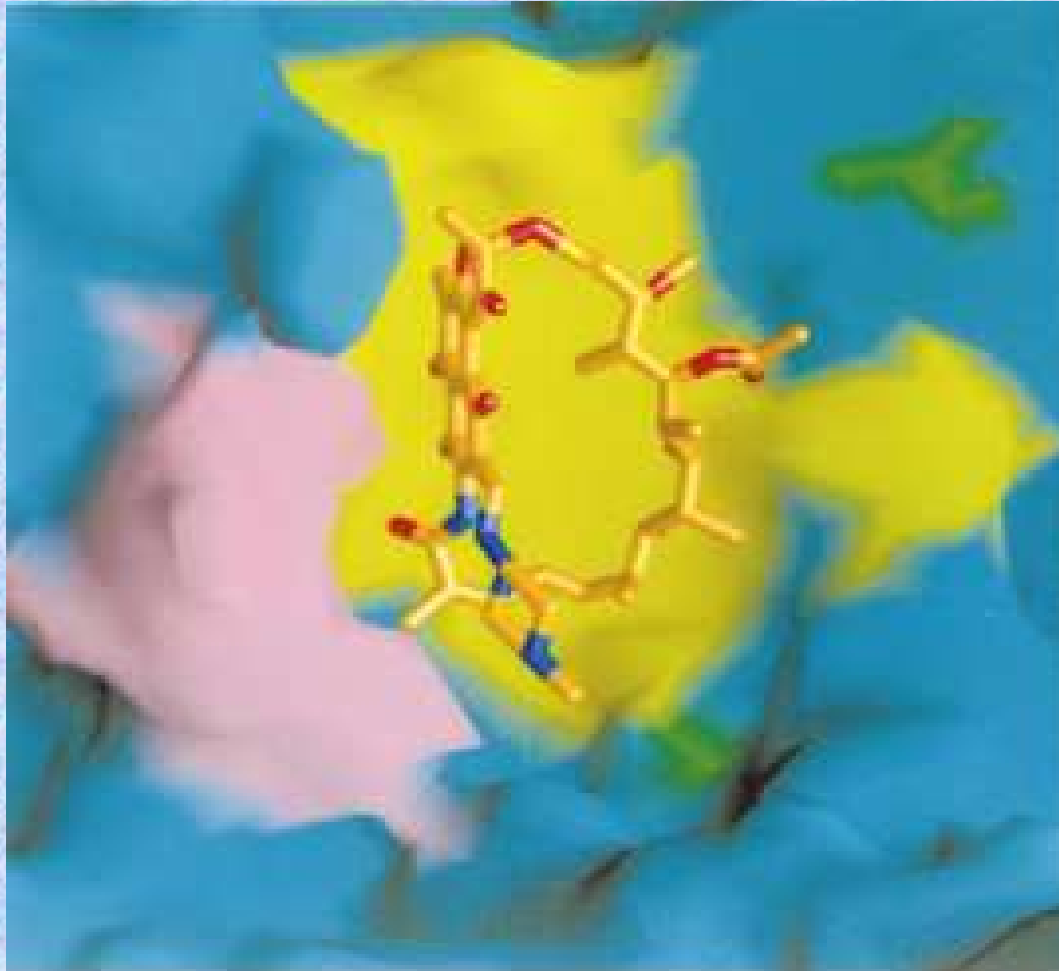
E.coli rpoB gene structure

Rif – sites responsible for resistance

White - non important sites;

Grey - conservative sites;

Second domain of rifampicine resistance site (505-537 triplets) of 35 MDR isolates



RIFAMPICINE MOLECULE AT POCKET OF THE RNA POLYMERASE β - SUBUNIT

Blue - surface of subunit

Yellow and green – sites responsible for rifampicine resistance

Rose – aminoacids, resistant to mutations

LOCATION, TYPE AND FREQUENCY OF MUTATION *rpoB* in 44 isolates of *M. tuberculosis*

AGC									
507		509		511		513		515	517
GGC	ACC	AGC	CAG	CTG	AGC	CAA	TTC	ATG	CAG
	508		<u>510</u>		512		514		<u>516</u>
	ACG		GAG 29,5%		GGC				GTC
	9,1%		TAG 11,4%		6,8%				9,1%
			AAG 6,8%						

				GGA 30,0%					
				GGC 9,0%	2,27%				2,3% TGC
				GCG 2,3%	AAC				27,2% TTG

Belarus

MUTATION FREQUENCY of *rpoB* gene

***M. tuberculosis* isolated from TB patients**

Amount mutations	Regions	Isolates	Changes amount	Isolates number
1 mutation	Minsk 11.36% Vitebsk 9.09% Mogilev 2.27% Brest 2.27% Grodno 0% Minsk obl 2.27%	12	6	74, 932, 455, 384, 414, 118, 367, 388, 446, 369, 23623, 407
2 Mutations	Minsk 9.09% Vitebsk 2.27% Mogilev 15.90% Brest 11.36% Grodno 4.54% Gomel 2.27% Minsk obl 2.27%	21	11	443, 507, 489, 2262, 414, 411, 440, 548, 7285, 1416, 238, 447 2331, 1217, 2715, 370, 139 408, 469, 24276, 442
3 Mutations	Minsk 4.54% Vitebsk 6.81% Mogilev 0% Brest 4.54% Grodno 0% Gomel 0% Minsk obl 2.27%	8	8	412, 402, 94, 85, 894, 453, 3255, 3246
4 Mutations	Minsk 2.27% Minsk obl 4.54%	3	2	571, 471, 368

	555555	555555
	001112	223333
	670263	691234
#E._coli_str._K12_substr._MG1655_K-12	FGQSDE	HRSALG
	■ ■ ■ ■ (G) ■ ■ ■ ■	
#M._tuberculosis_Belarus-469_Magilev_rpoBA	..L.W.
#M._tuberculosis_MDR-Belarus-3255_Bres	.SK..G	...R..
#M._tuberculosis_MDR-Belarus-n1217_BreG	..CR..
#M._tuberculosis_Belarus-24276_rpoBG	..LR..
#M._tuberculosis_MDR-Belarus-2262_Bres	..E..G	D..WV.
#M._tuberculosis_MDR-Belarus-1416_BresA	...WV.
#M._tuberculosis_MDR-Belarus-489_viteb	..E..G	D.....
#M._tuberculosis_MDR-932_rpoBG	D..WGR
#M._tuberculosis_Belarus-894_vitebsk_rpoB	..K..A	D..GG.
#M._tuberculosis_MDR-Belarus-507_Magil	..E..G	D..VG.
#M._tuberculosis_MDR-Belarus-442_MagilG	..LVG.
#M._tuberculosis_MDR-Belarus-453_rpoB	C....A	D.LWG.
#M._tuberculosis_MDR_rpoBG	D..WG.
#M._tuberculosis_MDR-Belarus-1414_rpoBG	L..WG.
#M._tuberculosis_MDR-Belarus_rpoBA	D..VG.
#M._tuberculosis_MDR-85-vitebsk_rpoB	..E..G	L..GG.
#M._tuberculosis_Belarus-455_Minsk_rpoB	..E..G	D..WG.
#M._tuberculosis_Belarus-139_rpoBVA
#M._tuberculosis_MDR-Belarus-_rpoBG	..L...
#M._tuberculosis_Belarus-7285_rpoBA	D.....
#M._tuberculosis_MDR-Belarus-94_rpoB	.SK..G
#M._tuberculosis_MDR-Belarus-408_rpoB	.A.G.G	.P..W.
#M._tuberculosis_MDR-Belarus-414_rpoB	..E..G	L.....
#M._tuberculosis_MDR-Belarus-407_rpoBVG	...VG.
#M._tuberculosis_MDR-Belarus-2548_M_rpoBA	D..VG.
#M._tuberculosis_MDR-443-Minsk_rpoB	..E..G	D..WG.
#M._tuberculosis_MDR-Belarus-468_rpoB	..E..G
#M._tuberculosis_MDR-Belarus-402_rpoB	.A.G.A	.PL...
#M._tuberculosis_MDR-Belarus-388_rpoBG	..L...
#M._tuberculosis_MDR-Belarus-412_rpoB	...G.A	.P....
#M._tuberculosis_MDR-Belarus-443_Minsk	..E..G	D..WG.
#M._tuberculosis_MDR-Belarus-369_Minsk	..E..G
#M._tuberculosis_MDR-Belarus-384-MinskG	L.....
#M._tuberculosis_MDR-Belarus-571_rpoB	..E..A	D..WG.
#M._tuberculosis_Belarus-414_Minsk_rpoB	..E..G	L.....

Mutations at changeable sites of β subunits rpoB gene MT

Анализ мутаций

- 506 – 1 - no resistance formation
- 507 – 4 - indirect interaction with rifampicine
- 510 – 15 – direct interaction with rifampicine
- 512 – 3 - indirect interaction with rifampicine
- 516 – 2 - direct interaction with rifampicine
- 523 – 11 – no resistance formation
- 526 – 19 – direct interaction with rifampicine
- 529 – 3 - no resistance formation
- 531 – 8 - direct interaction with rifampicine
- 532 – 20 – no resistance formation
- 533 – 19 – direct interaction with rifampicine
- 534 – 1 - no resistance formation

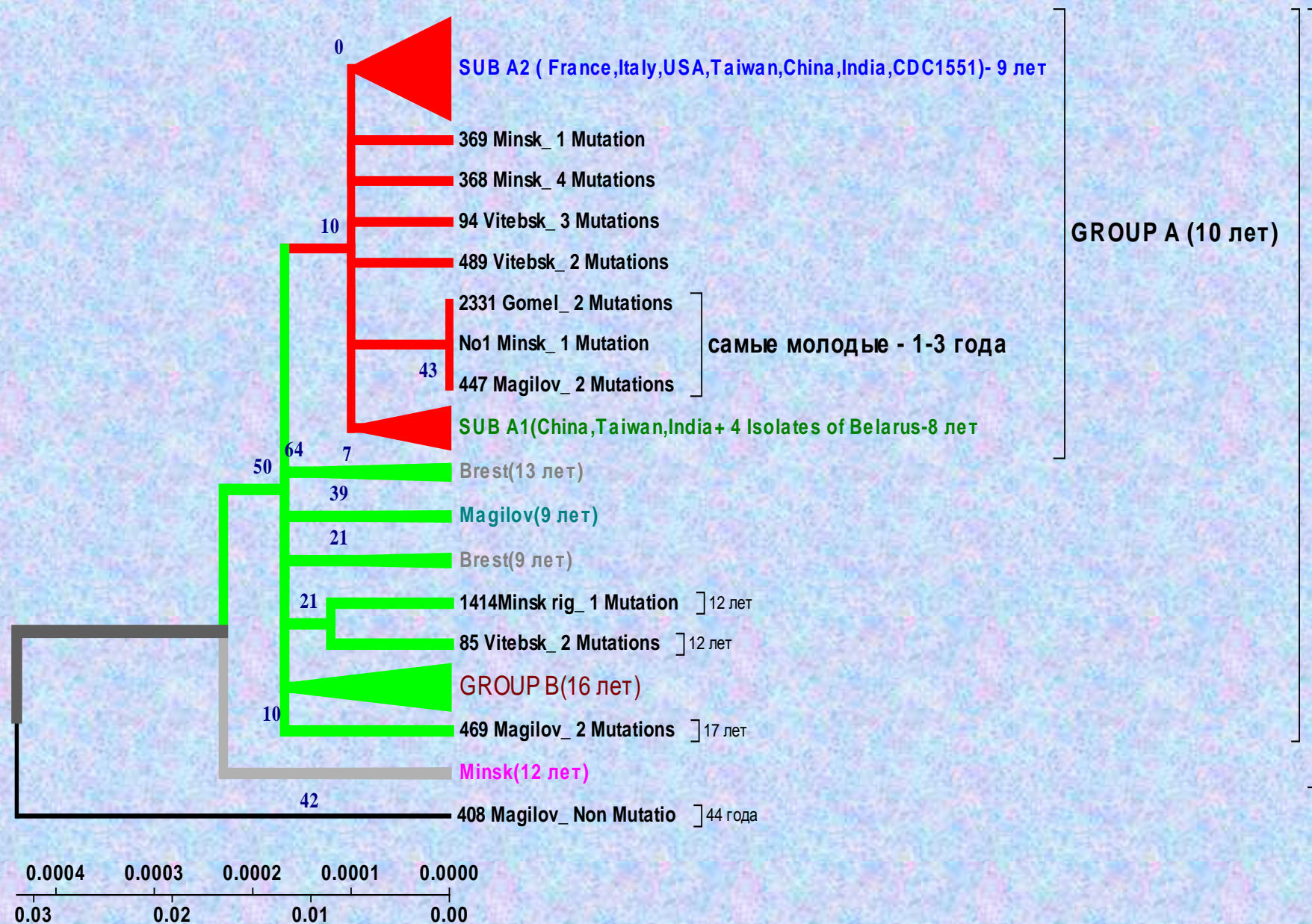
Основные мутации

Positions

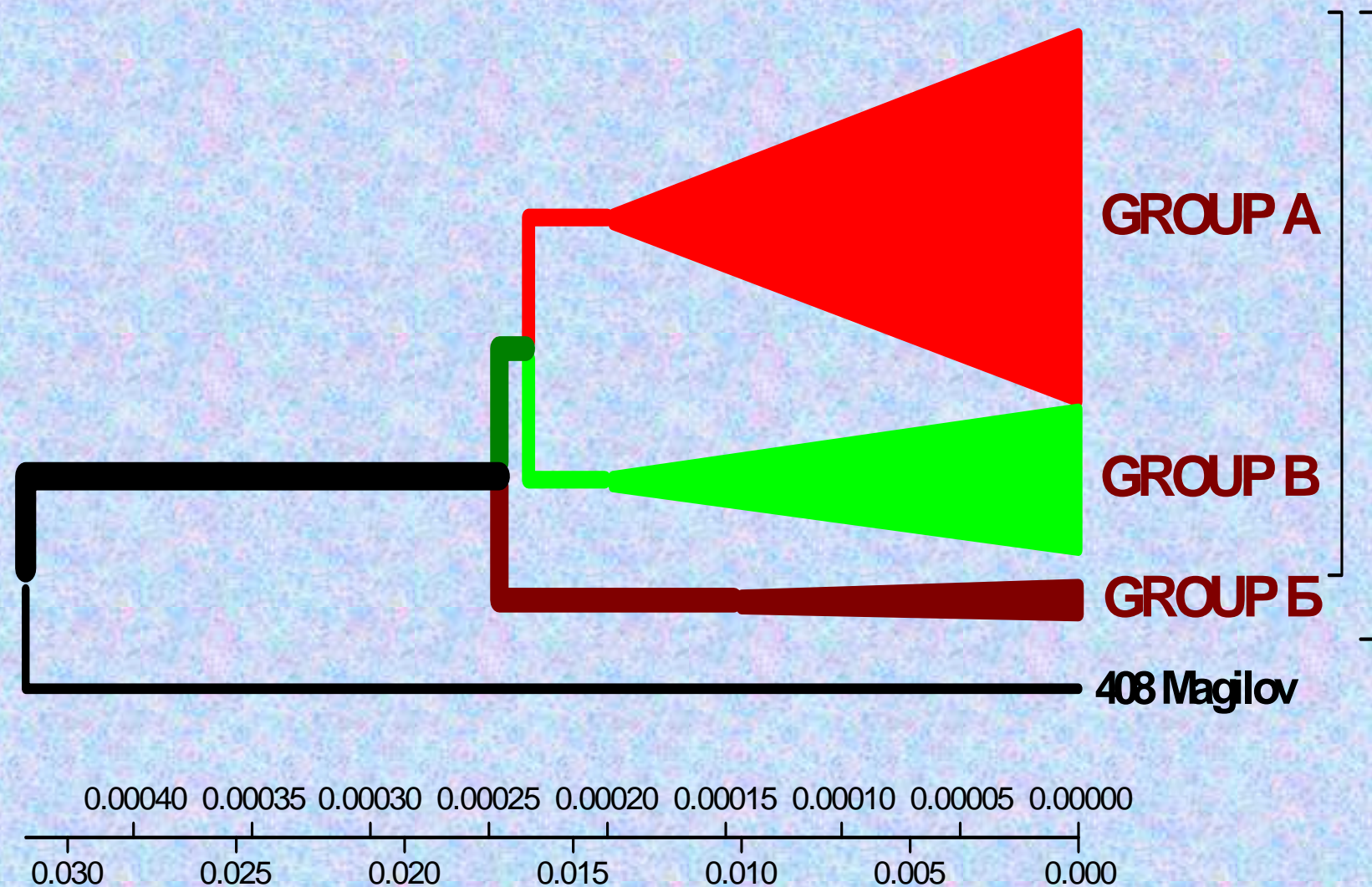
- 510 – 15 - 14,10%
- 523 – 11 - 10,34%
- 526 – 19 - 17,86%
- 531 – 8 - 7,52 %
- 532 – 20 - 18,80%
- 533 – 19 - 17,86%

86,5% of all significant for resistance formation mutations located at these sites

EVOLUTIONARY ANALYSIS OF **rpoB** gene SEQUENCES of 44 ISOLATES of MT

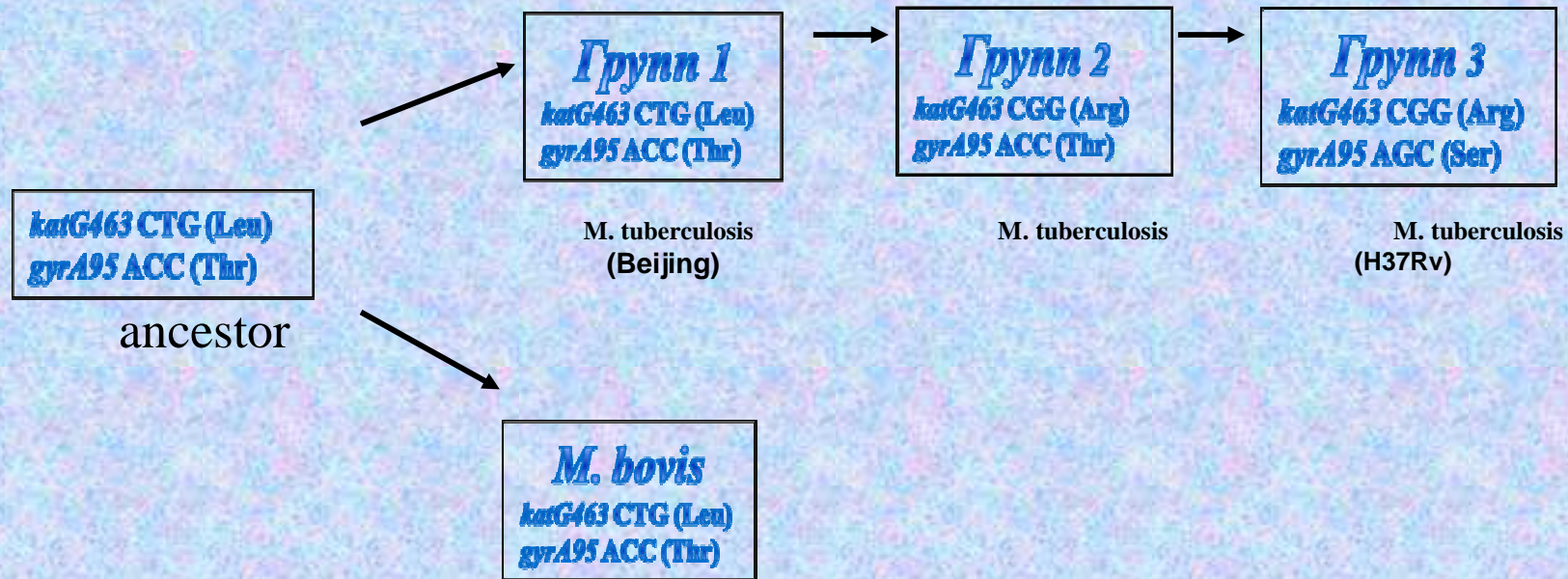


PHYLOGENETIC ANALYSIS SEQUENCES OF *rpoB* gene *M. tuberculosis* USING UPGMA METHOD



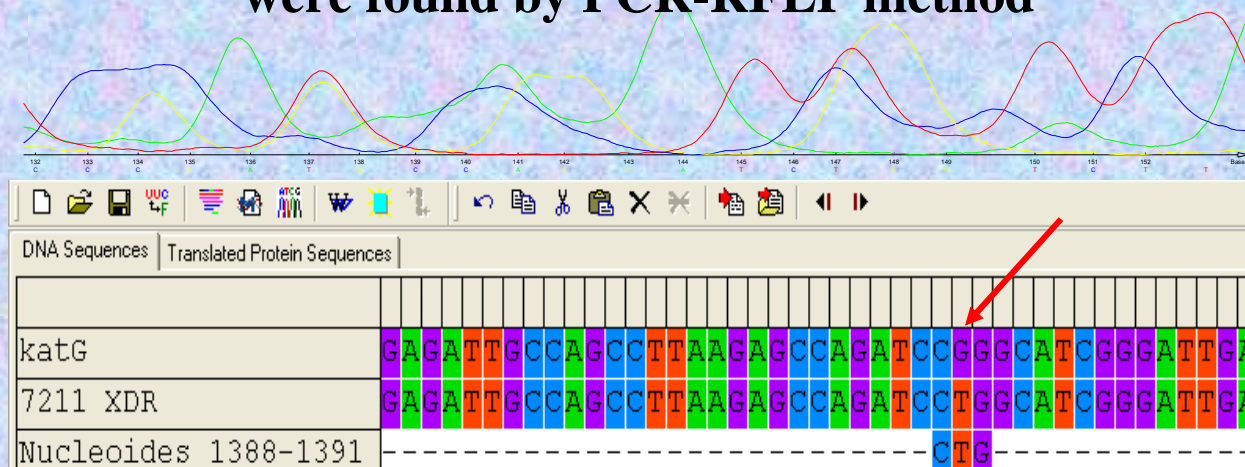
REGIONAL (GEOGRAFIC) ASSOCIATION OF rpoB- GENETIC GROUPS OF MT IN BELARUS



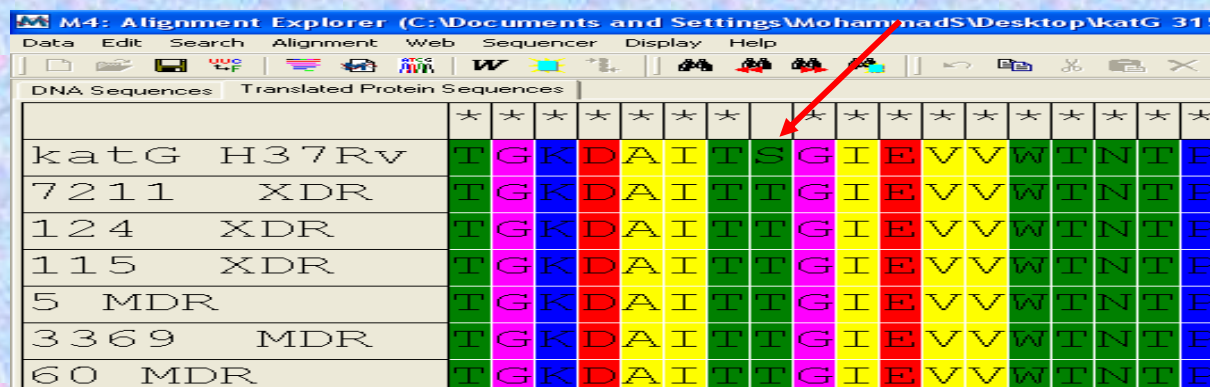


EVOLUTIONARY SCENARIA OF MICROORGANISMS *M. tuberculosis* complex and PRINCIPLE GENETIC GROUPS DETERMINATION BY SEQUENCING AND RFLP METHODS

Sequencing of amplicon *KatG* resistant and sensitive to isoniazide isolates detected 100% correspondence to those point mutations which were found by PCR-RFLP method



Amplicon **KatG463** (837 n.p.) sequencing with using primer for XDR isolets (G→ CTG).



A vtomatic sequencing amplicon **KatG315** in isolates (Ser→ Thr, AGC→ ACC)

MOLECULAR-GENETIC STRUCTURE OF CLINICAL ISOLATES *M.tuberculosis* USING CLASSIFICATION OF PRINCIPLE GENETIC GROUPS

ISOLATES	Description	GENETIC GROUPS		
		1	2	3
Standard	H37Rv , Academica	0%	0%	2(100 %)
Sensitive	n = 9	3 (33.3%)	4 (44.4%)	2 (22.2%)
MDR (n=104)	Minsk 1,2 (n=12)	9 (75 %)	2(16.6%)	1(8.3 %)
	Minsk obl (n=18)	10(55.5 %)	5 (27.7 %)	3 (16.6 %)
	Brest (n=4)	1(25%)	2(50%)	1(25%)
	Grodno obl.(n=10)	5(50%)	3 (30%)	2(20%)
	Vitebsk (n=7)	4 (57.1%)	2(28.5%)	1(14.3%)
	Vitebsk obl n= 11)	4 (36.3%)	4 (36.3%)	3(27.8%)
	Mogilev (n=9)	8 (88.9%)	1(11.1%)	0%
	Gomel (n=11)	6 (54.5%)	3 (22.3%)	2 (18.2%)
	Prisons (n=22)	10 (45,5%)	8 (36%)	4 (18%)
XDR	n = 31	15 (50%)	12 (40%)	4 (13%)
MDR- from Iran	n = 5	2 (40%)	2(40%)	1(20%)
Total	152	77 (50.6%)	48(31.6%)	27(17.8%)

M4: Alignment Explorer (C:\Documents and Settings\M.Setareh\Desktop\maghale TITOV whiB7\GENE ESLAH SHODEH khoshgel.mas)

Data Edit Search Alignment Web Sequencer Display Help

DNA Sequences Translated Protein Sequences

	* * * * *
whib7	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
Sens 264	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
Sens 253	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
Sens 282	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
Sens 238	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
MDR W411	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
MDR W71	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
MDR W600	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
MDR W1190	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
MDR 88	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
MDR7115	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
MDR 72	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 194x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 7211x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 12x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 111x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 1241x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 117x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 146x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 115x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 5X	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 124x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 3369x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 99x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *
XDR 1207x	V V L V P R T P R R L V L P C H V G D D L F A D T A G L E V A K L C V S C I R R Q C L A A A L R A E P G V G G E I F D G I V H K K P R G E P R R D A V A *

--//-- C--//--CvsCPIRRQC--//--GVWGG--//KRPRGRPRK_{DAVA}*

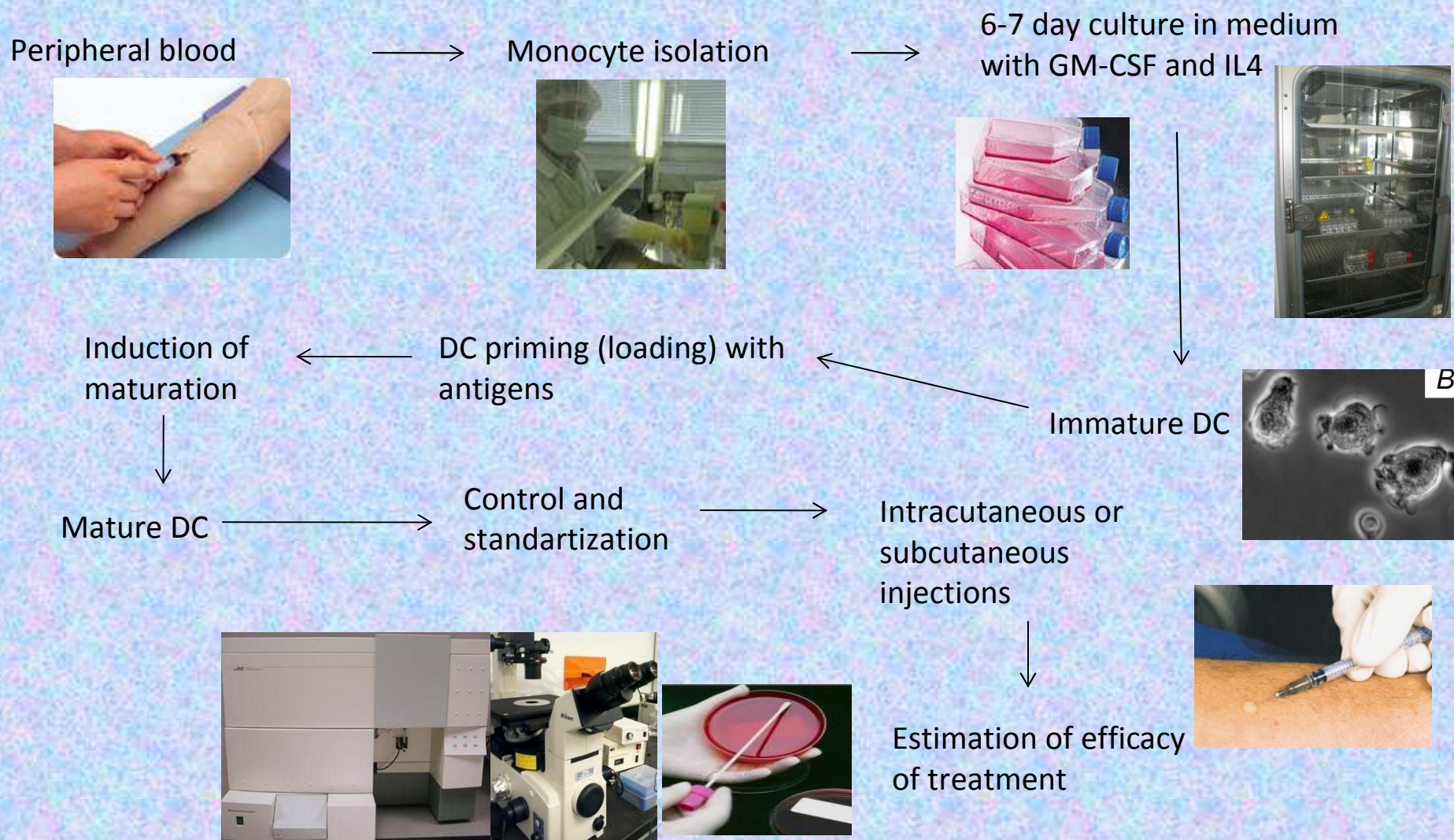
STRUCTURE of WhiB7 – protein in CLINICAL ISOLATES M.tuberculosis

We can see four cistein at conservative H T H- fragment which has terminal sequence G-(V)-W-G-G и C - terminal “AT-Hook”.

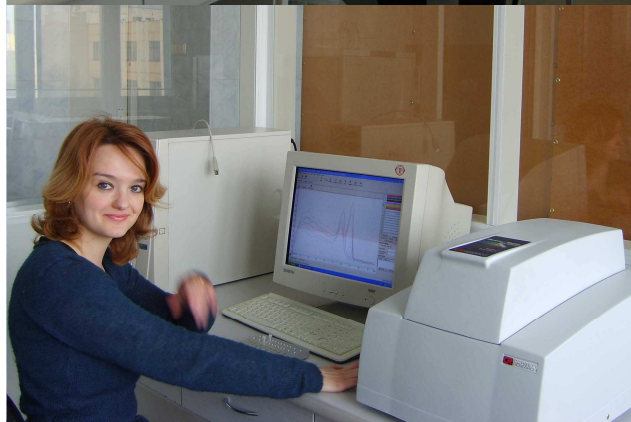
Comparative results of PCR for some genetic markers (*rpoB*, *whiB7*) used with the aim detection of *Mycobacterium tuberculosis*

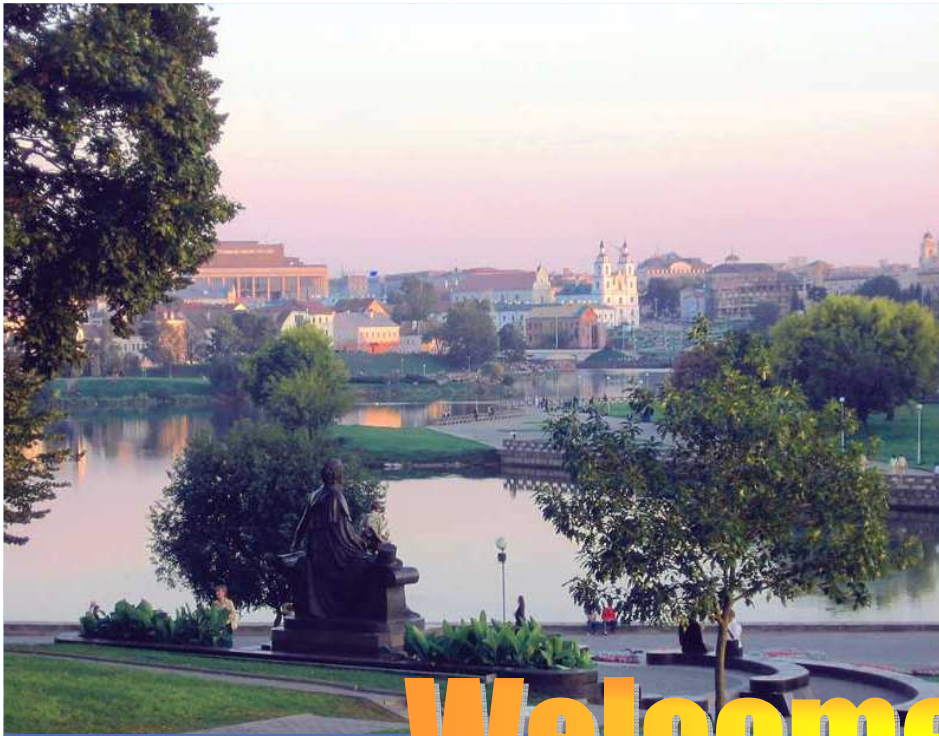
И з о л я т ы	<i>whiB7</i>	<i>rpoB</i>
Clinical isolates (n=33)		
Susceptible (6)	+	+
MDR (12)	+	+
XDR (15)	+	+
Standard non tuberculosis isolates		
<i>M. avium</i> ATCC 1603	--	+
<i>M. intracellular</i>	--	+
<i>M. fortuitum</i> ATCC 342	--	+
<i>M. terrae</i> ATCC 15755	--	+
<i>M. phlei</i>	--	+
<i>M. kansasii</i>	--	+
Non pathogenic isolates		
K Ch- IV группа	--	+
BZ/NC- IV группа	--	+
4 med - III группа	--	+
SIV III группа	--	+
B-ZO - III группа	--	+
VZK - IV группа	--	+
SPK- III группа	--	+
Control groups		
ZoP- <i>Candida rubrum</i> - as Control	--	--
Mk - <i>Candida rubrum</i> - as Control	--	--
<i>M. bovis</i>	+	+

Dendritic cells-based immunotherapy of chronic infections and cancer



laboratory of clinical and experimental microbiology





Welcome to Minsk



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