

In The Name Of God

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Medical sciences and Health Services

Collaborating Center for Tuberculosis
East Mediterranean Region

Mycobacteriology
Research Center

National Research Institute
Tuberculosis and Lung Disease

Collaborating Center for Tuberculosis
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تستهای حساسیت دارویی مایکوباکتریوم به روش مولکولار

Susceptibility Testing: Multiplex -PCR, PCR-SSCP and PCR -ELISA

Mycobacteriology Research Center (MRC)

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پرایمر های مورد نیاز
Primer

* *Primer for multiplex-PCR: Rifampicin Resistance (516,526,531)*

Primer name: rpoB516
Oligo sequence: rpoB516: CAGCTGAGCCAATTCATGGA

Primer name: rpoB526
Oligo sequence: rpoB526: CTGTCGGGGTTGACCCA

Primer name: rpoB531
Oligo sequence: rpoB531: CACAAGCGCCGACTGTC

Primer name: RIRm
Oligo sequence: RIRm: TTGACCCGCGCGTACAC

* *Primer for multiplex –PCR: Rifampicin Resistance (507,518,533)*

Primer name: rpoB507
Oligo sequence: rpoB507: GCGATCAAGGAGTTCGG

Primer name: rpoB518
Oligo sequence: rpoB518: TGAGCCAATTCATGGACCAGA

Primer name: rpoB533
Oligo sequence: rpoB533: CGCCGACTGTCGGCGCT

Primer name: RIRm
Oligo sequence: RIRm: TTGACCCGCGCGTACAC

* *Primer for multiplex-PCR: Rifampicin Resistance (511,513,522)*

Primer name: rpoB511
Oligo sequence: rpoB511: TTCGGCACCAGCCAGCT

Primer name: rpoB513
Oligo sequence: rpoB513: CACCAGCCAGCTGAGCC

Primer name: rpoB522
Oligo sequence: rpoB522: GACCAGAACAACCCGCTGT

Primer name: RIRm
Oligo sequence: RIRm: TTGACCCGCGCGTACAC

* *Primer for multiplex –PCR: Isoniazid resistance (KatG,inhA)*

Primer name: katGOF
Oligo sequence: katGOF:GCAGATGGGGCTGATCTACG

Primer name: katG5R
Oligo sequence: katG5R: ATACGACCTCGATGCCGC

Primer name: inhAP-15
Oligo sequence: InhAP-15: GCGCGGTCAGTTCCACA

Primer name: inhAPF2
Oligo sequence: inhAPF2: CACCCCGACAACCTATCG

* *Primer for Nested Allele Specific-PCR: Ethambutol Resistance*

Primer name: emb1 forward
Oligo sequence: emb1 f: GGGCGGGGCTCAATTGCC

Primer name: emb2 reverse
Oligo sequence: emb2 r: GCGCATCCACAGACTGGCGTC

Primer name: emb306 A
Oligo sequence: emb306 A: GACGACGGCTACATCCTGGGCA

Primer name: emb306B
Oligo sequence: emb306 B: GGTCGGCGACTCGGGCC

* *Primer for PCR-RFLP: Pyrazinamide Resistance*

Primer name: GYR-B forward
Oligo sequence: GYRB f: CCACCGACATCGGTGGATT

Primer name: GYR-B reverses
Oligo sequence: GYRAB r: CTGCCACTTGAGTTTGTACA

* *Primer for PCR-SSCP: Ciprofloxacin & Ofloxacin Resistance Resistance*

Primer name: GYR-A forward
Oligo sequence: GYRA f: CAGCTACATCGACTATGCGA

Primer name: GYR-A reverse
Oligo sequence: GYRA r: ATGAGGTACACCGAAGCCC

* *Primer for PCR-SSCP: Novobiocin and Clorobiocin Resistance*

Primer name: GYR-B forward
Oligo sequence: GYRB f: CCACCGACATCGGTGGATT

Primer name: GYR-B reverses
Oligo sequence: GYRAB r: CTGCCACTTGAGTTTGTACA

* *Primer for PCR-RFLP: Kanamycin and Amikacin Resistance*

Primer name: RRS1539
Oligo sequence: RRS1539: GGGGCGTTTTGCTGGTGCTCC

Primer name: RRS1096
Oligo sequence: RRS1096: GCGCAACCCTTGTCTCATGTTG

تهیه پرایمر
Primer Preparation

* زمانی که می خواهید پرایمر سفارش دهید سوالهای زیر را باید بدانید:

- سیکانس پرایمر
- مقدار آن
- روش تخلیص آن

**When you order primer from a commercial company, you should know the following Information:*

- Sequence (5'-3') of the primer, number of nucleotides
- The Unit size (e.g., 100 nmol)
- Purification standard (e.g., standard desalting or gel purified)

* زمانی که پرایمر مورد نظر از شرکت دریافت شد، اطلاعات زیر همراه پرایمر می باشد:

- وزن مولکولی
- مقدار GC
- درجه ذوب
- مقدار الیگونوکلئوتید ها

**The primer will then be synthesized and sent to you in a lyophilised (freeze-dried) form, together with the following data.*

- Molecular weight
- GC content
- Tm (50 mM NaCl)
- Amount of oligo (lyophilised). This could be expressed as OD260, nM and µg

About primer

مثلا پرایمر دریافتی شامل اطلاعات زیر است:

* *Stock solution*

- OD260 = 7.2
- Weight = 197.5 µg; 32.4 nmol
- Conc: 100 µM

برای تهیه stock solution با غلظت قید شده، به اندازه 100 µM آب مقطر اضافه کنید.

یعنی برای پرایمر فوق مقدار ۳۲۴ میکرولیتر آب اضافه می شود که غلظت پرایمر (۱۰۰ µM/µl) به دست می آید.

برای تهیه working solution با غلظت۱۰ µM: مقدار ۱۰ µl از محلول استوک پرایمر را با آب مقطر به حجم ۱۰۰ µl می رسانیم.

غلظت پرایمر با افزودن، یک میکرولیتر از محلول 10 µM primer در واکنش۲۰ µL، برابر با 10 Pmol می باشد.

Take for example a primer data sheet with the above information:

To make 100 µM/µl of stock solution; Check the weight of primer, (Here is 32.4 nmol).Then, based on this weight adds the 324 µl D.W to make a stock of 100µM/µl.

To make 10 µM of working solution: Take 10 µl of stock solution and bring the volume into 100 µl.

Reaction Mixture: Adding 1 µL (of the 10 µM of working solution), to a 20 µL PCR reaction will result in a final primer concentration of 10 Pmol of primer in 20 µL volume

Primer for multiplex-PCR: Rifampicin Resistance (516,526,531)

Primer name: rpoB516

Oligo sequence: rpoB516: 5'-CAGCTGAGCCAATTCATGGA-3'
Delivered: 9.5OD (259.7µg, 42.4 nmol) **Conce:** 100.0µM
MW: 6126 **Tm:** 57.3 **Synthesis Scale:** 0.04 **No.Bases:** 20 **GC%:** 50
Dissolve in: 424 µl .D.W

Primer name: rpoB526

Oligo sequence: rpoB526: 5'-CTGTCGGGGTTGACCCA-3'
Delivered: 8.4OD (248.7µg, 47.8 nmol) **Conce:** 100.0µM
MW: 5202 **Tm:** 57.6 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 64.7
Dissolve in: 478 µl .D.W

Primer name: rpoB531

Oligo sequence: rpoB531: 5'- CACAAGCGCCGACTGTC-3'
Delivered: 10.7 OD (307.1µg, 59.8 nmol) **Conce:** 100.0µM
MW: 5140 **Tm:** 57.6 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 64.7
Dissolve in: 598 µl .D.W

Primer name: RIRm

Oligo sequence: RIRm: 5'-TTGACCCGCGCGTACAC-3'
Delivered: 10.9 OD (324.1µg, 63.2 nmol) **Conce:** 100.0µM
MW: 5131 **Tm:** 57.6 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 64.7
Dissolve in: 632 µl .D.W

Multiplex-PCR: Rifampicin Resistance (516,526,531)

PCR Mixture for 25µl Reaction:

dNTP: (0.2 mM)1 µl
rpoB516:1 µl (10 pmol/ µl primer)
rpoB531 : 2 µl (10 pmol/ µl primer)
Mgcl2: 4 µl (5.5 mM)
DMSO: 0.5 µl (2%)
Note: PCR Buffer contains 1.5 mM Mgcl2.

UNG: 0.25 µl (0.25 U)
PCR Buffer:2.5 µl (1×)
rpoB526 : 1.5 µl (10 pmol/ µl primer)
RIRm : 2 µl (10 pmol/ µl primer)
Enzyme(Hot start taq):0.25 µl(1.25 U)
DNA Template :5 µl (50-100 ng)

PCR Cycle:

Initial denaturation 5 min95°c			
40 Cycles	**Step1**	**30 sec**	**95 ˚ C**
	Step2	**30 sec**	**68˚C**
	Step3	**30 sec**	**72 ˚C**
Final extension 7 min 72 ˚C			

PCR Product:

Primer rpoB516 and RIRm amplify a 218-bp fragment, Primer rpoB526 and RIRm amplify a 185-bp fragment and primer rpoB531 and RIRm amplify a 170 bp fragment.

Multiplex-PCR: Rifampicin Resistance (516,526,531)

∗ PCR Product:

Primer rpoB516 and RIRm amplify a 218-bp fragment, Primer rpoB526 and RIRm amplify a 185-bp fragment and primer rpoB531 and RIRm amplify a 170 bp fragment.

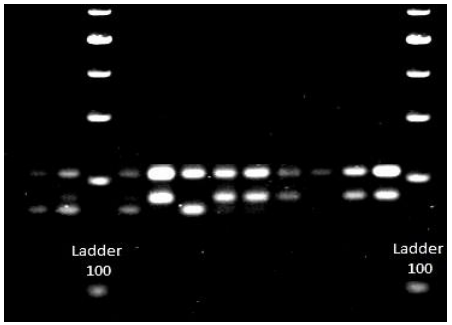
∗Evaluation of PCR-Product patterns:

After PCR, 7µl of PCR products (including loading buffer in PCR buffer) is examined for banding patterns by 8% Poly Acril amide gel electrophoresis.

Detection targets	Allele-specific primer (5′–3′)		Paired primer		Length of PCR product (bp)
*rpoB*516	*rpoB516*	CAGCTGAGCCAATTCATGGA	*RIRm*	TTGACCCGCGCGTACAC	218
*rpoB*526	*rpoB526*	CTGTCGGGGTTGACCCA			185
*rpoB*531	*rpoB531*	CACAAGCGCCGACTGTC			170

Photograph of PCR-Product using rpoB516, rpoB526, and rpoB531 and RIRm primer.

The 218-bp band represents *rpoB* codon 516-specific PCR represents *rpoB* codon 526-specific PCR product; and the 170-bp band, represents *rpoB* codon 531-specific PCR product. In case of a mutation existing in a given codon or region, no related allele-specific PCR product is generated.



Primer for multiplex-PCR: Rifampicin Resistance (507,518,533)

Primer name: rpoB507

Oligo sequence: rpoB507: 5'-GCGATCAAGGAGTTCGG-3'
Delivered: 5.8OD (163.µg, 26.5 nmol) **Conce:** 100.0µM
MW: 6173 **Tm:** 59.4 **Synthesis Scale:** 0.04 **No.Bases:** 20 **GC%:** 55
Dissolve in: 265 µl .D.W

Primer name: rpoB518

Oligo sequence: rpoB518: 5'-TGAGCCAATTCATGGACCAGA-3'
Delivered: 4.9OD (130.2µg, 20.2 nmol) **Conce:** 100.0µM
MW: 643.9 **Tm:** 57.9 **Synthesis Scale:** 0.04 **No.Bases:** 21 **GC%:** 47.6
Dissolve in: 202 µl .D.W

Primer name: rpoB533

Oligo sequence: rpoB533: 5'-CGCCGACTGTCGGCGCT-3'
Delivered: 4.2 OD (130.8µg, 25.3 nmol) **Conce:** 100.0µM
MW: 5163 **Tm:** 62.4 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 76.5
Dissolve in: 253 µl .D.W

Primer name: RIRm

Oligo sequence: RIRm: 5'-TTGACCCGCGCGTACAC-3'
Delivered: 10.9 OD (324.1µg, 63.2 nmol) **Conce:** 100.0µM
MW: 5131 **Tm:** 57.6 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 64.7
Dissolve in: 632 µl .D.W

Multiplex-PCR: Rifampicin Resistance (507,518,533)

PCR Mixture for 25µl Reaction:

dNTP: (0.2 mM)1 µl
rpoB507 : 2 µl (10 pmol/ µl primer)
rpoB533 : 0.5 µl (10 pmol/ µl primer)
Mgcl2: 4 µl (5.5 mM)
DMSO: 0.5 µl (2%)
Note: PCR Buffer contains 1.5 mM Mgcl2.

UNG: 0.25 µl (0.25 U)
PCR Buffer:2.5 µl (1×)
rpoB518 : 2 µl (10 pmol/ µl primer)
RIRm : 2 µl (10 pmol/ µl primer)
Enzyme (taq):0.25 µl (1.25 U)
DNA Template :5 µl (50-100 ng)

PCR Cycle:

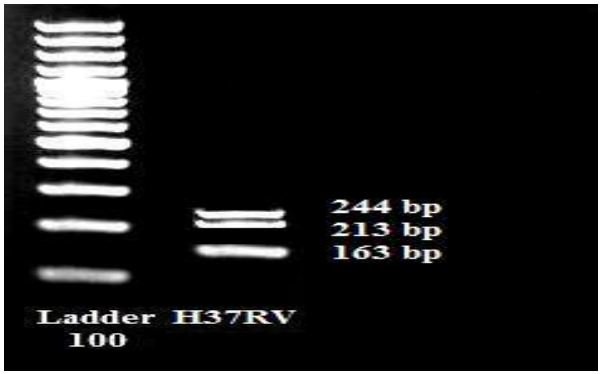
Initial denaturation 5 min95°c			
40 Cycles	**Step1**	**30 sec**	**95 ˚ C**
	Step2	**30 sec**	**68˚C**
	Step3	**30 sec**	**72 ˚C**
Final extension 7 min 72 ˚C			

PCR Product:

Primer rpoB507 and RIRm amplify a 244-bp fragment, Primer rpoB518 and RIRm amplify a 213-bp fragment and primer rpoB533 and RIRm amplify a 163 bp fragment.

Multiplex-PCR: Rifampicin Resistance (507,518,533)

Photograph of PCR-Product using rpoB507, rpoB518, and rpoB533 and RIRm primer.



The 244-bp band represents *rpoB* codon 507-specific PCR product; the 213-bp band, represents *rpoB* codon 518-specific PCR product; and the 163-bp band, represents *rpoB* codon 533-specific PCR product. In case of a mutation existing in a given codon or region, no related allele-specific PCR product is generated.

Evaluation of PCR-Product patterns:

After PCR, 7µl of PCR products (including loading buffer in PCR buffer) is examined for banding patterns by 8% Poly Acryl Amide gel electrophoresis. Fragments are visualized by ethidium bromide staining and UV light.

Detection targets	Allele-specific primer (5′–3′)		Paired primer		Length of PCR product
*rpoB*507	rpoB507	GCGATCAAGGAGTTCGG	RIRm	TTGACCCGCGCGTACAC	244
*rpoB*518	rpoB518	TGAGCCAATTCATGGACCAGA			213
*rpoB*533	rpoB533	CGCCGACTGTCGGCGCT			163

Primer for multiplex-PCR: Rifampicin Resistance (511,513,522)

Primer name: rpoB511

Oligo sequence: rpoB511: 5'- TTCGGCACCAGCCAGCT -3'
Delivered: 4.0OD (117.6µg, 22.9 nmol) **Conce:** 100.0µM
MW: 5131 **Tm:** 57.6 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 64.7
Dissolve in: 229 µl .D.W

Primer name: rpoB513

Oligo sequence: rpoB513: 5'-CACCAGCCAGCTGAGCC-3'
Delivered: 4.0 OD (116.5µg, 22.7 nmol) **Conce:** 100.0µM
MW: 5125 **Tm:** 60 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 70.6
Dissolve in: 227 µl .D.W

Primer name: rpoB522

Oligo sequence: rpoB522: 5'-GACCAGAACAACCCGCTGT -3'
Delivered: 6.2 OD (171.7µg, 29.8 nmol) **Conce:** 100.0µM
MW: 5767 **Tm:** 58.8 **Synthesis Scale:** 0.04 **No.Bases:** 19 **GC%:** 57.9
Dissolve in: 298 µl .D.W

Primer name: RIRm

Oligo sequence: RIRm: 5'-TTGACCCGCGCGTACAC-3'
Delivered: 10.9 OD (324.1µg, 63.2 nmol) **Conce:** 100.0µM
MW: 5131 **Tm:** 57.6 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 64.7
Dissolve in: 632 µl .D.W

Multiplex PCR: Rifampicin Resistance (511,513,522)

PCR Mixture for 25µl Reaction:

dNTP: (0.2 mM)1 µl
rpoB511 : 2 µl (10 pmol/ µl primer)
rpoB522 : 0.5 µl (10 pmol/ µl primer)
Mgcl2: 4 µl (5.5 mM)
DMSO: 0.5 µl (2%)
Note: PCR Buffer contains 1.5 mM Mgcl2.

UNG: 0.25 µl (0.25 U)
PCR Buffer:2.5 µl (1×)
rpoB513 : 1.5 µl (10 pmol/ µl primer)
RIRm : 1 µl (10 pmol/ µl primer)
Enzyme (taq):0.25 µl (1.25 U)
DNA Template :5 µl (50-100 ng)

PCR Cycle:

Initial denaturation 5 min95°c			
40 Cycles	**Step1**	**30 sec**	**95 ˚ C**
	Step2	**30 sec**	**68˚C**
	Step3	**30 sec**	**72 ˚C**
Final extension 7 min 72 ˚C			

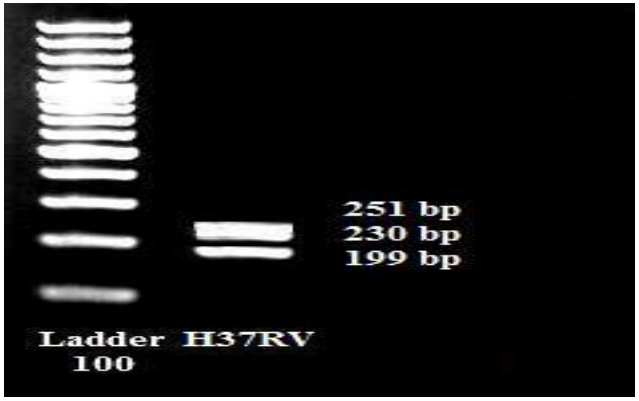
PCR Product:

Primer rpoB511 and RIRm amplify a 230-bp fragment, Primer rpoB513 and RIRm amplify a 251-bp fragment and primer rpoB522 and RIRm amplify a 199-bp fragment.

Multiplex PCR: Rifampicin Resistance (511,513,522)

Photograph of PCR-Product using rpoB511, rpoB513, and rpoB522 and RIRm primer.

The 230-bp band represents *rpoB* codon 511-specific PCR product; the 251-bp band, represents *rpoB* codon 513-specific PCR product; and the 199-bp band, represents *rpoB* codon 522-specific PCR product. In case of a mutation existing in a given codon or region, no related allele-specific PCR product is generated.



Evaluation of PCR-Product patterns:

After PCR, 7µl of PCR products (including loading buffer in PCR buffer) is examined for banding patterns by 8% Poly Acryl Amide gel electrophoresis. Fragments are visualized by ethidium bromide staining and UV light.

Detection targets	Allele-specific primer (5′–3′)		Paired primer		Length of PCR product
*rpoB*511	rpoB511	TTCGGCACCAGCCAGCT	RIRm	TTGACCCGCGCGTACAC	230
*rpoB*513	rpoB513	CACCAGCCAGCTGAGCC			251
*rpoB*522	rpoB522	GACCAGAACAACCCGCTGT			199

Primer for multiplex-PCR: Isoniazid resistance (KatG,inhA)

Primer name: katGOF

Oligo sequence: katGOF: 5'-GCAGATGGGGCTGATCTACG-3'
Delivered: 12.4 OD (345.7µg, 55.8 nmol) **Conce:** 100.0µM
MW: 6198 **Tm:** 61.4 **Synthesis Scale:** 0.04 **No.Bases:** 20 **GC%:** 60
Dissolve in: 558 µl .D.W

Primer name: katG5R

Oligo sequence: katG5R: 5'-ATACGACCTCGATGCCGC-3'
Delivered: 10.4OD (300.6µg, 55.2 nmol) **Conce:** 100.0µM
MW: 5118 **Tm:** 58.2 **Synthesis Scale:** 0.04 **No.Bases:** 18 **GC%:** 61.1
Dissolve in: 552 µl .D.W

Primer name: inhAP-15

Oligo sequence: InhAP-15: 5'-GCGCGGTCAGTTCCACA-3'
Delivered: 10.2 OD (297.8 µg, 57.6 nmol) **Conce:** 100.0µM
MW: 517 **Tm:** 57.6 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 64.7
Dissolve in: 576 µl .D.W

Primer name: inhAPF2

Oligo sequence: inhAPF2: 5'-CACCCCGACAACCTATCG-3'
Delivered: 11.2 OD (326.6µg, 60.8 nmol) **Conce:** 100.0µM
MW: 5374 **Tm:** 58.2 **Synthesis Scale:** 0.04 **No.Bases:** 18 **GC%:** 61.1
Dissolve in: 608 µl .D.W

Multiplex-PCR: Isoniazid resistance (KatG,inhA)

PCR Mixture for 25µl Reaction:

dNTP: (0.2 mM)1 µl

katGOF: 1 µl (10 pmol/ µl primer)

InhAP-15 :1 µl (10 pmol/ µl primer)

Mgcl2: 4 µl (5.5 mM)

DMSO: 0.5 µl (2%)

Note: PCR Buffer contains 1.5 mM Mgcl2

UNG: 0.25 µl (0.25 U)

PCR Buffer:2.5 µl (1×)

katG5R :1 µl (10 pmol/ µl primer)

inhAPF2:1 µl (10 pmol/ µl primer)

Enzyme (taq):0.25 µl (1.25 U)

DNA Template :5 µl (50-100 ng)

PCR Cycle:

Initial denaturation 5 min 95°c			
40 Cycles	**Step1**	**30 sec**	**95 ˚C**
	Step2	**30 sec**	**70 ˚C**
	Step3	**30 sec**	**72 ˚C**
Final extension 7 min 72 ˚C			

Multiplex-PCR: Isoniazid resistance (KatG,inhA)

PCR Product:

Primer katGOF and katG5R amplify a 292-bp fragment and Primer inhAP15 and inhAPF2 amplify a 270-bp fragment.

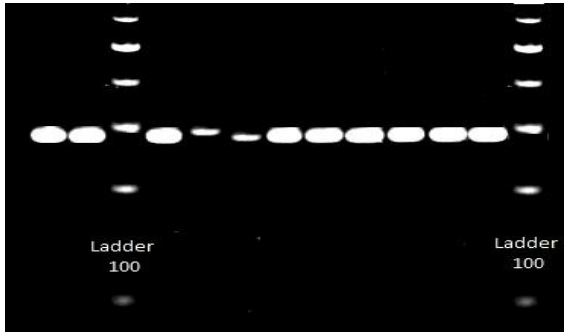
Evaluation of PCR-Product patterns:

After PCR, 7µl of PCR products (including loading buffer in PCR buffer) is examined for banding patterns by 8% Poly Acryl Amide gel electrophoresis. Fragments are visualized by ethidium bromide staining and UV light.

Detection targets	Allele-specific primer (5′–3′)		Paired primer		Length of CR product (bp)
*katG*315	katG5R	ATACGACCTCGATGCCGC	katGOF	GCAGATGGGGCTGATCTACG	292
mabA-inhA: −15	InhAP-15	GCGCGGTCAGTTCCACA	inhAPF2	CACCCCGACAACCTATCG	270

Photograph of PCR-Product using katGOF, katG5R, inhAP15 and inhAPF2 primer.

The 270-bp band, represents *katG* codon 315-specific PCR product and the 292-bp band, represents *inhA-mabA* promoter region specific PCR product. In case of a mutation existing in a given codon or region, no related allele-specific PCR product is generated.



Primer for Nested Allele Specific-PCR: Ethambutol Resistance

Primer name: emb1 forward

Oligo sequence: emb1 f: 5'-GGGCGGGGCTCAATTGCC-3'
Delivered: 4.0OD (132.2µg,) **Conce:** 100.0µM
MW: 5557 **Synthesis Scale:** 0.04 **No.Bases:** 18
Dissolve in: 240 µl .D.W

Primer name: emb2 reverse

Oligo sequence: emb2 r: 5'-GCGCATCCACAGACTGGCGTC-3'
Delivered: 6.0 OD (175.7µg, 27.5 nmol) **Conce:** 100.0µM
MW: 6392 **Tm:** 65.7 **Synthesis Scale:** 0.04 **No.Bases:** 21 **GC%:** 66.7
Dissolve in: 275 µl .D.W

Primer name: emb306 A

Oligo sequence: emb306 A: 5'-GACGACGGCTACATCCTGGGCA-3'
Delivered: 8.2 OD (232.3µg, 34.4 nmol) **Conce:** 100.0µM
MW: 6745 **Tm:** 65.8 **Synthesis Scale:** 0.04 **No.Bases:** 22 **GC%:** 63.6
Dissolve in: 344 µl .D.W

Primer name: emb306B

Oligo sequence: emb306 B: 5'-GGTCGGCGACTCGGGCC-3'
Delivered: 4.0 OD (120.7µg, 23.1 nmol) **Conce:** 100.0µM
MW: 5228 **Tm:** 64.8 **Synthesis Scale:** 0.04 **No.Bases:** 17 **GC%:** 82.4
Dissolve in: 231 µl .D.W

Nested Allele Specific-PCR: Ethambutol Resistance

In the first step a 325 bp fragment is amplified with emb1F and emb2 R primer.PCR compositions are as bellow.

PCR Mixture for 25µl Reaction:

dNTP: (0.2 mM)1 µl
emb1F : 0.5 µl (10 pmol/ µl primer)
emb2 R : 0.2 µl (10 pmol/ µl primer)
Mgcl2: 1.5 µl (1.5 mM)
PCR Buffer:2.5 µl (1×)
Enzyme (taq):0.25 µl (1.25 U)
DNA Template :5 µl (50-100 ng)

PCR Cycle:

Initial denaturation 5 min95°c			
30 Cycles	**Step1**	**30 sec**	**94 ˚C**
	Step2	**40 sec**	**65 ˚C**
	Step3	**30 sec**	**72 ˚C**
Final extension 10 min 72 ˚C			

Nested PCR Mixture for 25µl Reaction:

In the second step two 160 and 220 bp fragments are amplified with emb306A, emb306B and emb2 R primer in the case of sensitivity to Ethambutol. Existance of 160 bp or 220 bp fragment represents mutation in embB306 A. PCR compositions are as bellow.

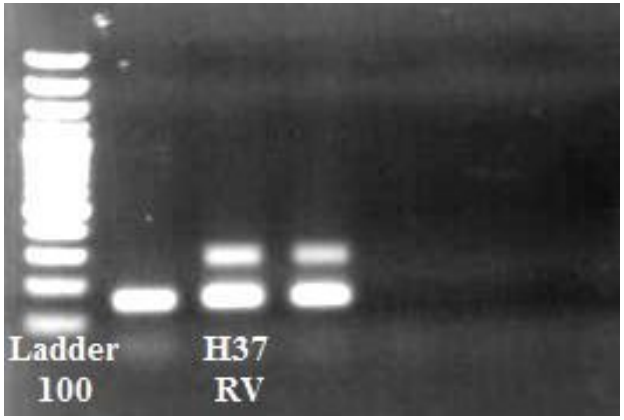
dNTP: (0.2 mM)1 µl
emb306A: 0.53 µl (10 pmol/ µl)
Emb2 R: 2 µl (10 pmol/ µl)
emb306B: 0.5 µl (10 pmol/ µl)
Enzyme (taq):0.25 µl (1.25 U)
PCR Buffer:5 µl (1×)
Mgcl2: 1.5 µl (1.5 mM)
DNA Template: 5 µl of first step PCR product

Nested Allele Specific- PCR: Ethambutol Resistance

PCR Product:

Primer emb1Fand emb2 R amplifies a 325-bp fragment.

Photograph of PCR-Product using emb306A, emb306B and emb2 R primer.



Evaluation of PCR-Product patterns:

After PCR, 5µl of PCR products plus 2µl of loading buffer (0.25% bromophenol blue, 40% sucrose in water) is examined for banding patterns by 1.5% Agarose gel electrophoresis. Fragments are visualized by ethidium bromide staining and UV light.

Primer for PCR-RFLP: Pyrazinamide Resistance

Primer name: Pnc A forward

Oligo sequence: Pnc A F: 5'-GGCGT CATGGACCTATATC-3'

Delivered: 6.8OD (194.9µg, 32 nmol) **Conce:** 100.0µM

MW: 6093 **Tm:** 59.4 **Synthesis Scale:** 0.04 **No.Bases:** 20 **GC%:55**

Dissolve in: 320 µl .D.W

Primer name: Pnc A reverse

Oligo sequence: Pnc A R: 5'-CAACAGTTCATCCCGGTTC-3'

Delivered: 6.2 OD (182.9µg, 32 nmol) **Conce:** 100.0µM

MW: 5724 **Tm:** 56.7 **Synthesis Scale:** 0.04 **No.Bases:** 19 **GC%:** 52.6

Dissolve in: 320 µl .D.W

PCR-RFLP: Pyrazinamide Resistance

In the first step a 672 bp fragment is amplified with pnc A f and pnc A r primer. PCR compositions are as bellow.

PCR Mixture for 50 µl Reaction:

dNTP: (0.2 mM)1 µl

pnc A f: 0.5 µl (10 pmol/ µl primer)

pnc A r: 0.8 µl (10 pmol/ µl primer)

Mgcl2: 2.5 µl (2.5 mM)

DNA Template :5 µl (50-100 ng)

PCR Buffer: 5 µl (1×)

Enzyme (taq):0.25 µl (1.25 U)

PCR Cycle:

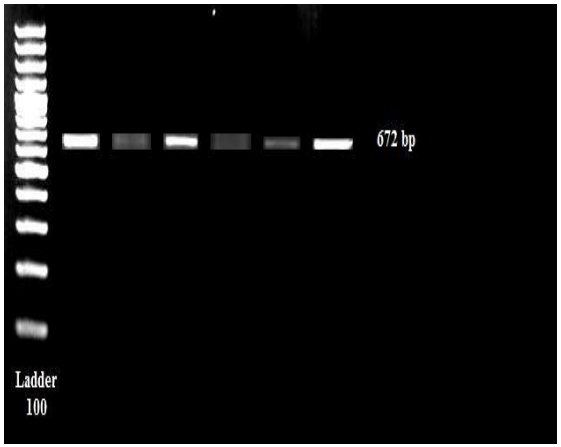
Initial denaturation 5 min 95°c			
30 Cycles	**Step1**	**30 sec**	**95 ˚C**
	Step2	**40 sec**	**60 ˚C**
	Step3	**60 sec**	**72 ˚C**
Final extension 10 min 72 ˚C			

PCR-RFLP: Pyrazinamide Resistance

PCR Product:

Primer pnc A F and pnc A R amplify a 672-bp fragment.

Photograph of PCR-Product using pnc A F and pnc A R primer.



Evaluation of PCR-Product patterns:

After PCR, 5µl of PCR products plus 2µl of loading buffer (0.25% bromophenol blue, 40% sucrose in water) is examined for banding patterns by 1.5% Agarose gel electrophoresis. Fragments are visualized by ethidium bromide staining and UV light.

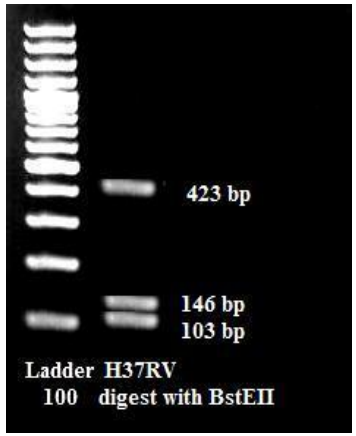
PCR-RFLP: Pyrazinamide Resistance

PCR-RFLP Reaction:

10 µl of PCR product (0.1-0.5 µg) is added directly to a mixture containing 1x restriction buffer and 1 µl of BstEII restriction enzyme. Then total volume of the mixture is added to 30 µl with distilled water. The mixture is incubated for 1-16 h at 37°C.

Detection targets	Allele-specific primer (5′–3′)		Paired primer		Length of PCR product
Pnc A	PncAF	GGCGT CATGGACCTATATC	Pnc A R	CAACAGTTCATCCCGGTTC	672

Photograph of PCR-Product digeted using BstEII restriction enzyme.



Evaluation of restriction patterns:

Finally digested PCR product is mixed with 5 ul of gel loading buffer (0.25% bromophenol blue, 40% sucrose in water) and 15 µl of the mixture is loaded onto a 3% agarose gel. Fragments are visualized by ethidium bromide staining and UV light. Susceptible strains produce 103,146 and 423 bp fragments. Existance of 146 and 526 bp fragments represent point mutation in codon 423 of the pncA gene.

Primer for PCR-RFLP: Kanamycin and Amikacin Resistance

Primer name: RRS1539

Oligo sequence: RRS1539: GGGGCGTTTTGCTGGTGCTCC
Delivered: 4.4 OD (136.7µg, 21.1 nmol) **Conce:** 100.0µM
MW: 6476 **Tm:** 65.7 **Synthesis Scale:** 0.04 **No.Bases:** 21 **GC%:** 66.7
Dissolve in: 211 µl .D.W

Primer name: RRS1096

Oligo sequence: RRS1096: GCGCAACCCTTGTCTCATGT TG
Delivered: 5.3 OD (118.6µg, 23.8 nmol) **Conce:** 100.0µM
MW: 6677 **Tm:** 62.1 **Synthesis Scale:** 0.04 **No.Bases:** 22 **GC%:** 54.5
Dissolve in: 238 µl .D.W

PCR-RFLP: Kanamycin and Amikacin Resistance

PCR Mixture for 50 µl Reaction:

dNTP: (0.2 mM)1 µl

RRS1096: 1 µl(10 pmol/ µl primer)

RRS1539: 1 µl (10 pmol/ µl primer)

Mgcl2: 10 µl (10 mM)

DNA Template :5 µl (50-100 ng)

PCR Buffer: 5 µl (1×)

Enzyme (taq):0.25 µl (1.25 U)

PCR Cycle:

Initial denaturation 10 min 95°c			
40 Cycles	**Step1**	**60 sec**	**94 ˚C**
	Step2	**60 sec**	**60 ˚C**
	Step3	**60 sec**	**72 ˚C**
Final extension 5 min 72 ˚C			

PCR-RFLP: Kanamycin and Amikacin Resistance

PCR Product:

The 300-bp DNA fragments carrying the rrs gene are amplified by primer RRS1096 and RRS1539 .Ribosomal binding of KM is affected by mutation in the 1400 region of the rrs gene and mutations in this region produce resistance to various aminoglycosides. MTB has only a single copy of the rrs gene, so such a point mutation results in resistance to AK-KM.

Evaluation of PCR-Product patterns:

After PCR, 5µl of PCR products plus 2µl of loading buffer (0.25% bromophenol blue, 40% sucrose in water) is examined for banding patterns by 1.5% Agarose gel electrophoresis. Fragments are visualized by ethidium bromide staining and UV light.

PCR-RFLP Reaction:

The most frequent substitution occurs at position 1400 and rarely substitution from C to T at position 1401. These mutants are distinguished from wild-type strains by digestion with the restriction endonucleases TaiI and Tsp45I. PCR-RFLP analysis. The PCR products are digested with the restriction endonucleases TaiI, Tsp45I, BstUI, and DdeI. 10 µl of PCR product (0.1-0.5 µg) is added directly to a mixture containing 1x restriction buffer and 1 µl of restriction enzyme. Then total volume of the mixture is added to 30 µl with distilled water. The mixture is incubated for 1-16 h at 37°C.

PCR-RFLP: Kanamycin and Amikacin Resistance

Detection targets	Allele-specific primer (5′–3′)		Paired primer		Length of PCR product
rrs gene	RRS1539	GGGGCGTTTTGCTGGTGCTCC	RRS1096	GCGCAACCCTTGTCTCATGTTG	300

Evaluation of restriction patterns:

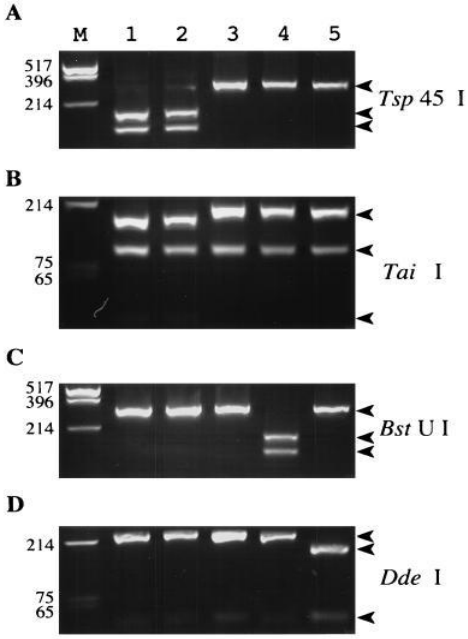
Digested PCR products are mixed with 5 ul of gel loading buffer (0.25% bromophenol blue, 40% sucrose in water) and 15 µl of the mixture is separated by electrophoresis on a 4% agarose gel. The resulting bands are then visualized by by ethidium bromide staining and UV light.

The PCR products obtained from wild-type are digested with both Tsp45I and TaiI. On the contrary, BstUI and DdeI digested the PCR product with the A-to-G mutation at position 1400 and the G-to-T mutation at position 1483 .The PCR-generated DNA fragment with this mutant sequence are digested into two fragments with lengths of 178 and 122 bp, respectively, whereas the PCR-generated DNA fragment with the wild-type sequence or some other mutant sequence are resistant to this enzyme. The mutation at position 1483 (from G to T) converts the PCR product into one that is digestible by the restriction endonuclease DdeI (recognition sequence, C^TRAG) into four fragments with lengths of 196, 48, 38, and 14 bp.

PCR-RFLP: Kanamycin and Amikacin Resistance

Photograph of analysis of the PCR products by PCR-RFLP analysis amplified using primer TBrrs1250 and TBrrivs38, digested with restriction endonucleases

(A) PCR-generated fragments are digested with Tsp45I. (B) PCR-generated fragments are digested with TaiI. (C) PCR-generated fragments are digested with BstUI. (D) PCR-generated fragments are digested with DdeI. Lane M, DNA marker; lane 1, PCR product from a kanamycin-susceptible isolate; lane 2, PCR product from a kanamycin-resistant isolate with the wild-type sequence; lane 3, PCR product from a kanamycin-resistant isolate with a mutation at position 1401 (C to T); lane 4, PCR product from a kanamycin-resistant isolate with a mutation at position 1400 (A to G); lane 5, PCR product from a kanamycin-resistant isolate with mutations at position 1401 (C to A) and position 1483 (G to T). Numbers on the left indicate the lengths (in base pairs) of the DNA marker (pUC118 HinfI digests).



PCR-SSCP: Ciprofloxacin & Ofloxacin Resistance

Primer name: GYR-A forward

Oligo sequence: GYRA f: CAGCTACATCGACTATGCGA
Delivered: 7.2 OD (197.5µg, 32.4 nmol) **Conce:** 100.0µM
MW: 6086 **Tm:** 57.3 **Synthesis Scale:** 0.04 **No.Bases:** 20 **GC%:** 50
Dissolve in: 324 µl .D.W

Primer name: GYR-A reverse

Oligo sequence: GYRA r: ATGAGGTACACCGAAGCCC
Delivered: 6.8OD (204.8µg, 35.2 nmol) **Conce:** 100.0µM
MW: 5811 **Tm:** 58.8 **Synthesis Scale:** 0.04 **No.Bases:** 19 **GC%:** 57.9
Dissolve in: 352 µl .D.W

PCR-SSCP: Ciprofloxacin & Ofloxacin Resistance

PCR Mixture for 50 µl Reaction:

dNTP: (0.4 mM)2 µl

GYRA f: 0.8 µl (10 pmol/ µl primer)

GYRA r: 0.8 µl (10 pmol/ µl primer)

Mgcl2: 1.5 µl (1.5 mM)

DNA Template :5 µl (50-100 ng)

PCR Buffer:2.5 µl (1×)

Enzyme (taq):0.25 µl (1.25 U)

DMSO: 2 µl (4%)

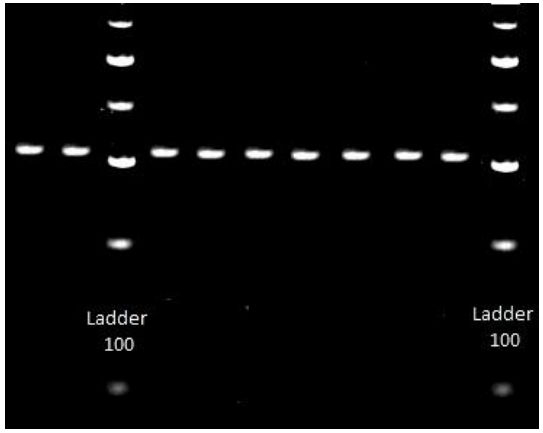
Note: PCR Buffer consists of 1.5 mM Mgcl2

Photograph of PCR-Product using GYR-A forward and GYR-A reverse primer.

PCR Product:

Primer GYR-A forward and GYR-A reverse amplify a 320-bp fragment.

The 320-bp band represents gyrA QRDR (Quinolone Resistance Determining Region) specific PCR product.



PCR-SSCP: Ciprofloxacin & Ofloxacin Resistance

PCR cycle:

Initial denaturation 5 min 95°C			
2 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **60°C** **72°C**
2 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **59°C** **72°C**
2 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **58°C** **72°C**
2 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **57°C** **72°C**
35 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **56°C** **72°C**
Final extension 7 min 72°C			

PCR-SSCP: Ciprofloxacin & Ofloxacin Resistance

Evaluation of PCR-Product patterns:

After PCR, 5µl of PCR products (including loading buffer in PCR buffer) is examined for banding patterns by 1.5% Agarose gel electrophoresis. Fragments are visualized by ethidium bromide staining and UV light.

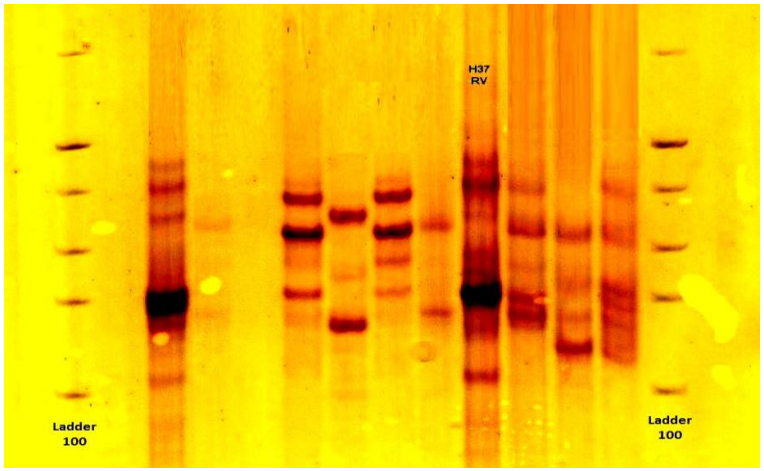
SSCP (Single Strand Conformation Polymorphism):

To characterize different mutations, 10 µl of PCR product is mixed with 1µl NaOH 1M, 3 µl of TBE1× and 6 µl of denaturation buffer including 0.05% bromophenol blue and 0.05% xylene-cyanol FF. Mixed PCR product is applied at 95˚c for 10 min and immediately put in ice then 7µl of denaturated PCR products is loaded on 12% SSCP gel (including1% Glycerol, 12% Acryl -Amid bis Acryl -Amid, TBE1× up to 40 ml).

Evaluation of PCR-Product patterns:

Mutations at gyr-A gene at positions 90, 91 and 94 are detected by loading a wild strain like H37RV on the same SSCP gel and competing wild type pattern with other strains patterns. Fragments are visualized by silver staining. Differentiation in strain pattern in contrast with wild type pattern implicates mutation at gyr-A QRDR region.

Image of PCR-Product patterns using SSCP method.



Primer for PCR-SSCP: Novobiocin &Clorobiocin Resistance

Primer name: GYR-B forward

Oligo sequence: GYRB f: CCACCGACATCGGTGGATT
Delivered: 5.8 OD (166.9µg, 32.4 nmol) **Conce:** 100.0µM
MW: 5789 **Tm:** 68 **Synthesis Scale:** 0.04 **No.Bases:** 19 **GC%:** 57.9
Dissolve in: 288 µl .D.W

Primer name: GYR-B reverse

Oligo sequence: GYRAB r: CTGCCACTTGAGTTTGTACA
Delivered: 7.9OD (227.1µg, 37.3 nmol) **Conce:** 100.0µM
MW: 6083 **Tm:** 55.3 **Synthesis Scale:** 0.04 **No.Bases:** 20 **GC%:** 45.0
Dissolve in: 373 µl .D.W

PCR Mixture for 50µl Reaction

dNTP: (0.4mM)2 µl
GYRB f: 1 µl (10 pmol/ µl primer)
GYRB r: 1 µl (10 pmol/ µl primer
Enzyme (taq):0.25 µl (1.25 U)
PCR Buffer:5 µl (1×)
Mgcl2: 0 µl (1.5 mM)
DMSO: 2 µl (4%)
DNA Template: 5 µl (50-100 ng)

PCR-SSCP: Novobiocin &Clorobiocin Resistance

PCR cycle:

Initial denaturation 5 min 95°C			
2 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **68°C** **72°C**
2 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **67°C** **72°C**
2 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **66°C** **72°C**
2 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **65°C** **72°C**
35 Cycles	**Step1** **Step2** **Step3**	**1 min** **1 min** **1 min**	**95°C** **64°C** **72°C**
Final extension 7 min 72°C			

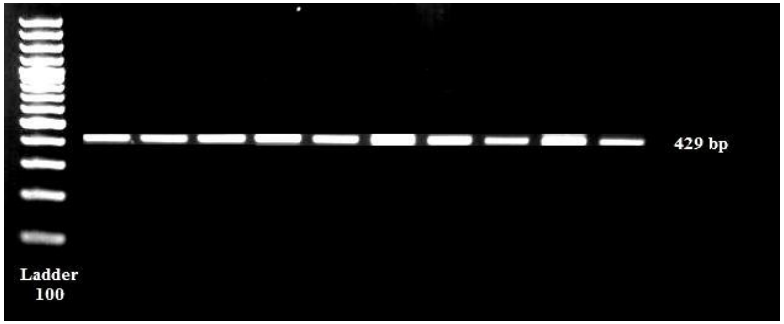
PCR-SSCP: Novobiocin &Clorobiocin Resistance

PCR Product:

Primer GYR-B forward and GYR-B reverse amplify a 429-bp fragment.

Photograph of PCR-Product using GYR-B forward and GYR-B reverse primer.

The 429-bp band represents gyrB QRDR (Quinolone Resistance Determining Region) specific PCR product.



Evaluation of PCR-Product patterns:

After PCR, 5µl of PCR products (including loading buffer in PCR buffer) is examined for banding patterns by 1.5% Agarose gel electrophoresis. Fragments are visualized by ethidium bromide staining and UV light.

SSCP (Single Strand Conformation Polymorphism):

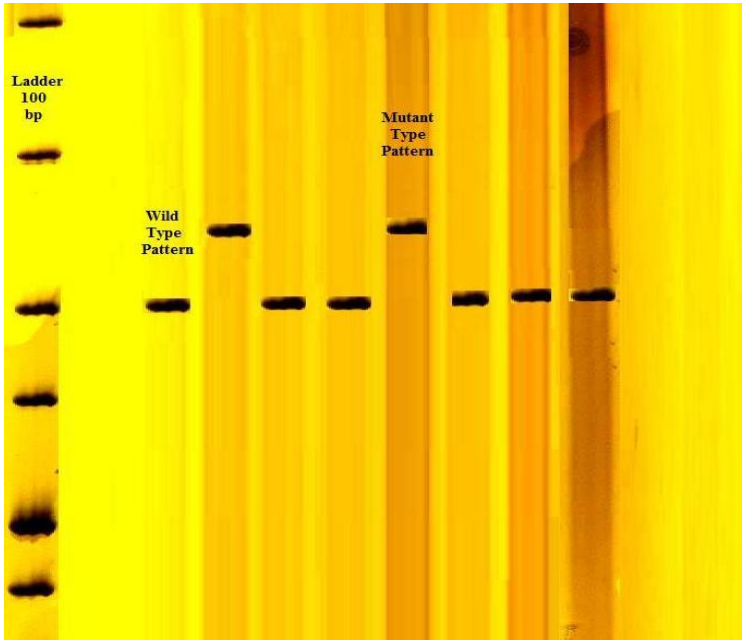
To characterize different mutations, 10 µl of PCR product is mixed with 1µl NaOH 1M, 3 µl of TBE1× and 6 µl of denaturation buffer including 0.05% bromophenol blue and 0.05% xylene-cyanol FF. Mixed PCR product is applied at 95˚c for 10 min and immediately put in ice then 7µl of denaturated PCR products is loaded on 12% SSCP gel (including1% Glycerol, 12% Acryl -Amid bis Acryl -Amid, TBE1× up to 40 ml).

PCR-SSCP: Novobiocin &Clorobiocin Resistance

Evaluation of PCR-Product patterns:

Mutations at gyr-B gene at positions 495,516 and 533 are detected by loading a wild strain like H37RV on the same SSCP gel and competing wild type pattern with other strains patterns. Fragments are visualized by silver staining. Differentiation in strain pattern in contrast with wild type pattern implicates mutation at gyr-B QRDR region.

Image of PCR-Product patterns using SSCP method.



PCR-ELISA: Isoniazid resistance

Step 1: DNA extraction

DNA was extracted by standard phenol-chloroform procedure.
If the quality of DNA is not satisfied, it will be purified with column.

Step2: PCR

The katG gene is amplified by two following primer:
katG 31F:
GGGCTTGGGCTGGAAGAG
katG 31R:
ACAACAGTTTCCTCGAGATCCTGT

Primer name: katG31F

Oligo sequence: 5′- GGGCTTGGGCTGGAAGAG-3′
Delivered: 4.0 (109µg, 19.3 nmol) Conce: 100.0µM
MW: 5661 Tm: 60.5 Synthesis Scale: 0.04µmol No.Bases: 18 GC%:66.7
Dissolve in: 193 µl .D.W

Primer name: katG 31R

Delivered: 4.8 (135.6µg, 18.6 nmol) Conce: 100.0µM
MW: 7288 Tm: 61 Synthesis Scale: 0.04µmol No.Bases: 24 GC%:45.8
Dissolve in: 186 µl .D.W

PCR-ELISA: Isoniazid resistance

Step 2: PCR mixture for 50µl reaction:

Digoxigenin-11-dUTP (DIG-dUTP): (0.01 mM) 0.5µl

dNTP: (0.2 mM) 0.5µl

MgCl2 :(3mM) 3µl

katG31F (30 pmol) 3 µl

katG31R (30 pmol) 3 µl

PCR buffer: 5µl

Taq DNA polymerase: 1U/ µl (0.25 µl)

Template (purified DNA): 50-100 ng

Note: dNTP is contained 0.2 mM of each dCTP,dGTP,dATP and 0.19 mM dTTP

PCR cycle:

Initial denaturation 10 min 95°C			
40	Step1 Step2 Step3	30 sec 30 sec 30 sec	95°C 68°C 72°C
Final extension 10 min 72°C			

PCR-ELISA: Isoniazid resistance

Step 3: hybridization and ELISA

1 labeled probe was used To detect the single nucleotide mutation in katG 315
Probe name: katG wt315
Oligo sequence: 5′-biotin- CGCGATCACCAGCGGCATCG -3′
Delivered: 1.2 OD (36.5µg, 5.6 nmol) Conce: 100.0µM
MW: 6543 Tm: 65.5 Synthesis Scale: 0.04µmol No.Bases: 20 GC%:70
Dissolve in: 56µl .D.W

The following hybridization steps is used as instruction of PCR-ELISA (DIG detection) kit (ROCH Company).

1. 1-30 µl of DIG-labeled PCR product is transferred to a sterile microcentrifuge tube.
2. Add 20 µl of denaturation solution (kit vial 1) to each reaction tube (including controls, and reagent blank). Mix, centrifuge and incubate 10 min at 15-25°C
3. Add hybridization solution contained probe to a final volume of 250 µl, and mix by vortexing.
4. Pipet 200 µl aliquot from each microcentrifuge tube in a well of supplied microplate strip.
5. Incubate the strips 1-3 h at 56°C on a MP shaker. Carefully remove the solution by aspirating away the buffer. Wash 3-5 times with 250 µl washing solution. After last wash step, aspirate the wells then invert the MP and tap it on a dry ,lint free, absorbent cloth.
6. Add 200 µl anti-DIG-POD. Incubate 30 min at 37°C on aMPshaker.
7. Repeat step 5
8. Add 200 µl of ABTS solution. Incubate in the dark 20-30 min at 37°C on a MP shaker until color becomes visible in the wells.
9. Read the absorbance of each well at 405 nm.

PCR-ELISA: Rifampicin Resistance

Step 1: DNA extraction

DNA was extracted by standard phenol-chloroform procedure.
If the quality of DNA is not satisfied, it will be purified with column.

Step2: PCR

The rpoB gene is amplified by two following primer:
RpoB-for1:
TGGTCCGCTTGCACGAGGGTCAGA
RpoB-rev1:
CTCAGGGGTTTCGATCGGGCACAT

Primer name: rpoB for1

Oligo sequence: 5′-TGGTCCGCTTGCACGAGGGTCAGA-3′
Delivered: 6.4 (184.4µg, 24.9 nmol) Conce: 100.0µM
MW: 7410 Tm: 67.8 Synthesis Scale: 0.04µmol No.Bases: 24 GC%:62.5%
Dissolve in: 249 µl .D.W

Primer name: rpoB rev1

Oligo sequence: 5′-CTCAGGGGTTTCGATCGGGCACAT-3´
Delivered: 5.4 OD (156µg, 21.1 nmol) Conce: 100.0µM
MW: 7385 Tm: 66.1 Synthesis Scale: 0.04µmol No.Bases: 24 GC%: 58.3%
Dissolve in: 211 µl .D.W

PCR-ELISA: Rifampicin Resistance

PCR mixture for 50µl reaction:

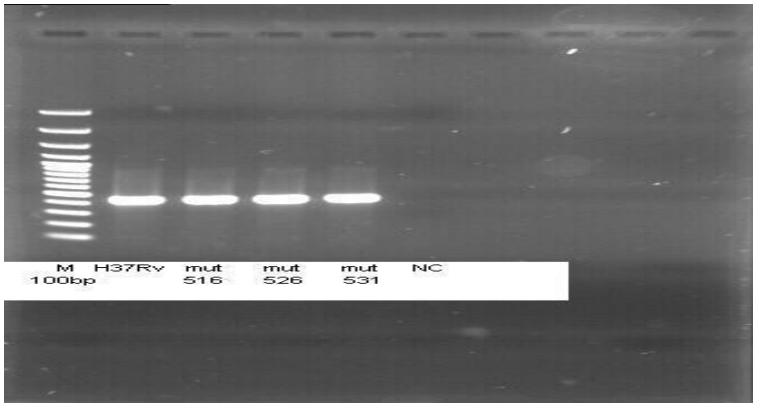
Digoxigenin-11-dUTP (DIG-dUTP): (0.01 mM) 0.5µl
dNTP: (0.2 mM) 0.5µl
MgCl2 :(3mM) 3µl
rpoB-for 1(30 pmol) 3 µl
RpoB-rev 1(30 pmol) 3 µl
PCR buffer: 5µl
Taq DNA polymerase: 1U/ µl (0.25 µl)
Template (purified DNA): 50-100 ng

Note: dNTP is contained 0.2 mM of each dCTP,dGTP,dATP and 0.19 mM dTTP

PCR cycle:

Initial denaturation 10 min 95°C			
40	Step1 Step2 Step3	30 sec 30 sec 30 sec	95°C 70°C 72°C
Final extension 10 min 72°C			

PCR product: The size of PCR product is about 420bp as the figure 1 shows



PCR-ELISA: Rifampicin Resistance

Step 3: hybridization and ELISA

12 labeled probe was used To detect the single nucleotide mutation in rpoB gene. 5 of them will be hybride wiht wild type and 7 will be hybrid with mutant strains.

The sequence and hybridization temprature of them is showed in following table.

Probe name	Sequence of probe	Tm temprature	Concentration of probe (µg/µl)
rpoB Wt1	Biotin-agccagctgagccaattc	56	3.94
rpoB Wt2	Biotin-tcatggaccagaacaacccg	56	1.185
rpoB Wt3	Biotin-cgctgtcggggttgac	56	2.70
rpoB Wt4	Biotin-ttgacccacaagcgccg	56	0.473
rpoB Wt5	Biotin-cgactgtcggcgctgg	56	0.41
rpoB Mt1a	Biotin-agccagccgagccaattc	56	0.648
rpoB Mt2b	Biotin-catgtaccagaacaaccc	56	106.4
rpoB Mt4a	Biotin-ttgacctacaagcgccg	56	4.03
rpoB Mt4b	Biotin-ttgaccgacaagcgccg	56	0.43
rpoB Mt4d	Biotin-ttgaccctcaagcgccg	56	0.72
rpoB Mt5a	Biotin-cgactgttggcgctgg	56	1.67
rpoB Mt5b	Biotin-cgactgtgggcgctgg	56	0.45

PCR-ELISA: Rifampicin Resistance

Probe name: rpoB wt1

Oligo sequence: 5′-biotin- agccagctgagccaattc -3′
Delivered: 1.3 OD (40µg, 6.7 nmol) Conce: 100.0µM
MW: 5924 Tm: 56 Synthesis Scale: 0.04µmol No.Bases: 18 GC%:55.6%
Dissolve in: 67µl .D.W

Probe name: rpoB wt2

Oligo sequence: 5′- Biotin-tcatggaccagaacaacccg-3´
Delivered: 1.5 OD (43µg, 6.6 nmol) Conce: 100.0µM
MW: 6535 Tm: 59.4 Synthesis Scale: 0.04µmol No.Bases: 20 GC%: 55%
Dissolve in: 66 µl .D.W

Probe name: rpoB wt3

Oligo sequence: 5'- Biotin-cgctgtcggggttgac-3'
Delivered: 1.0 OD (31.5µg, 5.9 nmol) Conce: 100.0µM
MW: 5385 Tm: 56.9 Synthesis Scale: 0.04µmol No.Bases: 16 GC%:68.8%
Dissolve in: 59µl .D.W

Probe name: rpoB wt4

Oligo sequence: 5'- Biotin-ttgacccacaagcgccg-3'
Delivered: 1.3 OD (40.5µg, 7.2 nmol) Conce: 100.0µM
MW: 5596 Tm: 57.6 Synthesis Scale: 0.04µmol No.Bases: 17 GC%:64.7%
Dissolve in: 72µl .D.W

PCR-ELISA: Rifampicin Resistance

Probe name: rpoB wt5

Oligo sequence: 5'- Biotin-cgactgtcggcgctgg-3'
Delivered: 1.3 OD (42µg, 7.8 nmol) Conce: 100.0µM
MW: 5370 Tm: 59.4 Synthesis Scale: 0.04µmol No.Bases: 16 GC%:75%
Dissolve in: 78µl .D.W

Probe name: rpoB mt1a

Oligo sequence: 5'- Biotin-agccagccgagccaattc-3'
Delivered: 1 OD (30.2µg, 5.1 nmol) Conce: 100.0µM
MW: 5909 Tm: 58.2 Synthesis Scale: 0.04µmol No.Bases: 18 GC%:61.1%
Dissolve in: 51µl .D.W

Probe name: rpoB mt2b

Oligo sequence: 5'- Biotin-catgtaccagaacaaccc-3'
Delivered: 1.6 OD (45.8µg, 7.8 nmol) Conce: 100.0µM
MW: 5877 Tm: 53.7 Synthesis Scale: 0.04µmol No.Bases: 18 GC%:50
Dissolve in: 78µl .D.W

Probe name: rpoB mt4a

Oligo sequence: 5'- Biotin-ttgacctacaagcgccg-3'
Delivered: 1.0 OD (29.9µg, 5.3 nmol) Conce: 100.0µM
MW: 5611 Tm: 55.2 Synthesis Scale: 0.04µmol No.Bases: 17 GC%:58.8
Dissolve in: 53µl .D.W

PCR-ELISA: Rifampicin Resistance

Probe name: rpoB mt4b

Oligo sequence: 5'- Biotin-ttgaccgacaagcgccg-3'
Delivered: 1.1OD (35µg, 6.2 nmol) Conce: 100.0µM
MW: 5636 Tm: 57.6 Synthesis Scale: 0.04µmol No.Bases: 17 GC%:64.7
Dissolve in: 62µl .D.W

Probe name: rpoB mt4d

Oligo sequence: 5'- Biotin-ttgaccctcaagcgccg-3'
Delivered: 1.2OD (39.1µg, 7.0 nmol) Conce: 100.0µM
MW: 5587 Tm: 57.6 Synthesis Scale: 0.04µmol No.Bases: 17 GC%:64.7
Dissolve in: 70µl .D.W

Probe name: rpoB mt5a

Oligo sequence: 5'- Biotin-cgactgttggcgctgg-3'
Delivered: 1.3OD (43.2µg, 8 nmol) Conce: 100.0µM
MW: 5385 Tm: 56.9 Synthesis Scale: 0.04µmol No.Bases: 16 GC%:68.8
Dissolve in: 80µl .D.W

Probe name: rpoB mt5b

Oligo sequence: 5'- Biotin-cgactgtgggcgctgg-3'
Delivered: 0.8OD (26.6µg, 4.9 nmol) Conce: 100.0µM
MW: 5410 Tm: 59.4 Synthesis Scale: 0.04µmol No.Bases: 16 GC%:75
Dissolve in: 49µl .D.W

PCR-ELISA: Rifampicin Resistance

The following hybridization steps is used as instruction of PCR-ELISA (DIG detection) kit (ROCH Company).

1- 1-30 µl of DIG-labeled PCR product is transferred to a sterile microcentrifuge tube.

2- Add 20 µl of denaturation solution (kit vial 1) to each reaction tube (including controls, and reagent blank). Mix, centrifuge and incubate 10 min at 15-25°C

3- Add hybridization solution contained probe to a final volume of 250 µl, and mix by vortexing.

4- Pipet 200 µl aliquot from each microcentrifuge tube in a well of supplied microplate strip.

Incubate the strips 1-3 h at 56°C on a MP shaker.

5- Carefully remove the solution by aspirating away the buffer.

Wash 3-5 times with 250 µl washing solution. After last wash step, aspirate the wells then invert the MP and tap it on a dry ,lint free, absorbent cloth.

6- Add 200 µl anti-DIG-POD. Incubate 30 min at 37°C on aMPshaker.

7- Repeat step 5

8- Add 200 µl of ABTS solution

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