

Nuove opportunità nella diagnostica di laboratorio della TBC e delle micobatteriosi

Enrico Tortoli

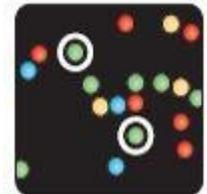
Tubercolosi e Micobatteriosi: un impegno globale
Ferrara, 31 Maggio 2018

Whole Genome Sequencing

- La possibilità di sequenziare interi genomi in tempi brevi, insieme allo sviluppo di software bioinformatici, costituiscono probabilmente l'innovazione tecnologica più importante del XXI secolo
- Fortunatamente il costo dei reagenti è diminuzione e la maggioranza dei tool bioinformatici sono addirittura gratuiti

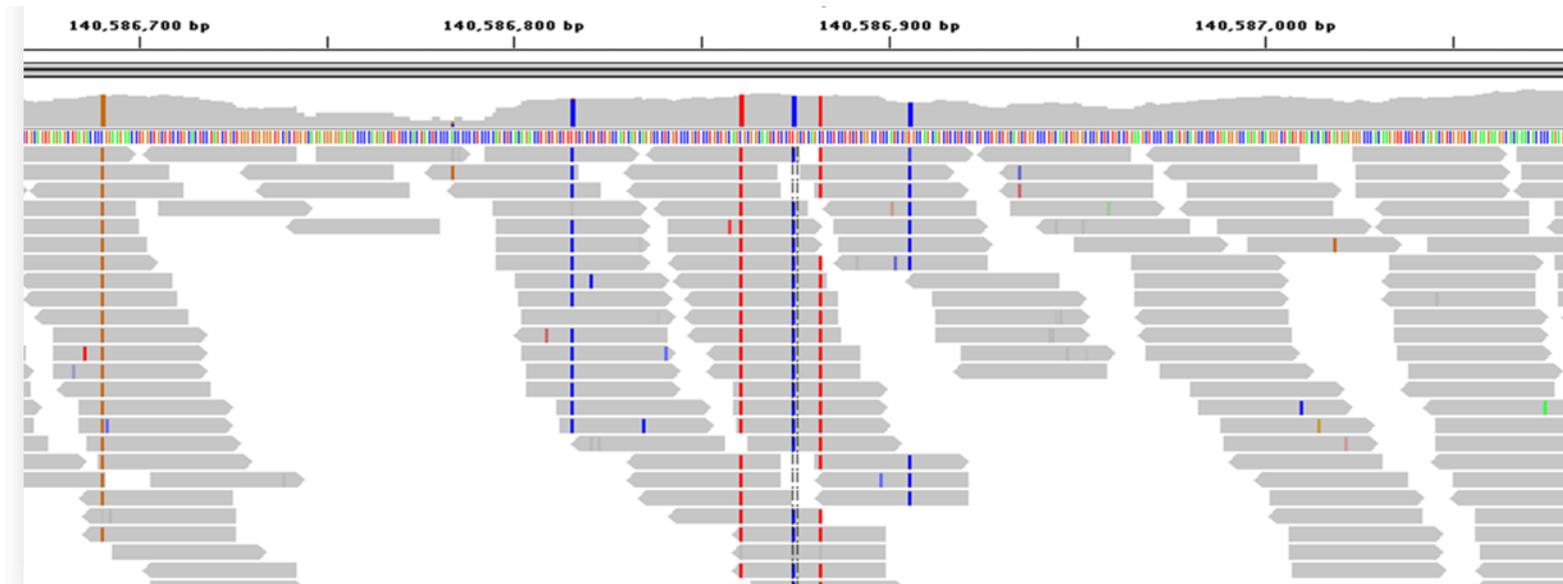
L'hardware in 2 parole

- Il genoma da amplificare viene frammentato
- I frammenti vengono fissati ad un supporto solido e legati ad adattatori tutti uguali fra loro (quindi amplificabili con un unico primer)
- Ad ogni aggiunta di un nucleotide il sistema scatta una "fotografia" in cui ciascun filamento è identificato dalle sue coordinate ed il tipo di nucleotide è identificato dal colore



Analisi delle sequenze

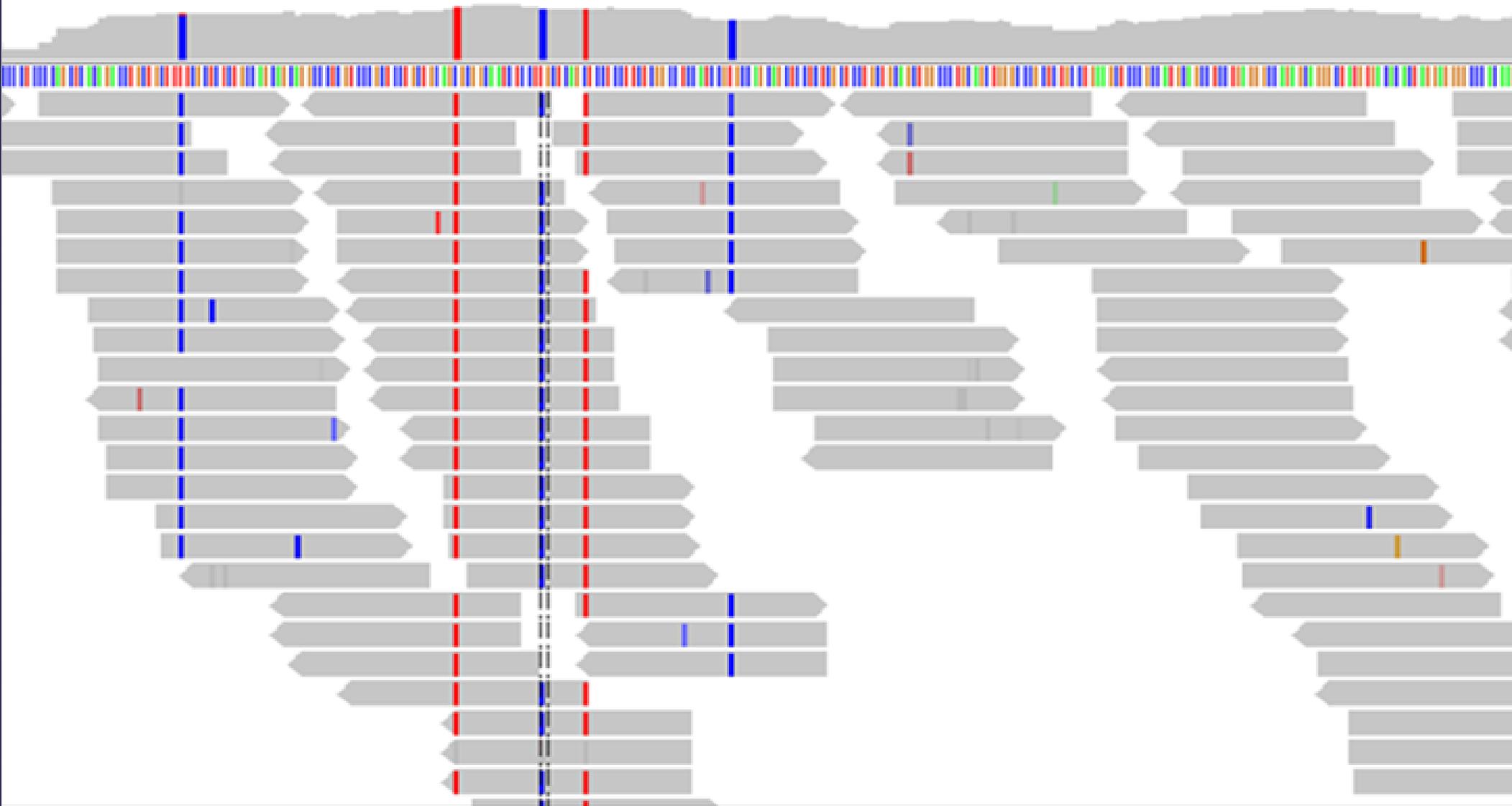
Le read vengono allineate ad un genoma di riferimento



140,586,800 bp

140,586,900 bp

140,587,000 bp



Analisi delle read

- **La pipeline bioinformatica rileva tutti gli SNP rispetto al ceppo di riferimento ed utilizza:**
 - gli SNP con significato filogenetico per assegnare il ceppo in esame ad una famiglia (o lineage)
 - gli SNP responsabili di farmaco-resistenza per predire la sensibilità ai farmaci di I e II linea
 - gli SNP senza significato noto vengono comunque rilevati

Materiali e metodi



- C R R Regione Toscana
- Periodo: Febbraio 2016 – Settembre 2017: 328 paz.
- MGIT positivo: 2 mL inattivati ed inviati per WGS
- Nessuna variazione rispetto alla normale routine (antibiogramma disponibile dopo 14,3 gg. dalla positivizzazione del MGIT)



Materiali e metodi

C R R Regione Toscana

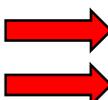
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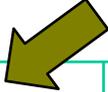
HSR



- Estrazione del DNA, preparazione delle library
- WGS (Illumina MiniSeq), analisi bioinformatica
- Risultato a 72h dall'arrivo del campione


Coverage	Lineage	RIF	INH		PZA	FQ		SLID / SM			EMB	SM	
		<i>rpoB</i>	<i>katG</i>	<i>inhA</i>	<i>pncA</i>	<i>gyrA</i>	<i>gyrB</i>	<i>rrs</i>	<i>eis</i>	<i>tlyA</i>	<i>embB</i>	<i>rpsL</i>	
17,98	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
73,06	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
61,51	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt	
67,35	Euro-American Superlineage	Glu721Lys	wt	wt	wt	Glu21Gln		wt	nd	wt	Leu11Leu	wt	wt
27,88	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	wt	Leu11Leu	wt	wt
69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	Thr1027Thr	wt	wt
1,36	nd	-	-	-	-	-		-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-		-	-	-	-	-	-
52,46	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
57,28	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp		wt	wt	g-10a	Leu11Leu	wt	wt
45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln		Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln		Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	wt	Leu11Leu	wt	wt
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-		-	-	-	-	-	-
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp		wt	wt	g-10a	Leu11Leu	wt	wt
47,9	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln		wt	nd	wt	wt	Cys361Ser	wt
80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	wt	Leu11Leu	wt	wt
98,91	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	a1008c	wt	Leu11Leu	wt	wt
81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln		wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	wt	Leu11Leu	wt	wt
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt	wt
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln		wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp		wt	wt	wt	Leu11Leu	wt	wt
91,81	Beijing	Ala1075Ala	Arg463Leu	c-15t	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Val163Ile	Leu11Leu	Asp534Asp	Lys43Arg



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		<i>rpoB</i>	<i>katG</i>	<i>inhA</i>	<i>pncA</i>	<i>gyrA</i>	<i>gyrB</i>	<i>rrs</i>	<i>eis</i>	<i>tlyA</i>	<i>embB</i>	<i>rpsL</i>
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61,51	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
67,35	Euro-American Superlineage	Glu721Lys	wt	wt	wt	Glu21Gln	wt	nd	wt	Leu11Leu	wt	wt
27,88	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	Thr1027Thr	wt
1,36	nd	-	-	-	-	-	-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-	-	-	-	-	-	-
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45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-	-	-	-	-	-	-
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
47,9	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln	wt	nd	wt	wt	Cys361Ser	wt
80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
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81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln	wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln	wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
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69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	Thr1027Thr	wt
1,36	nd	-	-	-	-	-	-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-	-	-	-	-	-	-
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45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-	-	-	-	-	-	-
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
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80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
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81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln	wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln	wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
91,81	Beijing	Ala1075Ala	Arg463Leu	c-15t	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	Val163Ile	Leu11Leu	Asp534Asp	Lys43Arg

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73,06	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
61,51	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
67,35	Euro-American Superlineage	Glu721Lys	wt	wt	wt	Glu21Gln	wt	nd	wt	Leu11Leu	wt	wt
27,88	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	Thr1027Thr	wt
1,36	nd	-	-	-	-	-	-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-	-	-	-	-	-	-
52,46	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
57,28	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-	-	-	-	-	-	-
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
47,9	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln	wt	nd	wt	wt	Cys361Ser	wt
80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
98,91	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	a1008c	wt	Leu11Leu	wt	wt
81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln	wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
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77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln	wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
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1,36	nd	-	-	-	-	-	-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-	-	-	-	-	-	-
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57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
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80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
98,91	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	a1008c	wt	Leu11Leu	wt	wt
81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln	wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln	wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
91,81	Beijing	Ala1075Ala	Arg463Leu	c-15t	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	Val163Ile	Leu11Leu	Asp534Asp	Lys43Arg

Coverage	Lineage	RIF	INH		PZA	FQ		SLID / SM			EMB	SM
		<i>rpoB</i>	<i>katG</i>	<i>inhA</i>	<i>pncA</i>	<i>gyrA</i>	<i>gyrB</i>	<i>rrs</i>	<i>eis</i>	<i>tlyA</i>	<i>embB</i>	<i>rpsL</i>
17,98	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
73,06	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
61,51	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
67,35	Euro-American Superlineage	Glu721Lys	wt	wt	wt	Glu21Gln	wt	nd	wt	Leu11Leu	wt	wt
27,88	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	Thr1027Thr	wt
1,36	nd	-	-	-	-	-	-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-	-	-	-	-	-	-
52,46	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
57,28	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-	-	-	-	-	-	-
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
47,9	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln	wt	nd	wt	wt	Cys361Ser	wt
80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
98,91	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	a1008c	wt	Leu11Leu	wt	wt
81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln	wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln	wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
91,81	Beijing	Ala1075Ala	Arg463Leu	c-15t	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	Val163Ile	Leu11Leu	Asp534Asp	Lys43Arg

Coverage	Lineage	RIF	INH		PZA	FQ		SLID / SM			EMB	SM
		<i>rpoB</i>	<i>katG</i>	<i>inhA</i>	<i>pncA</i>	<i>gyrA</i>	<i>gyrB</i>	<i>rrs</i>	<i>eis</i>	<i>tlyA</i>	<i>embB</i>	<i>rpsL</i>
17,98	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
73,06	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
61,51	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
67,35	Euro-American Superlineage	Glu721Lys	wt	wt	wt	Glu21Gln	wt	nd	wt	Leu11Leu	wt	wt
27,88	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	Thr1027Thr	wt
1,36	nd	-	-	-	-	-	-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-	-	-	-	-	-	-
52,46	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
57,28	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-	-	-	-	-	-	-
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
47,9	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln	wt	nd	wt	wt	Cys361Ser	wt
80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
98,91	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	a1008c	wt	Leu11Leu	wt	wt
81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln	wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln	wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
91,81	Beijing	Ala1075Ala	Arg463Leu	c-15t	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	Val163Ile	Leu11Leu	Asp534Asp	Lys43Arg



Coverage	Lineage	RIF	INH		PZA	FQ		SLID / SM			EMB	SM	
		<i>rpoB</i>	<i>katG</i>	<i>inhA</i>	<i>pncA</i>	<i>gyrA</i>	<i>gyrB</i>	<i>rrs</i>	<i>eis</i>	<i>tlyA</i>	<i>embB</i>	<i>rpsL</i>	
17,98	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
73,06	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
61,51	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt	
67,35	Euro-American Superlineage	Glu721Lys	wt	wt	wt	Glu21Gln		wt	nd	Leu11Leu	wt	wt	
27,88	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	Thr1027Thr	wt	
1,36	nd	-	-	-	-	-		-	-	-	-	-	
5,38	M. africanum West Africanum 2	-	-	-	-	-		-	-	-	-	-	
52,46	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
57,28	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp		wt	wt	g-10a	Leu11Leu	wt	wt
45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln		Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln		Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-		-	-	-	-	-	
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp		wt	wt	g-10a	Leu11Leu	wt	wt
47,9	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln		wt	nd	wt	wt	Cys361Ser	wt
80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
98,91	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	a1008c	wt	Leu11Leu	wt	wt
81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln		wt	wt	Leu11Leu	wt	wt	
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt	
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln		wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp		wt	wt	Leu11Leu	wt	wt	
91,81	Beijing	Ala1075Ala	Arg463Leu	c-15t	wt	Glu21Gln + Ser95Thr + Gly668Asp		wt	wt	Val163Ile	Leu11Leu	Asp534Asp	Lys43Arg



Coverage	Lineage	RIF	INH		PZA	FQ		SLID / SM			EMB	SM
		<i>rpoB</i>	<i>katG</i>	<i>inhA</i>	<i>pncA</i>	<i>gyrA</i>	<i>gyrB</i>	<i>rrs</i>	<i>eis</i>	<i>tlyA</i>	<i>embB</i>	<i>rpsL</i>
17,98	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
73,06	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
61,51	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
67,35	Euro-American Superlineage	Glu721Lys	wt	wt	wt	Glu21Gln	wt	nd	wt	Leu11Leu	wt	wt
27,88	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	Thr1027Thr	wt
1,36	nd	-	-	-	-	-	-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-	-	-	-	-	-	-
52,46	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
57,28	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-	-	-	-	-	-	-
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
47,9	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln	wt	nd	wt	wt	Cys361Ser	wt
80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
98,91	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	a1008c	wt	Leu11Leu	wt	wt
81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln	wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln	wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
91,81	Beijing	Ala1075Ala	Arg463Leu	c-15t	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	Val163Ile	Leu11Leu	Asp534Asp	Lys43Arg



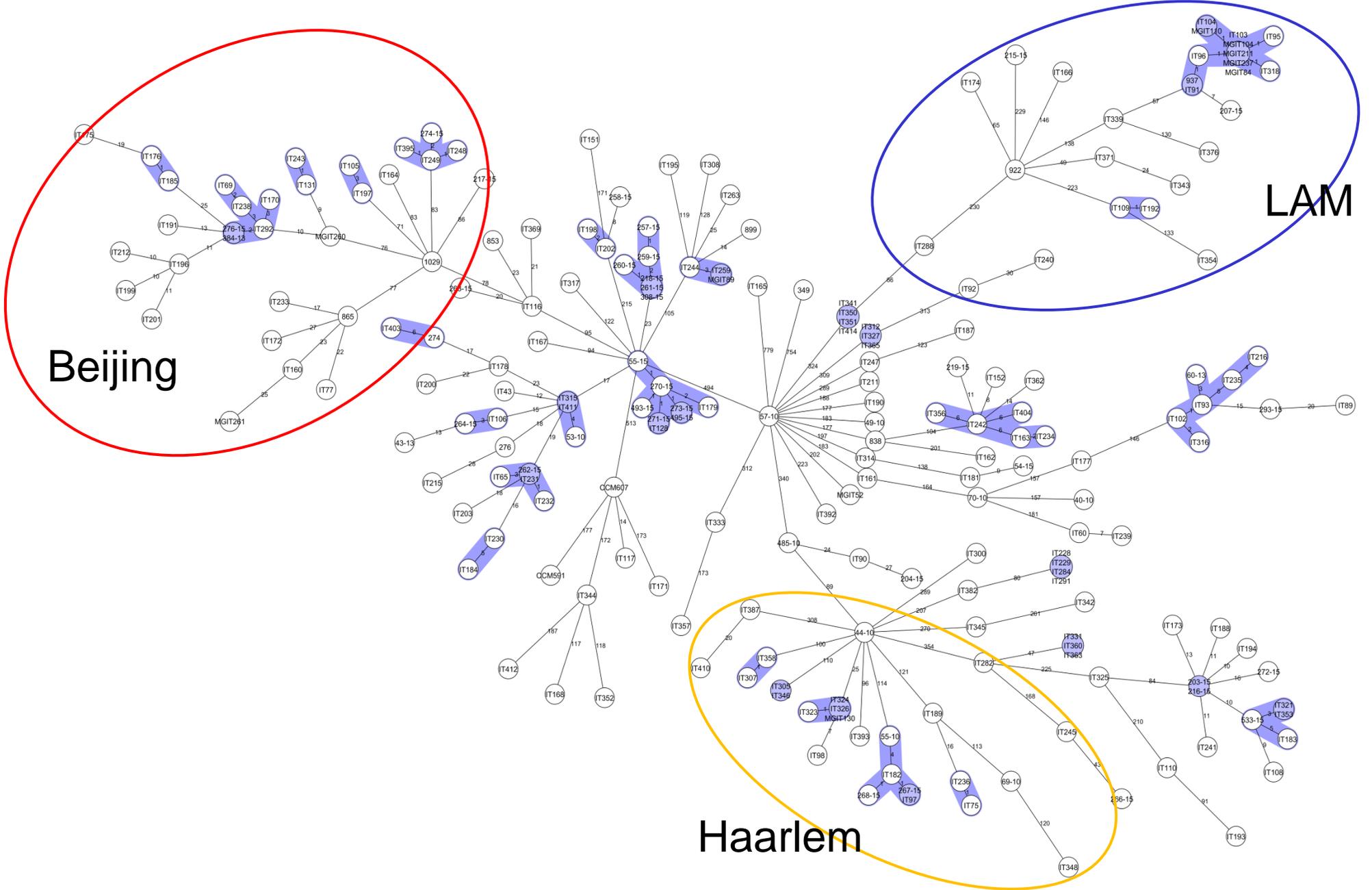
Coverage	Lineage	RIF	INH		PZA	FQ		SLID / SM			EMB	SM
		<i>rpoB</i>	<i>katG</i>	<i>inhA</i>	<i>pncA</i>	<i>gyrA</i>	<i>gyrB</i>	<i>rrs</i>	<i>eis</i>	<i>tlyA</i>	<i>embB</i>	<i>rpsL</i>
17,98	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
73,06	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
61,51	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
67,35	Euro-American Superlineage	Glu721Lys	wt	wt	wt	Glu21Gln	wt	nd	wt	Leu11Leu	wt	wt
27,88	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
69,68	Ural	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	Thr1027Thr	wt
1,36	nd	-	-	-	-	-	-	-	-	-	-	-
5,38	M. africanum West Africanum 2	-	-	-	-	-	-	-	-	-	-	-
52,46	Beijing	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
57,28	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
45,78	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
20,39	Euro-American Superlineage	Val50Gly + Ser450Leu	Ser315Thr	wt	Trp68Stop	Glu21Gln	Asn499Thr	wt	wt	Leu11Leu	Met306Val + Gly406Cys	Lys88Gln
51,56	Haarlem	Asp103Asp	Ala726Thr	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
83,93	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	Ile556Ile	wt	wt	Leu11Leu	wt	wt
0,78	nd	-	-	-	-	-	-	-	-	-	-	-
57,62	LAM	wt	Ser315Thr	wt	wt	Glu21Gln + Ser95Thr + Gly247Ser + Leu296Leu + Gly668Asp	wt	wt	g-10a	Leu11Leu	wt	wt
47,9	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln	wt	nd	wt	wt	Cys361Ser	wt
80,52	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
98,91	Euro-American Superlineage	wt	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	a1008c	wt	Leu11Leu	wt	wt
81,9	Euro-American Superlineage	wt	Gly305Gly	wt	His82His	Glu21Gln	wt	wt	wt	Leu11Leu	wt	wt
58,49	Haarlem	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
95,22	EAI	Ala1075Ala	Arg463Leu	wt	wt	Glu21Gln + Ser95Thr + Ala384Val + Ile614Ile + Gly668Asp	Met291Ile + Ala295Ala + Glu586Lys	g5t	wt	Leu11Leu	Glu378Ala	wt
77,83	Euro-American Superlineage	wt	Val545Val	wt	wt	Glu21Gln	wt	wt	c-100t	Leu11Leu	wt	wt
46,57	Euro-American Superlineage	Asp103Asp	wt	wt	wt	Glu21Gln + Ser95Thr + Leu205Val + Gly668Asp	wt	wt	wt	Leu11Leu	wt	wt
91,81	Beijing	Ala1075Ala	Arg463Leu	c-15t	wt	Glu21Gln + Ser95Thr + Gly668Asp	wt	wt	Val163Ile	Leu11Leu	Asp534Asp	Lys43Arg

Risultati 1/2

- Interpretabili 288/328 (coverage medio 73x)
- VPN per MDR 100%
- 11 MDR (100%)
- VPN per farmaci di I linea >96% (eccetto PIZ 63%)
- 11 ceppi sensibili ai farmaci di I linea, resistenti ai chinoloni
- Lineage prevalenti: Beijing, Haarlem, LAM

Risultati 2/2

- **Cluster analysis basata su**
 - **Core Genome Multilocus Sequence Analysis**
 - **SNP analysis**
 - **Clustering 12%; circa la metà confermati dal contact tracing tradizionale**
 - **7 cross contaminazioni di laboratorio**



Beijing

LAM

Haarlem

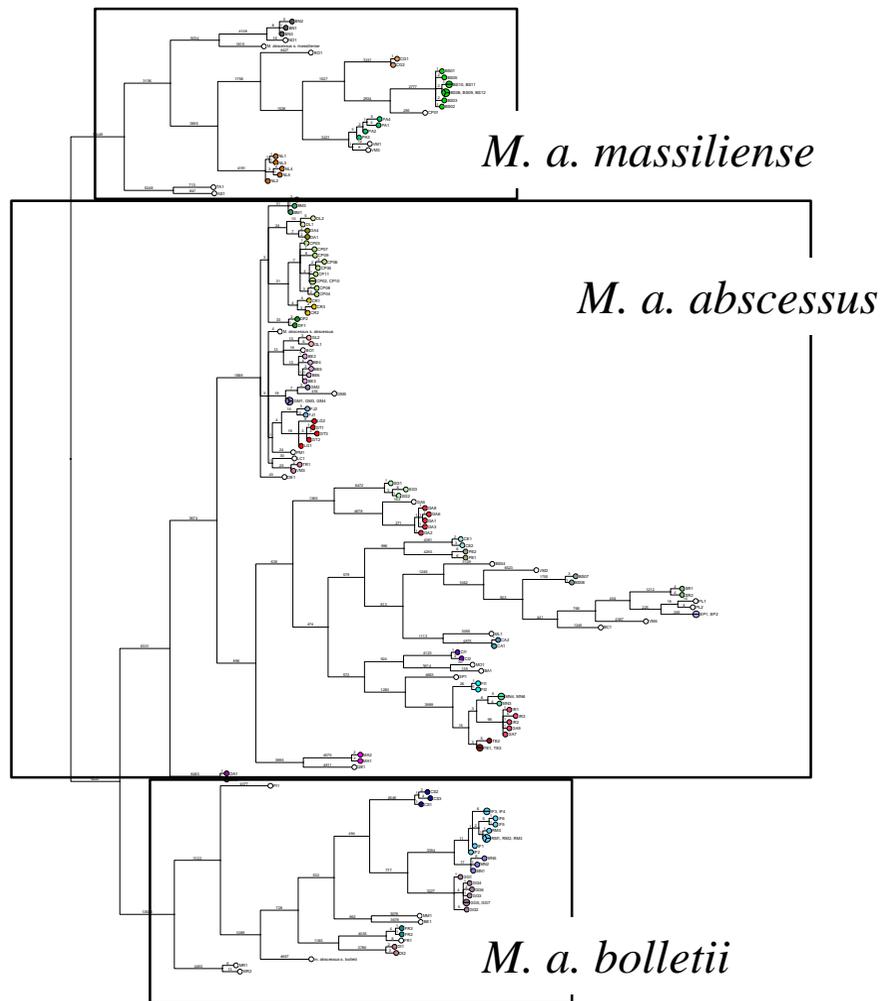
I limiti del sistema

- Alla base c'è sempre la coltura (MGIT) e nonostante ciò in circa il 10% dei casi il coverage non è risultato adeguato
- Nel MGIT positivo è sempre presente DNA umano e di altri batteri che viene tuttavia filtrato dal software
- Se una mutazione si trova in una regione con coverage molto basso può non venir rilevata

Conclusioni

- Costo del WGS di poco superiore a 100 €/ceppo
- Antibiogramma fenotipico necessario in una esigua minoranza dei casi
- Il sistema è già usato di routine in Gran Bretagna
- I dati ottenuti possono essere utilizzati per:
 - ottimizzazione della terapia a livello individuale
 - sorveglianza delle resistenze
 - sorveglianza delle trasmissioni a livello locale, nazionale ed internazionale

Fibrosi cistica e infezione da *M. abscessus*



- 163 genomi di 62 pazienti
- Tutte e 3 le sottospecie rappresentate (13, 66 e 21%)
- Dei 32 pazienti con almeno 3 isolamenti, 28 persistentemente infettati dallo stesso ceppo
- Presenza di ceppi clonali della subsp. *abscessus* circolanti a livello globale
- Mancanza di evidenze di trasmissione nosocomiale