

Tuberculosis: ¿un caso-una cepa? Análisis de casos con infecciones complejas desde un punto de vista microbiológico



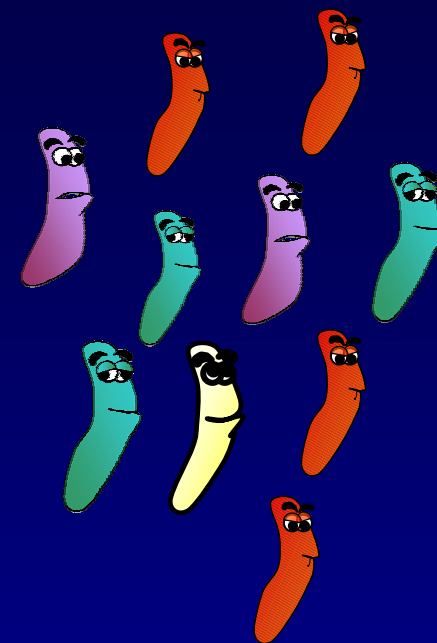
Darío García de Viedma
Servicio de Microbiología

XXXI Congreso SEMI
Oviedo, 17 Noviembre 2010

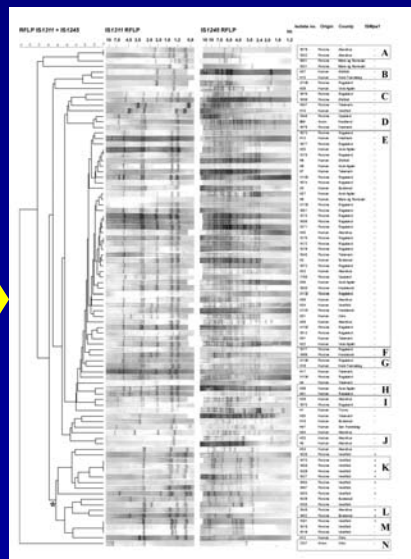
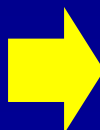
Epidemiología molecular tuberculosis



RFLP-IS6110
Spoligotyping
MIRU-VNTR
3R

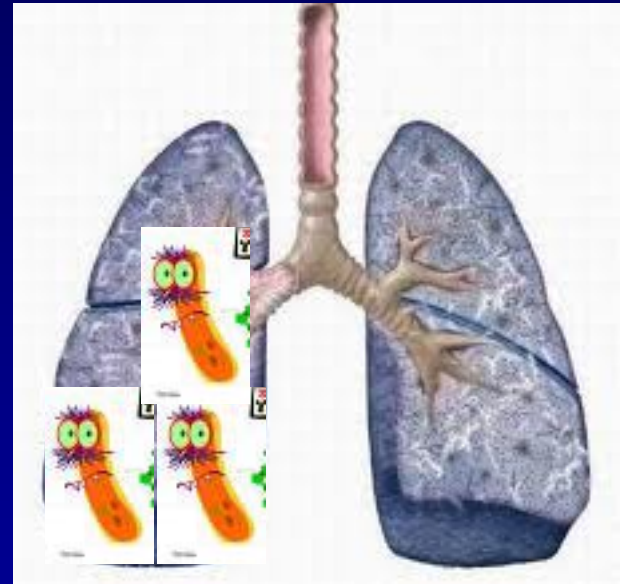
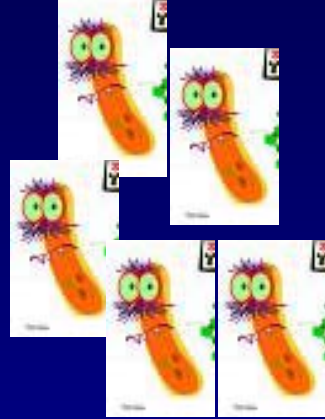


Cepas/Clones



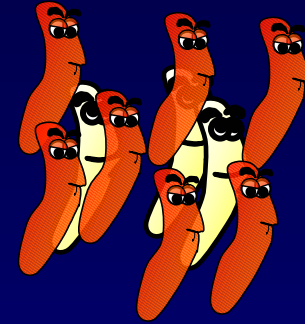
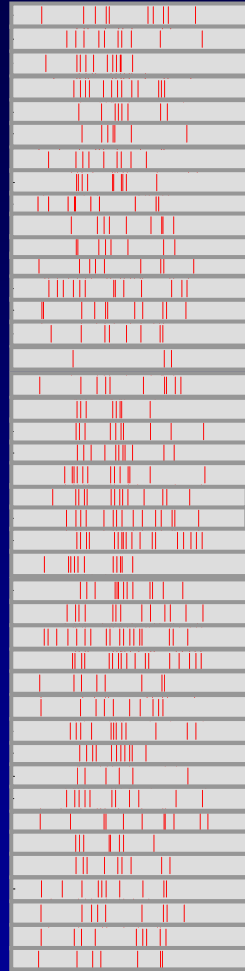
Dendrogramas
Identificar Clusters

Tuberculosis: un caso-una cepa





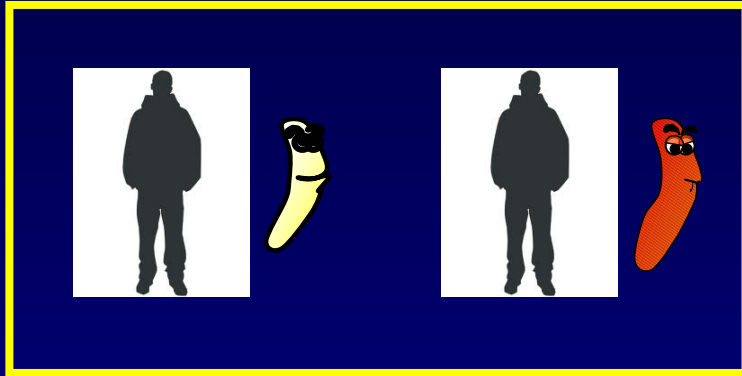
**Estudio de poblaciones
de casos de TB**



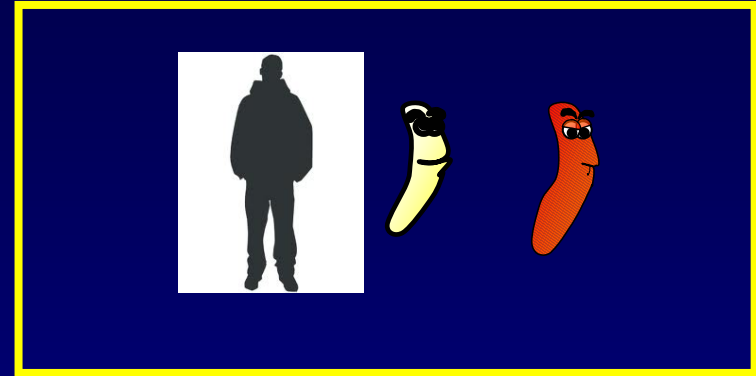
Complejidad clonal

**No cumplen paradigma de
UN CASO-UNA CEPA**

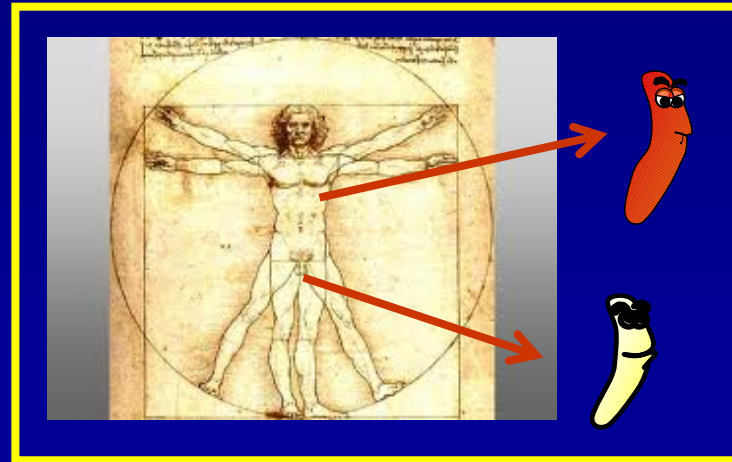
Rastreo de complejidad clonal en TB



Recurrencias



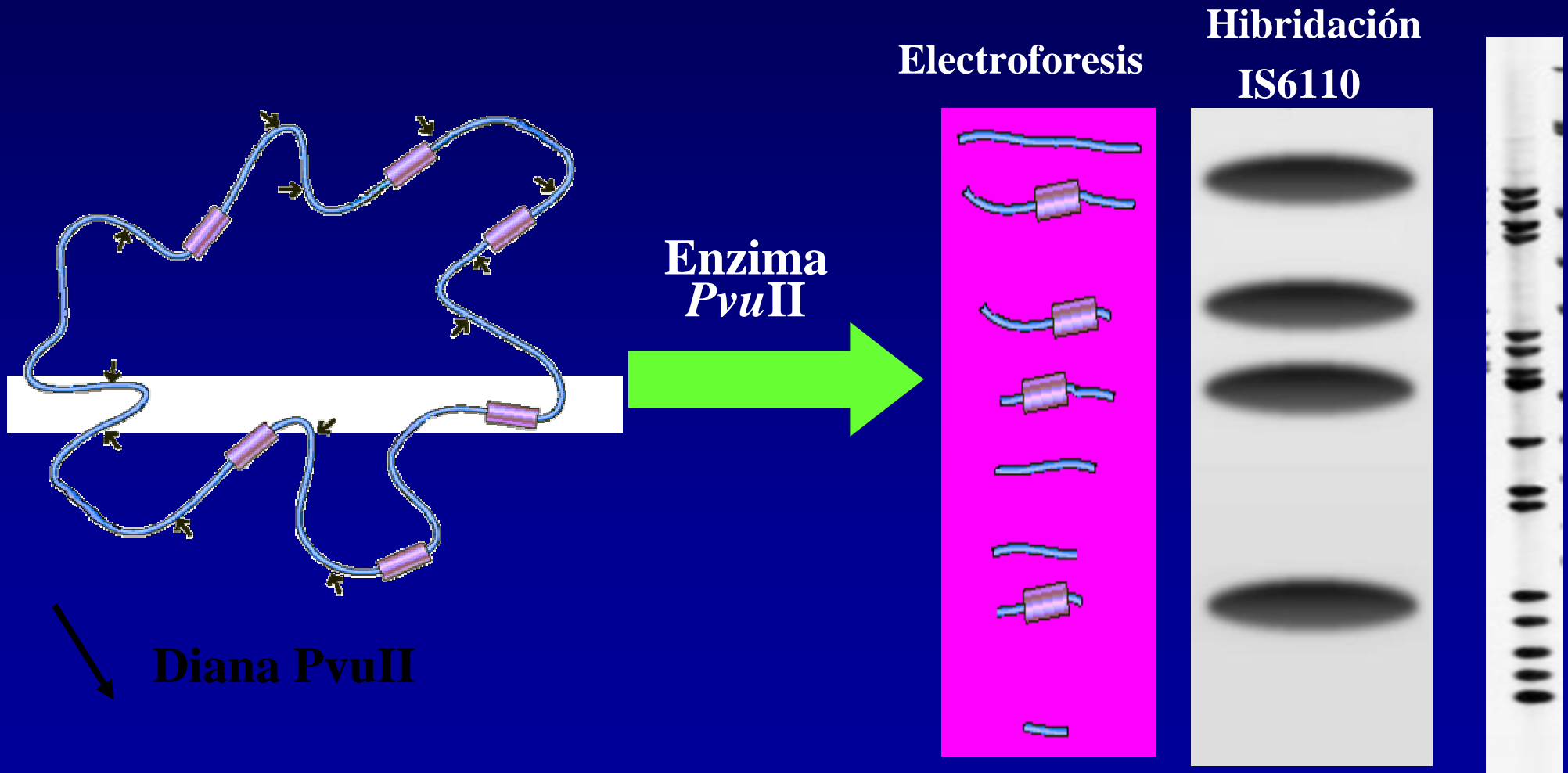
Infección respiratoria



Infección respiratoria + extrarrespiratoria

RFLP-IS6110 Restriction-fragment-length-polimorphism

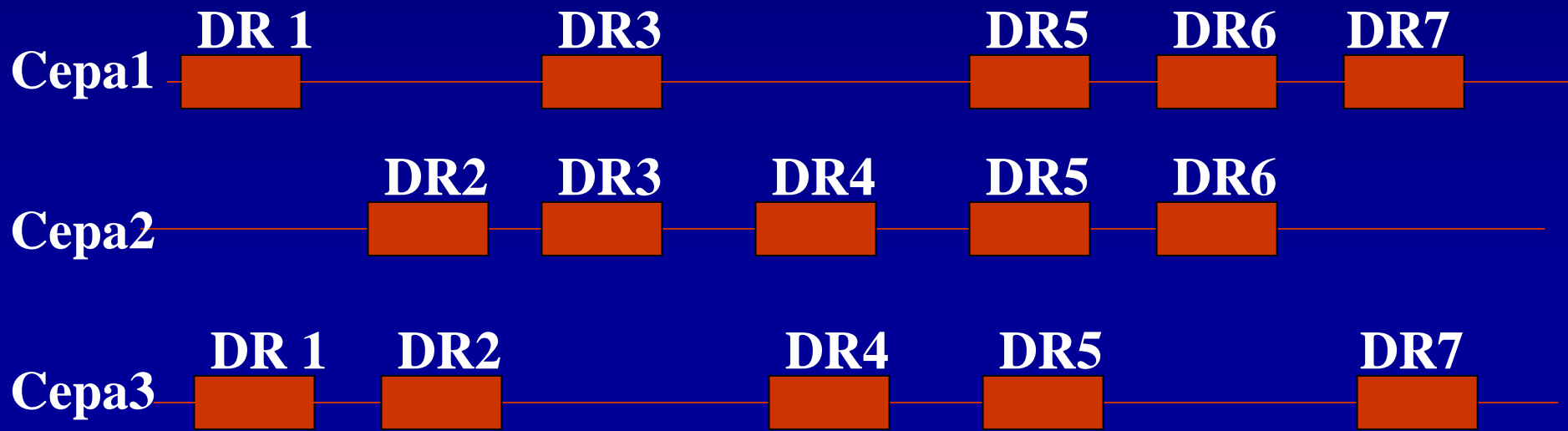
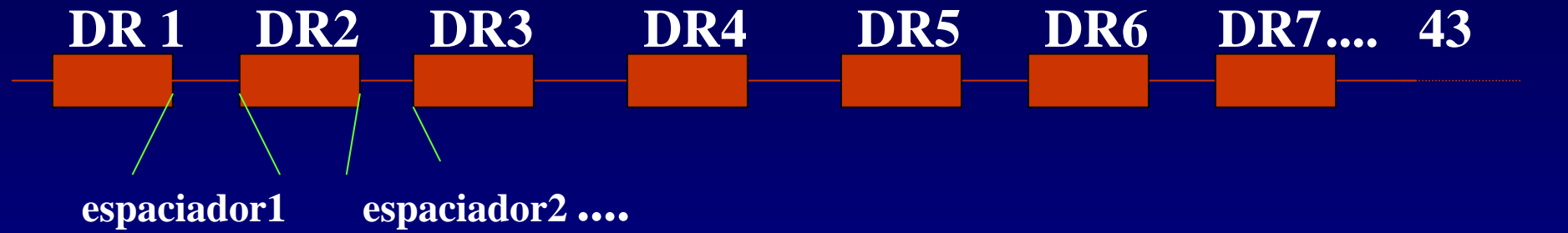
Analiza número y posición de IS6110



Spoligotyping: Spacers-oligo-typing

Analiza región DR (Repeticiones directas)

Spoligotyping



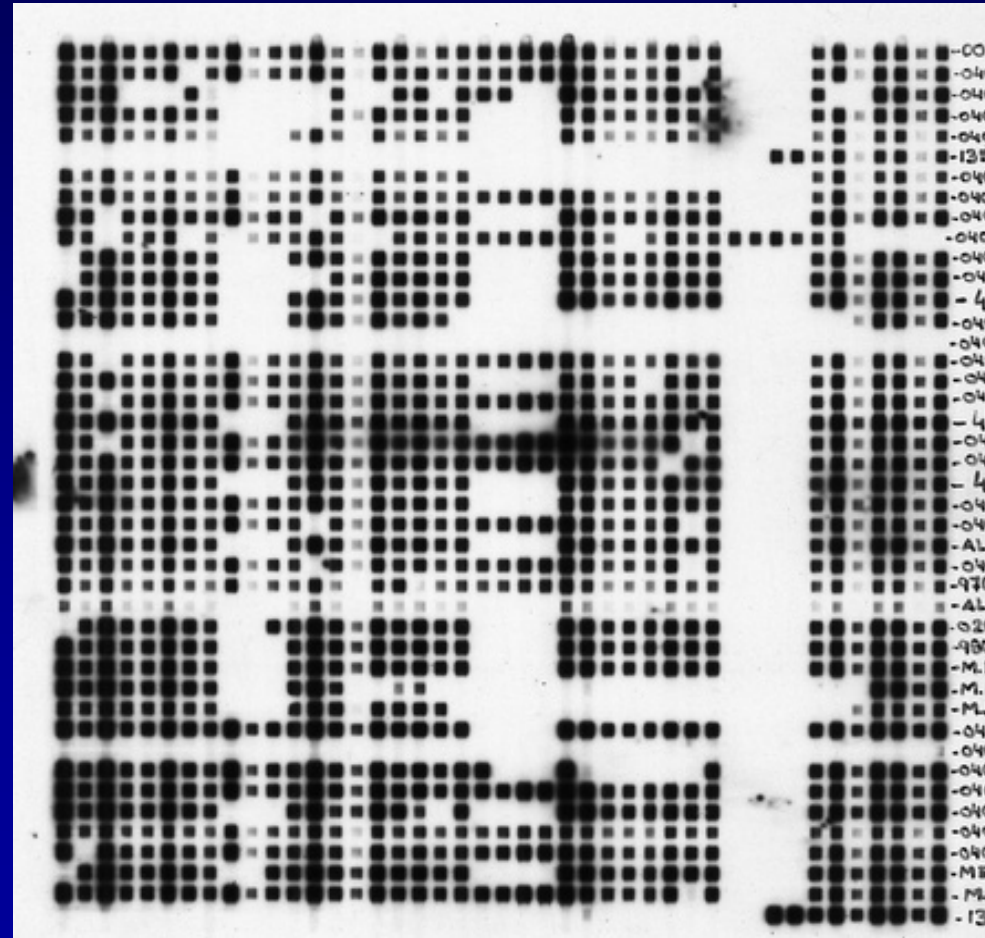
Spoligotyping

Sp:1, 2, 3, 4, 5, 6, 7, 8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27.....

PCR múltiple de los 43 espaciadores

Hibridación

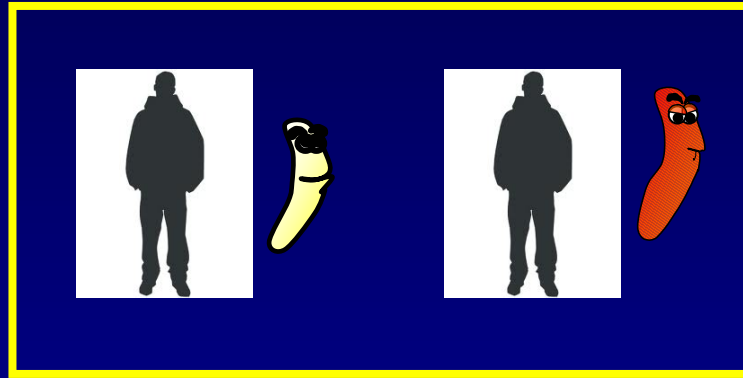
Comparación basada en presencia/ausencia de espaciador



Cepas

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15
- 16

TB Recurrente



TB Recurrente

Una infección por MTB “protege” frente a una nueva infección

6,7% de pacientes con un episodio de tuberculosis en últimos 12 años presentan un nuevo episodio recurrente



TB Recurrente

Análisis en
población

“no seleccionada”

VIH+

VIH-

Moderada incidencia

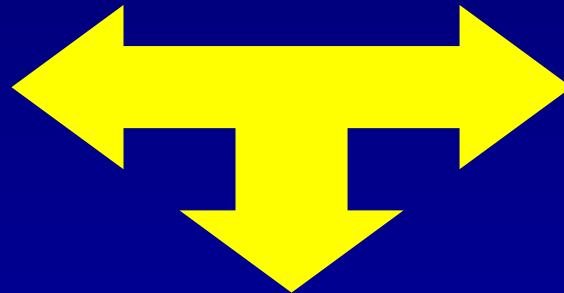
No alto riesgo de exposición

Retrospectivo 12 años

43 pacientes

Primer
episodio

Cepa1



Cepa2

Episodio
recurrente

Caracterización genotípica

¿Iguales o distintas?

TB recurrente

Demanda de análisis rápido de recurrencias

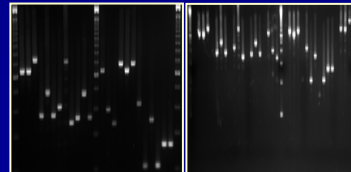
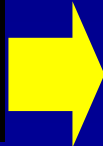
Episodio
previo



¿Reinfección/Reactivación?



Episodio
actual



TB recurrente

Demanda de análisis rápido de recurrencias

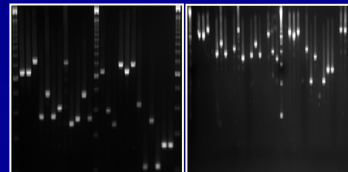
Episodio
previo



¿Reinfección/Reactivación?

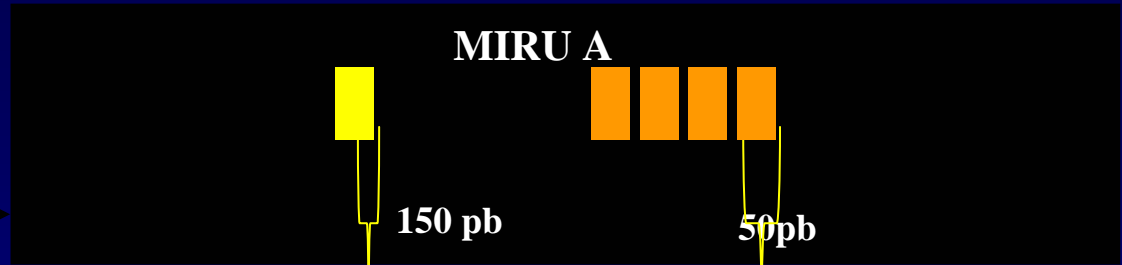
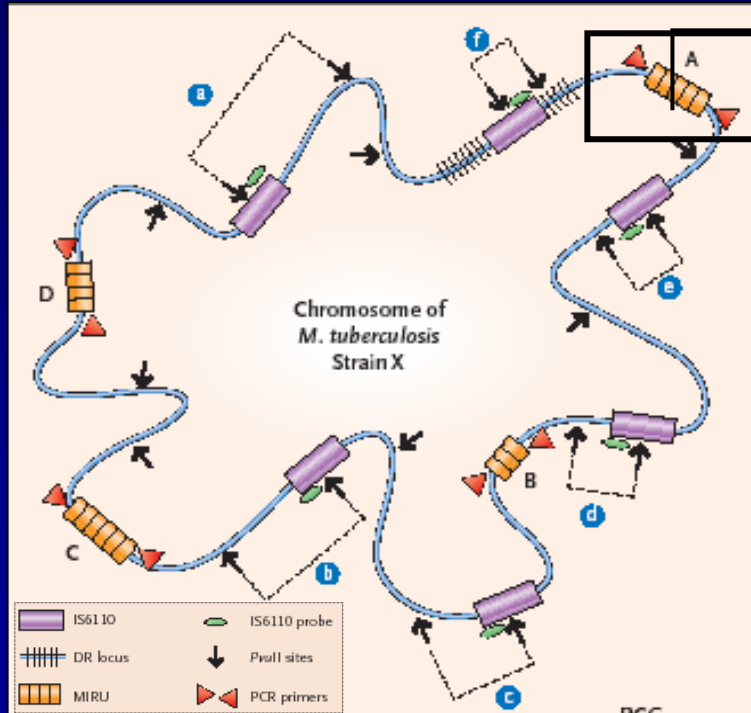


Episodio
actual



MIRU-VNTR

Mycobacterial Interspersed Repetitive Units- Variable Number Tandem Repeats

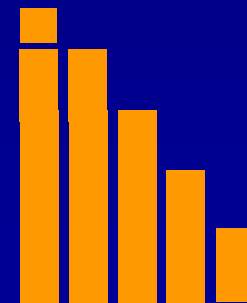


PCR electroforesis



Nº repeticiones

Cepa A
Cepa B
Cepa C
Cepa D
Cepa E
Cepa F



150 pb
200 pb
250 pb
300 pb
350 pb
400 pb

0
1
2
3
4
5

MIRUtipo: 246684848883478

TB recurrente

Demanda de análisis rápido de recurrencias

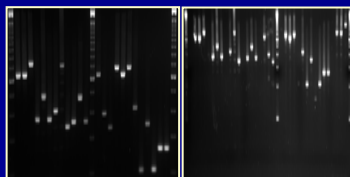
Episodio
previo



¿Reinfección/Reactivación?



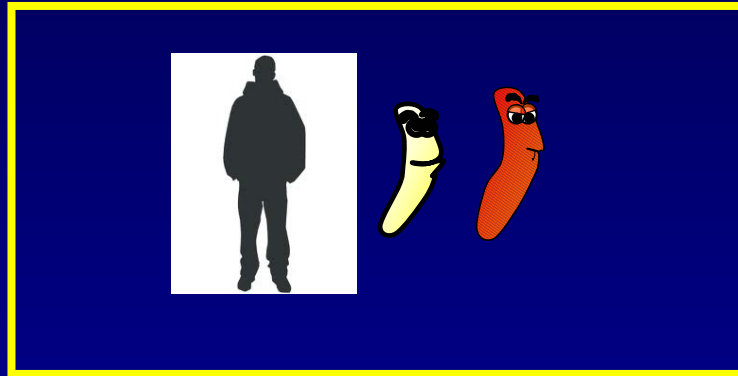
Episodio
actual



TB recurrente: Análisis rápido



Infección mixta



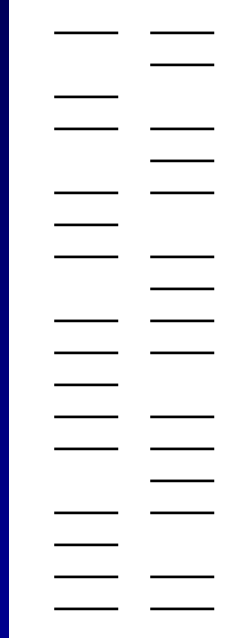
¿Infección mixta?



➔ Genotipado

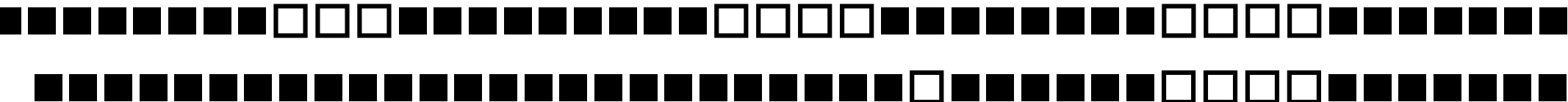
RFLP

Cepa1 Cepa2



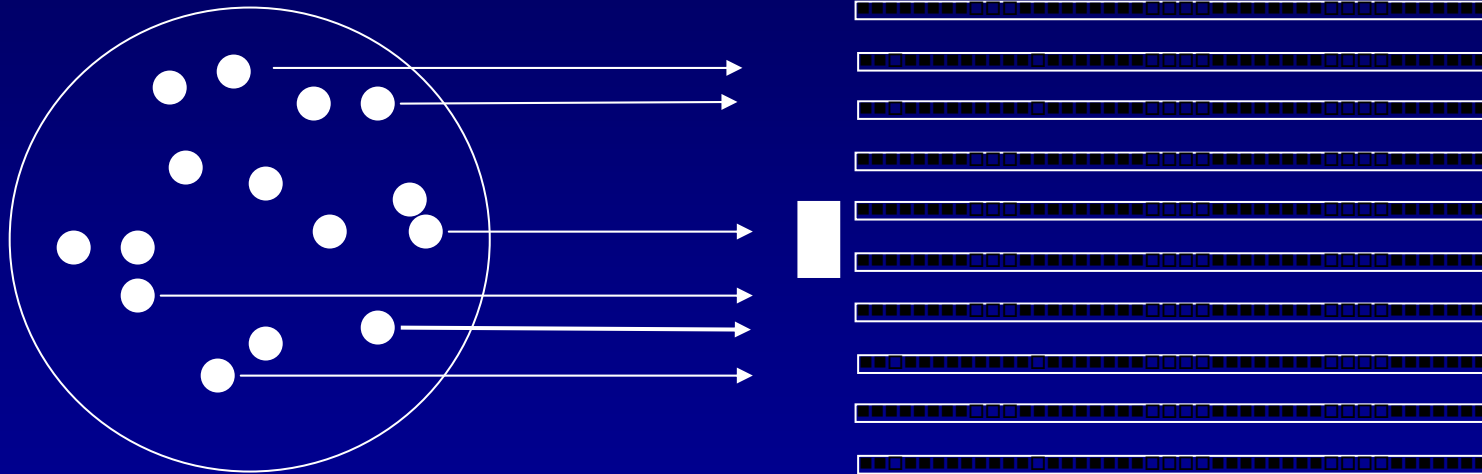
➔
**No es posible
identificar
presencia de más
de una cepa**

Spoligotipado

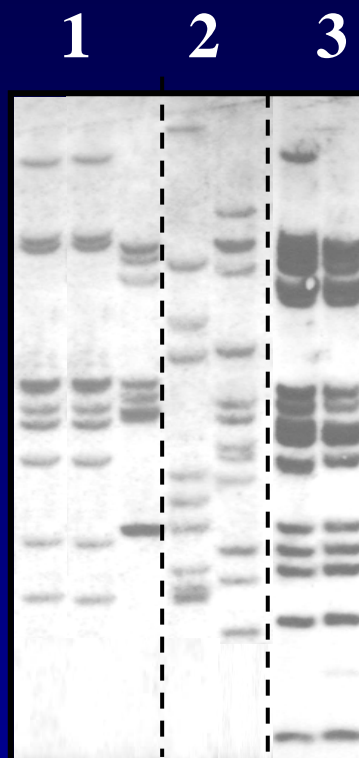


Infecciones complejas: Metodología

Análisis de múltiples colonias independientes



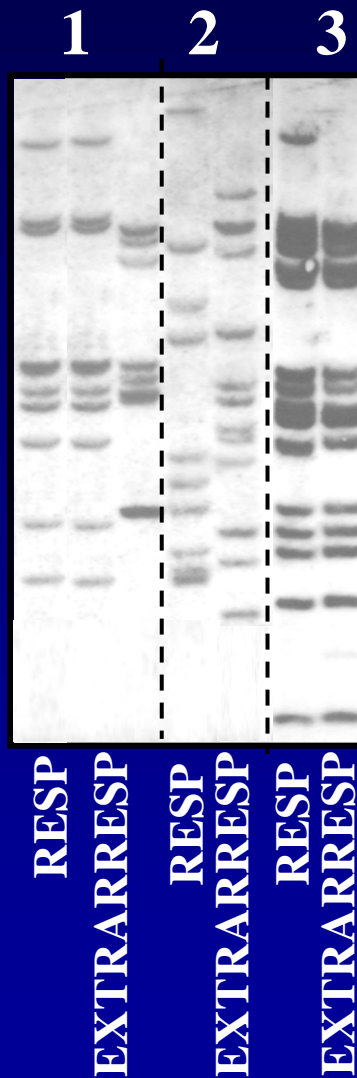
Infecciones complejas



50 pacientes retrospectivos

6% Coinfección con
dos cepas
simultáneamente

Infecciones complejas

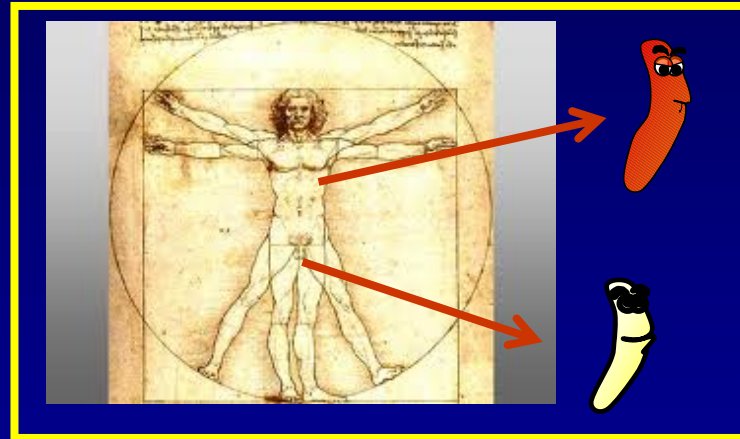


50 pacientes retrospectivos
Aislado resp + extrarresp

6% Coinfección con
dos cepas
simultáneamente

COMPARTIMENTALIZACION
DE LA INFECCION

Infección compartimentalizada



Infección compartimentalizada

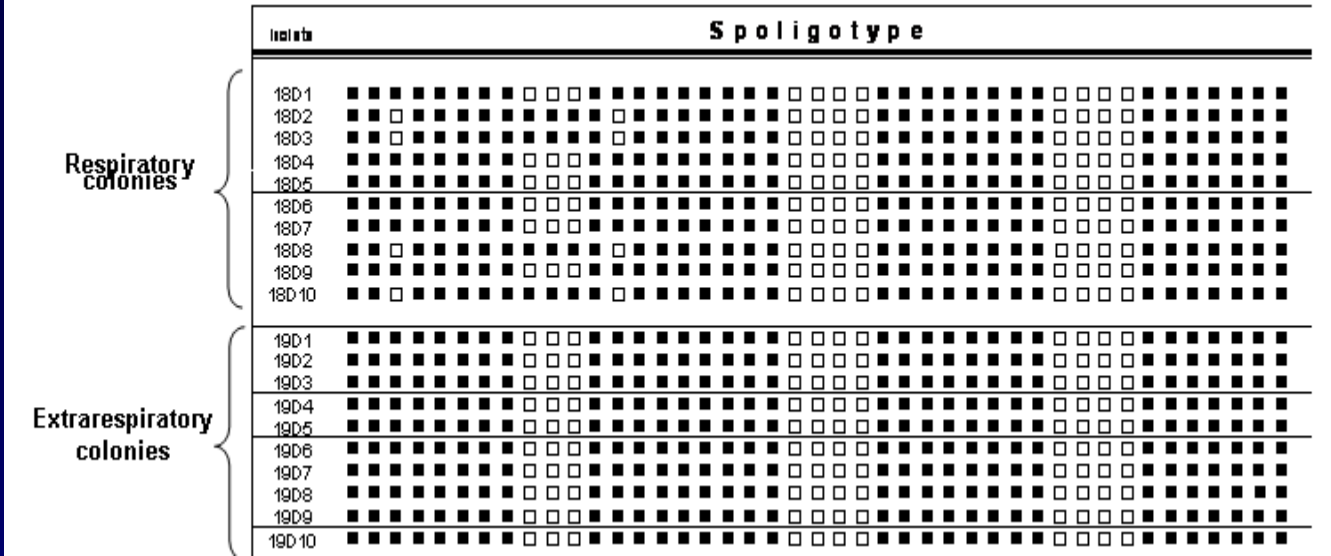
Patient	Sample	Isolate	Days Between Isolates	Number of different spacers	Spoligo type																							
13	BC, TTP	31C, 33C	6	0	[24 squares]																							
13	U	40C	3	5	[24 squares with 5 differences]																							
29	I	18D			[24 squares]																							
29	LA	19D	4	3	[24 squares with 3 differences]																							
32	I	33D			[24 squares]																							
32	PF	32D	24	1	[24 squares with 1 difference]																							

	Isolate	Spoligo type																							
Respiratory colonies	18D1	[24 squares]																							
	18D2	[24 squares]																							
	18D3	[24 squares]																							
	18D4	[24 squares]																							
	18D5	[24 squares]																							
	18D6	[24 squares]																							
	18D7	[24 squares]																							
	18D8	[24 squares]																							
	18D9	[24 squares]																							
	18D10	[24 squares]																							
Extrarespiratory colonies	19D1	[24 squares]																							
	19D2	[24 squares]																							
	19D3	[24 squares]																							
	19D4	[24 squares]																							
	19D5	[24 squares]																							
	19D6	[24 squares]																							
	19D7	[24 squares]																							
	19D8	[24 squares]																							
	19D9	[24 squares]																							
	19D10	[24 squares]																							

cepaA+B

cepaA

¿Competición? ¿Factores bacteriológicos?



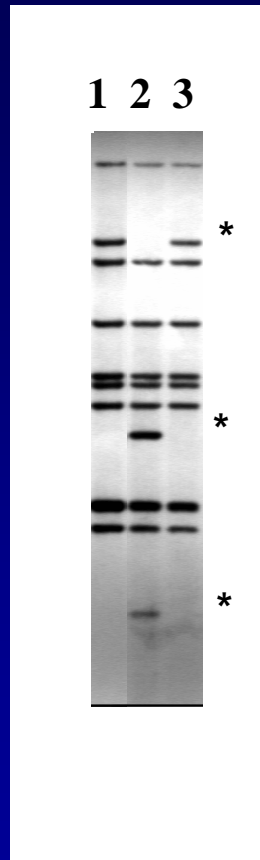
cepaA+B

cepaA

Infección de macrófagos humanos *in vitro* simultáneamente con las dos cepas



Compartimentalización



1: Respiratoria

2: Respiratoria

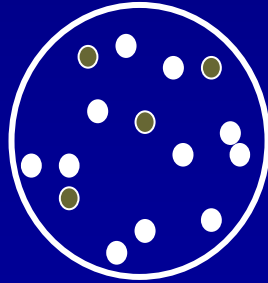
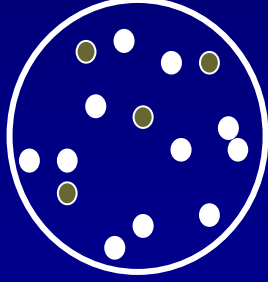
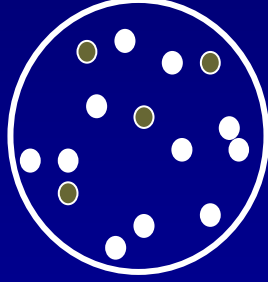
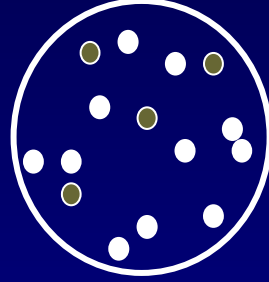
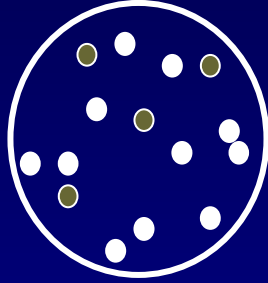
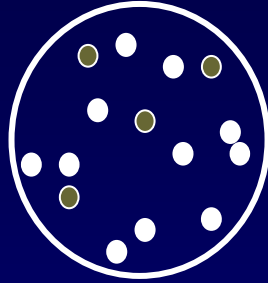
3: LCR

Cambios sutiles:

MICROEVOLUCION

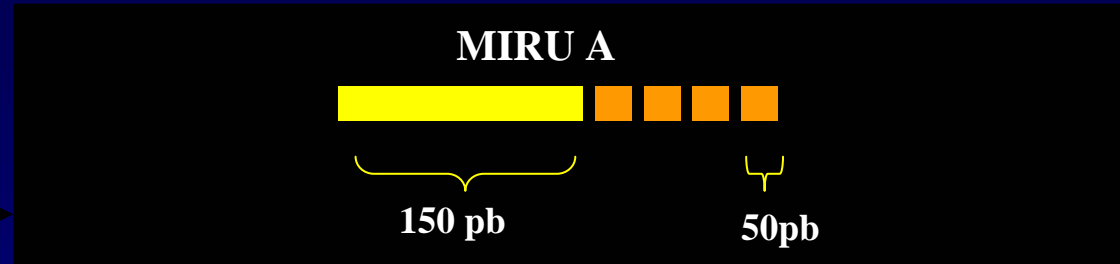
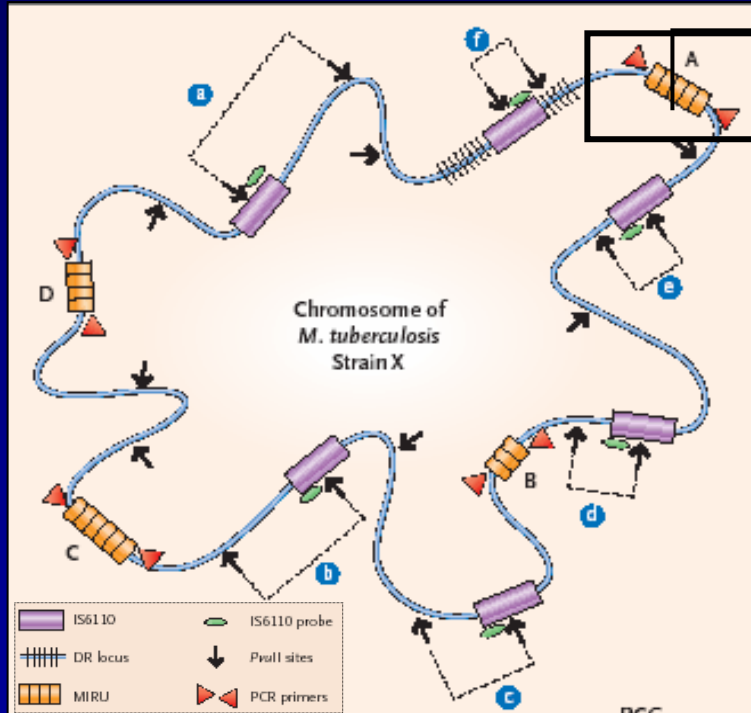
**modificación “comportamiento
infectivo” de cepa de MTB**

**Posible selección de cepa con mayor
capacidad de infección
extrarrespiratoria**



MIRU-VNTR

Mycobacterial Interspersed Repetitive Units- Variable Number Tandem Repeats

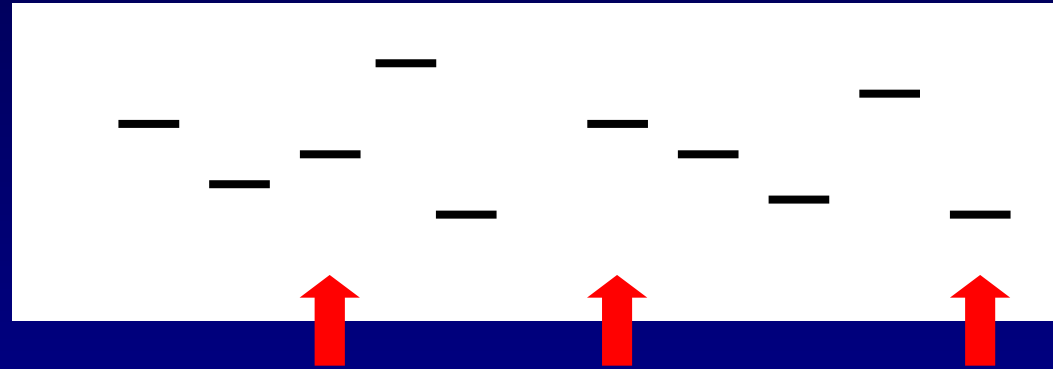
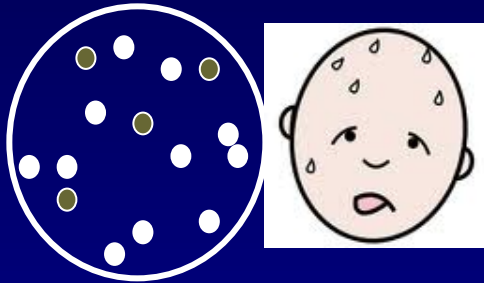


		PCR electroforesis	Nº repeticiones
Cepa A		150 pb	0
Cepa B		200 pb	1
Cepa C		250 pb	2
Cepa D		300 pb	3
Cepa E		350 pb	4
Cepa F		400 pb	5

MIRUtipo: 246684848883478

Infecciones complejas

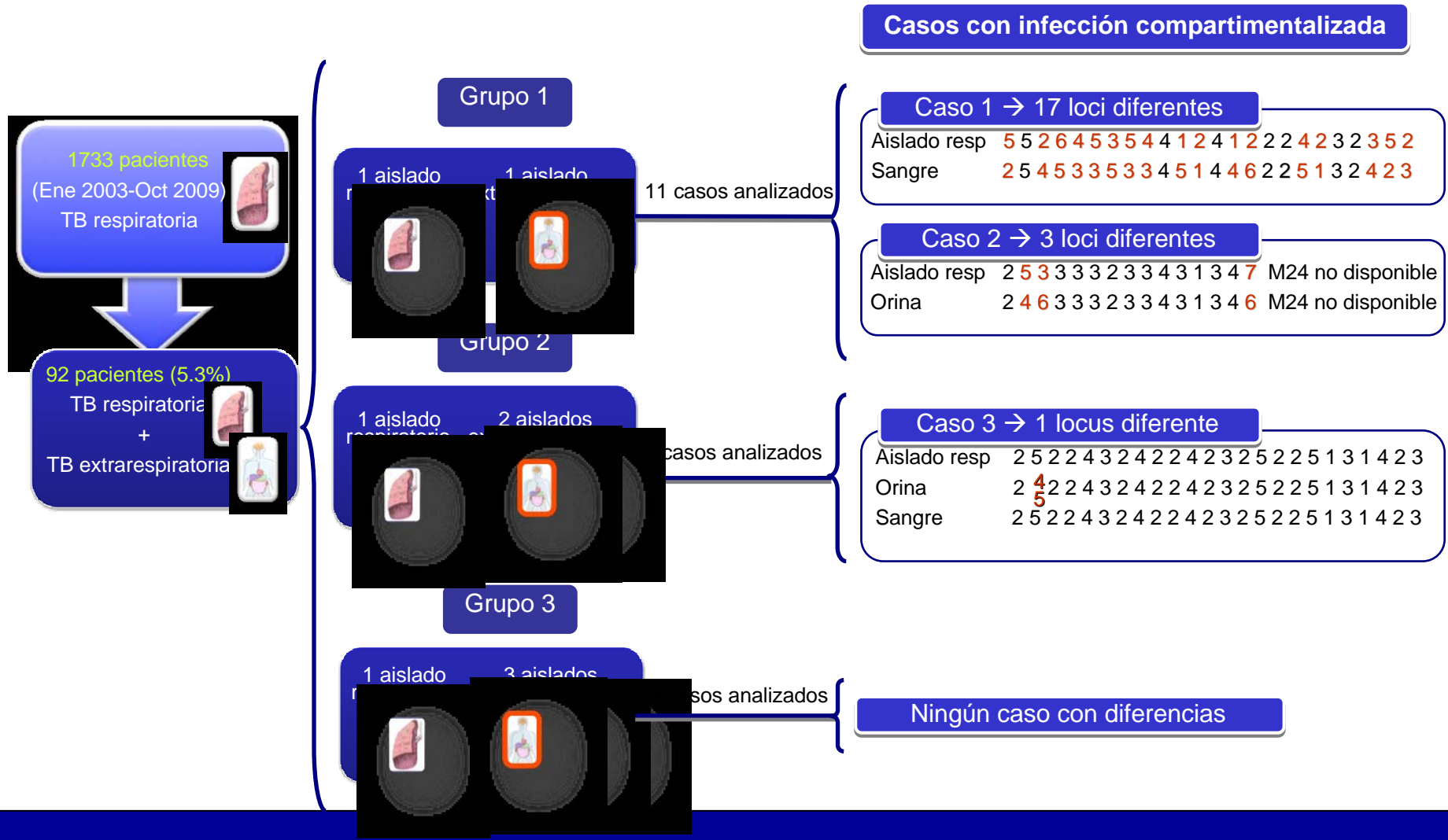
VNTR-MIRU y policlonalidad



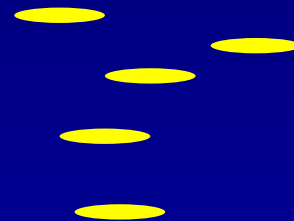
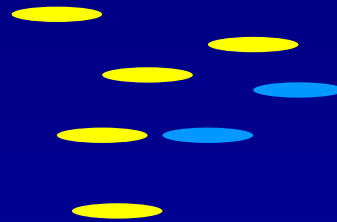
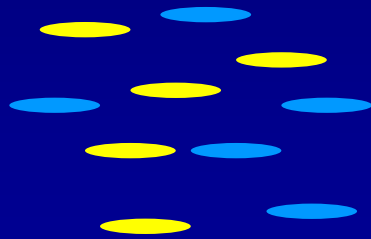
Infecciones complejas

**Población policlonal distinguible
sin analizar colonias aisladas**

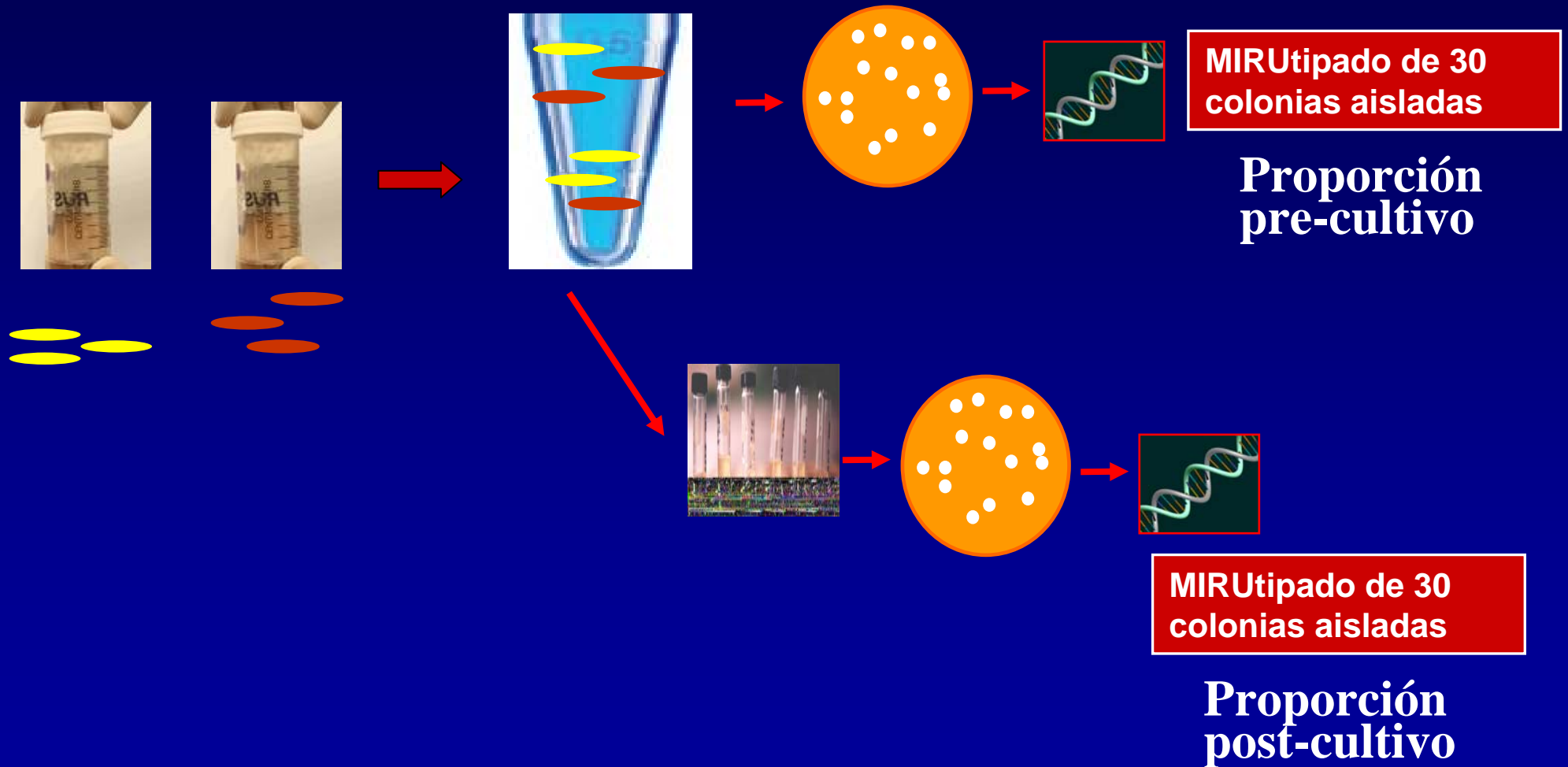
Infecciones complejas



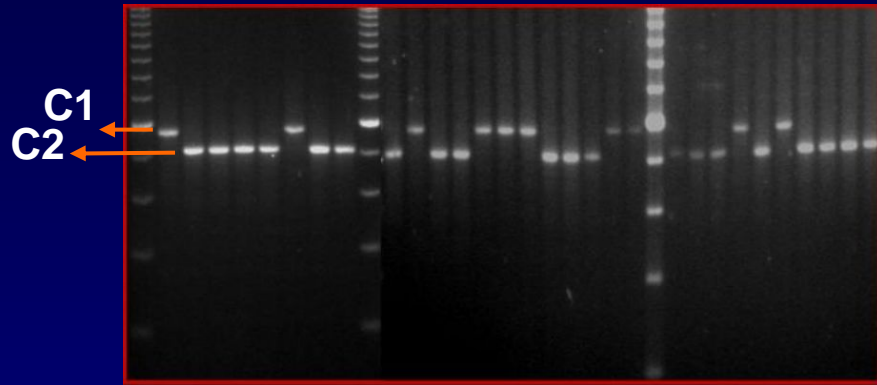
¿Puede el cultivo modificar la complejidad clonal existente en la muestra clínica?



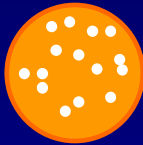
Efecto del cultivo sobre Esputos policlonales “artificiales”



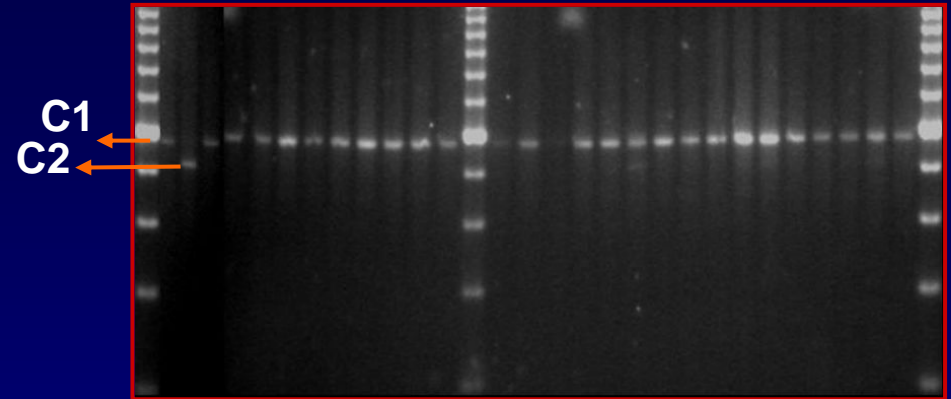
Antes del cultivo



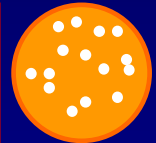
Cepa 1 (33.4%)
Cepa 2 (66.6%)



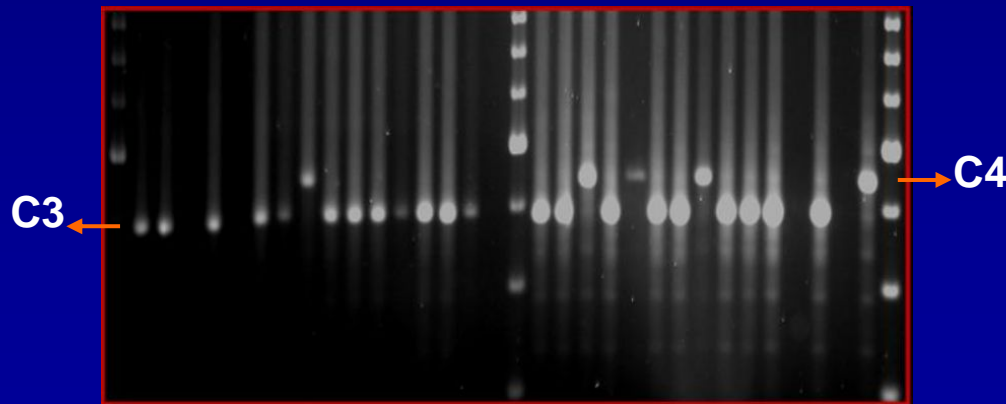
Después del cultivo



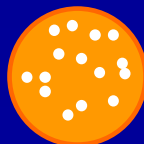
Cepa 1 (96.3%)
Cepa 2 (3.7%)



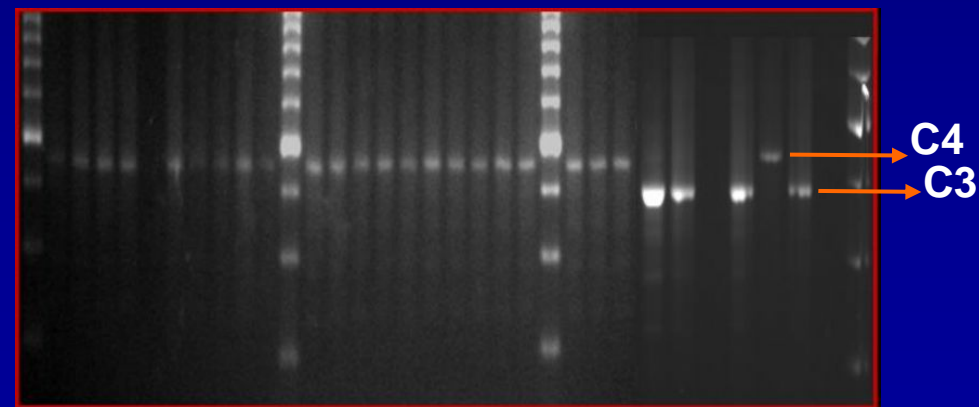
Antes del cultivo



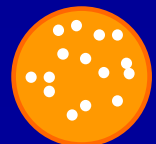
Cepa 3 (80.7%)
Cepa 4 (19.3%)



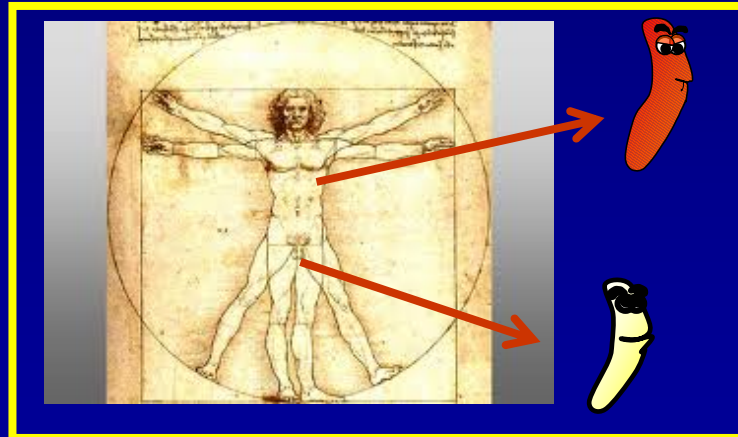
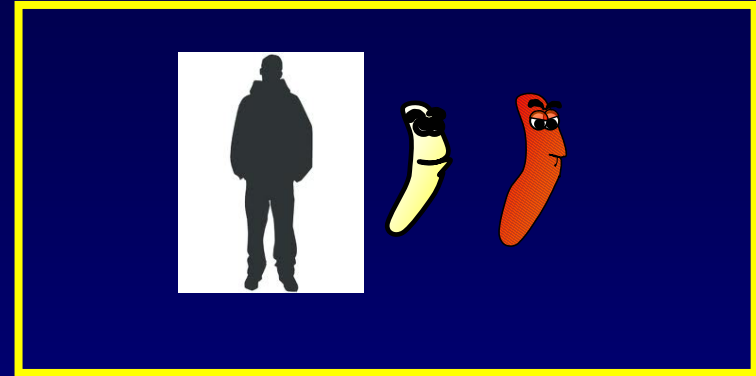
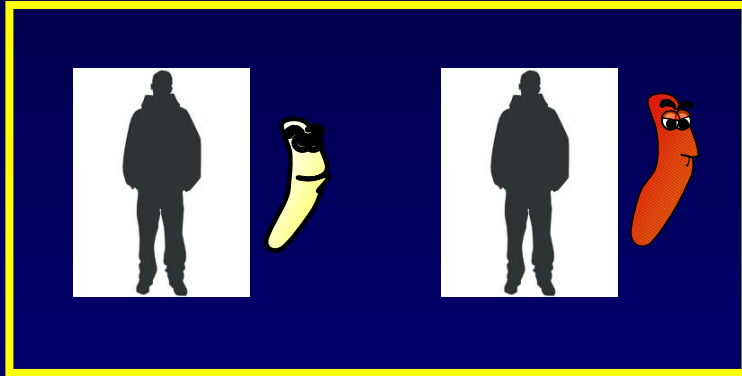
Después del cultivo

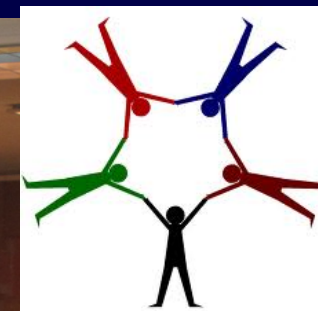


Cepa 3 (14.3%)
Cepa 4 (85.7%)



Rastreo de complejidad clonal en TB





Hospital General Universitario
Gregorio Marañón

Servicio Microbiología Clínica y Enfermedades Infecciosas