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GRAND ROUNDS

**Applying
Mycobacterium tuberculosis
Genotyping Results in
Local Program Activities**



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Introduction to Genotyping of *M. tuberculosis*

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Overview

- The Basics
- The Assumptions
- The Methods
- The Name Game



The Basics

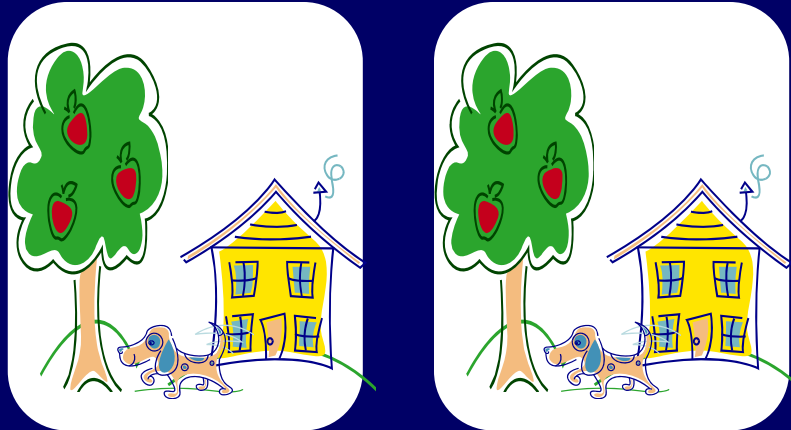
Genotyping is used to compare isolates and to answer the question “Are they the same or are they different?”.



Same or different?



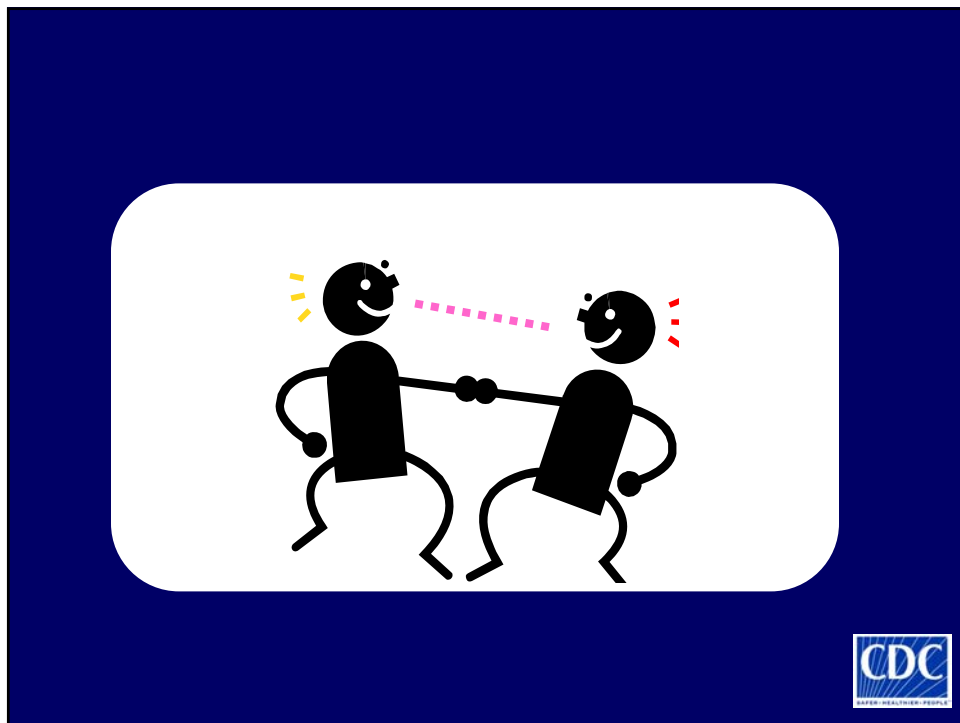
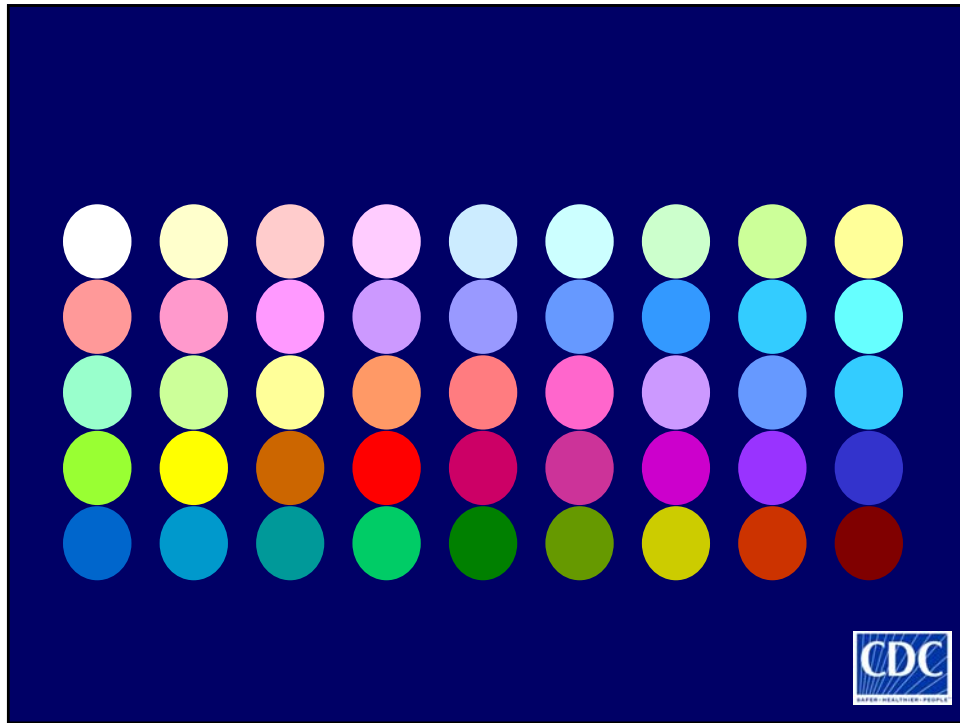
Same or different?

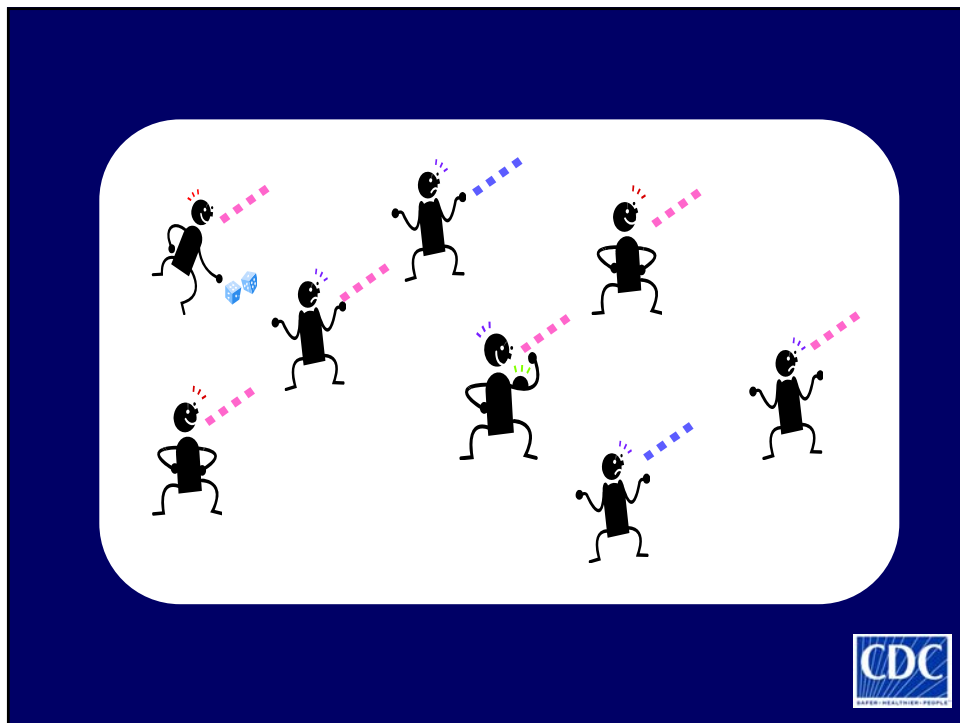
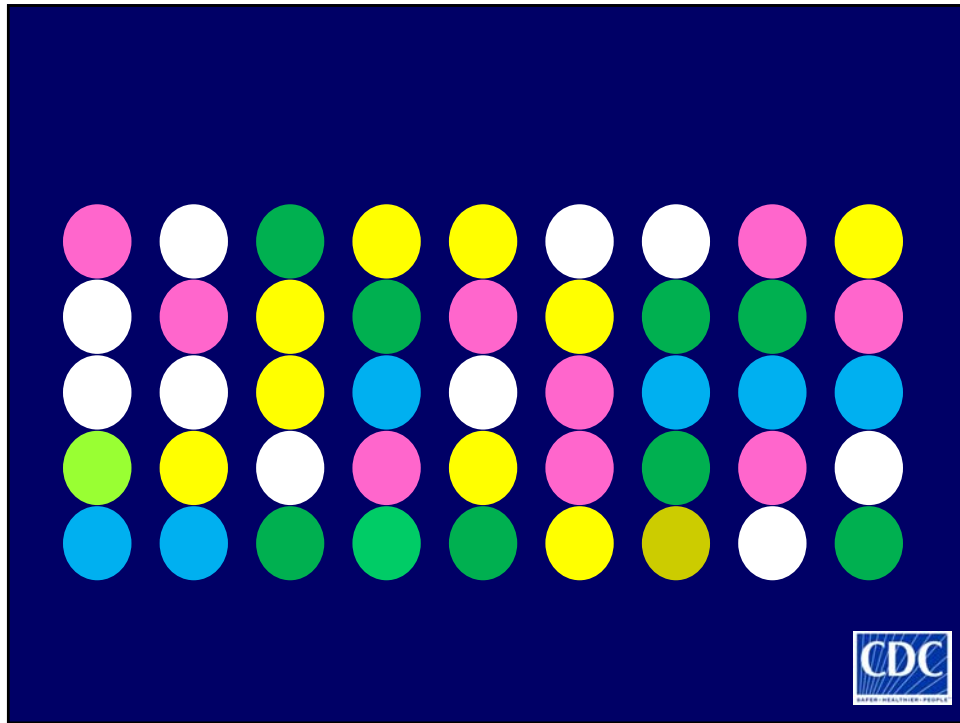


Assumption #1

There are so many different strains of *M. tuberculosis* circulating in a community that the best explanation for two patients being infected with the same strain is that the two patients share some type of relationship.

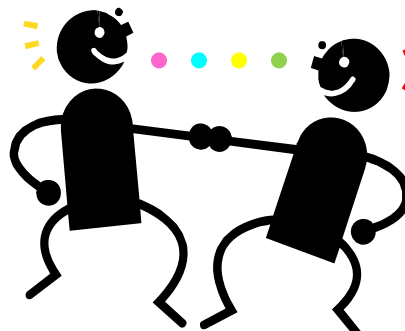






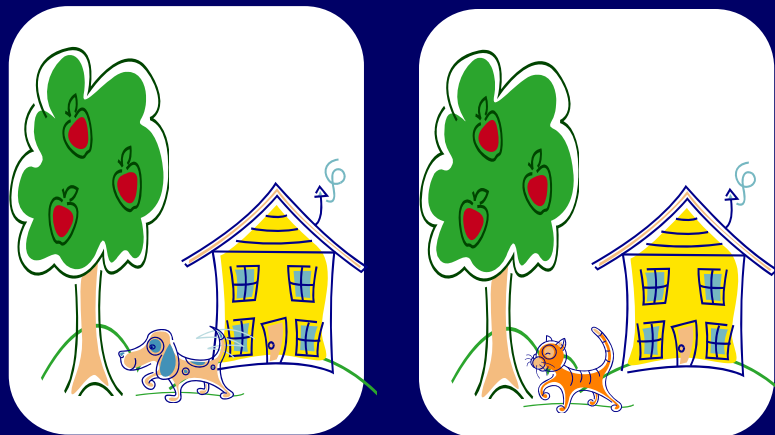
Assumption #2

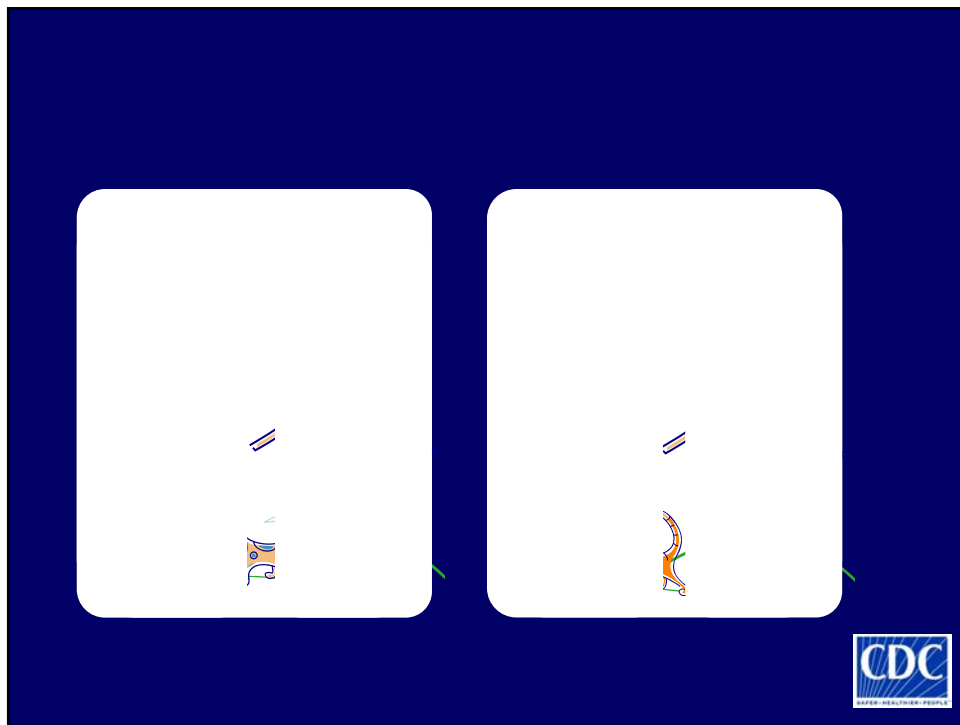
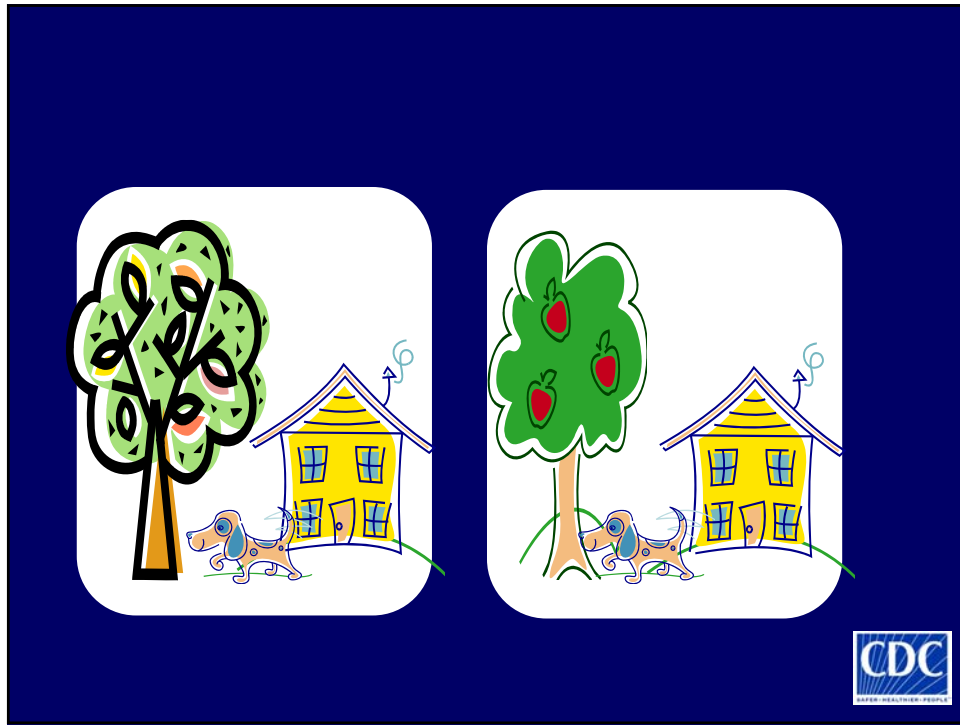
The genotyping method being used characterizes regions of the genome that change frequently enough to create diversity but not so frequently that it changes during a chain of transmission.

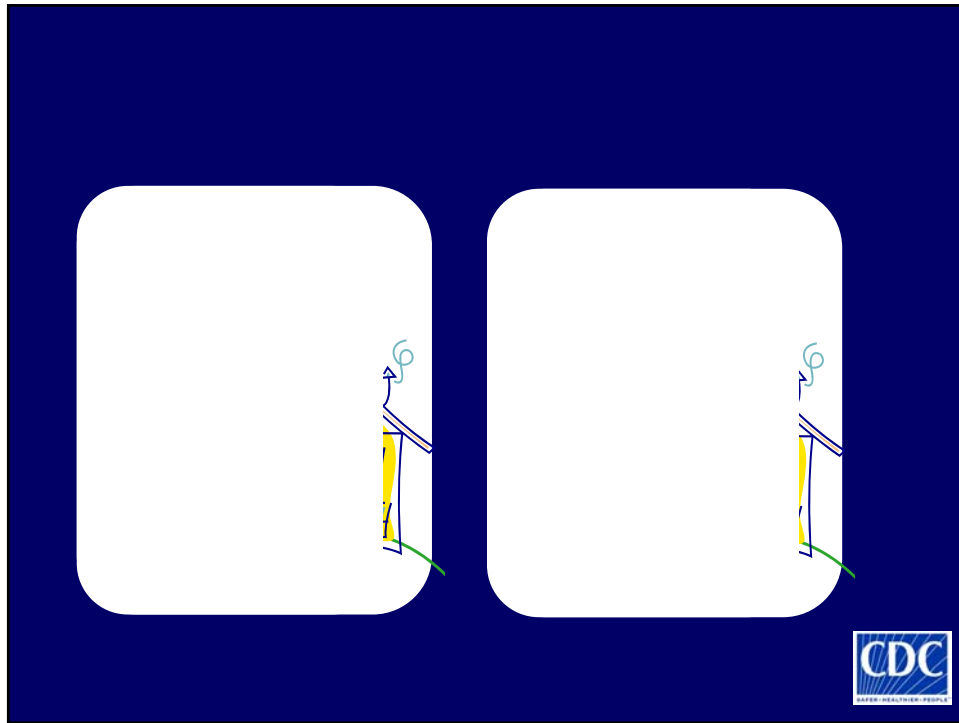


***M. tuberculosis* Genotyping**

- Characterize small segments of the *M. tb* genome (~1%)
 - Spoligotyping
 - MIRU-VNTR
 - IS6110-RFLP fingerprinting
- Why use multiple methods?





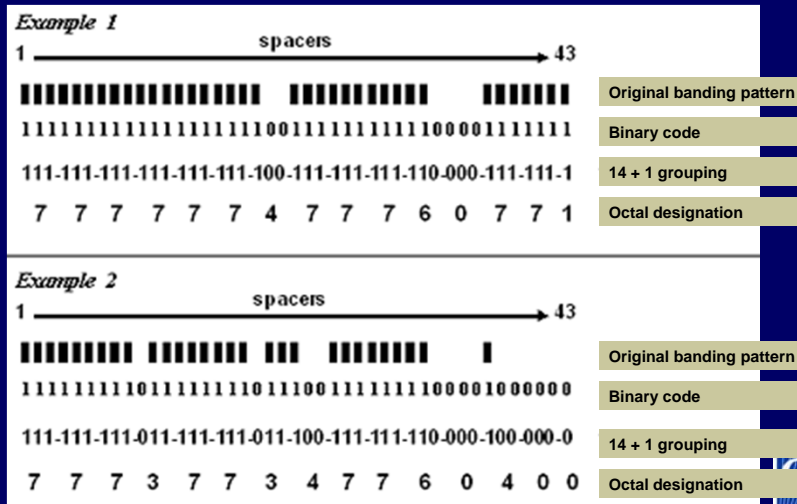


Spoligotyping

- Examines variation found in a single locus (direct repeat locus)
- Detects the presence or absence of 43 short spacer DNA sequences



Spoligotyping



Mycobacterial interspersed repetitive units variable number tandem repeats (MIRU-VNTR)

- Examines variation found in multiple loci distributed throughout the genome
- Determines the number of repeated units present at each locus



MIRU-VNTR

	MIRU locus name											
	02	04	10	16	20	23	24	26	27	31	39	40
# of repeats	2	3	2	2	3	4	2	5	3	3	2	2

MIRU: 232234253322

A = 10, B = 11, C = 12,
 "-" = no result obtained



Expanded MIRU-VNTR

- Original assay based 12 MIRU loci
- April 2009
 - Expanded to include 12 additional loci
 - MIRU2
- Total of 24 MIRU-VNTR loci

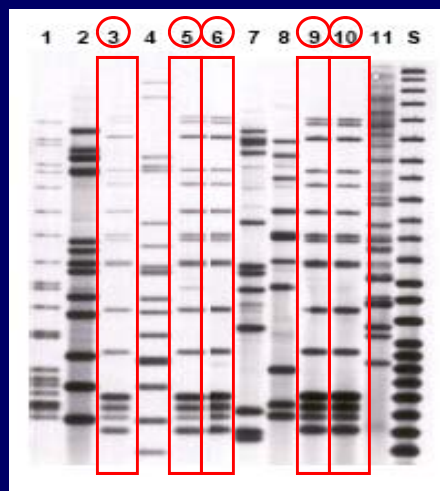


IS6110-RFLP fingerprinting

- Based on the variation found between strains in both the number of copies of IS6110 and their positions in the genome.



IS6110-RFLP



	A	B
spoligotype	000000000003771	777776777760601
MIRU1	224225173533	324325153323
MIRU2	112341156711	2463376589a2



	C	D
spoligotype	000000000003771	000000000003771
MIRU1	224225173533	224225173533
MIRU2	112341156711	112341156711

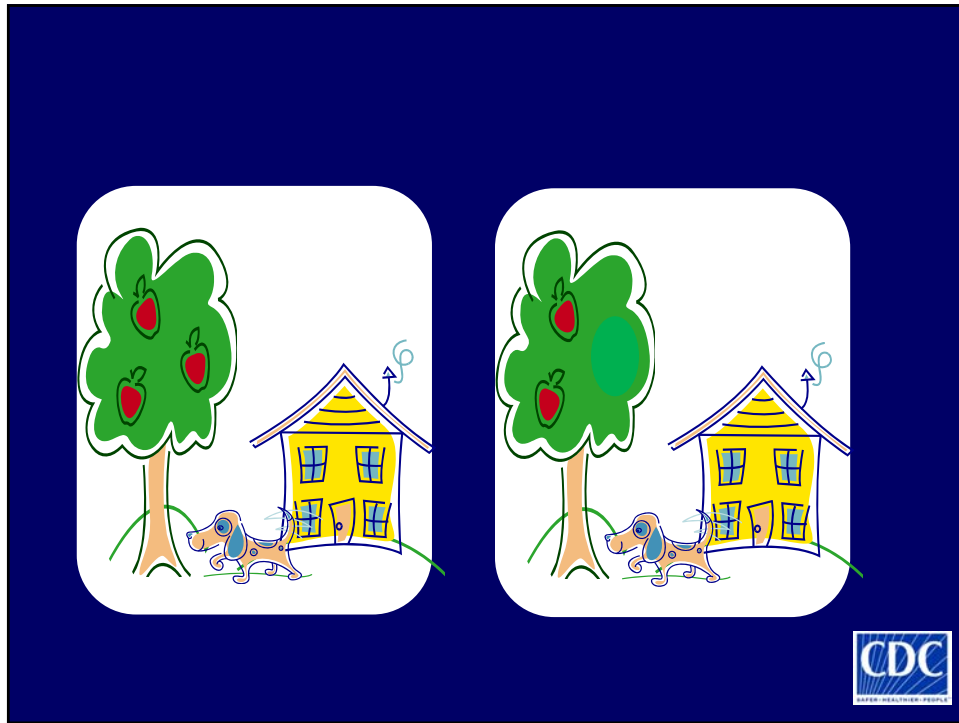


	C	D
spoligotype	000000000003771	000000000003771
MIRU1	224225173533	224225173533
MIRU2	112341156711	112341156711



	E	F
spoligotype	000000000003771	000000000003771
MIRU1	224225173533	124225173533
MIRU2	112341156711	112341156711





	G	H
spoligotype	000000000003771	000000000003771
MIRU1	-24225173533	224225173533
MIRU2	112341156711	112341156711

A table with three columns and four rows. The first column contains labels: spoligotype, MIRU1, and MIRU2. The second column is labeled 'G' and the third column is labeled 'H'. The table is set against a dark blue background with a CDC logo in the bottom right corner.

Nomenclature

	spoligotype	MIRU	PCRType	State cluster name	MIRU2	State cluster name 2
FL	000000000003771	224225153323	PCR00001			
GA	000000000003771	224225153323	PCR00001	GA_0002		
GA	000000000003771	224225153323	PCR00001	GA_0002		
GA	000000000003771	224225153323	PCR00001	GA_0002	123123123123	
GA	000000000003771	224225153323	PCR00001	GA_0002	223223223223	GA_00002_001
GA	000000000003771	224225153323	PCR00001	GA_0002	223223223223	GA_00002_001



National TB Genotyping Service (NTGS)

- CDC funded service to genotype one isolate from every culture-positive TB case in United States began in 2004
- 70,683 isolates have been genotyped (8/19/2011)





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