

INTRODUCTION TO **TUBERCULOSIS** **GENOTYPING**

Facilitator Guide

National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division of Tuberculosis Elimination



Introduction to Tuberculosis Genotyping

Facilitator Guide

Purpose

- The purpose of the *Introduction to Tuberculosis Genotyping slide set* is to provide basic education on tuberculosis (TB) genotyping and how it can be useful in TB prevention and control activities. The presentation is estimated to take about 30 minutes.
- This **facilitator guide** is to be used in conjunction with the *Introduction to Tuberculosis Genotyping slide set* to provide suggested wording to reinforce the messages in the presentation.

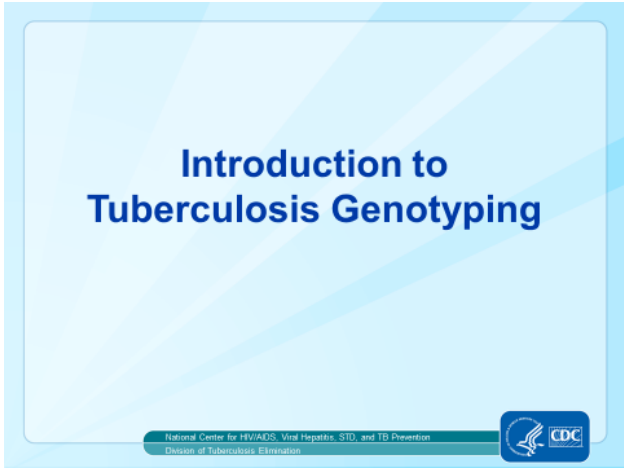
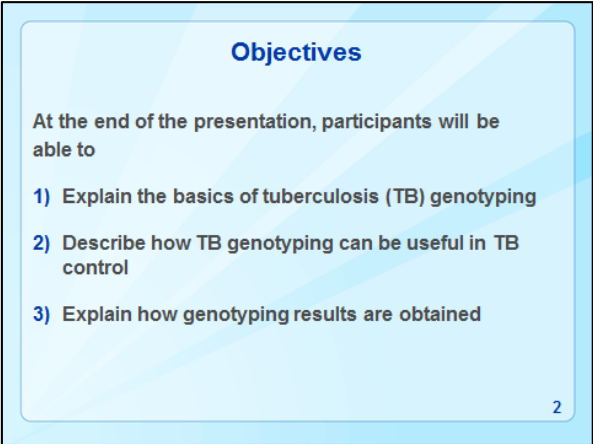
Audience

- The target audience for the *Introduction to Tuberculosis Genotyping slide set* is health care professionals who have basic or no knowledge of TB genotyping.
- The target audience for this **facilitator guide** is faculty and planners who will present the *Introduction to Tuberculosis Genotyping slide set*.

Acknowledgments

We wish to thank our colleagues from the Division of Tuberculosis Elimination for their collaboration in creating the *Introduction to TB Genotyping slide set* and facilitator guide.

- **From the Communications, Education, and Behavioral Studies Branch**
 - Peri Hopkins
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 - Anne Marie France
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	Slides	Speaker Notes
Slide 1		<p>Hi, welcome to today's presentation.</p> <p>For the next half hour, I will introduce you to tuberculosis (TB) genotyping.</p> <p>This course was designed for persons who work in the prevention and control of TB and who need to have knowledge of TB genotyping.</p> <p>Even if you've never heard of TB genotyping before this presentation, that's ok. This presentation is for you.</p>
Slide 2		<p>By the end of this presentation, you will be able to</p> <ol style="list-style-type: none"> 1. Explain the basics of TB genotyping. 2. Describe how genotyping can be useful in TB control. 3. Explain how genotyping results are obtained. <p>We will discuss two case scenarios that demonstrate the value that genotyping results can add when combined with information collected during contact investigations.</p>

QUESTION:
What is TB Genotyping?

- Laboratory method to detect TB infection
- A blood test to detect drug-resistant TB
- Laboratory approach to analyze genetic material (DNA) of *Mycobacterium tuberculosis* (*M. tuberculosis*)
- Tool to help understand transmission of *M. tuberculosis*
- Both c and d

DNA = deoxyribonucleic acid 3

Here is your first quiz question.

What is TB genotyping?

[If a clicker system is being used]

Please enter your choice on the clicker.

Or

[If no clicker system is being used]

Please raise your hand if you think TB genotyping is

A. A laboratory method to detect if someone has TB infection?

Or is it B, a blood test to detect if someone has drug-resistant TB?

Is genotyping C, a laboratory approach that analyzes the genetic material, or the DNA, of the bacteria that causes TB disease, *Mycobacterium tuberculosis*?

Do you think it is D, a tool to help understand transmission of *M. tuberculosis*?

Or who thinks the answer is both C and D?

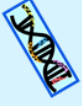
[Pause for responses]

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ANSWER:
TB Genotyping Is

- Laboratory method to detect TB infection
- A blood test to detect drug-resistant TB
- Laboratory approach to analyze genetic material (DNA) of *M. tuberculosis*
- Tool to help understand transmission of *M. tuberculosis*
- Both c and d

DNA = deoxyribonucleic acid 4



The correct answer is E.

TB genotyping is both

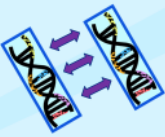
- A laboratory test used to analyze the genetic material of *M. tuberculosis*, and
- A tool to help understand *M. tuberculosis* transmission between patients.

Let's explore this a little further.

[Note to facilitator: If asked about DNA: DNA stands for 'deoxyribonucleic acid' and is an extremely long, double-stranded, nucleic acid molecule. DNA carries genes as segments along its strands.]

TB Genotyping

- Only for culture-confirmed TB
 - The technique requires material from a culture
- Matching genotypes may indicate that TB cases are related



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TB genotyping can only be done for TB cases with positive culture results. The technique requires use of material from a culture result.


Genotyping is not possible on negative culture results or for persons who have TB infection, but do not have TB disease.

If *M. tuberculosis* grows in the culture media, it is called an 'isolate'.

When two (or more) people have an isolate with a matching genotype, that indicates that the cases may be related and may be involved in the same chain of transmission.

When two TB patients have isolates with *nonmatching* genotypes, that indicates that they are probably not involved in the same chain of transmission.

Genotypes and *M. tuberculosis* Transmission



- Genotyping helps us understand transmission relationships between TB cases
- We expect genotypes from transmission-related TB cases to match

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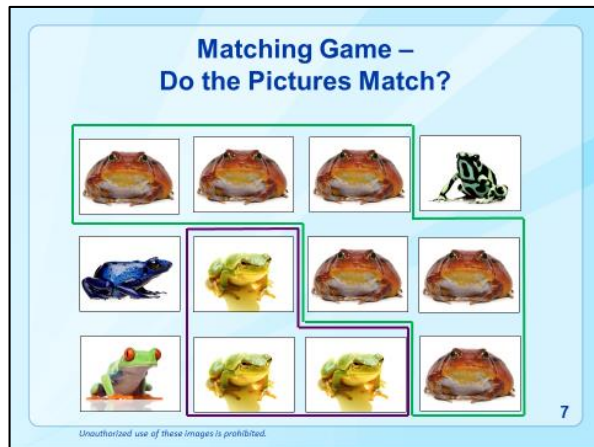
Genotyping can help us understand transmission relationships between persons with TB disease.

When one person with TB coughs and <<CLICK ONCE>>

Transmits the bacteria through the air to another person and that person develops TB disease, <CLICK TWICE>

Their TB bacterium is closely related, and the TB DNA will be almost identical. <CLICK ONCE>

So we expect genotypes from transmission-related cases to match, and that may indicate that there is a relationship between these patients.



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That may seem complicated, but let's put it into perspective.

The concept is actually very simple. The question is, 'Do the pictures match?'

TB genotyping is similar to the matching game some of us may have played as kids. Basically, we're looking for matches among all the pictures, or genotype results, we have.

So here, we have two groups of matching frogs, the green group and

<CLICK ONCE>

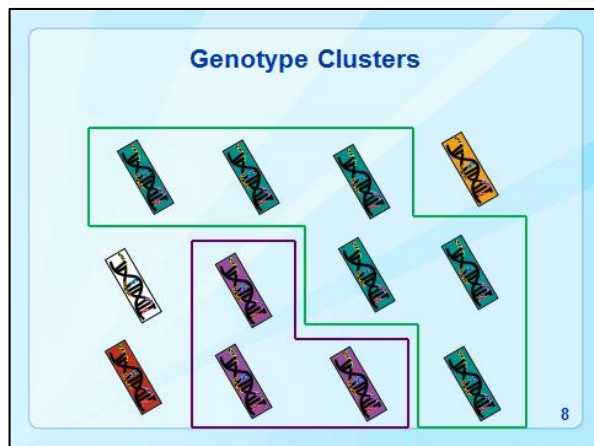
the orange group.

<CLICK ONCE>

And then there are a few outliers.

Which ones are not like the others? Right, the last two in the first column and the turquoise and black spotted frog in the top right corner.

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In TB genotyping, we're looking for groups of matching genotypes, or clusters.

So here we have two clusters,

<CLICK ONCE>

a purple one, and

<CLICK ONCE>

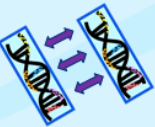
a teal one.

When two or more *M. tuberculosis* isolates match by genotype, they are referred to as a "genotype cluster."

A "cluster" means that the cases may be transmission-related.

How Can Genotyping be Useful in TB Control? (1)

- Assist with contact investigations
 - Confirm or refute patient connections
- Find previously unidentified contacts
- Detect and prevent outbreaks
- Refute outbreaks



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How can genotyping be useful in TB control?

TB genotyping can be beneficial in multiple ways.

First, TB genotyping can help in conventional TB contact investigations by

- Confirming that TB cases linked by contact investigation are related by transmission, and
- Refuting or disproving TB case connections.

If the genotypes do not match, the TB cases that may seem to be linked by contact investigation results are not actually linked by transmission.

TB genotyping can also help us find previously unidentified contacts and other TB case connections.

This could lead to the identification of

- A previously unknown source case, and
- Other locations where transmission may have occurred.

One of the major uses of TB genotyping is to help us detect and prevent outbreaks. By improved knowledge of how cases are linked and identifying infected contacts, you can prevent additional cases from occurring.

As mentioned, clusters of TB cases with matching genotypes may be related by recent transmission.

Genotyping can help refute outbreaks when a group of cases is suspected to be related by traditional epidemiology, but the genotypes do not match, which tells us that the cases do not actually represent an outbreak.

How Can Genotyping be Useful in TB Control? (2)

- Distinguish relapse from new infection
- Detect false-positive culture results

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Additionally, for a patient who is presenting with a second episode of TB disease, we can look at the genotypes to see if they are the same as when they were first diagnosed or different, thus helping to distinguish between a relapse and new infection.

Another advantage of genotyping is that an incorrect TB diagnosis based on a false-positive culture result will be identified more quickly.

False-positive culture results can be caused by various errors:

- Cross-contamination in the lab,
- Contamination of a clinical device, such as a bronchoscope, or
- Mislabeling of a specimen or other documentation errors.

Consequences of false-positive TB culture results include incorrect TB diagnosis and unnecessary treatment and wasted public health resources.

Through these different applications, TB genotyping

- Allows for a more comprehensive understanding of contact investigation results,
- Can prevent new TB cases,
- Distinguishes relapse of previous TB from a new infection, and
- Saves time and public health resources when a situation is determined not to be an outbreak.

****Animated Slide****

Let's start with a pretty common case scenario:

A local TB program is monitoring genotype results in their jurisdiction, say County A, and within a 4-month period, three people in the same town are diagnosed with TB.

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Two are members of a family: a mother and a 6-year old son.

<CLICK ONCE>

One other person, a neighbor, said she spent much of her time at that house during the day.

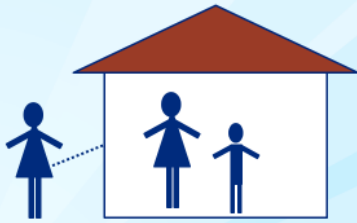
<CLICK ONCE>

So the conclusion of our epidemiologic investigation is that all three of the persons with TB spent most of their time together at the same house,

<CLICK ONCE>

And because of this, we strongly suspect that these three cases are related.

Case Scenario 1: A Household (1)



Persons diagnosed with TB spent most of their time together at the same house

- Likely related by transmission

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Slide 11

Case Scenario 1: A Household (2)

Genotype results for TB cases linked to household:

Mother	Genotype 1
Son	Genotype 1
Neighbor	Genotype 1

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Slide 12

Here are the genotype results for the three patients linked to this household.

We can see that TB bacteria from all of these patients had the same genotype result.

How would you interpret these genotype results?

Case Scenario 1: A Household (3) Interpretation of Genotyping Results

- All cases had matching genotypes
- All spent time together in the same house
- These cases were likely transmission-related

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The interpretation would likely be that, because we know that genotype results from all three cases matched,

And we know the mother, her son, and the neighbor all spent time together in that house and shared the same air space,

We assume that these three cases were likely related by transmission.

****Animated Slide****

Let's go back to the household and take a closer look at those cases.

The contact investigation activities for the mother did not find any other cases of active TB disease.

There were two other people in the household, **<CLICK ONCE >**

An adult male and a 7-year old girl. They both received a TB skin test, and both were diagnosed with TB infection (indicated as pink).

They were started on the 12-dose regimen of isoniazid and rifapentine one time a week by directly observed therapy at their home.

During interviews with the neighbor, she did not name any additional contacts outside of this household and said she lived alone.

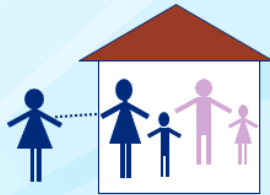
So, all in all, a fairly neat little scenario, and we think we're done, right?

Maybe not. Does anyone have any concerns about this situation?

Could there possibly be unnamed contacts?






Case Scenario 1: A Household (4) Back to the Household

- Contact investigation did not find any other cases
- Two other family members were diagnosed and treated for TB infection
- Neighbor with TB did not identify any other contacts aside from this family



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Case scenario 1: A Household (5)
Review of Genotype Data for County A – 2013

Mother	Genotype 1	
Son	Genotype 1	
Neighbor	Genotype 1	
Patient A	Genotype 1	
Patient B	Genotype 1	

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Yes, there could be more contacts and there could be more cases.

In fact, when County A looked at all cases with Genotype 1 in their jurisdiction during 2013, this is the list they discovered.

We already knew about the two family members and their neighbor.

But there are two other TB cases with this same genotype, Patient A and Patient B.

What does genotyping tell us here?

Case Scenario 1: A Household (6)
What do the Genotype Results Indicate?

- Five cases with matching genotypes within 6 months
- Cases may all be related by transmission, but
 - *When?*
 - *Where?*
- More information is needed

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The genotype results show that the five cases all had matching genotypes within one year, so they may all be related by transmission.

But we don't know the time or locations where transmission may have occurred for Patients A and B.

It's possible that they were infected many years ago, but it's also possible that they have been recently infected.

We need more information to try to determine if Patients A and B may be related by transmission to each other and to the other TB cases.

Case Scenario 1: A Household (7)
Next Steps

- Investigate to understand relationship of Patient A and Patient B to the other patients in the cluster
 - Identify likely locations of transmission
 - Determine if there are missed contacts
- Review
 - Public health records
 - Contact investigation logs
 - Estimated infectious periods
 - Re-interview TB patients and contacts

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Our next steps would be to investigate further.

If Patient A and Patient B are related in some way to each other or to the rest of the patients in the cluster (for example, if they attended the same social club that the mother attended), we may be able to identify


- Other potential locations of transmission, and
- Missed contacts who need testing.

To do this, we can do a careful review of

- Public health records,
- Contact investigation logs,
- Estimated infectious periods of the cases, and
- Information from interviews with TB cases and contacts in the genotype cluster.

****Animated Slide****

Case Scenario 2: A Workplace (1)



- Within one month
 - Three women diagnosed with TB
 - All work at the same casino
 - All work on the same evening shift
- One woman's boyfriend also diagnosed with TB

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Now for the second case scenario.

We're in a different state now; one that has legal gambling.

Within a single month, three women are diagnosed with TB.


All three women work in the same casino, and they all work the nightshift together.

Additionally, one woman's boyfriend is also diagnosed with TB.

<CLICK ONCE>

Case Scenario 2: A Workplace (2)
QUESTION:
 Are these TB cases related by transmission?

a. Yes
 b. No
 c. Maybe



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Do you think these TB cases are related by transmission?

[If a clicker is being used]

If you think yes, press A on your clicker.

If no, press, B.

If you think that maybe they are related by transmission, press C.

[If no clicker is being used]

Raise your hand if you think the answer is


- Yes?
- No?
- Maybe?

[Pause for responses]

****Animated Slide****

Case Scenario 2: A Workplace (3)
ANSWER:
 Are these TB cases related by transmission?

a. Yes
 b. No
 c. Maybe!



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If you answered C, you are correct.

These TB cases *may* be related by transmission.

But to determine that, we need more information.

Case Scenario 2: A Workplace (4)
Genotype Results for TB Cases Linked to Casino

Employee 1	Genotype 2
Employee 2	Genotype 3
Employee 3	Genotype 4
Boyfriend	Genotype 5



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Here are the genotype results for those four TB cases.

The TB bacteria cultured from Employee 1 was genotype number 2.

The TB bacterium from employee 2 was a different genotype, genotype number 3.

Employee 3 had genotype number 4, and

The boyfriend's genotype was number 5.

How would you interpret these results?

Case Scenario 2: A Workplace (5)
QUESTION:

How to interpret the genotype results?

- The genotype data are wrong
- The genotype data could be wrong, since cases are linked epidemiologically
- These cases are not related by transmission
- I don't know

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[If a clicker system is being used]

Please enter your choice on the clicker.

Or

[If no clicker system is being used]

Who thinks:

- The genotype data are wrong
- The genotype data could be wrong, since there's epidemiologic information that they're connected by transmission
- These cases are not related by transmission, or
- Who knows?

[Pause for responses]

Case scenario 2: A Workplace (6)
ANSWER:
How to interpret the genotype results?

- a. The genotype data are wrong
- b. The genotype data could be wrong, since cases are linked epidemiologically
- c. These cases are not related by transmission**
- d. I don't know

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****Animated Slide****

The correct answer is 'C'.

These cases are not related by transmission.

How do we know that?

[Pause for responses]

Case Scenario 2: A Workplace (7)
Interpretation of Genotype Results

- **Genotype results from all cases were different**
 - These cases are not related by transmission
 - This was a coincidence
- **Four contact investigations are needed**
 - Three in same work site
- **Not an outbreak**

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****Animated Slide****

The genotype results from each of these cases were different.

That tells us that these cases are not related by transmission.

Finding TB in four TB cases who happened to have had social connections was a coincidence.

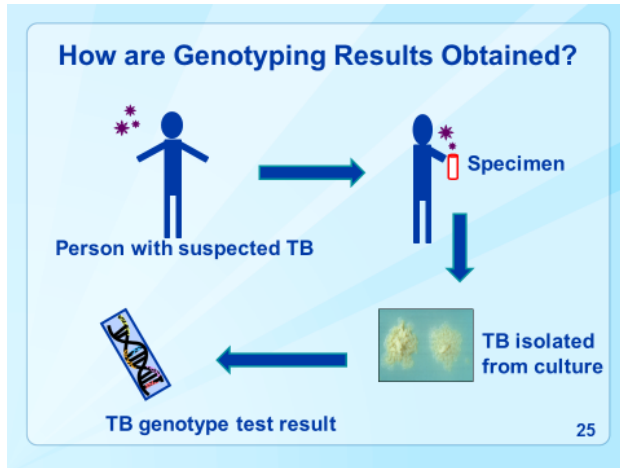
Rather than an outbreak investigation, this situation calls for four simultaneous contact investigations, with three in the same work site.

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This is NOT an outbreak **at this point**, and that is great news.

However, it is possible that future contact investigation activities uncover previously undetected cases.

****Animated Slide****



Now that you've seen how genotyping results can be used in a TB program, you may be wondering how genotyping results are obtained.

First, a person with suspected or confirmed TB provides a 'specimen.'

A specimen is a clinical sample (e.g., sputum, bronchial wash, urine, blood, cerebrospinal fluid, or tissues from organs or gastric aspirates).

Then the specimen is sent to a laboratory (hospital, private, or state lab) for culturing.

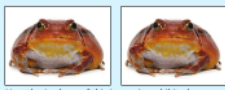
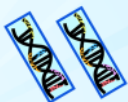
If *M. tuberculosis* grows in the culture media, it is called an 'isolate'.

Only isolates identified as *M. tuberculosis* can be genotyped.

Then, the isolate goes to a genotyping laboratory for testing and identification.

Take Home Points

- TB genotyping can be useful in TB control
 - Find additional contacts
 - Detect and prevent outbreaks
 - Refute outbreaks
- Interpreting genotyping results can be as simple as, “Do the pictures match?”



Unauthorized use of this image is prohibited.

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In conclusion, we have shared a lot of information with you about TB genotyping.

But the two main take home points are:

First, genotyping can be a useful tool in TB control in multiple ways, including

- Helping to find previously unidentified or unnamed contacts,
- Detecting and preventing outbreaks (for example, when a TB culture result is identified as being false-positive), and
- Refuting outbreaks.

When a suspected outbreak is refuted by genotyping, TB programs can save time and resources.

While a contact investigation for each TB case will be required, additional activities related to outbreak investigation and response will not be necessary.

The second take home point is that interpreting genotyping results can be as simple as, “Do the pictures match?”

If yes, the cases may be related by transmission, and additional investigation may be warranted.

If the pictures don’t match, the cases are not likely related by transmission, thus determining that it is not an outbreak, and further investigation activities may be avoided.

CDC Resources on Genotyping

- CDC TB genotyping website
 - www.cdc.gov/tb/programs/genotyping/default.htm
- TB genotyping best practices
 - www.cdc.gov/tb/publications/factsheets/statistics/Genotyping_BestPractices.pdf
- TB Genotyping Information Management System (TB GIMS)
 - <https://ajitv-nifm-web2.cdc.gov/TBGIMS/>
- Email CDC
 - tbgenotyping@cdc.gov

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This presentation is an introduction about genotyping and how it can be helpful to TB control. There is a lot more practical information out there to help programs interested in learning more and applying genotyping to their own TB control efforts.

Various resources are posted on the CDC Division of Tuberculosis Elimination website under genotyping, and additional resources are being developed.

You can access the TB Genotyping Best Practices document, which is a consensus document developed by CDC in collaboration with multiple state and local TB programs. This document describes specific steps and actions in applying TB genotyping effectively.

The Tuberculosis Genotyping Information System, or TB GIMS, is a web-based system that stores and manages genotyping data on TB patients in the United States.

Also, you can email CDC at tbgenotyping@cdc.gov

For local programs, your state genotyping program should be the first place to go for more information. We're happy to help you connect with them if you don't know who to contact.

Thank you!

For more information please contact the Centers for Disease Control and Prevention
1600 Clifton Road NE, Atlanta, GA 30329
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Web: <http://www.cdc.gov>

Or TBGenotyping@cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Division of Tuberculosis Elimination



Thank you very much for your interest.

I'm happy to try to answer any questions you may have at this time.

