

FEDERAL BUREAU OF INVESTIGATIONS**For Internal Release****Monday, August 18, 2008****FBI TO HOST ROUNDTABLE DISCUSSION
WITH D.R VAHID MAJIDI, ASSISTANT DIRECTOR OF THE
FBI WEAPONS OF MASS DESTRUCTION DIRECTORATE
AND DR. D. CHRISTIAN HASSELL, FBI LABORATORY DIRECTOR
REGARDING THE ANTHRAX INVESTIGATION****FBI National Press Office
Office of Public Affairs
935 Pennsylvania Avenue, N.W.
Washington, D.C.****10:00 A.M. EDT**

MR. KORTAN: My name is Mike Kortan from the FBI Office of Public Affairs, and I'm going to turn it over to Dr. Majidi here in a moment. Just to remind everybody, it's an on-the-record availability here as far as the two principles here, which we've described. And everyone else will be a background official that will support them.

We just remind everybody to use the microphones if you can when it gets to the question and answer point. The format's going to be Dr. Majidi is going to start off with an opening statement, an overview here. Dr. Hassell will introduce the panel members. And then we can begin a dialogue after that.

So Dr. Majidi.

DR. MAJIDI: Thank you. Good morning, ladies and gentlemen. I'm Vahid Majidi, the assistant director responsible for the FBI's Weapons of Mass Destruction Directorate. I would like to start today's session with a brief opening statement to define the scope of our roundtable discussion.

After nearly seven years of investigation, we have developed a body of power evidence that allows us to conclude that we have identified the origin and the perpetrator of the 2001 Bacillus anthracis mailing. The attribution process and identification of a specific perpetrator relies on the confluence of the intelligence investigation and forensic information.

It is the information from forensic data that determined the source of the 2001 Bacillus anthracis mailing was derived from the spore preparation known as RMR 1029 that was maintained at U.S. Army Medical Research Institute for Infection Disease, Fort Detrick, Maryland. From now on I'll refer to that as USAMRIID.

While there were countless investigative hours spent narrowing the field of suspects, we are here today to focus on the scientific aspects of this case. First of all, let me dispel some frequently repeated erroneous information.

For example, there were no intentional additives combined with the Bacillus anthracis spores to make them any more dispersible. Also, the purity of samples obtained from four letters were sufficiently different which allowed us to conclude that at least two different Bacillus anthracis batches were prepared from the original RMR 1029. This indicates that aliquots of RMR 1029 were removed and

cultured in at least two separate batches to produce the materials used in the mailings.

The FBI began this complex investigation by coordinating analysis of spore powders contained in the 2001 *Bacillus anthracis* mailings. We enlisted the help of many biodefense experts to assist our examinations, including those who had previously developed tests to differentiate strains of *Bacillus anthracis* and identify the spores in the letters as the AMES strain.

Other analytical strategies were employed to target the chemical and elemental profile of the spore powders. Specific techniques include: scanning and transmission electron microscopy, energy dispersive x-ray analysis, carbon dating by accelerator or mass spectrometry, and inductively coupled plasma optical emission and mass spectrometry.

Additional scientists from the Department of Defense and the Centers for Disease Control examined the spore material, and it was determined that there were many phenotypic variants within the samples. With generous support by both the National Institutes of Health and the National Science Foundation and other government agencies, FBI scientists worked with the Institute for Genomic Research to determine if genetic mutations were responsible for the altered appearance of the variants found in the *Bacillus anthracis* letters.

Several genetic mutants were discovered in these studies. FBI microbiologists contracted the assistance of several laboratories to develop high specific assays to detect four specific genetic mutations found in the *Bacillus anthracis* letters. The mutation detection assays were validated and used by the FBI Laboratory to examine the repository of *Bacillus anthracis* AMES that was collected through the course of this investigation.

This unprecedented scientific approach allowed the FBI to identify the potential sources of the *Bacillus anthracis* used to produce the 2001 spore powders, and through a comprehensive analytical approach, the investigators were provided with validated scientific data which linked the material used in the 2001 attack to materials from USAMRIID identified as RMR 1029.

It is important to emphasize that the science used in this case is highly validated and well accepted through the scientific community. The novelty is the application of these techniques for forensic microbiology. Today I'm very confident that the significant lessons learned from the 2001 *Bacillus anthracis* case have been rigorously evaluated by the FBI, and appropriate actions have been taken to safeguard the American public.

The FBI Laboratory has revolutionized the approach to nontraditional forensic samples, and has developed robust capabilities to collect and examine evidence containing biological, chemical, radiological, or nuclear materials. We have developed a strong partnership with the U.S. government laboratory complex, public health system, private industry, and academia to significantly enhance our capabilities dealing with future investigations.

The creation of the Weapon of Mass Destruction Directorate is another example of FBI's progressive approach focusing on prevention as well as investigations on all issues involving chemical, biological, radiological, and nuclear materials. Please note that there were many dedicated individuals, including prosecutors, scientists, investigators, analysts, support personnel, that all worked on this case. For the purpose of this meeting, however, the science and technology community within the Bureau has the lead, and if individuals from the sidelines are asked to provide additional background to any of your questions, please make sure to attribute all answers to the FBI Laboratory director, Dr. Hassell, sitting to my left.

Finally, I'm asking you to understand that this is the first step toward broader dissemination of scientific information surrounding this case. Additional information will be available through peer review publication, and I ask you to please respect the integrity of this process. In fact, several research projects related to the FBI's investigation have already resulted in peer review publications, and we will

provide you with that list. Additional publications will be available for peer review as more information from the investigation is released.

Before we open the floor for a question-and-answer session, we would like to introduce you to our distinguished panel. Today we have with us a small group of individuals representing the large cadre of non-Bureau scientists that helped us chart and navigate our scientific path through this unprecedented case. In the near future, after we work through each nondisclosure agreement and privacy issues, we will release the names of those key individuals who tirelessly worked with us on the 2001 Bacillus anthracis mailing.

To my left is the current FBI Laboratory director, Dr. Chris Hassell. Dr. Chris Hassell will introduce the panel members.

DR. HASSELL: Starting from my far left, we have Dr. Joseph Michael. He's a distinguished member of the technical staff at Sandia National Laboratories in Albuquerque, New Mexico. He currently works in the Materials Characterization Department of the Materials Science Center, where he develops and applies electron and ion microscopy to the characterization of materials. Dr. Michael is the co-author of a leading textbook on scanning electron microscopy. He assisted with elemental analysis, electron microscopy of the samples, and with the development of the strategies for analysis of the chemical and physical characteristics of the spore powders.

Next on the panel is Dr. Jacques Ravel. He's an associate professor of microbiology and a member of the Institute for Genome Studies at the University of Maryland School of Medicine. He also was formerly with the Institute for Genomic Research. His research focuses on the application of microbial genomics to several key areas, including microbial genome sequence comparative analyses, with a special emphasis on human microbial pathogens, including Bacillus anthracis. His work included genetic sequence analysis and characterization of genetic mutants in support of the FBI investigation.

Next, to my immediate left, is Professor Claire Fraser-Liggett. She's a professor of medicine and director of the newly created Institute for Genome Studies at the School of Medicine, University of Maryland in Baltimore, Maryland. She was previously the president and director of the Institute for Genome Studies, where she holds -- excuse me. I'm sorry -- where she led teams that sequenced the genomes of several microbial organisms, including important human and animal pathogens. This institute performed a genetic sequence analysis in support of the anthrax investigation.

To the right of Dr. Majidi is Dr. Rita COLWELL. She is currently a distinguished professor both at the University of Maryland, College Park, as well as at the Johns Hopkins University Bloomberg School of Public Health. She's also a senior advisor to Canon U.S. Life Sciences, Incorporated. From 1998 to 2004, she served as director of the National Science Foundation, which provided funding for much of the genetic sequencing efforts in support of the FBI investigation. She has served as president of the American Association for the Advancement of Science, the American Society for Microbiology, and she's a member of the National Academy of Sciences. In July of 2007, she received the National Medal of Science.

Next on the panel is Dr. James Burans. He is currently the associate laboratory director of the National Bioforensic Analysis Center. He's been in the forefront of the development of diagnostic assay techniques to identify and characterize biological threat agents. He led several scientific working groups that were assembled for the National Academy of Sciences, National Laboratories, and other federal R&D facilities.

And last, to my far right, is Dr. Paul Keim. Dr. Keim is Regents Professor of Biology, and he holds the Cowden Endowed Chair in Microbiology at Northern Arizona University. He's also director of the Pathogen Genomics Division at the Translational Genomics Research Institute. His research focuses on molecular genetics for a wide variety of organisms, including bacteria, fungi, and plants and animals. His work in support of the FBI included identification of the spore powders as the AMES strain of the Bacillus anthracis.

And like Dr. Majidi mentioned, behind me are many of the staff members from the FBI Laboratory who played a tremendous role in supporting this work, too. So they should be recognized as well.

DR. MAJIDI: The way we will work this morning's session is that I will ask each panel member to introduce themselves briefly, and give you an overview of exactly what area they worked on. After we are done, then the floor is open to questions from you. And I will be the moderator of this session. We'll try to focus all questions with regard to the science and technology aspects of this case only.

DR. MICHAEL: My name is Joe Michael. My area of expertise is electron microscopy. We apply electron microscopy to a range of cells to categorize materials. And our involvement was with respect to the physical chemical analysis of the attack materials.

DR. RAVEL: My name is Jacques Ravel. I'm an associate professor of microbiology at the Institute for Genome Sciences at the University of Maryland School of Medicine. Back in 2002, I was at the Institute for Genomic Research, also known as TIGR, where we as a team took on the responsibility to sequence some of the isolates of *Bacillus anthracis* that were provided to us by the FBI. We then sequenced them and kerotyped them genetically to find any kind of a genetic difference between the Nova strain that we sequenced at the same time, which is also known as the AMES ancestor strain. And we used those genetic differences to design some of the assays that were used in the investigation.

DR. FRASER-LIGGETT: I'm Claire Fraser-Liggett, currently professor of medicine and director of the Institute for Genome Sciences at the University of Maryland School of Medicine. I was previously the director of the Institute for Genomic Research. Since 1995, I've been involved in comparative microbial genomic studies, and with my colleagues at TIGR, including Dr. Jacques Ravel, have been involved in the development of a number of experimental and computational approaches for identifying and characterizing differences in sequence composition and functional characterization of those sequence variants in a number of human and animal pathogens and environmental organisms.

In October 2001, at the time of the letter mailings, we were finishing up the first study to sequence what at the time was the first reference sequence of *Bacillus anthracis* AMES. It was an isolate that had been provided to us by Portdown in the U.K. And as Jacques has described to you, following the letter attacks, our participation in the Amerithrax investigation expanded to begin to look at a number of additional samples and to begin to make genotype/phenotype characterizations.

DR. HASSELL: Thank you. Rita?

DR. COLWELL: I'm Rita COLWELL, a microbial systematist/microbial ecologist with a keen interest in molecular systematics. At the time of the anthrax incidents, I was the director of the National Science Foundation, and we had already a program in sequencing microbial genomes. It was clear to me that we must sequence the anthrax strains as soon as possible, and with the small grants for innovative research were able to respond to a proposal submitted by TIGR to fund the sequencing. The continuation of the activity was through a consortium of agencies -- NIH, DOE, Justice, FBI, et cetera -- focused on ensuring that sequencing of pathogens would continue, notably, the anthrax strains.

DR. BURANS: My name is Jim Burans. I'm a retired naval officer. I come from the Navy's biodefense community. During the early '90s, I helped to support some of the early work with the FBI on biocrime investigations as well as supporting forensic analysis for the U.N. Special Commission to Iraq. In the November 2001 time frame, I was asked to serve as a scientific consultant in the early stages of the Amerithrax case. And in the 2003 time frame, I helped to establish the National Bioforensic Analysis Center for the Department of Homeland Security to deal with the scientific investigative challenges of biocrime and bioterror investigations.

DR. KEIM: I'm Paul Keim. I'm a professor of microbiology at Northern Arizona University. I'm also director of the Pathogen Genomics Division at the Translational Research Institute in Arizona. And I'm an affiliate at Los Alamos National Laboratory. My role in this is that prior to 2001, we'd began

studying *Bacillus anthracis* and its worldwide population diversity. We developed techniques to DNA fingerprint at what now seem a rather crude level but was very highly sophisticated at the time, such that in October 2001, the Bureau came to us to identify the strain that was in the anthrax letters.

And we did identify the AMES strain. We spent a lot of time after that trying to understand exactly what the AMES strain was and what it wasn't. Using DNA sequences from TIGR, we were able to develop very precise identification of the AMES strain itself, going back into nature to characterize natural populations as well as many different laboratory isolates. We were responsible for doing a lot of the biosafety handling in the early days of the investigation. We acted as a repository for the strains that were subpoenaed and collected across the country, characterizing those as AMES or not AMES. And recently we've been involved with preparing DNAs in our biosafety laboratory that were then sequenced at TIGR.

DR. MAJIDI: Thank you, Paul. I just want to point out that we specifically sought you out because you are involved with well-known, well-respected scientific journals. And we wanted to give you ample time to ask as many detailed questions with regard to the science of this case. We definitely didn't want your voice to get lost in the signal-to-noise ratio of all the other journals. So this is your time, and I'll open the floor to questions.

QUESTION: Yudhijit Bhattacharjee from Science Magazine. Before we actually get into the details, I just wanted to ask you, Dr. Majidi, to list what you characterize as the scientific evidence in this case.

DR. MAJIDI: We looked at a number of different characteristics of the samples. And ultimately, what really drove us to RMR 1029 was the ability to sequence and identify genetics variation that resulted in different phenotypes which unequivocally took us to RMR 1029. We have obviously done a number of other analyses, elemental characterization, that drove us to conclude that there were no additives. We did various types of chemical analyses. And again, the techniques used were standard analytical laboratory techniques as well as various forms of microscopy. By and large, what drives us to RMR 1029 is the genetics information.

QUESTION: Would you take us on a step-by-step tour, starting from the sample in the letters to the flask at USAMRIID?

DR. HASSELL: This may be a group answer for this one. But let me step back also in the earlier question. Part of what we did was develop the process first before we really started into working on the samples themselves. So we got the panel members here and many others involved in setting out, if you have the evidence, what do you do with it? What's the most efficient way of analyzing? What's the most comprehensive way of making those determinations? So many, many discussions were held back in 2001 with several different working groups to do this. So we laid out the genetic way we would analyze it and the chemical, the physical methods there. So we put the process in place before we started doing anything, and we validated that process with the consensus of those scientists.

So because everyone here participated on this, I'm going to look for corrections as I go along here. But the samples were submitted for strain identification. That was the earliest step. And Dr. Keim was involved with that. Maybe you could give more detail and you could answer that?

BACKGROUND OFFICIAL: Yes. So the methods in 2001 were based upon hypervariable sequences that mutate very fast. *Bacillus anthracis* is a recently emerged clone, and so its genome is highly homogeneous compared to certain other pathogens. In the pre-genomics era, that was the way that we could do things. We could focus in upon these highly variable regions to get information about what a strain was and what it wasn't.

So we began characterizing it in that fashion. When the anthrax letters went out and we began receiving -- essentially every time a letter went out and isolates and a victim occurred, we received those isolates. And they would come to Flagstaff, where we would type them, subtype them with this approach. As the genomics came online and we began identifying single nucleotide polymorphism

snips, those then became really the assay for identifying what the AMES strain was and what it wasn't. That method, we published that in EID and in other places, so those papers will be made available at some point. But those methods ended up being incredibly accurate and precise and robust, very sensitive. And again, they were able to identify what the AMES strain was and what it wasn't. Maybe something that is lost in all this is there were many false alarms over the last seven years of anthrax outbreaks, and that these assays were able to limit the investigation and focus in upon what really was the AMES strain. And it allowed the federal government to not worry about things that were not the AMES strain.

DR. HASSELL: Thank you. And following that, there were some other studies to look at sort of the phenotypic variations within the samples. So we saw some variations in the different colonies that were unique to a certain subset of the samples that we had -- I'm sorry, to all of the evidentiary samples. They were common to all of those. And they were also found to be within a subset of the repository samples that we received from all the other laboratories.

We saw those phenotypic differences, like the difference in appearance, in how the colonies were looking and growing. Then we characterized those more fully and found the genetic mutations that were contained there. And then maybe I can turn it over to TIGR. Can you describe more what happened after that with characterizing those mutations?

BACKGROUND OFFICIAL: Yes. I think, again, just to step back, to put some of this later work in context, as I made reference to in my opening statement, with initial funding from the Office of Naval Research in 2001, we were completing the work on the first *Bacillus anthracis* genome sequence. And this project was to take that genome sequence, to complete closure where every base pair was determined without ambiguity. And we were very close to being done with that in October of 2001.

At the time of the anthrax letter attacks, as Dr. Colwell mentioned, we applied to NSF for SGER funding to do an initial comparison, which we completed very quickly and published in *Science* in 2002. I think that this was very important. We already knew from the work that had been done in Dr. Keim's lab that this was also a sample of *Bacillus anthracis* AMES. I think this was a very important first piece of information that set some of the strategy for this investigation going forward because this demonstrated to us that it was possible to identify high quality, reproducible polymorphisms when comparing two different samples of *Bacillus anthracis* AMES. And so that was very important information going forward. Had we done this comparison of two different samples of *Bacillus anthracis* AMES and found no differences whatsoever, I think we might have reconsidered the feasibility of going forward with the approach that we had taken. But we were very much encouraged with the genomics-based approach and its potential to reveal important information.

And as Paul has just described, then, we began to get samples that had come from the spore powders that had been collected as part of the ongoing investigation, and we took each of these through in terms of our routine sequence analysis process. As part of this, Jacques and some of his colleagues at TIGR developed a specific bioinformatics pipeline to rapidly identify potential polymorphisms. Again, one of the important things that we learned from our initial comparative study was that it was absolutely essential to be working with very high quality draft sequence, at a minimum, in order to reliably identify potential polymorphisms. We were able to convince ourselves, as part of the initial study, that low quality draft sequence could end up being a distraction because that were a sufficient number of sequencing errors, if you were working with low quality sequence, that could become a distraction. And we ended up -- you could end up chasing sequencing errors that turned out to be errors and not true polymorphisms.

So we were, as part of our initial studies, devising experimental and computational methods that allowed us to be much more efficient in our subsequent efforts. But as was made in Dr. Majidi's opening remarks or Dr. Hassell's remarks, the science that we were doing was -- we were not using new science. We were using science that we had had in place and had validated for many previous genome studies. We were now just applying this in a different way, with an eye towards identifying polymorphisms, sequence variants among the different samples of AMES that we were receiving.

DR. HASSELL: So then, just to bring us full circle, so then when the assays were developed, at the same time we were building a repository of samples from the whole population, or we sometimes use the term "universe," of people who had the AMES strain. We had that. We applied these assays to that. And there were really four assays that were developed that were highly specific. And I think it was just over a thousand samples. Within those samples, eight matched all four of those genetic markers. They were the markers for the mutation. Those eight are each traceable back from the investigation side back to RMR 1029. So finally, then, those four markers were also in all the samples from the evidence, from the letters themselves.

BACKGROUND OFFICIAL: I would like to add that there was an active consortium of agencies -- NSF, NIH, DOE, the intelligence community, Department of Justice, FBI, USDA, and DOD -- which acted as a source of advice. And this was a highly collaborative effort.

QUESTION: So these eight isolates that had all of the four mutations, you say that they were traceable to USAMRIID. Could you explain to me what that means?

DR. HASSELL: They were traceable through the investigative process. The science showed that they contained the markers.

QUESTION: I think there's --

DR. HASSELL: Well, I'm just saying that the way -- when we found out where those samples came from, they're from the individuals in the community who had those. That's where the investigation kicked in to say how they were related, how they were originally traced back in the investigation.

QUESTION: So it was more traditional police work that basically led you to believe that --

DR. MAJIDI: So let me go back to my original assertion, that any attribution is a confluence of three events: forensic information, investigative information, and intelligence information. So you can't really parse any one of these things and say which one really gave you that one last item.

By tracing back to the RMR 1029, what we say is that through various mechanisms, we were able to validate that the A samples had their origin from RMR 1029. We did that in a number of different methods. I don't want to go through all the specifics of the investigations because that's not the focus, but the simple check of a lab notebook would be one way to do it. The shipment records would be another way to do it. There's a number of methodologies which is not that focused in this discussion today.

BACKGROUND OFFICIAL: I heard a misconception in your question and that was, you used the word "islet" whereas you just used the word "sample," okay, and so when you think of these, you need to think of them as a collection of spores and that they contain these spore mutations that are being assayed for. An islet would be derived from a single cell, for example, and it might not contain all four. It could be the collection or the subpopulation that contains it.

QUESTION: Yeah. No, I hope I can be forgive that because I'm actually quoting from the affidavit which uses the term "1,000 islets." So I suppose instead of islets that should really be 1,000 samples stored at more locations.

DR. MAJIDI: That's why we are here today.

QUESTION: Sure.

DR. MAJIDI: Because there are some issues.

QUESTION: No, sure. I mean, I --

DR. MAJIDI: Your point is well taken and we'll try to answer as many of those as we can, yes.

QUESTION: And so just to finish up that strain of --

DR. MAJIDI: Sure.

QUESTION: -- of questioning, if you will, --

DR. MAJIDI: Sure.

QUESTION: -- so these eight -- the four mutations were found in eight of the 1,000 samples --

DR. MAJIDI: Right.

QUESTION: -- and using other investigative techniques, you were able to say conclusively that all of these eight had come from USAMRIID?

DR. MAJIDI: That's right.

QUESTION: Okay.

QUESTION: Amber Dance from Nature. Can you explain to me why the flash would have contained a mixture of samples instead of just studying a clone?

DR. MAJIDI: Sure. That's -- you know, it has to do with the genesis of the material itself.

BACKGROUND OFFICIAL: It was a collection of many different individual runs taken from USAMRIID as well as Dugway Proving Ground.

BACKGROUND OFFICIAL: Yeah. And not to make things more confusing, but it's not uncommon to see multiple morphologies from a single culture that can arise independently from a single culture.

DR. MAJIDI: So this really was not -- the RMR 1029, the way to look at it is that a combination of multiple AMES culture mixed together in one container. So that's why the variations that you see, the genetic variations you see is so uniquely RMR 1029 because of those subsamples that were mixed together to form RMR 1029.

QUESTION: But why would Dr. Ivins have been working with a mixture?

DR. MAJIDI: That was the material that was used at USAMRIID and it was used for a number of different reasons, for research and development. That was the gold standard for RID.

DR. HASSELL: Yeah. The motivation was to get a large -- I'm trying to figure out words that won't confuse it with others, but, you know, a large container, if you will, of concentrated spores so that it could be used as -- for other testing, for challenges, for vaccine investigations, for example. So there was a driver to have one central grouping.

DR. MAJIDI: I'm going to ask Dr. Hassell to give you the story of the birth of RMR 1029. How's that?

QUESTION: Okay.

BACKGROUND OFFICIAL: RMR 1029 was a sample of pooled spores that were the product of many production runs, production runs that were from Dugway Proving Grounds as well as productions of spores that were produced within each sample. It was a large pool of spores because they required a sufficient amount to conduct their vaccine challenge experiments. Those spores were used to -- in aerosol experiments to make sure that the vaccines that they were developing were efficacious and for other research.

So it's not just one culture. It is -- it was actually flasks of pooled spores.

QUESTION: And is that the standard way to work with anthrax, to have a pool of different strains together, stored in a flask?

BACKGROUND OFFICIAL: Not a pool of different strains together. It was all the AMES strain but it was multiple --

QUESTION: Okay.

BACKGROUND OFFICIAL: -- production runs in order to have a sufficient quantity of spores in order to conduct their experiments.

QUESTION: Christine --

DR. MAJIDI: You look confused. Did you want to follow up on that?

QUESTION: I'm going to ask a clarification question on that. Christine Piggee with Local Chemistry, American Chemical Society.

Are you saying that the mixture of different mutations of the same strain in the flask was an artifact of production?

BACKGROUND OFFICIAL: Eventually could have been the case.

QUESTION: So it was a mixture of substrains?

BACKGROUND OFFICIAL: Correct. That's right. So -- but remember, the majority of the material

is the wildtype AMES anthrax in that container and through various processes, you have the spore distinct mutation that appears in that flask.

Now, we don't know what the origin of the mutation is. We don't know if it started all in one batch or if it started in three of the combined batches or in two of the combined batches. We don't know. What we know is that RMR 1029 and its four genetic mutants within that container are uniquely RMR 1029. We will not find it in any of our others.

BACKGROUND OFFICIAL: Yeah. Understand that in the vaccine trials, this is a problem having a uniform challenge, you know, and so it was important to build a large enough stock so that you don't have to go back and reproduce it because when you do it, you might end up with a different substrain and hence not be able to compare the results of your experiments. So I think the presence of such a stock is not a surprise.

QUESTION: I'm sorry. If these mutations -- if some of these mutations arise automatically, I mean, I don't know how mutations arise because -- and it seems like that's an important question because, you know, the samples that you had have already undergone automatic mutation and the mutations that arose there, you know, happened to be the four mutations that you found in the flask.

BACKGROUND OFFICIAL: So we published several papers on this and in fact, you know, the theory of how mutations arise go back to the classic Gloria Dellbrick experiments and so at least the idea is, is that, experiments occur by random chance and different regions of the genome will mutate at different rates and that's been shown in this whole syntheses and so just by stochastic processes, you will end up with mutations occurring and depending upon the rate, the intrinsic rate of those, they'll happen faster or slower and some regions mutate faster and some mutate slower, and understand that a population of cells that has, you know, 10 to the 9th cells has actually undergone almost 10 to the 9th generations of growth and so that's a very large number and if you have 10 the 12th almost 10 the 12th generations of growth and so almost any mutation that you can imagine will arise during the growth of that.

The question is, is how soon does it arise and hence if it arises early, its frequency is going to be larger than if it arises late.

DR. MAJIDI: Let me see if I can ask you to just put a little more texture on that and talk about how stable these mutations are. Are these things just randomly appearing or are they stable over multiple generations, the particular four markers we saw?

BACKGROUND OFFICIAL: Well, the four markers that we saw have turned out to be stable over multiple generations and this was an assay that was -- this was something that was looked at very specifically because these mutations were deemed to be so potentially critical.

But if I might just make another more general comment to, I think, pick up on the discussion here, and it gets to the comments that Paul made. When I learned microbiology 30+ years now, it was clear that this notion of stochastic mutation was understood. We clearly didn't have genomic approaches to track these mutations, to measure them, but it's true not only in bacterial cultures, it's true in mammalian cell culture, and this has been well described in many, many publications, that repeated passage of bacterial cultures or mammalian cell lines tends to be associated with phenotypic changes.

This can be loss of virulence. It doesn't have to be, but when I was learning microbiology, I remember very distinctly what I learned was that in liquid culture, a large batch liquid culture should be considered whenever possible to be an end state in what you were doing and whenever possible you went back to low passage stocks as a starting point and from there, the next step was to plate out bacteria on a Petri dish or an appropriate solid substrate so you could actually look at to see what you had and colonies that appeared different would be, in most cases, apparent to the naked eye. They could have a different morphology. They could be pigmented or not pigmented, depending upon what you were working with, and it would be selection of a single colony that you would use to inoculate a flask.

It was considered bad technique to then take a liquid culture and use that for repeated inoculations because these stochastic mutations can accumulate and so if you think about it, if that's a natural process, but for all of the reasons why you don't in the process of vaccine development ideally want to be going back and generating multiple batches of material for development and testing, perhaps this puts all of this in a different context.

This is a trade-off between having enough material and some of the risks, if you will, or risks is perhaps not the best word, but some of the caveats that come with having to acquire that through the preparations of multi-flask use.

QUESTION: Thank you.

BACKGROUND OFFICIAL: I don't know if that helps.

QUESTION: That helps.

QUESTION: I'm going to follow up further on that. Rachel Erenberg from Science News.

And my understanding is both within the flask and within the Petri dish, you do get the stochastic mutations arising. How, in terms of doing sequencing, do you make sure -- how do you prevent actual growth of a dish because those mutations may arise in the course of the dish sitting in the cabinet? How do you know that the samples that you're sampling from, the time that you take the sample, are the same samples in terms of the genes, the genome snapshot that you're taking as they were when they arrived? How do you make sure they're the same?

BACKGROUND OFFICIAL: For essentially of the genome work that we have done, we have tried, I think in every case we have followed this protocol that I outlined, that when we have either prepared material ourselves or in the majority of cases they have provided material from outside collaborators, we have gone through this process of going back to low passage freezer stocks.

QUESTION: So what does that mean exactly?

BACKGROUND OFFICIAL: Clonal selection.

QUESTION: Sorry?

BACKGROUND OFFICIAL: Clonal selection.

BACKGROUND OFFICIAL: Clonal selection. It's really --

QUESTION: So you've got the addition of freezer that is highly --

BACKGROUND OFFICIAL: No, it's a slant or it's a frozen stock in a freezer that is -- it's going back to a stock that is not grown. Then you plate that material. It's plated out. A single colony is selected, grown as minimally as possible and for genome projects, you need a minimal amount of material. We're talking about a very different order of magnitude of material here for genome sequencing projects as compared to vaccine development efforts and that material is used for preparation of DNA, as a template for genome sequencing projects.

But you raise an important point, that there is the possibility that during that process, additional stochastic mutations might arise, but I think what's important to bear in mind is that if they do, these are not random mutations that come and go. If mutations arise, certainly over the time frames that we're talking about in terms of genetic analysis that we carried out, they should persist.

We're not talking about large numbers of passages where minority representations in a population would

be overgrown and the fact that these morphotypes that have been described were seen through repeated passage on plates could be identified through various assays that were developed gives us great confidence that in fact they were -- they represented stable components of these cultures. They were not -- they may have represented random mutations, but they were not random members of the population, if you will, that came and went at random.

DR. MAJIDI: Can I add one more item?

BACKGROUND OFFICIAL: I'd like to make a point of clarification. Many bacteria have phenotypic variations when you grow them as well as overnight in some bacteria. Anthrax is not known for that same practice. In fact, it's very homogeneous.

When one passes anthrax from one culture to another through passage, it's rare to experience any type of phenotypic variation or the mutations that we're talking about. In vaccine work, the Code of Federal Regulation actually limits for most bacteria the number of passages from your seed stocks to production of the vaccine to avoid this problem of mutation. Often that's as little as five to seven passages.

So in anthrax, it is very homogeneous. You don't see phenotypic variation and you rarely see mutations occurring with just a few passages. It was noteworthy that the anthrax powders that was in the letters had significant phenotypic variant numbers of phenotypic variants. This was unusual for anthrax and in particular unusual for AMES.

If one passes the 1981 AMES strain, the original AMES wildtype strain, one does not normally see mutations arising at a very high frequency. So it was noted that this was unusual in the anthrax powder.

QUESTION: Just for clarification because I've read different things. Did all eight samples have all four markers?

DR. MAJIDI: Yes.

BACKGROUND OFFICIAL: I just want to add one thing. One of the samples that was tested was actually DNA-extracted directly out of spores and those mutants. I think we should also clarify mutation is on the DNA. A mutant is a cell (inaudible). So this mutant was also found in the spore, in the DNA-extracted spore, meaning that those mutants then arise during the culture process.

Another point. In genome sequencing, often having a subpopulation variable that -- often talking less than one percent, it's never really a problem because when we sequence a genome, every bit of DNA is sequenced several times and from different molecules and those mutants actually don't even make it into the consensus sequence which was coming from the major component which is the wildtype.

QUESTION: And so what TIGR did was it sequenced the entire genomes of all the variants found in the sample?

BACKGROUND OFFICIAL: So we were handed over DNA. The DNA was compared with some of it in the lab. We sequenced for three of the letters, the wildtype genome, and two of the wildtype for each of the three letters, and then for only two letters we sequenced another different morphotype and only one, the fourth, the wildtype.

So in total, there was about 12 genomes. The wildtype was completely 100 percent identical in every way, every single one of the (inaudible), including the plasma, it was identical to the strain that we -- the genome that we pulled the AMES strain which is actually (inaudible) and the other morphotype had differences, unique differences and each of the morphotypes which were identical morphologically in each of the letters had similar differences on them in the genome sequence.

The sequencing that was performed was Sanger sequencing which at the time was routine and the most accepted and validated method. The advantage of Sanger is that we start from a clone and sequencing

