

2003 WORLD TROTTING CONFERENCE

SPEAKER: ROBERT WIEBE

TOPIC: DNA: in an Eggshell

DATE & TIME: Saturday July 26
8 am - 10 am

VENUE: Imperial Room, Royal York Hotel

PROFILE OF SPEAKER

ORGANIZATION:

☆ Maxxam Analytics

CURRENT POSITION:

☆ Business Development Manager

MAJOR CAREER / ACADEMIC ACHIEVEMENTS:

- ☆ Master of Science at the Ontario Veterinary College
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DNA Testing... In An Eggshell

(References are made to PowerPoint presentation slides, which follow the written portion of this presentation)

What I would like to give you is a perspective on DNA, both from a technical point of view but also from a logistical point of view and some of the things that happen that may impact on your business. I'm going to start by talking a little bit about the science of DNA and genetics as we as a laboratory see them. A little bit about DNA testing and how it's done including talking about the different types of technology. We're going to look at how that technology is applied to issues of identity and parentage. There is a lot happening in the industry now. Someone from another breeding association that we work with said, "When is DNA going to deliver on its promise of perfection?" And I will talk a little bit about why errors creep into DNA testing. I give you a perspective on some of the things your people may be dealing with. And then I will spend time at the end talking about what I see as being the future of DNA testing as it may apply to your industry.

Very quickly a horse has 32 distinct chromosomes and they essentially look like this and if you were to zoom in on a piece of a chromosome with a microscope it would look essentially like a ladder. And if you zoomed in on each run of the ladder, you would see there is a code embedded in there made up of 4 letters, and these letters are called bases. So this is essentially the structure of DNA from the point from the chromosome right now to each of the individual runs in this ladder. The code that is our DNA is billions of letters long and this code just repeats over and over again. Having said that, about 90% are junk and doesn't influence anything with respect to genetics. It's almost space filler. But the rest and I've indicated that with my blocks here. There are parts of DNA called genes and genes code for protein which are building blocks whether we are talking about metabolism or structure. Or whether that is protein components in bone or in muscle or in organs, the colour of your animal. All of that is coded for by genes that manifest themselves as protein and that is essentially what we talk about when we speak to phenotype. A large proportion of the DNA when you look at these 4 billion bases is the same whether we are talking about the individual sitting around the table, the horses at the track, or whether you go to different pedigrees or even different species. The work that has been done in genomics has indicated that there is about 95% similarity between horses earthworms and humans. So it's that little bit that is variable that is obviously quite important or I wouldn't be able to stand up here and talk. Having said that there are regions also that are highly variable not only within species but also between individuals. These variable regions tend to pop up not only in the places in the DNA that influence colour and things like that we can see as being differences, but also in these junk regions that I've spoken about. These animals if you were to look at them from a DNA perspective would be phenomenally similar, probably varying in much less than 1%, and yet you see the substantial difference in variability. The real question at hand for your industry is how do we identify animals? How do we use DNA as a tool for pedigree verification? As I said before a lot of the regions that we have looked at in the DNA are within the junk part, the part that doesn't code for anything. We happen to look at part of DNA that are called short tandem repeats (STR). It's a technology that we as scientists

are currently using. It is the current standard in your industry and in many industries for pedigree verification. So what do these look like? Short tandem repeats: if you look at a line of DNA code it looks something like that. It is essentially gibberish to just about anybody but if you look inside there what you see is a trend repeating itself (ex. AGAG) and that is where the name, short tandem repeats comes from. This is probably the best analogy that I have as to what a short tandem repeat really is. Essentially the analogy would be like this- As DNA scientists, who are very good at recognizing on a train the engine on the front of the train and the caboose at the back of the train and what varies between trains in the length in between and that is essentially analogous to what happens with the analysis of STR's. We look for STR's by saying we know where the front of it is and we know where the back of it is, but what varies between STR's, or what varies between you if we were to do STR's on humans or on your animals, would be the distance between these two marker points in the DNA. The way we go about analyzing this is a technology called gel electrophoresis. But that is not important. To look at this and see that on this complex picture that we have you'll see columns of results and each column represents one individual's profile. Looking at it up and down – that's one individual. If you look across, you'll see that in the area marked by those two lines, there's a concentration of what looked like loose spots, so those blue spots are representative of one individual test. So if we look in the area indicated by where those two things correspond, we get a picture of one test of what an animal looks like. And if we were to separate this whole picture, you would have what is referred to as an animal's DNA profile and within that you see there are the two spots for every test and those two spots are the results for the test. So let me go over this because I don't think I have been all that clear. We would test every individual with a number of DNA tests or DNA markers or STR's. For every test we are going to get 2 results and that becomes important when we talk about things like heredity. When we take those two bands that you saw there, and convert them into a numerical number. So if we were to look at DNA as a tool for identity, what we would say is we did one test and individual A had one piece of DNA that was 12, or one train if you will that was 12 cars long and another one that was 15 cars long or there were 15 repeats of this AGAG code that we saw earlier. And if we look at individual B, and they had a result of a 13 and 14. The conclusion that we as a laboratory would draw from that would be that these two samples came from different sources. Now let's say we tested another individual and compared it to A and it was exactly the same. The process that would go on in our mind as scientists is that unlike the last slide that we couldn't exclude the possibility that these two were from the same animal. At this point in time, all we can say is that they may come from the same source. There isn't a conclusion that is drawn here. We haven't done a definitive DNA test. We haven't done anything that would indicate these two samples are identical. In fact, when we get a match all we are able to do is move on from there to determine what the significance of that match is in a population. Having said that, what we're about to do is figure out how often this 12, 15 is going to appear in the population whether that's two people around the table, two horses on the track or whatever. The easiest way of doing this is to take an example that we can relate to. Let's assume that we were at a crime scene and there is a parking lot down the road here and there were 9 independent witnesses that came forward. And one of them stepped forward and said, I saw the car that did it, it was a Honda. And another witness said I don't know if it was Honda but I

know it was a red car. And another one said they seen that there was an in the license plate. And after all of these pieces of evidence, you begin to develop a description of this car that you are trying to find a match to if you will. Now if the police are trying to track this down, they may go to Honda and say from your market research how many cars on the road are Hondas, etc.? And as you go through each of these you begin to build an argument. If you had ever found that car you could say that there is a 1 in 1.8 billion chance that somehow this is not the car that we were looking for based on those descriptors that we had. If you were take those same descriptors and put them in the United States, you may get very different numbers. For example, maybe there are fewer Hondas etc. Overall, you get a different number of that probability. The things to take away from this are a DNA match, just like a descriptive match. It's a tool for excluding all of the animals, all of the cars that could not be the culprit. And people often when they are using DNA as a tool, they see it as a tool for finding the parent. What it's a tool for is excluding all the animals that could not be the parents, and the inference that is made from there is that in fact parentage is established. If we were to take the same slide and turn it over, we do a number of tests in DNA. I've mentioned them as STR's and markers, etc. and if we were to number those tests 1 through 9 and we do more than 9 tests and we get our DNA type which was the 12,15 which also can be expressed in letters, the same sorts of arguments can be made as with the car. What are the chances for test 2 for instance that an animal would have a DNA typing that was a K and an M. Maybe one in 3 animals. But essentially with this in hand and a database full of these results, we know that the likelihood that another animal is going to appear with this same DNA type is one in 2 billion. This is really a statistical argument. Not only that it is unlikely that there will be another animal out there, it is unlikely that there ever will be an animal that has the same unique identity as this test has.

Most of the work that we do in our lab speaks to parentage, but there is occasionally an issue of identity and it is becoming more of an issue specifically in the horse industry but also in livestock. In Canada, we are suffering an economic downturn associated with the BSE outbreak. We're trying to trace back identity of animals. Identity in horses is not been quite as much of an issue as it has been in livestock but I believe it is becoming more so. What I'm going to talk about now is a case that we've done in our laboratory that speaks to identity rather than parentage, which isn't what we normally do. Maxxam as you may or may not know also does a lot of the drug testing for the horse racing industry in Canada as well. And we had a case where a winning horse tested positive for a banned substance in a routine drug test. We were all shocked and amazed when the owner stepped forward and said that this must have somehow been a mistake, that there is no way that this animal would have been drugged. And so without his knowing that, and our cooperation with the racing authority, and I think this particular case was in thoroughbreds, we took the urine sample from our drug lab and took it out to our DNA lab and did DNA testing on this urine sample. Now this hadn't been done very much before. There was a small amount of cellular material in the urine. We were able to derive a DNA type and by comparing that back to the registry's database we were able to prove that the continuity had been maintained and that the result that was obtained applied directly back to the horse. Of course the regulator authorities took the appropriate action from there. So when we look at DNA as a tool for identification, I would be as bold as to say- there is no better tool as for accuracy in terms of identification in DNA. It's sort of

what DNA is all about, except when it comes to twins, which happen rarely. And I think the use of clones is going to have an impact on us even more. DNA, at least as it is practiced today, will not be able to distinguish between clones. What makes DNA very good as a tool is its permanent. An animal's DNA does not change over the course of its life. The DNA profile an animal is born with is the same DNA profile that an animal dies with and can be obtained from bones after death. The problem with DNA as a tool for identification is that often time's identification is required at a specific point in time. DNA at this point in time is not a real time tool for identification. This makes other tools such as tags or implants or other things that I know are issues of discussion around the table necessary. I would say that there is research underway to develop DNA testing methodologies that will bring the time for DNA testing down to hours instead of days. And I think this will be critical and open up an opportunity for wider use of DNA. There is also DNA technologies that are being developed that will allow testing to be on site. So it's not a matter of collecting the sample and shipping it off to the lab. You could in fact have an instrument on site that will provide the power and identity that is currently available only by sending a sample to the lab. In considering the use of tags and implants, and I know some jurisdictions are far ahead in this. My only point relative to DNA as a tool for identification is that I would strongly urge you in setting up a system that you establish an iron clad continuity between your DNA sample and the tag when its put in. Beyond that, these things become independent tools but together you can have, in essence, the best of both worlds. Having talked about DNA as a tool for identification, does anyone have any questions? I'd be happy to go back over things. I realize that I've moved fairly quickly. I can start again if you would like me to.

Question:

You have said that one of the real problems, or potential problems, was between twins and clones. As we all know USTA is involved in a case right now defining twins with multiple embryo transfers. Would the embryo transfers have the same problem?

Robert Wiebe:

No, the embryo transfers don't have the same problem. The issue with twins or clones from a DNA perspective has made or has bred the same animal twice. Embryo transfers is what the actual parentage is.

Question:

What they're arguing is that they did an embryo transfer from one breeding. They have two foals. The argument is now that they are twins. So would you have a problem testing those?

Robert Wiebe:

There is a variation on the testing we've done here that's called a twin's zygosity test. We could in fact for all of the markers that we have available to us to verify that these two animals had identical DNA profiles. If those profiles aren't identical, those animals are not twins. It's a fairly simple thing to do. We often have people coming to us on the human side of our business asking, "Are my twins identical twins?" If what you're asking is whether they're identical twins or whether they are fraternal twins. Fraternal will look

like siblings on our test and identical twins will look like identical animals. Am I getting closer to the answer to the question?

Question:

No actually I guess I was just asking... You were saying it's accurate except in the case of twins and clones, and my question was would it be accurate in the case of embryo transfers as it does in the same breed or would you have the same problem as when you have twins?

Robert Wiebe:

No, we would have the accuracy. We would be able to go back and determine parentage. We would be able to go back and answer the questions we are typically asked. What we can't do from a standpoint of identity is distinguish between twins and clones. If there were twins in front of me, I can't use DNA as a tool to say this is twin A or B and similarly with clones I couldn't tell the difference between them. Having said that, there will be tests that will be able to find these differences but they are just not available right now. Identical twins in fact are not identical over time.

Question:

Could you tell us something about the costs of the test?

Robert Wiebe:

The costs of the testing really are somewhere between the \$30 and \$40 mark. Our goal as a lab is to decrease that cost over time.

Question:

What about the developments lately, let's say the last couple of years. Have you been able to come down already?

Yes we have.

Question:

How much?

Since we began DNA testing almost 6 years ago, offering it as a commercial service, our price is half of what it was originally. And that is proportional to our savings and costs. We are looking forward over the next few years to some opportunities that will decrease that. DNA testing, like any technology, has certain aspects that decrease in price. As I get to the end of the topic, I will talk about the future of DNA testing; there may be other things that we can do to justify both in terms of our cost and their value. But in terms of identity and parentage you can work that into a currency that you want to choose. Are there any more questions?

Question:

Can we tell the difference between fraternal and identical twins? And also, can we tell whether they were twins in the sense that they were conceived at the same time or whether they're from the same year?

What DNA can never do is speak to the issue of time. So if part of this question is were they conceived at the same point in time? Any of those types of questions DNA cannot answer. It's not a tool that says, were two embryo transfers done from the same mare in the same year, or were three or ten etc? DNA does not put things into the context of time.

If we go back to the beginning of the talk, you will notice for every test there was two results. One of those results represents the part of that animal's DNA that was inherited from the maternal side and the other side represents DNA that was inherited from the paternal side. So one half of everyone's DNA is inherited from each parent. The logic behind finding parentage is much the same as it is in terms of identification. You actually deal with less power because you only get half as much information essentially from the test about the father as you do about identity. Let me go into this a little bit. If we have a mare and two candidate stallions with these DNA types that you see here. We look at the first foal. The way these tests are done is to look at the mare and the foal and say well if the foal's DNA, if those bands have to be accounted for by the parent's DNA, and we see that the top band in the foal corresponds to one of the bands that came from the mare, we have to then account for the lower band that came from the foal from the sire's DNA type. With sire #2, it has a band that corresponds with the lower band and so we would say that sire #2 in this case could not be excluded as the sire of this foal. When looking at sire #1, it brings up an interesting case from the stand point that that sire has a band that matches the foal, it doesn't account for the missing band that the foal has after you've taken into account the contribution of the mare. If we look at a different foal that could potentially is a sibling of the first one, you will see again that the top band that it requires came from the mare. The bottom one came from the sire as the first foal but again inheriting a different band, a different result. Moving on you can see that foal number 3 could inherit the lower band from the mother and the higher band from the father, and similarly you can see how these results play out. What you see here is that the same mating can produce 4 vastly different DNA profiles and speaks that all of these 4 foals could in fact be fraternal twins, but could not be identical. So the same mating at the same time could produce 4 different foals at one single test but these would not be considered identical twins. But again, across all of these tests, sire #2 is not excluded as the sire of the foal and sire #1 is.

In our DNA lab, we often talk about parentage verification. I'm ok with the use of that term as long as we realize that we are qualifying animals as parentage. Let me explain the difference that I'm deriving here. Parentage qualification demonstrates that the identified parent or parents could be the actual parents. That is to say that all of our DNA testing doesn't rule them out, but it probably rules out a lot of other animals. Parentage verification would seem to state that they are the actual parents. And in fact we can not do that. We can provide statistical arguments, etc. We can do all those sorts of things. But the percentage that we attach to our degree of confidence never crosses 100%. Again, if the sire has a twin, we couldn't separate those and so we can't speak to absolute verification of parentage, but we do speak to qualification of parentage. Having said that,

the term verification, as people use these terms they understand the things that we are saying or not saying when we report a result to our clients. In inbred populations, and we have some species we deal with, closely related full brothers, as bulls for instance, may both qualify as parents of the same animal. I don't think that's happened where we couldn't rule out another animal from qualifying in the equine industry but it does happen and it's something to keep in the back of your mind. When DNA was introduced into more routine use, probably 6 or 7 years ago, it was introduced in a lot of cases of planted blood typing because of the promise that it was a perfect technology. I was speaking at another conference to people on the cattle side of our business and they were asking, "when is DNA going to deliver on this promise of perfection that people were making?" I think you all understand what DNA can potentially do in terms of the services it's doing in pedigree verification and parentage verification. What I want to do is talk a little bit about some holes that still exist in DNA testing. What DNA can do and what DNA does on a routine basis is rule out all individuals that could not be the parents. If we throw enough DNA tests at a case, we will exclude all other animals and that is how we drive towards identity. But an ability of a set of DNA markers, to rule out all of these animals is actually based on the number of markers that we use and those specific markers. Again if we went back to the example of the car, simply saying that a car is a Honda does not make that a powerful test. But simply saying that the license plate has an A, etc becomes a very discriminating descriptor of that car. And again when all is said and done, we go back to a statistical argument and a balance of probabilities that says, if I'm bringing forward a result to you where the likelihood of another parent qualifying is one in a billion, I'm not making a statement that this is a parent, I'm leaving that inference to you. That's our job at the laboratory, and it's a pretty safe assumption given the power of the markers that can be thrown at a test. Maxxam currently has a total of 26 and there are actually 27 markers that are available for use in equine testing. There are some other things that happen and there are actually two of these that are very specific to the standardbred industry. There is a phenomenon called a null allele, and when we look at it in our system as scientists what we see is an animal that appears to have only one band. The logical first pass at interpreting this would say that both the mare and the stallion contributed the same DNA type to that animal. And it happens. But in fact what's happened there is another one that is sort of hiding in the test that we can't detect. When you go back to the train analogy, when we are doing this testing we have to find the engine on the train and the caboose on the train, and if we don't have both of those we can't do a test. There are certain animals on certain tests. It's as if we don't have the caboose. They have everything else in between and it hides our ability to see it but it is actually there. So what kinds of problems does this cause? Well if the only result (band) you see is the top one and an actual foal from this stallion inherits the second one, we will never see that as a typing. And so it looks like exclusion to us.

There are two things that I need to keep in mind to this and actually people using laboratories need to keep in mind. We know these DNA tests in our lab and a lot of other labs around the world. We know that in standardbreds there is a DNA test called HMS 7 that has a null allele in it, and so we don't use it. Having said that, some of our technical people have developed ways of looking at defining a train other than an engine on the front and a caboose on the back so that we can do our DNA test and find that hidden null allele if we need to. If you don't do the work and don't have the awareness in the

laboratory of these sorts of technical issues, or if the tests aren't redesigned, you will create errors in the historical database. There is another reason that DNA isn't perfect in this perspective and it deals with mutations. There are specific types of mutations that occur in STR's. Mutations are simply changes that the parent's type and the foal's type are different but they are in fact parents. So if we look at this potential mating between a mare and a sire with their appropriate DNA types, and we look at a foal. If the foal has one band that corresponds to a mare, and another one you would expect that would correspond to a sire, but in actual fact when it shows up in our laboratory and the results looks like this, there are a couple things that can happen. It may in fact be an exclusion, or it may be that it is passing on its genetics to the foal and there has been a mutation in the sire's DNA. And as a result, what we see is a mutation. What we then have to determine is if it's a real mutation. When we look at these two things, these null alleles and these mutations are referred collectively in our laboratory as single system inconsistency. Null alleles and mutations are phenomenally rare. We understand where and when null alleles will occur. Mutations occur at a maximum of probably one in a thousand matings. So we probably see one or two of these a week depending on our flow through. What they do is create a possibility of error and so how do we as a lab handle this degree of uncertainty? What we do is we've developed a set of rules, and one of the most important rules is if we see one test that excludes an animal but no others. We do not report that as an exclusion. It's an accepted norm in the industry that you need to see more than one test that excludes in order to exclude that animal as the sire. Behind that is a statistical argument. Given all the markers that we have, what is likelihood that this is the actual sire or that this is a mutation? And again, we need to have a technical knowledge of a test we are running in order to make this determination. And again, when we see bands and certain patterns, we are more likely to lean towards saying this is a mutation or this is an exclusion just based on our know-how and the 300,000 samples that we've typed in our laboratory. So with these two technical things behind us in terms of why DNA isn't perfect, I would say that single system inconsistencies however they come along, are not responsible for the problems that occur in DNA testing worldwide. One of the other problems is that not all laboratories are created equal. Part of the problem is a lot of you people have become aware of, when blood typing or serology labs made a transition to DNA labs, they didn't necessarily have the expertise to do that. Roughly the equivalent of a car manufacturer going into the business of manufacturing jet airplanes. One of the other issues is, and it goes back to the number of tests, there are some standard markers in use and I would expect that all of you who use DNA testing laboratories are familiar with the International society for animal genetics. And a number of years ago, they said they need to have some standardization. If we are going to be having DNA testing being done from around the world and animals moving from place to place, we need to have a standard to compare one to the other animal. And they went and took 9 of the tests (markers) and said these are our standards and any lab should be using these 9 markers. These 9 markers are good in terms of their ability to identify an animal. The power of a set of markers in identity far exceeds that of the ability for those markers to do parentage. So what you have is a set of markers that would be able to identify an animal anywhere in the world, but would not with the same power as required by your industry be able to resolve issues of parentage. But having these 9 set of markers as a standard have been critical and important for the industry and so with these in place it

should be the case the results are comparable between labs. But some of you know that it is now in fact the case. And there are reasons for this discrepancy. There are different kinds of equipment that are being used and they generate DNA profiles in slightly different ways. We've recently gone through a couple of cases where we've begun doing work for a registry that has some historical DNA types with another lab. Historically it's been the case that it would appear that the results are incompatible. But in fact they're not. You just need to do the translation that turns one set of results from one piece of equipment into another. They are in fact usable. The other discrepancies go to competency. As the technology is advanced, we are not making those same mistakes again. But the fact that we as an industry are more correct now than we were means that there are errors to be had historically. It's important that the results for these 9 markers be presented in a way that everyone can speak to again. If one laboratory is doing all of these 9 tests but reporting their results in a different language so to speak, it is going to be very difficult for that laboratory to interact. Again, these 9 markers although they are for identity, they are not enough for parentage. I would suggest that this is an area that our side of the industry needs to improve on. There are two checks in the system that are important. There are comparison tests. The international society for animal genetics that develop these 9 markers also runs a test where they send tests out to all participating laboratories and they can compare to see that Maxxam is the same as the laboratory that is servicing France etc. It's important to note that while we're not allowed to speak about other laboratories' results in this and to a large extent these tests are blind. Not all laboratories come up with the same test results, given the same marker, given the same test. And that's important to consider. The other thing that happens is often times a particular breed, whether it is in different countries or different jurisdictions will use different labs. And it is the sharing of types between those labs that bring out the inconsistency. If everything is done within the laboratory, and if you are using a laboratory that doesn't actually interact with other laboratories, it's errors will tend to be hidden until such time as you need to rely on a type from an outside laboratory. And that can create substantial problems. The third check on the system is the sire we typed or the foal that we typed a number of years ago when it becomes a sire. As we verify foals to it, that typing will be verified. If there are problems with the type originally, it will show up when all its foals exclude to it. So what would make DNA testing better. DNA laboratories do need to get better than they are. That's both in terms of technical know how and experience and technology development. DNA has become a very sexy science. There are a lot of companies that are trying to get into this market because they imagine it to be very large. People who understand DNA don't necessarily understand the impact of DNA on your industry or the impact of how animals are bred in your industry, on the ability of DNA to settle issues like parentage. It takes experience and a relationship, such as Maxxam that has with its clients, in order to develop that capability and experience to really effectively serve clients. And again technology development. We need to take those tests that are ambiguous and that are giving no real results, etc. and move the technology forward such that we are not relying on tests that may have the possibility of error. I would suggest also that the 9 markers that were decided on by the international society for animal genetics are not sufficient, and that more standard markers need to be identified, and that more comparison testing needs to take place, and that in fact the comparison testing needs to become more public. So that you, in making a decision about

a laboratory, or evaluating your current laboratory have the ability to see what its track record is. I don't think there is any value in the secrecy that currently exists around the issues of comparison testing. And these standards whether it is comparison testing, or the number of markers we use, or anything that we do, I believe needs to be more closely aligned with the needs of your industry. As your industry evolves, or as the needs of your industry change relative to things like cloning, or anything else, our industry has to be a bit more responsive than it has. Before I get into sort of what I see happening in the future of DNA testing, is there anything that I said that needs clarification or does anyone have a question?

Question:

Suppose an embryo transplant got 10 or 12 transfers from the same flushing in a mare. You have 10 or 12 foals that were all conceived at the same time and put into 12 surrogate mothers. Will DNA testing be able to separate those foals? Suppose all 12 of those foals were fillies, and they were all bred to the same stallion, does that complicate the identification by DNA to a large extent or not?

It does not, provided that there isn't a higher incidence of twins occurring when the mare is super ovulated. If she is producing 10 embryos, as long as they're not identical twins, that those embryos have not split post-conception on, those animals are siblings like any other sibling. And so yes, we can distinguish between them probably with the same degree of confidence that we do to any sibling foals that come out of a mating.

Question:

I don't know anything about the embryo situation whether they could be separated or they could be twins... Whether you could get 12 of them from one egg?

No. The incidence of identical twins of that technology is not higher. It is not higher than it is naturally. It's really not that much more complicated than if that mare had 10 foals in each of 10 years and they were siblings. That is essentially the case that we are trying to find out. It's simply trying to identify these animals as siblings. And we can do that; we can distinguish between them. And the second answer to your question is if they're bred subsequently to the same stallion or different stallions down the road. It would be as if they were different animals registered. I don't see a DNA problem with the situation that has been described.

Question:

Given that, are we taking identification on DNA far enough? Because you have eluded that you have 26 markers and we are only taking a certain number. If you can identify right now, it removes the difficulty of embryo transfers because you can't actually identify specific animals and they are uniquely different with the exception of twins. Are we taking the parental verification that we've got far enough at this stage or should we be going further and setting a standard for the future, which can actually individually identify each horse.

I believe that individual animal identification is going to become more critical as time goes by. I think advances in technologies are going to call for that in the case that you've described. I think it strengthens that argument that we need to have sufficient power in our DNA testing to go beyond what we expect to happen. So if there are 10 embryos flushed, I think that is a standard that we need to be able to separate those. We need to be able to separate 10 full siblings from each other. I'm just going to take one second to demonstrate. If we go back to this example, essentially these four foals and I could have drawn this out to 10, if you could look at the mare being flushed and the sire on the other side. If it was all from identical mating, on one test only, there are 4 different possibilities for DNA typings in these sibling animals. When we use 26 markers on this problem, I am quite confident that, provided they are not identical twins and again I don't think that is really an issue here, we will distinguish full siblings even in very closely bred lines.

Question:

The only thing I would say is that I agree there are a lot of possibilities but everytime you reduce one of those ten (multiples) it lowers the chances of differentiation. You took out one of them and you reduced it by 10 and now you're down to a lot less than 1.2 billion.

That's right. When we send a data file to your organization we don't report a statistic with it and this is exactly the reason that we don't do that. But if we look at full siblings and you realize that in any given test there is a 1 in 4 chance that two identical siblings will have the same type just given what's up here. There's a 25% chance of the animals matching at a specific test and we do 25 tests, that is 0.25 to the 25th. That is less than 1 in a million chance that sibling animals will wind up with the same DNA type. And that's the statistic we would use then. So test #1, we have a 25% chance that two animals from the flush will have the type that foal #1 has here. When we go to test #2, it would similarly have an independent 25% chance of lining up. It's important that they are independent. And we keep going on and on from there such that the chance that two siblings, essentially being identical is phenomenally low. I mean it just doesn't happen. There are similarities but there are also differences. Full siblings will look different in some way or another. And DNA is a more powerful tool than the visual observation is. This last slide is actually the last line. It has to reflect the needs of your industry. We need to understand your industry well enough to anticipate and deal with these cases. Now a case such as you described is not something that runs through our laboratory everyday. So if this is something that your organization is dealing with right now, I would encourage you to get in touch with us and we can help you develop a strategy on how to separate these things. Or at least I would be more than happy to have myself or one of our technical people provide you with a write up as to how or what DNA can and can't do and what strategies might be used to resolve this.

Question:

We're discussing this item in the studbook area. Do you test but don't necessarily report, the 26 markers at this point in time.

That is correct.

Question:

You've just told us that you can individually distinguish with the full use of the 26 markers individual animals produced by embryo transfer. We're faced with the problem of having this attacking a number of jurisdictions throughout the breeding world. It is with us already. We have restricted practices in trade, coming across into our industry at the moment. We got a request that you do provide the papers so that it will help us work through this issue at the moment because if we can't distinguish between horses, a lot of the argument as to one foal per mare disappears, and we need to have that technical certification that you are capable of identifying an individual standardbred. We can progress this further for our development because the cost of embryo transfer is significant in our country but people will only use it on the quality or high-end mares that they have, or for the welfare of the animal themselves which is paramount. So we would like to have that paper from you, plus the certification that you can do exactly that, individualize the horse.

So you would like us to essentially forward a paper on the discussion that we've had. Are there any other questions. There are things that are happening in the DNA industry that may impact on you. What I want to talk about a little bit is what possibilities are relative to these? What the economics of these are and the impact? There is a type of DNA testing called SNPS. And it's another tool for identification. There are DNA tests out that are going to speak more directly to the health of animals, and there are DNA tests that are coming out that I believe will speak more to the performance of animals. But let me start with SNPS. The long form of SNPs is a single nucleotide polymorph. If you look on the left-hand side here, which should be two identical strands of DNA, but at a certain point in time that CG is changed to an AT. It is not as complex as trying to explain what a STR is. Usually there are only two types here. So what you may have in this case is what is called the C type and the A type. The analysis of these at one point would have been very expensive but it is becoming less expensive. The problem is that, as I said, is that there are only 2 types, and with a STR there may be 10 types, 10 different bands that we were talking about. While these SNPS are less informative, you've got a possibility of A or B as your type versus a possibility of all the various lengths. That would dictate that you need more SNPS to equal the power of a STR. And because of that, it is more expensive technology. Right now the cost to a laboratory to do enough of these single nuclear-type poly morphism (SNPs) tests to equal the STR's that we are doing would be about \$75. These are still far off. Having said that, the basis of the technology, the type of equipment, the kinds of things that can be done, the price of SNPs at some point in time dip below the price of STR's. The critical question for your industry is will you be willing to switch technologies again? It's been a great example of a partnership that exists as we work with Standardbred Canada and USTA and going from the conventional blood typing to DNA typing, this would essentially be that all over again. And at what point in time would you, as an industry be willing to do that in exchange for the cost savings that go along with that? I believe that at some point in time we will reach a basement of maybe \$15 or \$20 per test. Don't quote me on this. I believe that some way at a certain volume that laboratories will be able to offer their DNA testing at the lower price. I believe that SNPS will be able to offer that testing at \$7 per test. The question is will there ever be a difference worthwhile to your industry and will it be worth the headache

of going back and starting over and retyping all the mares and stallions? And I'm going to leave that as a question that's hanging. I'm not convinced that there is value in livestock breeds where very few animals are tested proportionally relative to equine breeds. There may be some more application in cattle for instance but I don't know at this point in time that I could really step forward in front of you and make an argument that says you need to go back and retype the approximately 220,000 standardbred samples that sit in our freezer right now. I would have a hard time making that argument and saying that that is going to save you money in the long run. Everything that I've spoken about up until now has really been talking about what I have referred to as testing variability in the junk regions of DNA. That sort of betrays the value that DNA could offer to an industry. And I think one of the things that is going to become increasingly important is DNA testing and the variability of DNA as it relates to health. There are a lot of components of health or contributors to health that are determined by genetics. Immunity is determined by genetics, metabolism is determined by genetics. A lot of the things that make an animal healthy are determined by its genetics as opposed to its environment or management. So if we look at DNA testing by that logic, we would have a role to play in predicting the selection of animals that are likely to be more healthy. Pathogens, bacteria, viruses or what have you are also detectable by DNA based methods. So at this point in time, diagnostics is still a more complex thing, a lot of times done off site. The laboratory is not necessarily on the site where the examination is being done. I see DNA as evolving to the point where veterinary clinics would be able to have the ability to diagnose specific infections on site with the kind of accuracy that they currently are moving samples out of their laboratories to deal with. And I think this is important when you are looking at animals that are very valuable. A definitive diagnosis could be very important in treating that animal and important in speeding its recovery. As well, I think everyone is aware of the problems associated with overuse of antibiotics. I believe that this level of characterization of the pathogens will also facilitate the development of better antibiotics more geared to the genetics of the pathogens that they are meant to attack. So there is an evolving industry relative to pharmaceuticals and diagnostics that I see as being very exciting. The important thing to keep in mind is that there are very few diseases that some testing has been done already. The one test that has been done for a long time is HYPP testing in quarter horses. A disease that is more prevalent in quarter horses. It's a single test. It's a very simple test. And that's why it was on the scene very early. But the more complex diagnostic or predicted tests I think are still down the road. But as I said most testing right now is limited to simple diseases that are well characterized with single or few mutations. As DNA develops, it will be easier to test for more complex diseases and pathogens. About 2 and a half years ago, I was contacted by a start up company based in Los Angeles, and essentially what they wanted us to do was to be the DNA testing research arm of their company, which were essentially statisticians and what they wanted to do was take the 26 markers that we do and have us test in excess of 700 markers on a number of animals. And they were going to look at the results that we determined from those 700 markers on animals and correlate that back to how they performed on the track. To try and determine if there was DNA markers that could be identified that would correlate with speed, or winning or endurance or all those things that might be matched up. This kind of test is being used in other industries and the test is called a quantitative trait loci or locus (QTL). It is a tool for looking at complicated

quantitative traits. A quantitative trait is something not like colour where it is one or the other or easily identified, but something like speed where there is a continuous variation between animals, and endurance or various other things. Right now these are being introduced on quite a wide scale in livestock, marbling and meat. There is a QTL test for marbling and meat. There is a QTL test for tenderness in meat. And right now there is a QTL test being done in humans that will predict the likelihood of contracting certain diseases. I think all these speak to a direction that the industry is taking. These QTL's can also measure, as I said, speed, endurance, temperament and all those sorts of things. Why I think these things have the potential to be more important in some senses in your industry than they are in livestock is that this test for marbling accounts for 15% of the variability of marbling and meat. Well if I had two pieces of meat in front of me and the difference in marbling between these two pieces of meat is 5 or 10%, I'm not sure if I could visually tell the difference. But if I had a horse on the track that was 5 or 10% faster than the other horses on the track I would certainly be able to tell the difference. So whereas in livestock the gains are going to be little by little or bit by bit. If these tests have the same potential in your industry that they do where they are currently being used now, I think they will have a substantial impact on the industry. The question that I have is how would you deal with this issue? And there are two scenarios that I think need to be considered. One is that the test is widely available. That Maxxam or someone else makes this test available to all your breeders. Is regulation something that would contemplate or would you let this happen? But I think the more controversial and potentially the more problematic issue is what if this test is developed in secret? And what if there are people who are using this test? It will eliminate a level playing field and that fact is that you may not even know that this is happening. It may in fact be happening today. What it would look like in your industry is certain lines or certain animals that clearly have become much more than anyone expected, better than anything else that is out there.

When I talk about genetics, I talk about molecules. In your industry when you talk about genetics, you're talking about the traits that make an animal good. The failing in my industry is that we tend to see things too much at a molecular level. We think of it as being simpler than it is. So the people who put out a test for marbling or whatever that doesn't speak to the value of the industry is because they don't understand or they're not speaking to the complexity. And for me, if you look at something like speed, or metabolism, or how good a horse is to win, if you were to draw that in terms of all the things that might impact on that, and only look at the two way interaction so speed as it is influenced by metabolism or speed as it is influenced by endurance or anything else, what we have is a situation that is potentially too complex for DNA to handle on its own. This is something that is going to require the expertise of the industry. But having said that, to the extent that DNA can speak to a number of things, it will be an important tool. But it is important to realize that DNA is not a silver bullet. There will not be a DNA test that supplants the expertise that exists in your industry. I don't believe that. There are people in my industry that do. But I believe that DNA will become important in predicting the value of an animal. So the sale of an animal might be accompanied with a certificate that says it has the DNA profile that backs up with what the pedigree has been speaking to all along. Again, DNA is only a piece of that pie and only part of that triangle. Potentially you are going to have more to it than genetics management and environment. The

problem facing the DNA industry in dealing with this is how do you account for the large number of variables even if we were to confine it to the variables that are covered by genetics. How do we deal with all the complexity? Having said that, DNA is a huge and very fast moving industry. There are technologies being used in the pharmaceutical industry to figure out the complexities of disease and drug metabolism and the effective drug. I believe the technology that is being developed there is going to be transferred over to other areas. One of the things that is being used is called a DNA microarray. A DNA microarray is a DNA testing methodology that uses a silicone chip. The companies that are involved in producing these are biotech companies and IBM. And Hewlett Packard has a division that develops microarrays in conjunction with a biotech company out of LaHoya, California called Affymetrics. The questions that are still out there and I don't believe anybody is able to answer is who will fund the research that takes a very high-end technology and brings it into your world? And who will pay for this test if another issue always comes up? And again who will benefit in the long run? And until these questions are answered all of the things that could happen or are maybe likely to happen, and I'm not sure that they will happen without solid answers to those questions.

In summary, I think DNA is an extremely powerful tool for identification and parentage when qualified people in close communication perform it in a standardized way. In the future I think DNA is going to have a larger impact on more aspects of the racing industry in ways that I don't think either of us have thought about. I would suggest that at some point in time that it would be worth while to have people from our industry and people from your industry in a forum like this and concentrate on what impact this might have. I think we have a say in what happens in each other's industries. Are there any further questions or comments?

Question:

When you talk about using DNA to, are you talking about predicting the ability of a horse from DNA like a foal. You're going to sell a foal, or are you talking about the possibility of genetic engineering to some extent or both?

What I've talked about here speaks to accelerating gains that would come from natural breeding anyway or from the breeding strategies that are in place now. It's a tool for selection. It's not a tool as I've described it from manipulation. Having said that, manipulation is a whole other topic that was probably too big for a 2 hour session. But yes, I'm speaking to not genetically altering an animal but using genetics as a tool to select animals.

Questions:

I have a couple of very soft questions for you. How many different equine breeds does Maxxam test for? And from how many countries and approximately how many samples do you do a year? And what are you using to do the DNA? For the United States and for Canada, are you using hair samples, blood?

Sharon really heads up our efforts in dealing with customers on a transactional basis. Sharon how many equine breeds do we currently service?

Sharon:

Right now we have approximately 70 breeds that we deal with. I would say the majority of those would be equine. About 60 of them would be equine. I think the largest that we have right now are the standardbreds.

Rob Wiebe:

We passed the 200,000 Standardbred mark in about February this year. In addition to, we do typing throughout North America. We essentially, with the exception of quarter horses and some smaller breeds, we do all of the equine typing within Canada that there is to be done. We have relationship with Australia and last year we did samples from probably 4 or 5 different countries. Our business has some substantial interest coming out of South America right now that would expand our business down there. Can you help me out with the rest of the question?

Question:

Yes, Sharon for your testing what type of samples are you using?

Sharon:

The majority is hair samples with the horse industry. A lot of times we will go back to a stored sample with the thoroughbreds. Some of our other breeds, not necessarily equine. We have what we call FTA, which is a blood sample spotted onto a FTA card. Bone samples for deceased animals. They will send in a leg bone or a tooth. But the majority of the samples definitely are hair.

Question:

The next question I had was with respect to the automation of your laboratory. Are you finding that as time goes on and the world changes dramatically, are you able to automate your test efficiently so that us as the consumer can get a better price range?

Rob Wiebe:

There's that question again. And that's a very good question to follow up on a question about sample type. We have invested quite substantially in some research that is going on into automation. Some other ways of getting at the DNA in a sample. Hair has become the sample of choice especially in the equine industry because it is very easy to deal with. It is non-invasive. But in our laboratory what that translates into someone with a pair of forceps and scissors pulling out 3 hairs and cutting the roots into a tube. There is no way that we'll ever find a robot that will be able to select the root end of a hair and cut that into a tube. And that really to a large extent is a limiting step in our laboratory, so the ability to automate that is not that good. But having said that, there are technologies whether we're talking about SNPs but also with analyzing STR's that will allow us to automate the process. The problem is going to be one relative to cost of capital expense and volume and that's true of any business. We use equipment that has been around for awhile and as the rest of the DNA world has gone forward into these microarrays, the kinds of equipment we use become available on a used market, believe it or not in DNA, and we can get that at substantially decreased costs. And in fact one of the reasons I think

we've done well is we've not tried to jump ahead into new fancier pieces of equipment for doing the analysis stage of this. That has kept our costs down. There are companies that have made that step into the larger equipment and without the sample volume to sustain it, they're not running the equipment at the volume that is meant to be run at. They have equipment problems and capital issues to deal with. And that's essentially where we are right now. Having said that I see a couple of opportunities coming down the road where over the next 5 years, we would be able to build more automation into what we've done. And for us right now our focus on decreasing costs is really around a program that we have, SIGMA, and it's really an issue around increasing the efficiency and decreasing the kinds of errors that impact on our customers. And errors include the occasions when we miss our turnaround time. And by decreasing the number of times that we have to repeat a sample if it failed, by decreasing the time it takes to do a sample, we are probably building more efficiency into our system than we would get from the capital investment.

Question:

One last question from me and then we will ask for the general session for questions. What is an acceptable turnaround time for your equine clients? I'm curious because we might have some interesting comments from different countries.

Rob Wiebe:

That's interesting because most of our contracts are written as 10 or 15-day business day turnaround time. I know that our average is substantially less than that. So what's acceptable? Well if we are giving 5-day turnaround time then the acceptable is 5 days. If we hit 6 then we tend to get some phone calls. Acceptable is I believe what we're doing. Unacceptable I believe is one day longer. I think our average right now for standardbred turnaround time is less than 5 days.