Avoid Mistakes When Collecting DNA Samples

by ***Troy Smith***

You've heard plenty about DNA testing. You know DNA tests can be applied commercially to determine parentage and to manage genetic defects in cattle. You know that analysis of an animal's DNA can determine the presence of gene markers associated with specific performance or carcass traits. You also know DNA genotyping has advanced to where testing can reveal an animal's DNA profile — a summary of genetic influences on a variety of economically important traits.

And now you're ready to apply this rapidly advancing technology to your operation. Well, don't waste your time. Don't bother with collecting hair, blood, tissue or semen samples for DNA analysis. Don't do it, if you're not going to do it right. Sloppy sampling results in erroneous test results and misleading information, or no information at all.

> "We've got to slow down and do it right. All of it."
>
> — *Robert Weaber*

If you're serious about making DNA technology work to your advantage, avoid the all too common mistakes associated with collection, storage and shipment of DNA samples. They most often occur when people get in a hurry, so University of Missouri beef cattle geneticist Robert Weaber's advice is to "slow down." And, before you do anything else, make sure you know what kind of sample is required for the DNA test(s) you seek. Weaber also says sample requirements vary among laboratories providing genotyping services.

> It's the roots that the lab really needs. That bulb on the end of each hair contains a significant amount of DNA. And bigger is better, so coarse hair from the tail switch usually works well.

"Certainly, DNA can be obtained from blood or tissue samples, or from semen, but a laboratory may require a certain type of sample, depending on the kind of technology it uses. Certain sample types just work better for certain tests," says Weaber, noting that automated DNA isolation systems are designed for specific sample types.

"The number of tests to be performed may influence how much DNA a lab needs and the type of sample required," Weaber adds. "For example, two milliliters of blood will yield more DNA than a piece of skin."

Maxxam Analytics is the laboratory approved by the American Hereford Association (AHA) for DNA testing to determine parentage. Maxxam also performs tests for genetic defects including idiopathic epilepsy, dilutor gene and hypotrichosis. The laboratory, located in Guelph, Ontario, accepts a variety of sample materials for DNA analysis, including semen, blood and tissue, but the majority of tests are performed on hair samples. The reason could be that producers consider hair easier to collect and submit.

Hair collection

However, according to Weaber, a common mistake occurs when hair samples are submitted with no roots attached. It's the roots that the lab really needs. That bulb on the end of each hair contains a significant amount of DNA. And bigger is better, so coarse hair from the tail switch usually works well.

The hair should be dry and free of foreign matter, so brushing out the switch prior to collection is recommended. For an adequate sample, producers are advised to pull 20 to 25 hairs, making sure the roots are attached. When collecting hair root samples from a young calf whose hair is fine with small roots, it's generally recommended to include 30 to 40 hair roots.

Blood-spot cards

Blood-spot or FTA® cards are another frequently used method of submitting DNA samples. Inside each folded card, which resembles a matchbook, is a circle to which a few drops of blood are applied. The blood can be transferred from a needle-prick to the animal's ear or from blood drawn into a sterile syringe. The common mistake, says Weaber, is closing the card before the blood spot is dry. The blood sample should be allowed to air-dry, completely, before re-closing the card. Otherwise microbial growth can get started, and those microbes secrete enzymes which degrade DNA.

The same thing can happen if too much blood is applied to the card, making it soggy and slow to dry. Additionally, there may be a greater risk of cross contamination between blood-sodden cards

continued on page 150...

Hereford testing procedures

Hereford breeders wanting to get a female donor dam permitted or a sire permitted for artificial insemination (AI) use must have the animal DNA tested. Producers can also use DNA testing to parentage verify an animal before registering when the sire of the animal is in question.

Maxxam Analytics is the American Hereford Association's (AHA) official DNA lab. All animals that are tested through the lab will also be tested for genetic abnormalities.

The cost for DNA testing for less than 50 samples submitted at one time is $32/head for hair or $37/head for semen, blood or tissue. If a breeder submits more than 50 samples at one time, the fee is $19/head.

Here are the steps for each of the processes:

Permitting donor dams

- Call the AHA office, 816-842-3757, to request a DNA test kit. You will need the animal's registration number.
- AHA will send the producer a DNA Genetic Marker Test form. The form has a bar code specific to the animal. Producers cannot alter the form for another animal.
- Collect the sample and submit it to Maxxam (see "Instructions for obtaining hair samples").

Permitting AI sires

- Call the AHA office, 816-842-3757, to request a DNA test kit. You will need the animal's registration number. AHA will determine if the bull's parents have been tested and are on file. If the bull's sire and dam have not been tested, the breeder will need to collect and submit those samples as well.
- AHA will send the producer a DNA Genetic Marker Test form. The form has a bar code specific to the animal. Producers cannot alter the form for another animal.
- Collect the sample and submit it to Maxxam (see "Instructions for obtaining hair samples").

Genetic abnormality testing

- If a breeder wants to get an animal tested for a genetic abnormality, he can follow the same steps as those for permitting a donor dam. For more information about DNA testing procedures for Hereford breeders, call the AHA at 816-842-3757 and ask for Beverly Kincaid. HW

Instructions for obtaining hair samples

- Pull hair samples above the tail switch. Do not cut the hair. The hair root contains the material needed for DNA testing.
- Pull 20-25 hairs evenly and directly from the tail so the hair does not break. The switch must be dry and brushed clean of all debris. The lab suggests wrapping the hair around a pencil and then pulling.
- Place the hair in a straight line across the center of the form from the AHA for DNA testing. Keep the hair together with the roots to the left as noted on the form. Do not curl hair. Attach the center hair shafts to the form with tape.
- Fold the form as you would a business letter. Each sample has an individual envelope for mailing. If you have several to mail, put each sample in its individual envelope and then mail all the envelopes in one big envelope to save on postage. The address is located on the bottom of the form.

Note: It is important to only include hair from one animal in each kit. The lab cannot detect cross contamination of samples. The same form can be used for semen straw samples. Semen does not have to be frozen. Put the semen straw in a ballpoint pen casing, capped and taped on the form where it indicates to put the hair shafts. This procedure prevents the semen straw from breaking while being mailed. HW

...Collecting DNA continued from page 149

packaged together for shipment to the lab.

Some blood-spot collection kits provide a needle for pricking the animal's ear. Instructions call for pressing the collection card against the wound to collect the blood sample. Weaber says getting blood on the card might be easy enough, but confining it to that little circle can be pretty challenging.

"I prefer to use a two milliliter syringe to draw a little blood to place on the card, right where you want it," says Weaber. "But always use a new sterile syringe and needle for each animal to avoid cross-contamination of samples. Clean hands and clean instruments are really important when collecting any kind of sample."

Whole blood tips

When collecting a quantity of whole blood, each sample must be drawn into a "purple-top" collection tube. These tubes contain EDTA — an anticoagulant and preservative. If the wrong type of collection tube is used, the blood sample is likely to coagulate by the time it reaches the lab, preventing separation of DNA. Instructions for drawing blood from the jugular vein (neck) or the underside of the tail are available from laboratories that require whole blood samples or through Cooperative Extension Web sites.

"Don't forget," warns Weaber, "to invert the tube several times after collecting the blood sample to mix it with the anticoagulant."

An advantage shared by blood-spot cards and hair root samples is ease of shipping. No refrigeration is needed, but most laboratories recommend shipping samples within 48 hours of collection. Shipping whole blood is more tricky. Some labs call for blood to be packed with dry ice or "blue gel," but Weaber recommends shipping whole blood on wet ice for "next day" delivery.

"And don't ship on a Friday, or your samples may not be delivered until Monday. Shipping on Monday or Tuesday is best for midweek delivery," Weaber adds.

"Don't forget to invert the tube several times after collecting the blood sample to mix it with the anticoagulant."

— Robert Weaber

Punching it out

Tissue sample collection involves removal of a small skin sample. Pig ear-notchers have often been used to collect a sample from the animal's ear. Now available commercially are tissue-punch collection devices, which resemble ear tags used for identification purposes. When these are used properly, a bit of ear tissue is collected and sealed within a "tissue tag" bearing its own identification number and barcode.

There is a cost involved with the tag-type sampler and its applicator, but some producers prefer the convenience of this sample collection method. However, labs have reported the occasional receipt of tissue tags with no tissue inside — probably because the person didn't make sure the device went through the animal's ear. And another thing: since the ink can interfere with DNA tests, collecting a tissue sample from a tattooed portion of an ear should be avoided.

Before you package the semen for shipment, Weaber suggests placing the semen container inside an inexpensive pen (like a Bic® pen) whose ink cartridge has been removed. Or, the semen straw can be placed between two pieces of stiff cardboard before packaging for shipment.

Semen precautions

When you submit semen for DNA analysis, most laboratories request a straw of thawed semen. Use a proper shipping container to protect the straw against breakage. Before you package the semen for shipment, Weaber suggests placing the semen container inside an inexpensive pen (like a Bic® pen) whose ink cartridge has been removed. Or, the semen straw can be placed between two pieces of stiff cardboard before packaging for shipment. According to Weaber, refrigeration is not necessary and semen may be sent via regular mail.

Of all the mistakes made when collecting samples for DNA testing, Weaber believes the most common is data entry error in the field. Samples are mixed up and mislabeled. Numbers are transposed. A sample from a young calf is labeled with the dam's number. Any number of things can and will happen because of human error. At the end of the day, expert technique in sample collection doesn't count for much when the paperwork is a mess.

"We've got to slow down and do it right," states Weaber. "All of it." **HW**