

Take Time, Do It Right

If you've decided to collect samples for DNA testing, consider these tips for collecting samples that can lead to accurate results.

by **Troy Smith**

You've already 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.

If you are 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 advises, "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 says sample requirements also vary among laboratories providing genotyping services.

"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 (mL) of blood will yield more DNA than a piece of skin."

To help producers choose a laboratory, most breed associations maintain lists of

laboratories approved for performing various DNA tests according to association-approved standards and for providing reportable information. Five laboratories have been approved by the American Angus Association to perform DNA testing associated with the genetic defects arthrogyposis multiplex (AM) and neuropathic hydrocephalus (NH). They include MMI Genomics, AgriGenomics, Pfizer Animal Genetics, Igenity and GeneSeek Inc. MMI Genomics also serves as the only American Angus Association-approved provider of parentage verification testing.

Hair root samples

According to spokesman Jeremy Walker, a significant portion of DNA testing by GeneSeek's Lincoln, Nebraska-based laboratory involves hair root samples. A common mistake seen with this kind of sampling is that hair is submitted with few or no roots attached.

"It's the roots that we really need. That bulb on the end of each hair contains a significant amount of DNA," says Walker. "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 advisable. For an adequate sample, producers are advised to pull 20 to 25 hairs, making sure the roots are attached. The root ends of the hairs are then secured between the adhesive layers of the hair sample collector, which laboratories provide. The excess hair length is trimmed off, and the sample is placed in a resealable plastic bag.

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. For some tests, says Walker, a blood sample from calves may be preferable.

Blood-spot cards

Blood-spot or FTA® cards are another frequently used method of submitting DNA

Laboratory services

Following are names of laboratories that are approved by the American Angus Association for conducting tests for the genetic defects arthrogyposis multiplex (AM) and neuropathic hydrocephalus (NH). The availability of other testing services varies among the laboratories, as do their sample requirements. A summary of services and preferred sample types is provided, along with respective contact information to use when seeking further details.

- ▶ **MMI Genomics**, 1756 Picasso Ave., Davis CA 95618; 1-800-311-8808, ext. 3016; www.mmigenomics.com. MMI Genomics is the only Association-approved laboratory for parentage verification based on DNA analysis. Other testing services include coat color, horned/polled, marbling and tenderness. Blood-spot cards and semen samples are accepted.
- ▶ **AgriGenomics**, 2399 N. 1000 East Rd., Mansfield IL 61854; 217-762-9808; www.agrigenomicsinc.com. AgriGenomics provides DNA testing for genetic defects and coat color, accepting only whole blood or semen samples.
- ▶ **Pfizer Animal Genetics**, 333 Portage Rd., Kalamazoo MI 49007; 877-233-3362; www.pfizeranimalgenetics.com. Pfizer Animal Genetics offers testing for genetic defects, parentage, feed efficiency, marbling and tenderness. Hair root, blood-spot card and semen samples are accepted.
- ▶ **Igenity**, 4701 Innovation Dr., DB 101, Lincoln NE 68521; 877-443-6489; www.igenity.com. In addition to parentage, coat color, horned/polled and genetic abnormality testing, Igenity offers an Angus-specific genomic profile including 14 economically important traits related to maternal and reproductive characteristics, growth and performance and carcass merit. Results are incorporated into the Angus database for use in generating genomic-enhanced expected progeny differences (EPDs) (see "The route to genomic-enhanced EPDs," page 122). Most testing can be accomplished with FTA® cards, hair root, tissue-punch or semen samples. Whole blood also is accepted for genetic abnormality tests. Testing for bovine viral diarrhoea persistent infection (BVD-PI) requires a tissue-punch sample.
- ▶ **GeneSeek**, 4665 Innovation Dr., Suite 120, Lincoln NE 68521; 402-435-0665; www.geneseek.com. GeneSeek provides testing for parentage, genetic defects and coat color. Preferred sample types include hair roots, blood-spot card or tissue punch. Testing for BVD-PI is also available through GeneSeek.

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The route to genomic-enhanced EPDs

Through Angus Genetics Inc. (AGI), the American Angus Association has created a system for incorporating DNA technology into the process of genetic evaluation. The goal is to use genomic information collected through DNA analysis, along with the traditional performance data used in National Cattle Evaluation (NCE), to create improved tools for genetic selection. In other words, all available information will be combined to calculate genomic-enhanced expected progeny difference (EPD) values.

Toward that end, AGI has adopted the Igenity® Angus DNA profile as the means for analyzing 14 economically important traits: heifer pregnancy rate, docility, stayability, maternal calving ease, marbling score, ribeye area, fat thickness, carcass weight, tenderness, percent Choice (quality grade), yield grade, average daily gain, yearling weight and feed efficiency.

Results from participating breeders' cattle will be added to the Association's genetic database and used to calculate the enhanced EPDs, with initial emphasis on carcass traits. The genomic profile includes some traits for which there currently are no EPDs produced. When EPDs are developed for those traits, respective information from the profile will be included in the calculations.

Breeders choosing to participate must collect appropriate DNA samples and route them through AGI. Hair root collection cards and tissue-punch collection tags are acceptable sampling methods, but the preferred method of collecting and submitting samples is with blood-spot or FTA® cards. All sample collection materials may be obtained through AGI, either online or by contacting the Association office.

To obtain genomic profiles for specific animals, the animals must be identified in the Association database. It is recommended that this be accomplished online, along with submission of an order for the Angus DNA profile. Add-on tests for genetic defects and coat color are available. Testing for bovine viral diarrhea persistent infection (BVD-PI) is available, too, but requires a tissue-punch sample.

Samples should be sent to Angus Genetics Inc., 3201 Frederick Ave., Saint Joseph, MO 64506. Upon arrival, samples will be matched with profile orders, properly identified and forwarded to Igenity's laboratory for analysis. Results will be sent to AGI for use in generation of genomic-enhanced EPDs. Profile scores for any trait currently having no EPD will be reported to the breeder.

Step-by-step procedures for submitting samples for profiling are available through AAA Login, at www.angusonline.org. For more details or answers to your questions, contact your American Angus Association regional manager or call the office at 816-383-5100.

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samples. Inside of 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 here is closing the card before the blood spot is dry," Walker says. "The blood sample should be allowed to air-dry, completely, before reclosing the card. Otherwise, microbial growth can get started and those microbes secrete enzymes, which degrade DNA."

Walker says the same thing can happen if too much blood is applied to the card, making them soggy and slow to dry. Additionally, there may be a greater risk of cross-contamination between blood-soaked cards 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 2-milliliter (mL) syringe to draw a little blood to place on the card, right where you want it," Weaber says. "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."

Collecting blood in tubes

For DNA tests requiring a quantity of whole blood, each sample must be collected in 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," Weaber warns, "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.

Tissue samples

Gaining popularity for its relatively easy application is tissue sample collection. Initially, this involved removing a small skin sample from the animal's ear with a pig ear-notcher. Now commercially available are tissue-punch collection devices that resemble ear tags used for identification purposes. When 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 it seems to be an easier sampling method for some producers. However, we have received tissue tags with no tissue inside — probably because the person didn't make sure the device went through the animal's ear," Walker says. "Also, it's best to avoid taking a sample from a tattooed portion of an ear. Tissue samples containing tattoo ink don't work well for DNA testing."

Semen samples

When submitting semen for DNA analysis, most laboratories request a straw of thawed semen. Use a proper shipping container to protect the straw against breakage. Before packaging 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, Walker and Weaber agree 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," Weaber states. "All of it."

