Evaluating Red Angus Parentage Testing Policy

By RAAA Staff with Dr. Jim Gibb, Neogen

Introduction

Beginning with calves born in 2017, the Red Angus Association of America (RAAA) instituted a policy requiring that all sires of registered Red Angus calves be DNA parentage tested with results remaining on file with RAAA. This policy effectively brought all Association members into the business of DNA-parentage testing on at least a portion of their Red Angus inventory. The broad objective of this policy is to make the Red Angus database more accurate through increased pedigree verification. Studies have shown that breeder submitted/non-validated pedigree information typically contains a 10 to 20 percent error rate, meaning that pedigrees are reported incorrectly with an unacceptably high frequency. This problem reduces the accuracy of ongoing genetic evaluations, and can create

numerous other problems, including erosion in customer confidence in Red Angus cattle, and potentially, the breed as a whole.

When the RAAA Board of Directors implemented this policy, they recognized that 100 percent pedigree accuracy across the entire breed was an unattainable goal. However, they also believed that the use of sound science (DNA parentage verification) could substantially reduce the pedigree error rate over time. Although difficult to quantify precisely, there is ample evidence that current RAAA parentage-testing requirements have reduced pedigree errors on recorded Red Angus animals from approximately 15 percent prior to required testing implementation to less than 5 percent today.

The purpose of this non-technical evaluation is to provide perspective and answer commonly asked questions regarding RAAA's parent-verification policy, discuss the science behind current testing methods and explain how breed-wide pedigree errors are being reduced by applying well accepted, scientifically-based practices.

Overview of Bovine Parentage Testing (by Jim Gibb, Neogen)

Accurate pedigree records are essential to ensuring the integrity of beef cattle breed registries. Equally, if not more important, is the fact that rate of genetic progress is negatively impacted by incorrect sire and dam pedigree information. For example, misidentification rates of 7 to 15 percent in dairy cattle have been estimated to decrease genetic gain by 3 to 15 percent (Woodward and Van der Lende, 2008).

There are two categories of parentage testing: parentage verification and parentage determination. Parent verification (sire or dam) is the process of confirming that the alleged parent(s) is indeed the actual parent. Parent determination is the process of identifying the most likely parent from a group of possible parents.

Over recent decades, parentage testing has evolved from blood-typing to DNA testing. Blood typing was fairly accurate for excluding sires but was less effective for parent determination. In other words, blood-typing was mainly used to exclude sires versus identifying the correct sire from a large group of possible sires. Beef and dairy breed associations transitioned to DNA testing in the 1990s, with the use of micro-satellite technology, and began

shifting to SNP (Single Nucleotide Polymorphic) testing around 2005. By 2012, most major beef and dairy breeds had shifted to SNP parentage.

The move to SNPs provided several benefits. SNPs (also referred to as markers) are easier and less expensive to genotype. Plus, they are more accurate and can be incorporated into other DNA tests such as profiles for genomic-enhanced EPDs. SNP parentage testing has also greatly reduced the cost of parentage testing. Stand-alone, SNP parentage testing is about one-third the cost of blood-typing 30 years ago (without adjusting for inflation). It's even becoming common to include parentage at no extra charge when included with a comprehensive DNA profile used for GE-EPDs or a commercial profile like Red Navigator.

Accurate, low-cost parent determination has helped seedstock producers cut costs by enabling larger, more efficient multi-sire pastures and eliminating the waiting period between A.I. and bull turn-out. Commercial producers are also using sire determination to identity their top and bottom sires, those not getting cows bred, those that are causing calving problems, siring low-performing or siring abnormal calves. Multi-sire parentage also facilitates larger scale, more accurate genetic evaluation in commercial herds.

The keys to accurate parent verification and determination are accurate animal identification, DNA sample quality, the power of the parentage test itself and well-defined standards and processes in the lab. Undoubtedly, the highest quality samples are blood cards and tissue sampling units (TSUs). Blood samples have been used for years and are still a good choice, certainly better than hair. However, TSUs have become the sample type of choice. While more expensive than blood cards, TSUs are easier to use, less likely to get cross contaminated and are more efficient in the laboratory. TSUs are also very adaptable to electronic identification and record keeping. Nearly all long-time users of hair and/or blood cards that switched to TSUs choose never to go back. In addition to the ease of sample collection, TSUs yield more, higher-quality DNA which means fewer failures and retests, thus reducing the hassle and delayed results associated with pulling new samples.

Once samples arrive at the lab, DNA is extracted and the genotyping process begins. Today, most laboratories providing bovine parentage testing use a minimum of 96 SNPs, many of which were identified at the U.S. Meat Animal Research Center. Additional parentage SNPs have been identified in other

countries, which has enabled multiple laboratories to employ expanded parentage profiles that include approximately 120 or more SNPs. Neogen-GeneSeek now routinely uses up to 200 SNPs. The advantage of a 200 SNP profile is both accuracy and the ability to use the same profile across all breeds. For example, some of the SNPs in the original 96 SNP profile do not work well in Bos indicus-influenced animals (Brahman-type). The 200-marker parentage test performs well across all beef and dairy breeds, thus further improving efficiency.

Considerable care is taken in the laboratory to ensure accurate results. In addition to standard operating procedures (SOPs) in the lab, key factors such as minimum "call rates" and "compared loci" are critical benchmarks when validating and/or assigning parents. Parentage determination compares the parentage genotypes of the progeny with those of the possible sire and/or dam. The call rate is the percentage of markers in a sample that yielded genotypes. Due largely to the quality of the DNA that was extracted from a given sample, not all markers can be genotyped. In other words, higher-quality samples yield more and better-quality DNA, and ultimately a higher call rate. Lower call-rate samples are more likely to yield incorrect genotypes.

The number of compared markers is critical because it represents how many markers were successfully genotyped for both the candidate parent and calf. The next step is analyzing the compared markers to determine if the candidate parent is indeed the actual parent based on matched genotypes. Today's technology enables the evaluation of numerous possible sires and dams across hundreds of progeny in a matter of seconds.

It should be noted that there are two types of parentage testing, duo and trio. The assignment of the sire or dam to a calf is called a duo, whereas trio testing includes the calf, sire and dam. The latter is the most accurate parentage testing available. When combined with the use of today's most powerful DNA parentage profiles, trio testing can provide highly accurate results even in large-herd, multi-sire situations. Trio testing is recommended in herds with a higher level of line-breeding.

Table 1: Neogen/GeneSeek SOPs for parentage

Minimum compared markers:
78 markers compared
(100 for Bos indicus animals)

Maximum allowed exclusions:
Parent-Offspring Pairs:
78 to 100 markers - 1 exclusion allowed
101+ markers - 2 exclusions allowed
Trios analysis:
78 to 100 markers - 2 trio exclusions allowed
101+ markers - 3 trio exclusions allowed

In summary, bovine parent verification and determination has advanced significantly over the past few decades, making a significant leap forward with the advent of SNP marker technology. These advances provide seedstock (and commercial producers) with the ability to accurately assign parentage, leading to improved genetic evaluations (more accurate EPDs).

Successful parentage determination and validation processes start with accurate animal identification records and high-quality DNA samples for the progeny and all possible parents. Breeders working in close partnership with the Red Angus Association to confirm that all standards are met throughout the entire process is essential.

Current Red Angus DNA Policy

The RAAA Breeder's Guide outlines the rules and regulations for all sectors of the Association. The following details those specific to DNA, please reference the entirety of Section H in the Breeders Guide for complete RAAA DNA policies.

SECTION H - DNA TYPING RESOLUTION

- The Association's commitment and right to verify parentage of Red Angus animals, thus preserving the integrity of the pedigrees, is hereby affirmed.

That broad authority is vested in the Executive Committee of the Board of Directors and the Chief Executive Officer to continue the collection of DNA-typing data which is to be maintained as a source of reference as related to further developments occurring from time to time in the technological area of parentage verification.

4. That the RAAA has the authority to require DNA parentage on:

a. All bulls to be used from which the resultant calves are to be registered in the Red Angus Database.

- All bulls that are the source of semen for A.I., parent verification is required to the extent that the parent(s) SNP parentage genotypes are on file at an approved testing facility. A copy of the DNA record on such bulls must be on file with the Association as a requirement of progeny registration. DNA typing of the bulls to be used as A.I. sires will be at the submitting party's expense.

- Beginning with the 2017 calf crop, all bulls that are the source of natural mating (pasture breeding), parent verification is required to the extent that the parent(s) SNP parentage genotypes are on file at an approved testing facility. A copy of the DNA record on such bulls must be on file with the Association as a requirement of progeny registration. DNA typing of the bulls to be used as A.I. sires will be at the submitting party's expense. **(Rev. 6-15, beginning with calves born on or after January 1, 2017).**

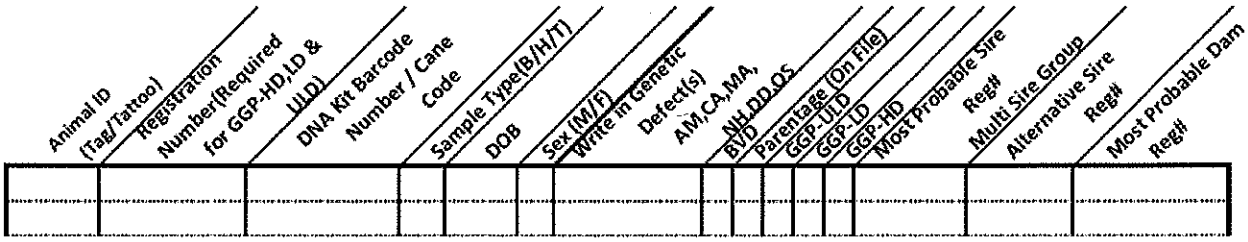
Red Angus DNA Protocols

- *DNA Order Form*

The order submission process as well as the information made available by the member to RAAA is an integral part of DNA parent verification. Red Angus has developed a DNA order form that is designed to guide members in providing the most accurate and complete submission information possible.

The Animal ID, Registration Number, Sex and Date of Birth are all categories that need to be filled out for each sample submitted. Even in the case that the animal is not yet registered, the remaining information helps prevent possible sample misidentification from incorrect labeling and/or other errors. A sample that has been labeled incorrectly will likely be tested to an incorrect mating and can lead to parentage results that are implausible or incorrect. Providing complete information for each sample can help the member and Association resolve any issues arising from sample identification problems.

Table 2: RAAA DNA Order Form



**See Complete DNA Order Form in Appendix*

The Most Probable Sire (A.I. or Herd bull), Alternative Sire (Clean-up or otherwise) and Most Probable Dam options are the most important pieces of information provided on any sample submitted for parentage analysis. The purpose of these informational entries is for the member to provide parent options that are precisely as stated "Most Probable." In cases where the individual is not registered, this information is pertinent to ensure accurate and conclusive parentage determination.

The Multi-Sire Breeding Group form included in DNA submission is helpful especially as an add-on to most probable parent options. These groups are meant to include no more than six sires to prevent unlikely and improbable bulls from being matched. While there are some operations that will have groups with more than six bulls as potential sires, it is still preferred that only the most likely/expected sire options are provided.

- *RAAA Processes and Lab Standards*

When members submit a DNA sample to RAAA and follow the requested protocol, they can be assured their samples are processed according to high standards at every phase, allowing them to receive the most accurate parentage results available.

All samples processed through the RAAA must receive a call rate of 90 percent or higher. Call rate, as mentioned previously, is the percentage of markers in a sample that yielded genotypes. Those that receive 89 percent or lower call rates will not receive results. These samples will be reported to the Association as *failed* and will not have a genotype cataloged in the system or available for comparison. This standard is a recommended practice by Neogen-GeneSeek and helps filter out low quality samples that could result in insufficient or inaccurate results when used for parent verification.

During the testing process of each sample, lab marker standards will be upheld for all parentage analysis. Once marker results are available from the lab, RAAA staff make comparisons using a *minimum* set of 78 markers (more when available). While the number of markers compared in each situation does differ, the minimum lab standard allows for the highest quality comparison between DNA-tested animals. All samples currently tested at Neogen-GeneSeek, through Red Angus will receive a genotype with 180-200 markers, and this number will increase even further in the future.

- *Data Reporting and Pedigree Changes*

The accuracy of DNA technology and precise testing protocols utilized by the RAAA allows for high quality DNA data reporting. With that said, there is no possibility for complete perfection, especially in any scenario that involves human data submission and processing. Red Angus takes multiple quality-assurance steps to eliminate any potential issues that could arise from incomplete and insufficient sample submission information.

The layout of the DNA order form is designed to avert the matching of calves to multiple sires or dams. However, this situation cannot be avoided when the information provided by the member requires that an animal must be tested to a large, unfiltered group of potential parents. It is <u>only</u> in this scenario, and when the dam sample is <u>not</u> available, that more than one sire can be specifically matched to a calf. Or vice versa with dams and sire.

In the instance that the group of potential sires/dams includes individuals that are related to the true parent of the offspring, then the calf can match to more than one potential parent. This is why the Association focuses on the Most

Probable parental options when testing each offspring sample. Nonetheless, there are instances that arise where a calf sample is submitted and only a large, non-filtered group of parents is provided. If such offspring are registered, the RAAA staff member will first test the animal to the registered parent(s). Should these options fail to produce a match, or if the calf is not registered, then there is no other choice but to test a large group of potential parents against the calf sample. If more than one matching sire is found, these bulls are not reported to the member. The calf results are listed as incomplete and a DNA sample from the dam is requested to resolve the issue. By requesting the dam sample, a trio verification can be completed on the calf, and the issue will virtually always be resolved with a very high degree of accuracy.

When registered animals are submitted to Red Angus for parentage verification or determination, RAAA staff ensure that the pedigree of the animal(s) is updated to match the DNA-based parentage results should they differ from the original pedigree. To determine the typical number of corrections completed by the Association, animals with DNA parentage results and those with pedigree changes were recorded for a recent four-week period (Table 3 on the following page).

It is a routine RAAA practice that all animals with either a DNA result of *Qualified (matching)* or *Excluded (not a match)* are then compared to the recorded pedigree of the animal. In the case that a different and *Qualifying* parent was found via DNA, the pedigree will be corrected to this parent. If the parent on the DNA results is *Excluded* and different from the pedigree, RAAA staff will check the sample to the parent on the pedigree and review sample identification with the member. If the parent on the DNA results is *Excluded* but is listed as the parent in the pedigree, the animal will go on hold (B status) until the pedigree exclusion is resolved.

Throughout a four-week tracking period, animals that completed testing and had pedigree updates (within that same week) were all previously registered without a sire and simply had the *Qualified* sire added to their pedigree by RAAA staff. Therefore, not a single pedigree was changed, only "completed," in the period of evaluation.

The number of retries were evaluated also during our tracking period. A retry is considered to be an animal that finished DNA testing, *Excluded* to a probable parent(s), was placed on hold, then was later resolved and

corrected in the Red Angus database. This process involves retrying the animal to different sire and/or dam options provided by the member. Animals retried to additional parent options are held to the same marker standards and protocols of all other samples. If/When a *Qualified* and probable sire and/or dam is found, the animals pedigree will be corrected.

Table 3: *Pedigree Evaluation by Week*

Week	March 12th	March 19th	March 26th	April 2nd
Animals Tested	308	123	286	150
Animals with Sires Added*	3	16	156	12
% Pedigrees Completed	0.97%	13.0%	54.55%	8.0%
Animals with Sires/Dams Changed (retries)	1	6	3	11
% Pedigrees Corrected (retries)	.32%	4.65%	1.04%	6.83%

*Animals originally registered by the member without a specific sire. These include multi-sire group registrations.

The table above shows that despite being the largest in terms of total animals tested, the first week of evaluation resulted in the fewest sire additions and retries. The three animals that had sires added were animals originally registered without a sire, in this case from three different breeders. The single retry animal was previously sire *Excluded* and upon finding the correct sire, was trio verified and updated in the Red Angus Database.

During the second week of evaluation, 16 individuals had sires added. The results on these 16 animals found all sires *Qualified* with zero (0) exclusions on more than 122 markers. Of the six retries, four of these animals were trio verified and the other two were sire verified with 115 markers. With these results, RAAA staff updated the pedigrees to the DNA *Qualifying* sire for all 22 calves.

The third week of sire additions comprised entirely of an order submitted by one member on their spring-born calves. Of the 156 animals that had sires added to their pedigree, 119 were trio verified. All three retries for that week were originally sire *Excluded* and then trio verified and thus had sires changed per these updated results.

The fourth and final week of tracking included 12 animals having sires added. These animals represented four different members and were all registered to Multi-Sire Groups (MSG) of three to four bulls. Every *Qualified* sire found, for each animal, was included in the animals MSG and therefore was added as the actual sire to all 12 pedigrees. Of the retries completed that week, four were previously sire *Excluded* and then trio verified and updated. One animal was originally sire and dam *Excluded* and then trio verified and had both sire and dam updated. The remaining six animals were previously sire *Excluded.* These animals were then sire *Qualified* per the retry, with 190-200 markers and updated accordingly.

In summary, the RAAA multi-week evaluation shows that 22 percent of DNA testing completed weekly is ultimately utilized by members to determine the sire of registered animals, which can be thought of as "filling in the blank" on the sire side of the pedigree. This 22 percent is the four-week average of the ***Animals with Sires Added*** that is shown in Table 3.

Out of all samples processed, 84 percent produced Qualified results, verifying the initially-submitted pedigree or "filling in the blank" as described above. Another 7 percent of sample results were initially *Excluded.* These will be retried and eventually verified, thereby reducing this small percentage even further. The remaining 9 percent is comprised of animals that have both sire and dam results of that are not on file - the majority of these are foreign animals (registered with another breed association) being used as parents.

Commonly Asked Questions

- **Can more than two sires 'qualify' to the same animal? Can qualified sires change based on trio analysis?**

Yes, in certain scenarios, it is possible that more than one sire will qualify to the same calf based on DNA analysis. Parent verification is based on the comparison of DNA markers between the sire and/or dam and the calf.

The calf receives 50 percent of its DNA from the sire and 50 percent from its dam. It must be understood that the DNA profile of the calf does not always match that of the sire or dam identically. Logically, and as seen in many cases, it will match partially to that of the sire and partially to that of the dam.

For example, one marker (out of all markers from the sample) for each animal may read as follows:

Calf: A/T Sire: **A**/A Dam: T/**T**

When making comparisons to sires alone, they can qualify with either zero or one exclusion - meaning that the compared DNA markers are either identical, or only vary in one place out of a minimum of 78 markers. See Table 1.

Without the availability of a DNA sample from the dam, it is not possible to tell whether a given exclusion is the result of genetic variation between sire and calf (that can be attributed to the dam's DNA), or if that exclusion truly means that the sire does not qualify.

This single-marker example illustrates how two sires can qualify to a calf:

Calf: A/T Sire 1: **A**/A Sire 2: **A**/G

Without the dam sample, there is no way to eliminate either sire as not being the true sire of the calf.

When parentage analysis is also completed for the dam of a calf, it is possible for sires that previously qualified to become excluded. To explain this situation briefly, when 100 percent of the possible genetic makeup of a calf is provided, any variation in the DNA markers between the sire and calf can be confirmed (or negated) as attributable to the dam of that calf.

For the example above, introduction of the dam sample allows Sire 2 to be eliminated and Sire 1 verified as the calf's actual sire.

Calf: A/T Sire 1: **A**/A Sire 2: A/G Dam: T/**T**

If the results of parent verification produce two or more sires who qualify with one exclusion, the provision of a DNA sample from the dam will determine the true sire of the calf.

See appendix for a more detailed and a real-world scenario involving the importance of trio verification in *The Importance of Dam Verification in SNP Parentage Testing: A Case Study.*

- **What are other beef breed association and A.I. company DNA protocols?**

The following cattle associations are known to have DNA protocols similar to, if not identical to, those of the Red Angus Association of America:

American Gelbvieh Association
American Hereford Association
American Simmental Association
North American Limousin Foundation

The same standards have been adopted by A.I. bull studs and are combined with the following requirements before a bull will be accepted as an A.I sire.

1. The bulls must have a DNA genomic profile
2. The bull must be parent verified (both sire and dam).
3. The bull must be tested free for genetic defects.
4.Once in possession of the company/stud, the animal is sampled and re-verified to his parents.

Large Commercial Parentage Testing Field Study Summaries

Eenennaam, A. L. Van, et al. "DNA-Based Paternity Analysis and Genetic Evaluation in a Large, Commercial Cattle Ranch setting." *Journal of Animal Science*, vol. 85, no. 12, 2007, pp. 3159–3169., doi:10.2527/jas.2007-0284.

"New SNP genotyping platforms continue to drive down the cost to generate SNP genotypes, and the future will undoubtedly see the introduction of inexpensive genotyping assays using high resolution SNP parentage panels. This will improve the accuracy of sire assignments and on-farm genetic evaluations and may result in progeny testing becoming an economically viable option for commercial ranchers. This case study illustrated some problems that may be encountered in paternity testing in large commercial herds. Field data are likely to include both missing sires and sires that did not produce any progeny. Low resolution marker panels and large cohorts of potential herd sires are particularly problematic and may result in sire-assignment errors and imprecise genetic evaluations. The frequency of sire mis-assignment can be minimized by using a high-resolution panel or by simple management practices that include dividing large herds into smaller

multiple-sire breeding groups with fewer sires while maintaining the same bull-to-female ratio, genotyping all potential bulls before breeding, sorting bulls into sire groups with divergent genotypes, keeping young bulls in separate breeding groups, and minimizing relatedness among bulls."

Baruch, E., and J. I. Weller. "Estimation of the Number of SNP Genetic Markers Required for Parentage Verification." *Animal Genetics*, vol. 39, no. 5, 2008, pp. 474–479., doi:10.1111/j.1365-2052.2008.01754.x.

"Using likelihood based 'importance-sampling' algorithms, Anderson & Garza (2006) found that 60–100 SNPs may allow accurate pedigree reconstruction, even in situations involving thousands of potential mothers, fathers and offspring for putative mother–father–offspring trios. Of course, pedigree reconstruction, among a number of possible alternatives, is the most difficult situation for parentage confirmation and requires more markers than simple rejection of a putative trio. Considering their lower error rates and much lower costs per genotype, SNPs are clearly replacing microsatellites as the marker of choice for parentage determination and confirmation. None of the previous studies attempted to estimate exclusion probabilities under the requirement of >1 conflict for exclusion. Considering that genotyping even a single pair of individuals for scenario 1 will require approximately 100 genotypes, the requirement of at least two conflicts is justified, even if genotyping error rates are in the range of 0.01–0.004, as most studies show."

Woodward, B.W., and T. Van der Lende. "Single Nucleotide Polymorphisms for Parentage Testing, Individual Identification and Traceability." 18 June 2008, doi:www.cabdirect.org/cabdirect/abstract/20103193088.

"In a large-scale project to evaluate parentage assignment in registered dairy cattle, 6,302 animals were genotyped in 1,639 herds. The IGENITY parentage and identity panel of 99 SNPs used for this project was a subset of those developed by Heaton et al. (2007). More than 85% of the animals had 80 or more successful genotype calls for comparison between sire and progeny; 11.8% had between 61 and 80 genotypes. The power of the SNP panel is evident in the distribution in number of exclusions: 76.2% with 0

exclusions, 5.2% with 1 exclusion, 1.1% with 2 exclusions, and 0.5% with 3. (In contrast, the percentage of samples with 1 or more exclusions genotyped with STRs is much higher.) The misidentification rate ranged from an average of 10.3% in herds with <100 cows to a high of 24.2% in herds with more than 2,000 cows; however, there were herds with a misidentification rate approaching 100%."

Conclusion. Current RAAA parent verification policy is sound, being based on widely-accepted scientific standards and DNA technology used throughout the beef and dairy cattle industry. No approach is perfect or completely foolproof. Human error is always a possibility. However, Red Angus is already benefitting from the Association's use of DNA-based parent verification via a significant reduction in breed-wide pedigree errors. Red Angus genetic evaluations and resulting EPDs directly benefit from these more accurate pedigrees. With additional advances in DNA technology in the years ahead, these benefits will only increase.

Appendix

- RAAA DNA Order Form
- The Importance of Dam Verification in SNP Parentage Testing: A Case Study

DNA Submission Instructions

Step 1: Complete RAAA DNA Order Form on Animal Information Tab

- Fill out Animal and Sample Information.
  - Non-registered calf samples: include all identifiying information (Date-of-Birth(DOB), ID and Sex).
  - Registered animals: include **REG #** and all identifiying information (DOB, ID, and Sex).
  - DNA Kit/Barcode can be found on the back of DNA card. Typically a 10 or 11 digit number.
  - If semen is provided as the DNA sample, the NAAB Semen Code is the DNA Kit/Barcode
- Select the Testing Option(s) for each sample.
  - **Genetic Defect Tests**: Request by writing the defect abbreviation(SEE BELOW) to be tested for.
  - **BVD**: Performed on hair or tissue ONLY. Request by marking X for animal(s) to be tested.
  - **Parentage**: Required for bulls, in order to register calves. Request by marking X for animal(s) to be tested.
  - **GGP**: ANIMAL MUST BE REGISTERED. See test details below. Request by marking X for animal(s) to be tested.
- Enter Potential Parents for samples selected for Parentage or GGP testing.
  - Most Probable Sire (A.I.), Alternative Sire (Clean-up) and Most probable Dam columns must be filled out.
  - If parent is not a RAAA registered animal, provide breed abbreviation with that assoc. registration #.
    Ex: Canadian Angus = CAN########, American Angus = UAN########, Simmental = USM########
  - If multiple sire or dam breeding groups were used please see Step 2.

Step 2: Complete Multi-Sire Breeding Groups Tab (if applicable)

- Create Breeding Group with ALL sires/dams who potentially parented the offspring and name (i.e 1, A, RED).
  - If parent is not a RAAA registered animal, provide breed abbreviation with that assoc. registration #.
    Ex: Canadian Angus = CAN########, American Angus = UAN########, Simmental = USM########
  - For submitted samples that apply, list Breeding Group name on Order Form in place of Alternative Sire etc.

Step 3: Submit Order to RAAA

- Mail completed and **signed** Order Form and Samples to RAAA Office:
  - **Attn: DNA: 18335 E. 103rd Ave, Suite 202 Commerce City, CO 80022**
  - E-mail completed Excel file to DNA@RedAngus.org

Test Pricing

Test Name	Abbrev. to mark on form	Common Name	Added to GGP	Price (each)
Genetic Defects				
Arthrogryposis Multiplex	AM	Curly Calf		**$20**
Contractural Arachnodactyly	CA	Fawn Calf		**$20**
Alpha-Mannisodosis	MA	--		**$20**
Neuropathic Hydrocephalus	NH	Waterhead	$10	**$20**
Osteopetrosis	OS	Marble Bone		**$20**
Developmental Duplication	DD	--	$20	**$25**
Parentage				**$15**
Bovine Viral Diarrhea (BVD) - **Hair or Tissue Sample Only**			$4*	**$7**
*discount price if added to Parentage or Defect test also				
GeneSeek Genomic Profiles (GGP): **Animal Must Be Registered**				
GGP-HD (High Density)				**$90**
Genomic EPD Enhancement, Parentage, OS and MA testing				
GGP-LD (Low Density)				**$48**
Genomic EPD Enhancement, Parentage, OS and MA testing				
GGP-ULD (Ultra-low Density)				**$34**
Genomic EPD Enhancement and Parentage				
Hair Sample - Lab Processing Fee				**$4**

RAAA DNA Order Form

GENESEEK

Name: _____ Member/Acct#: _____

Address: _____ Phone: _____

City: _____ State: _____ ZIP: _____ Fax: _____

**Email: _____ **USED FOR ALL ORDER COMMUNCATION UNLESS OTHERWISE STATED.

Testing must be paid for before samples can be submitted to the lab. Include check, to RAAA or call w/ card. Amount Due :$_____

Expected Tumaround Time: 20 Business Days. May be delayed if delivered samples are low quality and/or order form is not properly completed. Please review "DNA Sample Basics" to ensure a quality sample is submitted. Direct questions to the RAAA National Office. DO NOT contact the lab.

ANIMAL AND SAMPLE INFORMATION							TESTING OPTIONS						POTENTIAL PARENTS	
Animal ID (Tag/Tattoo)	Registration Number(Required for GGP)	DNA Kit Barcode Number / Cane Code	Sample Type(B/H/T)	DOB	Sex (M/F)	Genetic Defect(s) Write in Abbrev. for desired test	BVD	Parentage (On File)	GGP-ULD	GGP-LD	GGP-HD	Most Probable Sire Reg#	Alternative Sire Reg# or Breeding Group	Most Probable Dam Reg#

Terms: Upon submitting samples to the Red Angus Association of America (RAAA), the submitting party conveys ownership of the sample to RAAA. The submitting party agrees and acknowledges that RAAA intends to perform genetic testing on the submitted sample in an effort to describe the animal's genetics. The submitting party further agrees and acknowledges that it assumes all risks that are associated with the genetic testing results and any associated implications. RAAA has the right to make any results determined through the genetic testing publicly available. In the event that the submission of this/these samples results in a parentage exclusion(s) or identification of genetic defect carrier(s), the process through which owners/breeders are notified will follow RAAA Rules and Regulations. The submitting party agrees that all samples are submitted to RAAA in an effort to fulfill the RAAA mission of advancing the quality, reliability, and value of Red Angus and Red Angus influenced seedstock used in the commercial beef industry. Therefore, RAAA shall not be held responsible for damages of any kind, including but not limited to, lost profits, lost value of progeny, lost value of embryos and/or semen, interruption of business that may arise from the publication of genetic testing results. The term 'submitting party' shall mean any RAAA member/customer submitting a DNA sample and test request, as well as any RAAA member/customer submitting a DNA test request for a DNA sample that is already on file with RAAA.

Signature: _____

Effective Dec 15th 2017, Hair samples subject to $4 processing fee + test fee

Multi-Sire OR Dam Breeding Group Information

(Complete for Multi-Sire Pastures ONLY)

If sample is enclosed for sire/dam also list on Order Form and mark Parentage.

Breeding Group: ________

	Association	Reg #	Tattoo	Sample Enclosed?
Sire 1				
Sire 2				
Sire 3				
Sire 4				
Sire 5				
Sire 6				

Breeding Group: ________

	Association	Reg #	Tattoo	Sample Enclosed?
Sire 1				
Sire 2				
Sire 3				
Sire 4				
Sire 5				
Sire 6				

Breeding Group: ________

	Association	Reg #	Tattoo	Sample Enclosed?
Sire 1				
Sire 2				
Sire 3				
Sire 4				
Sire 5				
Sire 6				

Breeding Group: ________

	Association	Reg #	Tattoo	Sample Enclosed?
Sire 1				
Sire 2				
Sire 3				
Sire 4				
Sire 5				
Sire 6				

*This breeding group page can also be utilized for listing multiple dams.

The Importance of Dam Verification in SNP Parentage Testing: A Case Study

By: Fallon Flick, RAAA DNA Programs Coordinator

The significance of parent verifying calves in registered cattle herds cannot be stressed enough. In recent years the Red Angus Association of America has taken action to increase DNA requirements for registering calves with the Association.

The newest of these policies is requiring that all herd sires to have a DNA sample submitted for parentage testing, prior to any of their calves being fully registered with the RAAA. By submitting a DNA sample for a herd bull to undergo parentage testing, not only is his pedigree confirmed - the same possibility of verification for his offspring is also provided. When calves are verified to their sires, it ensures that performance data resulting from those calves is credited to the correct animal, and therefore improves the accuracy of EPDs.

There is no doubt that having DNA on file for all sires will assist in verifying resulting progeny. However, we must not forget about the portion of parent verification that lies with the dam. The only females required to have DNA samples on file with the RAAA are donors that are used for embryo transfer (ET) purposes. Despite this, many producers have been diligently submitting samples on all dams for use in parent verifying calves. This regime is key in determining if calves are twins or have been switched at birth. Most importantly, the availability of a dam sample allows for a complete trio analysis of the calf, sire and dam in question.

Case Study:

A recent DNA submission of 76 Red Angus bulls was analyzed through the RAAA. Of the samples submitted, 75 received data from the lab and were compared to sires and dams provided by the member for parent verification. Upon the completion of the parentage analysis testing, 38 calves were trio verified, 34 calves were sire verified, (dam samples were not on file), and three calves were excluded to their listed sires (dam samples were not on file).

Upon reporting these results to the member, a list of potential sires was provided in effort to find qualifying sires for the three calves that originally

sire excluded. A zero exclusion, qualifying sire was found for all three individuals. However, the member questioned the sire qualifications, and it was recommended that dam samples were submitted for trio analysis on all three calves. The member stated that the qualifying sires should be reflected on the pedigrees of the calves until dam samples were submitted and available for comparison.

The results for these three calves for order 683515 are summarized below.

Original Parentage Analysis Results:

	Barcode	Animal ID	Registration Number	Compared	Matches	Exclusions	Result
Offspring	Z000900034	7316					
Sire			1042135	81	74	7	Excluded
Offspring	Z000900049	7230					
Sire			1607674	110	98	12	Excluded
Offspring	Z000900073	7228					
Sire			3494198	94	90	4	Excluded

The following animals were not found for parentage analysis.

	Id Type	Ids
Dams	Registration Number	1322854, 1605055, 1605169

Original Reported Results:

Order #	Animal ID	Barcode	Sire 1	Sire 1 Result	Dam	Dam Result
683515	7228	Z000900073	3494198	Excluded	1322854	Not on File
683515	7230	Z000900049	1607674	Excluded	1605055	Not on File
683515	7316	Z000900034	1042135	Excluded	1605169	Not on File

Retried Parentage Analysis Results:

	Barcode	Animal ID	Registration Number	Compared	Matches	Exclusions	Result
Offspring	Z000900034	7316					
Sire		5504C	3494198	96	96	0	Qualified
Offspring	Z000900049	7230					
Sire		Y379	1441761	109	109	0	Qualified
Offspring	Z000900073	7228					
Sire			1042135	79	79	0	Qualified

The following animals were not found for parentage analysis.

	Id Type	Ids
Dams	Registration Number	1322854, 1605055, 1605169

Corrected Results per Sires Found:

Order #	Animal ID	Barcode	Sire 1	Sire 1 Result	Dam	Dam Result
683515	7228	Z000900073	1042135	Qualified	1322854	Not on File
683515	7230	Z000900049	1441761	Qualified	1605055	Not on File
683515	7316	Z000900034	3494198	Qualified	1605169	Not on File

With further evaluation of the situation the member chose to submit samples on the dams of the three calves in question, as well as new samples on the calves. The purpose of the second sample submission was to eliminate any possible errors in sample identification or data reporting and verify the qualifying sires found in the original order.

The re-submission of a calf sample when the DNA results are questioned by the member is always recommended by the RAAA DNA team. The RAAA encourages calf resubmission and, strongly suggests a dam sample be submitted for trio comparison. The reason for this is to eliminate any sample identification or contamination issues that may have altered the calf sample and to definitively verify the calf not only to a sire but also to the dam.

Upon completion of testing on the dam samples, the data was used in the parentage analysis tool along with the original calf samples submitted in order 683515. The results for the original calf samples from order 683515 and dam samples from order 687694 are summarized below.

Parentage Analysis Results:

	Barcode	Animal ID	Registration Number	Compared	Matches	Exclusions	Result
Offspring	Z000900034	7316					
Dam			1605169	105	102	3	Excluded
Offspring	Z000900049	7230					
Dam			1605055	110	110	0	Qualified
Offspring	Z000900073	7228					
Dam			1322854	103	96	7	Excluded

This "dam only" analysis above shows that two of the three calf samples do not match the expected or submitted dams. In turn, two of the three trio comparisons of the calf, the sire found to qualify, and new dam sample were excluded. The trio comparison results are summarized below.

Trio Analysis Results:

	Barcode	Animal ID	Registration Number	Compared	Matches	Exclusions	Result
Offspring	Z000900034	7316	3732725				
Dam			1605169	105	102	3	Excluded
Sire			3494198	96	96	0	Qualified
Trio				96	86	10	Excluded
Offspring	Z000900049	7230	3732701				
Dam			1605055	110	110	0	Qualified
Sire			1441761	109	109	0	Qualified
Trio				109	109	0	Qualified
Offspring	Z000900073	7228	3733011				
Dam			1322854	103	96	7	Excluded
Sire			1042135	79	79	0	Qualified
Trio				79	64	15	Excluded

With the data analyzed thus far, the 7316 and 7228 individuals would need to be re-evaluated with the following in mind.

1. The possibility of a calf-dam switch given date of birth and member records. The RAAA DNA team would suggest retrying the calf samples to other dams to eliminate or prove the switch. In the case that a probable and qualifying trio analysis was found, the calf would be parent verified. In the case that neither a qualifying trio is found or is unlikely, then the second point would be addressed.
2. The possibility that the original calf sample was contaminated or misidentified resulting in incorrect parentage analysis results. The RAAA DNA team would suggest that a tissue sample on the calf was taken and re-compared in order to eliminate any sample identification issues that could have caused unexpected or implausible results.

The uniqueness of this case is that a second calf sample was concurrently submitted and available for analysis at the time of the submitted dam sample (order 687694). The second calf samples were submitted and analyzed to the original trio that was listed in order 683515.

The results for these three calves in order 687694 are summarized below.

	Barcode	Animal ID	Registration Number	Compared	Matches	Exclusions	Result
Offspring	Z000900094	7316					
Dam			1605169	113	113	0	Qualified
Sire			1042135	88	88	0	Qualified
Trio				88	88	0	Qualified
Offspring	Z000900093	7230					
Dam			1605055	113	113	0	Qualified
Sire			1607674	114	102	12	Excluded
Trio				113	96	17	Excluded
Offspring	Z000900092	7228					
Dam			1322854	113	113	0	Qualified
Sire			3494198	104	104	0	Qualified
Trio				103	103	0	Qualified

The analysis of the second order brings to light the possibility of an original sample issue for calves 7316 and 7228. The second sample for these calves generated entirely different results than the first. Seeing that each sample not only verified to a questionable sire but excluded to the expected

dam, it is likely that the samples were incorrectly identified to each individual. In order to confirm this, the original sample for animal 7316 was compared to sire 3494198 and dam 1322854 and the original sample for animal 7228 was compared to the sire 1042135 and dam 1605169. The results of this analysis is shown below.

	Barcode	Animal ID	Registration Number	Compared	Matches	Exclusions	Result
Offspring	Z000900034						
Dam			1322854	105	105	0	Qualified
Sire			3494198	96	96	0	Qualified
Trio				96	96	0	Qualified
Offspring	Z000900073						
Dam			1605169	103	103	0	Qualified
Sire			1042135	79	79	0	Qualified
Trio				79	79	0	Qualified

This analysis confirms the possible sample misidentification with two of the three samples originally submitted. The assumption could be that the Z000900034 sample was actually taken from the 7228 calf and that the Z000900073 sample was taken from the 7316 calf. In the case that a second sample was not available to compare, and the first samples were analyzed with the above result it could also be possible that the dams of **7316** and **7228** swapped calves at birth. If the member could confirm that birth dates, pasture rotations (etc.) to eliminate the possibility of a calf switch, and prove that a sample identification issue was plausible, then the samples could be re-identified to the respective individual. If the member could not eliminate a possible calf switch, then a second sample would be requested to determine the possibility of incorrectly identified samples.

The second submission of the 7230 animal was compared to the qualifying sire found in order 683515. The summary of this analysis is below.

	Barcode	Animal ID	Registration Number	Compared	Matches	Exclusions	Result
Offspring	Z000900093	7230					
Dam			1605055	113	113	0	Qualified
Sire			1441761	113	113	0	Qualified
Trio				112	112	0	Qualified

The second sample submission matches the first sample results when compared as a trio to the dam expected and qualifying sire found. Since the original sire listed has been excluded, the sire difference could be due to an incorrectly recorded bull at the time of A.I., an assumed A.I. mating due to DOB that is now proven to be an early, natural calf or the result of another bull that was unknowingly exposed to the dam. Regardless of the situation, the trio comparison seen above verifies the calf to both sire and dam definitively.

The recent change in the RAAA DNA requirements, has established the ability to confirm lineage on animals of current and future generations. While this added availability can help solve the paternal portion of an animals' pedigree, we must not forget the importance of all animals and samples involved. The results of all DNA testing should always be evaluated with perspicacity. It has been proven that simple mistakes with sample identification alone can lead to troubling and debatable results. Despite this, DNA technology and trio verifications hold true as an extremely useful tool. This example only outlines one instance of a potential error in DNA collection and submission. It also reinforces the importance of sire and dam verifying calves.

Through the described analysis and testing the RAAA is confident in reporting and accepting the following data as DNA results for the respective animals.

Animal ID	Barcode	Sire	Sire Result	Dam	Dam Result
7728	Z000900092	3494198	Qualified	1322854	Qualified
7730	Z000900093	1441761	Qualified	1605055	Qualified
7316	Z000900094	1042135	Qualified	1605169	Qualified