DNA Testing
Alpha S1 Casein

Agenda

- Milk constituents and composition
- DNA, chromosomes and genes
- Alpha S1 Casein in dairy goats research
- ADGA testing procedure
- Results interpretation (using, predicting and submitting)
- Case study
- Live demo
- Resources
- Questions?

Milk Constituents

- Key components are water, protein, fat, lactose, vitamins and minerals
- Composition can be affected by various factors (species, breed, diet, stage of lactation, health, genetic factors, etc.)

Table 1: Major constituents of milk. Adapted from Jensen *et al.* (1991).

Compartment	Major constituents
Aqueous phase	Solution ash, Ca, Mg, PO_4, Na, K, Cl, CO_2, whey proteins (α-lactalbumin, β-lactoglobulin), lactoferrin, immunoglobulin, lysozyme, serum albumin, lactose, oligosaccharides, amino acids, urea, B-vitamins, ascorbic acid.
Colloidal dispersion	Caseins (α, β, κ), Ca, PO_4
Emulsion	Fat globules, triacylglycerols, fat soluble vitamins, cholesterol esters
Fat globule membrane	Milk fat globule membrane proteins, phospholipids, enzymes, trace minerals
Cells	Macrophages, neutrophils, lymphocytes, epithelial cells, leukocytes

Milk Composition Compare

- Composition Comparison

Table 2: Gross milk composition across different species. Adapted from Park *et al.* (2007) and USDA National Nutrient Database for Standard Reference. g and kJ are per 100g milk.

Nutrient	Unit	Goat	Sheep	Cow	Human
Water	g	87.03	80.7	88.13	87.5
Energy	kJ	288	451	255	291
Fat	g	4.14	7	3.25	4.38
Protein	g	3.56	5.98	3.15	1.03
Carbohydrate	g	4.45	5.36	4.8	6.89
Casein	%	2.4	4.2	2.6	0.4
Lactose	%	4.1	4.9	4.7	6.9

Milk Protein

- Protein
  - 80% of protein in milk is casein (4 types)
  - Casein characteristics and amount account for:
    - Cheese yield
    - Curd behavior (firmness and draining)
    - Ageing behavior
    - Milk allergies

Table 6: Milk protein composition across species. * % of total casein ** g per 100g milk. Total casein in goat milk represents around 80% of total milk protein. Table adapted from (Akers, 2002, Inglingstad et al., 2010, Farrell Jr et al., 2006)

Protein component	Goat	Cow	Human
Casein *			
αs1-casein	5-17	38	Trace
αs2-casein	6-20	10	Trace
β-casein	50	40	70
κ-casein	15	12	27
Whey protein **			
α-lactalbumin	0.12	0.12	0.18
β-lactoglobulin	0.22	0.33	Absent
Total Protein	2.7	3.3	0.9

Milk - Casein

- Average diameter of goat casein particles (micelles) are larger than those in cow milk
- Relationship between size of casein particles and curd firmness and set speed
  - Larger size equal decrease in firmness and set speed
  - Explains the delicate curd texture for goat milk
- Four types of casein (French study 1992)

CASEIN TYPE	Average relative contents in COW MILK %	GOAT MILK %	EXTREME VALUES IN GOAT MILK %
alpha S1	38	5.6	0-20
alpha S2	12	19.2	10-30
beta	36	54.8	43-68
kappa	14	20.4	15-29
Total casein content	*100%*	*100%*	

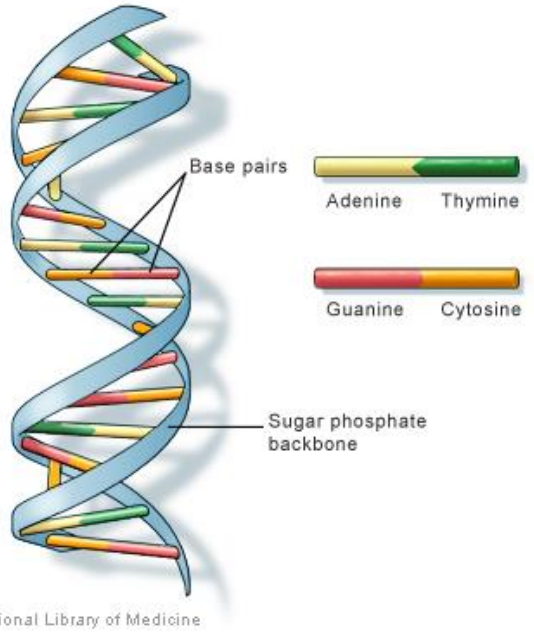
Genetics Definitions

- DNA
- Chromosome
- Gene

Definition - DNA

- DNA (deoxyribonucleic acid)

DNA is a double helix formed by base pairs attached to a sugar-phosphate backbone.

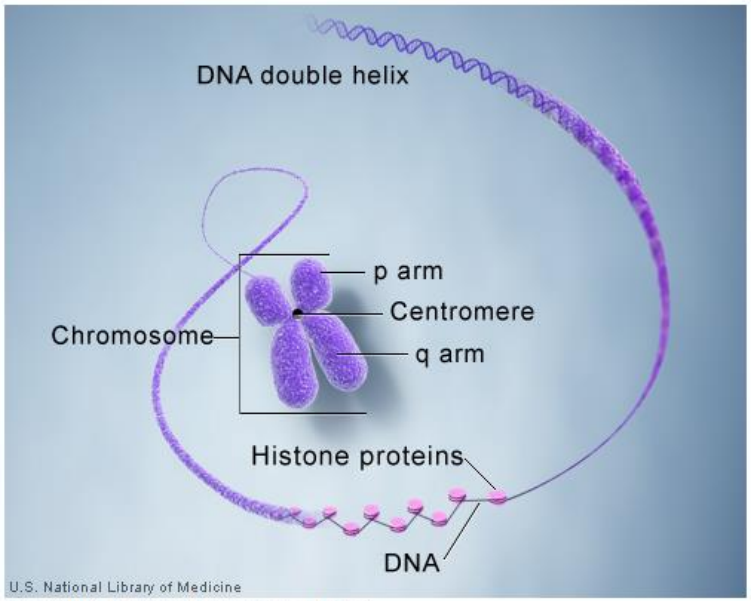


Credit: U.S. National Library of Medicine

Definition -Chromosome

- Individual segments of DNA. Contained in cell nucleus and can be seen during cell division.
- Goats have 30 chromosome pairs (60 total)
- Each chromosome has a unique shape

DNA and histone proteins are packaged into structures called chromoso

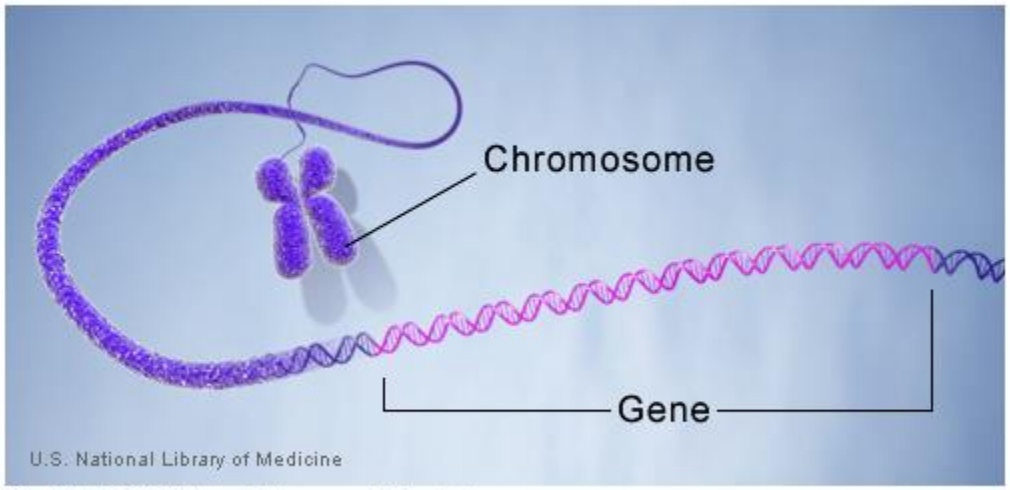


Credit: U.S. National Library of Medicine

Definition - Gene

- Basic unit of heredity made up of DNA
- Two copies of each gene, one from each parent
- Vary in size (length)

Genes are made up of DNA. Each chromosome contains many genes.



Credit: U.S. National Library of Medicine

Alpha S1 Casein - Variants

- The gene linked to Alpha S1 Casein (CSN1S1) is known
- Allele
  - One of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome.
- Variants (alleles) are grouped by:
  - Strong (3.5 g/l) – A, B, C, H, L
  - Intermediate (1.1 g/l) – E, I
  - Weak (.45 g/l) – F, D, G
  - Null (0 g/l) – 01, 02, N

Alpha S1 Casein Evolution

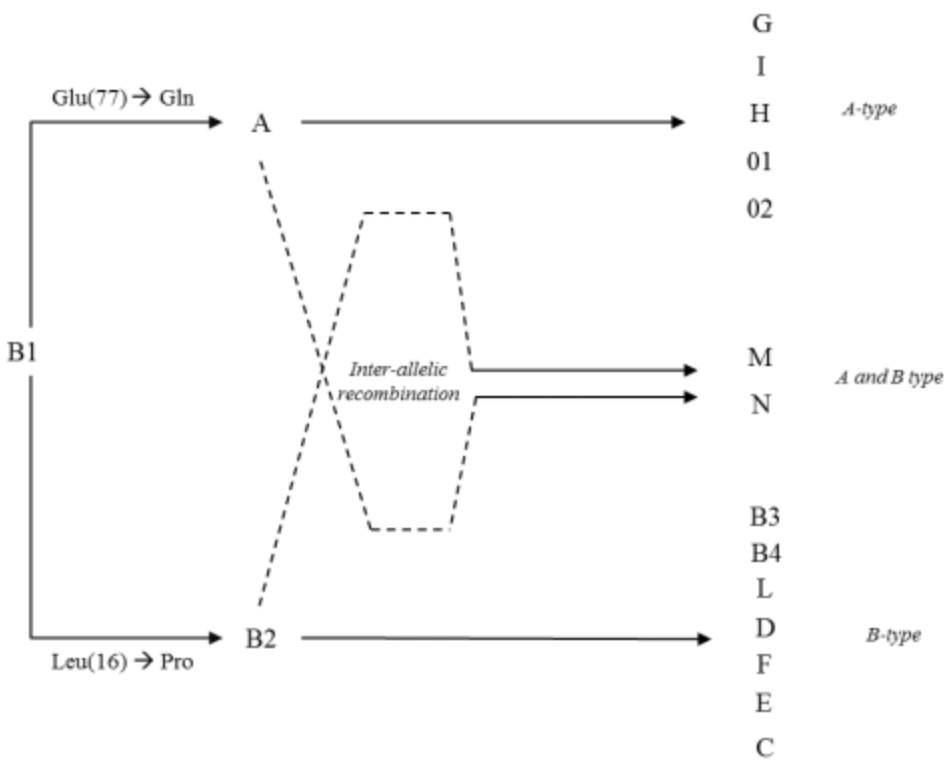


Figure 6: Schematic of proposed evolution of CSN1S1 alleles. Major amino acid changes between the A-type and B- type lineages are indicated. Adapted from Martin and Leroux (2000).

Alpha S1 Casein Change

Table 2
Effect of the α_{s1} *casein* genotype on protein content (g/kg) for a progeny-tested male population and estimated separately for the Saanen and Alpine breeds

Genotype group	α_{s1} *casein* genotype	Saanen	Alpine
Strong	*BC*	3.7	*
	AB	2.5	1.7
	BB	2.4	*
	AA	2.2	2.5
	AC	1.6	*
	CC	*	*
Intermediate	*CE*	1.0	*
	AE	1.0	1.0
	BF	0.6	0.9
	CF	0.6	*
	BE	0.5	1.1
	AF	0.5	0.7
	AO	*	*
	BO	*	*
	CO	*	*
Weak	*EE*	−0.7	0.2
	EF	−0.9	−0.4
	EO	*	*
	FF	*	*

* Effect was not estimated because no animals were recorded

Alpha S1 Casein Prevalence

From Caprine Alpha S1 Casein FB group database

Genotype	Alpine		Nigerian		Lamancha		Nubian		Saanen	
A/A	2	1.29	17	33.33	34	34.34	37	20.44	0	0.00
A/B	0	0.00	22	43.14	7	7.07	98	54.14	0	0.00
A/E	22	14.19	0	0.00	23	23.23	0	0.00	0	0.00
A/F	19	12.26	0	0.00	21	21.21	0	0.00	0	0.00
A/N	1	0.65	0	0.00	0	0.00	9	4.97	0	0.00
A/O1	1	0.65	0	0.00	0	0.00	0	0.00	0	0.00
B/B	4	2.58	10	19.61	1	1.01	20	11.05	9	7.03
B/E	5	3.23	2	3.92	0	0.00	1	0.55	39	30.47
B/F	14	9.03	0	0.00	1	1.01	4	2.21	16	12.50
B/N	0	0.00	0	0.00	0	0.00	12	6.63	0	0.00
E/E	13	8.39	0	0.00	1	1.01	0	0.00	23	17.97
E/F	39	25.16	0	0.00	5	5.05	0	0.00	24	18.75
E/O1	1	0.65	0	0.00	0	0.00	0	0.00	0	0.00
F/F	29	18.71	0	0.00	6	6.06	0	0.00	17	13.28
F/O1	5	3.23	0	0.00	0	0.00	0	0.00	0	0.00
Sample Count	155		51		99		181		128	

How to test

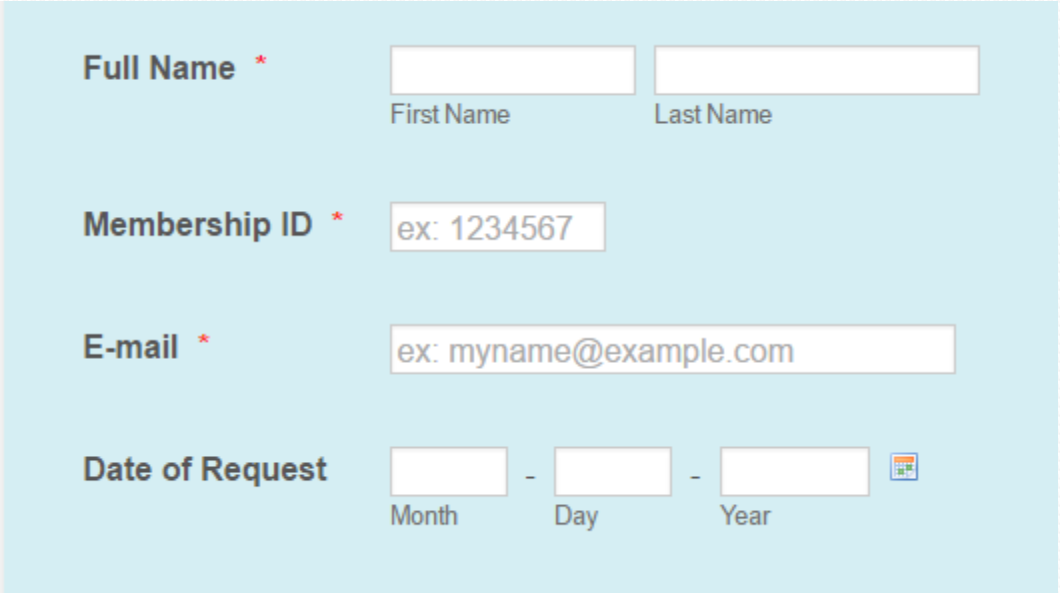
1. Request and pay for test
2. Receive test paperwork
3. Take sample
4. Send sample to lab
5. Receive results

Requesting a DNA Test

- You can request a test online at DNA Test Request Form
- The required info is:
  - Membership information
  - Payment
  - Animal ID and requested tests
  - Comments (optional)

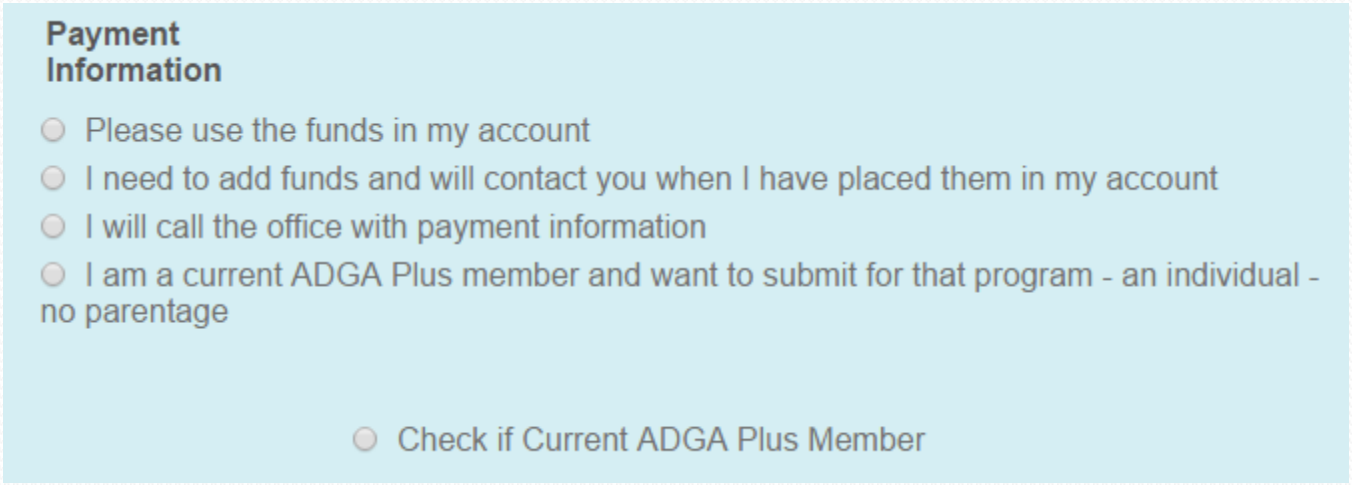
Request – Membership Info

- Required information is fairly self explanatory
- Fill out completely



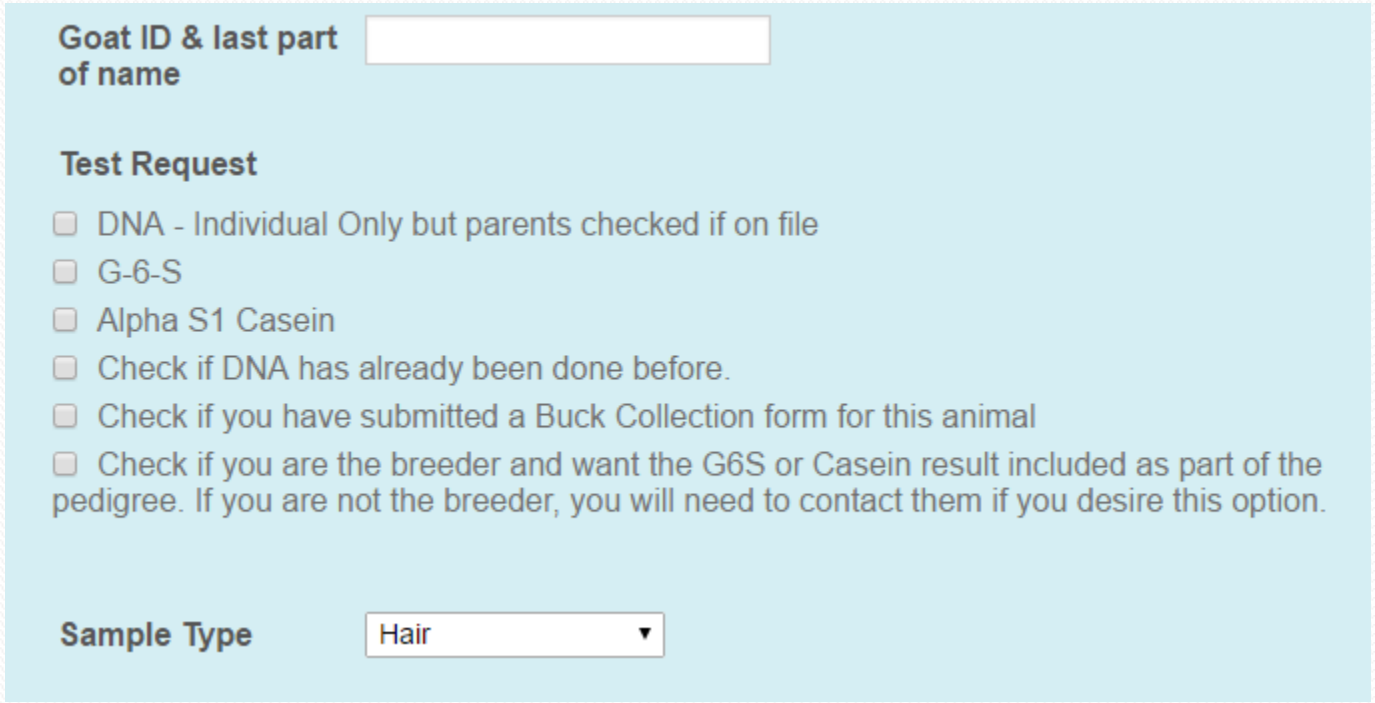
Request – Payment

- Check how you would like to pay for testing
- The first option is easiest (taking funds from your account)
- ADGA Plus members get discounted prices



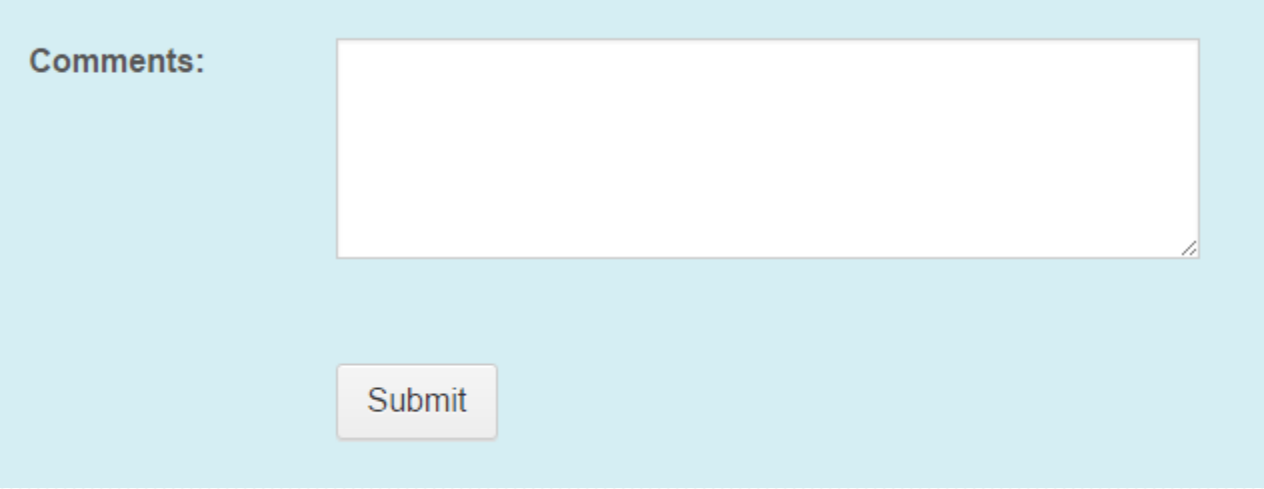
Request – Animal ID and Tests

- If registered, registration number and last part of name (ex. A1693170 – Amelie)
- If unregistered, RE/LE DOB Sex Breed (ex. GDM/H40 4/1/2016 Doe Alpine)
- Select tests
- Select sample type (hair, blood or semen)



Request – Comment and Submit

- Add comments for special handling or questions
- When all information is complete, press Submit and the form will be sent
- You will get a notification via email that the form has been received



Receive Test Paperwork

- After ADGA has processed your submission, they will mail a testing packet
- The packet contains:
  - Cover letter with instructions
  - VGL (Veterinary Genetics Lab) mailing label
  - Animal "ADGA DNA Record of Identification" (triplicate) with sample label

Sample

- Hair samples are recommended
  - Any age can be done easily
  - Guard hairs with **root bulbs** (20-40) are required
  - Place hair in a new, clean 4x9 envelope and seal
- Semen can also be used
  - Remove desired straw from tank and allow to air thaw
  - Place in a small Ziploc bag
- Complete sampling instructions are on each Animal ID form and can also be found online at: http://adga.org/performance-programs/dna-sample-collection/

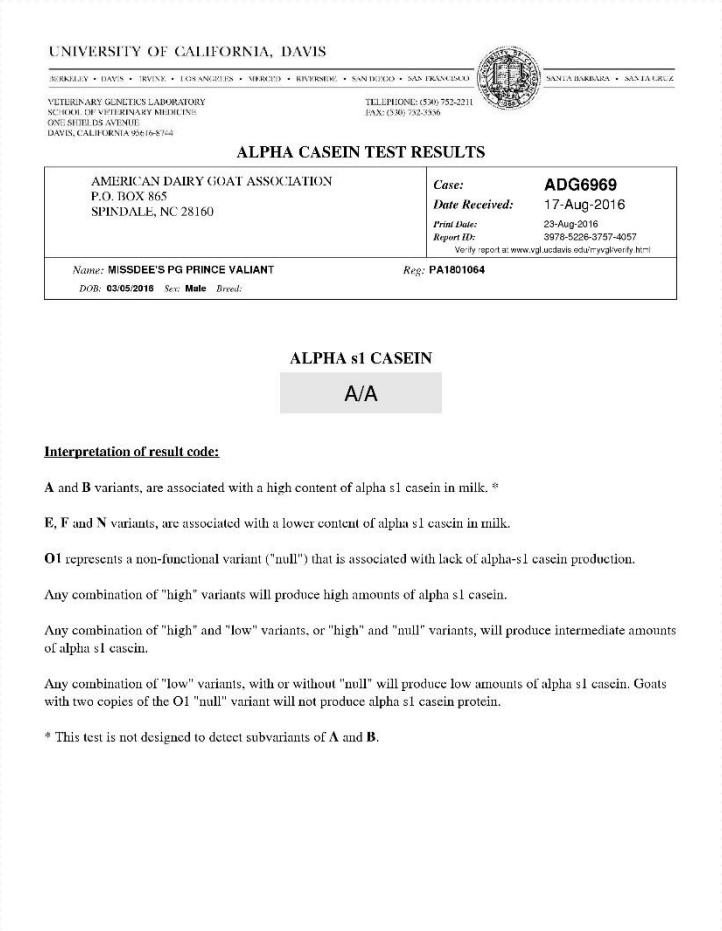
Send Sample to Lab

- Affix sample label to hair sample envelope
- Sign and date Animal DNA test ID forms
  - One for VGL
  - One for ADGA
  - One for owner (signature not necessary)
- Place one copy of test form and sample in large envelope (10 x 13 manila works well) and affix VGL mailing label
- Mail it off to VGL with proper postage
- Mail the ADGA copy to ADGA

Receiving Results

- Usually get results back about 2 weeks after you send sample off
  - Mail to VGL
  - Testing at VGL
  - Results to ADGA
  - Results to submitter
- You will receive results as a PDF attachment from ADGA
- PDF file contains certificates of test results

Results Certificate



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ALPHA CASEIN TEST RESULTS

AMERICAN DAIRY GOAT ASSOCIATION P.O. BOX 865 SPINDALE, NC 28160	*Case:* **ADG6969** *Date Received:* 17-Aug-2016 *Print Date:* 23-Aug-2016 *Report ID:* 3978-5226-3757-4057 Verify report at: www.vgl.ucdavis.edu/myvglverify.html

Name: **MISSDEE'S PG PRINCE VALIANT** *Reg:* **PA1801064**

DOB: **03/05/2016** *Sex:* **Male** *Breed:*

ALPHA s1 CASEIN

A/A

Interpretation of result code:

A and **B** variants, are associated with a high content of alpha s1 casein in milk. *

E, F and **N** variants, are associated with a lower content of alpha s1 casein in milk.

O1 represents a non-functional variant ("null") that is associated with lack of alpha-s1 casein production.

Any combination of "high" variants will produce high amounts of alpha s1 casein.

Any combination of "high" and "low" variants, or "high" and "null" variants, will produce intermediate amounts of alpha s1 casein.

Any combination of "low" variants, with or without "null" will produce low amounts of alpha s1 casein. Goats with two copies of the O1 "null" variant will not produce alpha s1 casein protein.

* This test is not designed to detect subvariants of **A** and **B**.

You have results. Now what?

- Interpretation
- Use
  - With production test results
  - Breeding decisions
- Predicting results
- Submit results to the Alpha S1 Casein database

Interpretation of Results

- Genotype expression
  - Strong (A/A, B/B, A/B)
  - Intermediate (A/E, B/E, A/F, B/F, E/E)
  - Weak 1 (A/N, A/O1, B/N, B/O1)
  - Weak 2 (E/F, F/F, E/N, E/O1, F/N, F/O1)
  - Null (N/N, O1/O1)
- There is no good or bad. It's informational.
  - For cheese making, strong genotype is preferred
  - For casein reduction, weak or null genotype is preferred
  - More work needs to done quantifying results

Using Results

- With production test results
  - Most labs will test milk samples for fat%, protein% and SNF
  - See how this correlates to alpha s1 casein results
- In breeding programs
  - If you are looking to increase cheese yield, try to use strong genotype sires and dams
  - Different breeds require different tactics
    - Swiss breeds, weak on strong genotypes would require generations of testing and culling
    - Lamancha, Nubian and Nigerian would require culling of intermediate, weak and null alleles. Much easier to maintain strong genotype

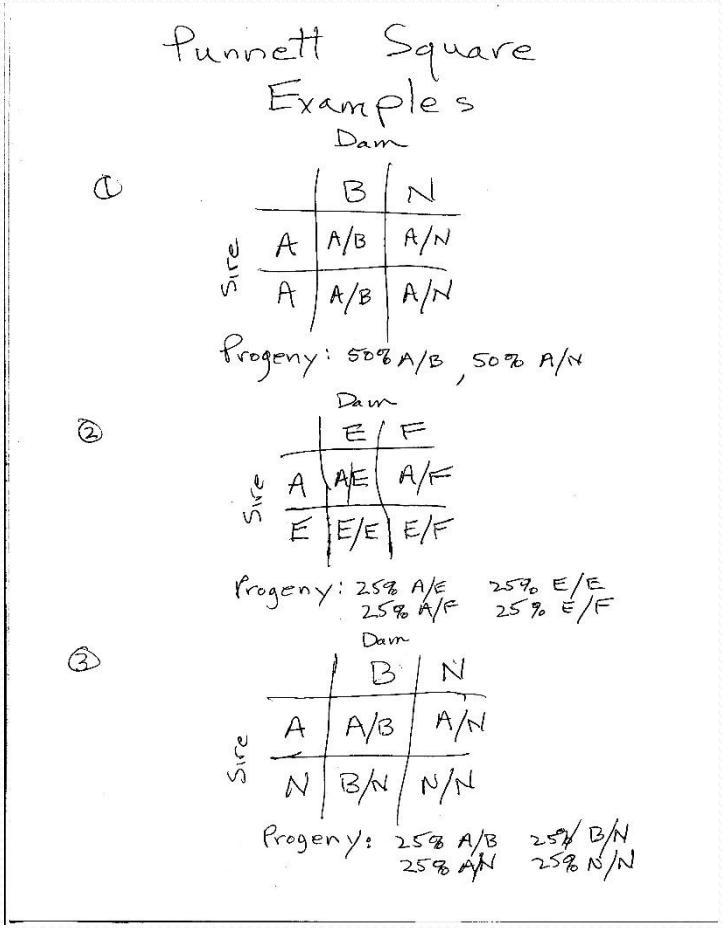
Predicting Results

- If you have sire and dam alpha s1 casein result
  - Use a Punnett Square to determine expected genotypes and probability
  - Use the Punnett Square calculator at:

    http://www.silverfallchinchilla.com/genetics/PunnetCalculator.asp

- If you have either the sire or dam, but not both
  - Can sometimes infer the missing result
  - Multiple progeny results sometimes make this easier

Prediction Examples



Submitting Results

- To ADGA
  - If you checked the box to have results included in pedigrees, your results will be public
  - Search at https://adga.org/casein-results/
- To the Caprine Alpha S1 Casein FaceBook group
  - Forward your casein results to geneder@wvi.com
  - They will be checked against existing pedigree records and hand entered
  - A searchable list is available at: Alpha S1 Casein Google Sheet

Case Study – Personal Experience

- Started Alpha S1 Casein testing in 2016
  - To date, all live bucks, does and collected AI sires have been tested
  - 63 total
- Have been on DHIR owner sampler since 2012
- Switched to DRMS 2016 and have been using PCDART for herd management
  - Added alpha s1 casein results as a user defined field
  - Created groups to quantify components of intermediate and weak genotypes
  - Created custom reports to track groups on test days

Case Study – Personal Experience

- Unique herd situation
  - All purebred French Alpine
  - Have worked with the same lines for many years
  - All milking does fed the same and freshen within a month or two
  - Eliminates factors due to breed and management differences
  - Can more readily compare different alpha s1 casein genotypes as they relate to milk and component production
- Run a custom test day report to compare different genotypic groups
  - Started doing this in October 2016
    - Intermediate group is consistently higher in fat%, Prot% and SNF%
  - Looking forward to tracking a whole lactation
  - Might add extra groups as needed

Case Study – Missdee's Alpines

Index	Barn Name	AS1C	Calv Date	Lct No.	Ref Age Yr-Mo	Days In Milk	Curr T.D. Milk	Curr T.D. %Fat	Curr T.D. %Prt	Curr T.D. %SNF	Fat Prot Ratio	Cur T D MUN
37	DANCER	EE	03/10/16	4	04-07	239	12.5	3.9	3.2	8.2	1.2	22
38	FLORA	FF	03/06/16	4	04-07	243	11.2	3.3	3.1	8.2	1.1	18
45	AMELIE	FF	03/13/16	2	02-07	236	9.9	3.1	3.3	8.5	0.9	21
39	BAI	EF	03/03/16	4	05-06	246	8.1	3.1	3.3	8.3	0.9	21
7	EVIE	EF	03/16/16	2	02-07	233	6.8	4.0	3.4	8.0	1.2	25
23	BEATRIX	EF	03/14/16	2	02-07	235	6.1	3.6	3.4	8.4	1.1	22
31	LAYLA	FF	04/26/16	1	01-07	192	4.5	3.5	3.3	8.2	1.1	21
36	SLANT	FF	04/27/16	1	01-07	191	4.4	1.9	3.0	7.8	0.6	19
26	VICKY	EF	04/18/16	1	01-07	200	4.3	3.6	3.8	8.7	0.9	26
29	ELECTRA	FF	03/18/16	2	02-07	231	4.2	3.9	3.5	9.0	1.1	24
12	CALISTA	EF	03/05/16	8	08-06	244	3.8	4.0	3.3	8.4	1.2	17
*****	11 cow average for major group 01 (			1,		1) Group						*****
				3		226	6.9	3.5	3.3	8.3	1.1	21
49	CHARLIZ	AE	04/20/16	1	01-07	198	8.4	4.0	3.6	8.8	1.1	19
18	PEPPER	AF	03/13/16	2	03-05	236	8.2	3.8	3.6	8.4	1.1	18
30	CHLOE	AE	03/15/16	1	02-07	234	7.0	4.5	3.4	8.4	1.3	18
22	LILAC	AF	04/20/16	1	01-07	198	6.6	5.4	4.0	9.0	1.4	22
28	LILY	AF	04/04/16	2	03-07	214	6.5	3.6	3.7	8.7	1.0	23
27	BONNY	AE	04/17/16	1	01-07	201	6.4	4.0	3.6	8.6	1.1	20
43	VELVET	AF	03/05/16	5	05-07	244	6.2	3.1	3.7	8.9	0.8	20
47	CELINE	AE	02/18/16	1	02-05	260	6.2	3.3	3.8	8.9	0.9	14
4	CORA	BF	03/30/16	1	02-06	219	5.3	3.4	3.2	8.3	1.1	19
41	DELILA	AE	03/12/16	2	02-05	237	4.3	4.8	4.1	9.4	1.2	19
42	CATALNA	AF	03/08/16	5	05-06	203	Dry					
*****	11 cow average for major group 02 (			2,		2) Group						*****
				2		222	6.5	4.0	3.7	8.7	1.1	19

Live Demo

- Punnett Square calculator
- Searchable alpha s1 casein results sheet
- PCDART integration with alpha s1 casein results

Resources

- ADGA Genetics website
  - http://adgagenetics.org
- American Dairy Goat Association website
  - http://adga.org
- Caprine Alpha S1 Casein FaceBook Group files section
  - https://www.facebook.com/groups/1753213781623522/files/
- Including α_{s1} *casein* gene information in genomic evaluations of French dairy goats
  - https://gsejournal.biomedcentral.com/articles/10.1186/s12711-016-0233-x
- Punnett Square Calculator
  - http://www.silverfallchinchilla.com/genetics/PunnetCalculator.asp
- Public group alpha s1 casein database
  - https://docs.google.com/spreadsheets/d/1_E76ePkoAiHojNswIKLGFqbNoyiP75KtJ65nBguat9Y/edit?usp=sharing

Questions?