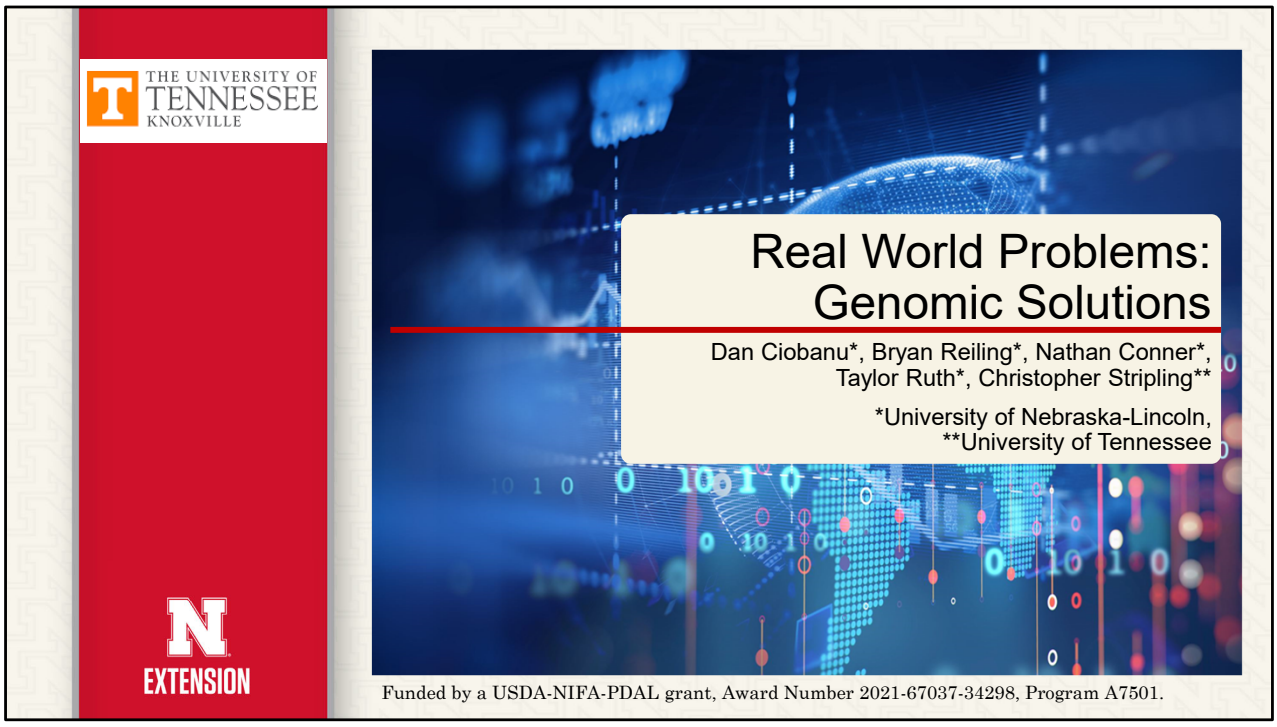
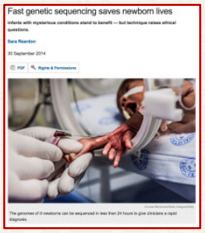


Real world problems; genomic solutions.

NEXT SLIDE

What problems might be solved with genomics?

- Human Health
  - Transform study, diagnosis, and treatment of cancer
  - Identify cause of rare genetic diseases
  - Enhance prenatal health assessments
  - Tailor medications to match genetic profile
  - Eventually … eliminate or cure disease with gene-editing technology



https://www.genome.gov/dna-day/15-ways

What problems might be solved with genomics?

Regarding human health, genomics has transformed (CLICK) the study, diagnosis, and treatment of cancer, (CLICK) has helped doctors identify the cause of rare genetic diseases, (CLICK) has enhanced prenatal health assessments, (CLICK) allows doctors to tailer medications to match genetic profiles, or in the case of cancer, doctors can monitor genetic changes in a patient's tumor to determine their treatment. This is known as precision medicine. (CLICK) and eventually, it is hoped that with gene editing technology, genomics might help eliminate or cure some diseases.

NEXT SLIDE

What problems might be solved with genomics?

- Protection & Security
  - Improve DNA-based forensic analyses

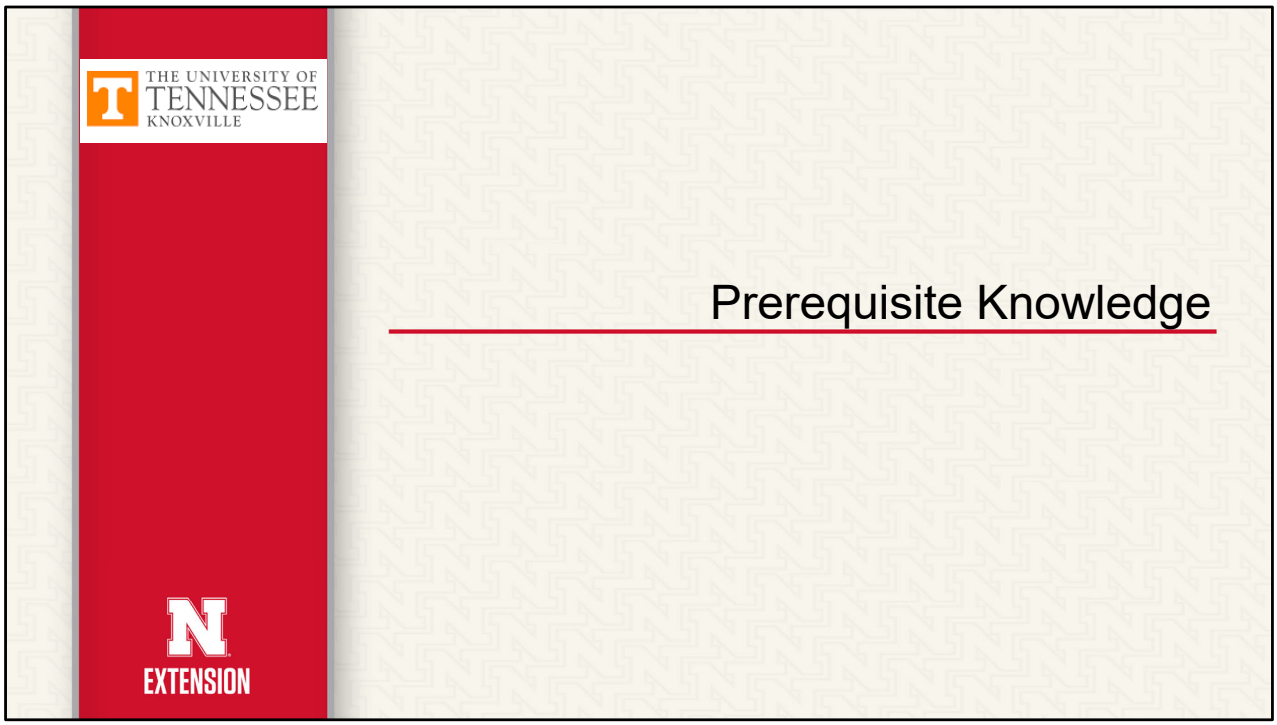
Credit: KTS Designs/shutterstock.com

- World Hunger
  - Empower farmers to improve the food supply

https://www.genome.gov/dna-day/15-ways

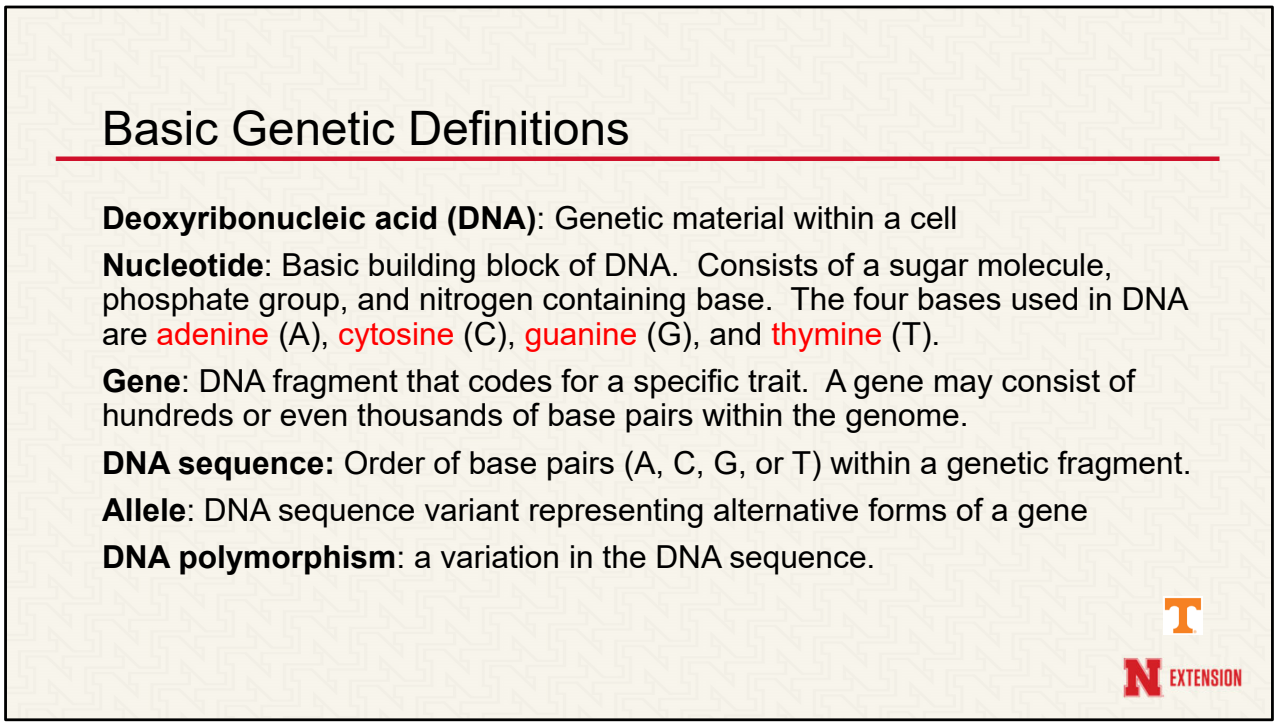
Genomics is helping the police through improved DNA-based forensic analyses, (CLICK) and genomics is empowering farmers and ranchers to improve the food supply. (CLICK), Meet Selz-Pralle Aftershock 3918. In 2017, this cow of superior genetics produced a world record 78,170 lbs of milk. That's nearly 10,000 gallons; more than a tanker truck can hold!

NEXT SLIDE



Prerequisite knowledge, for this lesson and inquiry-based learning activity.

NEXT SLIDE



Some basic genetic definitions

Click -- **Deoxyribonucleic acid (DNA)**: Genetic material within a cell

Click -- **Nucleotide**: Basic building block of DNA. Consists of a sugar molecule, phosphate group, and nitrogen containing base. The four bases used in DNA are adenine (A), cytosine (C), guanine (G), and thymine (T).

Click -- **Gene**: DNA fragment that codes for a specific trait. A gene may consist of hundreds or even thousands of base pairs within the genome.

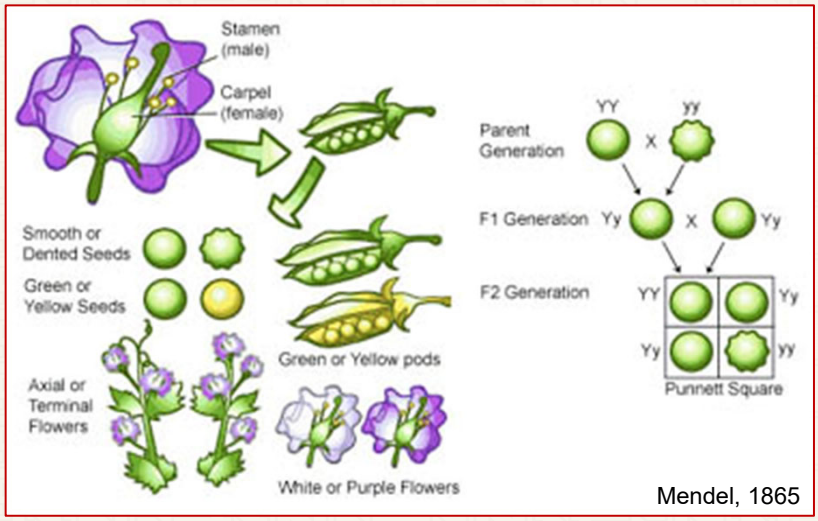
Click -- **DNA sequence:** Order of base pairs (A, C, G, or T) within a genetic fragment.

Click -- **Allele**: DNA sequence variant representing alternative forms of a gene

Click -- **DNA polymorphism**: a variation in the DNA sequence.

NEXT SLIDE

Basic Principles of Mendelian Genetics



I'm sure everyone has heard of Gregor Mendel, the father of genetics, who in 1865 established the laws of dominance and ressiveness with his experimentation with garden peas.

NEXT SLIDE

Single Gene Effects: Complete Dominance

Basic Genetics

- Example -- Hair color
  - B = Black (dominant)
  - b = Red (recessive)

Offspring inherit 1 allele from each parent for each gene

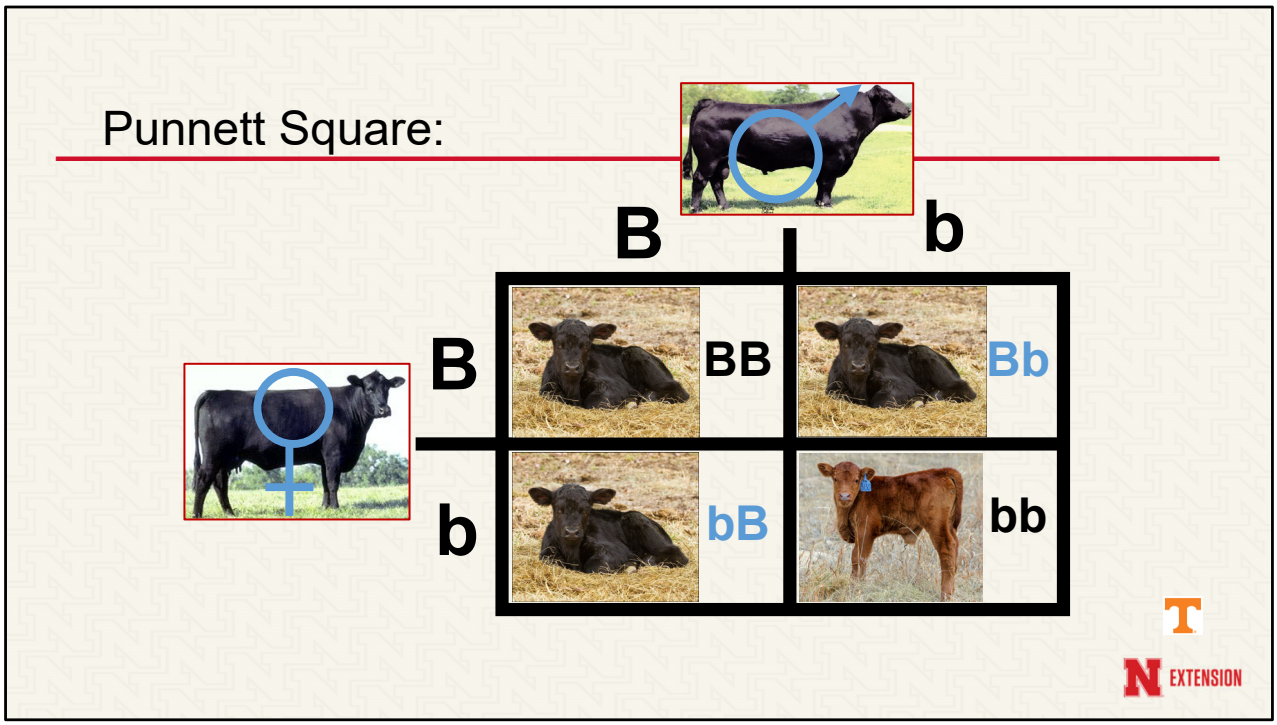
"BB" or "Bb"

"bb"

2 phenotypes
3 genotypes

Reviewing this concept with a more modern example using beef cattle, let's look at (CLICK) hair color. Black hair color is dominant, and red hair color is recessive. And because hair color is controlled by a single gene pair and the offspring will always (CLICK) inherit 1 allele from each parent for each gene, we effectively have (CLICK) two possible phenotypes, black and red, and 3 different genotypes.

NEXT SLIDE



Using a Punnett Square (CLICK), we can determine the probability of genotypic outcomes from the mating of two individuals. What is the probability of a red calf being born from the mating of two heterozygous, black parents (CLICK)? As you know, the offspring receive one allele from the sire and one allele from the dam. Thus, they could receive both dominant alleles, Big B, Big B (CLICK), and this calf would be homozygous black. However, they might receive the dominant black allele from one parent, combined with the recessive red allele from the other parent (CLICK). Half the offspring will have a heterozygous genotype. Finally, the offspring might receive a recessive red allele from each parent (CLICK), and this individual would be homozygous recessive, red. From this mating, on average, one quarter of the calves will be red.

I know that was relatively simple, and you likely learned how to use the Punnett Square in elementary school, but the principle illustrated by the simple Punnett Square is relevant to modern animal breeding decisions.

NEXT SLIDE

Genomics

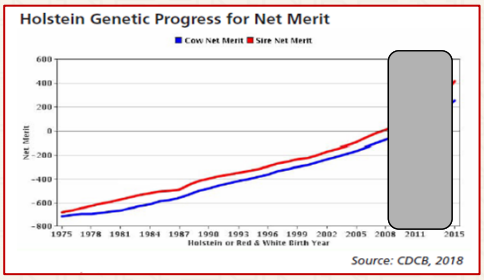
- Genomes sequenced
  - Humans: 1990-2003
  - Since … several animal & plant species sequenced
- Important component of modern animal & plant genetic improvement methods
  - Accurate genetic predications available at birth
  - As need for progeny testing is reduced, younger breeding stock can be used confidently to enhance genetic progress.

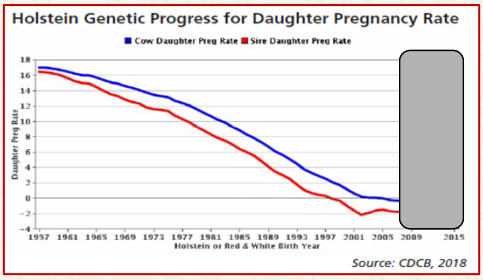
In the mid-1980s, geneticists believed that if the genome could be sequenced, it would potentially unlock genetic mysteries and open doors to possibilities for which we had never dreamt. Well, in 1990, scientists from across the world with backing by the U.S. government, sought to tackle what some believed might be the most daunting task ever undertaken …. to sequence the billions of nucleotides present within the (CLICK) human genome! This task was took 13 years, billions of dollars, and was the effort of hundreds of scientists. Yet today, the technology has improved so much that we have since sequenced (CLICK) the genome of several animal and plant species.

Today (CLICK), genomics is an important component of modern animal and plant breeding improvement methods for genomics provide us with accurate genetic predictions upon birth, and because the need for progeny testing is reduced, younger breeding stock can be used confidently to enhance genetic progress.

NEXT SLIDE

Genetic Evaluation: Pre- vs. Post-Genomics





- Pre-genomics
  - ↑ lifetime Net Merit
  - But ↓ daughter pregnancy rates
- Post-genomics (since 2009)
  - ↑↑ lifetime Net Merit
  - And ↑ daughter pregnancy rates

Holstein Foundation, March 2018
Understanding Genetics and the Sire Summaries

A genetic evaluation example, pre- vs. post-genomics. Usage of genomics to identify elite individuals within the dairy industry came to fruition in 2009. Prior to the use of genomics (CLICK), producers sought to increase lifetime net merit which is primarily a function of milk production. However, as producers selected for increased milk production, pregnancy rates continued to decrease as shown in the chart on the right. Since 2009 (CLICK), after incorporation and usage of genomics to identify genetically elite individuals, not only has lifetime net merit increased at an even faster rate, but daughter pregnancy rates have also increased!

NEXT SLIDE

Merchandising Genomics

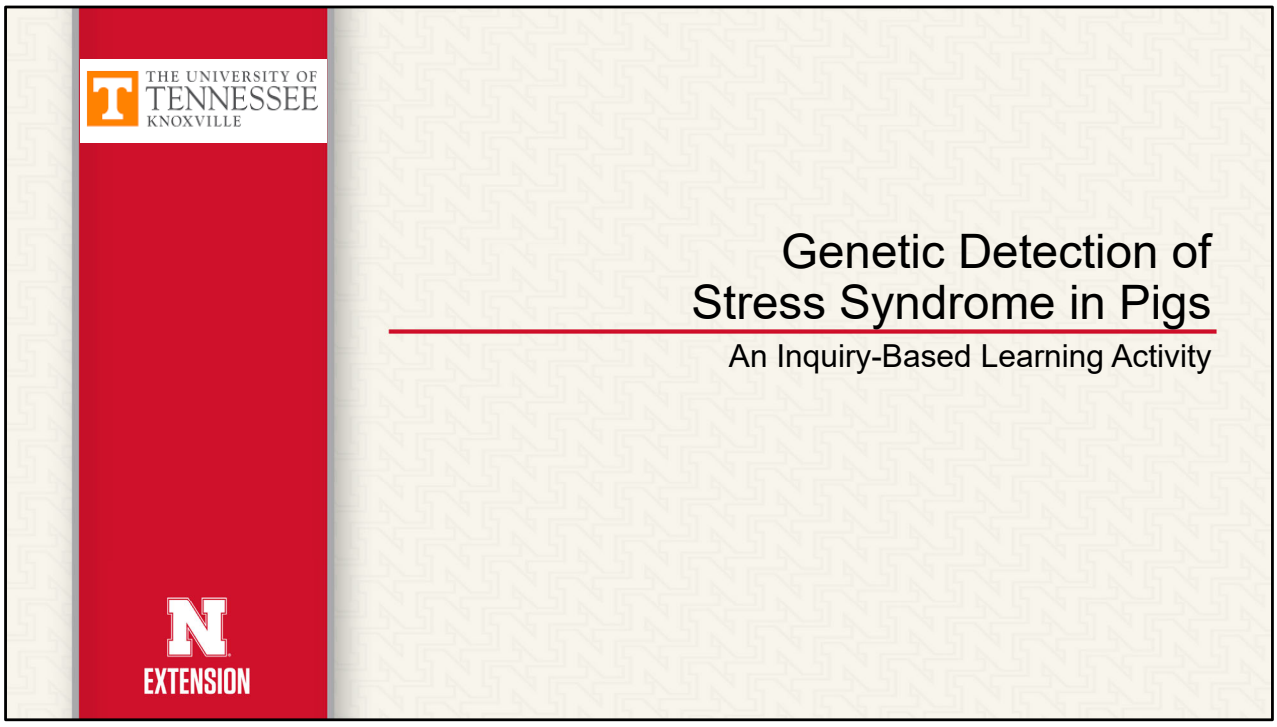
The "value" of genomics

- Meet Jedi Gigi
  - 2-month-old Holstein heifer
  - from Australia
  - Sold for $251,000
    - In January, 2017
  - To be imported to U.S.

It's payday: Callum Moscript and Declan Patten, of Warragul, with Lightning Ridge-CMD Jedi Gigi after she was sold for $251,000. Picture: Andy Rogers

What might be the value of genomics in farm animal production? Well (CLICK), meet Jedi Gigi. She was a 2-month-old Holstein heifer from Australia who sold for more than a quarter million dollars because of her genomic profile! From her, the buyers hope to extract eggs and embryos with superior genetics.

NEXT SLIDE

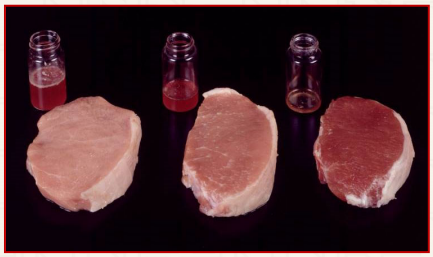


The inquiry-based learning activity associated with this module is related to Genetic Detection of Stress Syndrome in pigs.

NEXT SLIDE

Background Information

- What is Porcine Stress Syndrome (PSS)?
  - During a stressful event, pigs who have PSS may exhibit hyperthermia, open mouth breathing, skin blotching, muscle rigidity, loss of mobility, and even death.
  - Pigs with PSS may also produce a less desirable meat product that is pale, soft, and exudative (PSE)



EXTENSION

So, what is porcine stress syndrome or PSS?

CLICK – During a stressful event, such as handling & moving, transportation, or even showing at the fair, pigs who have porcine stress syndrome may exhibit hyperthermia, open mouth breathing, skin blotching, muscle rigidity, loss of mobility, and it can be fatal.

CLICK – Stress susceptible pigs may also produce a less desirable meat product that is extremely pale, could be almost white in color, that is very soft, and exudative. Exudative means that the muscle cannot hold water. And if it can't retain water, it will be very dry when cooked.

Overall, this stress syndrome was and is not good for any aspect of the industry.

NEXT SLIDE

Background Information

- How did PSS originate?
  - Ideally, we want pigs that are …
    - Fast-growing
    - Heavy-muscled & Lean

  - At some point, there was a single-point mutation
    - One nucleotide was exchanged for another.
    - The resulting recessive allele → more growth!
    - As continued to select for more growth & muscle,
      - the recessive allele became more prominent

  - The sinister downside
    - Homozygous recessive → PSS & PSE

How did this stress syndrome originate?

CLICK – Ideally, we want pigs that are fast-growing, heavy muscled, and lean. Those types of pigs are good for the producer, and they provide the desired product that most consumers want.

But, as producers and geneticists continued to select for faster growing and heavier muscled pigs (CLICK), at some point there was a single-point mutation. What that means is that one nucleotide (adenine, A, cytosine, C, guanine, G, or thymine, T was exchanged for another. Think about it, this is one nucleotide in a genome that contains billions. Anyway, this nucleotide exchange generated an allele or alternative form of a gene that functioned to generate even more growth. Hey, that's a good thing. Then, as we continued to select for more growth and muscle, this recessive allele became more prominent within the population.

CLICK – Unbeknownst to us at the time, however, was that this recessive allele that promoted increased growth and muscle had a sinister downside, and that was that these pigs would quickly show signs of stress and produced PSE pork.

NEXT SLIDE

Background Information

- Connection between PSS & Molecular Genetics
  - Discovered recessive allele on the Ryanodine receptor 1 (RYR1) gene responsible for PSS (i.e., the "stress gene")
  - Back to that "1 in a billion" mutation
    - At a specific loci on the gene, one base replaced another
      - Cytosine (C) codes for stress resistance (dominant)
      - Thymine (T) replaced Cytosine (C); T codes for stress susceptibility (recessive)
    - CC = stress resistant
    - CT = stress resistant carrier
    - TT = stress susceptible
- The Good News

What is the connection between Porcine Stress Syndrome and molecular genetics?

CLICK -- A guy by the name of Fujii, in 1991 discovered the recessive allele on the Ryanodine receptor 1 gene responsible for porcine stress syndrome. Today, it is more commonly referred to as the "stress gene".

CLICK – Now, back to that "1 in a billion" mutation. At a specific loci or location on the gene, one base replaced another. Cytosine which had been at this location, codes for stress resistance, and that is a dominant trait. Thymine, however, replaced Cytosine with this single point mutation known as a polymorphism. Thymine codes for stress susceptibility, which is recessive. The difference in the two alleles is essentially that single base pair.

CLICK – Thus, knowing that the offspring will receive one allele from the parent and one allele from the other parent, if they have CC at that location, the animal is homozygous dominant for stress resistance. A CT genotype means that the animal should be phenotypically stress resistant, but they will be carriers for stress susceptibility. If they have two T's, then those pigs are homozygous recessive for stress susceptibility, and they would be considered to be stress positive pigs.

CLICK -- The good news is that trough genomics and associated planned breeding programs, like what you'll do as part of this inquiry-based learning activity, the PSS allele has effectively been removed from most modern genetic lines of pigs.

NEXT SLIDE

Objectives

Students will be introduced to practical applications of molecular genetics and bioinformatics in animal breeding.

- Students will learn how to use genetic testing for detecting the cause of stress susceptibility in swine.
- Students will analyze results and identify genetic solutions to limit the effects of stress susceptibility in a swine herd.

Objectives of this inquiry-based learning activity is that you will be introduced to practical applications of genetics and bioinformatics in animal breeding.

CLICK – You will learn how to use genetic testing for detecting the cause of stress susceptibility in swine, and then (CLICK) will analyze results and identify genetic solutions to limit the effects of stress susceptibility in a swine herd.

NEXT SLIDE

IBL Activity, Part 1

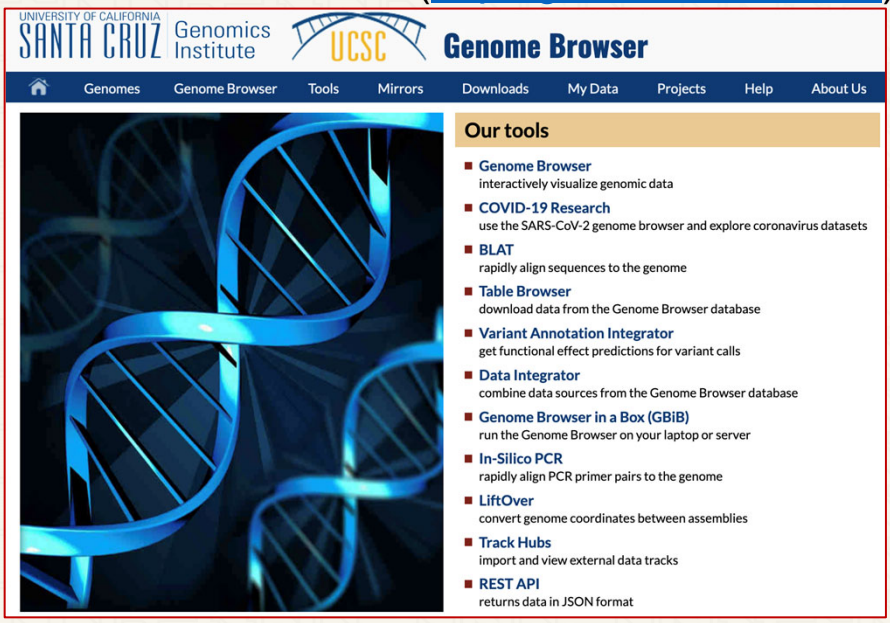
1) Assign the PSS RYR1 genotype to T.J. Tabasco
   a) Google it! Who is T.J. Tabasco?
2) Log into the UCSC Genome Browser
   a) www.genome.ucsc.edu

For part 1 of this activity, you will assign the PSS RYR1 genotype (that's the formal identification of the stress gene) to T.J. Tabasco. Who is T.J. Tabasco, you ask? Google it and write down what you learn on the associated worksheet.

CLICK -- Next, log into the University of California – Santa Cruz, genome browser.

NEXT SLIDE

UCSC Genome browser (http://genome.ucsc.edu/)



Your browser should take you to a site that looks like this. We will be using the Genome Browser, but before selecting that link, I wish to provide an analogy on how the genome browser works.

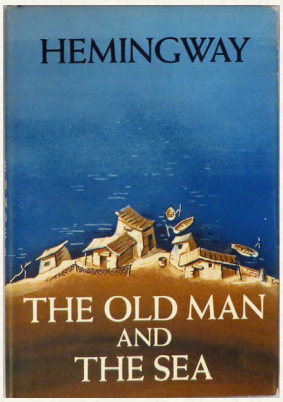
NEXT SLIDE

UCSC Genome browser: How does it work?

- It is like fast finding a sentence (DNA sequence) or a page number (chromosome and nucleotide positions) in a book (genome).

"Every day is a new day. It is better to be lucky. But I would rather be exact. Then when luck comes you are ready."

Ernest Hemingway,
The Old Man and the Sea

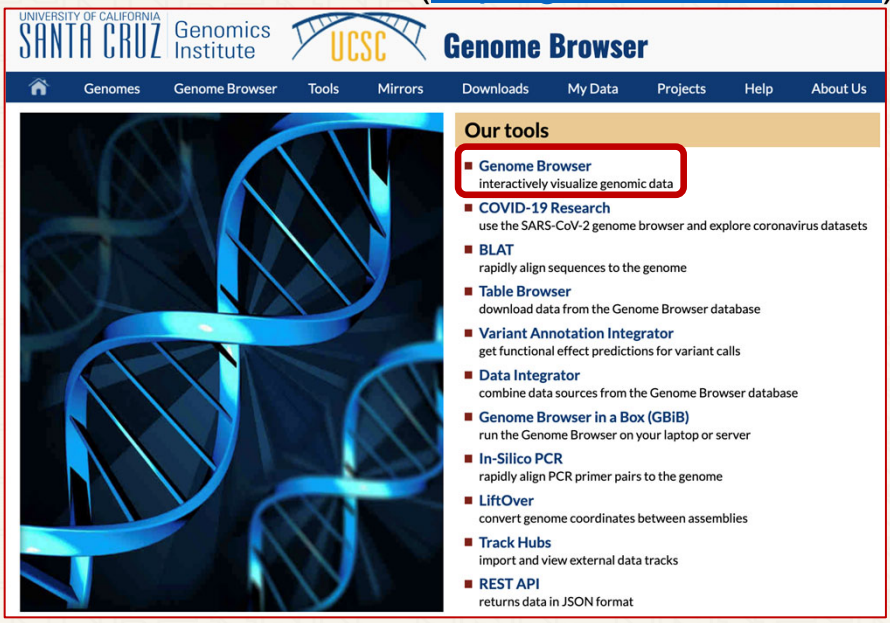


Pick a book, any book. Ok, I'll pick a book (CLICK), Ernest Hemingway's "The Old Man and the Sea". Now, if I were to challenge you to find a specific quote (CLICK) within this book, it would likely take you hours.

CLICK – The genome browser is effectively a "search engine" that functions to find a specific sentence or DNA sequence within a very large book, the genome.

NEXT SLIDE

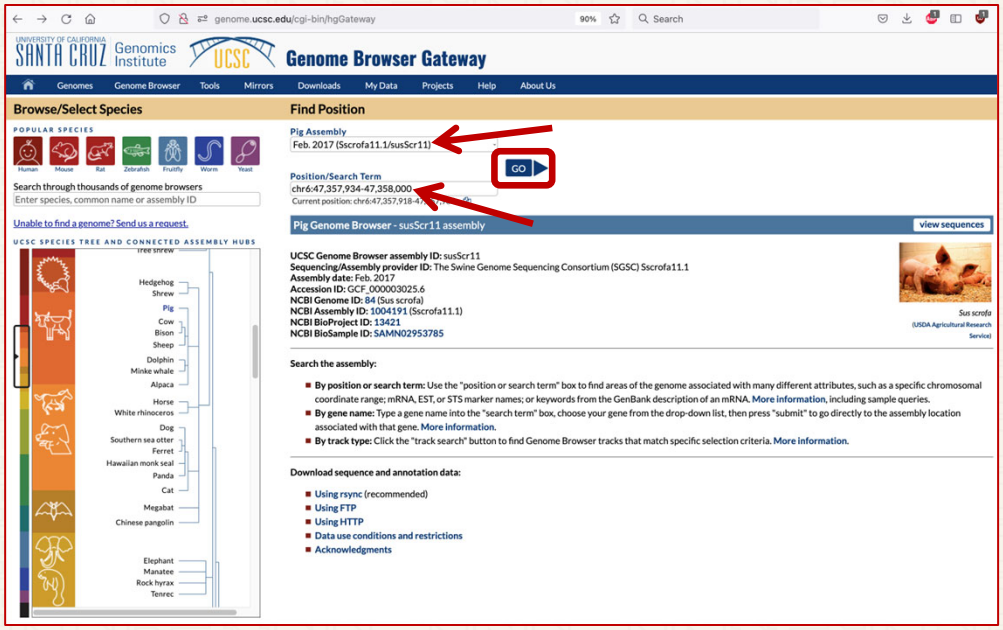
UCSC Genome browser (http://genome.ucsc.edu/)



Back to your computer, on this page, select (CLICK) the first link available to you, the genome browser.

NEXT SLIDE

Browsing the reference swine genome for the PSS RYR1 genotype



On this page that should appear, under Pig Assembly (CLICK), select Feb. 2017 from the dropdown menu. This is the latest information available regarding the swine genome.

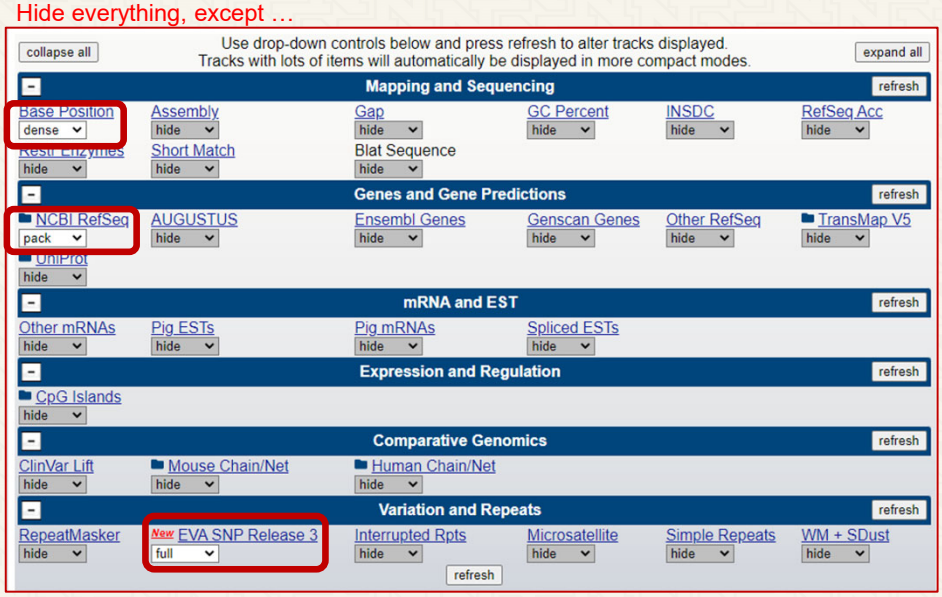
Under the Position/Search Term (CLICK), type in Chr6:47,357,934-47,358,000, or merely copy and paste from your electronic instructions.

Once those two items are in place, (CLICK), click on the “Go” button.

NEXT SLIDE

UCSC Genome browser: Display setup

Hide everything, except …

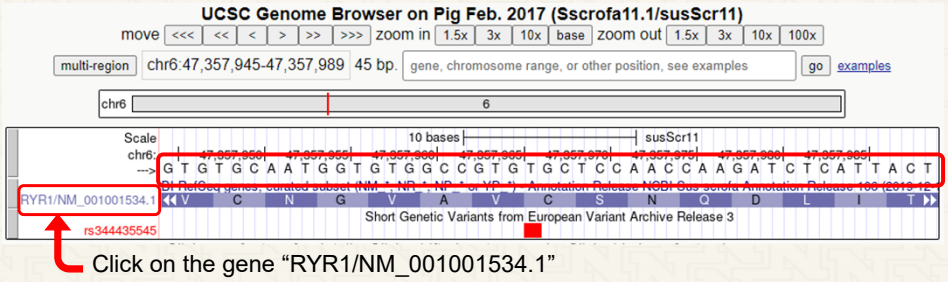


It's possible that you may need to clean up the display slightly to remove some excess "clutter" that we don't need. If needed, scroll down until you see this display set-up. We're simply going to HIDE everything except (CLICK) those three items that are identified on the screen. For base position, select "dense" from the dropdown menu. For NCBI Reference Sequence, select "pack", and for EVA SNP Release 3 at the bottom, select full. Then, click refresh.

NEXT SLIDE

Results

- Did T.J. Tabasco possess the DNA polymorphism (rs344435545)?
- What nucleotide is associated with this DNA polymorphism?
- Was T.J. Tabasco a carrier for stress susceptibility?



Click on the gene "RYR1/NM_001001534.1"

This output, which is located at the top of the page, shows the nucleotide sequence of T.J. Tabasco, our reference pig within the database, at a particular location (chromosome and base pair numbers) that we input.

You'll notice a couple of things. First, you should see a small red box. This red box functions to identify DNA polymorphisms. What is a polymorphism? That's where we had that single-point mutation where one base replaced another. Everyone of those that has ever been identified within the genome is given a name; that's the rs344435545 number. This particular polymorphism is associated with stress susceptibility. So, yes, T.J. Tabasco possesses this DNA polymorphism for stress susceptibility.

CLICK – What specific nucleotide is associated with this DNA polymorphism? For this strand of DNA, the browser provides us with the nucleotide sequence (CLICK). Now, identify the nucleotide directly associated with the polymorphism or red box. It's a T, for thymine.

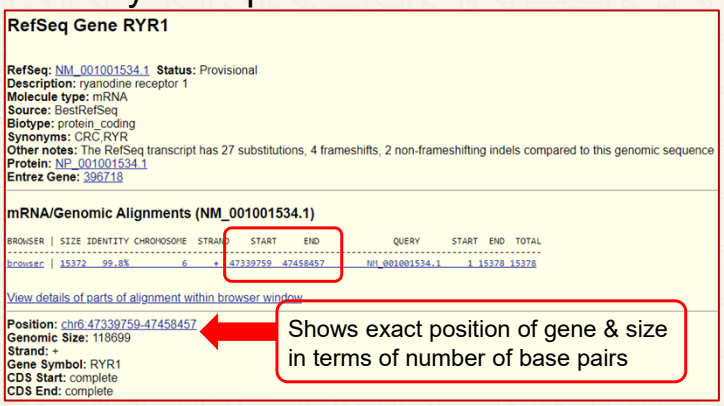
CLICK – So, was T.J. Tabasco a carrier for stress susceptibility? Yes, because of the T at this location.

Next, click on the gene itself (CLICK) identified as RYR1 ….

NEXT SLIDE

Results, cont.

- At what position is this gene located?
- How many base pairs are associated with this gene?

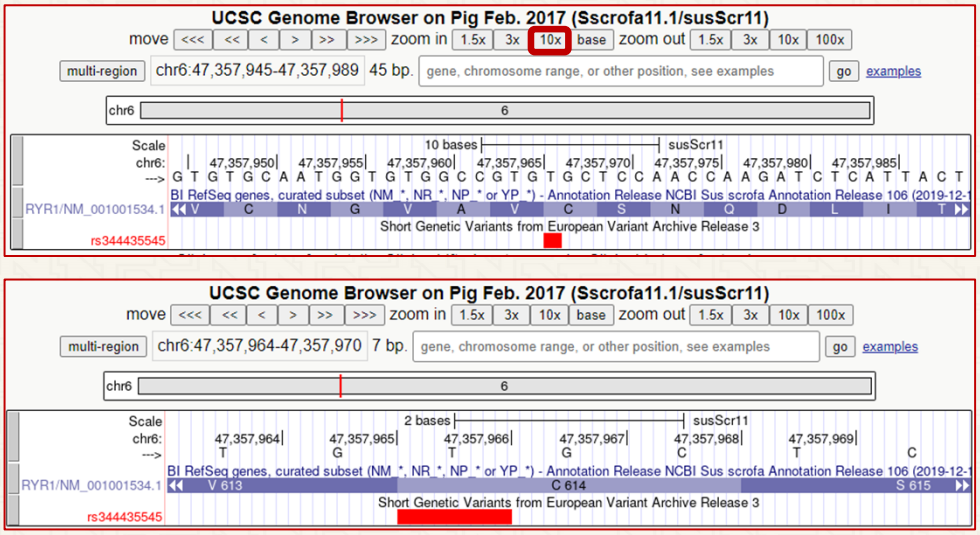


This resulting output provide additional information regarding the specific gene. It shows (CLICK) the exact position of this gene and size in terms of number of base pairs. It is located on Chromosome 6, starts at base pair number 47,339,759 and ends at base pair number 47,458,457. This gene possesses nearly 119,000 base pairs. And to think, it was merely the switch of one base pair that causes the porcine stress syndrome!

On your screen, to return to the previous page, simply click the back arrow.

NEXT SLIDE

Zoom IN

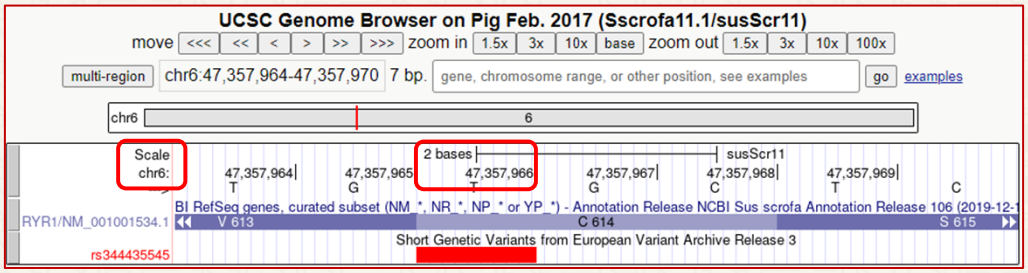


To get more detail, one can “zoom in” on the genome. Simply click (CLICK) on the 10X associated with “zoom-in” and you should see the magnified output (CLICK).

NEXT SLIDE

Zoom IN

- What is the exact location of the DNA polymorphism on the swine genome that causes stress susceptibility, PSS, and PSE?
- Google it! How many chromosomes are in the swine genome?
- Describe the overall enormity and complexity of the genome.



On this magnified output, you can identify the exact location of the DNA polymorphism on the swine genome that causes stress susceptibility, PSS, and PSE. That would be (CLICK) Chromosome 6, base pair 47,357,966.

CLICK -- I want you to “Google it” again – How many chromosomes are associated with the swine genome?

CLICK – Now knowing how many chromosomes are associated with the swine genome, and given what you now know about the numbers of base pairs involved with a single gene, describe the overall enormity and complexity of the genome?

NEXT SLIDE

IBL Activity, Part 2. An Animal Breeding Problem

- PSS symptoms observed when pigs transported between farms
- Breeding stock: 2 boars and 8 sows.
- Sequencing: DNA from all pigs was sent for sequencing.
- DNA sequences surrounding potential "stress gene" polymorphism obtained for all pigs

Now, let's apply this information to an animal breeding problem. A farmer noticed that some of the pigs in the herd developed symptoms associated with porcine stress syndrome when transported between farm sites. On this farm, there are 2 boars (male) and 8 sows (female). Tissue samples from these 10 pigs were sent for DNA sequencing to assess the role of the RYR1 DNA polymorphism (i.e., stress gene) on this farm.

NEXT SLIDE

Breeding Stock DNA Sequence Information

>boar_1

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGCGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

>boar_2

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGYGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

>sow_1

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGCGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

>sow_2

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGCGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

>sow_3

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGYGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

>sow_4

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGCGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

>sow_5

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGYGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

>sow_6

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGCGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

>sow_7

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGCGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

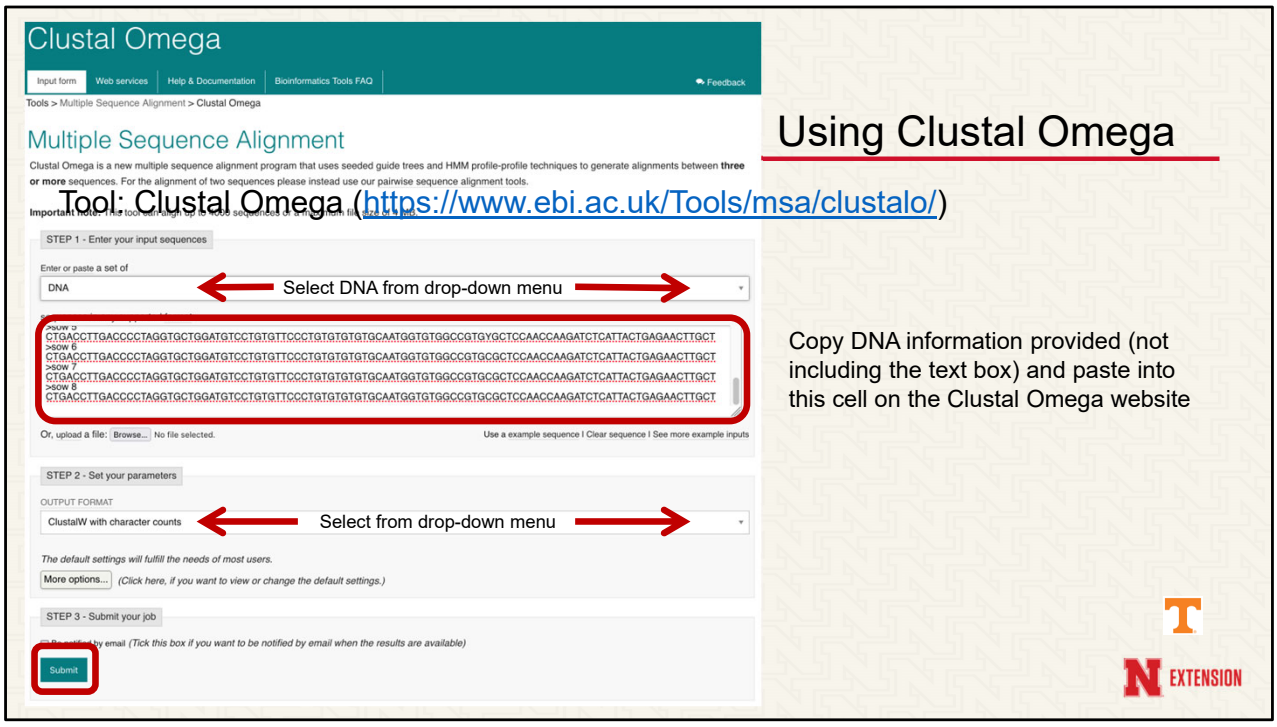
>sow_8

CTGACCTTGACCCCTAGGTGCTGGATGTCCTGTGTTCCCTGTGTGTGTGCAATGGTGTGGCCGTGCGCTCCAACCAAGATCTCATTACTGAGAACTTGCT

EXTENSION

This slide represents the DNA sequence information on each of the 10 pigs that was returned to you. Within your electronic instructions, you should have this same information.

NEXT SLIDE



For this exercise, we will use the Clustal Omega multiple sequence alignment tool. Click on the associated link within your electronic instructions (or copy and paste into your browser).

CLICK – Once done, it should take you to the Clustal Omega website.

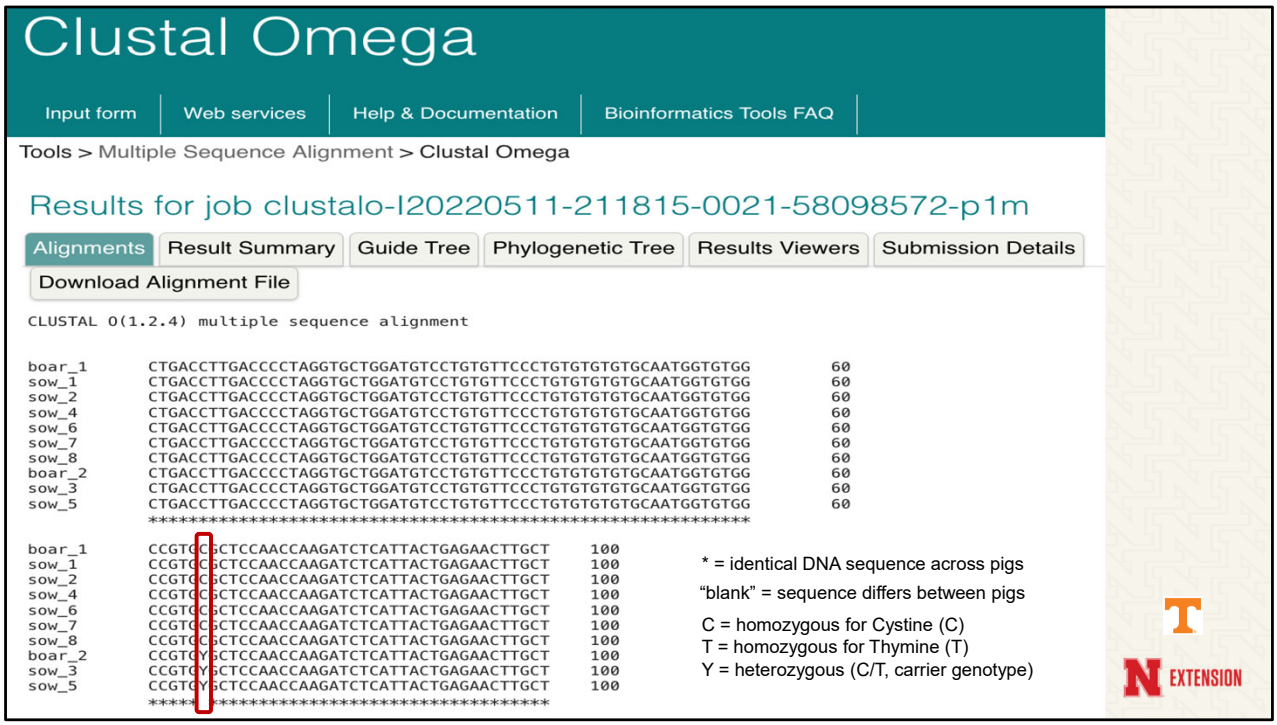
First, we need (CLICK) to select DNA from the first drop-down menu.

Next, you must copy the DNA information provided to you as part of the electronic instructions, not including the text box, and paste that information into this (CLICK) cell on the Clustal Omega website.

Third (CLICK), select “ClustalW with character counts” from this drop-down menu. This should be the default setting.

Once done, click (CLICK) submit. Results for this very small dataset should be returned to you within seconds.

NEXT SLIDE



This is a copy of your results. It may look intimidating, but it's actually very simple.

CLICK – First, the presence of an asterisk indicates that the DNA sequence was identical across all 10 pigs at that particular location. You can ignore all of that information. CLICK – In contrast, the presence of a blank indicates that there is a sequence variation between pigs. This is where you need to focus your attention (CLICK).

Finally, you know from your knowledge of basic genetics, that each animal possesses two alleles. Think of this page as showing you one copy, and the second copy is located immediately below. If you see a "C", (CLICK) it is implied that the animal has two copies of the Cystine nucleotide or is homozygous. Same thing for T for Thymine. If the output reveals a Y, then the animal is heterozygous and possesses one copy of Cystine and one Thymine.

NEXT SLIDE

Assignment of Individual Genotypes

Animal	Clustal Omega Output	Genotype	Description
Boar 1	C		
Boar 2	Y		
Sow 1	C		
Sow 2	C		
Sow 3	Y		
Sow 4	C		
Sow 5	Y		
Sow 6	C		
Sow 7	C		
Sow 8	C		

Your task is now to assign individual genotypes to each of the 10 pigs, and identify them as stress resistant, stress susceptible, or stress carriers. You can put me on pause now, if you like.

OK, hopefully, you have identified the proper genotypes for each pig (CLICK). Anything that had a C would be CC, anything that had a Y would be CT. Those animals that are CC (CLICK) are homozygous for stress resistance, and those that are CT are stress resistant, but carriers for stress susceptibility.

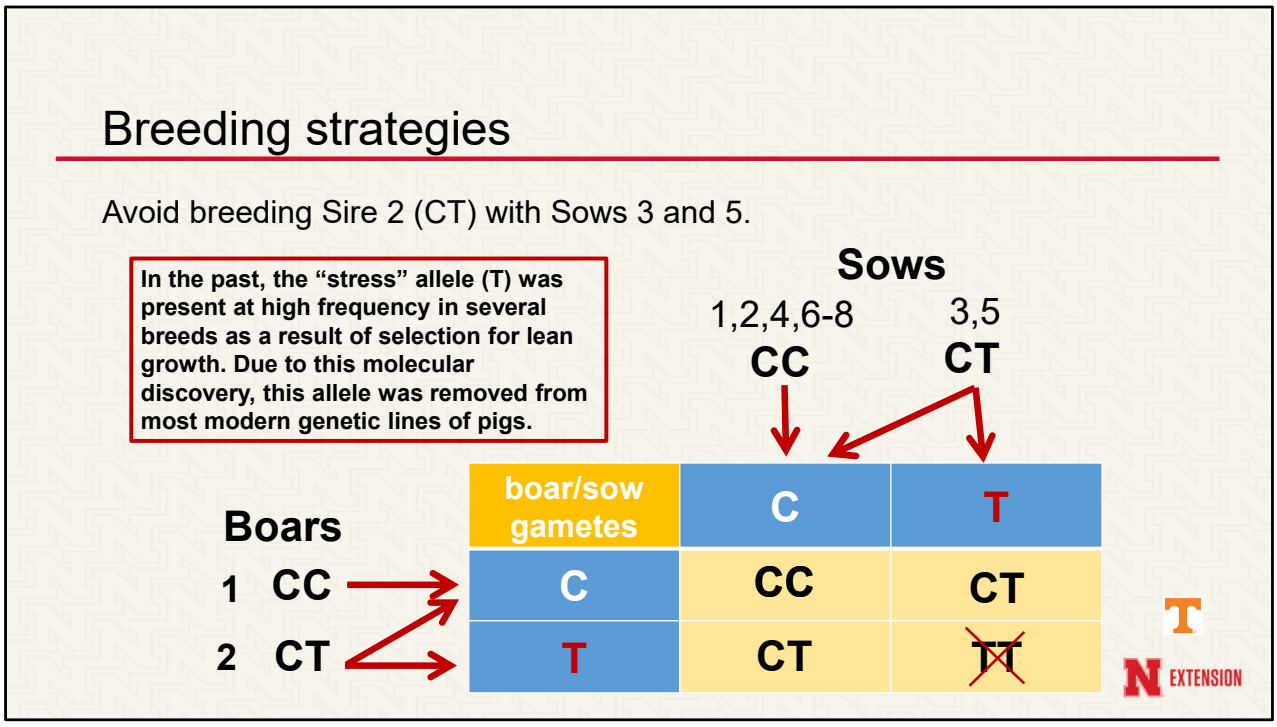
NEXT SLIDE

Application

- Develop mating strategies to limit negative impact of PSS
- PSS results from a nucleotide substitution (rs344435545)
  - Thymine (T) replaces Cytosine (C)
  - Thymine → stress susceptibility (recessive)
  - Cytosine → stress resistance (dominant)

Your task now, start working some Punnett Squares to develop mating strategies to limit the negative impact of porcine stress syndrome on this operation.

NEXT SLIDE



What are your breeding strategies? On this slide, I'm going to use a single Punnett Square to showcase the potential breeding implications associated with all possible matings of these 10 pigs.

CLICK – Boar 1 is homozygous CC. He will only ever transmit the C allele.

CLICK – Likewise, Sows 1,2,4,6,7, & 8 are homozygous CC. They will only ever transmit the C allele.

CLICK – All of the offspring associated with the mating of Boar 1 to Sows 1,2,4,6,7, or 8 will result in a CC genotype in the offspring. Those individuals are homozygous for stress resistance.

CLICK – Boar 2 is heterozygous CT. He might transmit the C allele, or he could transmit the T allele.

CLICK – Same would be true for Sows 3 & 5

From the mating of two heterozygous individuals, there is a 25% chance that the offspring will receive a C from both parents resulting (CLICK) resulting in a homozygous resistant genotype. Or, more likely (CLICK), they'll receive a C from one parent and a T from the other resulting in half the offspring being phenotypically resistant, but heterozygous carriers of the stress susceptible allele. And, unfortunately, a quarter of the offspring should inherit the T allele from each parent (CLICK). Those individuals will be stress susceptible. That, we need to avoid (CLICK).

CLICK – For this operation, our overall recommendation should be that this producer needs to avoid breeding Sire 2 with Sows 3 & 5.

CLICK – In the past, the “stress” allele was present at high frequency in several breeds as a result of selection for lean growth. Due to this molecular discovery, and strategic breeding programs, this allele has been removed from most modern genetic lines of pigs.

NEXT SLIDE

Reflection Questions

1) In general terms, describe the overall “size” of the swine genome?
2) What is the general purpose of the genomic browser, and how does it help geneticists?
3) Associated with this activity problem, why should Boar 2 not be mated to Sows 3 & 5?
4) Associated with this activity problem, what arguments can you provide for keeping the carrier animals?
5) Associated with this activity problem, what arguments can you provide for culling and replacing the carrier animals?

To wrap up this lesson, a few reflective questions for class discussion.

NEXT SLIDE

Application questions

1) In general, describe the structure & make-up of DNA.
2) In general, describe the rules of dominance vs. recessiveness.
3) How does the technology of genomics facilitate genetic progress?

And three application questions for discussion.

NEXT SLIDE

EXTENSION
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