# ELISA LAB

A bioscience collaboration

#### LAST TIME WE WERE TOGETHER...

#### Polymerase Chain Reaction (PCR)

- Using thermocycler, amplify (make more) DNA
- 30+ cycles of denaturation, annealing & elongation Remember the animation we watched last time
- <u>Polymerase</u> used to add nucleotides is Taq polymerase (an enzyme originally isolated from the bacterium Thermus aquaticus).
- "See" that DNA on gel by doing gel electrophoresis
  - Gel made of agar or polyacrylamide
  - Load DNA into gel
  - DNA is charged (+ or -?) so it migrates on gel to other electrode
  - Bands of DNA separate based on size





# TODAY WE LEARN <u>ELISA</u>

Enzyme-Linked Immunosorbent assay (ELISA)

- a test that uses antibodies and color change to identify a substance.
- You did the pre-lab (right?!?)
  - What will we be testing FOR?
  - Think about what the acronym ELISA means
    - Testing for presence (or absence) of an <u>antigen.</u>
    - Antigen could be found in blood sera, tissue sample or food sample.
- Look at overview protocol from pre-lab (page 29).
  - Read along with the protocol while watching this silent animation: <u>https://www.youtube.com/watch?v=6Ue1Hd3dyaQ</u>

# WHICH TECHNIQUE SHOULD I USE?

- To PCR or ELISA? That is the question.
  - Depends on what you are trying to accomplish.
  - You tell US:
    - what's the goal of PCR?
    - What's the goal of ELISA?
- Example of scientific study that used both techniques to confirm results in different ways:
  - Read abstract out loud
  - <u>http://www.biomedcentral.com/content/pdf/1746-6148-3-21.pdf</u>
  - Or if that link doesn't work: <u>http://www.biomedcentral.com/1746-6148/3/21/abstract</u>

#### OUR DISEASE IN QUESTION: BSE (BOVINE SPONGIFORM ENCEPHALOPATHY)

- Most commonly referred to as "Mad Cow Disease"
- Progressive neurological disorder of cattle
  - infection by transmissible agent called a prion.
     Nature of agent not well understood.
  - Most accepted theory is that agent is a modified form of a normal protein known as prion protein.
  - For reasons that are not yet understood, the normal prion protein changes into a pathogenic (harmful) form
  - Pathogenic form then damages the central nervous system of cattle.
- There have only been 5 <u>cases</u> of BSE in the United States:
  - 2003 = Washington State; 2005 = Texas; 2006 & 2017 = Alabama; 2012 = California;
- USDA announcement of 5<sup>th</sup> BSE case in the US: <u>https://www.aphis.usda.gov/aphis/newsroom/stakehold</u> er-info/sa\_by\_date/sa-2017/sa-07/bse-alabama



Photo credit: <u>http://stateofinnovation.com/sites/default/files/content/figures-</u> charts/111-08-prion.jpg

Info credit: <a href="https://www.cdc.gov/prions/bse/index.html">https://www.cdc.gov/prions/bse/index.html</a>

### CLINICAL SIGNS OF BSE

- changes in temperament (i.e. nervousness or aggression)
- abnormal posture; lack of coordination (ataxia); difficulty in rising
- decreased milk production
- loss of body weight despite continued appetite.
- Early in the clinical course of the disease, symptoms may be slight, undetectable or unrecognizable. Not all affected cattle display all signs of the disease.



Cattle such as the one pictured here, which are affected by BSE experience progressive degeneration of the nervous system.

# EMERGENCE OF BSE

- Research indicates that the first probable infections of BSE in cows occurred during the 1970's with two cases of BSE being identified in 1986.
- Possibly originated as a result of feeding cattle meatand-bone meal that contained BSE-infected products from a spontaneously occurring case of BSE or scrapieinfected sheep products. Scrapie is a prion disease of sheep.
  - Side note: last semester VetMed put Scrapie tags on sheep before they went to sale. All sheep entering the food chain must, by federal law, have a Scrapie tag so if there is an outbreak, the source is traceable.
  - What is a prion?
- Strong evidence & agreement that outbreak was then amplified and spread throughout the United Kingdom cattle industry by feeding rendered, prion-infected, bovine meat-and-bone meal to young calves.



Photo credit: @CAPSVetMed Slide info source: <u>https://www.cdc.gov/prions/bse/about</u> .html

#### FREQUENCY AND HUMAN IMPACT OF BSE

- The BSE epizootic epidemic in the United Kingdom peaked in January 1993 at almost 1,000 new cases per week. Over the next 17 years, the annual numbers of BSE cases has dropped sharply.
  - 11 cases in 2010
  - 225 cases in 200
  - 1,443 cases in 2000
  - 14,562 cases in 1995
- Regularly updated numbers of reported BSE cases, by country, are available on the website of the <u>Office International Des Epizooties</u>.
- Strong epidemiologic and laboratory evidence for causal association between a new human prion disease called <u>variant Creutzfeldt-Jakob disease (vCJD)</u>
  - First reported from the United Kingdom in 1996
  - Interval between the most likely period for the initial extended exposure of the population to
    potentially BSE-contaminated food (1984-1986) and the onset of initial variant CJD cases (19941996) is consistent with known incubation periods for the human forms of prion disease.
  - Invariably fatal brain diseases with unusually long incubation periods measured in years

Slide info source: <u>https://www.cdc.gov/prions/bse/about.html</u>

### OUR LAB SCENARIO TODAY...

Human analog? of BSE -> CJD

https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Fact-Sheets/Creutzfeldt-Jakob-Disease-Fact-Sheet

- Someone in the class has been infected with BSE
  - Did they eat some undercooked meat containing the prion?
  - Although humans can't pass CJD to each other, let's simulate an outbreak scenario of a prion disease. Maybe the infected person is shedding prions.
- You will "share simulated body fluids" with 3 different classmates. This needs to be done in a controlled manner, so await instructions...

#### DURING A 5 MINUTE INCUBATION...

• Go to the next slide for animation

### WHAT IS A COMMON MEDICAL APPLICATION OF ELISA?

Watch (stop around 3:39)

https://www.youtube.com/watch?v=aOfWTscU8YM

### REFERENCES

- <u>http://www.bseinfo.org/bseclinicalsignssymptoms.aspx</u>
- www.CDC.gov/bse

### VIEW NEXT SLIDE ONLY IF MORE EXPLANATION NEEDED...

### ANOTHER ANIMATION OF ELISA

- This one has antigen already bound to wells (different than our test today)
  - How does an Elisa test work?
  - https://www.youtube.com/watch?v=RRbuz3VQ100