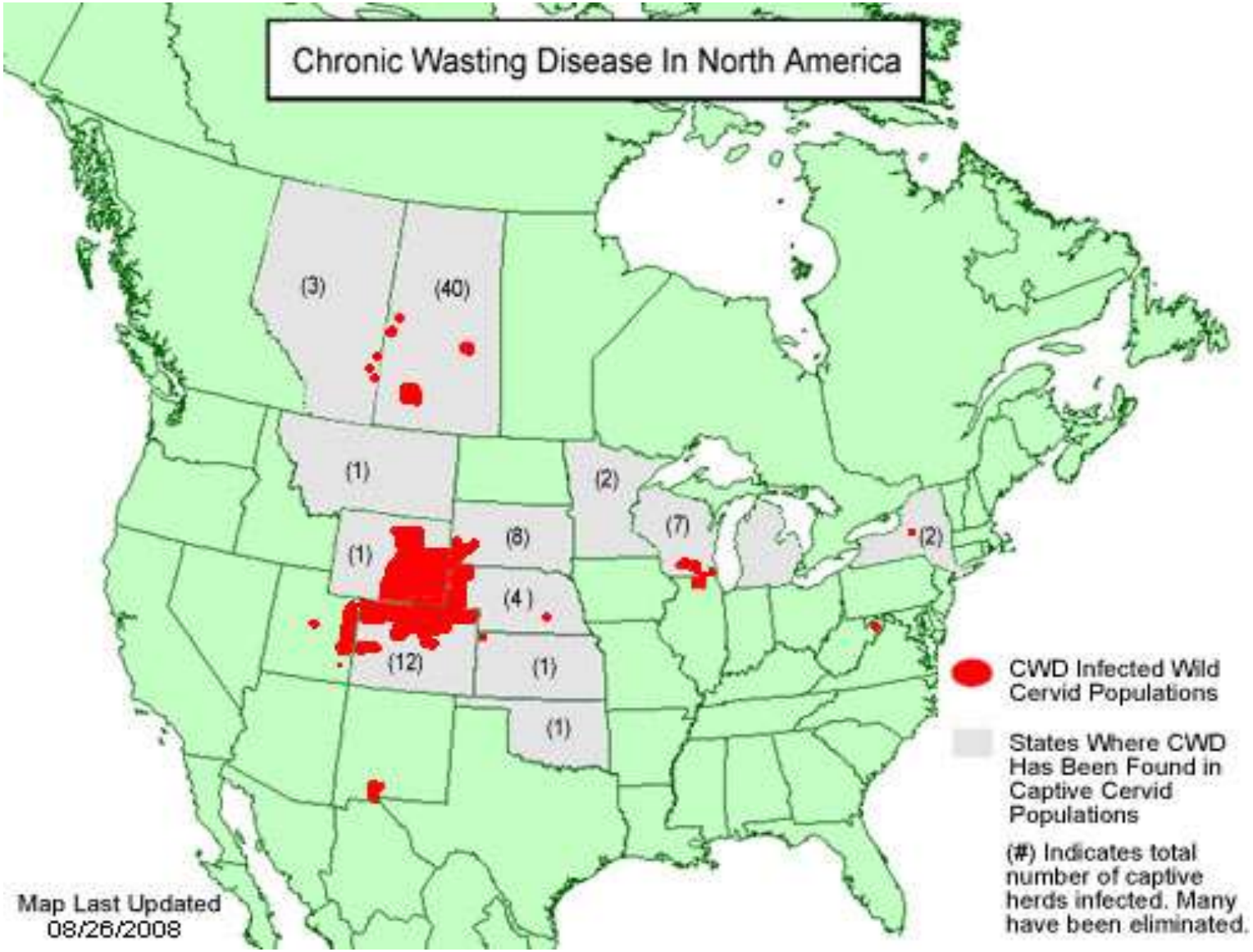
CHRONIC WASTING DISEASE

By: Alicia Gray



Map courtesy of the Chronic Wasting Disease Alliance.

THE DISEASE

- Prions
- TSE (Transmissible Spongiform Ecephalopathy)
- Chronic Wasting Disease

WHY WE CARE….

- Other Neurological Diseases
  - Such as
    - Alzheimers
    - Parkinsons Disease
    - MS
    - CJD (Creutzfeldt-Jacob's Disease)***

THE IDEA BEHIND OUR RESEARCH

- Difficulty of detecting the prion
  - No blood test
  - No urine test
  - Ideal tests do not detect the prion protein
- Biomarkers
  - Surrogate Protein Marker
- Generate dipstick test

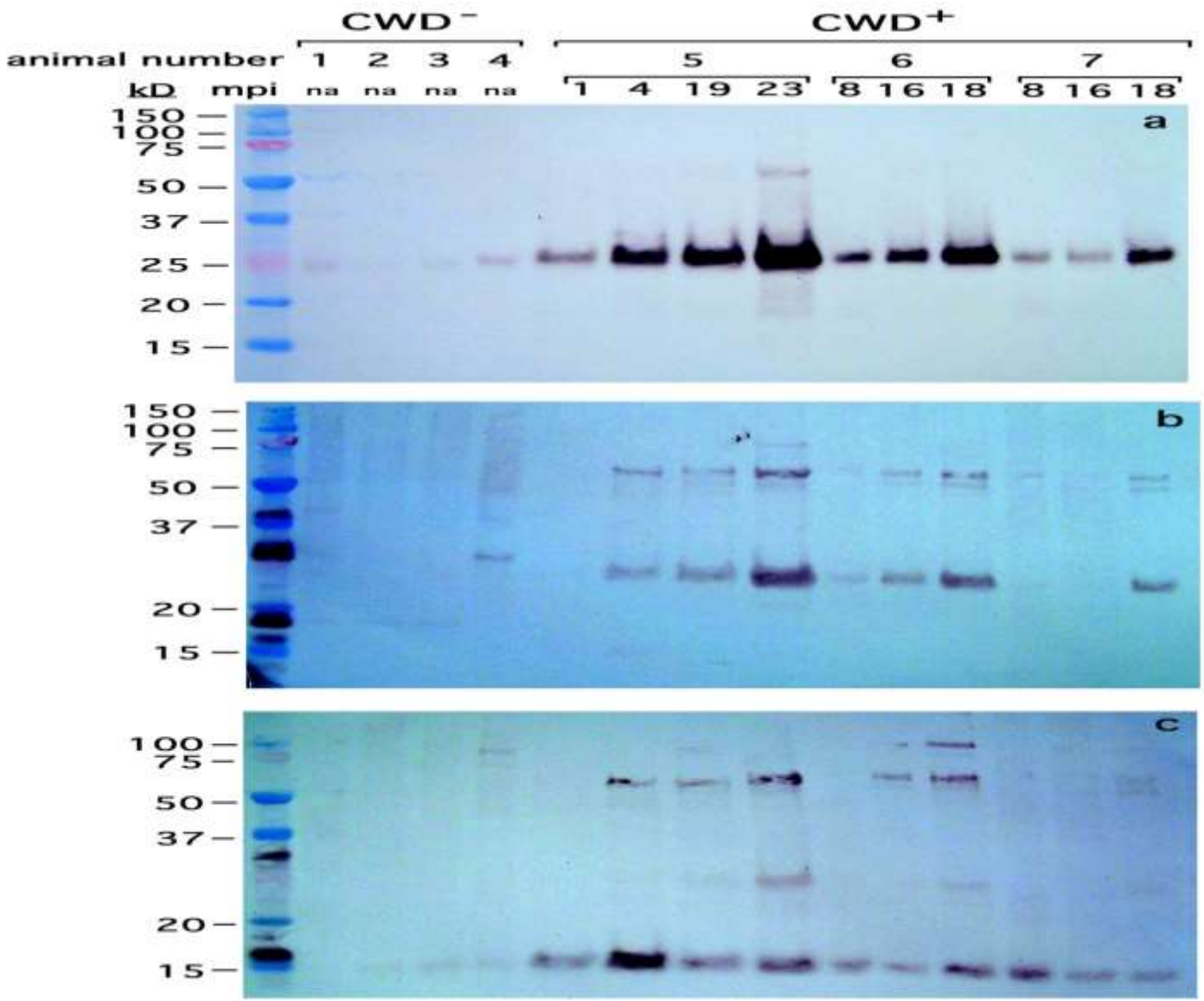


Figure 4. Detection of biomarkers for Chronic Wasting Disease in the urine of White Tail Deer. Proteins in urine collected from four different CWD- and three different CWD+ deer were separated by SDS-PAGE, transferred to nitrocellulose, then probed with antibodies against CWD10 (panel a), CWD2 (panel b), or CWD3 (panel c). Abbreviations: mpi – months post (CWD+) inoculation, na – not applicable. The first lane of each blot has protein molecular weight markers whose sizes (in kilodaltons – kD) are indicated on the left side that blot.

THE PROJECT

- Going back to DNA sequence
- To find the specific deer protein
  - Developing an antibody specific to the protein
  - Biomarker test

MY APPROACH

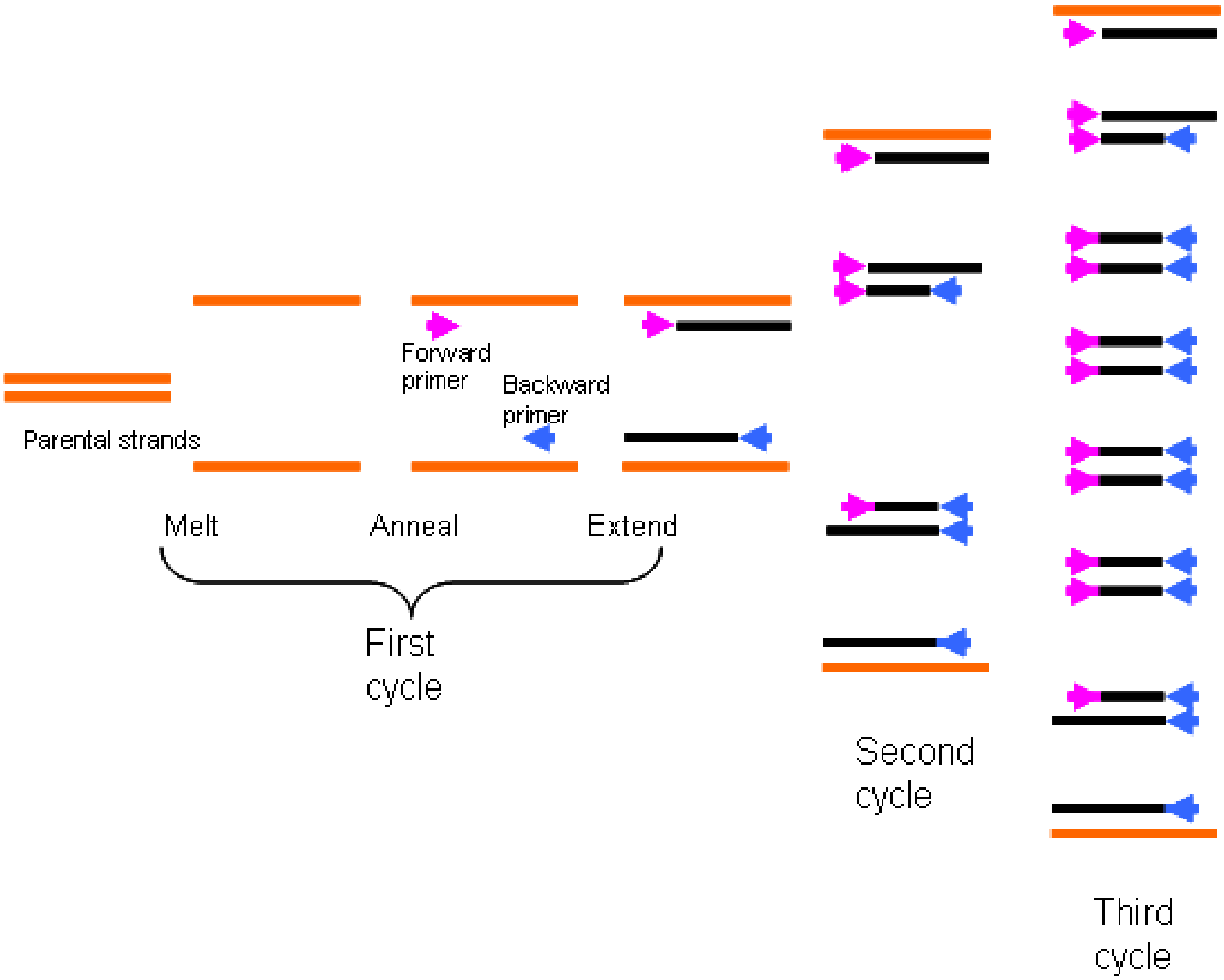
- Synthesizing degenerate primers based off of human, rat, bovine and mouse DNA
- With the primers, we would use PCR to determine if the primers could amplify anything similar to our known mammalian genes in the deer DNA.
- Sequencing to identify specific protein of interest
- Developing an antibody specific to that protein

DEGENERATE PRIMERS

- Degenerate primers are useful for pulling out one part of a gene sequence when you only know the gene sequence in related organisms.
- When designing my primers, I made it where the primers would match DNA, based off of the genomic DNA of the bovine, human, rat and mouse.
- Six primers were synthesized

PCR

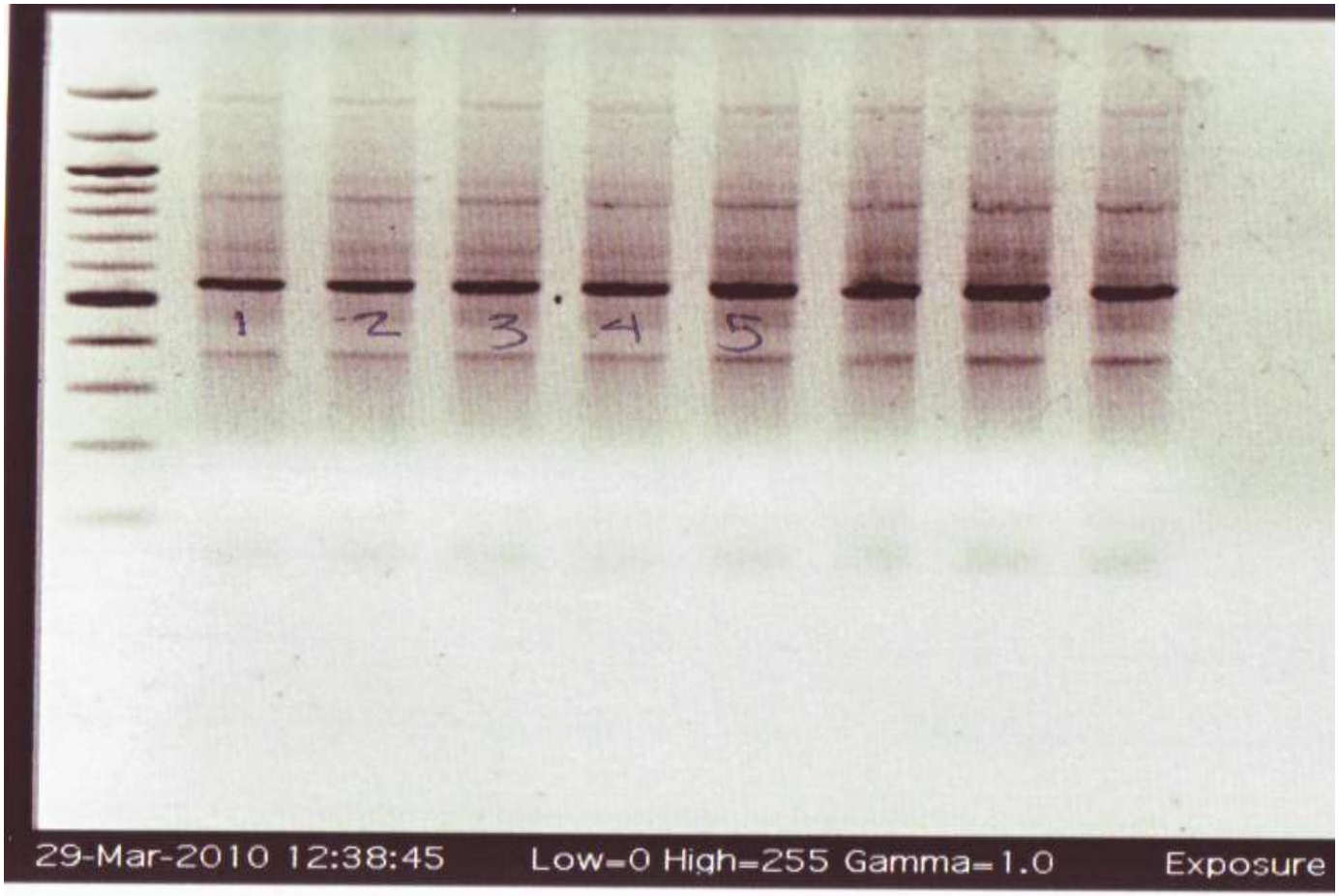
- PCR (Polymerase Chain Reaction) is a way to make a huge number of copies of a gene.
- Three steps in PCR
  - Denaturation
  - Annealing
  - Extension


Parental strands
Forward primer
Backward primer
Melt
Anneal
Extend
First cycle
Second cycle
Third cycle

ELECTROPHORESIS

- Agarose Gel
- Providing an electric current through the gel with the DNA put into certain wells.
- Successful PCR products were compared to DNA standards.
- Size

SUCCESS!!



SEQUENCING

- PCR products were submitted for DNA sequencing.
- Future
- From the segment…
  - DNA sequence ⟶ deduced protein sequence
  - Protein sequence ⟶ Peptide antigen
  - Peptide antigen ⟶ Species Specific Antibody

MY RESULTS

- Designed my primers
- Optomized PCR
- Generated PCR products
- Analyzed on Agarose gel
- Purified band of DNA
- Sent for sequencing

MY EXPERIENCE UNDER THE EPSCOR PROGRAM HAS:

- Given me a great insight of how research is conducted
- Given me a great perspective of Chronic Wasting Disease
- Introduced me to wonderful people!!

WORKS CITED

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