

Optimizing Fecal Sample Preparation to Determine Presence of Chronic Wasting Disease in White Tail Deer

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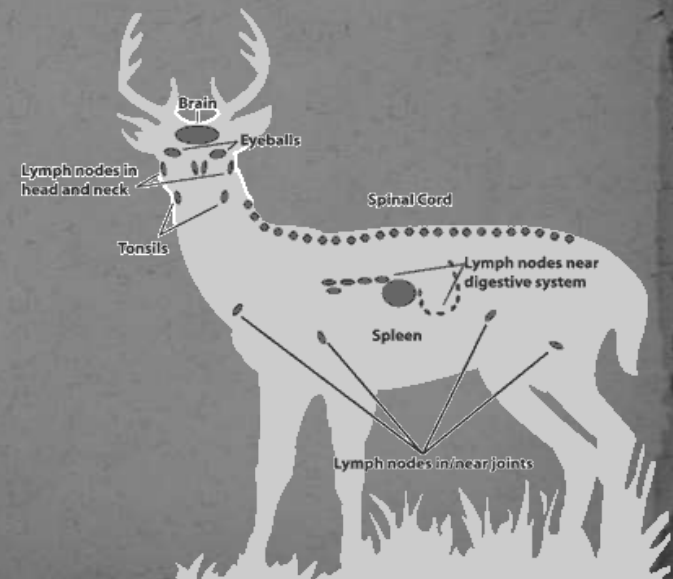
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Transmissible Spongiform Encephalopathy and Prion

- Transmissible spongiform encephalopathy (TSE) is a group of progressive diseases that affect the nervous systems and brains of many mammals, including human.
- Caused by accumulation of a misfolded isoform of the protein called prion protein (PrP)
- Prion is an infectious agent which forms aggregates in nervous tissues.

Prion(PrP)

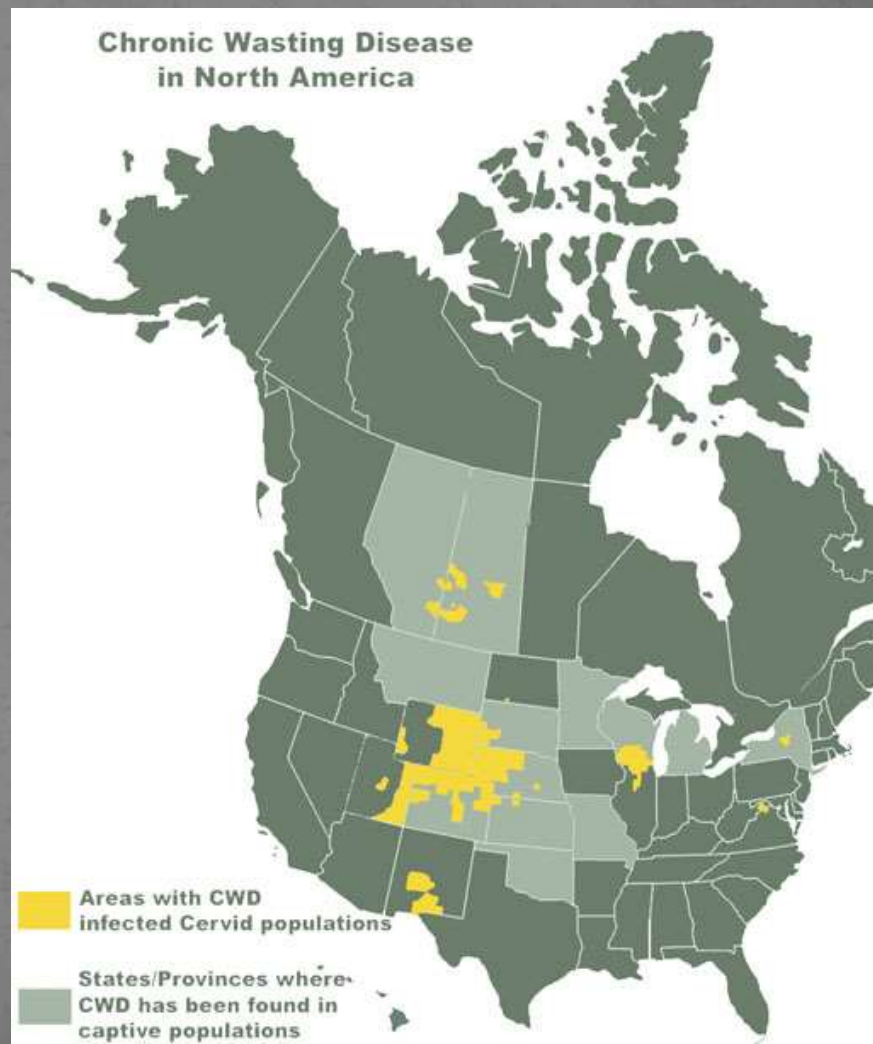
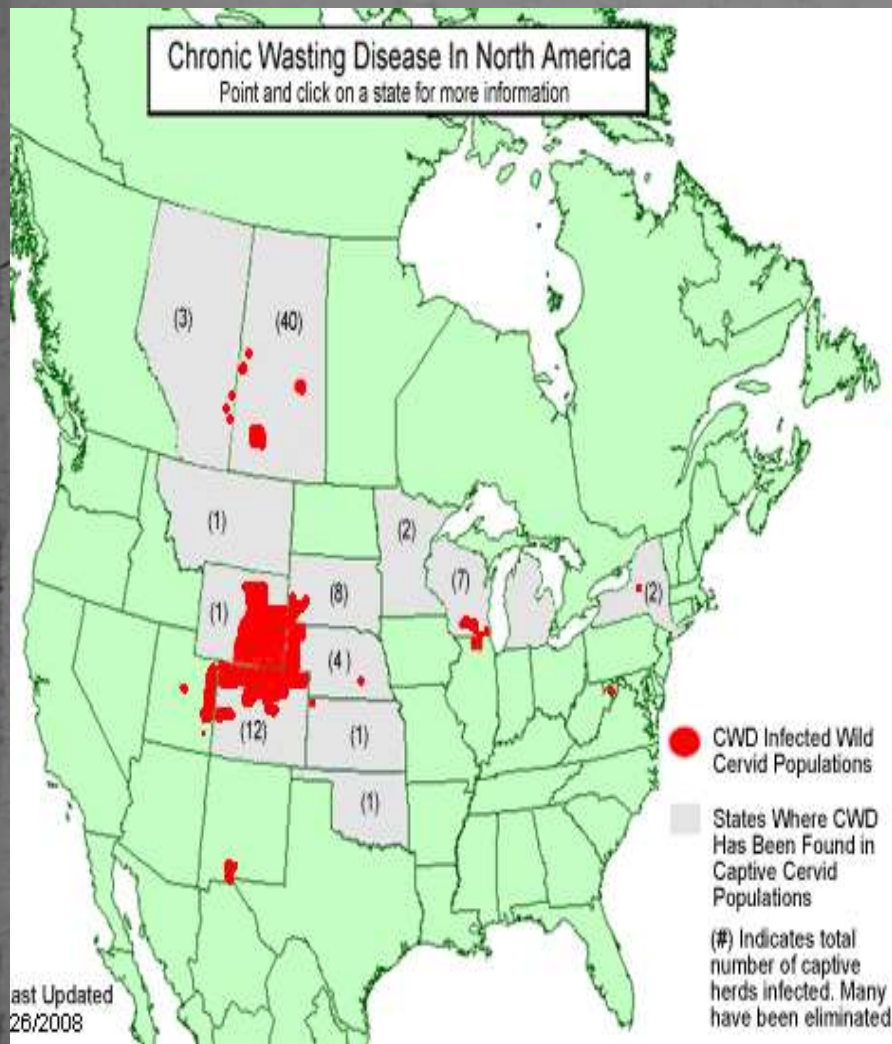
- Two protein conformations exist...
 - PrP_{sen} = native conformation of PrP present in all mammals and is **sen**sitive to digestion with protease. It is a membrane associated protein but its function is unknown.
 - PrP_{res} = misfolded conformation and is **res**istant to digestion with protease



Other Forms of Prion Diseases (TSEs)

- Mad Cow Disease (Bovine Spongiform Encephalopathy; BSE) → Cows and other mammals
- Scrapie → Sheep and goats
- Chronic Wasting Disease (CWD) → Deer and elk
- Creutzfeldt- Jakob Disease (CJD) → humans

CWD Distribution in the U.S.



Symptoms and Diagnosis

- Symptoms
 - Weight loss
 - Behavioral changes:
 - Decreased interactions with other animals
 - Listlessness
 - Lowering of the head
 - Blank facial expression
 - Repetitive walking in set patterns
 - A smell like meat starting to rot



Symptoms and Diagnosis

- Diagnosis
 - Post-mortem: detection of PrP_{res} and vacuolization in nervous tissues.
 - Ante-mortem: RAMALT (recto-anal mucosal associated lymphoid tissue) to detect PrP_{res}.

Biomarker- Advantages?

- Biomarkers= indicators of diseases/ conditions
 - Pregnancy
 - Albumin in urine to measure the kidney functions
 - ALT/AST level to evaluate the liver functions
 - many other...
- Easy, fast, non-invasive testing: use of urine/ feces/ blood



Some Biomarkers for detection of CWD

Biomarkers	Possible Functions
Dynamin 1	GTPase responsible for endocytosis
Calpain	Calcium dependent protease
TCP-1 α	A subunit of chaperonin involved in tubulin and actin folding

And others...

The Mission:

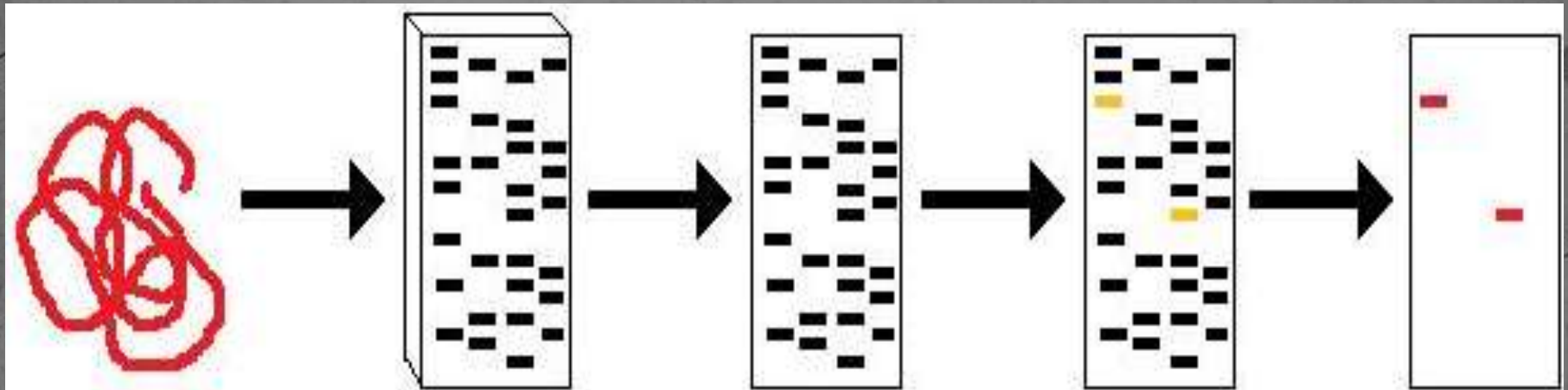
To detect these biomarkers in feces using
Western Blotting

Why feces?
-Easy to collect in the field.
-Non- invasive

For method development, we used feces samples from captive white tail deer that were inoculated with chronic wasting disease.

Western Blotting

Western blot is a common technique to detect a protein of interest in a sample using antibodies specific to the protein.



Protein

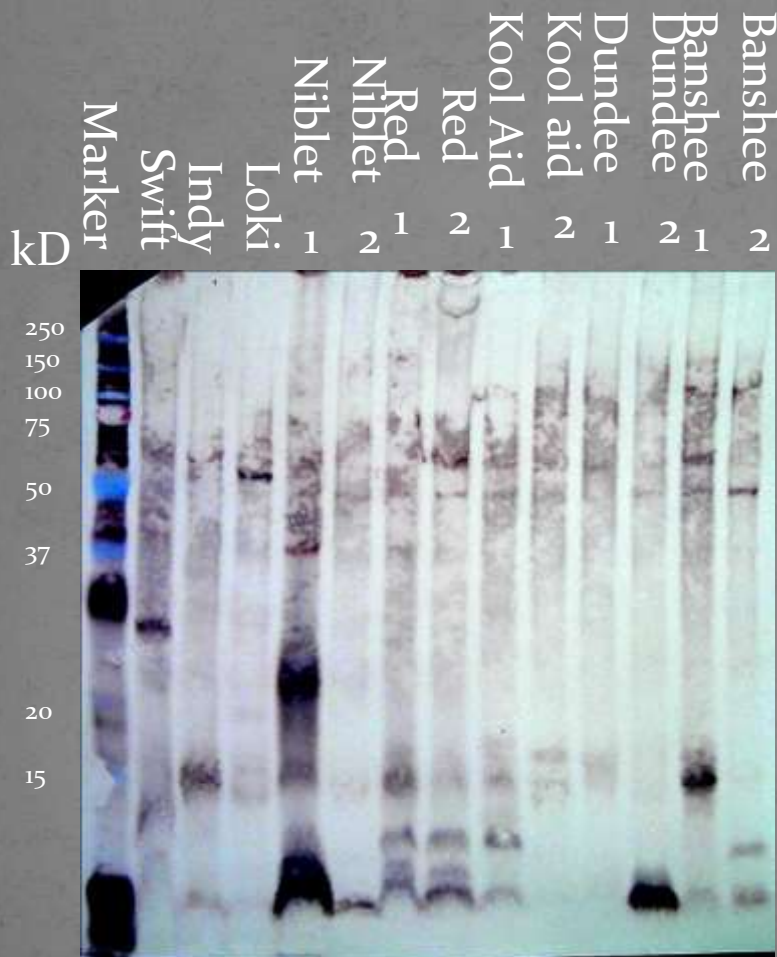
Separate
proteins on
SDS-PAGE

Transfer
proteins to
nitrocellulose
membrane

Membrane
is exposed
to specific
antibodies

Protein bands
detected by
specific
antibodies

Problems Encountered and Research Goals



This is very DIRTY!!

We would like to see nice clear bands...

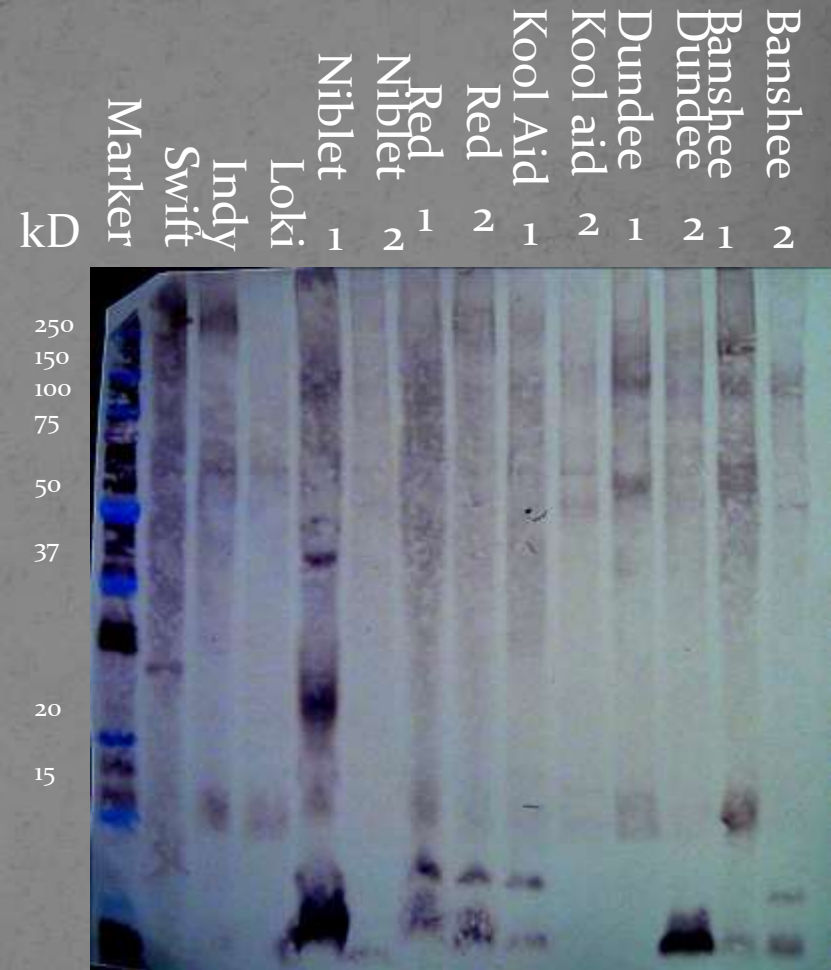
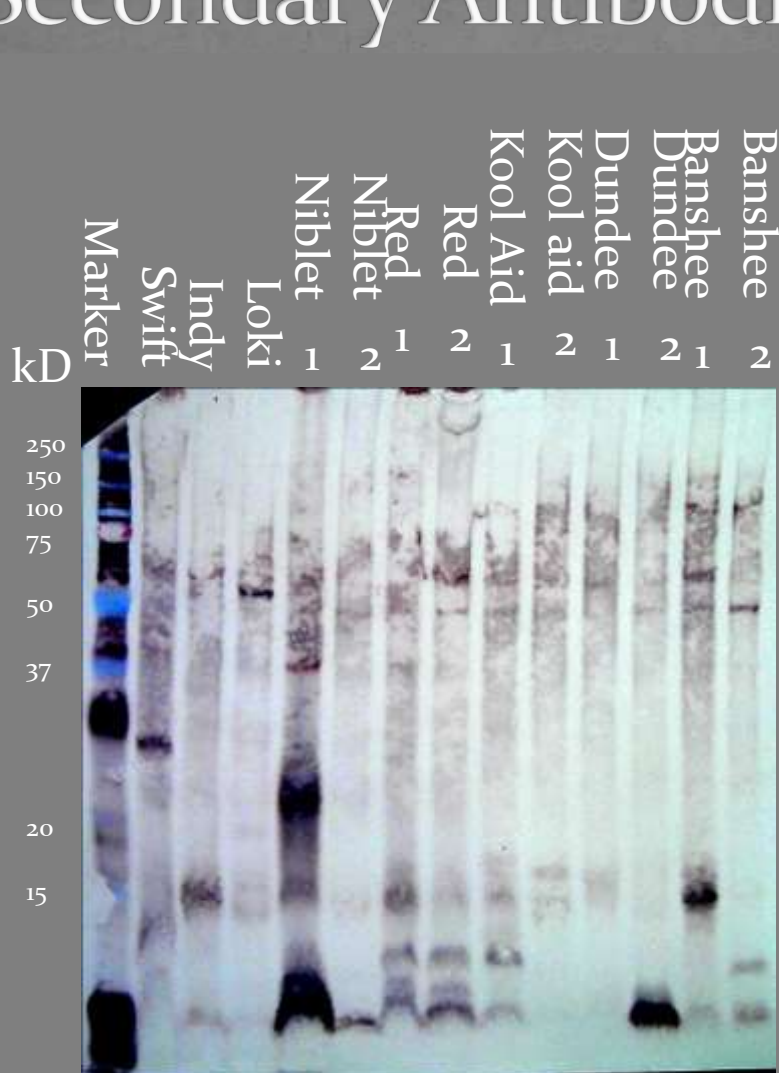
Our Goals:

To make this better looking...

By altering

- extraction buffer
- dilution of the primary and secondary antibodies
- Extraction time/ methods

Alteration 1: Dilution of Primary and Secondary Antibodies

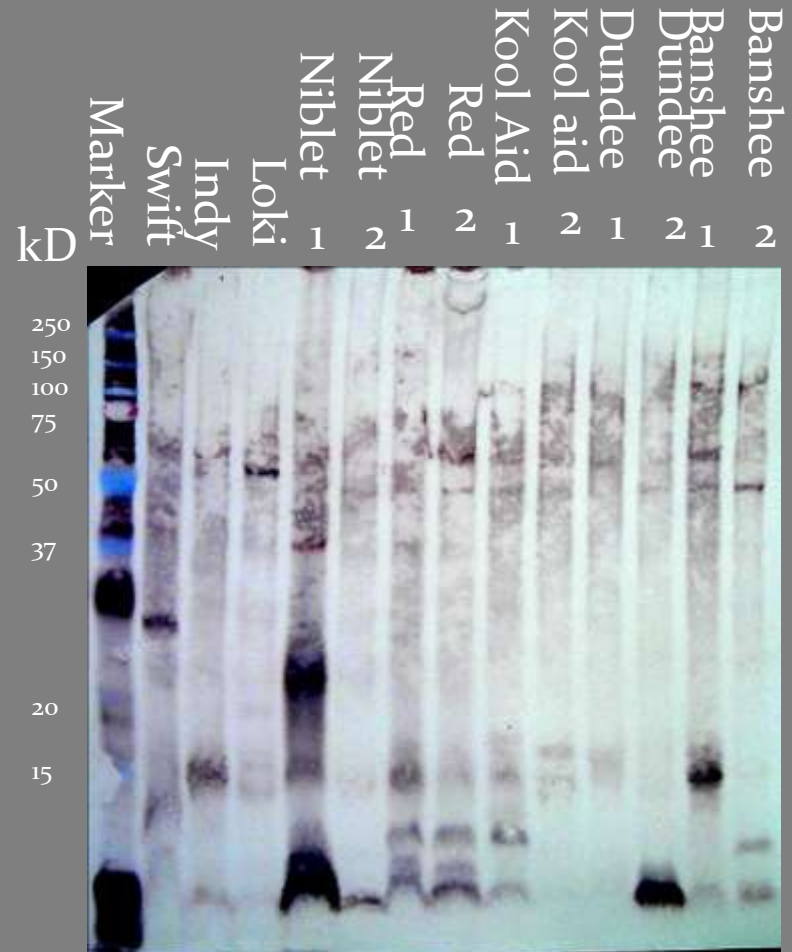


There is not much improvement.

Alteration 2: Extraction Time

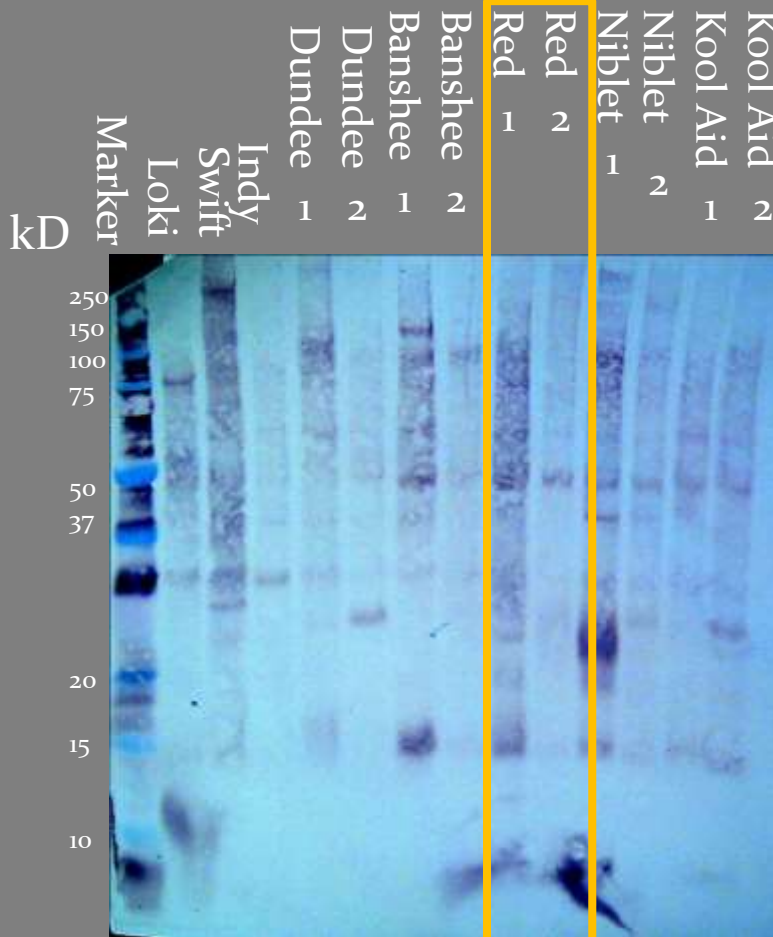
- We previously incubated the samples for 15 hrs to extract the mucus layers.
- In this experiment, 1, 3 and 5 hr extraction was performed.

1 hr extraction seemed to work the best.

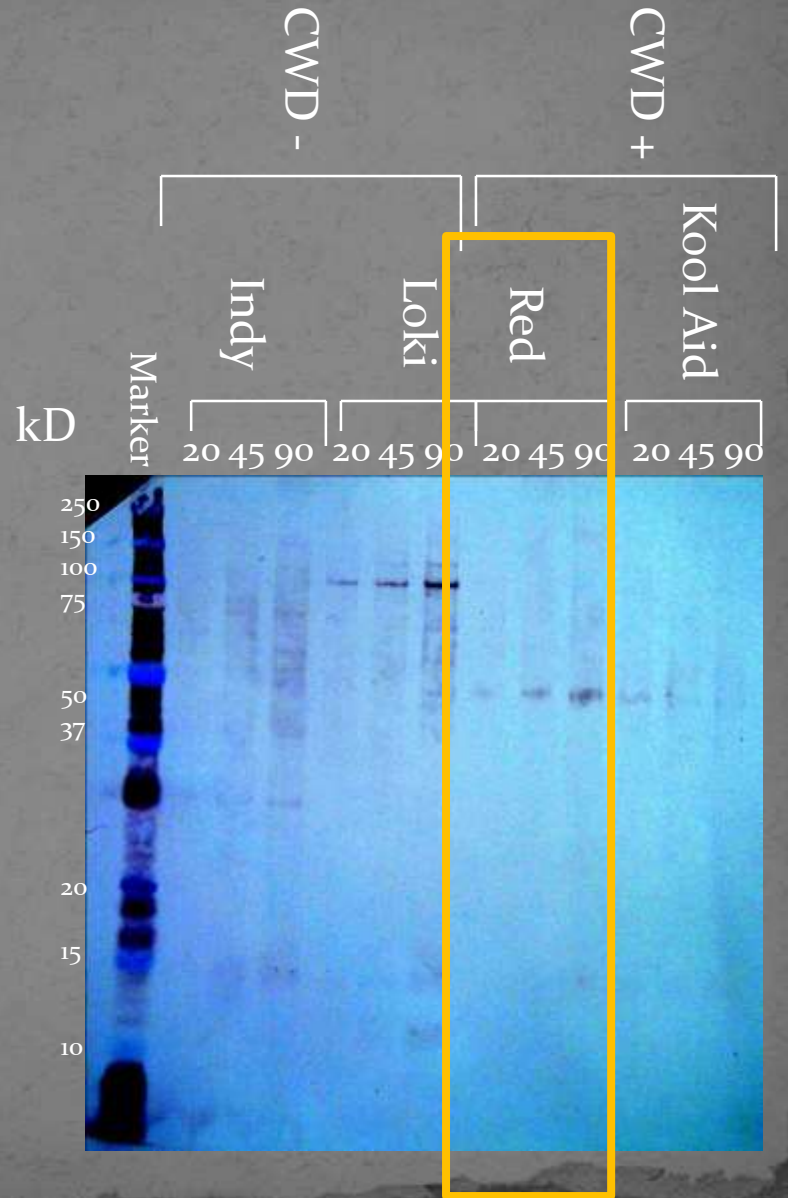


Alteration 3: Shaker

1 hr extraction on the tumbler shaker

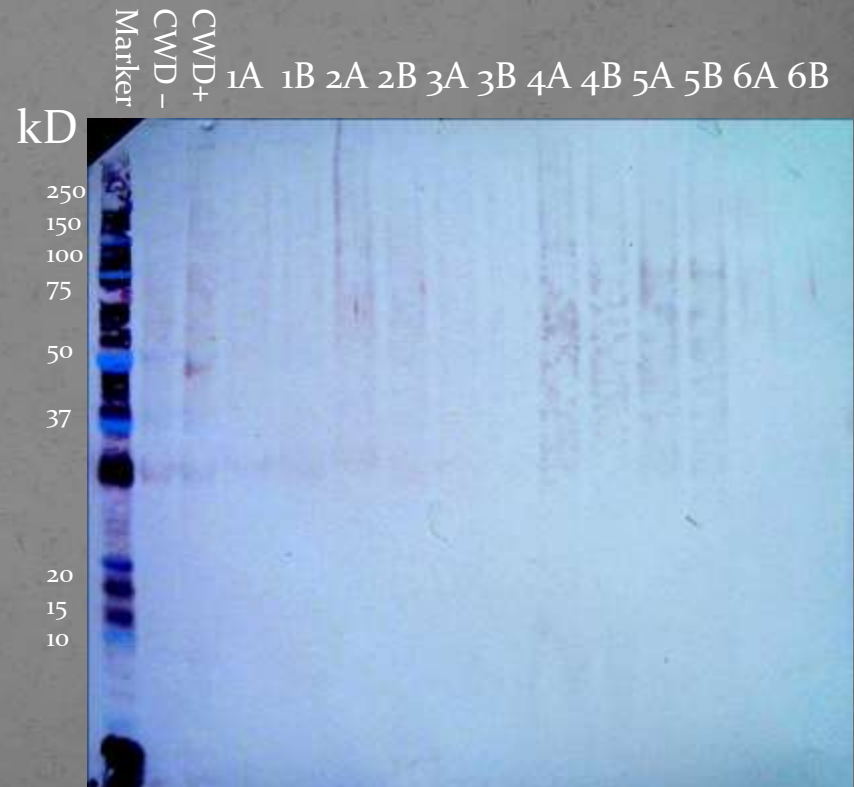


1 hr extraction on the rotary shaker



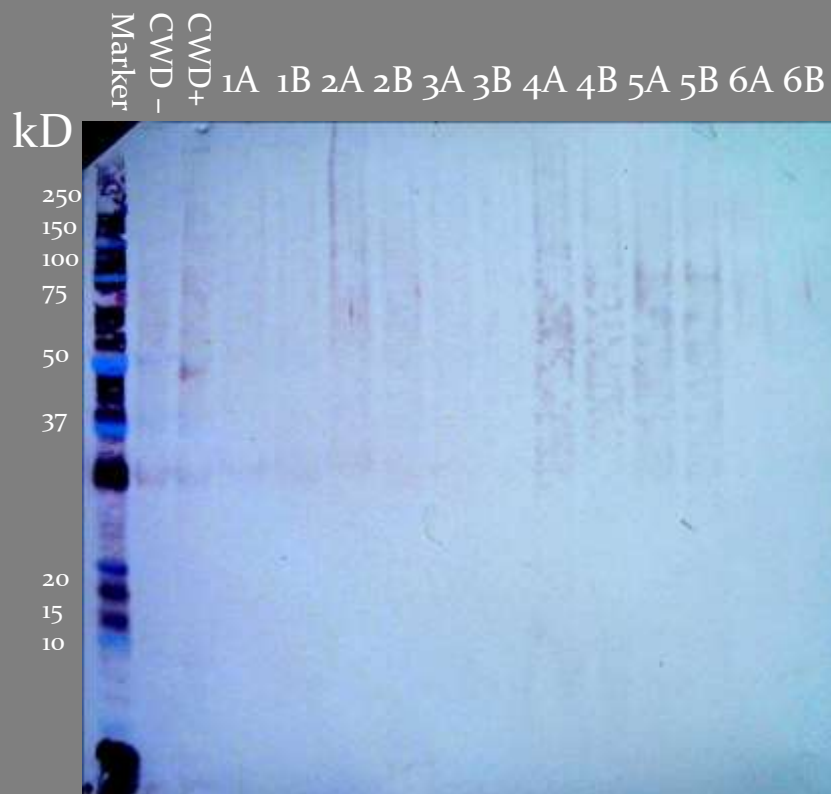
Unknown Samples

- Tried our methods on feces from deer of unknown CWD status.
- Blot was a little ugly.
- Decided to alter the buffer to have less salt and detergent.

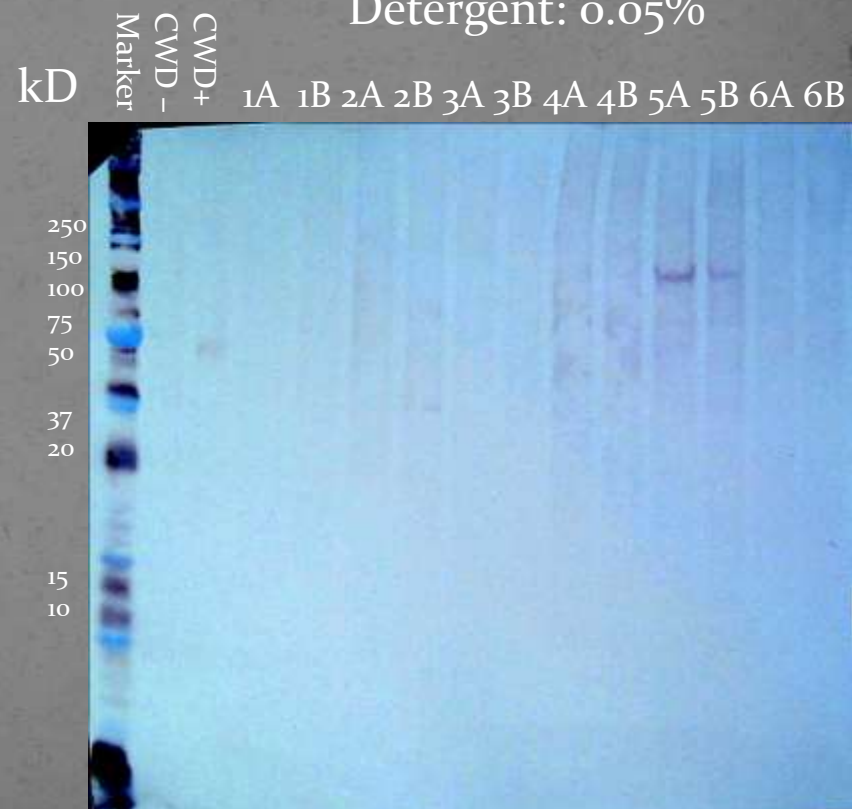


Alteration 4: Buffer

NaCl: 130 mM
Detergent: 0.5%



NaCl: 10 mM
Detergent: 0.05%



Conclusion

- Improved the method for protein extraction from deer feces for biomarker detection.
- Furthermore, these methods seemed to work for deer feces with unknown CWD status.
- This method has potential to serve as a non-invasive screen for CWD in the field.

Acknowledgement

- I would like to thank all the faculty members in Dr. Lewis' Lab, especially Ted John for walking through the experiments with me and being a great mentor.