Monoclonal Antibody Production Against Synthetic Peptides Representing PrP^c and Recombinant Prion Proteins (rPrP)

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-with-
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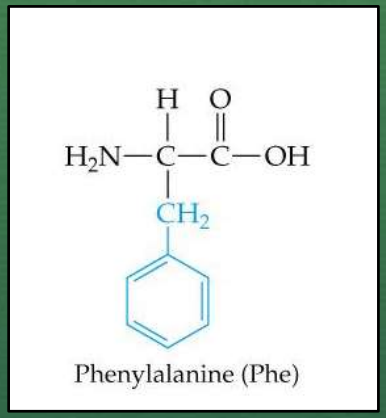
Introduction

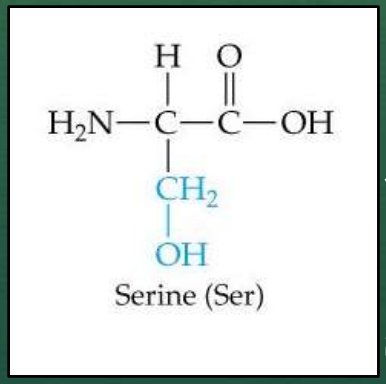
- Chronic wasting disease (CWD) is a slow, fatal neurodegenerative disorder that affects deer, elk, and moose
- CWD is thought to be caused by misfolded infectious isoforms of certain cellular proteins called prions

Photo: Delaware Game and Fish Department

Prion Protein

- Mule Deer prion protein variant
  - Amino acid position 225 – phenylalanine (F)/serine (S)
- Homozygous deer (225SS) are 30 times more likely to be CWD positive than heterozygous deer (225SF).
  - 225SS deer also have a shorter incubation period





Diagrams courtesy of: http://wps.prenhall.com/wps/media/objects/476/488316/Instructor_Resources/Chapter_19/FG19_06-10T02.JPG

Importance of Study

- The purpose of this study is to answer a question – Can monoclonal antibodies be produced against proteins encoded by the two alleles?
- Currently not possible to immunologically distinguish between the two proteins
- If we can distinguish between the two proteins, we will be able to study the difference in the susceptibility of mule deer to CWD

Previous Work

- We began using synthetic peptides that represented the prion protein as the immunogen
- Hybridomas -Two mice were vaccinated with synthetic peptides

Peptide F: Q-M-C-I-T-Q-Y-Q-R-E-**F**-Q-A-Y-Y-Q-R-G-A-S

Peptide S: Q-M-C-I-T-Q-Y-Q-R-E-**S**-Q-A-Y-Y-Q-R-G-A-S

Methods

- Harvested splenocytes were fused with SP2 myeloma cells to produce hybridomas

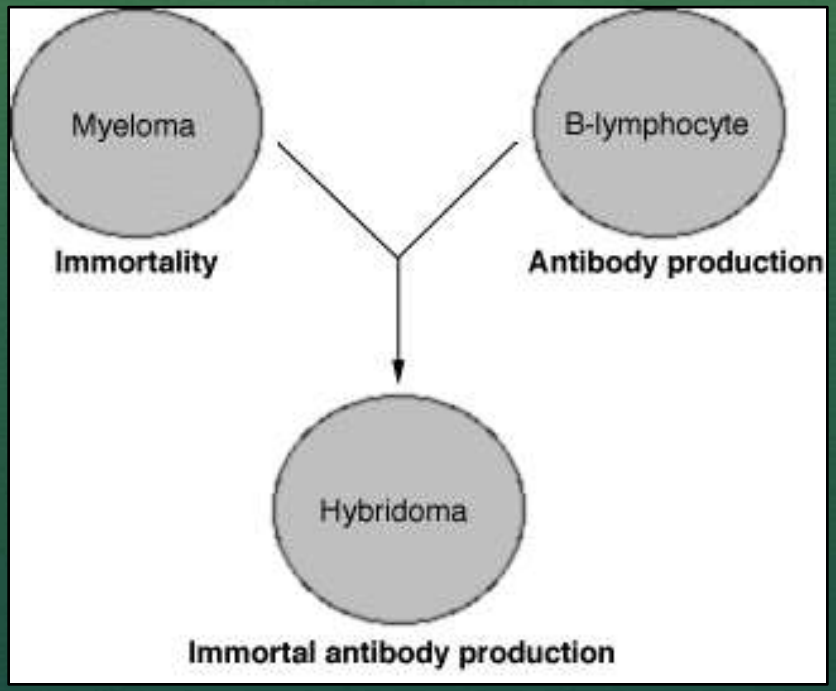
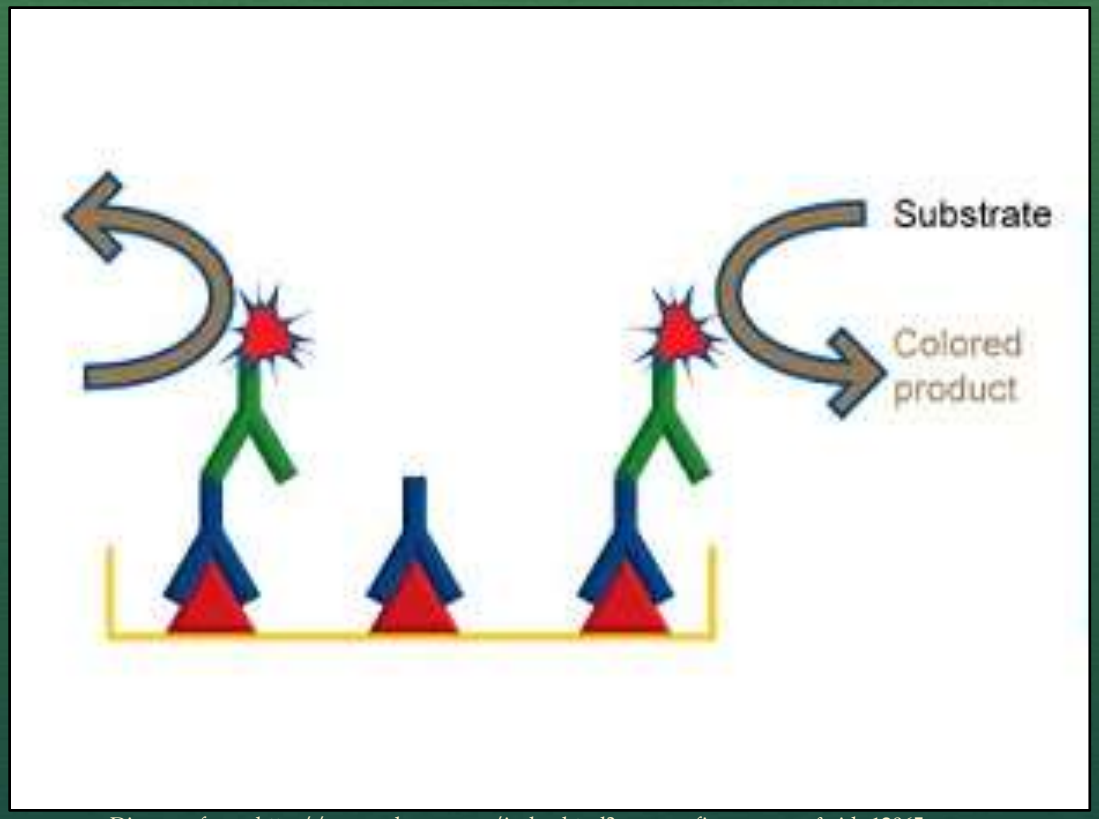


Diagram from:
http://www.bio.davidson.edu/Courses/Molbio/MolStudents/01kewestbrook/hybridoma.gif

Methods

- Indirect ELISA used to screen for antibodies



Synthetic Peptide

Produced Monoclonal Antibody

Conjugated Secondary Antibody

Horseradish Peroxidase

Diagram from: http://www.abcam.com/index.html?pageconfig=resource&rid=12065

Methods

- Western Immunoblots
  - Samples included recombinant proteins, PrP 225F (rPrP 225F) and PrP 225S (rPrP 225S)

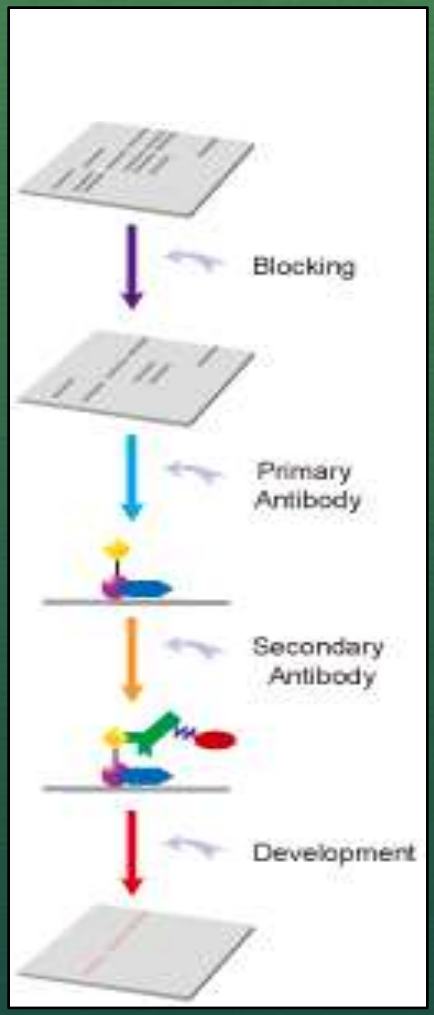


Diagram from: http://www.genscript.com/images/L00204-1.

Protein samples are separated by gel electrophoresis

Proteins are transferred to a PVDF membrane

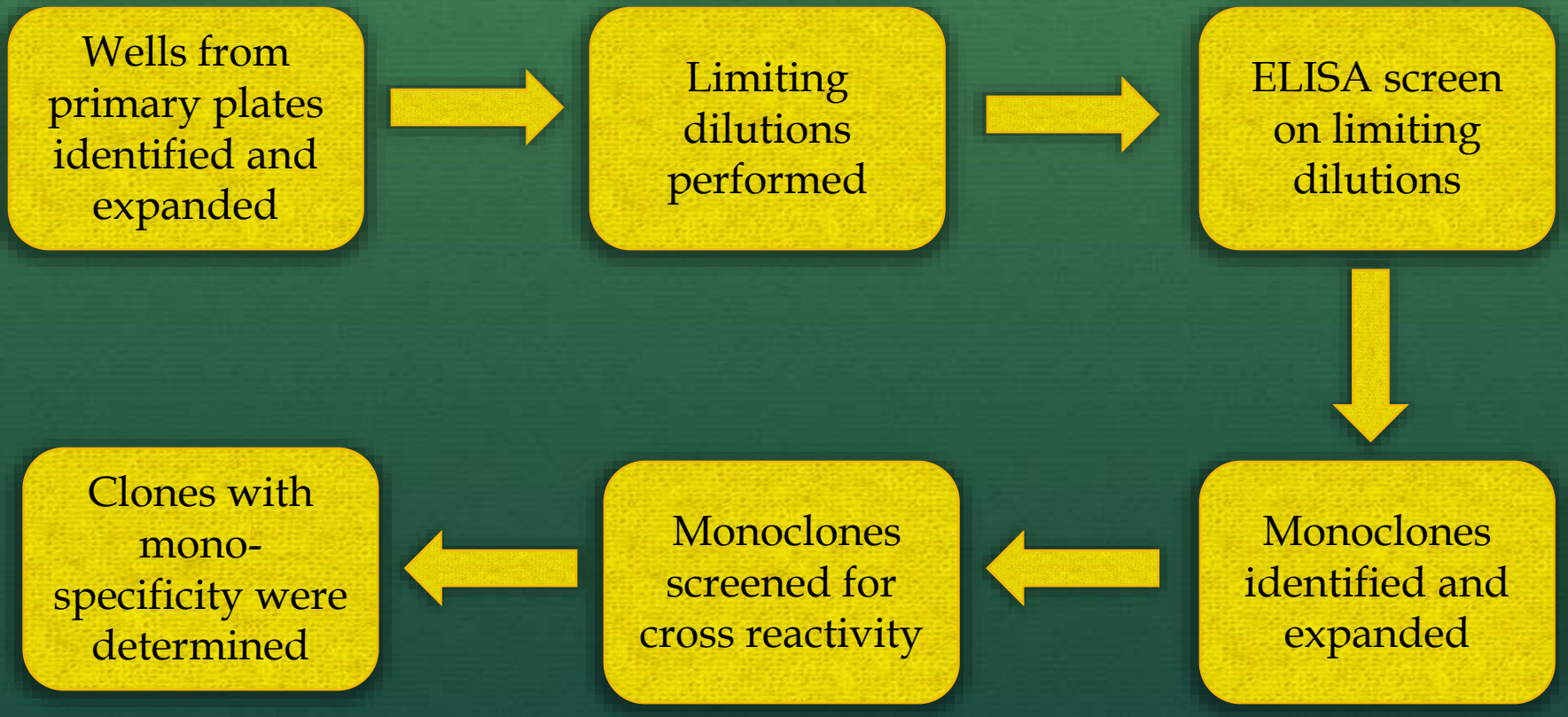
Monoclonal antibodies

Conjugated secondary antibody

Development of blot

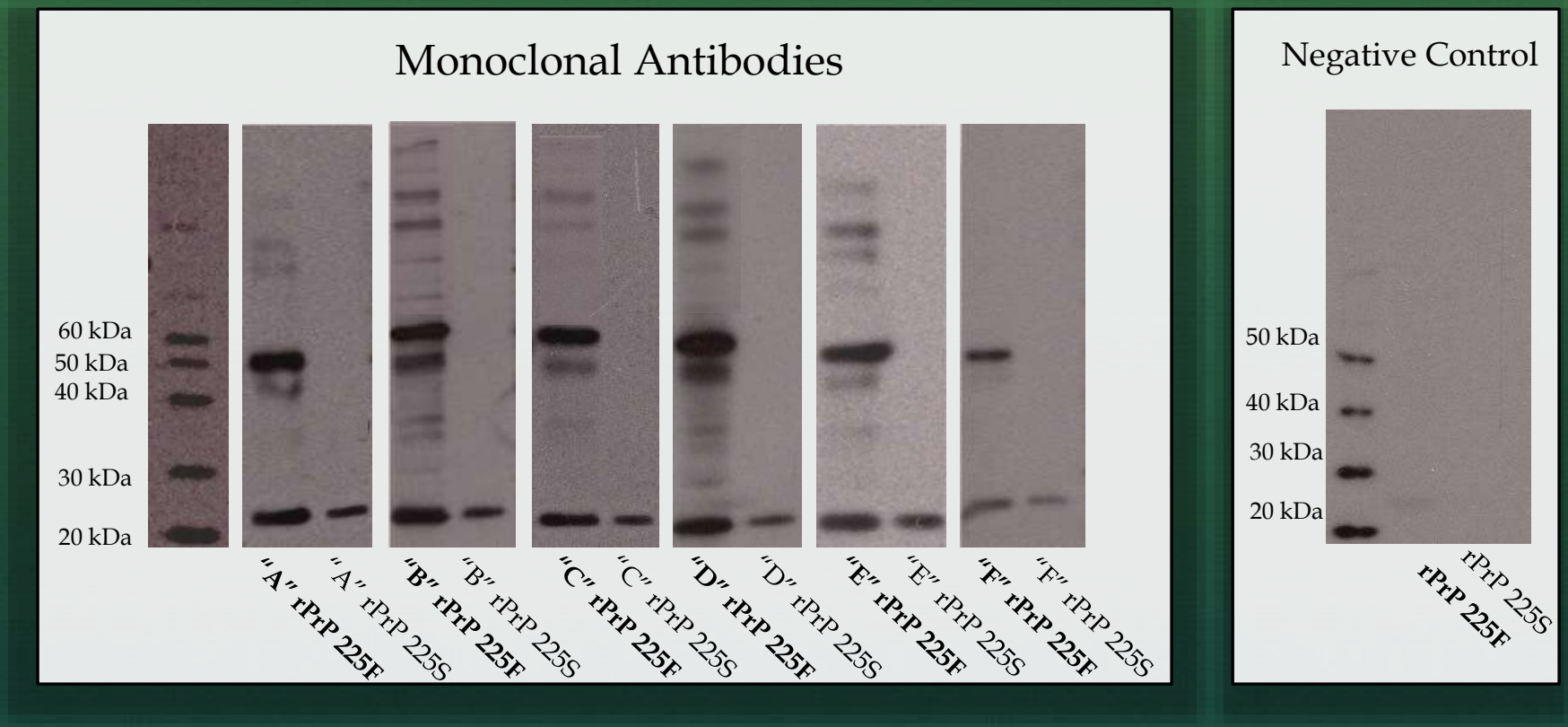
Previous Results

- ELISA



Previous Results

- Western Immunoblots
  - The antibodies do recognize both recombinant alleles



Conclusion

- ELISA – Antibodies recognize but do not distinguish between the two synthetic peptides
- Western Immunoblots – Antibodies recognize but do not distinguish between the two recombinant proteins
- Why did we get these results?

Hypothesis

- Hypothesis 1: We hypothesized that monoclonal antibodies can be produced against rPrP.

- Hypothesis 2: We also hypothesized that the monoclonal antibodies will be capable of distinguishing between the two alleles.

Current Results

- Screening of primary plates
  - 14 F clones
  - 12 S clones
- Limiting Dilutions

Further Work

- Cross reactive ELISA
- Use Western blots to confirm ELISA results
- Can it be used as a marker for PrP in diagnosis? Cost efficient for Wyoming State Vet Lab?

Acknowledgements

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Questions?