



Royal Veterinary College
University of London

Towards eradication of *Mycoplasma hyopneumoniae* from the UK pig herd

Production of an antibody to *Mycoplasma hyopneumoniae*

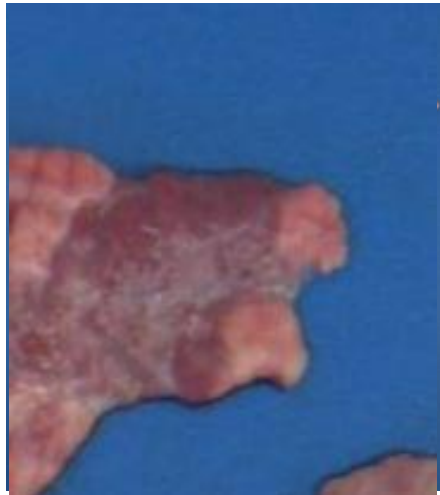
Veronica Brewster

Introduction

- Economically important multifactorial respiratory disease of pigs
- Control and eradication of *M. hyopneumoniae* rely on the availability of good diagnostic tests
 - Current diagnostics have many limitations
- Immunohistochemistry (IHC) identifies *M. hyopneumoniae* within a lung sample
 - Requires an antibody

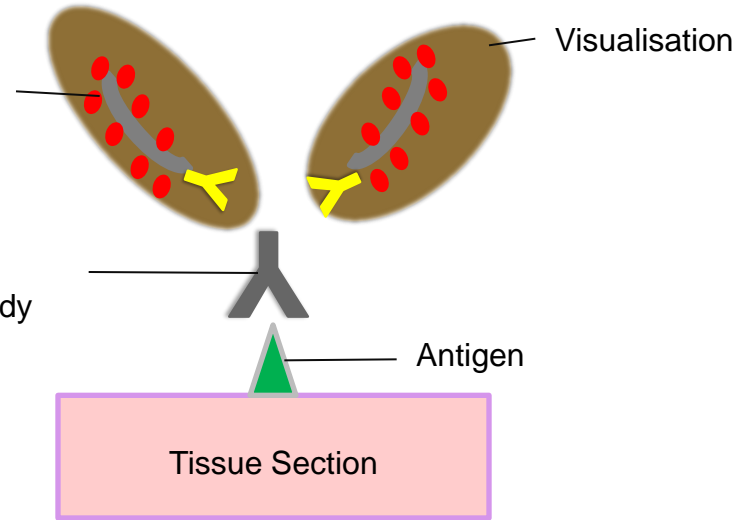


M & M: Background

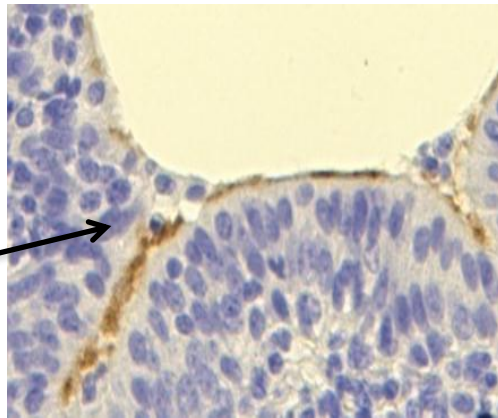


2°
Antibody

1°
Antibody



specific
staining



M & M: Steps to produce a polyclonal antibody

1. Synthesizing DNA by PCR

2. Inserting DNA into vector

3. Grow *E.coli* containing vector

4. Use PCR to confirm presence vector

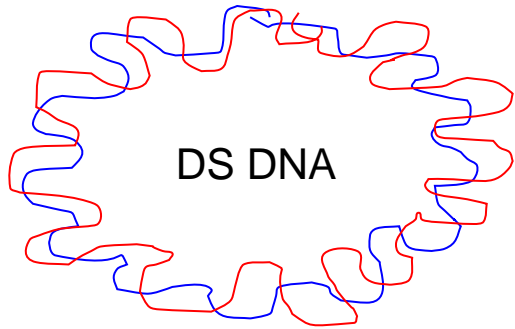
5. Purify vector & Inject into rabbit

6. Harvest serum containing polyclonal antibody

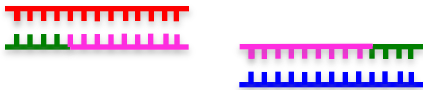
7. Purify polyclonal antibody



M & M: Synthesizing DNA by PCR



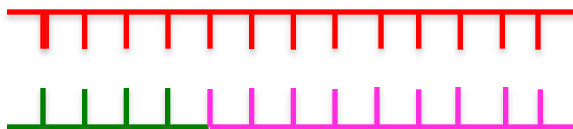
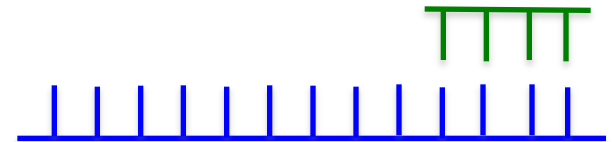
Separate DNA with heat



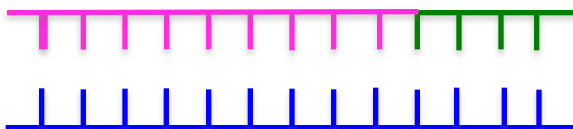
Repeated to produce lots of strands



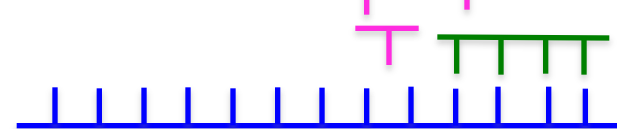
Attach primer to DNA



New DNA strand



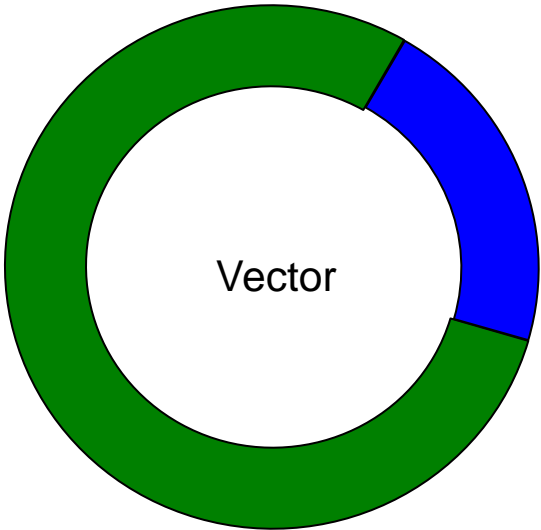
Nucleotides



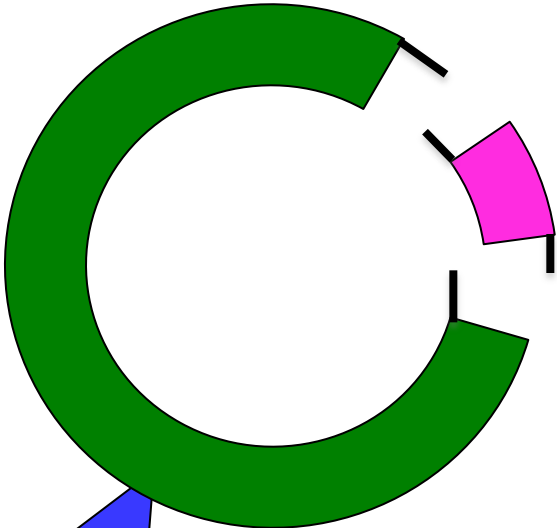
RVC



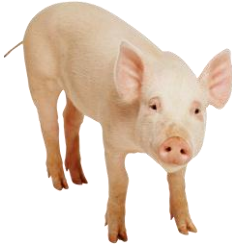
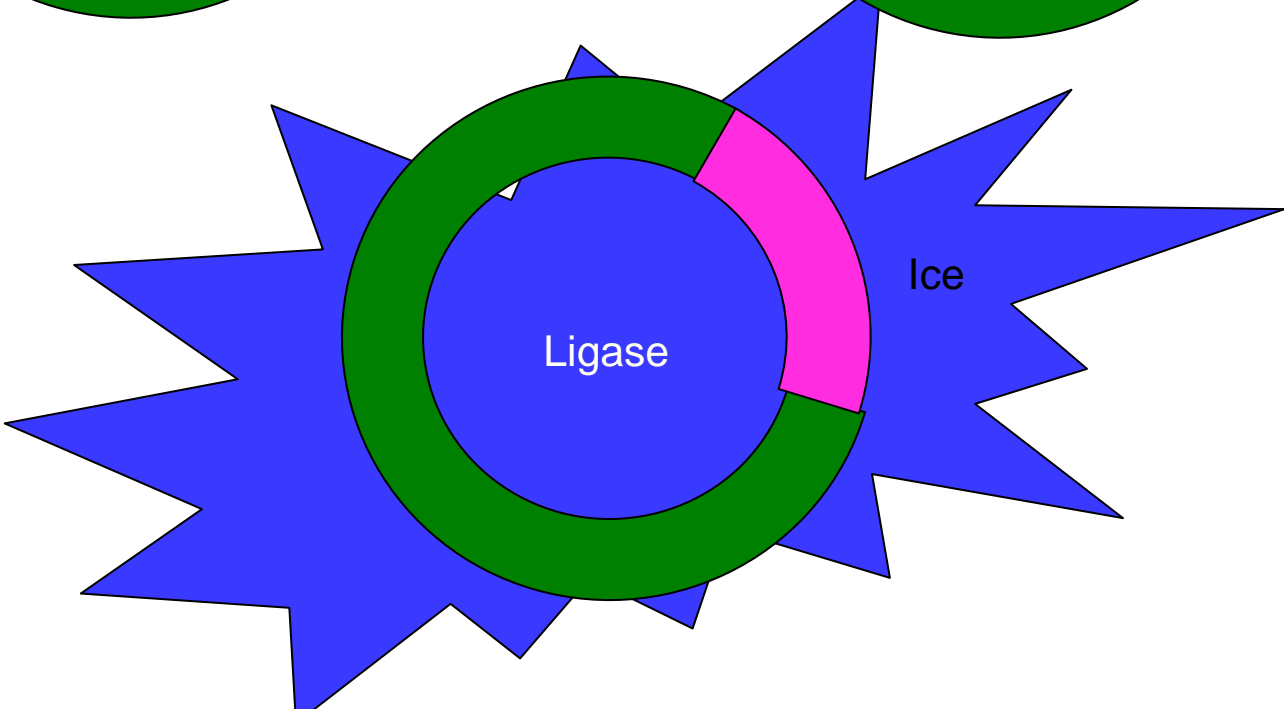
Material and Methods: Vector



Space to
insert
DNA

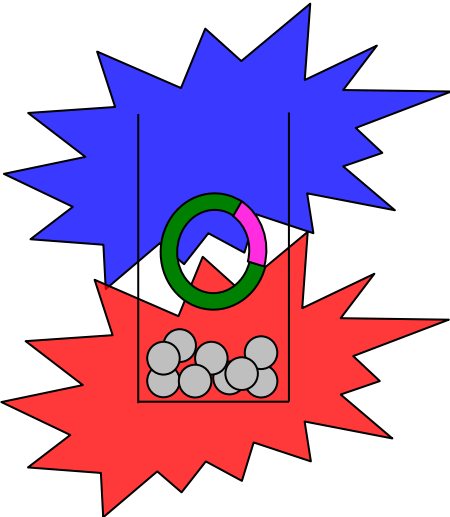


Digestion:
Cut the
ends

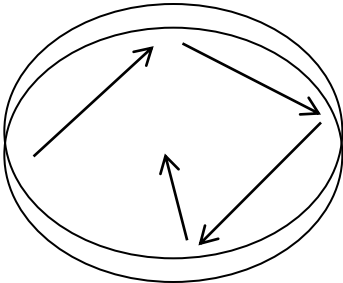


M & M: Growing *E. coli* & Colony PCR

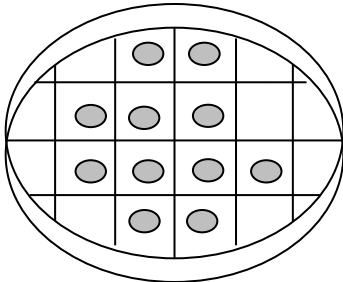
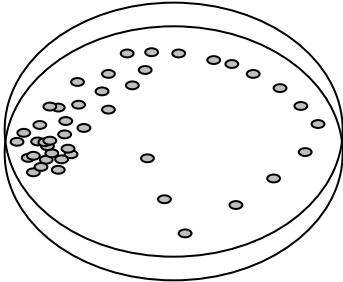
Transformation



Grow *E. coli* and plate



Pick Colonies



PCR

RVC



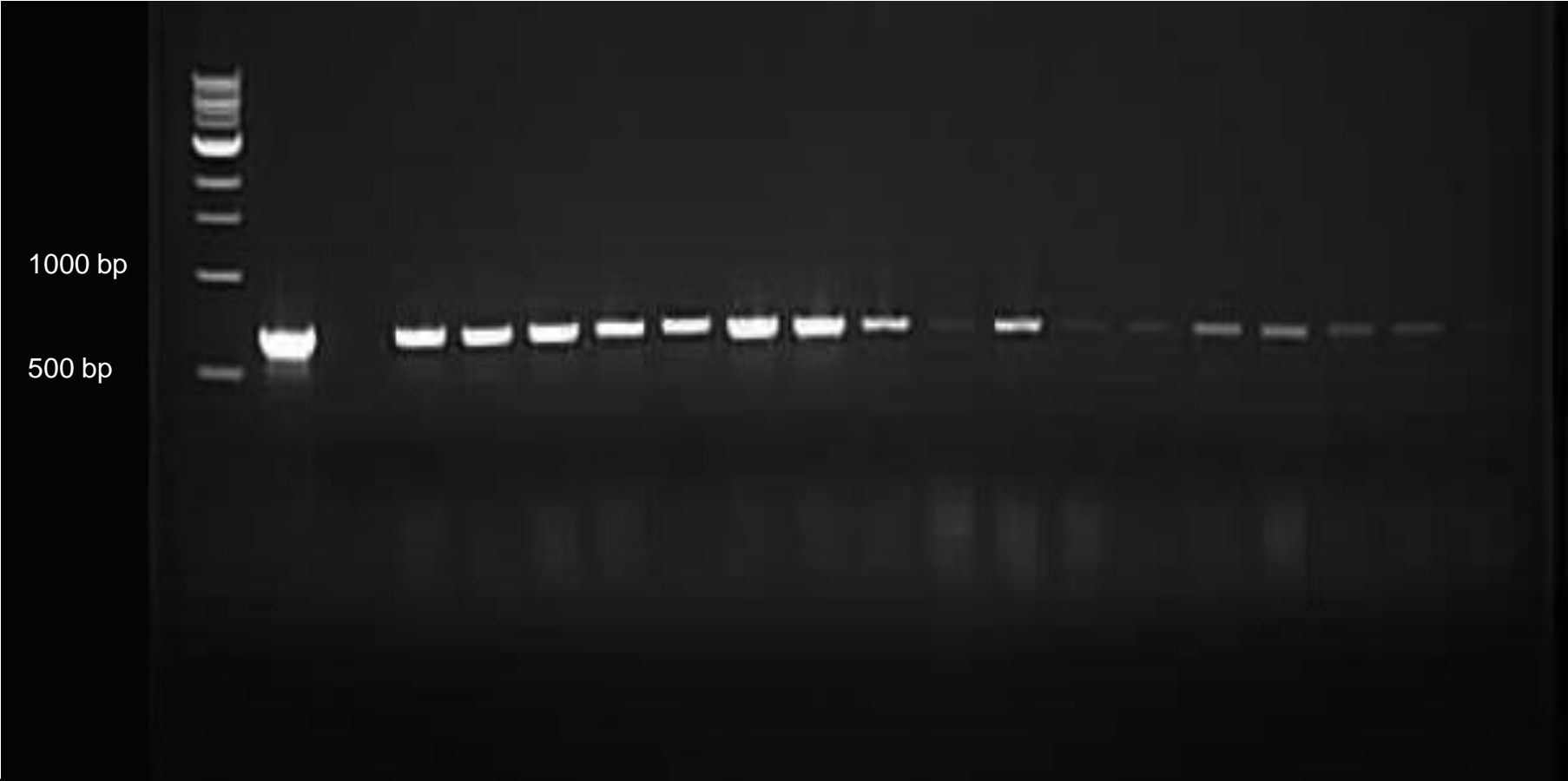
Results

- Synthesized DNA
- Inserted DNA into pMAL Vector
- Transformed DH5 alpha E. coli cells
 - Now contain pMAL vector
- Confirmed with colony PCR



Results Cont

1KB ladder +ve -ve 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



Next Steps

- Complete remaining steps to produce the polyclonal antibody
- Validate antibody using immunohistochemistry (IHC)
- Outsource production of monoclonal antibody



Industry Focus

- Correct diagnosis of EP is crucial
- The availability of a stand alone diagnostic test (IHC) will be invaluable to the industry
- Production of an antibody would ensure the longevity of the IHC
 - Would not have to rely on an outside source



Acknowledgements

- Supervisors
 - Dr Mandy Nevel
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- Sponsors

