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Identification and characterisation of immunoreactive antigens of mycoplasma hyopneumoniae

Jody Gorman
University of Wollongong

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**Identification and Characterisation
of Immunoreactive Antigens of
*Mycoplasma hyopneumoniae***



Jody Gorman (Wilton)
PhD candidate
University of Wollongong
2010

Declaration of Authenticity

This thesis is submitted in accordance with the regulations of the University of Wollongong in fulfillment of the degree of Doctor of Philosophy. It does not include any material previously published by another person except where due reference is made in the text. The experimental work described in this thesis is original, and has not been submitted for a degree to any other university.

Jody Gorman

Acknowledgements

I started this thesis over 12 years ago and two jobs, two kids, two very patient supervisors, an ever-loving family, many supportive friends and one super-supportive, patient and loving husband later, I am FINALLY submitting. That makes for a very long list of acknowledgements!

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A very big thank you to those mentioned and everybody else, who loved, supported, encouraged and helped me get to this point. I don't know what life without the incredible weight of an unfinished thesis is going to feel like but I suspect it will be pretty good!

I dedicate this thesis to my children Harrison and Ava.

Be everything you can be my beautiful babies!

List of Publications

Wilton, JL., Scarman, AL., Walker, MJ., and Djordjevic, SP. (1998) Reiterated repeat region variability in the ciliary adhesin gene of *Mycoplasma hyopneumoniae*. Microbiology. **144**:1931-43.

Matic, JN., **Wilton**, JL., Towers, RJ., Scarman, AL., Minion, CF., Walker, MJ., and Djordjevic, SP. (2003) The pyruvate dehydrogenase complex of *Mycoplasma hyopneumoniae* contains a novel lipoyl domain arrangement. Gene. **319**:99-106.

Djordjevic, SP., Cordwell, SJ., Djordjevic, MA., **Wilton**, JL., and Minion, FC. (2004) Proteolytic Processing of the *Mycoplasma hyopneumoniae* Cilium Adhesin. Infect Immun. **72**:2791-802.

Jenkins, C., **Wilton**, JL., Minion, FC., Falconer, L., Walker, MJ., and Djordjevic, SP. (2006) Two Domains within the *Mycoplasma hyopneumoniae* Cilium Adhesin Bind Heparin. Infect Immun. **74**:481-7.

Wilton, JL., Jenkins, C., Cordwell, SJ., Falconer, L., Minion, CF., Oneal, DC., Djordjevic, MA., Connolly, A., Barchia, I., Walker, MJ., and Djordjevic, SP. (2009) Mhp493 (P216) is a proteolytically processed, cilium and heparin binding protein of *Mycoplasma hyopneumoniae*. Mol Microbiol. **71**:566-582.

Jenkins, C., Seymour, L., Deutscher, A., Falconer, L., Stewart, K., **Wilton**, JL., Minion, FC., Rhode, M., Eamens, G., Redcliffe, L., Walker, MJ., and Djordjevic, SP. (submitted) *Mycoplasma hyopneumoniae* triggers the deposition of fibronectin at the site of colonisation.

Abstract

M. hyopneumoniae is the causative agent of porcine enzootic pneumonia (PEP) and inflicts a severe economic burden on swine production. Colonisation involves adherence of *M. hyopneumoniae* to the porcine respiratory epithelia in a process believed to be mediated by surface proteins capable of binding target cells. This thesis involved the identification and characterisation of several immunoreactive *M. hyopneumoniae* antigens in an effort to increase the current understanding of the surface topography of this organism.

The first antigen identified in this research was the now well-characterised cilium adhesin of *M. hyopneumoniae*, P97. P97 was found in earlier research to contain two regions of reiterated repeats (RR1 and RR2). The repeats were found in this research to differ in number amongst strains of *M. hyopneumoniae* from different geographical localities and did not appear present in related porcine mycoplasmas *M. hyorhinis* or *M. flocculare*. Sequences like the proline rich tandem repeats of 5 and 10 amino acids found in RR1 and RR2 respectively are typically involved in protein-protein interactions. Proline rich repeats are usually highly immunogenic (Williamson, 1994) providing the recombinant 35 kDa antigen (encompassing the repeats) produced in this work with vaccine potential.

The second antigen cloned and characterised in this research was identified as a fragment of *M. hyopneumoniae* pyruvate dehydrogenase E1 α (PdhA). Its intracellular location was indicated in trypsinisation studies of whole *M. hyopneumoniae* but its

identification in the insoluble phase as well as the aqueous phase of triton extractions raises the question of a dual function; a phenomenon reported for PdhA in *M. pneumoniae* (Layh-Schmitt *et al.*, 2000) and for NrdF in *M. hyopneumonia* (Fagan *et al.*, 1996).

The final antigen characterised in this research was identified as a 216 kDa adhesin-like protein of *M. hyopneumoniae* (P216). Four recombinant P216 antigens, covering 75% of the molecule, were cloned, expressed, purified and used to raise antisera in rabbits. P216 was found to be rapidly processed to cleavage fragments P120 and P85, both located on the surface of *M. hyopneumoniae*. A putative transmembrane domain was identified in P120 providing it with a mechanism for cell surface attachment. It has been hypothesized that P85 maintains its association with the cell surface through a KEKE motif and the two regions of glutamine repeats identified in its' sequence. Heparin binding of all three recombinant P216 fragments suggests that P120 contains at least two heparin-binding domains and P85 at least one, but further studies are required to define the exact location of these sites.

This research provided a foundation for continued research on P97 and P216, which remain the only two cilium adhesins of *M. hyopneumoniae* identified to date. Defining the colonisation mechanism of *M. hyopneumoniae* has implications for the treatment and prevention of disease, therefore research in this area provides an improved understanding of pathogenesis and potential vaccine development.

Table of Contents

Declaration	ii
Acknowledgements	iii
Dedication	v
List of Publications	vi
Abstract	vii
Table of Contents	ix
List of Figures	xiii
List of Tables	xv
Abbreviations	xvi
 Chapter One: <i>Mycoplasma. hyopneumoniae</i> as the causative agent of Porcine Enzootic Pneumonia	 1
1.1. The Mycoplasmas	2
1.1.1. As a Genus of the Class Mollicutes	2
1.1.2. Morphology and Ultrastructure	4
1.1.3. Genome Structure and Organisation	6
1.1.3.1. Comparative Genomics	8
1.1.4. Membrane Proteins	10
1.1.4.1. Mycoplasmal Adhesins	12
1.2. <i>Mycoplasma hyopneumoniae</i>	16
1.2.1. Pathogenesis of disease caused by <i>M. hyopneumoniae</i>	17
1.2.1.1. Attachment Mechanism	19
1.2.2. Porcine Enzootic Pneumonia (PEP)	20
1.2.2.1. Treatment and Control of Disease	23
1.2.2.2. Resistance to Reinfection: Cell Mediated vs Humoral Immunity	25
1.2.3. Vaccination Against PEP	27
1.2.3.1. Commercial Vaccines	28
1.2.3.2. Experimental Vaccines	30
1.2.3.3. Recombinant DNA and Sub-unit Antigen Vaccines	31
1.3. Aims and Objectives	35
 Chapter Two: General Material and Methods	 36
2.1. Materials	37
2.1.1. Biological Material	37
2.2. Methods	40
2.2.1. Culture Methods	40
2.2.1.1. Bacterial Cell Culture	40
2.2.1.2. Mycoplasma Cell Culture and Harvest	40
2.2.2. Methods for DNA Analysis	41
2.2.2.1. Extraction of Genomic DNA from Mycoplasma	41
2.2.2.2. Extraction of Plasmid DNA from Bacteria	42
2.2.2.3. Genomic DNA Digestion	43
2.2.2.4. Plasmid DNA Digestion	43
2.2.2.5. Oligonucleotide primers	44
2.2.2.6. Polymerase Chain Reaction (PCR)	44
2.2.2.7. Agarose Gel Electrophoresis	44

2.2.2.8.	Purification of DNA from Agarose	45
2.2.2.9.	Preparative Agarose Gel Electrophoresis for DNA Fractionation	46
2.2.2.10.	Southern and Colony Hybridisations	47
2.2.2.11.	Cloning into pPCR-Script	48
2.2.2.12.	Cloning into pQE9 for expression in <i>E. coli</i>	49
2.2.2.13.	Cloning from N-terminal Sequence Data	50
2.2.2.14.	DNA Sequence Analysis	50
2.2.2.15.	DNA sequence Assembly and Bioinformatics Analysis	52
2.2.3.	Methods for Protein Analysis	52
2.2.3.1.	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)	52
2.2.3.2.	Preparation of Antisera	53
2.2.3.3.	Immunoblot Analysis	54
2.2.3.4.	Two-Dimensional Gel and Immunoblot Analysis	55
2.2.3.5.	N-terminal Protein Microsequencing	56
2.2.3.6.	Protein Expression and Purification	56
2.2.3.7.	Trypsin Treatment of <i>M. hyopneumoniae</i> Cells	57
2.2.3.8.	Triton X-100 and X-114 Extractions	57
Chapter Three:	Cloning and Characterisation of the Strain J Cilium Adhesin	59
3.1.	Introduction	60
3.2.	Methods	63
3.2.1.	Random Cloning and Preliminary Characterisation of the 3' end of the Strain J Adhesin Gene	63
3.2.1.1.	Cloning of the 3' end of the <i>M. hyopneumoniae</i> strain J Adhesin Gene	63
3.2.1.2.	Protein Expression and Purification of the 28 kDa Adhesin Antigen	64
3.2.1.3.	Preparation of Antisera Against the 28 kDa Adhesin Antigen	64
3.2.2.	Cloning and Sequence Analysis of the 5' end of the Strain J Adhesin Gene	65
3.2.3.	Nucleotide Sequence Accession Number of the J Strain Adhesin	66
3.2.4.	Cloning and Sequence Analysis of the Reiterated Repeat Regions	66
3.2.5.	Southern Hybridisation Analysis Using a Radiolabelled Probe	67
3.2.6.	Sub-unit Antigen ELISA	67
3.3.	Results	69
3.3.1.	Preliminary Analysis of the <i>M. hyopneumoniae</i> Strain J Adhesin	69
3.3.1.1.	Surface location of the <i>M. hyopneumoniae</i> Strain J Adhesin	69
3.3.1.2.	Strain Variation in the Adhesin Antigen	71
3.3.1.3.	Repeat Region Variability Leading to Strain Variation in the Adhesin	73
3.3.2.	Cloning and Sequence Analysis of the 5' end of the <i>M. hyopneumoniae</i> Strain J Cilium Adhesin Gene	75
3.3.2.1.	Cloning of the 5' end of the Gene Encoding the J Strain Adhesin	75
3.3.2.2.	Sequence Analysis of the J Strain Adhesin	77
3.3.3.	Computational Analysis of the Deduced Amino Acid Sequence of the J Strain Adhesin	79
3.3.4.	Alignment of P94 with P97 and Mhp1	81
3.3.5.	Cloning and Sequence Analysis of the Repeat Regions from Geographically Diverse Strains of <i>M. hyopneumoniae</i>	83

3.3.6.	Expression of an Adhesin Antigen Encompassing RR1 and RR2	85
3.3.6.1.	Primer Design and PCR Amplification	86
3.3.6.2.	Cloning for Expression	86
3.3.7.	Immunogenicity of the Adhesin Antigen	90
3.4.	Discussion	92
Chapter Four: Cloning and Characterisation of an Immunoreactive Antigen of <i>M. hyopneumoniae</i>		98
4.1.	Introduction	99
4.2.	Methods	101
4.2.1.	Preparation of Antisera Against the Recombinant Antigen	101
4.3.	Results	102
4.3.1.	Selection of the Immunoreactive Antigen and N-terminal Sequencing	102
4.3.2.	Southern Hybridisation Analysis Using the Degenerate Oligonucleotide	102
4.3.3.	Cloning and Preliminary Characterisation	104
4.3.3.1.	DNA Fractionation and Southern Hybridisation Analysis	106
4.3.3.2.	Cloning the Gene Encoding the Immunoreactive Antigen	106
4.3.3.3.	DNA Sequence Analysis of pJW3	109
4.3.3.4.	Alignment of Translated PdhA Clone Sequence with Homologous Proteins	109
4.3.3.5.	Sequence Analysis of the Genes Encoding PdhA and PdhB in <i>M. hyopneumoniae</i>	111
4.3.3.6.	Southern Hybridisation analysis of <i>pdhA</i> and <i>pdhB</i> in Strains of <i>M. hyopneumoniae</i> and Related Porcine Mycoplasmas <i>M. hyorhinis</i> and <i>M. flocculare</i>	114
4.3.4.	Further Characterisation of PdhA	116
4.3.4.1.	Cloning for Expression of a Recombinant PdhA Antigen	116
4.3.4.2.	Induction and Purification of the PdhA antigen	118
4.3.4.3.	Production of Rabbit Antiserum Against the PdhA Antigen	120
4.3.4.4.	Cellular location of PdhA	120
4.3.4.5.	Presence of PdhA Among Geographically Diverse Strains of <i>M. hyopneumoniae</i> and Related Porcine Mycoplasmas	122
4.3.4.6.	Inability of Naturally Infected Pigs to Recognise Recombinant PdhA	122
4.4.	Discussion	126
Chapter Five: Cloning and Characterisation of a 216 kDa Adhesin-like Protein of <i>M. hyopneumoniae</i>		132
5.1.	Introduction	133
5.2.	Methods	135
5.2.1.	Tryptic Digestion and Selection of Fraction 2 Antigens	135
5.2.2.	PCR	135
5.2.2.1.	Inverse PCR	135
5.2.2.2.	Gradient PCR	136
5.2.3.	Cloning into pET161	137
5.2.4.	Post Two-Dimensional Gel Separation Analysis	138
5.2.5.	Heparin binding	139
5.2.5.1.	Heparin blots	139
5.2.5.2.	Heparin binding assays	139
5.2.6.	Growth Assay	141

5.3.	Results	142
5.3.1.	Cloning and Preliminary Analysis	142
5.3.1.1.	Southern Hybridisation Analysis with the Degenerate Oligonucleotide	142
5.3.1.2.	DNA Fractionation and Southern Hybridisation Analysis Identifying the Fragment for Cloning	144
5.3.1.3.	Cloning the Fragment into pPCR-Script™	144
5.3.1.4.	Sequence Analysis of the Cloned Fragment	146
5.3.2.	Inverse PCR to Obtain the Full Gene Sequence	148
5.3.2.1.	Digestion of Chromosomal DNA and Southern Hybridisation	148
5.3.2.2.	Inverse PCR	150
5.3.2.3.	DNA Sequence Analysis of the Amplified Inverse PCR Product	150
5.3.3.	Access to <i>M. hyopneumoniae</i> Genome Database to Sequence the 5' End of the Novel Gene	153
5.3.3.1.	Amplification and DNA Sequence Analysis of the 5' End of the p216 Gene from Strain Beaufort	153
5.3.3.2.	DNA Sequence Assembly and Analysis of the p216 Gene from Strain Beaufort	154
5.3.3.3.	Protein Analysis of P216 from strain Beaufort	156
5.3.4.	Alignment of P216 from Strains Beaufort, 232, J and 7448	158
5.3.5.	Cloning and Expression of a C-terminal P216 Antigen from Strain Beaufort	163
5.3.5.1.	Primer Design and PCR Amplification	163
5.3.5.2.	Cloning for Expression	165
5.3.5.3.	Expression of the P216 Antigen	167
5.3.6.	Cloning and Expression of P216 Antigens from Strain 232	167
5.3.6.1.	Primer Design and PCR Amplification	169
5.3.6.2.	Cloning for Expression	169
5.3.6.3.	Expression of the P216 Antigens	170
5.3.6.4.	Purification of the P216 Antigens	172
5.3.7.	Generation of P216 Antiserum in Rabbits	172
5.3.8.	Proteomic Analysis of P120 and P85	174
5.3.9.	Characterisation of P216	177
5.3.9.1.	Presence of P216 Among Geographically Diverse Strains of <i>M. hyopneumoniae</i> and Related Porcine Mycoplasmas	177
5.3.9.2.	Cellular location of P216	179
5.3.9.3.	2D Immunoblotting Studies of P120 and P85	179
5.3.9.4.	Processing of P216 During Different Stages of the Growth Cycle	182
5.3.9.5.	Heparin Binding of P216	182
5.4.	Discussion	187
	Chapter Six: General Discussion and Future Work	194
	References	202
	Appendices	216

List of Figures

Figure	Page
Chapter 1: <i>Mycoplasma. hyopneumoniae</i> as the causative agent of Porcine Enzootic Pneumonia	
1.1 A phylogenetic tree showing the taxonomy of the mycoplasmas and their relatedness to Gram-positive bacteria.	3
1.2 Positioned of <i>M. hyopneumoniae</i> on the ciliated tufts among the porcine cilia.	18
Chapter 3: Cloning and Characterisation of the Strain J Cilium Adhesin	
3.1 Gene map of the cloned <i>M. hyopneumoniae</i> fragment of pAS1.	70
3.2 Surface location and strain variation of the cilium adhesin.	72
3.3 Repeat region variability in the adhesin antigen.	74
3.4 Gene map of the adhesin showing primers used for PCR and sequencing	76
3.5 PCR amplification and cloning of the 5' end of the adhesin gene.	78
3.6 Computational analysis of the deduced amino acid sequence of the strain J adhesin.	80
3.7 Protein alignment of the J strain adhesin with P97 and MHP1	82
3.8 Predicted amino acid sequences of PCR amplification products spanning RR1 and RR2 from eight strains of <i>M. hyopneumoniae</i> originating from different geographic localities.	84
3.9 Amplification of an adhesin gene fragment for cloning and expression.	87
3.10 Cloning the adhesin gene fragment for expression and purification.	89
3.11 Potential of the adhesin as a vaccine antigen.	91
Chapter 4: Cloning and Characterisation of an Immunoreactive Antigen of <i>M. hyopneumoniae</i>	
4.1 Selection of antigen for cloning from a 2D immunoblot of TX114 extracted <i>M. hyopneumoniae</i> with porcine convalescent sera.	103
4.2 Southern hybridisation analysis of the degenerate oligonucleotide against <i>EcoRI</i> digested genomic DNA from <i>M. hyopneumoniae</i> .	105
4.3 Agarose gel electrophoresis and Southern hybridisation analysis for identification of the fragment of interest in <i>EcoRI</i> digested and fractionated <i>M. hyopneumoniae</i> DNA.	107
4.4 Colony and Southern hybridisation analysis to confirm cloning.	108
4.5 Sequence analysis of the 2.5 kb clone insert.	110
4.6 ClustalW alignment of translated PdhA clone sequence with homologous mycoplasma proteins.	112
4.7 Southern hybridisation analysis of <i>pdhA</i> and <i>pdhB</i> in strains of <i>M. hyopneumoniae</i> and related porcine mycoplasmas.	115
4.8 Amplification of a <i>pdhA</i> gene fragment for expression.	117
4.9 Cloning for expression of a PdhA antigen.	119
4.10 Purification of the PdhA antigen and generation of antiserum in rabbits.	121
4.11 Cellular location of PdhA in <i>M. hyopneumoniae</i> .	123
4.12 Presence of PdhA among geographically diverse strains of <i>M. hyopneumoniae</i> and related porcine mycoplasmas.	124

Chapter 5: Cloning and Characterisation of a 216 kDa Adhesin-like Protein of *M. hyopneumoniae*

5.1	Southern hybridisation analysis of the degenerate oligonucleotide against <i>Eco</i> RI digested genomic DNA from <i>M. hyopneumoniae</i> .	143
5.2	Agarose gel electrophoresis and Southern hybridisation analysis for identification of the fragment of interest in <i>Eco</i> RI digested and fractionated <i>M. hyopneumoniae</i> DNA.	145
5.3	Colony and Southern hybridisation analysis to confirm cloning.	147
5.4	Sequence analysis of the 3.7 kb <i>Eco</i> RI insert of pJW6.	149
5.5	Restriction of Beaufort DNA for Inverse PCR.	151
5.6	Inverse PCR to obtain the entire gene sequence encoding the novel antigen.	152
5.7	PCR of the 5' end of the novel gene from strain Beaufort.	155
5.8	Prediction of a transmembrane domain in P216.	157
5.9	Amino acid alignment of P216 from strain Beaufort, 232, J and 7448.	159
5.10	PCR of a <i>p216</i> gene fragment for cloning and expression.	164
5.11	Cloning of a <i>p216</i> fragment into pQE9 for expression.	166
5.12	Cloning P216 antigens from <i>M. hyopneumoniae</i> strain 232.	168
5.13	Cloning and expression of <i>p216</i> fragments from <i>M. hyopneumoniae</i> strain 232.	171
5.14	Purification of P216 fragments from strain 232..	173
5.15	Generation of P216 antisera.	175
5.16	Peptide mass mapping of P216.	176
5.17	Immunoblot analysis of cell lysates of different strains of <i>M. hyopneumoniae</i> and related porcine mycoplasmas.	178
5.18	Localization of P216 cleavage products on the surface of <i>M. hyopneumoniae</i> .	180
5.19	Two-dimensional gel electrophoresis and immunoblot analysis of P216 cleavage products P120 and P85.	181
5.20	Immunoblot of cell lysates of synchronised cultures of <i>M. hyopneumoniae</i> strain J.	183
5.21	Binding of P216 to biotinylated heparin in ligand blots.	185
5.22	Binding of heparin to recombinant fragments F1 _{P216} –F3 _{P216} .	186
Appendices		
A1	Circular map and polylinker sequence of the pQE9 vector (Qiagen).	220
A2	Circular map and polylinker sequence of the pPCR-Script Amp SK(+) cloning vector (Stratagene).	221
A3	Circular map and polylinker sequence of the pCR2.1 cloning vector (Invitrogen).	222
A4	Circular map and polylinker sequence of the pET161 cloning Vector (invitrogen).	223

List of Tables

Table		Page
Chapter 1: <i>Mycoplasma. hyopneumoniae</i> as the causative agent of Porcine Enzootic Pneumonia		
1.1	Surface proteins observed in a number of mycoplasma species.	12
Chapter 2: General Material and Methods		
2.1	Bacterial strains used in this study.	37
2.2	Plasmids used in this study.	38
2.3	Mycoplasma species and strains used in this study.	39
Chapter 4: Characterisation of <i>M. hyopneumoniae</i> ribosomal protein L7/L12		
4.1	N-terminal sequence data and degenerate oligonucleotide probe generated for cloning the gene encoding the antigen of interest.	102
Chapter 5: Cloning and Characterisation of a 216 kDa Adhesin-like Protein of <i>M. hyopneumoniae</i>		
5.1	N-terminal sequence data and degenerate oligonucleotide probe generated for cloning the gene encoding the antigen of interest.	142

List of Abbreviations

ABTS	2,2'-azino-bis(3-ethylebenzthiazoline-6-sulfonic acid) diammonium salt
ANGIS	Australian National Genomic Information Service
ATCC	American Type Culture Collection
bp	base pairs
CSPD	disodium 3-(4-methoxy spiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-yl)phenylphosphate
COG	clusters of orthologous groups
DAB	diaminobenzidine
DIG	digoxigenin
DMEM	Dulbecco's modified Eagle medium
DNA	deoxyribonucleic acid
°C	degrees celsius
2D	two-dimensional
ECM	extracellular matrix
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme linked immunosorbent assay
FCS	foetal calf serum
g	gram
<i>g</i>	g forces for centrifugation
GAGs	glycosaminoglycans
h	hour
IFN-γ	interferon gamma
Ig	immunoglobulin
IPTG	isopropyl-β-D-thiogalactopyranoside
kb	kilobase pairs
kDa	kilo daltons
L	litre
LB	Luria Bertani
m	milli
M	molar
mA	milliamps
MALDI-TOF	matrix-assisted laser desorption ionization-time-of-flight
μ	micro
min	minute
mL	milliliter
MS	mass spectrometry
MSCRAMMs	microbial surface components recognizing adhesive matrix molecules
n	nano
NCBI	National Centre for Biotechnology Information
NrdF	R2 sub-unit of ribonucleotide reductase
OD	optical density
ORF	open reading frame
PBS	phosphate buffered saline
PBST	phosphate buffered saline with tween 20
PCR	polymerase chain reaction
Pdh	pyruvate dehydrogenase

PEP	porcine enzootic pneumonia
pH	picohenry
PRDC	porcine respiratory disease complex
PRR's	proline rich repeats
PVDF	polyvinylidene difluoride
rpm	revolutions per minute
RR1	repeat region 1
RR2	repeat region 2
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
RT	room temperature
sec	second
TBE	tris-borate EDTA
TBS	tris buffered saline
TCA	tricarboxylic acid
TX-100	triton X-100
TX-114	triton X-114
V	volts
v/v	volume per volume
w/v	weight per volume