RESEARCH REPORT
SWINE HEALTH

Title: Development of protein based ELISA for the rapid strain specific identification of PRRSV and responding to novel emerging variants. **NPB #06-132**

Investigator: Dr. Julian A. Hiscox

Institution: Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK

Date Submitted: 30th May 2008.

Industrial Summary:

The aim of this project was to develop and evaluate diagnostic reagents, tools and methodologies to identify both generic and specific strains of PRRSV as well as future proofing this technology for the rapid identification of new and emerging strains. The plan of work and objectives consisted of expressing different PRRSV proteins which might be used to detect strain specific and non-specific variants. The candidate proteins selected were the virus genome binding protein (nucleocapsid (N) protein) and surface proteins of the virus particle (e.g. GP5 protein). Apart from traditional expression technologies the focus of this research was to pioneer the use of recently developed rapid *in* vitro (i.e. test tube) protein expression systems to demonstrate proof of principal in identifying new strains of PRRSV. Traditional virus protein expression technologies were used to produce differentially modified proteins (as occurs in the infected cell) for use in ELISA and to act as comparison for proteins produced in the rapid expression systems. The data indicated that whilst protein modification resulted in increases in efficiency for ELISA detection, the rapid protein expression system was by far the most efficient way of producing protein quickly. Indeed from receipt of viral gene sequence purified protein suitable for ELISA was produced within 5 days in the rapid system, compared to approximately up to a month for lead in times for conventional protein expression. As highlighted in our publication describing rapid protein expression, this technology thus holds great promise for responding rapidly to detect new and emerging strains of PRRSV. Future work highlighted in the grant and planned for these PRRSV proteins was to characterize them at the cell biological level in order to better understand their roles in the life cycle of PRRSV and for exploiting these biological properties for vaccine development. This work was conducting in collaboration with the bio-imaging unit at the University and was the first to characterize the real time trafficking of N protein around the cell. As highlighted our the publication of this work, this information can be rapidly exploited for the development of growth attenuated recombinant vaccines.

Contact: Dr. Julian A. Hiscox, University of Leeds, j.a.hiscox@leeds.ac.uk.

Scientific Abstract:
The major aim of this project was to develop and evaluate diagnostic reagents, tools and methodologies to identify both generic and specific strains of PRRSV as well as future proofing this technology for the rapid identification of new and emerging strains. The project focused on evaluating and characterising various virus proteins and protein expression systems for use in ELISA. Two proteins were immediately selected as being of interest. The viral RNA binding protein, nucleocapsid (N) protein and one of the major virus surface glycoproteins, GP5 protein. These undergo post-translational modification in the host cell. Many protein based ELISAs are based on E.coli expressed protein and do not take this into account. Using gene sequence provided by our collaborator at the University of Nebraska-Lincoln, the respective genes were cloned into a variety of different expression vectors for evaluation of different ways of making modified and unmodified protein. In the first methodology, proteins were expressed using two different vector systems utilising a histidine tag (for nickel affinity purification) in E.coli, which is commonly used for expression strategies. In the second methodology these proteins were expressed in insect (Sf9) cells and mammalian cells. This had the added advantage over E.coli based systems that the proteins would be modified post-translation and thus would mimic those found in virus infected cells and perhaps would have greater immunogenic coverage. In the third methodology proteins were expressed with fluorescent labelled tags for identification and isolation in mammalian cell culture and subsequent enrichment using fluorescent activated cell sorting (FACS) to select positive expressing cells. In the fourth methodology proteins were expressed using rapid large scale *in vitro* translation systems which have recently been developed. Although unmodified proteins are produced, these systems offered the added advantage of rapid protein expression and purification as many of the gene cloning steps could be eliminated as well as some of the purification steps. In addition, recombinant protein expression in bacteria/cell based systems often fails or is less than efficient due to toxicity; the *in vitro* translation systems eliminated this concern. A large number of reagents in terms of gene expression constructs for the N and GP5 proteins were generated in this project including several different truncation variants of N protein. All of the resulting proteins had different tags to aid purification, for example from histidine tags to fluorescent labelled proteins for enriching positive expressing cells and immuno-affinity purification. Good protein yields were generated from histidine tagged proteins expressed in E.coli and insect cells, and from mammalian cells. Although proteins were obtained from enriched cells expressing fluorescent labelled tags, yields were low. Poor production of proteins in mammalian cells was probably a result of protein toxicity rather than an expression/purification problem *per se*. Overall, high level expression and purification of both histidine tagged N protein and GP5 protein was obtained. Evaluation as reagents in ELISA using sera from PRRSV infected swine indicated that although the modified proteins expressed in mammalian cells were the better immunogenic reagent the rapid and efficient production of proteins using the *in vitro* translation system may outweigh this, as highlighted in our publication describing these methodologies. Finally, live cell imaging data utilising the fluorescent labelled N proteins defined trafficking of the protein in the cell in real-time - the first description of this. As highlighted in our publication of this work, this information can be rapidly exploited for the development of growth attenuated recombinant vaccines.

Introduction:

This aim of this project was to develop diagnostic reagents and tools to identify both generic and specific strains of PRRSV as well as future proofing this technology for the rapid identification of new and emerging strains. The plan of work consisted of expressing different PRRSV proteins for use in ELISA. The candidate proteins selected were the nucleic acid binding protein, nucleocapsid (N) protein (which is conserved) and thus may potentially detect all strains and a surface protein of the virus particle (GP5 protein), which is variable and therefore may be specific. Apart from traditional expression technologies this application pioneered the use of recently developed rapid protein expression systems to demonstrate proof of principal in identifying new strains of PRRSV using protein based ELISA. As part of these expression systems, the two proteins were characterised in the mammalian cell.

The outcome of the project is science that can be directly applied to the marketing of protein based ELISA kits for the rapid use in the identification of new PRRSV threats. Scientifically the project investigated whether different modifications to proteins in the infected cell alter their antigenicity – and hence ability to be used as reagents. In addition, reagents in the form of purified protein were generated for the further characterization of PRRSV by structural molecular and cellular biology in the wider PRRSV research community. These reagents have been exchanged between the two collaborating sites and as part of the publications generated are freely available to the wider academic community.

Objectives (from the proposal):

Objective One: Expression and purification of post-translationally modified and non-modified PRRSV proteins by expression in E.coli, Sf9 (insect) and mammalian cells (porcine and non-porcine cell lines). The proteins selected are the nucleocapsid and GP5 proteins. This objective will generate proteins for testing via ELISA-Objective Three.

Objective Two: Expression and purification of proteins via Proteomaster technology. This will generate proteins for testing via ELISA – Objective Three.

Objective Three: Comparison of the relative efficiency of modified and non-modified proteins to detect strain variants of PRRSV and whether proteins generated using Proteomaster technology are compatible for this technology. This will demonstrate that protein based ELISA can be used for the differential identification of PRRSV strains and can be used for the rapid identification of new and emerging strains.

Materials & Methods:

Gene cloning and validation: The genes encoding the N and GP5 glycoprotein of PRRSV strain FL12 derived from the University of Nebraska-Lincoln highly pathogenic PRRSV infectious clone were sub-cloned into a TA vector (Invitrogen) by PCR. The rationale for selecting this suite of proteins is that N protein, as it is an RNA binding protein, is moderately conserved and depending on modification could detect all variants of PRRSV, whereas the membrane and GP5 proteins are more variable and therefore have the potential to be strain specific. All of these proteins produce an immune response, e.g. GP5 protein. The primers used in the PCR stage contained appropriate restriction enzymes for the restriction and ligation of the PRRSV gene from the TA-vector to a modified form of the pTri-Ex vector (Novagen) (this two-step approach was more robust than direct PCR cloning). This vector allowed for the production of recombinant protein in E.coli, insect (Sf9) and mammalian (MARC-145, LLC-PK1 and 3D4/31) cells through the use of appropriate promoters for each cell type. All vectors were sequenced in both directions and the resulting protein evaluated by SDS-PAGE using Coomasie and silver staining as well as western blot (using his-tagged antibody). Additionally we used several other cloning methodologies to maximize project success; including a vector which was for expression in E.coli only (pDest, Invitrogen) and fine tuned for satisfying Objective Two and also a vector which added a fluorescent tag(s) to the appropriate protein (pEGFP, pECFP and pDsRed, Clontech) for fluorescent activated cell sorting.

Protein expression and purification: To express proteins in Sf9 cells, pTriEx vectors were used to produce a recombinant baculoviruses using a variation of the FasBac system (Invitrogen). To obtain purified protein from E.coli Tuner (DE3) pLacI (Novagen) cells were transformed with the appropriate vectors and, protein expression was induced with IPTG (isopropyl-β-D-thiogalactopyranoside). Mammalian cells were transfected with the appropriate vectors using either Lipofectamine 2000 or PEI. Sf9 cells were infected with the recombinant baculoviruses and incubated for 72 h. In all cases, cells were harvested by centrifugation, resuspended in 20 mM phosphate pH 7.4, 0.5 M NaCl, 10 mM imidazole, and sonicated. The supernatant was collected after centrifugation at 20,000 g for 30 min, and treated with RNase A (10 μg/ml, Sigma) for 1 h on ice before loading onto Ni2+-NTA column (Novagen). Unbound and non-specifically bound proteins were washed

away with serial dilutions (10, 50, 100, 150 mM) of imidazole in 20 mM phosphate pH 7.4, 0.5 M NaCl. The recombinant proteins were eluted with 20 mM phosphate pH 7.4, 0.5 M NaCl, 200 mM imidazole. The protein concentration was determined by spectroscopy at OD280 using elute buffer as blank reference. If necessary either gel filtration was used to further purify the protein of interest or column chromatography for separation of proteins between 3-70kDa. Eluted fractions were analyzed for purity by SDS-PAGE.

In vitro translation: The Roche ProteoMaster instrument was used for RTS 100 and RTS 500 scale protein preparations.

Fluorescent activated cell sorting and confocal microscopy: Fluorescent activated cells sorting (FACS) on a BD FACSAria was used to enrich cells expressing tagged N or GP5 proteins. These proteins were further characterised by confocal microscopy using live cell imaging to confirm expression and biological activity. Fluorescent loss in photo-bleaching (FLIP) and FRAP (fluorescent recovery after photo-bleaching) was used to analyse fluorescent labelled N protein in real time.

Results:

Objective 1: For expression and purification of N protein and GP5 protein in E.coli, insect, mammalian and rapid translation systems several different expression vectors were generated and these are detailed in Table 1 for N protein and Table 2 for GP5 protein. (Note that several truncation variants of N protein were generated and these are currently being characterised. The rationale for this is to test the hypothesis that N proteins – or parts thereof, from different PRRSV N proteins could be expressed as a single peptide to develop a universal test reagent for all strains). As part of this objective these vectors were then used to express N protein or GP5 protein in either E.coli, insect or mammalian cells for subsequent purification. For example, Figure 1 and Figure 2 show the expression and purification of N protein and sub-region variants, respectively, from E.coli. Proteins in both Figures were stained with a Coomassie equivalent stain and indicate good yields of protein purification.

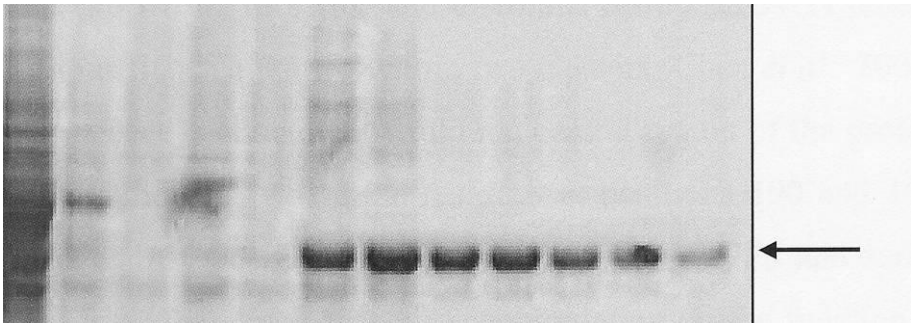


Figure 1. Expression of pTri-Ex-N in E.coli showing the various stages in purification. Basic expression with no purification is to the extreme left and the final purified product is shown to the extreme right (arrowed). Purification was achieved through the use of nickel affinity columns and involved the addition of increasing concentrations of a chemical called imidazole (left to right). The purified protein is arrowed.

Table 1. N gene constructs generated in the study.

Construct	Use
N protein:	
pTopo-N for fluorescent proteins (x3)	These are the sub-clones to allow ease of cloning into the appropriate final vector. All vector inserts were sequenced.
pTopo-N1 (x3, for pECFP, pEGFP and pDsRed)	
pTopo-N2 (x3, as above)	
pTopo-N3 (x3, as above)	
pTopo-N, N1, N2 and N3 for pTri-Ex	
pTopo-N for pDest	
pDest-N	For expression of N in the Proteomaster.
pTri-Ex-N	For expression of N protein in E.coli, insect and mammalian cells.
pTri-Ex-N1	For expression of the N-terminal part of N protein in E.coli, insect and mammalian cells.
pTri-Ex-N2	For expression of the central region of N protein in E.coli, insect and mammalian cells.
pTri-Ex-N3	For expression of the C-terminal part of N protein in E.coli, insect and mammalian cells.
pDest-N1, N2 and N3.	For expression in the Proteomaster.
pEGFP-N	Fluorescent tagged N protein for expression in mammalian cells.
pECFP-N	
pDsRed-N	
pEGFP-N1	Fluorescent tagged N-terminal part of N protein for expression in mammalian cells.
pECFP-N1	
pDsRed-N1	
pEGFP-N2	Fluorescent tagged central part of N protein for expression in mammalian cells.
pECFP-N2	
pDsRed-N2	
pEGFP-N3	Fluorescent tagged C-terminal part of N protein for expression in mammalian cells.
pECFP-N3	
pDsRed-N3	
pEGFP-N1N2	Fluorescent tagged N and central part of N protein for expression in mammalian cells.
pECFP-N2N3	Fluorescent tagged central and C-terminal part of N protein for expression in mammalian cells.

Table 2. GP5 gene constructs generated in the study.

Construct	Use
GP5 protein:	
pTopo-GP5 for fluorescent proteins (x3)	These are the sub-clones to allow ease of cloning into the appropriate final vector. All vector inserts were sequenced.
pTopo-GP5 for pTri-Ex	
pTopo-GP5 for pDest	
pTri-Ex-GP5	For expression of GP5 protein in E.coli, insect and mammalian cells.
pEGFP-GP5	Fluorescent tagged GP5 protein for expression in mammalian cells.
pECFP-GP5	
pDsRed-GP5	
pDest-GP5	For expression of GP5 in the Proteomaster.

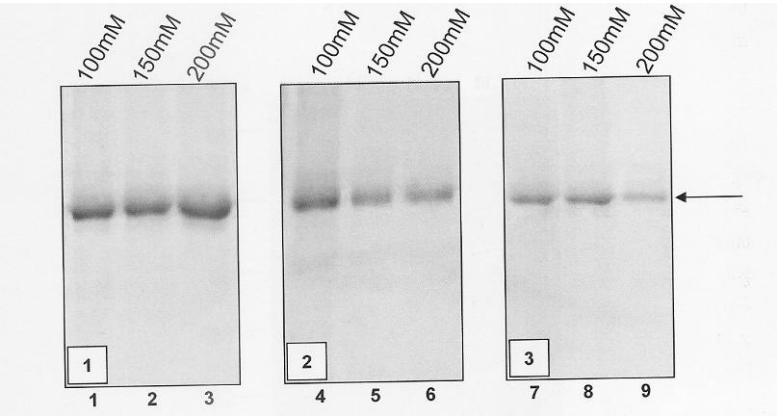


Figure 2. Expression and purification of pTri-Ex-N1 (gel 1), pTri-Ex-N2 (gel 2) and pTri-Ex-N3 (gel 3) in E.coli. Purification was achieved through the use of nickel affinity columns. Various concentrations of imidazole (indicated above) were used to optimize the yields of the different proteins (arrowed).

Both N protein and GP5 protein were expressed and purified from mammalian cells using both his-tag/nickel affinity and enrichment using FACS and immuno-precipitation to the fluorescent antibody. For example, Figure 3 shows the expression of various N protein fluorescent tagged vectors and also a sub-region (to the central part of N protein) construct in mammalian cells. As a spin off of characterization these were used to study the trafficking of PRRSV N protein within the cell in conjunction with the bio-imaging facility. Figure 4 illustrates the methodology used to develop enrichment of positive expressing cells using fluorescent activated flow cytometry.

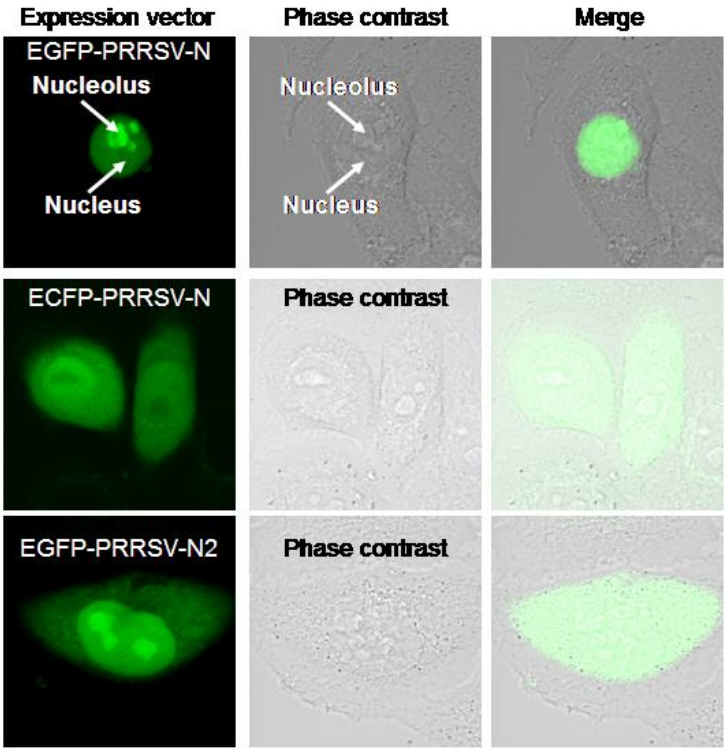


Figure 3. Live cell confocal imaging of pEGFP-N, pECFP-N and pEGFP-N2 in LLC-PK1 cells, shown is the fluorescent signal, phase contrast and merged image. For reference, various parts of the cell are labeled. This study has been conducted for all the appropriate proteins described in Tables One and Two.

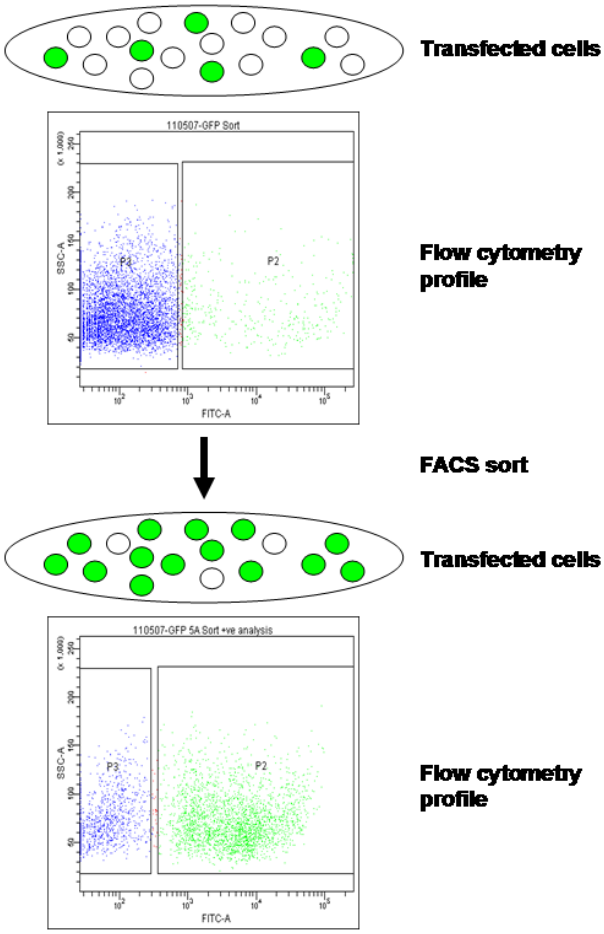


Figure 4. Diagram and raw data presentation showing the enrichment of LLC-PK1 cells expressing pEGFP-N protein. The initial starting population (top) after transfection is about 10% of cells expressing the protein (green) as determined by flow cytometry. Cells not expressing the protein are shown in purple in the flow profile. After one round of FACS there are many more cells (~80%) now expressing the EGFP-N protein. However, yields of purified proteins were low.

The expression of N protein in the various systems was then compared to that from virus infected cells (Figure 5). As controls the mobility of N protein from infected cells was compared to the mobility of N protein from purified virions, N protein taken from virions absorbed onto cells at non-physiological temperatures and in the presence of cycloheximide, N protein expressed in E.coli (therefore non-phosphorylated) and over-expressed in mammalian cells (therefore phosphorylated by cellular kinases) (in these cases the histidine tagged was removed through cleavage). In a separate experiment all samples were treated with calf intestinal alkaline phosphatase (CIAP) to remove any phosphate residues and thus confirm this modification was present. The data indicated that the mobility of N protein in infected cells was identical to that found in mature virions and purified recombinant N protein from mammalian cells. CIAP treatment resulted in faster migrating species which also had identical mobility to N protein expressed and purified from E.coli. This indicated that N protein in infected cells and purified virions were phosphorylated and (by inference due to their identical apparent mobility) possessed the same number of phosphate residues. Interestingly, N protein from absorbed virions had two mobility patterns; one equivalent to N protein expressed in E.coli and an intermediate migration pattern between that and the phosphorylated species found during infection. Treatment of these samples with CIAP gave species with equivalent mobility to E.coli, again indicating the presence of phosphate.

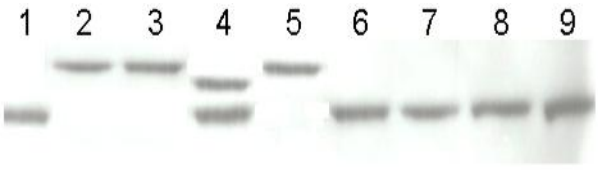


Figure 5. Western blots of PRRSV N protein expressed in E.coli (Lane 1), porcine kidney cells (Lane 2), from purified virions (Lane 3), from absorbed virus (Lane 4) and infected cells PRRSV (lane 5). CIAP treatment of N protein expressed in porcine kidney cells (Lane 6), from purified virions (Lane 7), from absorbed virus (Lane 8) and infected cells (lane 8)

GP5 protein was also expressed and characterised in a similar manner. For example, Figure 6 shows the live mammalian cell expression of GP5 tagged to the fluorescent reporter protein DsRed in the presence and absence of EGFP-N protein. Similar to this latter protein the DsRed tag was used in FACS to enrich mammalian cells expressing GP5.

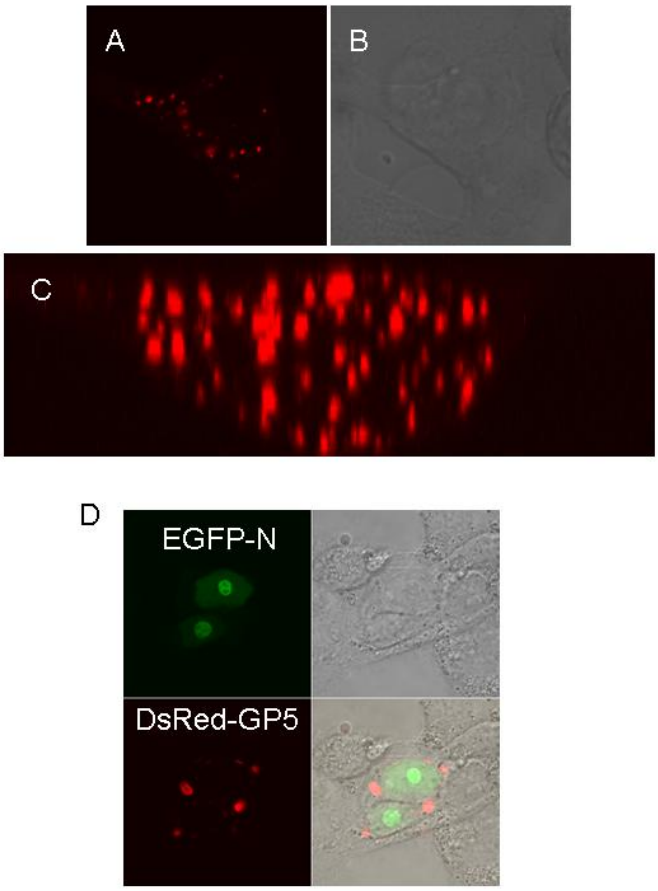


Figure 6. Confirmation of expression of DsRed-GP5 in live cells (A), for orientation (B) is the transmission phase image. (C) is the 3D-reconstruciton of DsRed-GP5 showing a section through a cell. (D) shows the co-expression of EGFP-N and DsRed-GP5 in live cells. The purpose of this was to develop methodologies to allow the co-expression and enrichment of virus proteins. However, yields of protein were low.

Objective 2: In this objective the Roche ProteoMaster instrument was used for the expression and purification of N protein and GP5 proteins using the pDest-N and pDest-GP5 constructs, respectively. Two different yields were used. For optimization the RTS100 reagent was used this provided protein on the order of 16 μg per reaction and was sufficient to develop histidine tagged purification strategies. Once these were established then the RTS500 reagent was used and led to the production of approximately 40 mg of protein per reaction. Figure 7 shows an example of the optimization of purification of histidine tagged N protein using the RTS system and Figure 8 shows the expression and purification of the N protein sub-region constructs using the ProteoMaster. Likewise histidine tagged GP5 was expressed and purified using this system, in this case gel filtration chromatography was used to enrich the sample of purified protein (Figure 9).

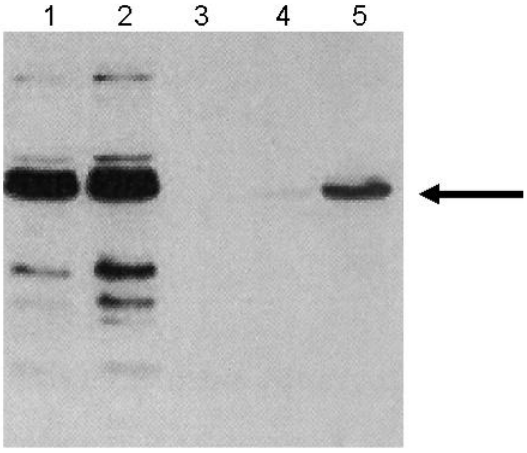
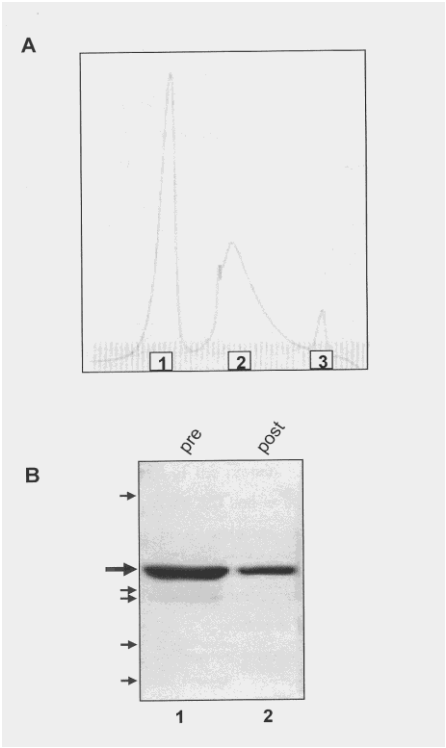


Figure 7. SDS-PAGE analysis of the optimisation of purification of histidine tagged PRRSV N protein using the RTS system. Lanes 1 and 2 showing elution with either 150 mM or 250 mM imidazole, respectively. Lane 3 and 4 are wash steps with 50 mM imidazole and purified protein shown in Lane 5.



F*igure 8. SDS-PAGE analysis of purified sub-regions of PRRSV N protein using the RTS system, N-terminal region (lanes 1 and2), central region (lanes 3 and 4) and C-terminal region (lanes 5 and 6).*

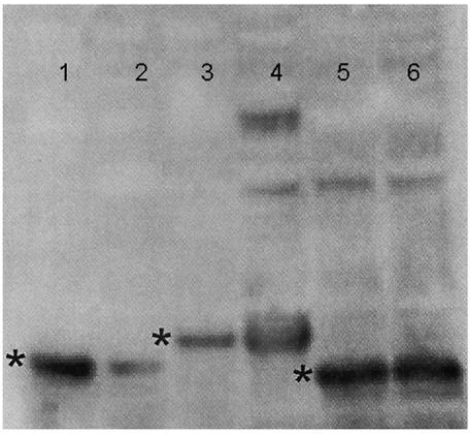


Figure 9. Gel filtration of PRRSV GP5 protein expressed using the RTS system. Protein samples purified using affinity chromatography were further purified using a 70 ml Superdex 75 column. After column equilibration, pooled protein samples were injected onto the column and separated according to size. Fractions were collected and analysed for protein content by A_{280} absorbance, the resulting trace (A) was used to determine which fractions contained recombinant GP5 protein. (B) GP5 proteins contained within fractions eluted with peak 1 were separated by SDS-PAGE (lane 2) alongside pooled pre-purified protein (lane 1) and proteins visualised by Coomassie staining. GP5 protein is indicated by a large arrow and contaminating proteins highlighted with smaller arrows.

Objective 3: The recombinant proteins generated in Objectives 1 and 2 were then screened using appropriate potential divergent antisera against PRRSV (North American and European). Both N protein and GP5 proteins generated in this study were effective in ELISA and highlights of this research with specific examples are provided. The major research finding was that protein generated in the RTS system was an effective peptide/protein that could be used in ELISA and that the N-terminal region of the N protein had an almost identical antigenic capacity to the full length N-protein which was used as a positive control (Figure 10).

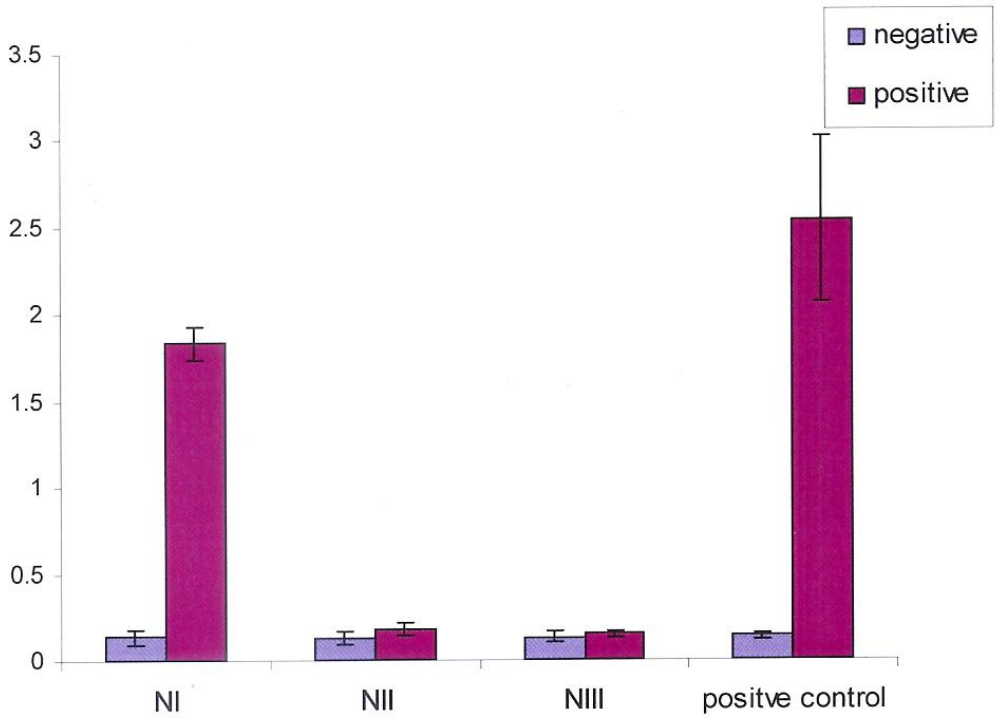


Figure 10. Evaluation of the antigenic capacity of the PRRSV N protein regions and N protein. Purified N protein regions and N protein generated using the RTS system was used as the coating antigen in diagnostic ELISA in three separate assays, average absorbance at 550 nM are shown with error bars representing deviation from the mean. Antigens were incubated with negative (blue bars) or positive (violet bars) PRRSV antisera and signals detected with conjugated secondary antibody.

The different recombinant histidine tagged proteins/antigens produced in the four different systems, e.g. mammalian cells, E.coli, RTS system and insect cell systems, were compared in their ability to act as antigens. In this case the amount of each protein used to coat the ELISA plate was equilibrated. For example, for histidine tagged GP5 protein the data indicated that GP5 protein expressed in mammalian cells was a better antigen than those of the other three expression systems. However, both the proteins produced and purified from insect cells and the RTS system still gave strong reactivity (Figure 11).

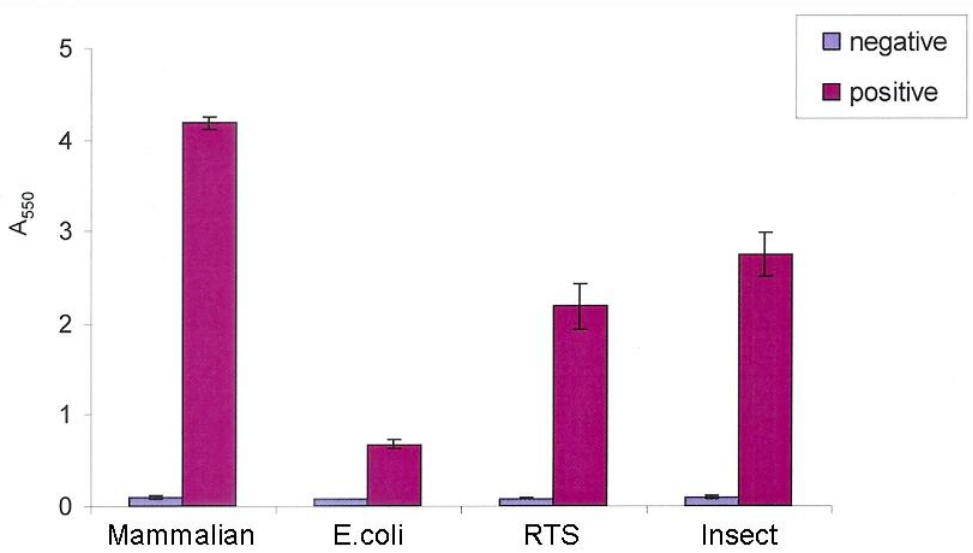


Figure 11. Evaluation of the antigenic capacity of histidine tagged PRRSV GP5 protein produced by different systems (indicated). Average absorbance at 550 nM are shown with error bars representing deviation from the mean. Antigens were incubated with negative (blue bars) or positive (violet bars) PRRSV antisera and signals detected with conjugated secondary antibody.

Both the full length histidine tagged GP5 and N protein antigens were evaluated against antisera for North American and European strains of PRRSV (Table 3). The data indicated that in general N protein recognised both strains, but with a preference for the North American sera whereas the GP5 protein recognised the North American derived sera only i.e. was above the threshold level. In this experiment previously purified coronavirus histidine-tagged nucleocapsid proteins (infectious bronchitis virus (IBV) and severe acute respiratory syndrome coronavirus) were used as negative controls to check for cross-reactivity, they did not cross react with PRRSV sera, likewise neither PRRSV GP5 nor N protein reacted with antisera derived from poultry in infected with IBV, indicating that these two PRRSV proteins are specific for PRRSV antibodies.

Table 3. Analysis of the ability of expressed North American histidine tagged N and GP5 to recognise North American and European PRRSV sera. S/P ratio is shown.

Expressed protein	North American	European
N – mammalian	1.67	1.28
N – E.coli	0.62	0.34
N – RTS	1.03	0.74
N – insect	1.25	1.07
GP5 – mammalian	2.21	0.19
GP5 – E.coli	0.98	0.11
GP5 – RTS	1.16	0.12
GP5 – insect	2.01	0.13

Note: Means values from triplicate assays were used to calculate S/P values. The cut off point for a positive signal was above 0.19.

Two publications have so far resulted from these results and include appropriate acknowledgement of research funding:

Spencer, K. A., F. A. Osorio and **J. A. Hiscox.** 2007. Recombinant viral proteins for use in diagnostic ELISAs to detect virus infection. Vaccine **25:** 5653-5659.

This paper describes the different expression methodologies and highlights the use of the RTS-ProteoMaster system.

You, J. H., G. Howell, A. K. Pattnaik, F. A. Osorio and **J. A. Hiscox.** 2008. A model for the dynamic nuclear/nucleolar/cytoplasmic trafficking of the porcine reproductive and respiratory syndrome virus nucleocapsid protein based on live cell imaging. Virology. doi:10.1016/j.virol.2008.04.037. In press.

This paper describes the trafficking of PRRSV N protein in the cell in real time and made use of the fluorescent vectors generated in this study and was as a result/spin-off of their characterisation. It was done in collaboration with the bio-imaging facility at the University of Leeds.

We are currently preparing one more publication based upon the use of the ProteoMaster system for PRRSV antigen preparation.

Discussion:

A large number of versatile constructs were generated in this study which have a wide variety of used in protein purification in mammalian, E.coli, cell free/RTS ProteoMaster, and insect based systems. By far the best system was the conventional use of histidine tagged proteins for nickel affinity purification. The fluorescent labelled proteins were easy to detect (by fluorescence microscopy) and the mammalian cells that expressed these proteins easy to enrich – by fluorescent activated cell sorting. The rationale for this was that expression of recombinant proteins in mammalian cells is sometimes poor due to low transfection efficiencies. Being able to positively select cells that express proteins e.g. using FACS and fluorescent tags, would work around this problem. However, recovery of purified proteins using IPs on the fluorescent tag was inefficient. This may be improved in the future by also incorporating a histidine tag and using this for purification. Undoubtedly the results demonstrated that PRRSV proteins generated in mammalian cells and therefore fully post-translationally modified were the better antigens for diagnostic ELISA. However, proteins produced in the other three systems could still be used. Surprisingly the cell free/RTS ProteoMaster strategy gave better results that the E.coli based system, despite the former being based on the latter (i.e. translation extracts prepared from E.coli). However, the RTS system by its very nature of having a semi-permeable membrane and reaction vessel aids in the purification of the protein, and results in less contamination. The only apparent disadvantage of the RTS system is cost, with the generation of approximately 240 mg of protein costing approximately $3000. However, this can be off set because far less material is required for purification, and it may be a robust and reliable method with little optimisation required.

For future work we would wish to pursue several avenues of research as a result of this project:

First and foremost this would be a thorough analysis of the antigenic potential of PRRSV N protein, narrowing down the specific regions involved in antigen recognition and whether these are conserved between different strains or not. The advantage of this is that relatively large epitopes at high concentration could be expressed using the RTS system, which cannot be done using synthetic systems. Subsequent to the initiation of this study several other cell based systems are now available including those to help with proper folding of the protein (important for the epitope recognition) and expression in eukaryotic extracts for maximum protein yield.

Second, to exploit the RTS system using mock-field based trials to investigate how long it would actually take to go from PRRSV field isolate to having a prepared protein based ELISA system and the costs involved to see whether this was an economically feasible approach compared to conventional cloning based strategies – which arguably take longer to implement.

Third, we were pleasantly surprised by the spin off observation on the trafficking of PRRSV N protein and as highlighted in the paper in press. This was mentioned in the original proposal under future potential avenues. Therefore we would wish to further exploit this finding for the development of growth attenuated live recombinant vaccines which are deficient in N protein trafficking.