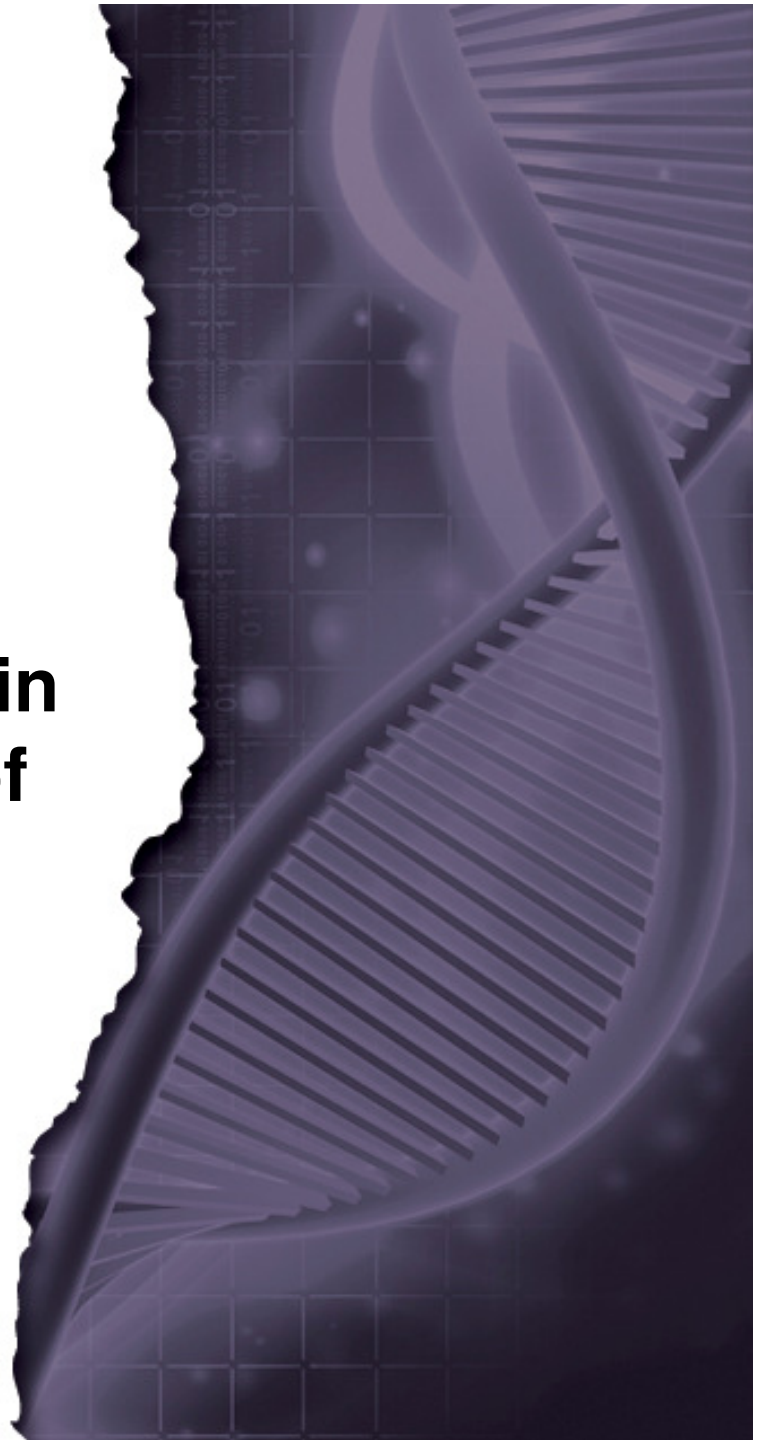


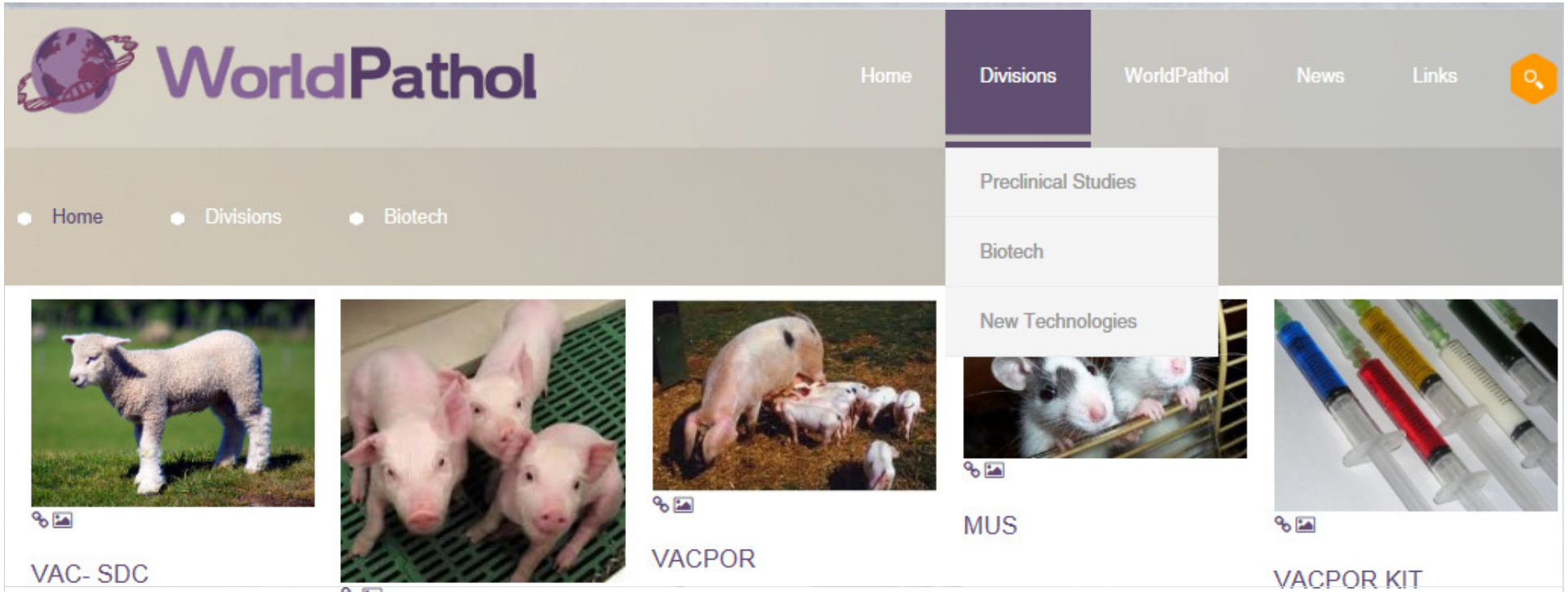
"Use of ORF-1 product Rep in prevention and diagnosis of Porcine circovirosis"

Silvia Pellicer. WorldPathol S.L. (Spain)

4th International Conference on

Vaccines and Vaccination

24-26 September 2014. Valencia (Spain)



WorldPathol biotech develops new drugs and biological products on request

www.worldpathol.com

PORCINE CIRCOVIRUS DISEASES (PCVD)

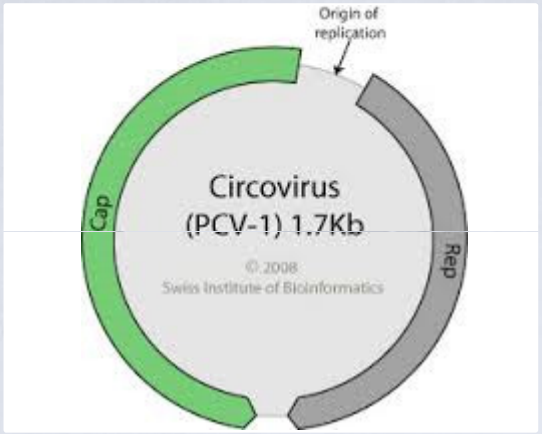
PMWS (*Postweaning multisystemic wasting sindrome)* is one of the main causes of economical losses in swine production market.

- Diagnosis: detection of **PCV2** in the lesions (Porcine circovirus type 2).
- PCV2 infection is wide spread in farms.
- Only pigs with a high PCV2 load develop the illness due to defective humoral response.



PCV2

Small non-enveloped virus single stranded DNA belonging to *Circoviridae* family.



Genomic organization (ss DNA):

ORF-1: Rep

ORF-2: Cap main immunogenic antigen of the virus

CURRENT VACCINES

- Based on inactivated PCV2
- Based on recombinant subunit ORF-2 (Cap)
- Based on non pathogenic strain PCV1 displaying pathogenic ORF-2 (Cap)

WHAT DO WE WANT?

- Produce a vaccine to prevent PCVD based on subunits
- Produce a diagnosis kit to differenciate vaccinated from infected animals (DIVA)

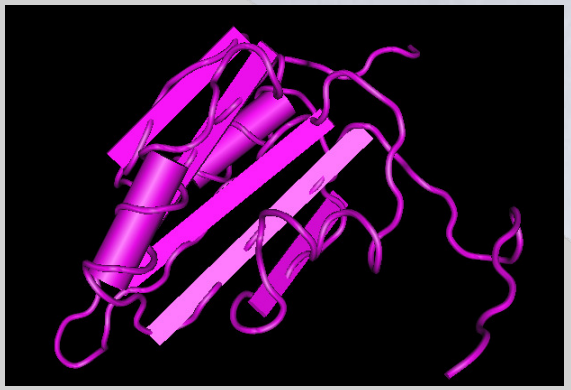


WHY REP?

Pérez-Martín, E. et al., 2008.
"Development of two *Trichoplusia* ni larvae-derived ELISAs for the detection of antibodies against replicase and capsid proteins of porcine circovirus type 2 in domestic pigs".
J Virol Methods. 154(1-2), 167-74

107 pigs sera analysed
PMWS affected animals had lower anti-Cap and anti-Rep antibodies.

REP features



Vega-Rocha *et al*. 2007

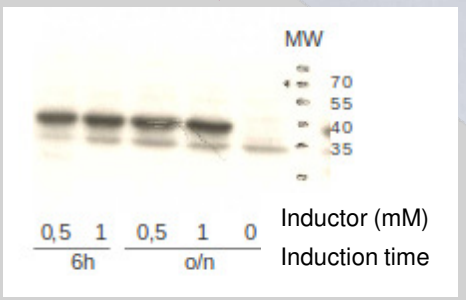
- ✓ Product of ORF-1
- ✓ Essential for virus replication

Aminoacids number	314
Molecular Weight	35,78 kDa
Isoelectric point	8,41

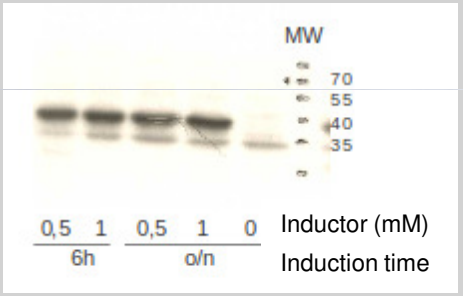
STRATEGY

- Optimization of nucleotide sequence of Rep (ORF-1) for expression in *E. coli.*
- Construction of a plasmid for Rep overexpression in *E. coli*.
- Optimization of Rep overexpression and purification.

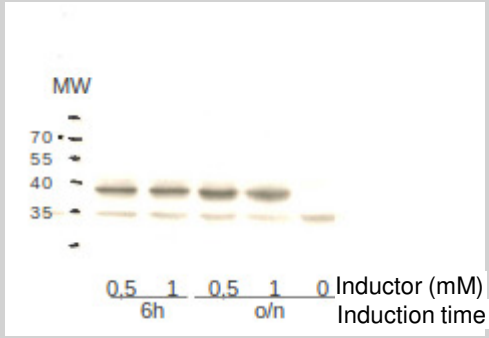
E.COLI OVEREXPRESSION OPTIMIZATION



STRAIN 1



STRAIN 2



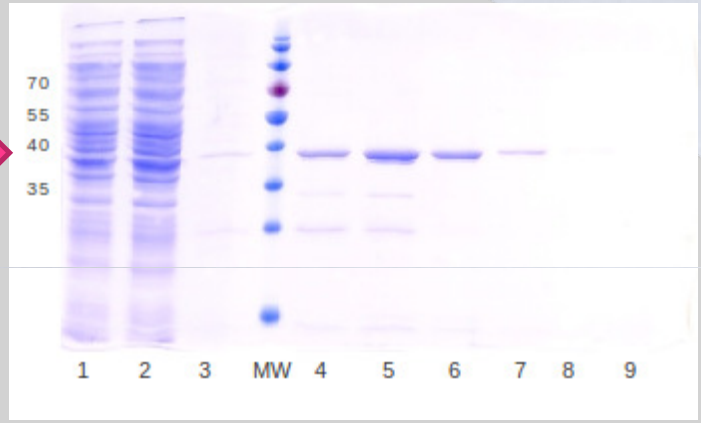
STRAIN 3

BEST CONDITION

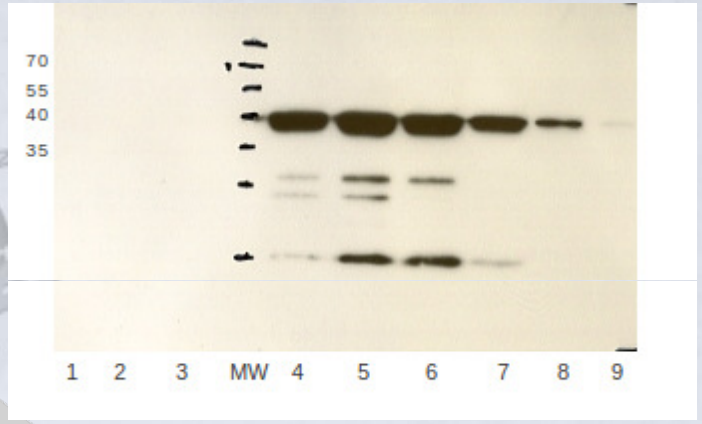
Strain 1
Overnight
1mM inductor
37ºC

E.COLI PURIFICATION OPTIMIZATION

COOMASSIE STAIN



WESTERN BLOT



- ✓Affinity cromatography
- ✓Good efficiency
- ✓Purity about 90%

NEXT STEPS:

- ✓ Purification of Rep in medium-large scale.
- ✓ Development of ELISA for Product Quality Assurance.
- ✓ *In vivo* assays to test immunogenicity of obtained Rep.

THANK YOU!

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WorldPathol



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