

# Improving diagnostic tools for suspected Cat-scratch disease cases

# Cat-scratch disease (CSD)

- Results from being scratched by a cat carrying *Bartonella henselae* infected fleas

Symptoms include:

- Bumps/blisters at site of injury
- Swollen lymph nodes (lymphadenopathy)



# CSD Diagnosis

PCR via lymph node aspirate or blood sample:

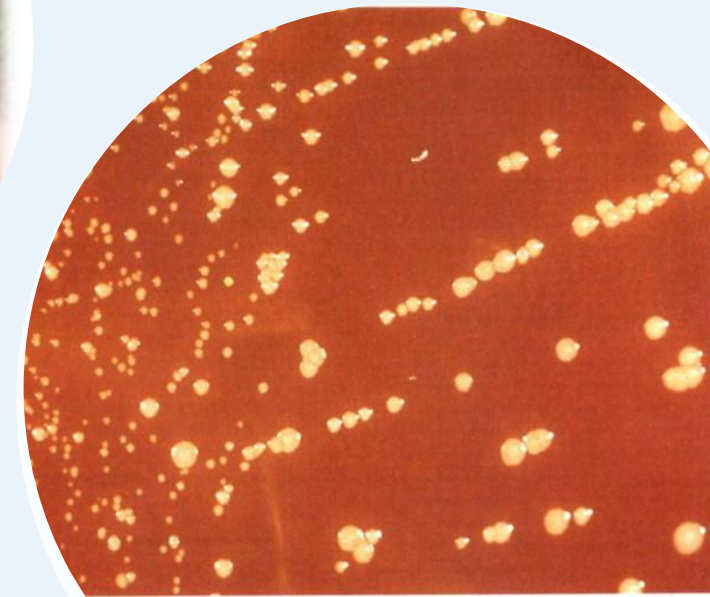
- Large needle not ideal in children
- Poor blood test sensitivity

Culture:

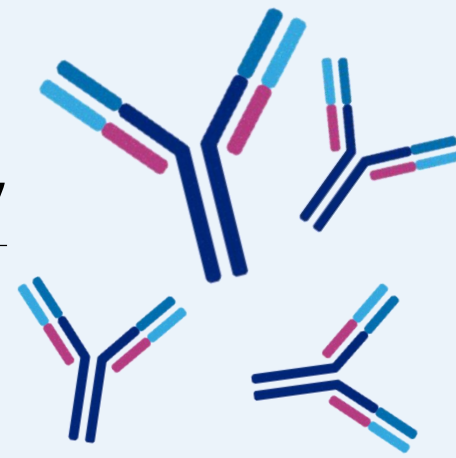
- Takes ~3 weeks

Serology:

- Mainly immunofluorescence assays
- Antigens differ between batches
- Disadvantages to all methods. There is need for a new test.



# Aims and objectives of the study



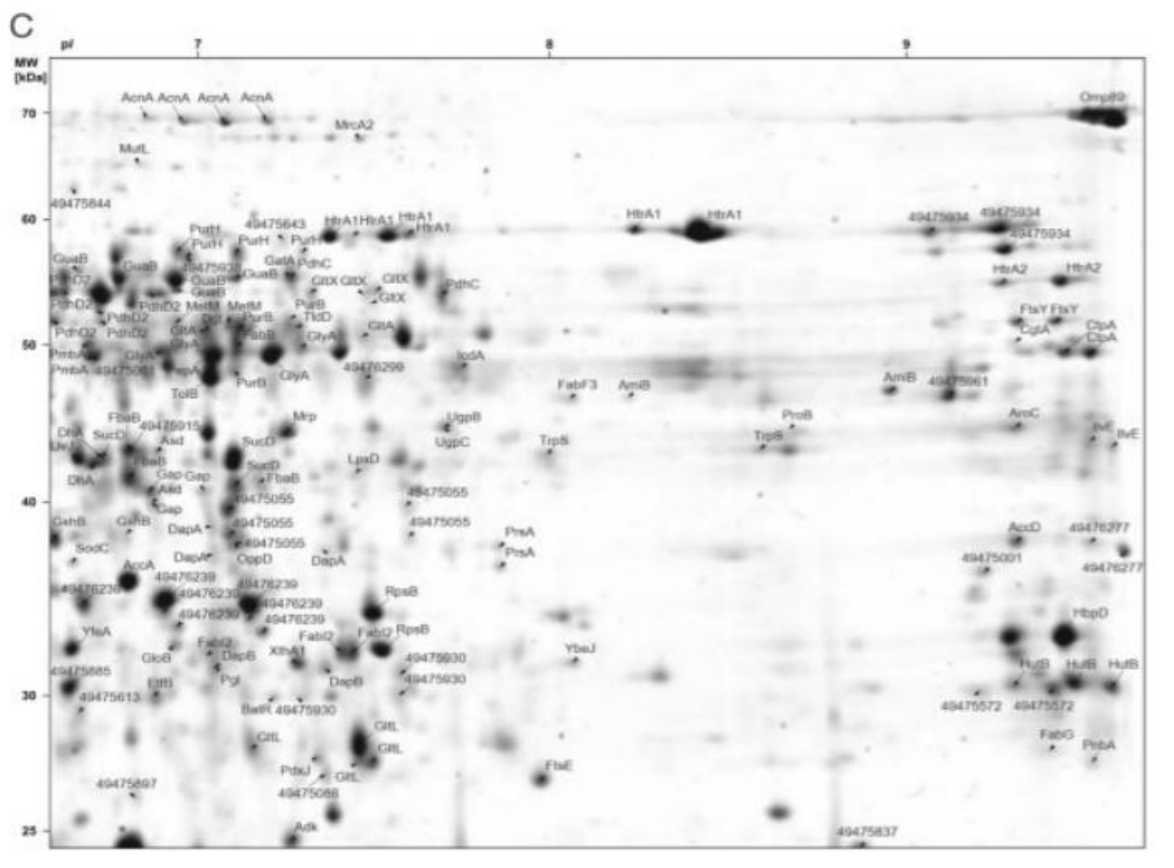
## Aim:

- Produce a better diagnostic test for suspected CSD patients based on detecting *B. henselae* antibodies in patient sera.

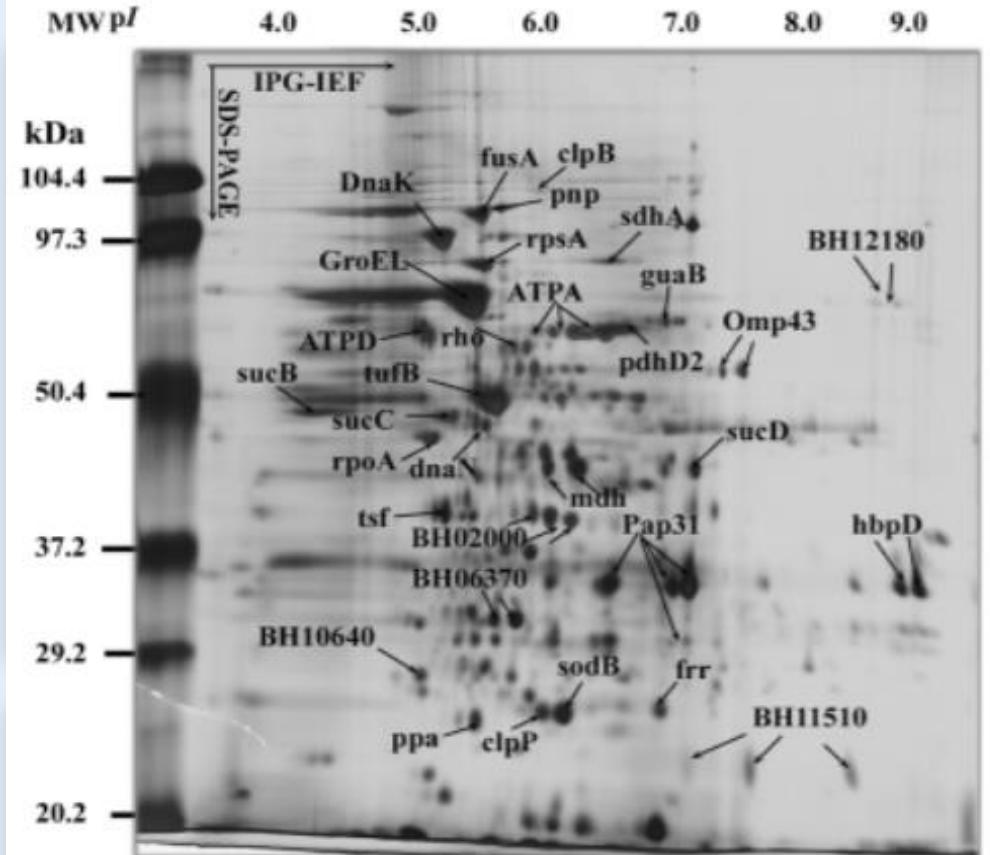
## Objectives:

- Can we create recombinant versions of immunogenic *B. henselae* proteins?
- Do the recombinant proteins retain immunogenicity?

# Previous work, leading to the study



(Eberhardt *et al*, 2009)



(Saisongkroh *et al*, 2010)

- Whole proteome analysis of *B. henselae* via SDS-page
- Immunoblotting performed to find immunogenic proteins
- AtpD, GroEL, PPI and P26 identified across both papers as immunogenic

# Lab 1

- Identification of relevant genes in *B. henselae* genome sequence
- Designing their primers for PCR in Lab 2

Looked at 4 genes coding for the proteins of interest:

- P26
- AtpD
- PPI
- GroEL

The screenshot shows the Artemis software interface for analyzing the *B. henselae* genome. At the top, the menu bar includes 'File', 'Select', 'View', 'Goto', 'Edit', 'Create', 'Write', 'Run', and 'Display'. Below the menu, the 'EMBL Entry' is identified as 'AJ007747'. The main window displays a genomic map with several genes represented by colored bars: BbLPS1.01 (green), BbLPS1.02 (cyan), BbLPS1.03 (cyan), BbLPS1.04 (cyan), BbLPS1.05 (cyan), BbLPS1.06 (cyan), and BbLPS1.07 (cyan). Below the map, a sequence view shows the DNA sequence with a highlighted region in cyan. The sequence is as follows:

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G S R I A T S P S P F R A R P T W L E G A W R T R S R Q S N H L
D L V + R Q A R R H F A P G R R G S V V R G A R D P G N R I C M
. I S Y E D E P V A I S R Q A D V A Q W C V A H A I P A I E Y A G
GGATCTCGTATAGCGACAGCCCGCTCCGATTTCGCCAGCCGACGCTGCTCAGTGGTCCGTCGCCACCGCCATCCCGCCCAATCGAATATGCTCC
CCTAGAGCATATCCCTCTTCCGCGCAGCCGCTAAGCCGCGCTCCGCTCCACCGAGTCACCAAGCACCGCGTCCGCTAGCCCGCTTAGCTTATAAGACC
. D R I A V L G D G N R A L G V H S L P A H R V R D R C D F I S A
I E Y L S L G T A M E R W A S T A * N H T A C A I G A I S T A P
S R T Y P C A R R W K A G P R R P E T T R P A R S G P L R I H Q
```

Below the sequence, a table lists features for the highlighted region:

CDS	1	827	BbLPS1.01, probable formyl transferase, partial CDS, len: >270
misc_feature	135	440	Pfam match to entry PF00551 formyl_transf, Formyl transferase
CDS	824	1612	BbLPS1.02, unknown, len: 262 aa
CDS	1409	2328	BbLPS1.03, unknown, len: 239 aa
RBS	2313	2317	possible RBS upstream of BbLPS1.04
CDS	2325	3254	BbLPS1.04, probable formyl transferase, len: 309 aa; similar t
misc_feature	2514	2855	Pfam match to entry PF00551 formyl_transf, Formyl transferase,
RBS	3264	3267	possible RBS upstream of BbLPS1.05
CDS	3277	4101	BbLPS1.05, probable formyl transferase, len: 274 aa; some simi
misc_feature	3541	3798	Pfam match to entry PF00551 formyl_transf, Formyl transferase,
RBS	4088	4094	possible RBS upstream of BbLPS1.06

Artemis software used to analysis *B. henselae* genome

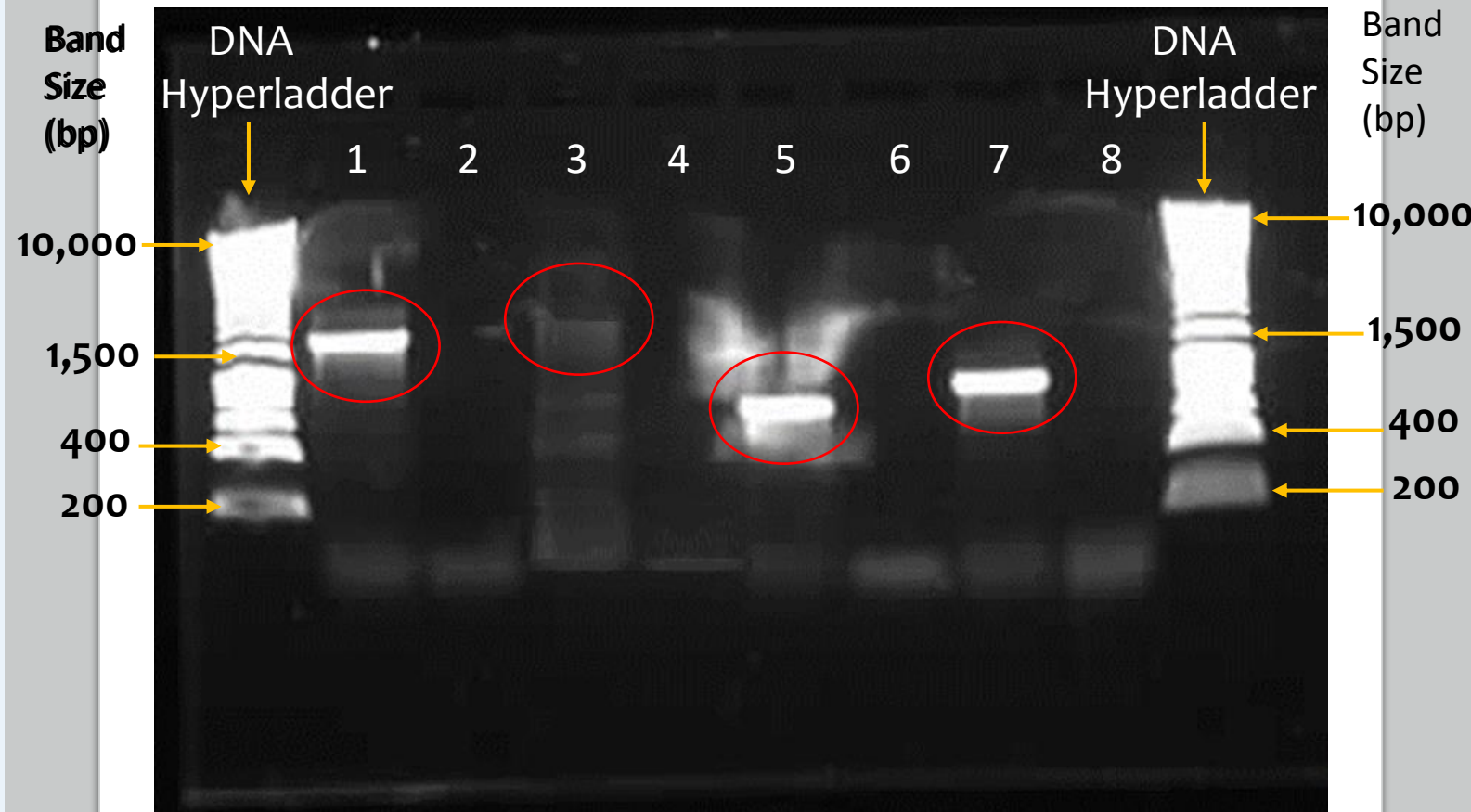
# Lab 2

- PCR to amplify ORFs identified in lab 1
- Clone ORFs into TOPO vector
- Transformed into *E. coli* and verified presence by culturing *E. coli* onto agar

Table 1: Expected band sizes of PCR products/Genes

Gene	Expected size (bp)
<i>atpD</i>	1,596
<i>groEL</i>	1,644
<i>p26</i>	738
<i>ppi</i>	954

## Electrophoresis visualisation of PCR amplified products



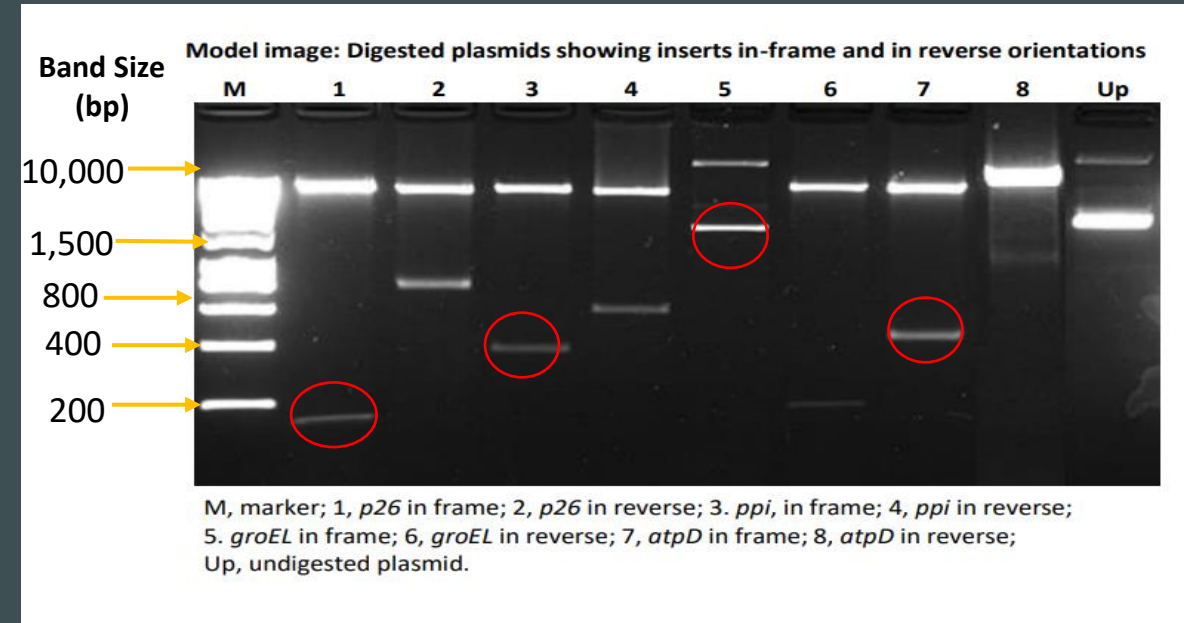
(1) *atpD* +ve (2) *atpD*-ve (3) *groEL* +ve (4) *groEL*-ve (5) *p26* +ve (6) *p26* -ve (7) *ppi* +ve (8) *ppi* -ve.

# Lab 3

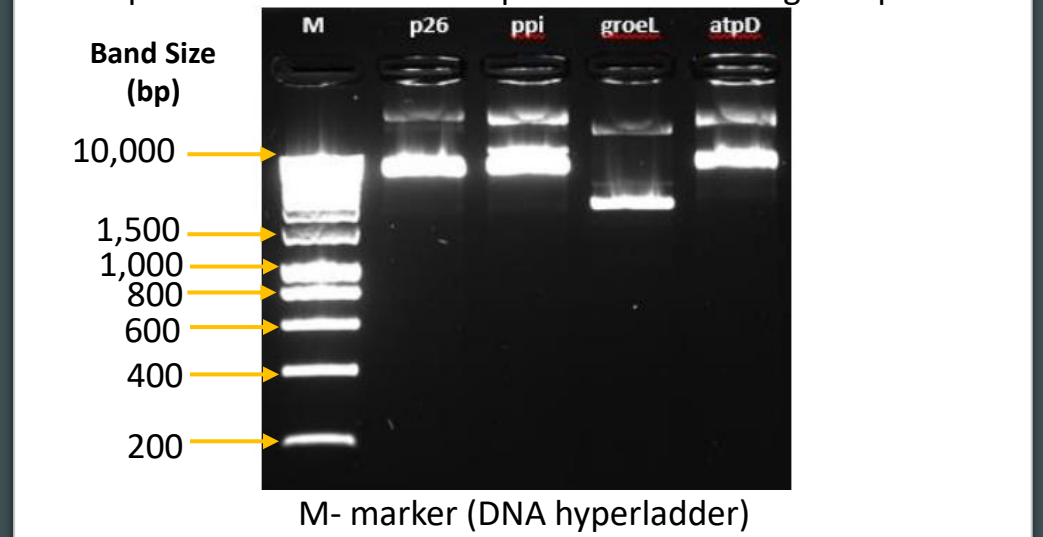
- Verify presence and orientation of the insert of the vector from lab 2
- Must be in forward orientation to allow for protein expression

Table 2: Expected band sizes of gene inserts in forward or reverse orientation

Gene	Expected size of forward orientation (bp)	Expected size of reverse orientation (bp)
<i>p26</i>	165	765
<i>ppi</i>	409	607
<i>groEL</i>	1,627	209
<i>atpD</i>	460	1,404



Electrophoresis visualisation of plasmids containing PCR products

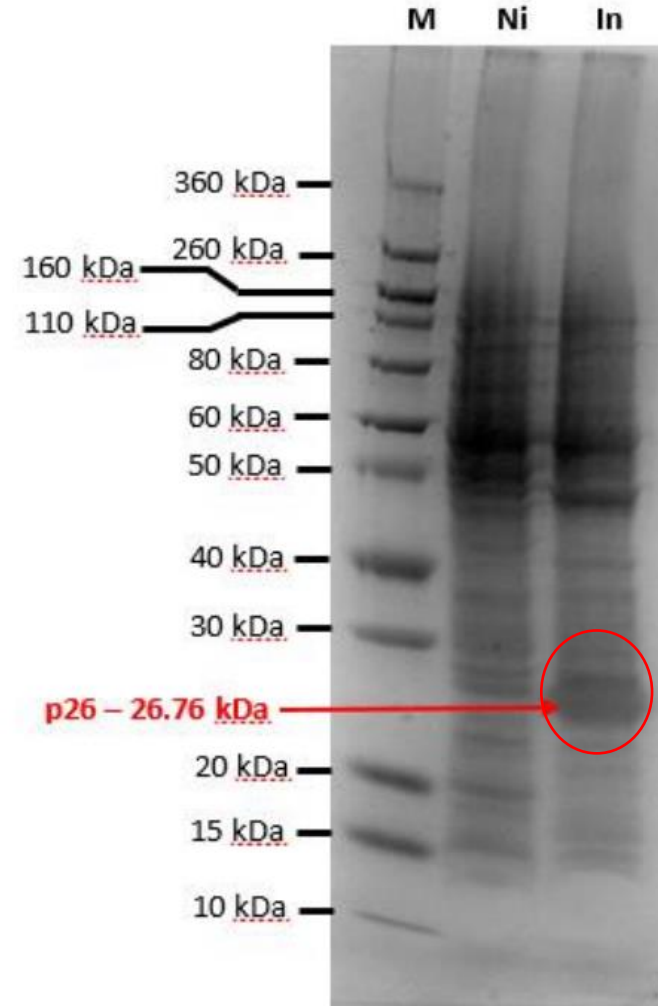




# Lab 4

- Induce expression of recombinant protein P26
- Non-induced control to show SDS-page had worked
- SDS-page to confirm recombinant protein expression

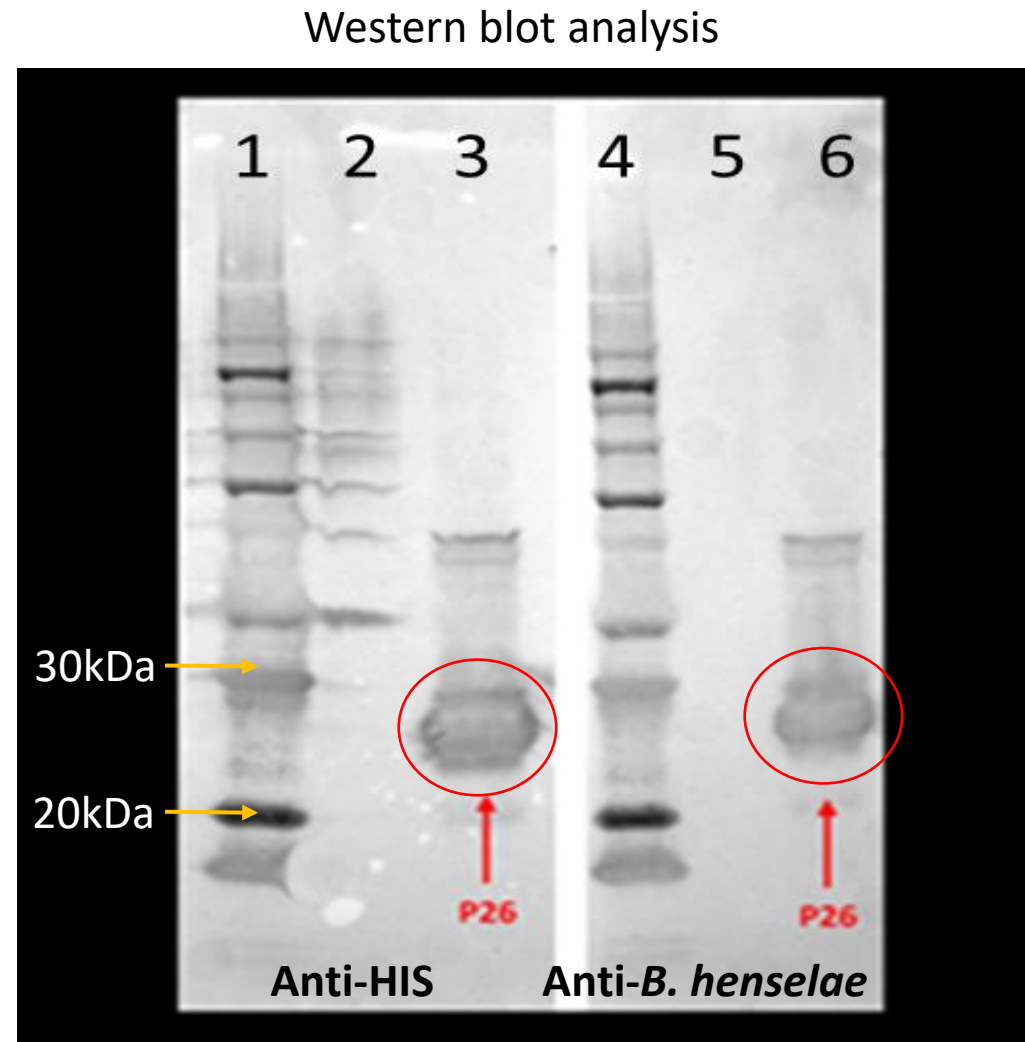
## SDS-Page electrophoresis confirmation of recombinant proteins



**M-** marker (size ladder) **Ni-** non-induced expression **In-** induced protein expression

# Lab 5

- Verify the presence of the recombinant proteins through HIS tag detection
- Test their immunogenicity via a western blot



(1&4)- Protein ladders (2&5)- non-induced P26 recombinant expression (3&6)- induced P26 recombinant expression

# Findings so far

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- P26 recombinant protein retains its immunogenicity and can be used in further experiments for CSD diagnosis
- P26 has the potential to be used as an antigen in a novel serodiagnostic assay for CSD

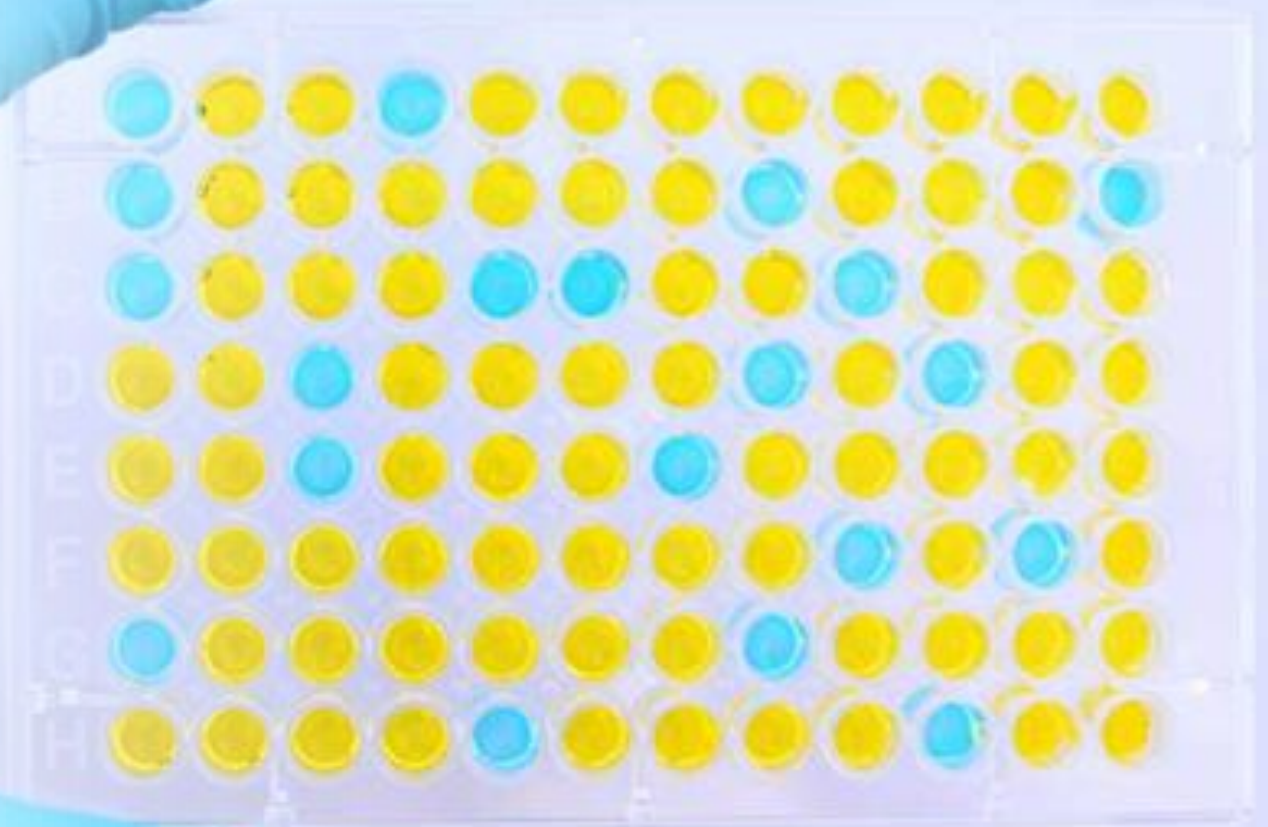


# Follow on study

- Aim: create a serodiagnostic test using recombinant P26 protein

## Objectives:

- Test recombinant P26 on more CSD patient sera
- Evaluate recombinant P26's sensitivity and specificity for potential use of an ELISA
- Begin initial steps of ELISA development



# Follow on study

Test on more sera samples and evaluate the sensitivity and specificity of recombinant P26 for potential use as an ELISA:

**Table 3.** Sensitivities and specificities of ELISA testing on CSD samples

Antigen	Definite serum sample	Immunoglobulin (Ig)	Se	Sp	Reference
<i>B. henselae</i>					
OMP	CSD with lymphadenitis, cat contact	IgM	48	98.2	Giladi <i>et al.</i> (2001)
		IgG	75	99.5	
		IgM and IgG	85	97.7	
Whole cells	CSD with PCR positive	IgM	45	98	Herremans <i>et al.</i> (2007)
		IgG	32	98	
		IgM and IgG	59	98	
Whole cells	CSD with PCR positive	IgM	65	91	Vermeulen <i>et al.</i> (2007)
		IgG	28	91	
		IgM and IgG	77	82	
Whole cells	<i>B. henselae</i> with PCR positive	IgM	56.3	98.4	Herremans <i>et al.</i> (2009)
		IgG	35.8	97.6	
Recombinant <i>B. henselae</i> protein					
r17-kDa protein	<i>B. henselae</i> IFA titers 1 : 128 to 1 : 1024	IgG	71.1	93	Loa <i>et al.</i> (2006)
rGroES	<i>B. henselae</i> IFA titers 1 : 256	IgG	80	15	McCool <i>et al.</i> (2008)
rRpL		IgG	78	59	
rBepA		IgG	86	44	
rGroEL		IgG	80	30	
r17-kDa protein	<i>B. henselae</i> IFA positive	IgM	100	97.1	Hoey <i>et al.</i> (2009)

(Saisongkorh *et al.*, 2010)

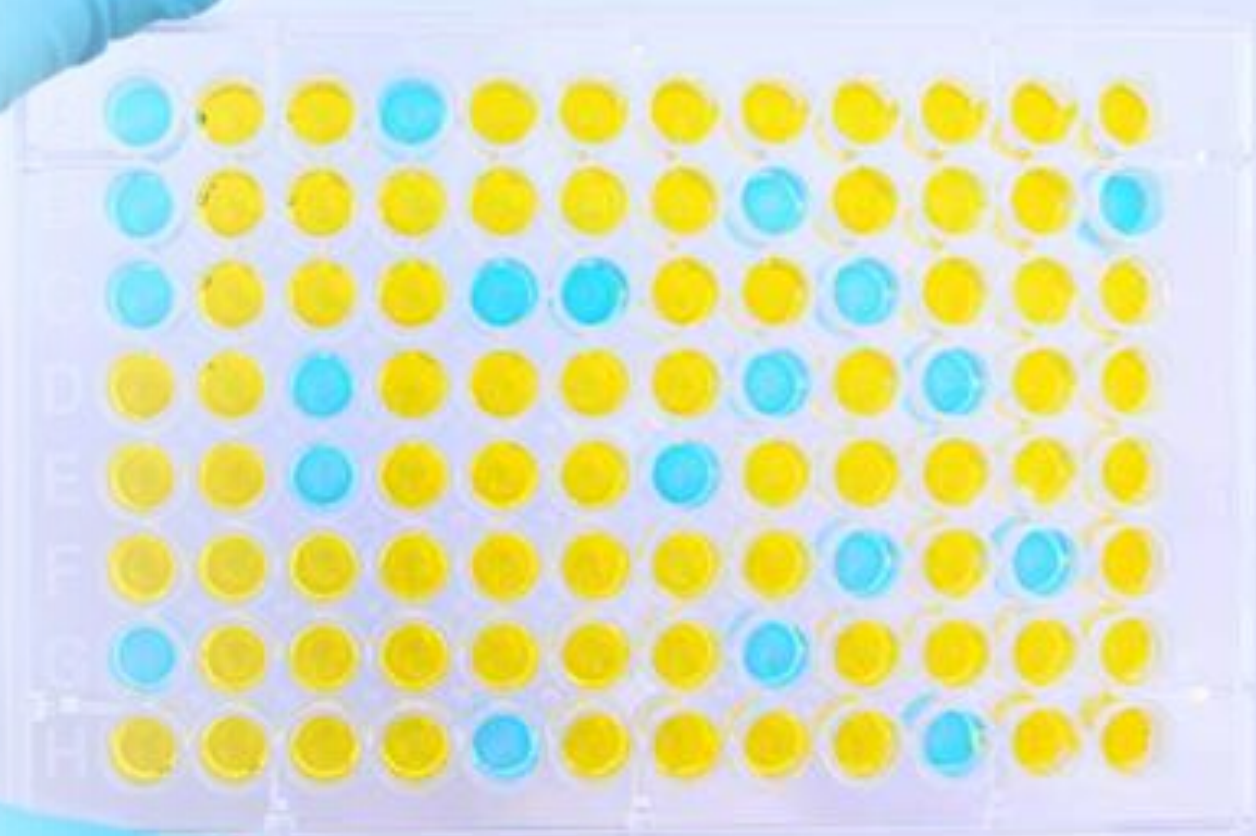
- Gather known CSD serum samples
- Gather known negative CSD samples
- Collect known CSD sera from Hansmann *et al* who has 29 known CSD serum samples (Hansmann *et al.*, 2005).
- Collect known negative sera samples from 41 lymphoma patients in collaboration with a previous study (Ferrara *et al.*, 2014).

# Follow on study

Several papers have conducted similar studies on different recombinant *B. henselae* proteins such as groEL and 17-kDa. (Loa *et al*, 2005) (McCool *et al*, 2008).

ELISA development small initial steps:

- Purify recombinant P26 protein by affinity chromatography on Ni-NTA resin
- Quantify purified protein by bicinchoninic assay (McCool *et al*, 2008)
- Test differing protein volumes to put in ELISA wells, ranging from 0.3-10µg/mL (Loa *et al*, 2005)



# References

- Eberhardt, C., Engelmann, S., Kusch, H., Albrecht, D., Hecker, M., Autenrieth, I. and Kempf, V., 2009. Proteomic analysis of the bacterial pathogen *Bartonella henselae* and identification of immunogenic proteins for serodiagnosis. *PROTEOMICS*, 9(7), pp.1967-1981.
- Ferrara, F., Di Niro, R., D'Angelo, S., Busetti, M., Marzari, R., Not, T. and Sblattero, D., 2014. Development of an enzyme-linked immunosorbent assay for *Bartonella henselae* infection detection. *Letters in Applied Microbiology*, 59(3), pp.253-262.
- Hansmann, Y., DeMartino, S., Piemont, Y., Meyer, N., Mariet, P., Heller, R., Christmann, D. and Jaulhac, B., 2005. Diagnosis of Cat Scratch Disease with Detection of *Bartonella henselae* by PCR: a Study of Patients with Lymph Node Enlargement. *Journal of Clinical Microbiology*, 43(8), pp.3800-3806.
- Loa, C., Mordechai, E., Tilton, R. and Adelson, M., 2006. Production of recombinant *Bartonella henselae* 17-kDa protein for antibody-capture enzyme-linked immunosorbent assay. *Diagnostic Microbiology and Infectious Disease*, 55(1), pp.1-7.
- McCool, T., Hoey, J., Montileone, F., Goldenberg, H., Mordechai, E. and Adelson, M., 2008. Discovery and analysis of *Bartonella henselae* antigens for use in clinical serologic assays. *Diagnostic Microbiology and Infectious Disease*, 60(1), pp.17-23.
- Saisongkorh, W., Kowalczywska, M., Azza, S., Decloquement, P., Rolain, J. and Raoult, D., 2010. Identification of candidate proteins for the diagnosis of *Bartonella henselae* infections using an immunoproteomic approach. *FEMS Microbiology Letters*, 310(2), pp.158-167.