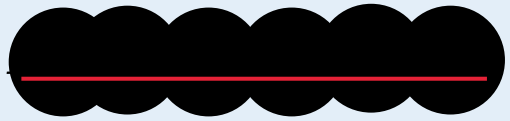


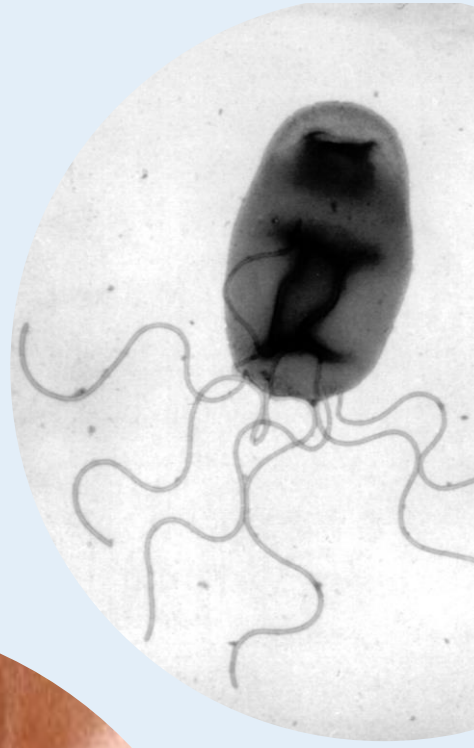
Improving diagnostic tools for suspected Cat-scratch disease cases

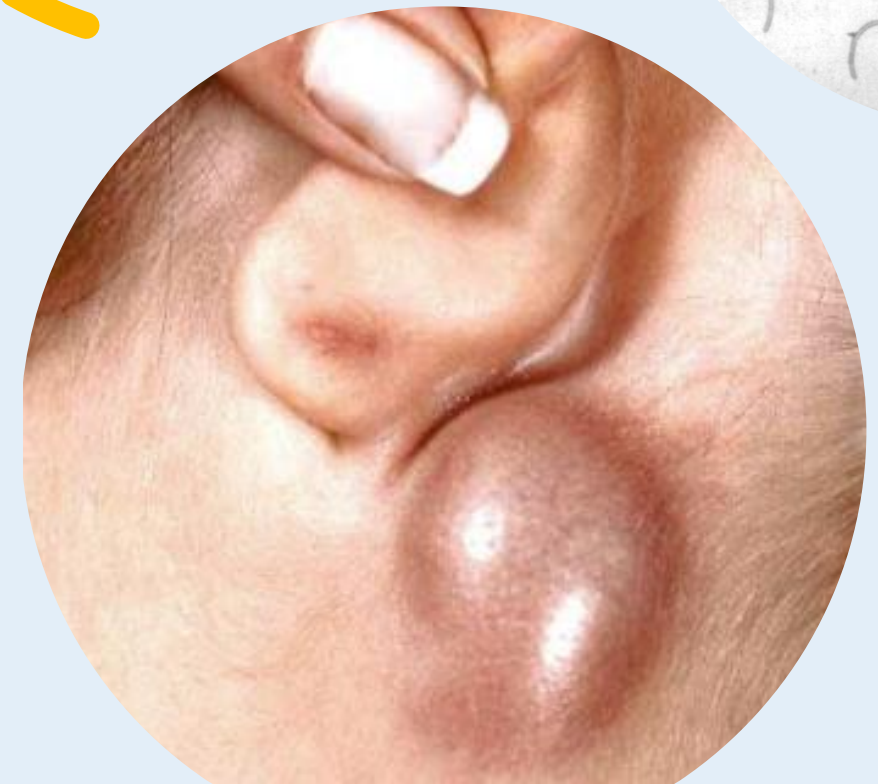
Cat-scratch disease (CSD)

- 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:

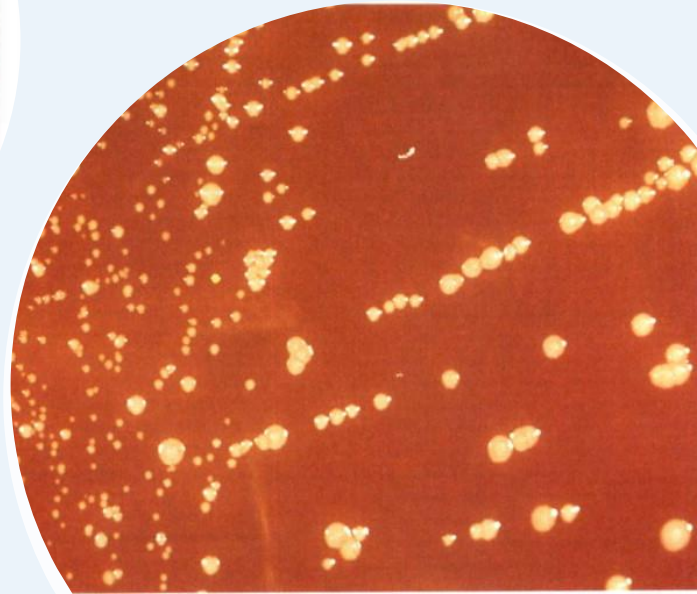
- Large needle not ideal in children
- Poor blood test sensitivity

Culture:

- Takes ~3 weeks

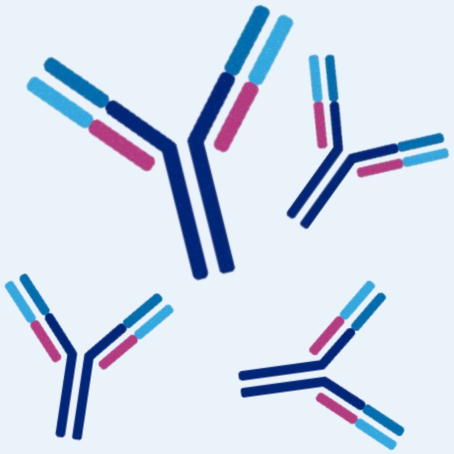
Serology:

- Mainly immunofluorescence assays
- Antigens differ between batches



- Disadvantages to all methods. There is need for a new test.

Aims and objectives of the study



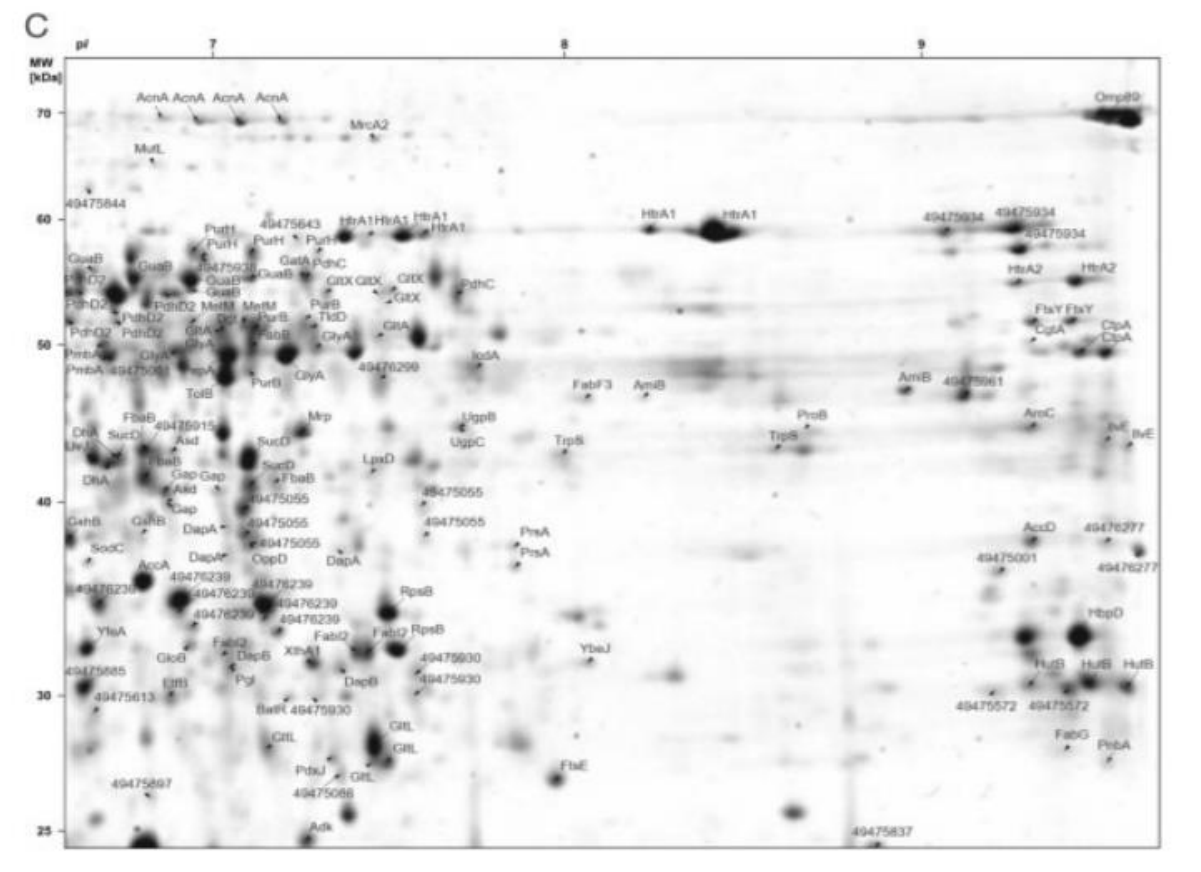
Aim:

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

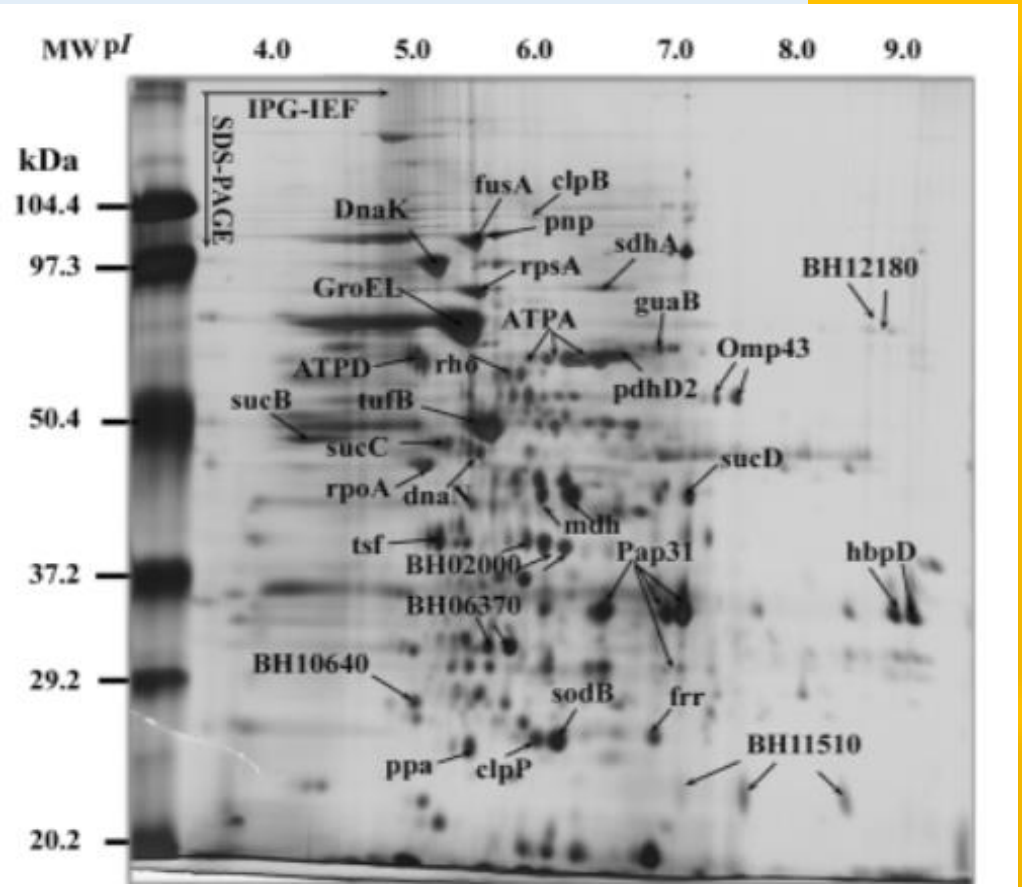
Objectives:

- 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)



(Saisongkorh *et al*, 2010)

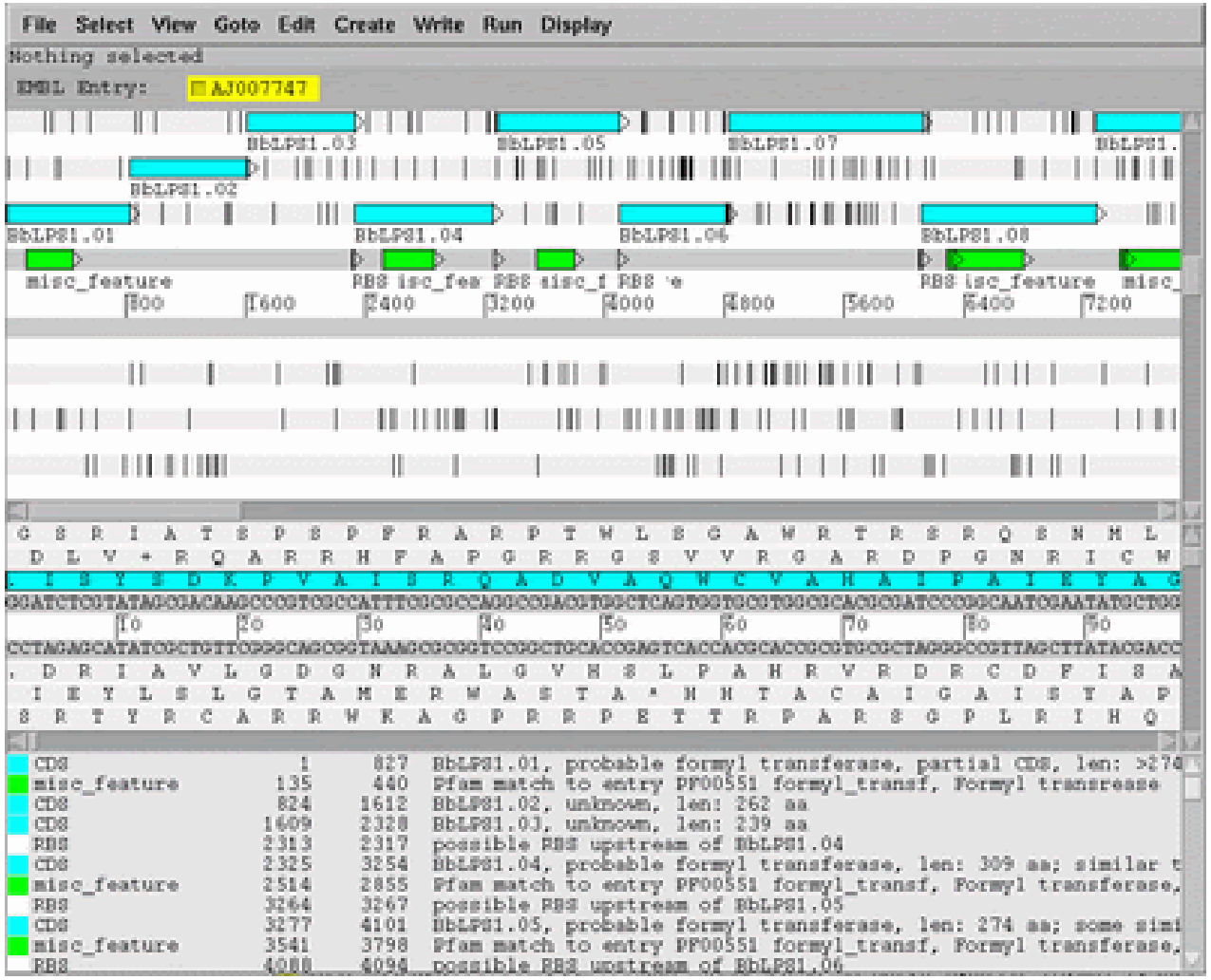
- Whole proteome analysis of *B. henselae* via SDS-page
- Immunoblotting performed to find immunogenic proteins
- AtpD, GroEL, PPI and P26 identified across both papers as immunogenic

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*



Artemis software used to analysis *B. henselae* genome

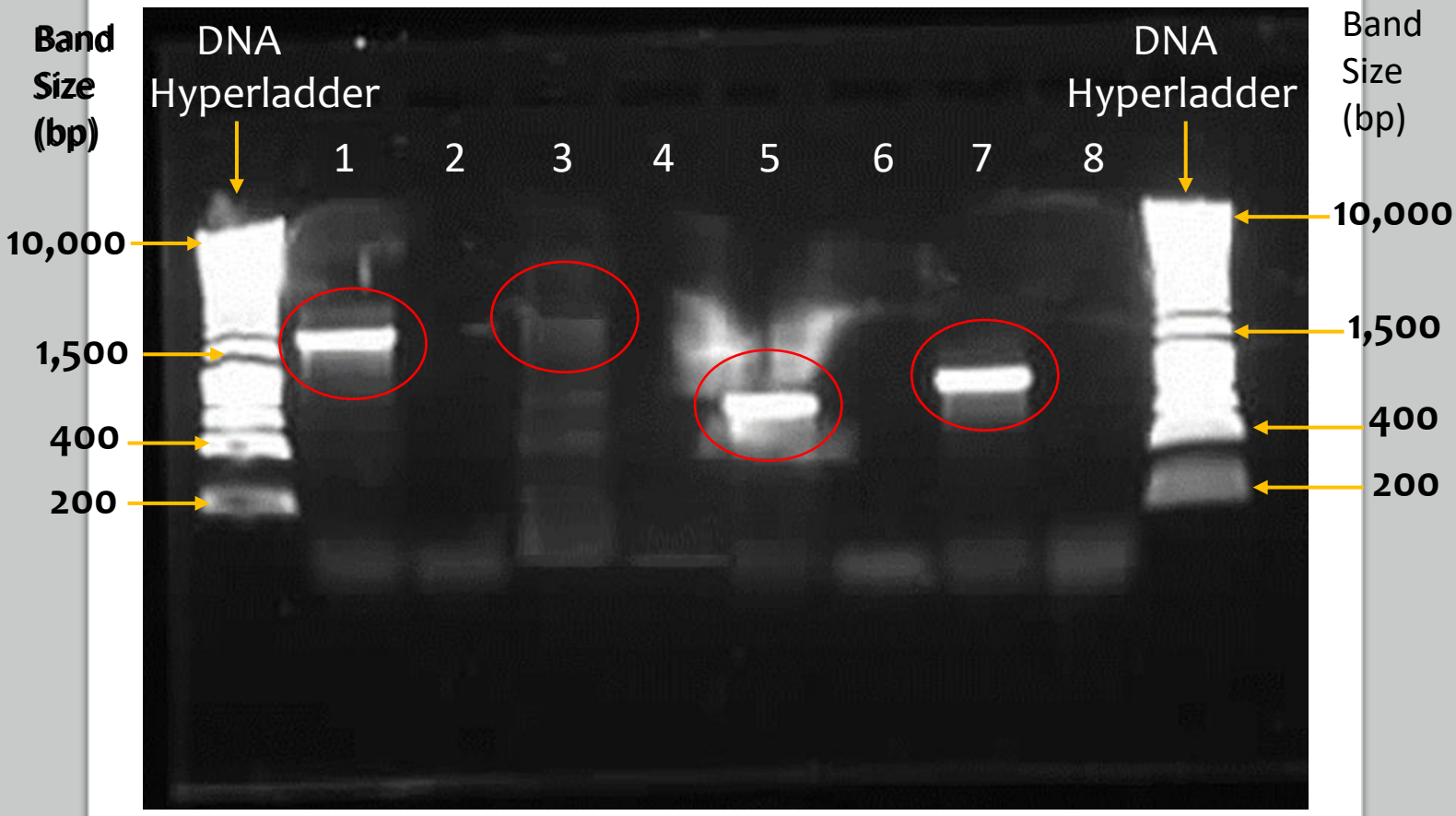
Lab 2

- 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)
atpD	1,596
groEL	1,644
p26	738
ppi	954

Electrophoresis visualisation of PCR amplified products



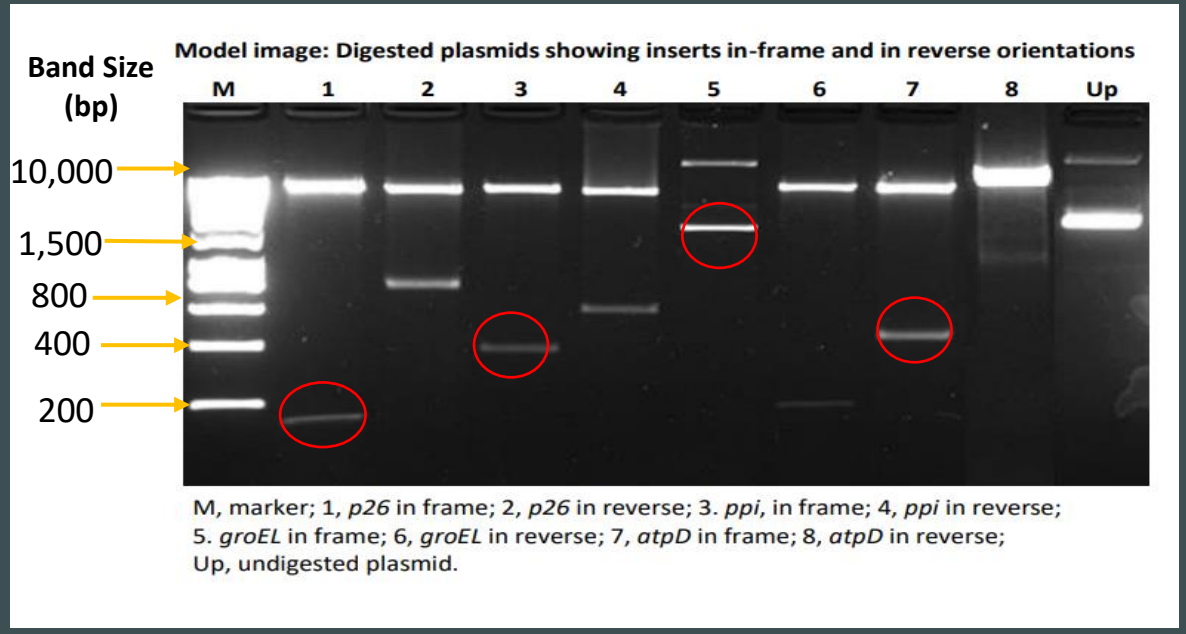
(1) atpD +ve (2) atpD-ve (3) groEL +ve (4) groEL-ve (5) p26 +ve (6) p26 -ve (7) ppi +ve (8) ppi -ve.

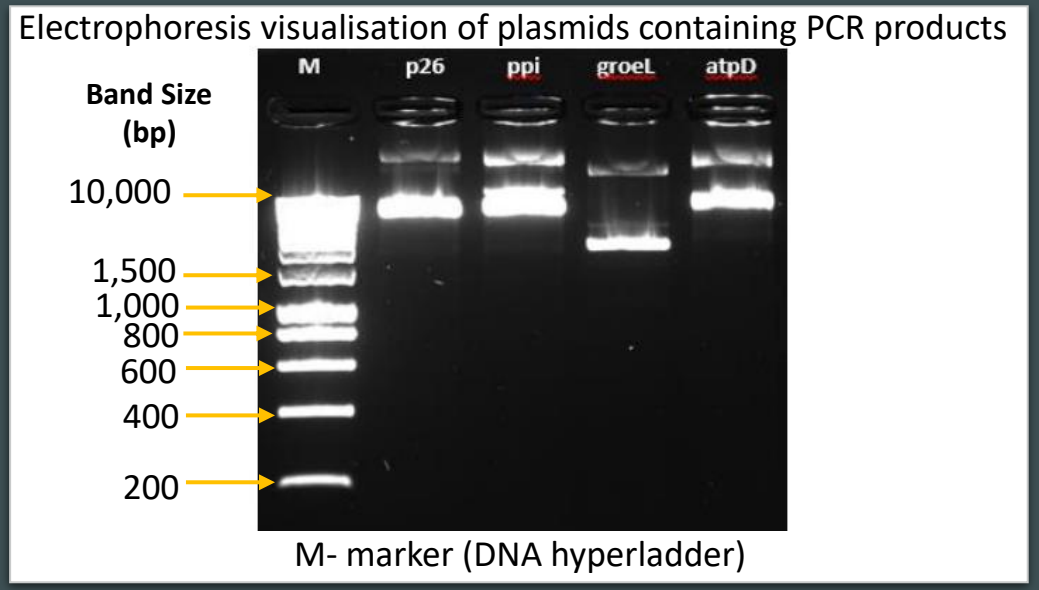
Lab 3

- 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)
p26	165	765
ppi	409	607
groEL	1,627	209
atpD	460	1,404

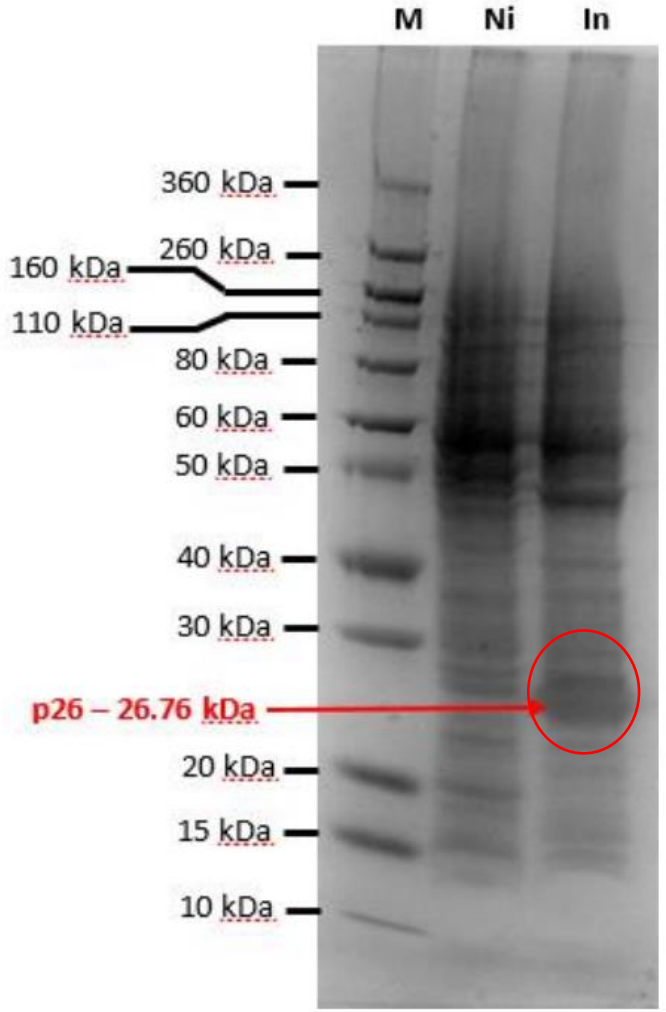




Lab 4

- 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

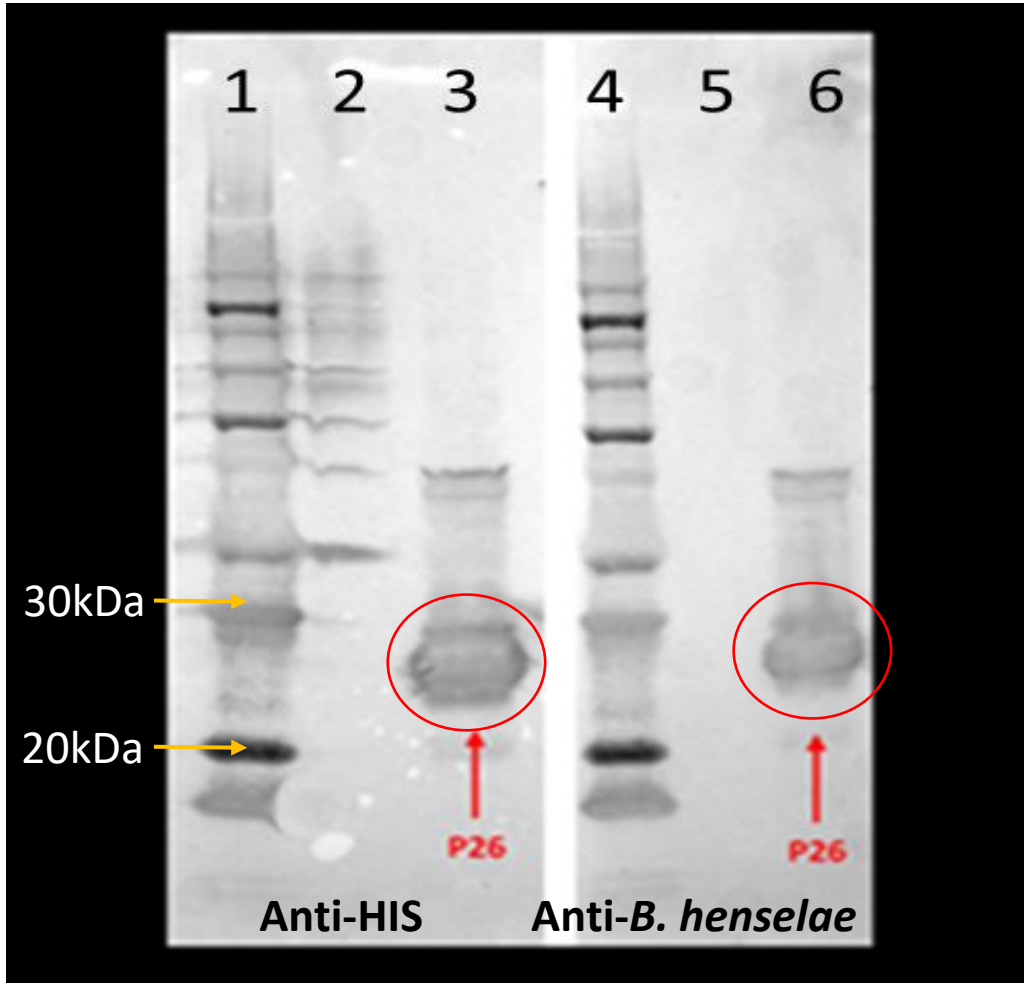


M- marker (size ladder) **Ni**- non-induced expression **In**- induced protein expression

Lab 5

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

Western blot analysis



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

Findings so far

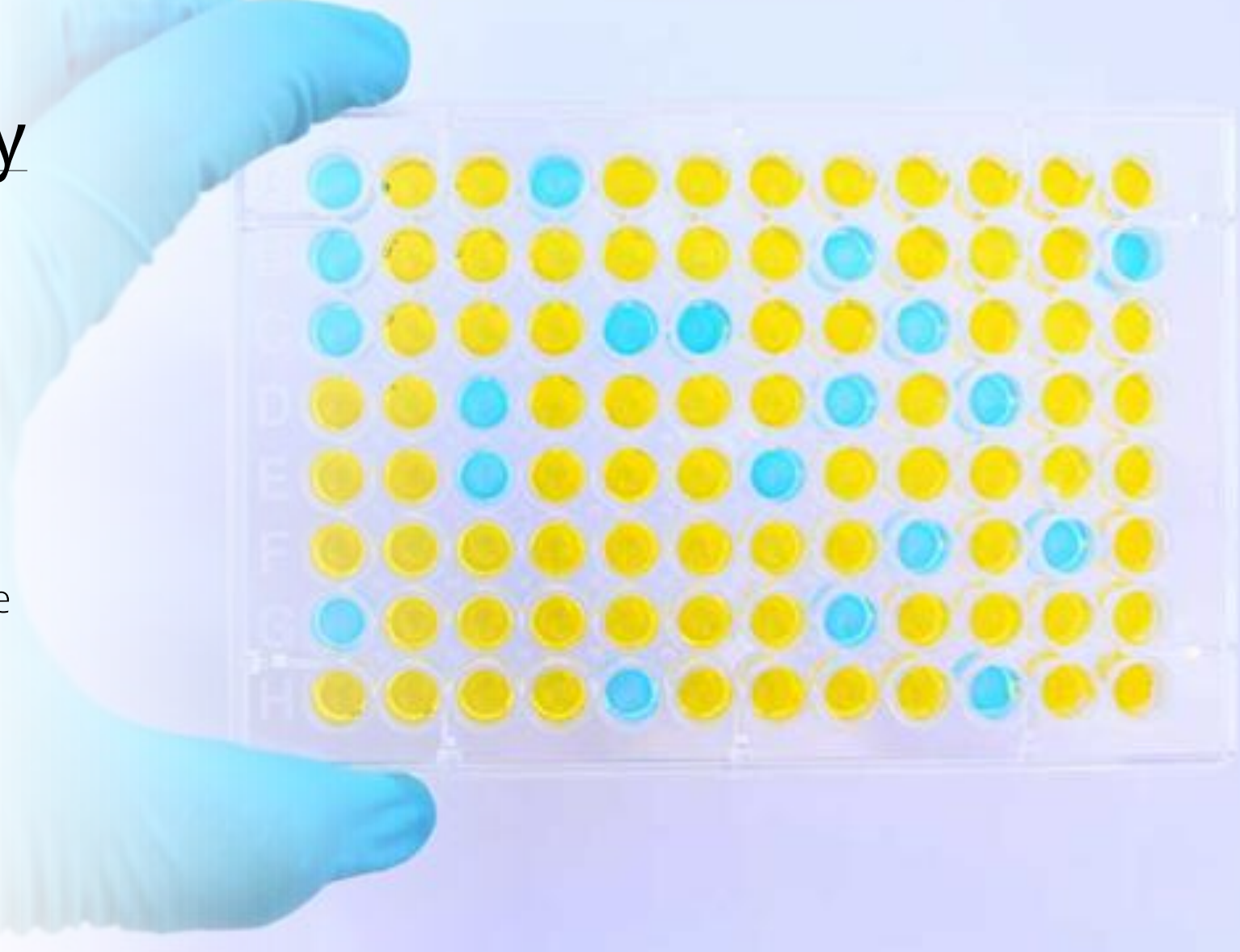
- P26 recombinant protein retains it immunogenicity and can be used in further experiments for CSD diagnosis
- P26 has the potential to be used as an antigen in a novel serodiagnostic assay for CSD

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

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

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

(Saisongkorh *et al*, 2010)

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

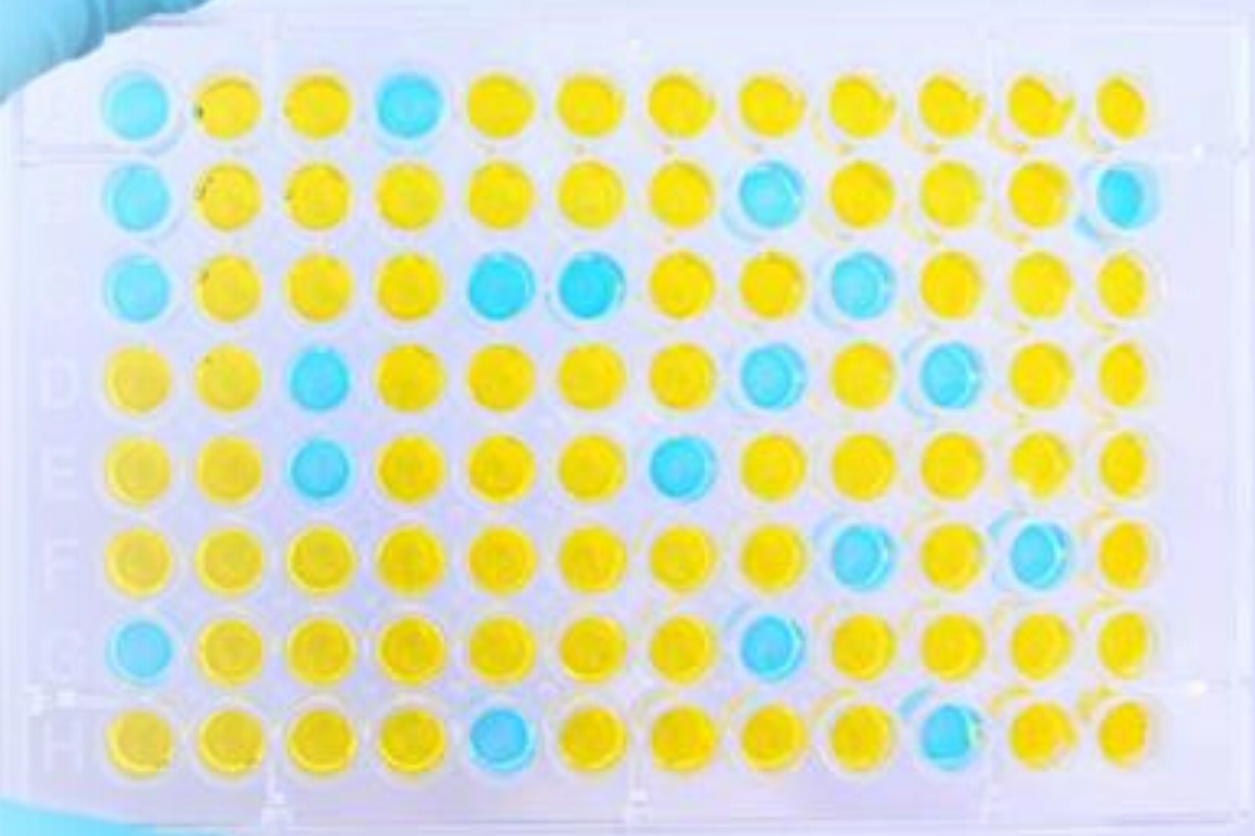
- Gather known CSD serum samples
- Gather known negative CSD samples
- Collect known CSD sera from Hansmann *et al* who has 29 known CSD serum samples (Hansmann *et al*, 2005).
- Collect known negative sera samples from 41 lymphoma patients in collaboration with a previous study (Ferrara *et al*, 2014).

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)



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