

Q FEVER SUBUNIT VACCINE

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Q Fever Subunit Vaccine. (May 2015)

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Q fever is a globally important zoonotic infection caused by the obligate intracellular bacterium *Coxiella burnetii*. Since *C. burnetii* is both an occupational hazard to humans interacting with naturally infected animals and a potential biothreat agent, a safe and effective vaccine is an important goal. Despite one being approved for use in Australia (Q-Vax®), this has not been licensed elsewhere due to complications with pre-sensitised individuals. As a result, research into developing a safe and effective vaccine against Q fever remains a priority in reducing the global impact this bacteria has on both livestock and human health. The goal of this project is to isolate and purify dominant antigenic *Coxiella* proteins expressed in *Escherichia coli*. These proteins will then be used as subunit vaccines that will be test the ability to confer protective immunity against Q fever in a mouse model large particle aerosol infection.

NOMENCLATURE

Luria-Burtani broth with Kanamycin- LB Kan

Base pairs- bp

Supernatant- SN

Kilodaltons- kDa

Isopropyl β -D-1-thiogalactopyranoside- IPTG

Small Ubiquitin-like Modifier- SUMO

Polymerase Chain Reaction- PCR

Enzyme-Linked Immunosorbent Assay- ELISA

Histidine- HIS

CHAPTER I

INTRODUCTION

Coxiella burnetii is a small, Gram-negative bacterium that is the etiological agent of Query fever (Q fever). This obligate intracellular pathogen is capable of withstanding extreme environmental pressures; high temperatures, ultraviolet light exposure, osmotic pressure, and standard disinfectants. *C. burnetii* has a very low infectious dose of 1-10 bacteria and can form a spore-like small cell variant to stay dormant in the environment if a host is not available. Once inside a eukaryotic cell, the bacterium forms an acidic vacuole and uses it to multiply. The pathogen undergoes antigenic phase variation. Phase I is the most virulent in animals. A switch to phase II is the result of the surface lipopolysaccharides truncation which results in reduced virulence (1).

As the causative agent of Q fever, a zoonotic bacterial disease, it is an important public health goal to have a vaccine that can confer protective immunity for individuals at high risk for coming into contact with the bacteria. Since livestock are the natural reservoirs of the bacteria, those who work with livestock have the greatest risk of infection (3). Infection often occurs from inhaling contaminated aerosols containing from bodily fluids or feces of an infected animal (3). At a more global scale, "*C. burnetii* is also classified as a "Category "B" critical biological agent" by the Centers for Disease Control and Prevention and is considered a potential weapon for bioterrorism" (3). *C. burnetii* has a low mortality rate (1-11%), but there has been a steady increase in the number of cases each year. It is essential to have protective measures against this bacterium that is so infectious and has the potential to be used as a biological weapon. It is

especially important to be able to prevent infection of our military that cannot afford to have an outbreak of Q fever while deployed.

Diagnosis of Q fever is difficult and is often confused for the flu due to its similar primary symptoms; fever, vomiting, and headache. Consequently, patients are often misdiagnosed and do not receive the appropriate treatment. There are two forms of the infection, acute and chronic. Acute Q fever is normally mild but some experience complications such as pneumonia, hepatitis, or meningoencephalitis (1). Chronic Q fever occurs in less than five percent of those with a previous acute infection. It can develop weeks or years later and normally affects pregnant women, immunosuppressed people, or those with a pre-existing heart defect (5). Chronic Q fever is severe and causes life-threatening damage to the lungs or most commonly to the heart (endocarditis) (3).

Currently there is a whole cell vaccine against *C. burnetii* licensed for human use in Australia which is effective in providing protective immunity, but they are not approved in the U.S. due to their reactogenic effects. The whole cell vaccine induces local to severe systemic reactions in individuals that have been previously exposed to *C. burnetii* (2). This means that a skin test must be given to patients prior to immunisation. It is too costly and time consuming to test each individual for previous exposure to *C. burnetii* (3). Therefore, a safe and efficient vaccine is needed to provide immunity and prevent infection without the risk of adverse reactions.

The goal of the project is to create a recombinant protein subunit vaccine that can elicit an adaptive immune response to successfully generate a protective antibody and T cell population.

T cells are thought to be necessary for a robust immune response that will produce memory cells for future encounters with the bacterium (7). CD8 T cells are effective against intracellular pathogens. Previous studies have found some *Coxiella burnetii* proteins stimulated an antibody response after the whole cell vaccination was given. Information on the proteins cross reactivity, function in the cell, and potential for native purification were used to choose a small group of candidate proteins. Once the proteins have been successfully expressed in *E. coli* and purified they will be tested for immunological responses in a mouse model system.

CHAPTER II

METHODS

Literature Review of Subunit Vaccine Candidates

In order to choose proteins that could potentially be antigenic, a review of some previously compiled data was done. Some early studies had shown that protection could be provided by subunit protein vaccines, including Williams et al. Which identified a *Coxiella burnetii* porin protein (P1) that provided protection to mice from a lethal challenge (8). Another protein that provided full protection in guinea pigs and mice was a large GroEL protein (67 kDa) (10). Purified *Coxiella burnetii* LPS has also been shown to provide protection in mice (9). However in a sublethal challenge model in BALB/c mice a singular protein were not found to provide protection, so a combination of dominant antigens was postulate to provide significant protection (11). The most important quality in a protein for our project was the ability to be purified so that immunogenic analysis could be performed. All four proteins chosen had previously been purified. Other qualities that were compared included if there was reactivity with hyperimmune sera, the molecular mass, and if the protein had been characterized in previous studies. Since a combination of proteins is most likely what will be needed for full protection, a variety of proteins with different qualities were favorable. The first protein chosen for this project, CBU_0891, fit the criteria because it was previously in a vaccine trial, has infection derived reactivity with human sera, and was characterized as an exported membrane associated protein composed of 312 amino acids (6). Our second antigen, CBU_1249, also has reactivity with human sera and is composed of 203 amino acids (6). It was chosen as a candidate to add diversity of function, it is annotated as a DNA-binding protein. CBU_1115 is annotated as a hypothetical protein comprised of 105 amino acids and has reactivity with human sera. This

candidate was chosen mainly because it has been previously tested as a Q fever diagnostic (6). The final candidate, CBU_1716, is predicted to be a glycine cleavage T protein composed of 391 amino acids, it also adds variation to the functions of each candidate protein (6). Since these proteins will most likely be combined in a later study to test for protection, a variation of functions could be an advantage when stimulating an immune response.

PCR Primers

Primers were designed by copying the gene sequence and amino acid sequence of each candidate protein into Microsoft word. The amino acid sequence was then copied into predisi.com to predict if there was a sec dependent secretion peptide on the *C. burnetti* sequence. If a sec sequence was present, it was highlighted on the sequence in Word and base pairs after the sequence were designed into the primer. A predicted primer around 15-25 base pairs was copied into an oligocalculator, such as <http://www.basic.northwestern.edu/biotools/oligocalc.html>. Bases were added or taken away until the melting temperature 50°C was reached. The reverse primer was designed using the same process and then putting the primer in a reverse compliment calculator, http://www.bioinformatics.org/sms/rev_comp.html. *C. burnetii* gene sequences of interest were amplified using PCR. Reagents used for PCR are from the pET SUMO TA cloning kit (Invitrogen) with the addition of the previously designed primers and template DNA. Amplification was performed in a 50 µl reaction volume consisting of 1µl template DNA, 5 µl 10X PCR Buffer, 0.5 µl dNTP mix (10mM), 1 µl primer 1, 1 µl primer 2, 40.5 µl RNase free water, and 1 µl Taq DNA Polymerase.

Transformations

The amplified PCR product was cloned into the SUMO vector through a ligation reaction (Figure 1.1). Ligation reagents from the pET SUMO TA cloning kit were combined with fresh PCR product to a reaction volume of 10 μ l; 1 μ l fresh PCR product, 1 μ l ligation buffer, 2 μ l SUMO vector, 5 μ l water, 1 μ l T4 DNA Ligase. The ligation reaction was incubated overnight at 15°C. The construct from the ligation was transformed into One Shot Mach1-T1 competent *E. coli* cells. Competent cells were thawed on ice, 2 μ l of the ligation reaction was added to the competent cells and incubated on ice for 30 minutes. Heat shock was used for 45 seconds in the heat block at 42°C, then cells were put back on ice for 2 minutes and 250 μ l of warm S.O.C. was added. Cells were incubated at 37 °C on the rotator for 1 hour, plated on warm LB Kan plates (50 μ g/ml) with 250 μ l room temperature S.O.C., and incubated overnight at 37°C.

Colony PCR was used to analyze transformants. Each sample colony was added to 50 μ l 5 % Chelex and incubated at 100°C for 10 minutes. This was then spun down for 3 minutes at 16,000 x g. The colony PCR reaction consisted of 3 μ l colony and Chelex mixture, 5 μ l 10x PCR buffer, 0.5 μ l dNTP mix (10mM), 1 μ l SUMO forward primer 1, 1 μ l gene specific reverse, 38.5 μ l RNase free water, and 1 μ l Taq DNA Polymerase. Colony PCRs were visualized with a 1% agarose gel. The GeneJet plasmid prep mini kit (Thermo Scientific) was used isolate the plasmid for transformation and to prepare colony PCR confirmed constructs for sequencing.

Chromatograms and sequences were analyzed to confirm the plasmid was present and in the correct orientation.

Protein Purification

Sequence confirmed constructs were transformed into BL21(DE3) One Shot *E. coli* cells (Invitrogen). The constructed plasmids have an inducible promoter that expresses the gene product in the presence of Isopropyl β -D-1-thiogalactopyranoside (IPTG). The sequence confirmed constructs were cultured by inoculating 50 ml LB with Kanamycin (50 μ g/ml) with one single colony and incubating overnight at 37 °C. Fresh LB Kan (500mL) was inoculated with 2.5ml of overnight culture and grown to an OD of 0.8 (about 1 hour and 15 minutes). Culture was then induced by adding 500 μ l IPTG and growing 4 hours at 37 °C. Cells were harvested by centrifuging at 10,000 rpm for 20 minutes. The pellet was washed with PBS and stored in 1 mL fractions at -80°C until purification. The pellet was thawed and microcentrifuged at 6000 rpm for 5 minutes, then resuspended into a buffer mixture consisting of 886 μ l suspension buffer, 100 μ l lysozyme (fc 1 mg/mL), 0.4 μ l benzonase nuclease, and 10 μ l protease inhibitor (1x). Different suspension buffers were used depending on the type of purification, the standard suspension buffer contained 50mM Tris, 50 mM NaCl and was at a pH of 7.5. Cells were disrupted by sonicating with a 3mm microtip in 5 minute cycles of 10 seconds on, 5 seconds off until the solution was clear. The solution was centrifuged at 16,000 x g for 20 minutes at 4°C. The soluble lysate was separated from the pellet and both were purified using HisPur Ni-NTA Resin batch method. This method uses an affinity column with nickel resin that binds to the 6xhis tag that is on each protein. Purified protein was analyzed using an SDS Page Gel.

CHAPTER III

RESULTS & DISCUSSION

PCR Primers

Coxiella burnetii antigenic candidates were chosen based on an extensive review of the literature. Primers were designed to recombinantly express these proteins in a pET SUMO *E. coli* expression system, Figure 1.1 is the vector map of pETSumo from Invitrogen. The vector contains a lac operon, His tag, and a Kanamycin resistance gene. Figure 1.2 shows *Coxiella burnetii* genes that were amplified using PCR and then run on a 1% agarose gel; CBU_0891 (939 bp) and CBU_1143 (351 bp) are in the correct vicinity of their molecular weight. However, CBU_1249 (612 bp), and CBU_0612 (498 bp) are a little higher than they should be. Figure 1.3 is a PCR of four additional *Coxiella burnetii* genes; CBU_0781 (1017 bp) and CBU_0140 (1242 bp) are at the correct molecular weights, the band at CBU_0311 (759 bp) is around 1000 base pairs which is too high, and CBU_0383 (639 bp) did not show up at all. The PCR for CBU_0383 was performed again with a PCR program changing the melting temperature to 51 °C, which was the melting temperature for this particular primer. The PCR was successful and the band was at the correct molecular weight marker as seen in Figure 1.4.



Figure 1.1 From Invitrogen. Plasmid map of vector used to clone *Coxiella burnetii* genes and express recombinant proteins.

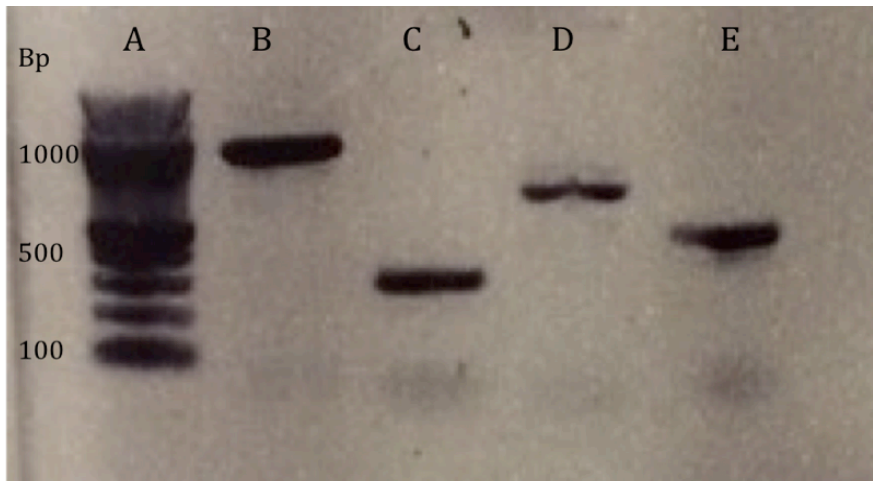


Figure 1.2 PCR product on 1% agarose gel **A** DNA ladder **B** CBU_0891 (939 bp) **C** CBU_1143 (351 bp) **D** CBU_1249 (612 bp) **E** CBU_0612 (498 bp).

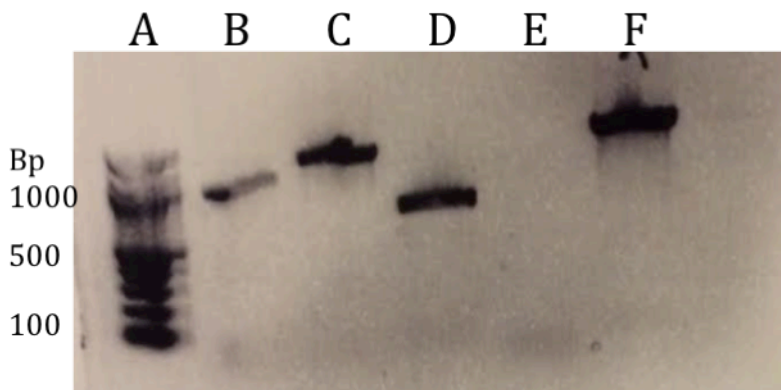


Figure 1.3 PCR product on 1% agarose gel. **A** DNA ladder, **B** CBU_0781 (1017 bp), **C** CBU_0140 (1242 bp), **D** CBU_0311 (759 bp), **E** CBU_0383 (639 bp), **F** positive control.

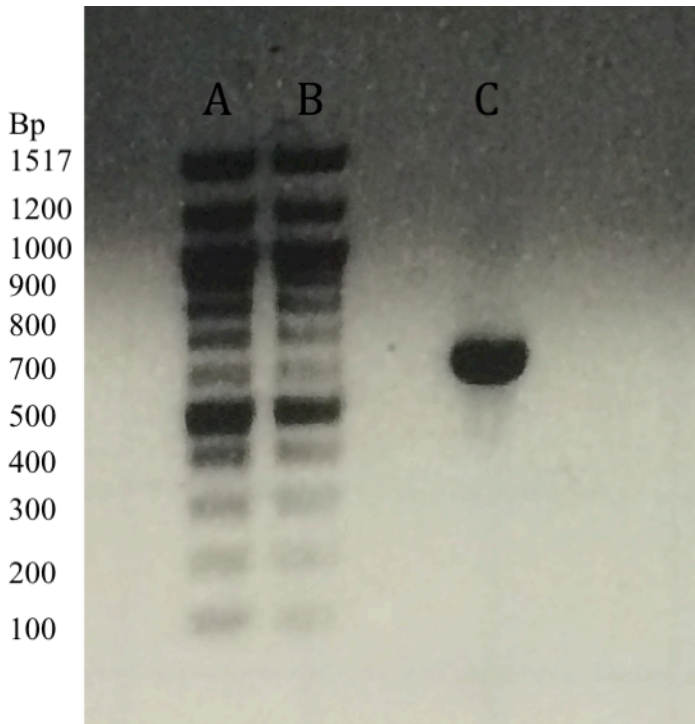


Figure 1.4 PCR product on 1% Agarose gel
A&B DNA ladder **C** CBU_0383 (639 bp).

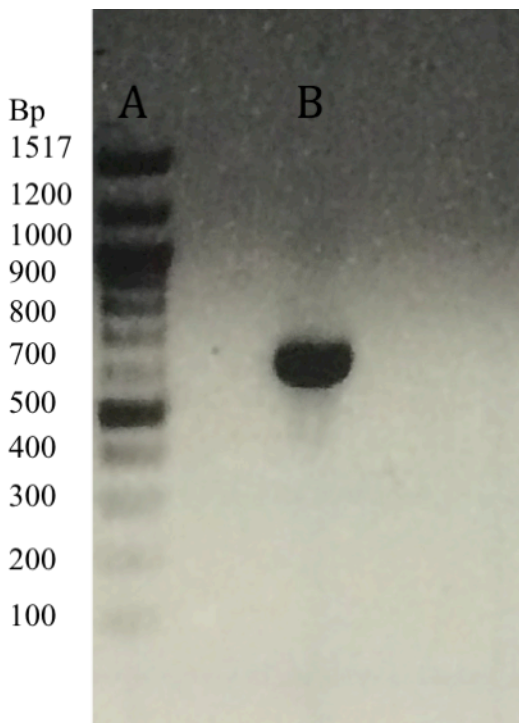


Figure 1.4 PCR product on 1% Agarose gel
A DNA ladder **B** CBU_0383 (639 bp).

Transformations

Plasmids transformed into Mach1 *E. coli* cells grew small, white circular colonies with no discernable elevation. Colony PCR of a few colonies from each plate was used to confirm the plasmid was present. The primers used for the colony PCR reaction only read in one direction, therefore products are only observed if the construct is in the correct orientation. Colony PCR confirmed constructs were sent for sequencing. Figure 2.1 is a colony PCR of plasmid CBU_0891 six colonies from the transformation were selected. All colonies from CBU_0891 showed the plasmid was present and these were sent off for sequencing. Figure 2.2 shows a colony PCR of CBU_1249. Three lanes D, E, and F show a faint band and these three colonies were sent off for sequencing.

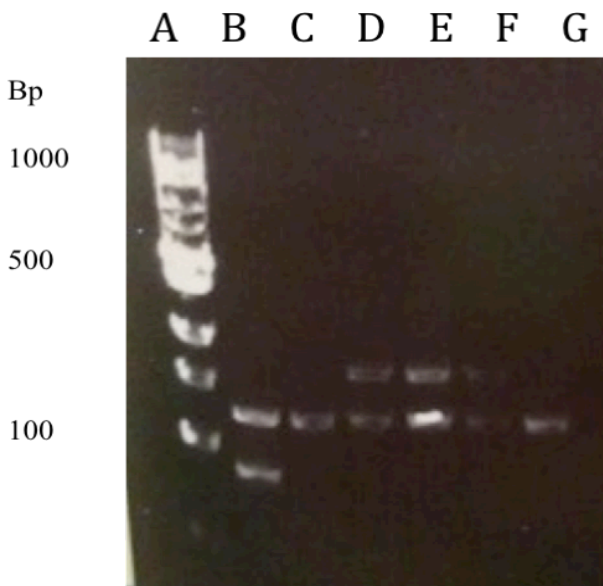


Figure 2.1 Colony PCR product on 1% agarose gel. **A** DNA ladder, **B-G** CBU_891 colonies 1-6 (939 bp).

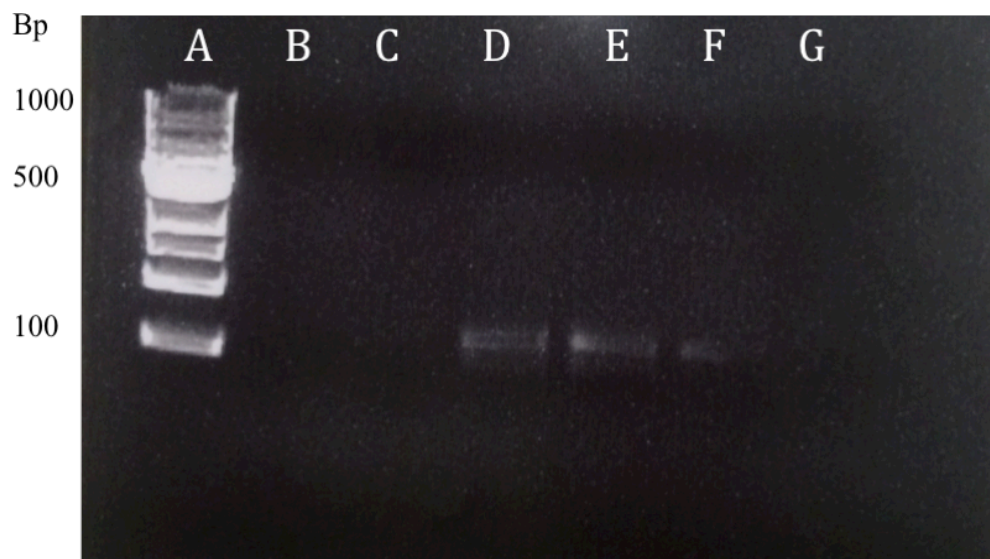


Figure 2.2 Colony PCR on 1% agarose gel A DNA ladder B-G CBU_1249 colonies 1-6 (612 bp).

Protein Purification

The constructed plasmids have an inducible promoter that expresses the gene product in the presence of IPTG. Cultures grown in the presence of IPTG were compared with uninduced cultures by running them on a 4-12% Bis-Tris protein gel. Figure 3.1 shows these eight fractions from four separate inductions. Although lane B is smudged, when comparing it to the induced lane C it is evident that more protein was expressed in the induced culture. When looking at the gel at 55 to 60 kDa there is a band that wasn't present in the uninduced lane, the induced lane also has thicker bands at 38 and 50 kDa which means more expression occurred. When comparing lanes D and E/ F and G a new band of expression occurred around 20 kDa and 30 kDa respectively. Lanes H and I did not have any new bands of expression in the induced lane, but the induced lane had thicker bands and therefore more expression of protein. Purifications under all conditions were done using a nickel packed affinity column, which binds to the 6xhis tag in each protein. Purifying with denaturing versus native conditions for CBU_1716 and CBU_1115 was not successful; therefore a salt gradient purification was performed on each to determine if that was a better choice for this particular protein. Figure 3.2 shows the protein gel

of the salt gradient purification done on CBU_1716 protein. The gel had protein at the correct molecular weight when including the SUMO tag that is still on the protein, 38 kDa plus 11 kDa (Sumo) makes the protein around 50 kDa. Although protein was purified at each concentration of salt, the lowest salt concentration, 0.1 M NaCl acquired the most protein. Figure 3.3 is the protein gel of the salt gradient purification for CBU_1115. At the highest salt concentration, 1M KCl, a faint band is seen just below 31 kDa. CBU_1115 protein should be around 20 kDa including the sumo tag. This protein purification was not successful and a pH gradient will be the next purification tried on CBU_1115.

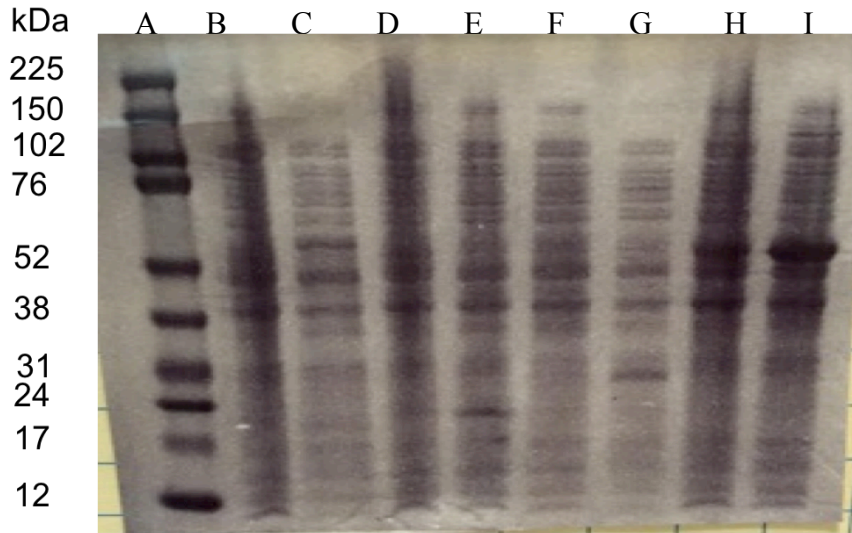


Figure 3.1 Coomassie blue stained 4-12% Bis-Tris protein gel. A Protein Plus Ladder, B&C CBU_0891 uninduced & induced, D&E CBU_1249 uninduced & induced, F&G CBU_1115 uninduced & induced, H&I CBU_1716 uninduced & induced.

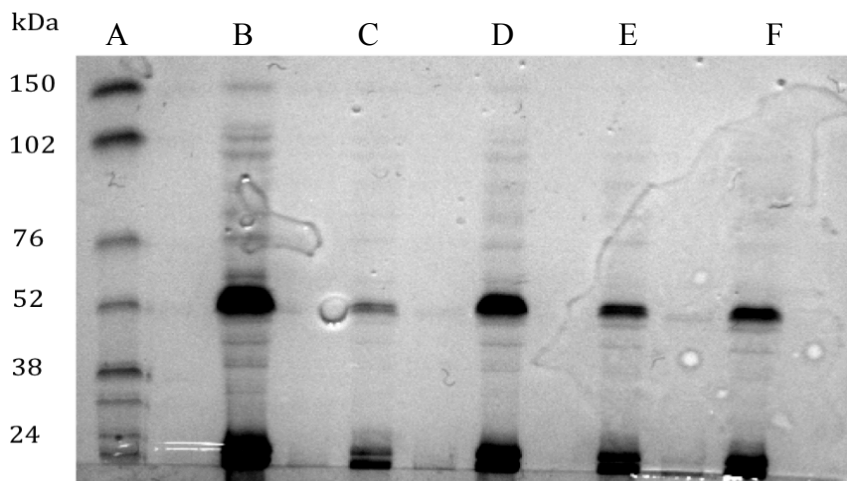


Figure 3.2 Coomassie blue stained 4-12% Bis-Tris protein gel, Salt gradient purification of CBU_1716 (50 kDa). A Protein Plus Ladder B 0.1 M NaCl C 0.5 M NaCl D 1M NaCl E 0.1 M KCl F 1M KCl

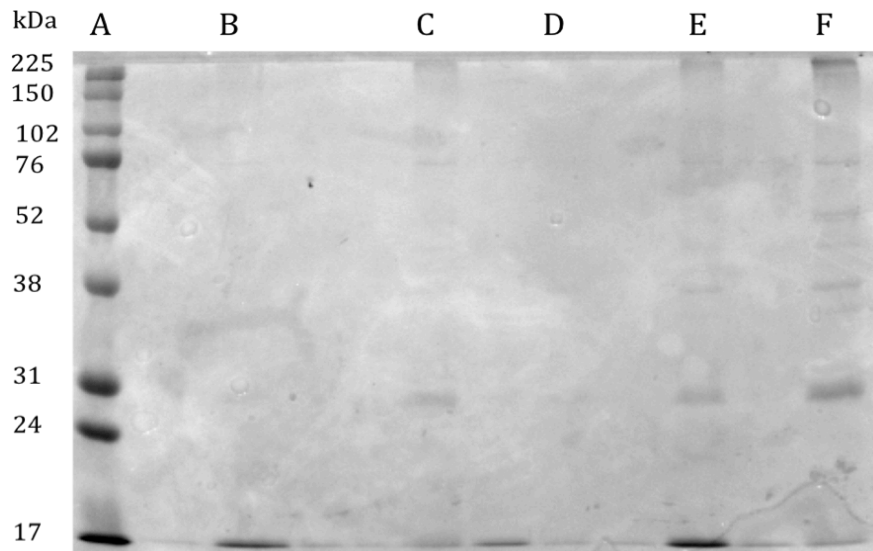


Figure 3.3 Coomassie blue stained 4-12% Bis-Tris protein gel, Salt gradient purification of CBU_1115 (20kDa). **A** Protein Plus Ladder **B** 0.01 M NaCl **C** 0.5 M NaCl **D** 1M NaCl **E** 0.1 M KCl **F** 1M KCl

Immunization

In order to test the efficacy of each vaccine, the purified proteins combined with an adjuvant, CpG, will be subcutaneously injected into female BL57/6 mice. This adjuvant favors a Th1 immune response. This will be followed by a boost 2 weeks later, and resting for an additional 5 weeks to allow innate activation to subside. The challenge will be done with 1×10^5 *C. burnetii* RSA 493. The mice will be euthanized after 2 weeks and serological responses will be measured for specific antibody titers, immunoglobulin antibody class switching, and T cell memory recall. These tests will help determine the type of immune response induced. If successful, the mice that were vaccinated with the recombinant protein vaccines would demonstrate a similar protective response to the positive control mice that were vaccinated with the whole cell vaccine. While the negative control animals will manifest disease.

CHAPTER IV

CONCLUSION

During the cloning process, a total of 14 primers were designed. PCR cloning was unsuccessful for CBU_1645, CBU_1184, CBU_1143, and CBU_0952. The primers for each of these need to be redesigned. Colony PCR from the transformations was only successful for CBU_0891 and CBU_1249. This technique was hard to perform accurately and sequencing was also done on CBU_1115 and CBU_1716 constructs that were not verified by colony PCR. The sequencing results confirmed each of the four constructs (CBU_0891, CBU_1249, CBU_1115, and CBU_1716) were successfully cloned into the pET SUMO vector and transformed into *E. coli* for expression of protein. Each of the four protein candidates have been recombinantly expressed in *E. coli* by an inducible promoter. Each initial induction successfully produced enough protein for purification with the Ni resin affinity column. Purification of these proteins is still ongoing as each protein is different and requires a different purification protocol. *C. burnetii* proteins are difficult to purify due to their extreme isoelectric points, this is most likely due to the intracellular lifestyle of *C. burnetii* thriving at a low pH. Several different methods of purification are being used on each candidate antigen. CBU_1716 has been successfully purified using a salt gradient purification. This protein will be added to the ongoing immunization study that was designed to test the protective efficacy of subunit antigens against a *C. burnetii* aerosol challenge. Once the other candidates are purified they will also be added to an immunization and protection study.

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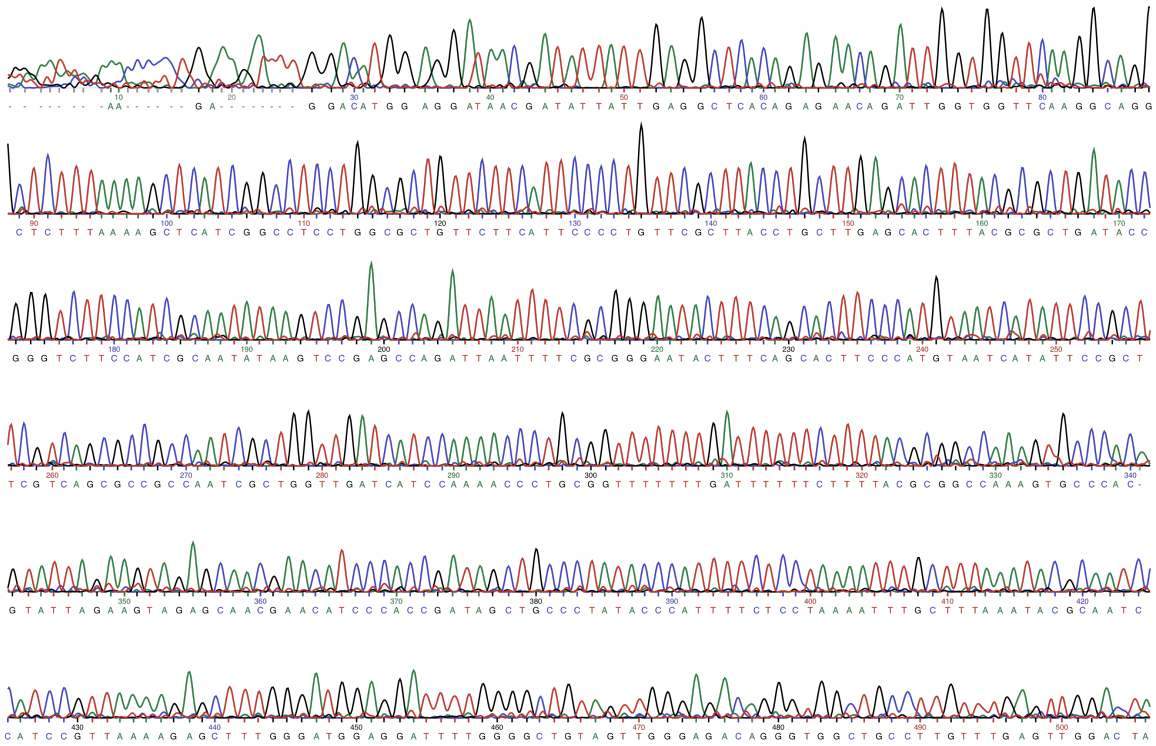
APPENDIX

Candidate Antigen	Forward Primer	Reverse Primer
CBU_0140	ATG CCG AAA AAT CCA GAT AAA	TCAAAAATTTCTTGGAACCA
CBU_0311	GGC GGT CCT GAT ATC C	TTAGATATTGAACAGGTAATTA
CBU_612	CAA ACG GTT GGG CTT GTC	TTATTTCAATGCTGATACAAC
CBU_0781	ATG AGT AGA CGT GAG ACT	TCACCGAGGACTAGACA
CBU_0891	GTG AAA CGG TAT CTT TTT TTT	TCAAGGCAGGCTCTTTAAAA
CBU_0952	GAA AAT CGC CCC ATT TTA A	CTATTTATTTAAAAAAGTCGCC
CBU_1115	ATG TCA AAA TTT TTA CAC CGT	TCAAGATTTATTCTCTCTATC
CBU_1143	CAA GCG CCG CAT CCG	TTAAATCGAATCGATCGTGC
CBU_1184	TTG CGC TTA CAA AGA GTC T	TTAGGAAGGAATTTCTGG
CBU_1249	ATG ACG GAA AAT GCG ATC	TCAATGATTAGTTCCTGAAAAA
CBU_1645	ATG ATA GAA AAA TCG CAA CG	TCATTCCTGCGAAGCAG
CBU_1716	ATG CCT TTG CAT TAT GGA TC	TCAAATGTTTTTTTACCACG
CBU_1718	ATG GCT GCA AAA GTT TTA AAA	TTACATCATGCCGCC
CBU_1910	GCC CCC TCT CAA TTC A	TTACTTTTCTACCCGGTC

Sequencing Data CBU_0891

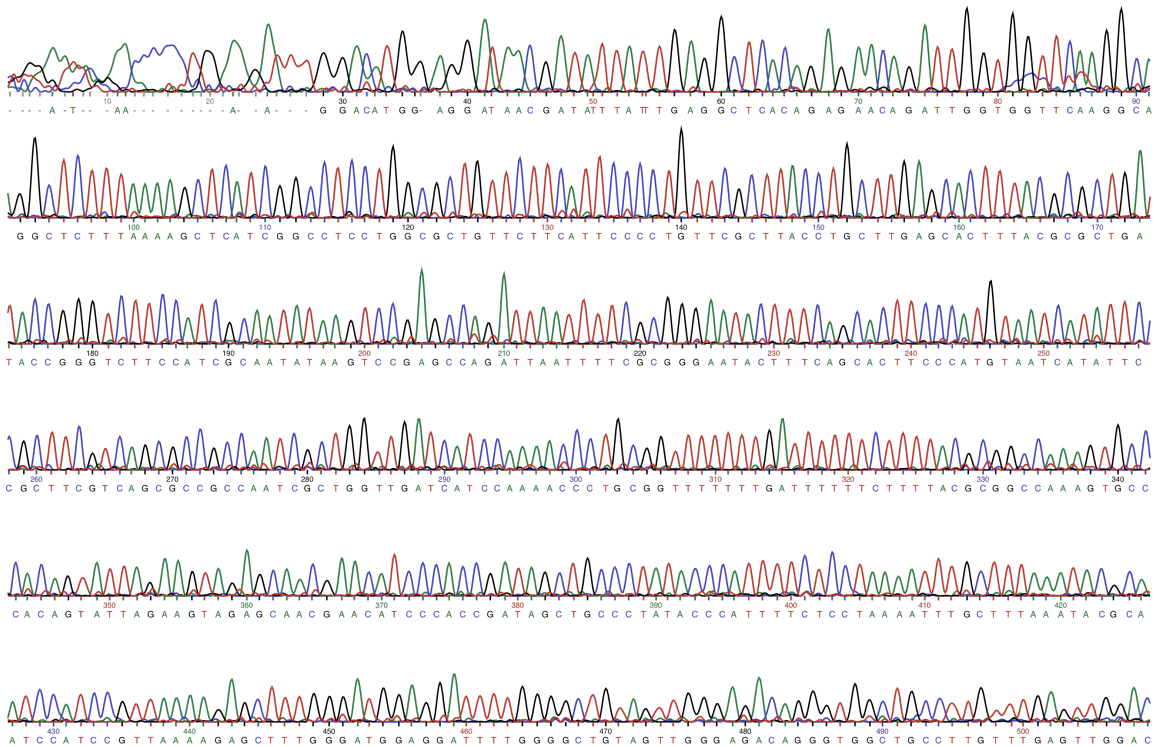
Samples: 11210
Bases: 760
Average spacing: 15

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Samples: 11121
Bases: 899
Average spacing: 13

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CBU_0891_Forward Sequence

NNNNNNNNAANNNNNNNGANNNNNNNNGGACATGGAGGATAACGATATTATTG
AGGCTCACAGAGAACAGATTGGTGGTTCAAGGCAGGCTCTTTAAAAGCTCATC
GGCCTCCTGGCGCTGTTCTTCATTCCCCTGTTTCGCTTACCTGCTTGAGCACTTTA
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TTA

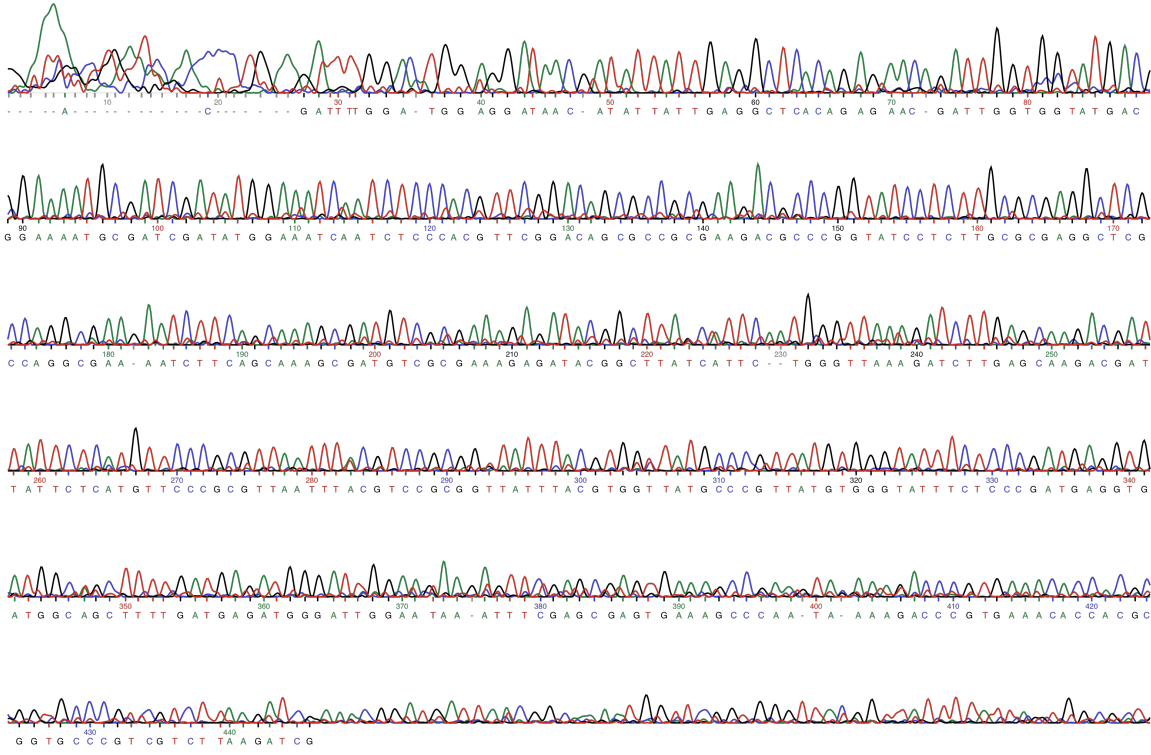
CBU_0891_Reverse Sequence

NNNANTNNNAANNNNNNNNNANNANNNGGACATGGNAGGATAACGATATTTA
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ATCGGCCTCCTGGCGCTGTTCTTCATTCCCCTGTTTCGCTTACCTGCTTGAGCACT
TTACGCGCTGATACCGGGTCTTCCATCGCAATATAAGTCCGAGCCAGATTAATT
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TCNGCCTGAANCATAGCCCGAANAGTCANGNNTCACAACCGCGTTANCGGATG
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NNNTCTNNAGGGGTNNNGNNNNNNNANNNNNCN

Sequencing Data CBU_1249

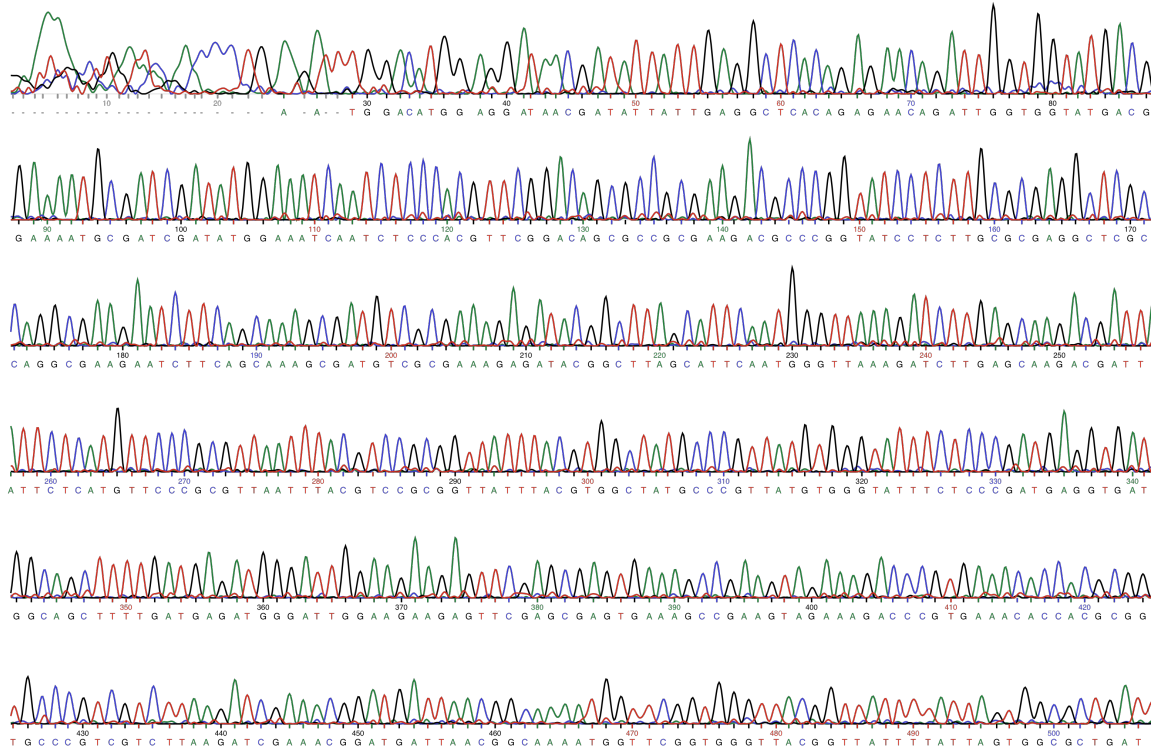
Samples: 11208
Bases: 447
Average spacing: 26

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Samples: 11047
Bases: 912
Average spacing: 13

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CBU_1249_Forward Sequence

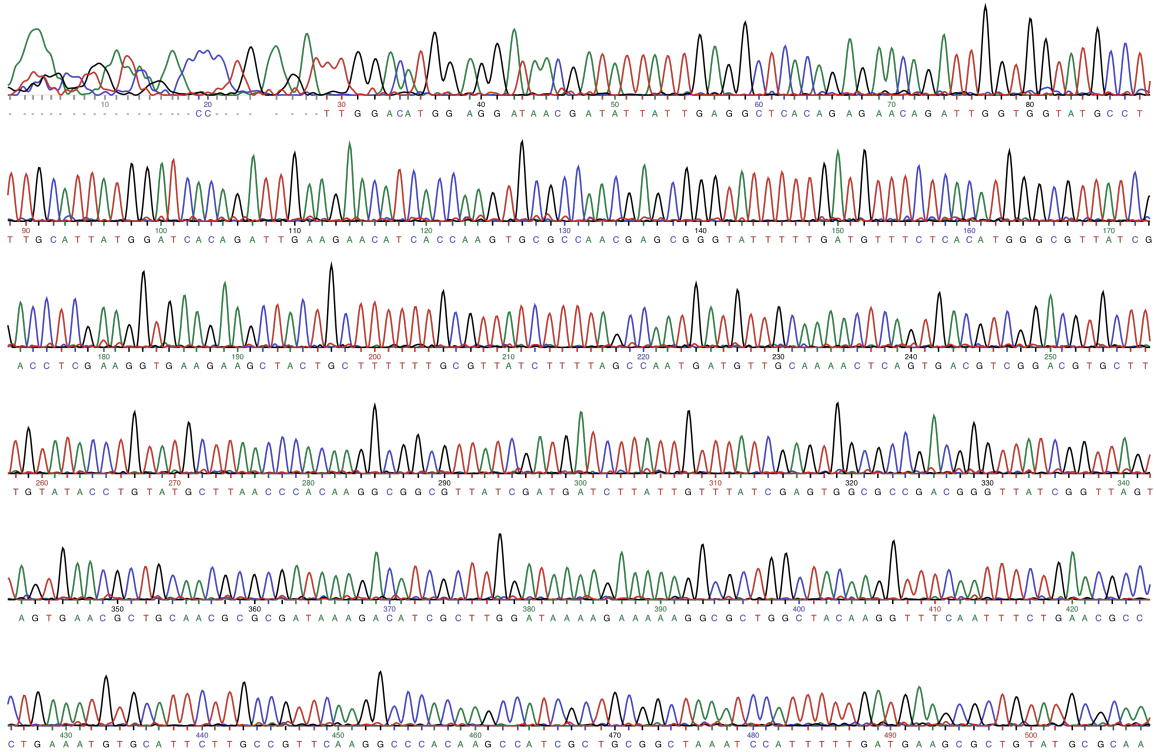
NNNNNANNNNNNNNNNNNNNCNNNNNNNGATTTTGGANTGGAGGATAACNATA
TTATTGAGGCTCACAGAGAACNGATTGGTGGTATGACGGAAAATGCGATCGA
TATGGAAATCAATCTCCCACGTTCCGGACAGCGCCGCGAAGACGCCCGGTATCC
TCTTGCGCGAGGCTCGCCAGGCGAANAATCTTCAGCAAAGCGATGTCGCGAA
AGAGATACGGCTTATCATTNNTGGGTAAAGATCTTGAGCAAGACGATTATT
CTCATGTTCCCGCGTTAATTTACGTCCGCGGTTATTTACGTGGTATGCCCGTT
ATGTGGGTATTTCTCCCGATGAGGTGATGGCAGCTTTTGATGAGATGGGATTG
GAATAANATTTTCGAGCGAGTGAAAGCCCAANTANAAAGACCCGTGAAACACC
ACGCGGTGCCCGTCGTCTTAAGATCGA

CBU_1249_Reverse Sequence

NNNNNNNNNNNNNNNNNNNNNNNNNANANNTGGACATGGAGGATAACGATATTA
TTGAGGCTCACAGAGAACAGATTGGTGGTATGACGGAAAATGCGATCGATAT
GGAAATCAATCTCCCACGTTCCGGACAGCGCCGCGAAGACGCCCGGTATCCTCT
TGCGCGAGGCTCGCCAGGCGAAGAATCTTCAGCAAAGCGATGTCGCGAAAGA
GATACGGCTTAGCATTCAATGGGTAAAGATCTTGAGCAAGACGATTATTCTC
ATGTTCCCGCGTTAATTTACGTCCGCGGTTATTTACGTGGCTATGCCCGTTATG
TGGGTATTTCTCCCGATGAGGTGATGGCAGCTTTTGATGAGATGGGATTGGAA
GAAGAGTTCGAGCGAGTGAAAGCCGAAGTAGAAAGACCCGTGAAACACCAC
GCGGTGCCCGTCGTCTTAAGATCGAAACGGATGATTAACGGCAAATGGTTC
GGTGGGTTACGGTTATTTTATTAGTGGCGCTGATTGTCTCTGTGGGCGTTTGGT
GGCAAGGCAAAAAACATATTCTTTGGGCAATTGCAATCAGTTATTTCTTCGCC
CGCAAGNAGAATTTGCCCTTAAACAAACGTCAGTGAATTCAGTGAATACTA
AATAAAACGATCAACACAAATTTGACGGAGCAACCCACGGGCCATAAAATA
CAAGAATAAAGATAACTTTTTTCAGGAACTAAATCATTGAAGACAAGCTTTAG
GGTATTTTATTCNGGCGNNAAGTNGCTGTCNNGGTGATTGCTGNCTACTTTANT
CGTANCNNCCACCACCACCACCTGAGATCCNGCTGCTTAANCAAAGCC
CNGANNNNNCTGNNNNGNCNNCTTNCCCACCNNNCTGNAGCNNNNNNNCNN
ANNNNNNAANCCCCCNTNNG

Sequencing CBU_1716

Samples: 10910
Bases: 8960
Average spacing: 13
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11/5/2014



CBU_1716_Reverse Sequence

NNNNNNNNNNNNNNNNNNCCNNNNNNNNNTTGGACATGGAGGATAACGATATTATTGAGGGCTCACA
GAGAACAGATTGGTGGTATGCCTTTGCATTATGGATCACAGATTGAAGAACATCACCAAGTGCG
CCAACGAGCGGGTATTTTTGATGTTTCTCACATGGGCGTTATCGACCTCGAAGGTGAAGAAGCT
ACTGCTTTTTTTCGTTATCTTTTAGCCAATGATGTTGCAAACTCAGTGACGTCGGACGTGCTT
TGTATACCTGTATGCTTAACCCACAAGGCGGCTTATCGATGATCTTATTGTTTATCGAGTGGC
GCCGACGGGTTATCGGTTAGTAGTGAACGCTGCAACGCGGATAAAGACATCGCTTGGATAAAA
GAAAAGGCGCTGGCTACAAGGTTTCAATTTCTGAACGCCCTGAAATGTGCATTCCTTGCCGTTT
AAGGCCACAAGCCATCGCTGCGCTAAATCCATTTTTGATGAAGCGCTGTATGCGCAATTGGA
AGCCCTTAAACCTTCCACTTTATTTTCATCACCGACGCGAGATTTGCAAATAGCCCGAACAGGC
TACACGGGTGAAGATGGANTTGAATAATTGTCGCCGCTTCTCGCGGACGGATCTTTGGGCGC
GCTTCGTTTACCAGGGAGTGAACCTTGTGGTTTAGGAGCACGCGATACCTTGCCTTAGAANC
NNGTTTAAATTTTATATGGNACAGATATGGGACGAAACGACTTCTCNTTTTAAATTTCCAATCTC
AGCTGGACGGTGTCCNGGNACGACNCGGNTCGNAATTNATANGTCGNNNNNCNCTTGGNAAAAA
CNANTTNGAACNANNAATNGNCCNNAANAANCANTCTTANNCGGGNTTTNNNNNN