（Abroad・Domestic）Official trip report form（Student） 2014/06/10 (Year/Month/Day)

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Laboratory	Division of Collaboration and Education, CZC
Year (Grade)	D4
Destination	Obihiro University of Agriculture and Veterinary Medicine. Japan
Period of trip	January 9 to March 7, 2014
Purpose of trip	Internship

Summary of activities （about 800 words, provide photos, tables and figures that clearly show the activities during the period）

The purpose of my internship was to acquire additional skills in Molecular epidemiology of Protozoan disease, especially African Trypanosomiases.

Why Obihiro University?

- The **National Research Center for Protozoan Diseases** (NRCPD)
  Obihiro University has a specialist center for protozoan diseases.
  -*Trypanosoma:* given that my primary interest is Trypanosomiasis, It was an opportunity for me to benefit from their expertise.
  -Tick-borne diseases: Obihiro also specialiazes in Tick-borne diseases, which is my second interest.
- Prof. Noboru Inoue: Prof. Inoue is an expert on Protozoan diseases especially *Trypanosoma* and has worked in many countries including many *Trypanosoma* projects in Africa where the disease is endemic. Therefore, I needed to acquire his expertise.

Cloning and Expression of an uncharacterized surface protein of *Trypanosoma congolense* and potential use as a diagnostic antigen

Activities

I undertook several activities listed below:

- Trypanosome culture
- DNA extraction
- DNA amplification and gel extraction
- Cloning and sequencing

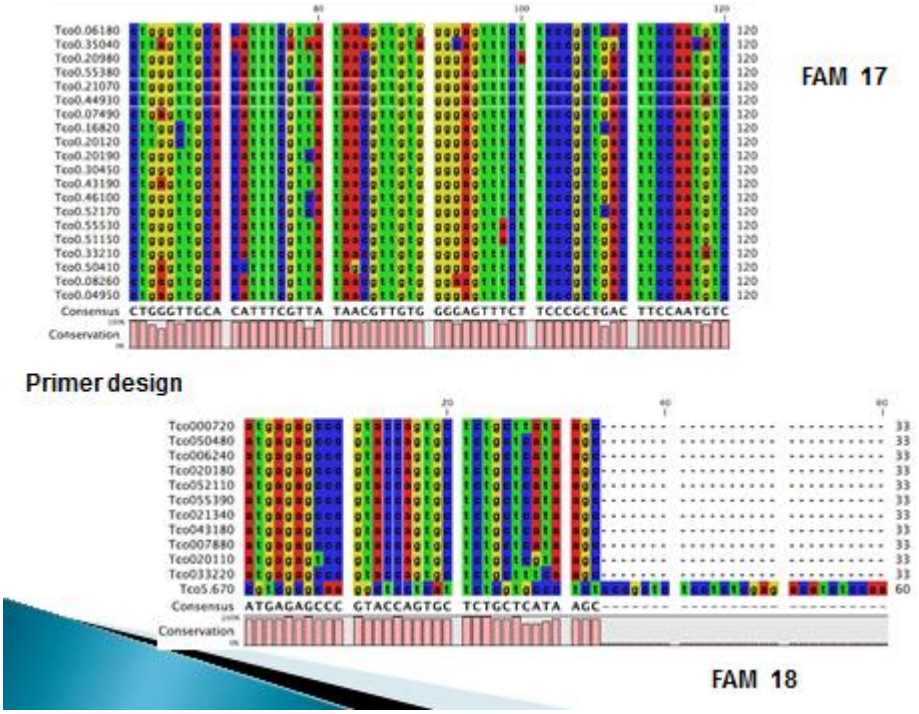
- Recombinant protein expression

I was also exposed to several activities below necessary for the continuity of my project but were not achievable in Obihiro due to the limited time of 2 months. However, I have to undertake these activities in my future endeavours now that I'm aware of them.

- Polyclonal antibody production
- Southern blot analysis
- Western blot analysis
- Indirect fluorescent antibody test (IFAT)
- Mice infections
- ELISA
- Data management and analysis

Following a report: In a cell-surface phylome analysis of African Trypanosomes (Jackson *et al.*, 2013), the cell surface proteins with putative surface functions were characterized. Therefore, I intended to study and characterize unknown *T. congolense* cell surface proteins with a potential application as diagnostic antigens for *T. congolense*. I set out to select and study Family 17 & 18 *Trypanosoma congolense* genes.

Primer design



I designed primers for Family 17 and Family 18 *T. congolense* cell surface genes.

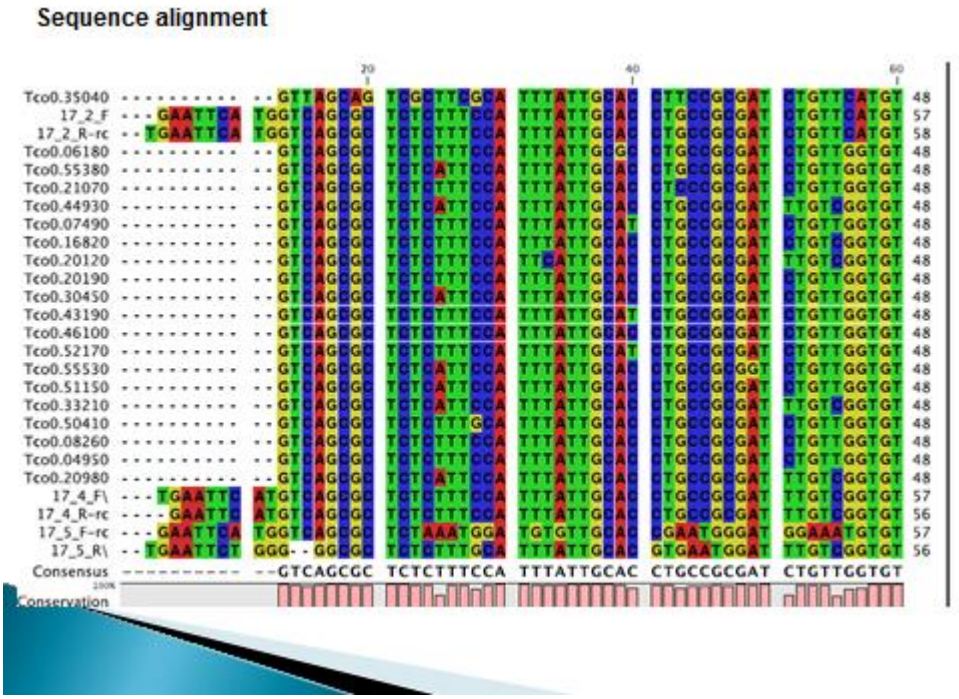
Fam 17 genes are conserved so we designed a common primer, Fam 17 common. Fam 18 genes are conserved too except Tco5.670 a specific gene.

So I designed a common Fam 18 primer in addition to a specific Tco5.670 primer. We incorporated restriction enzyme sites ECOR1 and NOT1. The primers were designed for PGEX 6P.1 expression vector and GST Tag.

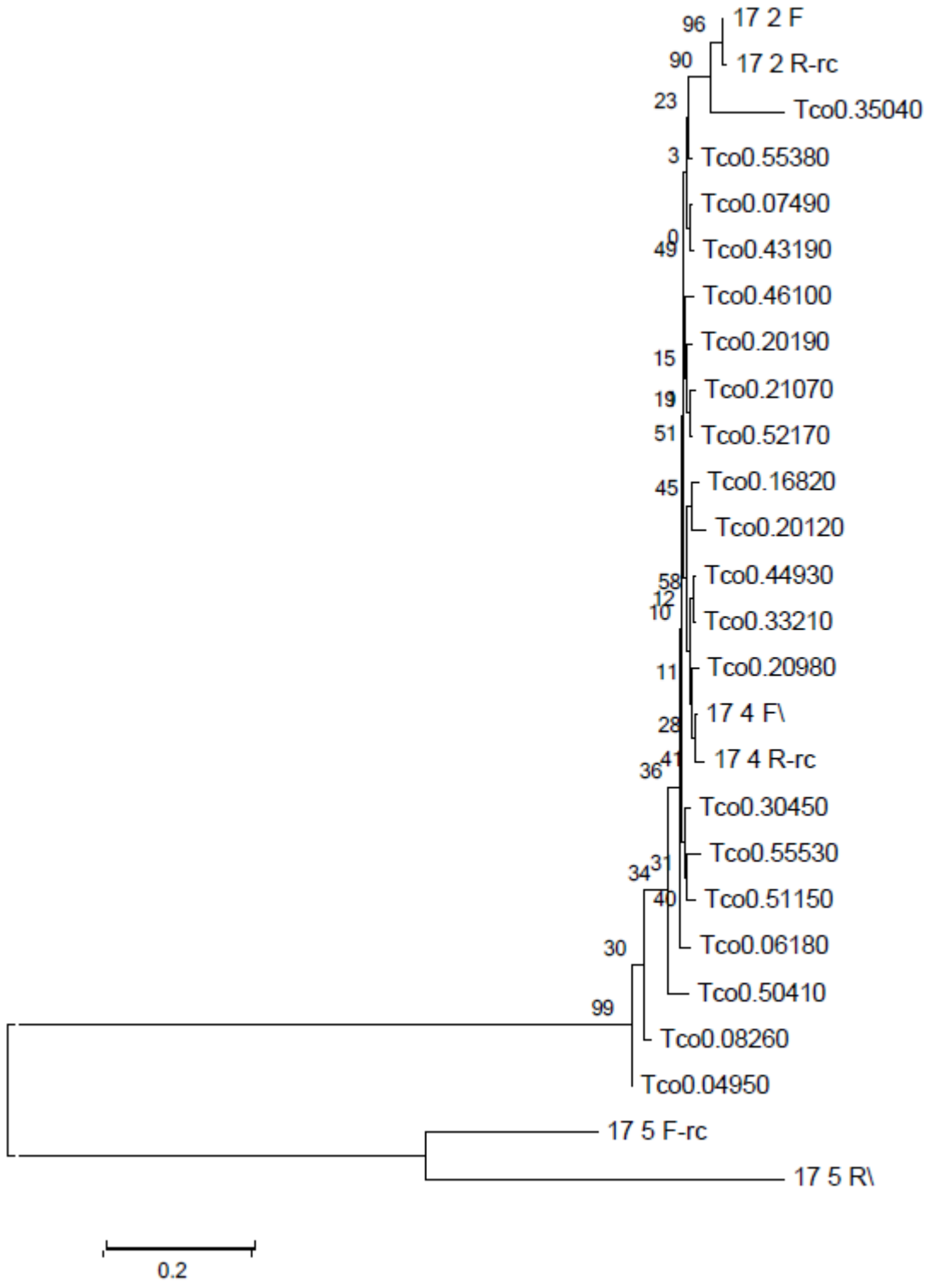
This was followed by PCR, cloning and sequencing.

For PCR, I amplified our target genes using Taq Polymerase and Phusion™ High-Fidelity DNA Polymerase enzymes in parallel. However, Phusion polymerase failed to amplify so the subsequent amplifications employed Taq polymerase.

For cloning, Taq amplicons require TOPO TA Cloning® Five-minute cloning of *Taq* polymerase-amplified PCR products kit employing Ampicillin antibiotic during protein expression while Phusion amplicons would have required Zero Blunt® TOPO® PCR Cloning Kit Five-minute cloning of blunt-end PCR products kit employing Kanamycin antibiotics during protein expression.



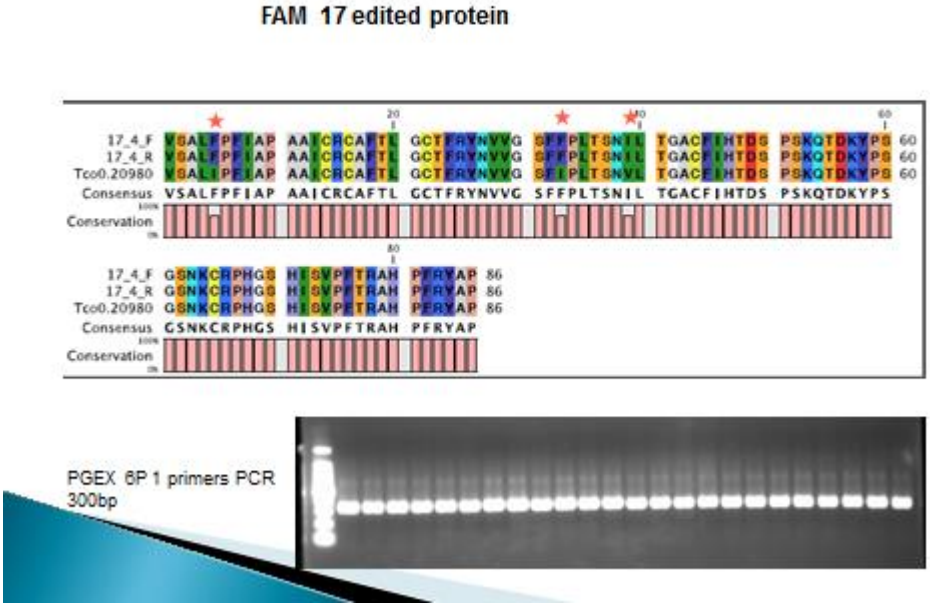
I succeeded with only Fam 17 clones. We aligned Fam 17 sequences with other *T. congolense* sequences. Clones 17- 2, 17- 4 and 17-5 Forward and reverse sequences. Fam 17 clones were a perfect match with reference *T. congolense* sequences in the Database.



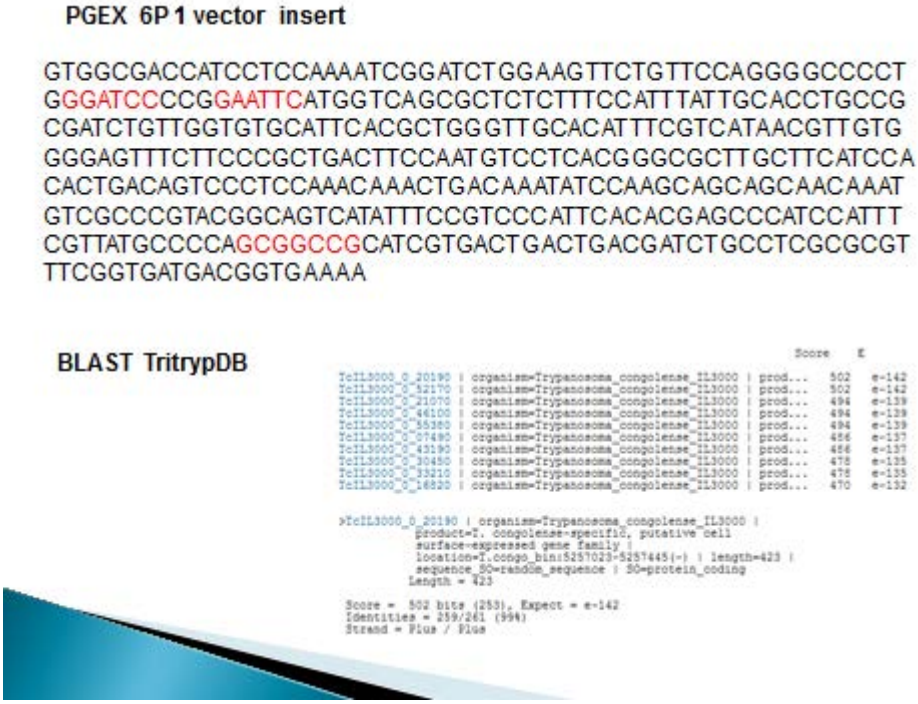
Phylogenetic Tree of Fam 17. Clones Fam17-2, Fam 17-4 and Fam 17-5. Forward & Reverse sequences. Our Fam 17 sequences/clones, Fam 17-2, Fam 17-4 and Fam 17-5 formed three distinct clades and were a bit different/distant from other *T. congolense* sequences. Fam 17-5 was very distant forming a distant/distinct branch followed by Fam 17-2 which formed a terminal clade. Fam 17-4 clustered in the center of other *T. congolense* sequences but formed

a separate clade all together.

Fam 17 proteins compared with other *T. congolense* proteins gave only 3 amino acid difference. Clone 17-4 F&R.



Small scale protein expression in PGEX 6P.1 expression vector and BL21 *E. coli* competent cells. I incorporated *Eco*R1 and *Not*1 Restriction enzyme sites in our primer design.



Sequencing the PGEX 6P.1 primers PCR, the sequence contained the target restriction enzyme sites ECOR1, NOT1 and ATG start codon. So the target gene was inserted into the Vector and *E. coli.* On BLAST analysis in TritrypDB, our sequence was similar to *T. congolense* TcIL3000 with high statistical significance.

But on doing SDS-PAGE, there was no target band at position 37kDa which was our expected protein size (11kDa protein plus 26kDa GST Tag).

Further works:

Before I change expression vector, I try just transformation to other strain of *E. coli* (for example *Rossetta*, *Origami*). Some kinds of protein is improved of their expression efficiency.

- I have to change the protein expression system to PET vector
- Or other expression systems such as Yeast and Mammalian cells.

Discussion

- The cell surface of *Trypanosoma*, like many protistan blood parasites, is crucial for mediating host-parasite interactions and is instrumental to the initiation, maintenance and severity of infection.
- Genes expressed on the trypanosome cell surface are instrumental in causing disease and sustaining infection by resisting the host immune system.
- Genes predicted to encode cell surface proteins (with putative surface functions & with predicted cell-surface expression) of *T. brucei* with those from two related African trypanosomes, *T. congolense* and *T. vivax* were compared.
- Early assays using polyclonal antibodies raised against crude trypanosomal antigen preparations were found to detect antigen in animals infected with *Trypanosoma.*
- Later, the species specificity of the assay was improved following the development of monoclonal antibodies as capture antibodies that recognized antigens
- Currently, there is a promising use of recombinant antigens to improve on the available trypanosome cell lysate to detect antibodies (Goto *et al*., 2011; Nguyen *et al*., 2012).

Internship benefit

- Additional molecular biology skills acquired. To attain my goal of Protein expression, I had to undertake several other upstream techniques/procedures. This was important

because I got to learn various techniques.

- Protein analysis skills. Originally, I had no prior knowledge working with proteins. However, in Obihiro I acquired knowledge of protein analytical skills.
- I hope to develop diagnostic proteins/antigens for trypanosomiasis diagnosis and control in Uganda.

Approval of supervisor	Institution・Official title・Name： 印

※1 Send the electronic file to the Leading School section, International Affairs Office, also submit the original print out with seal of supervisor to the Leading School section, International Affairs Office.

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