Use of *Pichia pastoris* to Analyze Protein Growth and Expression in Comparison to Standard BL21(DE3) E. coli

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Introduction

T-Cell Acute Lymphoblastic Leukemia (acute meaning that it is aggressive and progresses quite rapidly) is the overproduction of white blood cells, particularly immature T-cells which are called T-Lymphoblasts. These leukemic cells which circulate in the blood system, are potentially fatal if not treated in a timely manner. In accordance to Silvia Buonamici's article (2009), these leukemic cells can use C-C Chemokine Receptor 7 (CCR7) to direct their migration through the blood brain barrier via a process called chemotaxis. In this case cells expressing CCR7 are attracted to CC-Chemokine Ligand 19 (CCL19) which is located on the cells inside of the barrier. By making their way across the barrier, these leukemic cells can remain inaccessible to chemotherapies that are given to children, since the chemotherapy normally circulates through the blood.

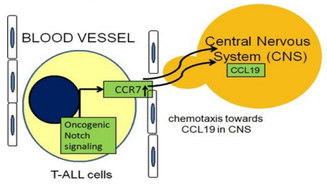

Figure 1. Movement of T-ALL Cells to CNS via chemotaxis through CCR7 and CCL19

For physicians to be able to reach the cells that have traveled to sites in the body, behind the blood brain barrier, many children who have T-Cell acute lymphoblastic leukemia are being treated through the use of intrathecal chemotherapy. In this mode of treatment, the protocol involves the injection of a drug directly into the cerebrospinal fluid inside of the central nervous system (CNS). The problem with this type of treatment is that this can lead to CNS Prophylaxis Disease, which creates long-term neurotoxicity that can lead to cognitive impairment, endocrine disorders and a short stature. Due to the nature of the blood brain barrier, it is difficult to for physicians to maintain a proper dosage of chemotherapy drugs in the brain without too much destruction of normal tissues. Therefore, radiation therapy is also used as a treatment. With radiation therapy, the physician tries to "burn" the most rapidly dividing cells to death. In the brain the idea is that the leukemic cells are the most rapidly dividing, and therefore are most susceptible to the effects of chemotherapy.

Long term effects from this type of treatment can include developmental defects, behavior defects, headaches, cognitive impairment, stunted growth, and even more devastating- new cancerous brain tumors. For many children under the age of 21, this type of therapy is very toxic as it has the potential to interfere with organ system function such as poor cardiac health. Overall, once the patients recover, these treatments can lead to morbid side effects, that interfere with maintaining a regular lifestyle for the rest of the patient's life.

Objective

It is critical that a different solution is discovered in order to treat T-ALL. Even though these treatments may be considered successful, the development of a safer method could hopefully eliminate the need for treatments such as radiation therapy or intrathecal injections. This can allow for a higher chance of a normal life post-treatment and even more so a reduced chance of recurrence of the leukemic T-Cells.

Hypothesis

Dr. Vines' lab has worked intensely to develop a systemic antagonist to bind to CCR7. Currently, they are trying to use the protein 8-83, which is a truncated form of CCL19 with the first 7 amino acids eliminated, in order act as an antagonist. This would block CCR7's attraction to CCL9 and ultimately leave the T-Cell ALL to flow freely in the bloodstream rather than cross through the blood brain barrier to hide. However, Vines' lab has not been able to successfully induce consistent protein expression or purification.

Based on this, my partner and I believe that by experimenting with different expression systems, we should be able to yield a consistent protein expression to lead to purification of 8-83. More specifically, we hypothesize that the yeast, *Pichia pastoris*, will be a more appropriate host for the expression of 8-83. This is because in similar studies, *P. pastoris* has been used to express 5P12-RANTES to act as an HIV inhibitor (Cerini, 2015). Furthermore, we hypothesize a nutrient rich media that best helps *Pichia Pastoris* to grow will improve protein expression.

Methodology

In order to successfully sub-clone into the *Pichia pastoris* we would have to first design primers in order to flank 8-83 with the BsaI restriction site which will allow us to ligate into the pJAG vector through seamless cloning. The method we would use for seamless cloning would be the Golden Gate Assembly which allows for digestion and ligation to occur in a 30 minute time frame in one micro centrifuge tube. After ligation of the 8-83 into pJAG, we would transform the vector into E. coli to grow up and isolate colonies to ensure correct orientation of the sequence. To express the protein in the *P. pastoris*, we will electroporate the linearized pJAG vector into *P. pastoris* to allow incorporation into the genome at the Histidine (His) locus. This disrupts expression of His allowing us to isolate successfully integrated vectors for further analysis. Once we confirm successful integration, we will expand those isolates (colonies) and induce with methanol and analyze for 8-83 expression. We expect greater than 95% purity with functional protein folding since the 8-83 would be in the supernatant.

Results

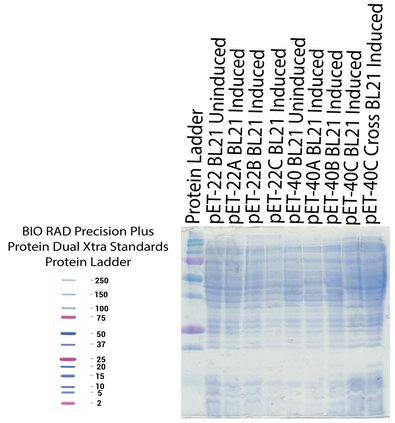

Figure 4. SDS-PAGE shows that there may be possible leaky expression. Otherwise, the results are inconclusive,

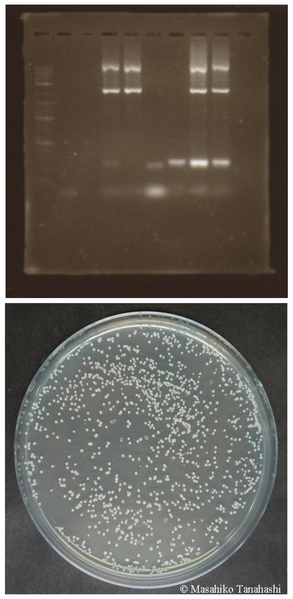

Figure 5. Gel containing primers and 8-83. Results show that the negative controls (lanes 2, 3, 6, and 7) are contaminated except lane 3. Thus, the remaining results are unreliable.

Figure 6. This is what our *Pichia pastoris* should look like after 4-5 days from streaking it with YPD media.

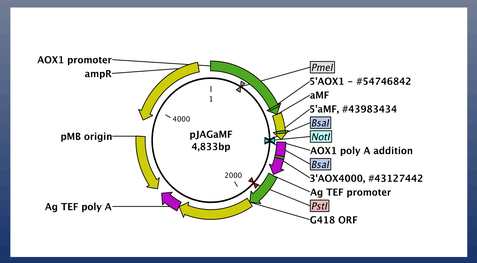

Figure 2. *Pichia pastoris yeast vector, pJAG*

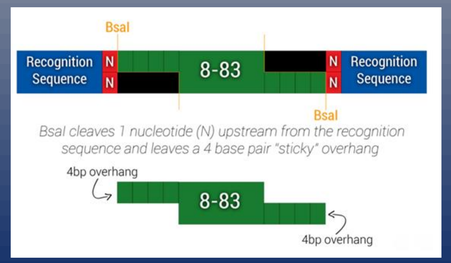

Figure 3. BsaI restriction endonuclease cutting 8-83

Discussion/Future Directions

We did make progress understanding much about the use of a yeast expression system, however, we were not able to fully complete our experiment. Despite this, the project will be taken over by one of our mentors and it is expected that he will carry out some of the processes that we learned about but did not go through; such as homologous recombination and the use of electroporation to incorporate the pJAG vector into the *P. pastoris* genome. Although we did not obtain any specific findings in relation to our experiment in order for us to determine whether it is a better expression system than bacteria, our overall research did lead towards this idea. Based on a project conducted by Oliver Hartley and several colleagues, he was able to express 5P12-Rantes, an HIV inhibitor, (which is similar to 8-83 in his experiment) through the use of *P. pastoris* as his expression system. Moreover, there are also different benefits based on the type of cell type of yeast: eukaryotic, just like mammalian cells. Because it is eukaryotic, it contains common organelles with human cells that produce CCL19. It also leads to less toxicity which gives a higher chance of expression rather than killing off the cells.

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