Comparison of Protein Expression in the Cytoplasm vs Periplasm in E. Coli

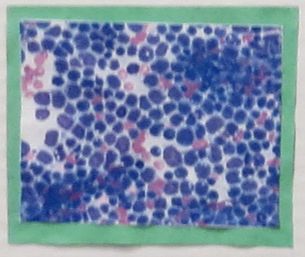
Objective

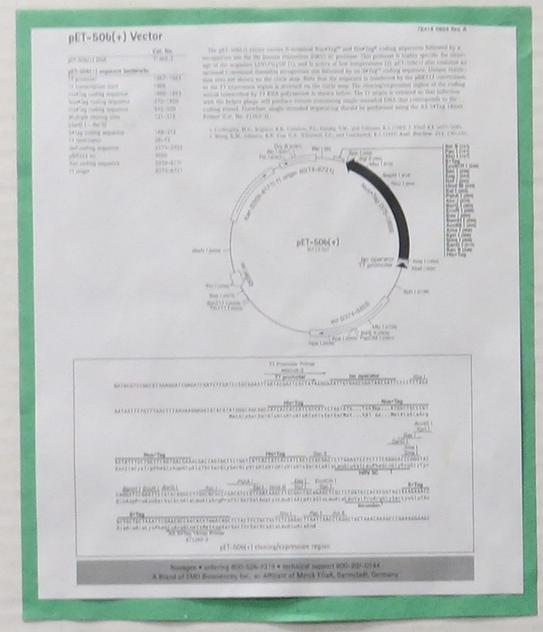
Dr. Charlotte Vines' lab is working on a CCR7 antagonist originally described by Pilkington, to block the CCR7 directed migration of T-ALL into the brain. This antagonist, 8-83, is generated by cutting off the first seven amino acids of CCL19. The goal of this study is to develop a CCL19 antagonist and thus prevent leukemia cells from entering the brain.

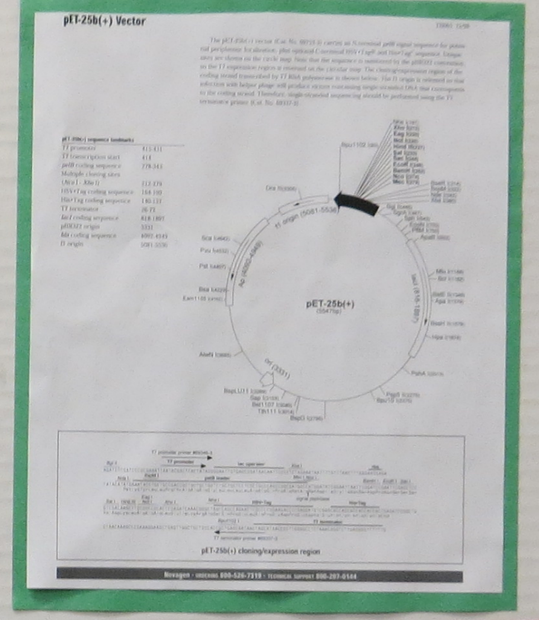
Hypothesis

Dr. Vines' groups had problems making and purifying this antagonist in bacteria from the periplasmic space of E. Coli.

We hypothesize that by expressing the antagonist 8-83 in the cytoplasm of E. Coli (in the Shuffle strain that allows disulfide bond formation in the cytoplasm) and not the periplasm, protein expression and folding might be more effective.





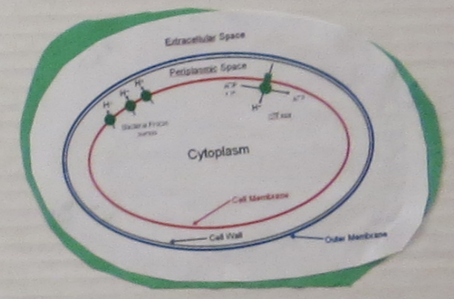


Background Information

T-cell acute lymphoblastic leukemia (T-ALL) is a type of cancer that is characterized by an over production of white blood cells, mainly in the bone marrow. This year, there is an estimate of 6,950 diagnoses, 60% of the cases will be children under the age of 21. If not treated, T-ALL has the potential to rapidly spread from the blood stream to several organs in the body including the brain where T-ALL can hide from treatment.

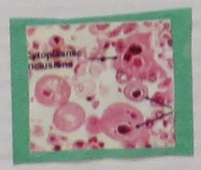
Current treatments for T-ALL are chemotherapy and radiotherapy that can reduce the amount of cancerous cells however it can also kill healthy cells. Although these treatments might be effective, there is a chance for leukemia relapse. Thus there is a need to develop more effective and less aggressive treatments.

It is well known that T-ALL cells use C-C Chemokine Receptor 7 (CCR7) to get into the brain (Buonoamici & Lanis, 2009) via signaling of the C-C chemokine ligand 19 (CCL19) from the brain. It is needed to stop the migration of T-ALL into the brain so that leukemic cells can be more easily killed in the blood stream.



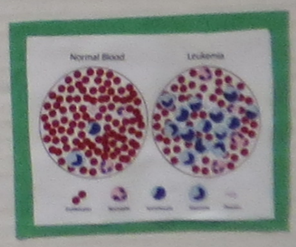
Methods

1. Transform (insert DNA into bacteria). We will transform the vectors (circular DNA) pET-25 and pET-50 that contain the 8-83 DNA insert into the DH5α and Shuffle *E. coli* strains. pET-25 was selected because it has a pelB sequence that will promote periplasmic localization of 8-83, while pET-50 has a cytoplasmic localization signal to induce expression of a soluble protein in the cytoplasm.
2. Purify the vectors by mini prepping them. A series of buffers will be used to break open the cells and to purify DNA.
3. We will use the Nano drop to confirm the purity of the DNA.
4. We will run a gel diagnostics which is the process where we run an agarose gel to examine whether the plasmid has the size expected. That is, the plasmid should have 5.5 kilobase pairs that corresponds to the vectors plus 8-83.
5. We will plate the cells with LB media and antibiotics and place them in an incubator.
6. We will inoculate a colony and grow it by shaking it in an incubator at 37°C and 250 rpm.
7. We will measure the optical density of E. Coli cultures to determine growth characteristics and the log stage of growth. Optical density is a method for calculating the concentration of E. Coli and their rate of growth.
8. We will induce 8-83 expression at the log (exponential) stage of growth (0.8). Induction will be achieved exposing E. Coli to isopropyl β-D-1 thiogalactopyranoside (IPTG) for 4 hours so that transcription of 8-83 can occur.
9. We will run our Sodium Dodecyl Sulfate Polyacramdie gel electrophoresis (SDS-PAGE) to examine whether the 8-83 was produced by E. Coli.
10. We will purify 8-83 by using the His-tag by using a nickel column. The His-tag will bind to the nickel column and thus 8-83 will remain in the column while will be able to get rid of other molecules. Next, we will use imidazole to get the His-tag with the 8-83 off the column.



Future Directions

Once we get the protein we will purify and use a migration assay to examine if 8-83 binds to CCR7 and stops migration of T-cells towards CCL19.



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