Examining the impact of Glucose on Protein Expression of Unstable, "Leaky" Plasmids in *E. Coli*

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Background

T-Cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive type of cancer with a poor prognosis whose treatment may have aggressive long term effects. T-ALL is a type of cancer circulating in the blood and most commonly found amongst children. T-ALL is the product of the overproduction of immature lymphocytes from bone marrow. Currently, the standard treatment is an intensive, lengthy chemotherapy, with a duration of 2.5-3 years, which ultimately leads to devastating side effects that can have negative impacts on the patient's quality of life.

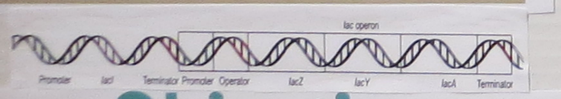
Studies have found that T-ALL cells have the ability to migrate from the bloodstream into the CNS and cross the blood brain barrier via the C-C chemokine receptor 7 (CCR7) via signaling of its ligand C-C chemokine ligand 19. Once the T-ALL access the CNS these cells hide from chemotherapy. This poses a threat to the patient due to a relapse and effecting remission. The goal of the present study is to stop the migration of T-ALL into the CNS via development of an antagonist of CCL19. This antagonist is 8-83 which will bind to CCR7 and acts as a blockade and prevent T-ALL from hiding in the brain. ("What is Leukemia?" Nauroz, Syed)

Targeting the central nervous system (CNS) which can successfully eliminate T-ALL but the patient experiences severe morbid side effects with this process, and might eventually end in relapsation. Along with chemotherapy, radiation is widely used to stop the rapid growth of cells, however, this procedure also affects healthy cells. Another treatment can involve bone marrow transplant, however, rejections of this procedure are commonly seen in older recipients and immune system failure. Bone marrow transplants are also associated with high doses of radiation which declines the patient's mortality rate. Chemotherapy has as a final goal to prevent the leukemic cells or lymphocytes from entering the CNS.

Statistics about acute lymphocytic leukemia

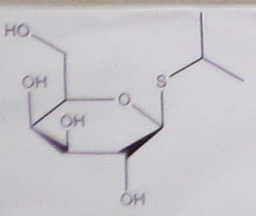
The American Cancer Society's estimates for acute lymphocytic leukemia (ALL) in the United States for 2016 (including both children and adults) are:

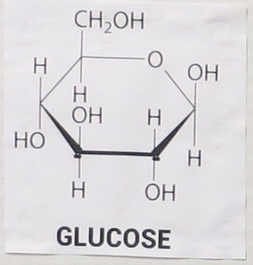
About 6,590 new cases of ALL (3,590 in males and 3,000 in females)About 1,430 deaths from ALL (800 in males and 630 in females) The risk for developing ALL is highest in children younger than 5 years of age.



Objective

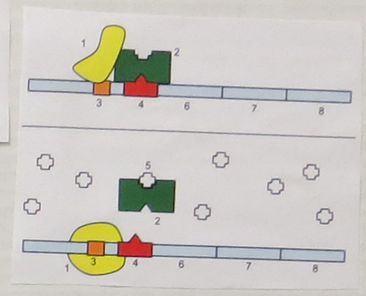
The overall objective is trying to use protein with 8-83 to act as antagonists to block CCR7 from reaching CCL19. This would prevent T-ALL to pass the blood brain barrier and hide from chemotherapy treatment. However, the narrowed objective of this study is to investigate whether 1% glucose administered in media (TB) at the time of induction has an effect on the stability of vectors pET-20, pET-22 and pET-23 in order to accomplish active 8-83 protein.





Hypothesis

The addition of 1% glucose to growth media will reduce instability of "leaky" plasmids and thus lowering protein toxicity in E. Coli



The lac operon Top: Repressed, Bottom: Active
1: RNA Polymerase; 2: Repressor; 3: Promoter; 4: Operator, 5: Lactose; 6: lacZ; 7: lacY; 8: lacA

Applying the study

To express the 8-83 antagonist to CCR7 and act as a blockade which keeps CCL19 from binding together we will be using pET-22, pET-20 and pET-23. pET vectors contain different sequences adjacent to the cloning sites that encode a number of peptide tags which helps protein localization, detection and/or purification. Degrees of toxicity will vary from protein to protein.

The use of Glucose

The method focused on this research project is the addition of 1% glucose to the culture medium. The chosen media is Terrific Broth due to the enriched medium that helps recombinant strains of E.Coli maintain an extended growth phase.

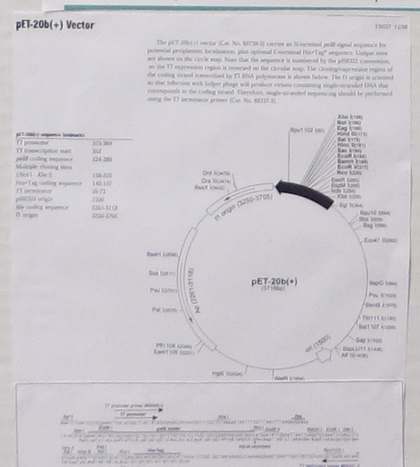
The purpose of exposing the cells to glucose is to repress induction of 8-83 expression by the lac promoter by lactose. "In the presence of glucose, cAMP levels are low, so transcription from the lac promoter is low." This is called the glucose effect or catabolite repression. (pET System Manual 12).

Unstable Plasmids

pET-23(low copy) and pET-20(low copy) are going to be compared to the protein growth of pET-22(low copy). These vectors were chosen due to instability from uncontrolled induction. Many promoters are not securely regulated and even display a degree of expression before exposing the cells to an inducer. Furthermore, since lac promoters are "leaky" the plasmids can become unstable and at times there is loss of the plasmid itself. This leads to culture overgrowth by cells that do not have a plasmid.

What is Toxicity?

It is noted that recombinant protein is carried through recombinant DNA, which implies two segments of DNA are present in a plasmid, expressed in *E.Coli* may inhibit the normal function of the cell and be considered "toxic" to bacteria. Toxicity of the target protein can cause low expression levels or no expression can be observed. The protein's toxicity can mark itself on different levels: incomplete repression of protein expression, constitutive expression of a repressor protein and etc (EMBL on Protein Expression)



T1: Induced for OD readings / Glucose addition
T2: Induced for comparison
T3: Uninduced with Glucose
T4: Uninduced for Uninduced control

Methods

1. T1,T2,T3,T4: inoculate single colony from each transformation Plate into 4 tubes with 10-15 mL TB or 2xYT (12 total)
2. T1,T2,T3,T4: grow overnight 37 degrees Celsius at 250 rmp or 24-36 hours at 30 degrees Celsius at 250 rmp
3a. Morning: Check OD levels, if above 0.8: add more media, if below 0.8 wait.
3b. T1,T2,T3,T4: Use 1mL place in microcentrifuge tube for miniprep
-Quantify DNA (nanodrop)
-Gel diagnostic (1% agarose gel electrophoresis) to verify
4. Once all ODs equal 0.8 (T1,T2,T3,T4) take 1 mL each into labeled microcentrifuge tubes
4a. T1,T3: add 1% glucose for induction
5. -T1,T2: Induce with 0.4 mM IPTG 4 hrs, 37 degrees Celsius at 250 rpm
-T3,T4: 37 degrees Celsius at 250 rpm with T1,T2
6. Run SDS page with T1,T2,T3,T4: Coomassie stain to verify protein expression

Materials

*materials include but are not limited to

Use water bath to heat shock the cells and thus transform them. Grow bacteria using plate for media growth and antibiotics. A shaker/incubator is used for bacterial overnight culture growth along with microcentrifuge tubes to resuspend bacterial cells. Different pipette extraction measurements to transfer media/DNA/buffers into sample tubes, plates or other equipment.

Use miniprep kit to purify DNA. Use nanodrop machine to quantify purity of the DNA; agarose gel diagnostic to examine the digest and vector size by comparing it to a DNA ladder. Use of spectrophotometer to check OD levels. Use IPTG for induction; use SDS page to examine protein expression purify protein using his-tag.

Expected Results

Few proteins are found to be toxic for the cells and are the cause for no growth and are likely to kill them, it is expected that growth of the cells in glucose will determine if the plasmid, DNA or 8-83 protein is "toxic." If the plasmid is not toxic but the 8-83 protein is toxic, it is expected that almost immediately after induction the E. Coli cells will die and the expression levels will be very low (Protein Expression- *E.Coli*/Decreasing Protein Toxicity).

In order to decrease the effects of protein toxicity either periplasmic expression or expression in inclusion bodies may be necessary. Other factors that will be explained in the research experiment is if there is any significance between toxicity and localization (periplasmic vs cytosolic). We expect to find information because of the abstract , "Sequences determining the cytoplasmic localization of a chemoreceptor domain," written by Seligman L, Bailey J, and Manoil C.

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