Cloning and co-expression of Leishmania PTW/PP1 regulatory complex proteins

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Abstract

The purpose of this experiment is to explore the structure of the PYW/PP1 protein complex, which is linked to the synthesis of Base J, a modified base found in all trypanosomes and more specifically the trypanosome, Leishmania. Leishmania is a parasitic bacteria responsible for the infectious disease Leishmaniasis. Leishmaniasis is spread by the bite of sandflies and comes in two forms, cutaneous and visceral. The cases of cutaneous Leishmaniasis are estimated at 700,000–1,200,000 cases and 200,000–400,000 for visceral Leishmaniasis. Visceral Leishmaniasis can cause the swelling of internal organs, fever, weight loss, and low blood count. Without treatment of visceral Leishmaniasis, the disease is typically fatal. However, by researching the PTW/PP1 complex, the process of the synthesis of Base J might be understood and allow for the development of a drug to treat or even potentially cure Leishmaniasis.

Introduction

The objective of this experiment is to find the structure of the PYW/PP1 complex by determining the relationships between the proteins, PNUTS, JBP3, GTIP1, PP1c, and GT by taking their genes from genomic DNA, expressing them in vectors and using the expression vectors to produce the proteins that will then be tested for a relationship. The corresponding genes and vectors are shown below.

Investigation Objective

To create expression vectors that will express this protein complex, several intermediate steps are required.

1. E. coli transformed with plasmid DNA, grown and purified.
2. Genes of interest amplified from gDNA.
3. Plasmids and genes of interest cut with restriction enzymes to produce complementary overhangs.
4. Genes of interest will be ligated into the relevant plasmid.
5. Ligated vectors will be transformed into E. coli and grown.

Discussion

Positive control was grown with un-cut plasmid with active antibiotic resistance without insert and grew successfully. No DNA control suggests the work was not contaminated. Negative control of cut plasmid should have deactivated antibiotic resistance but was not effective. Experimental plates show successful creation of expression vectors.

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References