CALIFORNIA STATE SCIENCE FAIR
2015 PROJECT SUMMARY

Name(s)

Sophia M. Hewitt

Project Number

S1514

Project Title

Optimizing Chromosomal Exposure for Fluorescence in situ Hybridization in Tetrahymena thermophila

Abstract

Objectives/Goals

The objective was to test whether a greater drop height onto slides before QFISH would expose more fully the chromosomal DNA.

Methods/Materials

Cell samples of Tetrahymena thermophila were grown for 2 weeks in an incubator. They were centrifuged, the supernatant was removed,various chemicals were added, including formaldehyde to fix the cells, and then 80 ul of the cell sample were deposited onto each of 5 different slides, with each sample dropped from different heights (0 cm, 10 cm, 20 cm. 30 cm, and 40 cm). Probes tagged with FITC and coding for T. thermophila telomeres (AACCCCAACCCC...) were added, and the slides were stored at 37 degrees Celsius for two days to give the probes time to hybridize. After this, VectaShielc, which contained DAPI, was added. The slides were observed under a confocal laser scanning microscope. The DAPI staining was detected (although the FITC tagged probes did not). Due to limited time on the microscope, only slides of 0, 30, and 40 cm could be observed. Computer software was used to analyze the results.

Results

The DAPI staining hilighted the macronucleus, and diameters from the drop heights of 0, 30, and 40 cm were compared, revealing no substantial difference. There was a lot of autofluorescence on the same channel as the FITC, so there were no results from the telomere probes.

Conclusions/Discussion

Based on this study, dropping cells from different heights does not seem to affect the diameter of their nuclei. Thus, the heights would not make probes more likely to attach to the DNA, observing results using FISH would not be any easier, and the results would not be improved. However, a small sample size could be skewing the results. Also,when running FISH on T. thermophila, FITC is not a good fluorescent marker. More research is necessary.

QFISH is a good method for measuring accurately the lengths of specific target DNA, lixe telomeres. A simple method for getting better results would be very beneficial in a lot of studies - for example, measuring to see if one had shortened the telomeres, another project I would like to work on. The idea behind this project was that dropping the cells from higher up would expand the nucleus, thus making more room for the probes to attach and brightening overall results. I will attempt to find a working method of improving my results and, using this, I hope to measure telomere lengths.

Summary Statement

For my project, I tested whether dropping cells of Tetrahymena thermophila from different heights onto slides before QFISH would expand the nucleus and provide more room for the probes to attach to the DNA, leading to more fluorescence.

Help Received

Research performed at LA Biohackers (public biology lab); Cory Tobin (supervisor, advice, assistance when handling formaldehyde); my parents drove me and got supplies; Beckman Biological Imaging Facility, CalTech (allowed me to use their microscope for 90 minutes without assistance)