CALIFORNIA STATE UNIVERSITY SAN MARCOS

PROJECT SIGNATURE PAGE

PROJECT SUBMITTED IN PARTIAL FULLFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

PROJECT TITLE: Optimizing Micro-particle Bombardment Parameters for the Marine Algae Emiliania Huxleyi

AUTHOR: Gabriella Mireles

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THE PROJECT HAS BEEN ACCEPTED BY THE PROJECT COMMITTEE IN
PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF
SCIENCE IN BIOTECHNOLOGY.

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PROJECT COMMITTEE CHAIR

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Abstract

Optimizing Microparticle Bombardment Parameters for the Marine Algae *Emiliania huxleyi*

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Professional Master’s Degree Program

California State University San Marcos

*Emiliania huxleyi* is numerically the most abundant coccolithophorid and is found throughout the world with the exception of polar sea waters (Marsh, 2003). The genome of *E. huxleyi* was sequenced in collaboration between Dr. Betsy Read’s lab at California State University San Marcos and the U.S. Department of Energy (Read et al., 2013). The function of the vast majority of genes in the *E. huxleyi* genome is unknown. Being able to characterize these genes will give insight into the biological processes occurring in the cell. *E. huxleyi* transformation has not yet been successful which may be due to either the DNA delivery method or plasmid instability. For these reasons, the overall objective of this Semester in Residency (SIR) project is to develop successful DNA delivery procedures for *E. huxleyi* using fluorescently labeled oligonucleotides using a Design Of Experiment (DOE) approach. Micro-particle bombardment involves shooting gold microcarriers coated with DNA toward target cells. There are various parameters that can be manipulated with the aim of successfully introducing microparticles into *E. huxleyi* cells. Those tested herein include microcarrier size, helium pressure, gap distance, and target distance. Successful cell uptake was determined by analyzing cells after bombardment using flow cytometry. The one conclusion that can be drawn from both sets of bombardment experiments is that target distance is likely to have a statistically significant effect on bombardment using the Biolistics® PDS-1000/He Particle Delivery System (Bio-Rad Laboratories, 2012). Although it is still unknown whether increasing the target distance has a positive or negative effect on the bombardment process. The work carried out herein will pave the way for future studies aimed at developing a stable transformation system in *E. huxleyi*. 
Optimizing Micro-particle Bombardment Parameters for the Marine Algae *Emiliania huxleyi*  

**GABRIELLA MIRELES**

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DR. BETSY READ  
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Overview

- Introduction
  - Background
  - Significance

- Material and Methods
  - Preparation of Bombardment Components and cells
  - Bombardment and Recovery

- Results
  - Alexa Fluor 647 labeled DNA cellular uptake
  - Alexa Flour 488 labeled DNA cellular uptake

- Discussion and Conclusion

- Questions
E. Huxleyi is a single-celled microalgae

- Calcium Carbonate shell
- Wide distribution & most abundant
Significance

- Sequenced genome
  - 142 Mb
  - ~30,000 genes

- Characterizing gene function
  - Gene silencing and overexpression

- Launch new areas of research such as:
  - Subcellular localization
  - Manipulation of metabolic pathways
  - Exploration of RNAi pathway
Micro-particle Bombardment

• Introduction of high velocity micro-particles coated with DNA into living cells (Sanford, 1990).

1. Chlamydomonas reinhardtii
2. Volvox carteri
3. Dunaliella salina
4. Gracilaria changii
5. Laminaria japonica
6. Phaeodactylum tricornutum
7. Navicula saprophila
8. Cyclotella cryptica
9. Euglena gracilis
10. Porphyridium sp.
11. Cylindrotheca fusiformis
12. Haematococcus pluvialis
13. 'Chlorella' kessleri
14. Chlorella sorokiniana
Specific Aims

- **Aim 1:**
  - Develop optimal micro-particle bombardment (bombardment) parameters using a Design Of Experiment (DOE) approach

- **Aim 2:**
  - Independently validating the bombardment parameters
Material and Methods
Preparation for Bombardment

PDS-100/He Particle Delivery System

Bombardment Accessories

Consumed during bombardment

- Rupture disks
- Retaining cap
- Gold microcarriers
- Stopping screens
- Macrocarriers
- Macrocarrier holder
- Macrocarrier cover lid
- Brass adjustable nest
Inside the bombardment chamber

- Gap Distance
- Target Distance

Diagram labels:
- Gas Acceleration Tube
- Rupture Disk
- DNA-coated Microcarriers
- Stopping Screen
- Target Cells

Before and After images showing the process.
## DOE Results with Alexa Fluor ® 647

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Gap Distance (Inches)</th>
<th>Target Distance (cm)</th>
<th>Rupture Disk (psi)</th>
<th>Microcarrier Size (µm)</th>
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</thead>
<tbody>
<tr>
<td>Std 15</td>
<td>1/8</td>
<td>12</td>
<td>1550</td>
<td>1.6</td>
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</tbody>
</table>
Statistical Analysis with Alexa Fluor ® 647

Pareto Chart

- A: Gap Distance
- B: Target Distance
- C: Rupture Disk
- D: Microcarrier Size

Significant
Possibly Significant
Not Significant
Independent validation
## DOE Results with Alexa Fluor ® 488

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Gap Distance (Inches)</th>
<th>Target Distance (cm)</th>
<th>Rupture Disk (psi)</th>
<th>Microcarrier Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 17</td>
<td>1/4</td>
<td>6</td>
<td>1350</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Statistical Analysis with Alexa Fluor ® 488

Pareto Chart

A: Gap Distance
B: Target Distance
C: Rupture Disk
D: Microcarrier Size

Significant
Possibly Significant
Not Significant
Discussion & Conclusion

- Confirmed bombardment obstacle – Reproducibility

- Both sets of data confirm – target distance is most likely a statistically significant effect
  - Inconclusive whether increasing target distance has a positive or negative effect on the bombardment process

- Future work:
  - Increase range of rupture disks
  - Type of microcarrier
  - Increase the amount of DNA
Questions