Characterization of Genomic Insertion Site in Chlamydomonas Mutant

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ABSTRACT

Our study utilizes *Chlamydomonas reinhardtii* CMJ030 mutant strain derived from the parental strain CC-4533 obtained from the Chlamydomonas Collection and Resource Center [1]. This mutant was engineered through the electroporation of DNA cassettes (using pMJ013b for LMJ.SG0182 mutants or CIB1 for LMJ.RY0402 mutants), impeding resistance to the antibiotic paromomycin. The CMJ030 strain exhibits versatility in growth modes—photoautotrophic, mixotrophic, and heterotrophic—and displays mating type minus, normal motility and lipid storage, high transformation efficiency, and robust recovery from cryogenic storage in liquid nitrogen. The primary goal of our research is to extract and analyze DNA from the *Chlamydomonas* CMJ030 mutants to pinpoint and characterize the genomic insertion site(s). This objective will be achieved by employing bioinformatics tools for PCR mapping [2]. Our methodology encompasses several stages: initially, cell propagation and isolation into single colonies on agar plates to encourage distinct colony formation; subsequent DNA isolation from these colonies; PCR amplification using specific primers (oMJ282 and oMJ284) to target the locus of interest in both wild-type and mutant strains; amplification of cassette-genome junctions; sequencing of the PCR products to gather critical DNA sequences indicative of the insertion sites; and finally, alignment of these sequences against the reference genome to validate the insertion site.

INTRODUCTION

- The test subject of this experiment is a species of green algae, *Chlamydomonas reinhardtii* and mutants derived from the parental strain.
- *Chlamydomonas reinhardtii* is an ideal model organism to study genetics.
- The insertion of the CIB1 cassette in the CMJ030 mutants providing resistance to the paromomycin [2].
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- The wild type and the mutants will be subjected to the Polymerase Chain Reaction (PCR). The region of interest will be amplified, allowing us to map the location of the CIB1 cassette.

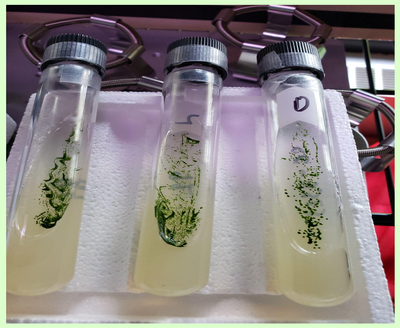

Figure 1 A. *Chlamydomonas reinhardtii* wild type and CMJ030 mutants.

HYPOTHESIS

The preliminary data suggest that the insertion site is located on chromosome 13 in the CMJ030 mutant LMJ.RY0402.189391. We hypothesize that the insertion site will be located on a different chromosome.

MATERIALS AND METHODS

- The cells were propagated in two types of growth media, TAP and HS agar.
- The cells were inoculated onto agar plates and streaked into single colonies, to rise distinctively.
- DNA samples were extracted from the wild type and the CMJ030 mutants.
- Utilized bioinformatics resources to analyze the genomic data of the CMJ030 mutants
- The CMJ030 mutants were subjected to virtual PCR in preparation for step 3 of the experiment, **figure 1B**.

FULL WRITTEN METHODS ➡

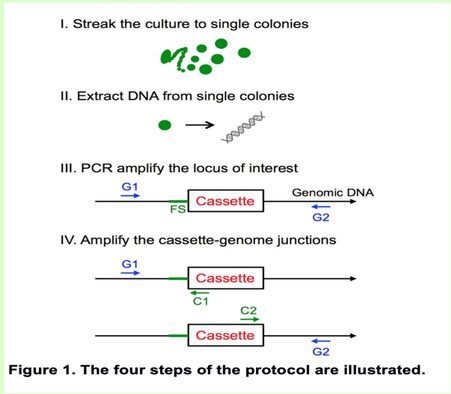

Figure 1. The four steps of the protocol are illustrated.

Figure 1B. Experimental procedure overview Different stages of the experiment required different materials and approaches [2].

RESULTS

Figure 2. *Chlamydomonas reinhardtii* propagation and streaking. The parental strain cells and CMJ030 mutants were inoculated onto agar plates using the methods listed in step 1 of the protocol [2].

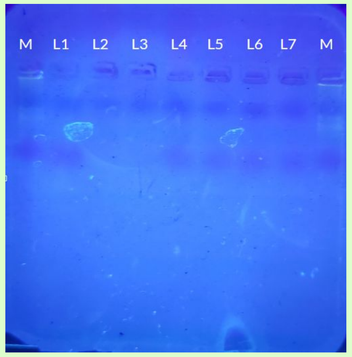

Figure 3. Gel electrophoresis of DNA extraction. Results shows no DNA bands from the mutant in step 2. Upon further inspection, we hypothesized that this occurred because the concentration of DNA extracted was too low.

Sample name	Original sample conc.	Original sample conc. units	Sample Volume (uL)	Dilution Factor
2B	Out of range	ng/uL	10	20
1B	0.706	ng/uL	10	20
0B	Out of range	ng/uL	10	20
2A	1.32	ng/uL	10	20
1A	2.73	ng/uL	10	20
0A	0.848	ng/uL	10	20

Table 1. DNA concentration analysis
Determining the dsDNA broad range concentrations via fluorescence to identify the double-stranded DNA concentration extracted from mutant and the wild type cells.

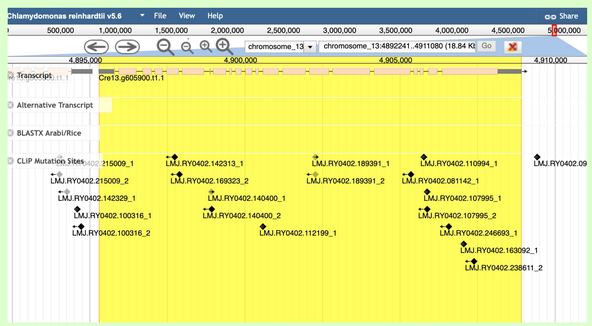

Figure 4. *Chlamydomonas reinhardtii* reference genome[3]. Identifying and analyzing the orientation of the mutant LMJ.RY0402.189391 genome in the *Chlamydomonas reinhardtii* v5.6 reference database[3].

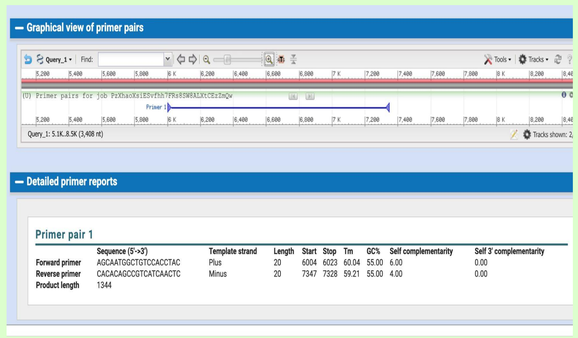

Figure 5. Virtual PCR. Virtual simulation of Polymerase Chain Reaction on DNA sequences in mutant LMJ.RY0402.189391.

CONCLUSION

- During propagation and inoculation **figure 2**, the cells were placed into two different types of growth media, TAP and HS agar. The cells grew in the TAP media, but they did not grow in the HS media. This information was allowed us to grow the cells efficiently and to proceed into the next stages of the experiment, DNA isolation.
- We modified the DNA isolation protocol from in step 2 **figure 1B**. We used a Qiagen Dneasy kit protocol to isolate DNA from the cells[5].
- During DNA isolation **figure 3**, the concentration of the isolated DNA was low, and we were unable to view the DNA bands after the gel electrophoresis. To analyze the DNA concentration we performed the fluorometry using the dsDNA broad range fluorophores to identify the concentration of the extracted dsDNA T**able 1.**
- Utilizing bioinformatic resources [3,4], we were able to examine the genome of the mutants and identify the orientation of the CIB1 cassette in the Chlamydomonas genome.
- Prior to conducting PCR, we used virtual PCR figure 5 , in preparation for the procedure in step 3 **figure 1B** of the protocol. The DNA amplicon product for mutant LMJ.RY0402.189391 is 1344 bp in length.

FUTURE DIRECTIONS

The wild type and the CMJ030 mutants will be subjected to the wet lab PCR experiment, gel electrophoresis of the amplified DNA. Then 1344 bp DNA bands will be gel purified and the DNA will be sent out for sequencing. Return DNA sequencing data will be used to align DNA against the Chlamydomonas reference genome.

REFERENCES

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