Characterization of Genomic Insertion Site in Chlamydomonas Mutant

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Our study utilizes *Chlamydomonas reinhardtii* CMJ030 mutant strain CC-4533 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 mutant to pinpoint and characterize the genomic insertion site. This objective will be achieved by employing bioinformatics tools and 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.

Literature cited:

1. Zhang, R., Patena, W., Armbruster, U., Gang, S. S., Blum, S. R., & Jonikas, M. C. (2014). High-Throughput Genotyping of Green Algal Mutants Reveals Random Distribution of Mutagenic Insertion Sites and Endonucleolytic Cleavage of Transforming DNA. *The Plant Cell*, *26*(4), 1398–1409. https://doi.org/10.1105/tpc.114.124099

2. Ivanova, N., & Zhang, R. (n.d.). *Instructions for characterizing insertion sites by PCR*. https://www.chlamylibrary.org/files/Instructions%20on%20PCRs%20to%20check%20the%20insertion%20site.pdf