SAND2013-7719P

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Analysis of *N. salina* Ponds Using RFLPs in Two Variable Regions

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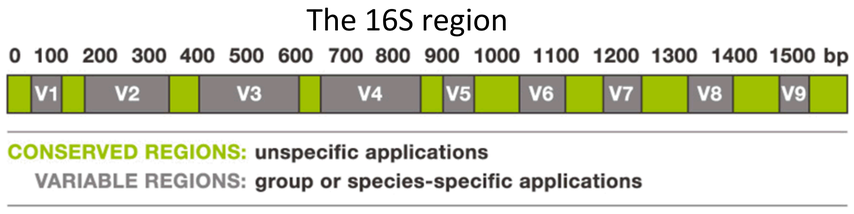
INTRODUCTION

The development of alternative energy sources such as biofuels is crucial to national security and economic interests. *N. salina* is an alga species that has the potential to be used as a source in the future with further development and analysis. Therefore, the preservation and growth of healthy cultures of the species is critical. To maintain the health of *N. salina* ponds, an understanding of the satellite prokaryotic and eukaryotic species inside the ponds is required. This project aimed to identify unique species inside of the ponds that may eventually be analyzed to understand how and why *N. salina* ponds crash.

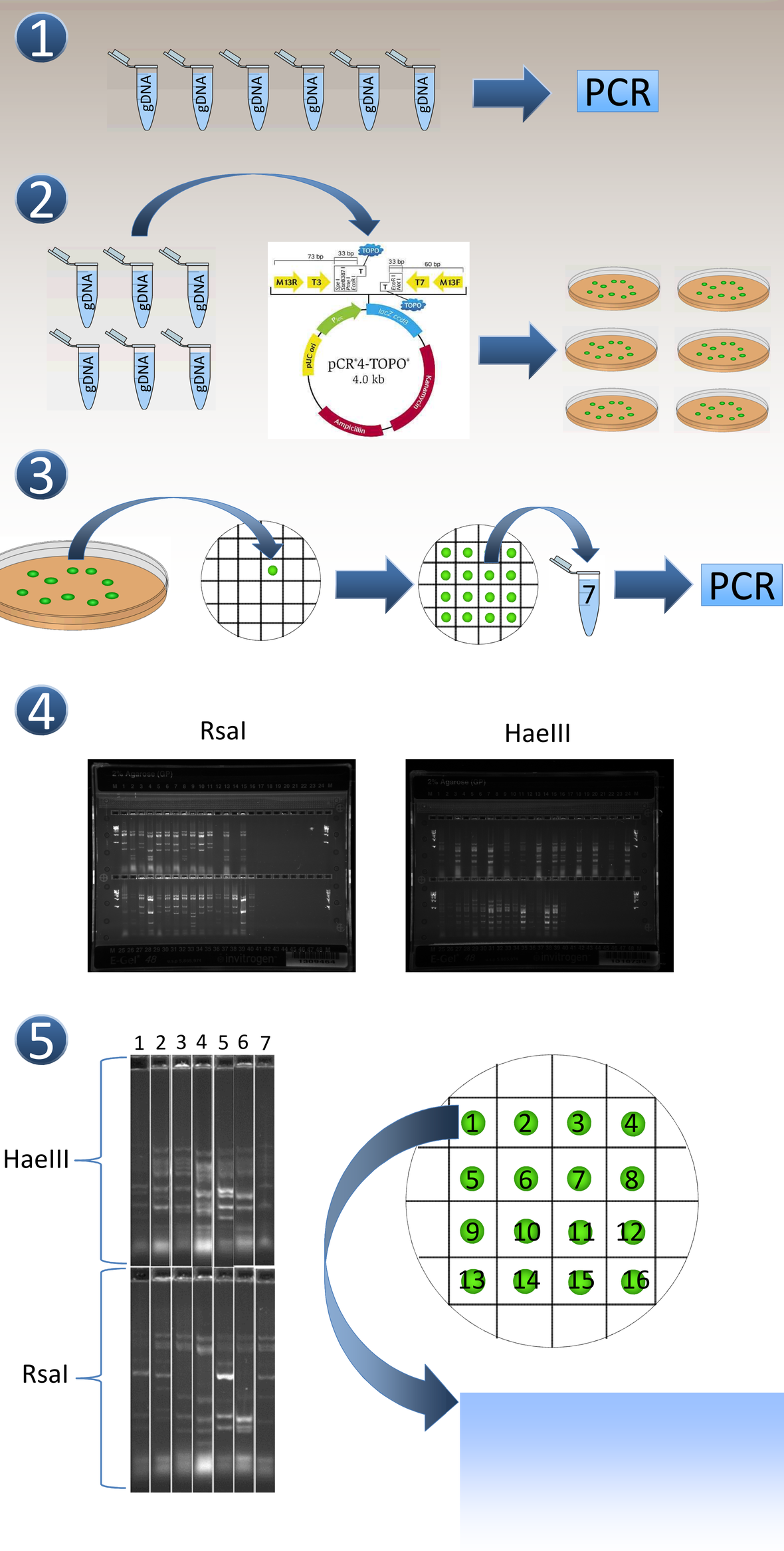
1. gDNA is obtained from six purified pond samples. The gDNA volume is split in two then amplified; half is used for prokaryotic analysis and half for eukaryotic, with different primers for each type. The primers in the prokaryotic samples surround the 16S region while the primers in the eukaryotic surround the 18S variable region.

The 16S region



2. The gDNA is transformed into a vector that selects for ampicillin and kanamycin.
3. Colonies are picked from the plates, organized into separate plates, then put into a corresponding PCR tube and amplified using colony PCR.
4. The contents of the colony PCR are split—half is restricted with the enzyme HaeIII and half with RsaI—and analyzed using restriction fragment length polymorphisms (RFLP). The restriction digest is run next to a ladder in a 2% agarose gel with ethidium bromide.
5. Together, the sets of two enzyme digests can be compared against each other to find unique clones. Different band patterns after being restricted by the two enzymes point to unique species. Almost 70 different prokaryotic species were found. The unique clones are then sent in for sequencing, and the sequence data is used to determine the identities of the species.

PROJECT TIMELINE



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SAND No. 2011-XXXXP