Ciliate genome remodeling

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Introduction

Ciliates, single celled organisms, contain two types of nuclei: micronuclei and macronuclei. Macronuclear chromosomes contain functional genes, while micronuclear chromosomes contain fragmented genes interrupted by non-coding genetic material [4].

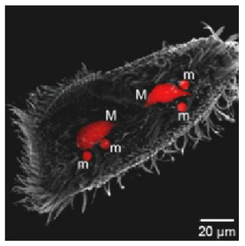

Figure 1: Macronucleus. micronucleus.

A number of genes identified so far are not only fragmented, but scrambled in the micronuclear DNA [3].

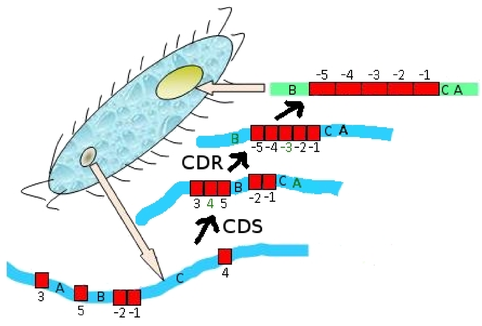

Figure 2: CDR and CDS operations decrypting micronuclear DNA

Decryption of the α-Telomere Binding Protein gene requires context-directed swaps (CDS), while Actin I requires CDS and reversals (CDR) due to the presence of inverted segments represented by negative numbers (Fig.2). Our lab analysis of these two genes uses polymerase chain reaction, electrophoresis, and sequencing to identify intermediates for comparison with known sequences available at GenBank.

Objectives

- Experimentally observe ciliate computational biases
- Characterize permutations invertible by ciliate operations
- Create software for analysis of the ciliate decryptome

Laboratory Methods

S. Lemnae DNA from several stages of micronuclear decryption was analyzed. For weak DNA signals standard PCR techniques were enhanced by repeated PCR or nested PCR (Fig. 4). Multiple products per gel electrophoresis lane (Fig. 5) were cut out and individually reprocessed. Sequencing was done by Genewiz, Inc.

Results & Discussion

The actin I gene contains negative segments, and must be resolved using some CDR. However, as a first move, CDS and CDR are both valid choices. If CDR is done first, the region [-3 -2 -1 9], identifiable with primer AMD-39, will be amplified and visible in the gel. If CDS takes place first, an 89 intermediate will exist instead. In Figure 3B, lanes 8-12 containing DNA with primer AMD-39 showed banding while lanes 3-7 containing DNA with primer AMD89 did not, indicating a preference for CDR. In Figure 3C, lanes 5 and 6 also showing banding for AMD-39 and not for AMD89.

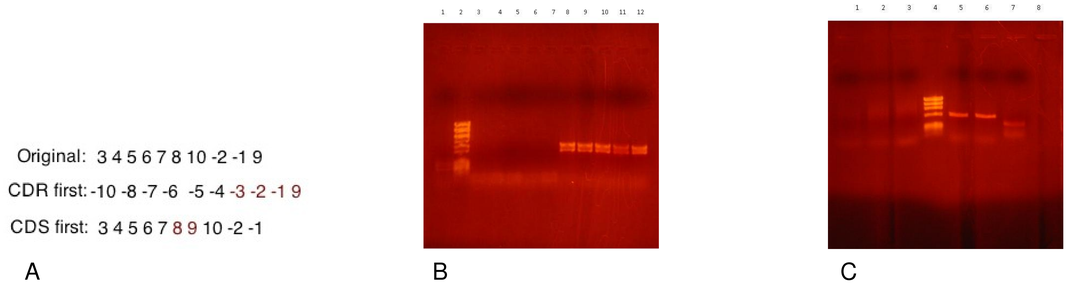

Figure 3: (A)Actin I sequence (B)PCR products of old and new (C)DNA with -39 and 89 primers

Theorem 1 *The only fully reduced CDS invertible unsigned permutation of length 2n for which any pointer pair supports CDS is of the form* $[1, 3, 5, \cdots, 2n-1, 2, 4, 6, \cdots, 2n]$.

Theorem 2 *CDS inverts a fully reduced CDS invertible unsigned permutation of the form* $[1, \cdots, n]$ *in the minimal possible number of block interchanges.*

Christie [1] found the least number of block interchanges inverting unsigned permutations. The concept of the breakpoint graph[2] was adapted to accomodate CDR and CDS, allowing permutations to be resolved using the graph and its algorithm for CDS and CDR.

Our program, "simpl," has 3 modules. Basic simpl finds and displays all paths to fixed points for a given signed sequence. simpl.decision reads batch input of sequences from a file, determining if each can be inverted via CDS and CDR. simpl.ies finds all intermediate sequences, representing internal eliminated sequences with letters to aid in primer design and PCR verification of intermediates.

Figure 4: Primers MD812 and IDJE11

Figure 5: Multiple PCR products

Future Work

- Expand PCR work to comprehensively test intermediates for all paths in the α-TBP, actin I, and DNA polymerase genes
- Investigate using breakpoint graphs to solve the invertibility decision problem
- Integrate GenBank sequence data into simpl, aiding in primer design and interpretation of sequencing results

Acknowledgements

- Prof. Jim Smith, Boise State University, for consultation and use of his laboratory
- Prof. Hans Lipps and Dr. Franziska Jönsson, University of Witten/Herdecke, Germany, for a gift of *Stylonychia lemnae*DNA, for PCR advice and for Figure 1.
- Boise State University Mathematics REU 2012
- NSF REU funding via grant DMS 1062857
- NIH INBRE funding NIH grants: National Center for Research Resources P20 RR016454 NIGMS P20 GM103408

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