

# 5<sup>th</sup> Annual Joint Bioinformatics Workshop

July 19, 2005  
2229 Seamans Center  
University of Iowa

by

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***Identification of an efficient approach to identifying effective antisense  
nucleic acids to target a mRNA molecule***

## ABSTRACT

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The efficient identification of sequences in mRNAs that are effective targets for antisense oligodeoxynucleotides (ODNs) in vivo is an important goal. In vivo tests of antisense ODNs are time consuming and expensive. Therefore we investigated computational and in vitro methods to identify antisense sequences that are effective in vivo. The SIP24/lcn2 mRNA sequence was used for this analysis. Several computational programs were tested and found to predict a range of potential antisense sequences. As an in vitro test of antisense activity, we determined the ability of the SIP24/lcn2 mRNA to hybridize with a microarray of 20mers complementary to sequential sequences in the mRNA. Hybridization was performed under non-denaturing conditions and at salt concentrations that resemble those in the cell. From these preliminary computational and in vitro studies seven oligodeoxynucleotides (ODNs) were chosen to be tested in vivo as antisense ODNs to target the SIP24/lcn2 mRNA of the mouse HC11 cell line. Each of the three microarray-chosen ODNs decreased the SIP24 protein level as demonstrated by Western blot analysis. Also, they caused a reduction in the SIP24/lcn2 mRNA level as determined by real-time RT-PCR. These results show that the in vitro microarray analysis accurately predicted appropriate sequences for effective antisense ODNs in vivo whereas the predictions from computational analyses were more varied.