Introduction
The ability to quickly identify cross-hybridized DNA duplexes is important in the design of DNA libraries for microarrays, mutation analysis, DNA computing, and combinatorial group testing. These applications rely on hybridization of perfect Watson-Crick complements to ensure predictable results. Cross-hybridized (CH) strands, strands that do not represent perfect complements, will result in false signals.

Since our ultimate goal is to construct libraries of oligonucleotides in which no strands form CH duplexes, we designed a way to detect CH strands in pools of up to 16 different non-hybridizing strands using the fluorescent dye SYBR Green I.

The mathematical theory Group Testing to Annihilate Pairs \(^1\) was used to determine how many pools were needed based on a given probability of error (0.05), to identify the cross-hybridized strands.

Methods
- A library of thirty-three 16-mers was designed using the software SynDCode.
- Known CH strands (With a 1 bp bulge near the center) were added to the library.
- AG values for all potential combinations of strands were calculated with the software Pairfold.
- Strands were annealed slowly and SYBR Green I was added.
- Annealed strands were melted over a 30-degree window and fluorescence was monitored at 520 nm in a real-time PCR thermalcycler.

SYBR Green Assay
Fluorescence absorbance
Temperature (°C)

Questions
- Can SYBR Green I detect mismatches?
- What types of mismatches are detectable?
- How many pools do we need to test to identify the cross-hybridizing strands? (currently being investigated)

Applications
- DNA nanotechnology
- DNA computation and code word design
- Microarrays (gene chips and DNA chips)
- Group testing

Conclusions
- SYBR Green I is able to detect one CH pair within a pool of 16 non-hybridizing strands.
- We have validated a DNA library.
- We have demonstrated that a one base bulge is identifiable within a pool of 16 strands
- At a probability of 5% error, only 60 pools are necessary to identify the CH strands.

References
- \(^1\) Bishop, M. et al., Group Testing to Annihilate Pairs Applied to DNA Cross-Hybridization Elimination Using SYBR Green I, in press
- http://syndcode.geneseo.edu/
- Pairfold program http://nar.oxfordjournals.org/cgi/content/full/31/13/3416

Acknowledgements
- This work was funded by the National Science Foundation Biomathematics Initiative Project #0436298
- We thank Kayla Nimmo, SUNY Geneseo ’06 for help with experiments.

Example of Cross Hybridized Duplex

Example of Watson-Crick Duplex

Group Testing
• Looking for an object (CH duplex) that will manifest itself

SYBR Green I
• Binds to the minor groove of double-stranded (ds) DNA
• Fluorescence increases markedly when bound to ds DNA

Pool with CH duplex
Typical results are shown for a few pools; over 100 pools were tested.

No CH duplexes