Contact us for more information on how you can perform better PCR design and analysis.
Phone: 734-222-9080
Email: info@dnasoftware.com

HOW TO ELIMINATE FALSE POSITIVES IN PCR DESIGN

ARE YOU USING THE WRONG PCR DESIGN TOOL?

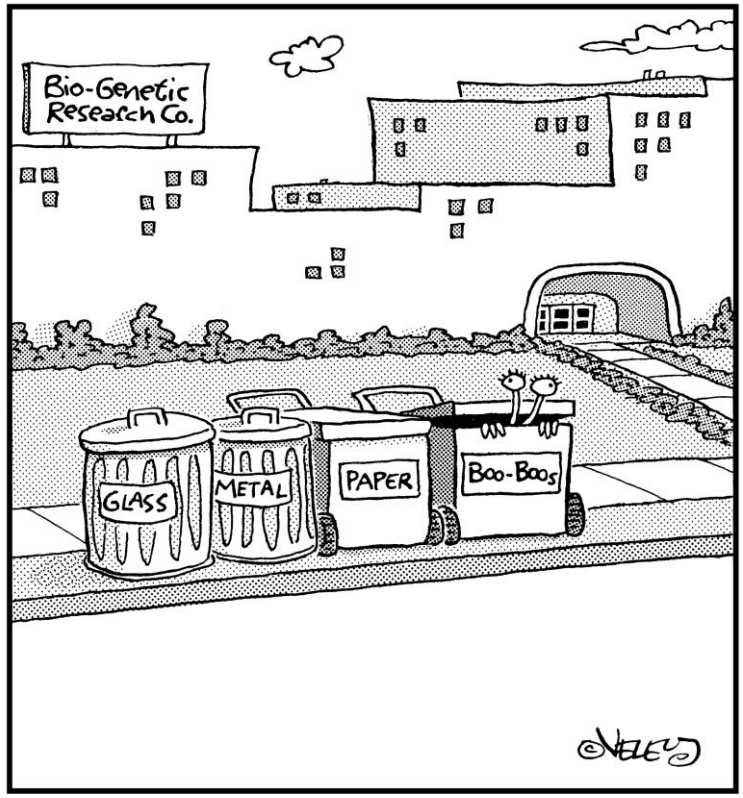
The most widely used bioinformatics tool in the world is NCBI BLAST. The BLAST algorithm is used to query an oligonucleotide sequence against a library of genomes to determine sequence similarity, which infers common evolutionary ancestry, and by extension, function. Yet laboratories all over the world struggle to design highly sensitive PCR assays using BLAST as false positives are almost impossible to evaluate.

This eBook quickly and easily summarizes the challenges associated with false positives in PCR assay design in BLAST.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990) J. Mol. Biol., 215, 403.

A FAMILY OF HAMMERS INCLUDES MEMBERS WITH DIFFERENT FUNCTIONS.

The most widely used builders tool is the hammer. A claw hammer can pound nails into wood and remove them. A sledge hammer is used to demolish a structure and a ball-peen hammer is most useful for metal-working jobs. All of these items is a member of the hammer family, yet each has a specific purpose in which it is most useful. Attempting to “get by” with the wrong tool just because the correct tool is not available may result in unexpected or even unexplained results.



Home.howstuffworks.com/home-improvement/constructionmaterials/5-tools-for-a-builders-toolbox.htm

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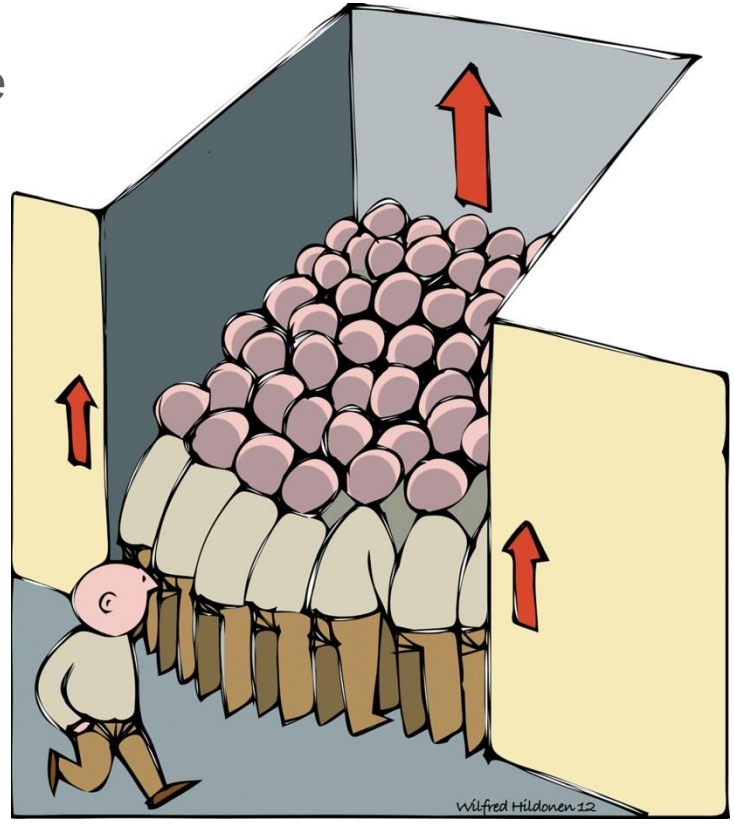
HERD MENTALITY EQUALS LOW PERFORMING PCR ASSAYS.

Again, the BLAST algorithm is the most widely used bioinformatics tool in the world, but rules to detect sequence similarity in BLAST cannot be used to evaluate mishybridization and crosshybridization which will always lead to false positives, thereby reducing the sensitivity of PCR assay design. Inversely, the application of thermodynamic stability to your design will reveal salt and temperature specific hybridization events that could easily be redesigned *in silico*, thereby negating the trial-and-error cycle of oligonucleotide design that most scientists employ as their go-to-strategy.



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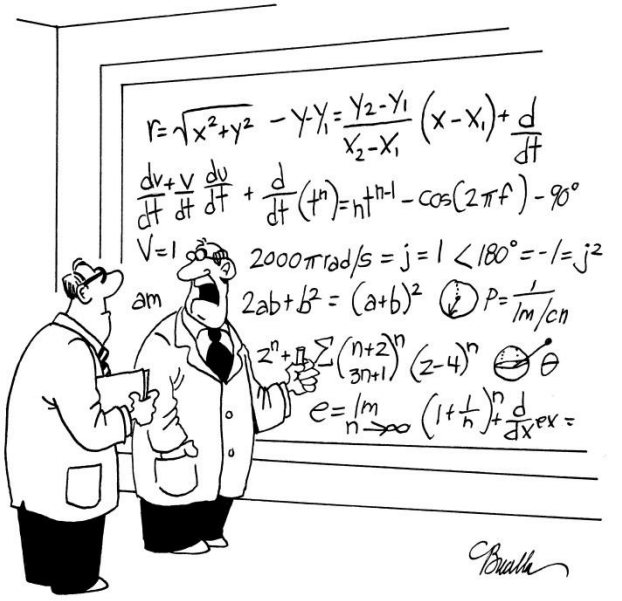
HOW MANY BAD ASSAY DESIGNS MAKE A GOOD ASSAY DESIGN?

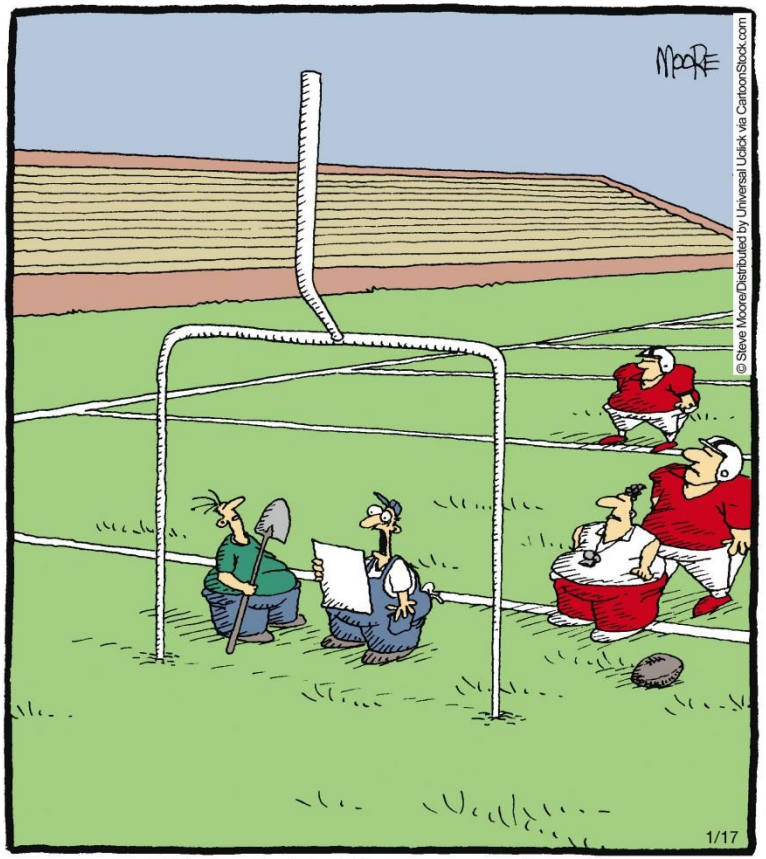
The rules to detect sequence similarity in BLAST do not equal the rules used to calculate the thermodynamic stability of matched and mismatched basepairs, or modified basepairs, to duplex stability. For instance, the mismatches G/T, G/A and G/G will promote mishybridization or crosshybridization to varying degrees in varying matched basepair sequence contexts, which will lead to reduced sensitivity and false positives. The application of a thermodynamic model to PCR assay design will characterize these contributions to false positives so that the sensitivity of an assay design can be maximized.



"It's a government funded study to find out how many wrongs make a right."

UNEXPECTED STRUCTURES AFFECT THE OUTCOME.

The rules to detect sequence similarity in BLAST do not equal the rules used to calculate the inhibitive thermodynamic effects of oligonucleotide secondary structures such as hairpins, bulges, dangling ends and a variety of other folding patterns that lead to sub-optimal primer binding on the target. This situation will certainly result in unexpected primer extensibility and false amplicons of varying lengths and concentrations. The application of thermodynamic stability to the PCR assay design will characterize unwanted oligonucleotide structures, thereby preventing a delay of game or a missed deadline penalty.



"He's right. We screwed up."

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INFORMATION OVERLOAD LEADS TO UNEXPECTED DELAYS.

The rules to detect sequence similarity in BLAST do not lend themselves to customized and targeted searches where users use giant databases to generate irrelevant data and information overload. The application of thermodynamic stability allows users to query more specific and predefined temperature and salt conditions to yield more specific and high-value hits that more rigorously define and model false positive behavior in PCR assay designs.

"Let's hold off making a decision until we have even more information we don't really need."

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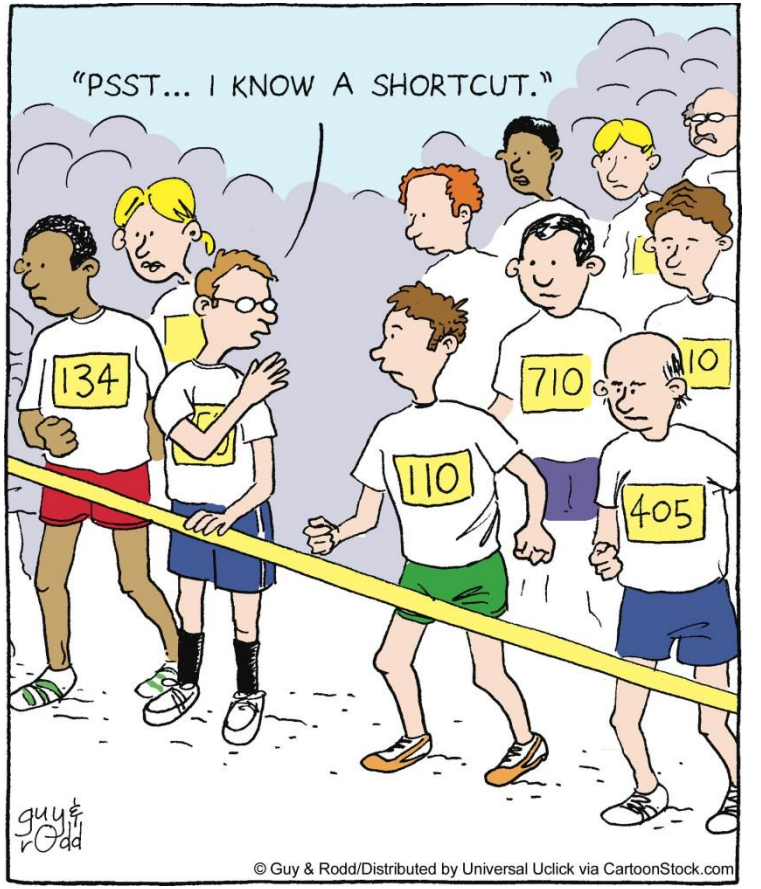
BENEFITS OF APPLYING A THERMODYNAMIC MODEL TO PCR ASSAY DESIGN.

- No more trial-and-error PCR assay design.
- Improved specificity
- Improved sensitivity
- Eliminate false positives
- Reduce meaningless data



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About the Author

John SantaLucia
CEO and Founder, DNA Software

A world-renowned expert in nucleic acid hybridization, Dr. John SantaLucia served as CSO since co-founding the company in 2000. In August, 2010, Dr. SanatLucia took the reins of DNAS as president and CEO, providing the vision and leadership for the company's technologies and marketplace opportunities.

Education:

post-doctoral - Biophysical Chemistry; University of California, Berkeley

Ph.D. - Physical Chemistry; University of Rochester, Rochester, NY

BS - Chemistry; Clarkson University, Potsdam, NY

About DNA Software

DNA Software™ has world-leading expertise in the science of DNA.

We offer a portfolio of technologies that enable our customers to develop diagnostic assays and therapeutics with unrivaled sensitivity and specificity.

For over 14 years, we've earned the trust of leaders in the life sciences community.

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