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New virtual tool may provide more accurate diagnosis of genetic mutations

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DNA sequencing to detect genetic mutations can aid in the diagnosis and selection of treatment for cancer. Current methods of testing DNA samples, Sanger sequencing and pyrosequencing, occasionally produce complex results that can be difficult or impossible to interpret. Scientists at the Johns Hopkins University School of Medicine have developed a free software program, Pyromaker, that can more accurately identify such complex genetic mutations.

Pyromaker is a web-based application that produces simulated pyrograms based on user input including the percentage of tumor and normal cells, the wild-type sequence, the dispensation order, and any number of mutant sequences. Pyromaker calculates the relative mutant and wild-type allele percentages and then uses these to generate the expected signal at each point in the dispensation sequence. The final result is a virtual trace of the expected pyrogram.

The researchers validated Pyromaker against actual pyrograms containing common mutations in the KRAS gene, which plays an important role in the pathogenesis of a variety of tumors. The actual pyrograms and virtual pyrograms were quantitatively identical for all mutations tested.

They then demonstrated that all codon 12 and 13 single and complex mutations generate unique pyrograms. However, some complex mutations were indistinguishable from single base mutations, indicating that complex mutations may be underreported. Working with two

complex pyrograms that were difficult to interpret initially, the researchers identified five approaches to resolve them: Sanger sequencing alone, hypothesis testing with Pyromaker, Pyromaker iterative mutation re-creation, melting curve analysis, and TA cloning with Sanger sequencing.

Senior author James R. Eshleman, MD, PhD, Professor of Pathology and Oncology, Associate Director, Molecular Diagnostics Laboratory, Johns Hopkins University School of Medicine, explains, "User-directed hypothesis testing allows for generating virtual traces that can be compared to the actual data to clarify ambiguous results from pyrosequencing and the Sanger method. Alternatively, Pyromaker can quickly and efficiently test the possibilities that can explain a complicated polysequencing result." Both strategies were able to successfully identify the complex mutations.

TA cloning and sequencing also provided unequivocal interpretation, but this method is labor intensive, risks plasmid contamination of the laboratory, may delay reporting, and is not routinely used in most clinical diagnostic laboratories.

"Although pyrosequencing and Sanger sequencing are both powerful tools to resolve most mutations, for certain complex cases, neither of them alone is enough to provide a definitive interpretation," notes Dr. Eshleman. "Additional methods, such as Pyromaker analysis or TA cloning and sequencing, allow one to definitively diagnose the variant allele. Pyromaker is available free online and can be accessed from any computer with internet access. Iterative Pyromaker analysis is the least expensive and fastest method to resolve these cases."

More information: Pyromaker has been made freely available at pyromaker.pathology.jhmi.edu .

The article is "A Virtual Pyrogram Generator to Resolve Complex Pyrosequencing Results," by G. Chen, M.T. Olson, A. O'Neill, A. Norris-Kirby, K. Beierl, S. Harada, M. Debeljak, K. Rivera-Roman, S. Finley, A. Stafford, C.D. Gocke, M-T. Lin, and J. R. Eshleman (doi: 10.1016/j.jmoldx.2011.12.001). *The Journal of Molecular Diagnostics*, Volume 14, Issue 2 (March 2012)

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