

Simple, sensitive, and cost-effective assays for analyzing Fragile X-related disorders

August 12 2016

Fragile X syndrome, the most common heritable cause of intellectual disability and a frequent cause of autism, is characterized by abnormalities of the FMR1 gene that are difficult to analyze. Preclinical studies of Fragile X and the Fragile X-related disorders are hampered by the lack of low-cost and sensitive yet simple methods. National Institutes of Health (NIH) researchers have now developed a set of assays that are robust, cheap enough for routine research use, and may be suitable for initial patient screening, according to a new report in *The Journal of Molecular Diagnostics*.

Fragile X-related disorders result from expansion of a hyper-variable and methylation-prone trinucleotide-repeat tract in the FMR1 gene. Patients with Fragile X syndrome typically have more than 200 repeats whereas individuals with 55 to 200 repeats are at risk for Fragile X-associated [primary ovarian insufficiency](#) and Fragile X-associated tremor/ataxia syndrome.

"Careful analysis of the total number of repeats, the number of interruptions in the repeat tract, and the [methylation status](#) of the FMR1 gene is important for a proper understanding of an individual's risk of transmission of larger alleles to their offspring and to their personal risk of disease pathology. It is also critical for addressing a number of important research questions," explained Karen Usdin, PhD, Senior Investigator and Chief of the Gene Structure and Disease Section, Laboratory of Cell and Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, NIH (Bethesda, MD).

The new assays have the ability to amplify alleles with up to approximately 1,000 repeats, even in samples from patients who are mosaic, ie, who have a mixture of cells with different repeat numbers. The assays are sensitive enough to analyze saliva samples with minimal purification. Testing can be completed within a timeframe similar to that of recent commercial diagnostic assays (less than 24 hours to determine repeat size and/or methylation status, less than 24 hours to determine the interruption status and percent methylation) and are comparable in terms of hands-on time required. These assays make it possible to detect even small changes in DNA methylation, making them useful in the hunt for new drugs to reverse the effects of repeat expansion.

"The basic assays for repeat number, methylation status, and the number of uninterrupted repeats cost less than \$5, the mark typically considered the threshold for population-based screening," said Bruce Hayward, PhD, a Senior Research Fellow in the Laboratory of Cell and Molecular Biology and the report's first author.

To develop these assays, the researchers modified an established PCR assay that was capable of sizing only small alleles to enable it to size larger alleles. "Realizing the potential broader utility of the sizing assay, we then expanded its abilities to include both methylation and interruption status. We showed that these assays perform robustly even in the most challenging of situations," said Dr. Hayward.

The researchers hope that these assays will be used by many laboratories that are studying the events associated with early embryonic development and the effect of repeat length and methylation status on gene expression and differentiation. "Without the ability to verify CGG-repeat number and methylation status, it is impossible to distinguish between bona fide developmentally-regulated changes and artifacts arising from the instability in repeat number and methylation commonly associated with these cells," said Dr. Usdin.

More information: *The Journal of Molecular Diagnostics*. [DOI: 10.1016/j.jmoldx.2016.06.001](https://doi.org/10.1016/j.jmoldx.2016.06.001)

Provided by Elsevier

Citation: Simple, sensitive, and cost-effective assays for analyzing Fragile X-related disorders (2016, August 12) retrieved 6 May 2024 from <https://medicalxpress.com/news/2016-08-simple-sensitive-cost-effective-assays-fragile.html>

This document is subject to copyright. Apart from any fair dealing for the purpose of private study or research, no part may be reproduced without the written permission. The content is provided for information purposes only.