murdoch children's research institute

Methylation Specific-Quantitative Melt Analysis

Novel Fragile X Syndrome Diagnostic Test

The opportunity

Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability and co-morbid autism (1/4000 births) caused by abnormal methylation of the FMR1 gene. FXS diagnosis and prognosis for type and severity of clinical involvement is significantly complicated by cellular mosaicism that may go undetected by standard testing.

Interest in epigenetics, and in particular DNA methylation, is growing exponentially as more and more diseases are linked to changes in the "epigenome". Changes in methylation have been linked to diseases such as cancer, lupus, and a range of birth defects.

In the clinic, DNA methylation is being used to determine residual disease in prostate cancer patients and inform drug responsiveness in glioma patients.

Researchers at MCRI have developed an assay and analysis method that has a quantitative limit of 2% and a qualitative limit of 1% for methylation at a target site within a DNA sample and enables high throughput screening in a 'closed tube' format.

The technology

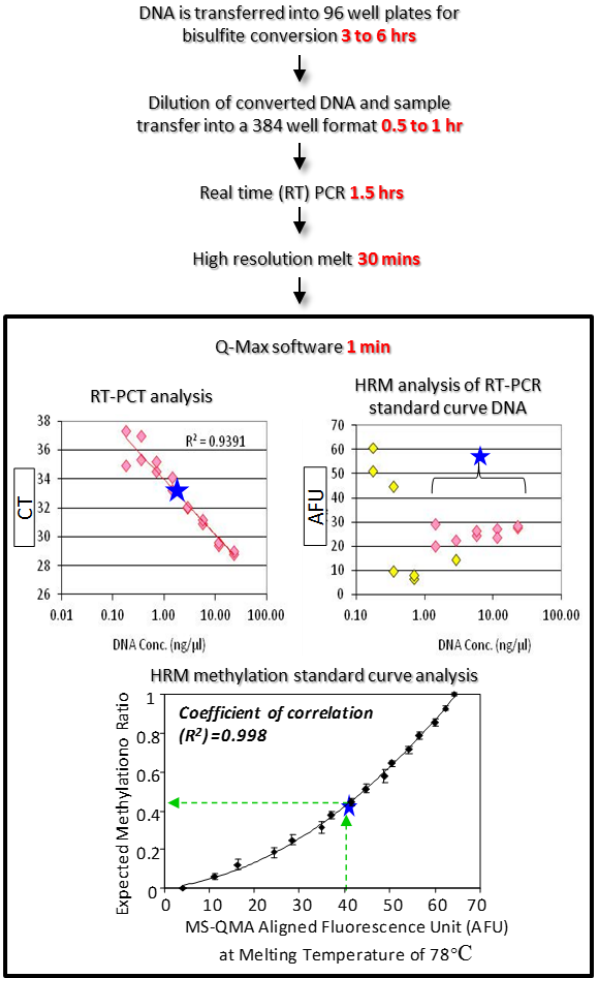
MS-QMA is a highly sensitive and low-cost molecular assay which combines the qualitative strengths of high resolution melt technology and the high-throughput, quantitative real-time PCR standard curve to provide accurate quantification of DNA methylation in a single assay. For FXS diagnosis, the MS-QMA assay quantitates methylation of target regions within the promoter region of the FMR1 gene (FREE2 biomarkers located at the FMR1 exon 1 / intron 1 boundary). MS-QMA offers the following features and benefits over the current market leading technologies:

- Detects low level mosaicism which is often missed by standard testing in typical Full Mutation (FM) males and in Pre-Mutation (PM) individuals.
- Low reagent costs (<$10 AUD).
- Time and labour efficiencies (Single day automated and semi-automated protocol options available)
- Methylation specific, so does not pick up Pre-Mutation and Grey Zone alleles that do not cause FXS. This represents major cost savings through reduced genetic counselling for incidental findings.
- Correlated with IQ and ADOS (Autism Spectrum Disorder severity) in females and males, to provide prognostic information; information that cannot be provided by any other FMR1 test developed to date.
- Multiple tissues can be detected at low analytical sensitivity sensitivity of 1% to 2% abnormal methylation (buccal, blood, saliva and dried blood spots, newborn blood spots).
- Q-MAX software developed for straightforward data interpretation and reporting
- High-throughput: 200 samples per run in 3 to 5 hours from DNA to final result.
  In comparison southern blot takes 1 to 2 weeks for 15 samples; other triple primed / long range PCR commercial tests take 2 to 3 days for 200 samples
- Validated on more than 4,000 samples; published in leading journals (Clinical Chemistry; Expert Reviews in Molecular Medicine, etc).
- Currently in further development for quantitative methylation testing of imprinting disorders including Prader Willi Syndrome, Angelman Syndrome, and Chromosome 15 q Duplication Syndrome

The novel method referred to as MS-QMA, combines the qualitative strengths of high resolution melt technology and the high-throughput, quantitative real-time PCR standard curve to provide accurate quantification of DNA methylation in a single assay.

MS-QMA has been shown to correlate with the more cumbersome reference methods of Southern blot and MALDI-TOF MS, and even to perform better with low quality DNA, e.g. DNA extracted from newborn blood spots. Analysis of MS-QMA raw data is by a custom designed computer algorithm, which simultaneously performs all analysis steps. The algorithm is available in the form of a user-friendly desktop application called Q'Max.

The performance of the method was assessed in Fragile X Syndrome (FXS), a neurodevelopmental disorder that is complex and heterogeneous in both clinical phenotype and epigenotype.



The present method was able to differentiate FXS affected individuals from controls, with sensitivity, specificity, positive and negative predictive values between 92 and 100%. The method was also shown to be sufficiently sensitive to provide prognostic information, as methylation ratios were significantly correlated with various measures of intellectual impairment (e.g. verbal IQ impairment P=0.002).

Applications

MS-QMA has the potential to be a powerful research tool for investigators examining the relationship between DNA methylation and disease.

MS-QMA has an immediate application in the diagnosis of FXS and other developmental delay conditions. This also includes potential applications in newborn screening, prenatal testing and use in prognosis.

The method also has the potential to form the basis of diagnostic/prognostic tests for other conditions associated with aberrant DNA methylation, as well as companion diagnostics.

Development stage

MS-QMA and the FREE biomarkers have been extensively published and validated.
Analysis of MS-QMA raw data is by a custom designed computer algorithm, which simultaneously performs all analysis steps. The algorithm is available in the form of a user-friendly desktop application called Q'Max.

Opportunity for partnership

The Murdoch Children's Research Institute is seeking a partner to develop the technology as a research tool, together with the Q'Max desktop application.

We are also seeking a partner to co-invest in the development of the technology for clinical applications.

Intellectual Property

Murdoch Childrens Research Institute currently has 3 families covering this technology, after having let one lapse. Of the 3 patent families, 2 are for the FREE diagnostic biomarkers (we had let another FREE application lapse), and the other remaining is for the MS-QMA method. Intellectual property, in the form of copyright, also exists in the Q'Max desktop application. The details regarding our 3 Patent Families are:

1)
Patent Family for an assay for determining epigenetic profiles of markers of Fragile X alleles (PCT/AU2010/000169), which is directed towards assessing methylation of FREE1 and FREE2, its status internationally is:
Australia (2010215061): Accepted
Israel (214690): Accepted
Europe (10743317.9): Accepted
USA (13/202,085): Pending

2)
Patent Family for the treatment and diagnosis of epigenetic disorders and conditions (PCT/AU2011/001024), directed towards assessing methylation of FREE2(D) and FREE2(E) regions which have utility for prognosis of late-onset conditions. The status internationally is:
Europe (11815911.0): Pending
US (14/932,634): Pending
Australia (2011288917): Granted

3)
Patent Family for an assay for quantitating the extent of methylation of a target site (PCT/AU2014/000044), this is directed towards the MSQMA assay and the use of this assay in diagnosis:
Europe (14743573.9): Pending
US (14/763,485): Pending
Australia (2014210369): Pending

Key publications

Godler DE et. al. Early Detection of Fragile X Syndrome: Applications of a Novel Approach for Improved Quantitative Methylation Analysis in Venous Blood and

Newborn Blood Spots. Clinical Chemistry (2014) v. 60, p.963-973.

Aliaga SM et al. 2016 Identification of Males with Cryptic Fragile X Alleles by Methylation-Specific Quantitative Melt Analysis. Clin Chem. February 2016 vol. 62 no. 2 343-352

Inaba Y. et at. 2014 Early detection of fragile X syndrome: applications of a novel approach for improved quantitative methylation analysis in venous blood and newborn blood spots. Clin Chem. 2014 Jul;60(7):963-73.

Contact: Dr Joanne Boag
Head of IP & Licensing – Biotech & Medtech
P +613 8341 6445
Joanne.boag@mcri.edu.au

Jonathon Morison
Business Development Manager
P +613 9936 6316
Jonathon.morison@mcri.edu.au