qPCR-HRM

Application in Molecular Diagnostics & Microbiology

1. Genotyping of IFN-λ3 gene associated with HCV clearance

5. Identification and antimicrobial susceptibility testing integrated

3. Staphylococcus epidermidis genotyping

2. Speciation of Gram-negative Bacteria

4. Drug susceptibility test for Mycobacterium leprae directly from clinical sample

Techtum Lab, 2018
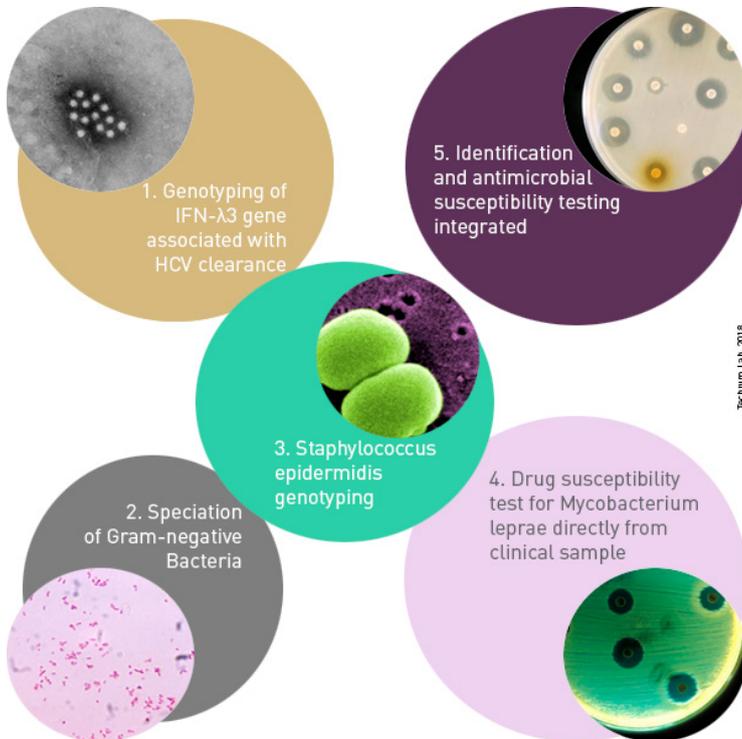
Recent publications on High Resolution Melting (HRM) analysis showed HRM as a massively cost-effective and powerful method for identification, mutation screening, gene scanning, and genotyping.

We compiled some new techniques and advances within the field that you will find useful when implementing HRM analysis:

1. IFN-λ3 SNPs were genotyped using HRM. Result showed differences in levels of IFN-λ3.

    Grzegorzewska et al, 2017. Poland. ⇓link

2. Biochemical profiling is the typical method but it is time consuming, which is bad since treatment is needed. HRM provides greater multiplexing capability and identifies *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Salmonella Sp*, and *Acinetobacter baumannii*, and a generic Gram-negative specific 16S rRNA control.

    Edwards et al, 2018. UK and Malawi. ⇓link

3. qPCR target class I or II SNPs in housekeeping genes, EvaGreen is used. With post-qPCR, HRM provides information which *Staphylococcus epidermidis* belong to which genotypes.

    Larssen et al, 2018. Norway. ⇓link

4. qPCR-HRM able to identify mixed infections of susceptible and resistant *Mycobacterium leprae*. This tool can be utilized as a large-scale and inexpensive drug resistance surveillance tool.

    Araujo et al, 2017. Brazil, US, Belgium and Japan. ⇓link

5. HRM enables broad bacterial identification or antimicrobial susceptibility testing. Bacterial identification protocol based on 16S rRNA is automated. Test completed really quick within 6,5 hour.

    Athamanolap et al, 2017. US. ⇓link

Instrument and reagent

Magnetic Induction Cycler (Mic) is both a PCR and qPCR cycler with multiplexing capacity. It can be equipped with either 2 or 4 optical channels. The latest optical technology integration in Mic enables its user to perform HRM analysis on any of its channel. When running HRM assay in this 2 kg compact cycler, you can use dye such as SYTO© 9, EvaGreen, or LC© Green. In addition, it has fast cycling capability so you will get quality data fast!

Standard Applications
Genotyping for SNPs or insertion-delection (InDel)
Quantifying somatic mutations or epigenetic methylation levels
Sequence matching for drug resistance bacteria
Gene scanning for new mutations

Mentioned in...
Clinical Chemistry, Goh et al, 2016 ⇓link - HRM on Mic provides best spatial separation of InDel polymorphisms compare to Rotor-Gene 6000 and CFX Connect

qPCRBIO HRM Mix uses a novel SyGreen 2 dye, which unlike other dsDNA binding dyes, this can be used at saturating concentrations without inhibiting PCR. Almost no optimisation is needed. This mix is sensitive to discriminate class I to IV mutations as well as CpG methylation differences.

Standard Applications
SNP genotyping
Gene scanning
CpG methylation analysis
Efficient specific amplification from GC and AT rich sequences