High Resolution Melt Curve Analysis- An Innovative Approach for Molecular Diagnosis

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Abstract—*The High Resolution Melting (HRM) is a homogeneous, extremely powerful technique for SNP genotyping, mutation scanning and sequence scanning in DNA\samples. Enabled by the recent availability of improved double-stranded DNA (dsDNA)–bindingdyes and next-generation real-time PCR instrumentation, High Resolution Melting Analysis is based on PCR melting (dissociation) curve techniques.High Resolution Melting Analysis (HRM) is a post PCRmethod. During processing, special saturation dyes known as Intercalating dyes, are added to the reaction, that fluoresce only in the presence of double stranded DNA. The region of interest within the DNA sequence is first amplified using the polymerase chain reaction. As the amplicon (amplified product) concentration in the reaction tube increases the fluorescence exhibited by the double stranded amplified product also increases. After the PCR process the HRM analysis begins. In this process the amplicon DNA is heated gradually from around 50° Cup to around 95°C. As the temperature increases, at a point the melting temperature of the amplicon is reached and the sample DNA denatures and the double stranded DNA melts apart. Due to this the fluorescence fades away. This is because in the absence of double stranded DNA the intercalating dyes have nothing to bind to and they only fluoresce at a low level. This observation is plotted showing the level of fluorescence vs the temperature, generating a Melting Curve. Even a single base change in the sample DNA sequence causes differences in the HRM curve. Since different genetic sequences melt at slightly different rates, they can be viewed, compared, and detected using these curves. Melt curves generated after High Resolution Melting analysis is normally plotted with fluorescence on the Y axis and temperature on the X axis. These are similar to real-time PCR amplification plots but with the substitution of temperature for cycle number.*