Melt curve analysis

FIELD OF THE INVENTION

The present invention relates to melt curve analysis. In particular, the present invention relates to a method, to an apparatus and to a computer program for analyzing a signal descriptive of melt curve data.

BACKGROUND

Melt curve analysis and HRM (high resolution melting) analysis are commonly used methods for detecting and analyzing the presence of nucleic acid sequences in a sample. The analysis is normally performed immediately after PCR amplification of the nucleic acids and relies on monitoring fluorescence of the reaction solution as a function of temperature. The fluorescent molecules used can be double-stranded DNA binding fluorophores or fluorescently labeled probes.

Typically almost fully saturating dsDNA binding fluorophore is required for a successful HRM analysis. This requirement limits the selection of possible fluorophores as many of them would be required in concentrations too high to allow efficient amplification.

Traditional HRM analysis is not well suited for quantitative analysis. As an example, in some cases it would be beneficial to be able to quantitatively assess the relative abundance of a mutant sequence in a heterogeneous sample. This type of information would be valuable for example in analysis of acquired single nucleotide polymorphisms (SNPs) related to cancer and analysis of aneuploidy.

In addition, the resolution capability of traditional HRM restricts its use e.g. in detecting homozygous SNP mutants as the differences in melting temperatures are minor.

SUMMARY

~~Therefore, it is an object~~ Embodiments of the present invention ~~to~~ provide an analysis technique that is suitable for quantitative analysis of nucleic acid molecules and that enables detection and/or identification of nucleic acids in a sample at a good resolution (in terms of differences in melting temperature).

~~The objects of the invention are reached by a method, by an apparatus and by a computer program as defined by the respective independent claims.~~

According to a first aspect of the present invention, there is provided a method for analyzing a melt curve characterizing the melt of a solution comprising one or more populations of nucleic acid molecules and a constant number of fluorophores of at least first type, the method comprising obtaining a fluorescence signal descriptive of melt curve data over a temperature range, the fluorescence signal representing the intensity of the light emitted by said fluorophores as a function of temperature, modeling the fluorescence signal at a plurality of temperatures within the temperature range as a sum of a first signal component representing the combined light intensity emitted by unbound fluorophores of said first type in the solution at a given temperature and a set of one or more second signal components, each representing the combined light intensity emitted by said fluorophores bound to the respective nucleic acid molecule population at the given temperature, wherein the first signal component is provided as a product of a first term representing the relative number of unbound fluorophores of said first type at the given temperature and a second term representing the emission efficiency of an unbound fluorophore of said first type at said given temperature, and wherein each second signal component is provided as a product of a respective third term representing the relative number of said fluorophores bound to the respective nucleic acid molecule population at the given temperature and a respective fourth term representing the emission efficiency of said fluorophore bound to the respective nucleic acid molecule population at said given temperature, and utilizing numerical analysis to determine the values of said first, second, third and fourth terms at said plurality of temperatures such that the difference between the fluorescence signal and the modeled fluorescence signal meets a predefined criterion.

According to a second aspect of the present invention, there is provided an apparatus for analyzing a melt curve characterizing the melt of a solution comprising one or more populations of nucleic acid molecules and a constant number of fluorophores of at least first type, the apparatus comprising at least one processor and at least one memory including computer program code for one or more programs, the at least one memory and the computer program code configured to, with the at least one processor, cause the apparatus to perform at least the following: obtain a fluorescence signal descriptive of melt curve data

over a temperature range, the fluorescence signal representing the intensity of the light emitted by fluorophores of said first type as a function of temperature, model the fluorescence signal at a plurality of temperatures within the temperature range as a sum of a first signal component representing the combined light intensity emitted by unbound fluorophores of said first type in the solution at a given temperature and a set of one or more second signal components, each representing the combined light intensity emitted by said fluorophores bound to the respective nucleic acid molecule population at the given temperature, wherein the first signal component is provided as a product of a first term representing the relative number of unbound fluorophores of said first type at the given temperature and a second term representing the emission efficiency of an unbound fluorophore of said first type at said given temperature, and wherein each second signal component is provided as a product of a respective third term representing the relative number of said fluorophores bound to the respective nucleic acid molecule population at the given temperature and a respective fourth term representing the emission efficiency of said fluorophore bound to the respective nucleic acid molecule population at said given temperature, and utilize numerical analysis to determine the values of said first, second, third and fourth terms at said plurality of temperatures such that the difference between the first fluorescence signal and the modeled fluorescence signal meets a predefined criterion.

In this regard, a novel method for analyzing a melt curve characterizing the melt of a solution comprising one or more populations of nucleic acid molecules and a constant number of fluorophores of at least first type is provided. Said method comprises obtaining a fluorescence signal descriptive of melt curve data over a temperature range, the fluorescence signal representing the intensity of the light emitted by said fluorophores as a function of temperature, modeling the fluorescence signal at a plurality of temperatures within the temperature range as a sum of a first signal component representing the combined light intensity emitted by unbound fluorophores of said first type in the solution at a given temperature and a set of one or more second signal components, each representing the combined light intensity emitted by said fluorophores bound to the respective nucleic acid molecule population at the given temperature, wherein the first signal component is provided as a product of a first term representing the relative number of unbound fluorophores of said first type at the given temperature and a second term representing the emission efficiency of an

unbound fluorophore of said first type at said given temperature and wherein each second signal component is provided as a product of a respective third term representing the relative number of said fluorophores bound to the respective nucleic acid molecule population at the given temperature and a respective fourth term representing the emission efficiency of said fluorophore bound to the respective nucleic acid molecule population at said given temperature, and utilizing numerical analysis to determine the values of said first, second, third and fourth terms at said plurality of temperatures such that the difference between the fluorescence signal and the modeled fluorescence signal meets a predefined criterion.

The method may comprise modeling each of said third terms as a product of the overall number of binding locations for the respective nucleic acid molecule population at said given temperature and the value of a first parametric function that is descriptive of the occupancy level of said overall number of binding locations as a function of the relative number of unbound fluorophores in the solution, wherein said overall number of binding locations is determined by a second parametric function that is descriptive of the melting probability of the respective nucleic acid molecule population as a function of temperature, and