

R documentation

of 'meltR.A.Rd'

September 14, 2022

meltR.A

Fit absorbance melting curves to obtain thermodynamic parameters

Description

Automates the trivial but time-consuming tasks associated with non-linear regression. Calculates extinction coefficients, subtracts out the baseline buffer readings, and calculates the strand concentration, Ct, in each sample. Then uses three non-linear regression methods to calculate thermodynamic parameters. Method 1 fits each melting curve individually then reports the average H and S from all of the curves. Method 2 calculates the Tm for each melting curve, and calculates thermodynamic parameters by fitting the relationship between Tm and Ct. Method 3 calculates thermodynamic parameters with a global fit, where H and S are constant between isotherms and the baselines are allowed to float. Also includes an algorithm that optimizes the mole ratio of fluorophore labeled strands to quencher labeled strands.

Usage

```
meltR.A(  
  data_frame,  
  blank = "none",  
  NucAcid,  
  wavelength = 260,  
  concT = 90,  
  fitTs = NULL,  
  methods = c(TRUE, TRUE, TRUE),  
  Tm_method = "nls",  
  Mmodel,  
  Tmodel = "VantHoff",  
  Save_results = "none",  
  file_prefix = "Fit",  
  file_path = getwd(),  
  auto.trimmed = FALSE,  
  Silent = FALSE  
)
```

Arguments

<code>data_frame</code>	data_frame containing absorbance melting data
<code>blank</code>	The blank sample for background subtraction, or a list of blanks to apply to different samples for background subtraction. "none" to turn off background subtraction. If there is a single blank in the data set, the identity of the blank, for example, <code>blank = 1</code> or <code>blank = "blank"</code> . If there are multiple blanks in the data, <code>blank = list(c("Sample 1", "Blank 1"), c("Sample 2", "Blank 2"))</code> and so on. Sample identifiers should be what they are in the data frame. If you need to figure out what the sample identifiers are, run <code>unique(df\$Sample)</code> , where <code>df</code> is the name of the R data frame you are using, in your R console.
<code>NucAcid</code>	A vector containing the Nucleic acid type and the sequences you are fitting for calculating extinction coefficients. Examples: <code>c("RNA", "UUUUUU", "AAAAAA")</code> , <code>c("DNA", "GCTAGC")</code> , etc... . For a custom extinction coefficient enter "Custom" followed by the molar extinction coefficients for every nucleic acid in the sample. For example, <code>c("Custom", 10000, 20000)</code> .
<code>wavelength</code>	The wavelength you are using in the data set in nm. Options for RNA: 300, 295, 290, 285, 280, 275, 270, 265, 260, 255, 250, 245, 240, 235, and 230 nm. Options for DNA 260 nm. Most accurate at pH 7.0.
<code>concT</code>	The temperature used to calculate the NucAcid concentration. Default = 90.
<code>fitTs</code>	Option to only fit certain temperature ranges for melting curves. Either a vector or a list. If this is set to a vector, meltR.A will only fit temperatures in this range for all melting curves Example = <code>c(17, 75)</code> . If set to a list of vectors, meltR.A will change what values are fit for each curve. Example, <code>list(c(0,100), c(17,75), , c(0,100))</code> . The length of this list has to be equal to the number of samples that will be fit.
<code>methods</code>	what methods do you want to use to fit data. Default = <code>c(TRUE, TRUE, TRUE)</code> . Can be true or false. Note, method 1 must be set to TRUE or the subsequent steps will not work.
<code>Tm_method</code>	either "nls" to use the Tms from the fits in Method 1, "lm" to use a numeric method based on linear regression of fraction unfolded calculated with method 1, or "polynomial" to calculate Tms using the first derivative of a polynomial that approximates each curve.
<code>Mmodel</code>	The molecular model you want to fit. Options: "Monomolecular.2State", "Monomolecular.3State", "Heteroduplex.2State", "Homoduplex.2State".
<code>Tmodel</code>	The thermodynamic model you want to fit. Options: "VantHoff". Default = "VantHoff".
<code>Save_results</code>	What results to save. Options: "all" to save PDF plots and ".csv" formatted tables of parameters, "some" to save ".csv" formatted tables of parameters, or "none" to save nothing.
<code>file_prefix</code>	Prefix that you want on the saved files.
<code>file_path</code>	Path to the directory you want to save results in.
<code>auto.trimmed</code>	Ignore this argument unless you are writing auto baseline trimmers
<code>Silent</code>	TRUE to not print data in your console. Default = FALSE.

Value

A list of data frames containing parameters from the fits and data for plotting the results with `ggplot2`.

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