R documentation

of 'meltR.A.Rd'

October 25, 2022

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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 baslines 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,
  outliers = NA,
  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

data_frame	data_frame containing absorbance melting data
blank	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, he identity of the blank, for example, blank = 1 or blank = "blank". If there are multiple blanks in the data, blank = list(c("Sample 1", "Blank 1"), c("Sample 2", "Blank 2")) 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 unique(df$Sample), where df is the name of the R data frame you are using, in your R console.
NucAcid	A vector containing the Nucleic acid type and the sequences you are fitting for calculating extinction coefficients. Examples: c("RNA", "UUUUUU", "AAAAAA"), c("DNA", "GCTAGC"), etc... . For a custom extinction coefficient enter "Custom" followed by the molar extinction coefficients for every nucleic acid in the sample. For example, c("Custom", 10000, 20000).
wavelength	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.
concT	The temperature used to calculate the NucAcid concentration. Default = 90.
outliers	A vector containing the identifiers of the outlier samples that you want to remove.
fitTs	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 = c(17, 75). If set to a list of vectors, meltR.A will change what values are fit for each curve. Example, list(c(0,100), c(17,75), ... , c(0,100)). The length of this list has to be the equal to the number of samples that will be fit.
methods	what methods do you want to use to fit data. Default = c(TRUE, TRUE, TRUE). Can be true or false. Note, method 1 must be set to TRUE or the subsequent steps will not work.
Tm_method	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.
Mmodel	The molecular model you want to fit. Options: "Monomolecular.2State", "Monomolecular.3State", "Heteroduplex.2State", "Homoduplex.2State".
Tmodel	The thermodynamic model you want to fit. Options: "VantHoff". Default = "VantHoff".
Save_results	What results to save. Options: "all" to save PDF plots and ".csv" formated tables of parameters, "some" to save ".csv" formated tables of parameters, or "none" to save nothing.

file_prefix	Prefix that you want on the saved files.
file_path	Path to the directory you want to save results in.
auto.trimmed	Ignore this argument unless you are writing auto baseline trimmers
Silent	TRUE to not print data in your console. Default = FALSE.

Value

A list of data opject containing raw data, data, transformation, fit objects, and statistics from the fits plotting, exporting, and advanced analysis.

- 1. Summary - A data frame containing the thermodynamic parameters from each method.
- 2. Method.1.indvfits - A data frame containing the thermodynamic parameters from the individual fits.
- 3. Range - A data frame containing fractional error between Method 1, 2, and 3 for each thermodynamic parameter.
- 4. Derivatives.data - A data frame containing the first and second derivatives for each sample containing RNA.
- 5. Method.1.data - A data frame containing the raw data from method 1 and the model.
- 6. Method.1.fit - A list of nls objects containing the fits obtained from fitting melting curves individually. Fit statistics can be extracted here.
- 7. Method.2.data - A data frame containing the raw data from method 2 and the model.
- 8. Method.2.fit - A nls object containing the fit obtained from fitting the relationship of Tm and Ct.
- 9. Method.3.data - A data frame containing the raw data from method 3 and the model.
- 10. Method.3.fit - A nls object containing the fit obtained from fitting the raw data.

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