User manual for calculating the thermodynamic stability (ΔG) of RNA/DNA duplexes, DNA/DNA duplexes and the difference between DNA/DNA and RNA/DNA duplexes (DNA/DNA - RNA/DNA) by thermogenome.pl

The free energy (ΔG) necessary to unwind polynucleotide duplexes with defined length can be calculated from the measured values of entropy (ΔS) and enthalpy (ΔH) for the 10 possible nearest-neighbour DNA/DNA[1] interactions, and the 16 possible RNA/DNA[2]. interactions. The perl script "thermogenome.pl" calculates the thermodynamic stability (ΔG) of RNA/DNA duplexes, DNA/DNA duplexes and the difference between DNA/DNA and RNA/DNA duplexes (DNA/DNA - RNA/DNA). ΔG of the nearest-neighbour interactions was calculated by using Kowalski's sliding-window approach[3]. Published values of ΔH and ΔS (at 37 °C and 1 M salt concentration) for each nearest-neighbour interaction for DNA/DNA duplexes and RNA/DNA duplexes were used.

You can run the script by writing "perl thermogenome.pl confugration_file.txt" at the "command line". You can omit "perl" if you are running Mac OS/Linux, but make sure you have made thermo_bias.pl executable.

The configuration file contains all the parameters needed by the program and should be as follows:

InputFile=
OutputFile=
Start=
Window=
MaxLength=
Use=

All parameters should follow the = sign with no space.

"InputFile" and "OutputFile" should contain the path to the input file and output file respectively.

The sequences in the Input file, should be in fasta format

"Start" is the offset from which thermodynamic values will be calculated for each sequence in the input file. It usually is 0.

"Window" is the length of nucleotides for which the thermodynamic stability is calculated at each step.

"MaxLength" is the length to which thermodynamic stability will be calculated for each

sequence.

“Use” is the duplex composition for which you want to calculate thermodynamic stability - if you want to calculate:

RNA/DNA duplex stability, you should set “Use=RD”,

DNA/DNA duplex stability you should set “Use=DD”.

DNA/DNA - RNA/DNA duplex stability you should set “Use=T2”. If you have set T2 “Window” and “Start” are automatically assumed to be 9 and 0.

References:

1. SantaLucia, *J. Jr.* A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proc. Natl Acad. Sci. USA* **95**, 1460–1465 (1998).
2. Sugimoto, N. *et al.* Thermodynamic parameters to predict stability of RNA/DNA hybrid duplexes. *Biochemistry* **34**, 11211–11216 (1995).
3. Huang, Y. & Kowalski, D. WEB-THERMODYN: Sequence analysis software for profiling DNA helical stability. *Nucleic Acids Res.* **31**, 3819–3821 (2003).