Melting tempeature

There are two algorithms for melting temperature calculation:

- Rough
- Primer 3

Note, that Rough works only for DNA alphabet, it does not work for DNA extended and RNA alphabets. Primer3 works for all extended alphabets.

Rough

The melting temperature is calculated as follows. For sequences of length 15 or longer:

$$T_m = 64.9 + 41 * (nG + nC - 16.4) / (nA + nT + nG + nC)$$

For shorter sequences:

$$T_m = (nA + nT) * 2 + (nG + nC) * 4$$

Here "nA", "nT", "nC", "nG" denote the number of the corresponding nucleotide.

Primer 3

This calculation algorithm is borrowed from the Primer3 package. The algorithm uses the nearest-neighbor (NN) model or the formula from Bolton and McCarthy, PNAS 84:1390 (1962) (as presented in Sambrook, Fritsch and Maniatis, Molecular Cloning, p 11.46 (1989, CSHL Press)). The algorithm has the following parameters:

- *DNA concentration* (nanomolar) - a value to use as nanomolar concentration of each annealing oligo over the course the PCR. This parameter corresponds to 'c' in equation (ii) of the paper [SantaLucia (1998) A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. Proc Natl Acad Sci 95:1460-1465 http://www.pnas.org/content/95/4/1460.full.pdf+html], where a suitable value (for a lower initial concentration of template) is "empirically determined".
- *Monovalent concentration* (millimolar) - the millimolar concentration of monovalent salt cations (usually KCl) in the PCR.
- *Divalent concentration* (millimolar) - the millimolar concentration of divalent salt cations (usually MgCl^(2+)) in the PCR.
- *DNTP concentration* (millimolar) - the millimolar concentration of the sum of all deoxyribonucleotide triphosphates. A reaction mix containing 0.2 mM ATP, 0.2 mM CTP, 0.2 mM GTP and 0.2 mM TTP would have this value equals to 0.8.
- *DMSO concentration* (%) - the concentration of DMSO in percent.
- *DMSO factor* - The melting temperature of primers can be approximately corrected for DMSO:

  $$T_m = T_m(\text{without DMSO}) + \underline{\text{DMSO factor}} * \text{DMSO concentration}$$

- *Formamide concentration* (mol/l) - The concentration of formamide in mol/l. The melting temperature of primers can be approximately corrected for formamide:

  $$T_m = T_m(\text{without formamide}) + (0.453 * GC_{\%} / 100 - 2.88) * \underline{\text{Formamide concentration}}$$

- *NN Max Length* - the maximum sequence length for using the nearest neighbor model. For sequences longer than this, algorithm uses the "GC%" formula from Bolton and McCarthy, PNAS 84:1390 (1962):

  $$T_m = 81.5 - \text{DMSO concentration} * \text{DMSO factor} + 0.453 * (GC_{\%} - 2.88) * \text{Formamide concentration} + 16.6 * \log_{10}(\text{Monovalent concentration} / 1000) + 0.41 * GC_{\%} - 600 / \text{Length}$$

- *Thermodynamic table* - specifies the thermodynamic table for the melting temperature calculation:
  - Breslauer - method for Tm calculations from the paper[Rychlik W, Spencer WJ and Rhoads RE (1990) "Optimization of the annealing temperature for DNA amplification in vitro", Nucleic Acids Res 18:6409-12 http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=2243783]. and the thermodynamic parameters from the paper[Breslauer KJ, Frank R, Blcker H and Marky LA (1986) "Predicting DNA duplex stability from the base sequence" Proc Natl Acad Sci 83:4746-50 http://dx.doi.org/10.1073/pnas.83.11.3746]
  - SantaLucia - method for Tm calculations and the thermodynamic parameters from [SantaLucia JR (1998) "A unified view of polymer, dumbbell and oligonucleotide DNA nearest-neighbor thermodynamics", Proc Natl Acad Sci 95:1460-65 http://dx.doi.org/10.1073/pnas.95.4.1460]
- *Salt Correction Formula* - specifies the salt correction formula for the melting temperature calculation:
  - Schildkraut - [Schildkraut, C, and Lifson, S(1965) "Dependence of the melting temperature of DNA on salt concentration", Biopolymers 3: 195-208 (not available on-line)]
  - SantaLucia - [SantaLucia JR(1998) "A unified view of polymer, dumbbell and oligonucleotide DNA nearest - neighbor thermodynamics", Proc Natl Acad Sci 95:1460-65 http://dx.doi.org/10.1073/pnas.95.4.1460]
  - Owczarzy - [Owczarzy, R., Moreira, B.G., You, Y., Behlke, M.A., and Walder, J.A. (2008) "Predicting stability of DNA duplexes in solutions containing magnesium and monovalent cations", Biochemistry 47 : 5336 - 53 http://dx.doi.org/10.1021/bi702363u]