Tm Calculator v 1.12.0

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Help

The NEB Tm calculator is intended for use in estimating the optimal annealing temperature for PCR with NEB polymerases. Tm values are calculated using thermodynamic data from Santa Lucia [1] and the salt correction of Owczarzy [2]. For Phusion® DNA Polymerases, the salt correction of Schildkraut [2] is used.

The calculator supports primer sequences containing a limited number (max 3) ambiguous/degenerate base codes. In these cases, the Tm shown will reflect the minimum Tm from the set of all sequences derived by expansion of the ambiguity codes. Ta (annealing temperature) in these cases is calculated based on the minimum Tm values of the primer pair. In single pair mode, the individual expanded sequences are available and annotated with the specific Tm and % GC for the sequence.

The calculator will accept internal dU in the input; however, it does not have thermodynamic data for internal dU residues, so dU is treated as T for all calculations. The error incurred in doing so is unknown, though the chemical similarity of dU and dT might suggest that it will be small.

Batch Processing

In batch mode, the NEB Tm calculator will process multiple pairs of primer sequences and provide a tabular output. Primer pairs may be entered directly into the text box, copied and pasted into the text box, or loaded from a local file. In many modern browsers, a file can be dropped onto the file selection/browse button from the desktop. After processing, results may be downloaded in tab-delimited format as a plain text file. In some browsers, file download will trigger display of the output in a new tab. The data may be copied from that tab or saved using the browser's **File Save As ...** menu function. **Note that input lines that do not match the expected format (see below) are skipped and not shown in the output.** Errors and warnings are listed and attached to each line in the output. The output is best viewed by pasting it into a spreadsheet.

Primer pairs (one pair per line) are expected to be in the following format:

ID1 *[separator]* ***Primer1 sequence*** *[separator]* ***ID2*** *[separator]* ***Primer2 sequence*** with ID2 being optional.

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P1fwd, AGCGGATAACAATTTCACACAGGA, P1rev,
GTAAAACGACGGCCAGT
P1fwd; AGCGGATAACAATTTCACACAGGA; P1rev;
GTAAAACGACGGCCAGT
```

where the separators can be commas or semicolons. Tab-separated (tsv), comma-separated files (csv) or semicolon-separated data exported from Microsoft Excel spreadsheets is acceptable. The primer sequence must contain only A,C,G,T or spaces. **Please note that tabs cannot be entered directly into the text area, so manual entry should use semicolons or commas as separators.** Input lines containing only one primer will not be used to calculate Ta. The entire input file must use the same format for input.

How do I calculate just the Tm for a list of sequences (not pairs)?

The NEB Tm calculator is designed to recommend optimal annealing temperatures for primer pairs. To get Tm values for a list of single primers, enter them one per line (ID1; Sequence1). The software will process the line as having an invalid second primer and will not calculate a Ta, but will calculate the Tm of the first primer.

Why is the primer Tm (or annealing temperature) different from other Tm calculators?

The NEB Tm calculator is designed to take into account the buffer conditions of the amplification reaction based on the selected NEB polymerase. Many Tm calculators do not, relying instead on a default salt concentration. The annealing temperature for each polymerase is based on empirical observations of efficiency. The optimal annealing temperature for high fidelity hot start DNA polymerases like Q5 may differ significantly from that of Taq-based polymerases.

Why is my primer Tm (or annealing temperature) slightly different from what I calculated a few years ago?

The calculations in the NEB Tm calculator were modified in 2016 to correct an error that was decreasing the effective primer concentration to one quarter of its input value. The correction raised Tm values by roughly 2°C for all polymerase reaction buffers except Phusion, which used a different algorithm.

Because the Q5 annealing temperature adjustment was determined based on empirical observations, Ta values for Q5 products have not been modified. Please note that Tm values obtained from other calculators generally underestimate the Tm for use with Q5. If you obtain Tm values from another calculator, we suggest raising your annealing temperature to at least Tm + 3. However, for best results when using Q5, we still recommend using the annealing temperature provided by the NEB Tm Calculator.

Please contact NEB technical support if you need additional guidance.

Tm Calculation Method

The general format for T_m calculation is

$$T_m = \frac{\Delta H^o}{\Delta S^o + R \cdot \ln C_p} - 273.15$$

where C_p is the primer concentration, ΔH^o is enthalpy ($cal \cdot mol^{-1}$), ΔS^o is entropy ($cal \cdot K^{-1} \cdot mol^{-1}$) and R is the universal gas constant (1.987$cal \cdot K^{-1} \cdot mol^{-1}$). ΔH^o and ΔS^o are computed

from experimentally derived values for these parameters using the nearest-neighbor model, summing over all dinucleotides in the primer sequence. 273.15 is subtracted to convert from Kelvin to Celsius.

In the NEB Tm Calculator, T_m is computed by the method of SantaLucia [1] as

$$T_m = \frac{\left(\Delta H_i^o + \Delta H^o\right) \cdot 1000}{\Delta S_i^o + \Delta S^o + R \cdot \ln C_p} - 273.15$$

where the primer concentration C_p is assumed to be significantly greater (6x) than the target template concentration. ΔH^o and ΔS^o are computed using the nearest-neighbor model values outlined in [1]. ΔH_i^o and ΔS_i^o are adjustments for helix initiation [1]. The factor of 1000 is included to convert enthalpy values reported in $kcal \cdot mol^{-1}$ to $cal \cdot mol^{-1}$.

The T_m, as calculated above, assumes a 1 M monovalent cation concentration. This value is adjusted to reaction buffer conditions using the salt correction of Owczarzy as outlined in [2]

$$T_m \text{ (corrected)} = \frac{1}{\frac{1}{T_m} + \left[(4.29 \cdot f_{gc} - 3.95) \cdot \ln(m) + 0.94 \cdot (\ln(m))^2\right] \cdot 10^{-5}}$$

where f_{gc} is the fractional GC content, and m is the monovalent cation concentration.

For Phusion® polymerases, the T_m is adjusted to reaction buffer conditions using the salt correction of Schildkraut as outlined in [2]

$$T_m \text{ (corrected)} = T_m + 16.6 \cdot \ln(m)$$

where m is the monovalent cation concentration.

While the method and data of SantaLucia are preferred, it was necessary to use the alternate salt correction to allow compatibility with recommendations provided by Thermo Fisher Scientific.

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1. SantaLucia (1998) PNAS 95:1460-5
2. Owczarzy et al (2004) Biochem 43:3537-54