

## Tm Calculator Instructions for Use

### 1. Select Polymerase/Mix:

- a. Choose the polymerase or mix you intend to use from the dropdown menu.

### 2. Modify Primer Concentration:

- a. Adjust the (final) primer concentration as needed. The default setting is 500 nM, suitable for most PCR Biosystems products.

### 3. Input Primer Sequences:

- a. Standard bases (A, G, C, T, and U) are accepted.
- b. Up to 5 degenerate bases per primer are allowed. Permissible degenerate bases include N, S, W, M, K, Y, R, B, D, H, and V.
- c. Alternatively, select standard primers from the dropdown list.
- d. An annealing temperature will be displayed only if two primers are added.

### 4. Batch Mode:

- a. Use batch mode to calculate the Tm for more than two primers simultaneously.
- b. Add multiple primers into the input box or modify the sample .csv file with your primers and names, then upload via "Choose File".
- c. Primer names must be provided in batch mode.
- d. Primer names may include alphanumeric characters, dashes (-), underscores (\_), and periods (.).

### 5. Batch Mode Input Types:

#### Non-Interleaved Mode:

- a. Each primer pair should be entered on a single line: "Primer 1 Name", "Primer 1 Sequence", "Primer 2 Name", "Primer 2 Sequence".
- b. Separate primer names and sequences with semi-colons (;) or commas (,), but be consistent.
- c. For spreadsheet import, enter primer names and sequences for each pair in four neighbouring cells in the same row.

#### Interleaved Mode (default):

- a. Enter one primer name and sequence per line (either directly in the input box, separating primer names from each sequence with a comma (,) or semi-colon (;), or via spreadsheet).
- b. The calculator treats every two consecutive primers as a pair.
- c. The calculator does not consider overlapping pairs. For example, the 1st and 2nd primers form a pair, as do the 3rd and 4th primers. However, the 2nd and 3rd primers will not be paired.

#### Note:

This calculator is designed for use with PCR Biosystems polymerases and mixes listed in the "Product" dropdown menu. It may not predict the correct annealing temperature for products from other manufacturers.