

Oligo annealing temperature calculation.

Ms Alex Aitken

$$T_m = 61.5 + (0.41 * [\% G+C]) - (500/\text{length})$$

$$T_{\text{ann}} = T_m - 5C$$

Note: it is also possible to alter the annealing temperature – for the initial 4 cycles use a temperature which is higher than subsequent cycles – this will ensure that the initial copies of the PCR product (although lower in number) will be of a high fidelity than subsequent cycles. Theory - Once a pool of the correct PCR product has been established this will be the optimal template for the final cycling and so yield a more uniform product.