

Setting Thermal Profiles

Thermal profiles always consist of at least three basic parts, an isothermal phase, a cycling phase and a melt phase. To remove any of these uncheck the check box at the bottom right of the specific step.



Isothermal Phase

Many taq polymerases in modern reagent systems require an isothermal phase to activate the enzyme. Where complex genomes are involved, an extended isothermal step prior to cycling will ensure good denaturation of the template. This phase is always defined by the denaturation temperature of the first cycle in the cycling phase. The length of this phase maybe altered in intervals of 30 seconds up to 10 minutes by dragging the slider bar to the right of the step; refer to the documentation supplied with the specific reagent system you are using.

Cycling Phase

To alter the cycling conditions in the profile, simply drag the cycle profile to change the temperature or dwell time for either the denaturation, annealing or extension phase.

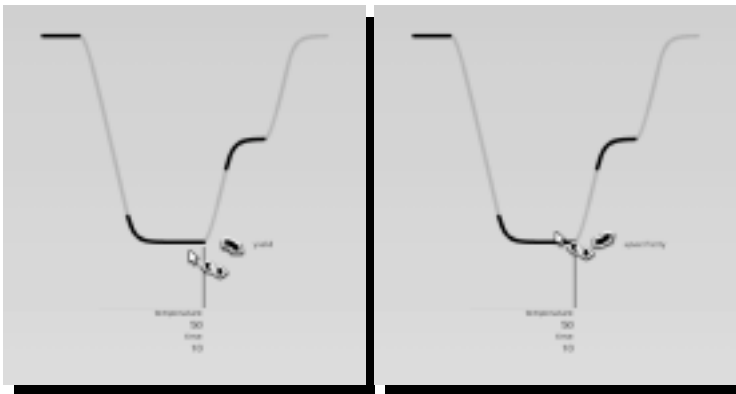


A single cycle is always defined by denaturation, anneal and extension phase. These have a temperature and a dwell time associated with them. To alter these simply



click on the element you want to modify and drag it to the new position. Changes to the dwell time and dwell temperature may be modified interactively.

Touch-up and touch-down gradients may be added to the annealing stage of a specified cycling step. To add a gradient simply select the annealing part of the cycle while holding the shift key and drag the mouse up or down to either increase yield or specificity.



Typically gradients are applied to enhance yield or to enhance specificity. Decreasing the annealing temperature over successive cycles generally increases yield since during the later cycles there is a proportionally higher amount of amplicon from which to initiate amplification. However, where a reaction is prone to artifacts this method may result in an increase in artifactual amplifications. Increasing the annealing temperature over successive cycles makes the reaction more specific preventing the occurrence of artifactual amplification.

As well as three temperature cycling Evocycler™ can also perform two temperature cycling where the extension dwell is removed. This is ideal for small amplicons of less than 100bp, and can significantly reduce the time taken for perform the amplification. To select two temperature cycling simply select the icon on the right hand side of the cycle. The software automatically adjusts all of the other parameters accordingly.

Additional Steps may be added by holding shift down and selecting the cycle that you wish the new step to start from.



Melt Phase

EvoCycler™ automatically performs a melt at the end of the PCR assay. This is used to generate information about the amplicons produced during the reaction and to create the virtual gel image. To alter the temperatures used simply type in the two boxes that appear at the start and at the end of the melt step in the profile.

Above the cyclor icon in the GUI, is a clock that shows the current time and the expected time of the run, represented as a sweep. This gives an easy quick glance indication of run time. This time is automatically updated as the profile is modified.

Quickstart™

Evocycler™ has a quickstart function that automatically loads the selected profile when the software is started. This is particularly useful if the instrument is used to routinely run a single amplification, saving time opening the same methodology at the start of each run.