



Touchdown PCR: using the decrement temperature programming feature of Techne thermal cyclers

■ Introduction

One of the most common problems encountered in PCR, especially when amplifying products from genomic DNA, is the presence of non-specific products or primer-dimers. Since these are formed by mis-priming events and are generally shorter than the desired target they can be preferentially amplified at the expense of the target and rapidly overwhelm the reaction. The problem becomes worse if there is only a small amount of target template present in the sample.

In general, the way to overcome amplification of non-specific products is to optimise the PCR. This can include testing various concentrations of the reaction components such as Mg^{2+} , dNTPs, primers and template. However one of the most important parameters is the annealing temperature of the reaction. This can be determined theoretically using various software programmes to calculate the melting temperature (T_m) of the primers.

The T_m is defined as the temperature at which half the strands are single stranded and half are double stranded when base paired to a complimentary strand. The annealing temperature for PCR is usually set at about 4 to 5°C below the theoretical T_m of the primers and the primer pairs used in a reaction should ideally not differ in T_m by more than 5°C.

However the behaviour of primers in the actual reaction is difficult to predict. A rapid way to determine the ideal annealing temperature is to use a temperature gradient across the thermal cycler block for the annealing step. The PCR products are then run on an agarose gel to visualise the bands. The temperature of the block column giving the best yield of specific product can be determined by viewing the gradient calculator on the instrument. This temperature is then used as the annealing temperature for subsequent PCRs with these primers. The Techne TC-512, TC-5000 and TC-3000G all have gradient blocks which will allow the user to do this.

Without a gradient block, annealing temperature optimisation can be a lengthy process, requiring repeated runs to test each different annealing temperature. However there is a method that can be used to increase the specificity of the reaction without actually determining the optimal annealing temperature. This method is known as touchdown PCR¹.

■ Touchdown PCR

In touchdown PCR the annealing temperature is gradually decreased during the cycling process. At the beginning of the cycling stage, the annealing temperature is set 5 to 10°C higher than the T_m of the primers. While the temperature is high, this favours only the most specific base pairing between the primer and template and therefore only specific products will be amplified.

In subsequent cycles the temperature is decreased in small amounts so that by the end of the amplification stage, the annealing temperature is 2 to 5°C below the T_m . Since the specific products have already been amplified and are present in excess, these will be preferentially amplified at the lower, more permissive annealing temperatures.

Programming might at first appear complex; however this is made simple with the increment/decrement time/temperature feature available on all Techne thermal cyclers.

■ Increment/decrement time/temperature

Under normal circumstances, the hold temperature of all steps is constant throughout a stage. However, it is possible to automatically increment or decrement the temperature of a specified step of a cycled stage. To do this, an initial temperature and a final temperature need to be defined for the step. During the cycling stage the temperature will increase or decrease by an amount depending on the number of cycles in the stage.

For example if the initial temperature is 60°C and the final temperature is 50°C and there are 11 cycles, the first cycle will have a hold temperature of 60°C (the first cycle is never incremented/decremented, only subsequent cycles); the second cycle will have a hold temperature of 59°C and so on until the 11th cycle which will have a hold temperature of 50°C. Therefore the temperature increment or decrement per cycle (Δt) is given by:

$$\Delta t = (\text{Initial temperature} - \text{Final temperature}) / (\text{Number of cycles} - 1)$$

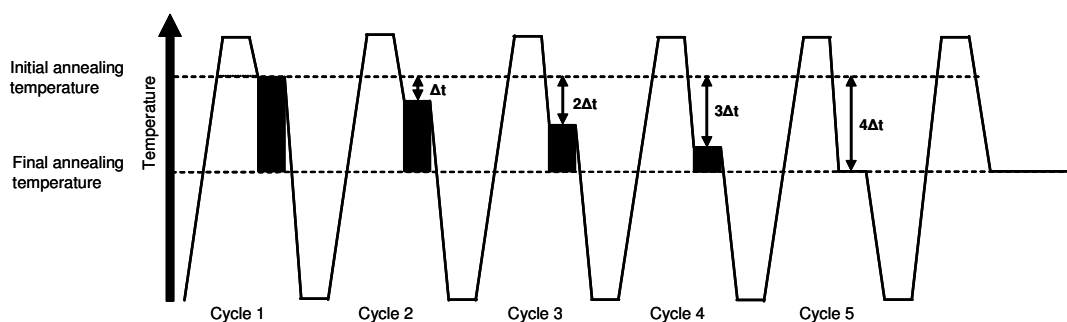


Figure 1: Decremental temperature function.

Increment/decrement time works in a similar way except that the hold time is increased or decreased with each cycle. This can be useful in applications such as long PCR.

■ Programming for Touchdown PCR

The following examples demonstrate how to program the various Techne thermal cyclers for touchdown PCR.

TC-5000 and TC-512

1. Open the 3 STEP PCR TEMPLATE and touch EDIT.
2. Adjust the default parameters as required and set the number of cycles for the amplification stage.
3. Touch the first segment of the amplification stage to edit the required denaturation temperature and hold time.
4. Touch the second, annealing, segment to edit the decrement temperatures for the annealing step. Do this as follows:
 - a. Touch the Inc/Dec button.
 - b. On the top line where it says 'First', enter the starting annealing temperature, 5 to 10°C higher than the T_m of the primers. Next, enter the hold time.
 - c. On the next line where it says 'Last', enter the final annealing temperature, 2 to 5°C lower than the T_m of the primers. Next, enter the hold time. Touch OK to accept.
5. Touch the third segment to edit the temperature and hold time for the extension step.
6. Edit the remaining defaults as required.
7. Touch 'Save As' to save and give the program a name.



TC-4000, TC-412, TC-3000, TC-3000X and TC-3000G

1. Open the 3 STEP PCR TEMPLATE and select 'Copy program'. Give the program a suitable name.
2. Adjust the default parameters as required and set the number of cycles for the amplification stage.
3. Edit the first step of the amplification stage to the required denaturation temperature and hold time.
4. Scroll down to the second, annealing step and press the enter key to go into edit mode.
5. Next, press the pause key. The prompt at the bottom of the screen will ask if you want to change step. Press the enter key for yes.
6. The cursor will flash at the beginning of the step. Press the up or down arrow key until you see 'fst' which indicates the first or starting annealing temperature.
7. Edit the temperature so that it is 5 to 10°C higher than the T_m of the primers. Enter the required hold time.
8. Press enter until the line flashes then scroll down to the next line which begins with 'lst' which indicates the last or final annealing temperature.
9. Edit the temperature so that it is 2 to 5°C lower than the T_m of the primers. Enter the required hold time.
10. Press enter until the line flashes then scroll down to edit the temperature and hold time for the third, extension step.
11. Press enter until the line flashes then scroll down to edit the remaining defaults as required.
12. When finished, press the end key to save and confirm by pressing the enter key.

■ **Conclusions**

All Techne thermal cyclers have flexible programming options which allow the user to set up just about any thermal cycling profile. The increment/decrement time and temperature features simplify programming for complex applications such as touchdown PCR, allowing the user to easily edit existing programs with these features.

■ **References**

1. R.H. Don, P.T. Cox, B.J. Wainwright, K. Baker and J.S. Mattick. "Touchdown" PCR to circumvent spurious priming during gene amplification. *Nucl. Acids. Res*: **19**, 4008.