Protocol P01-002B

Long PCR: Programing increasing hold times

Prime

Introduction

Many applications in PCR are simply to identify the presence or absence of a particular target in a complex pool of DNA. This usually requires amplification of only a short fragment of the DNA for identification purposes, usually between 100 and 500bp in length. However in some cases it may be required to amplify longer segments of DNA, or even complete genes, in order to clone them and determine their function. In such instances it may be necessary to amplify lengths of 5 to 10 kb or even longer. This is known as long PCR.

Long PCR

As well as amplifying long stretches of DNA, long PCR also needs to be accurate. For this reason, many protocols use a mix of thermostable DNA polymerases, usually *Taq* DNA polymerase for high processivity and another DNA polymerase such as *Pfu* or *Pwo* with 3'-5' proofreading ability. Proofreading polymerases remove any misincorporated bases which may halt extension of the PCR product, therefore allowing longer primer extension than can be achieved with *Taq* alone.

Due to the use of proofreading polymerases in the reaction, the enzyme must not be mixed with the primers until immediately before thermal cycling as it will degrade them. Alternatively, a hot start procedure can be used whereby the polymerase is added either during a programmed hot start step or during the first annealing/extension step.

Primers for long PCR tend to be slightly longer (up to 35 bases) than those for standard PCR (18-22 bases). This allows for a higher annealing temperature in the PCR and a combined annealing/extension step at 68°C is often used. Due to the size of the product, long annealing/extension times are required. This is generally around 25 to 35s per kb in the first 10 to 15 cycles. In the subsequent 15 to 20 cycles the extension time is increased by 15 to 20s per cycle to allow for the increased amount of product. This can be programmed using the increment time feature available on all the Techne Prime thermal cyclers.

Increment/decrement time/temperature

Under normal circumstances, the hold time of a particular step is constant for each cycle within a stage. However, it is possible to automatically increment or decrement the hold time of a specified step of a cycled stage. To do this, it is simply a matter of activating the increment/decrement function and setting the time increase per cycle. Note: this feature cannot be set unless the stage has >1 cycle programmed.

The final hold time can be calculated as follows:

Final hold time (s) = Initial hold time(s) + [(Number of cycles -1) x ΔT(s)]

Where ΔT(s) is the time increment/decrement in s.

Increment/decrement temperature works in a similar way except that the temperature of a step is increased or decreased with each cycle. This can be useful in applications such as touchdown PCR.

Programming for Long PCR

The following steps demonstrate how to program the 3Prime, Prime and Prime Elite thermal cyclers for long PCR.

3Prime

1. First ensure the number of cycles for the stage has been set then enter the required initial hold time for the step.
2. Touch the step **Time** button. This will open the time screen (Fig. 1).
3. Touch **Increments and Decrements**.
4. Touch the button next to **Increase Per Cycle** to turn it **ON**.
5. Next touch the time button to set the increase per cycle (Fig. 2).

A new screen will open which is similar to the simple time entry screen.

6. Enter the required value followed by **OK**.

If an entered time value is invalid, a prompt will indicate the acceptable range.

7. Touch **Accept** to return to the previous screen.
8. Touch **OK** to accept the modification and return to the program.

The time value will now appear orange in the programming screen indicating that it contains a modified function (Fig. 3).

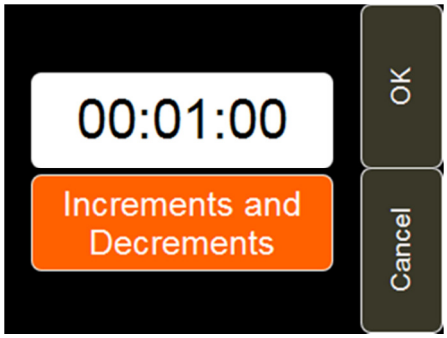

Fig. 1: Increments and Decrements.

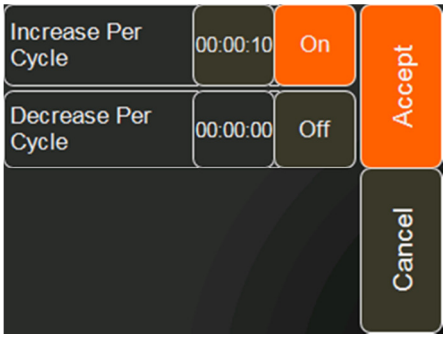

Fig. 2: Turn on Increase Per Cycle.

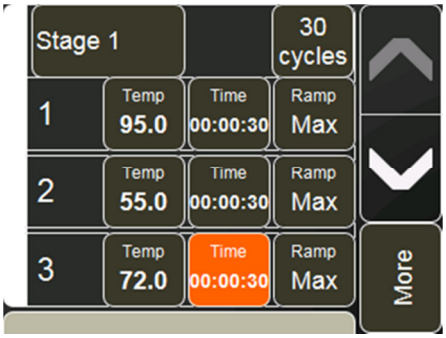

Fig. 3: Modified step shown in orange.

Prime and Prime Elite

1. First ensure the number of cycles for the stage has been set then enter the required initial hold time for the step.
2. Touch the step **Time** button. This will open the time entry screen (Fig. 4).
3. For an increase in time per cycle, touch the button below **Increase Per Cycle** to turn this function **ON**.
4. Touch the time entry button below this.

A time entry screen will open. The maximum increase in hold time per cycle (based on the number of cycles and the specification limits of the unit) will be shown (Fig. 5).

5. Enter the value required followed by **OK**.

If an entered time value is invalid, a prompt will indicate the acceptable range.

6. Touch **OK** again to return to the programming screen.

The time value will now appear orange in the programming screen indicating that it contains a modified function (Fig. 6).

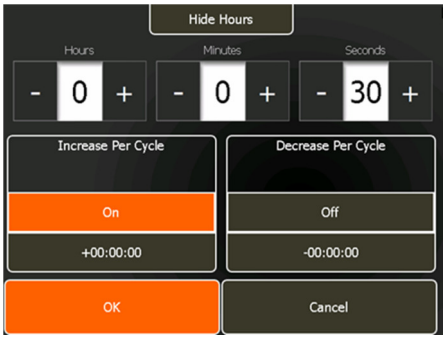

Fig. 4: Increments and Decrements.

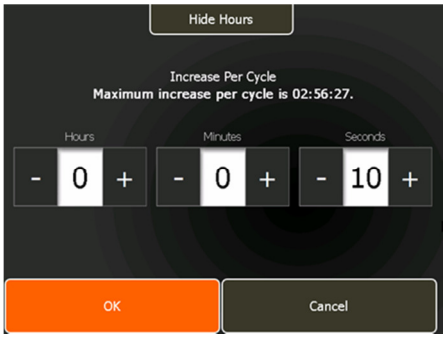

Fig. 5: Enter the required Decrease Per Cycle.

Fig. 6: Modified step shown in orange.

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 long PCR, allowing the user to easily edit existing programs with these features.