Introduction

In the modern laboratory, speed is often of the essence and any process capable of saving valuable research time is an advantage. One recent advance in this direction is the development of fast PCR protocols which require either specialised PCR reagents and/or modified thermal cycling steps.

The factors that determine the length of a PCR run are:

- the time required for initial denaturation/enzyme activation,
- the number of steps and cycles in the program,
- the hold times of the steps,
- the ramp rates between the steps,
- the final extension.

All these factors need to be considered when optimising for fast PCR protocols. This application note demonstrates the time savings that can be made by changing some of these parameters to successfully reduce the total reaction run time.

Methods

PCRs were carried out as described in the legends to Figures 1, 2 and 3 using 20µl reaction volumes. 2x PCR Master Mix with 1.5mM $MgCl_2$ (ABgene, AB-0575-DC) was used as the standard PCR mix and AmpliTaq Gold® Fast PCR Master Mix, UP (2x), (Applied Biosystems, 4390937) as the fast PCR mix. For gel analysis, one third of each reaction was loaded and run on 2% agarose gels and the products visualised by Ethidium bromide staining.

Effect of ramp rate

Ramp rates are an important part of the PCR as primer annealing and product extension will occur during these phases. Therefore simply running an existing optimised program on a faster ramping thermal cycler may not necessarily give the same results. To test this, a fairly robust PCR amplifying a 231bp fragment of the pBR322 plasmid vector was run in parallel in a Prime Elite with a maximum ramp rate of 5°C/s and a TC-512, which has a maximum heating ramp rate of 3.6°C/s. Results are shown in Figure 1.

For this particular reaction, the results demonstrate no appreciable differences in yield or sensitivity between the two thermal cyclers. However the time saved on running the reaction on a faster ramping unit was considerable: the program took only about 1 hour to complete when using the Prime Elite compared to 1h 30 min in the TC-512; a saving of around 30 min on the run time entirely due to the increased ramp rate.

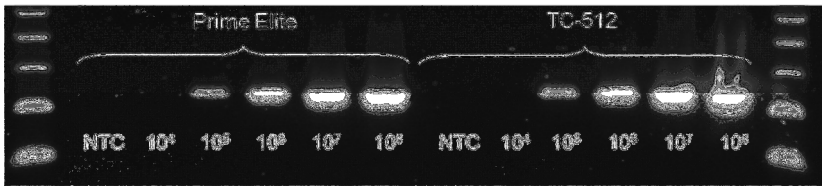

Figure 1: 231bp fragment of pBR322 amplified using the standard PCR mix. **Program:** initial denaturation 94°C for 5 min; 35 cycles of 94°C for 15s; 58°C for 20s; 72°C for 20s; final extension of 72°C for 5 min. The numbers shown are the copies of template added to each 20µl reaction. NTC is a no template control.

Changing the protocol

Another way to shorten run times is to modify the standard protocol by reducing the number of steps and/or hold times of individual steps. If primers have T_m values above 60°C it may be possible to change from a 3-step to a 2-step PCR, combining the annealing and extension steps. Figure 2 shows a temperature gradient optimisation experiment changing from a 3-step to a 2-step protocol for primers with a T_m of approximately 64°C. The reaction amplifies both 400bp and 100bp fragments of the ALU insertion of the human tissue-type plasminogen activator (TPA) gene found on chromosome 8 of human genomic DNA. Note that changing from three to two steps decreased the amount of the larger PCR product, although there was no noticeable difference in yield of the 100bp product. The overall run time saved in changing the protocol was 22 min (52 min run time for the 2-step protocol *vs*. 1h 14 min for 3 steps).

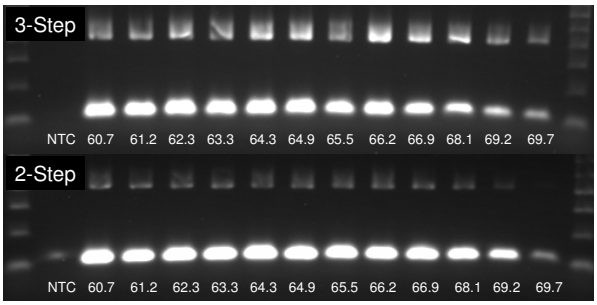

Figure 2: 100bp and 400bp fragments of the ALU insertion amplified using the standard PCR mix. **3-step program**: initial denaturation 94°C for 5 min; 35 cycles of 94°C for 15s; 65°C for 30s (gradient of 10°C); 72°C for 30s; final extension of 72°C for 5 min. **2-step program**: as for 3-step omitting the 72°C for 30s step. The numbers shown are the column temperatures of the gradient.

Changing the mix

There are many reagents on the market specifically formulated for fast PCR. These contain specialised enzymes and stabilisers to facilitate rapid ramping and short hold times. Figure 3 shows the amplification of the 231bp pBR322 fragment, comparing the use of the standard PCR mix with the fast PCR mix when running a fast protocol on the Prime Elite. The fast mix gave improved yield and sensitivity over the standard mix in a fast protocol; although the overall yield was, by comparison, lower than when using a standard protocol with longer hold times for each step (compare with Figure 1). The total run time using the fast protocol was around 40 minutes compared to the standard run time of about 1 hour on the Prime Elite. Note that the run time could be reduced further by choosing a fast PCR mix with a shorter enzyme activation time.

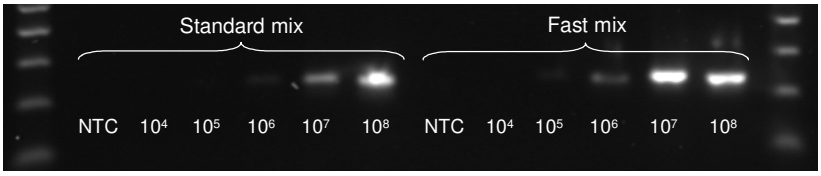

Figure 3: 231bp fragment of pBR322 amplified using the standard PCR mix and fast PCR mix: **Fast program:** initial denaturation 95°C for 10 min; 35 cycles of 96°C for 3s; 58°C for 3s; 68°C for 5s; final extension of 72°C for 10s. The numbers shown are the copies of template added to each 20µl reaction. NTC is a no template control.

Conclusions

Techne thermal cyclers offering a fast ramp rate such as the Prime Elite and its predecessor the TC-PLUS, offer significant time savings for PCR. Modifications to the program and changing to a fast PCR mix can further shorten run times, although there may be some compromises in yield and sensitivity which would need to be taken into consideration if speed of the PCR is of primary importance over these factors. Further reaction optimisation may overcome these. Table 1 summarises the time savings made using the experiments described.

Protocol change	Time saved (approx.)	Compromise
Transfer from a standard ramping instrument to a fast ramping instrument. (Conventional 3-step PCR program)	30 minutes	May need additional optimisation of the reaction.
Change from 3-step to 2-step program. (Fast ramping thermal cycler)	20 minutes	May not be suitable for longer PCR products.
Change from a 3-step program to a fast 3-step program using a special fast PCR mix. (Fast ramping thermal cycler)	20 minutes	May be less yield and sensitivity than conventional programs.

Table 1: Summary of the approximate time saved in making the protocol changes as described.

The Techne Prime Elite thermal cycler gives the user all the features required to make best use of these fast methodologies by offering flexible programming and rapid block ramp rates of up to 5°C/s.