

GUIDELINES FOR FAST PCR

The actual run time of a PCR protocol/program is determined by:

1. The number of steps and cycles.
2. The amount of time spent at each temperature step.
3. The time it takes for the instrument to reach the set (incubation) temperature (i.e. the ramp time).

There are essentially three ways to make a PCR protocol/program faster:

1. Speed up temperature ramping (i.e. to use a fast instrument).
2. Use a fast polymerase (with enhanced functionality, giving a higher processivity).
3. Alter the PCR protocol to shave time off of temperature steps.

General procedure when shaving time off of temperature steps:

1. When using a hot-start enzyme, the initial denaturation time is critical for enzyme activation and depends on the choice of enzyme. Select an enzyme with a short activation time¹.
2. Make sure you are using a fast polymerase (with a higher processivity).
3. Start by testing your original protocol, with a fast polymerase, on your conventional thermal cycler/PCR instrument. If successful, transfer your protocol to AmpXpress.
4. Repeat the original protocol, with the fast polymerase, on AmpXpress.
5. If successful transfer you could start to shave off time.
 - a. The first thing to test is to reduce denaturation (during cycling) to 0 seconds.
 - b. If successful, elongation can also be reduced. Some enzymes have an extension rate up to 1 kb/second.
 - c. As a final step, finish by testing shorter annealing times. This is the most critical step and may not be possible to shorten. Minimum annealing time depends on buffer choice and primer design.

A typical PCR protocol comprises 30–40 cycles and contains the following steps:

Step	Conventional PCR instrument	AmpXpress and standard Taq	AmpXpress and a fast polymerase
Initial template denaturation/ enzyme activation	94-95°C for 5 - 15 min	95-98°C for 60 sec	95-98°C for 10 - 30 sec
Denaturation of template	15–30 sec at 94-95°C	0 sec at 96-98°C	0 sec at 95-98°C
Annealing of primers	15–90 sec	10–20 sec	3–10 sec
Extension of product	15–90 sec	10–20 sec	1–10 sec
Final extension (to improve detection on EtBr-agarose gels)	72°C for 10 min	72°C for 2 min (only necessary for sequencing and cloning)	72°C for 30 sec (only necessary for sequencing and cloning)
Total run time	1.5–3.0 hours	25–35 minutes	15–25 minutes

¹ Enzymes that require excessive time (10-15 minutes) for activation should preferably not be used for fast PCR. If e.g. 10 minutes is required to activate a particular enzyme AmpXpress will not change that fact, rather the customer should look for a more rapid enzyme.

FAST PCR SUMMARY

PCR run times can be reduced from 1.5–3.0 hours to 15–25 minutes simply by using AmpXpress together with a fast polymerase.

SOME OPINIONS ON FAST PCR

“...the improvements in PCR instrumentation’s ability to change temperatures quickly must be matched simultaneously with improved enzymatic abilities.”

“As fast PCR evolves, all the components must improve together for the technique to continue as a true advance.”

Biocompare.com, Technology Spotlight by Caitlin Smith, January 21, 2008

“It is important to note that when hold times are decreased to <15 s, it becomes critical that the instrument and consumables are engineered such that the sample reaches the specified temperature and remains at that temperature for the programmed time. In a typical PCR system, it may require ≥ 15 s for the sample to actually reach the set temperature, and thus fast protocols often exhibit unacceptable variability across the sample block, or from run to run. Studies using in-sample temperature probes have confirmed wide variation in actual sample temperatures for many PCR systems.”

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“As instrumentation ramp rates continue to improve, the move to protocol modifications using wild-type enzymes can increase the chances of reaction failure and poor performance.”

Biocompare.com, John Foskett, technical director at Kapa Biosystems