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TC-512

Application note: A01-001A

Optimisation of PCR using the block gradient

PCR optimisation

Although primers are usually supplied with theoretical annealing temperatures, these can be calculated in various ways and are often specified for specific salt concentrations. Too low an annealing temperature can produce non-specific products whereas if the temperature is too high the PCR yield can be low. An annealing temperature optimisation step can often avoid these problems and is especially important when changing a sensitive assay from one thermal cycler to another. Block tolerances can vary and small differences in temperature may affect results, therefore using the gradient feature of a thermal cycler, the PCR can be optimised for each particular instrument.

Block gradient

When programming a gradient on the TC-512 or its predecessor, the Touchgene Gradient, the set temperature is the temperature in the middle columns of the block and the gradient is the variation at the two extremes, with the left hand column (column 1) being the coolest and the right hand column (column 12 in a 96-well block) the hottest.

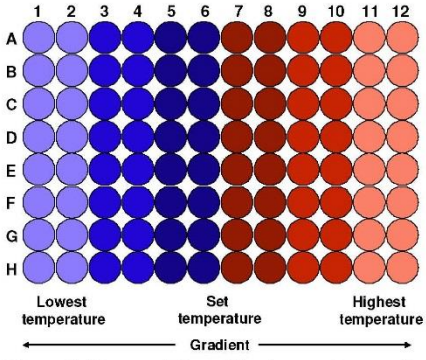

Figure 1: Representation of the temperature gradient across a 96-well block.

The TC-512 is capable of producing a gradient of temperatures across its block by using the four independent heating channels that divide the block into quarters from left to right. The gradient function controls each of these channels at a different temperature, producing a near perfect linear gradient across the block.

The maximum temperature gradient which can be set is 30ºC (16ºC for a 384-well block); the lowest temperature is 20ºC and the hottest temperature is 70ºC. Examples are given in the table below:

Lowest temp. (ºC)	Set temp. (ºC)	Highest temp. (ºC)	Gradient (ºC)
20	35	50	30
40	55	70	30
45	55	65	20
50	55	60	10

Table 1: Examples of possible block gradients that can be programmed into the TC-512.

It is important to remember that the greater the gradient, the poorer the uniformity across the block and the difference in temperature between individual columns could be greater than 2.5ºC. Therefore if a wide gradient is used initially, fine-tuning may be required to establish the optimum temperature for primer annealing.

Programming a gradient

As a starting point, a gradient should be set up such that it spans the calculated annealing temperature of the primers +/-10ºC. This range can then be further reduced to fine-tune the assay. This step can be combined with various primer concentration combinations to optimize annealing temperature and primer concentration simultaneously.

To program a gradient in the annealing step:

1. Open the required program or copy/create a new program.
2. Press “Edit” and then press the annealing step which is to have the gradient. This will open the “Edit program function” screen.
3. The first temperature is the set temperature for the centre of the block.

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4. Scroll across to the gradient symbol and enter the range of the gradient. E.g. 20ºC will mean the block will have a gradient in temperature approximately +/-10ºC either side of the set temperature.

Segment 58ºC ⊿20ºC 00m 30s MAX ºC/s

5. Exit and save the program.

Once the experiment has run, the samples can be visualised on an agarose gel to assess the results. Optimal annealing temperature and primer concentrations will give the band with the highest intensity (yield) with no non-specific products.

The figure below illustrates an idealised optimisation experiment with a 20ºC gradient around a set annealing temperature of 58ºC using a 96-well block. The experiment uses three different primer concentration combinations. It can be seen that the primer concentrations of combination 2 at the annealing temperature in column 7 gives optimal amplification.

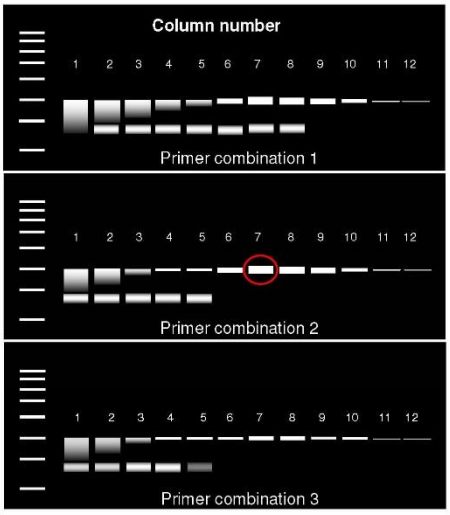

Figure 2: Idealised representation of an annealing temperature and primer concentration optimisation experiment. The sample amplified under optimal conditions is circled in red.

Gradient calculator

While the gradient is mainly linear there are small variations at the extremes of the gradient due to thermal losses from the hottest edge of the block and a gain of heat from the adjacent column at the coldest edge. This is the reason why the first column does not reach the lowest ideal temperature of the gradient and the last column cannot reach the highest temperature.

A gradient calculator is included in the TC-512 software which is used to display the actual temperatures of the block for any given gradient. The values are based on tests and calculated from the percentage of the gradient seen in each column. The gradient calculator is located in the System User main menu.

An example of the gradient calculator for the 96-well block used in the experiment opposite is shown below. The gradient is 20ºC with a set temperature of 58ºC; the block ranges from 49.6ºC in column 1 to 66.4ºC in column 12.

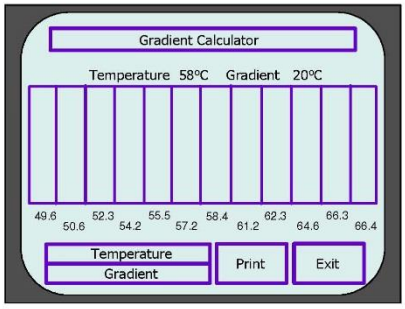

Figure 3: The gradient calculator on the TC-512.

- Selecting "Temperature" allows the mid temperature to be set.
- Selecting "Gradient" allows the temperature variation across the block to be defined.
- It is possible to print the column and gradient information by selecting "Print".

The gradient calculator indicates that the annealing temperature in column 7 is 58.4ºC. This experiment can now be fine-tuned using the optimised primer concentration and narrowing the temperature gradient.

Conclusions

The gradient function of a thermal cycler such as the TC-512 is a useful feature which can improve the results of a PCR by allowing a simple optimisation step that can be performed in a single run. It could also be used to perform the initial optimisation of a number of different assays with various sets of primers, thus saving the user a significant amount of time.

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