Whitepaper CRISPR Assay Setting

Unique Temperature per Cycle Control Algorithm for Friendly CRISPR Assay Setting

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-related genome editing technology has become the most eye-catching life science innovation in the past few years. With these systems, researchers can permanently modify genes in living cells and organisms and, in the future, make it possible to correct mutations at precise locations in the human genome in order to treat genetic diseases.

Throughout the process of a CRISPR system assay and downstream genome editing detection, precise reaction temperature control is a crucial element for the successful outcome. Not only should the steady reaction temperature be accurately maintained, but the cooling rate in some steps should also be controlled. For example, the temperature of a reaction mixture is requested to be heated to 95°C and then cooled to 85°C at a ramp rate of 1°C/sec, then slowly cooled down to room temperature at a ramp rate of 0.3°C/sec. Utilizing a thermal cycler, a so-called PCR machine, to perform the assay temperature control would be a good idea.

Thermal cyclers are designed to heat or cool the reaction mixture to a certain temperature and hold the temperature steadily for certain periods of time. This makes them the ideal instrument to perform temperature control for assays that need to be carefully taken care of. Some thermal cyclers can be adjusted to heat or cool at a slower ramp rate to accommodate the needs of the tasks mentioned above. However, due to the fact that the heating or cooling is not taking place at a constant rate (see Figure 1), it is not easy to precisely define the ramp rate needed for the task.

Take the task for cooling from 85°C to room temperature at a ramp rate of 0.3°C/sec as an example. By adjusting the ramping rate to 0.3°/sec, you can simply set up an 85°C step, followed by a 25°C step and let it run. The cooling rate of your reaction mix may be more than 0.3°C/sec at the beginning and less than 0.3°C/sec at the end. The other way to control slow ramping is by changing the temperature setting for a certain step after each cycle.

In this way, you can achieve precise time and temperature control throughout the entire duration, but the programming process may be a bit more complicated. You’ ll need to configure a program containing two or more temperature steps (so that you can repeat or cycle them) and change their temperature setting after each repeat (cycle).

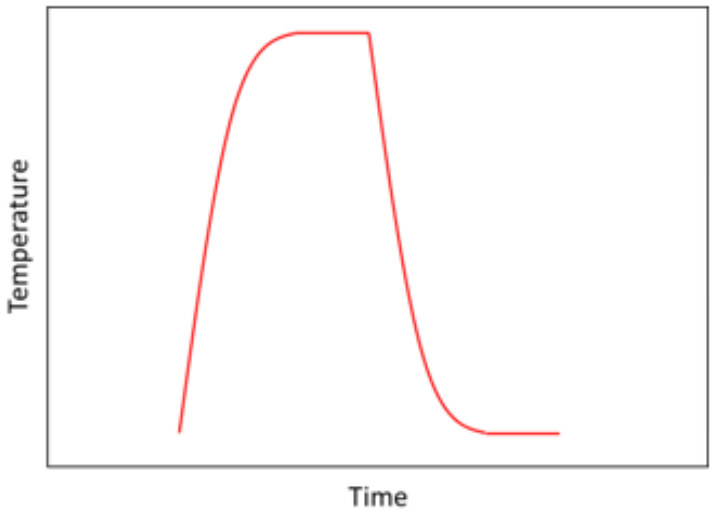

Figure 1. Typical ramping curve

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Let's use the example mentioned above again. With an 85°C reaction mixture, you need to set an 84.7°C, 2 sec step and an 84.1°C, 2 sec step and set a -1.2°C/cycle temperature decrement, then repeat these two steps for 50 times. In this way, you'll get precisely -0.6°C cooling every 2 sec. If you want to have smoother temperature change, you can even set an 84.7°C, 1 sec step and an 84.4°C, 1 sec step, a -0.6°C/cycle temperature decrement, then repeat these two steps for 100 times. Most cyclers have a cycle number limit, so you may need to add more temperature steps and reduce the cycle numbers (see Table 1). You need to consider the number of steps, the temperature difference between each adjacent step, the per cycle temperature decrement setting and cycle numbers.

Table 1

Repeat	Temperature	Hold Time
100 cycles, -0.6°C/cycle	84.7°C	1 sec
	84.4°C	1 sec

Repeat	Temperature	Hold Time
50 cycles, -1.2°C/cycle	84.7°C	1 sec
	84.4°C	1 sec
	84.1°C	1 sec
	83.8°C	1 sec

The TurboCycler Lite thermal cycler from Blue-Ray Biotech provides a new option for slow ramp control. By allowing to repeat a single temperature step, you just need to set the per cycle temperature decrement to meet the cooling rate requirement and repeat this step until you get the final temperature. If the cycle number needed exceeds the cycle number limit (99 cycles for TurboCycler Lite), just add another set of repeat (see Table 2).

Table 2

Repeat	Temperature	Hold time
99 cycles, -0.3°C/cycle	84.7°C	1 sec
99 cycles, -0.3°C/cycle	55°C	1 sec
2 cycles, -0.3°C/cycle	25.3°C	1 sec

The advanced slow ramp control makes the TurboCycler Lite an ideal instrument for CRISPR-related assays. It's also equipped with a full temperature range lid heater which can be beneficial for use as an incubator for NGS sample pretreatment and other experiments.

Quickly-evolving biotechnology developments simplify and speed-up researchers' work on exploring the truth of nature. As a member of the life science community, Blue-Ray Biotech promises to develop the most innovative laboratory instruments and provide the best service to support scientists to fulfill their dream.