# Thermal Cycler

It can also be named 'Thermocycler', 'PCR machine' or 'DNA amplifier'. There are 2 BIO-RAD S1000 thermal cycler in our lab. They are almost identical. S1000 has a lot of functions, of which we typically just a small part. It can only take 0.2 mL PCR tubes

## General Pre-Setting

There are a lot of previous protocols, which can be used either directly or with some minor modification. Before modification, making a copy is recommended

1. Lid temperature. Set it 5 °C above the maximum tube temperature. This is for avoiding the drying of liquid during the temperature ramps
2. Initial heating. Set it at 75 – 95 °C so that the DNA strands can fully melt for annealing. Not needed for incubations
3. Actual temperature ramp. The logic of the S1000 is a temperature with a duration. The resolution is 0.1 °C and 1 s. For example, 95 °C for 1 min
4. The increment or decrement is realised by 'Go To' function with + or – per cycle. For example, step 2 can be '95 °C for 1 min with -0.1°C per cycle'. Step 3 can be 'Go To step 2 for 500 times'. As a result, we have -0.1°C per 1 min, from 95 °C to 45 °C
    a. There is a temperature per time in the setting. Based on Haorong Chen's experience, it is not as good as temperature per step. Again, explorations are always welcome
5. The ending temperature is normally set as 25 °C, and can go as low as 20 °C. Below that is generally unnecessary. At the very end, hold the temperature at 4 °C forever (0 for duration)

## Regular Annealing and Incubation

Prepare mixture in 0.2 mL PCR tubes. Don't add more than 100 µL liquid in a single tube. If necessary, separate into 2 or more tubes. Place them in Block A or B and shut the lid

1. Twist the knob and make sure there is one clicking sound indicating the lid is tight
2. Use ' run' to select the protocol and execute in on Block A or B, depending on the place of the tubes. The volume doesn't matter and can just go with the default (50 µL)
3. Once it starts, use the function button on the left to change the view and see if the temperature ramp is correct. If not, cancel it as early as possible to avoid lost of time and material
4. The time estimated by the machine is in accurate. Better estimation is by running the protocol and know. The actual time needed is always about 1.5 times the time estimated

## Special Tricks

Sometimes we need operation between different temperature ramps. Some special tricks can make the tasks a lot easier

1. Go to the next step. In the view of current running protocol, press 'enter' can pop some options out. Press go to the next step can skip the current step. For example, step 2 is 95 °C for 30 min and step 3 is 65 °C for 30 min. When in step 2 for 20 min, it can go directly into step 3 by going to the next step
2. Add holding at certain temperature forever. If adding strands at certain temperature is needed, hold the temperature at that forever and make sure it is holding by the view. Twist the knob in the opposite direction and open the lid. Try to add strands fast as the

temperature can drop really quickly when the tube is outside the thermal cycler. Use the 1st trick once the tube is back to the cycler

## UVP lamp (model UVGL-25)

The lamp can emit UV light centred around 254 and 366 nm which may be switched by applying short- and long-wavelength settings, respectively. It is used as the source of UVC and UVA. UVB can be found in Spectroline TE-312S UV Transilluminator

**Preparation**

Use Homemade quartz tube (inner cross-section size: 2 × 4 mm) for holding liquid in place. Unlike a glass tube, the quartz tube is transparent in the UV spectrum, thus suitable for UV irradiation

1. Dilute the sample to 2 nM 20 – 40 μL using MES buffer and keep the solution in quartz tubes
2. If there is any additive to the DNA solution, add it. For example, mix saturated TP1 solution with DNA solution for a final volume ratio of TP1 solution to mixture at 1:10
3. Find the partial cut pipette tip box for 20 μL tips. Attach the quartz tubes to the back side of the covering

**UV Irradiation**

Place the pipette tip box with tubes at about 1 cm in front of the UV source.

1. The timer could be set by on the AC power or just by phone/computer
2. In order to avoid all other lights, cover the box and the UV source with opaque material'