

Thermal Cyclers

Eppendorf Mastercycler Gradient Pro (PSC 555, 659, NSC 338, 340, 488)
Eppendorf Mastercycler Gradient (NSC 460, Kell 405)

Every time you use the thermal cycler, you MUST sign the log book.

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Eppendorf Mastercycler Gradient Pro

1. Cover plate with film or caps. 10 ul volume is OK.
2. Turn on instrument back left by plug. User name: Biology; no password. OK (tab)
3. Lift lid handle and push back. Load plate. Pull lid forward and lower handle completely – will lock in place.
4. The screen is NOT touchpad – use arrows and tabs to select or move cursor. It also is NOT attached permanently to the instrument. Be careful that you don't drop it.
5. Add a folder for your lab if you want or just select Biology folder. DO NOT create a folder for yourself. You can put a folder for yourself IN the lab folder.
6. Select new. – can name your program and add comments. OK
7. Edit. Skeleton program that you can change. Blue highlighted step can be edited. Use arrows to move between steps. Use arrows to move between temp, time and # of cycles. Press enter to move to next step. The instrument sees everything between dotted lines as 1 item.
8. Make any changes you need to and then press Enter after each change.
9. Gradient cycle – select Options tab. If you don't see the Options tab, press the << tab. You can select increments to increase or decrease in temperature or time. Use arrows to get to box (next to gradient picture). Press enter to check. Now you can see some new tabs. Select Show Gradient to see temperatures or times you have setup. Just remember, change temperature in gradient page NOT in “show gradient” page.
10. You can change ramp rate with keypad or slide bar. Header – can change temperature mode from fast (default) to safe and standard. These change the dwell time – makes sure the well is the same temperature as block. Use down arrow to get to temp mode; Enter; use arrows to select
11. Exit – scroll to Biology/Enter. Select shutdown tab. Select shutdown tab.
12. #1 in center of instrument just means instrument #1.

Mastercycler Gradient Thermal Cycler

To Edit an Existing Program and Save as a New Program

1. Turn on (Power in back left hand side).
2. Main menu; use Arrows; go to Files; Press Enter.
3. Arrow to Load; press enter.
4. Arrow to choose program you want to edit; Press Enter. Sometimes when you press Load the list of programs doesn't come up, it only asks you to save the previous program. Press SEL to change the Yes to No and press Enter. If this doesn't work the first time, repeat.
5. Arrow down to parameter line that you want to change.
6. Arrow over to part of the parameter line you want to change. Use keypad to make changes, press Enter.

7. DEL: deletes the line that the cursor is on.
8. INS: to insert a line above the cursor.
9. When done, press Exit.
10. Save: Yes. Press Enter to save; if you do not want to save, press SEL to change the highlighted Yes to No and press Enter.
11. Change name – use a name and a code to identify your file.

To Start a Run

1. Turn on (Power in back left hand side).
2. Arrow to Start; press Enter.
3. Scroll to file you want; press Enter.
4. Program is automatically tested and starts. Press OPT to see finish time.

To Stop. Press Start/Stop and then Enter.

To set up a Standard PCR program

1. Select Files; Press Enter.
2. Select standard; press Enter.
3. Change parameters using the arrows to move the cursor and then make changes using the keypad, Press enter before changing another line. See editing and existing program above to add or delete lines.
4. At the end of the program: Go To – select which line to go to for multiple cycles (usually step 2, after the initial longer denaturation step).
5. Set Rep – select the # of cycles to repeat.
6. Add a Final Extension step if you want to. This is not included in the Rep cycles.
7. SEL, select until find Hold. Enter temp. you want your samples to hold until you get them – usually 4°C.
8. Exit.
9. Save: Yes; press Enter.
10. Change name – use your name and a code to identify file.

To set up a Gradient PCR program

1. Select file; press Enter.
2. Select New; press Enter.
3. Move cursor to lid using arrows; move across to lid and type in 105; press Enter. Leave No Wait and Auto.
4. Arrow down; SEL and a line for Temp. and Time appears above end.
5. Initial Denaturation. Enter Temp. and move cursor across to enter Time. Press Enter.
6. Denaturation. SEL. Enter Temp and Time and press Enter.
7. Gradient. SEL. Enter Temp and Time. Press Enter. Move cursor back up to line you just entered. Select OPT and 5 additional options appear. Arrow up and arrow over so that cursor is under Temp. and then arrow down to the 5 options. If you don't move the cursor under Temp. before going down, the gradient parameters will disappear. Arrow down to $G = __ ^\circ\text{C}$. The only one you need to change is $G = 0.0^\circ$. The other options are: $+0.0^\circ$ is for temperature increment, the temp. for each cycle is increased or decreased by the value entered; $+0:00$ is the time increment, for each cycle the time is extended or reduced by this value; $R = 3^\circ/\text{s}$ is the ramp value, how fast the cycler ramps up or down, it is usually set at $3^\circ/\text{sec}$.; G = the gradient, each column is set at a different temp., the center of the block is the Temp. entered at the beginning of the parameter line. G tells the thermal cycler how much of a spread across the block the gradient will go with the lowest temp on the left and the highest temp. on the right. For example, $G = 10^\circ\text{C}$, with a Temp. of 50°C means that the gradient will spread 10°C above 50°C and 10°C below 50°C . with column 7 closest to 50°C .
8. Extension. Add an Extension step the same way you entered the denaturation step.
9. SEL until Go To appears in the parameter line. Enter which line to go to for multiple cycles (usually step 2, after the initial longer denaturation step). Set Rep – select the # of cycles to repeat. Press Enter.
11. Add a Final Extension step if you want to. This is not included in the Rep cycles. Press Enter.

12. SEL, select until find Hold. Enter temp. you want your samples to hold until you get them – usually 4°C. Press Enter.
13. Exit.
14. Save: Yes; press Enter.
15. Change name – use your name and a code to identify file.

To see Temp. Range of Gradient

1. Main menu; select OPTIONS, press Enter.
2. Select Gradient; press Enter
3. Change parameters to match your run. For example: Tube Size = 0.2 ml; T = 50°C; G = 10°C. Press Enter.
4. No printer so you have to write temp. for all 12 rows down. You also can't access this function during the run. Be sure to put in gradient range – enter 10 (for 10 above and 10 below middle temp).

Hint for gradient run to find optimal annealing temp. Set up tubes in every other row. You will still get a nice temp. range, but will save template and reagents.