

## Appendix V

### MASTER CYCLER QC INSTRUCTIONS AND FORM

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This QC should be performed every 6 months. Readings must be within  $\pm 1^{\circ}\text{C}$  of the  $99^{\circ}\text{C}$  set temperature and within  $\pm 0.4^{\circ}\text{C}$  of the  $50^{\circ}\text{C}$  and  $72^{\circ}\text{C}$  set temperatures.

Before QC of the thermal cycler is done, the reading of the digital thermometer must be compared to that of the NIST.

	1	2	3	4	5	6	7	8	9	10	11	12
A	X											
B		X	X								X	
C							X					
D												
E					X							
F						X						
G		X									X	
H												

Place 0.2 mL tubes with holes in the caps containing  $50\mu\text{L}$  water in the thermal cycler in the wells indicated with an "X" in the above diagram. Set the thermal cycler to incubate at  $99^{\circ}\text{C}$ . Do not use the heated lid. Allow the thermal cycler two minutes to heat up and then put the probe from the digital thermometer into a tube. Close the thermal cycler lid. When the temperature has stabilized, record the temperature and put the probe into the next tube. Continue until the temperatures in each tube have been read.

Repeat this process for  $72^{\circ}\text{C}$  and  $50^{\circ}\text{C}$ , critical temperatures in PCR. Record all results on the QC worksheet and keep in the Mastercycler QC logbook.

**Quick QC:** Use the following method every other week when thermal cycler is in heavy use.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		X									X	
C												
D												
E					X							
F												
G		X									X	
H												

Check the digital thermometer against the NIST as stated above. Set the cycler to incubate at  $50^{\circ}\text{C}$ . Place tubes with holes in the top containing  $50\mu\text{L}$  water in the marked wells of the cycler (indicated above). Use the digital thermometer to read the temperature when it has stabilized in each tube. Repeat this process for  $72^{\circ}\text{C}$ . Record all results on the QC worksheet and keep in the Mastercycler QC logbook.

Date: \_\_\_\_\_

Technician: \_\_\_\_\_

Temperature	Well position	Mastercycler display temperature	Digital thermometer temperature	Valid range (°C)	Pass/Fail	
99°C	A1	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B2	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B3	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B11	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	C7	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	E5	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	F6	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	G2	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	G11	°C	°C	98 - 100	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
72°C	A1	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B2	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B3	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B11	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	C7	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	E5	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	F6	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	G2	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	G11	°C	°C	71.6 - 72.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
50°C	A1	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B2	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B3	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	B11	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	C7	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	E5	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	F6	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	G2	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail
	G11	°C	°C	49.6 - 50.4	<input type="checkbox"/> Pass	<input type="checkbox"/> Fail

Notes: