Routine control of real time PCR cyclers using the SureCycle® kit (Art. No. F4001) – Quick step guide

Note: Two consecutive qPCR runs need to be carried out.

Part 1 – Determination of the optimal denaturation

1 Master mix

Prepare the master mix for 20 reactions

Components of the master-mix	Amount per reaction	20 reactions (with 10 % excess)
Reaction Mix	19.3 µl	424.6 µl
Taq Polymerase	0.7 µl	15.4 µl
Total volume	**20 µl**	**440 µl**

2 Cycler setup

Program the cycler with the SureCycle®
Profile 1 Denaturation

	SureCycle Profile 1 (with 10 % excess)	
Initial Denaturation (HOLD)	1 min, 95 °C	
Denaturation	15 sec, 95 °C	45 cycles
Annealing/Extension	30 sec, 63 °C	
Cooling	10 sec, 40 °C	
Temperature Transition Rate/ Ramp Rate	Maximum	

3 Pipetting scheme

When using a blockcycler:

- Pipette 4 x 4 wells in the corner positions (highlighted in blue) – control reactions
- 4 wells in the center (highlighted in red) – reference reactions

When using a rotorcycler:

- Pipette 20 reactions
- 4 reactions are used as reference reactions (position 1 - 4 highlighted in red)
- The rest is positioned according the rotor size (highlighted in blue) – control reactions

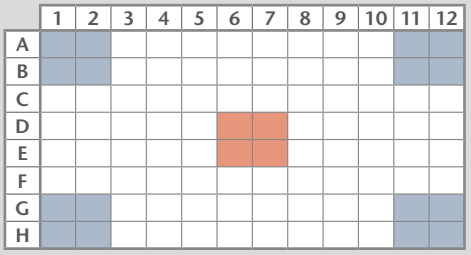

Fig. 1: Blockcycler pipetting scheme in 96-well format: 5 x 4 wells

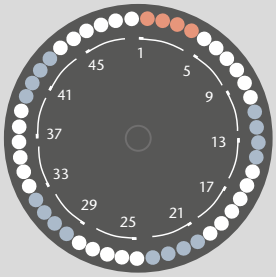

Fig. 2: Rotorcycler – 5 x 4 wells (example RIDA®CYCLER – 48 well rotor)

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4 Start denaturation verification run

5 Result Interpretation of SureCycle® Profile 1 Denaturation

VIC-channel:

- Calculate the mean value of the four reference reactions – red marked wells
- The target range of control reactions is defined as ±2 Cp of the reference reactions (mean value)
- See also table 1

FAM channel:

- All results should be negative
- In case control or reference reactions show positive Cp-values, check table 2 in the manual

	1	2	3	4	5	6	7	8	9	10	11	12
A	26.8	26.6									26.8	26.4
B	26.8	26.8									26.9	26.7
C												
D						26.9	27.0					
E						27.0	27.0					
F												
G	26.7	26.8									26.8	26.7
H	26.6	26.8									26.6	26.7

Fig. 3: Example calculation SureCycle® Profile 1 Denaturation, VIC-channel
Mean value D6/D7/E6/E7: 27.0
Taget range ±2 Cp: 25.0 - 29.0

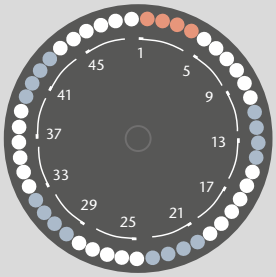

Fig. 4: Example calculation SureCycle® Profile 1 Denaturation, VIC-channel
Mean value Pos. 1 - 4: 27.0
Taget range ±2 Cp: 25.0 - 29.0

Table 1: Result interpretation of profile 1 – VIC- channel

VIC-channel Denaturation-System			
Reference reaction	**Control reaction**	**Result**	**Consequence for further analysis**
Positive	Cp within target range	Correct	Real-time PCR instrument temperature system works correctly
Positive	Cp out of target range	Incorrect	Denaturation temperature too low potential false PCR-results
Negative	Cp; no target range	Incorrect	Denaturation temperature too low potential false PCR-results

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Part 2 – Determination of the optimal annealing

6 Master mix

Prepare the master mix for 20 reactions

Components of the master-mix	Amount per reaction	20 reactions (with 10 % excess)
Reaction Mix	19.3 µl	424.6 µl
Taq Polymerase	0.7 µl	15.4 µl
Total volume	**20 µl**	**440 µl**

7 Cycler setup

Program the cycler with the SureCycle®
Profile 2 Annealing

	SureCycle Profile 2 (with 10 % excess)	
Initial Denaturation (HOLD)	1 min, 95 °C	
Denaturation	15 sec, 92 °C	45 cycles
Annealing/Extension	30 sec, 60 °C	
Cooling	10 sec, 40 °C	
Temperature Transition Rate/ Ramp Rate	Maximum	

8 Start annealing verification run

Use same recommended pipetting scheme from above (step 3)

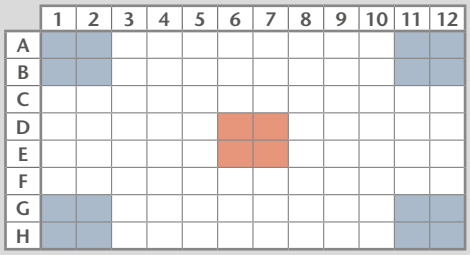

Fig. 5: Blockcycler pipetting scheme in 96-well format: 5 x 4 wells

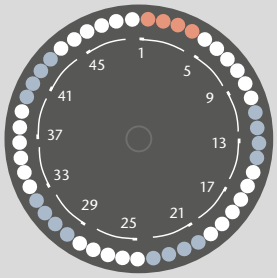

Fig. 6: Rotorcycler – 5 x 4 wells (example RIDA®CYCLER – 48 well rotor)

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9 Result Interpretation of SureCycle® Profile 2 Annealing

FAM-channel:

- Calculate the mean value of the four the reference reactions – red marked wells
- The target range of control reactions is defined as ±2 Cp of the reference reactions
- See also table 2

VIC-channel:

- All results should be negative
- In case control or reference reactions show positive Cp-values, check table 4 in the manual

	1	2	3	4	5	6	7	8	9	10	11	12
A	26.8	26.6									26.8	26.4
B	26.8	26.8									26.9	26.7
C												
D						26.9	27.0					
E						27.0	27.0					
F												
G	26.7	26.8									26.8	26.7
H	26.6	26.8									26.6	26.7

Fig. 7: Example calculation SureCycle® Profile 2 Annealing, FAM-channel
Mean value D6/D7/E6/E7: 27.0
Taget range ±2 Cp: 25.0 - 29.0

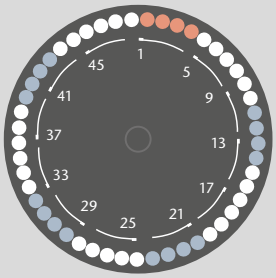

Fig. 8: Example calculation SureCycle® Profile 2 Annealing, FAM-channel
Mean value Pos. 1 - 4: 27.0
Taget range ±2 Cp: 25.0 - 29.0

Table 2: Result interpretation of profile 2 – FAM- channel

VIC-channel Denaturation-System			
Reference reaction	**Control reaction**	**Result**	**Consequence for further analysis**
Positive	Cp within target range	Correct	Real-time PCR instrument temperature system works correctly
Positive	Cp out of target range	Incorrect	Denaturation temperature too low potential false PCR-results
Negative	Cp; no target range	Incorrect	Denaturation temperature too low potential false PCR-results

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