

Standard thermal cycling conditions for PACE genotyping reactions

Step	Description	Temp.	Time	N°. Cycles
1	Enzyme activation	94°C	15 min	1
2	Template denaturation	94°C	20 secs	10
	Annealing and extension	65-57°C	60 secs (drop 0.8°C per cycle)	
3	Denaturation	94°C	20 secs	30
	Annealing and extension	57°C	60 secs	

Should clusters still require tightening to ensure more accurate data analysis, the following recycling conditions can be used

Step	Description	Temp.	Time	N°. Cycles
Recycling	Template denaturation	94°C	20 secs	3
	Annealing and extension	57°C	60 secs	