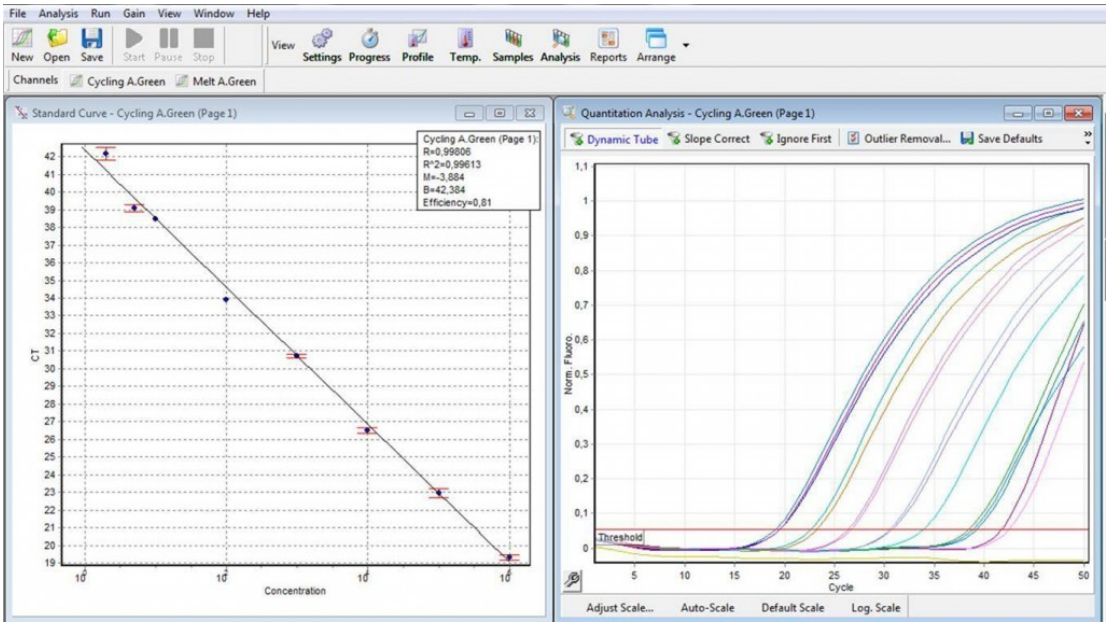
The new primer pair (pH207 and pAZ102-E) was evaluated by SYBR®Green qPCR assay performed on a qPCR platform Rotor-Gene 6000 (Corbett Life Science, Australia). Reactions were achieved in a final volume of 15 µl containing 2 µl of the appropriate template (serial dilutions of plasmid in rat DNA), 1X Brilliant II SYBR® Green QPCR Master Mix (Agilent Technologies) and 500 nM of each of the primers.

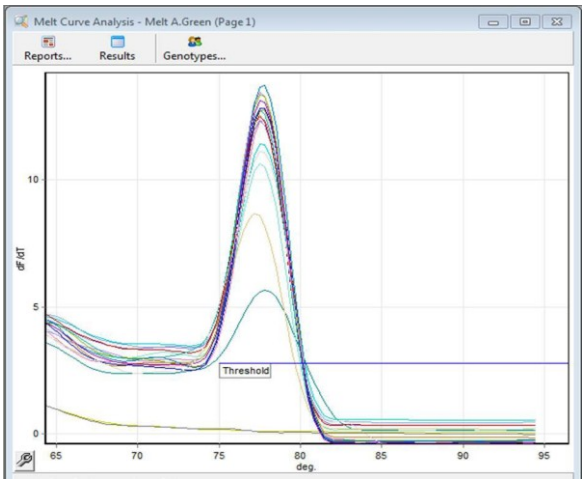
qPCR conditions are the following:

step		temperature	duration
1	Activation	10 min.	95 °C
2	Denaturation	10 sec.	95 °C
3	Annealing	15 sec.	59 °C
4	Signal acquisition		
5	Elongation	24 sec.	72 °C
6	Goto step 2 => 49 X		
7	Melting curve from 64 °C to 95 °C		

Real-time PCR standard curves and amplification curves for consecutive 6-fold dilutions of standard templates



Melt analysis of amplification products



Quantitative results for consecutive dilutions of standard templates

Name (Plasmids copy number + rat DNA)	Type	Ct
2	Standard	41,75
2	Standard	42,52
5	Standard	38,92
5	Standard	39,28
10	Standard	38,48
100	Standard	33,91
1000	Standard	30,58
1000	Standard	30,81
10000	Standard	26,38
10000	Standard	26,65
100000	Standard	23,24
100000	Standard	22,69
1000000	Standard	19,07
1000000	Standard	19,48
1000000	Standard	19,45
NTC-TE	Negative Control	