Spartan DX-12™ Melt Curve Functionality

SL Prevost, HM Bernatchez, KA Jackson, NA Arbour, CJ Harder
Spartan Bioscience Inc.

Here we demonstrate melt curve functionality across several Spartan DX-12™ instruments.

Introduction

Melting curve analysis following real-time PCR has become a standard method for verification of the specificity of real-time PCR amplification. This method has the advantage of not relying on open tube procedures such as gel electrophoresis. As the melting temperature of an amplicon is dependant on GC content, length, and sequence, different amplicons can have different melting temperatures, and this property can be used to distinguish desired product from undesired products (1).

The Spartan DX-12™ performs melt curve analysis at step sizes between 0.1°C-1.0°C/step. Ramp rate during a melt curve on the Spartan DX-12™ is linear, and is dependant on the step size selected (see Table 6 for rates).

The purpose of this study was to determine the repeatability, reproducibility, precision and speed of generation of melt curve data across several Spartan DX-12™ instruments.

Materials and Methods

DNA preparation

A synthetic plasmid construct was designed in-house and produced for Spartan Bioscience by GENEART Inc. (Toronto). Plasmid DNA was received at a stock concentration of 1.5 mg/ml, reconstituted to 1.5 mM stock in sterile water, aliquoted and stored at -20°C.

Input DNA

Plasmid DNA was diluted to a concentration of 0.75 ng/µl and 2 µl were used per 50µl reaction.

Real-time PCR

Oligonuceotide primers designed against the Norovirus Open Reading Frame exon 1-2 junction (ORF 1-2) were used (2,3). The primer sequences are shown in Table 1.

Components of the real-time amplification mixture are listed in Table 2. Forthy-eight replicate samples were amplified in 50 µl volumes on the StepOne instrument (ABI). Reactions were then pooled and re-aliquoted into 20 µl volumes in Spartan tubes (20 µl PCR Tube Assembly, Tubes - Cat. No.01004153 and Caps - Cat. No. 01004155). Melt curves were run across 12 wells and 5 Spartan DX-12™ instruments. Table 3 shows the cycling parameters used for amplification on the StepOne (ABI) (2-temperature program). Melt curves were performed at 0.1°C/step, 0.2°C/step, 0.5°C/step, and 1.0°C/step on the Spartan DX-12™ instruments.

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
COG1 primers	CgY Tgg ATg CgN TTY CAT gA	CTT AgA CgC CAT CAT CAT TYA C	85

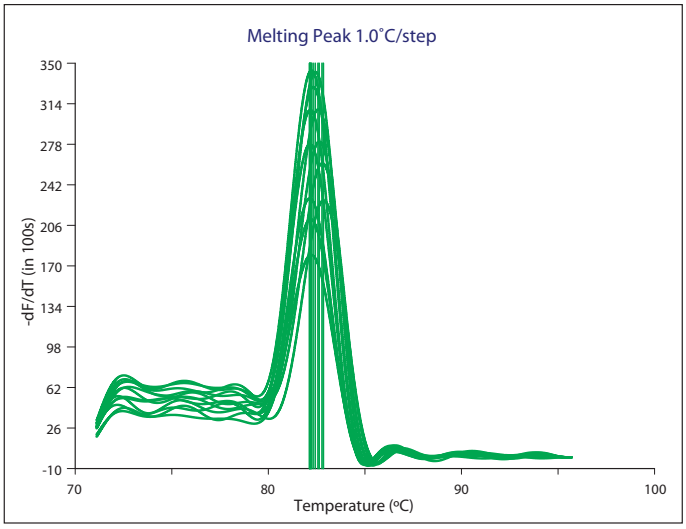
Table 1. Primer sequences and amplicon sizes.

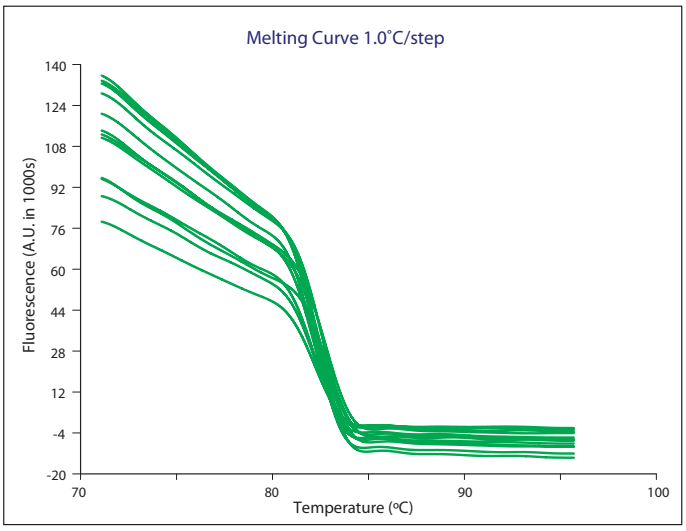
Component	Final amount
10X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen)	1 X
$MgCl_2$ (Invitrogen)	2.5 mM
dNTP mix (Invitrogen)	0.125 mM
Taq DNA polymerase (Invitrogen)	1 U
SYBR Green (Invitrogen)	0.5 X
PCR primers (IDT)	0.2 µM
Template DNA	2 µl
Sterile water	up to 50 µl
Total reaction volume	**50 µl**

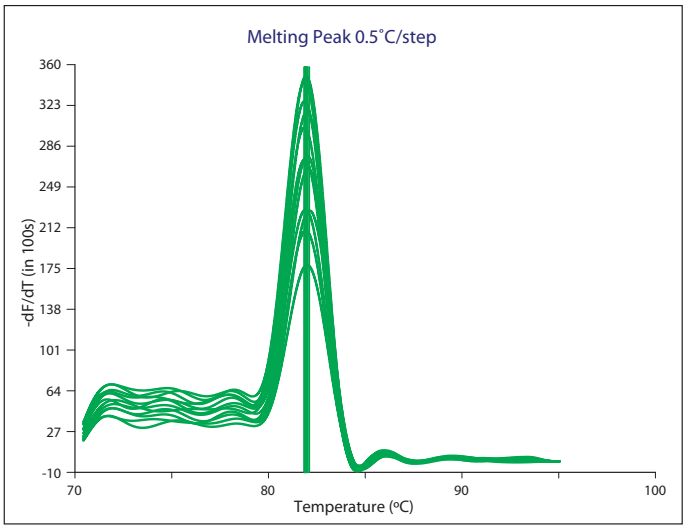
Table 2. Components of PCR amplification mixture.

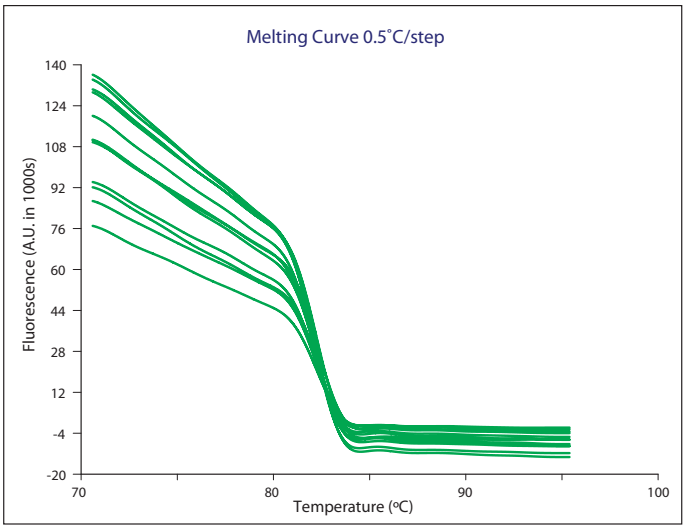
Step	Temperature	Time	Cycles
Initial denaturation	97°C	60 s	1
Denaturation	97°C	45 s	50
Annealing/extension	58°C	45 s	50

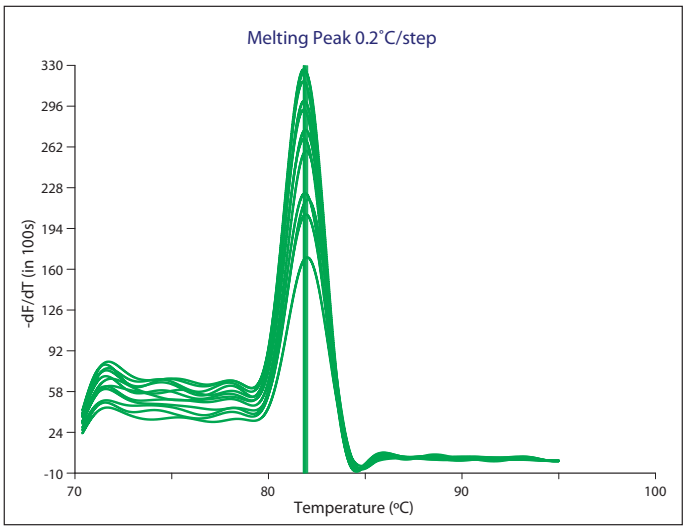
Table 3. Cycling parameters.

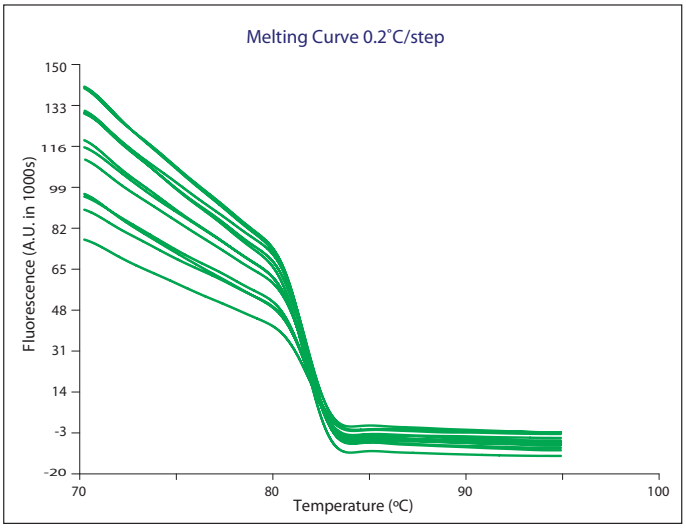












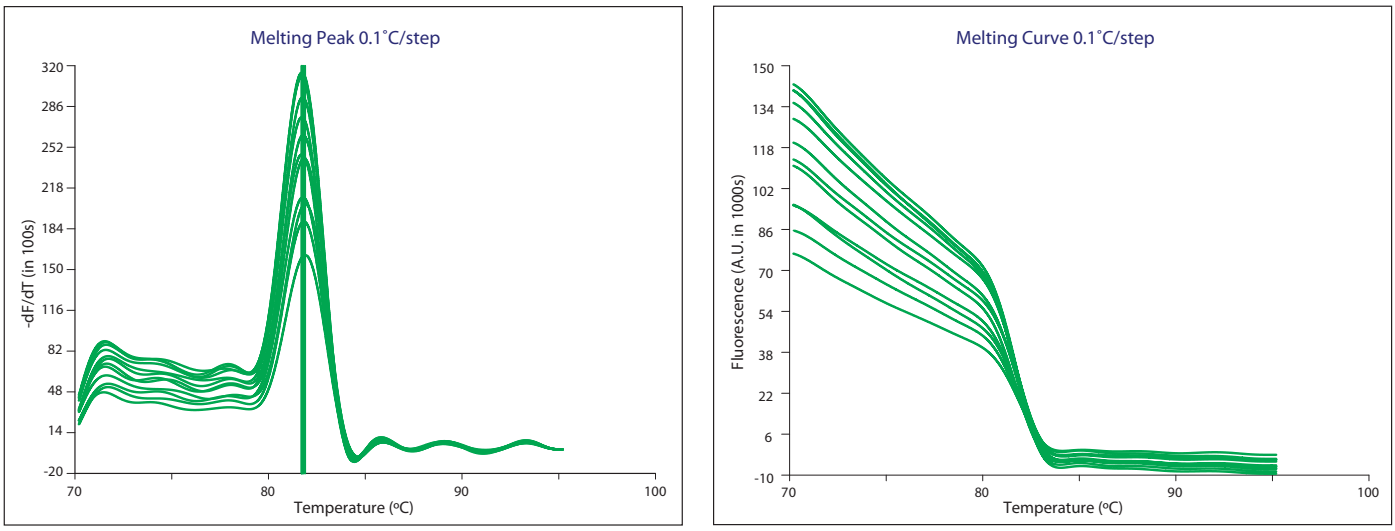


Figure 1. Melt curve and melt peak plots across 12 wells of the same instrument for 1.0°C/step, 0.5°C/step, 0.2°C/step and 0.1°C/step.

DNA analysis

Fluorescence data was transferred from the Spartan DX-12™ and graphed using the Spartan Graphing & Analysis Software (ver. 3.10).

Results

Figure 1 shows plots of melt curve and melt peak data across step sizes of 1.0°C-0.1°C.

Intra-instrument repeatability tests on the Spartan DX-12 show consistent results across all 12 wells. Standard deviations of melt peaks (Tm values) across 12 wells are between ± 0.39-0.51 for 1.0°/step, ± 0.07-0.11 for 0.1°C/step (Table 4).

Inter-instrument testing also shows consistent results across instruments, demonstrating excellent reproducibility. Tm values across 72 wells of 6 different instruments were 82.42°C ± 0.34, 82.12°C ± 0.14, 81.84°C ± 0.11 and 81.68°C ± 0.10 for 1.0, 0.5, 0.2 and 0.1°C/step respectively (Table 5).

Total run time calculations (Table 6) show the differences in melt curve run times from 1.0°C/step - 0.1°C/step, ranging from 5 min 33 sec to 23 min 33 sec respectively, with run

Step Size (°C/step)		Instrument 1	Instrument 2	Instrument 3	Instrument 4	Instrument 5	Instrument 6
1.0	Average Tm Value	82.36	82.50	82.47	82.40	82.37	82.4
	Standard Deviation	0.24	0.35	0.51	0.48	0.18	0.11
0.5	Average Tm Value	82.21	82.14	82.08	82.04	82.1	82.13
	Standard Deviation	0.10	0.14	0.18	0.18	0.10	0.10
0.2	Average Tm Value	81.94	81.87	81.72	81.83	81.80	81.88
	Standard Deviation	0.08	0.10	0.08	0.08	0.08	0.08
0.1	Average Tm Value	81.78	81.70	81.63	81.65	81.66	81.68
	Standard Deviation	0.08	0.10	0.07	0.07	0.07	0.09

Table 4. Melt curve Tm intra-instrument repeatability (across 12 wells).

Step Size (°C/step)	1.0	0.5	0.2	0.1
Average Tm	82.42	82.12	81.84	81.68
Standard Deviation	0.34	0.14	0.11	0.10

Table 5. Inter-instrument melt curve reproducibility.

times increasing with decreasing ramp rates.

Discussion and Conclusions

These results demonstrate that the Spartan DX-12™ is capable of achieving excellent inter- and intra-instrument melt curve repeatability and reproducibility. These results validate the thermal stability and functionality of the Spartan DX-12™, and indicate that the instrument performs both precise and fast melting-curve analysis.

In addition, these results demonstrate that Tm precision at 0.5°C/step (SD ± 0.14) is more than sufficient for most

Step Size (°C)	Transition (°C)	Total Time (min)	Rate (°C/min)
1.0	40	5.3	0.13
0.5	40	7.3	0.09
0.2	40	13.3	0.05
0.1	40	23.3	0.03

Table 6. Estimated Melt Curve total run times and temperature transition rates.

References

1. Ririe KM et al. (1997). Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Analytical Biochemistry*. 245(2): 154-60.
2. Rolf KJ *et al.* (2007). An internally controlled, one-step, real-time RT-PCR assay for norovirus detection and genogrouping. *Journal of Clincal Virology*. 39(4): 318-21.
3. Kageyama T *et al.* (2003). Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *Journal of Clinical Microbiology*. 41(4): 1548-57.