Thermal cycler amplification robustness: a comparison of several models

Introduction

The ability of a thermal cycler to amplify difficult targets uniformly across a sample block is a critical factor for many researchers. Ideally, a thermal cycler should produce high yields for a variety of difficult-to-amplify targets, including GC-rich and long templates. This study compares the ability of several thermal cyclers to uniformly amplify a selection of difficult templates.

Materials and methods

The instruments tested in this study are shown in Table 1. The same equipment, methods, and reagents were used for each instrument. A successful PCR experiment is shown by a clear, neat band on an electrophoresis gel. The intensity of the bands are not measured or compared.

Table 1. Instruments tested for amplification robustness.

Manufacturer	Model name	Cat. No.
Thermo Fisher Scientific	Applied Biosystems™ ProFlex™ 96-Well PCR System	4484075
Thermo Fisher Scientific	Applied Biosystems™ SimpliAmp™ Thermal Cycler	A24811
Thermo Fisher Scientific	Applied Biosystems™ MiniAmp™ Plus Thermal Cycler	A37835
Thermo Fisher Scientific	Applied Biosystems™ MiniAmp™ Thermal Cycler	A37834
Thermo Fisher Scientific	Applied Biosystems™ 2720 Thermal Cycler	4359659
Agilent	SureCycler™ 8800 Thermal Cycler	G8800A
Bioer	GeneMax™ Thermal Cycler	BYQ6067
Bio-Rad	C1000 Touch™ Thermal Cycler with 96-Well Fast Reaction Module	185-1196
Bio-Rad	T100™ Thermal Cycler	186-1096
Eppendorf	Mastercycler™ Nexus Gradient	6331 000.017
Eppendorf	Mastercycler™ Pro S	6325 000.510
SensoQuest	Labcycler Gradient	011-101, 012-103
Takara	Dice™ Touch	TP350

AT-rich template (28.2% GC content)

A single bulk reaction was prepared using Applied Biosystems™ AmpliTaq Gold™ 360 Master Mix (Cat. No. 4398876) according to the standard protocol, with primers targeting the *RB1* oncogene. The forward primer (5′-TGTAAAACGACGGCCAGTCCTTAGGTGGAT-CAGCTGGGTG-3′) and reverse primer (5′-CAGGAAA-CAGCTATGACCTCAATGAATTTGCATTTGTTGCAT-3′) were used at 0.2 µM, and the template, Human Genomic DNA (Roche, Cat. No. 11691112001), was used at 1 ng/µL. The bulk reaction was split among 32 wells to test for amplification consistency. The thermal cycling protocol consisted of a primary stage at 94°C for 10 min followed by a secondary stage consisting of 32 cycles at 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, before a final stage at 72°C for 7 min.

GC-rich template (80.4% GC content)

A single bulk reaction was prepared using AmpliTaq Gold 360 Master Mix with 360 GC Enhancer reagent and primers targeting the *CCNE* (cyclin-E1) gene. The forward primer (5′-TGTAAAACGACGGCCAGTAGCCAGGAAGAT-GGCCGATG-3′) and reverse primer (5′-CAGGAAACAGC-TATGACCTTCCGCGCGCAGGGATTT-3′) were used at 0.2 µM, and the human genomic DNA template was used at 1 ng/µL. The bulk reaction was split among 32 wells to test for amplification consistency. The thermal protocol was the same as that used for the AT-rich template.

Dual-target amplification

A single bulk reaction was prepared using AmpliTaq Gold 360 Master Mix according to the standard protocol, with primers that produce a high molecular weight (HMW) product and low molecular weight (LMW) product. The forward primer (5′-TGTAAAACGACGGCCAGTTCTCTTG-CCTCAGCCTCCCA-3′) and reverse primer (5′-CAG-GAAACAGCTATGACCTTAAGCTGTTTCCATAGGGT-GACACA-3′) were used at 0.2 µM, and the human genomic DNA template was used at 1 ng/µL. The bulk reaction was split among 32 wells to test for amplification consistency. The thermal protocol was the same as that used for the AT-rich template.

Long template (12,342 bp)

A single bulk reaction was prepared using the Applied Biosystems™ SequalPrep™ Long PCR Kit (Cat. No. A10498) according to the standard protocol, with primers targeting the *BRCA2* gene. The forward primer (5′-CTCCCCCACAAAAAGGGGACAAAGC-3′) and reverse primer (5′-ACAAACTCCCACATACCACTGGGGG-3′) were used at 2 µM, and the human genomic DNA template was used at 2.5 ng/µL. The bulk reaction was split among 32 wells to test for amplification consistency. The thermal protocol consisted of a primary stage at 94°C for 2 min followed by a secondary stage consisting of 35 cycles at 94°C for 10 sec, 61°C for 30 sec, and 68°C for 14 min and 20 sec, before a final stage at 72°C for 5 min.

Data acquisition and analysis

After amplification, long PCR products were loaded onto an Invitrogen™ E-Gel™ *48* 1% Agarose Gel (Cat. No. G800801). All other assays were loaded onto an E-Gel *48* 2% Agarose Gel (Cat. No. G800802). The reactions were subjected to electrophoresis under standard conditions, with the Invitrogen™ 100 bp DNA Ladder (Cat. No. 15628019), and gel images were acquired using a constant exposure time and aperture size. After imaging, bands were quantified using Thermo Scientific™ myImageAnalysis™ Software (Cat. No. 62237).

Results

The 13 instruments were tested using the four PCR experiments as listed above. In many cases, certain wells failed to amplify any PCR product. The wells that failed to amplify in each assay are tallied in Table 2.

Table 2. Tally of wells that failed to produce bands.

Manufacturer	Instrument	AT-rich template	GC-rich template	Dual target, HMW	Dual target, LMW	Long template (*BRCA2*, 12,342 bp)
Thermo Fisher Scientific	ProFlex	0	0	0	0	0
Thermo Fisher Scientific	SimpliAmp	0	0	0	0	0
Thermo Fisher Scientific	MiniAmp Plus	0	0	0	0	0
Thermo Fisher Scientific	MiniAmp	0	0	0	0	0
Thermo Fisher Scientific	2720	0	0	0	0	2
Agilent	SureCycler 8800	0	0	0	0	7
Bioer	GeneMax*	1	0	1	1	4
Bio-Rad	C1000 Touch	1	0	0	0	1
Bio-Rad	T100*	2	0	0	0	7
Eppendorf	Mastercycler Nexus Gradient*	0	0	0	0	3
Eppendorf	Mastercycler Pro S	1	0	1	1	1
SensoQuest	Labcycler Gradient	0	0	0	0	7
Takara	Dice Touch*	1	2	0	0	12

* Evaporation occurred in corner wells.

Discussion

Thermal cycler performance is crucial when amplifying difficult or long DNA templates. Temperature accuracy and stability make it possible to successfully amplify difficult or long DNA templates, which require extended hold times. Multiple or smeared bands, rather than a single target band, were observed when using some thermal cyclers. If the block temperature is not uniform, nonspecific binding during the annealing step may occur, generating unwanted amplicons in addition to the target amplicon. Refer to **thermofisher.com/thermalcycleraccuracy** for more information.

Evaporation can occur in corner wells during a long temperature hold protocol. A heated lid with positive and constant pressure minimizes sample evaporation and ensures well-to-well reproducibility. Learn more at **thermofisher.com/thermalcyclerevaporation**.

The ability of a thermal cycler to uniformly amplify reactions is pivotal. In this study, we have shown that the quality of PCR amplification varies between thermal cyclers. This was carried out as a side-by-side comparison while utilizing the same reaction chemistry.

For best results, we recommend using a thermal cycler that produces a high yield for difficult-to-amplify targets but also does not sacrifice integrity and consistency of amplification. The combined results for each assay category suggest that the Applied Biosystems™ thermal cyclers meet these criteria.

Appendix

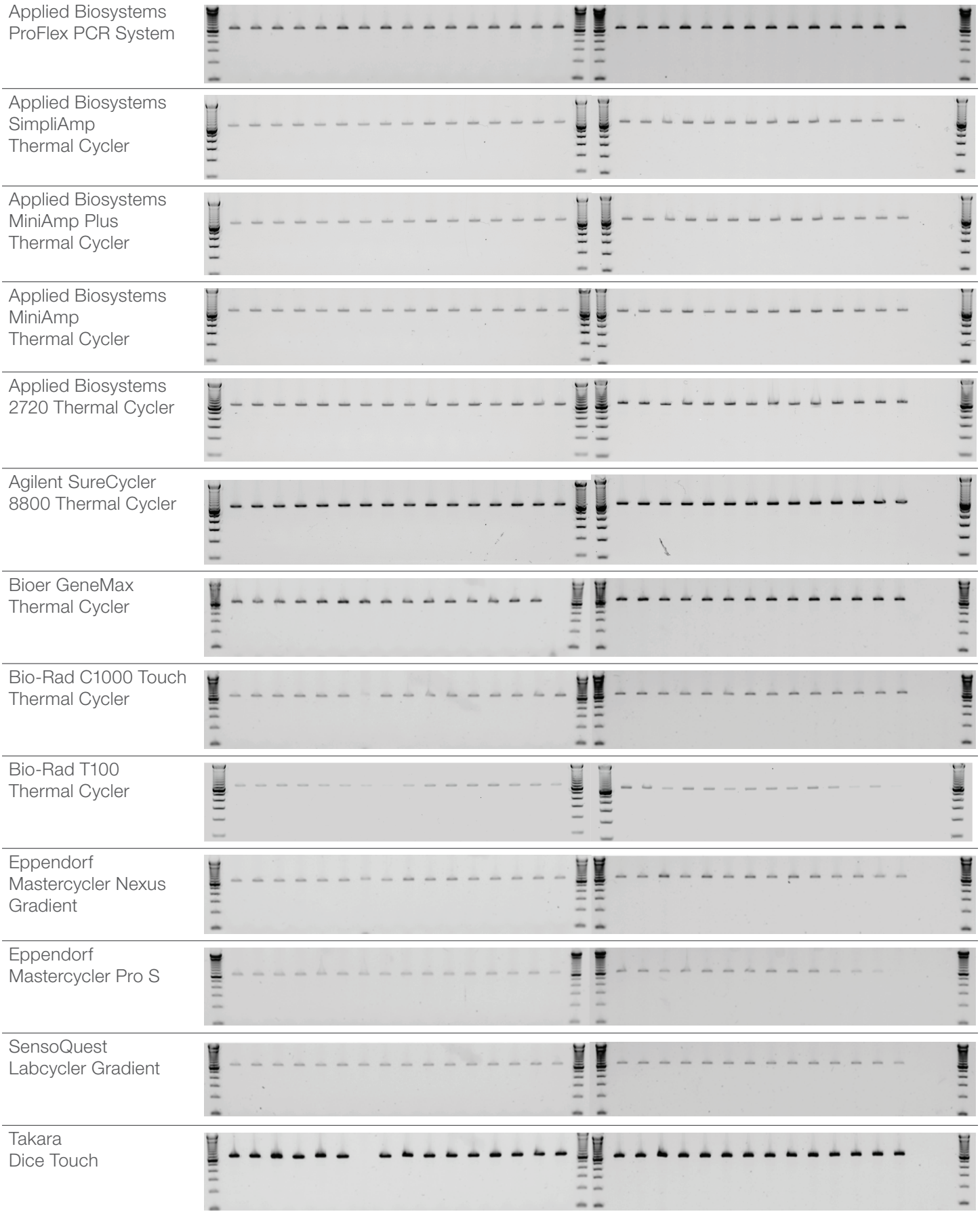

Figure 1. Gel images of PCR products following amplification of AT-rich template.

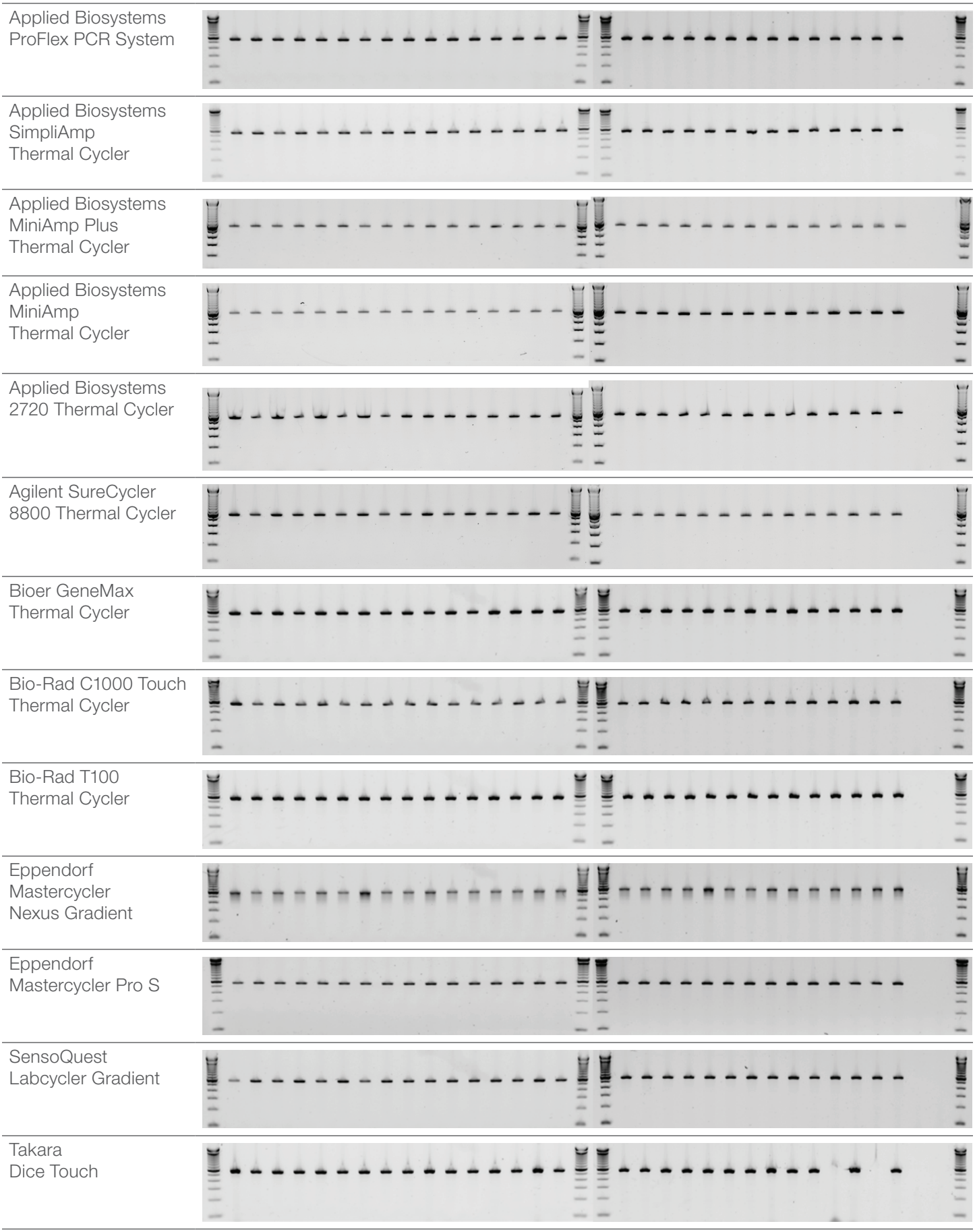

Figure 2. Gel images of PCR products following amplification of GC-rich template.

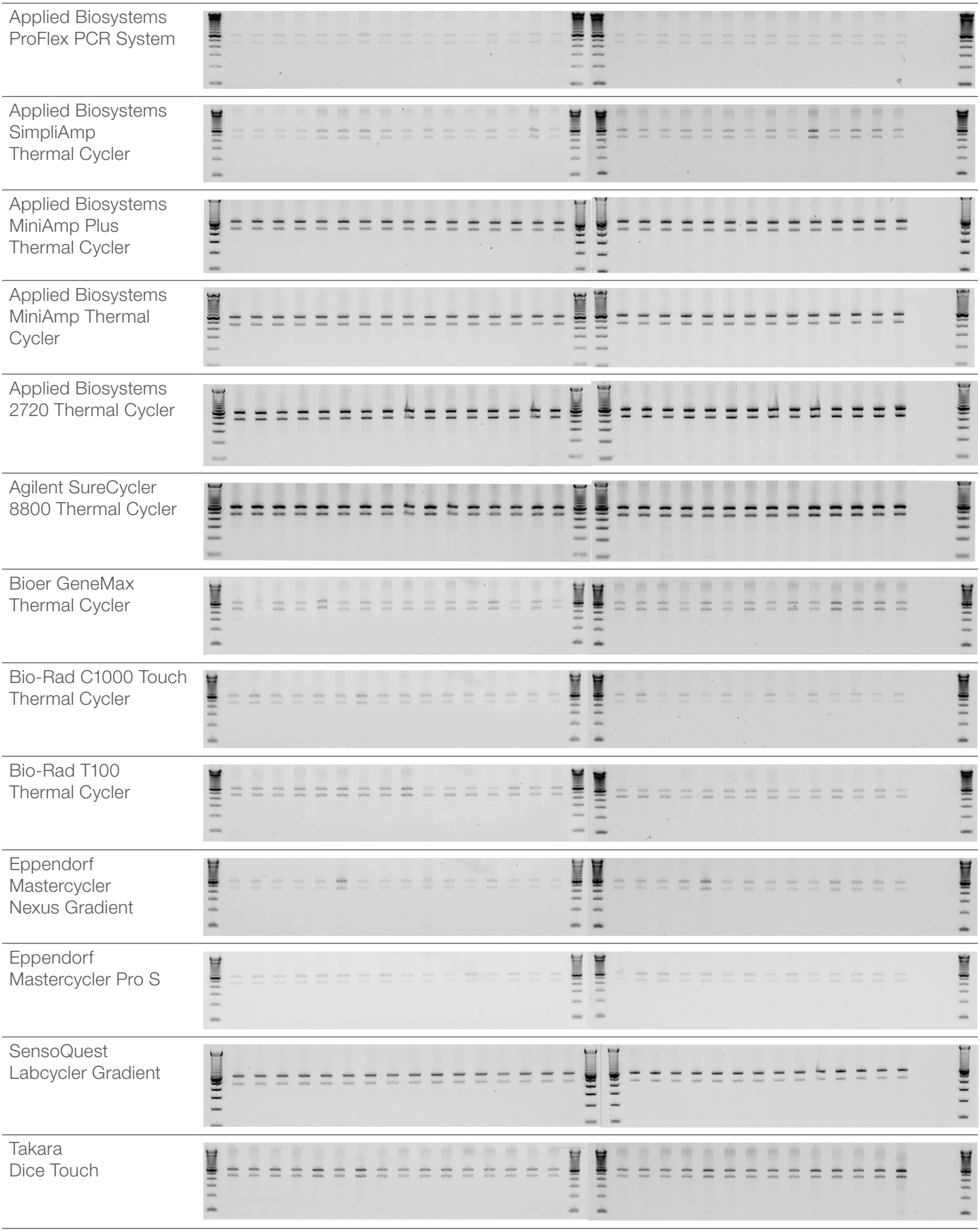

Figure 3. Gel images of dual-target PCR products.

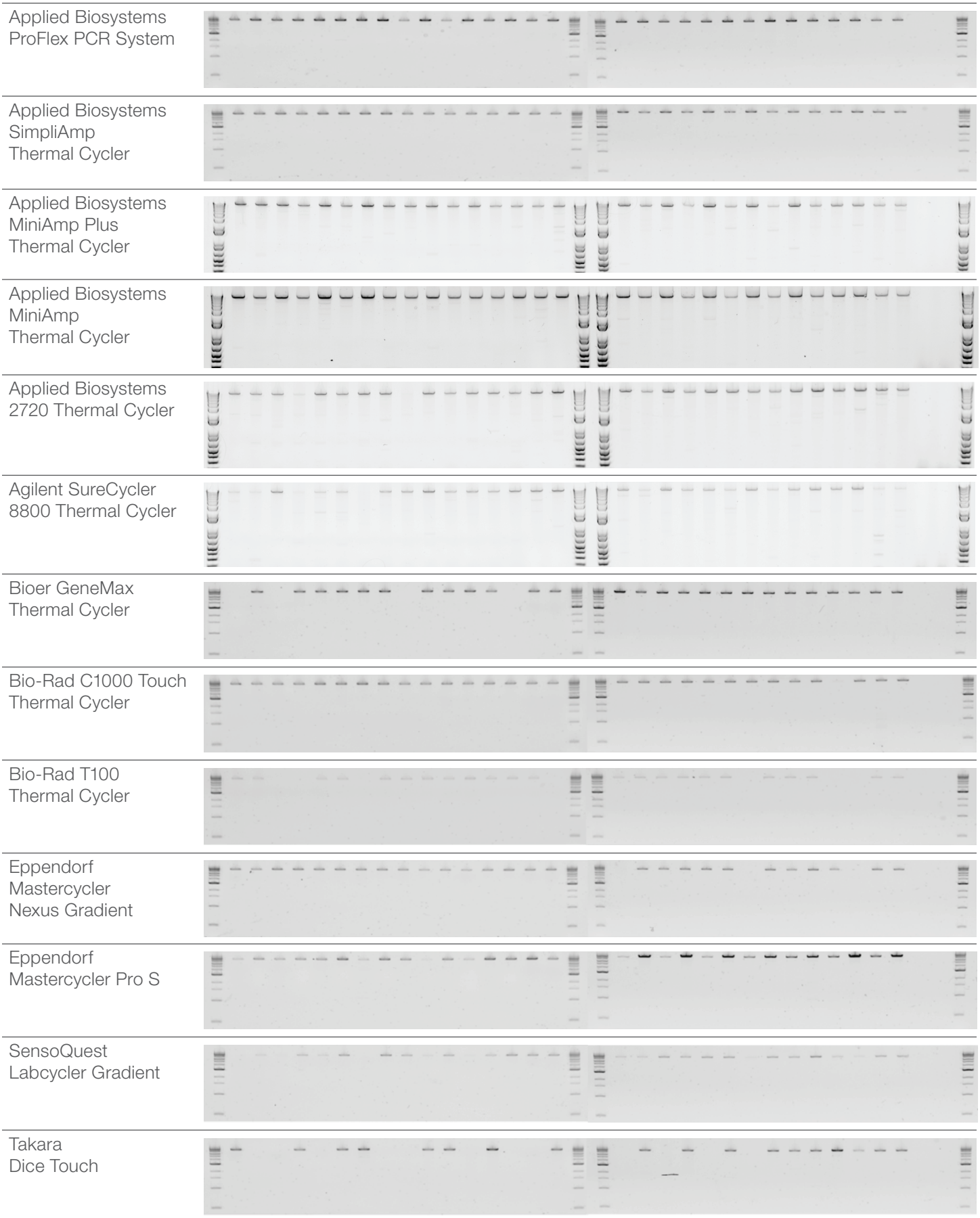

Figure 4. Gel images of long PCR products following amplification of the *BRCA2* gene.

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High GC

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	M
TE	TE	TE	TE	DNA	A1	B5	A2	B6	A3	B7	A4	B8	C9	D1	C10	D2	C11	D3	C12	D4	DNA	TE	TE	TE	TE
M	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	M
TE	TE	TE	TE	DNA	E6	E7	F9	G1	F10	G2	F11	G3	F12	G4	H5	H6	H7	H8	E5	E8	DNA	TE	TE	TE	TE

High AT

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	M
TE	TE	TE	TE	DNA	A5	B9	A6	B10	A7	B11	A8	B12	C1	E9	C2	E10	C3	E11	C4	E12	DNA	TE	TE	TE	TE
M	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	M
TE	TE	TE	TE	DNA	D6	D7	F1	G5	F2	G6	F3	G7	F4	G8	H9	H10	H11	H12	D5	D8	DNA	TE	TE	TE	TE

Dimer

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	M
TE	TE	TE	TE	DNA	A9	B1	A10	B2	A11	B3	A12	B4	D9	E1	D10	E2	D11	E3	D12	E4	DNA	TE	TE	TE	TE
M	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	M
TE	TE	TE	TE	DNA	C6	C7	F5	G9	F6	G10	F7	G11	F8	G12	H1	H2	H3	H4	C5	C8	DNA	TE	TE	TE	TE

Figure 5. Gel layout and plate coordinates of 2% E-Gel *48* Agarose Gel for amplification of GC-rich template, AT-rich template, and dual target (dimer). DNA refers to the 100 bp DNA Ladder; TE refers to TE buffer.

BRCA2

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	M
TE	TE	TE	TE	DNA	A1	B1	A2	B2	A11	B11	A12	B12	G1	H1	G2	H2	G11	H11	G12	H12	DNA	TE	TE	TE	TE
M	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	M
TE	TE	TE	TE	DNA	A5	D5	A6	D6	A7	D7	A8	D8	H5	H6	H7	H8	C6	C7	C5	C8	DNA	TE	TE	TE	TE

Figure 6. Gel layout and plate coordinates of 1% E-Gel *48* Agarose Gel for long-range amplification of *BRCA2* template. DNA refers to the 100 bp DNA Ladder; TE refers to TE buffer.

Find out more at **thermofisher.com/thermalcyclers**

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