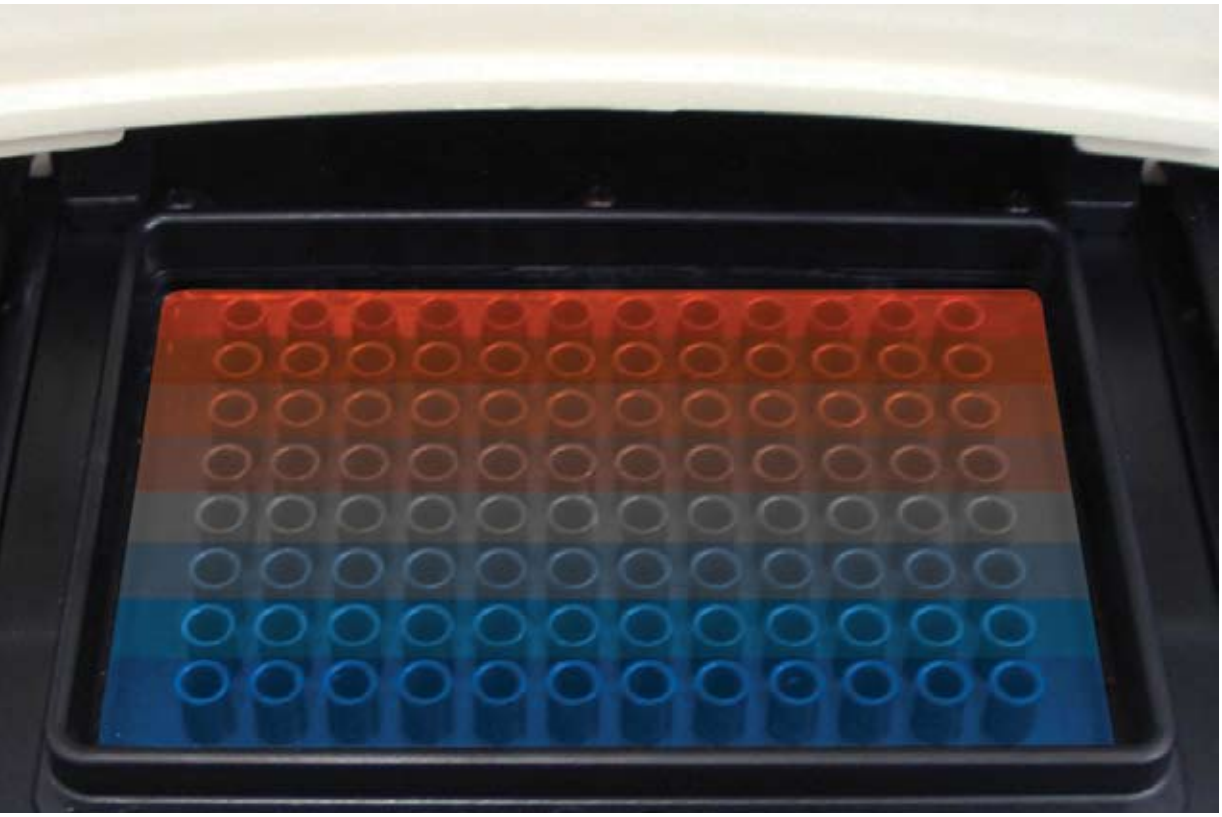

Thermal Gradient Feature
Maximize Your Optimization Power

Optimize Reactions in a Single Run

- Optimizing incubation temperatures improves speed and specificity of PCR and real-time PCR, especially with SYBR Green assays
- Thermal gradient feature allows optimization of denaturation, annealing, or extension temperatures in one experiment
- Multizone temperature control ensures accuracy and reproducibility for dependable results
- Dynamic ramping keeps incubation times constant
- Thermal gradients are available on all of Bio-Rad's thermal cyclers and real-time PCR systems

BIO-RAD

Thermal Gradient Feature

Maximize Your Optimization Power

Using a temperature gradient allows you to evaluate 8 or 12 annealing, polymerization, or denaturation temperatures in a single run, depending on the cycler model. A gradient feature is available with all Bio-Rad thermal cyclers, allowing easy optimization of PCR and real-time PCR protocols. The thermal gradient can be used to optimize reaction conditions in a single run, identify the best annealing temperature for multiple primer sets, perform reactions that require different annealing temperatures at the same time, and more.

Single Run Optimization of PCR Protocols

PCR and real-time PCR protocols can be optimized in just one run. Depending on the instrument used, up to 12 samples per temperature may be run, allowing optimization of different primer sets (Figure 1) or different reaction conditions, such as dNTP, primer, Mg^{2+} concentration (Figure 2), or polymerase used.

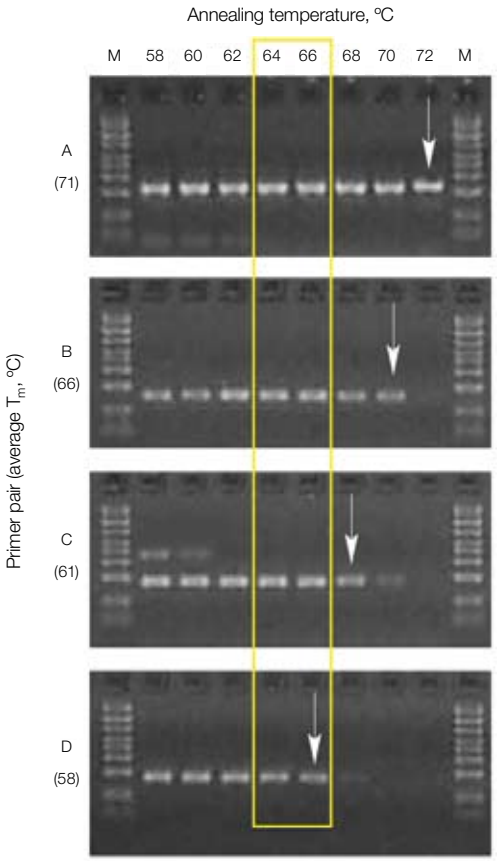

Fig. 1. Gradient optimization of different PCR reactions. All reactions were evaluated in a single run. Four different primer sets (A, B, C, and D) were designed and tested for amplification. Arrows indicate the annealing temperature that provided the highest specificity while maintaining good yield. Yellow box indicates optimal temperatures. M, markers.

Single Annealing Temperatures

Running a gradient PCR for new primer sets allows you to identify a single annealing temperature that will provide efficient, specific amplification of all targets. For example, Figure 1 shows that primer pair B amplifies properly between 58–70ºC, while pair A amplifies optimally between 64–72ºC, due to some primer-dimers below 64ºC. Nonspecific amplification is seen for pair C at 58 and 60ºC, but not above this temperature. Finally, pair D amplifies correctly between 58–66ºC. Time can be saved by running these reactions together with a common annealing temperature between 64–66ºC.

Multiple Annealing Temperatures

You can take advantage of the thermal gradient to perform different PCR reactions (same dwell times, different annealing temperatures, up to 12 samples per reaction) in the same run. For example, if a gradient from 55 to 65ºC is programmed for the annealing step, up to 12 samples can be run in row A with an annealing temperature of 65ºC, another 12 samples in row D at 61ºC, 12 more in row F at 57ºC, and another 12 at 55ºC in row H.

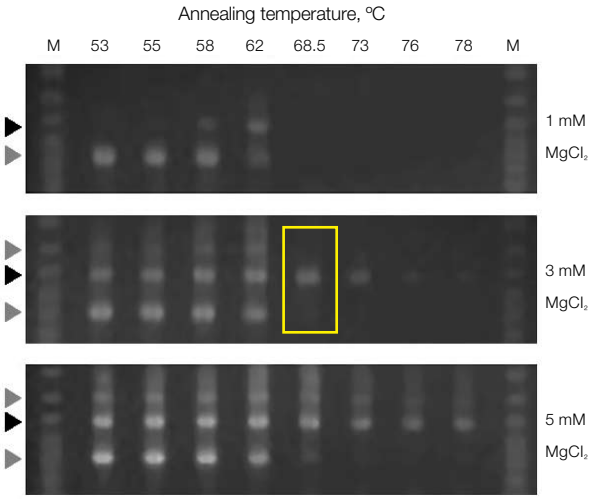

Fig. 2. Temperature and $MgCl_2$ effects on PCR. All reactions were evaluated in a single run. The specific product (▶) is obtained at good yield only at very specific conditions (T_a = 68.5ºC and 3.0 mM $MgCl_2$). Upper and lower bands (▶) are unwanted PCR products. Yellow box indicates optimal temperature. M, markers.

Faster Optimization of Fast PCR Protocols

A thermal gradient can be used to find the highest possible annealing/extension temperature and the lowest possible denaturation temperature to minimize temperature excursions, thereby reducing overall protocol run times.

The following simple strategy can quickly optimize a PCR reaction for minimal hold times, minimal ramping time, and shortest overall run times (Table 1).

- Begin with this fast PCR protocol template: 98°C, 30 sec; then 35 cycles of 92°C, 1 sec, xx°C, 15 sec, where xx = temperature gradient; then 72°C, 1 min
- Use a temperature gradient (for example, 0–10°C above the lowest primer T_m) to find the highest possible annealing/extension temperature
- Perform a second run with a temperature gradient (for example, 85–95°C) to find the lowest possible denaturation temperature during cycling

For more information, request technical bulletin 5362 or BioRadiations 118.

Table 1. Example optimization strategy for fast PCR.

Optimization Stage	PCR Protocol	Run Time
Before optimization	95°C, 3 min; then 35 cycles of 95°C, 15 sec, 60°C, 30 sec, 72°C, 30 sec; then 72°C, 10 min	88 min
Hold times reduced and annealing and extension steps combined using guidelines	98°C, 30 sec; then 35 cycles of 92°C, 1 sec, 60°C, 15 sec; then 72°C, 1 min	60 min
Gradient used to minimize temperature excursions	98°C, 30 sec; then 35 cycles of 90°C, 1 sec, 65°C, 15 sec; then 72°C, 1 min	36 min

Robust Real-Time Allelic Discrimination Assays

In allelic discrimination assays, using a thermal gradient can be used to identify the annealing temperature that will ensure amplification of only a specific allele by each primer pair (Figure 3) (Ugozzoli et al. 2004). For more information, request technical bulletin 3024.

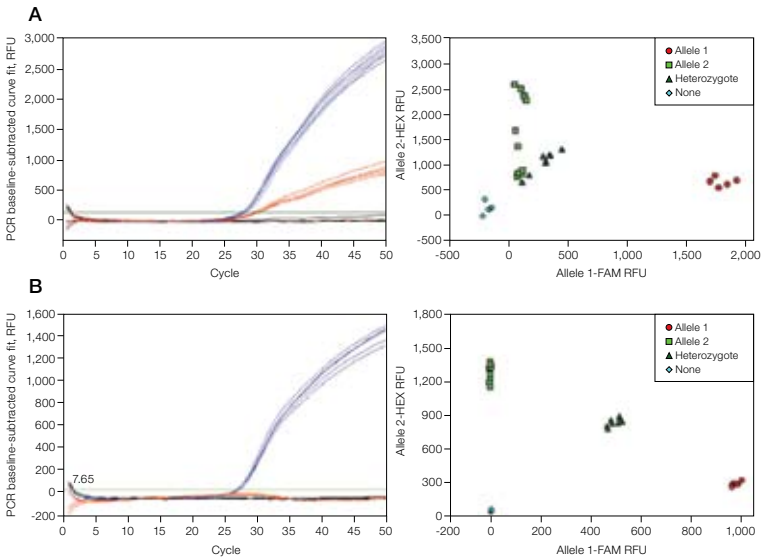

Fig. 3. Gradient optimization of real-time PCR allelic discrimination assays. An annealing temperature gradient from 55 to 70ºC was used. Only the wild type (WT) probe amplification is shown. **A**, WT probe detects amplification of WT (■) but also mutant (■) homozygous samples at annealing temperatures of 55, 56.1, 57.9, or 60.5ºC (left). This results in an allelic discrimination plot with scattered clusters where some samples are difficult to genotype (right); **B**, WT probe detects only WT samples at 64.3ºC (mutant samples are not detected) (left). Subsequent allelic discrimination analysis shows compact clusters that allow unambiguous genotyping (right).

Assurance of the Highest Reproducibility and Uniformity

Multizone temperature control across the block, with up to four embedded thermosensors, ensures high reproducibility of the gradient from run to run and from instrument to instrument. Moreover, this multisensor design guarantees the highest level of temperature uniformity across the block in nongradient protocols (Figure 4). Nongradient blocks usually have simpler designs with only one or two sensors and cannot provide the same level of temperature uniformity as the Bio-Rad gradient block design.

Reference

Ugozzoli LA et al., Real-time genotyping with oligonucleotide probes containing locked nucleic acids, Anal Biochem 324, 143–152 (2004)

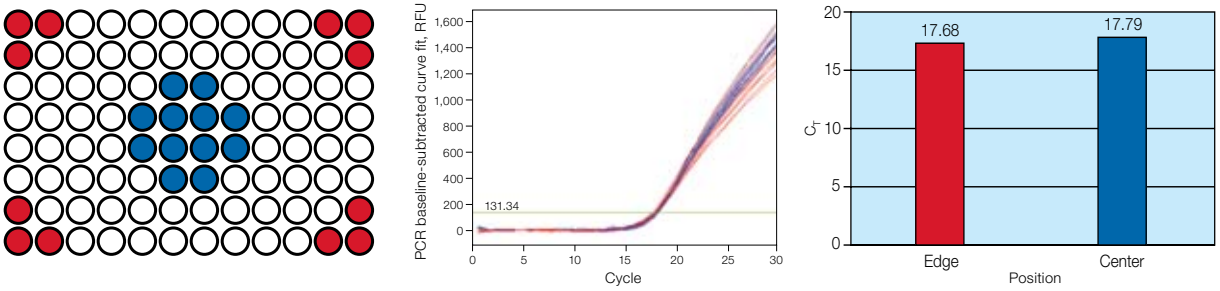

Fig. 4. Uniformity of a real-time PCR assay. Replicate samples were amplified in an iCycler iQ® cycler in the center wells (■) or edge wells (■) of a 96-well block (left panel) and detected in real-time with a specific probe (center panel). C_T values were statistically compared using a Mann-Whitney *U* test to show no significant differences ($\alpha = 0.01$) (right panel).

Specifications

	MJ Mini™ Cycler	MyCycler™ Cycler	iCycler® Cycler	DNA Engine® Cycler	DNA Engine Dyad® Cycler	Dyad Disciple™ Cycler	DNA Engine Tetrad® 2 Cycler
							
Sample capacity	48 x 0.2 ml tubes; tube strips or plates	96 x 0.2 ml tubes; tube strips or plates	Depends on reaction block used	Depends on reaction block used	Depends on reaction block used	Depends on reaction block used	Depends on reaction block used
Speed of ramping	Up to 2.5°C/sec	Up to 2.5°C/sec	Up to 3.3°C/sec	Up to 3°C/sec*	Up to 3°C/sec*	Up to 3°C/sec*	Up to 3°C/sec*
Temperature range	0–99°C	4–100°C	4–100°C	0–105°C*	0–105°C*	0–105°C*	0–105°C*
Temperature accuracy	±0.2°C	±0.5°C	±0.3°C	±0.3°C	±0.3°C	±0.3°C	±0.3°C
Temperature uniformity	±0.4°C	±0.5°C	±0.4°C	±0.4°C	±0.4°C	±0.4°C	±0.4°C
Dimensions (W x D x H)	19 x 32 x 20 cm	24 x 44 x 21 cm	26.4 x 54.6 x 23 cm	24 x 35 x 25 cm	47 x 29 x 21 cm	46 x 28 x 21 cm	47 x 61 x 21 cm
Weight	4.1 kg	10 kg	10 kg	5.8 kg**	10.4 kg**	10.1 kg**	21.6 kg**
Display	64 x 128 LCD	12 cm (4.7") high-resolution 1/4 VGA	1/4 VGA screen	20 x 4 alphanumeric LCD	320 x 240 pixels 256 colors	No display PC controlled	320 x 240 pixels 256 colors
Memory	400 typical programs	99 typical programs	255 typical programs	400 typical programs	1,000 typical programs	No intrinsic memory — relies on DNA Engine Dyad or PC	1,000 typical programs
Resumes after power outage	Yes	Yes	Yes	Yes	Yes	PC controlled	Yes
Gradient feature	Yes	Yes	Yes (96-well block)	Yes (96-well block)	Yes (96-well block)	Yes (96-well block)	Yes (96-well block)
Gradient layout	Rows	Rows	Rows	Columns	Columns	Columns	Columns
Number of temperatures	8	8	8	12	12	12	12
Gradient accuracy	±0.2°C	±0.5°C	±0.3°C	±0.3°C	±0.3°C	±0.3°C	±0.3°C
Row/column uniformity	±0.4°C	±0.5°C	±0.4°C	±0.4°C	±0.4°C	±0.4°C	±0.4°C
Gradient range	35–99°C	30–99°C	40–99°C	30–105°C	30–105°C	30–105°C	30–105°C
Temperature differential	1–16°C	1–25°C	1–25°C	1–24°C	1–24°C	1–24°C	1–24°C
Real-time PCR system upgrade path	MiniOpticon™	—	MyiQ™ (one color) iQ™5 (5-color)	Chromo4™	—	Chromo4 (up to 2 modules)	—
Gradient available for real-time PCR	Yes	—	Yes	Yes	—	Yes	—
Ordering Information							
Thermal cyclers	PTC-1148	170-9703, MyCycler 170-9707, gradient upgrade	170-8720, iCycler 96-well thermal cycler	PTC-0200G, chassis ALS-1296G, 96-well block	PTC-0220G, chassis ALS-1296G, 96-well block	PTC-0221G, chassis ALS-1296G, 96-well block	PTC-0240G, chassis ALS-1296G, 96-well block
Real-time upgrades			170-9775 MyiQ 170-9785 iQ5	CFB-3240G Chromo4 detector		CFB-3240G Chromo4 detector	

* Some thermal cycler specifications do not apply to dual-block and Slide Chambers™. For specifications for these units, go to **www.bio-rad.com/amplification/**
** Chassis only. 96-well Alpha™ unit weighs 3.2 kg.

SYBR is a trademark of Invitrogen Corporation.

Notice regarding Bio-Rad thermal cyclers and real-time systems.
Purchase of this instrument conveys a limited non-transferable immunity from suit for the purchaser's own internal research and development and for use in applied fields other than Human In Vitro Diagnostics under one or more of U.S. Patents Nos. 5,656,493, 5,333,675, 5,475,610 (claims 1, 44, 158, 160–163 and 167 only), and 6,703,236 (claims 1–7 only), or corresponding claims in their non-U.S. counterparts, owned by Applera Corporation. No right is conveyed expressly, by implication or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5' nuclease methods. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Bio-Rad's real-time thermal cyclers are licensed real-time thermal cyclers under Applera's United States Patent No. 6,814,934 B1 for use in research and for all other fields except the fields of human diagnostics and veterinary diagnostics.

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