Application Note

Maximising performance of **PCRBIO DNA Markers** in agarose gel electrophoresis

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Introduction

DNA ladders are essential molecular tools for estimating the size of DNA fragments during agarose gel electrophoresis, a routine technique in molecular biology. These ladders contain DNA fragments of known sizes, allowing researchers to determine the approximate length of DNA samples by comparison. Accurate performance of DNA ladders is critical for reliable data interpretation, particularly in applications such as genotyping, cloning, and verifying the success of PCR amplification. Variability in ladder performance can arise from multiple factors, including the gel matrix, staining method, and electrophoretic conditions.

Gel staining dyes are equally pivotal in visualising DNA fragments through their ability to bind to nucleic acids and fluoresce under UV or blue light. The compatibility of these dyes with different DNA ladders, agarose quality and concentration, and duration of electrophoresis can significantly influence the clarity and sharpness of bands. Therefore, a systematic evaluation of DNA ladder performance under various agarose concentrations and staining conditions is necessary to identify optimal combinations that ensure reproducibility and resolution.

This application note focuses on assessing the performance of PCRBIO DNA Markers (Ladders I-IV) using six different gel staining dyes on a range of agarose gel concentrations and running times. By examining these variables, we aim to provide a practical guide for achieving consistent and high-resolution results in gel electrophoresis across diverse experimental setups when using PCRBIO DNA Ladders.

Materials and Methods

Reagents

Top Vision agarose from Thermo Fisher Scientific was used for gel preparation. All gels were run in 1x TAE (Tris-Acetate-EDTA) Buffer. PCRBIO DNA Markers, Ladder I (100 bp – 10 kb, cat. no. PB40.11), Ladder II (250 bp – 10 kb, cat. no. PB40.12), Ladder III (50 bp – 1500 bp, cat. no. PB40.13), and Ladder IV (100 bp – 1500 bp, cat. no. PB40.14) were tested with the following dyes:

1. SYBR™ Safe DNA Gel Stain, 10000x (Thermo/ Invitrogen)
2. EcoDye™ Nucleic Acid Staining Solution, 10000x (BIOFACT Co.)
3. MIDORI Green Advance DNA Stain, 25000x (Nippon Genetics)
4. MIDORI Green Xtra DNA Stain, 25000x (Nippon Genetics)
5. GelRed® Nucleic Acid Stain, 10000x (Merck/ Millipore)
6. GelRed® Prestain Loading Buffer with Orange Tracking Dye, 6x (Biotium)

Experimental conditions

Ladder dilutions were prepared by adding 10 µL ladder stock to 90 µL Milli-Q water along with 20 µL of 6x Sample Loading Buffer A.

Gels were prepared by dissolving agarose in TAE buffer, followed by the addition of staining dye as required according to the manufacturer's instructions. Dyes were added directly to the agarose gel, except

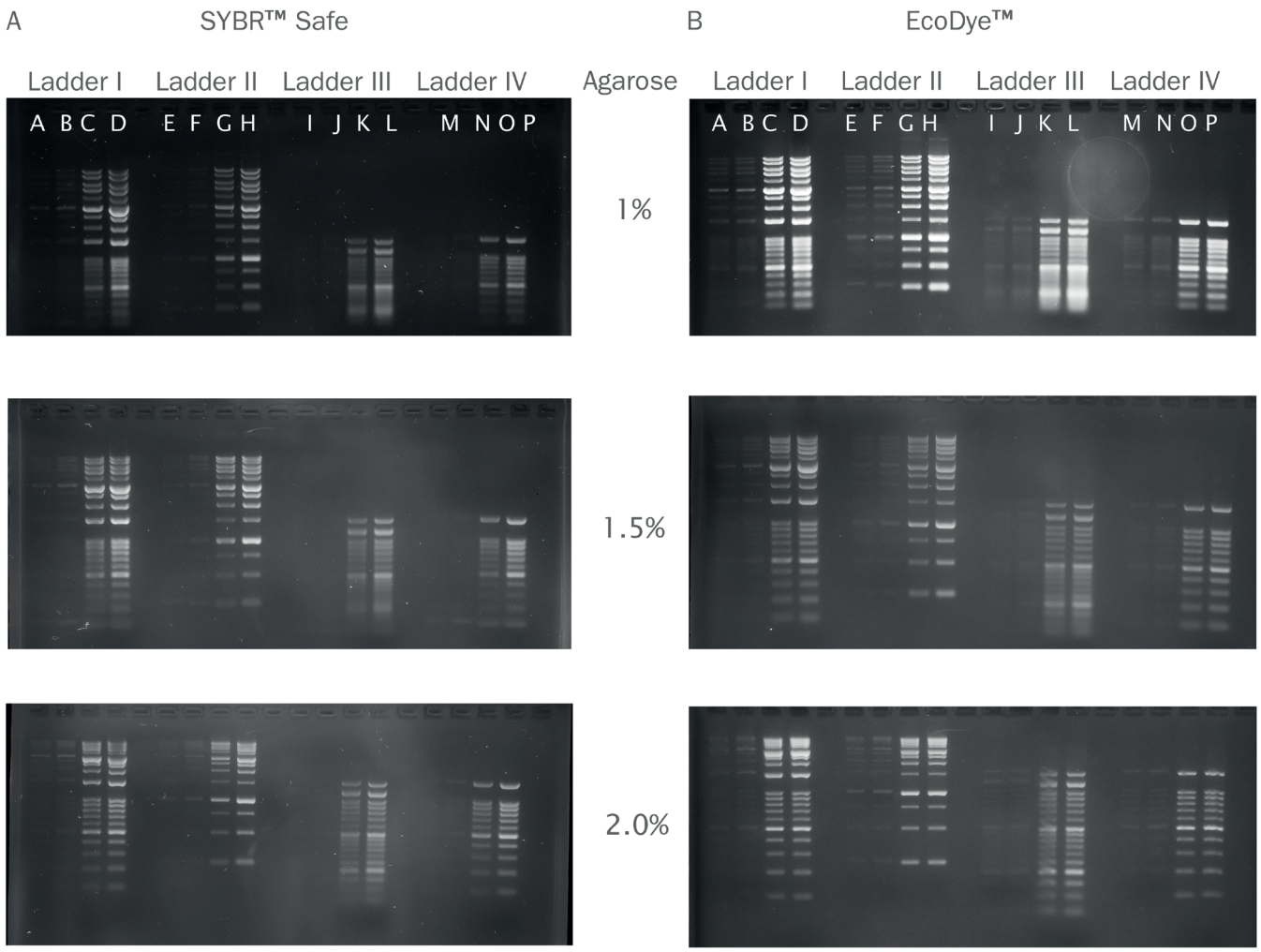

Figure 1. PCRBIO DNA Markers stained with SYBR™ Safe and EcoDye™.

PCRBIO Ladders I–IV were run on 1%, 1.5%, and 2% agarose gels stained with SYBR™ Safe (A) and EcoDye™ (B). Electrophoresis was performed at 90 V for 60 minutes. Lanes A and B contain 5 µL and 10 µL of 1:10 diluted Ladder I, respectively, while lanes C and D, respectively, contain 5 µL and 10 µL of undiluted Ladder I. The same loading pattern applies to Ladders II (lanes E–H), III (lanes I–L), and IV (lanes M–P).

for GelRed® Loading Buffer, which was added directly to the ladder samples prior to loading.

1%, 1.5%, 2% agarose gels were prepared. 5 µL and 10 µL of both stock and the 1:10 dilution of each DNA ladder were added to each gel and dye combination. All gels were run at a steady voltage of 90 V for 20–90 minutes, with images captured at intervals throughout each run.

Results and Discussion

Effects of ladder dilution

Diluting all ladders 1:10 generally resulted in weak or undetectable bands (Fig. 1 and 2), except for GelRed dyes, which generated well-defined, visible, bands even with the lowest amount of ladder loaded (Fig. 3). Conversely, undiluted ladders provided sharper and more consistent results with most dyes (Fig. 1 and 2), but the use of GelRed® Stain led to poorly resolved bands or smears (Fig. 3).

Effect of different gel dyes

- SYBR™ Safe: Provided good overall band intensity and resolution when undiluted ladders were used. Ladder III showed poor separation, particularly for low molecular weight bands on both 1% and 1.5% agarose gels. It did provide better resolution on 2% agarose (Fig. 1A).
- EcoDye™: Produced strong signal and very good band separation with all undiluted ladders. Undiluted Ladder III showed good separation on both 1.5% and 2% agarose gels, but lower molecular weight bands were not readily distinguishable on a 1% gel (Fig. 1B)
- MIDORI Green Advance: Showed results very similar to EcoDye™, with strong signal and good separation for all undiluted ladders, except for Ladder III on 1% and 1.5% agarose gels. Ladders I, II, and IV showed very similar results regardless of whether 5 or 10 µL were loaded per lane (Fig. 2A).
- MIDORI Green Xtra: Resulted in strong signal when using undiluted ladders. Both 5 and 10 µL of

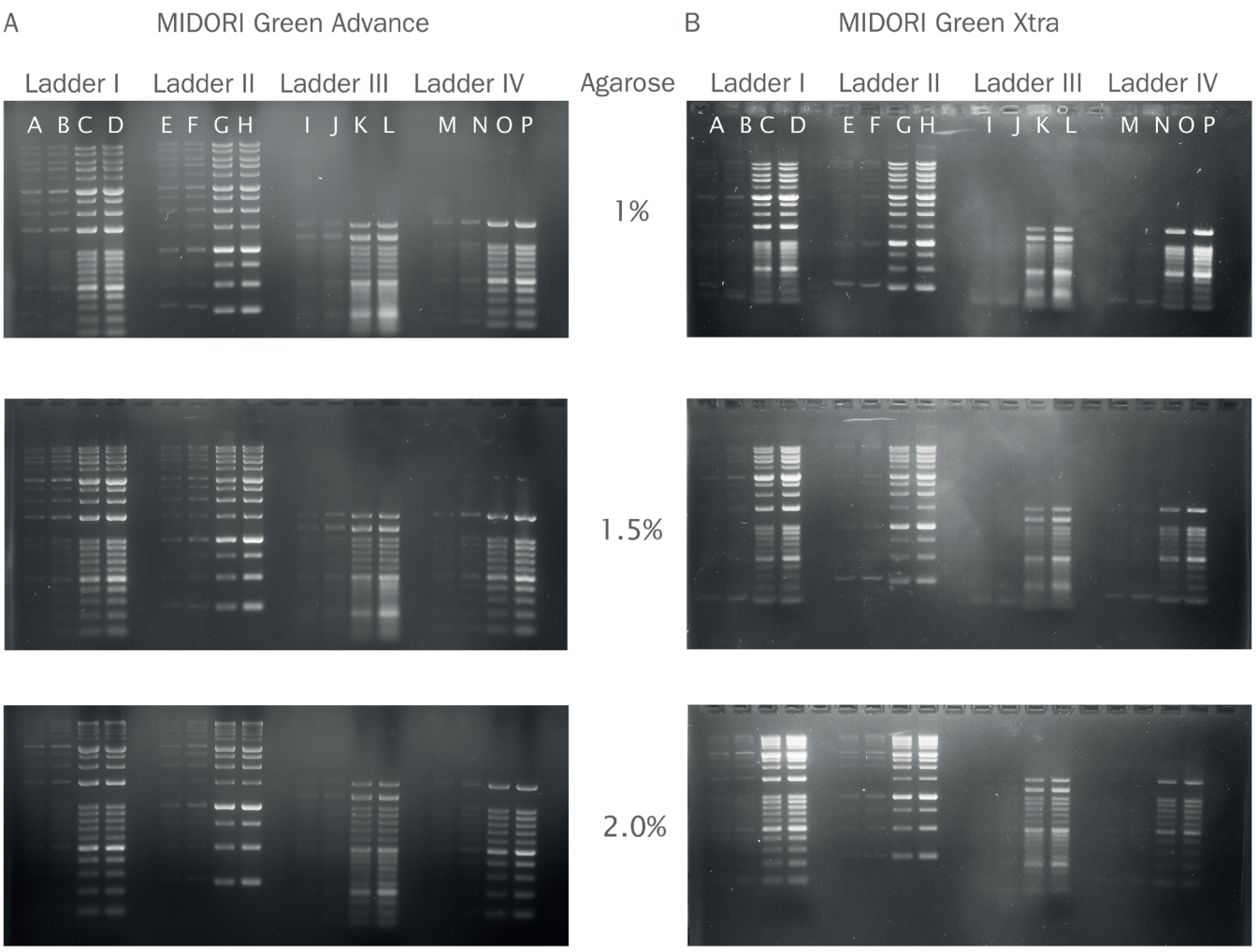

Figure 2. PCRBIO DNA Markers stained with MIDORI Green Advance DNA Stain and MIDORI Green Xtra Stain.

PCRBIO Ladders I–IV were run on 1%, 1.5%, and 2% agarose gels stained with MIDORI Green Advance (A) and MIDORI Green Xtra (B). Electrophoresis was performed at 90 V for 60 minutes. Lanes A and B contain 5 µL and 10 µL of 1:10 diluted Ladder I, respectively, while lanes C and D, respectively, contain 5 µL and 10 µL of undiluted Ladder I. The same loading pattern applies to Ladders II (lanes E–H), III (lanes I–L), and IV (lanes M–P).

undiluted ladders have similar results. Ladders had poor band separation on both 1% and 1.5% gels but did separate reasonably well on 2% agarose. Ladder IV also separated poorly on a 1% agarose gel but showed reasonable separation on 1.5% and 2% gels. In general, the challenge using this dye is that for long run times, the intensity of bands drops significantly to nearly undetectable (Fig. 2B).

- GelRed® Stain: Showed high signal intensity and sharp band resolution when diluted ladders were used. All ladders were nicely resolved at all agarose concentrations. Ladder III gave best results in gels with 1.5% agarose or more and running for at least 60 min (Fig. 3A). Separation further improved up to 90 min without loss in intensity.
- GelRed® Loading Buffer: Had good fluorescence and had the most consistent band resolution with both diluted and undiluted ladders across all agarose concentrations tested. Ladder III showed reduced resolution on 1% and 1.5% agarose, when not diluted. Best results with this ladder were obtained when loading 10 µL of a 1:10 dilution. A significantly faster run of the ladders was achieved when more DNA was loaded on the gel, with 10 µL undiluted ladder being apparently faster that that obtained with 5 µL, and this in turn was faster than the 2 diluted samples (Fig. 3B). Therefore, although the appearance of the ladders is probably the best with this dye, results depend on the amounts of DNA loaded, making analysis of the band of interest harder than with other staining methods.

Effect of agarose concentration

As expected, each ladder behaved differently on the various agarose gel concentrations tested. Ladders I and II span a wider range of molecular weights (up to 10 kb) and were thus favoured by lower agarose concentrations. Conversely, ladders III and IV span lower molecular weights (up to 1500 bp) at a higher resolution and thus were favoured by high agarose concentrations (Fig. 1-3).

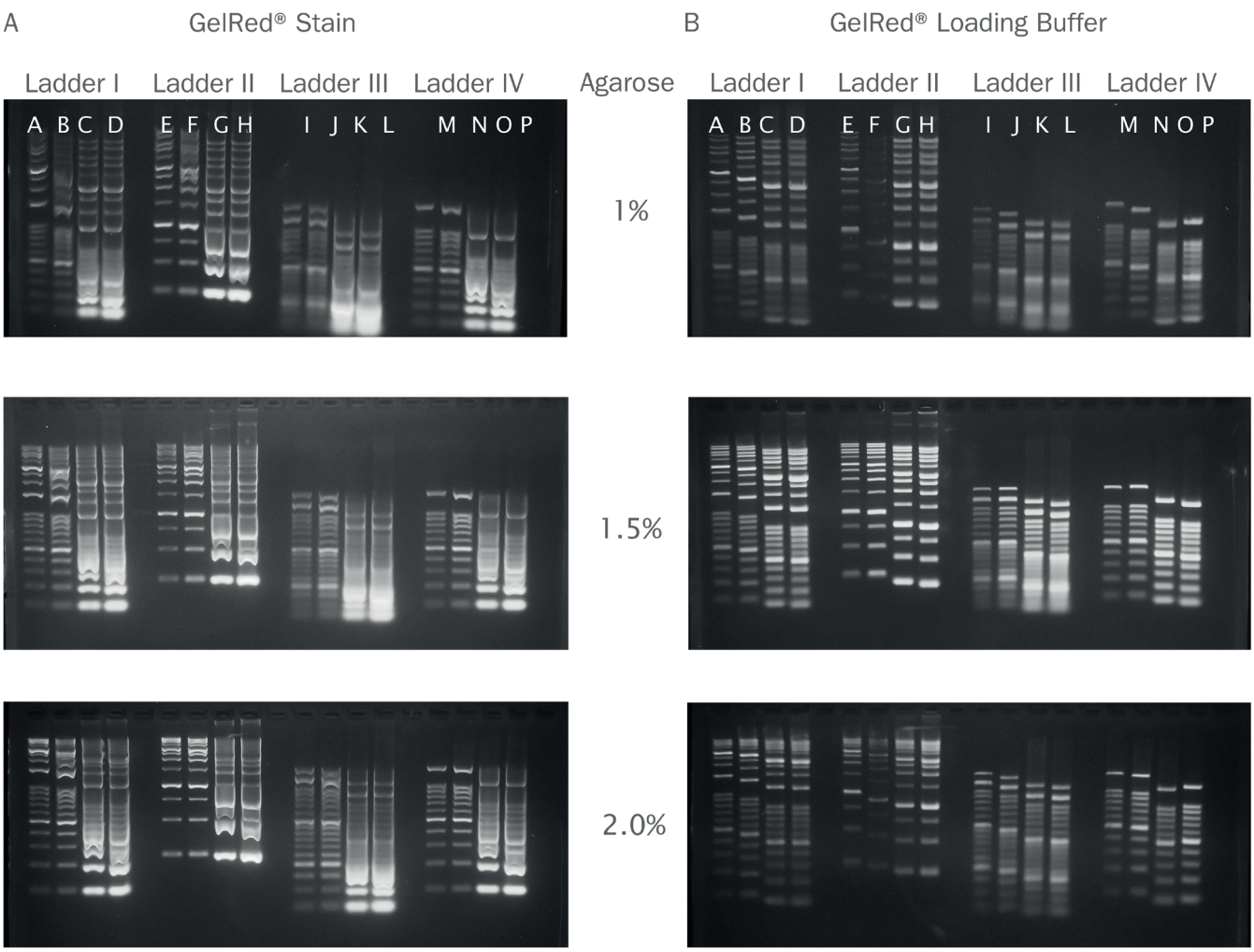

Figure 3. PCRBIO DNA Markers stained with GelRed® Stain and GelRed® Loading Buffer.

PCRBIO Ladders I–IV were run on 1%, 1.5%, and 2% agarose gels stained with GelRed® Stain (A) and GelRed® Loading Buffer (B). Electrophoresis was performed at 90 V for 60 minutes. Lanes A and B contain 5 µL and 10 µL of 1:10 diluted Ladder I, respectively, while lanes C and D, respectively, contain 5 µL and 10 µL of undiluted Ladder I. The same loading pattern applies to Ladders II (lanes E–H), III (lanes I–L), and IV (lanes M–P).

- 1% Agarose: Favoured the resolution of larger fragments but compromised lower bands. Ladders I, II, and IV showed good separation of most bands with all the tested dyes. Some titration of the amount of ladder was needed to ensure all bands were discrete. Ladder III ran poorly on this agarose concentration, due to the high number of fragments present. Best results for this ladder on this gel concentration were obtained when running 10 µL of 1:10-diluted ladder with GelRed® Loading Buffer or 5 µL of ladder stock with EcoDye™. However, even under these conditions, bands below 500 bp did not resolve well.
- 1.5% Agarose: Offered the most uniform resolution with all ladders, although titration of ladder amount was necessary to generate best results. Ladder III had poor resolution below 500 bp in combination with the SYBR™ Safe and MIDORI Green Xtra dyes.
- 2% Agarose: Enhanced the resolution of smaller fragments and therefore was the best concentration to use for Ladder III and IV. Ladder I and II did not resolve well above 2-3 kb, with best results for these regions obtained when using MIDORI Green Advance, GelRed® Loading Buffer, and to a lesser extent MIDORI Green Xtra. Loading diluted ladders resulted in poor to no band detection with all dyes, except for the GelRed dyes.

Effect of run time

Longer run times favour band separation for all ladders on all agarose concentrations and dye combinations. This is relevant for the lower molecular weight Ladders III and IV, which are susceptible to poor separation at short run times on less than 2.0% agarose gels. Conversely, run time also impacts the broad molecular weight Ladders I and II on higher agarose concentrations as the larger bands separate poorly at agarose concentrations above 1.5%. For instance, bands above 1 kb in Ladders I and II, and most of the bands of Ladders III and IV remain clumped together and appear as a blur or smear after 30 minutes of electrophoresis with SYBR™ Safe (Fig. 4A, top) and with GelRed® Loading Buffer (Fig. 4B, top) on 2% agarose gels. However, all ladders are fully

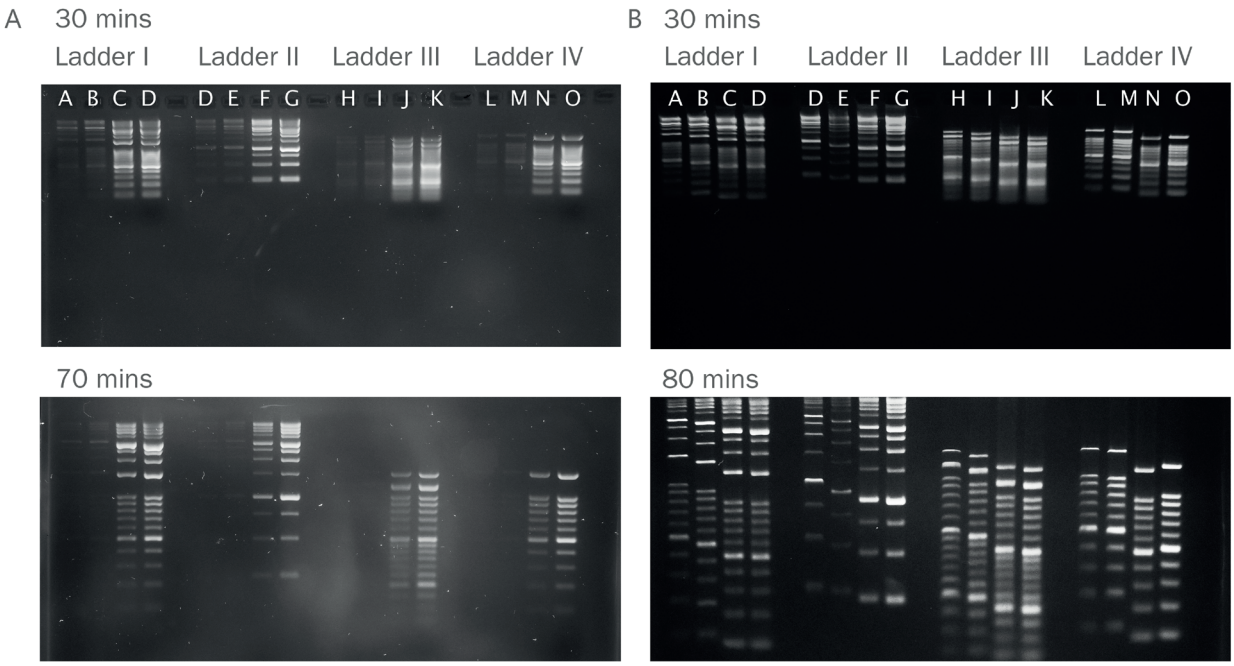

Figure 4. Effect of runtime on PCRBIO DNA Markers.

PCRBIO Ladders I–IV were run on 2% agarose gels stained with SYBR™ Safe (A) and GelRed® Loading Buffer (B). Electrophoresis was performed at 90 V for 30 min (top images) and 70 or 80 min as indicated (bottom image). Lanes A and B contain 5 µL and 10 µL of 1:10 diluted Ladder I, respectively, while lanes C and D, respectively, contain 5 µL and 10 µL of undiluted Ladder I. The same loading pattern applies to Ladders II (lanes E–H), III (lanes I–L), and IV (lanes M–P).

resolved with longer runs for both dyes (Fig 4, bottom panels). This highlights the importance of run time in achieving good band separation, particularly when visualising shorter DNA fragments. This highlights the importance of run time in achieving good band separation, particularly when visualising shorter DNA fragments.

Important to note is the fact that if on one hand longer run times may help the separation of the bands, for some of the dyes tested a visible reduction of signal was seen, so a good balance between time and visibility must be looked for when using EcoDye™, and both MIDORI Green dyes.

The effects of gel run time on all agarose concentrations in combination with each of the tested dyes is summarised in (Table 1). Recommended run times for sufficient separation of all bands in each ladder are shown in minutes. Values for SYBR™ Safe, EcoDye™, and both MIDORI Green dyes are estimated for 5-10 µL of undiluted ladder, whereas times for the GelRed® Loading Buffer and GelRed® Stain are shown for 5-10 µL of the 1:10 diluted ladder.

Recommendations for best performance of different ladder types

Optimal ladder loading

Use of undiluted ladders (5–10 µL) is recommended for clear and consistent results with all green dyes tested and agarose concentrations. For GelRed® Stain and Loading Buffer, loading 5-10 µL of 1:10-diluted ladder will yield best and most accurate results.

Increasing the amount of ladder loaded on a gel causes a shift in the migration of ladder bands of the same molecular weight. This is more pronounced with some dyes (*e.g.*, GelRed® Loading Buffer) than others. Thus, care should be taken when comparing apparent molecular weight of DNA targets estimated in comparison to different amounts of DNA ladder.

Optimal agarose concentration

Ladder I and II: Are best used on gels with ≤1.5% agarose concentration. Running these ladders above this concentration will prevent separation of higher molecular weight bands and is not recommended.

Ladder III and IV: Resolve well on ≥1.5% agarose gels.

Ladder III does not resolve well below 500 bp on 1.5% agarose gels, except when 10 µL of diluted ladder is used with GelRed dyes or 10 µL of undiluted ladder is used with the MIDORI Green Advance stain. This ladder is ideal for high-resolution applications and should only be used when detailed band separation below 1500 bp is required and ideally run on 2-2.5% agarose gels. Longer running times are required for optimal separation of lower molecular weight fragments.

Agarose concentration	SYBR™ Safe	EcoDye™	MIDORI Green Advance	MIDORI Green Xtra	GelRed® Stain	GelRed® Loading Buffer	Comment
				Ladder I			
1.0%	60	50	40	40	40	40	
1.5%	50	50	40	30	40	40	
2.0%	70	>80	40	60	70	70	N.R.
				Ladder II			
1.0%	50	40	30	30	40	30	
1.5%	50	50	40	30	40	40	
2.0%	60	80	30	50	80	70	N.R.
				Ladder III			
1.0%	>90 F	>90	>80	>60 F	>80	80	N.R.
1.5%	>70	60	80	>60 F	50	60	N.R.
2.0%	50	30	50	40	50	40	
				Ladder IV			
1.0%	60	60	40	40	40	50	
1.5%	60	40	40	40	40	40	
2.0%	50	30	30	30	40	30	

Table 1. Time (minutes) to distinguish all bands in each PCRBIO DNA Marker, when run at 90 V on a 14 cm gel.

For GelRed® Stain and GelRed® Loading Buffer time is estimated for 5-10 µL diluted ladder, for all other dyes for 5-10 µL undiluted ladder stock. Times preceded by ">" indicate that bands did not separate well up to this point, "F" denotes bands became too faint to observe after this time point. "N.R." denotes that running the indicated ladder is not recommended at that specific agarose gel concentration.

Ladder IV showed well-separated bands across all conditions but performed best with 2% agarose and GelRed® Stain. This ladder also resolves well on 1.5% and is recommended for most applications requiring basic size estimation of low molecular weight (<1500 bp) bands. It is a good alternative to Ladder III if extremely precise fragment size estimation isn't required.

Optimal gel staining dyes

GelRed® Loading Buffer provided the best balance of signal and resolution across all gel concentrations and amounts of ladder loaded, although the DNA concentration-dependent run may cast some doubts on the real size of the fragment of interest.

GelRed® Stain worked well when diluted ladder were used and enabled good band resolution and uniform intensity across the different gel concentrations. As such, it is probably the best option for use with PCRBIO DNA Markers and is also cost-effective because only small volumes of dye are required per gel.

SYBR™ Safe, EcoDye™ and MIDORI Green dyes all give good results when undiluted ladders are used, but care should be taken to run the various ladders on the recommended agarose concentrations (see points above). Also important to note, the intensity of the bands will decrease with longer runs more than with the GelRed dyes.

Conclusion

PCRBIO DNA Markers, when used under optimised conditions, deliver reliable performance for diverse molecular biology applications. Based on these findings, researchers can tailor their gel electrophoresis setups to meet specific experimental needs, optimise the presentation and interpretation of their results.

Ordering Information

Please reach out to our team with any queries or to get a quote for PCRBIO DNA Markers by email: info@pcrbio.com. Please refer to Table 2 below for available pack sizes and catalogue numbers.

Product	Lanes	Cat. No.
PCRBIO Ladder I (100bp - 10kb)	100	PB40.11-01
	500	PB40.11-05
PCRBIO Ladder II (250bp - 10kb)	100	PB40.12-01
	500	PB40.12-05
PCRBIO Ladder III (50bp - 1500bp)	100	PB40.13-01
	500	PB40.13-05
PCRBIO Ladder IV (100bp - 1500bp)	100	PB40.14-01
	500	PB40.14-05
Sample Loading Buffer	1 mL	PB40.61-01
	5 mL	PB40.61-05

Table 2. PCRBIO DNA Marker pack sizes and catalogue numbers