DNA Dye Detection Limits using Azure cSeries Imagers

Introduction

Gel-based detection of DNA was first described in 1972 by Aaij and Borst[1] and has gone on to become a cornerstone technique of molecular-biology. With the development of increasingly advanced and sensitive DNA technologies, reliable, accurate quantification of low concentration DNA products is becoming increasingly desirable.

The traditional method for gel-based DNA detection is to add ethidium bromide (EtBr) during gel casting. However, the use of EtBr is falling out of favor due to the mutagenic characteristics of EtBr arising from its intercalation of double stranded DNA which may affect DNA replication or transcription.

Additionally, although EtBr is not classified as hazardous waste at low concentrations many organizations treat it as such, often at great expense. Whilst staining of gels post-run with EtBr can reduce the amount of hazardous waste generated, it does not address issues arising from exposure of workers to a mutagen. Because of this a variety of non-mutagenic DNA dyes have been developed; however, unless they are capable of matching, or improving on the sensitivity of EtBr then their use is unlikely to gain traction. With the rise and advancement of digital imaging technology and techniques, the use of non-mutagenic DNA dyes ("safe" stains) has increased in popularity do to their combined ease of use and dynamic range. Therefore, we assessed the limit of detection (LoD) for various DNA dyes using cSeries imagers from Azure Biosystems.

Methods

Sample Preparation

New England Biolabs Quick Load 1kb DNA Ladder, which features known concentrations of DNA for each band, was serially diluted 1:1 in water and 6X gel loading dye (See Table 1 for dilutions).

Gel Casting and Running

0.8% agarose gels in 1x TAE were cast, 10 µL of each dilution was loaded and gels were run in 1x TAE for 120 to 150 minutes at 70V. One gel was cast with EtBr at a dilution of 1:10,000, all other gels were cast without dye and post-stained. Post-staining was performed with a 1:10,000 dilution of either EtBr, EZ-Vision® Bluelight, SYBR® Gold, SYBR® Green or SYBR® Safe in TAE buffer for 30 minutes.

Gel Imaging

Following staining, gels were imaged on both the Azure c200 and c600 imagers. Gels stained with EtBr were imaged using the UV transilluminator (302 nm), all other gels were imaged using Epi-Blue lights with an orange filter. In all instances images were captured using auto-exposure settings. No difference in sensitivity was detected between images generated by the Azure c200 or c600 confirming that any limit of detection differences are dye dependent.

Band	4kb	3kb	2kb
Lane	Mass (pg) in 10 µL		
1	4125	15625	6000
2	2063	7813	3000
3	1031	3906	1500
4	516	1953	750
5	258	977	375
6	129	488	188
7	64	244	94
8	32	122	47

Table 1. DNA ladder dilutions.

Results and Conclusion

In this note the sensitivity of various DNA dyes was assessed. A 1kb DNA ladder of known concentration was serially diluted and samples were resolved by agarose gel electrophoresis. One gel was cast with EtBr with all others post-stained. Sample gel images displaying the 2kb band are shown (Figure 1) along with a summary of DNA dye LoDs (Table 2).

Briefly, post-staining of gels with EtBr did not provide the same LoD as casting gels with EtBr in situ. All alternative DNA dyes displayed a similar LoD to EtBr post-stained when observing larger DNA fragments. Whilst EtBr displayed an enhanced LoD, when detecting smaller fragments, compared to EZ-Vision® Bluelight, SYBR® Gold and SYBR® Green; SYBR® Safe demonstrated an identical LoD.

These results suggest that labs looking to reduce worker exposure to mutagenic EtBr whilst also reducing the amounts of hazardous waste produced do not have to accept a DNA dye with a lower limit of detection.

Reference

1. Aaij, C. & Borst, P. The gel electrophoresis of DNA. *Biochim Biophys Acta* 269, 192-200 (1972).

Band	4kb	3kb	2kb
Stain	Limit of Detection (pg)		
EtBr Cast in Gel	32	122	47
EtBr post-stained	64	244	94
EZ-Vision® Bluelight	32	244	188
SYBR® Gold	32	122	94
SYBR® Green	32	244	94
SYBR® Safe	32	122	47

Table 2. Summary of DNA dye LoDs. Gray shading indicates highest limit of detection.

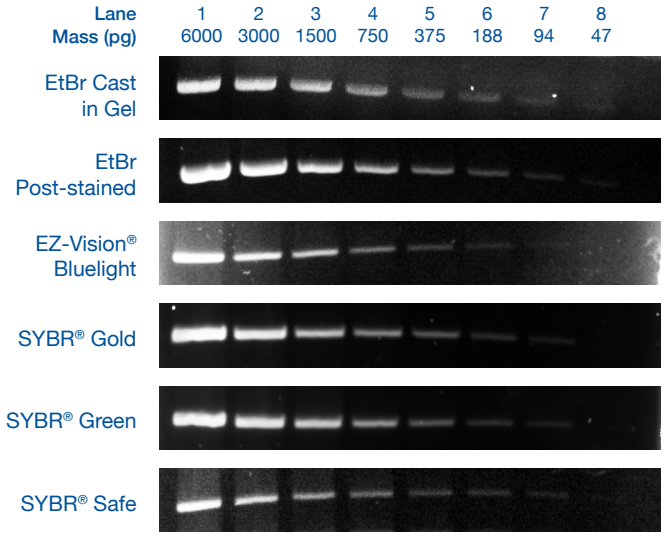

Figure 1. Samples gel images. Representative blots showing 2kb band of DNA ladder.

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