Problem or Question	Comments and Suggestions/Answers
Smeared or smiling DNA band(s) or discrepant DNA migration	Many customers use GelRed precast gels for convenience. However, because GelRed and GelGreen are high affinity dyes designed to be larger dyes to improve their safety, they can affect the migration of DNA in precast gels. Some samples, such as restriction digested DNA may migrate abnormally in GelRed precast gels. The following modifications may improve band resolution in precast gels. 1. Reduce the amount of DNA loaded. Smearing and smiling is often caused by overloading of DNA. The recommended loading amount for ladders and samples of known concentration is 50-200 ng/lane. For samples of unknown concentration, try loading one half or one third of the usual amount of DNA. 2. Reduce the amount of GelRed in the gel, for example use 0.5X instead of 1X final concentration of GelRed. 3. Pour a lower percentage agarose gel. Higher molecular weight DNA separates better with a lower percentage gel. 4. Change the running buffer. TBE buffer has a higher buffering capacity than TAE buffer. 5. To avoid any interference the dye may have on DNA migration, we recommend using the post-staining protocol. If your application requires loading more than the recommended amount of DNA, use the post-staining protocol.
Weak fluorescence, decreased dye performance over time, or film of dye remains on gel after post-staining	The dye may have precipitated out of solution. 1. Heat GelRed solution to 45-50^{o}C for two minutes and vortex to dissolve. 2. Store dye at room temperature to avoid precipitation.
Does post-staining require a de-staining step?	No, but de-staining can be performed if background is high.
Can GelRed and GelGreen be used to stain ssDNA or RNA?	GelRed and GelGreen can be used to stain both ssDNA and RNA, but GelRed is about 5 times more sensitive for single-stranded nucleic acids than GelGreen. Titration assays using a fluorescence microplate reader showed that the fluorescence signal of GelRed bound to ssDNA and RNA is about half that of GelRed bound to dsDNA.

What instruments can be used to detect GelRed and GelGreen?	GelRed is compatible with a standard UV transilluminator (302 or 312 nm). GelGreen has sufficient absorption between 250-300 nm and a strong absorption peak at around 500 nm. GelGreen is compatible with a 254 nm UV transilluminator or a gel reader with visible light excitation such as a Dark Reader or a 488 nm laser gel scanner.
What emission filters are suitable for use with GelRed and GelGreen?	Use the ethidium bromide filter for GelRed; use a SYBR Green or yellow filter for GelGreen. Alternatively, a long-pass yellow filter can be used with both GelRed and GelGreen. Please review the emission spectra for GelRed and GelGreen for more specific wavelengths.
Can I make GelRed/GelGreen gels ahead of time and store them for later use?	GelRed and GelGreen dyes are stable. You can store premade GelRed/GelGreen gels for later use as long as the integrity of the agarose gel does not deteriorate. We recommend storing gels at 4 °C in the dark.
What is the stability of GelRed/GelGreen in molten agarose? Can I store GelRed/GelGreen in molten agarose and pour gels as needed?	GelRed is more stable than GelGreen. We do not recommend storing GelRed in molten agarose for more than a few days.
Can I reuse a GelRed/GelGreen precast gel after running samples?	No. We do not recommend that GelRed/GelGreen gels be reused after electrophoresis because the staining intensity can be decreased with sequential electrophoresis.
Can I re-melt a GelRed/GelGreen gel and cast again?	Yes, but it may be necessary to add some more dye to the re-melted gel for the best signal.
How should I dispose of GelRed and GelGreen?	GelRed and GelGreen passed the EPA regulated Title 22 test. Some facilities have approved the disposal of GelRed and GelGreen directly down the drain. However, because regulations vary, please contact your safety office for local disposal guidelines. Please review the GelRed/GelGreen safety report for more detailed information.
Are GelRed and GelGreen compatible with downstream applications such as cloning, ligation and sequencing?	Yes. We recommend Qiagen or Zymoclean gel extraction kits or phenol-chloroform extraction to remove the dye from DNA. Some users have reported performing PCR on

	DNA in the presence of GelRed with no purification step, for example by incubating GelRed-stained gel slices in TE buffer to extract DNA by passive diffusion for use in PCR, or by using a few microliters of molten agarose from GelRed-stained gel slices containing DNA for PCR.
How safe is GelRed/GelGreen?	In AMES and related tests, GelRed and GelGreen were shown to be much safer alternatives to EtBr and SYBR dyes. Nevertheless, please exercise safe laboratory practices when using these reagents.
Where can I find more information about the safety of GelRed/GelGreen?	Please visit our website www.biotium.com to download a comprehensive safety report.
What is the lower detection limit of GelRed/GelGreen?	GelRed and GelGreen are ultra-sensitive dyes. Some users have reported being able to detect bands containing less than 0.1 ng DNA. However, the sensitivity of the staining will depend on the instrument capability and exposure settings.
What is the binding mechanism of GelRed/GelGreen?	GelRed and GelGreen most likely bind by a combination of intercalation and electrostatic interaction.
In which direction does GelRed/GelGreen migrate?	GelRed and GelGreen do not migrate through the gel as easily as EB. It is not necessary to add additional dye to the running buffer, and the gel will be stained more homogenously than that of EtBr.
Do GelRed and GelGreen need to be used in the dark?	GelRed and GelGreen are stable dyes. You can use the dyes in room light. However, we recommend storage of the dyes in the dark between uses. We have had a customer report using GelRed with success after accidentally leaving it in ambient light for one month.
How much GelRed/GelGreen do I need to use?	Dilute the 10,000X stock 10,000-fold for 1X precast gels (for example, 5 uL for a 50 mL gel), or 3,333-fold for a 3X post staining solution (15 uL for a 50 mL solution).
Can GelRed/GelGreen post-staining solution be reused?	Yes. However, if the sensitivity decreases, use a fresh solution of the dyes.
Can GelRed be used for Electrophoretic Mobility Shift Assay and Pulse Field Gel	Yes, GelRed can be used with EMSA and PFGE gels by post-staining.

Electrophoresis?	
Can GelRed/GelGreen be used for Southern or Northern blotting? Will it interfere with transfer or hybridization?	GelRed has been validated for Southern blotting (Plant Cell Report DOI:10.1007/s00299-011-1150-7). We recommend using the post-staining protocol for blotting applications.
Can GelRed be used in formaldehyde based RNA gels?	Yes, customers have reported using GelRed in glyoxal and formaldehyde agarose gels for precast staining of RNA.
Can GelRed be used for polyacrylamide, DGGE, EMSA or PFGE (pulse-field) gels?	Yes. Please use the post-staining procedure.
Can GelRed be used for Comet Assay?	Yes.
Is GelRed compatible with alkaline gel running buffer (30mM NaOH, 1mM EDTA)?	Yes.
Can GelRed be used for cesium chloride gradient purification of DNA?	Customers have reported using GelRed in cesium gradients. To extract GelRed from DNA after cesium banding, we recommend add SDS to a final concentration of 0.1% before butanol extraction. Alternatively, chloroform can be used instead of butanol for extraction.
What purification protocols are recommended to remove GelGreen/GelRed after staining?	Customers report good results using ZymoClean Gel DNA Recovery Kit from Zymo Research, GenElute Agarose Spin Column from Sigma, PureLink Quick Gel Extraction kit from Life Technologies, Illustra GFX PCR DNA and Gel Band Purification kit from GE Healthcare and High Pure PCR Product Purification Kit from Roche Applied Sciences.
Why do you offer GelRed and GelGreen in DMSO or water? Is there a difference between the dye in DMSO and water?	The water formulation is a newer and improved product compared to the stock in DMSO. We recommend using dyes in water to avoid the potential hazards of handling DMSO, which can be absorbed through the skin. We continue to offer dyes in DMSO because some users do not wish to alter their established laboratory protocols. Based on internal testing, both formulations perform similarly.