Protocol: qPCR with dsGreen

dsGreen is a very sensitive dye for the detection of double stranded DNA (dsDNA). Both dyes are used for non-specific detection of amplification in realtime qPCR experiments.

1. If the dsGreen reagent was stored below 20 °C, defrost it and keep it at room temperature. For rapid thawing, the reagent can be heated up to 50 °C.
2. Calculate the volumes of reagents required for the reaction according to the manufacturer's instructions to PCR reagents. Please note that the dsGreen (100x in stock) must be diluted in a PCR master-mix of up to 1x concentration.
3. Prepare a 1x master mix containing no DNA according to the manufacturer's instructions to PCR reagents, add necessary amount of dsGreen. Keep the master mix and PCR tubes on the ice if you use Taq-polymerase without hot start.
4. Transfer the master mix to tubes or plates and add DNA.
5. Proceed with amplification according to your instrument manufacturer.

Notes:

Always include positive and negative controls in your qPCR experiments. The temperature program for qPCR amplification does not differ from a standard PCR program for the given template and primers. For detection, the FAM channel should be used. In order to be able to distinguish a specific product from primer dimers, use the melting curve step in your PCR program.

Lumiprobe Corporation
201 International Circle, Suite 135
Hunt Valley, Maryland 21030
USA
Phone: +1 888 973 6353
Fax: +1 888 973 6354
Email: order@lumiprobe.com

Lumiprobe GmbH
Feodor-Lynen-Strasse 23
30625 Hannover
Germany
Phone: +49 511 16596811
Fax: +49 511 16596815
Email: de@lumiprobe.com

Lumiprobe RUS Ltd
Kotsyubinsky street, 4
121351 Moscow
Russian Federation
Phone: +7 800 775 3271
Email: ru@lumiprobe.com